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EVOO Research's Got Talent 2020

I Edition

Book of Abstracts

Bari, Italy, 20-22 January 2020

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social and education sciences, sustainability, circular economy and landscape,

nutraceutical and health properties and nutrigenomics.

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Program

EVOO Research's Got Talent 2020

Bari Italy - 20 – 22 January 2020 https://evooresearchgottalent.wordpress.com/

DATE	HOUR	LOCATION and CHAIRMAN	ACTIVITY
19 January 2020	19.00	BUO Restaurant- Bari	Registration and welcome cocktail
20 January 2020	8.30	**Conference Location** -Isolato 47 Str. Lamberti, 16- Bari Old City.	**Institutional greetings and presentation of the congress**
20 January 2020	9.00 - 10.20	**Chairman** Sacchi Raffele University of Studies "Federico II Naples" Italy	**Oral session: 20 minute speech** Francesco Bimbo Nicola Caporaso Marika Cariello Sara Carpi
20 January 2020	10.20 - 11.00	Conference Location	*Coffee break*
20 January 2020	11.00 - 12.20	**Chairman** Poiana Marco University of Studies "Mediterranea" Reggio Calabria Italy	**Oral session: 20 minute speech** Giovanni Caruso Lorenzo Cecchi Rita Celano Danny Cliceri
20 January 2020	12.20 - 13.20	**Chairman** Poiana Marco University of Studies "Mediterranea" Reggio Calabria Italy	**Oral session: 20 minute speech** Pasquale Crupi Alessandra De Bruno Stefania De Santis Pierfrancesco Deiana
20 January 2020	13.30 -14.30	Restaurant	*Lunch*
20 January 2020	15.00 – 17.00	**Chairman** Clodoveo Maria Lisa University of Studies "Aldo Moro " Bari Italy	**Dott. Ssa L. Santarnecchi. Polytechnic of Milan** *European financial support for young researchers: when are you ready for this challenge?*
20 January 2020	17.00 -18.00	**Chairman** Cavalluzzi Maria Maddalena University of Studies "Aldo Moro " Bari Italy	**Prof. A. Sannino -University of Salento** *A challenging workplace situation in lab: balancing the ability to work in independently and ability to work in team*
20 January 2020	18.00 -19.00		**Prof. N. Coniglio-University of Bari** *Learning from failure*
20 January 2020	21.00	Restaurant	Dinner EVOO experiences- Terranima-Bari

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DATE	HOUR	LOCATION	ACTIVITY
21 January 2020	9.00 - 10.20	**Chairman** Ocaña Moral. M. Teresa University of Jaen Spain	**Oral session: 20 minute speech** Elia Distaso Graziana Difonzo Valentina Fanelli Dennis Fiorini
21 January 2020	10.20 - 11.00	Conference Location	*Coffee break*
21 January 2020	11.00 - 12.20	**Chairman** Franchini Carlo University of Studies "Aldo Moro " Bari Italy	**Oral session: 20 minute speech** Federica Flamminii Silvia Grassi Lorenzo Guerrini Olusola Samuel Jolayemi
21 January 2020	12.20 -13.20	**Chairman** Aprea Eugenio University of Trento Italy	**Oral session: 20 minute speech** Maja Jukić Špika Carmen Lammi Francesco Longobardi M. Carmen López de las Hazas
21 January 2020	13.30 -14.30	Restaurant	*Lunch*
21 January 2020	15.00 – 17.00	**Chairman** Amirante Riccardo Polytechnic of Bari Italy	**Dott. P. Tamburrano – Polytechnic of Bari** *The Marie Skłodowska-Curie actions Individual Fellowships: fundamentals to apply to the call*
21 January 2020	17.00 -19.00	**Chairman** Fracchiolla Giuseppe University of Studies "Aldo Moro " Bari Italy	**Dott.ssa ML Divella- University Politecnica delle Marche** *Creative skills and ability to formulate new problems and ideas.*
21 January 2020	19.00 -20.00	**Chairman** Fracchiolla Giuseppe University of Studies "Aldo Moro " Bari Italy	**Prof.ssa A. Manuti- University of Bari** *A challenging workplace situation in lab: competitiveness and agonism*
21 January 2020	21.00	Restaurant	Dinner EVOO experiences

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DATE	HOUR	LOCATION	ACTIVITY
22 January 2020	9.00 - 10.20	**Chairman** Mulinacci Nadia University of Studies of Florence Italy	**Oral session: 20 minute speech** Maria Paciulli Diamantakos Panagiotis Elisa Pannucci Maja Podgornik
22 January 2020	10.20 - 11.00	Conference Location	*Coffee break*
22 January 2020	11.00 - 12.20	**Chairman** Casiraghi Ernestina University of Studies Milan Italy	**Oral session: 20 minute speech** Aimilia Rigakou Annalisa Silenzi Federico Stilo Annia Tsolakou
22 January 2020	12.20 -13.20	**Chairman** Casiraghi Ernestina University of Studies Milan Italy	**Oral session: 20 minute speech** Enrico Valli Alessandro Vivaldi Jing Yan
22 January 2020	13.30 -14.30	Restaurant	*Lunch*
22 January 2020	15.00 – 17.00	**Chairman** Corbo Filomena University of Studies "Aldo Moro " Bari Italy	**Dott. G. Torrisi European Research Council Executive Agency** *European Research Council (ERC) support for young researchers: Starting Grants and Consolidator Grants.*
22 January 2020	17.00 -18.00	**Chairman** Corbo Filomena University of Studies "Aldo Moro " Bari Italy	**Prof. A. Moschetta-Universsity of Bari** *How important is ethics in research?*
22 January 2020	18.00 -19.00	**Chairman** Clodoveo Maria Lisa University of Studies "Aldo Moro " Bari Italy	**Prof.ssa T.Gallina Toschi-University of Bologna** *Ability to create an international partnership able to meet the challenge of European call*
22 January 2020	21.00	Restaurant	Dinner EVOO experiences EVOO Excellence in Research Award Ceremony

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meeting with the Italian stakeholder

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Index of abstract

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GI'S Implicit Price Drivers: the Italian EVOO Market

Bonanno, A.[1], *Bimbo, F.*[2], Costanigro, M.[1], Viscecchia, R.[3]

[1]*Department of Agricultural and Resource Economics, Colorado State University, Fort Collins, CO, USA 80524.*
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[3]*Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Università degli Studi di Foggia, Via Napoli 25, Foggia 71122, Italy.*

Keywords: Geographical Indications, EVOO, Hedonic Price Model.

Objectives

Agri-food companies can use their brand to market the food products or complying with geographical indication (GIs) production standards and use collective labels, alternatively, they can use both individual brands than collective labels. Individual and collective labels add value to food products by signaling the reputation of sellers or of the coalition to which farmers belong to. Thus, this work aims to assess to what extent GIs add value to the product having after one has accounted for the individual brand name, and once assess the GI premium price we explore its drivers.

Methods

We use a scanner database of Extra-Virgin Olive Oil sales in the Italian market, an hedonic price model and an Unconditional Quantile Regression estimator to assess the dispersion of GIs' implicit prices along two dimensions: for each GI across the price distribution; and at specific points of the price distribution across GIs. Then we regress the implicit prices versus market structural variables and GI related features in order to assess in the what measure the latters contribute to explain the GI's added value.

Results

Large implicit price heterogeneity is detected for the many PDO/PGI available in the market as well as at the different price levels. The larger the number of operators, the smaller is the GIs IPs across the entire price distribution while higher is the number of GI products sold in a market (region), larger is their implicit price most likely related to higher consumers' awareness to GI products. GIs sold in many regions as well as under multiple brands benefit of lower premium price.

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Innovations in extra virgin olive oil quality assessment and uses: new analytical methods and new "eating experience"

Caporaso N.

Department of Food Sciences, School of Biosciences, The University of Nottingham, Nottingham (UK)
Department of Agricultural and Food Sciences, University of Naples "Federico II", Naples (Italy)

Keywords: Extra virgin olive oil, analytical methods, non-destructive analysis, olive oil quality, molecular gastronomy, innovation.

Abstract

This contribution will present some new insight into innovative methods for detecting and assessing food quality and composition, with its possible application for extra virgin olive oil (EVOO) quality assessment. In particular, rapid and non-destructive methods based on NIR spectroscopy and hyperspectral imaging can be conveniently applied to several stages of the olive production chain, from the field assessment of the fruit quality, to the on-line implementation of such technologies in the olive mill, to the final product and even directly from the bottled product on the shelf. In this context, data analysis (chemometrics) is of paramount importance, and calibrations have be built carefully, with innovative methods to update them remotely.

Innovations also exist in the field of EVOO not only in the analytical methods but also in its use as an ingredient. Both in traditional and new consumer countries, the interest for EVOO as opposed to other fats has fostered the creation of new dishes and the pairing with other ingredients in order to create "new eating experience", also in the so-called Molecular Gastronomy discipline. The great variability of EVOO sensory characteristics, depending on variety, location and technological factors, allowed innovative chefs to investigate new pairing concepts based on the flavour network. The latest research in this context will be presented and discussed, with the aim to fostering the debate and thus valorise EVOO for new culinary applications.

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Peripheral blood mononuclear cells (PBMC) transcriptomic profile in healthy subjects and metabolic syndrome patients after acute intake of extra virgin olive oil

Cariello M.

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Keywords: Extra Virgin Olive Oil, nutrigenomics, PBMC, gene expression

Objectives

Extra virgin olive oil (EVOO) contains high levels of monounsaturated fatty acids (MUFAs; oleic acid: 70–80%), and a number of biologically active minor components (phenolic compounds) playing a role in health promotion. EVOO consumption has been associated with reduced cardiovascular risk but molecular mechanisms underlying its beneficial effects are not fully understood. Here we aimed to identify genes and miRNAs expression changes mediated by acute high- and low-polyphenols EVOO intake.

Methods

Pre- and post-challenge gene and miRNAs expression analysis was performed on the peripheral blood mononuclear cells (PBMCs) of 12 healthy subjects and 12 patients with metabolic syndrome (MS) through microarray and RTqPCR.

Results

In healthy subjects, acute intake of EVOO rich in polyphenols (Coratina cultivar) ameliorated glycaemia and insulin sensitivity and modulated the transcription of genes and miRNAs involved in metabolism, inflammation and cancer switching PBMCs to a less deleterious inflammatory phenotype; weaker effects were observed in patients with MS as well as in healthy subjects following low-polyphenol EVOO (peranzana cultivar) challenge. Our study shows that acute high-polyphenols EVOO intake is able to modify the transcriptome of PBMCs through the modulation of different pathways associated with the pathophysiology of cardio-metabolic disease and cancer. These beneficial effects are maximized in healthy subjects, and by the use of EVOO cultivars rich in polyphenols. Nutrigenomic changes induced by EVOO thus legitimate the well-known beneficial effects of EVOO in promoting human health and, potentially, preventing the onset of cardiovascular disease and cancer.

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The extra-virgin olive oil polyphenols oleocanthal and oleacein counteract inflammation-related gene and miRNA expression in adipocytes

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Keywords: EVOO; gene expression; inflammation adipocyte; obesity; oleocanthal; oleacein; miRNA; NF-κB

Objectives

Inflammation of the adipose tissue plays an important role in the development of several chronic diseases associated with obesity [1-2]. Polyphenols of extra virgin olive oil (EVOO), such as secoiridoids oleocanthal (OC) and oleacein (OA) have many nutraceutical proprieties [3-9]. However, their roles in obesity-associated adipocyte inflammation, the NF-κB pathway and related sub-networks have not been fully elucidated. Here, we investigated impact of OC and OA on the activation of NF-κB and the expression of molecules associated with inflammatory and dysmetabolic responses.

Methods

To this aim, fully differentiated Simpson-Golabi-Behmel syndrome (SGBS) adipocytes were pre-treated with OC or OA before stimulation with tumor necrosis factor (TNF-α).
Levels of mRNA gene expression as well as cell and exosomal miRNAs were measured by real-time PCR. Being the transcription factor NF-κB a master regulator of the expression of genes and miRNAs, we examined the effect of OC and OA on the ability of TNF-α to activate NF-κB in human adipocytes. Finnaly, in order to rationalize the experimental results obtained, molecular modeling studies, including docking, molecular dynamics simulations and ligand-protein binding energy evaluations, were carried out.

Results

EVOO polyphenols significantly reduced the expression of genes implicated in adipocyte inflammation (interleukin-1β (IL-1β), cyclooxygenase-2 (COX-2)), and angiogenesis (vascular endothelial growth factor (VEGF) and its receptor kinase insert domain receptor (KDR), metalloproteinase-2 (MMP-2)), oxidative stress (NADPH oxidase), leukocytes chemotaxis and infiltration i.e. monocyte chemoattractant protein (MCP-1), C-X-C Motif Ligand 10 (CXCL-10), macrophage colony-stimulating factor (M-CSF). Accordingly, miR-155-5p, miR-34a-5p and let-7c-5p tightly connected with the NF-κB pathway, were deregulated by TNF-α in both cells and exosomes. The miRNA modulation by TNF-α was significantly counteracted by EVOO polyphenols.
Computational studies suggested a potential direct interaction between OC and NF-κBat the basis of its activity.

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This study demonstrates that OC and OA counteract adipocyte inflammation attenuating NF-κB activation. Therefore, these compounds could be novel dietary tools for the prevention of inflammatory diseases associated with obesity.

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Precision olive growing: a modern management for an ancient tree

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Keywords: Canopy volume, leaf area index, NDVI, unmanned aerial vehicle

Objectives

Biophysical and geometrical parameters are crucial for olive orchard management as they are related to vegetative growth, fruit yield and quality. Since measuring these parameters manually is labor intensive, unmanned aerial vehicles (UAV) have been proposed as alternative due to their great flexibility in flight scheduling and the increasing availability of dedicated miniaturized sensors. The aim of this work was to assess the ability of an UAV platform and VIS-NIR camera techniques in estimating biophysical and geometrical parameters of olive tree in intensive olive orchards located in Tuscany and Sicily.

Methods

The capability of VIS-NIR cameras and UAV platforms in determining biophysical and geometrical canopy parameters was tested in three olive orchard located in Tuscany and Sicily, characterized by different cultivars and planting distances. Images were acquired using an S1000 UAV octocopter equipped with a consumer photo-camera (RGB) and a multispectral camera (NIR-RG) mounted on a 2-axis stabilized gimbal. The multispectral images were first mosaicked, then georeferenced and orthorectified by using the known ground-referenced points. The Normalized Difference Vegetation Index was calculated employing the map algebra technique implemented in GIS software. Canopy height, diameter and the three-dimensional canopy volume were reconstructed starting from the point cloud and the digital surface model (DSM). In order to test the ability of UAV imagery to estimate the biophysical and geometrical tree parameters, the data sets obtained from the RGB-NIR cameras (tree canopy height, diameter, volume and NDVI) were compared with the ground measurements (tree canopy height, diameter, volume and LAI) by linear regression.

Results

The NDVI and canopy volume estimated by UAV-NIR camera technique was well correlated with on-ground measurements, confirming previous findings. The slight underestimation of the UAV-estimated canopy volume can be reasonably interpreted as the result of the irregular shape of the olive canopy and higher accuracy of the structure from motion technique with respect to the on-ground-measured values. The estimation of specific geometric parameters, such as canopy height and canopy projection area, also proved successful. Our results confirm that the combined use of VIS-NIR cameras and UAV platforms can be used to adequately estimate biophysical and geometrical parameters such as LAI, tree height, and canopy volume in high-density olive orchards.

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Optimization of HS-SPME-GC-MS Quantitation of Volatile Compounds and Application to more than 1000 Samples for Supporting the Panel Test in Virgin Olive Oil Classification and For Authentication of the Geographic Origin

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Keywords: sensory analysis, sensory defects, chemometrics, rancidity indices, microbiological indices

Objectives

Extra Virgin Olive Oil (EVOO) is considered as the highest quality product among edible oils, and economic frauds regarding false claim of geographical origin and commercial category cannot be fully avoid to date [1,2]. The use of HS-SPME-GC-MS analysis for quantification of volatile organic compounds (VOCs) has gained great attention in the last years and the possibility of using reliable chemical data from this analysis, has been recognized as more and more crucial for support both the virgin olive oil classification and the authentication of its geographical origin [3].

In this study, we collected and analyzed more than 1000 virgin olive oil samples over three olive oil campaign, with the aim of developing chemometric approaches for the quality control of virgin olive oil. To reach this goal, we optimized and validated the HS-SPME-GC-MS quantification of 73 Volatile Organic Compounds (VOCs) of virgin olive oil by using several internal standards. The validated method was the used for the analysis of approx. 1200 oil samples; the obtained set of data has been analyzed using several statistical tools in order to propose reliable and robust approaches suitable to support the panel test in virgin olive oil classification and to authenticate the geographical origin of virgin olive oils from the main worldwide producing countries.

Methods

More than 1000 virgin olive oils from different geographical origin (Italy, Spain, Greece, Tunisia, Portugal) and category (EVOO, VOO, LVOO) were collected in the olive oil campaign 2016/17, 2017/18, 2018/19, and analyzed by both HS-SPME-GC-MS and Panel Test. The quantitative analysis was optimized using up to 11 internal standards and the validated method was applied to the samples using a 6890N GC system equipped with a MS detector, model 5975 by Agilent. A HP-Innowax capillary column 50 m × 0.2 mm ID, 0.4 µm DF was employed. A SPME fiber 50/30 µm DVB/CAR/PDMS was exposed under orbital shaking at 400 rpm for 20 min in the headspace of a 20 ml screw cap vial fitted with a PTFE/silicone septa containing 4.3 g of sample and 0.1 g of internal standard solution vial, then the adsorbed VOCs were desorbed in the injection port of the GC system. Mass detector worked in scan mode within the range of 30-350 Th, 1500 Th/s at ionization energy of 70 eV. Each VOC was quantified using a calibration curve in which the area ratio (ratio between areas of that VOC and the selected ISTD) was plotted versus the amount ratio.

Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), Analysis of Variance (ANOVA) and *t*-test were employed for data analysis, aiming at building the chemometric approaches above mentioned.

Results

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The method for quantification of 73 VOCs was optimized and validated using 11 internal standard and selecting the more suitable one for each of the quantified VOCs, allowing obtaining reliable data in wide ranges of calibration in spite of the several critical issues pointed out for quantitative analysis by HS-SPME-GC-MS. The method was then used for analyzing the selected samples for building chemometric approaches aimed to:

- Supporting the panel test in virgin olive oil classification (n=1223)

- Authenticate the geographical origin of virgin olive oils from the main worldwide producing countries (n=1217).

The proposed approaches were built and internally validated using a training-set, and then externally validated using a set of independent samples. The PCA-LDA model gave the best results for supporting the panel test in virgin olive oil classification, while a simplified model allowed obtaining a very good predictive capability only using data from 10 VOCs, thus strongly simplifying the analytical work.
Our data also allowed pointing out octane, heptanal, pent-1-en-3-ol, *Z*-3-hexenal, nonanal and 4-ethylphenol as the more suitable VOCs for discriminating the different classes of virgin olive oils (i.e., extra virgin olive oil and virgin olive oil defective for different kinds of defects).
The ANOVA-LDA model, only using 25 selected VOCs was the best approach for authentication of the geographical origin of virgin olive oils from the main worldwide producing countries, with a prediction capability higher than 97% for some specific origins.
In conclusion, reliable and robust chemometric approaches suitable for virgin olive oil quality control have been proposed based on quantitative data obtained by an optimized HS-SPME-GC-MS quantitation method, developed using several internal standard. The proposed approaches allow protecting consumers and producers from incorrect claiming of commercial classification and geographic origin.

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Novel insights into analysis of phenolic secoiridoids in extra virgin olive oil

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Keywords: extra virgin olive oil; phenolic secoiridoids; health claim; (+) and (−)-UHPLC-HRMS analysis; artefacts

Objectives

Extra virgin olive oils (EVOOs) containing more than 5 mg/20 g tyrosol, hydroxytyrosol, and their secoiridoids can be recognized by health claims related to the protection of blood lipids from oxidative stress. Therefore, a reliable, accurate, and standardized analytical procedure is needed to determine these markers of EVOO quality. In order to overcome the limitations of current methods, a detailed investigation of sample preparation and chromatographic conditions was performed by UHPLC-UV-HRMS. The main aims were therefore: i) to enhance the chromatographic resolution; ii) to investigate the formation hemiacetals and acetals from secoiridoids during the sample preparation; and iii) to define the most reliable conditions to provide an analytical method with suitable accuracy and repeatability.

Methods

EVOO phenolic compounds were extracted following the IOC method. To determine the total amount of free and linked p-HPEA and 3,4-DHPEA in EVOO phenolic extracts, the acid hydrolysis procedure was used. The qualitative determination of EVOO phenolic profile was made by UHPLC-HRMS analyses. Compound were characterized according to the corresponding HRMS spectra, accurate masses, characteristic fragmentation, and retention time. For quantitative determination of *p*-HPEA, 3,4-DHPEA and secoiridoids in EVOO phenolic extracts and hydrolysates, the UHPLC-UV analyses was used. *p*-HPEA and 3,4-DHPEA were employed as reference standards.

Results

The use of a not acidified UHPLC gradient provided single sharp peaks for oleocanthal and oleacin. UHPLC-HRMS/MS investigation revealed the formation of dimethyl-acetal, methyl-hemiacetal and monohydrate derivatives of secoiridoids in aqueous methanolic solution, making less accurate and reproducible the measurements. Acetonitrile proved a suitable solvent to avoid the formation of dimethyl-acetals and methyl-hemiacetals and extract efficiently EVOO phenols. Secoiridoid contents of EVOO samples were determined before and after acid hydrolysis. Both methods afforded comparable levels, highlighting the usefulness of acid hydrolysis in routine analyses. Use of tyrosol and hydroxytyrosol equivalents provide more accurate quantitative data.

The improved procedure defines the most accurate and reproducible conditions for analysis of healthy and functional EVOO phenols.

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Individual variability in the perception of extra-virgin olive oil: relations between critical oral sensations and flavor determinants

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Keywords: Dynamic perception, volatile organic compounds, individual variability

Objectives

The perception of oral sensations critical for product acceptability show individual variability, generating different sensory sensations among different individuals. Extravirgin olive oil (EVOO) induces three of the most critical oral sensations: bitter, pungent and astringent. A different individual sensitivity to these sensations suggests a different perception of EVOO during consumption, with a possible effect on flavour perception.
In this context, the release kinetics of volatile organic compounds (VOCs) from EVOO matrix within the oral cavity should be accounted for considering the complex processes influencing the evolution of sensory attributes detected by tasters. These effects are typically considered in sensory and instrumental analyses in a static point of view, suggesting the adoption of methods that allow a measure during consumption, in order to better understand EVOO perception processes. Among sensory methods, Temporal Check-All-That-Apply (TCATA) is an affirmed and robust technique to measure the perception of multiple attributes during consumption. Regarding instrumental analysis, Proton Transfer Reaction-Mass Spectrometry coupled to Time-of-Flight (PTR-ToF-MS) analyser offers the possibility to follow the VOCs release in the mouth in real time.
Considering these assumptions, the aim of the study was to investigate individual differences in temporal perception of sensation that are critical for EVOO acceptability and explore if the perceived differences for critical sensations have an effect on the temporal perception of flavor attributes, in relation to the contribution of VOCs released.

Methods

Nine samples of Italian EVOO P.D.O. were selected on the base of their sensory variability and absence of sensory defects. The individual sensitivity of 19 subjects (mean age: 45.2, 9 women) for bitterness, pungency and astringency was measured with a suprathreshold intensity test evaluating for each attribute the intensity on LMS of 5 water solutions (10ml) at increasing concentration and in double reply.
Subsequently, in the first phase of evaluation, all subjects familiarized themselves with the sensory properties of EVOO samples and were trained to the T-CATA method. After evaluation of panel performances on the use of 7 descriptors (bitter, pungent, astringent, almond, artichoke, grass and tomato), the panel evaluated 6 of the 9 samples (3 g) for 90 seconds in triple reply., presented in a randomized order in sensory booths under red light.
In the second phase of evaluation, a subgroup of 10 subjects (mean age: 41.5, 5 women) differing for individual sensitivity toward critical sensation was involved in a T-CATA with the remaining 3 samples, following the same tasting procedure of the previous phase.

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In parallel with tasting, VOCs released during consumption were collected fitting a single-usage ergonomic nose-piece rubber tube into the nostrils of the assessors. The nose-piece was connected to the PTR-ToF-MS in order to perform in vivo the VOCs analysis.

Results

Hierarchical cluster analyses on intensity ratings in water solutions allowed to obtain two clusters for each critical sensation. Bitterness and astringency were the attributes with the higher variability among clusters. Considering the entity of the variability and cluster size, two clusters of subjects varying for the sensitivity toward bitterness were selected: high bitter tester (HBT) (8 subjects, mean age: 47.8, 3 females) and low bitter taster (LBT) (11 subjects, mean age: 43.2, 6 females).

For each attribute, temporal curves for the 6 samples from the first phase were compared among LBT and HBT clusters, highlighting an effect on the critical sensation during the evaluation. Compared to LBT, HBT selected with a higher frequency the attributes bitter (mean area: LBT = 4137, HBT = 5379, p-value = 0.002; max citation proportion: LBT = 73, HBT = 88, p-value = 0.001) and astringent (mean area: LBT = 1732, HBT = 3963, pvalue < 0.001; max citation proportion: LBT = 73, HBT = 88, p-value < 0.001). In the case of pungency, HBT resulted higher than LBT in term of mean area of the curves (LBT = 4079, HBT = 4999, p-value = 0.002), while the maximum of citation proportion resulted not significantly different (LBT = 79, HBT = 81, p-value = 0.661).

The arbitrary time frame of maximum global impact of critical attributes was identified after visual inspection of the TCATA curves (20 to 50 seconds). Within this time interval, flavor attributes resulted to be influenced differently on the base of the cluster: while tomato, almond and grassy attributes tend to be suppressed in intensity by a higher perception of critical attributes, the artichoke attribute tends to be exalted.

Results on the relation between VOCs and sensory perception are currently under analyses at this stage of the study and will be reported in next communications.

Conclusions

In the current state, the study highlighted that the oral sensations critical for acceptability may be perceived differently during consumption on the base of individual sensitivity toward critical oral sensations, with a notable variability in term of bitterness and astringency. This effects resulted to have a role in the temporal perception of specific flavour attributes (e.g. tomato) and consequently on the consumption experience, aspect to be considered in order to interpret and meet consumers' preferences.

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Flavored olive oil produced by different techniques: organoleptic and nutraceutical quality

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Keywords: *Origanum vulgaris L. Thymus vulgaris L. Olea europaea L.* Total phenol content. Radical scavenging activity

Objectives

The goal of the study was to verify the effect of three different flavoring treatments of olive oil on the quality of the product, assessed by means of the chemical characteristics, the phenol composition, and the radical scavenging activity (RSA) of the resulting oils.

Methods

The flavoring methods consisted in 1) the infusion of two herbs (thyme or oregano) into the oil, 2) the addition of herbs to the crushed olives before the malaxation step during the extraction process, and 3) the implementation of the ultrasound before the olive paste malaxation. Afterwards, quality indices, polyphenols by Ciocalteu reagent and HPLC-UV-MS/MS analysis, and RSA by reduction of DPPH in methanol were measured.

Results

The less favorable method was the addition of herbs directly to the oil. A positive effect was achieved by the addition of herbs to the olive paste further improved by the employment of ultrasound. The last two methods allow to produce oils "ready to sell", instead the infused oils need to be filtered. Moreover, a significant increment of phenolic content and radical scavenging activity of olive oils was determined, too. The increments were higher when oregano is used instead of thyme. Ultrasound inhibited the olive polyphenoloxidase, the endogenous enzyme responsible for olive oil phenol oxidation.

In conclusion the olive paste mixed with herbs before malaxation was revealed as the most favorable method due to the best efficiency, reduced time consumption and minor labor, enhancing the product quality of flavored olive oil.

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Evaluation of enrichment with Phenolic Extract from olive oil mill wastes in different model system

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Keywords: Antioxidant Activity, Enrichment, Functional Food, Olive Mill Waste Water, Pomace.

Objectives

The production of olive oil is accompanied by the generation of aqueous and solid wastes that represent a serious environmental problem due to its highly polluting organic load arising from polyphenol content with low biodegradability. At the same time, Olive Mill Waste Waters (OMWW) could be valorized for their extraordinary content of bioactive compounds (about 30 phenolic compounds), therefore as a potent source of natural antioxidants. The recovery of phenol compounds contributes to the sustainability of olive sector reducing the waste environmental impact and allowing to obtain extracts which can be antioxidant ingredients to be used in food industry and other ones. The main objective of the research was to optimize the recovery of phenols from wastes (pomace and water) produced in the South of Italy and obtain functional food by the use of the obtained phenolic extracts (PE).

Methods

The Olive Oil Waste (pomace and water) of Carolea and Ottobratica cultivars were obtained during different crop seasons (2016-2017-2018), produced according to a three-phase centrifugation process. Pomace extraction was carried out using the analytical methodology described by Lafka et al. 2011, appropriately modified. The method reported by De Marco et al. (2007) was instead performed for OMWW with some modifications. Other extraction methods with different solvents and dynamics have been also applied. Antioxidant activity, Total and individual phenolic compounds content were evaluated for each extract. Subsequently, the PEs with the best characteristics were used to enrich two different matrices: sunflower oil and water. The sunflower oil was enriched with 50 mg of hydroxytyrosol L^{-1} of extract and lecithin; the enriched water (EW) was obtained by adding of Tyrosol (50 and 100 mg L^{-1}). Physicochemical and antioxidant determinations were performed to verify the effectiveness of the enrichment.

Results

Higher yield of phenolic compounds was obtained from OMWW than from olive pomace, with values higher than 17 g L^{-1}, and a significant amount of tyrosol and hydroxytyrosol, whereas 1.7 g gallic acid kg^{-1} were extracted from the pomace extract. Considering the high total phenol content phenolic composition of OMWW compared to olive pomace extract, this extract was considered a valuable sources of antioxidant component for next addition to model food. The antioxidant stability of enriched sunflower, was evaluated and the principal results confirmed a good stability of the sample after the enrichment and also during the storage. The evaluation enriched water model system, showed a maintaining of antioxidant capacity after the adding of the low-phenolic extract. This research demonstrates the possibility to valorize the olive wastes as source of antioxidants that can be successfully used for functional foods production.

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Intestinal deletion of Stearoyl-CoA Desaturase-1 induces inflammation and tumorigenesis reversed by dietary oleic acid

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Keywords: Scd1, oleic acid, nutraceutics, human health

Objectives

Stearoyl-CoA Desaturase 1 (SCD1) is an enzyme catalyzing the biosynthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA) that can be either derived from the diet or synthesized de novo, with oleic acid as the main product. Given the putative role of fatty acids in the regulation of cellular proliferation, inflammation and cancer metabolism, we focused on the role of Scd1 and its product oleic acid in intestinal physiological and pathological state.

Methods

We generated mice with a selective disruption of Scd1 in the intestinal epithelium (iScd1–/– mice) on a C57BL/6 background; iScd1+/+ mice were used as controls. We also generated iScd1–/–ApcMin/+ mice to study cancer susceptibility. Mice were fed a chow, oleic acid-deficient, or oleic acid-rich diet. Intestinal tissues were analyzed by histology, Real-time Polymerase Chain Reaction, immunohistochemistry, mass spectrometry, and flow cytometry and tumors were quantified and measured.

Results

Ileal mucosa of iScd1–/– mice showed a reduction of the Δ9 desaturation ratio with a lower proportion of palmitoleic and oleic acids and an accumulation of stearic acid relative to control mice. Ileal tissues from iScd1–/– mice had also an increased expression of inflammatory markers and crypt proliferative genes compared to control mice. The iScd1–/–ApcMin/+ mice developed more and larger tumors than iScd1+/+ApcMin/+ mice. iScd1–/–ApcMin/+ mice fed with oleic acid-rich diet had reduced intestinal inflammation and significantly lower tumor burden compared with mice fed a chow diet. Overall, we demonstrated that intestinal SCD1 is required for the synthesis of oleate in the enterocytes and the maintenance of fatty acid homeostasis. Importantly, dietary supplementation with oleic acid reduces intestinal inflammation and tumor development in mice.

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Characterization and valorisation of Sardinian olive germplasm

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Keywords: local varieties; phenolic compounds; fatty acids; squalene; α-tocopherol; growing season

Objectives

The ongoing research project aims first to valorize the rich Sardinian olive germplasm, with a special regard to the less known local varieties, trying to identify the specific favourable features. Principally, the research aims to describe in dept the chemical composition of the mono-varietal virgin olive oils obtained from 21 local varieties, and compare them both with each other and with 5 well widespread Italian cultivars, used as a term of comparison. Knowledge of the characteristics of monovarietal VOOs could provide helpful guidelines to local producers on the selection of the most proper varieties. The rediscovery and enhancement of the peculiar characteristics of regional olive germplasm is a great opportunity for the enhancement of traditional cultivation areas and, perhaps, for the promotion of second level designations inside the regional label PDO "Sardegna".

Methods

The research took place in the olive germplasm collection of the University of Sassari (Oristano: 39°54′12″ N, 8°37′19″ E) under controlled environmental, agronomic, and technological conditions. Olive samples from adult trees have been harvested during 4 harvest seasons (from 2015 to 2018), and the oil extraction process was carried out through a low scale industrial mill "Sintesi 80" Mori TEM (Tavernelle Val di Pesa, Italy). In order to evaluate properly the chemical and nutraceutical properties of VOOs, phenolics, squalene and tocopherols were determined by HPLC-DAD, whereas fatty acid composition by GC-MS.

Results

A detailed description of monovarietal VOOs chemical composition was provided. All the varieties analysed, both local Sardinian and Italian, revealed high content of bioactive molecules. Varietal differences and similarities observed were in line with those described by genetic studies that stress on the presence of varietal groups mainly related to the principal Sardinian varieties: Bosana, Tonda di Cagliari, Semidana, and Nera di Oliena. These varietal groups, together with other local varieties, expressed some specific characteristics: Bosana group and Sivigliana da Olio had the highest phenolic content; Nera di Oliena group and Corsicana da Olio were the varieties with the highest oleic acid relative content; Tonda di Cagliari and similar varieties were good sources of squalene.

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A numerical analysis as innovation accelerator: the design of an innovative ultrasound device for improving extra virgin olive oil yield and quality

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Keywords: Numerical simulation-based design; cavitation; ultrasound treatment; extra virgin olive oil.

Objectives

This work describes how 3D multiphase fluid dynamic simulations were employed in the design process of an innovative device, named Sono-Heat-Exchanger (SHE), that represents the first in the World continuous full-scale device combining the effects of a heat-exchanger with those of plate-shape ultrasonic transducers. The SHE is a revolutionary device able to drastically impact on the Extra Virgin Olive Oil (EVOO) extraction process, because it allows, for the first time, the simultaneous increase of the extraction yield and the phenols content. A thorough numerical analysis was used for deeply studying the complex interaction between the non-Newtonian olive oil paste and the oscillating pressure waves generated by cavitation phenomena, which are induced by the ultrasound treatment. The calculations aimed at evaluating the flow parameters able to influence the process, thus avoiding expensive and time-consuming preliminary experimental tests for defining the optimal characteristics of the SHE. So that, the numerical analysis turned out to be essential in bridging the technology readiness "valley of death", advancing the device's TRL - Technology Readiness Level - beyond 6 and reaching level 9.

Methods

The SHE is composed of a couple of concentric annular sections. The olive paste flows into the external annular section, while water flows through the internal annular section to control the temperature of the olive paste. Outside of the external annular flow section, a plate-shape transducer is placed on each side of an octagon-shaped surface. The SHE allows to condition the olive oil paste with an ultrasound treatment, keeping, at the same time, its temperature under control and within the most favourable range for the EVOO extraction. As a result, it is possible to increase the extraction yield without the need of increasing the process temperature and affecting the product quality.

The fluid dynamic analysis was performed by means of the commercial software Ansys Fluent 17,1. The flow was modelled as laminar, due to the high viscosity of the olive paste. The olive oil paste was modelled as non-Newtonian compressible-liquid, following the Tait equation. In order to simulate the unsteady cavitating flow, the Schnerr-Sauer model was adopted. The oscillating frequency of the transducers was set to 23 kHz and a time step equal to a tenth of a single oscillation period was adopted. A whole structured dynamic grid was used to mesh a quarter of the geometry, for reducing the computational time. The best compromise suggested by a grid convergence analysis consisted of about one million of elements.

Results

In the case of the olive paste, ultrasound-induced cavitation promotes the disruption of tissue structures, enhancing the release of nutraceutical compounds. This means that everything happens at very small scales and in a very short time. The numerical approach allowed to

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take a closer look at the phenomena occurring within the device, providing an analysis that would have resulted prohibitive to perform by means of a solely experimental-based approach.
Initially, the vapour appears in regions located extremely close to the transducer surfaces. Once formed, the vapour bubbles start to increase, as could be inferred from the fact that the vapour fraction increased accordingly in the simulations. An interesting result is that, in a given point, cavitation occurs with a periodicity that does not necessarily match the transducers oscillating frequency. This behavior is due to the fact that the pressure waves produced by a single transducer are not free to propagate in the liquid medium, but they experience many complex interactions with other negative or positive pressure waves generated both by wall reflections and other adjacent transducers. The study of such interactions allowed the definition of the optimal cross-section height for the SHE, as the best compromise between the need of maximize the ultrasound effects and that of avoiding undesired interference effects between adjacent transducers

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Strategies to promote the olive oil chain sustainability: the recovery of valuable compounds from waste and by-products

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Keywords: by-products, olive leaves, olive pomace, functional compounds

Objectives

By-products and waste from both olive orchards and the olive oil industry are an important environmental issue. Despite the low value assigned to wastes, these are rich in useful functional compounds. Thus, our principal aim was the valorization of olive chain wastes and by-products by means of functional compounds extraction from olive leaves and pomace and the subsequent use as to improve the shelf-life of foods as to assay the biological activity.

Methods

OLE (olive leaf extract) was produced at laboratory scale, thus chemically characterized for its polyphenols profile by UHPLC-ESI-MS*n*. Moreover, with the aim of testing the effectiveness of OLE in extending foods shelf-life, these were added in baked goods and olive-based vegetable products.by monitoring oxidation pathway, sensory parameters and microbiological aspects. The effect of OLE cytoprotection was assessed in renal cells treated with cadmium. Moreover, β-sitosterol and α-tocopherol extraction from olive pomace and quantitation were carried out by means of GC-FID.

Results

The baked snacks added of OLE showed a significantly lower level of volatile compounds originated from lipid oxidation and an increased antioxidant activity than control. In olive based-paste, the main microbial groups were negatively affected by OLE addition, including the spoilage microorganisms. Moreover, the toxic cellular responses due to cadmium were counteracted by co-treatment with OLE and exerted antioxidant actions.

The extraction of β-sitosterol and α-tocopherol from olive pomace (from two and three phases oil extraction systems) by means of SC-CO2 was optimized and the compounds were quantified by GC-FID.

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A robust DNA isolation protocol from commercial EVO oil for PCR-based approaches for traceability and authentication

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Keywords: DNA extraction protocol; traceability; authentication; genetic tagging; SSRs; SNPs

Objectives
Extra virgin olive oil (EVOO) has elevated commercial value due to its health appeal, desirable characteristics and quantitatively limited production, and thus it has become an object of intentional adulteration. As EVOOs on the market might consist of a blend of olive varieties or sometimes even of a mixture of oils from different botanical species, an efficient DNA fingerprinting method is indispensable to check the varietal composition of the blend.
Methods
Analyses were performed on three EVOO samples, provided by an Italian mill. The DNA was isolated through a timely and effective protocol developed after a comparison between four publicly available DNA extraction protocols. Then, in order to verify the effectiveness of the DNA extraction protocol herein proposed, we performed the analysis of DNA polymorphisms of nine simple sequence repeat (SSR) coupled with eight SNP markers.
Results
Genetic traceability of EVOOs requires DNA to be extracted from commercial olive oils, which are all subjected to a filtration process in order to satisfy consumers' demand (i.e., clear oil). To identify an effective and robust DNA extraction protocol from filtered commercial oils, four protocols were tested and compared each other. However, none of these proved to be highly reproducible. Therefore, we decided to improve one of them with some key modifications in order to enhance the quality and quantity of the extracted DNA while reducing the starting material, reagents, costs and time of extraction. The developed DNA isolation protocol was robust and highly reproducible. The SSR markers were able to define genetic patterns of the three EVOOs, and the SNP markers were used to verify the presence of Italian olive cultivars among the most representative used in the PDO and PGI certifications.
The proposed investigation strategy might favor producers in terms of higher revenues as well as consumers in terms of price transparency and food safety.

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Development of methods and their application to move toward a wider understanding of the different aspects contributing to extra virgin olive oil quality

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Keywords: extra virgin olive oil; quality; authentication; fingerprinting; chemical analysis; sensory analysis; prebiotic activity

Objectives

The studies carried out these last years were aimed: 1) to develop new methods to analyse bioactive substances found in extra virgin olive oil (EVOO), as polar phenolics and squalene; 2) to assess differences in terms of quality between low price and high price EVOOs (LEVOOs and HEVOOs, respectively) found on the Italian market, by performing a comprehensive chemical and sensory characterization; 3) to fingerprint monovarietal EVOOs from five cultivars from Marche region, highlighting chemical and sensory peculiarities and investigating the prebiotic activity.

Methods

Samples of LEVOOs (15, costing 3.60-5.90 euro/L) and HEVOOs (51, costing 7.49-29.80 euro/L), and OOs (10, costing 3.59-5.59 euro/L), were purchased in local supermarkets. MEVOOs (a total of 79 samples) from the cultivars (cvs) Ascolana tenera (ASC), Coroncina (COR), Mignola (MIG), Piantone di Mogliano (MOG), and Raggia (RAG) from Marche region (Italy) were provided from local small enterprises in Marche Region (Italy). Chemical (fatty acid and volatile substances composition, tocopherol and polar phenolic substances composition and quantity, squalene, acidity, peroxide value and antioxidant activity), sensory and statistical analyses were performed as reported in [1]. Prebiotic activity was evaluated by a semi-continuous fermentation system inoculated with a pool of human faeces under anaerobic conditions, to mimic a human colonic fermentation [2], and assessed by monitoring bifidobacteria, lactobacilli, and clostridia and short chain fatty acids (SCFAs) using real time -PCR and gas-chromatography respectively, during 24h fermentation of each previously digested EVOO.

Results

The newly developed method to quantify squalene in EVOO and OO allows to prepare and analyse the sample with a total time of about 15 min and presents good values for the validation parameters, also in terms of sensitivity, sufficient to quantify squalene also in OOs, where the concentrations are much lower [3]. The application of the method to characterize MEVOOs from the different cvs, highlighted clear differences, confirmed in a two years production. Then, a new method to analyse olive oil polyphenols has been developed due to their well-known positive contribution to the oil healthy, sensory and technological quality and to the lack of a well-established method for their quantification [4, 5]. Application of the method to MEVOOs highlighted features specific to the cvs investigated; e.g. ASC resulted to be characterized by high relative content of pinoresinol and RAG by acetoxypinoresinol, significantly higher as compared to the other cvs. In the comparison between HEVOO, LEVOO and OO, one of the interesting outcome was how the ratio [(hydroxytyrosol

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(hyty)+tyrosol (ty))/(hyty+ty+secoiridoid derivatives)] can help also for authentication purposes, since OO resulted to have significantly higher values as compared to EVOOs [1]. Then, also the higher values found in HEVOO as compared to LEVOOs, indicate that this ratio could play a role even within the EVOO quality assessment. Sensory analysis highlighted a significant difference between MEVOOs produced by Italian small enterprises and industrial EVOOs where 50% of the industrial EVOOs did not result to have a sufficient score to be acknowledged as extra virgin. Then, the prebiotic activity of selected MEVOOs were investigated [6]. Several samples resulted to have an interesting activity, similar or even greater than that shown by inulin, in increasing a selective growth of lactobacilli and a production of total SCFA as fermentation end-products, thus providing another contribution to the many recognised health beneficial effects of high quality EVOO in our diet.

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Design and exploitation of olive by-products phenolic extracts functional ingredients

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Keywords: olive leaves, phenolic extract, encapsulation, functional ingredient, food antioxidant

Objective

The main goal of the research is the valorization and exploitation of olive by-products which represent an effective source of phenolic compounds. Among olive by-products, the attention was mainly focused on olive leaves and the experimental design was structured on a three-step plan: 1. evaluation of the compositional and technological properties of olive leaf phenolic extracts (OLE); 2. development and characterization of OLE-based functional ingredients by means of microencapsulation; 3. Exploitation of OLE-beads for the enrichment of real food matrices (mayonnaise).

Methods

1. A water and water-ethanol olive leaf extract (OLE) were characterized for their composition and technological functionality (i.e. water/oil holding ability, air/water surface activity and emulsifying capacity at pH 4.5 and 7) as well as their chemical stability over time. 2. OLE enriched alginate based microparticles, obtained by emulsion/gelation technique, were characterized in terms of encapsulation efficiency (EE%), particle size, optical and confocal microscopy, FT-IR, thermic analysis, swelling behavior and release kinetics. 3. Mayonnaise systems, i.e. control and fortified with pure (Mayo+OLE) or encapsulated OLE (Mayo+Alg/Pec), were characterized in order to asses the major factor affecting the shelf-life at time zero and during storage at 10, 20 and 30°C (i.e. particle size, color, flow behavior, tribology, primary and secondary oxidation products and sensory evaluation.

Results

1. The extraction solvent influenced the mineral and pigments contents and the antiradical activity. All the phenolic extract tested for surface activity showed a typical dose-dependent behavior; with the extracts Eth30 at pH 4.5 and Eth70 at pH 7 which resulted to be the most surface active. The model emulsions enriched with Eth30 at pH 4.5 showed a monomodal particle size distribution (PSD) with a mean diameter centered on 2 μm and a dimensional range within 0.93-6.30 μm. 2. Alginate based microparticles with a mean diameter lower than 100 μm were produced; the addition of biopolymers increased the EE%; alginate-pectin microspheres exhibited the highest EE% value (≈ 80%). FT-IR spectra and DSC thermogram evidenced the presence of new molecular interactions. A correlation between swelling and release was observed; the main contribution for polyphenols release from the beads was given by diffusional processes, as confirmed by the diffusion and relaxation constants obtained by data modelling with Peppas-Sahlin equation. 3. Mayonnaise shelf-life results showed a general chemical and physical stability reduction over time, in particular mayo control sample at 35°C with respect to the Mayo+OLE and Mayo+Alg/Pec enriched mayonnaise that, on the contrary, highlight good stability towards oxidation phenomena. Particle size distribution of mayonnaise samples (PSD) showed two populations and a shift

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towards lower size for the Mayo+Alg/Pec. Results about flow behaviour highlight significant differences in terms of apparent viscosity ($p<0,05$) between the system Mayo-Alg+Pec with respect to both control and Mayo-OLE probably due to the interactions between lipid droplets and alginate/pectin microparticles which could exert a thickening effect.
This research suggests that olive leaves extracts (OLE) can exploit important technological functionality in complex food systems and can be considered a promising multifunctional ingredient that can help in the production and stabilization of complex food products like emulsions. Emulsification/gelation can be a promising technique to design functional ingredient for food application, since it can provide enough small microspheres, a satisfying EE% and the possibility to achieve a tailored release of the bioactive compound. Moreover, this study represents the first step for new challenges regarding the production of healthy and functional mayonnaise sauces.

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Green approaches for olive and oil quality evaluation

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Keywords: Image Analysis, Near Infrared Spectroscopy, olive quality, olive maturity, oil quality

Objectives

In the oil industry, fast and sustainable approaches for the quality control of raw materials and final products are fundamental in vision of new digital industrial technology. Besides, the final products obtained should achieve full customer satisfaction for the most varied uses without wastage of resources and energy. These challenges are well answered by two green techniques, image analysis and infrared spectroscopy (IR), whose potential is well known at research level, even if they are still slightly applied in the oil industry.
Thus, the main objective of this work is to present successful applications of image analysis and IR for olive and oil quality evaluation.

Methods

Along three harvesting years (2016, 2017 and 2018) olives at different maturation stages were collected. In details, 13 cultivars of olives were harvested in four different Italian regions (Abruzzo region, AR; Calabria region, CR; Apulia region, PR; Sardinia region, SR) during the harvesting season (September - December). For each sampling time, both maturity stage and chemical parameters were evaluated. For the maturity evaluation, olive images were acquired and elaborated in order to extract an average value of the red channel to be used for assigning olives to three maturity classes. For quality characterisation, moisture (%), dry matter (%), oil content (%), soluble solids (°Brix), total phenolic content (TPC, mgGA/kg) and antioxidant activity (%) were determined.
The NIR analysis was performed in diffusive reflectance by a FT-NIR spectrometer (MPA, Bruker Optics) equipped with an integration sphere (12500-4000 cm-1). A total of 303 aliquots of olives were analysed and divided into calibration and validation set.
Partial Least Squares – Discriminant Analysis (PLS-DA) classification models based on FT-NIR data were built to predict the maturity classes identified by imaging.
Furthermore, PLS regression models were developed using NIR spectra in order to obtain prediction curves for all the measured chemical indexes.
In another application, IR techniques were used to study the oxidative stability of coldpressed vegetable oils during storage. For the purpose, three cold-pressed seed oils (sunflower – SUN; hempseed – HEM; camelina – CAM) were stored under accelerated conditions (65°C in a forced-draft oven) up to 28-30 days. IR spectra (FT-NIR, MPA, Bruker Optics, 12500-4000 cm-1; FT-IR, Vertex, Bruker Optics, 4000-700 cm-1) were collected during storage every three-four days, for a total of 9-10 sampling points. The same samples were characterized in terms of fatty acid composition, acidity, and tocopherol content and evaluated for peroxide value and p-anisidine value. The IR data, after suitable pre-treatments, were analysed by Principal Component Analysis (PCA).
The PC1 scores obtained were normalised (0-1) and evaluated in function of storage time to be compared with the analysed oxidative indices.

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Results

In the first application, average red channel values extracted from each olives' image allowed to identify three maturation classes based on skin colour to be used as a priori information for the PLS-DA models: green (G), green-black (GB), and black (B). The PLS-DA models gave promising results, being the average cross-validation sensitivity 80.1% and the specificity 75.9%. Better results were obtained on specific cultivar, even in prediction. Moreover it was possible to select specific wavenumbers on the basis of which the low cost, simple prototype to be used directly on field was built.

The regression models built to predict, by FT-NIR spectroscopy, moisture (39.3-87.2%), dry matter (12.8-60.7%) and oil content (1.9-25.4%) gave optimal figures of merit, being the RCV2 above 0.75 and the RMSECVs lower than 4.29%. Promising results were obtained for the models developed for soluble solids (1.7-20 °Bx), TPC (2487.8-59689.4 mgGA/kg) and antioxidant activity (2.42-161.1%). All the obtained results demonstrate the feasibility of selecting olives in a fast and green way with the aim of improving and control EVOO production.

As for the second application, the kinetic study on the oxidation of cold-pressed seed oils gave good results when modelling PC1 scores obtained from both FT-NIR and FT-IR data on SUN and HEM, whereas unsatisfactory results were obtained for CAM, probably due to the lower oxidation level reached during the accelerated storage of this oil. The same approach could be applied to EVOO to evaluate its quality during storage.

The obtained results pave the way for the implementation of imaging and IR approaches along all the EVOO production chain, for the prediction of relevant quality characteristics at industrial level. These approaches could provide the oil sector with fast, green and nondestructive methods capable of simultaneous estimation of a wider number of quality parameters.

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Filtration of olive oil: characterization of turbidity and qualitative effects during the storage

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Keywords: biophenols hydrolysis; fusty defect; high pressure processing, shelf-life; volatile compounds

Objectives

The study has two main aims: i) characterize the water-solids dispersion suspension system responsible for the olive oil cloudy appearance, and ii) measure the qualitative effects of the turbidity during the storage in oil treated with different stabilization techniques.

Methods

Six batches of olive oil were treated in 4 different ways, namely filtration (to remove water and solids), freeze-drying (to remove only the water), filtration with wool glass (to remove only the solids) and no treatment. Turbidity, water content, water activity, solid particle content and total microorganisms were measured in each sample. Furthermore, FFA, peroxides, UV indexes, biophenols, volatile profile and panel tests were used to chemically characterise the oils. Finally, images of the olive oil turbidity were obtained using the confocal laser scanning microscopy. Then, 4 batches of olive oil were treated in a full factorial experiment with filtration and high pressures (HP). Thus, olive oils underwent to 4 different treatments: untreated, filtration and no HP (allowed to obtain the limpid olive oil), HP and no filtration (allowed to obtain a cloudy olive oil without microorganisms), and filtration and HP (to check for interaction between treatments). The obtained oils were analysed after 15 days, 1 month and 6 months of storage. Turbidity, water content, water activity, solid particle content and total microorganisms were measured in each sample. Furthermore, FFA, peroxides, UV indexes, biophenols, volatile profile and panel tests were performed on each oil.

Results

The turbidity characterization revealed a high variability among the six measured olive oils. Particularly, different proportions between water and solid particles were measured in the oil. Furthermore, water was found in from of free drops and bound to solids in different ways in the olive oil. Consequently, each turbidity degree could be related with a different risk for the olive oil quality during the storage. For example, olive oils with high water activity, high microbiological contamination and low phenolic content are more prone to qualitative decrease during the storage. The shelf-life test of olive oil filtered and treated with HP allowed to measure the chemical changes during the storage and to isolate the effects of microorganisms. Moreover, both techniques were able to provide an olive oil without microorganism activity. In the untreated samples, the high level of yeast contamination and the high value of water activity value was related to the quick formation of off-flavour aroma compounds and to the fusty defect appearance. Furthermore, the high value of water activity affected the LOX pathway compounds, leading to the decrease of E-2-hexanal and of the fruity positive attribute, and increased the hydrolytic degradation rate of secoiridoids. Hence, through the removal of the water, filtration does not allow the microorganism activity preventing the formation of the fusty defect, and reduce the rate of qualitative parameters decrease.

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Effect of free and encapsulated olive leaf extracts on salad dressing stability.

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Keywords: Salad dressings, encapsulation, double emulsion, oxidative stability, antioxidants, rheological properties.

Objectives

This study aims at evaluating the effects of free and encapsulated olive leaf extracts on salad dressing properties and stability during storage at 4°C. The exploitation of these extracts obtained from olive oil by-products can help in improving sustainability of the production process, while enhancing the oxidative stability of food.

Methods

Salad dressings were formulated as both single (O/W) and double-emulsion (W/O/W) systems containing 75% and 25% aqueous and oil phases, respectively. Both systems were prepared with the addition of free or encapsulated olive leaf extracts, in order to obtain a final total phenolic content of 160 mg/kg. Besides, a single and a double-emulsion dressing without phenolic extracts were also produced as control. The dressings were placed in airtight containers flushed with nitrogen and stored at 4ºC, for up to 90 days. Total phenolic content (TPC), ABTS and DPPH antioxidant activity, peroxide value (PV), double emulsion yield, pH, and rheological properties (consistency coefficient and flow behavior index) of the dressings were determined periodically.

Results

Over the course of storage period, the retention of phenolic content was above 90% in all the enriched dressings. Therefore, significant ABTS and DPPH radical scavenging and reducing capacities were also retained, assuring the oxidative stability of the samples. Actually, changes in PV were more pronounced in control samples than in the enriched ones. All the samples maintained their acidic pH over the storage period. The double emulsion control sample showed a yield higher than those of the enriched dressings. Phenolic enrichment significantly ($P<0.05$) improved the consistency coefficient and flow behavior index of the dressings, with encapsulated extract exhibiting higher structuring effects due to the alginate microspheres. In general, the type of extract (free or encapsulated) rather than the different emulsion system was the most significant factor influencing chemical and physical characteristics of enriched salad dressings.

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Can texture profile analysis be a tool for cultivar diversity estimates and how does olive texture reflects on oil traits?

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Keywords: hardness, the size of the milled tissue, oil yield, ripening, sensory analyses

Objectives

The main objective was to determine the textural profile of selected olive cultivars and to gain knowledge about the influence of the fruit maturity degree and changes in the physico-chemical composition on the texture parameters. The present study also aimed to define the influence of the milled tissue size on the oil yield and sensory characteristics of obtained virgin olive oils with the hypothesis that texture properties affect the degree of tissue fragmentation and thus the content of separated olive oil.

Methods

Fruits (10 kg) of four cultivars of *Olea europaea* L (Oblica, Lastovka, Levantinka, Coratina cvs.), were harvested at green stage of ripening, during September 2019 in the olive orchard located in Kaštela, Croatia. In addition, fruits of Oblica cv. were harvested as half-ripe and ripe. One hundred olive fruits, from each cultivar, were subjected to texture profile analyses (TPA) measurement by texture analyzer (TA Plus; Lloyd Instruments, Fareham, UK) equipped with a 500 N load cell (XLC -0500-A1; Lloyd Instruments) and cylindrical probe (FG/CY3; Lloyd Instruments). The experimental data were analyzed by Nexygen Plus 3 software. Prior to TPA, fruit length (cm), width (cm), and mass (g) was measured. Olive dry matter content was analyzed according to HR EN ISO 665 (2004) standard.

Olive's form each cultivar were divided into two subsamples and processed by centrifugal extraction using laboratory oil mill Abencor (mc2, Ingenierias y Sistemas, Sevilla, Spain). Two subsamples of olive fruits were milled at two different particle diameters by the hammer crusher, after which oil processing was carried out under the same conditions (Jukić Špika et al., 2015). Three replications of each subsample per cultivar were done.

The oil yield (%) was calculated from the weight of the olive paste dough to be mixed and the weight of the oil obtained. The VOO sensory analysis panel of the Institute for Adriatic Crops performed quantitative descriptive analysis of VOO samples. Analyses were carried out according to modified International Olive Council methodology (IOC, 2007, Jukić Špika et al., 2019).

Data were subjected to one-way ANOVA (cultivar influence) and two-way ANOVA, (cultivar × size of tissue milling) followed by Tukey's HSD test ($p \leq 0.05$). Sample distribution and relationship between samples was estimated by principal components analysis (PCA). The obtained data were analyzed using Statistica software version 11.0 (StatSoft, Inc. USA, 2012).

Results

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Texture profile analyses (TPA) parameters measured for our samples were hardness, springiness (or elasticity), cohesiveness, adhesiveness, gumminess and resilience. Majority of TPA parameters differed significantly among cultivars (except for adhesiveness), among which highest variability (F value) was observed for hardness.
Along ripening Oblica cv., which can be described as fruits of high hardness, middle high cohesiveness, middle springiness and low gumminess, was monitored. Fruit ripeness changed fruit physicochemical composition and induce softer fruits with slashed cohesiveness and gumminess. Results of data analysis (two-way ANOVA) shows significant difference in oil yield emerged between cultivars and between the sizes of tissue milling. Cultivar turned out to be more important factor, Levantinka and Coratina having higher values, while Lastovka had significantly the lowest oil yield. However, significant interaction was observed between the main factors ($F = 48.18$, $p \leq 0.0001$).
The size of tissue milling elicited significant differences yielding less oil milling Lastovka fruits on smaller particles, but no differences were recorded at other studied cultivars.
According to principal component analysis, Lastovka cv. was separated by having the highest springiness and resilience. Altered physicochemical and textural Oblica cv. fruits, by increased maturity stage, yield higher oil content, but the sizes of tissue milling of more mature fruits of this cultivar did not cause changes in oil yield.

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EVOO-polyphenol extracts exert hypocholesterolemic effects through the modulation of the LDLR pathway: in vitro and cellular mechanism of action elucidation

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Keywords: *Hypocholesterolemic activity, EVOO polyphenols extract, LDLR-pathway*

Objectives

Cardiovascular disease (CVD) is a leading cause of death worldwide. Many risk factors are responsible for the development of this multifactorial disease and hypercholesterolemia is one of those. Many in vitro and in vivo human and animal studies have shown that EVOO improves the lipid profile by increasing HDL-cholesterol and reducing LDL-cholesterol and triglyceride levels, and inhibits human lipoprotein oxidation, making LDL less atherogenic. However, the molecular mechanism through which it modulates the cholesterol metabolism has not been fully elucidated.

Based on these considerations, in order to link the EVOO polyphenols extract profiles and compositions with the nutraceutical properties focusing on their potential hypocholesterolemic effect, a deeper mechanistic investigation was carried out in order to find out how EVOO-polyphenol extracts may modulate the activity of the key targets involved in the cholesterol metabolism.

Methods

To achieve this goal, HepG2 cells were treated with two EVOO-polyphenol extracts and their ability to modulate the LDLR pathway via HMGCoAR and PCSK9 regulation was investigated using a combination of molecular and functional techniques. In parallel, this study has been performed using statin, the main used cholesterol-lowering drug, as reference compound.

Results

Findings clearly indicate that through the direct ability of EVOO polyphenols extracts to inhibit the HMGCoAR activity, an up-regulation of the SREPB-2 transcription factor was observed at intracellular level. Thus, the augmentation of the SREBP-2 led to an increase of total LDLR protein levels, which determines from a functional point of view to an improved ability of human hepatic HepG2 cells to uptake LDL from the extracellular environment with a final *in vitro* hypocholesterolemic effect. In addition, results suggest that the cholesterol-lowering property of EVOO polyphenols extracts is also exerted by the slightly modulation of the PCSK9 pathway.

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New Spectrophotometric Methods for the Determination of Peroxide Value in Extra-Virgin Olive Oils

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Keywords: Olive oil; Peroxide value; Green chemistry; UV spectrophotometry

Objectives

The official method used for the determination of the peroxide content in olive oil (PV) is based on iodometric titration, i.e. on the redox reaction between the hydroperoxides of the oil with excess potassium iodide; the latter, in an acidic environment, releases molecular iodine in a stoichiometric quantity:

$ROOH + 2I^- + 2H^+ \rightarrow ROH + I_2 + H_2O$

The iodine released is complexed by starch which acts as an indicator. By quantifying the iodine liberated with sodium thiosulphate, the concentration of hydroperoxide can be calculated.

$I_2 + 2NaS_2O_3 \rightarrow Na_2S_2O_6 + 2NaI$

One of the well known problems of this method is the co-presence of the interfering reaction due to the oxidation of iodide by oxygen present in solution, causing an overestimation of the PV.

$4I^- + O_2 + 4H^+ \rightarrow 2I_2 + H_2O$

In addition, the accuracy of the method is influenced by a variety of experimental factors such as skill of operator, light, temperature, sodium thiosulfate decomposition. Furthermore, the iodine released in the organic phase can also react with unsaturated lipids and cause an underestimation of the concentration of peroxides. Finally, this method requires relatively long analysis times, large amounts of sample and organic solvents with a high environmental impact. Considering these disadvantages, a novel approach based on the spectrophotometric detection of the I_3^- was developed.

Methods

0.10 g of sample were added with 1 mL of 0.5 % HCl in ethanol, 0.10 mL of saturated KI solution and incubated for 5 min. Then, the resulting solution was filtered and the I3- generated following the oxidation-reduction reaction between iodide and lipidic hydroperoxides was measured at 353 nm.

Results

A matrix calibration curve, obtained by spiking a purified olive oil (ROO) sample with increasing quantity of a standard solution of tert-butylhydroperoxide (TBHP), obtaining oil calibrants with PV of 1.0, 2.0, 4.0, 6.0. 8.0 and 10 meqO_2/kg, showed a good linear response (R^2=0.99), with variation coefficients less than 5% (n=3) and limit of detection (LOD) and quantification (LOQ) of 0.2 and 0.5 meqO_2/Kg, respectively. Finally, olive oil samples were analyzed by the proposed method and a good correlation with the official iodometric titration method was obtained. In conclusion the developed spectrophotometric procedure allowed the quantitative determination of the lipidic hydroperoxides in olive oils even at the first moments of oxidation with different advantages respect to the official method in terms of analytical performances, extraction solvent (substitution of chloroform with ethanol), ease of use, and reduction of sample amounts and solvent volumes.

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Searching for hydroxytyrosol gene targets

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Keywords: Hydroxytyrosol, Olive oil

Objectives
Food and bioactive compounds are able to modulate the gene expression and may play a role on disease prevention. Olive oil, and concretely its main phenolic acid hydroxytyrosol (HT) are widely recognized by their protective effects. In the present study we identify by in *silico* methodologies the common differentially expressed genes and proteins after in vivo HT supplementation; then, differentially expressed genes and proteins were validated in rodent liver samples from different laboratories.
Methods
Transcriptomic and proteomic *in vivo* studies supplemented with HT or its precursors (oleuropein and secoiridoids) reported in Scopus and PubMed databases were selected to identify common lipid metabolism-related genes and proteins. Venn diagrams were generated by means of in-house R scripts to find intersections among differentially expressed genes. Different rodent liver samples obtained from previous studies of different groups were employed to validate the selected common genes and proteins.
Results
We found 4 mutual differentially expressed genes (Efr3, Kctd2, Plscr1 and Scl37a4) after HT supplementation from the five sets of data. Also, 18 genes involved in lipid metabolism were also selected for validation in liver samples. Regarding common differentially expressed proteins, only Hspd2 and Actn4 were modulated by three studies while 20 different proteins were common to at least 2 studies. Unfortunately, none of the proteins analyzed in the livers of the HT cohorts were validated. Despite the number of articles investigating the role of HT on gene expression, the integrative data to identify the consistent targets of HT revealed that Fgf21 and Rora can be potential targets of HT.

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Olive leaves polyphenols as active ingredients to prolong oxidative stability of biscuits

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Keywords: olive by-products, antioxidants, microencapsulation, bakery products, shelf life

Objectives

Natural antioxidants can be easily extracted by agroindustrial by-products, as olive leaves, rich in polyphenols. These molecules, with known antioxidant activity, may be proposed as active ingredients to retard fat oxidation during food storage. However, these bioactive compounds have poor stability when exposed to high temperature, pH and oxygen. Encapsulation into "microgels" is a promising technique to protect those molecules. The aim of this project was the addition of olive leaves phenolic extracts, in a free form or encapsulated, to biscuits formulations, and the evaluation of their effect on fat oxidation during accelerated storage.

Methods

Control biscuits containing 30% fat (butter) were prepared and analyzed in comparison to test biscuits containing 300 ppm of olive leaves polyphenols in a free form or encapsulated in microbeads of sodium alginate and pectin. The amount of polyphenols was chosen on the base of a preliminary acceptability test with 30 untrained panelists.

Control and test biscuits were packed in heat-sealed trays made of PE, PET and EVOH and stored for 15 days under controlled conditions. The accelerated storage conditions set up in a thermostatic chamber were: Temperature, 40°C; Relative Humidity, 50%; Lighting, Day Light Lamp. Biscuits were analyzed for determination of total phenolic compounds by means of Folin Ciocalteau assay and antioxidant activity by means of DPPH assay. Oxidative stability was monitored using the Oxitest reactor (VELP Scientifica), based on accelerating fat oxidation at high temperature and oxygen pressure. The level of fat oxidation was measured by peroxide value. Each analysis was conducted on biscuits after 0, 5, 10, 15 days of storage.

Results

No differences were observed both for total phenol content and antioxidant activity on the biscuits enriched with free or encapsulated polyphenols after baking; lower levels were instead measured for the controls.

An increment of about 8% of the oxidative stability was observed for the biscuits enriched with free polyphenols in comparison to controls, and about 35% between biscuits containing encapsulated polyphenols and controls, probably because of the slower release of the bioactive molecules from the encapsulation system. These data were confirmed by the trend of the peroxide value.

During storage a progressive reduction of total phenols content and antioxidant activity was observed, in parallel with the increase of the oxidative indices. These trends resulted faster for control biscuits in comparison to the enriched ones. Moreover the biscuits enriched with encapsulated polyphenols resulted more resistant the ones containing the free forms.

This study proposes promising applications of polyphenols extracted from olive leaves as ingredients to prolong the shelf-life of bakery products.

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Aqueous extraction of oleocanthal and its formulation in pharmaceutical tablets and injection solutions for clinical trials.

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Keywords: *Olive oil; Oleocanthadiol; extraction; clinical trials; tablets, injection solution*

Objectives

The phenolic fraction of the extra virgin olive oil (EVOO) has been studied over the last two decades due to its important health protective properties. Numerous studies deal with the beneficial properties of each phenolic constituent, pointing out the need of new, innovative green extraction methods for the extraction of the phenolic fraction. My research project is expanding to various aspects of the olive oil phenols field: the biosynthesis of phenols in the olive fruit, the extraction from olive oil and the preparation of pharmaceutical formulations. The main objective of my research was to prepare the first pharmaceutical formulations of oleocanthal to be used for phase I clinical trials. To achieve this objective, it was first necessary to find the appropriate source and method of extraction of oleocanthal from olive oil. For this purpose, I used available information from the database that has been created in our lab including results from 7,000 olive oil samples, and the conclusions from our experience in the olive oil production process in order to maximize the concentration of specific phenols in olive oil. The production of high phenolic EVOO allowed us to develop new methods for the extraction and formulation of olive oil phenols and more specifically of Oleocanthal to be used for clinical trials.

Methods

After screening the phenolic profile of thousands of olive oils from our lab database, extracting olive oil from hundreds of olive fruit samples in our lab-scale olive mill from different varieties and in different malaxation conditions we were able to maximize the concentration of specific olive oil phenols in our samples.

Olive oil extraction from 'Lianolia' variety olive fruits from the region of island of Corfu, Greece was performed in the early ripening degree, at 29 oC for 50 min. The phenolic fraction of the high phenolic EVOO was analyzed by qNMR. The olive oil sample was extracted in an one-step extraction process affording an aqueous solution of oleocanthadiol, which is the hydrated water-soluble form of oleocanthal. The oleocanthadiol solution was concentrated to a maximum of 3g/L. This solution was directly used for the preparation of injection solution. Another part of the solution was formulated using fluid bed technology in tablets containing 10 mg per tablet.

Results

Appropriate malaxation temperature and malaxation time enhanced the formation of oleocanthal in the high phenolic EVOO. The sample we extracted from the olive fruits contained Oleocanthal as the major constituent (>90%) in high concentration (>3,000 mg/Kg). The production of this specific type of high phenolic EVOO allowed us to develop a green, cost effective and sustainable method for the extraction of oleocanthal that is described in a relative patent. The hydration of Oleocanthal to oleocanthadiol led us to

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produce an aqueous solution which can be used directly as injection solution after condensation. The aqueous solution of oleocanthadiol was also formulated in tablets of 10mg/tablet. Both formulation were used in clinical trials against prostate cancer. Patients receiving i.v. dose of 200 mg per day or in-prostate injection up to 10 mg/day did not present any side effects.

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Determination of antioxidant and antimicrobial activities of hydroxytyrosol-enriched extract from olive mill wastewaters.

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Keywords: *Olea europaea* L., olive mill wastewaters, polyphenols extraction, hydroxytyrosol, circular economy.

Objectives

Olea europaea L. is among the most important crop in Mediterranean countries. During olive fruits processing many by-products are generated as olive mill wastewaters (OMWW). The recovery of HTyr from OMWW has attracted much interest for both the demonstrated biological activities of HTyr and the environmental benefits that could be achieved by a more ecological disposal of this by-product, thus meeting the principles of circular economy. Hence, the aim of the study was the *in vitro* evaluation of antioxidant and antimicrobial activities of a standardized HTyr-enriched extract (HTE). HTE, product of an eco-friendly, patented extraction procedure from OMWW, was chemically characterized and the activities were correlated with those of the HTyr standard.

Methods

HTE, obtained through an eco-friendly, patented process based on membrane technology, was chemically characterized by NMR spectroscopy and HPLC/DAD/MS analysis. The antioxidant activity was evaluated using ABTS assay. Synthetic HTyr, produced in our laboratory by a patented procedure, was used as reference compound. Moreover, the HTE antimicrobial activity against the two olive pathogens *Pseudomonas savastanoi* pv. *savastanoi* (Pss) and *Agrobacterium tumefaciens* (At) was evaluated by subculture method and compared with those of the HTyr standard.

Results

Considering the biological properties of HTyr, there have been many efforts to obtain HTyr-enriched extracts from natural sources for industrial applications. HTE was obtained from OMWW through a process based on membrane technology, defined as BAT (Best Available Technology) and recognized by the EPA (Environmental Protection Agency). The chemical characterization allowed to identify HTyr as the main constituent of the extract along with other low molecular weight phenols. HTE showed an antioxidant activity higher than HTyr standard. HTE was tested for its antimicrobial activity on two olive tree pathogens, *Pseudomonas savastanoi* pv. *savastanoi* (Pss) and *Agrobacterium tumefaciens* (At). Pss, in particular, is the etiological agent of the olive knot disease responsible of important production losses. Results showed significant *in vitro* antimicrobial activity against Pss and At, in both cases higher than HTyr alone, suggesting a role also of the minor phenolic components, which act synergistically with HTyr. In conclusion, a sustainable separation system allowed the production of an extract enriched in HTyr from OMWW. HTE has proven potent antioxidant activity and the antimicrobial activity against the pathogens tested highlighted a potentiality of HTE for plant disease control. A clearer definition of the

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modality of action that takes place in the antimicrobic mechanism would greatly help in defining more effective formulations.

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The possibility of using two-phase olive-mill waste in the light of circular economy

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The results of this research have been provided for possible publication into International Journal of Agricultural Sustainability.

Keywords: two-phase olive mill waste, exploitation, direct land spreading

Objectives

Two-phase olive-mill waste (OMW) has high organic-matter content (90% of dry matter). Therefore, its use has been proposed to improve the characteristics of the Mediterranean soil, which is low in organic matter. As there is little information available about two-phase OMW produced in northern Mediterranean countries, the aim was to determine its composition and evaluate its use as a soil amendment in northern Mediterranean olive groves.

Methods

Sampling of the two-phase olive-mill waste

To characterize two-phase OMW, 14 samples were taken from seven two-phase olive mills in Slovenian Istria during the olive harvesting in 2016 and 2017. The samples were then used for determination of their main characteristics (levels of the mineral nutrients: total nitrogen, phosphorous, potassium, and calcium; the toxic elements iron (Fe) and copper (Cu) and the dry matter, crude protein, fat and phenolic compounds).

Experimental site and soil amendments

This study was carried out across four different nonirrigated production olive groves on eutric cambisol in Slovenian Istria, with an overall plantation density of 300 plants ha^{-1} (trees spaced at 6 m × 5 m). Each grove represented one replication with two treatments. Therefore, eight plots with 10 olive trees each and the vegetative and productive characteristics representative of the entire area were selected: four were amended with 80 m^3ha^{-1} of two-phase OMW, while the other four served as the control.

Soil analysis

To determine the differences between the soil of the control and amended (experimental) plots, fresh soil samples were collected three times (March 21st, 2017; November 30th, 2017; March 30th, 2018). For each soil sample from each plot, measurements were taken for pH, exchangeable K (K_2O), total organic carbon (TOC) and total nitrogen (TN) and the C/N ratios were calculated as well.

Statistical analysis

All of the statistical analyses were performed using the statistical software package R.

Results

This study shows that the characteristics of two-phase OMW produced in northern Mediterranean countries are similar to those of other countries. Furthermore, the calcareous characteristics of the Mediterranean soil can reduce its phytotoxic effects,and might thus represent a natural system for two-phase OMW treatment. Phenolic compounds in the two-phase OMW are rapidly decomposed, and the soil has high buffering capacity. The combined application of two-phase OMW and mineral fertilizer to olive groves on eutric cambisols has

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positive effects on the physical, chemical and biochemical properties of the soil. Therefore, direct land spreading of two-phase OMW represents an environment-friendly practice for exploitation and recycling of these organic materials, even though disposal of two-phase OMW is considered to be a serious environmental issue due to its phenolic compounds and organic acids.

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Innovative methods for the selective isolation of olive phenolic ingredients and their impact on the sensory properties of olive oil

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Keywords: *phenolic compounds, olive oil, sensory properties, NMR, oleuropein aglycon, oleocanthal*

Objectives

The use of olive oil for therapeutic purposes and as a product of high nutritional value has been known since ancient times. Dioscorides had observed the positive effects of raw olive oil on health and had highlighted its bitter and spicy taste. Nowadays, Extra Virgin Olive Oil is a food with an officially recognized health claim in the EU. It contains health protecting ingredients belonging to secoiridoids, such as derivatives of oleuropein and ligstroside. The already known derivatives are conjugated secoiridoid phenols, such as oleocanthal, oleacein, oleuropein aglycons and ligstroside aglycons. This health claim refers to the presence of the above phenols in the olive oil at a specific concentration. Phenols are contained in olive oil in a wide range of concentrations and it has been proven that they have beneficial properties both as ingredients of olive oil and as pure chemical substances tested in studies in cells and experimental animals, results which have been published in a significant number of scientific papers. The content of phenols in olive oil, fruits and leaves depends particularly on the variety of the olive tree and their harvest season. The first aim of this study was the selective isolation, the spectroscopic characterization and the development of quantitative analytical procedures for the phenolic ingredients from extra virgin olive oil, olive fruits and leaves by qNMR. The second aim was the preparation of olive oils selectively containing only one of the isolated phenols at different concentrations and in parallel a series of olive oils having different concentrations of total equivalents of phenols. The final target of this study was to investigate the correlation of bitterness and pungency (spicy) that Dioscorides had first identified in raw olive oil with the content of specific phenolic components in olive oil and construct an equation according to which the taste of the olive oil will be predicted according to the content and type of phenolic ingredients.

Methods

In the context of my thesis, in our laboratory, we have developed methods of isolating and quantifying each phenolic ingredient in different varieties of olive oil, leaves and fruits. Selective extraction of phenols from the appropriate products of olive tree has been proven to be an abundant source of their production. Thus, the isolation of phenols with selective extraction and chromatography methods could be a more efficient and cost-effective way of producing them than their synthesis. We selected the part of the plant to be extracted in order to isolate the various phenols individually. **In total we developed four specific methods for the isolation of pure oleocanthal, oleacein, oleomissional and oleuropein aglycon described in details in two patents.** We then diluted each independent isolated phenol in olive oil of zero phenolic content and prepared olive oil samples containing each phenol separately at different concentrations (0-1000 mg/Kg). In parallel, through screening of thousands of samples we selected olive oil samples that contained all phenols at equal levels

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and prepared olive oil samples with different concentrations ranging from 0-2000 mg/Kg. All the above olive oils were organoleptically evaluated by a certified sensory panel.

Results

Through investigation among hundreds of samples of the main Greek olive tree varieties and olive parts or products (olive oil, fruits and leaves), we identified which variety and which part of the plant was the most appropriate to be extracted in order to obtain the specific phenol or phenols we wish to isolate on a large scale: pure oleocanthal, oleacein, oleomissional and oleuropein aglycon. After the preparation of the pure compounds we proceeded with the preparation of the olive oils containing the desired types and concentrations of phenols. The sensory evaluation of the olive oil samples led to an exponential equation of the correlation of total phenol concentration with the cumulative score of bitter and pungent. This is the first time that such an equation has been constructed. The bitterness and pungency scores of Extra Virgin Olive Oil can now be reliably predicted based on the phenolic content. Concerning the impact of each individual phenol the results we obtained were as follows: The bitter taste of the olive oil, is mainly influenced by the concentration of the monoaldehyde form of the oleuropein aglycon and to a less extend by that of oleocanthal. Regarding the pungency, it is mainly related with the oleocanthal content and less to the oleuropein aglycon and oleomissional. Concerning the sum of bitterness and pungency score, the oleuropein aglycon predominates, in contrast with oleacein. The production of olive oils with high phenolic content but reduced bitterness and pungency should have as target the reduction of the oleuropein aglycon concentration.

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MaestraNatura: a health literacy intervention to promote good food choices. Focus on EVOO quality.

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Keywords: Public health, Health Literacy, Food education

Objectives

Extra virgin olive oil (EVOO) is universally recognized as a symbol of the Mediterranean diet and its effects on human health have been largely demonstrated by numerous relevant intervention studies. Nevertheless, consumer awareness on the importance of using quality EVOO is still very superficial. EVOO still remains a marginal product and unfortunately it is still considered just a seasoning like many others. As often happens in the nutritional field, transferring information from the scientific community into one's daily habits is often difficult. Although the science of nutrition continues to progress and gains more and more credibility as a fundamental weapon of prevention, there are still strong resistances and difficulties to overcome. First, low level of health literacy (HL) of consumers. HL is considered a determinant of health, given the demonstrated influence on correct lifestyles, adherence to therapies, and on appropriate access to health services.
Dietary behaviors, like all behaviors, are acquired through a gradual process that begins early in life and in which the individual biological component is strongly conditioned by numerous external factors, such as family habits and socio-economic conditions. Therefore, to protect the health of adults it is necessary to intervene in children, trying to improve their HL skills and to develop a scientific thought. For this purpose, school plays obviously a pivotal role. The purpose of this project is therefore to implement EVOO knowledge through HL intervention, in primary and secondary schools' students, by using MaestraNatura (MN) program, a new methodology able to fill the gaps in children knowledge on nutritional issues. In particular, the project aim at extending correct knowledge about the origin and function of different food and at developing students' scientific thought through an experiential inductive teaching method.

Methods

The sample of students of the project is from different Institutes distributed throughout the national territory. MN is an educational program coordinated by the Italian National Institute of Health in collaboration with the Ministry of Health and the Ministry of Education. MN was implemented and tested from 2012 to 2018 in six Italian regions by involving 2797 classes, 1726 teachers and 55,000 students aged 6-13. MN has already given evident results in transferring the nutritional principles contained in the Food Guide Pyramid, compared to traditional food education programs. Considering this, through the teaching modules of MN, it is proposed to implement the knowledge about quality EVOO.

Results

The final objective of the project is to evaluate the effectiveness of HL intervention programs to promote nutritional health and improve consumer awareness about the importance of making informed food choices.

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Long-term studies on virgin olive oil volatiles: untargeted and targeted fingerprinting by comprehensive two-dimensional gas chromatography with mass spectrometry

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Keywords: Extra Virgin Olive Oil, GCxGC-TOFMS, Combined Untargeted and Targeted UT Fingerprinting, data alignment, long term studies

Objectives

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) is a technique that provides highly informative fingerprinting for food characterization. However, 2D fingerprints consistency can be affected by variables correlated to both the analytical platform and the experimental parameters adopted for the analysis making challenging cross-comparative studies extended over long-time range.
This study focuses on the combined untargeted and targeted (UT) fingerprinting of volatiles from extra-virgin olive (EVO) oils and proposes an effective work-flow to correct fluctuations that may occur during long-term studies and that affect patterns alignment and response consistency.

Methods

Volatiles from high-quality EVO oils are sampled by headspace solid phase micro extraction (HS-SPME) with a DVB/CAR/PDMS df 50/30 µm 2 cm length fiber and analyzed by GC×GC-TOF MS featuring tandem ionization on a polar × medium polarity column set-up. 2D-pattern misalignments are simulated by changing chromatographic settings (modulation period M_P and ^{2}D column dimensions) while MS response fluctuations are induced by adopting different tuning and acquisition modes.

Results

Misalignments, caused by chromatographic setup changes, impact on ^{1}D and ^{2}D retention times (^1t_R, ^2t_R) and peak-width while MS tuning and acquisition mode impact on analytes absolute and relative response. A strategy is then proposed to define a) a minimal signal-to-noise ratio (SNR) threshold, for consistent extraction of MS features to be adopted for UT fingerprinting, b) a minimal direct match factor (DMF) similarity value to improve the specificity of the matching, and c) a minimal distance threshold to guide the matching transform toward a 100% of true-positive matches. Once designed, the work-flow is applied to a target template of known analytes with a full supervision of the analyst. On the other hand, a fully automated and unsupervised procedure is then applied to automated for the UT feature template by adopting previously optimized parameters. For both targeted and UT templates, the percentage of matching between misaligned patterns reaches 95%. Finally, to compensate for detector response fluctuations, different normalization approaches are examined: normalization on total or partial response and normalization on multiple Internal Standards ISs. Although the first two approaches result attractive, being simpler and less time-consuming, results are not satisfactory as those obtained by multiple ISs normalization.

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The latter performs better for those analytes showing higher response fluctuations due to the pressure-drop applied.
In conclusion, this study shows that, thanks to a careful optimization of "smart templates" parameters, a full metadata transfer for targeted and untargeted features can be achieved even when dramatic misalignment occurs on complex 2D-patterns.

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EVOO Phenolics and shelf-life. Significance, preservation and transformation.

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Keywords: EVOO, shelf-life, stability, phenolics, oleocanthalic acid, NMR

Objectives

The goal of this study was to explore the chemical transformations that affect the phenolic content of extra virgin olive oil under three different storage conditions. Oleocanthal oleacein, ligstroside aglycone and its isomer forms oleokoronal and ligstrodials, as well as the aldehydic form of oleuropein aglycone and its corresponding isomers, oleomissional and oleuropeindials are the major phenolic substances that contribute to the health claim beared by some EVOOs under specific conditions and are being monitored in this project. What happens to oleocanthal and oleacein? Do they maintain their antioxidant and health protecting properties, regardless of the temperature they are stored in? Do they transform into other substances and lose the health protecting attributes? These questions are to be answered, so that we know how and when a EVOO can maintain the health-claim label.

Methods

29 different EVOO samples from 8 different varieties and 9 different geographic regions were analyzed using q-1H-NMR and a liquid-liquid extraction protocol (Karkoula *et al*, 2012) in order to determine the concentration of oleocanthal, oleacein, oleuropein aglycone and ligstroside aglycone. The samples were stored in dark glass bottles, without oxygen, in three different temperatures: room temperature (20 oC), common fridge (4 oC) and freezer (-18 oC) for 12 months. Their phenolic profile was monitored every three months using the same techniques and the phenolic concentration was determined by measuring each substance separately. In order to study the different patterns that occurred from our results, we developed a prediction model that, using mild heating, can predict in a few days the concentration of tyrosol and hydroxytyrosol derivatives after a year of storage at room temperature. Changes that were observed in the phenolic fraction of the samples during their storage, led to the isolation and structure elucidation of oxidation and hydrolysis products by chromatographif and spectroscopic methods.

Results

As expected, the temperature variations had a significantly different effect in the phenolic profile of the samples. During room temperature storage, most of the samples lost 51% of the oleacein contained, either by oxidation or hydrolysis mechanisms, and 40% of their oleocanthal content. On the other hand, samples that were stored at 4 oC maintained their phenolic content, losing only 12.5% of oleacein and 11.8% of oleocanthal. Similar results were obtained from the freezer stocked samples, with 8% and 6,4% oleacein and oleocanthal losses respectively. The aldehydic form of ligstroside aglycone along with its isomer form oleokoronal and ligstrodials, as well as the aldehydic form of oleuropein aglycone and its corresponding isomers, oleomissional and oleuropeindials were included in the phenolic content measures, as tyrosol and hydroxytyrosol derivatives that also contribute to the European Regulation 432/2012 health claim. Samples stored at room temperature reserved

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about 54% of their original phenolic content, fridge stored samples retained 85% of their phenolic concentration and the samples stored in the freezer showed only 10% losses on the health protecting substances contained. Overall, it needs to be communicated that an EVOO sample must be bottled with >500 mg/kg health claim contributing ingredients, so it can be maintained >250 mg/kg for 12 months after.
By monitoring the changes that some samples demonstrated during their 12 month storage, some new chemical structures made their appearance on the 1H-NMR spectra. Freshly produced olive oil, when produced properly, does not contain them, and these substances had to be isolated and described. To study these compound and in order to predict the impact of storage on the phenolic compounds, we developed a prediction model, showing in only 14 day period the phenolic profile of an EVOO sample after 12 months of room temperature storage. By using this prediction model, oleocanthalic acid, a product of oleocanthal oxidation was isolated and described for the first time, along with oxidation and hydrolysis products of oleocanthal and oleacein.

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Olive tree status in response to irrigation with saline reclaimed water and deficit strategies

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Keywords: acidity, fruit weight, oil extractability, oleic acid, polyphenol, peroxide, total production, Leaf and soil nutrients, Gas Exchange,Water potential

Objectives

The 70% worldwide surface of olive orchards is irrigated. The evaluation of non-conventional water resources and water-saving techniques has gained importance during the last decades in arid and semiarid environments. Water is the most limiting factor in oliviculture of semiarid areas. Evaluating alternative water sources feasibility, as municipal wastewater, combined with deficit irrigation strategy is becoming a challenge.
According to our knowledge, there are no information published about the effects of i) the irrigation with DW (tertiary water treated by an innovative system) or of the combination of both water sources (desalinated water and RW) with the RDI strategy and, ii) these strategies on cultivar 'Arbosana', which is the variety object of study of this work.
Our study is the first that assess physiological effects of irrigation with saline reclaimed water combined with deficit irrigation strategies during 2017 and 2018. Besides, this work intends to investigate the effects of the use of desalinated and saline reclaimed water combined with two irrigation strategies: full irrigation and RDI on i) olive production, ii) fruit maturity indices, iii) olive nutrient and iv) oil yield and oil quality in young trees.

Methods

Experimental Site and Plant Material

The study was conducted at an experimental site located in the southeast of Italy (Bari, Apulia Region) (41°06′41″N, 16°52′57″E) (5 m above sea level) during 2017 and 2018. The crop used was 2 years self-rooted olive trees (cv Arbosana) planted on not covered 100-L polyethylene pots (diameter, 50 cm; height, 65 cm). Pots were on the ground with a 1.85 × 2.10 m planting system in rows-oriented N-NE to S-SW. The soil texture within the first 90 cm depth was classified as loam (44.78 % sand, 12.32 % clay, and 42.90 % silt) (USDA textural soil classification).

Irrigation treatments

Two irrigation water sources were examined. The first was DESERT desalinated water (DW), obtained by treating secondary wastewater coming from Bari wastewater treatment plant with ECw 1.2 dS m-1 with ultrafiltration, active carbon and reverse osmosis till reach ECw of 1 dS m-1).
DESERT (Low-cost water DEsalination and SEnsoR Technology) is an innovative water desalination and sensor technology compact module for continuously monitoring water quality has been developed in the framework of the DESERT European project (Water JPI, 2016) with participating partners from Italy, Spain and Belgium. DESERT technology, in order to contrast water scarcity and to increase the water quality, enhances the energy savings using solar energy to treat the non-conventional water. The second one was saline Reclaimed

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Water (RW) obtained by mixing the secondary wastewater (ECw 1.2 dS m-1) with the brine produced on the DESERT prototype till reaching an ECw of 3 dS m-1).
Two irrigation treatments were established for each water source. The first treatment was a full irrigation control (FI) irrigated throughout the growing season to fully satisfy crop water requirements (100% ETc). The irrigation volume has been calculated by the water balance method, with restitution of 130% crop evapotranspiration (ETc) lost in each irrigation interval. The second one was a regulated deficit irrigation (RDI) treatment with an irrigation regime similar to FI, except during the second stage of moderate fruit growth, near the initiation of the first stage of oil accumulation, when it received half the water as applied to the FI (50% ETc). This RDI period was chosen because it corresponded to approximately the end of maximum rate of pit hardening and before the rapid phase of fruit growth and oil accumulation begins, thus avoiding the fruit set period (Stage 1), when olive trees are more sensitive to water stress.
Different measurements were performed:
Irrigation quality, Olive production, ripening indices and industrial transformation, Total production, Ripening indices determination, Olive industrial transformation, Chemical oil analysis, Legal quality parameters, Fatty acid composition, Determination of total phenolic compounds and pigments, Physiological measurements, Leaf measurements.

Results

This study evaluated the effects of irrigation with two water sources: low-cost water DEsalination and SEnsoR Technology (DESERT) desalinated water (DW) ECw ~1 dS m−1) and reclaimed water (RW) (ECw ~ 3 dS m−1) combined with two irrigation strategies: full irrigation (FI) (100% of ETc) and regulated deficit irrigation (RDI, 50% of ETc) on fruit yield, ripening indices oil yield, quality of oil, physiological status, leaf and soil nutrients of trees cv Arbosana planted in Mediterranean conditions.
Our results showed that RW without water restrictions increased the fruit yield by 35% due to a slight increase in the fruit weight and, mainly, to a greater fruit set than the control trees; although this did not result in a higher oil yield (g tree−1) since the oil content per fruit dry weight was reduced. The RDI strategy did not decrease the fruit yield despite the fact that olive weight tended to decrease, and it increased the oil yield by ~14.5%. The combination of both stresses (RW and RDI) neither decreased the fruit yield; however, it significantly reduced oil yield (25% less in 2018) since oil content per fruit dry weight was strongly reduced (40%) compared to control trees. Both RDI treatments, regardless water source, determined acidity levels in olive paste lower than in FI treatments; however, it reduced oil extractability and fatty yield. The finding about oil quality indicated that olive exposure to RW, regardless of the water amount, decreased oil quality mainly due to the reduction of oleic acid and the increase of C18:2/C18:3 ratio and peroxides; on the contrary, both RW and RDI improved the total polyphenols. In all cases, the parameters met the legislation. In short, with appropriate management, RW and RDI have great potential to manage oil olive production; nevertheless, studies subjected to long-term use of these techniques should be experienced to ensure the sustainability of oil yields and quality.
Besides, our results showed that saline RW caused significantly higher soil concentration of valuable agronomic macro- and micro-nutrients such as N, K, P, Ca, Mg, Fe and Zn than DW. Moreover, RW also determined a high soil content on Na and Cl- which fortunately did not give rise to a phytotoxic accumulation in olive leaves. Finally, RW irrigation decrease K, Mg, Ca and Fe concentrations in soil solution, reducing the capacity of roots to absorb these

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essential nutrients without diminishing the K+ /Na+ ratio. This fact showed a certain salt tolerance of the olive crop. Thus, in terms of leaf nutrients, RW resulted in an improvement in total N, P and Zn, and in NO3- and PO4-3 anions.
Regarding water status, saline stress induced by RW did not reduce the stem water potential and gas exchange parameters, except during months with higher evaporative demand in 2017 when trees irrigated with RW closed partially their stomata to avoid vascular damage by vessel cavitation. Water stress induced by RDI decreased stem water potential and increased significantly water use efficiency, mainly in RW-RDI in 2018.
These results highlight that, in semiarid areas, RW combined with RDI can be a future practice on olive irrigation, but long-term studies to establish suitable management practices must be developed.

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Discrimination of virgin olive oils with different geographical origin: a rapid untargeted chromatographic approach based on volatile compounds

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Keywords: Virgin olive oil; Geographical origin; Untargeted approach; Volatile compounds.

Objectives

For ensuring that consumers are not misled, the EU Reg. 29/2012 establishes that *"Extra virgin and virgin olive oil shall bear a designation of origin on the labelling"*. This means that for extra virgin (EVOOs) and virgin olive oils (VOOs) commercialized within the EU, it is mandatory to specify the geographical provenance on the label of the product following specific rules. However, the regulation does not specify an official analytical procedure to verify the conformity of the label-declared geographical origin; this has raised the interest of researchers to develop an effective method to ensure the traceability. During the last years, different analytical techniques, including chromatographic ones, have been investigated in order to find useful markers; innovative instrumental approaches have been tested to deal with the need for simple, rapid, and environmentally friendly techniques (Melucci et al, 2016; Valli et al., 2016). The aim of this work was the application of flash gas chromatography, combined with data elaboration by two different classification techniques, for rapid discrimination of EVOOs and VOOs according to geographical provenance.

Methods

A total of 210 EVOOs and VOOs with a different geographical origin ("EU" for oils from EU member states; "Extra-EU" for oils from outside EU countries; "Blends" for samples obtained by mixing oils from different EU state members or oils g from EU and outside EU states) were collected directly from companies. The analysis of volatile compounds was carried out by the Flash Gas Chromatography Electronic Nose Heracles II (Alpha MOS, Toulouse, France). Each sample was analysed in triplicate. For the data analysis, full chromatograms were processed by applying chemometric elaborations with an untargeted approach. As data pre-treatment, chromatograms were aligned by COW algorithm and autoscaled. Two different statistical techniques were used to classify samples according to their geographical origin, the first (PLS-DA) based on a linear approach, and the second (artificial neural network, ANN) on a non-linear approach. The two models were also validated using an external data set.

Results

For both elaborations, satisfactory results, in terms of percentages of samples correctly classified, were obtained: PLS-DA (external validation) allowed classification of 89% and 81% of "EU" and "Extra-EU" samples, respectively; for ANN (testing set) the percentages were equal 93.2% and 88.8%, respectively. The external validation percentages were lower compared to those obtained for the cross-validation as expected, but the results can be considered more robust since they were obtained considering the 25% of samples that were not used to build the model. The Variable Importance in Projection score obtained by the PLS-DA confirmed that a specific section of the chromatogram has a major contribution to

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sample discrimination. This approach is promising to discriminate EVOOs and VOOs according to the labelled geographical provenance. The methodology is fast (around 200 sec for each chromatographic run) and easy to use (no sample treatment is required). The results obtained herein sustained the use of multivariate chemometrics with untargeted detection of volatile compounds as a powerful traceability tool to discriminate EVOOs and VOOs of different origin.

This work, recently submitted to a scientific journal, was supported by the project OLEUM "Advanced solutions for assuring the authenticity and quality of olive oil at a global scale", which has received funding from the European Commission within the Horizon 2020 Programme, GA no. 635690. The information expressed reflects the author's views; the EC is not liable for the information contained herein.

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Valli, E., Bendini, A., Berardinelli, A., Ragni, L., Riccò, B., Grossi, M., & Gallina Toschi, T. (2016). European Journal of Lipid Science and Technology, 118, 1601-1619.

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The sound of olive oil

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Keywords: density; fatty acids; rheology; ultrasonic velocity

Introduction

Extra virgin olive oil (EVOO) adulteration is considered a common fraudulent practice in the olive oil industry. In order to assess EVOO authenticity, a large number of potential techniques for EVOO characterization have been reported. Sound properties, however, have hardly been explored for oil authentication to date. Listening to the sound of oils may provide a rapid and non-destructive alternative for EVOO analysis. The aim of the study was to explore the sound characteristics of EVOO and other vegetable oils for potential use in EVOO authentication and to examine the underlying causes for the ultrasonic velocity differences between the oils.

Objectives

The aim of the study was to explore the sound characteristics of EVOO and other vegetable oils for potential use in EVOO authentication and to examine the underlying causes for the ultrasonic velocity differences between the oils.

Methods

A pulse-echo ultrasonic system was developed to measure the sound characteristics of EVOO and other oils. Eighty oil samples, including 30 EVOO, 15 refined OO, 15 pomace OO, 10 rapeseed oil, 5 sunflower oil and 5 peanut oil samples, were analyzed for their sound properties, which were compared with their viscosities, densities and fatty acid Material and Methods: A pulse-echo ultrasonic system was developed to measure the sound characteristics of EVOO and other oils. Eighty oil samples, including 30 EVOO, 15 refined OO, 15 pomace OO, 10 rapeseed oil, 5 sunflower oil and 5 peanut oil samples, were analyzed for their sound properties, which were compared with their viscosities, densities and fatty acid compositions.

Results

The ultrasonic velocity of EVOO decreased linearly with increase in temperature and the temperature coefficient of ultrasonic velocity in EVOO amounted −2.92 m·s−1·°C−1. The ultrasonic velocity of EVOO differed significantly from those of pomace OO and the oils of other botanical origin, but not from the velocity of refined OO. Ultrasonic velocity was positively correlated with the density and negatively correlated with the viscosity of the oils. The higher density and lower viscosity of the oils were in turn related to a higher unsaturation degree of the oils. Hence, oils with a higher proportion of unsaturated fat presented higher densities and lower viscosities, which resulted in higher ultrasonic velocity values.

Conclusion

EVOO can be identified from oils of other botanical origin and pomace OO according to its own distinct sound. The ultrasonic pulse echo system allows rapid and non-destructive

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analysis, which is a first step towards an interesting new tool in the EVOO authentication toolbox.

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Francesco Bimbo		The research interests in which Dr. Bimbo is currently involved are two: the first research area is about the organization and the economy of the food-supply chains, with a focus on short food-supply chains. The second area of research is on the economics of credence attributes on food products. Such subjects are explored from different angles, using multiple types of data (e.g., market data, survey data and data collected through the use of virtual simulated reality), data are then analyzed through the use of empirical econometrics approaches.
Nicola Caporaso		My research background focuses on understandingthe link between food processing, its chemical composition and its final quality. I have worked on extra virgin olive oil, food flavour, antioxidant activity and sensory analysis. I have also investigated the use of rapid non-destructive methods (NIR hyperspectralimaging) for the analysis of key quality constituents in a variety of granular food products including wheat, cocoa, coffee and others.
Marika Cariello		I am Marica Cariello, I am currently a postdoctoral research fellow at University of Bari. My activity is focused on nutrigenomic effetcs of extravirgin olive oil (EVOO) in heatlhy subjects and metabolic syndrome patients. Furthermore, I studied the effects of several apulian EVOO monocultivar on intestinal inflammation.
Sara Carpi		Sara Carpi is Researcher in Pharmacology at the Department of Pharmacy, University of Pisa (Italy). During her post-doc fellowship, she has been awarded with research fellowships of Italian Pharmacology Society and Merck & Co. She spent a period of study in the ImmunoViroTherpay Lab, Department of Pharmacy of University of Helsinki (Finland), supported by an EMBO fellowship. Her current research is focused on the evaluation of genetic and epigenetic modulation induced by natural compounds, focusing on nutrigenomic and nutriepigenetic.
Giovanni Caruso		Giovanni Caruso is Senior Researcher at the Department of Agricultural, Food and Environmental Sciences of the University of Pisa and his research activity focuses on the physiology of fruit trees and orchard management, with particular emphasis on olive. Research interests: irrigation, soil management, fruit physiology and oil quality. Other investigations included the monitoring of carbon fluxes in a fully-productive olive orchard under different soil management systems. Recent works include the use of UAV for the estimation of the biophysical and geometric parameters of grapevines and olive trees.

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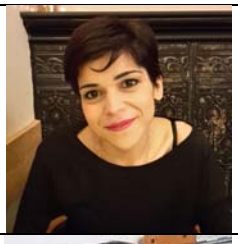
Lorenzo Cecchi		I'm Lorenzo Cecchi, from Florence (Italy); I have a degree in chemistry, I worked about 9 year in the olive-oil field, as analyst, researcher and olive oil taster, then I attended the PhD course in the Food area. My main skills in the Virgin Olive Oil field concern phenolic compounds, volatile compounds and their relation to the sensorial analysis and the re-use of the olive oil byproducts. I'd like to give my contribute to improve the average quality of extra virgin olive oil, also in collaboration with producer and researchers from all over
Rita Celano		My name is Rita. I'm from Salerno. All my study path from graduation to doctorate I did at the University of Salerno. I work in the field of food chemistry and analytical chemistry, in particular I am fascinated by mass spectrometry. I hope to make my small contribute to scientific research with my work.
Danny Cliceri		Danny from Florence, researcher in Sensory and Consumer Science with a passion for nature. I would like to use my knowledge to promote more sustainable diets and sustain the biodiversity through the valorization of local ingredients and food products.
Pasquale Crupi		Pasquale Crupi is a Research Scientist at CREA-VE, Council for Agricultural Research and Economics - Viticulture and Enology, Italy. His research is mainly focused on chemical characterization of metabolites in food matrices. From 2007 to 2013 he was member of Italian delegation to the International Organisation of Vine and Wine (OIV). From 2006 he is a Lecturer for many post-graduate courses. He is the author of more than 100 publications
Alessandra De Bruno		I am a young researcher who has been studying the Calabrian olive sector, since 2009. I have a conscientious, hard-working, meticulous and ambitious character". I am very pleased to presented my work to EVOO Research's Got Talent 2020.
Stefania De Santis		I am a senior researcher on a fixed-term contract at the Department of Pharmacy-Drug Science of the University of Bari Aldo Moro (Italy). I am creative and meticulous in my work with good problem-solving and team-working skills. As a researcher, I am highly motivated and my ambition is to contribute with my findings towards the prevention and/or treatment of chronic inflammatory disorders in humans by the use of nutraceuticals and functional foods

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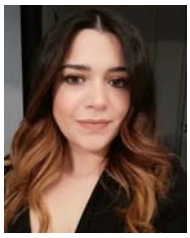
Pierfrancesco Deiana		My name is Pierfrancesco Deiana, 30 years old, from Sassari, Italy. I have recently finished my PhD a the University of Sassari, focusing on the valorization of the Sardinian Olive germplasm, with a specific concern on virgin olive oils composition. My ambition is to provide a deep and complete characterization of the Sardinian olive varieties and to contribute to the enhancement of the Regional and Italian olive oil sector.
Elia Distaso		He is currently a post-doctoral researcher at the Polytechnic of Bari. As mechanical engineer, his research is devoted to the study of energy generation and conversion systems. Currently, a large part of his activity is dedicated to the development of an innovative ultrasound-based technology for the Extra-Virgin Olive Oil extraction process. Such a technology has been designed to induce controlled cavitation phenomena in the olive oil paste with the aim of obtaining a simultaneous increase in the oil extraction yield and in its nutraceutical content. Among the most relevant achievements of this activity, there is the first in the world full-scale continuous device for the EVOO industry employing a coupled ultrasonic and thermal conditioning of the olive oil paste.
Graziana Difonzo		I am Graziana Difonzo, a post doc researcher at University of Bari (Italy). The main object of my work is the extraction of functional compounds from olive oil waste and by-products and the subsequent physico-chemical characterization and use in foods. My mission is the valorization of waste and by-products by means of their exploitation in different fields such as agro-food and pharmaceutical.
Valentina Fanelli		I am a molecular biologist with a PhD in plant biotechnology. I have spent the last few years on olive and grapevine biodiversity exploration and discovery. Moreover, I have worked on methods and protocols aiming to efficiently isolate DNA from food matrix, such as olive oil, in order to perform traceability based on molecular markers. I am interested in setting up innovative methods for molecular food traceability and study in genes involved in desirable agronomic traits.

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Dennis Fiorini		My name is Dennis Fiorini. I am a food chemist and I work at the University of Camerino (Italy) where I teach and I carry out research in the field of Food Chemistry. Within my work, research is my main passion, with the development of new methods to analyse substances that have specific interest in food (e.g. for their sensory or healthy properties) and also with their application to explore the wonderful world of food quality
Federica Flamminii		I am a PhD student in food science at the University of Teramo, my research activity is focused on the valorization of olive by-products as important functional ingredients sources. This experience will allow to improve my career prospects as a future researcher in the olive oil sector.
Silvia Grassi		I carried out my studies at the Faculty of Food and Agriculture, University of Milan, where I got a BSc in Food Service Science and Management, a MSc in Human Nutrition and Food Science and a PhD in Technological Innovation for the Food, Agricultural and Environmental Sciences. I currently hold a postdoc position at the Department of Food, Environmental and Nutritional Sciences of the University of Milan. Within my postdoc project I deal with the application of the most important sensing techniques, coupled with multivariate data analysis, for food process and quality evaluation. Even though, my research in the field of EVOO is still at the beginning, I believe that the sensing techniques I have experience in look promising along the EVOO supply chain, from the olive maturation stage determination directly in the field to the EVOO quality assessment.
Lorenzo Guerrini		I'm a post-doc researcher focusing on biosystem engineering as main research topic at University of Florence. During my career, I spent several times in the study of the technologies for extra virgin olive oil extraction. My master's degree thesis, as well as his PhD thesis involved the olive oil production, and particularly, the malaxation and the oil storage respectively. During my 10 years of research activity, I was involved in several research projects, most of them on extra virgin olive oil processing and storage.
Olusola Samuel Jolayemi		Sam' is a young, resilient, "Mediterranean-diets-loving", Nigerian Food Scientist, who thrives better working in team. He is a perceptive communicator who desires to work with institutions renowned for giving people unparalleled opportunities to build their careers and capabilities.

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Maja Jukić Špika		PhD Maja Jukić Špika, Dynamic and versatile Scientific Associate from Institute for Adriatic Crops and Karst Reclamation, Split, Croatia, with extensive detailed research and experimental experience focusing on olive texture attributes, bioactive compounds and sensory analyses of virgin olive oil. Last ten years is working on multidisciplinary projects aiming at conservation of olive plant genetic resources, its phentyping and genotyping, developing the methodology for reliable determination of the olive oil origin, and other projects thought we are getting to know and discover olive and VOO and the various factors that affect oil uniqueness.
Carmen Lammi		Carmen Lammi (F) is researcher (RTD-B) in Food chemistry, Department of Pharmaceutical Sciences (DISFARM), Faculty of Pharmacy, University of Milan, Italy (UMIL). Major research interests: Plant proteins and peptides: development of innovative functional foods and dietary supplements for the prevention of chronic diseases and other pathologies; Plant proteins and peptides: structure elucidation, investigation of the biological activity by cellular studies, absorption and bioavailability evaluation at intestinal level, development of analytical methods by mass spectrometry, shot-gun proteomics, 2D-electrophoresis, identification of food allergens, peptidomics; Phytochemicals: development of innovative analytical methods and investigation of the biological activity by cellular studies, absorption and bioavailability evaluation at intestinal levels
Francesco Longobardi		Francesco Longobardi is assistant professor is researcher at the Department of Chemistry of the University of Bari. The research ctivity is now focused on the use of techniques of nuclear magnetic resonance (NMR) and isotope ratios mass spectrometry (IRMS) for the characterization of the geographical origin and for the study of issues related to the quality and safety of agricultural and food production. He published papers (or book chapters) in scientific journals and books relevant to the above topics, and is co-author of 2 patent. His greatest aspiration is to become a musician

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M. Carmen López de las Hazas		My name is M.Carmen López de las Hazas, I'm from Madrid. I love the research, I'm a fan of pipette! I'm very motivated by the science! Furthermore, I'm multitasking and highly adaptable!
Maria Paciulli		Italian enthusiastic Food Scientist. Expert in physical and thermal properties of olive oil. My interests are in the sustainability of the olive oil sector starting from the olive processing to the by-products valorisation.
Diamantakos Panagiotis		My name is Panagiotis Diamantakos and I m coming from Athens, Greece. I m a chemical engineer and the last 7 years i ve worked in the Pharmaceutical school of Athens , on olive oil analysis with NMR, and extraction methods for olive oil phenolics. My ambition is to explore new aspects of olive oil chemistry, to highlight the importance of olive oil as a healthprotective food but also to highlight the importance of olive oil phenolic ingredients through clinical trials
Elisa Pannucci		I am Elisa Pannucci, I am currently a postdoctoral research fellow at University of Tuscia. My activity is focused principally at recovering and reusing polyphenols from olive (Olea europaea L.) oil by-products. My efforts are mostly focused on evaluating the biological activities of hydroxytyrosol, a low molecular weight phenol found in olives and therefore in olive oil by-products.
Maja Podgornik		Maja Podgornik graduated at the Biotechnical Faculty of theUniversity of Ljubljana, where she also received her PhD with doctoral dissertation. In the framework of national and international projects, she has worked at the Institute for Oliveculture, Science and research Centre of Koper since 2005. Maja Podgornikis a member of the Scientific Council and the head of the Institute for Oliveculture. Since 2018 is also Head and national coordinator of the public service in the olive production. Main research fields are agronomy and technology of olive growing and Mediterranean agricultural plants. She works in the field of plant protection, irrigationmanagement, climatic change and new technologies in agriculture production.

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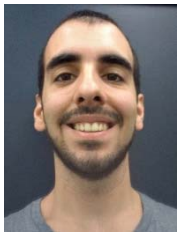
Aimilia Rigakou		My name is Aimilia Rigakou and I am PhD Candidate in the department of Pharmacy at the National and Kapodistrian University of Athens. I enjoy working in the laboratory and contribute to the evolution of this domain of research. I am also interested to meet other young olive oil researchers from around the world, exchange opinions, and enrich my knowledge in this field. Finally, besides research I deal with the art.
Annalisa Silenzi		I am Annalisa Silenzi, a Dietitian and Nutritionist with a Master of Science in Food sciences and Human Nutrition. I focused my attention firstly in the clinical field then, I dedicated myself to nutrition in terms of public health, developing an interest in health literacy and food literacy. Currently I am part, as a PhD student, of the research team of the Gender Prevention and Health Unit of the Center for Gender Medicine at the Italian National Institute of Health (Istituto Superiore di Sanità), where I am working on research projects aimed at promoting correct lifestyles through food education and scientific dissemination.
Federico Stilo		Federico Stilo's PhD project deals with food volatilome, especially Italian extra-virgin olive oil's one, with particular emphasis on sensory active volatiles responsible for positive and negative attributes. The toolbox to achieve project objectives includes comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry, automated headspace sampling, and dedicated data processing tools for effective chemical fingerprinting and profiling.
Annia Tsolakou		My name is Annia Tsolakou, I am from Greece, I am a Biotechnologist with postgraduate studies in Pharmacognosy and specialized in EVOO phenolics.My ambition is to uncover the mechanisms and genetical characteristics that define the existence of different olive oil phenolic profiles
Enrico Valli		As Assistant Professor at the Department of Agricultural and Food Sciences of the University of Bologna, my main research interests lie in instrumental and sensory analysis of extra virgin olive oil; my ambition is to carry out useful investigations for helping to evaluate and assure its quality and genuineness.

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Alessandro Vivaldi		I am Alessandro Vivaldi from The Department of Agricultural and Environmental Sciences of the University of Bari Aldo Moro. My current position is Assistant Professor. I have developed wide research on reclaimed water reuse as an irrigation source for agriculture. In particular, I have investigated the effects of reclaimed water on tree crops, considering plant physiology response, soil chemical properties, leaf mineral status, crop production, and fruit quality and safety. My principal ambition is to make agriculture more competitive, greener, fair and innovative.
Jing Yan		Jing YAN is a PhD student in Wageningen University and Research. Her research topic is to investigate fraud in the extra virgin olive oil supply chain, which includesthe identification of vulnerable points and development of novel fraud detection methods. During her PhD project, four novel techniques were developed to distinguish EVOO from lower grade olive oils and from oils of other botanical origins. All the techniquesare not easy to circumvent by fraudsters. The efforts dedicated to the development of novel fraud detection techniques contribute to the EVOO authenticity.

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