

## CONGRESSO SISSG 2018 BARI ELENCO POSTER

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ANA = Analitica ed autenticità, CON = Contaminanti, BAK = Sostenibilità e lipidi e prodotti da forno, COP = Utilizzo e valorizzazione dei sotto prodotti, TEC = Tecnologie per gli oli ed i grassi, SL = Shelf life

## **SESSIONE ANALITICA ED AUTENTICITA'**

### **ANA-3**

#### **Application of chemical and thermal analysis for the evaluation of traceability of Italian extra virgin olive oil**

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**Keywords:** olive oil, DSC, fatty acids, geographical origin, cultivars

#### **Objectives**

The aim of this work, supported by AGER foundation (Project "SOS Sustainability of the Olive Oil System", grant no. 2016-0105), is to select chemical (fatty acids) and thermal predictors, obtained by means of differential scanning calorimetry (DSC), able to discriminate among extra virgin olive oils (EVOO) belonging from different minor cultivars of different Italian regions. The use of DSC as complementary technique to those traditionally employed in this sector will be thus proposed

#### **Methods**

Thirteen extra virgin olive oils belonging from minor olive cultivars of three different Italian regions were analyzed: two from Apulia (Oliva Rossa, Cima di Melfi and Bambina), three from Sardinia (Corsicana da Olio, Semidana and Sivigliana), three from Abruzzo (Dritta, Tortiglione and Gentile de L'Aquila) and four from Calabria (Ottobratica, OttobraticaCalipa, Tonda di Filogaso, Ciciarello).

For each of the selected cultivars the drupes were harvested at three different ripening times, at intervals of 15 days, starting about one month before the traditional harvest time.

For each sample fatty acids were determined as FAMES by capillary GC analysis after alkaline treatment. The results were expressed as area normalization in percent (%).

Thermal properties upon cooling and heating were measured by means of DSC Q100 (TA Instruments, New Castle, DE, USA) in a range of temperature from 30 to -80°C and vice versa at a scanning rate of 2°C/min. Temperatures and enthalpies of the transitions were recorded.

ANOVA and Principal component analysis (PCA) were conducted by means of the Statistical Software SPSS (version 25) to discriminate or find relationships among the samples according to specific variables. The experimentation has been conducted over two crop years.

#### **Results**

Several differences were evidenced between the analyzed EVOOs, with good correlations between the chemical and thermal measurements. Saturated and monounsaturated fatty acids resulted respectively related to the onset temperatures of the cooling and heating transitions, being instead enthalpy not discriminating among the samples. PCA analysis helped in identifying the variables able to separate the oils according to their cultivar and geographical origin.

DSC resulted to be a useful tool to obtain a unique fingerprint of the oils, useful to identify their botanical and geographic origin.

The experimentation will be conducted over three years, to confirm the data and open a good way for the characterization and thus recover and valorization of the minor olive cultivars, botanical heritage of the Italian regions.

### **ANA-4**

#### **FAST AND GREEN FT-IR CLASSIFICATION OF EXTRA VIRGIN OLIVE OIL BASED ON ETHYL ESTER CONTENT.**

Giacomo Squeo<sup>(1)</sup>, Silvia Grassi<sup>(2)</sup>, Roccangelo Silletti<sup>(1)</sup>, Carmine Summo<sup>(1)</sup>, Vito Michele Paradiso<sup>(1)</sup>, Antonella Pasqualone<sup>(1)</sup>, Cristina Alamprese<sup>(2)</sup>, Francesco Caponio<sup>(1)</sup>

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**Keywords:** FT-IR, ethyl esters, olive oil, classification

#### **Objectives**

Ethyl esters are mandatory parameters to be checked for extra virgin olive oil commercial classification. Their determination is complex, time consuming, and expensive. Moreover, many toxic solvents are required. Thus, our aim was the development of a model based on FT-IR spectroscopy able to classify virgin olive oils in two classes: extra and non-extra products.

## Methods

A set of 119 virgin oils were analysed for the content of ethyl esters; besides, their FT-IR spectrum was collected (6 per sample), in the range 4000-600  $\text{cm}^{-1}$ , with a 4  $\text{cm}^{-1}$  resolution. After averaging, spectra were subjected to smoothing and Standard Normal Variate (SNV) pre-treatments and used to develop classification models by means of Linear Discriminant Analysis (LDA). The 30 variables most relevant for discrimination were selected by applying the Select algorithm implemented in V-Parvus software. Models were validated by internal and external validation.

## Results

Preliminary results showed good classification ability of the developed model. An average correct classification of 98%, 92%, and 88% for calibration, cross-validation and prediction, respectively, was obtained. At this point, the method seems to be useful for fast screening purposes. In future perspectives, sample set broadening and use of the selected variables for the development of regression models for the quantification of ethyl esters could be considered.

## ANA-5

### La spettroscopia NIR per determinare il grado di maturazione delle olive: confronto tra uno spettrofotometro VIS/NIR e uno FT-NIR

Alessio Tugnolo <sup>(1)</sup>, Cristina Alamprese<sup>(2)</sup>, Roberto Beghi<sup>(1)</sup>, Silvia Grassi <sup>(2)</sup>, Ernestina Casiraghi<sup>(2)</sup>, Valentina Giovenzana<sup>(1)</sup>,

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*(2) Università degli Studi di Milano, Dipartimento di Scienza per gli Alimenti, la Nutrizione e l'Ambiente,*

**Parole Chiave:** Spettroscopia, tecnologia green, maturazione, olive

### Obiettivi

Negli ultimi anni, nel settore olivicolo è cresciuto l'interesse verso lo sviluppo di metodiche analitiche alternative a quelle tradizionali, più rispettose dell'ambiente e in grado di fornire risposte rapide e applicabili anche in campo. In questo contesto, la spettroscopia NIR si rivela uno strumento efficace. L'obiettivo di questo lavoro è stato lo sviluppo di applicazioni basate sulla spettroscopia vis-NIR e FT-NIR per monitorare la maturazione delle olive in campo. La rapida valutazione dell'indice di maturazione è infatti determinante per individuare il momento più opportuno per la raccolta delle olive, al fine di ottenere una buona resa produttiva e una buona qualità dell'olio.

Questo lavoro è parte di un progetto AGER (Progetto Agroalimentare e Ricerca), denominato "Sustainability of the Olive-Oil System - S.O.S.", il cui obiettivo è quello di migliorare e rafforzare la sostenibilità della filiera dell'olio extravergine di oliva in Italia.

### Metodologia

Sono state analizzate cinque cultivar di olive (Cima di Melfi, Oliva Rossa, Corsicana, Semidana e Sivigliana) raccolte a diversi stadi di maturazione. Le olive sono state classificate in base all'indice di maturazione utilizzando due metodi diversi e sono stati inoltre acquisiti gli spettri vis-NIR (400–1023 nm), con strumento da campo, e FT-NIR (900–2560 nm) mediante spettrometro da banco. Dopo pretrattamento mediante smoothing, i dataset ottenuti da ciascuno strumento (1020 spettri) sono stati analizzati mediante Principal Component Analysis (PCA). Sugli spettri pretrattati mediante smoothing, Standard Normal Variate (SNV) e trasformazione in derivata prima è stato poi applicato il metodo di classificazione PartialLeastSquare – Discriminant Analysis (PLS-DA), con l'obiettivo di creare modelli in grado di classificare correttamente le olive in 4 classi di maturazione (Classe 1-> olive totalmente verdi; Classe 2-> olive con meno del 50% di superficie invaiata; Classe 3-> olive con più del 50% di superficie invaiata; Classe 4 -> olive totalmente invaiate.). Sono stati elaborati due dataset, uno completo, contenente gli spettri di tutte le olive analizzate, e un dataset specifico per le olive della cv. Cima di Melfi (180 spettri).

### Risultati

Dall'analisi qualitativa, mediante PCA, si è individuato un andamento dei campioni di olive analizzati in funzione dell'indice di maturazione (PC1) e una suddivisione per provenienza e/o varietà (PC2). Per

quanto riguarda la classificazione, i migliori valori di accuratezza in validazione sono stati registrati per gli spettri acquisiti nel vis-NIR e pretrattati mediante SNV, sia per la classificazione delle 1020 olive totali ( $95.20 \pm 4.88\%$ ), sia per quelle appartenenti alla cv. Cima di Melfi ( $92.33 \pm 5.86\%$ ). Con gli spettri FT-NIR si sono registrati dei valori di accuratezza inferiori, pari a  $84.41 \pm 6.11\%$  per gli spettri totali (pretrattamento di smoothing) e a  $82.98 \pm 7.65\%$  per quelli della cv. Cima di Melfi (pretrattamento SNV).

La classificazione delle olive in campo mediante applicazione della spettroscopia NIR appare quindi possibile in modo rapido, accurato ed oggettivo.

## **ANA-6**

### **Confronto tra l'impatto ambientale dell'analisi tradizionale e mediante spettroscopia nir sull'oliva**

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**Parole Chiave:** Life Cycle Assessment, oliva, spettroscopia

#### **Obiettivi**

Le analisi che tradizionalmente si effettuano sulle olive richiedono l'utilizzo di molteplici strumentazioni, lunghi tempi di analisi, l'utilizzo di solventi potenzialmente dannosi, sia per la salute umana che per l'ecosistema, e la distruzione del campione. Le stesse analisi effettuate con una tecnologia ottica, non distruttiva (spettroscopia nella regione del visibile e vicino infrarosso, Vis-NIR), permettono la stima dei parametri di interesse, in modo molto rapido, con una sola lettura, anche condotta dagli addetti alla raccolta. L'unico aspetto laborioso è la costruzione di robuste calibrazioni. Il grande vantaggio sarebbe un ridotto impatto ambientale della tecnologia ottica, la cui valutazione, a confronto con i metodi tradizionali, è lo scopo di questo lavoro. Esso è parte di un progetto AGER (Progetto Agroalimentare e Ricerca), denominato "Sustainability of the Olive-Oil System - S.O.S.", il cui obiettivo è quello di migliorare e rafforzare la sostenibilità della filiera dell'olio extravergine di oliva in Italia.

**Metodologia** Le analisi tradizionali per la valutazione qualitativa delle olive individuate come riferimento sono state: contenuto di acqua, olio e fenoli totali. Il confronto è stato effettuato per le stesse determinazioni mediante analisi ottica. Il metodo Life Cycle Assessment (ISO 14040:2006, ISO 14044:2006), è stato applicato per valutare l'impatto ambientale mediante un preciso schema di esecuzione. L'approccio utilizzato (*from cradle to grave*), ha considerato tutti gli input e output di ogni analisi, tenendo conto della strumentazione necessaria per l'esecuzione (estrazione materie prime, costruzione, utilizzo e smaltimento), dei solventi utilizzati (ottenimento solvente, utilizzo e smaltimento), delle risorse energetiche necessarie. Inoltre, per la strumentazione Vis-NIR sono state considerate le analisi necessarie per la calibrazione dello strumento. I dati raccolti sono stati elaborati con il software SimaPro.

#### **Risultati**

Il confronto tra l'impatto ambientale delle analisi tradizionali e quello delle analisi ottiche ha evidenziato un netto vantaggio nell'applicazione della strumentazione ottica la quale, non solo garantisce la non distruzione del campione e un risultato in tempi brevi ma, ha un impatto sull'ambiente 36 volte inferiore rispetto alle analisi tradizionali di riferimento. Se le maggiori voci di impatto ambientale per le analisi tradizionali sono la corrente elettrica, la strumentazione e le sostanze chimiche, per l'analisi non distruttiva, l'impatto ambientale è dettato maggiormente dalla necessità di calibrare lo strumento, attività che richiede di effettuare su un relativamente elevato numero di campioni (500 per la calibrazione iniziale, 200 per il mantenimento) sia le analisi tradizionali che le letture ottiche (Vis-NIR).

## **ANA-7**

**Title.** Validation of an off-line SPE-GC-FID method for the determination of n-alkanes in vegetable oils.

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Keywords: n-alkanes, Solid Phase Extraction (SPE), GC-FID, Vegetable oil, on-line LC-GC

**Objectives** The main objective of the work was the validation of a rapid and solvent-sparing off-line SPE-GC-FID method for the determination of endogenous n-alkanes in vegetable oils and comparison with the on-line LC-GC-FID method. The distribution of n-alkanes may be a very good fingerprint useful to distinguish between different vegetable oils and to determine adulteration of high quality oils with other cheap and low-quality oils.

**Methods** For n-alkane evaluation, a suitable mixture of internal standards was added to an aliquot of the sample, then SPE cartridge packed with silver silica gel was carried out and n-alkanes were eluted with n-hexane. The analytical determination was performed by capillary GC, applying large volume injection with the retention gap technique. Solvent and matrix-matched calibration curves were obtained in the range 0.05-50  $\mu\text{g/g}$  and repeatability was assessed by performing 6 replicate analysis on the same extra virgin olive oil.

**Results** The method showed good performance characteristics: the linearity coefficients ( $R^2$ ) obtained with the minimum square method were equal to 0.99; recoveries in matrix ranged from 86 to 99%, being practically quantitative at the lower spiking level. Different oils showed typical n-alkane profiles. Also the amount of total n-alkane differed significantly: the highest concentration was found in an extra virgin avocado oil (595  $\mu\text{g/g}$ ), while the rest of the samples revealed amounts ranging from 6  $\mu\text{g/g}$  (refined palm olein oil) to 95  $\mu\text{g/g}$  (desterolized and deodorized high oleic sunflower oil). Results obtained were comparable to those obtained with the on-line method. The off-line method was only slightly less repeatable (RSD 5.4%) than on-line method (RSD 2.8%).

## **ANA-8**

**Two dimensional gas chromatography: a powerful tool for discriminating VOCs in monovarietal and commercial extra virgin olive oil**

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**Keywords:** EVOO, two dimensional gas chromatography, VOC

### **Objectives**

The unique and special flavour of EVOO can be attributed to VOCs such as the C6 and C5 aldehydes, which are responsible for green aromatic notes, but also terpenes and sesquiterpenes, which tend to generate greater aromatic complexity in quality oils. 15 monovarietal EVOOs produced from different cultivars (purchased directly from producers) were compared with 25 blended commercial EVOOs (purchased in national grocery stores). The aim of the study was to demonstrate the possibility of discriminating between these two classes of samples based on VOC composition.

### **Methods**

Extra virgin olive oil VOCs were analysed with HS-SPME-GCxGC-TOF-MS<sup>[1]</sup>. A total of 1254 putative compounds were detected, with 123 being significantly different in monovarietal and commercial oils. Compound identity was confirmed with Kovats retention index and by comparing spectra compounds to NIST 2.0, Wiley 8 FFNSC 2 (Chromaleont, Messina, Italy).

### **Results**

Principal component analysis (PCA) showed clear separation between the two classes of samples. Specifically, commercial samples were more influenced by compounds such as alcohols, acids (acetic acid and formic acid) and other acetates frequently responsible for certain unpleasant notes. The monovarietal samples were more influenced by compounds deriving from LOX pathways (aldehydes

C6 and C5), as well as terpenes and sesquiterpenes, such as valencene, copaene and ocimene for example, which generally generate pleasant wood, green and lemon notes.

Furthermore, differences in the composition of monovarietal samples were evaluated. Two varieties (Casaliva and Ottobratico) were clearly distinguished from the rest of the monovarietal samples, underlining that the cultivar and pedoclimatic conditions can influence VOC composition.

The preliminary results show that HS-SPME-GC×GC-TOF-MS is a fast and very powerful tool for revealing differences between the aroma of various EVOO classes.

[1] G. Purcaro, C. Cordero, E. Liberto, C. Bicchi, and L.S. Conte, (2014). Toward a definition of blueprint of virgin olive oil by comprehensive two-dimensional gas chromatography. *J. Chromatogr. A* 1334, 101–111.

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## **ANA-10**

### **Stable isotope ratio analysis for characterizing PDO extra-virgin olive oil ‘Garda’**

Federica Camin<sup>1</sup>, Luana Bontempo<sup>1</sup>, Luca Ziller<sup>1</sup>, Pietro Franceschi<sup>1</sup>, Mauro Nisi<sup>2</sup>, Furio Battelini<sup>2</sup>

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**Keywords:** <sup>2</sup>H/<sup>1</sup>H, <sup>13</sup>C/<sup>12</sup>C, <sup>18</sup>O/<sup>16</sup>O, IRMS, origin traceability

### **Objectives**

PDO extra-virgin olive oils are premium products with higher quality which command a higher price. Stable isotope ratio analysis of <sup>13</sup>C/<sup>12</sup>C, combined with or without analysis of <sup>18</sup>O/<sup>16</sup>O and <sup>2</sup>H/<sup>1</sup>H proved to be a good tool for characterising geographical origin, as isotope ratios change according to latitude, suggesting distance from the sea and environmental conditions during the growing of plants (water stress, atmospheric moisture and temperature) as co-factors of variability.

Objective of this study was to define the stable isotope ratio profile of the PDO extra-virgin olive oil ‘Garda’.

### **Methods**

Around 80 samples of extra-virgin olive oil produced in the area of Garda Trentino (Italy) were collected in 2 seasons (2016 and 2017) and analysed.

The C isotope ratio of bulk oil has been determined by EA-IRMS (Elemental Analyser-Isotope Ratio Mass Spectrometry) directly on the raw sample without any need of pre-treatment. The analysis of H and O stable isotope ratios of bulk dehydrated oil has been performed via TC (Thermal Conversion)/EA-IRMS. The H and C isotopic values of the four main fatty acids (linoleic, oleic, palmitic and stearic acids) have been determined using IRMS coupled with GC (Gas Chromatography), after transesterification of oil.

### **Results**

The Garda PDO extra-virgin olive oil has very characteristic and typical H, C and O stable isotope ratios, because it is produced in an area with a unique microclimate. On this basis, it can be distinguished by the extra-virgin olive oils produced in the Mediterranean areas.

The H and C stable isotope ratios of the main fatty acids can be further markers of geographical traceability.

### **Acknowledgment:**

The study has been funded by the project ‘Innovazione e Ricerca per l’Olio Extravergine dell’Alto Garda Trentino, Agraria Riva del Garda, Ricerca Industriale L.6 (2016-2019)’

## SESSIONE CONTAMINANTI

### CON-4

#### **MOSH/MOAH negli oli extravergini di oliva: aspetti tecnico-analitici e ricorrenza nei campioni reali.**

Luisi Arianna, Sulpasso Michele

*Chemiservice srl*

**Keywords:** Idrocarburi minerali (MOH), idrocarburi aromatici, analisi, fonti di MOH, esposizione alimentare umana,

#### **Objectives**

I MOSH (MineralOilsaturatedHydrocarbons) e MOAH (MineralOilaromaticHydrocarbons) fanno parte della vasta e complessa classe di composti degli Oli Minerali. Le cause che portano alla presenza di oli minerali MOSH e, in parte dei MOAH negli oli vegetali, sono molteplici: contaminazione ambientale in campo (gas di scarico, fumi di combustione presenti nell'atmosfera), contaminazione da materiali da imballaggio in diretto contatto con semi/frutti oleaginosi durante le fasi di stoccaggio/trasporto, trattamento con particolari pesticidi. Negli oli vegetali, un'ulteriore particolare causa della loro presenza è dovuta anche ai processi di pressione (oli lubrificanti), raffinazione (passaggio su terre decoloranti) e confezionamento. Occorre considerare che attualmente mancano indicazioni normative ufficiali relative ai limiti massimi dei livelli degli oli minerali tollerabili negli alimenti; manca un metodo ufficiale standardizzato per la distinzione tra le varie frazioni di oli minerali.

#### **Methods**

Il Laboratorio Chemiservice si avvale dell'applicazione di un metodo validato internamente e accreditato presso l'ente di accreditamento italiano ACCREDIA che si è rivelato perfettamente in linea con i metodi di analisi citati dall'EFSA, sia sotto il profilo della procedura analitica sia sotto il profilo delle prestazioni. Principio del metodo: l'olio, addizionato di opportuni standard di riferimento interni (C40-tetracontano e perilene) e surrogati viene frazionato mediante cromatografia su colonna di gel di silice idratato; se ne ricavano due frazioni contenenti rispettivamente i MOSH e i MOAH che vengono analizzate mediante gascromatografia in colonna capillare con rivelazione a ionizzazione in fiamma (GC-FID).

#### **Results**

Dall'indagine statistica effettuata su campioni analizzati dal Laboratorio Chemiservice relativi al periodo settembre 2017-gennaio 2018 (circa 200 campioni), negli oli extravergini di oliva, riscontriamo i seguenti risultati. Nella valutazione statistica sono state considerate le seguenti frazioni: MOSH (C10-C35), MOAH (C10-C35).

MOSH (C10-C35) - Nel periodo compreso tra settembre 2017 e gennaio 2018 sono stati analizzati quasi 200 campioni di olio extravergine di oliva. Dai dati raccolti si evince che quasi il 40% dei campioni analizzati presentano concentrazioni di MOSH superiori a 10 mg/kg con un valore medio di 12,9 mg/kg.

MOAH (C10-C35) - Dai dati raccolti si evince che quasi la metà dei campioni analizzati presentano concentrazioni di MOAH superiori a 1 mg/kg con un valore medio di 1,5 mg/kg.

Nella presentazione di ottobre verrà riportato un aggiornamento di tali valutazioni estendendo il periodo di acquisizione dei dati a giugno 2018. Si riporteranno anche valutazioni su oli prodotti/commercializzati in paesi extra Europei.

### CON-6

#### **Fitofarmaci negli oli vegetali: aspetti tecnico-analitici, aspetti normativi e ricorrenza nei campioni reali.**

Paola Paolillo, Arianna Luisi

*Chemiservice srl*

**Keywords:** Fitofarmaci, analisi multiresiduale, olio di oliva, oli vegetali, analisi cromatografica, rivelatore di massa triplo quadrupolo

#### **Objectives**

I residui di pesticidi sono presenti negli alimenti e nei mangimi a causa dell'uso di prodotti fitosanitari su colture o prodotti alimentari utilizzati per la produzione di alimenti e mangimi. Al fine di garantire un livello elevato di protezione del consumatore, a livello europeo sono fissati i limiti di legge, i cosiddetti "livelli massimi di residui" o "LMR". Gli LMR definiscono la concentrazione massima di residui di pesticidi consentiti negli alimenti e nei mangimi. Tali limiti normativi sono stabiliti dal regolamento (CE)



n. 396/2005. Attualmente sono in vigore LMR armonizzati per oltre 500 pesticidi. Per i pesticidi non menzionati esplicitamente nella legislazione sui LMR è applicabile un LMR di default di 0,01 mg / kg, un livello pari al limite di quantificazione (LOQ) ottenibile con i metodi analitici utilizzati per l'applicazione dell'MRL. Il regolamento (CE) n. 396/2005 fornisce inoltre il quadro giuridico per le attività di controllo dei residui di antiparassitari che devono essere svolte dagli Stati membri al fine di far rispettare gli LMR. L'oggetto è la presentazione del quadro normativo comunitario e nazionale vigente in relazione ai limiti massimi di residui (LMR) nei semi e frutti oleaginosi e possibilità di applicazione ai prodotti trasformati. Panoramica sulla normativa vigente in alcuni dei paesi maggiori importatori di olio di oliva provenienti da agricoltura convenzionale. Cenni ai metodi analitici normalizzati applicati nel laboratorio Chemiservice. Statistica sull'incidenza dei fitofarmaci negli oli di oliva e in altri oli vegetali alimentari di maggior interesse commerciale.

### **Methods**

Per la determinazione della concentrazione delle sostanze attive nell'olio di oliva e negli oli vegetali destinati al consumo umano sono stati utilizzati due metodi diversi entrambi normalizzati. Per un gruppo ristretto di campioni sono stati messi a confronto i risultati ottenuti con i due metodi

### **Results**

Valutazione dei risultati analitici di più di 3000 campioni per anno per un periodo compreso tra il 2015 e il 2017. Quasi il 99 % degli oli di oliva analizzati sono risultati conformi ai limiti legali; nel periodo 2015-2016 circa il 50 % dei campioni non presentavano livelli quantificabili di fitofarmaci, nel 2017 la percentuale di campioni con tracce di fitofarmaci non quantificabile è scesa a circa 35%.

Applicazione di entrambi i metodi analitici ad un gruppo di campioni di oli vegetali: le due metodiche hanno restituito a risultati confrontabili statisticamente; presentazione dei vantaggi e degli svantaggi dell'applicazione delle due metodiche. Esposizione delle problematiche sia legate all'applicazione della normativa comunitaria ai prodotti trasformati sia alla non-armonizzazione delle normative vigenti in paesi di importazioni non comunitari.

## **SESSIONE OLI E GRASSI IN PRODOTTI DA FORNO**

### **BAK-3**

#### ***Study of the antioxidant potential of tyrosyl oleate in a real lipid matrix***

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**Keywords: tyrosyl oleate, oxidation process, tarallini**

#### **Objectives**

The aim of this research was the study of the antioxidant potential of a lipophenol, tyrosyl oleate, obtained from the reaction between oleic acid and tyrosol. The antioxidant effect of tyrosyl oleate was studied in tarallini, typical Italian bakery product, was carried out through the study of the oxidation process.

#### **Methods**

The reaction between tyrosol and oleic acid was carried out according to Fernandez et al. (2012) and after purification through chromatographic column, different percentage of tyrosyl oleate were used in tarallini formulation. Formulation of tarallini was made according to the traditional receipt using wheat flour, salt and sunflower oil to obtain the control sample (CS); based on sunflower oil content 1, 4 and 7% of tyrosyl oleate was added (1TO, 4TO and 7TO). The oxidation process was studied, immediately after the formulation through an accelerated oxidation analysis using the Oxitest<sup>®</sup> instrument (Riciputi, 2017). Then at different storage time (T0, T15, T30, T37 and T45) peroxide value, spectrophotometric analysis according to Shantha& Decker method (1994), Oxidized Fatty Acids (OFA) (Verardo et al., 2010) and volatile compounds (Marzocchi et al., 2017) originated from lipid oxidation, with innovative gas chromatographic techniques, were determined in the different samples.

#### **Results**

Analysis by Oxitest<sup>®</sup> allowed to discriminate between the control sample and the three samples with tyrosyl oleate; in fact, already in 1TO sample the IP value was more than twice (13.58 h) of CS (6.10 h); in the 4TO and 7TO samples the IP value reached 22.34 h and 25.28 h, respectively. For the peroxide value, at T0 and T15 all the samples did not show significant differences ( $p < 0.05$ ) having all the values under the legal limit of 20 meqO<sub>2</sub>/ kg of fat. At T30, instead, CS and 1TO exceed the legal limit (79.6 and 49.0 meqO<sub>2</sub>/ kg of fat, respectively), while 4TO and 7TO registered a peroxide value of 17.3 and 16.1 meqO<sub>2</sub>/ kg of fat. These two samples exceed the legal limit after 45 days of storage (T45); so, in the worst condition (sunflower oil and storage at environmental conditions) the addition of tyrosyl oleate can slow down the lipid oxidation process. CS registered significant higher ( $p < 0.05$ ) OFA content rather than all the samples with tyrosyl oleate (0.60 mg FA/100 mg FAME); 7TO sample showed the significantly lowest values than 1TO and 4TO at T30 (0.41, 0.37 and 0.31 mg FA/100 mg FAME, respectively). The concentration of volatile compounds originated from lipid oxidation increased with the increasing of shelf life. Tarallini made with tyrosyl oleate shown a significantly lower concentration of these compounds than CS for all the storage time; hexanal, the major representative compound of lipid oxidation, was the preponderant compound. Considering that the tarallini were made with one of the most oxidable oil, sunflower oil, and stored at room temperature without any modified atmosphere; the presence of tyrosyl oleate allows to counteract lipid oxidation and extend the shelf life of tarallini added with it.

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## SESSIONE UTILIZZAZIONE E VALORIZZAZIONE DEI SOTTOPRODOTTI DELLE FILIERE OLI E GRASSI ALIMENTARI

### COP-3

#### **Waste and by-product from olive oil production as source of functional compounds**

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**Keywords:** by-products, olive leaves, oleuropein, olive pomace,  $\beta$ -sitosterol, shelf-life, antioxidants

#### **Objectives**

A large amount of wastes and by-products are generated during olive oil production process such as olive leaves and pomace. Since the importance of some molecules from vegetables, particularly from olives, has been highlighted, olive leaves and pomace are potential rich sources of phenolic compounds, endowed with a wide array of biological activities and also of valuable lipophilic molecules. If the olive leaves are a good source of oleuropein and flavonoids, the olive pomace represents a good source of  $\beta$ -sitosterol, squalene and terpenic compounds. For these reasons our aims are the extraction and the use in foods and in biological systems of the above-mentioned compounds.

#### **Methods**

The extracts obtained from olive leaves were characterized for the phenolic profile by UHPLC-ESI-MS whereas the lipophilic molecules from olive pomace were detected by gas chromatography after saponification and derivatisation. The foods (salty snacks and olive-based paste) enriched with olive leaves extracts (OLE) were monitored with antioxidant activity assays, volatile compounds determination and microbiological analyses. Intracellular ROS (Reactive Oxygen Species) content was evaluated with the fluorescent probe dihydrorhodamine-123 in epithelial cells exposed to OLE.

#### **Results**

The salty snacks added of OLE showed a significantly lower level of volatile compounds originated from lipid oxidation and an increased antioxidant activity (which improved the oxidative stability of snacks) than control snack without OLE. OLE influenced the microbial growth in olivebased paste during storage. The pre-treatment of epithelial cells with OLE lowered the intracellular ROS content. Moreover, squalene,  $\beta$ -sitosterol and terpenic compounds were detected in

### COP-4

#### **Technological functionality of olive leaves phenolic extracts**

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**Keywords:** olive by-products, olive leaves, technological functionality, surface properties, emulsifying capacity.

#### **Objectives**

Most of olive by-products, such as olive leaves, are still undervalued despite their high potential as a source of functional compounds. However, to incorporate such compounds as active ingredients in complex food systems, it is of primary importance the knowledge of their technological functionality which represented, thus, the objective of this work. To this aim, three olive leaves phenolic extracts obtained by either pure water or two different water:ethanol ratios (Eth0, Eth30, Eth70), as described by Difonzo et al. (2017), were characterized for some technological properties such as surface activity at the air/water interface, emulsifying capacity, water/oil holding ability and flow behaviour.

## Methods

The surface tension was evaluated by means of a tensiometer and a dose-dependent curve was obtained for each phenolic extract at pH 4.5 and pH 7. The emulsifying activity was evaluated by measuring the particle size and distribution of oil-in-water model emulsions: the dispersed phase, represented by sunflower oil, varied from 5% to 20% (w/w) while two buffered solution at pH 4.5 and 7 were used as continuous phase. A concentration of 0.3% (w/v) of each extract was added to the water phase. The flow behavior was investigated by means of a rheometer equipped with concentric cylinders while water and oil holding capacity (WHC/OHC) were determined by previous solubilization of each extract either in water or in oil followed by centrifugation.

## Results

The phenolic extracts exerted a significant surface activity showing a typical dose-dependent behavior; in particular the extracts Eth30 at pH 4.5 and Eth70 pH 7 resulted to be the most surface active. Surface properties were affected by pH. Such results were confirmed also by the emulsifying capacity: the model emulsions enriched with Eth30 at pH 4.5 showed a monomodal particle size distribution with a mean diameter of 5  $\mu$ m and higher resistance towards physical instability when compared to the other systems. No significant differences were observed in their flow behavior while WHC and OHC were higher for the Eth70 extract.

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## COP-5

### Catalysts development for biomass conversion into chemicals and fuels

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**Keywords:** tunable catalyst, trans-esterification, fatty acids esterification

### Objectives

In this communication we describe our approach to solving the problem of the simultaneous *trans*-esterification of lipids (that require a basic catalyst) and esterification of FFAs (that require an acid catalyst), using mixed oxides *ad hoc* prepared. New mixed oxides based on calcium, cerium and aluminium have been used as catalysts in the reaction of *trans*-esterification of oils of different composition and quality, namely: i) extra virgin olive oil as a test case (low acidity) and ii) non-edible oil (high acidity). Among the several catalysts used, 12CaO 7Al<sub>2</sub>O<sub>3</sub> 7CeO<sub>2</sub> has shown unprecedented properties in terms of activity and resistance in the simultaneous *transesterification/esterification*.

### Results

In this work we have prepared new mixed oxides based on ceria loaded with calcium oxide or alumina either separately or in combination, by using a dry-technique that avoids the use of water and the formation of large volumes of waste water. The different acid-base properties in function of the composition allow to produce catalysts able to convert directly in one pot process a low quality oil rich in FFAs into a valuable product. The choice of ceria as acidic support was suggested by our previous experience on its use in catalysis.

Al<sub>2</sub>O<sub>3</sub> was selected for its stabilization properties. As we have already shown (Aresta et al., 2010) in combination with CeO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub> modulates the oxidative properties of the latter towards organics by interacting with the surface and making more difficult the surface oxygen-transfer. In this work, each oxide has been used alone to show its catalytic capacity towards the same substrate, and couples of oxides or quaternary oxides have been used. CaO Al<sub>2</sub>O<sub>3</sub> has also been used, that may not be considered a real mixed oxide. CaO CeO<sub>2</sub> and CaO Al<sub>2</sub>O<sub>3</sub> CeO<sub>2</sub> oxides with variable composition have

been tested in order to demonstrate the effect of the acid and basic components on real mixtures of lipids and FFAs.

#### Conclusions

The new mixed-oxides catalysts used in this work are quite adaptable to convert bio-oils of different composition lipids-FFAs. A further improvement is represented by the catalytic extraction that avoids the preliminary extraction of bio-oil and produces FAMEs and glycerol quite pure for further applications. The catalyst  $12\text{CaO} \cdot 7\text{Al}_2\text{O}_3 \cdot 7\text{CeO}_2$ , is active especially in the conversion of biooils with high FFAs content. It can be easily prepared by HEM and calcined at 823 K, that reduced the environmental impact of catalyst preparation. It is easily recovered and can be re-used several times reducing the environmental impact of the catalyst disposal. The fact that the whole process does not use water is another quite positive aspect, as water use and processing is a quite sensitive parameter in industrial processes. As an additional benefit, bio-glycerol produced in this way is salt-free and water-free, that reduces the need of costly and high energy purification techniques, making it more easily usable in further conversion technologies such as the biotechnological conversion into added value chemicals and monomers for polymers (e.g. 1,3-propandiol), an application that is salt sensitive. The excess methanol used in the conversion of lipids and FFAs is not wasted as it can be easily recovered and re-used.

## **SESSIONE TECNOLOGIE PER GLI OLI ED I GRASSI**

### **TEC-2**

#### **Industrial demonstration of megasonics technology for enhanced oil recovery**

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**Keywords:** MEGASONICS, ULTRAOUND, EXTRACTABILITY, YIELD, QUALITY

#### **Objectives**

The goal of research was to demonstrate the effects of high-frequency ultrasound treatment, post malaxation, on olive oil quality and extractability, in an industrial olive oil mill context.

#### **Methods**

A megasonic system was developed to verify the effects of high frequency ultrasound technology in an olive oil extraction process, post malaxation. Megasonic system was designed for scale up trials employing 9 kJ/kg (600 kHz) ultrasound energy inputs. Trials with Barnea and Picual varieties were conducted in a single processing line, with and without megasonic application, using batches of 3.6 ton of olives. The experimental tests were carried out in Australia at the Boundary Band mill (Boort, VIC – Australia).

#### **Results**

The results shown a significant improvement of the extractability. Additional trials involving the megasonic treatments of pastes, previously malaxed with enzymes addition, trials concerning the malaxation reduction time from 90 to 60 min, also shown positive effects of the megasonics technology in an industrial setting

### **TEC-3**

#### **Combined machines by using ultrasounds, microwave and heat exchanger to improve the olive paste conditioning: impact on olive oil quality and yield**

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**Keywords:** heat exchanger, microwave, ultrasound, malaxation, rheology, olive oil, quality

#### **Objectives**

In this paper an industrial combined plant assembled by a low frequency ultrasound device, a microwave apparatus and a heat exchanger were employed and implemented in an industrial olive oil plant to improve the conditioning of the olive paste.

#### **Methods**

Four different conditioning conditions were compared to the traditional one. The extractability index (E), rheological parameters and olive oil quality were determined.

The use of heat exchanger only for olive paste conditioning leads to a low value of extractability.

#### **Results**

By placing the heat exchanger and the traditional malaxer in series, it is possible to obtain the same quantitative performances, reducing the conditioning time from 40 min to 20 min. By using a microwave system in series with a heat exchanger, it is possible to reduce the conditioning time considerably to just 4 min, obtaining an entirely continuous process. Combining heat exchanger, microwave, ultrasound a slight increase of extractability was found. Finally, the use of alternative conditioning technologies, alone or in combination, are able to save the lipophilic antioxidant furniture while, on the contrary, brought to a

reduction of hydrophilic antioxidant. The cavitation effect of ultrasound is able to overcome this drawback.

#### **TEC-4**

#### **Design and testing of a full-scale scraped surface heat-exchanger for the thermal conditioning of olive paste coupled with a passive malaxer.**

**Maria Lisa Clodoveo**

Keywords: heat exchange, energy efficiency, malaxer, olive oil extraction process

#### **Objectives**

The aim of the project was to design and test a low-cost and high-efficiency system composed by a scraped heat-exchanger coupled with passive malaxers. The developed system is useful to warm up and knead the olive paste simultaneously. Respect to the heat-exchangers already commercialized, this prototype is equipped with a scraping blades that increase the convective movements, reducing the process time, optimizing the sustainability of the continuous olive oil extraction system. The innovative device, placed downstream the crusher, was dimensioned to allow a fast warming up the olive paste reducing the malaxing time. The passive hermetic malaxer is a cylindrical tank equipped with rotating blades. Respect to the traditional malaxer, the passive one is built excluding the water jacket simplifying the constructive aspects. Both the heat exchange and the passive malaxers are thermally insulated to avoid any possible thermal losses towards the external environment, including all the pipes through which the olive paste passes when already heated. The thermal conditioning allows both cooling and warming.

#### **Methods**

The thermal power transferred to the olive paste can be calculated knowing its mass flow rate, the specific heat capacity and the difference of temperature between outlet and inlet.

Assuming that the thermal losses towards the external environment are negligible thanks to the efficient thermal insulation, the amount of heat power transferred to the oil paste can be considered equal to the amount of heat power taken from the water stream. The thermal power to be exchanged between the oil paste and the water flux needs to be achieved by using an appropriate heat transfer surface area. In fact, the value of the thermal power is equal to the global heat exchange coefficient multiplied by the overall heat transfer surface area and the logarithmic mean temperature difference. With regard to the global heat exchange coefficient, it depends on the convective coefficients of water and olive paste. The convective coefficients of olive paste depends on scraper velocity. This consideration depends on the knowledge that the Nusselt number can be retrieved according to the Reynolds number. The thermal power loss from the passive malaxer can be evaluated by means of the Fourier's Law.

#### **Results**

The innovative low cost device (TRL9) allows a fast warming up the olive paste reducing the malaxing time and with a minor energy employment, assuring satisfactory oil yields.