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The impact of innovative processing technologies and chemometric methods on virgin olive oil quality - a review

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Virgin olive oil production and usage have increased in recent years. Only mechanical or other physical processes are used to extract olive oil from olive fruit. As an alternative to traditional methods, new processing strategies such as high pressure ultrasonic (HPU), microwave (MW), pulsed electric field (PEF), and ultrasounds (US) have been developed to improve the quality, physicochemical and nutritional properties of oils while reducing processing time and energy consumption. The top two olive oil qualities are extra-virgin and virgin olive oils that have unique physical, chemical, and sensory properties. Reference methods for determining quality parameters take a long time, utilise expensive and dangerous chemicals, require an analyst knowledge, require a sample preparation, and may have constraints that limit their use for a precise and thorough quality management of olive oil. Recently developed analytical processes include NIR, MIR, FT-NIR, Raman, NMR spectroscopy, mass spectrometry and chromatographic techniques with Chemometric analysis (PCA, PLS-R, PLS-DA, cluster) to assess the quality characteristics of olive oils quickly and accurately.

This review highlights the significant advancements in virgin olive oil quality assessment and emphasizes the valuable contributions of innovative processing technologies, analytical methods, and chemometric techniques in this field.

Keywords: Virgin olive oil, Innovative olive oil processing techniques, Chemometric, quality control

1. INTRODUCTION

Olive oil "obtained from the fruit of the olive tree (*Olea europaea* L.) only by washing, decantation, centrifugation and filtration, described as a fragrant, transparent, yellowish-green liquid by International Olive Council (IOC) [1]. The high yield olive oil production begins with harvesting the fruits at their optimum stage of ripening Traditional olive oil extraction process includes separating the leaves, washing and processing the fruits using mills. There are roughly 12 000 olive oil mills worldwide, with over 80% using centrifugation methods for olive oil extraction. The olives are ground and crushed to make the olive paste, which is then subjected to the malaxation process, and the oil will be extracted by using hydraulic presses, centrifugation (two or three-phase system). Then, olive oil is separated from the aqueous phase through centrifugation to be filtrated, stored and packaged [2]. Olive oil is probably an item that has been made and consumed since ancient times. An increase in interest in this oil has been observed and identified with the Mediterranean diet. Olive oil is widely popular in the Mediterranean region, and its consumption has increased globally as a result of its health benefits and sensory properties [2, 3]. World production of olive oil reach about 3 215 000 tones. Turkey is the world's fourth-largest olive oil-producing nation with 235 700 tons of olive oil produced in the 2021/22 crop year (IOC,

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2022). Contablsumption of olive oil in IOC member countries is 2 054 000 tones, while non-member countries consumed 1 071 000 tons of olive oil. Total consumption is expected at around 3 125 000 tones (-4.4%) [4] IOC for the year 2022. Thus, although only 5% of the oil produced in the world is olive oil, its consumption has doubled in the last two decades. Although Spain is the biggest oil producing country, Greece (20 L) is the biggest oil consuming country. Other countries are Spain (14.2), Italy (11.3), Portugal (8), Syria (4.9), Morocco (4.7), Tunisia (3.8) and Turkey (2.2) litres [4-6]. Olive oil quality and purity requirements, as well as safety issues, are specified by numerous legislation and agencies, such as the EU Regulations, as a natural product produced using "only mechanical means" from olive drupes. Legislation adequately describes the authenticity of olive oil. The top two olive oil qualities are extra-virgin and virgin olive oil, which have distinct physical, chemical, and sensory features. The IOC and European regulations are used to classify olive and olive pomace oil [2]. The quality of olive oil is determined by various factors, including geographical location, climate, crop season, tree age, field operations and treatments, and the time and activities used for olive harvesting, processing technology, process variables, and storage conditions. These variables influence olive oil properties such as fatty acid profile, phenolic contents, and sensory qualities. Controlling these variables precisely can result in an optimisation of olive oil quality, production yield, economics, and environmental impact [3], [7-9] grown in five olive orchards with different soil type (Sandy, Clay, Stony, Brown, Limestone and Gypsum.

2. ORGANIC OLIVE OIL PROCESSING

"Organic farming is an agricultural method that aims to produce food using natural substances and processes" [10, 11]. Organic olive oil should be obtained from organic crops, according to EU rules and regulations (EEC regulation 848/2018). According to the reference legislation, the general objectives of organic production are to maintain the health of soil, plants, water, and animals; to promote biological diversity; to use natural resources (energy, water, air, etc.); to obtain high quality extra virgin olive oil; and to satisfy the consumer's demand for organic olive oils that are better for their health and do not harm the surrounding environment. This law also prohibits the use of GMO products (from seeds to feed), and all products of chemical origin are prohibited in the organic olive oil production line. The logo can only be used on products that have been certified as natural by means of an authorised corporate enterprise or body. This is a useful tool for producers to realise certified organic products and for farmers wanting to market them throughout all EU countries. Although, organic farming is more expensive and more complex than conventional agriculture [12] since it offers several advantages, such as minimising all forms of pollution and producing food of high quality. Health is considered to be the competitive advantage of organic olive oil since It may include higher levels of polyphenols and squalene, but most significantly, it is grown and produced without the use of chemicals or GMO goods [3]. Thus, consumers pick organic foods, because they probably incorporate fewer agrochemicals, hormones, and artificial additives in contrast to conventional foods. However, PDO certification is preferred more for their higher perceived quality and consumer interest [13, 14]. In fact, Carzedda recently noticed that origin attributes like PDO or PGI have positive effect on a preference while the organic attribute is not highly valued by Italian consumers [11, 13] for extra virgin olive oil. The world's organic agricultural lands for olive cultivation are 20%. Europe and Africa provide 70% and 30% of the organic olive production, respectively. The organic olive production in Tunisia (254547), Italy (230671), Spain (194249), Turkey (81747) and Greece (50181) hectares. The organic field used in olive cultivation has increased almost three times [13]. Organic olive is a very important product both globally and in Turkey where 8% of olive orchards are kept organically and 2% of whole production is organic olive oil.

Organic farming in Turkey is validated by private Control and Certification agencies in accordance with the Organic Farming Regulation (No. 5262/2004). The Turkish Ministry of Food, Agriculture and Livestock accredited control and Certification private bodies. Organic olive tree production provides numerous advantages, both environmentally, socially, and economically with health and environmental protection, economic benefits for producers and consumers. Furthermore, both cooperatives have joined the organic olive and olive oil markets. Taris previously manufactured organic virgin olive oil, and Marmarabirlik entered the market with organic table olives in 2015 [12]. The organic olive oil production may significantly contribute to the nation's economy with a high added value if the proper safeguards are taken and knowledge is transferred to olive producers [15].

3. NEW EMERGING NON-THERMAL OLIVE OIL PROCESSING METHODS

Consumers want food with a better organoleptic character and more health and nutritional benefits. Simultaneously, there is an increasing awareness of the environmental sustainability of products and processes. They can shorten processing time, increase extraction yield (through rapid mass transfer), preserve sensory qualities (by non-thermal processing), and reduce or eliminate the usage of solvents, all while saving energy. Electro-technologies (pulsed electric field (PEF), high voltage electrical discharge (HVED), Ohmic heating, non-thermal plasma), electroma-

gnetic radiation technologies such as microwaves (MW), radiofrequency drying, pulsed light (PL), high pressure (HPP) or ultrasound (US) processing are the examples of these new technologies. Some of these technologies have the crucial attribute of being non-thermal procedures due to their capability to work at low-mild temperatures, which may be important for virgin olive oil because it is particularly sensitive to temperature during processing [16-18]. New emerging technologies low in energy usage and environmentally friendly processing strategies to reduce the negative effects of traditional olive processing methods, meet rising consumer demand for more natural products with fewer additives and preservatives, while also providing availability, freshness, and safety [16, 18], [25-28] mostly applied before malaxation [3]. They can be successfully implemented in EVOO processing to improve, shorten, or replace the malaxation step, or to eliminate the pre-heating of olive paste; however, they must be carefully designed to produce olive oils with the desired characteristics, as too much power and/or too long treatment times can affect the quality and stability of EVOO components [20]. Malaxation, which includes mixing crushed olive paste to facilitate oil drop coalescence, is critical during olive oil extraction process [16, 29]. Malaxation is the bottleneck in the continuous extraction process since the mechanical crusher and centrifugal separators are continuous devices but the malaxer is a batch equipment. Furthermore, due to a poor ratio between its large volume and tiny surface area, the malaxer has a low heat transfer coefficient and is an inefficient heat exchanger [17, 30]. Oil yield, along with oil quality, is an important component in the oil extraction process. Using current technology and despite ongoing improvement, the extractability ranges between 80% to 90%, as some of the oil is lost in the pomace and waste water, i.e. 10 to 20% [27]. Recent researches have been directed toward the development of malaxing equipment, capable of converting the batch malaxing step into continuous process thereby shortening the malaxation time, decreasing malaxation temperature while increasing both the yield and the quality (more flavour and aroma, less bitterness, a better yield, more antioxidants, and a longer shelf life) in obtained virgin olive oil [17, 18, 27, 31]. Utilisation of new emerging technology could allow for a decrease in malaxation temperature ranging from 26 to 15°C without affecting extraction yield, influencing the functional and nutritional value of the olive oil due to the low temperature of the olive paste, olive oil is produced rather than standard thermal processing methods [31]. Some of the selected studies have been given at Table I.

The traditional method for EVOO extraction includes a malaxation process, which boosts yield by around 5% when compared to non-malaxated olives, however the temperature and time of malaxation can affect the quality of olive oils. Innovative moderate approaches have been presented to boost EVOO output without a negative impact on the quality parameters. Recently, Angeloni et al [18] found that the HPP treatment had no detrimental impact on olive oil quality, however HVED had a negative impact on olive oil quality. Amirante et al. [17] found that using a Sono Heat Exchanger (SHE) in conjunction with US enhanced oil extractability by 5% and polyphenol content by 12% when compared to EVOO samples extracted in the traditional way. Navarro et al. [19] discovered that up to 25% more oil may be extracted, providing enrichment of phenolic and volatile components while having no effect on the physicochemical parameters or tocopherol content and causing no faults or off-flavours of olive oil produced by PEF treatment. Nardella et al [20] HPU increased oil extractability by up to 20%, but the impact of HPU on olive oil quality and chemical composition is contradictory, particularly in the case of micro-components, such as polyphenols, tocopherols, and volatile compounds, due to the various transformations that occur during olive oil production and processing parameters, such as power, temperature, and time of treatment, as well as oxygen concentration in the headspace during malaxation, olive variety, and climate. Thus, an HPU treatment might be designed to increase antioxidants and volatile while limiting oxidative and thermal losses during extraction of olive oil. Amarillo et al., [21] Conditioning olive paste using microwaves and megasonics boosted olive oil extractability by up to 2.4% while having no negative influence on the extra virgin olive oil's sensory or chemical quality criteria (fermentative and oxidative). Servili et al. [24] When utilised at 3.5 bar power, low frequency high power ultrasound boosted oil extractability by up to 4.6% while causing no changes to the legal quality metrics and having a favourable impact on the phenolic composition of EVOO. Stilliatano et al. [25] assessed the efficiency of heating of paste before malaxation and a using vacuum decanter to avoid the final vertical centrifugation on olive oil yield, quality, and economic and environmental impacts (life cycle costing (LCC) and life cycle assessment (LCA). When the innovative system was used, oil quality improved with lower peroxide content and higher contents of chlorophylls, total polyphenols, and tocopherols, as well as antioxidant activity, but with lower oil yields than when the conventional system was used. The economic results revealed that the innovative system had the highest extraction cost as well as the lowest profitability, even though a positive return on investment feasibility can be achieved due to an increase in olive oil selling prices, which could be significant for the sustainability evaluation of innovations in the olive oil industry. US can be applied to olive paste to release oil quickly from vacuoles with a shorter malaxation time, low manufacturing cost, and high extraction yield, because of its mechanical effect on cell membranes [17, 27], [32-34]. Due to the combined effects of higher

Table I - Some Innovative Non-Thermal Processing Technologies for Obtaining Virgin Olive Oil

Technology	Objectives	Parameters	Results	References
HE and US	Sono heat exchanger combined with ultrasound applied malaxation	Olive oil quality, yield, oil extractability, phenolics compounds, fluid dynamic analysis	Increase in oil extraction (5%), polyphenols (12 %) at 23–27°C	[17]
HPU, HVED	High pressure ultrasound, high voltage electrical discharge applied before malaxation	Olive oil quality (FFA, PV, K_{232}, K_{270}, ΔK), phenolic compounds, volatiles	HVED caused increase in anisidine value (50 %), fall in biophenol concentration (5 to 10 %), 15 % in volatiles at 19 °C	[18]
PEF	Pulsed electric field applied before malaxation/malaxation time and temperature	Olive oil quality, yield, oxidative stability, phenolic compounds, volatiles, tocopherols	PEF increased oil yield up to 25%, 10–17% higher oleacin and oleocanthal and hex-2-enal content, unaltered physicochemical parameters, tocopherols below 20 °C	[19]
HPU	Ultrasound applied before malaxation/frequency, malaxation time	Olive oil quality, yield, phenolic compounds, volatiles, consumer perception	HPU increase the oil extractability up to 20 %, the effect on olive oil quality and chemical composition contradictory	[20]
MW and MSW	Microwave and megasonic wave applied malaxation/ malaxation time	Olive oil quality, yield, oxidative stability, ethyl esters and wax content, polyphenols, tocopherols	MW caused increase in oil extractability 2.4 %, does not negatively impact on the sensory chemical quality	[21]
US	Ultrasound assisted PDO malaxation	Consumer perception, sensory properties	Extra-virgin olive oil extracted through ultrasounds may be, generally, accepted by consumers.	[22]
US	Ultrasound assisted malaxation	Volatiles, phenolic compounds, antioxidant activity	Increase of the yield has not impaired any difference in chemical composition, sensory characteristics, the nutraceutical properties of EVOO produced by US and heat exchanger	[23]
US	Low frequency high power ultrasound applied malaxation / different levels of pressure	Olive oil quality, yield, oil extractability, phenolic compounds, volatile compounds	Ultrasound technology İncreased oil extractability to 4.6 %, no alterations to quality parameters, showed a positive impact to the phenolic composition at 3.5 bar	[24]
-	Low Oxygen Pressure milling, vacuum malaxation	Quality, and olive oil extraction yield	Innovative plant resulted in olive oil with a significant increase in quality, with lowest in extractability, unfavourable in life cycle costing and life cycle assessment	[25]
Microwave, Megasaound	Microwave and megasound applied malaxation/ malaxation time	Olive oil quality, oil extractability, phenolic compounds, volatile compounds	Combined continuous MW and megasonic conditioning technology to fasten the olive oil extraction, to enhance yields, and total phenolic content	[26]
US and PEF	US and PEF technologies in an industrial olive oil mechanical extraction plant	Olive oil Yield and Rheological Characteristics of olive paste	US and PEF system before malaxation increased oil extractability of up to 3.7 % and oil yield up to 0.54 %. Caused a slight decrease in viscosity	[27]

processing temperatures and cavitation, HPU processing, particularly to aid malaxation, tends to greatly improve oil production and increase oil extractability [20]. PEF is effective for reversible or irreversible cell membrane permeability by subjecting olive paste to an electric field, which causes pores in cell membranes. The potential of PEF and HPP applications is to increase extraction yield and lower malaxation time and getting olive oil with a high phytonutrient content, including bioactive and antioxidant components, as well as health-promoting characteristics [27, 28, 35] since they have enhancing ability for mass transport thus improving the extractability of intracellular bioactive compounds from olive paste. These technologies are also effective at increasing the oxidative stability of olive oils without any negative impact on their flavour, colour, and consistency. These technologies have a significant effect on oil quality parameters, nutritional and sensory features such as no changes in the fatty acid content and volatile components of the virgin olive oil, but they resulted in an increase in tocopherol, chlorophyll, and carotenoid contents, yield,

extractability while reduced in malaxation time [17]. Romaniello et al. [27] found that the application of the US and PEF systems prior to malaxation boosted oil extractability to 3.6 to 3.7% and yield to 0.5 to 0.4%, respectively. Microwaves are non-ionising electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz that can be used to shorten the period of malaxation while also enhancing oil release [30]. It is an innovative extraction technology applied during the extraction of olive oil, that causes disruption of cell wall materials facilitating the release of high-quality oil with low energy requirement, and has significant environmental impact and low financial costs, due to heating [18, 26, 35].

Leone et al. [26] discovered that new conditioning of olive paste employing MWs, MS, and their combination at an industrial scale had no significant effect on extra virgin olive oil quality and related chemical and sensory descriptors. A combination of continuous MW and megasonic conditioning technology allows for faster olive oil conditioning, higher yields, and oils with a higher overall phenolic content.

By-products of the Olea europaea L. processing industry are rich in various bioactive compounds such as polyphenols, anthocyanins, tannins, flavonoids, and dietary fibre (pectin) can be recovered and reused for a variety of applications. Traditional extraction procedures such as the use of organic solvents and filtration processes (membrane) Soxhlet, hydro-distillation, and solvent extraction methods can be used to separate these bioactive compounds from olive by-products [36, 37] but applied at considerably high temperatures. However, green extraction technologies like high-hydrostatic pressure, and ultrasound-assisted extraction, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), pulsed electric field, Radiofrequency drying, high voltage electrical discharge, and super-heating are all methods of low temperature extractions. SFE and PLE (critical fluid extraction and pressurised liquid extraction) may also be used to concentrate these valuable by-products [36, 38]. Based on the results of economic and environmental studies, the most efficient and profitable extraction method was found to be the innovative green technologies despite their high extraction cost and their low or zero negative environmental impacts [25].

4. QUALITY CONTROL OF VIRGIN OLIVE OIL WITH CHEMOMETRIC METHODS

Virgin Olive oil is a special food with a high price. Adulteration, mislabelling, mischaracterisation, and fake origin are all examples of variables that affect authenticity. Therefore, quality and safety as well as the trade in vegetable oil products, depend greatly on the detection of quality and fraud [39]. Previous studies are concentrated on classification, monitoring of adulteration, characterisation of cultivars and determination of the geographic origin of olive oil. Even if virgin olive oil analysis remains a cornerstone in terms of diagnosing possible fraud, there is a need for the development of new procedures for assessing quality, authenticity and also determining geographical and botanical origin [39, 40]. Traceability is another important quality that must be secured throughout the full virgin olive oil acquisition process, encompassing the four fundamental stages (harvesting, milling, storage, and packaging) to ensure the product's identification and manufacturing chain [41].

The free acidity (FA), peroxide value (PV), and specific extinctions (k232, k270, and k) obtained according to the official methods under the codes COI/T.20/Doc/N°34, N°35, and N°19, respectively, are well-known chemical parameters used to determine the quality and authenticity of OO and to classify VOO grade [47]. IOC and EC have both approved that chemical parameters along with sensory properties (fruity attributes, defects) are the most common parameters that define the olive oil quality. International Olive Council establishes moisture and volatile matter, insoluble impurities in light petroleum, flash point, trace metals (for iron and copper), fatty acid ethyl esters and biophenol contents as set as additional quality criteria. Moreover, oxidative stability, chlorophyll and carotenoid content, and the bitterness index [1, 42] and contaminants (such as pesticides or mycotoxins, among others) are also included for the evaluation of the quality and minimising associated risks of olive oil [1, 3, 5]. There is growing interest in novel, robust, quick, and cost-effective analytical methods for investigating olive oil quality for authentication and traceability. Because several of the official analytical procedures used for the certification of virgin olive oils have limitations in certain aspects, a number of alternative analytical methods and approaches have been proposed over the last decade [1, 5]. The analytical procedures (including sample preparation, analysis, data acquisition and processing) have been developed and proposed to quality control of virgin olive oil are; Vibrational spectroscopic techniques (Near-infrared (NIR) Visible/ Near Infrared (Vis/NIR), Fourier transform infrared (FT-IR) and Fourier transform-Raman (FT-Raman): Mass spectrometry (electrospray ionization (ESI), Atmospheric pressure photoionisation ion (APPI), Matrix-assisted laser desorption/ionisation (MALDI), fingerprinting method based on MALDI-TOF MS); Chromatographic techniques (GC and HPLC) and other analytical approaches (voltammetry, DSC and e-sense techniques) [42] have been used. These techniques have been used for the determination of acidity, peroxide value, iodine value, anisidine value, malondialdehyde, soap contents within a single measurement to determine olive oil quality with significantly good results in a very short time [43-48]. These new analytical procedures could determine and predict several pa-

rameters because they offer important advantages such as no need of reagents, rapid measurements and fast data acquisition, relatively low cost, easy samples handling because of their non-destructive nature (analysis is performed directly on intact samples or with only minimal sample preparation) [49]. The produced analytical data (spectroscopic, chromatographic, isotopic, sensorial, etc.) are often multivariate data matrices which demand appropriate Chemometric analysis that allow to be used as rapid screening techniques compared to the standard reference methods for determining the quality and authenticity of olive oil [44, 47]. Thus, various procedures have been created and proposed for the quality control of virgin olive oil, as well as discrimination and classification are shown at Table II.

Azazian et al. [45] created FT-NIR calibration models to assess virgin olive oil quality by evaluating thirteen parameters, including five major FAs and DAG and FFA concentrations. Alamprese et al. [50] Image analysis was used to create FT-NIR PLS-DA calibration models for determining the olive ripening degree. PLS-DA models developed independently for olive origin yielded prediction sensitivity and specificity values greater than 81%. As a green, non-destructive, extremely dependable technology for optimising virgin and extra virgin olive oil quality, such a tool can be used for sorting olives right at the mill's entry or even

Table II – Recent Analytical Methods used with Chemometric Techniques for Virgin Olive Oil Quality Control

Methods	Objectives	Parameters and Data Processing	Results	References
FT-NIR	Quality and Authencity of EVOO	Volatile aldehydes and ketones, triacylglycerol, diacylglycerols, free fatty acids, phenolics, and water, PLS	FT-NIR calibration models allow assess the authenticity and freshness of EVOOs, the linoleic acid composition (15 to 21 %) of the oil, and establish the volatile and water content	[45]
FT-NIR	Quality of olives	Olive ripening degree, PLS-DA	FT-NIR PLS-DA calibration models confirmed by common visual evaluation of maturity index up to 81 %.	[50]
NIR	Quality	Moisture content, PLS	FT-NIR with PLS calibration models correlated well with KF reference values up to 95 %	[51]
HPLC-ESI-MS	Characterization	Phenolic and tocopherol compounds, pigments, oxidative stability, triacylglycerol, and fatty acid compositions, PCA and HCA	PCA and HCA can explain the variability of the oil composition according to the cultivar	[52]
HS-SPME-GC-MS	Authentication	Volatile compounds, LDA, PLS-DA	LDA and PLS-DA Chemometric models can be used to discriminate monocultivar virgin olive oils based on their volatiles up to 94 % confidence level.	[53]
HS-SPME-GC-MS	Quality assessment	Volatile compounds, PCA, HCA, LDA, kNN	PCA and HCA were applied for clustering virgin olive oils and LDA, kNN, and SVM predictive models correctly classified the oils up to 88.1%.	[54]
UHPLC-UV/Vis	Authentication	Polar fraction of olive oils, PLS-DA, SVM, SIMCA	UHPLC-UV/Vis spectrums are transformed to instrument-agnostic fingerprints by using SIMCA, PLS-DA and SVM models to discriminate virgin olive oils	[55]
Flash GC	Quality grades	Volatile fraction fingerprints of virgin olive oils, PLS-DA	Volatile fraction of virgin olive oils analysed by flash gas chromatography to predict the commercial category of olive oils by using PLSDA models classifying up to 85 %	[56]
Flourescence Spectroscopy	Quality	Acidity, peroxide value, K232, 270, ΔK, tocopherols, PLSR	PLSR models with 0.9 R^2 values were found at excitation at 350 nm, for correctly measuring physiochemical properties and tocopherol contents of virgin olive oils	[57]
HPLC-DAD	Variety Origin	Fingerprints of phenolic fraction, PCA, PLS-DA, SIMCA, kNN	Virgin olive oils accurately classified between 92.23 and 94.17%, best results were obtained with SIMCA models	[58]

*PCA: Principal Component Analysis, PLS: Partial Least Square, DA: Discriminant Analysis, R: Regression, HCA: Hierarchical Clustering Analysis, LDA: Linear Discriminant Analysis, kNN: k-nearest neighbour, SIMCA: Soft independent modelling of class analogy,

in the field. Moisture and volatile matter are olive oil quality characteristics, according to CODEX, and the creation of a quick screening method for moisture assessment is critical [51]. Karunathilaka et al. [51] The NIR method presents a clear time and cost saving alternative to the KF method and would be appropriate for routine screening applications provided calibration is effectively achieved, as FT-NIR PLS calibration models gave a sensitivity up to 95% when compared to the reference laboratory Karl-Fischer method. Baccori et al. [52] investigated the selection of novel feral olive cultivars for oil production with high oil quality. The results show that the chemical properties of the oils under consideration vary greatly. The statistical analysis (PCA and HCA) can explain the variation in oil composition by cultivar. They determined that feral olive oils are a new edible oil source that is high in natural bioactive components. Cecchi et al. [53] found that volatiles that have a considerable influence on customer preferences influenced by the varietal origin of virgin Olive Oil (VOO). The LDA and PLS-DA algorithms properly classified 94% of the oils into three clusters based on their volatile components that will help against fraud and to increase the value of monocultivar extra virgin olive oils. Gerhardt et al. [54] used HS-GC-IMS to analyse non-targeted volatile organic compound (VOC) profiles in order to differentiate between virgin olive oils of different classification. PCA and HCA were used to cluster virgin olive oils, while LDA, kNN, and SVM were employed to develop predictive models predicting the oils volatiles correctly up to 88.1%. Perez Beltrean et al. [55] The polar fractions of several olive oil samples analysed by (NP) UHPLC-UV/Vis are translated to instrument-agnostic fingerprints using PLS-DA and SVM models obtained before and after signal instrumentation. This will enable the development of multivariate classification models that can be exported between laboratories and generally deployed in routine laboratories to readily authenticate olive oil. Barbierei et al. [56] Using PLSDA models, the volatile fraction of virgin olive oils was analysed by flash gas chromatography to predict the commercial category of olive oils, and 331 olive oil samples were accurately classified ranging from 72 to 85%. Baltazar et al. [57] set multivariate models to evaluate the physicochemical properties and antioxidant content (tocopherols) of extra virgin olive oils from fluorescence spectra obtained at 326 nm, 350 nm, and 365 nm excitation wavelengths. In evaluating the physiochemical characteristics and antioxidant content of virgin olive oils, PLSR prediction models were created reaching 0.9 R^2 values excitation at 350 nm. Bajoub et al. [58] investigated the phenolic constituents of 140 extra-VOO samples from seven olive fruits and the results statistically processed for varietal authentication purposes using PCA, PLS-DA, SIMCA, and k-NN. Overall classification accuracy for the authentication of virgin olive oil samples was between 92.23 and 94.17%, with the best results achieved by using SIMCA models for classification. Furthermore, computer vision, machine olfaction technology, electronic tongues and dielectric spectroscopy, differential scanning calorimetry, and voltammetry are used to determine parameters such as acidity, peroxide indexes, ripening indexes, organoleptic properties, fatty acids, volatiles, the melting point, odours, flavour and minor components in olive fruit, olive slurry, and olive oil to determine quality, adulteration, freshness stage and to predict sensory attributes of olive oil [47, 48, 59].

5. CONCLUSIONS

In recent years, the quality assessment of virgin olive oil has received increasing attention due to its nutritional and health benefits, as well as its economic importance. Olive oil authentication is vital not solely for consumers, but additionally for suppliers, retailers, regulatory agencies, and administrative authorities. The production process of extra virgin olive oil is conducted, as known, only by means of mechanical-physical methods applied to the treatment of the olive fruit. Various emerging processing Technologies suc as US, PEF, and MW have been developed and applied to enhance the quality of virgin olive oil during or instead of malaxation. These technologies can improve the yield, purity, stability, and preserve the natural compounds present in the virgin olive oil. The quality and safety of olive oils are regulated by governing organisations such as the IOC and the EU using physicochemical characteristics and sensory attributes. Analytical methods play a crucial role in assessing the quality parameters of virgin olive oil. Recent advancements in spectroscopic (NIR, MIR, FT-NIR, Raman), techniques have enabled the identification and quantification of minor constituents, volatile compounds, and sensory attributes with greater accuracy and sensitivity. These comprehensive analysis allows for a more detailed understanding of the composition and quality of virgin olive oil. Chemometric techniques have emerged as powerful tools to analyze and interpret the vast amount of data generated from innovative processing technologies and advanced analytical methods. These techniques, including PCA, PLS-R, and cluster analysis, can identify patterns, correlations, and outliers in the data, providing valuable insights in optimizing processing conditions, predicting sensory attributes, and determining the authenticity and geographical origin and quality of the virgin olive oil.

Systems for process monitoring, quality control, quality prediction of virgin olive oil can be utilised for relevant parameters in both industry 4.0 and 5.0. Overall, by investigating the impact and effectiveness of innovative processing technologies, analytical methods, and chemometric techniques, contributes to the understanding of how these advancements can enhance the quality of the virgin olive oil.

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Evaluation of the nutritional value of couscous dish with emphasis on its lipid profile and quality

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Couscous is an ancient meal available almost anywhere in North Africa. It is well-known as a staple meal in Algeria, with several variations in terms of ethnic cuisine and preparation. For this study, we chose a standard couscous recipe that includes all the elements used in the making of a couscous-based dish (meat, vegetables, and legumes) in order to investigate the lipid profile, nutrient composition and define the adequate serving size for a complete couscous dish. This study focused on the lipid composition and lipid health indices of this popular dish as fat can contribute to the increase of nutrition related diseases, which have been prevailing lately. Our results showed that, a nutritionally adequate serving size of couscous providing 942 Kcal energy, contains 10.26 g of protein, 28.13 g of fat and 162 g of carbohydrates, is equal to 270 g. The fat content of the studied couscous was estimated to 10.42 per 100 g and was related to the amount of cooking oil used and also to the fat content of beef, which is the main ingredient of the couscous dish. The lipid profile showed that most of the fatty acids were polyunsaturated with moderate amounts of monounsaturated and saturated fatty acids. This reflected on the atherogenic and thrombogenic indices which were found to be low 0.556 and 07,67 respectively; this is due to the low saturated fatty acids that are the main contributors to atherogenicity and thrombogenicity, cholesterol was also low at 0.829 mg/100g. Couscous proved to be a good source of dietary fibres 52.11 g per serving. Vitamin B12 was not detected, but each vitamin B1, B3, B6, A and D was found in various amounts, 4.120; 3.286; 0.754; 0.189; 0.226 mcg/100g respectively. Beef and ghee might be at the origin of vitamin D which rarely occurs in food other than seafood. Although there are many variations in this popular dish, this study gives an overall idea of the nutritional value of similarly composed couscous-based dishes.

Keywords: Fat, Fatty acid profile, Cholesterol, Fat health indices, couscous.

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INTRODUCTION

Couscous is an ancient meal found almost anywhere in North Africa. It is well-known as a staple meal in Algeria, with several variations in terms of ethnic cuisine and preparation. Wheat flour and semolina are the main ingredients, with a lower amount of barley and corn added in a good amount [1]. A couscoussier is a traditional double-chambered food steamer used to cook couscous by North Africans (in Amazigh and Arabic cuisines) and nowadays by people all over the world. The steam-cooking pot is couscoussier in French, Taseksut in Amazighi, and Kaskas in Arabic [2, 3]. The origins of the word couscous are unknown. Some reckon it's onomatopoeic, a linguistic approximation of the hissing sound produced as steam goes through the holes in the pot. There are other theories as well, but no one can deny that the dish is of Berber origin. Even if everyone understands what is meant with couscous, it is usually eferred to with another name. Some Berbers

refer to it as sikuk; some as sksu in the Souss region, ta'am in Algeria, and kouski in Tunisia. There are also variations outside of the Maghreb [4]: Couscous can be prepared in various ways most of which contain a kind of protein (meat, chicken, or fish), beans (fava beans, chickpeas), and vegetables (onions, courgettes, tomatoes, carrots, etc), also; several types of fat can be used in couscous dish preparations like olive oil. The added fats are chosen based on their availability and consumption at family gatherings [4].

Fatty acid (FA) analysis is a quick and accurate way to determine the FA composition of fats and oils [5]. The FA catalogue is divided into three categories based on the number of double bonds: saturated FA (SFAs), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA) [27]. The presence of SFA may have a negative impact on several factors related to cardiovascular disease (CVD) and atherosclerosis, with C14:0 and C16:0 fatty acids being among the most atherogenic, whereas C18:0 is thought to be neutral in terms of atherogenicity but thrombogenic [6], however; PUFA, particularly omega-6 (ω-6) and omega-3 (ω-3) FAs are proving indispensable in a properly maintained ratio for numerous beneficial health functions [7].

A large-scale prospective cohort study found that high cholesterol consumption was associated with increased mortality risk and CVD mortality or v. However, Pan et al. [8] found that dietary cholesterol consumption was not associated with dyslipidaemia or serum lipids. The degree of CVD risk varies depending on the type and level of dietary FAs [9, 10]. To avoid many health problems, various important nutritional indices are frequently used to describe the FA composition of foods and to evaluate their nutritional value. Index of atherogenicity (IA) and Index of Thrombogenicity (IT) are the most commonly used nutritional indices to determine lipid quality and FA composition because they outline significant implications and provide clear evidence [10].

Vitamins are families of extremely complex molecules that are organic in nature, present in food in small amounts, and necessary for a regular metabolism. A deficiency in vitamins may lead to complex diseases, but when these nutrients are replenished, the deficiency symptoms are resolved [11]. The prevalence of these deficiencies varies from one population to another for example studies targeting different groups of the Algerian population have shown that 40% of pregnant women were below the recommendations for Vitamin C, D, A, E, B1, B2, B3, B6, B9 and B12 [12].

Since the traditional making diagram of couscous has already been identified and how couscous based dishes, as described by Chemache et al. [3], are made, we have chosen a standard couscous recipe for this study that includes all the elements stated above in order to investigate the nutrient composition and define a nutritionally adequate serving size for a complete dish of couscous. Additionally, a focus was put on the lipid composition, as lipids contribute greatly to numerous nutrition-related diseases, as well as the lipid health indices of this popular dish, which are often used to analyse the FA content of meals, assess the nutritional value of FAs, and investigate their potential application in the treatment and prevention of diseases, this study also addresses some vitamins of the B complex, vitamin A and D, to give a more broad and complete image of the nutritional aspect of couscous.

MATERIALS AND METHODS

SAMPLE PREPARATION

The raw materials and ingredients were sourced and collected from local markets in Amman, Jordan, as they were available with the same specifications as in Algeria. The dish was prepared according to a local Algerian recipe including the ingredient quantities used, cooking time, and temperature.

The following procedure and ingredients were used to make the dish: 500 g of chopped beef was sautéed in the bottom part of the cooking pot in 30 g of ghee and 15 g of maize oil, 200 g of finely chopped onions, followed by 300 g of tomato puree, 30 g of tomato paste and 100 g of soaked chickpeas were added to the meat along with 1 teaspoon (± 4.2g) each of the following spices: paprika, turmeric, black pepper, ginger powder, Rass el Hanout. Salt to taste. 1000 ml of boiling water are added to the pot and the mixture is left to simmer for 2 hours on medium high heat. Within those 2 hours, 500 g of couscous gains are soaked in 250 g of water salted to taste and put in the upper part of the cooking pot to stem to 15 min the steaming process is repeated three times at the end of which 65 g of extra virgin olive oil is added to the couscous gains which are then set aside. At the 2h mark 150 g each of the following vegetables: carrots, courgettes and green beans were added and simmered for another 30 mins. Steamed couscous gains and the meat vegetable stew mixture are combined to make the complete dish. Each dish batch was homogenised after preparation and cooking and then divided into sub-samples prepared for the analysis and kept in airtight plastic containers in the freezer until the time of analysis at the Department of Nutrition and Food Technology Laboratory, The University of Jordan.

PROXIMATE ANALYSIS

Moisture, crude protein (N × 6.25), total lipid, crude fibre and ash contents were determined according to the standard procedures of AOAC [13]. Nitrogen Free Extract was obtained by difference (NFE = 100% - [protein+fat+ash+fibre%]). Energy values were calculated by multiplying carbohydrates, protein, and fat content by their respective energy conversion factors of 4, 4, and 9. Samples were analysed in triplicates.

FATTY ACID PROFILE ANALYSIS

Fatty acid methyl esters of the homogenised samples extracted fat were prepared according to the method described by Glass and Christopherson [14]. In summary, 100 mg of the extracted lipids were dissolved in 3 ml of hexane (Gas Chromatography grade) and 120 µl internal standard (i.e., nonadecane methyl ester) and 200 µl of 2N methanolic potassium hydroxide were added, and vortexed to reach a clear solution (approximately 1 min). A 200 µl of acetic acid was then added to the mixture and shook for 1 minute. The prepared methyl esters were analysed using capillary GLC column (Restek, Rtx-225, USA, cross-bond 90%-cyanopropylmethylpolysiloxane, 100 m, 0.25 µm) immediately after esterification by injection of 1µl of the hexane layer through the injection port of the GLC (model GC-2010, Shimadzu. Inc., Koyoto, Japan). The initial oven temperature was 165°C, held for 4 minutes, increased at a rate of 2°C/min to 180°C, increased at a rate of 5°C/min to 230°C, and then held for 6 minutes, for a total program time of 36 minutes. The injector temperature was 250°C, the FID temperature was 260°C, the flow rate was 1 ml/min Helium, and the split ratio used was 80. The fatty acids methyl esters (FAMEs) were identified using chromatogram of fatty acids standard.

CHOLESTEROL CONTENT DETERMINATION

Cholesterol determination was done after enzymatic hydrolysis and oxidation according to Trinder [15] using suitable ready kit R-Biopharm (Boehringer Mannheim- R-Biopharm, Germany (Cholesterol Colorimetric method for the determination of cholesterol in foodstuffs and other materials). The colorimetric indicator was lutidine-dye (3,5-diacetyl-1,4-dihydrolutidine) the formation of which was stoichiometric to the amount of cholesterol and was measured by the increase of light absorbance in the visible range at 405 nm. The spectrophotometer (Perkin-Elmer, Cleman Instruments Division 55-215) was set to Zero on the blank at 405 nm absorbance.

INDICES OF LIPID HEALTHY QUALITY

From the data collected on the fatty-acid composition, the Index of atherogenicity and Index of thrombogenicity were calculated according to Garaffo et al. [16] using the following formulas:

Index of atherogenicity (IA):

$$IA = [(4 \times C14{:}\,0) + C16{:}\,0 + C18{:}\,0] \,/\, [\Sigma PUFA + \Sigma PUFA - n6 + \Sigma PUFA - n3]$$

Index of thrombogenicity (IT):

$$IT = \frac{C14{:}0+C16{:}0+C18{:}0}{0.5\times MUFA+0.5\times PUFA\text{-}n6+3\times PUFA\text{-}n3/PUFA\text{-}n6}$$

VITAMIN ANALYSIS

The method described by Albawarshi et al. [16] was followed to determine water soluble vitamins in our samples. The concentrations of vitamins in the extracts were determined using Thermo Scientific DionexUltiMate® 3000 High-Performance Liquid Chromatography (HPLC) system consisting of a LPG 3400 SD pump, ACC- 3000 autosampler, and photo diode array detector (DAD). Reverse phase-HPLC with ACE C18-AR (250 × 4.6 mm; 5 µm) column was used. Gradient mobile phase consisted of 0.03% TFA in water (pH 2.6, B) and acetonitrile (A) was employed. The injection volume was 20 µl, the flow rate was 0.9 ml/min, and the column temperature was 25°C. The signal (peak area) of each vitamin was obtained using DAD at three wavelengths; 265 (B1, B3), 280 (B6), and 361(B12) nm.

The concentrations of A and D vitamins in the extracts were determined using methods described by Dionex [17]. The same HPLC device with a different Reverse phase-HPLC with Acclaim TM C8 120 Å and column (4.6 × 250 mm; 5 µm) was used. The mobile phase consisted of 98.5% methanol and 1.5% deionized water in isocratic elution. The injection volume was 20 µl, the flow rate was 1.0 ml/min and the column temperature was 25°C. The signal (peak area) of each vitamin was obtained using DAD at two wavelengths of 265 (D) and 325 (A) nm [18].

DETERMINATION OF PORTION SIZE

From the results obtained after proximate analysis the serving size for couscous was determined by equivalent of 10 g of protein per serving, was determined by calculation using Microsoft excel, then weighted using a kitchen scale and photographed for visual reference at a height of 0.54 meter from the sample and an angle of 45° with a camera quality of 24 megapixels [19].

STATYSTICAL ANALYSIS

All measurements were performed in triplicates and the mean values were declared. T test was performed using JMP (release 10, SAS institute, Cary, NC) was carried out to determine any significant differences between the fatty acids. Least significant difference (LSD) at a 5% level of probability was determined to separate differences in the proportions of fatty acids in the dish.

RESULTS AND DISCUSSION

PROXIMATE ANALYSIS

Results of the proximate analysis are shown in Table I. Fat content was found to be 10.42%; which was relative to the amount of cooking oil added and the fat rendered from the type of animal protein used (in this case beef). In terms of quantity, the dietary lipid intake will vary according to the many factors that influence energy intake, namely age, gender, height, and

Table I - Proximate composition (%) and Energy content (Kcal)* of couscous dish

Macronutrient	Values per 100 g
Fat (%)	10.42 ± 0.23
Protein (%)	3.80 ± 0.21
Fiber (%)	19.30 ± 0.36
Carbohydrates (%)	60.00 ± 0.76
Ash (%)	6.49 ± 0.01
Moisture (%)	74.06 ± 0.07
Energy (Kcal)	348.96 ± 0.05

*Values are means of triplicate determination ± SD (Standard deviation).

Table II - Fatty acid profile (g/100g total FA), lipid health indices and cholesterol content* of couscous dish

Component	Content (couscous)
SFA	28.595 ± 0.001
Myristic acid (C14:0)	3.988^{c} ± 0.001
Palmitic acid (C16:0)	15.068^{a} ± 0.001
Heptadecanoic Acid (C17:0)	0.442^{f} ± 0.0001
Stearic acid (C18:0)	7.250^{b} ± 0.001
Arachidic acid (C20:0)	0.629^{d} ± 0.001
Behenic acid (C22:0)	0.486^{e} ± 0.001
MUFA	28.652 ± 0.001
Palmitoleic acid (C16:1)	1.343^{b} ± 0.001
Margaric acid (C17:1)	0.198^{c} ± 0.001
PUFA	40.073 ± 0.00
Linoleic acid (C18:2)	39.763^{a} ± 0.00
γ-linolenic acid (C18:3)	0.730^{b} ± 0.00
IA	0.556 ± 0.01
IT	0.767 ± 0.01
Cholesterol (mg/100g)	0.829 ± 0.001

*Values are means of triplicate determination ± SD (Standard deviation).
SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
IA: Index of Atherogenicity, IT: Index of Thrombogenicity.
^{a,b,c} Means with different superscripts are significantly different ($P<0.05$).

weight. Dietary fat is digested more efficiently than dietary carbohydrate, according to findings from nutrient utilisation studies and epidemiological research. Weight-loss intervention studies, on the other hand, show that hypocaloric high-carbohydrate diets do not result in more weight loss than hypocaloric high-fat diets. Individuals wanting to maintain or reduce weight should focus on managing their calories [20].
When using the Dietary Reference Intakes (DRIs) for protein and the estimated energy requirements (EERs), the percentage of total energy required in the form of protein increases with age, is higher for women than men, and is higher for small compared to large adults at any age; it also depends on the total physical activity and thus energy intake [21].
When comparing the nutritional habits in Algeria among five other Mediterranean countries and also with the various official recommendations and the 'Mediterranean diet' as originally described. Karamanos et al. [22] found that protein contribution (%) to the energy intake varied very little, ranging from 13.4% in Greece to 18.5% in Italy - Rome. In the case of this dish of couscous, the contribution of protein in energy is very small as containing only 3.8 g/100 g.
The fibre content of our dish was very high, this adds consistence to the dish which produces a feeling of fullness, because fibres are resistant to digestion in the stomach, therefore, even though caloric intake may be similar, distention resulting from an increased fibre intake leads to a greater feeling of satiety [23]. Couscous contained 19.3 g fibre per 100 g, this amount covers approximately 90% of the DRIs and Adequate Intake (AI) which are set to 25 and 21 g/day, respectively. Evidence showed that recommended intakes of dietary fibre can provide prebiotics to the diet which lead to improvements in gut health, elimination of allergies, and prevention of infections [24].
Acceptable Macronutrient Distribution Range (AMDR) was set for total digestible carbohydrate based on the role of carbohydrates as a source of energy to maintain body weight. The AMDR for children and adults is 45% to 65% of total calories. The Recommended Daily Allowance (RDA) was set using a coefficient of variation of 15% [100 g/day + 2(15)] to yield an RDA of 130 g/day for children and adults [25]. Consequently, the quantity of carbohydrates will differ from one person to another. Although not exceeding the RDA, the amount of carbohydrates in our dish stays relatively high, with 60 g per 100 g. This might be problematic when crafting a meal plan for a diet as a portion of the calories will be covered by carbohydrates to the detriment of other macronutrients, although there is no counter-indication to high carbohydrate diets being used for weight loss and weight maintenance [26].
Moisture content was found to be 74.06% for couscous, this is due to the addition of a certain amount of water during the cooking process to achieve the desired consistency. Ash content is reflective of the mineral content and was found to be 6.49%.

FATTY ACID COMPOSITION, LIPID HEALTH INDICES, AND CHOLESTEROL CONTENT OF COUSCOUS

This study provided a nutritional evaluation of the FAs found in couscous and its health-related lipid indices, with focus on the FAs contributing in the calculation of the lipid health indices as well as the cholesterol content as shown in Table II. The analysis was made with T-test for a unique sample, the significance was found in all parameters ordered from the highest value "a" to the lowest.
Due to the combination of fats from both animal and vegetal origins used in the making of this dish, results showed diversity in the percentages of FAs. The examined dish contained moderate amounts of MUFAs. The PUFAs were predominant compared to MUFAs and SFAs.

The SFA found in the greatest quantity was palmitic acid (15.068 ± 0.001), followed by stearic acid (7.2507 ± 0.001), and myristic acid (3.988 ± 0.001). However, arachidic acid, behenic acid, and heptadecanoic acid were all present in low amounts. This is in accordance with the SFA composition found in beef as shown by Wood et al. and Daley et al. [27, 28]. This FA account raises cholesterol activity from beef and beef products, increasing cardiovascular illnesses as a result [29]. Palmitic acid, according to Musaiger et al. [30], is a major cholesterol-raising SFA in the diet. Stearic acid was also found in couscous, but it had no effect on total cholesterol or lipoprotein cholesterol levels in humans, also no atherogenic effect when consumed [31]. Replacing SFA with protein, especially plant protein, may reduce CVD risk [32].

As shown in Table II, the most abundant MUFA was oleic acid, this may be due to the use of a mix of maize oil and olive oil in the cooking process which abundantly contain oleic acid. This is in accordance with White et al. and López-Miranda et al. [33, 34]. Moreover, Alagawany et al. and Skřivan et al. [7, 35] reported that oleic acid was the most abundant FA found in olive oil and animal fat (i.e., beef meat).

Diets rich in MUFAs have been shown to decrease low-density lipoprotein (LDL) cholesterol and yield better lipid profiles. Normal subjects and type 2 diabetes patients have improved glucose metabolism. When MUFAs are used instead of CHO, the insulin demand and plasma glucose levels are both decreased. In normotensive and hypertensive patients, a 31% drop in systolic and diastolic blood pressure was seen after the implementation of a high MUFA diet [36].

As PUFAs accounted for more than 30% the FA composition in our dish, the most abundant was C18:2 (ω-6): linoleic acid. This might be due to the use of maize oil in the preparation of the dish; as it is the highest FA component of maize oil as stated by White et al. [33]. Additionally, this amount of omega 6 could be attributed to the high content of chickpeas with omega 6, which are used as an ingredient in couscous cooking [37]. Thereby, our dish may be considered a good source of PUFAs which contribute to the composition of all cell membranes, regulates cell signalling pathways, cellular activities, and gene expression by maintaining homeostasis for proper membrane protein activity and influencing membrane fluidity [38]. Consequently, the amount of omega 6 FAs was relatively high. The consumption of omega 6 rich diet has been linked to low-grade inflammation, oxidative stress, endothelial dysfunction, and atherosclerosis [39].

Nutritional indices are often used to analyse the FA content of meals, assess the nutritional value of FAs, and investigate their potential application in the treatment and prevention of disease. The IA indicates the relationship between the sum of the major SFAs and the major unsaturated FA classes [28]. The IT, which reveals a predisposition to produce clots in blood vessels, was created using IA by Chen and Liu [10]. The link between pro- and anti-thrombogenic (MUFA and PUFA) FAs is what is conveyed by this [40]. The main SFA favouring lipid adhesion to cells of the immunological and circulatory system are C14.0 and C16.0 which are considered pro-atherogenic and pro-thrombogenic, whereas C18:0 is thought to be neutral in terms of atherogenicity but thrombogenic [6].

The IA and IT were relatively low (0.556 ± 0.086 and 0.767 ± 0.078, respectively). Lower IA and IT levels imply better protection against coronary heart disease. IA values of 1.0 in the human diet are suggested from a nutritional standpoint [41]. In our dish, it did not exceed 0.6. This was possibly due to its high quantities of MUFAs and PUFAs, which are thought to be anti-atherogenic and anti-thrombogenic because they limit plaque aggregation and reduce levels of each esterified FA, cholesterol, and phospholipids, inhibiting the development of micro- and macro- coronary disorders [42]. Due to the fact that both IA and IT can be used to assess the potential impact of FA composition on cardiovascular health, consuming foods with FA compositions that have lower IA and IT offers superior nutritional value and may reduce the incidence of coronary heart disease. The suggested values from IA and IT, however, have not yet been provided by any organiation [43].

While a cholesterol RDI of 300 mg/day was recommended by Reiter-Brennan et al. [44], our couscous dish only contained 0.829 mg/100 g; which is negligible compared to the RDI. Exposing cholesterol to elevated temperature during cooking in the presence of MUFAs or PUFAs causes oxidation at variable degrees and hence, the higher the unsaturation degree of FAs, the higher the cholesterol oxidation thus leading to lower total cholesterol content [45, 46]. Dietary cholesterol is positively correlated to plasma cholesterol in some cases as there are high responsive and low responsive subjects [45, 47]. According to results of epidemiology studies, high plasma cholesterol level, especially high level of LDL, is directly associated with CVD [48]. Our results suggest that couscous present a low risk due to its negligible cholesterol content.

MICRONUTRIENTS

Thiamine or vitamin B1 retention is widely employed as an indicator of cooking losses in meat because thiamine is the nutrient most susceptible to thermal degradation and leaching from meat [49]. Since our dish was cooked in its broth without being discarded at the end, it was able to maintain a 4.12 mcg of vitamin B1.

Reportedly, thermal processing can also enhance the bioavailability of vitamin B6 (niacin) and carotenoids by releasing them from entrapment in the plant matrix [50]. This aligns with our results as we were able to

Table III – Vitamins concentrations (mg/100g) in couscous dish

	Vitamins					
	B1	**B3**	**B6**	**B12**	**A**	**D3**
Content (mg/100g)	4.120 ± 0.50	3.286 ± 0.06	0.754 ± 0.90	-	0.189 ± .20	0.226 ± 0.10

Values are means of triplicate determination ± SD (Standard deviation).

Table IV - Nutrition facts (contents of each fat, protein, fiber, carbohydrates, energy, saturated fatty acids, monounsaturated fatty acids, poly unsaturated fatty acids) for one serving of couscous (i.e., 270 g).

Nutrient	**Amount per serving**
Fat (g)	28.134
Protein (g)	10.260
Fiber (g)	52.110
Carbohydrates (g)	162.000
Energy (kcal)	942.000
SFA(mg)	8.040
MUFA(mg)	8.000
PUFA(mg)	11.300

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Figure 1 - Serving size for couscous (270 g).

detect the presence of vitamin B6, B3 and vitamin A although in low amounts (Tab. III). As illustrated in Table III, the amount of vitamin D detected in couscous was 0.226 mcg ± 0.125 per 100 g. Vitamin D is found in modest levels in beef mostly in the form of vitamin D3 and its metabolite 25(OH)D3 [51] Ghee also contains a small amount of vitamin D estimated at 0.326 mcg/100g [52]. The presence of vitamin D in our final dish may be due to the use of these two ingredients. Vitamin B12 on the other hand was not detected, this was probably due to thermal processing.

For the sake of the experiment, the prepared samples were stored in the freezer compartment of a refrigerator (as mentioned in the materials and methods section). Freezer compartments of refrigerators generally do not allow for temperature below – 18°C to be reached. Fluctuations in the freezing temperature might be responsible for the significant losses of vitamins in meats and lower losses in vegetables [53]. Our results show that couscous can contribute to the intake of some vitamins of the B complex along with vitamin A and D

SERVING SIZE

Dietitians use the notion of portions to assess the calories and macronutrient content of food taken in practice [54]. A serving is a standard measure of food with known calorie and macronutrient composition, whereas a serving size is a comparable measure of food. A serving of cooked rice, for example, has about 15 grams of carbohydrate, 3 grams of protein, and 1 gram of fat, for a total of 80 calories [48, 55]. Half cup is the measure of cooked rice (serving size) corresponding to a serving. Dietitians depict food portions using household food models, often known as 'handy measures' (food measurement instruments used to quantify portion sizes of food) [53, 56]. Weighing foods before eating them is inconvenient and inefficient. As a result, nutrient information on commonly consumed composite traditional Algerian foods with defined recipes, as well as an appropriate and reliable tool to assess and quantify their servings, has become critical, as it has been seen in countries such as South Africa, where photographs of food portion sizes are available [57].

Our results showed that the adequate serving size for couscous is 270 g from a nutritional point of view as depicted in the photograph (Fig. 1). This serving size provides the consumer with 10 g of protein which was used as a reference to define the serving because the contribution of protein in energy was found to be very small with it containing only 3.8 g/100 g. by determining the serving to 10g of protein we raised the caloric contribution of protein to 10% thus for the serving to be nutritionally adequate as per the recommendation of the UK department of Health which states that at least 10% of the total daily caloric intake should be from protein to prevent nitrogen loss. [58]

It is noticeable that most of the 942 kcal in a serving of couscous is provided mostly by the 162 g of carbohydrates and 28.134g of fat (Tab. IV). The amount of fiber per serving which is 52.110g gives bulk to the dish, making the consumer feel full for longer periods of times. It prevents overeating and contributes to a healthy microbiota.

CONCLUSION

To summarise, a serving of couscous provides good amounts of macronutrients, micronutrients, and a large quantity of fibre that contributes to creating substance making the dish a balanced meal of its own and contributes to a healthy microbiota. The quantity and quality of fat found in the study on couscous could be attributed to the vegetable oil and animal fat used in the cooking process. This research, however, is subject to several limitations as was based on only one recipe and it is known that there are multiple ways to prepare couscous. Although the findings of this study offer a better understanding of the composition of similar couscous dishes with a recipe close to the one used, further studies should be considered to cover the many versions of the dish, other parameters that contribute to the national aspects of foods such as glycaemic index and glycaemic load can also be investigated in the future.

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Profiling fatty acids, sterol, tocopherol and bioactive properties in roasted hazelnuts and their skin oil grown under organic and conventional cultivation systems

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This study aimed to to compare the fatty acids, sterol, tocopherol, phenolic substance concentrations and antioxidant activities of the skins of roasted hazelnuts grown with organic and conventional systems. In addition, lipid profiles of kernels were determined. Natural hazelnuts were separated from their skin by roasting at the temperature (140°C) and time (30 min.) commonly used in the industry. According to the study results, organic hazelnut skin (OS) had higher phenolic substance (294.58 versus 251.37 mg GAE/g) and antioxidant activity (546.72 versus 450.6 mg TE/g for CUPRAC, 498.78 versus 390.06 mg TEAC/g for ABTS) than that of conventional. OS contained lower amounts of unsaturated fatty acids (UFA), total sterol and total tocopherol that of conventional ones. On the other hand, there was a skin like trend among the kernels, except that no difference in tocopherol accumulation was observed. Regardless of the production system, the skin was found to be a source of phytosterols and tocopherols more than 10 times greater than the kernel. In conclusion, OS, which are by-products for the organic hazelnut industry, have the potential to be used for nutritional enrichment in organic food formulations due to its high phytochemical content.

Keywords: hazelnut skin oil, lipid characteristic, organic nut, conventional farming, bioactive compounds, antioxidant properties, roasting

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1. INTRODUCTION

Hazelnut is one of the most popular nuts worldwide due to its pleasant aroma, rich nutritional content, fat-soluble bioactive substances, phenolics and phytochemicals [1]. Although hazelnut is consumed naturally (with skin), consumers mostly prefer the roasted (without skin) form because it is more aromatic. Therefore, roasting is the most used processing method in the hazelnut industry. With the roasting process, the skin, which is defined as the brown layer that completely covers the kernel and which constitutes 2.5% of the fruit weight, is separated [2]. It is known that phytochemicals on the outer layers of fruits and vegetables protect against oxidative stress, therefore many bioactive substances such as phenolic compounds are concentrated in the outer parts, and this is also true for hazelnuts [3]. For these reasons, hazelnut skin is very rich in fat-soluble bioactive substances and phenolics [4]. These compounds have protective properties against the damaging effects of free radicals and are known to decrease the risks of some illnesses likek some types of cancer, coronary heart disease and type-2 diabetes [1]. The skin produced as a result of the roasting is the most considerable by-product of the hazelnut processing industry. It is thought that this by-product, which is extremely helpful in terms of nutrient content, should be evaluated and studies are being carried out on it. For example, it has been determined that hazelnut skin added to the formulation at 1-3% rates improves the cooking properties and antioxidant properties of chicken burgers, and it has

been emphasised that it can be used successfully as natural ingredients in the production of burgers [5]. Roasted hazelnut skin was used for nutritional enrichment in the production of fresh egg pasta [6], and it was shown that the membrane could be used as an alternative antioxidant source.

Organic foods are associated with superior nutritional properties and non-contaminated sustainable farming practices by consumers, so the demand for fresh and processed organic foods is constantly increasing [7]. Due to the increasing needs, organic hazelnut cultivation has doubled in the last five years, reaching 21,500 tons, and meeting 3.5% of all hazelnut production. This rate is 1.5% when all foods are averaged [8, 9]. This data shows us that the demand for organic hazelnuts is more than twice the average of all organic foods.

All components used in the formulation of processed organic foods must be organic. Even though organic hazelnut skins can be used in organic food formulations for many different purposes such as nutrient enrichment, antioxidant, phenolic substance source, colouring, an overall screening of the lipid and bioactive properties of organic hazelnut skins is lacking in literature. Besides, although phenolic substance, flavonoids and antioxidant capacity studies in conventional-natural hazelnut skins [2, 10] and polyphenol and tocopherol studies in conventional-roasted Polish hazelnut skins [11] were carried out, no study was found in the literature on the fatty acid, tocopherol and sterol profile of conventional-roasted first quality Turkish hazelnut skins. The primer purpose of this research is to characterise the lipid and bioactive properties of roasted hazelnut skins cultivation by organic and conventional methods. For this aim, fatty acid, sterol, tocopherol profiles, total carotenoid, phenolics, flavonoids and antioxidant activity analyses were performed on hazelnut skin samples. In addition, lipid characteristics of roasted hazelnuts were also measured.

2. MATERIALS AND METHODS

2.1. COLLECTION AND ROASTING OF HAZELNUT SAMPLES

Organic hazelnut samples were obtained from farmers producing organic hazelnuts within the extent of the Organic Agriculture Project realized under the title of community certification by Keşap Chamber of Agriculture (Keşap, Giresun, Turkey). Conventional hazelnuts were obtained from growers engaged in conventional production from the same region. Organic nut samples were obtained from orchards inspected by the international certification company (ECAS Company, Antalya, TURKEY). Giresun Quality (First Quality) hazelnuts harvested in 2020 were used as research material. Samples randomly selected from orchards were picked in the second week of August, after their outer green husks turned yellow and the moisture decreased to 30%. The drying process was carried out for 3 days between 09:00 and 20:00 at room temperature conditions (average temperature 24.7°C). The samples were laid on a 5 × 5 m jute cover in the concrete ground and mixed 5 times a day during the drying period. After 20:00 in the evening, each group was gathered in the centre and covered with nylon to prevent moisture entering from outside. At the end of the drying period, the humidity decreased in all samples below 6% and the samples were kept at -18°C until the day of analysis. The hazelnuts, which were dried and brought to the laboratory, were mixed equally and a single sample was obtained for roasting. After the hazelnuts were separated from their shells by hand, they were roasted in a conventional oven (Ecocell, Germany) for the time and temperature frequently used in the industry (140°C - 30 min). Roasting treatment had three replicates and all analytical measurements were performed in duplicate. Roasted nuts were stored at -18°C until the day of analysis.

2.2. METHODS

2.2.1 Moisture content and oil extraction

Shelled hazelnuts used in the analysis were hand-cracked and separated from their shells. The moisture content of hazelnuts and their skins were calculated by determining the weight loss resulting from keeping the samples in an oven heated at 103°C for approximately 4 hours. Oil extraction was performed with a test scale screw press device with a 3 kW regulatable speed. The screw turning speed was adjusted at 60 rpm to extract 80% of the total oil [7].

2.2.2 Determination of fatty acids

The fatty acid profile of the hazelnut oil was defined after fatty acid methylation according to the method ISO 12966-2 [12]. Briefly, 0,1 g of nut oil extracted by pressing was placed into a tube. The oil was shaken by adding 5 mL of heptane as solvent and 0.5 mL of 2 N potassium hydroxide solution in methanol. The solvent in the resulting mixture was removed with anhydrous sodium sulphate. After the prepared mixtures were hold for 1 minute, samples were analysed using gas chromatography-flame ionisation detector (GC-FID) (Perkin Elmer, Autosystem GLX, Shelton, USA) equipped with SP™ - 2560 [100 m × 0.25 mm × 0.2 µm (Supelco, Bellefonte, USA)] column. Data were evaluated with Total Chrome Navigator and explained as % fatty acid.

2.2.3 Indices

Oleic acid/linoleic acid ratio (O/L) and iodine value (IV) [13] rates were calculated according to the equations below.

$$O/L = \text{oleic acid}/\text{linoleic acid} \quad (1)$$

$$IV = (\text{palmitoleic acid} \times 1.901) + (\text{oleic acid} \times 0.899) + (\text{linoleic acid} \times 1.814) + (\text{linolenic acid} \times 2.737) \quad (2)$$

2.2.4 Determination of sterol profile

The sterol profile of the nut oils was performed by according to the procedure reported by Demirtas et al. [14]. First of all, α-cholestanol solution used as internal standard was prepared at a concentration of 1000 mg/L. 1 ml of this solution was taken and 0.5 g was mixed with hazelnut oil extracted by pressing method. The mixture was taken into a screw capped tube and saponification reaction was carried out with potassium hydroxide solution prepared in 10 mL methanol at 80°C for 60 minutes. The mixture was then extracted 3 times in succession using 5 mL of n-hexane solvent. The resulting extract solution was treated with nitrogen gas until the volume was below 10 mL. After the mixture was extracted 3 times using 5 mL of distilled water, the volume of the organic phase was made up to 10 mL with n-hexane. The water in the mixture was evaporated with anhydrous sodium sulphate. 0.5 mL of the obtained extract was placed into a vial and 250 µL of bis (trimethylsilyl) trifluoroacetamide / trimethylchlorosilane (4:1, v/v) and 250 µL of pure pyridine were joined and derivatized at 60°C for 15 minutes. The resulting mixtures were studied with GC-FID and SE-54 (5%-phenyl-1%-vinylmethylpolysiloxane), 30 m × 0.32 mm × 0.25 µm (Agilent, Santa Clara, CA, USA) column. Data were evaluated with Total Chrome Navigator and explained as mg/100 g oil.

2.2.5 Determination of tocol profile

Tocol isomers of oils samples extracted from hazelnut kernels and their skin was performed according to the method reported by Demirtas et al. [14]. In summary, 1 g of hazelnut oil extracted by pressing method was supplemented into a tube and then 25 mL of heptane was added, and the mixture was shaken for 10 minutes. The resulting extract was filtered using 0.45 µm pore size syringe filter before injecting into HPLC (High Performance Liquid Chromatography). Analysis of samples was carried out using HPLC (Agilent Series 1100, Waldbronn, Germany) with a fluorescence detector and normal phase column (5µm LiCrosorb Si60 25 cm × 4.6 mm i.d., HiChrom Ltd., Theale, UK). Results with Chemstation were explained as mean values ± standard deviation as mg/100 g oil.

2.2.6 Spectrophotometric assays

2.2.6.1 Extraction of bioactive substances

For extraction, 3 g of each nut sample was weighed. It was sonicated for 30 minutes at 45°C using an ultrasonic water bath (Kudos, Shanghai Kudos Ultrasonic Instrument Co., Ltd., China) with 10 mL of 80% ethyl alcohol. After centrifugation, the supernatant was taken to another tube and 10 mL of 80% ethyl alcohol was added to the remaining pulp and sonicated at 45°C for 15 minutes. After centrifugation, the supernatant was separated and 10 ml of 80% ethyl alcohol was added to the remaining pulp and sonicated for 5 minutes at 45°C. The supernatants were combined and made up to 25 ml with 80% ethanol and used in total phenolic, total flavonoid and antioxidant activity analyses.

2.2.6.2 Determination of total phenolics, total flavonoids and antioxidant activities (ABTS and CUPRAC methods)

Biochemical properties were determined in hazelnut kernel and hazelnut skins. Total phenolic, total flavonoids and antioxidant activity (according to ABTS and CUPRAC assays) were detected as biochemical properties. All measurements were made using a spectrophotometer (Shimadzu, Japan). The total amount of phenolic substances was performed with the Folin-Ciocalteu reagent according to the procedure reported by Mayda et al. [15]. Total flavonoids were detected according to the method described by Mayda et al. [15]. Total phenolics as gallic acid equivalents (mg GAE/g) and total amount of flavonoids was explained as mg catechin equivalent (mg CE/g). Antioxidant activity was measured using the ABTS [15] and CUPRAC [16] assays and explained as Trolox equivalent antioxidant capacity (TAC) per g (mg TEAC/g) and mg Trolox equivalent (mg TE/g), respectively.

2.2.6.3 Extraction and determination of total carotenoids

For carotenoid analysis, 250 mg of each sample was weighed and crushed in a mortar with the help of liquid nitrogen. The homogenates were transferred to 15 mL centrifuge tubes, 5 mL of pure methyl alcohol was added, and sonicated for 15 minutes in an ultrasonic homogenizer (Bandelin MS72, Berlin, Germany). After sonication, it was kept at +4°C for 1 night and then filtered and analysed. The absorbances of the extracts were read by the spectrophotometer at 663 nm, 645 nm and 440.5 nm. Firstly, the amounts of chlorophyll a and chlorophyll b, and then the total amount of carotenoids were calculated using the formulas below [17].

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{V}{(1000 \times w)}$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{V}{(1000 \times w)}$$

$$\text{Total carotenoid} = 46.95 \times (A_{440.5} - 0.268 \times \text{chlorophyll a+b})$$

w: weight by grams for extracted;
v: final size of extracted;
A: absorbance.

2.3. STATISTICAL EVALUATION

A one-way analysis of variance (ANOVA) and the Pearson correlation test were carried out on software, R version 4.1.1. Principal components analysis (PCA) has performed by using JMP version 16 and two principal components were extracted. The results were explained as mean values ± standard deviation (n = 3).

3. RESULTS AND DISCUSSION

3.1. FATTY ACID PROFILE

Moisture and oil content of organic roasted hazelnut (OH), conventional roasted hazelnut (CH) organic roasted hazelnut skin (OS) and conventional roasted hazelnut skin (CS) are given in Table I. While there was no effect of the production system on moisture in both skin and kernel, it was observed that organic samples contained more oil (OH, CH, OS, CS, respectively, 57.30, 56.63, 11.61, 10.58%) ($P<0.05$). In addition, the oil rate in the skin was approximately 5 times lower than in the kernel in both production systems. Tunçil [18] (57.90%) in kernel, Ivanovic et al. [10] (12.03%) and Tunçil [18] (17.80%) in the skin reported similar oil ratios.

Fatty acid profiles of kernel and hazelnut skins are presented in Table I. Eleven different fatty acids were detected in OH and CHs. While the major fatty acid in the kernel was oleic acid, it was followed by linoleic, palmitic and stearic acids, the ratio of other fatty acids remained below 0.2%. Similar rankings were found by Amaral et al. [1] in Portuguese hazelnuts. Except for stearic acid, other major fatty acids (oleic, linoleic and palmitic acid) were determined to be influenced by the production system ($P<0.05$). The production system had a statistically significant effect on only palmitoleic acid, one of the minor fatty acids. In OH, lower unsaturated fatty acids (UFA) from lower oleic and linoleic fatty acids and higher SFA from higher palmitic acid were detected. Again, due to the lower oleic acid content of OH, the PUFA (polyunsaturated fatty acids) rate was found to be lower.

In the hazelnut skin, 14 different fatty acids were determined as three more than the kernel (pentadecanoic, lignoceric, lauric acid). As in fruit, fatty acids other than oleic, linoleic, palmitic and stearic acids, which are the dominant fatty acids, remained below 0.2%. However, there were significant variations in the fatty acid profile of the hazelnut skin compared to the kernel. Regardless the production system, it was observed that the oleic acid level of the skin was found at about 10% less than the kernel, and the linoleic acid was more than twice as high. It was also seen that the amount of palmitic acid was slightly higher, while the other fatty acids were at equal levels. Consistent with our data, Özdemir et al. [4] reported that oleic acid is the major fatty acid in hazelnut skin (75.12%), followed by linoleic (16.0%), palmitic (6.8%) and stearic acid (1.2%). Although Özyurt et al. [19]

Table I - Moisture, fatty acid composition (%) and oxidative stability indices of oils extracted from organic and conventional roasted hazelnut and their skins

Parameters	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
Moisture	2.10±0.04a	2.21±0.04a	6.36±0.10a	6.92±0.06a
Total oil	57.30±0.06a	56.63±0.36b	11.61±0.07a	10.58±0.01b
Pentadecanoic acid	nd	nd	0.01±0.00a	0.01±0.00a
Palmitic acid	5.54±0.01a	4.81±0.01b	8.74±0.001a	6.43±0.07b
Palmitoleic acid	0.14±0.00a	0.11±0.01b	0.11±0.00a	0.09±0.00a
Heptadecanoic acid	0.04±0.00a	0.04±0.00a	0.03±0.00a	0.04±0.00a
Stearic acid	2.30±0.00a	2.34±0.00a	1.34±0.00b	1.66±0.02a
Oleic acid	84.70±0.03b	85.34±0.01a	75.53±0.01b	76.54±0.48a
Linoleic acid	6.86±0.11b	6.94±,0.01a	13.54±0.04b	14.55±0.11a
α-Linolenic	0.06±0.00a	0.06±0.00a	0.10±0.01b	0.13±0.00a
Arachidic acid	0.11±0.00a	0.10±0.01a	0.11±0.00a	0.15±0.00a
Eicosenoic acid	0.11±0.00a	0.12±0.00a	0.09±0.00a	0.14±0.00a
Behenic acid	0.01±0.00a	0.01±0.00a	0.04±0.00a	0.06±0.00a
Miristic acid	0.02±0.00a	0.02±0.00a	0,05±0,01a	0.04±0.00a
Lignoseric acid	nd	nd	0.06±0.00a	0.01±0.00b
Lauric acid	nd	nd	0.02±0.00a	0.03±0.00a
SFA	8.02±0.01a	7.32±0.02b	10.40±0.01a	8.52±0.09b
MUFA	84.95±0.03b	85.57±0.01a	75.73±0.01b	76.77±0.48a
PUFA	6.92±0.01b	7.00±0.01a	13.64±0.01b	14.68±0.11a
UFA	91.87±0.03b	92.56±0.01a	89.37±0.01b	91.45±0.58a
İndices				
UFA/SFA	11.46±0.02b	12.65±0.03a	8.59±0.02b	10.73±0.10a
O/L	12.35±0.01a	12.30±0.02a	5.58±0.00a	5.26±0.01b
IV	88.85±0.02b	89.51±0.01a	92.68±0.01b	95.37±0.62a

All datas are expressed as means ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at $P<0.05$. nd: not detected. SFA: saturated fatty acids, MUFA: mono unsaturated fatty acids, PUFA: poly unsaturated fatty acids, UFA: unsaturated fatty acids, O/L: oleic acid/linoleic acid, IV: iodine value.

Table II - Sterol composition (mg/100g) of oils extracted from organic and conventional roatsted hazelnut and their skins

Sterols	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
Campesterol	3.99±0.05b	4.77±0.21a	22.32±0.49b	37.88±1.77a
Stigmasterol	0.68±0.07b	0.94±0.17a	35.97±0.89b	73.86±0.31a
Δ7-stigmastenol	1.93±0.17a	1.96±0.11a	6.74±0.09b	10.30±1.18a
β-sitosterol	74.09±0.96b	86.47±0.05a	646.48±15.80b	897.29±13.90a
Sitostanol	1.63±0.46a	1.69±0.09a	126.88±0.30b	189.30±1.47a
Δ5-avenasterol	3.15±0.53a	3.44±0.37a	134.54±0.64b	200.60±0.31a
Δ7-avenasterol	0.38±0.07b	0.59±0.03a	3.99±0.85a	5.58±0.83a
Total sterol	85.80±0.96b	99.91±0.16a	968.60±6.26b	1415.60±13.10a

All datas are expressed as means ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at P<0.05.

made a similar ranking, they reported the amount of oleic acid slightly higher than the results of this work (80.52%).

While it was seen that the production system affected all the dominant fatty acids in the skin, only α-linoleic and lignoceric acids were found to affect the minor fatty acids (P<0.05). Higher levels of oleic (76.54-75.53%, respectively), linoleic (14.55-13.54%) and stearic acid (1.66-1.34%) and lower levels of palmitic acid (6.43-8.74%) accumulated in CS than OS. As a result, it was observed that there was more UFA in CS compared to OS (91.45-89.37%, respectively), as in kernel. Despite the lower stearic acid content of OS, it was determined that the SFA level was higher due to the higher palmitic acid content. Again, it was observed that the MUFA level was high owing to the excess quantity of oleic acid in the conventional samples. The fatty acid composition is affected by variety, harvest time, drying process, harvest year, geographical origin, environmental conditions, preservation and processing conditions [20]. As all other conditions are the same, greater oil accumulation and difference in fatty acid profile in organic samples may be due to differences in the cultivation system.

3.2. OXIDATIVE STABILITY EVALUATION

Due to the change caused by the production system in the composition of fatty acids, significant differences have emerged in the degree of unsaturation of lipids and, accordingly, in their susceptibility to oxidation. Various methods exist for determining the reactivity and susceptibility to oxidation of lipids are discussed below. Low UFA/SFA rate is thought to be a long shelf life in hazelnuts [21]. It was observed that the UFA/SFA ratio of organic samples was lower in both kernel and skin (OH, CH, OS, CS, 11.46, 12.65, 8.59, 10.75, respectively) (P<0.05). It is particularly interesting that OS has a much lower UFA/SFA ratio compared to others. Göncüoğlu Taş and Gökmen [22] reported the UFA/SFA ratio as 9.2, and Ghirardello et al. [23] as12.03, which is comparable to our study results. The oleic/linoleic (O/L) rate is a considerable standard for evaluating kernel qualification, and a higher O/L value means better oxidative stability [13, 24]. While the O/L values of OS and CS were determined as 5.58 and 5.26, respectively, it was determined that the difference was significant and but there was no difference in kernel. The iodine value (IV) is a indicator of the grade of unsaturation of oils. High IV is a marker that oils are more reactive, less stable, and more sensitive to rancidity and oxidation [13]. It was determined that conventional samples had higher IV values in both kernel and skin (OH, CH, OS, CS, 88.85, 89.51, 92.68, 95.37, respectively) (P<0.05). In kernel, Karaosmanoğlu and Üstün [24] (89.81-94.40) and Belviso et al. [13] (89.84-86.94) reported comparable results with our study.

It can be said that organic samples may be highly resistant to lipid oxidation and have more shelf life because of lower UFA/SFA, IV and higher O/L values in both kernel and skin. Regardless the production system, it can be said that the skin is more sensitive to oxidation compared to kernel due to low O/L, close levels of UFA/SFA and high PUFA/SFA, IV values. The oxidation ratios of fatty acids are almost 1:10:100:200 for stearic, oleic, linoleic and linolenic acids, respectively [25]. In relation to this, organic samples with lower linoleic acid levels are more stable to oxidative changes. In addition, this information can explain that the skin may be less durable than hazelnuts, because there was approximately twice as much linoleic acid in the skin than in the kernel.

3.3. STEROL PROFILE

Seven sterols identified in organic and conventional hazelnut and their skins are presented in Table II. β-sitosterol, the highest amount of sterol in roasted hazelnuts, met 86% of the total. β-sitosterol was detected at the levels of 74.09 and 86.47 mg/100g in OH and CH, respectively, and the difference was statistically significant (P<0.05). Similar to our work, Amaral et al. [1] reported that the most abundant sterol in Portuguese hazelnuts is β-sitosterol. β-sitosterol was followed by campesterol and Δ-5avenasterol, while others (stigmasterol, Δ7-stigmastenol, sitostanol, Δ7-avenasterol) comprised less than 5% of total sterol. Total sterol amount of OHs (85.80 mg/100g) was found to be significantly lower than CHs (99.91

mg/100g) (P<0.05). Since other sterols are not affected by the production system, the difference in total sterol may be due to the lower amount of β-sitosterol and campesterol in organic samples. Alasalvar et al. [25] determined total sterol and β-sitosterol levels in Tombul hazelnut as 113.52 and 105.48 mg/100g, consistent with our data. In a research carried out on Polish hazelnuts, 7 different phytosterol and 3 different stanols were reported, while the total sterol was found to be in the range of 130.32-152.22 mg/100g, slightly higher than our results [26].

Although β-sitosterol is the most abundant sterol in the skin as in kernel, its ratio in total sterol is lower (OS: 66%, CS: 63%). Total sterol was 11 times higher in OS (968.60mg/100g) and 14 times higher in CS (1415.60 mg/100g) than kernel. It is also quite interesting that sitostanol was found to be 77 and 112 times higher in OS and CS than in OH and CH (respectively). It was determined that individual sterols and total sterols, except for Δ7-avenasterol, which is found in small amounts in the skin, accumulate less in the organic production system compared to the other one (P<0.05). Individual and total sterols in hazelnuts can be affected by cultivar, geographic origin, harvest time and environmental factors [25, 27]. As the conditions listed above were the same for all samples, this work shows that the production system can also have an effect on the level of sterols.

Total sterol content of OS was 1.65, 12.96, 10.99, 52.64 times higher than the sterol contents reported by Alberici et al. [28] for corn (587 mg/100g), olive (74.7 mg/100g), rapeseed (88.1 mg/100g) and soybean oils (18.4 mg/100g), respectively. According to the results of the study, organic production was led to slight decrease the amount of sterol. However, despite this decrease, OS can be said to be a richer source of phytosterols when compared to the oils given in the above work [28]. The sterols found in plant sources have high anticancer properties in human metabolism, in addition to lowering LDL cholesterol, preventing diabetes, enhancing immunity [1]. Therefore, the inclusion of OSs in organic food formulations may increase the potential health benefits of the food in which it is used.

3.4. TOCOPHEROL PROFILE

The individual and total tocopherol contents of organic and conventional hazelnuts and their skins are given in Table III. Three isomers of tocopherol have been determined and identified in hazelnut kernel. Among the tocol isomers determined in OH and CH, α-tocopherol (53.89, 54.71 mg/100g, respectively) is the most abundant isomer, constituting 85 and 98% of the total, followed by γ-tocopherol (7.30, 3.19 mg/100g, respectively) and β-tocopherol (1.67-1.68 mg/100g, respectively). Stuetz et al. [29] reported the α-tocopherol amount of hazelnuts as 40.06 mg /100g oil. Ciemniewska-Zytkiewicz et al. [26] determined that α-tocopherol, the most higher level of tocopherol in Polish hazelnut varieties, constituted 86-90% of the total tocopherol, followed by γ-tocopherol (6-10%) and β-tocopherol (2.9-3%). While it was understood that the production system did not affect the total tocopherol amount of hazelnuts (P>0.05), it was observed that CHs had slightly more α and γ-tocopherol (P<0.05). Even though there was no significant difference between the production systems in terms of total tocopherol content, it was determined that CHs had a higher Vitamin E effect due to higher α-tocopherol content.

Unlike kernel, α, β and γ-tocopherol were detected in the skin, as well as δ-tocopherol. α-tocopherol (320.31, 380.65 mg/100g, respectively) was the most abundant in both OS and CS, followed by γ-tocopherol (282.14, 294.66 mg/100g, respectively), δ-tocopherol (22.20, 23.27 mg/100g, respectively), and β-tocopherol (18.67, 22.78 mg/100g, respectively). Our results are comparable to Özdemir et al. [4]. Göncüoğlu Taş and Gökmen [2] reported that α-tocopherol (16.82, 44.39 mg/100g) and total tocopherol (22.61,59.35 mg/100g) obtained from different hazelnut cultivars were much lower than our results. In OS and CS, the ratio of α-tocopherol, which is the dominant isomer, in total tocopherol decreased

Table III - Tocopherol composition (mg/100g), vitamin E (mg/100g) content of oils extracted and total carotenoid content (mg/g) from organic and conventional roasted hazelnut and their skins

Parameters	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
α-tocopherol	53.89±0.35b	54.71±0.21a	320.31±2.15b	380.65±13.94a
β-tocopherol	1.67±0.11a	1.68±0.07a	18.69±0.06b	22.78±0.39a
γ-tocopherol	7.30±0.08a	3.19±0.03b	282.14±1.82b	294.66±6.70a
δ-tocopherol	nd	nd	22.20±0.09b	23.27±0.35a
Total tocopherol	62.98±0.53a	59.58±0.17a	643.34±3.92b	721.34±21.48a
Vitamin E	55.28±0.39b	55.78±0.39a	356.20±2.35b	419.45±14.78a
Total carotenoid	1.59±0.24a	2.01±0.27a	7.13±0.59a	7.33±1.03a

All datas are expressed as means ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at P<0.05. nd: not detected.

Vitamin E (expressed as α-tocopherol equivalents). The conversion factors for vitamin E activity were as follows: α-Tocopherol × 1.00, β-tocopherol × 0.40, γ-tocopherol × 0.10, δ-tocopherol × 0.01 [27].

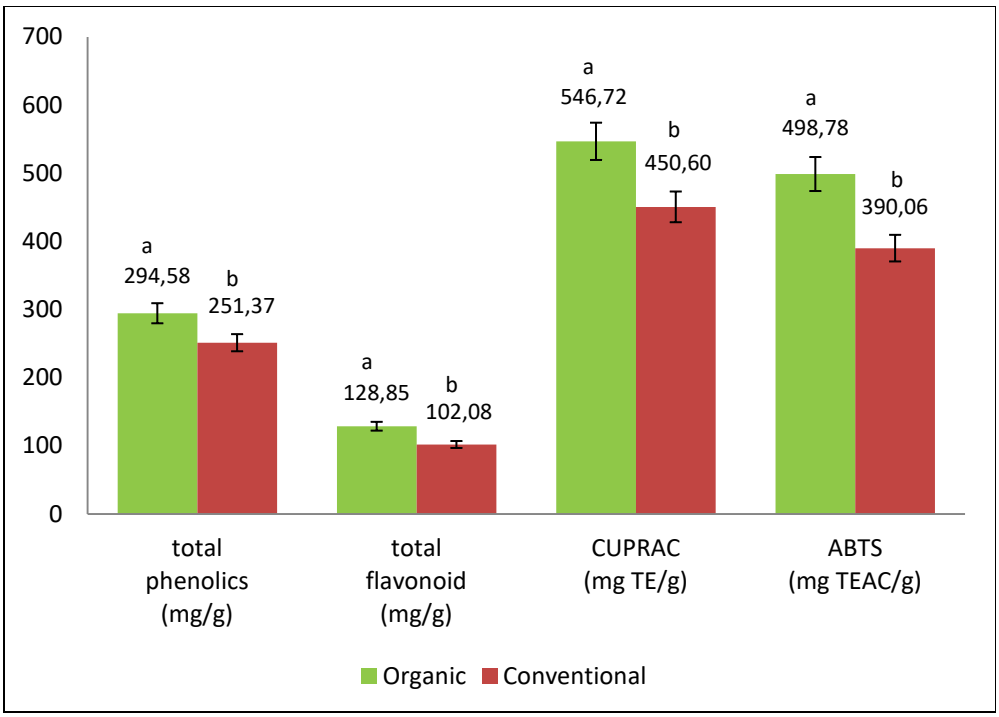

Figure 1 - Total phenolics, total flavonoids and antioxidant activities of organic and conventional roasted hazelnut skins by different tests such as CUPRAC and ABTS. Different letters indicate of statistical difference according to one-way ANOVA test at $P < 0.05$.

compared to kernel (50-52%), while the ratio of γ-tocopherol increased (43-40%). It was determined that CSs were higher than OSs in all tocopherol isomers and accordingly in terms of total tocopherol amount and vitamin E activity ($P<0.05$). The knowledge in literature that the stressful conditions inherent in the organic cultivation system support the synthesis of bioactive compounds could not be confirmed for tocopherol and phytosterol in this study [30]. Fertilisation is an important factor affecting the biosynthesis of bioactive compounds such as carotenoids and tocopherols in fruits [25]. This fact could explain the greater tocopherol amount observed in conventionally grown hazelnut skins in this work.

Tocopherols are known as fat-soluble antioxidants found in different amounts in all vegetable oils [31]. There is strong evidence that they protect fats from oxidative degradation and show the vitamin E activity in the human body, playing an important role in the prevention of some chronic health problems such as heart disease and certain cancer types [25]. Compared to kernel, OS and CS's were 5.90, 6.95 times for α-tocopherol, 11.19-13.5 times for β-tocopherol, 38.6-92 times for γ-tocopherol, 10.21-12 times for total tocopherol and 6.44-7.50 times for vitamin E effect was found to be higher. On the other hand, while it was determined that approximately 4 times more carotenoids were accumulated in the skin than in the kernel, it was determined that the production system did not affect it ($P>0.05$).

Organic and conventional hazelnut skins with high tocopherol content can be used to increase the oxidative stability of the foods, to contribute to the antioxidant capacity and to increase the amount of tocopherols. Considering that all additives to be added to processed organic foods must be organic, OSs can be used to increase the shelf life, tocopherol and carotenoid content of many organic foods.

3.5. TOTAL PHENOLICS, TOTAL FLAVONOIDS AND TOTAL ANTIOXIDANT ACTIVITIES (DPPH and ABTS TESTS)

To date, the total phenolic contents (TPC) and antioxidant properties of many organic and conventional foods have been investigated and it has been reported that organic samples contain higher phenolic substances and show antioxidant activity [32, 33]. In our study, TPC of hazelnut skins were 294.58 mg GAE/g and 251.37 mg GAE/g for OS and CS, respectively (Figure 1). Based on our study data, it was understood that the production system affected the TPC of hazelnut skins and OS contained 17% more. Moreover, the total flavonoid content (TFC) of the skins was determined, and OS (128.85 mg CE/g) was found to contain 26% more TFC than CS (102.08 mg CE/g) ($P<0.05$). It is estimated that exposure of plants to aggressive and stressful conditions leads to the induction of secondary metabolic forming, resulting in greater speed and amount of synthesis of phenolic substances [30]. Furthermore, the exposure pathogenic pressure of plants in organic agriculture where chemical pesticides is not used can also lead to a rise in phenolic synthesis [33]. Due to the nature of organic agriculture, plants are exposed to stress. The

higher level of TPC and TFC synthesis in OS may be owing to these causes. Studies in which hazelnut skin phenolics were determined have been carried out in the literature and quite different results have been reported. For example, Göncüoğlu Taş and Gökmen [2] detected TPC and TFC in Tombul hazelnut skin as 142.2 mg GAE/g and 57.0 mg CE/g, Ivanovic et al. [10] as 706.0 mg GAE/g and 477.7 mg CE/g, respectively. These differences in the literature may be related to different extraction conditions and solvents. In this study, the antioxidant activities of OS and CS were determined using CUPRAC and ABTS methods (Figure 1). The antioxidant activity of OS was found to be 21% and 28% higher than CS in the CUPRAC and ABTS method, respectively ($P<0.05$). Some scientists have determined a strong relationship between the phenolic substance concentration and antioxidant capacity in hazelnuts [34]. Our study confirmed this information and similarly positive correlations were found between TPC and CUPRAC ($r = 0.991$) and ABTS ($r = 0.687$). This correlation may explain the higher antioxidant activity of OS because OS had more TPC than CS. As a result, it can be said that the use of pesticides in conventional production reduces the TPC of hazelnut skins, and accordingly, the antioxidant activity decreases. Similar to our study, Murathan et al. [32] reported that organic samples showed higher antioxidant activity in almond hull.

3.6. PRINCIPAL COMPONENT ANALYSIS

PCA biplot indicates both PC points of samples and loadings of variables and provided better understanding of the correlation between fatty acids, sterol, tocopherol and bioactive compounds of hazelnut skins cultivation by different methods. According to PCA results, the first two components were explained

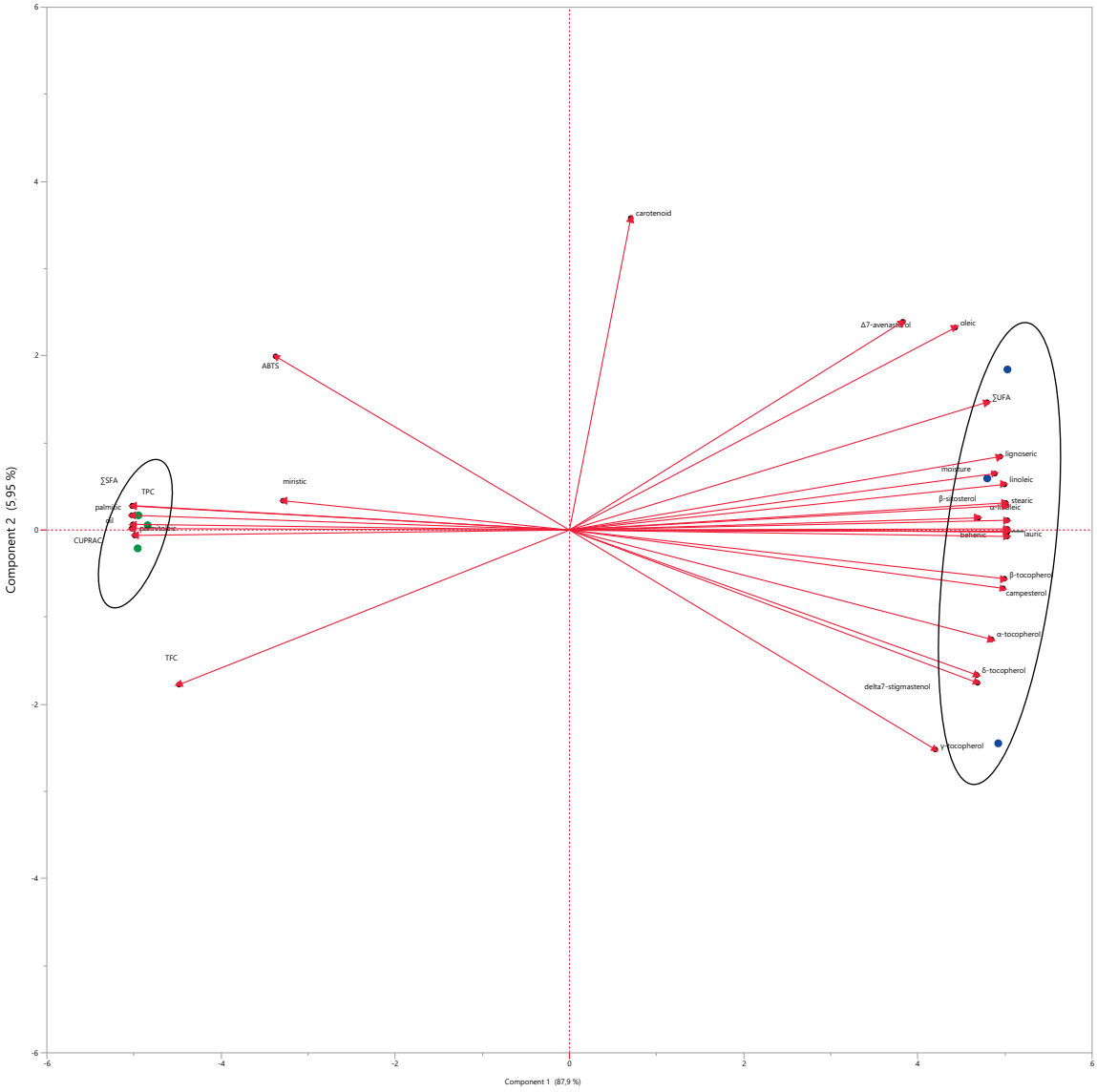

Figure 2 - Principal Component Analysis biplot displaying scores of roasted hazelnut skins (organic samples: green, conventional samples: blue) and loadings of variables (red vectors). SFA-saturated fatty acids, UFA-unsaturated fatty acids. TPC-total phenolics, TFC-total flavonoids, ABTS and CUPRAC-antioxidant activity.

88.6% of the data (Figure 2). Component 1 (PC1) accounted for 87.9% of the total variance, while Component 2 (PC2) accounted for 5.9%. Samples of both production methods are obviously separated from each other along PC1 on the score plot. All organic samples were collected on the negative part of PC1, while all conventional samples were on the positive part. According to PCA results, OS was grouped with TPC, TFC, antioxidant activity (ABTS, CUPRAC), SFA, palmitic acid, palmitoleic acid, oil; CS was grouped mainly with UFA, oleic acid, β-sitosterol and α-tocopherol. A significant positive correlation was seen between TPC, CUPRAC, and ABTS. Therefore, the methods used to evaluate antioxidant activity (ABTS and CUPRAC) appear to be related to TPC. In this study, the principal component and ANOVA analysis results support each other.

4. CONCLUSIONS

This is the first study to comprehensively elucidate the influence of the cultivation system on the fatty acid, sterol and tocopherol profile, oxidative stability, phytochemical content (carotenoid, TPC, TFC) and antioxidant activity of hazelnut skin. Evaluation of the main compounds of the skins obtained from hazelnuts produced under conventional and organic farming practices revealed significant differences. In general, significantly higher TPC, TFC, antioxidant activity (ABTS and CUPRAC), linoleic acid and SFA, equal levels of carotenoids, whereas lower levels of UFA, total sterol and total tocopherol were detected in organic hazelnut skins than conventional ones. Depending on the difference in fatty acid composition, it has been observed that organic hazelnut skins may be more durable to lipid oxidation and therefore have a greater shelf life. Although the production system did not affect the amount of tocopherol in the kernel, a parallel change with the skin was observed in other parameters. On the other hand, the skin had quite different fatty acid composition due to its lower oleic acid and higher linoleic acid content than the kernel. Again, the skin contained approximately 13 times sterol, 11 times tocopherol and 4 times carotenoids compared to the kernel. According to the results of this study, organic hazelnut skin is a very rich source of antioxidants, tocopherols and phytosterols. Therefore, organic hazelnut skins may be proposed as a source of phytochemicals for nutritional enrichment in processed organic food formulations and in nutraceutical applications.

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Orange seeds as juice solid waste: microwave and oven roasting, composition, bioactive properties, fatty acid profiles and principal component analysis

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One of the important waste materials of the citrus juice industry, its seeds are a potentially valuable resource and are also a cause of major environmental problems. Evaluation of the parts of herbal products other than the edible part is one of the current paramount issues. However, for oilseeds to be used effectively and beneficially in various fields, it is considered useful to determine the bioactive properties, phenolic components, fatty acids and mineral contents of seeds and oils as a result of heat treatment. The oil contents of unroasted and roasted-orange seeds were determined between 42.55 (unroasted) and 45.56% (oven), respectively. While total phenol amounts of the orange seeds are found between 115.79 (control) and 133.89 mg GAE/100g (microwave), total flavonoid contents of orange seeds were recorded between 22.02 (control) and 150.83 mg/100g (oven). Also, antioxidant activities of seeds were measured between 3.42 (control) and 3.87 mmol/kg (microwave). The relationship between the antioxidant activity of the seeds and their bioactive components was linear. In general, an increase was observed in the amount of phenolic compounds in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). Gallic acid and 3,4-dihydroxybenzoic acid contents of orange seeds were identified between 5.33 (control) and 45.92 (oven) to 10.01 (control) and 15.14 mg/100g (oven), respectively. While oleic acid contents of the oils obtained from unroasted and roasted orange seeds are identified between 24.44% (microwave) and 24.81% (oven), linoleic acid results of oils were detected between 39.00% (oven) and 39.23% (microwave). The amount of fatty acids of orange seed oils fluctuated depending on the type of roasting and statistically significant differences were monitored between the amounts of fatty acids ($p<0.05$). K, Cu, Ni, Zn and B contents of orange seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control.

Keywords: orange seed, waste, roasting, bioactive compounds, antioxidant activity, polyphenols, fatty acids, elements, HPLC, GC, ICP-AES

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1. INTRODUCTION

The agricultural industry creates a serious waste problem in the food and agriculture field as wastes such as seeds, fruit and peels, roots, bark, and leaves, which are generated as waste from fruit and vegetable processing, are mostly discarded [7]. Citrus seeds waste is a potentially valuable resource, and also cause major environmental problems [1-3]. Significant amounts of citrus seeds emerge as waste in citrus processing plants, making it difficult to dispose of them [1,2,4,5]. Solid waste, which is one of the most important environmental problems of today, is left randomly to the environment and its type is increasing day by day. Evaluation of the parts other than the edible part of plant products is one of the important issues of today [5,6]. Many physical and chemical properties of some seed and plant oils

consumed as edibles are close to one another. But, it is considered that it may be beneficial to determine the minor components of the oil extracted from the seeds in order to use oilseeds effectively and beneficially in various fields [1,13,19-21]. Most common fats or oils are a mixture of very small amounts of lipid components and triglycerides. Fatty acids in different lipid molecules have different nutritional importance. The fatty acid profile of citrus seed oil was found to be similar to that of most edible oils [1,12-15,19,20]. Citrus seed is a waste product that can be used after being separated from the shell and pulp [14]. The most important citrus fruits are orange, mandarin, lemon and goldentop fruits, which are used especially in fruit juice production, as well as citrus and citrange species, which are important rootstocks of these species. One of the important waste materials of the citrus juice industry is the seeds. The use of wastes produced in large quantities from fruits daily by the agricultural industry is limited to the animal feed industry or these wastes thrown into the environment cause significant environmental damage. Studies have focused on the determination of bioactive properties, nutritional values, vitamins, minerals, and other beneficial components of such wastes [22, 23]. Recently, the focus has been on the evaluation of waste materials and by-products from food processing. This allows for highly available resources and ultimately the production of a variety of new foods. It is getting more and more difficult to solve the problems arising from industrial wastes. Therefore, more efforts are needed to develop by-products and wastes with nutritional and industrial potential [14]. Apart from the edible part of citrus fruits, the waste part, which is mostly composed of peels and seeds, is used in the treatment of various diseases among the public [8]. Fruit and vegetable peels are rich in bioactive components such as polyphenols and carotenoids, which are called phytochemicals and have various positive effects on health [9,10]. Plant seeds are usually the source of essential oils for nutritional and industrial fields [12]. Today, the waste of one factory can be the raw material of another industry. Therefore, due to the nutritional properties of by-products such as shells and seeds, which are produced as waste in food processing industries, they can be a valuable source of by-products [14,16-18]. Phenolic substances are important for human health due to its effects on taste and odour formation, participation in colour formation and change, antimicrobial and antioxidative effects. Phenolic compounds have positive effects on nutritional physiology [11]. In order to evaluate waste materials and by-products such as orange seeds from food processing, it is necessary to increase the shelf life by removing the water in the seeds, to add flavour and aroma to the seeds, and to destroy some harmful substances in its composition with the effect of heat. As a result of these processes, the changes in the phytochemical contents of the orange seeds of the microwave and oven processes were observed. Therefore, it is necessary to develop by-products and wastes with nutritional and industrial potential and to allow them to be used as food supplements. The disposal of by-products in food processing has become one of the most common problems in the industry [16,17]. Therefore, investigating the use of citrus seeds in human and/or animal diets has become an important issue. There are many studies that determine the bioactive components in the seeds of citrus fruits, which are grown at a significant level in Turkey, reveal their comparative advantages, and reveal the effect of different heat treatments on the determination of the amounts of phenolic compounds with antioxidant properties. The aim of this study: 1- determination of the physico-chemical properties, total phenol, total flavonoid amounts, antioxidant capacity, phenolic and fatty acids of orange seed; 2- the effect of roasting in microwave and oven was compared on the bioactive properties and phytochemicals of seeds.

2. MATERIAL AND METHODS

2.1. MATERIAL

Orange (*Citrus sinensis* L.) fruits (50 kg) obtained from Mersin (Büyükeceli-Gülnar) in Turkey in 2021 were used. The seeds obtained from the orange fruit were cleaned with tap water and dried in atmospheric weather conditions. The seeds were dried by arranging them in a single layer on a cloth. The seeds were mixed at regular intervals during the drying process. The dried seeds were powdered in a laboratory mill and stored in hermetically sealed coloured glass jar at 4°C until analysis.

2.2 METHODS

2.2.1 Heat treatment

Ground orange seeds were subjected to a heat treatment prior to analysis (except Control). The orange seeds were heated in an oven at 120°C for 50 min; in a microwave at 900W for 7 min.

2.2.2 Moisture content

The moisture contents of the orange seeds were determined by the KERN & SOHN GmbH infrared moisture analyser [24].

2.2.3. Oil content

After grinding the roasted and unroasted orange seeds, 10 g were weighed into the Soxhlet cartridge and placed in the Soxhlet apparatus. Petroleum ether was used for oil extraction. The extraction process continued for 5 hours at 50°C. This period was completed, the petroleum ether was evaporated in the evaporator. After oil extraction, possible particles were removed by filtering, the crude oil content (%) was determined. [24].

2.2.4 Extraction procedure

Extraction was made according to the method determined by Garcia-Salas et al [25]. 2 g samples were taken from the grinded orange seeds, mixed with 10 ml of methanol, and the mixture was stirred by vortex for 1 minute. Then, after the solution was sonicated for 30 min, it was centrifuged at 4500 rpm for 10 min. The resulting supernatants were collected and concentrated at 37°C. After the volume of the extracts was made up to 10 ml, they were filtered. All analyses were performed in 3 replications.

2.2.5 Total phenolic content

The total phenolic results of the orange seeds were recorded by the Folin-Ciocalteu reagent according to study stated by Yoo et al. [26]. FC (1 ml) and Na_2CO_3 (10 ml) were added to extract and mixed with vortex. The deionised water was added until the final volume was 25 ml and kept in the dark for 1 h. The absorbance was measured at 750 nm in a spectrophotometer. A calibration curve was prepared with gallic acid (0-200 mg/ml) as the standard. The results are shown as mg gallic acid equivalent (GAE)/100 g.

2.2.6 Total flavonoid content

The orange seed extract (1 mL) was mixed with 0.3 ml of $NaNO_2$, 0.3 ml of $AlCl_3$ and 2 ml of NaOH, respectively, and kept in the dark for 15 min. The absorbance of mixture was measured at 510 nm using spectrophotometer. The results are given as mg quercetin (QE)/100g [27].

2.2.7 Antioxidant activity

1.1-diphenyl-2-picrylhydrazyl (DPPH) was used for the antioxidant capacity of orange seed extracts [28]. The extract was added to 2 ml of a methanolic solution of DPPH, followed by vortexed and kept in the dark for 30 min. The absorbance was read at 517 nm. The results obtained was stated as mmol trolox (TE)/kg.

2.2.8 Determination of phenolic compounds

HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 x 250 mm) column was used for chromatographic separation of phenolic compounds of seed extracts. The mobile phase was a mixture of 0.05% acetic acid in water (A) and acetonitrile (B) with the flow rate of 1 ml/min at 30 °C. The injection volume was 20 µl. The peaks were taken at 280 using a PDA detector. The elution programme was employed: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The total running time per sample was 60 min.

2.2.9 Fatty acid composition

The orange seed oil was esterified according to the ISO-5509 (1978) method. Fatty acid methyl esters of samples were analysed using gas chromatography (Shimadzu GC-2010) equipped with a flame-ionisation detector (FID) and capillary column (Tecnocroma TR-CN100, 60 m x 0.25 mm, film thickness: 0.20 µm). The temperature of the injection block and detector was 260ºC. The mobile phase was nitrogen with 1.51 ml/min flow rate. The total flow rate was 80 ml/min and split rate was also 1/40. The column temperature was programmed 120ºC for 5 minutes and increased 240ºC at 4ºC/min and held 25 minutes at 240ºC.

2.2.10 Determination of mineral

After orange seeds were dried at 70ºC until reaching a constant weight, they were ground in a laboratory-type mill. About 0.5 g of ground seeds was burned by using 5 ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a microwave system. A 40-cell microwave was used to ensure the reliability of the analysis. After the volumes of the dissolved samples were made up to 20 ml with deionised water, the element concentrations in the samples were analysed by Inductively coupled plasma optical emission spectrometry (ICP-AES; Varian-Vista Model) equipment [30].

Working conditions of ICP-AES:

Instrument: ICP-AES (Varian-Vista)
RF Power: 0.7-1.5 kw (1.2-1.3 kw for Axial)
Plasma gas flow rate (Ar): 10.5-15 L/min. (radial) 15 " (axial)
Auxiliary gas flow rate (Ar): 1.5 "
Viewing height: 5-12 mm
Copy and reading time: 1-5 s (max.60 s)
Copy time: 3 s (max. 100 s)

2.3 Statistical Analyses

After averaging the triple analysis data for all treatments, the mean values were exposed to analysis of variance. Significant differences between raw (control), roasted kernels and roasting type results were calculated by Duncan's Multiple Range test ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF ORANGE SEEDS

The results of bioactive compounds and antioxidant activities of orange seeds roasted in the oven and microwave are shown in Table 1. Results obtained with the bioactive compounds and antioxidant activity values of orange seeds exhibited some changes depending on roasting types. The moisture and oil results of unroasted (control) and roasted-orange seeds were calculated between 2.93% (oven) and 7.66% (control) to 42.55% (control) and 45.56% (oven), respectively. While the total phenolic results of the orange seeds change between 115.79 (control) and 133.89 mg GAE/100g (microwave), total flavonoid results of unroasted and roasted-orange seeds were determined between 122.02 (control) and 150.83 mg/100g (oven). Antioxidant activities of seeds were

Table I - Some chemical and bioactive compounds of orange seeds roasted in microwave and oven

Process	Moisture content (%)	Oil content (%)	Total phenolic content (mg/100 g)	Total flavonoid content (mg/100 g)	Antioxidant activity (mmol/kg)
Control	7.66 ± 0.63a*	42.55 ± 2.55c	115.79 ± 2.65c	122.02 ± 4.68c	3.42 ± 0.02c
Microwave	3.68 ± 0.14b	43.47 ± 0.10b	133.89 ± 2.14a	143.45 ± 4.75b	3.87 ± 0.00a
Oven	2.93 ± 0.33c	45.56 ± 0.73a	121.03 ± 8.95b	150.83 ± 3.79a	3.56 ± 0.14b

* values within each column followed by different letters are significantly different at P < 0.05.

Table II - Phenolic compounds of orange seeds roasted in microwave and oven

Phenolic compounds (mg/100 g)	Control	Microwave oven	Oven
Gallic acid	5.33 ± 0.45c*	38.18 ± 2.79b	45.92 ± 4.78a
3,4-Dihydroxybenzoic acid	10.01 ± 2.49c	11.47 ± 1.31b	15.14 ± 0.12a
Catechin	18.73 ± 0.61b	17.96 ± 4.23c	25.48 ± 4.67a
Caffeic acid	2.08 ± 0.67c	4.18 ± 0.60a	3.79 ± 1.16b
Syringic acid	3.37 ± 1.20c	4.28 ± 0.22ab	4.37 ± 0.57a
Rutin	10.15 ± 0.08b	10.40 ± 0.71a	9.83 ± 3.33c
p-Coumaric acid	1.60 ± 0.42c	3.59 ± 0.78b	3.86 ± 0.65a
Ferulic acid	2.13 ± 0.59bc	3.74 ± 0.95a	2.78 ± 0.92b
Resveratrol	0.75 ± 0.10c	1.32 ± 0.38a	1.27 ± 0.25b
Quercetin	4.02 ± 1.98b	6.23 ± 1.40a	3.06 ± 0.82c
Cinnamic acid	0.73 ± 0.29a	0.53 ± 0.07b	0.45 ± 0.17c
Kaempferol	0.92 ± 0.51c	2.40 ± 0.71a	1.76 ± 0.55b

* values within each row followed by different letters are significantly different at P < 0.05.

measured between 3.42 (control) and 3.87 mmol/kg (microwave). The moisture amount of orange seeds decreased with microwave and oven roasting, while the oil content of seeds partially increased. This increase is probably due to the evaporation of water in the seed during roasting and the increase in dry matter content. In addition, bioactive compounds and antioxidant activities of orange seeds increased with the roasting process compared to the control. The highest total phenol and antioxidant activities were established in orange seeds roasted in the microwave. A linear relationship between the antioxidant activity results of the seeds and their bioactive components was monitored. Statistically significant changes among the chemical properties and antioxidant activity results of orange seeds were monitored depending on the type of roasting ($p<0.05$). Microwave and oven roasting increased both the bioactive component amounts and antioxidant activity values of orange seeds. The oil results of orange and tangerine seeds were reported as 17.01% and 15.87%, respectively [14]. In another study, the oil results of citrus seeds obtained from Turkey and Vietnam were determined to be 45.1-58.8% and 32.1-54.8%, respectively [31]. The oil content of citrus seeds was determined between 34.92 and 41.66% [32]. The total phenolic content of oil extracted from orange seeds was 1152.88 mg GAE/kg [32]. The oil contents of citrus seeds growing in Egypt changed between 40.2 and 45.5% [13]. The rate of oil result of citrus seeds varied between 33.4% and 41.9% [33]. The oil results of mandarin and bitter orange seeds were recorded as 27.61% and 36.42%, respectively [34]. The total phenolic contents of orange seed oils were 1152.88 mg GAE/kg [32]. Moulehi et al. [35] reported that the total polyphenol result of Mandarin (*Citrus reticulata*) seed was established between 0.68 and 2.11 mg GAE/g DW. The total phenolic results of orange seed was determined between 10.9 - 39.4 mg GAE/g (DW) [36]. While total phenolic results of *Citrus* seeds change between 411.43 (lemon) and 814.84 mg GAE/100 g (bitter orange), total flavonoid results of *Citrus* seeds were monitored between 97.84 (grapefruit) and 126.48 mg/100 g (lemon), respectively [34]. Antioxidant activity of orange fruit seed was determined as 94.10 µmol Trolox/100 g [37]. Also, Özcan et al. [34] determined between 53.27 (mandarin) and 74.21% (lemon) antioxidant activity in *Citrus* seeds. Kumar and Sharmai [38] measured between 94.87 (*C.limetta*) and 97.82 µmol/g (*C.sinensis*) antioxidant activities in few Citrus seeds. The results we obtained regarding the bioactive components of orange seeds showed some changes when compared to the values of previous works. These differences could be probably attributed to harvest time, ripeness, growing conditions, and climatic factors.

3.2 PHENOLIC COMPOUNDS OF ORANGE SEEDS

The quantitative results of phenolic constituents of unroasted and roasted orange seeds are given in Table 2. The predominant phenolics of unroasted and roasted orange seeds were gallic acid and 3,4-dihydroxybenzoic acid (Fig.1). The phenolic constituent results of the seeds differed according to the roasting type compared to the control. Gallic acid and 3,4-dihydroxybenzoic acid contents of orange seeds were established between 5.33 (control) and 45.92 mg/100g (oven) to 10.01 (control) and 15.14 mg/100g (oven), respectively. Also, while catechin results of orange seeds vary between 17.96 (microwave) and 25.48 mg/100g (oven), syringic acid results of seed samples were monitored between 3.37 (control) and 4.37 mg/100g (oven). Rutin and quercetin results of

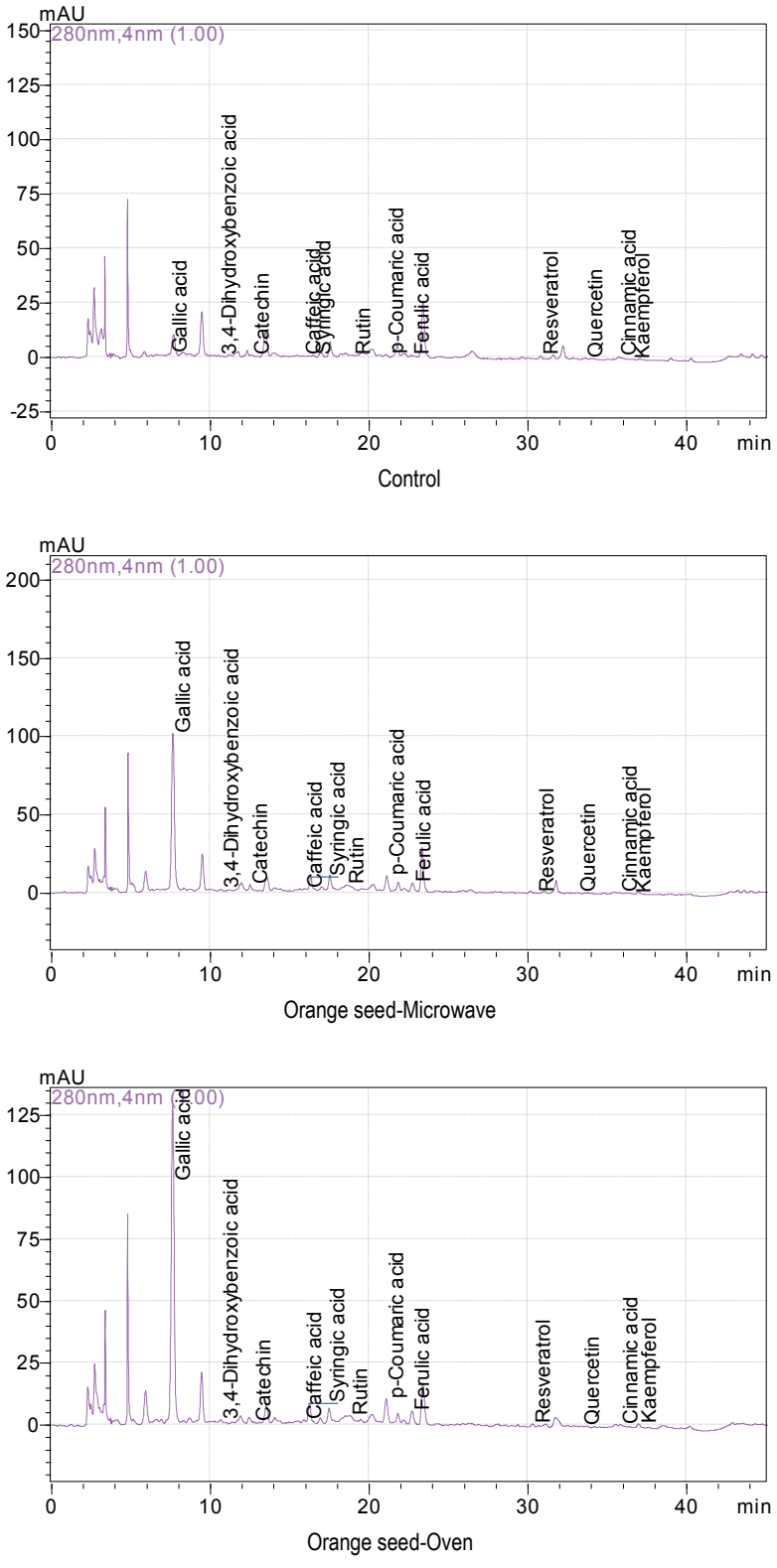

Figure 1 - Phenolic chromatograms of orange seeds

unroasted and roasted orange seeds were recorded between 9.83 (oven) and 10.15 mg/100g (control) to 3.06 (oven) and 6.23 mg/100g (microwave), respectively. Also, while caffeic acid results of seeds are monitored between 2.08 (control) and 4.18 mg/100g (microwave), ferulic acid results of the orange seeds were established between 2.13 (control) and 3.74 mg/100g (microwave). *p*-Coumaric acid values of the seeds changed between 1.60 (control) and 3.86 mg/100g (oven). The highest kaempferol and resveratrol (2.40 mg/100g and 1.32 mg/100g) were identified in orange seed roasted in microwave. In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). However, the routine and resveratrol contents of microwave roasted seeds were higher when compared to the results of control and oven roasted seeds. The amounts of most phenolic compounds roasted in the microwave were higher than those roasted in the oven. The higher phenolics of roasted orange seeds compared to the control may have been caused by Maillard reaction products during roasting, oil oxidation and caramelisation in the seed. Statistically significant differences were monitored between the results of phenolic compounds depending on the type of roasting ($p<0.05$). Silva and Jorge [37] reported that the predominant phenolic compounds of the citrus fruit seed oils were salicylic acid, quercetin and *p*-coumaric acid. It has been reported that genotype and environmental factors have a significant effect on the phenolic profile of citrus seeds, which are rich in *p*-coumaric, ferulic acid and caffeic acid [35,39]. When the results are compared with the previous studies, although the dominant phenolic component is the same, the amounts may differ. These changes may be due to variety, genetic structure, climatic factors, maturation, and harvest time.

3.3 FATTY ACID PROFILES OF THE OILS EXTRACTED FROM ORANGE SEEDS

The quantitative amounts of fatty acid profiles of the oils obtained from unroasted and roasted orange seeds are illustrated in Table 3. Stearic, palmitic, oleic and linoleic acids are the key fatty acids of orange seed oils (Fig. 2). Palmitic and stearic acid results of the orange seed oils were detected between 26.67% (oven) and 26.84% (unroasted) to 6.02% (unroasted) and 6.04% (oven), respectively. While oleic acid contents of the oils extracted from unroasted and roasted orange seeds are identified between 24.44% (microwave) and 24.81% (oven), linoleic acid results of orange seed oils were monitored between 39.00% (oven) and 39.23% (microwave). Also, linolenic acid results of the orange seed oils varied between 3.04% (control) and 3.17% (microwave). Arachidic acid contents were found below 0.41% in all oil samples. In general, the amount of fatty acids of the unroasted orange seed oils was slightly increased compared to the control. While the results of some fatty acids were different, some were found to be similar. The amount of fatty acids of orange seed oils differed according to the type of roasting, and these differences in fatty acids were found to be statistically significant ($p<0.05$). The seed oils of orange contained 38.26% linoleic, 24.89% oleic, 28.12% palmitic, 4.34% stearic, 2.58% linolenic and 0.55% arachidic acids [14]. Citrus seed oil contained 76.19% linoleic, 13.87% oleic, 6.76% stearic and 2.40% palmitic acids [40]. Park et al. [41] determined that palmitic, stearic, oleic, linoleic and linolenic acid contents of lemon (*Citrus limon*) seed oil changed between 11.68-16.86%, 1.95-3.41%, 11.10-18.65%, 15.51-27.03% and 1.50-5.54%, respectively. The orange seed oil contained 26.42% palmitic, 5.20% stearic, 23.04% oleic, 40.19% linoleic, 3.92% linolenic and 0.38% arachidic acid [32]. Orange seed oil contained 26.2% palmitic, 5.8% stearic, 26.5% oleic, 37.4% linoleic, 3.1% linolenic and 0.4% arachidic acids [37]. Citrus seed oils contained 33.2% to 36.3% linoleic, 24.8% to 29.3% oleic and 23.5% to 29.4% palmitic acids as the key fatty acid found [33]. Oleic and linoleic acid results of some *Citrus* seed oils varied between 21.84% and 27.58% to 33.94% and 38.67%, respectively [34]. Saidani et al. [21] verified that oils extracted from Tunisian citrus seeds are mostly constituted of triacylglycerols that are rich in unsaturated fatty acids. Valencia seed oil contained palmitic acid (47.33%), stearic acid (7.50%), oleic acid (18.40 %) and linoleic acid (l 8.39%) as the major fatty acids and less amount of myristic (1.55%), palmitoleic (5.04%) acids were the minor fatty acids

Table III - Fatty acid composition of the oils extracted from orange seed roasted in microwave and oven

Fatty acids (%)	Control			Microwave oven			Oven		
Palmitic	26.84	±	0.25a*	26.73	±	0.48b	26.67	±	0.08c
Stearic	6.02	±	0.02c	6.03	±	0.07b	6.04	±	0.01a
Oleic	24.67	±	0.09b	24.44	±	0.16c	24.81	±	0.05a
Linoleic	39.03	±	0.13b	39.23	±	0.22a	39.00	±	0.04b
Arachidic	0.40	±	0.00b	0.41	±	0.01a	0.40	±	0.00b
Linolenic	3.04	±	0.01c	3.17	±	0.01a	3.09	±	0.01b

* values within each row followed by different letters are significantly different at $P < 0.05$.

[42]. The fatty acid composition of seed oils can be affected by climatic factors such as location, precipitation and temperature, and harvest time [43]. The results showed some fluctuations depending on the processing conditions. These differences are likely due to agricultural factors, physiological factors, fruit maturity, temperature, other climatic factors and harvest time and agriculture applications.

3.4 ELEMENT CONTENTS OF ORANGE SEEDS ROASTED IN MICROWAVE AND OVEN

The effects of microwave and oven roasting on the element contents of protocol seeds, which are produced as industrial fruit juice production waste, are given in Table 4. Depending on the roasting type, differences were observed in the macro and micro element contents of the orange seeds when compared to the control. P and K amounts of orange seeds were recorded between 2939.01 (microwave) and 3012.91 mg/kg (oven) to 9477.58 (control) and 10265.58 mg/kg (microwave), respectively. In addition, while Ca contents of seed samples are found between 2.49 (Oven) and 2.75 mg/kg (control), Mg amounts of orange seeds were reported between 321.85 (Oven) and 357.68 mg/kg (control). The highest S amount was determined in control seed sample. Although the Fe contents of orange seeds were statistically different ($p<0.05$), the results were close to each other. But, there was no statistically significant difference between the Fe contents of the control and microwave

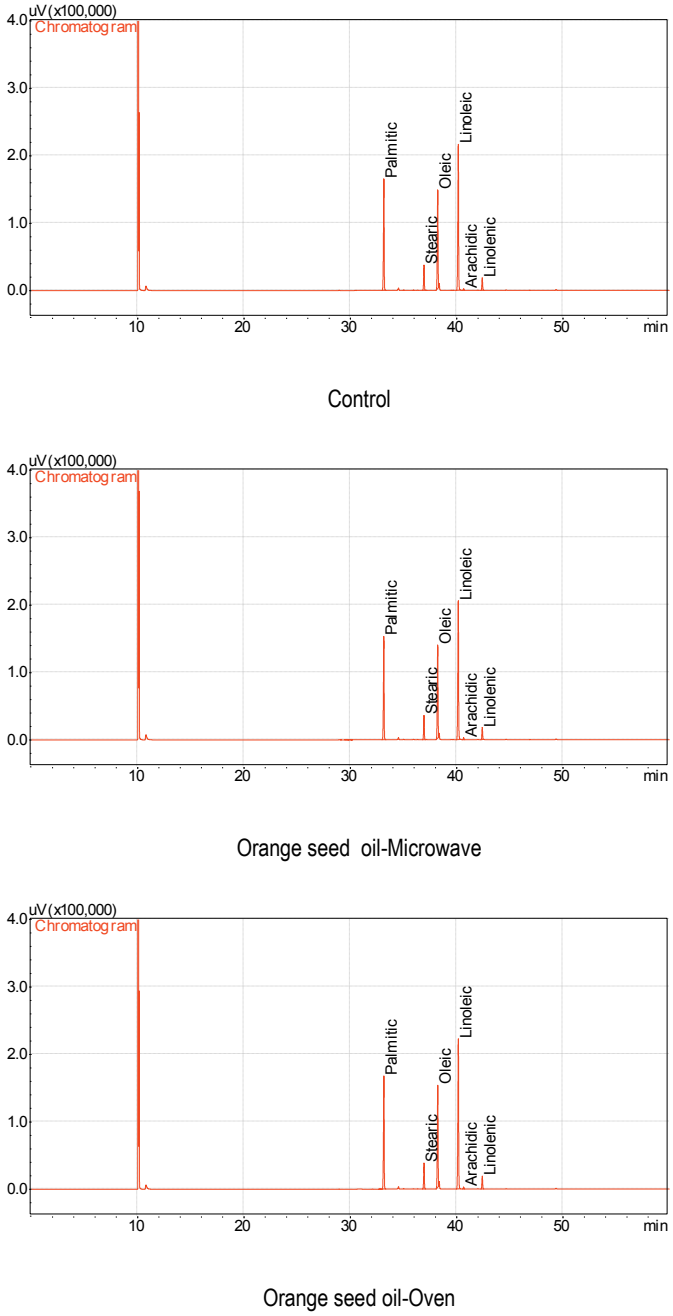

Figure 2 - Fatty acid chromatograms of the oils extracted from orange seeds

Table IV - Mineral contents of orange seeds roasted in microwave and oven (mg/kg)

Samples	P	K	Ca	Mg	S	Na	Fe	Cu	Mn	Ni	Zn	B
Control	2955.46 ±28.16b*	9477.58 ±189.12c	2.75 ±0.01a	357.68 ±0.44a	319.14 ±1.18a	51.22 ±0.20ab	7.75 ±0.30a	2.08 ±0.03b	1.46 ±0.02a	0.20 ±0.02	2.72 ±0.16b	6.54 ±0.09b
Microwave (900 W/7 min)	2939.01 ±36.21c	10265.58 ±839.77a	2.71 ±0.20ab	349.79 ±27.10b	311.75 ±30.34b	52.01 ±4.93c	7.74 ±0.70a	2.35 ±0.09a	1.38 ±0.09b	0.35 ±0.02	2.87 ±0.43a	6.77 ±0.69a
Oven (120°C/50 min)	3012.91 ±30.62a	9863.12 ±1354.47b	2.49 ±0.21c	321.85 ±28.91c	289.59 ±20.19c	52.91 ±7.11a	7.29 ±0.58b	1.96 ±0.09c	1.28 ±0.17c	0.32 ±0.03	2.47 ±0.18c	6.04 ±0.39c

* values within each row followed by different letters are significantly different at $P < 0.05$.

roasted orange seeds. Cu and Zn amounts of orange seeds were measured between 1.96 (Oven) and 1.46 mg/kg (control) to 2.47 (Oven) and 2.87 mg/kg (microwave), respectively. As seen in Table 4, K, Cu, Ni, Zn and B contents of seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control. The probable reason why the element contents of the orange seeds roasted in microwave and oven differ compared to the control may be due to the applied heat treatment parameters (temperature/time) and some analytical conditions. Orange seeds contained 40 mg/100g P, 45 mg/100g Ca, 66 mg/100g K and 18 mg/100g Na [13]. Orange seed cake contained 0.006 ppm Ca, 1.53 ppm Mg, 0.02 ppm Na, 7.33 ppm K, 0.02 ppm Fe and 0.01 ppm Cu [44] [45] reported that The seeds of "late Valencia" and "Blood orange" orange varieties contained 22.80 and 25.20 mg/100g K, 13.45 and 11.76 Na, 93.85 and 82.60 Ca, 33.86 and 27.74 P, 0.78 and 0.87 Fe. The Mn, Cu, Ni, Cd, Cr, Zn, Ca, Mg, Na, K contents of the orange seeds were 0.13 mg/100g, 0.27 mg/100g, 0.03 mg/100g, 0.04 mg/100g, 0.15 mg/100g, 0.63 mg/100g, 31.00 mg/100g, 1.02 mg/100g, 55.56mg/100g and 57.50mg/100g [46]. The sweet orange seed contained 132.53 ± 0.2 mg/100 g Ca, 375.23 ± 0.2 mg/100 g P, 85.63 ± 0.2 mg/100 g Mg, 2.53± 0.1mg/100 g Zn, 51.43 ± 0.2 mg/100 g Na and 12.46 ± 0.2 mg/100 g Fe [47]. Özcan and İnan [48] determined 451.60 mg/kg-2739.30 mg/kg Ca, 886.99-1708.00 mg/kg Mg, 2443-3939 mg/kg P, 23.43-35.87 mg/kg Zn in the seeds of several orange varieties. The element contents obtained from orange seeds differed from the results of previous studies. These differences may be due to the orange variety, harvest time, applied heat treatments and types.

3.5 PRINCIPAL CONSTITUENTS ANALYSİS (PCA) OF PHENOLIC COMPOUNDS OF ORANGE SEEDS

The Principal Component Analysis (PCA) was applied to assess the effect of heating on phenolic components of orange seeds, which are given in Fig 3. PC1 explained about 67.166% of variability. PC2 exhibited about 32.834% of variability. PC1 was identified with gallic acid (0.993), caffeic acid (0.972), syringic acid (0.999), *p*-coumaric acid (0.999), resveratrol (0.990) and kaempferol (0.875). Moreover, rutin (0.991) and quercetin (0.987) were the main variables on PC2.

4. CONCLUSION

The results regarding the bioactive compounds, antioxidant activity values, phenolics and fatty acids profiles of orange seed and oils showed some changes depending on the type of roasting. In addition, bioactive compounds, antioxidant activity values of orange seeds increased with the roasting process compared

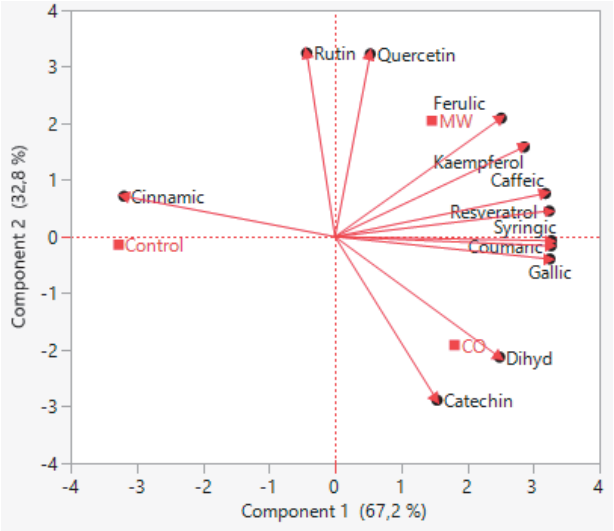

Figure 3 - Biplot graph drawn with results of PCA

to the control. The highest total phenol and antioxidant activities were established in microwave roasted orange seeds. The relationship between the antioxidant capacity of the seeds and their bioactive components was linear. In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). In general, an increase was observed in the amount of phenolic constituents in microwave and oven-roasted orange seeds compared to the control (except for catechin, rutin and quercetin). However, the routine and resveratrol contents of microwave roasted seeds were higher when compared to the results of the control and oven roasted seeds. The amounts of most phenolic compounds roasted in the microwave were higher than those roasted in the oven. The higher phenolics of roasted orange seeds compared to the control may have been caused by Maillard reaction products during roasting, oil oxidation and caramelisation in the seed. Statistically significant differences were monitored between the results of phenolic compounds depending on the type of roasting ($p<0.05$). The amount of fatty acids of orange seed oils differed according to the type of roasting, and these differences in fatty acids were found to be statistically significant ($p<0.05$). In general, the amount of fatty acids of the unroasted orange seed oils was slightly increased compared to the control. While the amounts of some fatty acids were different, some were found to be similar. The result of fatty acids of orange seed oils fluctuated depending on the type of roasting and statistically significant differences were determined between the results of fatty acids. K, Cu, Ni, Zn and B contents of seeds roasted in microwave were higher than those roasted in control and oven. In addition, P, K, Na and Ni contents of oven-roasted orange seeds were found to be higher when compared to the control.

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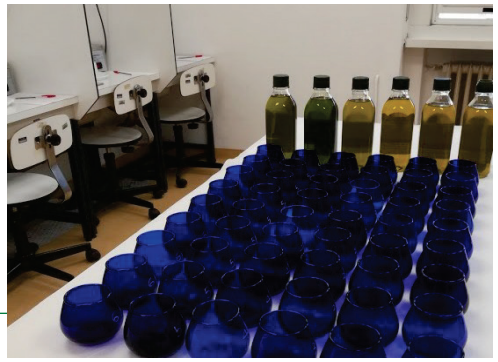
Reg. UE 2022/2104 and 2022/2105 establish the chemical-physical parameters and methods for quality control of olive oil.
The organoleptic assessment (Panel test) contributes to the definition of the quality of the oil, the Regulation classifies virgin olive oil in the categories:

- EXTRA VIRGIN OLIVE OIL
- VIRGIN OLIVE OIL
- LAMPANTE OLIVE OIL

according to the intensity of the defects and of the fruitness perceived, as determined by a group of tasters selected, trained and monitored as a panel, using statistical techniques for data processing.
It also provides information on the organoleptic characteristics for optional labeling.
The organoleptic assessment is qualified by a level of reliability comparable to that of the analytical tests.
Our Panel is recognized by the IOC (International Olive Council), by the Italian Ministry of Agricultural, Food and Forestry Policies as a tasting committee in charge of the official control of the characteristics of virgin olive oils and designation of origin (D.O.) oils.
The organoleptic assessment is accredited by ACCREDIA (Italian Accreditation Body).
The Panel serves industry, production consortia, certification bodies and large-scale distribution.



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Expert Sensorial Analysis and Head of Panel Test
Team Chemistry, Technology and Food Safety

Short note

Chemical Composition and Cytotoxicity of the Essential Oil of *Rothmannia schoemannii* Tirveng

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The chemical composition of the essential oil of *Rothmannia schoemannii* Tirveng. (Rubiaceae) was investigated for the first time. The essential oil was obtained by hydrodistillation and fully characterised by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). In total, 28 components were identified in the essential oil, which made up 99.6% of the total oil. The essential oil is composed mainly of β-eudesmol (30.8%), α-cadinol (18.2%), caryophyllene oxide (6.5%), δ-cadinene (5.5%), and γ-eudesmol (5.4%). The essential oil exhibited cytotoxic activity against J5 (human hepatocellular carcinoma) and A549 (human lung adenocarcinoma) cells with IC_{50} values of 112.5 and 98.5 ug/mL, respectively.

Keywords: Essential oil, *Rothmannia schoemannii,* Rubiaceae, hydrodistillation, cytotoxicity.

1. INTRODUCTION

Rothmannia Thunb. is a genus of trees and shrubs belonging to the tribe Gardenieae, subfamily Ixoroideae of the family Rubiaceae. There are about 40 species of *Rothmannia*, occurring in tropical and subtropical Africa, Myanmar, the Seychelles, Indochina, the Andaman Islands, South China, the Malay Peninsula, Java, Sumatra, Borneo and Papua New Guinea [1]. Previous phytochemical investigations reported the abundance of iridoids, triterpenoids, and flavonoids [2-4]. *Rothmannia schoemannii* Tirveng. is locally known as *bengkil* in Borneo. It is native to Borneo, Peninsular Malaysia, Thailand, Sumatra, and Java. It can be found in undisturbed mixed dipterocarp and keranga forests up to 700 m altitude. The leaf has been used for kidney pain and diarrhoea with blood, drinking the leaf juice is said to help during childbirth [5]. Essential oils as secondary metabolites involve complex mixtures of natural compounds with versatile organic structures representing useful medicinal properties [6]. Essential oils are important natural sources and are used as raw materials to produce fragrance compounds in cosmetics, as flavouring additives for food and beverages, as scenting agents in a variety of household products, and as intermediates in the synthesis of other perfume chemicals [7]. Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activities, most notably antibacterial, antifungal, and antioxidant properties [8]. Regarding the essential oil composition of the genus *Rothmannia*, the literature search did not reveal any report on the essential oil composition of the genus except for *R. macrophylla*, which has been reported by us [9]. Hence, the aim of the study was to evaluate the chemical composition and cytotoxicity of the essential oils of *R. schoemannii*.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Sample of *R. schoemannii* was collected from Fraser Hill, Pahang (Latitude:

3°42'42.72"N Longitude: 101°44'11.6"E) in January 2023. The voucher specimens (SA-12-63) have been identified by Shamsul Khamis and deposited at UKMB Herbarium.

2.2. ISOLATION OF ESSENTIAL OILS

The fresh leaves of *R. schoemannii* (300 g) were weighed and then subjected to hydrodistillation using a Clevenger-type apparatus. A hydrodistillation run time of 4 hours was used to obtain the optimum yield without drastically affecting the oil components. The obtained oil was then dried using anhydrous magnesium sulphate, weighed, and stored in dry amber vials at 4°C until analysis. The average yield of oil was calculated as the percentage weight by weight (% w/w) of the plant material.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas Chromatography with Flame Ionisation Detection (GC-FID) analysis was performed on an Agilent Technologies 7890B and an Agilent 7890B FID equipped with HP-5 column (30 m long, 0.25 µm film thickness and 0.25 mm inner diameter). At a flow rate of 0.7 mL/min, helium was used as a carrier gas. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was maintained at 50°C, then slowly increased to 280°C at 5°C/min and lastly detained isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percents were reported as means ±SD of triplicate [10]. Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped with HP-5MS column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min [11]. For GC-MS detection, an electron ionisation system with an ionisation energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from m/z 50-400 amu. To determine the chemical components of essential oil, standards (major components) need to be injected, together with the correspondence of retention indices. The data of mass spectra were compared with those occurring in Wiley, NIST08, and FFNSC2 libraries [12]. Each peak was considered the same response factor for all components for semi-quantification of essential oil components. Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

2.4. CYTOTOXICITY

Human hepatocellular carcinoma J5 and human lung adenocarcinoma A549 were obtained from ATCC (Rockville, MD, USA) and propagated in RPMI-1640 medium supplemented with 10% heat-inactivated FCS and 2 mM L-glutamine (Life Technologies, Inc., MD), and cultured in a 37°C, 5% CO_2 incubator. The cytotoxicity of the essential oil was assessed using the alamarBlue® proliferation assay according to a protocol from AbD Serotec. Cells (3000 cells/well) were incubated with either essential oils (dissolved in DMSO, final 0.1% DMSO in medium) or vehicle control (0.1% DMSO) for 24 h and 48 h, followed by replacing with fresh medium containing 10% alamarBlue® reagent for an additional 6 h. The absorbances at 570 nm and 600 nm were measured by a microplate reader. All values are given as means ± SD of 3 independent experiments [13].

2.5. STATISTICAL ANALYSIS

Data obtained from essential oil analysis and cytotoxicity were expressed as mean values. The statistical analyses were carried out by employing one-way ANOVA ($p < 0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

3. RESULTS AND DISCUSSION

The essential oil was obtained at a 0.2% (w/w) yield. The identified components in the essential oil are listed in Table 1, according to their Kovats indices (KI) on an HP-5 column. The essential oil of *R. schoemannii* revealed the presence of 28 components with a percentage of 99.6%. The essential oil was characterised by a high concentration of oxygenated sesquiterpenes (69.9%) followed by sesquiterpene hydrocarbons (26.8%). The essential oil was demonstrated by its richness in β-eudesmol (30.8%), α-cadinol (18.2%), caryophyllene oxide (6.5%), δ-cadinene (5.5%), and γ-eudesmol (5.4%). Meanwhile, other minor components detected in the oil exceeding 2%, were β-caryophyllene (3.2%), α-gurjunene (2.9%), spathulenol (2.8%), α-copaene (2.5%), germacrene D (2.5%), elemol (2.5%), α-amorphene (2.4%), β-cubebene (2.2%), and globulol (2.1%). In comparison to the previous study, β-eudesmol has also been reported as the major component in *R. macrophylla* which constituted of 20.9% [9]. Meanwhile, the chemical differences in the essential oil composition of plant species concerning their geographical origins and harvesting season have been reported, showing that the chemical and biological diversity of aromatic and medicinal plants depend on factors such as cultivation area, climatic conditions, vegetation phase, and genetic modifications. In fact, these factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic components [14, 15].

For the evaluation of the cytotoxicity of *R. schoemannii* essential oil, we tested its effect on the viability of two human cancer cell lines: J5 (human hepatocellular

Table I - Chemical components identified in from *R. schoemannii* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identifications[c]
1	Camphene	946	944	0.5	RI, MS
2	*α*-Terpinene	1014	1016	0.4	RI, MS
3	Limonene	1033	1032	0.2	RI, MS
4	Borneol	1165	1165	1.0	RI, MS
5	Terpinen-4-ol	1175	1172	0.8	RI, MS
6	*α*-Cubebene	1345	1345	0.5	RI, MS
7	Cyclosativene	1370	1369	0.2	RI, MS
8	*α*-Ylangene	1372	1373	0.4	RI, MS
9	*α*-Copaene	1375	1374	2.5	RI, MS
10	*β*-Cubebene	1388	1387	2.2	RI, MS
11	Longifolene	1407	1407	1.0	RI, MS
12	*α*-Gurjunene	1409	1410	2.9	RI, MS
13	*β*-Caryophyllene	1415	1417	3.2	RI, MS
14	*α*-Humulene	1435	1436	1.8	RI, MS
15	Aromadendrene	1439	1439	1.5	RI, MS
16	*α*-Amorphene	1480	1483	2.4	RI, MS
17	Germacrene D	1485	1484	2.5	RI, MS
18	**δ-Cadinene**	1520	1522	**5.5**	RI, MS, Std
19	*α*-Calacorene	1544	1545	0.2	RI, MS
20	Elemol	1548	1548	2.5	RI, MS
21	Palustrol	1565	1567	0.4	RI, MS
22	Spathulenol	1575	1577	2.8	RI, MS
23	**Caryophyllene oxide**	1582	1582	**6.5**	RI, MS, Std
24	Globulol	1590	1590	2.1	RI, MS
25	Ledol	1600	1602	1.2	RI, MS
26	**γ-Eudesmol**	1630	1630	**5.4**	RI, MS, Std
27	**β-Eudesmol**	1648	1649	**30.8**	RI, MS, Std
28	**α-Cadinol**	1650	1652	**18.2**	RI, MS, Std
	Monoterpene hydrocarbons			1.1	
	Oxygenated monoterpenes			1.8	
	Sesquiterpene hydrocarbons			26.8	
	Oxygenated sesquiterpenes			69.9	
	Total identified (%)			**99.6**	

RI: based on comparison of calculated RI with that reported in Adams; MS: based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases; Std: based on comparison with authentic/standard compounds

[a]Linear retention index experimentally determined using a homologous series of C_6-C_{30} alkanes.

[b]Linear retention index taken from Adams, Wiley, NIST08 and literature.

[c]Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

carcinoma) and A549 (human lung adenocarcinoma) cells. Cells were incubated with various concentrations of essential oils for 48 h, and then the cell viabilities were measured by the alamarBlue® proliferation assay. The results showed that treatment for 48 h reduced the viability of J5 cells and A549 cells, with IC_{50} values around 112.5 and 98.5 µg/mL, respectively. This represents the first report of the cytotoxic activities of *R. schoemannii* essential oil against liver and lung cancer cells.

β-Eudesmol, a sesquiterpenoid alcohol, has been reported to exhibit diverse biological and therapeutic activities. It shows potent antiproliferative activity against cholangiocarcinoma (CCA), liver cancer, leukaemia, and melanoma cells [16]. Previous research on CCA cells in vitro and in animal models has shown promising anti-CCA activity of β-eudesmol. It induced apoptosis in CCA cell lines through the activation of caspase-3 and 7 [17]. The expression of the detoxifying enzymes heme oxygenase (HO)-1 and NAD(P)H quinone dehydrogenase (NOQ)-1 in CCA cells is also suppressed by β-eudesmol [18]. The antiproliferative activity of β-eudesmol against CCA cells is attributed to its inhibitory activity on STAT1/3 phosphorylation and NF-κB expression [19]. In a xenografted nude mouse model of CCA, a high dose of β-eudesmol (100 mg/kg body weight for 30 days) prevented tumour volume and lung metastasis [20]. Based on these previous reports on β-eudesmol, the cytotoxicity in this study might be explained by the presence of β-eudesmol. This study shows that the high content of this component obtained in the essential oil may contribute, at least in part, to the activity ascribed to the plant.

4. CONCLUSIONS

In this study, the GC-FID and GC-MS analysis of the essential oil allowed us to identify Oxygenated sesquiterpenes as the major group components with

the presence of β-eudesmol, α-cadinol, caryophyllene oxide, δ-cadinene, and γ-eudesmol. as the most abundant components. In addition, according to cytotoxicity, the essential oil revealed significant activity with IC_{50} value 98.5-112.5 µg/mL. The species might be a source of natural products for further investigation into the development of new antiproliferative agents, which could be used as natural additives in the food, cosmetic, and pharmaceutical industries. Thus, further phytochemical and biological studies should be carried out to identify their active constituents and toxicities.

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Short note

Effect of gamma irradiation and cooking on the physico-chemical properties, nutrients, and anti-nutrients compositions of egusi melon (Citrullus vulgaris) seeds

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The effect of gamma irradiation and cooking on the physico-chemical and functional properties of "egusi" melon as well as its impact on quality attributes of the oil were investigated. The seeds of "egusi" melon were divided into five portions. One part was cooked, one irradiated at 5KGy, another was irradiated at 10KGy, and one part was cooked and irradiated at 10KGy as a double treatment method. The last part was left as is (raw) for reference. Irradiation was done using Cobalt-60 as gamma radiation source. Proximate compositions, mineral contents, amino acid contents, oil quality attributes, as well as anti-nutritional factors were determined for all samples using standard methods. Results revealed that gamma irradiation caused a decrease in values of moisture from the raw sample (5.80) with cooked, 10KGy and 5KGy irradiated samples of "egusi" melon showing reduced moisture contents of 4.27, 4.27 and 4.14% respectively. Protein decreased from 32.20% in raw "egusi" melon seeds to 29.77% and 29.47% in the 10KGy and 5KGy irradiated samples respectively. Mineral content of "egusi" melon seeds were affected by irradiation at 10 and 5kGy where the values of potassium increased from 14.93 in the raw to 20.88 in the 10KGy irradiated and 22.83 in the 5KGy irradiated samples. For the quality attributes of "egusi" melon oil, gamma irradiation caused significant decrease ($P \leq 0.05$) in iodine value and significant increase in acid and peroxide values. The treatments had profound effects on the Amino Acid Contents of "egusi" melon. Cooking increased the level of amino acids except for glutamic acid which decreased from 14.26 to 13.63 when cooked. Irradiation also had this effect, increasing in all but glutamic acid (14.26 to 13.83) but the combined treatment (cooking and Irradiation) showed an increase in glutamic acid content. Lysine, Histidine, Valine, and Leucine decreased when subjected to the combined treatment. The study showed that irradiation only caused little changes in the nutrient composition of "egusi" melon at low dosage highlighting its potential as a preservation and disinfection treatment for "egusi" melon.

Keywords: Gamma irradiation, domestic cooking, "egusi" melon seed, oil extract.

INTRODUCTION

Irradiation is a general term for the deliberate exposure of materials to radiation energy. Irradiation is often called 'cold pasteurisation' because of the significant reduction of several pathogenic microorganisms, negligible loss of nutrients and minimal changes in sensory attributes [1]. Food irradiation is done mostly by using Cobalt-60 Gamma rays because of its deep penetration which enables the administration of treatment to entire industrial pallets or totes, reducing the need for material handling making it the preferred method by most processors. Food irradiation is a beneficial technology providing food with good shelf life and safety but could, on the other hand, cause both physiological [2] and biological changes that may disrupt the nutritional value and organoleptic properties of the irradiated foods [3]. Processing of food by Ionizing radiation, depending on the dose, kills some or all the harmful bacteria and other pathogens present. This prolongs the shelf-life of the food

especially in cases where microbial spoilage is the greatest concern. Irradiation has also shown to delay the sprouting of vegetables or the ripening of fruits.

MATERIALS AND METHODS

Melon ("egusi") seeds (*Citrullus vulgaris*) used for the study were purchased from a local market called Oja-Oba in Akure, Ondo state, Nigeria. Seeds were screened to remove bad ones, dehulled mechanically and further screened. The seeds were then divided into five parts (the raw; cooked; cooked and irradiated at 10kGy; irradiated at 5 and irradiated at 10KGy respectively). The gamma irradiation was conducted at the Shedan Science and Technology Complex (SHESTCO), Abuja, using cobalt-60 irradiation facilities at 5kGy and 10kGy respectively. They were all dried to constant weight in an oven at 60°C, ground using mechanical blender, put in an air-tight container, and stored in a dry cool environment for further analysis. All chemicals and reagents used in the study were of an analytical grade.

PROXIMATE COMPOSITION DETERMINATION

Proximate composition of samples was determined using AOAC [4] methods of analysis. Carbohydrate was determined by a different method.

MINERAL CONTENT DETERMINATION

Minerals of each sample (Ca, Mg, Zn, Fe) were determined by Flame Atomic Absorption Spectrophotometer as described by AOAC [4]. Potassium and Sodium contents were determined using flame photometer. A weighed 0.5 g of each sample was digested in 6.5 ml of acid solution (HNO_3, H_2SO_4, $HClO_4$ in ratio of 5:1:0.5). The resulting solution was heated until white fumes appeared. The clear solution was diluted up to 50 ml with distilled water and filtered with Watman filter paper no. 41. The standard calibration curves were obtained for the elements of interest using prepared standard working solutions. The concentration of a particular element in a sample was determined by absorption using the calibration curves. Cathode lamps were used as a radiation source. This experiment made use of Air acetylene gas. Other elements in the sample will generally not absorb the chosen wavelength and thus will not interfere with the measurement which makes this method highly selective and sensitive.

Physicochemical properties like Acid Value, Saponification Value, Iodine Value, Peroxide Value and Free Fatty Acids were determined using the A.O.C.S. [5] methods of analysis.

DETERMINATION OF WATER AND OIL ABSORPTION CAPACITIES

The method of Prinyawiwatkul *et al.* [6] with modifications was used in the determination of water and oil absorption capacities. Each flour sample (1.0 g) was thoroughly mixed with 10 ml of deionised water (Density = 1.00 g/cm^3) or oil (Density = 0.9095 g/cm^3) in 10-ml centrifuge tubes. Suspensions were stirred intermittently over a 30 min period at room temperature (25°C) and then centrifuged at 12,000 g for 30 min at 25°C. The supernatant was decanted into a 10 cm graduated measuring cylinder. The volume of supernatant of water and oil absorption capacities were then calculated. The bonded oil and water were determined by difference and converted to grams. The percentage of oil and water absorption capacity were expressed as g/g % of the flour sample. Each flour sample was analysed in triplicates.

ANTI-NUTRITIONAL FACTOR DETERMINATION

The antinutritional factors of the oils were determined by the method of Inuwa *et al.* [7].

Oxalate determination: the oxalate content of the samples was determined using titration method. 2 g of each sample was placed in a 250 ml volumetric flask suspended in 190 ml distilled water. 10 ml of 6 M HCl solution was added to each of the samples and the suspension was digested at 100ºC for 1h. The samples were then cooled and made up to the 250 ml mark of the flask. The samples were filtered and duplicate portions of 125 ml of the filtrate were measured into beakers and four drops of methyl red indicator were added, followed by the addition of concentrated NH_4OH solution (dropwise) until the solution changed from pink to yellow colour. Each portion was then heated to 90ºC, cooled and filtered to remove the precipitate containing ferrous ion. Each of the filtrates was again heated to 90ºC and 10 ml of 5% $CaCl_2$ solution was added to each of the samples while stirring consistently. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatants were decanted, and the precipitates completely dissolved in 10 ml 20% H_2SO_4. The total filtrate resulting from the digestion of 2 g of each of the samples were made up to 200 ml. Aliquots of 125 ml of the filtrate were heated until near boiling and then titrated against 0.05 M standardised $KMnO_4$ solution to a pink colour which persisted for 30 sec. The oxalate contents of each sample were calculated.

Phytate determination: the phytate contents of each of the samples was determined through phytic acid determination. This entails weighing of 2 g of each sample into 250 ml conical flasks. 100 ml of 2% conc. HCl was used to soak the samples in the conical flask for 3 h and then filtered through a double layer filter paper. 50 ml of each of the filtrates were placed in a 250 ml beaker and 107 ml of distilled water added to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added to each sample solution as an indicator and titrated with a standard iron chloride solution which contained 0.00195 g iron/ml and the end point was indicated by a brownish-yellow colouration that persisted for 5 min. The percentage

Table I - Effect of gamma irradiation on the proximate composition of melon (Egusi) (%)

Test	Raw	Cooked	C+I	10KGy	5KGy
M.C	3.80 ± 0.01[a]	4.27 ± 0.01[c]	4.46 ± 0.06[b]	4.27 ± 0.01[c]	4.14 ± 0.02[d]
ASH	4.59 ± 0.08[c]	3.76 ± 0.03[d]	3.41 ± 0.02[e]	6.15 ± 0.04[a]	5.47 ± 0.01[b]
FAT	48.92 ± 0.01[a]	47.00 ± 0.12[b]	45.44 ± 0.03[c]	44.71 ± 0.02[c]	49.31 ± 1.15[a]
C.F	2.31 ± 0.03[d]	4.34 ± 0.29d[a]	4.02 ± 0.01[b]	3.00 ± 0.01[c]	3.07 ± 0.01[c]
PROTEIN	32.20 ± 0.02[b]	31.88 ± 0.03[c]	35.32 ± 0.02[a]	29.77 ± 0.05[d]	29.47 ± 0.02[e]
CHO	9.99 ± 0.06[e]	13.29 ± 0.02[c]	11.78 ± 0.01[d]	16.36 ± 0.02[a]	13.99 ± 0.01[b]

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different ($P \leq 0.05$)
Keys: M.C = moisture content, C+I = Cooked and irradiated at 10kGy, C.F = Crude fibre

phytic acid was calculated.
Tannins determination: one gram of each sample was dissolved in 10 ml distilled water, agitated, and left to stand for 30 min at room temperature. Each sample was centrifuged, and the supernatant recovered. 2.5 ml of the supernatants were transferred into 50 ml volumetric flasks. Similarly, 2.5 ml of standard tannic acid solution was measured into a separate 50 ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to 50 ml in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tannin was calculated by the difference in absorbance against calibration graph.

DETERMINATION OF AMINO ACID PROFILE

The amino acid profiles were determined using the methods described by Spackman *et al.* [8]. The samples were dried to constant weight, defatted, hydrolysed and evaporated in a rotary evaporator. They were then loaded into the Technicon Sequential Multi-Sample Amino Acid Analyser (TSM).

DEFATTING SAMPLE

A known weight of the sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 volume/volume mixture) using Soxhlet extraction apparatus as described by AOAC [4]. The extraction lasted for 15 hours.

NITROGEN DETERMINATION

A small amount (200 mg) of ground samples was weighed, wrapped in Whatman filter paper (No.1) and put in a Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium oxide (SeO_2) in ratio of 10:5:1, was added into the flask to facilitate digestion. Anti-bumping granules (4 pieces) were added.
The flask was then put on Kjeldhal digestion apparatus for 3 hours until the liquid turned light green, the digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. A 10 ml aliquot of the diluted solution with 10 ml of 45% sodium hydroxide added was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green or methyl red indicator until about 70 ml of distillate was collected.
The distillate was then titrated against standardised 0.01 M hydrochloric acid until a grey coloured end point. The percentage nitrogen in the original sample was calculated using the formula:

$$\text{Percentage Nitrogen} = \frac{(a-b)x0.01x14xVx100}{WC}$$

Where:
a = Titre value of the digested sample
b = Titre value of the blank sample
V = Volume after dilution (100ml)
W = The dried sample weight (mg)
C = 10 ml aliquot of the sample
14 mg = Nitrogen constant in mg
100 = Conversion factor to percentage

RESULTS AND DISCUSSION

The effects of gamma radiation on the proximate composition of "egusi" melon seeds are presented in Table I. A decrease in moisture content is observed for all treatments. This reduction is however negligible. This agrees with the observations made by Rady *et al*. [9] who reported that gamma radiation has little effect on moisture content of oil seeds. Minimal changes were also observed in the fat contents of the samples. This is also in line with the findings of Siddhuraju *et al.* [10] and Seda *et al*. [11] on different plant materials. There were great increases in crude fibre contents of the treated seed in contrast to the report of Bhat *et al.* [12] who reported a decrease in crude fibre and ash contents of velvet bean seeds when irradiated.
Table II shows the effects of gamma radiation on the functional properties of "egusi" melon seeds. Oil absorption capacity was slightly reduced when irradiated at 5KGy but increased as the dosage was increased to 10KGy. Abu *et al.* [13] reported that the oil absorption capacity of Cowpea seeds was not affected at low doses (2KGy). Water absorption capacity improved slightly at the 5KGy irradiation level

Table II - Effect of gamma irradiation on functional properties of melon (Egusi) seeds

Tests	Raw	Cooked	C+I	10KGy	5KGy
E.C(%)	34.00 ± 0.12^{d}	38.00 ± 0.13^{b}	36.00 ± 0.12^{c}	40.34 ± 0.68^{a}	36.00 ± 0.12^{c}
L.G.C(%)	6.34 ± 0.48^{a}	6.34 ± 0.48^{a}	6.34 ± 0.48^{a}	6.34 ± 0.48^{a}	6.34 ± 0.48^{a}
F.C(%)	10.03 ± 0.23^{b}	2.20 ± 0.36^{d}	8.54 ± 0.63^{c}	12.82 ± 0.34^{a}	8.54 ± 0.25^{c}
W.A.C(ml/100g)	120.00 ± 0.12^{d}	140.00 ± 0.12^{c}	200.00 ± 0.12^{b}	300.00 ± 0.12^{a}	140.00 ± 0.12^{c}
O.A.C(ml/100g)	320.01 ± 0.12^{a}	205.00 ± 0.12^{c}	115.00 ± 0.12^{d}	310.00 ± 0.12^{b}	104.96 ± 0.06^{e}

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different (*P≤ 0.05*)
Keys: E.C = Emulsion capacity; L.G.C = Least gelation capacity; F.C = Foaming capacity; C+I = Cooked and irradiated 10kGy; W.A.C = Water absorption capacity; O.A.C = Oil absorption capacity

Table III - Effect of gamma irradiation on minerals of Egusi melon (mg/100g)

Tests	Raw	Cooked	C+I	10KGY	5KGY
K	14.93 ± 0.01^{e}	25.38 ± 0.01^{a}	22.65 ± 0.01^{c}	20.88 ± 0.01^{d}	22.83 ± 0.02^{b}
Na	1.71 ± 0.02^{e}	2.50 ± 0.02^{b}	2.27 ± 0.06^{c}	2.01 ± 0.04^{d}	2.61 ± 0.01^{a}
Ca	5.58 ± 0.02^{e}	12.84 ± 0.04^{a}	11.57 ± 0.04^{b}	6.81 ± 0.01^{d}	8.50 ± 0.01^{c}
Mg	3.30 ± 0.03^{e}	5.04 ± 0.01^{a}	4.47 ± 0.01^{c}	4.08 ± 0.02^{d}	4.98 ± 0.03^{b}
Zn	4.70 ± 0.01^{a}	3.36 ± 0.05^{c}	3.07 ± 0.03^{d}	3.48 ± 0.02^{b}	3.03 ± 0.02^{e}
Fe	1.58 ± 0.04^{d}	1.32 ± 0.13^{e}	1.70 ± 0.15^{c}	2.67 ± 0.03^{a}	2.26 ± 0.01^{b}
P	2.71 ± 0.01^{e}	3.57 ± 0.15^{b}	3.23 ± 0.02^{d}	3.32 ± 0.01^{c}	4.18 ± 0.02^{a}

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different (*P≤ 0.05*)
Key: C+I = Cooked and irradiated at 10kGy

and was greatly increased at 10KGy irradiation levels. This may be due to an exposure to non-polar protein sites as reported by Zayas [14]. Gamma irradiation increased the emulsion capacity of "egusi" melon. The variations in emulsion properties may be attributed to protein aggregation as well as surface hydrophobicity which affect emulsifying properties in different ways [15]. No significant changes were observed in the least gelation capacities of both cooked and irradiated sample of "egusi" melon.

In Table III, there was an increase in the mineral contents of the treated "egusi" melon samples after treatment with the cooked sample having the highest values except in the case of Iron where the cooked sample had the least content. Generally, "egusi" melon seeds treated with the lower irradiation dose of 5KGy had higher mineral contents compared to those treated with the higher dose (10KGy). This is in accordance with the findings of Hassan *et al.* [16] who reported an increase in the mineral contents of maize and sorghum after irradiation.

Table IV shows the amino acids contents of raw and processed melon seeds while Table V shows the percentage compositions of different types of amino ac-

Table IV: Effect of gamma irradiation on the Amino acids of melon (Egusi). (mg/g protein)

Tests	Raw	Cooked	C+I	10KGY
LYSINE	3.71 ± 0.13^{b}	4.20 ± 0.21^{a}	2.91 ± 0.18^{c}	3.98 ± 0.18^{ab}
HISTIDINE	2.08 ± 0.22^{b}	2.80 ± 0.23^{a}	1.75 ± 0.05^{b}	2.52 ± 0.13^{a}
ARGININE	7.16 ± 0.23^{c}	8.35 ± 0.16^{b}	8.86 ± 0.06^{a}	8.01 ± 0.08^{b}
ASPARTIC ACID	6.43 ± 0.21^{b}	9.73 ± 0.10^{a}	9.44 ± 0.21^{a}	9.67 ± 0.16^{a}
THREONINE	2.76 ± 0.15^{b}	3.12 ± 0.20^{b}	3.67 ± 0.12^{a}	2.90 ± 0.21^{b}
SERINE	2.88 ± 0.24^{c}	3.57 ± 0.18^{b}	4.76 ± 0.17^{a}	3.19 ± 0.31^{c}
GLUTAMIC ACID	14.26 ± 0.23^{b}	13.63 ± 0.14^{c}	16.88 ± 0.21^{a}	13.83 ± 0.18^{c}
PROLINE	2.98 ± 0.17^{c}	3.65 ± 0.18^{b}	4.46 ± 0.17^{a}	3.20 ± 0.20^{c}
GLYCINE	3.71 ± 0.20^{b}	4.99 ± 0.22^{a}	4.09 ± 0.26^{b}	4.60 ± 0.13^{a}
ALANINE	3.64 ± 0.22^{c}	4.10 ± 0.21^{b}	4.68 ± 0.18^{a}	3.91 ± 0.17^{bc}
CYSTINE	1.07 ± 0.16^{a}	1.40 ± 0.19^{a}	1.53 ± 0.22^{a}	1.33 ± 0.24^{a}
VALINE	4.48 ± 0.19^{b}	5.01 ± 0.16^{a}	3.21 ± 0.23^{c}	5.01 ± 0.23^{a}
METHIONINE	0..95 ± 0.25^{a}	1.26 ± 0.23^{a}	1.31 ± 0.24^{a}	1.10 ± 0.18^{a}
ISOLEUCINE	3.65 ± 0.18^{b}	3.99 ± 0.09^{ab}	4.34 ± 0.18^{a}	3.96 ± 0.14^{ab}
LEUCINE	5.73 ± 0.13^{b}	6.22 ± 0.11^{a}	5.01 ± 0.09^{c}	6.02 ± 0.22^{ab}
TYROSINE	2.43 ± 0.21^{b}	2.74 ± 0.06^{b}	4.36 ± 0.14^{a}	2.75 ± 0.23^{b}
PHENYLALANINE	3.90 ± 0.22^{b}	4.11 ± 0.10^{b}	5.25 ± 0.32^{a}	3.98 ± 0.19^{b}

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different (*P ≤ 0.05*)
Key: C+I = Cooked and irradiated at 10kGy

Table V - Summary of amino acids composition of the total Egusi melon fractions

Amino acid	Raw	Cooked	C+I	10KGy
Total Amino Acid (TAA)	71.82	82.87	86.51	79.96
Total Essential Amino Acid (TEAA)	37.92	43.2	42.2	41.56
TEAA/TAA (%)	52.79	52.12	48.78	51.97
Total Non-Essential Amino Acid (TNEAA)	33.9	39.67	44.31	38.4
Total Sulphur Amino Acid (TSAA)	4.78	2.66	2.84	2.43
% Cystine (TSAA)	22.38	47.36	46.12	45.26
Total Aromatic Essential Amino Acid phe.+tyr. (ArEAA)	6.3	6.85	9.61	6.73
Total Acidic Amino Acid (TAAA) % Glu.+ Asp.	28.8	28.18	30.42	29.38
Total Basic Amino Acid (TBAA) % Lys. +Arg. + Hist.	18.03	18.52	15.62	18.14
Total Neutral Amino Acid (TNAA) %	58.27	61.26	60.61	60.93
Ratio of TEAA **:** TNEAA	1.1	1.1	0.8	1

Table VI - Effect of gamma irradiation on the Anti-nutritional factor of Egusi melon (g/100g)

Tests	Raw	Cooked	C+I	10KGy	5KGy
OXALATE	0.04 ± 0.01^{a}	0.01 ± 0.00^{c}	0.01 ± 0.01^{c}	0.02 ± 0.05^{b}	0.04 ± 0.00^{a}
TANNIN	0.09 ± 0.03^{a}	0.03 ± 0.02^{bc}	0.01 ± 0.02^{c}	0.04 ± 0.01^{b}	0.09 ± 0.01^{a}
PHYTATE	5.34 ± 0.12^{a}	4.58 ± 0.16^{c}	4.41 ± 0.12^{c}	4.87 ± 0.12^{b}	1.62 ± 0.12^{d}
PHATIC ACID	18.95 ± 0.02^{a}	16.48 ± 0.08^{b}	15.66 ± 0.06^{c}	17.30 ± 0.01^{b}	5.77 ± 0.02^{d}

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different ($P \leq 0.05$)
Key: C+I = Cooked and irradiated

Table VII - Effect of gamma irradiation on physico-chemical properties of Egusi melon.

	Raw	Cooked	C+I	10kGy	5kGy
A.V (mgNaOH/g)	1.01 ± 0.07^{d}	1.08 ± 0.08^{d}	1.69 ± 0.02^{c}	1.92 ± 0.13^{b}	2.14 ± 0.07^{a}
S.V (mgNaOH/g)	304.02 ± 0.11^{c}	306.13 ± 0.06^{b}	307.03 ± 0.06^{a}	306.27 ± 0.06^{b}	306.08 ± 0.05^{b}
I.V (g/100g)	192.20 ± 0.12^{a}	164.31 ± 0.13^{b}	126.42 ± 0.11^{c}	101.01 ± 0.08^{e}	119.13 ± 0.08^{d}
FFA (% oleic acid)	0.52 ± 0.04^{c}	0.58 ± 0.17^{c}	0.79 ± 0.08^{b}	0.85 ± 0.09^{ab}	1.02 ± 0.10^{a}
P.V (mEq/kg)	21.30 ± 0.13^{c}	20.31 ± 0.09^{e}	20.72 ± 0.12^{d}	23.06 ± 0.14^{b}	25.11 ± 0.13^{a}

Values are expressed as means ± Standard Deviation
Means in the same row with different superscripts are significantly different ($P \leq 0.05$)
Keys: A.V = acid value, S.V = saponification value, I.V = iodine value, F.F.A = free fatty acid, P.V. = peroxide value

ids. The essential amino acids composition appears adequate making up about half of the total amino acids content of each "egusi" melon treatment. Lysine, being a limiting essential amino acid in plant proteins [17], is 3.71 mg/g in the raw sample, 4.20 mg/g in the cooked sample, 2.91 mg/g in the cooked and irradiated sample and 3.98 mg/g in the10kGy irradiated sample. Glutamic acid accounted for approximately 14%, 13%, 16%, and 13% of the total amino acid contents of raw, cooked, cooked and irradiated, as well as irradiated at 10kGy samples respectively. The concentrations of methionine were found to be the lowest of all the amino acids (0.95-1.31%). This confirms previous observations that sulphur-containing amino acid concentrations are not very high in most cereals and oilseeds [18]. In a 1997 study, Taha [19] found that glutamic acid, leucine and arginine were predominant among amino acids of soya protein, accounting for over 35% of the total amino acid content, whereas methionine and cystine accounted for only 2.5%. The study also showed that Lysine, Isoleucine, Phenylalanine, Valine, Tyrosine, Arginine and Histidine were significantly destroyed only by radiation doses of 20-40kGy. The small amino losses in this study might be a result of a radiation-induced splitting of peptide bonds with a formation of free radicals. It may also stem from deamination-decarboxylation of some of the amino acid linkages, followed by a chain of chemical reactions [20].

The effects of Gamma radiation on the antinutritional constituents of "egusi" melon are presented in Table VI. Irradiation significantly lessened phytic acid content. This agrees with the findings of Doudu *et al*. [21] who reported that cooking and irradiation caused significant reduction in phytic acid levels of sorghum. Using both treatments further reduced the phytate content. This reduction may be due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by radiation [22]. Tannin and Oxalate contents were also decreased by cooking and irradiation. The combined treatment showed the greatest antinutrient reduction. Table VII shows the effect of Gamma radiation on the physico-chemical properties of "egusi" melon. The results indicate that cooking and/or irradiation caused a decrease in iodine value. For acid and peroxide values, the irradiated samples had higher contents to the non-irradiated ones. Similar findings were obtained by Zeb and Ahmed [23] who reported that the iodine value of sunflower and soybean

oil decreased significantly with high gamma radiation (15 and 20 KGy). The decrease in iodine value may be attributed to the engagement of the unsaturated fatty acid double bonds [24]. For saponification value, no significant differences were observed between the contents of the cooked and irradiated samples although all treatments showed a slight increase in value. This result is also in agreement with the report of Zeb and Ahmed [21]. In the tropics, where vegetable oils are the most commonplace dietary lipid, it has been observed that a free fatty acid (FFA) content of cooking oil within the limits of 0.0-3.0% is desirable [25]. The low levels of FFA in all the "egusi" melon seeds investigated indicate that the oils from these seeds are good and may store for long periods without going rancid.

CONCLUSIONS

Irradiation at doses 5KGy and 10KGy had no major adverse effect on the chemical composition of "egusi" melon seeds. The mineral and amino acid contents were also improved. Irradiation was effective in decreasing phytic acid and tannin contents of the seeds. However, susceptibility to oxidative rancidity of irradiated samples increased as irradiation dose increased. Low doses are recommended for better preservation and longer shelf life. This study has clearly demonstrated that gamma irradiation is a safe and successful method to preserve and improve the nutritional value of "egusi" melon seeds and enhance the properties of its oil.

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Enterprise Europe Network (EEN)

Anno 2023
(aggiornato al 31 dicembre 2023)

Progetto BORO20220316027

Romanian developer of a powerful natural polyphenol antioxidant is looking for partners under commercial or supplier agreement

The Romanian company has developed the most powerful natural polyphenol antioxidant with a concentration of polyphenols that is 30 times higher than that of olive oil. The company is interested in identifying international business partners from the agro-food and farmaceuticals sectors interested in buying the product to be used an antioxidant in their production process. Cooperation will be based on commercial or supplier agreements.
Dead-line for EOIs: 27/03/2024

Progetto BRPL20230523006

Polish distributor and importer of healthy food, coffee and cleaning products is looking for new foreign products to include into their portfolio and introduce them on the Polish market

Polish company, located in the north-eastern part of Poland, is a well-established distributor (wholesaler) and importer of a variety of fast-moving consumer goods (FMCG). Among others, the company is particularly interested in Italian products, including "pasta", "passata", pesto. In general, the company is looking for new products (that are not yet available in Poland) belonging to following categories: household chemicals, groceries (cappuccino, cocoa, drinking chocolate, tea, sweets, spices), bio products. Envisaged type of partnership: commercial agreement.
Dead-line for EOIs: 24/05/2024

Progetto BRPT20230809030

Portuguese company seeks manufacturing partners to produce and/or market an innovative portable food box via licensing / manufacturing agreements

Aware of the critical role that packaging plays in the process of conveying the value of food products to consumers, this Portuguese-based company has developed a line of portable food boxes with a revolutionary design that builds the bridge between sustainability and cost effectiveness, allowing users to eat without compromising their freedom. The Portuguese company has recently innovated a new line of portable food boxes that facilitate the handling and transport of the packaging. They are seeking experts/manufacturers with the capacity to develop products with new shares, sizes and materials. It will be necessary to carry out prototyping on emerging materials, including compostable, biodegradable and recycled solutions. The ideal partner will be able to offer small order quantities to start with and possibly have in-house R&D capability or connection with certified laboratories to test new materials. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 8/08/2024

Progetto TOIL20230730003

Looking for new extrusion technology, raw materials and additives for manufacturing of Israeli breakfast cereals

An Israeli company is looking for a technology, base materials and additives to produce breakfast cereals via an extrusion process. Ideally, the technology should lead to the healthier product in terms of energy, salt and sugar content and functional additives. The technology requested should be at the stage of concept, R&D work or fully developed / ready to launch. Looking for commercial agreement with technical assistance, license agreement & manufacturing agreement.
Dead-line for EOIs: 29/07/2024

Progetto BRFR20230828003

A French manufacturer of high-quality home textiles is looking for sustainable packaging for their products

The company is a French manufacturer of home textiles, funded in 1808 and managed for over 2 centuries by 8 generations of the same family. They select noble wools all over the world (merino, alpaca, camel, yack, cashmere etc.) to turn them into blankets, scarves, shawls, duvets, pillows etc. Brun de Vian-Tiran factory has succeeded in maintaining the excellence of its fabrics through two centuries by encouraging their employees' preservation of skilled know-how and their high levels of care and attention. The factory has now the title of Living Heritage Enterprise. Respect for nature is at the heart of their activity. They only work with natural fibers. Going even further, the factory makes use of products which are in conformity with the criteria for the OEKO-TEX® STANDARD 100 label, and part of their production has already received this official label. The company is now looking for a partner who should be able to provide 35 000 packaging items per year: 5 000 pillows, 10 000 duvets and 20 000 blankets. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 27/08/2024

Progetto BRAT20230801027

Austrian SME is looking for producers of agricultural feed and food raw materials under supplier agreement or distribution services agreement

The Austrian SME was founded in Innsbruck in 2019 and specialises in trading hemp derivates

for the food, feed and cosmetic industry. In recent years, they also added oilseeds, oils and nuts to their portfolio. In sum, they offer a vast portfolio of oilseeds, nuts, cereals and oils to satisfy their clients demands. The company is currently seeking to expand its network of producers of.oilseeds, cereals, dried and dehydrated fruits. They are also looking for new suppliers of refined oils such as sunflower, rapeseed, soya, palm, grape seed, hemp. The company requires raw materials that comply with EU food and feed regulations. As a trading company, the sole purpose of their purchases is resale to their network of customers across Europe. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 17/09/2024

Progetto BOEE20230908008

A novel Estonian start-up in food business, specialized in development of natural functional drinks, is looking for collaboration in crafting innovative science-based functional beverage companies.

This Estonian company seeks strategic partners across different countries to expand their innovative agri-food solutions into new markets. They focus on creating science-backed, low-sugar, and gut-friendly functional beverages, utilising fermented vegetable juices like carrot and beetroot, catering to health-conscious consumers. They are searching for partners with a presence in the local food and beverage industry, including distributors and drinks producer with manufacturing capabilities.
Dead-line for EOIs: 24/09/2024

Progetto BRSE20231201006

Swedish Company - Global Leader in Dietary Supplements Manufacturing Seeks Contract Manufacturing Partnerships Worldwide

Headquartered in southern Sweden, is a leading player in the food industry, specializing in the manufacture of dietary supplements. With an extensive international presence in countries like Sweden, Norway, China, India, the Middle East, and Africa, The Swedish Company is actively expanding its reach in Europe, Asia, and Africa. The company aims to establish strategic partnerships with contract manufacturers possessing GMP and Halal certifications to enhance its its global market share
Dead-line for EOIs: 17/11/2024

Progetto RDRCO20231221024

Colombian foodtech is in search of partners to collaborate in the creation of research, development, and innovation (R&D&I) projects, as well as to identify financing opportunities in the agri-food sector.

The foodtech is an entity with over 21 years of experience in the agro-industrial sector. Seeking to contribute through a comprehensive portfolio of services that encompass research in globally relevant thematic areas, laboratory testing services, knowledge transfer activities, design and development of food products, specialized consulting, as well as the formulation and execution of research, technological development, and innovation (R&D&I) projects.
Dead-line for EOIs: 12/01/2025

Progetto BOES20240108005

Spanish company specialised in the design and manufacture of ingredients and additive formulations for the meat industry is seeking manufacturer partners

This enterprise operates a production center dedicated to the fabrication of exclusive blends comprising ingredients and additives. These functional mixtures serve as intermediate components for the production of diverse meat products. Additionally, the company extends specialized technical guidance concerning ingredient application and the manufacturing processes associated with its products. It functions as a strategic collaborator in the product development initiatives of any manufacturer.
Dead-line for EOIs: 07/01/2025

Progetto BOBG20231129016

Bulgarian company is offering organic certified walnuts and walnut oil

A Bulgarian company is offering organic walnuts and walnut oil, produced in Bulgaria. The company is looking for importers, distributors, and buyers.
Dead-line for EOIs: 28/11/2024

Per ricevere ulteriori informazioni e per entrare in contatto con i soggetti titolari degli annunci si prega di inviare una mail al seguente indirizzo: ***susy.longoni@mi.camcom.it*** *specificando il/i codice/i progetto di vostro interesse.*

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I fattori relativi al clima e al suolo stanno acquisendo un ruolo sempre più importante e cruciale nell'ambito delle performance produttive della vite e della sua resistenza nei confronti degli stress sia biotici che abiotici. Il cambiamento climatico, ormai conclamato, li sta rendendo ancora più critici di un tempo tanto da aumentare la fragilità dei vigneti odierni.
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POTATURA DELL'OLIVO. METODO COLP

AUTORE:
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Il Metodo COLP (Conoscenza, Osservazione, Lettura, Potatura) è una forma di allevamento cespugliata piramidale dove ogni parte di pianta dialoga in equilibrio con le altre. Sviluppato in anni di sperimentazione con l'obiettivo di mantenere, miglio-

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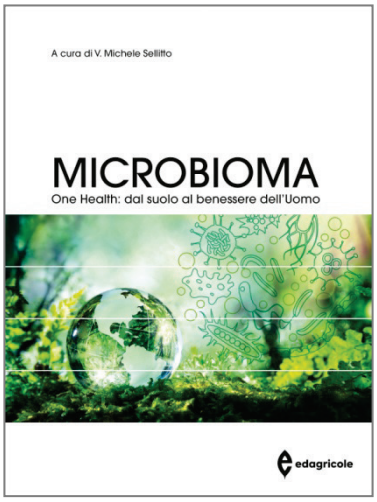

MICROBIOMA
One Health: dal suolo al benessere dell'Uomo

A CURA DI:
SELLITTO VINCENZO MICHELE
AUTORI VARI

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L'obiettivo del volume è quello di introdurre il concetto di microbioma all'interno di un nuovo paradigma in cui il suolo è considerato un organismo vivo in un ecosistema agrario totalmente interconnesso, di cui l'essere umano e gli animali sono parti integranti.
Paradigma che è alla base della visione olistica One Health, approccio ideale per raggiungere la salute globale, secondo il quale in un ambiente sano l'Uomo è sano e le interazioni tra i microbiomi ne sono causa e conseguenza.
Vengono perciò approfondite le dinamiche pianta-suolo-microrganismi partendo dal concetto di Soil Health e analizzando gli scambi fra i microbiomi del suolo con le colture e gli animali e le relative tecnologie applicate ai processi agricoli e ai sistemi alimentari.
Vengono introdotti il microbioma umano e le correlazioni microbiologiche che determinano la genesi di patologie innescate dal disequilibrio del microbioma intestinale, correlazioni che portano a considerare l'Uomo come un "superorganismo", la cui salute dipende dal mantenimento di un'armonia complessa che stiamo solo oggi cominciando a capire.

INDICE: La genesi di un suolo - La vita nel suolo - Microbiota del suolo e One Health - Il microbioma per il sequestro del Carbonio - Interazioni nella rizosfera - Questioni di Quorum - Le basi genetiche dell'interazione pianta-microbioma - Ciclo rizofagico: processo fondamentale per un'agricoltura sostenibile - Miglioramento del microbioma simbionte delle leguminose - I benefici delle micorrize e del Wood Wide Web - Dal microbioma all'olobionte per un'agricoltura sostenibile - Microbiomi e biotecnologie: realtà e sfide future - Microbioma e Agricoltura, trend e prospettive - Microbiomi e prodotti fermentati: qualità, sicurezza e salute - Il microbiota umano: ruolo e funzioni - Il microbiota umano e la patologia metabolica - Microbioma di suolo, piante e Uomo: il ruolo del sistema redox - Interazioni tra il microbioma del suolo e quello dell'Uomo - Teoria e tecniche di bioinformatica: l'analisi del microbioma.

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11 - 14 February 2024 | Graz, Austria

The International Symposium on Microbial Lipids started is an international conference in Vienna in 2010. It brings together 80–100 international scientists, including PhD students and postdocs, for scientific exchange in the field of microbial lipids. The conference covers key topics ranging from fundamental research on microbial lipids and their modification to exciting biotechnological applications. Topics include microbial lipid biosynthesis, membrane-associated processes, lipidomics, and enzymatic lipid modification.

While the supply of fats and oils for food and feed applications is covered well from plant sources, microbial lipid production is of high interest if very specific lipid structures such as poly-unsaturated fatty acids or low-availability terpenoids are required. Moreover, the application of oleaginous yeasts or the production of algal lipids pave new ways in upcycling agricultural side streams and even propose to lower the CO2 load in our environment.

The session 'Biomembranes and Membrane-associated processes' will focus on processes in and at biomembranes, with a special focus on the roles of membrane proteins and enzymes. Particular attention will be given to groundbreaking new approaches that enable control over cellular functions by modulating microbial lipid compositions, help unravel the essence of life by engineering minimal cells from lipid-based membrane compartments, and allow the analysis of lipid metabolism by cutting-edge lipidomics and functional proteomics.

Microbial lipids impacting higher eukaryotic cells are of high interest. Although microbial lipid membranes have been in the focus of experimental and theoretical biophysics for decades, many molecular details of membrane transport, membrane signaling, and membrane-based biocatalysis remain poorly understood. This session will highlight recent breakthroughs that provide unprecedented molecular level insights into these key physiological processes.

Enzymatic lipid modification has been a highly dynamic field in the last years. While lipases have already found broad application, enzyme discovery and enzyme engineering led to the development of highly efficient biocatalysts for the modification of fatty acids and other biobased hydrophobic molecules, which is expected to lead to various new processes. In particular, enzymatic lipid modification holds great potential for the utilization of lipids as renewable resources.

The aim is to establish the International Symposium on Microbial Lipids as a regular platform for the global networking of scientists in the field of microbial lipids.

More information and program:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11544&sp_id=1

Black Sea Grain – 2024

27 - 28 February 2024 | Prague

Premiere event for the global leaders of grain & oilseed industry since 2004

Black Sea Grain offers 2 days of exclusive agri market analytics and insights from the top industry experts, effective networking and meetings with players across the value chain, top managers, decision-makers and opinion leaders.

The conference will bring together producers and crushers of grains & oilseeds, commodity traders, agri-food, logistics and finance sectors, government authorities and industry associations, to establish effective interaction in commodity supply chains that start in Ukraine and develop globally.

This year Black Sea Grain will collaborate with the 8th Prague Karlsbourse to encourage closer cooperation of Ukrainian and European operators, explore new ways of doing grain business and responding to the ongoing market change all together.

Key issues:

- Global agricultural market trends. 2024 crop forecasts
- Supply chain transformation. Competition between origins
- Black Sea & Danube grain and oilseeds market – trade flows, S&D forecast, price trends
- Logistics & cargo insurance. War risk management. Aiming at efficient, safe and sustainable multimodal logistics for the European and Ukrainian markets
- Ukraine/EU integration. Regional cooperation – turning challenges into opportunities
- Agri-food. New economic, production and consumer trends
- Global weather alarm. How to ensure sustainable crop production and quality? Agri inputs sector
- Asia & Africa: current and forthcoming demand expectations.

See more on:
https://ukragroconsult.com/en/conference/black-sea-grain-2024/about

Olio Officina Festival 2024

29 February - 2 March | Milano, Italy

Olio Officina Festival is a cultural project aiming to affirm a solid culture of oil, as well as vinegar and

any other condiment, as well as the valorization of a healthy and correct diet intended as a preventive medicine and source of well-being.
The event is addressed both to experts and general public and offers workshops, round tables, dialogues, interviews, cooking courses, tasting sessions, short tasting courses for beginners, art exhibitions, video screenings, shows, performances, book presentations.
The language of the event is Italian.
More information: https://www.olioofficina.it/olio-officina-festival/format.htm

POC 2024

4 - 6 March 2024 | Kuala Lumpur, Malaysia
Bursa Malaysia Derivatives presents the 35th Palm & Lauric Oils Price Outlook Conference & Exhibition (POC2024).
For over 30 years, this world-renowned event is the most anticipated annual congregation of key decision makers and thought leaders in the global edible oils industry. The event provides valuable networking opportunities among its participants and a forum to deliberate on topics surrounding the supply and demand of major edible oils, the industry's most pressing issues, market trends and trade possibilities.
The POC2024 on-line networking system is a very useful tool for delegates attending the Conference. This new feature allows all confirmed delegates, speakers and guests to contact each other, organise meetings and network before, during and after the event using this easy to use, private messaging system.
Discover more on https://www.pocmalaysia.com/

European Algae Industry Summit

17 - 18 April 2024 | London, UK
The Summit's 12th edition will bring together business executives, academics, policy makers, and stakeholders from the European algal industry. The summit will provide attendees a forum to exchange information, discuss recent developments, and examine possibilities as well as challenges connected to algae production, cultivation, and applications.
The summit will include interactive sessions, talks by experts in the field, networking opportunities, and exhibitions of cutting-edge algae-related goods and technologies. Participants will gain valuable insights to the current situation of the algae industry, new trends, and collaborative efforts aimed at advancing the sector in terms of its viability as an industry and environmental.

Key topics

- Current Market Overview of the European Algae Industry
- Policy and Regulations Shaping the Algae Industry
- Cultivating a Sustainable Future
- Exploring Fermentation
- Revolutionizing Aquaculture Through Algal Based Solutions
- Expanding Customer Demand for Algae Products and Solutions
- Algae Strain Identification, Selection and Genetic Modification
- The Application of Algae Throughout the Food and cosmetic industry

Who Will Attend?

- Algae Producers and Cultivators
- Marketing for Algae Based Products
- Scientific Officers
- Market Consultants
- Policy Makers
- Representatives From the EU
- Technology and Equipment Developers / Suppliers
- Representatives from the food and cosmetic industry
- Green Technology and Biotechnology Investors
- Algae Product Distributors
- Feed and Aquaculture Professionals

For more information visit:
https://www.wplgroup.com/aci/event/european-algae-industry-summit/

IEEE 2024 International Workshop on Measurements and Applications in Veterinary and Animal Sciences

22 - 24 April | Turin, Italy
The aims of the IEEE International Workshop on Measurements and Applications in Veterinary and Animal Sciences (MeAVeAS) are to comprehensively explore the various aspects of interactions among the realms of instrumentation and measurement, bio-engineering, material science, chemical and biological measurements, and the field of veterinary medicine and animal and food science.
Metrology applied to medical and biological disciplines is an ancient and multidisciplinary science of which there is evidence already from ancient Egypt, when a measure called "cubit", which included the distance between the hand and elbow of individuals, was used to classify morphologically the people, referring to them the reference measure represented by the "cubit" of the pharaoh.
Metrology studies measurements of magnitude in order to have official references (units of measurement) to refer to, to identify measurement systems, means and methods for following measurements of various kinds.
The Workshop provides a platform for researchers, veterinarians, and animal scientists to engage in the exchange of ideas and information, establish connections and collaborations, and stay updated

on innovations in the fields of veterinary medicine and animal science.
MeAVeAS is dedicated to bringing together professionals working on the development of instrumentation and measurement methods, not only for veterinary medicine but also for ecology and for food and animal science. Our focus extends to various aspects, including cutting-edge technologies for monitoring the health of animals, metrology-assisted production in the food industry, sensors and associated signal conditioning techniques tailored for veterinary medicine and animal science, as well as calibration methods for electronic test and measurement applications in these fields.
A Call for papers and a Call for Special sessions are foreseen. Special Sessions have the primary objective of establishing mini-workshops dedicated to specific topics. Within these sessions, researchers with shared interests can collectively expand their knowledge, engage in fruitful discussions, exchange ideas, and foster collaborative endeavors.
Discover more on the web site:
https://www.meaveas.org/home

4th Oils & Fats Exhibition And Conference Bangladesh - 2024

25 - 27 April 2024 | Dhaka, Bangladesh

Oils & Fats Exhibition and Conference Bangladesh offer a supreme platform to showcase your innovative Oils & Fats solutions to South Asia's audience of industry professionals. Connect with key decision-makers, build valuable partnerships, and gain unparalleled visibility in the rapidly growing Oils & Fats market in Bangladesh. Don't miss the opportunity to develop your brand, expand your network, and stay at the lead of the Oils & Fats industry. Oils & Fats Expo Bangladesh is concurrent with the "12th International Grain Tech Bangladesh" exhibition.
More information and program updates: https://www.oilfatbd.com/

2024 AOCS Annual Meeting & Expo

28 April - 1 May 2024 | Montréal, Québec, Canada

Beyond Chemistry: Solving Complex Problems Together
The AOCS Annual Meeting & Expo brings together thousands of chemists, engineers, technologists, and researchers focused on the science and technology of fats, oils, proteins, surfactants, and related materials.
The 2024 theme, Beyond Chemistry: Solving Complex Problems Together, embraces the work of a diverse range of specialists who address global problems — and improve the lives of people around the world.
Co-located with the 2024 Sustainable Protein Forum and featuring additional short courses, this year's annual meeting will bring together our largest ever community of inspired minds.
The annual meeting meeting technical program features 80+ sessions across 10 interest areas for specialists in fats, oils, proteins, surfactants, and related materials. This comprehensive two and a half day program offers hundreds of presentations and posters from chemists, engineers, technologists, nutritionists, and researchers from around the world — including unique multidisciplinary sessions that bring together multiple interest areas to find solutions to shared problems.
2024 Annual Meeting Session Topics:

- Analytical
- Biotechnology
- Industrial Oil Products
- Lipid Oxidation and Quality
- Phospholipid
- Processing
- Protein and Co-Products
- Surfactants and Detergents

This year's student competitions will take place in-person in Montreal. All students who submit an accepted abstract to one of the competitions and attend the annual meeting will be eligible. Presenters must be a student at the time of the abstract's submission.
AOCS is looking for unique perspectives and expertise to support our technical services and education programs. Committees and expert panels will be open for participation in order to show how they work.

Short courses:

- *Edible Fats and Oils Refining | 27-28 April*

Delve into the intricacies of edible oil refinement in this 2-day short course. This course emphasizes practical applications and optimization, enabling participants to make informed decisions in their plant operations and refine edible oils effectively.

- *Lipid Oxidation in Foods | 28 April*

This comprehensive course delves into the challenges posed by lipid oxidation in food systems and technologies that can help control these detrimental reactions to decrease food waste and increase food safety.
For more information visit:
https://annualmeeting.aocs.org/

14th ICIS World Surfactants Conference

9 - 10 May 2024 | Jersey City, NJ

Bringing together industry professionals from all corners of the surfactants value chain for an informative and valuable two days, the ICIS World Surfactants Conference is now in its fourteenth year.With the demand for surfactants continuing to grow, it is more important than ever for industry professionals to stay informed about developments in the field. This year's conference will once again provide a trusted and valuable platform for

sharing knowledge, connecting peers, and helping leaders stay up to date on the latest trends and innovations in the surfactants industry. The conference is aimed at those looking to gain critical insight into the surfactants industry and the pain points stakeholders are having. With the addition of speakers from across the value chain industry groups:

- Consumer Brands and End Users
- Chemical Producers
- Distributors and Traders
- Converters and Processors
- Industry associations

Learn. With an insightful agenda planned, you will hear from those at the forefront of the industry who are making decisions that will impact the wider market.

Exclusive insight into the following topics:

- Supply and Demand Dynamics
- Sustainability
- Surfactant Innovations
- Regulation and Policies

Netwoking. We understand that the surfactants industry is complex and involves a range of professionals from different parts of the value chain. That's why we focus on bringing together a diverse community across the industry, to ensure that our conference provides a valuable and meaningful experience for all in attendance.

By gathering a diverse group of experts and providing ample opportunities for connection and collaboration, we strive to create a dynamic and productive environment that supports the success of everyone who attends.

Collaborate. Foster collaboration with industrial peers and work towards achieving sustainability targets and overcoming market challenges, through new insight gained at the conference and the networking opportunities available to those in attendance.

5th MS-Wine Day

22 - 24 May 2024 | Asti, Italy

Thanks to its application versatility and its innovative potential, mass spectrometry offers advanced tools for the compositional characterization of oenological matrices and the traceability of wine products: it allows the accurate evaluation of quality with the analysis of aromatic volatile compounds, polyphenols and antioxidant compounds present in wines and grapes, proves to be fundamental for the identification of contaminants, such as pesticides and mycotoxins, and represents an indispensable tool for the monitoring and optimization of production techniques, the study of vine diseases and the monitoring of aging and fermentation processes.

The Mass Spectrometry Division of the Italian Chemical Society has established the series of "MS-Wine Day" conferences every two years with the aim of creating a stable scientific reference point of synergy between Public Bodies, Private Bodies and Companies operating in the analytical sector -oenological.

These events have the aim to connect experts, researchers and professionals, promoting discussions focused on the potential and benefits of mass spectrometry in the wine industry. During the event the applications of mass spectrometry will be explored in the fermented beverage sector in general, thus broadening the horizon of discussion and research.

Main topics

- Wine and Fermented Beverages quality
- Traceability and Counterfeit in Fermented Beverages
- Metabolomic and Proteomic Profiles in Wine, Beer, and Spirits
- Cutting-Edge MS Techniques Applied to Enology
- Monitoring Oenological Processes and Fermentation
- Identification of Contaminants and Faults in Alcoholic Beverages

More information and program on the web site:
https://www.spettrometriadimassa.it/Congressi/5MSWineDay/index.html

Oleofuels 2024

12 - 13 June 2024 | Milan, Italy

Following the success of Oleofuels 2023 which brought 300+ senior level industry professionals to Seville, Spain in June, ACI organize the 15th edition of the event for professionals and experts in the field of oleofuels, providing a unique platform for networking and knowledge exchange.

In this two-day conference, industry leaders, manufactures, researchers, policymakers, and market experts will come together to discuss the latest advancements, challenges, and innovations in the field of oleofuels. The event will feature informative presentations, interactive panel discussions, and engaging networking sessions.

The conference offers a valuable opportunity to gain insights into the current market trends, learn about the most recent technological developments, and explore potential collaborations within the industry. It will provide participants with an in-depth understanding of the global oleofuels market, its future prospects, and the regulatory framework shaping the industry.

Key topics:

- Oleofuel Global Outlook, Trends & Divers
- Exploring Growing Mandates and Legislations in Europe: What Impact Will This Have on the European Market?
- Overcoming Feedstock Challenges: Sourcing & Supply

- Moving Towards a More Cohesive Future
- Supply Chain Management
- Case Studies: FAME vs HVO
- Optimising New Technologies
- Decarbonising the Transport Sector: Road
- Decarbonising the Transport Sector: Aviation
- Decarbonising the Transport Sector: Maritime

For more information and update visit:
https://www.wplgroup.com/aci/event/oleofuels/

5th International Symposium on Lipid Oxidation and Antioxidants

08 - 10 July 2024 | Bologna, Italy

Euro Fed Lipid organise the upcoming 5th ISLOA, which will take place in the fascinating city of Bologna.

Lipid oxidation is a critical area of research with far-reaching implications in various fields, including food science, nutrition, pharmaceuticals, and health. The role of antioxidants in mitigating lipid oxidation is equally significant.

The congress aims to bring together experts, scholars, researchers, and professionals from around the world to exchange knowledge, share their latest findings, and foster collaboration in this important domain.

The meeting will cover, among others, the following topics:

1. Innovative methods for lipid oxidation and antioxidant evaluation (e.g. fluorescent probes, mass spectrometry, lipidomics, NMR);
2. Elucidation of lipid oxidation and antioxidant mechanisms (e.g. free radical chemistry, multiphasic systems);
3. Lipid oxidation and antioxidant effects in real systems (e.g. bulk oil, emulsions, oleogels, food, recycled oils);
4. Protein oxidation in lipid-containing model systems and food: oxidative interactions and antioxidant effects;
5. Nutritional and physiological effects of oxidized lipids and antioxidants.

For more information and update visit:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11650&modus=

8th MS Food Day

16 - 18 October 2024 | Brindisi, Italy

High quality, healthy and safe food, with good nutritional and sensory characteristics, are necessary for a satisfactory quality of life.

For this reason, authentication and traceability of foodstuffs, characterization of food components, quality control, identification and quantification of additives, allergens, chemical and microbiological contaminants, preservation of food components during storage and processing, packaging technology, determination of nutritional and sensory properties, are essential for citizens and consumers with widespread consequences on health, and on agriculture, industry and economy.

In all these aspects mass spectrometry plays a key role. The impressive evolution of its applications, methods, instrumentation and technology yielded highly sensitive, specific, fast, robust and validated methods that are fundamental tools in food science and technology.

The 8th MS Food DAY is a biannual conference focused on all topics related to the use, methods and applications of mass spectrometry in food.

It represents an excellent occasion for presenting the state-of-the-art of mass spectrometry in food chemistry & technology, along with the latest innovations and novelty in instrumentation and applications.

As already testified by the successful previous editions, this will also create the optimum opportunity to meet the needs and opportunities of academic institutions, research and control institutions, private enterprises and food companies.

The conference will include plenary lectures, oral and poster communications.

Topics include:

Innovations in food science applications of MS

- Food Authenticity & Traceability
- Food Safety
- Food Quality
- Food and Health
- Functional Food & Nutraceutical
- FoodOmics
- Sensomics
- Oils and fats
- Artificial intelligence
- Machine learning in MS
- MS on Food Big Data Handling
- Food Packaging
- Process monitoring

Methodological and instrumental developments

- High resolution Mass Spectrometry
- High-throughput techniques
- Ambient Mass Spectrometry
- Isotope Ratio Mass Spectrometry
- Stable isotopes
- Direct Injection/Infusion Mass Spectrometry
- Ion sources and mass analysers

For more details visit:
https://www.spettrometriadimassa.it/Congressi/8MS-FoodDay/

Olio Officina Festival

2024 CONDIMENTI PER IL PALATO & LA MENTE
TREDICESIMA EDIZIONE

Olio Musicale

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Author instructions

La Rivista Italiana delle Sostanze Grasse (RISG) welcomes research, experimental or technological papers, short communications, reviews articles on edible and industrial oils and fats of vegetable and animal origin, soaps, detergents, surfactants, cosmetics and toiletries, mineral oils, lubricants.

The manuscript will be evaluated by a team of referees whose opinion is essential for acceptance for publication. We shall ask you to indicate three names of qualified experts as a referee.

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