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RISG
LA RIVISTA ITALIANA DELLE
SOSTANZE GRASSE

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GRAFICA, IMPAGINAZIONE E STAMPA

Ancora srl
Via Benigno Crespi, 30
20159 Milano

DIRETTORE RESPONSABILE: P. ROVELLINI
REDAZIONE: C. ZIGLIANI

REDAZIONE

Chiara Zigliani
risg@mi.camcom.it

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DIREZIONE E REDAZIONE
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Ai Lettori della RIVISTA ITALIANA
delle SOSTANZE GRASSE

Siamo giunti al centenario della nostra Rivista e ritengo che questo periodico rappresenti anche l'anima della nostra azienda, racchiudendo il principio della nostra *mission* che, nonostante le numerose trasformazioni, non è mai cambiato: essere un centro di riferimento nazionale di ricerca, innovazione e trasferimento tecnologico.

Per capire la storia della RISG non si può prescindere dalla storia della nostra azienda, che in parte trae origine dalla istituzione di una scuola laboratorio per le industrie chimiche degli oli e dei grassi a seguito dell'intesa fra ambienti tecnico-scientifici e industrie nazionali. L'iniziativa partì dall'Unione Saponieri Italiani che aveva sede centrale a Milano e che deliberò nel 1903 di dar vita a una scuola di saponeria a sostegno di questa branca rimasta stazionaria e troppo chiusa al progresso scientifico. Nel 1906 si costituì la Scuola-Laboratorio e Stazione Sperimentale per l'Industria degli Oli e dei Grassi, che nel 1919, in considerazione dei risultati ottenuti, venne nominata Regia Stazione Sperimentale per l'Industria degli Oli e dei Grassi con Decreto Luogotenenziale del 2 febbraio n. 637 e nel 1952 assunse la denominazione di Stazione Sperimentale per le Industrie degli Oli e dei Grassi, sotto la presidenza di Enrico Mattei.

Dal 2011, questa è confluita in Innovhub SSI, dove le competenze specialistiche sono state integrate con quelle delle altre Stazioni Sperimentali lombarde, concorrendo a definire l'attuale articolazione dell'azienda. Questo importante processo di integrazione ha rappresentato un notevole valore aggiunto, creando sinergie fondamentali per l'ulteriore sviluppo trasversale delle attività e dei servizi messi a disposizione del comparto industriale. Oggi, grazie a un approccio multidisciplinare, siamo in grado di spaziare in più ambiti, dal settore alimentare alle risorse energetiche e al manifatturiero avanzato, con particolare attenzione all'impatto ambientale. Ma il nostro obiettivo resta il medesimo, quello che ha caratterizzato la nostra nascita e che ci permetterà di affrontare con entusiasmo i prossimi 100 anni: promuovere qualità e innovazione come elemento cardine di uno sviluppo sostenibile.

Massimo Dal Checco
Amministratore Unico di Innovhub SSI

In occasione del centesimo anno di attività della Rivista Italiana delle Sostanze Grasse, mi sembra importante ripercorrere le fasi di questo giornale scientifico e portarle all'attenzione di coloro che le hanno sempre seguite con interesse e che sono stati testimoni del rapido evolversi degli aspetti tecnologici e scientifici che hanno influenzato anche la crescita culturale della nostra Azienda.

La rivista nasce come Bollettino nel 1921, su idea dell'allora Direttore della Stazione Sperimentale, Prof. Fachini. Cercando tra i vecchi documenti ho ritrovato che nel corso del tempo il nostro periodico, nato come "Bollettino degli oli e dei grassi", ha cambiato

spesso il nome, fino al 1961, quando divenne quello attuale "**La Rivista Italiana delle Sostanze Grasse**". Solo per due anni, durante la guerra, non fu pubblicato e questo spiega perché siamo arrivati al volume n. 100 proprio quest'anno.

Gli argomenti trattati con continuità fin dalla sua origine riguardano soprattutto l'olio di oliva in tutti i suoi aspetti e caratteristiche, la ricerca sugli oli di semi, di sansa e oli esterificati, lo studio della composizione e dei componenti minori delle sostanze grasse, la tecnologia per la loro produzione. La rivista si è occupata spesso anche di argomenti scientifico-tecnologici riguardanti i detergenti e le sostanze tensioattive in genere sulla base dell'esperienza acquisita dai ricercatori dell'Istituto. Numerosi articoli hanno riguardato aspetti legati all'inquinamento delle acque da parte dei detergenti ad uso domestico e industriale e alla loro biodegradabilità. Nel corso degli anni, la Rivista allargò i suoi orizzonti anche con la stesura di Norme analitiche nei campi delle sostanze grasse.

Riprendo e faccio anche mie le parole che ho ritrovato e che erano del Prof. Enzo Fedeli, il quale scriveva in un editoriale del 1983: "*60 anni di vita compie questo nostro 1983, anni di storia tumultuosa come ben sai, che tuttavia le hanno lasciato un'ansia di sopravvivere, di vivere per servirti nel tuo lavoro quotidiano. Non è una data che possa passare sotto silenzio, la sua vitalità è prova della nostra vitalità, del nostro desiderio di fare credendo in un futuro migliore per tutti, una speranza nelle difficoltà del presente. Direttori della Rivista, autori che le hanno dato prestigio e contenuto, redattori che l'hanno esteticamente fatta piacevole, possano, ciascuno lasciando un'orma e un sostegno per l'attività futura: auguri per cento di questi giorni*".

Dal 2020 ho assunto con estremo piacere e interesse l'incarico di Direttore di questa Rivista, riferimento nazionale di notevole importanza. In questi anni ho potuto contare sulla notevole esperienza della segreteria nella persona di Franca Paparella, sul validissimo Comitato di Redazione e sul Comitato Scientifico di esperti Referee, così come sui numerosi ricercatori nazionali e internazionali che chiedono di pubblicare i loro ultimi lavori di ricerca.

Buon Centenario Rivista Italiana delle Sostanze Grasse!

Pierangela Rovellini
Direttore Editoriale RISG

A Franca Paparella, che ha terminato il suo percorso lavorativo presso Innovhub SSI, va il nostro ringraziamento per il tempo dedicato alla Rivista.

Grazie Franca per l'ottimo lavoro che hai saputo condurre ogni giorno con passione, determinazione, precisione e creatività. Questi sono i valori che rappresentano la nostra Azienda e tu li hai perseguiti in maniera esemplare.

Grazie anche per l'impegno impiegato a trasferire il tuo lavoro e a permetterne la continuità, dimostrando che le conoscenze non ci appartengono ma devono diventare le conoscenze di tutti.

Oggi non è solo un traguardo, ma anche l'inizio della realizzazione di tutti i tuoi sogni. Grazie!

Il Comitato di Redazione RISG

Composition of cold-pressed hemp seed oils: key elements of quality and authenticity

Mara Mandrioli[1]
Matilde Tura[1] ✉
Enrico Valli[1,2]
Tullia Gallina Toschi[1,2]

[1] Department of Agricultural and Food Sciences, Alma Mater Studiorum Università di Bologna, Bologna, Italy

[2] Interdepartmental Centre for Industrial Agrofood Research, Alma Mater Studiorum – Università di Bologna, Cesena, Italy

✉ CORRESPONDING AUTHOR:
Dr. Matilde Tura, PhD.,
Department of Agricultural and Food Sciences,
Alma Mater Studiorum – Università di Bologna,
Viale Fanin 40, 40127,
Bologna, Italy
Phone: +39 051 20 9 6015
Email: matilde.tura2@unibo.it

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With the promotion of the agro-industrial chain of *Cannabis sativa* L., the presence on the market of food products obtained from industrial hemp has become more frequent and relevant. Among these, it is important to mention cold-pressed hemp seed oil, which, in relation to specific sensory quality and nutritional characteristics, is certainly one of the most widespread. In order to guarantee consumers regarding its safety, quality and authenticity, it is essential to know the key compositional parameters, their variability and the analytical methods that can be used to detect them. In this review article, all the evidence from the literature, useful to define a quality regulatory framework for the product category "cold-pressed hemp oil" according to the basic criteria of the Codex Alimentarius and some related considerations such as the seed conservation methods, the fundamental variables of the production process and the safety of the edible oil obtained, this last in relation to the legal limit (7.5 mg/kg), expressed as the sum of Δ^9 – tetrahydrocannabinol (acid plus neutral form), are discussed. Nowadays, apart this last legal obligation, there is no specific and harmonized EU legislation to define the quality and authenticity of cold-pressed hemp seed oil. In order to help achieve this objective, this review presents a discussion of the data from the literature and provides interpretative elements. The path and information described herein were keys in drafting the commercial standard entitled "Cold-pressed hemp oil obtained from *Cannabis sativa* L. seeds - Characteristics and methods of analysis" UNI-11876:2022.

Keywords: Hemp seed oils, cold-pressing, vegetables oils, *Cannabis sativa* L , authenticity

1. INTRODUCTION

As a consequence of the resumption of the traditional cultivation and supply chain of hemp (*Cannabis sativa* L.), given by Law n. 242 of 2nd of December 2016 [1], in the last decade there has been an increase in Italy from 27 to 603 dedicated hectares [2], especially in historically vocated areas. The varieties of industrial hemp allowed for cultivation are those registered in the Common Catalogue of Varieties of Agricultural Plant Species, as required by Directive 2002/53/EC [3]. Currently, 101 varieties of hemp [4] are registered in the catalogue, and from which it is permissible to produce food, in particular seeds, flour obtained from seeds, and oil obtained from seeds as defined in Annex 1 of the Decree of the Ministry of Health of 4 November 2019 and in the EU regulation [5,6]. For a certain period, hemp seeds were considered as a by-product of the fiber plant, and were often destined only for animal feed. Recently, thanks to the many research studies in this field, it has been shown that hemp seeds and the oil obtained from them are food products with a high nutritional and health value. The oil content and composition of the hemp seed are influenced by genetics and environmental factors. The seed contains over 30% oil, 25% protein, dietary fiber, vitamins, and minerals [7]. The most commonly used method for pressing the achenes is cold pressing.

The Codex Alimentarius defines cold-pressed oil as an oil obtained only through mechanical processes, such as extrusion or pressure without the application of heat and without altering its characteristics, which can only be purified by washing with water, decanting, filtration, and centrifugation [8]. Furthermore, cold pressing technology is advantageous as it is environmentally friendly, requires less energy than solvent-based systems/refining process, and represents a guarantee for the maintenance of nutrients [9] and specific sensory aspects that are peculiar and more intense in cold-pressed and not refined edible oils. In particular, hemp seed oil (HSO) has been described as dark green to light yellow-green in color, with a nutty flavor and sometimes with a slightly bitter aftertaste. However, for the evaluation of the sensory profile and quality, in order to describe positive attributes and defects, it is essential to refer to a common and shared vocabulary. For this purpose a specific lexicon with 45 descriptors was recently developed, together with a sensory wheel and a tasting sheet to allow training of different panels and to harmonize a procedure for sensory evaluation [10]. The lipid profile of cold-pressed HSO is characterized by the prevalence of polyunsaturated fatty acids (PUFA), followed by monounsaturated fatty acids (MUFA) and to a lesser extent by saturated fatty acids (SFA), mainly represented by linoleic acid, α-linolenic acid, oleic acid, γ-linolenic acid, and palmitic acid. The presence of small amounts of stearidonic acid is also peculiar in HSO, since this fatty acid is generally not present in common plant species cultivated for the production of vegetable oils. Typical sources of stearidonic acid derive from fish products, such as fish oils and microalgae, but recently, given the great interest, research has also highlighted significant amounts in some plant species, in particular the *Boraginaceae* family, such as Echium oil, extracted from the seeds of *Echium plantagineum* [11] and in small quantities in borage oil and black currant oil [12]. Cold-pressed HSO shows a ratio of ω-6/ω-3 fatty acids between 2.5:1 and 3:1, which is considered optimal from a nutritional point of view while, on the other hand, it is also extremely sensitive to oxidative degradation, auto-oxidation, photo-oxidation, and enzymatic oxidation with a risk of consequent rancidity of the product. In cold-pressed HSO there are also minor components such as phytosterols, tocopherols, chlorophylls, and carotenes which, due to their respective concentrations, can play an important role in exercising antioxidant or pro-oxidant activity. According to Reg. (EC) 1924/2006 [13], several nutritional and health claims are related to the fatty acid profile and in this paper the potential application to HSO will be discussed. For example, the claims are: *i*) source of omega-3 fatty acids, allowed if the product contains at least 0.3 g of alpha-linolenic acid per 100 g and per 100 kcal or at least 40 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; *ii*) rich in omega-3 fatty acids, allowed if the product contains at least 0.6 g of alpha-linolenic acid per 100 g and per 100 kcal or at least 80 mg of the sum of eicosapentaenoic acid and docosahexaenoic acid per 100 g and per 100 kcal; *iii*) rich in polyunsaturated fats, allowed only if at least 45% of the fatty acids present in the product derive from polyunsaturated fats and provided that the polyunsaturated fats provide more than 20% of the energy value of the product; *iv*) rich in unsaturated fats, allowed only if at least 70% of the fatty acids present in the product derive from unsaturated fats and provided that the unsaturated fats provide more than 20% of the energy value of the product. To regulate the concentration of cannabinoids, there are legal restrictions, and lately the European Parliament has voted in favor of restoring the authorised Δ^9-tetrahydrocannabinol (THC) level on the field in industrial hemp from 0.2% to 0.3%, in hemp seeds Reg. (EU) 1393/2022 [6] establishes the limit of 3.0 mg/kg, while for HSO the limit raises to 7.5 mg/kg, a value that refers to the sum of delta-9-tetrahydrocannabinol (Δ^9-THC) and delta-9-tetrahydrocannabinolic acid (Δ^9-THCA). With reference to the synthesis of cannabinoids, some authors report their presence in the hemp seeds only as a consequence of cross-contamination deriving from contact with the glandular trichomes of cannabis inflorescences [14,15,16]. In particular, in the basal part of the glandular trichomes, the resin containing cannabinoids is synthesized and accumulates, above the cells of the spherical head, inside the outer membrane (cuticle) that covers the head. Sometimes this membrane opens allowing the resin to flow out and seep onto adjacent plant tissues, such as seeds [17]. Therefore, there would not be intrinsic presence of phytocannabinoids in hemp seeds [18,19,20], but some traces of these compounds can transfer to them through this mechanism, and consequently can be found in the oil extracted as a result of cross-contamination. It is also of fundamental importance to follow, during all stages of cold-pressed HSO production, careful and detailed technological practices aimed at: *i)* not triggering irreversible oxidative phenomena to preserve all the antioxidant components; and *ii)* avoiding possible contamination of unwanted substances to obtain a safe and high quality product. The quality, authenticity, and purity control of edible oils, even in a forensic view, is carried out through evaluation of a number of highly diagnostic parameters [21]. The composition in fatty acids, the content in minor components, such as phytosterols and tocopherols, are indicative and specific, both in terms of quality and quantity, of the botanical species of origin, while basic quality parameters such as free acidity and peroxide value allow evaluation of degradation status (hydrolytic or oxidative) of the oil. For evaluation of these parameters and their relative methods, references can be found in the current national and international regulations, being

Reg. (EU) 2022/2104, related to the characteristics of olive oils and olive pomace oils, and one of the cornerstones as well as the Codex Alimentarius [8]. In this work, an extensive bibliography has been collected on the characterization of cold-pressed HSO in order to collect and discuss all the useful data to define the quality parameters, as indicated and required by the general rules relating to vegetable oils, considering that, at present, a specific and harmonized regulation for cold-pressed HSO at the EU level is not present. Thus, the aim of the present paper is to provide a guideline for the main analytical reference parameters that are useful to define the quality and authenticity of cold-pressed HSO. Moreover, the references collected and discussed in this review were useful to draft a commercial standard UNI 11876:2022 [22], titled "Cold-pressed hemp oil obtained from the seeds of *Cannabis sativa* L. - Characteristics and methods of analysis".

2. HEMP SEEDS QUALITY FROM HARVESTING TO STORAGE

Seeds quality is essential to produce a good quality oil, whose value of free acidity and number of peroxides respect that indicated by the Codex Alimentarius [8]. In particular, it is very important to monitor selected environmental parameters, such as temperature, relative humidity, and storage time, as well as to check other parameters specifically related to seeds, i.e., moisture content and presence and percentage of impurities (dockage). These parameters are fundamental to obtain a low value of free acidity of the freshly produced oil (hydrolytic quality) and to avoid oxidation and fermentation or mold, which would also lead to sensory deterioration of the product. In general, hemp seeds are harvested when about 70% are ripe and the average moisture content is around 15-24%. Harvested seeds usually contain up to 15% impurities (green leaves, buds, stems, weed seeds, and foreign materials) and this dockage is often not cleaned in the pre-storage phase, which can last up to a year before processing. Due to the large presence of foreign bodies and the high moisture content at the time of harvest, hemp seeds can easily deteriorate and there can also be a greater presence of contaminating bacteria after harvest. In general, for different crops, in order to reduce storage loss, guidelines for more correct storage have been developed, e.g. rye [23], canola [24,25], durum wheat [26], and pinto beans [26], but, to date, there are no nationally or European guidelines for the correct storage of hemp seeds. Currently, several hemp growers use the guidelines for canola seeds, because hemp seeds have about 30-36% oil content, which is similar to that of some varieties of canola. However, high oil canola contains more than 42% [25,27], while the oil content of hemp seeds is about 36% [7]. In addition, it is necessary to consider that hemp seeds contain 15% of saturated fatty acids, which is lower than that of olive or canola oil, while polyunsaturated fatty acids are about 85% and, as is known, these compounds are easily prone to oxidation [28,29]. Additionally, other compounds, such as proteins, are present in hemp seeds and they are different than those in canola. These differences in the composition of hemp seeds compared to other oil seeds also affects their conservation. Finally, canola seeds usually have less than 5% of foreign bodies, which is lower compared to hemp seeds [30]. The main factors affecting the quality of stored oil seeds are temperature, moisture content or relative humidity, and storage time [29]. It is recommended to control the relative humidity inside the storage tanks, which must be equal to or less than 70% (water activity 0.7) because most fungi, molds, and bacteria do not develop in these conditions of low relative humidity. Seeds stored in lower relative humidity conditions have a longer shelf-life with little deterioration. In fact, when oil seeds are stored at high humidity, unbound (free) water is available for the development and multiplication of microorganisms. Seeds stored at higher temperatures always have higher rates of respiration than those stored at lower temperatures and therefore germinate more easily [31]. When temperatures are below 5°C, most molds do not grow [29]. Therefore, to correctly store hemp seeds temperature and humidity are the main factors to be monitored [23,26,32]. The deterioration can cause damage to the seeds, worsening their quality and, consequently, the quality of the oil produced. In particular, as mentioned before, lipid oxidation and the increase in the concentration of free fatty acids are linked to the deterioration of the seed [30]. Moreover, seed germination is the external biological expression of its physiological, biochemical, and morphological changes [33,34]. For this reason, 20% germination or the presence of visible mold is usually used as a cornerstone of guidelines for the safe storage of oilseeds [35]. In an intact, undamaged, and well-preserved seed, enzymatic hydrolysis does not occur or occurs to a very limited extent. In the oil industry, free acidity is one of the main oil quality parameters. This is because an increase in free acidity indicates an increase in the concentration of free fatty acids in the oil, often resulting from poor conservation of the raw material. Furthermore, these fatty acids, not bound in triglycerides, are more susceptible to oxidation, thus making the oil even more sensitive to oxidative phenomena. Moreover, these changes in the lipid composition determine a degradation of the sensory characteristics and the formation of unpleasant odors, as well as a decrease in its nutritional properties.

A study conducted by Jian and colleagues (2019) [30] showed that hemp seeds can be stored at 30°C for up to 10 weeks, regardless of the percentage of dockage, if the seeds have a moisture content of 9%. In order to store hemp seeds up to 6 months, it is essential to have a moisture content less than 8%.

Moreover, they determined an equation to calculate the storage time:
ɵ=a-b*T-(c-d*T)*MC

Where:
ɵ= is the storage time that does not cause deterioration of the seeds expressed in weeks;
T= temperature (°C);
MC= seeds humidity (%);
a,b,c,d= regression parameters.

This equation indicates that both T and MC have a linear influence on the storage time and the interaction between T and MC also has an influence on the correct storage period of the seeds. The predicted storage times may show a prediction error of approximately 1.14 weeks with respect to the storage times experimentally measured by the authors. Moreover, the method reported by ASTM established a protocol to determine the spoilage in hemp seeds based on their color [36].

3. COLD-PRESSED HEMP SEED OIL COMPOSITION

a. Fatty acid profile

The qualitative and quantitative determination of the total fatty acids defines the characteristic compositional profile of the oil, typical of botanical species, and therefore represent an important parameter of authenticity. Analytical determination is generally carried out by gas chromatography after derivatization of fatty acids in the corresponding stable and volatile methyl ester derivatives, as reported in the following methods: UNI EN ISO 12966 [37], Reg. (EU) 2022/2104 [38], NGD C41-1976 [39], and NGD C42-1976 [40]. Compared to other vegetable oils, HSO has the highest percentage, on average near 80%, of polyunsaturated fatty acids (PUFA) and linoleic acid (18:2 ω-6), with contents ranging from 50% at 70%; it is the most represented one, followed by α-linolenic acid (18: 3 ω-3), with percentages from 15% to 34% and γ-linolenic acid (18:3 ω-6) [7]. Peculiar, as reported in the introduction, is the presence of stearidonic acid (18:4 ω-3) in small amounts, from 0 to 2%. The ratio between the ω-6 and ω-3 series of fatty acids (Table I), currently between 2.5:1 and 3.4:1 in HSO, is perfectly in line with the dietary recommendations expressed by the FAO. According to Reg. (EC) 1924/2006 [13] relating to nutrition and health claims applicable to specific food products, HSO has a fatty acid profile that could bear the following claims: source of ω-3 fatty acids, rich in ω-3 fatty acids, rich in polyunsaturated fats, and rich in unsaturated fats.

b. Sterol composition

Similar to what was mentioned above for the determination of total fatty acids, the qualitative and quantitative profile of the sterol fraction also contained in the unsaponifiable fraction of the hemp seed oil is typical and related to the botanical origin, thus representing a very important parameter that can confirm the purity or authenticity of the product. The sterol determination method involves saponification, extraction of the unsaponifiable fraction, purification, and derivatization before instrumental gas chromatography analysis, as described in the methods indicated in UNI EN ISO 12966 [37], Reg. (EU) 2022/2104 [38] and NGD C 71 - 1989 [48], NGD C 72 – 1989 [49], and AOCS Ch 6-91 [50]. In cold-pressed HSO, total sterol ranges from 3425 to 6719 mg/kg, being β-sitosterol the most represented (70%), followed by campesterol (15%) and δ5-avenasterol (7%) [51,56] (Table II). In contrast to what is mainly cited in the literature, Schlag et al. (2022) [57] reported that the presence of lanosterol, as being not singularly quantifiable due its low concentration and the coelution with β-sitosterol, but, given the contradiction in the quantification (β-sitosterol in the author's table is reported as lanosterol); given that this evidence was not confirmed, the data of Schlang were not included in Table II. In order to compare the sterol profile with other vegetable oils, the qualitative profile of cold-pressed HSO could be similar to that of sesame oil, except for brassicasterol which was found in HSO (but less than 0.2%) only by Kostadinovic et al. (2015) [53]. While the acidic composition can be influenced by genetic manipulations, the sterol composition reports a lower variability, thus having great value in revealing mixtures with oils of different botanical origin or non-compliant, in particular in terms of total amount, as a consequence of refining treatments.

c. Stigmastadienes content

Refining impacts on the minor components in the case of sterols present in the unsaponifiable fraction, especially in the bleaching and deodorization phases, there is the elimination of the alcoholic group in position 3 and the formation of the double bond in position 3,4 with the consequent dehydration and elimination of a water molecule. This leads to the formation of a steradiene hydrocarbon. Cold-pressed vegetable oils, being not refined, should not contain steradiene hydrocarbons (i.e. stigmastadienes). Therefore, this analytical parameter represents an index of refining and/or possible mixture with refined oils to protect the genuineness of cold-pressed oils. The determination of stigmastadienes is carried out by gas chromatography, as described in methods UNI EN ISO 15788-1 [58], Reg. (EU) 2022/2104 [38]. Reg. (EU) 2022/2104 and Reg. (EU) 2022/2105 [59] indicate a limit of ≤0.05 mg/kg referring to extra virgin and virgin olive oils, in "cold-pressed oils" the natural presence of stigmastadienes is not justified and there is no legislation referable to this parameter. Therefore, a limit equal to ≤0.10 mg/kg could represent a reference, as already reported in some industry standards referring to pressure oils [60]. In our laboratory, we analyzed

Table I - Fatty acid composition of cold pressed hemp seed oil (the table indicates different significant digits, in accordance with each original publication)

Fatty acid	Leizer et al. (2000) [19]	Parker et al. (2003) [41]	Dubois et al. (2007) [42]	Callaway (2004) [7]	Tura et al. (2023) [43]	Rovellini et al. (2013) [44]	Siano et al. (2018) [45]	Tura et al. (2022) [46]	Occhiuto et al. (2022) [47]	
							Cv.Fedora	Cv.Futura 75	Cv.Futura 75	Cv.Futura 75
	%	%	%	%	%	%	%	%	%	%
Myristic acid (C14:0)	-	-	-	-	0.03-0.04	0.03	-	-	0.02	0.05
Pentadecanoic acid (C15:0)	-	-	-	-	0.01	0.02	-	-	-	-
Palmitic acid (C16:0)	5-7	6.26	6.3	5	4.95-7.12	6.07	7.15	7.68	6.95	8.67
Palmitoleic acid (C16:1)	-	-	-	-	0.10-0.37	0.14	-	0.14*	0.11	0.11
Heptadecanoic acid (C17:0)	-	-	-	-	0.03-0.05	0.03	-	0.05	0.03	0.05
Stearic acid (C18:0)	1-2	2.72	2.8	2	1.69-2.55	2.38	2.73	2.73	2.68	3.76
Oleic Acid (C18:1)	8-13	11.72	12.1	9	6.87-12.33	10.26	12.75	14.19*	12.31	16.73
Linoleic acid (C18:2)	52-62	59.96	55.9	56	38.48-52.16	55.75	56.08	55.84	56.16	51.39
Alfa-Linolenic acid (C18:3)	12-23	19.33	19.7	22	11.02-17.40	17.37	14.89	16.36	17.74	15.36
Gamma-Linolenic acid C18:3)	3-4	-	2.8	4	0.98-4.43	4.65	3.03	1.10	2.33	2.03
Stearidonic acid (C18:4)	-	-	-	2	0.20-1.50	1.48	-	0.77	0.81	0.56
Arachidic acid (C20:0)	0.39-0.79	-	0.7	-	0.03-0.06	0.87	0.89	0.32	0.50	0.73
Eicosenoic acid (C20:1)	0. 51	-	-	-	-	0.4	0.26	0.37	0.24	0.33
Eicosadienoic acid (C20:2)	-	-	0.8	-	0.03-0.06	-	1.03	-	0.14	0.24
Behenic acid (C22:0)	-	-	0.3	-	0.16-0.29	0.34	0.20	0.30	-	-
Erucic Acid (C22:1)	-	-	-	-	0.01	0.03	-	-	-	-
Lignoceric acid (C24:0)	-	-	-	-	0.07-0.13	0.17	-	0.15	-	-
Σ-SFA		8.98		7	6.89-9.47	9.86	10.97	11.23	9.68	12.53
Σ-MUFA		11.72		9	6.97-20.84	10.91	13.01	14.70	12.65	17.17
Σ-PUFA		79.29	79.1	84	59.11-70.38	79.25	75.03	74.07	77.67	70.31
Σ-PUFA/ Σ-SFA							6.84			
ω6		59.96	59.4	60		60.40			59.12	54.39
ω3		19.33	19.7	24		18.85			18.55	15.91
ω6/ω3			2.8	2.5	2.60-3.67	3.20		3.32	3.19	3.42

*Sum of the isomers

Table II – Sterol composition of cold pressed HSO. The table shows different expression of quantitative data and significant digits, in accordance with each original publication. Moreover, a conversion was made for all the reported data in mg/kg, apart for Rovellini et al. (2013) [44], for which the relative % was left. Data of Monserrat de la Paz et al. (2014) [51] are referred to a refined oil.

Sterol	**Matthäus & Brühl (2008) [52]**		**Rovellini et al. (2013) [44]**	**Monserrat de la Paz et al. (2014) [51]**	**Kostadinovic et al. (2015) [53]**	**Siano et al. (2018) [45]**	**Blasi et al. (2022) [54]**	**Gutièrrez Luna et al. (2022) [55]**
	average	**range**				**Cv.Fedora**		
	(mg/kg)	(mg/kg)	Relative%	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Cholesterol	34	22-74	0.3	0.92	14.78		8.60-13.03	-
Brassicasterol	-	-	-	-	1.72	-	-	-
24-metylencholesterol	40	25-81	0.7	7.05	29.57		-	-
Campesterol	709	257-1001	16	505.69	504.5	117.4	345.1-813.8	365.3
Campestanol	11	0-19	0.3	-	52.96		17.79-50.52	-
Stigmasterol	133	97-181	2.2	100.23	90.10	28.2	50-247.5	43.6
d7-Champesterol	26	12-39	0.6	-	33.36		-	-
d5 23 Stigmastadienol	-	-	-	-	-		-	-
Chlerosterol	36	23-74	0.9	58.39	21.32		10.43-52.19	-
β-Sitosterol	3191	2704-4434	67.8	1905.07	2311.35	530.4	1510-4010	2753.6
Sitostanol	76	39-127	0.9	-	52.96		19.77-67.60	-
d5 Avenasterol	336	209-572	7.4	142.80	219.06	72.6	142.56-528.44	243.3
d7-9(11)Stigmastadienol	-	-	-	-	-		-	-
d5-24Stigmastadienol	54	36-65	1.1	31.97	11.35		16.77-70.90	-
d7-Stigmasterol	30	19-47	-	21.74			-	-
d7-Stigmastenol	-	-	0.7	-	53.30		26.77-180.12	-
d7-Avenastrerol	51	32-79	1.1	19.87	28.89		42.96-93.02	27.0
Total sterols	**Matthäus & Brühl (2008) [52]**		**Rovellini et al. (2013) [44]**	**Monserrat de la Paz et al. (2014) [51]**	**Kostadinovic et al. (2015) [53]**	**Siano et al. (2018) [45]**	**Blasi et al. (2022) [54]**	**Gutièrrez Luna et al. (2022) [55]**
	average	**range**				**Cv.Fedora**		
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)		(mg/kg)
		4727,00	3922-6719	4393	2793.73	3425,22	-	2199-5891

the stigmastadienes in commercial cold-pressed HSO and found values around 0.09-0.14 mg/kg (data not published).

d. Tocopherols composition

Vegetable oils contain the presence of tocopherols and their corresponding tocotrienols which differ in the unsaturated lateral racemic chain. The various botanical species are characterized by the presence of almost all eight molecular species. The presence of these compounds is important as they have a strong antioxidant activity by acting as a "scavenger" against lipid oxidation. Tocopherols, in particular α-tocopherol, possess vitamin activity, thus further enhancing the nutritional value of the oily matrix. The analytical determination of tocopherols and tocotrienols is performed by applying chromatographic methods as indicated in UNI EN ISO 9936 [61], UNI/TS 11825 [62].The literature highlights that in cold-pressed HSO the most represented tocopherol is γ-tocopherol with content from 625.3 to 924.5 mg/kg, followed by α-tocopherol with 17-77.6 mg/kg, δ-tocopherol with 25-40.2 mg/kg, and β-tocopherol with 0.1-5.8 mg/kg (Table III). Liang et al. (2015) [56] reported that total tocopherols range from 800 to 1500 mg/kg with 85-91% represented by γ-tocopherol. Tocotrienols are not present, and only Blasi et al. (2022) [54] reported the presence of α-tocotrienol in some samples. However, there are also very distant values in the literature such as those reported by Siano et al. (2019) [45] who found in the Fedeora cultivar very low contents of α-tocopherol and γ-tocopherol equal to 2.7 and 5.0 mg/kg, respectively. The quali-quantitative determination of tocopherols is not a regulated parameter by national or international law for this oil. However, it represents an indicator of genuineness as it is known that the oils obtained from cold pressing keep the contents of the bioactive constituents almost unchanged; while refining treatments, especially due to temperatures reached of 110-120°C, determine their degradation. Thus, the presence of tocopherols in concentrations not in line with the characteristic profile can be attributable to refining or mixing treatments of different or refined oils. Furthermore, given that the presence of a profile in predominantly unsaturated fatty acids in the oily matrix causes a lack of stability, it is very important to preserve, during all stages of production, all the components that exert a strong antioxidant activity to extend the shelf-life of the product and maintain its nutritional properties.

e. Free acidity

This determination provides a measure of free organic acidity, highlighting the level of hydrolytic degradation. The analysis is carried out by titration as indicated in the methods described in UNI EN ISO 660 [63], Reg. (EU) 2022/2104 [38], NGD C 10 -1976 [64], and AOCS Cd 3d-63(03) [65]. The oil dissolved in a suitable solvent is titrated with a strong base in the presence of indicator. The result is expressed as an acid number or the mg of KOH necessary to neutralize the fatty acids present in 1 gram of oil. It is also possible to express the value as a percentage of fatty acid, usually the most representative of the matrix, e.g. oleic acid, palmitic acid. The Codex Alimentarius establishes an acidity limit of 4.0 mg KOH/g oil for cold-pressed and virgin oils [8]. The results reported in the literature are unexpected and show that this parameter, despite having a well-defined legal limit, is often widely exceeded (Table IV). Spano et al. (2020) [67] found that 3 of the 9 oils analyzed had contents beyond the limit, and Calzolari et al. (2021) [68] found 33 of 45 oils had values over the limit, reaching out-of-control results with values up to 17.24% if expressed as oleic acid. This parameter represents an evident criticality in the production of HSO. The consequence of these results can be attributable to critical aspects present in the production chain. Of primary importance is the good quality of the seeds, which must be harvested at the right degree of ripeness and humidity, well preserved and intact, in order to avoid, due to the presence of water or enzymatic substances deriving from the breakage of the seed, the triggering of unwanted reactions. During the ex-

Table III – Tocopherol composition of cold-pressed hemp seed oil (in the table different significant digits are indicated in accordance with what reported the authors and for uniformity of the results in the table all values have been converted into mg/kg).

Tocopherol	Rovellini et al. (2013) [44]	Kostadinovic et al. (2015) [53]	Siano et al. (2018) [45]	Blasi et al. (2020) [54]	Tura et al. (2022) [46]	Occhiuto et al. (2022) [47]	Tura et al. (2023) [43]
			Cv.Fedora		Cv.Futura 75		
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
α-tocopherol	37	17	2.7	3.5-77.6	38.73	39.2-47.7	14.6-53
β-tocopherol		2	-	3.7-5.8	-	0.1-2.1	-
ɣ-tocopherol	858(*)	649	5.0	625.3-1013.2	794.66	774.3-924.5	594-967
δ-tocopherol	33	25	-	14.0-35.1	29.22	3.2-40.2	19.6-50.3
Total (mg/kg)	928	697	-	655.0-111.08	-	816.9-1014.5	-

(*) β-tocopherol + ɣ-tocopherol

Table IV – Free acidity (different significant digits are indicated in accordance with what reported by the authors).

	(% Oleic acid)	(mg KOH/g)
Rovellini et al. (2013) [44]	0.49	
Tura et al. (2022) [46]		1.78
Occhiuto et al. (2022) [47]	0.58-0.65	
Kostadinovic et al. (2015) [53]	0.91	
Mikulcovà et al. (2017) [66]		0.7
Spano et al. (2020) [67]	0.40-17.24	
Calzolari et al. (2021) [68]	0.81-16.69	
Tura et al. (2023) [43]	0.45-2.31	0.89-4.58

traction phase, due to the high pressure exerted, a rise in temperature inevitably occurs, which can exceed 70°C. These reactions and thermal stress cause the triggering of hydrolytic degradation and its consequent evolution. Compliance with this parameter can be improved through careful selection and storage of seeds, followed by temperature control during the extraction process.

f. Peroxide value

The determination of the peroxide value (PV) expresses the degree of oxidative degradation of the oil. Oxidative rancidity is a phenomenon that mainly affects unsaturated fats and is strongly catalyzed by the action of light which acts, in the presence of oxygen, on the double bonds to form radical compounds that trigger reactions and lead to the formation of hydroperoxides, aldehydes, ketones, and short-chain carboxylic acids that give the characteristic rancid scent. The analysis is carried out through iodometric titration as indicated in the methods described in UNI EN ISO 3960 [69], Reg. (EU) 2022/2104 [38], NGD C 35 -1976 [70], and AOCS Cd 8b-90(03) [71]. Hydroperoxides, being primary oxidation products, react with a potassium iodide solution, and the titration of the developed iodine indirectly reports the concentration of the hydroperoxide content. The result is expressed as milliequivalents of active oxygen per kilogram of oil mEq O_2/kg. The Codex Alimentarius establishes a peroxide number limit of 15 mEq O_2/kg oil for cold-pressed and virgin oil. In the literature, PV values for HSO have ranged from 1.55 to 28.2 (mEq O_2/kg) [43,44,46,47,53,67], while Piskernik et al. (2021) [72] reported values between 23.7 and 77.2 (mmol/kg). Similar to what reported for the acidity parameter, for PV several authors also found values higher than the legal limit in the cold-pressed HSO sold on the market. Also in this case, all the critical points mentioned above (paragraph 2.5) contribute to triggering the oxidative phenomenon by promoting the formation of hydroperoxides in the presence of oxygen. It is possible to find very low peroxide values even in the presence of a marked manifest rancidity when the kinetics of the reaction has already evolved to the formation of secondary oxidation products, and in particular aldehydes and ketones. For this purpose, in order to have a accurate evaluation of the oxidative state of the oil matrix, it is advisable to consider the parameter of p-anisidine number. This could prove useful to evaluate the resistance to forced oxidation using Rancimat, not as a reference parameter, but as a useful element for producers and packers in order to not reduce shelf-life expectations too much.

g. Phytocannabinoid content

In HSO it is possible to find measurable quantities of Δ^9-THC resulting from the contact of the seeds with the bracts and leaves of the inflorescence. In oils that undergo refining processes, Δ^9-THC is removed, while for oils obtained by cold pressing this does not happen. Therefore, in order to keep Δ^9-THC levels below the legal limits, it is essential to clean the seeds. In fact, setting up a washing process of the seeds before extraction of the oil allows producing an oil with low amounts of THC, removing this cannabinoid from the surface of the seeds before pressing them. Furthermore, considering that the varieties of hemp admitted to cultivation (Common Catalog of Varieties of Agricultural Plant Species) are characterized by a low Δ^9-THC content, the risks of a cold-pressed HSO with a high content of these cannabinoids are reduced [73]. However, it is essential during quality control of cold-pressed HSO to monitor the cannabinoid content, in particular in relation to Δ^9-THC and tetrahydrocannabinolic acid (THCA). The method for the determination of Δ^9-THC, its precursors, and other cannabinoid compounds in food products containing hemp is described in EU Recommendation No. 2115/2016 [74], which provides for the chromatographic separation technique coupled with mass spectrometry (LC-MS or GC-MS) after purification treatment [liquid-liquid extraction (LLE) or solid phase extraction (SPE)]. However, given that not all laboratories have such sophisticated and expensive instrumentation, which also requires high professionalism for use, many validations of alternative methods that exploit GC-FID and HPLC techniques have been published. The German Pharmacopoeia has also adopted a Δ^9-THC quantification method based on extraction with solvent ethanol, followed by liquid chromatography with UV detector. Through these methods, it is possible to carry out the separation and quali-quantitative evaluation of all the main phytocannabinoids present. Reg.(UE) 1393/2022 defined a limit for the presence of Δ^9-THC in HSO equal to a maximum of 7.5 mg/kg, which is intended as the sum of the concentrations of the trans-Δ^9-THC substance and its inactive acid precursor (Δ^9-THCA-A). The literature highlights the presence of cannabinoids in HSO, and the most represented compounds are CBDA, CBD, Δ^9-THC, and Δ^9-THCA. Some authors have also indicated the presence of CBDA, CBG and CBN [15] (Table V). Regarding cannabinoid exposure assessment, in 2015 EFSA [76] established an acute reference dose (ARfD) for Δ^9-THC of 1 µg/kg body

Table V – Cannabinoid composition of cold-pressed hemp seed oil (different significant digits are indicated in accordance with what reported the authors and for uniformity of the results in the table all values have been converted into mg/kg).

Cannabinoids	Tura et al. (2023) [43]	Leizer (2000) [19]	Matthaus & Brühl (2008) [52]	Citti et al. (2018) [15]	Nigro et al. (2022) [75]
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
CBDA	4.25-91.6	-	-	<LOQ 821.1	180
CBD	ND-22.2	10	-	<LOD - 1056	
CBDV	-	-	-	<LOQ - 75.39	
CBG	-	-	-	<LOD - 1.381	
CBN	-	-	-	<LOQ -12.05	
Δ^9-THC	ND-5.29	ND	11-117	<LOD -1.804	
Δ^9-THCA	ND-5.0	-	-	<LOQ - 9.462	
Δ^9-THC+Δ^9-THCA	ND-10.30	-	-	-	

weight. Subsequently, the BfR (Federal Institute for Consumer Health Protection and Veterinary Medicine, 2021) [77] suggested a case-by-case toxicological assessment of foods containing HSO based on the ARfD. In 2020, in a scientific report from EFSA, for the scenario on HSO, [78], acute exposure to Δ^9-THC was estimated down, highlighting that for "high consumers" acute exposure to total Δ^9-THC in adults ranged from 3 to 21 µg/kg of body weight and in "other children" from 7 to 59 µg/kg of body weight. In the same report, the EFSA also recommended carrying out studies on the stability of Δ^9-THC and on the conversion of Δ^9-THCA during food processing (e.g. cooking). Nonetheless, with the limit of Δ^9-THC currently fixed at 7.5 mg/kg in HSO and assuming the consumption of an oil that reaches this limit, the maximum amount of this oil for a person weighing 75 kg would be 10 g/day. Kladar et al. (2021) [73] highlighted that cold-pressed HSO were the second most Δ^9-THC containing food product group and Steinmetz et al. (2022) [79] that only 4 of 102 HSOs samples analyzed had a low to moderate risk of inducing harm through typical dietary exposure to Δ^9-THC. However, assuming a reasonable usage equal to 38.21 g of HSO and 25.37 g of cold-pressed HSO (corresponding to 41.2 and 27.4 ml, respectively) an oil could be consumed daily without significant concern. The present scenario indicates that further investigation of Δ^9-THC in HSOs, guidelines for its production, and compliance with legal limits will be essential to protect consumer health and promote commercialization of a safe product, uniform in quality. Moreover, the European Commission has established that cannabidiol (CBD) can be considered as a novel food, although due to the significant uncertainties and data gaps related to certain effects on humans, the panel concluded that the safety of CBD as a novel food cannot be currently established.

h. Color

The evaluation of oil color is usually carried out by measurement of CIELab color space parameters, i.e. the color coordinates a* and b* and the psychometric index of lightness L*. These color parameters are frequently used as an index of oil quality. There is no reference legislation to define the color of seed oils, and the guidelines of the Codex Alimentarius (2021) [80] indicate that the color, odor, and taste of each product shall be characteristic of the designated product. The color of the oils mainly depends on the presence of two pigments, carotenoids and chlorophylls [81,82]. Their concentration in the raw material depends on the variety, degree of ripening of seeds, and climatic features during plant growth. Stress damage and senescence also affect their content [83]. Cold-pressed HSO has a color that varies from light green to very intense green. This color is a consequence of the considerable quantities of pigments, and in particular chlorophylls which are co-extracted during pressing. On the other hand, some authors reported that the the color of HSO can be yellow or green [84,85] in relation to the content of chlorophylls [86]. It is essential to highlight that several studies have reported that HSO contains a large amount of chlorophylls that can affect oxidative stability and lead to rancidity [15]. These natural pigments act as powerful prooxidants, increasing the susceptibility to photo-oxidation of the oils when exposed to light and promoting change from the intensive dark green color to yellow [83]. A recent study carried out on two cold-pressed HSO obtained from fresh and stored seeds of the Henola cultivar showed significant differences relating to the measurement of color; in particular, the parameter a* showed correlation between storage time of the seeds and a decrease in green color, precisely in line with the oxidation of chlorophyll [87]. The concentration of these pigments in the finished product, on the other hand, is mainly affected by the storage conditions; the oxidative and degradative processes that the oils undergo during storage alter the content of carotenoids and chlorophylls. Recent studies have shown that these compounds can be

considered as quality indicators for the finished product [88]. In addition, Matthäus & Brühl (2008) [52] reported that high-quality virgin HSO is distinguished by a light to dark green color, highlighting that the color turns yellow during storage. Moreover, previous published scientific papers showed that the value of yellowness and lightness of HSO increased during storage, which were related to changes in the color of oils during storage and effective indicators of difference in quality [89]. Several authors have investigated the color of HSO, in particular in relation to different pre-treatment on seeds (such as roasting of the seeds) [90], oil extraction process (e.g. ultrasound-assisted, supercritical extraction, etc.) [91,92], or oil oxidative stability (e.g. addition of essential oils to hemp seed oil) [89]. Moreover, color represents one of the most immediate sensory properties of food and is decisive in the consumers' choice; also, for this reason, the possibility of measuring color objectively is of great importance [88,93].

4. CONCLUSION

In this review, the aspects of quality and authenticity of cold-pressed HSO have been overviewed. The analytical parameters of acidity, number of peroxides, fatty acids, tocopherols, sterols, stigmastadienes, and phyto-cannabinoids represent a fundamental reference for their definition. It has been shown that many cold-pressed HSO exceed the acidity value foreseen by the Codex Alimentarius (4 mg KOH/g of oil), which is due to incorrect conservation of seeds or processing and present a detectable amount of Δ^9-THC. With reference to the latter parameter, the new legal limits recently introduced (7.5 mg/kg of oil) mitigate the concern for regulatory compliance in the market, but careful monitoring and updated research will be always necessary to guarantee any safety aspect. The data collected in this review have been used to draw up the UNI 11876:2022 standard, which represents a valid reference for those who produce and sell HSO. This information can be relevant to take care of any technological phase of production, and in particular the harvesting, storage, and extraction phase of seeds in order to preserve the sensory and nutritional characteristics of the final oil and to obtain high and harmonized quality on the market.

BIBLIOGRAPHY

[1] Legge 2 Dicembre 2016, n.242. Disposizioni per la Promozione della Coltivazione e della Filiera Agroindustriale della Canapa (16G00258), GU Serie Generale n. 304 del 30-12-2016.

[2] ISTAT, Dataset : Coltivazioni Italia, available online here http://dati.istat.it/Index.aspx?QueryId=33707

[3] COUNCIL DIRECTIVE 2002/53/EC of 13 June 2002 on the common catalogue of varieties of agricultural plant species

[4] INFORMATION FROM EUROPEAN UNION INSTITUTIONS, BODIES, OFFICES AND AGENCIES. EUROPEAN COMMISSION, COMMON CATALOGUE OF VARIETIES OF AGRICULTURAL PLANT SPECIES Supplement 2021/6 (Text with EEA relevance) (2021/C 211/01)

[5] Decreto del Ministero della Salute 4 novembre 2019. Definizione di livelli massimi di tetraidrocannabinolo (THC) negli alimenti (20A00016), GU n.11 del 15-1-2020.

[6] COMMISSION REGULATION (EU) 2022/1393 of 11 August 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of delta-9-tetrahydrocannabinol (Δ9-THC) in hemp seeds and products derived therefrom.

[7] J.C. Callaway, Hempseed as a Nutritional Resource: An Overview. Euphytica, 140, 65-72, (2004)

[8] Codex Alimentarius, "Standard for named vegetable oils", Codex Stan 210-1999. Amended in 2005, 2011, 2013, 2015, 2019, 2021.

[9] M.M.Özcan, K. Ghafoor, F. Al Juhaimi, I.A.M. Ahmed, E.E. Babiker, Effect of Cold-Press and Soxhlet Extraction on Fatty Acids, Tocopherols and Sterol Contents of the Moringa Seed Oils. South African Journal of Botany, 124, 333-337, (2019)

[10] M. Tura, M. Mandrioli, E. Valli, C. Dinnella, T. Gallina Toschi, Sensory wheel and lexicon for the description of cold-pressed hemp seed oil. Foods, 12, 661, (2023)

[11] M.Y. Abeywardena, M. Adams, J. Dallimore, S.M. Kitessa, Rise in DPA Following SDA-Rich Dietary Echium Oil Less Effective in Affording Anti-Arrhythmic Actions Compared to High DHA Levels Achieved with Fish Oil in Sprague-Dawley Rats. Nutrients, 8, (2016)

[12] M. Mir, Echium Oil: A Valuable Source of n-3 and n-6 Fatty Acids. OCL - Oleagineux Corps Gras Lipides, 15, 252-256, (2008)

[13] REGULATION (EC) No 1924/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 20 December 2006 on nutrition and health claims made on foods.

[14] K. Ning, C. Hou, X. Wei, Y. Zhou, S. Zhang, Y. Chen, H. Yu, L. Dong, S. Chen, Metabolomics Analysis Revealed the Characteristic Metabolites of Hemp Seeds Varieties and Metabolites Responsible for Antioxidant Properties. Frontiers in Plant Science, 13, (2022)

[15] C. Citti, B. Pacchetti, M.A. Vandelli, F. Forni, G. Cannazza, Analysis of Cannabinoids in Commercial Hemp Seed Oil and Decarboxylation Kinetics Studies of Cannabidiolic Acid (CBDA). Journal of Pharmaceutical and Biomedical Analysis, 149, 532–540, (2018)

[16] M. Abdollahi, F. Sefidkon, A. Peirovi, M. Calagari, A. Mousavi, Assessment of the Cannabi-

noid Content from Different Varieties of Cannabis Sativa L. during the Growth Stages in Three Regions. Chemistry and Biodiversity, 18, (2021)
[17] E. Small, S.G.U. Naraine, Size Matters: Evolution of Large Drug-Secreting Resin Glands in Elite Pharmaceutical Strains of Cannabis Sativa (Marijuana). Genetic Resources and Crop Evolution, 63, 349-359, (2016)
[18] F. Grotenhermen, M. Karus, D. Lohmeyer, THC-Limits for Food: A Scientific Study. Journal of the International Hemp Association, 5, 101-105 (1998)
[19] C. Leizer, D.M. Ribnicky, A. Poulev, D. Dushenkov, I. Raskin, The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition. Journal of Nutraceuticals, Functional and Medical Foods, 2, 35-53,(2000)
[20] C. Alasalvar, S.K. Chang, B. Bolling, W.Y. Oh, F. Shahidi, Specialty Seeds: Nutrients, Bioactives, Bioavailability, and Health Benefits: A Comprehensive Review. Comprehensive Reviews in Food Science and Food Safety, 20, 2382-2427, (2021)
[21] T. Gallina Toschi, A. Bendini, J. Lozano-Sánchez, A. Segura-Carretero, L. Conte, Misdescription of edible oils: Flowcharts of analytical choices in a forensic view. European Journal of Lipid Science and Technology, 115(11), 1205-1223, (2013)
[22] UNI 11876:2022, "Oli e grassi vegetali ed animali e derivati - Olio di canapa di spremitura a freddo, ottenuto dai semi di Cannabis sativa L. - Caratteristiche e metodi di analisi" (2022)
[23] G. Sathya, D.S. Jayas, N.D.G. White, Safe Storage Guidelines for Rye. Canadian Biosystems Engineering / Le Genie des biosystems au Canada, 50, 1-8, (2008)
[24] G. Sathya, D.S. Jayas, N.D.G. Safe Storage Guidelines for Canola as the Seeds Slowly Dry. Canadian Biosystems Engineering / Le Genie des biosystems au Canada, 51, (2009)
[25] K. Sun, F. Jian, D.S. Jayas, N.D.G. White, Quality Changes in High and Low Oil Content Canola during Storage: Part I - Safe Storage Time under Constant Temperatures. Journal of Stored Products Research, 59, 320-327, (2014)
[26] U. Nithya, V. Chelladurai, D.S. Jayas, N.D.G. White, Safe Storage Guidelines for Durum Wheat. Journal of Stored Products Research, 47, 328-333, (2011)
[27] F. Jian, D. Divagar, J. Mhaiki, D.S. Jayas, P.G. Fields, N.D.G. White, Static and Dynamic Methods to Determine Adsorption Isotherms of Hemp Seed (Cannabis Sativa L.) with Different Percentages of Dockage. Food Science and Nutrition, 6, 1629-1640, (2018)
[28] N. Izquierdo, R. Benech-Arnold, D. Batlla, R.G. Belo, J. Tognetti, Seed Composition in Oil Crops. Oilseed Crops, 34-51, (2017)
[29] J. Singh, S. Paroha, R.P. Mishra, Factors Affecting Oilseed Quality during Storage with Special Reference to Soybean (Glycine Max) and Niger (Guizotia Abyssinica) Seeds. International Journal of Current Microbiology and Applied Sciences, 6, 2215-2226, (2017)
[30] F. Jian, M.A. Al Mamun, N.D.G. White, D.S. Jayas, P.G. Fields, J. McCombe, Safe Storage Times of FINOLA® Hemp (Cannabis Sativa) Seeds with Dockage. Journal of Stored Products Research, 83, 34-43, (2019)
[31] F. Jian, D.S. Jayas, Understanding the Initiation and Development Hotspots in Storage-Grain Ecosystems, 25, 1-10, (2014)
[32] P.R. Rani, V. Chelladurai, D.S. Jayas, N.D.G. White, C.V. Kavitha-Abirami, Storage Studies on Pinto Beans under Different Moisture Contents and Temperature Regimes. Journal of Stored Products Research, 52, 78-85, (2013)
[33] S.A. Al-Yahya, Effect of Storage Conditions on Germination in Wheat. Journal of Agronomy and Crop Science, 186, 273-279, (2001)
[34] A.A. Kandil, A.E. Sharief, M.S. Sheteiwy, Effect of Seed Storage Periods, Conditions and Materials on Seed Quality of Some Soybean Cultivars. International Journal of Agriculture Sciences, 5, 339-346, (2013)
[35] N. White, R. Sinha, W. Muir, Intergranular carbon dioxide as an indicator of biological activity associated with the spoilage of stored. Canadian Agricultural Engineering, 24(1), (1982)
[36] ASTM, Designation: D8400-21, Standard Guide for Assessing Spoilage of Hemp Seed Intended for Human. 6-9, (2022)
[37] UNI EN ISO 12966-2:2017, Oli e grassi animali e vegetali - Gas cromatografia di esteri metilici di acidi grassi - Parte 2: Preparazione di esteri metilici di acidi grassi.
[38] COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards marketing standards for olive oil, and repealing Commission Regulation (EEC) No 2568/91 and Commission Implementing Regulation (EU) No 29/2012.
[39] NGD C 41-76. Preparazione degli esteri metilici di acidi grassi (da impiegarsi nelle analisi gascromatografiche)
[40] NGD C 42-76. Determinazione della composizione degli acidi grassi mediante analisi gascromatografica
[41] T.D. Parker, D.A. Adams, K. Zhou, M. Harris, L. Yu, Fatty Acid Composition and Oxidative Stability of Cold-Pressed Edible Seed Oils. Journal of Food Science, 68, 1240-1243, (2003)
[42] V. Dubois, S. Breton, M. Linder, J. Fanni, M. Parmentier, Fatty Acid Profiles of 80 Vegetable Oils with Regard to Their Nutritional Potential. Euro-

pean Journal of Lipid Science and Technology, 109, 710-732, (2007)
[43] M. Tura, M. Mandrioli, E. Valli, T. Gallina Toschi, Quality Indexes and Composition of 13 Commercial Hemp Seed Oils. Journal of Food Composition and Analysis, 117, 105112, (2023)
[44] P. Rovellini, L. Folegatti, D. Baglio, S. De Cesarei, P. Fusari, S. Venturini, A. Cavalieri, Caratterizzazione Chimica Dell'olio Ottenuto Dalla Spremitura a Freddo Dei Semi Di Cannabis Sativa L. Riv. Ital. Sostanze Grasse, 90, 139-152, (2013)
[45] F. Siano, S. Moccia, G. Picariello, G.L. Russo, G. Sorrentino, M. Di Stasio, F. La Cara, M.G. Volpe, Comparative Study of Chemical, Biochemical Characteristic and ATR-FTIR Analysis of Seeds, Oil and Flour of the Edible Fedora Cultivar Hemp (Cannabis Sativa L.). Molecules, 24, 1-13, (2019)
[46] M. Tura, M. Mandrioli, E. Valli, R.C. Rubino, D. Parentela, T. Gallina Toschi, T. Changes in the Composition of a Cold-Pressed Hemp Seed Oil during Three Months of Storage. Journal of Food Composition and Analysis, 106, 104270, (2022)
[47] C. Occhiuto, G. Aliberto, M. Ingegneri, D. Trombetta, C. Circosta, A. Smeriglio, Antioxidant Activity of Two Hempseed Oils and Their Byproducts after Cold Pressing. Molecules, 27, 3431, (2022)
[48] NGD C 71-89. Determinazione della composizione degli steroli mediante gascromatografia con colonna capillare
[49] NGD C 72-89. Determinazione del contenuto di steroli mediante gascromatografia con colonna capillare
[50] AOCS Ch 6-91 Determination of sterol composition and content.
[51] S. Montserrat-De La Paz, F. Marín-Aguilar, M.D. García-Giménez, M.A. Fernández-Arche, Hemp (Cannabis Sativa L.) Seed Oil: Analytical and Phytochemical Characterization of the Unsaponifiable Fraction. Journal of Agricultural and Food Chemistry, 62, (2014)
[52] B. Matthäus, L. Brühl, Virgin Hemp Seed Oil: An Interesting Niche Product. European Journal of Lipid Science and Technology, 110, 655-661, (2008)
[53] S. Kostadinović Veličkovska, L. Brühl, S. Mitrev, H. Mirhosseini, B. Matthäus, Quality Evaluation of Cold-Pressed Edible Oils from Macedonia. European Journal of Lipid Science and Technology, 117, 2023–2035, (2015)
[54] F. Blasi, C. Tringaniello, G. Verducci, L. Cossignani, Bioactive Minor Components of Italian and Extra-European Hemp Seed Oils. Lwt, 158, 113167, (2022)
[55] K. Gutiérrez-Luna, D. Ansorena, I. Astiasarán, Fatty Acid Profile, Sterols, and Squalene Content Comparison between Two Conventional (Olive Oil and Linseed Oil) and Three Non-Conventional Vegetable Oils (Echium Oil, Hempseed Oil, and Moringa Oil). Journal of Food Science, 87, 1489-1499, (2022)
[56] J. Liang, A. Appukuttan Aachary, U.T. Holladder, Hemp Seed Oil: Minor Components and Oil Quality. Lipid Technology, 27, 231-233, (2015)
[57] S. Schlag, Y. Huang, W. Vetter, GC/EI-MS Method for the Determination of Phytosterols in Vegetable Oils. Analytical and Bioanalytical Chemistry, 414, 1061-1071, (2022)
[58] EN ISO 15788-1:2001. Animal and vegetable fats and oils - Determination of stigmastadienes in vegetable oils - Part 1: Method using capillary-column gas chromatography (Reference method) (ISO 15788-1:1999).
[59] COMMISSION IMPLEMENTING REGULATION (EU) 2022/2105 of 29 July 2022 laying down rules on conformity checks of marketing standards for olive oil and methods of analysis of the characteristics of olive oil.
[60] P. Bondioli, Oli di semi di spremitura: nuovi orizzonti alla luce delle recenti variazini normative. Innovhub-SSI-SSOG, Convegno AITA Oli e Grassi nell'Industria Alimentare Milano, 18 Febbraio 2020.
[61] ISO 9936:2016. Animal and vegetable fats and oils — Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography.
[62] UNI/TS 11825:2021. Oli e grassi vegetali - Determinazione dei Tocoferoli e Tocotrienoli mediante cromatografia liquida ad alta pressione in fase inversa (RP-HPLC) e detector fluorimetrico.
[63] UNI EN ISO 660:2020. Oli e grassi animali e vegetali - Determinazione del numero di acidità e dell'acidità.
[64] NGD C 10 -1976. Acidità.
[65] AOCS Cd 3d-63(03) Determination of acidity
[66] V. Mikulcová, V. Kašpárková, P. Humpolíček, L. Buňková, Formulation, Characterization and Properties of Hemp Seed Oil and Its Emulsions. Molecules, 22, 1-13, (2017)
[67] M. Spano, G. Di Matteo, M. Rapa, S. Ciano, C. Ingallina, S. Cesa, L. Menghini, S. Carradori, A.M. Giusti, A. Di Sotto, S. Di Giacomo, A.P. Sobolev, G. Vinci, L. Mannina, Commercial Hemp Seed Oils: A Multimethodological Characterization. Applied Sciences (Switzerland), 10, 1-15, (2020)
[68] D. Calzolari, G. Rocchetti, L. Lucini, S. Amaducci, The Variety, Terroir, and Harvest Types Affect the Yield and the Phenolic and Sterolic Profiles of Hemp Seed Oil. Food Research International, 142, 110212, (2021)
[69] ISO 3960:2017. Animal and vegetable fats and oils - Determination of peroxide value - Iodometric (visual) endpoint determination.
[70] NGD C 35 -1976. Determinazione del numero

di perossidi.
[71] AOCS Cd 8b-90(03) Determination of Peroxide Value.
[72] S. Piskernik, A. Levart, M. Korošec, K. Perme, J. Salobir, T.P. Žontar, Fatty Acid Profiles, Nutritional Quality and Sensory Characteristics of Unconventional Oils and Fats on the Slovenian Market. Journal of Food and Nutrition Research, 60, 373–383. (2021)
[73] N. Kladar, B.S. Čonić, B. Božin, L. Torović, European Hemp-Based Food Products – Health Concerning Cannabinoids Exposure Assessment. Food Control, 129, (2021)
[74] COMMISSION RECOMMENDATION (EU) 2016/2115 of 1 December 2016 on the monitoring of the presence of Δ9-tetrahydrocannabinol, its precursors and other cannabinoids in food.
[75] E. Nigro, M.T. Pecoraro, M. Formato, S. Piccolella, S. Ragucci, M. Mallardo, R. Russo, A. Maro, A. Di Daniele, S. Pacifico, Cannabidiolic Acid in Hemp Seed Oil Table Spoon and Beyond. (2022)
[76] Scientific Opinion on the Risks for Human Health Related to the Presence of Tetrahydrocannabinol (THC) in Milk and Other Food of Animal Origin. EFSA Journal 2015, 13, 4141, doi:10.2903/j.efsa.2015.4141.
[77] BfR. Opinion No. 006/2021 issued 17 February 2021. The BfR Recommends Acute Reference Dose as Basis for Assessing Hemp-Containing Foodstuff; Bundesinstitut für Risikobewertung (BfR): Berlin, Germany, 2021.
[78] EFSA Journal 2020;18(1):5953 Acute human exposure assessment to tetrahydrocannabinol (Δ9-THC) https://doi.org/10.2903/j.efsa.2020.5953
[79] F.P. Steinmetz, G. Nahler, J.C. Wakefield, How safe are hemp-based food products? A review and risk assessment of analytical data from Germany. Nutrition & Food Science, (ahead-of-print), (2022)
[80] FSANZ Food Standards Code. Food Standards Australia New Zealand 2016, 2003.
[81] A. Subratti, L.J. Lalgee, N.K. Jalsa, Liquified Dimethyl Ether (DME): A Green Solvent for the Extraction of Hemp (Cannabis Sativa L.) Seed Oil. Sustainable Chemistry and Pharmacy, 12, (2019)
[82] F. Ramos-Escudero, M.L. González-Miret, A. Viñas-Ospino, M. Ramos Escudero, Quality, Stability, Carotenoids and Chromatic Parameters of Commercial Sacha Inchi Oil Originating from Peruvian Cultivars. Journal of Food Science and Technology, 56, 4901-4910, (2019)
[83] L. Izzo, S. Pacifico, S. Piccolella, L. Castaldo, A. Narváez, M. Grosso, A. Ritieni, Chemical Analysis of Minor Bioactive Components and Cannabidiolic Acid in Commercial Hemp Seed Oil. Molecules, 25, (2020)
[84] S.S. Borhade, Chemical Composition and Characterization of Hemp (Cannabis sativa) Seed oil and essential fatty acids by HPLC Method. Archives of applied science research, 5(1), 5-8. (2013)
[85] B.D. Oomah, M. Busson, D.V. Godfrey, J.C. Drover, Characteristics of hemp (Cannabis sativa L.) seed oil. Food chemistry, 76(1), 33-43, (2002)
[86] W. Golimowski, M. Teleszko, D. Marcinkowski, D. Kmiecik, A. Grygier, A. Kwaśnica, Quality of Oil Pressed from Hemp Seed Varieties:'Ealina 8FC','Secueni Jubileu'and 'Finola'. Molecules, 27(10), 3171, (2022).
[87] M. Islam, Y.V. Rajagukguk, A. Siger, J. Tomaszewska-Gras, Assessment of Hemp Seed Oil Quality Pressed from Fresh and Stored Seeds of Henola Cultivar Using Differential Scanning Calorimetry. Foods, 12, (2023)
[88] A. Mondragón-Valero, D.S. Hernández, I.R. Guillamón, G.H. Hernández, I. López-Cortés, Factors that influence color degradation in extra virgin olive oils. Science within Food: Up-to-date Advances on Research and Educational Ideas, 81.
[89] M. Moczkowska, S. Karp, O.K. Horbanczuk, M. Hanula, J. Wyrwisz, M.A. Kurek, Effect of Rosemary Extract Addition on Oxidative Stability and Quality of Hemp Seed Oil. Food and Bioproducts Processing, 124, 33-47, (2020)
[90] E.E. Babiker, N. Uslu, F. Al Juhaimi, , I.A. Mohamed Ahmed, K. Ghafoor, M.M. Özcan, I.A. Almusallam, Effect of Roasting on Antioxidative Properties, Polyphenol Profile and Fatty Acids Composition of Hemp (Cannabis Sativa L.) Seeds. Lwt, 139, 1-6, (2021)
[91] M.Esposito, L. Piazza, Ultrasound-Assisted Extraction of Oil from Hempseed (Cannabis Sativa L.): Part 1. Journal of the Science of Food and Agriculture, 102, 732-739, (2022)
[92] A. Aiello, F. Pizzolongo, G. Scognamiglio, A. Romano, P. Masi, R. Romano, Effects of Supercritical and Liquid Carbon Dioxide Extraction on Hemp (Cannabis Sativa L.) Seed Oil. International Journal of Food Science and Technology, 55, 2472-2480, (2020)
[93] D. Escolar, M.R. Haro, A. Saucedo, J. Ayuso, A. Jiménez, J.A. Alvarez, Color Determination in Olive Oils. Journal of the American Oil Chemists' Society, 71, 1333-1337, (1994)

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Influence of olive fly (*Bactrocera oleae*) on the phenolic composition and antioxidant activity of four Algerian olive cultivars

Lynda Medjkouh-Rezzak[a]
Abderezak Tamendjari[a] ✉
Soraya Mettouchi[a]
Khalida Bouarroudj-Hamici[a]
Maria Beatriz Oliveira[b]

[a] Laboratoire de Biochimie Appliquée
Faculté des Sciences de
la Nature et de la Vie
Université de Bejaia, Algérie

[b] REQUIMTE/LAQV,
Department of Chemical Sciences
Faculty of Pharmacy
University of Porto, Portugal

✉ CORRESPONDING AUTHOR:
Email: abderezakt@yahoo.fr

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The olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is one of the main pests of the olive tree which can affect the production and quality of the products. The phenolic compounds are important biological constituents and play a significant role in the susceptibility or tolerance of a cultivar to fly attack. This work aimed to study the influence of the attack of this pest on the phenolic composition and the antioxidant activity of four Algerian olive cultivars. The weight, maturity index, attack rate, phenolic profile, and antioxidant activities (Reducing power, ABTS assay and Chelating capacity) of the olives of the cultivars were determined. Phenolic compounds were determined by HPLC.
The results showed that the size of the fruit (weight) was significantly correlated with the attack ($r = 0.91$). The phenolic composition was significantly affected; the total losses of polyphenols were maximal in infested olive samples of Ferkani (52.64%) and Souidi (42.71%). Consequently, the antioxidant activities evaluated by different methods decreased significantly, the losses reached 86%. The values of the Relative Antioxidant Capacity Index (RACI), which represent the average scores of the antioxidant activities of each sample, showed that the varieties have different sensitivities. The lowest scores were recorded by attacked olives. The results confirmed the importance of healthy fruit in obtaining products with a high level of phenolic compounds.
Keywords: *Bactrocera oleae,* olives, phenolic compounds, HPLC, antioxidant activity.

1. INTRODUCTION

Olive trees have been growing throughout the Mediterranean basin for between six and seven millennia. During the colonisation period (16-18th centuries) all the regions of the world with a similar Mediterranean-type climate experienced planting by Spanish, Italian or French settlers. It was domesticated as the Oleaster [1, 2] and its cultivation spread to regions where the wild olive tree (oleaster) cannot thrive. They are grown for oil and canned fruit production; very little cultivation has a decorative purpose [3].
Bactrocera oleae (Rossi) (Diptera: Tephritidae), the olive fruit fly, is a key pest of Olea europea particularly in the Mediterranean area where more of the 90% worldwide olive cultivation takes place. This pest can develop 2-5 generations/year, and due to the feeding activity of larval instars on fruits, it is capable of strongly affecting quality and quantity of the olive production [4]. Damages appear during fruiting, when the insect females lay their eggs in the olive fruit pulp and, subsequently, larvae feed and grow in the fruit issues inducing serious losses, both qualitative and quantitative, to the fruit and oil production [5]. During larval development, pulp consumption destroys several tissues in the olive fruit, which leads to a lipolytic reaction between lipases and triacylglycerols, therefore arising the amount of free fatty acids in the olive oil [6]. Moreover, fly infestation increases olive oil acidity and peroxide value, as well as musty and earthy off-flavours, extensively reducing oil quality (e.g., down-

grading extra virgin olive oil to less valuable categories). Indirect effects are mainly due the presence of necrotic areas and microorganisms in feeding tunnels [7].
Tolerance to the olive fly was complex [8]. Many factors are involved: mechanical barriers (e.g., aliphatic waxes), chemical factors (e.g., oleuropein, cyanidin), morphological characteristics (e.g., fruit size) and their combination. Also, the relative importance and contribution of these factors is not yet fully clarified [9 - 11].
Olive (*Olea europaea* L.) fruits contain numerous secondary metabolites, primarily appreciable amounts of phenolic compounds which are particularly interesting for their nutraceutical properties [5]. These antioxidant compounds have numerous human health benefits and are important in the plant defence against pathogens and insects. The objective of this work is to study the relationship between fruit weight and its attack rate by the pest, and to assess the influence of the fruit fly *Bactrocera oleae* on phenolic compounds and antioxidant activity.

2. MATERIAL AND METHODS

2.1. SAMPLING

Olive fruits of *Abani* (A), *Ferkani* (F), *Rougette de Mitidja* (R) and *Souidi* (S) cultivars were collected manually from the trees in the Olive production station in Takarietz (Sidi-Aich, southern Béjaia) in Algeria in 2014 (located at 36°, 36', 47" north and 4°, 41', 18" east, at the altitude of 111m).

2.2. FRUIT WEIGHT

The fruit weight of the studied cultivars was determined as the weight of 100 drupes randomly picked from aliquots of samples previously homogenised [12].

2.3. DETERMINATION OF MATURITY INDEX (MI)

The maturity index is determined according to the formula established [13]. This formula is based on a punctuation system corresponding to each stage of coloration of the pericarp and the mesocarp.

2.4. DETERMINATION OF ATTACK RATE (AR)

The attack rate of samples was determined by calculating the number of olives attacked in a batch of 100 olives taken randomly after harvesting. It is calculated using the formula described [14].

2.5. SORTING AND PREPARATION OF OLIVE SAMPLES FOR THE DIFFERENT ANALYZES

After determining the maturity index and % infestation (larvae + pupae + number of exit holes), the olives are divided into 3 lots: lot 1: healthy olives (which are not attacked by *B. oleae*) Called S; Lot 2: natural olives (reflecting the real attack rate of the fruit) called N, lot 3: only olives attacked (each olive has at least one exit hole) called A. In this work, only the olives were studied after their lyophilisation no oil extraction was performed.
The preparation of the olives for the various analysis was carried out in the Applied Biochemistry Laboratory. The olive powder was obtained by lyophilisation according to the following steps:
First, the olives were cut into thin pieces and frozen at (-80°C);
The second step consists of lyophilization at (-58°C);
Finally, grinding in an electric mixer was carried out and then the sample was stored at (-18°C) to preserve the composition of the olives.

2.6. PROFILE OF PHENOLIC COMPOUNDS BY HPLC

The solid-liquid extraction method was used for the extraction of phenolic compounds according to the method described by Mc Donald et al. [15]. The freeze-dried olives were macerated in MeOH-water, stirred then centrifuged. The pellet was recovered for a second extraction and the supernatant was washed in triplicate with hexane to remove all traces of lipid. The hydrophilic phase was recovered by decantation and then filtered.
The chromatographic analysis was carried out in an integrated HPLC system equipped with an LC-NetII / AD43, an AS-2057 automatic sampler, a PU-2089 PLUS pump, a CO-2060 PLUS thermostat column, a multi-wavelength diode Network detector MD-2018 (DAD) connected in series to a fluorescence detector FP-2020 PLUS (Jasco, Japan).
A Zorbax SB-C18 column (250 × 4.6 mm, 5 mm) from Agilent Technologies (Waldbronn, Germany) was used for the separation of the compounds, according to the conditions described [16], with some modifications.
A solvent gradient system consisting of acetic acid in water (5% v/v) (eluent A) and methanol (eluent B) was used as follows: 0': 15% B; 10': 28% B; 15': 28% B; 16': 30%; 40': 40% B; 45': 45% B; 60': 100% B. The elution is carried out at 30°C, using a flow rate of 1 mL / min, the injection volume being 20 µL. The chromatograms were recorded at 240 nm, 280, 320 and 335 nm, based on the maximum absorption wavelengths of each compound analysed [17]. In addition, hydroxytyrosol and tyrosol were followed by fluorescence (λexc: 280 nm, λem: 330 nm) [18]. The chromatographic data were analysed using PDA-Borwin Controller software (JMBS, France). The compounds were identified by chromatographic comparison with authentic standards and by their UV spectrum.

2.7. ANTIOXIDANT ACTIVITIES

2.7.1. Reducing power

The reducing power of the samples was determined [19]. Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) solutions were prepared and added to 1 mL of each sample. After

incubation during 20 min at 50°C, 2.5 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 1500 g for 10 min. An aliquot (2.5 mL) of the upper layer of the solution was mixed with 2.5 mL of ultrapure water and 0.5 mL of FeCl solution (0.1%). The absorbance of each mixture was measured at a wavelength of 700 nm. The increase in the absorbance values can be correlated with the reducing power that was expressed as mg caffeic acid equivalents (CAE) per 100 g of DM.

2.7.2. ABTS assay

The antioxidant activity of olive extracts was determined using a 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonicacid (ABTS) radical cation discoloration assay [20]. Succinctly, 3.9 mL of diluted ($ABTS^{+\bullet}$) solution was added to 100 µL of a phenolic fraction or Trolox. The mixture absorbance was read at 734 nm, at 30°C, exactly after 6 min of the initial mixing.

2.7.3. Chelating capacity

The chelating capacity of the methanolic extracts of four Algerian olive cultivars was determined [21]. This method is based on the inhibition of the formation of the Fe (II)-Ferrosine complex after the treatment of the samples with the Fe^{2+} ions. Five hundred microliters of the extract solutions were added to 100 µL of FeCl (0.6 mM) and 900 µL of methanol. After 5 min of incubation, 100 microliters of ferrosine (5 mM) were added and the mixture was stirred and allowed to react for 10 min to allow the complexing of the residual iron. The absorbance of the ferrozine - Fe^{2+} complex was measured at 562 nm.

2.7.4. Relative antioxidant capacity index (RACI)

The results of the antioxidant activity obtained by the above-described chemical methods were integrated by calculating the Relative Antioxidant Capacity Index (RACI). The RACI index allows the comparison of antioxidant capacity derived from different chemical methods (Reducing power and Chelating capacity).
To calculate the relative index of the antioxidant capacity of each sample, we started by calculating the standard score according to the following formula:

$$scorestandrds = \frac{x - \mu}{\sigma}$$

Where:
X = is the raw data,
$\mu$is = the mean, and σ is the standard deviation [22].

2.8. STATISTICAL ANALYSIS

The statistical analysis was carried out using the software Statistica 5.5. For each parameter, the analysis of the variance (ANOVA) was used, followed by the Newman & Keuls test with a confidence level of 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. FRUIT WEIGHT (FW), MATURITY INDEX (MI) AND ATTACK RATE (AR) OF OLIVE FRUITS

The fruit weight (FW), the attack rate (AR) and the maturity index (MI) of the unsorted olive samples of the four cultivars studied are summarised in Table I. It appears that the variety exerts a significant effect on fruit weight ($p \leq 0.05$). Rougette de Mitidja has the highest weight (2.81 g) while Souidi has the lowest one (0.97 g).
The size of the fruit has a significant influence on the susceptibility of olives to attacks by B. oleae. The lowest attack rate (21%) was recorded by the Souidi variety which has the smallest fruits (0.97 g). Conversely, heavy olives were the most attacked. A significant positive correlation ($r = 0.91$) was noted between attack and the weight of fruits. Our results agreed with those of several authors who found that the fly prefers large-fruited varieties for egg laying [23 - 25]. Also, a relationship was highlighted between olive size and the percentage of fly attack; the largest olives exhibited the highest infestation [26, 27, 11].
Cultivar and maturation were crucial aspects in the olive fly preference [28]. The susceptibility of 20 most widely distributed mill and table olive Spanish varieties was studied [29]. Even though the olive fruit fly damaged all varieties, significant differences in susceptibility were detected among the mill olive and among the table olive varieties. Even though the diameter and oil content were positively correlated with B. oleae fruit infestation (correlation coefficients ranged between 0.5 and 0.95), their work reveals that other yet-unknown factors may influence B. oleae oviposition preferences.
Some of the factors related to fruit traits that possibly play a role include fruit size and mass, colour, fruit exocarp hardness, surface covering (mainly of aliphatic waxes), phenological stage of the crop, and chemical composition of olive fruits [30]. Recently, it was reported that *B. oleae* adult females mainly rely on olfactory cues, namely volatile organic compounds

Table I - Fruit weight (FW), attack rate (AR) and maturity index (MI) of four Algerian olive cultivars.

	FW (g)	AR (%)	MI
Abani	1.61±0.1 (b)	34.67±0.94 (b)	5.74±0.012 (c)
Ferkani	2.35±0.05 (c)	44.67±6.85 (b)	3.34±0.097 (a)
Rougette de Mitidja	2.81±0.05 (d)	65.33±9 (c)	4.67±0.008 (b)
Souidi	0.97±0.02 (a)	21±0.82(a)	6.54±0.008 (d)

The mean within each column labeled by different letters indicate a significant difference ($P < 0.05$).

Table II - Individual and total phenolic compounds of four Algerian olive cultivars (mg/Kg DM).

Variété	State	HT	Tyr	Ole.	Ver	AC	L-7-G	Rut.	A-7-G	Total HPLC
Abani	S	103.91±1.52(de)	30.97±0.51(d)	41.48±1.48(a)	1265±15.63(g)	0.00	165.48 ± 2.51(ef)	263.97±0.33(d)	3.96±0.05(c)	1875.17±12.84(c)
	N	93.68±1.61(de)	24.15±1.09(c)	92.27±3.90(a)	1062.67±19.20(i)	0.00	157.94±9.38(ef)	259.04±1.48(d)	4.05±0.21(c)	1693.79±36.87(d)
	A	54.05± 1.73(be)	21.99±0.44(bc)	60.62±1.37(a)	840.56±11.91(e)	00	151.32±2.67(e)	211.77±9.04(c)	3.84±0.10(c)	1344.95±26.38(b)
Ferkani	S	17.98±0.19(a)	16.45±0.99(ab)	3789.72±40.37(f)	1458.63±16.87(j)	0.00	171.58±6.13(f)	141.09±1.41 (b)	2.14±0.03(a)	5597.60±65.97(j)
	N	10.86±0.28(a)	12.26±0.20(a)	2626.96±104.46(e)	926.98±32.07(f)	0.00	147.84±2.71(e)	119.92±4.62 (b)	2.07±0.00(a)	3846.89±143.94(h)
	A	9.87±0.14(a)	13.07±0.09(a)	1721.39±49.49(d)	713.12±26.55(d)	0.00	109.79±0.49(c)	82.06±2.31(a)	2.00±0.01(a)	2651.30±79.07(f)
Rougette de Mitidja	S	374.89±14.74(f)	152.27±5.49(f)	1386.19±29.23(c)	2530.67±48.67(k)	0.00	130.45±5.50(d)	121.36±2.51 (b)	3.80±0.07(c)	4699.64±90.19(i)
	N	71.45±0.58(cd)	33.76±1.61(de)	1314.85±7.29(b)	1769.17±159.83(f)	0.00	92.55±5.27(bc)	132.48±7.15 (b)	3.56±0.20(c)	3417.83± 153.47(e)
	A	55.2± 2.42(g)	37.72±1.63(e)	1086.14±47.97(c)	1185.09±7.28(h)	0.00	95.35±2.58(bc)	86.67±2.68(a)	4.62±0.20(d)	2550.79±31.18(g)
Souidi	S	112.36±3.72(d)	11.81±0.25(abc)	65.14±0.84(a)	478.59±1.86(c)	24.63±0.17(d)	62.09±0.60(a)	428.51± 1.87(f)	2.06±0.05(a)	1185.21±6.39(b)
	N	72.70±0.30(cd)	19.66±0.44(c)	51.84±1.74(a)	393.26±10.00(b)	13.49±0.63(c)	102.86±3.73(bc)	444.74±13.21 (f)	2.26±0.05(a)	1100.81±28.15(b)
	A	33.39± 0.93(ab)	13.19±0.25(a)	24.69±0.02(a)	166.14±3.64(a)	11.83±0.22(b)	85.22±2.14(b)	341.62±7.88 (e)	2.88±0.02(b)	678.95±14.54(a)

The mean within each column labeled by different letters indicate a significant difference (P < 0.05). State of olives – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A). Phenolic compounds: AC, cafeic acid; HT hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein;Ver, verbascosid; Rut, rutin; L-7-G, Luteolin-7-glucoside; A-7-G, apigenin-7-glucoside;

produced by the tree [31]. Correlation between infestation level during olive maturation and the aromatic hydrocarbon toluene from olive leaves from different cultivars had been observed previously [9].

3.2. PHENOLIC COMPOSITION

The chromatographic analysis of olives phenolic extracts showed a qualitative composition of phenolic compounds almost similar for all the samples, but different from a quantitative point of view. Eight compounds hydroxytyrosol, tyrosol, oleuropein, verbascosid, caffeic acid, luteolin, rutin and apigenin were identified (Table II).

By comparing total polyphenol levels, for all studied varieties, *Ferkani* had the highest grade (5597.6 mg EAG / kg) and *Souidi* had the lowest (1185.21 mg EAG / kg). A significant difference (*p = 0.05*) was noted among cultivars. It is important to note that the different varieties studied didn't have the same degree of maturity (it is 3.34 for *Ferkani* cultivar and 6.54 for the *Souidi* cultivar). A negative correlation was noted between maturity and phenolic content (*r = -0.99*). The polyphenol content decreases progressively during the maturation process [32], this decrease can reach 30% depending on the variety [33]. The values obtained in this study were far inferior to those found by Ben Othman et al. [34] which is 17600 mg / kg MS.

The total polyphenol losses are maximal in the 100% attacked sample from *Ferkani* cultivar, it was 52.64% followed by the *Souidi* cultivar 42.71%. In the two remaining cultivars *Rougette de Mitidja* and *Abani*, the respective losses were 30, 28%.

The maximum losses recorded in our study are much higher than those found by Koprivnjak et al. [35], which are 21% in the Istarska variety from Croatia, and lower than the value found in the Chemlali variety from Tunisia of 83% [36]. This is due to the specific phenolic profile of olives, which depends on the variety [37]. This difference is due to, according to Koprivnjak *et al.* [35], fruit properties that are not conducive to larval development, and low volume of the mesocarp of infested fruit, which is the reason for the low degradation of polyphenols.

The two phenolic alcohols; hydroxityrosol (3, 4-DHPEA) and tyrosol (p-HPEA) have the highest levels in the *Rougette de Mitidja* cultivar (representing 19.37% and 6.33% of the total polyphenol contents respectively). They decrease drastically with the infestation level. A significant negative correlation was noted between the attack and these two compounds. The *Rougette de Mitidja* variety was the most affected by the attack and had the highest loss rates of 85.88% and 75.05% for hydroxytyrosol and tyrosol respectively, followed by of *Abani* cultivar (47.98% and 29% respectively).

Oleuropein varied quantitatively from one variety to another, ranging from 3.6% to 67.7% of the total phenols for the *Abani* and *Ferkani* cultivars respectively.

Ferkani cultivar has an oleuropein content two times higher than the *Rougette de Mitidja* cultivar despite the cultivars have almost the same maturity index and *Abani* and *Souidi* cultivars with advanced maturity stages have the lowest levels. In general, cultivars with a small-size fruit have higher concentration of oleuropein compared to large-sized fruit cultivars during developmental stages [38]. On his part, Bianchi, reported that at the beginning of ripening, oleuropein was the most abundant compound in olives and its concentration reached up to 14% of the dry matter of young fruit [39]. This decrease of oleuropein during maturation inversely was correlated with the increase in oleuropein derivatives, especially hydroxytyrosol. The losses, which vary according to the cultivar, were very pronounced in the Souidi (62.1%) and Ferkani (54.58%) cultivars, while the rest of the varieties recorded values below 50%.

According to Spadafora et al. [40], the defence molecules in olives were phenols synthesised and accumulated in fruit tissues during growth and maturation. The main defence component among these phenols was the phenolic β-glucoside secoiridoid, oleuropein, a bitter molecule characteristic of olives. This compound possessing antioxidant and antimicrobial activity has been referred to as a defence molecule against insect attack. When the olive tissues are injured by pathogens or by mechanical damage, β-glucosidase, belonging to the family of glucohydrolase enzymes, specifically hydrolysing oleuropein to produce highly reactive molecules. The olives contain large amounts of β-glucosidase, which specifically hydrolyses oleuropein. Gucci et al. [41], claimed that the main phenolic compounds affected by olive fly infestation were the secoiridoids. Gomez-Caravaca et al. [42], reported significant losses of simple phenols, lignans and secoiridoids.

All the varieties studied showed appreciable levels of verbascosid. *Rougette de Mitidja* cultivar was characterized by the highest content 2530.67 mg/kg, followed by *Ferkani* and *Abani* cultivars with respective contents of 1458.63 and 1062.67 mg/kg. The lowest value was found for the *Souidi* cultivar (478.59 mg/kg). Substantial losses were recorded and up to 65.29% in the *Souidi* cultivar. The two cultivars *Rougette de Mitidja* and *Ferkani* also have high loss rates, which were 53.17% and 51.11%, respectively. As for *Abani* cultivar, it showed only a loss of 20.90%.

Caffeic acid was present in trace amounts in most of the studied cultivars, except for the *Souidi* cultivar, which had 24.63 mg / kg. The most important loss of caffeic acid was recorded with the *Souidi* variety, which was 51.97% for the sample attacked 100%.

Three flavonoids were determined in the four analysed olive cultivars: luteolin (L7G), rutin and apigenin (A7G). Luteolin quantitatively occupied the second position of the total flavonoid content after rutin. *Ferkani* had the highest content (171.58 mg/kg) followed by the *Abani* and *Rougette de Mitidja* cultivars with respective grades of 165.48 mg/kg and 130.45 mg/kg. Concerning *Souidi* cultivar, it had only an amount of 62.09 mg/kg.

Rutin was the main flavonoid in the analysed olive varieties. The maximum level was recorded in the *Souidi* variety (428.51 mg/kg) followed by the *Abani* cultivar (263.97 mg/kg). However, the two remaining varieties, *Ferkani* and *Rougette de Mitidja*, showed only 141.09 and 121.36 mg/kg respectively. These results lead us to conclude that rutin is present in larger quantities in small olive varieties. Significant losses were reported of rutin for some olives varieties up to 41.84% for *Ferkani*. For *Abani*, *Rougette de Mitidja* and *Souidi* cultivars, the respective losses were: 19.77%, 28.58% and 20.28%.

Apigenin, the minor flavonoid of the analysed olives cultivars, was identified at very low levels. The studied

Table III - Antioxidant activities of four Algerian olive cultivars

Variety	State	Reducing power (mg CAE / 100 g of DM)	ABTS assay (%)	Chelating capacity (mg EEDTA/100g DM)
Abani	S	206.42±1.79(f)	70.14±10(f)	32.51±0.12(ef)
	N	149.90±6.87(e)	63±1.82(de)	28.43±0.43(cd)
	A	66.57±6.46(b)	56.19±0.89(c)	22.29±0.05(ef)
Ferkani	S	359.67±11.15(h)	56.24±2.36(c)	32.72±1 .74(ef)
	N	238.51±6.21(g)	48.24±2.75(b)	26.76±0.24(bc)
	A	80.94±4.88(c)	40.76±1.50(a)	22.72±0.09(a)
Rougette de Mitidja	S	680.56±2.95(i)	62.86±0.71(de)	41.8±0.07(h)
	N	136.97±4.12(e)	46.33±0.47(b)	36.71±2.88(g)
	A	94.83±3.1(d)	38±0.61(a)	29.89±0.05(de)
Souidi	S	146.55±3.10(e)	65.24±0.99(e)	35.01±0.2(fg)
	N	57.95±3.77(b)	60.52±0.95(d)	29.92±1.49(de)
	A	45.02±2.95(a)	53.67±1.75(c)	24.83±0.55(ab)

The mean within each column labeled by different letters indicate a significant difference ($P < 0.05$). State of olives – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A).

Table IV - Correlation matrix between phenolic compounds and antioxidant activity.

	VAR	A	MI	HT	Tyr	Ole	Ver	AC	L-7-G	Rut	A-7-G	TP	RP	ABTS	CC
VAR	1,000														
A	0,613*	1,000													
MI	0,106	-0,610*	1,000												
HT	0,623*	-0,175	0,820*	1,000											
Tyr	0,948*	0,528	0,271	0,674*	1,000										
Ole	-0,328	0,465	-0,966*	-0,937*	-0,441	1,000									
Ver	0,538	0,454	-0,379	0,139	0,261	0,152	1,000								
AC	-0,471	-0,688*	0,704*	0,197	-0,207	-0,503	-0,907*	1,000							
L-7-G	-0,290	-0,233	-0,270	-0,188	-0,526	0,217	0,606*	-0,457	1,000						
Rut	-0,330	-0,807*	0,898*	0,489	-0,127	-0,759*	-0,650*	0,905*	0,207	1,000					
A-7-G	0,869*	0,318	0,247	0,746*	0,753*	-0,481	0,723*	-0,491	0,139	-0,179	1,000				
TP	-0,200	0,511	-0,986*	-0,838*	-0,382	0,963*	0,407	-0,695*	0,406	-0,853*	-0,267	1,000			
RP	-0,152	0,427	-0,916*	-0,709*	-0,395	0,854	0,591	-0,790*	0,612*	-0,835*	-0,103	0,956*	1,000		
ABTS	-0,152	-0,775*	0,790*	0,622*	-0,125	-0,776*	-0,079	0,459	0,323	0,767*	0,233	-0,705*	0,526	1,000	
CC	0,720*	0,641*	0,088	0,346	0,850*	-0,176	-0,007	-0,082	0,705*	-0,161	0,392	-0,210	0,335	0,395	1,000

* Signifiant at P<0.05.
Var: variety, A: attack; MI: maturity index; HT: hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein; Ver: verbascosid; AC: cafeic acid; HT: hydroxytyrosol; Tyr: tyrosol; OLE: oleuropein; Ver: verbascosid; Rut: rutin; L-7-G: Luteolin-7-glucoside; A-7-G: apigenin-7-glucoside; Rut: rutin; L-7-G: Luteolin-7-glucoside; A-7-G: apigenin-7-glucoside; TP: total phenol; RP: reducing power; CC: chelating capacity

cultivars have only very low levels, ranging from 2.06 to 3.96 mg/kg for *Souidi* and *Abani* cultivars respectively. The losses, in all the analysed cultivars, do not exceed 6.5% (noted for the *Ferkani* cultivars).

The main reasons for the loss of biophenols according to Koprivnjak et al. [35] are most likely an increase in endogenous polyphenoloxidase activity due to the damage of the cellular structure and the exposure to oxygen due to exit holes on the surface of the fruit. The changes induced by the attack of the olive fly on the expression of some key genes in the biosynthesis of volatile and phenolic compounds, such as lipoxygenase, beta-glucosidase, and polyphenol oxidase, have been analysed in olives of three cultivars (Picual, Manzanilla, and Hojiblanca) [43]. The results showed a strong induction of a new olive polyphenol oxidase gene (oeppo2) which explains the reduction of phenolic content in the oils obtained from infested fruits and suggest the existence of a PPO-mediated oxidative defence system in olives.

3.3. ANTIOXIDANT ACTIVITIES

The results of the antioxidant activity of the studied olive samples measured by three different methods are summarised in Table III.

3.3.1. Reducing power

The capacity of the olive samples to reduce Fe^{3+} to Fe^{2+} varied widely according to the fly attack degree. Values found in this study (Table III) decreased in attacked samples of all cultivars (from 680.56 to 94.83 mg CAE per 100 g of DM for *Rougette de Mitidja*, from 359.67 to 80.94 mg CAE per 100 g of DM for *Ferkani* from 206.42 to 66.57 mg CAE per 100 g of Dry Matter (DM) for *Abani*, and, finally, from 146.55 to 45.02mg CAE per 100 g of DM for *Souidi* cultivar). Losses of activity were about 86%, 77%, 69 and 67% for *Rougette de Mitidja*, *Ferkani*, *Souidi* and *Abani* respectively. *Rougette de Mitidja* being more susceptible to a fly attack. The *B. oleae* attack influences significantly the reducing power values of olives. The drastically decrease of the reducing power activity of olive from the attacked samples was due to the decrease in antioxidants (positive correlation was noted between reducing power and phenolics, $r = 0.96$), used probably to protect the fruit against the action of *B. oleae* larvae.

3.3.2. ABTS assay

Phenolic extracts of four studied cultivars showed a high scavenging capacity estimated according to the ABTS-RSC assay. The values determined (Table III) showed a decreasing tendency in the olives (from 70.14 to 56.19%, from 56.24 to 40.76%, from 62.86 to 38% and from 65.24 to 53.67% for *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively). The changes verified can be justified by the decreasing levels of available antioxidants, as explained previously. It is noteworthy that activity losses estima-

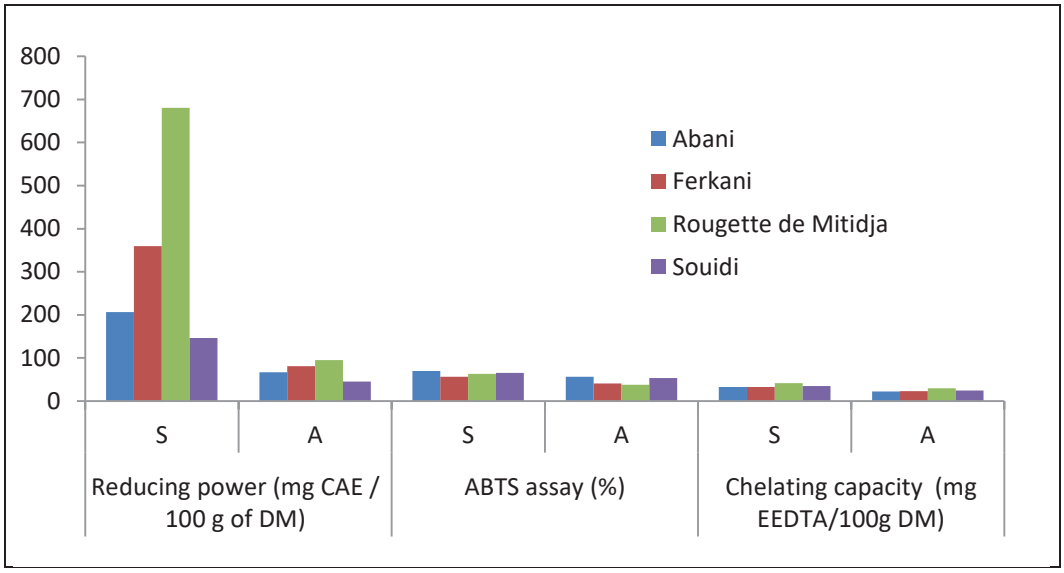

Figure 1 - Antioxidant activities of healthy and infested samples of studied cultivars.
S: Healthy olives; A: attacked olives

ted by ABTS-RSC assay in attacked samples were 19.89%, 27.27%, 39.55% and 17.73% in *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively. The determined ABTS values exhibited a similar behaviour described for the reducing power. Medjkouh et al. [44] have found same results in another study on the antioxidant activity of olive oils from olives attacked by *B. Oleae*.

3.3.3. Chelating capacity

Phenolic extracts of studied olive cultivars showed an important chelating capacity. Olives exhibited almost the same activity, which was from 32.51, 32.72, 41.8, 35.01 mg EDTA/100 g DM for *Abani*, *Ferkani*, *Rougette de Mitidja* and *Souidi* cultivars respectively. Losses recorded in this study were very close and ranged from 28.49%, 29.08%, 30.56% and 31.44% for *Rougette de Mitidja*, *Souidi*, *Ferkani* and *Abani* respectively.

Antioxidant activity decreased drastically in samples infested by the olive fruit fly comparatively to the healthy samples as showed in Figure 1. This is due to the significant losses of antioxidants in the olive fruits. Janji et al. [45] reported in their work the effect of infestation by the olive fruit fly *Bactrocera oleae Gmel* on the stability of olive oil. The latter recorded a clear decrease following the great losses of polyphenols, tocopherols, and pigments (chlorophyll and carotenoids).

3.4. RELATIVE ANTIOXIDANT CAPACITY INDEX (RACI)

The values of the Relative Antioxidant Capacity Index (RACI), which represent the average scores of the antioxidant activities of each sample, were shown in Figure 2.

The RACI was validated as a reference for ranking samples according to their antioxidant potential which

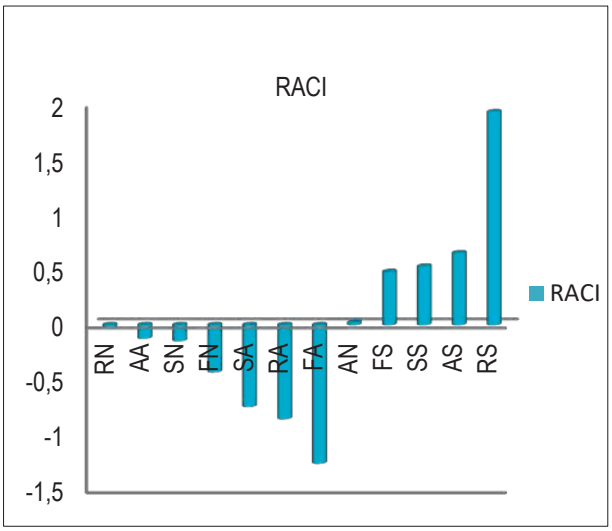

Figure 2 - Relative Antioxidant Capacity Index (RACI) of olive fruits samples. Cultivars (first letter) – Abani (A); Ferkani (F), Rougette de Mitidja (R), Souidi (S); State of olives (second letter) – healthy olives (S), natural (reflecting the real attack rate of the fruit) (N), only attacked olives (A).

results from the combination of all the methods used, because it makes the comparison of the data which should follow a normal distribution more reliable.

From this figure we can affirm that the extract of healthy olives of the *Rougette de Mitidja* cultivar marked the superiority in its contribution to all the tests, mentioning an RACI of +1.993. The lowest RACI value was recorded by the extract of attacked olives of the *Ferkani*, attacked variety (-1.262).

The order of classification can be given as follows: [(*Ferkani*, attacked), -1.262] < [(*Rougette de Métidja*, attacked), -0.858] < [(*Souidi*, attacked), -0.745] < [(*Ferkani*, natural), -0.430] < [(*Souidi*, natural), -0.145] < [(*Abani*, attacked), -0.123] <[(*Rougette de Métidja*,

natural), -0.036] < [(*Abani*, natural), +0.02] < [(*Ferkani*, healthy), +0.477] < [(*Souidi*, healthy), +0.526] < [(*Abani*, healthy), +0.647] < [(*Rougette de Métidja*, healthy), +1.930].

Most of the positive RACI values were recorded by the healthy samples for the four varieties. Among the natural samples, only the Abani variety showed a very small positive value (0.020). This can be elucidated by the diversity of their phenolic compounds, which differ in their quantities and relativities.

4. CONCLUSION

The current work yielded information on the olive fruit fly on antioxidants and the antioxidant activity of four olive varieties grown in Algeria.

Antioxidant potential was reduced due to the loss of antioxidant compounds, as it is the case of phenolic compounds, namely hydroxytyrosol, tyrosol and oleuropein, as already witnessed and reported in this study. Olives with an infestation higher than 20% have a loss rate between 30% and 52%.

Regarding olive pests and diseases, olives are primarily affected on the economic field since significant losses are entailed each year in the olive fruit production. The quality and composition of olive oils are significantly modified by the olive fly. The actions of olive flies are so serious that olive oils are often downgraded and this has a negative impact on the international market.

REFERENCES

[1] G. Besnard, B. Khadari, P. Baradat, A. Bervillé, Olea europaea phylogeography based on chloroplast DNA polymorphism. Theoretical and Applied Genetics104, 1353-1361, (2002).

[2] C. Breton, M. Tersac, A. Bervillé, Genetic diversity and gene flow between the wild olive (oleaster, *Olea europaea* L.) and the olive: several Plio-Pleistocene refuge zones in the Mediterranean basin suggested by simple sequence repeats analysis. Journal Biogeography 33, 1916-1928, (2006).

[3] A.J. Bervillé, C.M. Breton, Genetic and environmental features for oil composition in olive varieties. Oléagineux-Corps gras-Lipides 21, D504, (2014).

[4] A. Malacrinó, L. Schena, O. Campolo, F. Laudani, V. Palmeri, Molecular analysis of the fungal microbiome associated with the olive fruit fly *Bactrocera oleae.* Fungal Ecology 18, 67-74 (2015).

[5] F. Alagna, M. Kallenbach, A. Pompa, F. De Marchis, R. Rao, I.T. Baldwin, G. Bonaventure, L. Baldoni, Olive fruits infested with olive fly larvae respond with an ethylene burst and the emission of specific volatiles. Journal *Integr*ative Plant Biology 58, 413-425, (2016).

[6] K. Brkić Bubola, M. Krapac, B. Sladonja, Influence of olive fruit fly attack on quality and composition of '*Rosinjola*' virgin olive oil. Acta Horticulturae 1199 , 489-496, (2018).

[7] F. Grasso, M. Coppola, F. Carbone, L. Baldoni, F. Alagna, G. Perrotta, A.J. PeÂrez-Pulido, A. Garonna, P. Facella, L. Daddiego, L. Lopez, A. Vitiello, R. Rao, G. Corrado, The transcriptional response to the olive fruit fly (*Bactrocera oleae*) reveals extended differences between tolerant and susceptible olive (*Olea europaea* L.) varieties. PLOS One 12(8)*,* e0183050, (2017).

[8] G. Corrado, A. Garonna, CG.L. Cabanás, M. Gregoriou, G.P. Martelli, K.D. Mathiopoulos. Host Response to Biotic Stresses In: E. Rugini, L. Baldoni, R. Muleo, L. Sebastiani, Editors. The Olive Tree Genome. Springer International Publishing 75-98, (2016).

[9] R. Malheiro, S. Casal, S.C. Cunha, P. Baptista, J.A. Pereira. Identification of leaf volatiles from olive (*Olea europaea*) and their possible role in the ovipositional preferences of olive fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). Phytochemistry 121, 11-19, (2016).

[10] R. Rizzo, V. Caleca, A. Lombardo. Relation of fruit color, elongation, hardness, and volume to the infestation of olive cultivars by the olive fruit fly, *Bactrocera oleae*. *Entomol Exp et Appl.,* 145, 15-22, (2012).

[11] N. Garantonakis, K. Varikou, A. Birouraki. Interaction between Bactrocera oleae (Diptera: Tephritidae) infestation and fruit mineral element content in Olea europaea (Lamiales: Oleaceae) cultivars of global interest. Applied Entomology and Zoology 51 (2), 257-265, (2017).

[12] B. Jiménez, A. Sánchez-Ortiz, M.L. Lorenzo, A. Rivas, Influence of fruit ripening on agronomic parameters, quality indices, sensory attributes and phenolic compounds of *Picudo* olive oils. International Food Research Journal 54, 1860-1867, (2013).

[13] International Oleicole Council. Guide pour la détermination des caractéristiques des olives à huile. 2011, n° 1.

[14] V. Kaul, A.K. Tiku, Shankar, Uma.; Monobrullah, Md. Green Stink Bug (Hemiptera: Pentatomidae) Record as a New Pest of Olive in India. Journal of Asia-Pacific Entomology 10(1), 81-83, (2007).

[15] S. Mc Donald, P-D. Prenzler, M. Antolovich, K. Robards. Phenolic content and antioxidant activity of olive extracts. Food chemistry, 73, , 73-84, (2001).

[16] M.N. Franco, T. Galeano-Díaz, Ó. López, J.G. Fernández-Bolaños, J. Sánchez, C. De Miguel, V. Goulas, P. Charisiadis, I.P. Gerothanassis, G.A. Manganaris, Classification, Biotransformation and Antioxidant Activity of Olive Fruit Biophenols: A Review. Currents Bioactive Compounds 8, 232-239 (2012).

[17] M. Araújo, F.B. Pimentel, R.C. Alves, M.B.P.P. Oliveira, Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. Trends Food Science and Technology 45, 200-211, (2015).
[18] H.K. Obied, Jr. D.R. Bedgood, P.D. Prenzler, K. Robards, Chemical screening of olive biophenol extracts by hyphenated liquid chromatography. Analytica Chimica Acta 603, 176-189, (2007).
[19] H. Wang, M. Zhao, B. Yang, Y. Jiang, G. Rao, Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. Food Chemistry 107, 1399-1406, (2008).
[20] A. Nakbi, M. Issaoui, S. Dabbou, N. Koubaa, A. Echbili, M. Hammami, N. Attia, Evaluation antioxidant activities of phenolic compounds from two extra virgin olive oils. Journal Food Composition and Analysis 23, 711-715, (2010).
[21] K. Le, F. Chiu, K. Ng, Identification and quantification of antioxidants in Fructuslycii. Food Chemistry 105, 353-363, (2007).
[22] T. Sun, S.A. Tanumihardjo, An Integrated Approach to Evaluate Food Antioxidant Capacity. Journal Food Science 72, 159-165, (2007).
[23] R. Rizzo, V. Caleca, Resistance to the attack of *Bactrocera oleae* (Gmelin) of some Sicilian olive varieties. In Proceedings of Olivebioteq 2006, Second International Seminar "Biotechnology and quality of olive tree products around the Mediterranean Basin" November 5th-10th, Mazara del Vallo, Marsala, Italy 2, 291-298, (2006).
[24] M. Lysandrou, Fruit Flies in the Mediterranean and Arab World: How Serious a Threat are they and How Can we Minimize their Impact. Arab Journal of Plant Protection 27(2), 236-239, (2009).
[25] X.G. Wang, M.W. Johnson, K.M. Daane, V.Y. Yokoyama, Larger olive fruit size reduces the efficiency of Psyttalia concolor, as a parasitoid of the olive fruit fly. Biological Control 49, 45-51, (2009).
[26] R. Antonelli, F. Chesi, Relation between some physical variables and the probability of olive fruit fly infestation on drupe of the var. *Frantoio*. Frustula Entomologica 8, 601-611, (1985).
[27] H.A. Mesbah, B. Megda, The insect pests of olive trees in Siwa Oasis with special reference to Dacus oleae, Saissetia oleae and certain beneficial insects. *JOBAZ,* Egyptian German Society of Zoology 21, 167-185, (1996).
[28] R. Malheiro, S. Casal, L. Pinheiro, P. Baptista, J.A. Pereira, Olive cultivar and maturation process on the oviposition preference of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae). Bulletin of Entomological Research 109, 43-53, (2019).
[29] E.C. Quesada-Moraga, S. Cubero-González, G. Casado-Mármol, A. Ariza Fernández, M. Yousef. Field evaluation of the susceptibility of mill and table olive varieties to egg-laying of olive fly. Journal Applied Entomology 142, 1-10, (2018).
[30] M. Hladnik, A review of plant protection against the olive fly (*Bactrocera oleae* (Rossi, 1790) Gmelin) and molecular methods to monitor the insecticide resistance alleles. Acta Agriculturae Slovenica 109(1), 135-146, (2017).
[31] G. Giunti, O. Campolo, F. Laudani, G. Massimo Algeri, V. Palmeri, Olive fruit volatiles route intraspecific interactions and chemotaxis in *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) females. Scientific Reports 10, 1666, (2020).
[32] E. Gimeno, A.I. Castellote, R.M. Lamuela-Raventós, M.C. de la Torre, M.C. López-Sabater, The effects of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol, and β-carotene) in virgin olive oil. Food Chemistry 78, 207-211, (2002).
[33] I.D. Rojnićn, R. Bažok, J.I. Barčic, Reduction of olive fruit fly damage by early harvesting and impact on oil quality parameters. *European Journal Lipid Science and Technology* 116, (2014).
[34] N. Ben Othman, D. Roblain, N. Chammen, P. Thonart, M. Hamadi, Antioxidant phenolic compounds loss during the fermentation of *Chétoui* olives. Food Chemistry 116, 662-669, (2009).
[35] O. Koprivnjak, I. Dminić, U. Kosić, V. Majetić, S. Godena, V. Valenčič, Dynamics of oil quality parameters changes related to olive fruit fly attack. European Journal Lipid Science and Technology 112, 1033-1040, (2010).
[36] F. Mraicha, M. Ksantini, O. Zouch, M. Ayadi, M. Bouaziz, S. Sayadi. Effect of olive fruit infestation on the quality of olive oil from *Chemlali* cultivar during ripening. Food Chemical Toxicology 48, 3235-3241, (2010).
[37] A.F. Vinha, F. Ferreres, B.M. Silva, P. Valentão, A. Gonçalves, J.A. Pereira, M.B. Oliveira, R.M. Seabra, P.B. Andrade. Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. Food Chemistry 89, 561-568, (2005).
[38] V. Goulas, P. Charisiadis, I. Gerothanassis, G. Manganaris, Classification, biotransformation and antioxidant activity of olive fruit biophenols: A review. Currents Bioactive Compounds 8, 232-239, (2012).
[39] G. Bianchi. Lipids and phenols in table olives. European Journal Lipid Science and Technology 105, 229-242, (2003).
[40] A. Spadafora, S. Mazzuca, F.F. Chiappetta, A. Parise, A.M. Innocenti. Oleuropein-specificβ-glucosidase activity marks the early response of olive fruits (*Olea europaea*) to mimed insect attack. Agricultural Science in China 7, 703-712, (2008).

[41] R. Gucci, G. Caruso, A. Canale, A. Loni, A. Raspi, S. Urbani, A. Taticchi, S. Esposto, M. Servili, Qualitative changes of olive oils obtained from fruits damaged by Bactrocera oleae (Rossi). International Journal Horticultural Science 47, 301-306, (2012).
[42] A.M. Gomez-Caravaca, L. Cerretani, A. Bendini, A. Segura-Carretero, A. Fernandez-Gutierrez, M. Del Carlo, D. Compagnone, A. Cichelli. Effects of fly attack (Bactrocera oleae) on the phenolic profile and selected chemical parameters of olive oil. Journal Agricultural Food Chemistry 56, 4577-4583, (2008).
[43] A. Notario, R. Sánchez, P. Luaces, C. Sanz, A.G. Pérez. The Infestation of olive fruits by bactrocera oleae (Rossi) modifies the expression of key genes in the biosynthesis of volatile and phenolic compounds and alters the composition of virgin olive oil. Molecules 27*(5),* 1650, (2022).
[44] L. Medjkouh, M.A. Nunes, A. Tamendjari, S. Keciri, J. Santos, M.B.P.P. Oliveira. The effect of the olive fruit fly (*Bactrocera oleae*) on quality parameters, and antioxidant andantibacterial activities of olive oil. Food Function 7, 2780-2788, (2016).
[45] G. Janji, M. Almalim, A. Arab, Z. Hadj Abdou, Effect of infestation with olive fruit fly *Bactrocera oleae* Gmel on the stability of olive oil. University of Aleppo Research 95, 1-20 (2019).

FT-NIR spectra analysis and processing to determine the quality parameters of various edible oils and chicken fat

Askim Akca Mecit[1]
Turkan M. Keceli[2] ✉

[1] Pilyem, Doyum Pilic Ltd.
Adana, Turkey

[2] Cukurova University,
Faculty of Engineering,
Department of Food Engineering,
Adana, Turkey

Valorisation of chicken fat as a fat source in chicken meat products or as a low-cost source of biodiesel could be a viable option for the poultry industry's long-term sustainability and pollution reduction. The acidity and peroxide levels of culinary oils and fats are important grading and safety factors. FT-NIR techniques with chemometric treatment are a rapid, reliable, and convenient alternative to wet-chemical characterisation by reference analysis. This research demonstrated that using FT-NIR spectroscopy (1122-902 nm) and (1090-898 nm) with PLS-R, PCA, and Discriminant Analysis (DA) was sufficient to analyse data, predict, and discriminate edible oils and chicken fat according to their quality parameters regardless of whether they are present in low or high amounts. The PLS-R regression models can predict FFA and PV because they have a perfect agreement with reference analysis (R^2, 0.94 and 0.99) and have RPD >2 showing FT-NIR is suitable for quality control applications of edible oils and chicken fat. DA was able to discriminate between the groups chicken fat and virgin olive oil, from other edible oils with a 98% accuracy, based on their FFA and PV by both methods. The FT-NIR method with a multivariate approach is an excellent alternative to reference methods, using a small sample and no chemical, fast, reliable, and as green technique that could be used as a quality control tool for both predictions of quality and discrimination purposes.

Keywords: FT-NIR, Reference Analysis, Quality parameters, FFA, PV, Multivariate methods, PCA, Discriminant Analysis

INTRODUCTION

Edible oils and fats are obtained from the extraction of oilseeds (peanut, soybean, sunflower, and so on), fruits (coconut, olive, and palm) or animal tissues. Oils and fats are used mainly for edible purposes, as ingredient or raw material, and additive in food and feed production to improve the quality and taste and to provide essential nutrient and energy, consumed as human food. Edible oils and animal fats are utilised as fat spreads, cooking fats, frying oils, salad oils, mayonnaise etc., either directly or after proper modifications. The remaining minor parts of oils and fats are processed into a variety of oleo chemicals, which are utilised as surfactants, used in pharmaceutical industries, used as animal feed and as a biodiesel [1, 2]. Chicken fat creating an environmental problem is usually considered as waste and thus it is discarded. However, it can be an alternative to edible oils and fats in food processing and can contribute to the development of a sustainability of poultry industry [3, 4]. Chicken fat can be used to increase plasticity when mixed with other solid fats [3] and can be converted to biodiesel. Oils and fats may have differences in their qualities that significantly affects their stability, reactivity, and processing. Monitoring changes and quality of oils and fats during processing and storage is very important from a quality, functionality, economic value, and food safety point of view. The nutritional value,

✉ CORRESPONDING AUTHOR:
Turkan M. Keceli,
Email: tkeceli@cu.edu.tr
Phone: +90 322 3386173
Fax: +90 322 3386614

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freshness and quality of edible oils greatly affected by acidity and peroxide value that also affects human health and may cause problems during processing [5]. Acidity is determined by acid/base titration and may change during storage, processing, heating, or frying due to time, temperature, and moisture content. Besides, the acidity of edible oils shows hydrolysis or lipolysis, thus it is a direct measure of the quality [6] and tendency for rancidity. Oils and fats containing high amounts of free fatty acids (FFAs) are more prone to oxidation and produce rancidity, since FFAs are less stable, and thus it greatly affects quality and commercial value of oils and fats [7] as well as for their classification [8]. FFA content of edible oils and fats are reduced during refining and biodiesel production [4, 7]. The peroxide value (PV) being normally determined by titration is an indicator of freshness, it reflects oils oxidative level and thus its tendency to become rancid and therefore, it is a very important quality parameter for food safety [9]. Oxidative degradation generates a negative impact on flavour, shelf life, and nutrition of oils and fats [10, 11]. Peroxide value is below 10 meq O2/kg for fresh oils and if PV as high as 100 meq O2/kg might be the cases of food poisoning [10]. The American Oil Chemists Society (AOCS) and International Olive Council (IOC) have recommended standard titrimetric methods for measuring FFA and PV of oils and fats [12, 13]. These titrimetric methods have some disadvantages since they are time-consuming, laborious, tedious and can result in health and environmental problems, inconvenient for on-line monitoring, expensive, poorly reproducibile, and less sensitive [5, 7, 14-16]. These chemical methods require large amounts of organic solvents, toxic and carcinogenic reagents that cause health concerns and environmental disposal problems and difficulty in distinguishing the end-point with dark coloured oils and fats and largely dependent on the skills of the analyst [5, 11]. Therefore, reliable, fast and safe analytical methods are required to determine quality parameters of oils and fats due to differences in composition, production, refining, blending, or adulteration [16] that should be addressed by the official authorities and producers [16, 17]. The Fourier transform Near infrared (FTNIR) technique combined with chemometrics has been developed as an analytical tool for determination of oils and fats quality. It is a rapid technique (takes few minutes) and reduces the use of toxic solvents, pollution-free, safe to use, helps environmental protection, it is economical, a simple operation even for untrained staff, efficient and allows online, off-line and at-line detection of quality parameters for use of quantification of various oil parameters including acidity based on C-H stretching and peroxide value based on COO stretching [2, 5, 11, 18] . The FT-NIR technique could determine and predict several parameters such as acidity, peroxide value, iodine value, anisidine value, malondialdehyde, soap contents within a single measurement [6,7,18-20] providing a great amount of information which is useful for determination of quality of oils and fats. However, NIR spectra have wide and overlapping bands due to the similar nature of oils and fats, therefore needs chemometric methods such as principal component analysis (PCA) and Partial Least Square regression (PLS-R) need to be used to detect spectral differences by computing latent variables, known as loadings spectra, that are related to the component of interest for evaluation of data and interpretation of quality parameters [21, 22, 23]. Thus, chemometric analysis methods has been frequently developed for the rapid and online FT-NIR spectroscopic detection system for food quality, safety and control and has been used for the discrimination of edible oils and fats [19, 20], classification [16] and to distinguish animal fats from different species [1]. There are many uses for FTNIR spectroscopy in determining the origin of edible oils and performing general analysis in edible oils and fats. Putri et al. [5] tested some quality parameters like acidity, peroxide, and saponification values in patin fish oil with the FTIR spectroscopy combined with Principal Component Regression (PCR) and (PLS-R) providing a high correlation coefficient (R^2) reached up to 0.99 with FT-NIR range 721 to 2950 cm-1. Galbraith et al. [10] used NIR to build regression models to predict and determine peroxide value of the various edible oils within NIR range of 3799-14,998 cm^{-1} and RMSEP ranged between 1.9 to 2.50. Jiang et al. [17] was able to have excellent performance in predicting acid value of edible oils during storage with MPA based strategy, R^2 = 0.92 and RPD was 2.82 by NIR with a range of 1150-1700 nm. Also, Kaufmann, et al. [23] have used PLS calibration model for acidity prediction in palm oil, achieving R^2 = 0.97 using most relevant wavelengths range of 1,100 to 1,500 nm. Thus, previous studies consisted of quality parameters such as acidity [6, 7, 17, 21, 23] and peroxide values [5, 9, 11, 20] of single type oils and fats. To best of our knowledge, the FT-NIR method has not been applied for determination of the free acidity and peroxide value to assess chicken fat quality. Therefore, the aim of this study is to investigate, compare and highlight the potential of the FT-NIR spectroscopic techniques to monitor free acidity and peroxide values of various common edible oils and chicken fat both by reference analysis and FT-NIR technique and to construct a reliable multivariate model to predict and discriminate edible oils and chicken fat according to their quality parameters.

2. MATERIALS AND METHODS

2.1. MATERIALS

Sunflower oil, corn oil, virgin, and the olive oil with three different brands were purchased from local markets in 1 kg/bottle and chicken fat was provided from Pilyem Feed factory, Turkey. All edible oils and

chicken fats were stored at 5±1°C, respectively in the dark until the related analysis.
The chemicals and solvents used throughout the study were HPLC grade. N-hexane, chloroform, ethyl acetate, ethanol, HCl, acetic acid, sodium thiosulphate, KI, acetone, KOH, and phenolphthalein were obtained from Merck (Darmstadt, Steinheim Germany).

2.2. METHODS

2.2.1. Analytical Measurements

The free acidity as a percentage of oleic acid (% w/w) and the peroxide value as meq O_2 kg^{-1} were determined, according to Ca 5a-40 and Cd 8-53, respectively, described in American Oil Chemist Society [12] official reference methods.

2.2.2. FT-NIR Spectroscopy

The FT NIR spectra of edible oils and chicken fat were measured as indicative of quality parameters of edible oils and chicken fats. The spectrophotometer was a Multi-Purpose Analyzer (MPA) Fourier Transform Near Infrared Transmittance FT-NIR (Bruker Optics, Ettlingen, Germany) fitted with an inGaAs detector and thermostated between 5 and 35°C. The FT-NIR spectra were acquired with a 10 kHz scanner velocity from 12500 (2500 nm) to 4000 (800 nm) cm^{-1}, with 5 scans per spectrum and an 8 cm^{-1} resolution. In 30 seconds, the entire sample FT-NIR spectrum was captured. Chicken fat was heated to 50°C to guarantee that it was completely melted, translucent as described in [21] before scanning. The cell components were washed in warm water, rinsed with acetone, and dried after each sample. Each edible oil and chicken fat spectra was collected in triplicate. OPUS program fully GMP compliant, fully 21 CFR part 11 compliant from Bruker was employed for data acquisition. Treatment of data OPUS/Quant 2 was used to carry out the NIR calibration process (Bruker Optics GmbH, Ettlingen, Germany). Multiple components can be quantified within a single spectrum using software applications.

2.2.3. Chemometric Analysis

The mean and standard deviation of three measurements were used to calculate the results. Oils and fat FT-NIR spectra in the range of 12.500 (800 nm) –4.000 (2500 nm) cm^{-1}. A paired sample t-test ($p<0,05$) and z-score was used to compare the data obtained by reference and FT-NIR method. PCA-Correlation, PLS-Regression, and Discriminant Analysis (DA) were used to assess the quality by both official reference and FT-NIR methods using XLSTAT 2022.1.1.1251, Addinsoft, New York, NY, USA software package.

2.2.4. Data Analysis (PCA), Model Performance (PLS-R) and Discrimination (DA)

PCA was used initially to examine the possible classification of the various edible oils and fats with a full correlation since it enables reducing variable dimensions for samples clustering. The first principal component, PC1, covers the maximal information direction and is orthogonal (that is, explains complementary information) to PC2. PCA is an unsupervised exploratory method that is linear combinations of the original variables. If they are closer they are more similar, if they are further apart they more distinct in the score plots. Thus, the plots can be used to deduce sample differences and similarities. Samples to the right of the scores plot, for example, will often have a large value for variables to the right of the loadings plot and a small value for variables to the left of the loadings plot [18]. The model performances FT-NIR-PLS-R models of edible oils and chicken fat were evaluated based on determination of correlation coefficient R^2, RMSE and RPD values of the calibration models of PLS-R. The R^2 is an indicator of the goodness of fit between the predicted and reference values for each quality parameter (free acidity and peroxide value) and it may change between 0 and 1 indicating fitness of the models [14, 21]. The ratio of the standard deviation of the reference data divided by the standard error of prediction is known as RPD (Ratio of Performance Deviation) is also used to check the accuracy of the prediction models that have been constructed. According to extensive research, a PLS model with an RPD value between 2.0 and 3.0 is regarded as a decent PLS model and adequate for analytical purposes [14]. The Discriminant Analysis (DA) model was built by using the backward stepwise analysis option (within-class covariance matrices are assumed to be equal) was performed to discriminate edible oils and chicken fats according to their quality criteria.

3. RESULTS AND DISCUSSION

3.1. FT-NIR SPECTRA AND REFERENCE ANALYSIS VALUES OF FREE ACIDITY AND PEROXIDE VALUE OF EDIBLE OILS AND CHICKEN FAT

Free fatty acidity is used to constitute the quality and the classification of edible oils. Hydroperoxides are formed due to oxidation of fatty acids and are measured through peroxide value. Both are the most significant parameters to determine quality of edible oils and fats [21, 22]. FT-NIR spectra pre-processing (normalisation) it is necessary to evaluate the results by using multivariate methods for interpretation [23] and to detect spectral differences by computing latent variables (loading spectra) that are related to the free fatty acidity and peroxide value of oils and chicken fat [21]. Figure 1 shows FT-NIR spectra of chicken fat and edible oils.
FT-NIR spectra obtained at 5450 and 4490 cm^{-1} (1090-898 nm) and 5612 and 4509 cm^{-1} (1122-902 nm) and the peaks at can be appointed to the first overtones of C-H stretching and C=O stretching vi-

brations, respectively. FT-NIR spectra of edible oils and chicken fat showed that the most intense absorption bands at 5650 and 4490 cm^{-1} corresponds to free fatty acidity and peroxide value (Fig. 1). Multiple components can be quantified within a single spectrum using software applications [21, 23-25]. Edible oils and chicken fat have similar spectra due to their nature (Fig. 1). The spectra obtained between 5612 to 4500 was used for FFA and 5450 to 4489 was used for PV determinations in FT-NIR after 1st normalization process in this study. The reference titrimetric method data was incorporated into the Bruker FT-NIR system to establish a spectral library for the creation of a quick, non-destructive approach to test oils and chicken fat quality. Table I shows the FFA and peroxide values of edible oils and chicken fat that are determined both by reference and NIR methods.

The results showed that there were variations in the free fatty acid and peroxide value contents of oils and fats obtained from three different brands (Tab. I). The free fatty acid level of the edible oils and fats ranged between 0.28 (corn oil) to 9.63 (chicken fat) % (as oleic acid) determined both by reference analysis and FT-NIR method, respectively. It was found that the virgin olive oil had the highest and corn oil had the lowest peroxide value of 17.91 and 11.48 $meqO_2$ $kg^{-1}$of determined by both reference analysis and FT-NIR method, respectively (Tab. I). According to Table I p-value is 0.05 and edible oils did not exhibit significant differences in PV values between reference and FT-NIR method showed in FFA content of corn oil and chicken fat ($p<0,05$). Z-scores were between -0.13 and -0.17 respectively confirming the accuracy of the FT-NIR-method against reference method for FFA and PV determination. Our results agree with [22, 26, 27] found no significant differences between reference method and FT-NIR method for (fat, protein, and water) composition as well as quality parameters of oils and fats including FFA, PV.

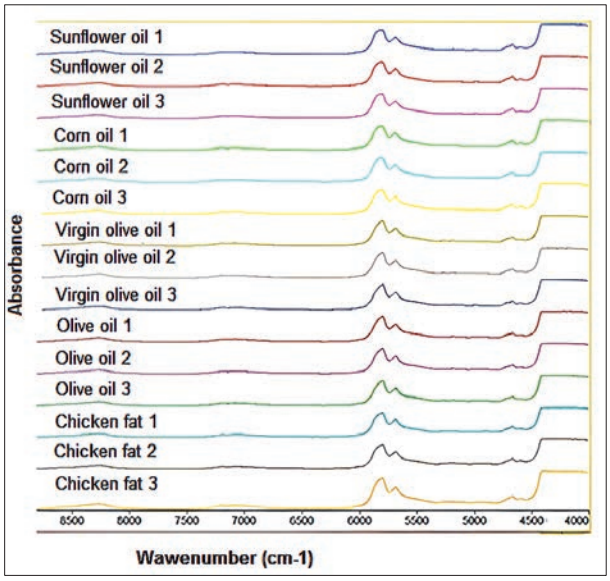

Figure 1 - FT-NIR spectrum of edible oils and chicken fat. Spectra were obtained in the transmittance mode using 6.5 mm i.d. glass vials and accumulating 5 scans per spectrum and a resolution of 8 cm^{-1}. Spectra were shifted on the y-axis to clearly show their characteristic bands. Inset: Differences between the different types of edible oils and chicken fat were analyzed in the interval from 4600 to 8500 cm^{-1} (833 - 2500 nm).

3.2. PREDICTION MODELS AND COMPARISON OF FT-NIR AND REFERENCE METHODS

FT-NIR spectroscopy coupled with is a factorial multivariate calibration method such as Partial least square

Table I - Comparison of Free Fatty Acidity and Peroxide Values of Edible Oils and Chicken Fat by Reference Titrimetric Method and FT-NIR Spectroscopic Method

Edible oils & Fats		Free Fatty Acidity (% as oleic acid)	Peroxide Value (meq O_2 kg^{-1} oil)
Chicken Fat	Reference	9.15±2.81	12.87±5.01
	FT-NIR	9.63±3.32	12.98±4.88
	p-value	0.11	0.80
Corn Oil	Reference	0.28±0.14	12.87±0.76
	FT-NIR	0.46±0.28	11.48±2.13
	p-value	0.37	0.27
Olive Oil	Reference	0.59±0.29	12.84±7.18
	FT-NIR	0.74±0.36	13.67±6.35
	p-value	0.03*	0.54
Sunflower Oil	Reference	0.50±0.16	17.08±2.19
	FT-NIR	0.56±0.43	15.94±4.35
	p-value	0.00**	0.56
Virgin Olive Oil	Reference	1.31±0.17	16.80±11.16
	FT-NIR	0.99±0.19	17.91±12.45
	p-value	0.00**	0.50
	z-score	-0.13	-0.17

*<0.05 **<0.01

(PLS-R) were found to be effective in building the calibration models from variables that has been extensively used for the quality parameters of edible oils [28-30]. The PLS-R models using the FT-NIR spectra for the prediction of the content of the free fatty acids and peroxides found in edible oils and chicken fat was developed by using cross validation (Jacknife), standardised coefficient models. Correlation plots and PLS-R model data are given in Figure 2a-b.

FT-NIR spectra were evaluated by using PLS-R calibration models to correlate and predict free fatty acidity and peroxide values for both official reference analysis and FT-NIR method. Although there is a clear difference between FFA content of chicken fat (9.6%, the highest) and corn oil (0.3%, the lowest) determined by both method, good regression models could be obtained through PLS-R regression, cross validated models by using standardised coefficients. This clearly indicates that both models used for determining and predicting the values of free fatty acidity and peroxide values of edible oils and chicken fat are acceptable models to be predicted or measured accurately by using the FT-NIR Method no matter whether they are present in high or low amounts.

PLS-R regression for free fatty acidity and PV in edible oils and chicken fat evaluated by PLS-R-FT-NIR and official reference method provided equations were as follows; FFA (FT-NIR) = -0,027+0,55* REF FFA with correlation coefficient R^2 = 0.99, RMSE = 0.35 and RPD = 3.1 and PV (FT-NIR) = -0,89 + 0,52* REF PV with correlation coefficient R^2 = 0.94, RMSE = 1.57 and RPD = 2.4, respectively. Our results clearly showed that good prediction models can be developed using the PLS-R technique for the prediction of quality parameters of edible oils and chicken fat since R^2 values were very close to 1 (0.99 and 0.94, respectively) (Fig. 2a, b). PLS regression models can result in accurate models even though the constituents concentrations vary [31] for different types of oils used [10]. While FFA values range between 0.3 to 9.6% and peroxide values between 11.5 to 17.9 meq O2/kg, RMSE values vary between 0.35-1.57. These results clearly show a good prediction models for FFA and PV were obtained from FT-NIR and reference methods. Similar results were also obtained by [31] FFA values ranged between 0.27-11.70%, RMSE values were obtained as 0.47 and 0.61. Our results were in accordance with the previous studies estimated with similar R^2 values found between 0.85 to 0.99 and 0.81 to 0.99 [14, 22, 24, 32, 33] for FFA and peroxide values of olive oil and other edible oils. The RPD values for free fatty acidity and PV values of edible oils and chicken fat were found between 3.1 and 2.4 respectively. This clearly indicates, in addition to R^2 values, that both models used for determining and predicting the values of free fatty acidity and peroxide values of edible oils and chicken fat are acceptable models and free fatty acid and peroxide value of the oils and chicken fat can be predicted accurately by using the FT-NIR method compared to the reference method. In fact, it may be difficult to obtain RPD values higher than 3, because of the sample preparation, presentation, or difficulty with reference testing, and a sample set with minimal variability. RPD > 3.0

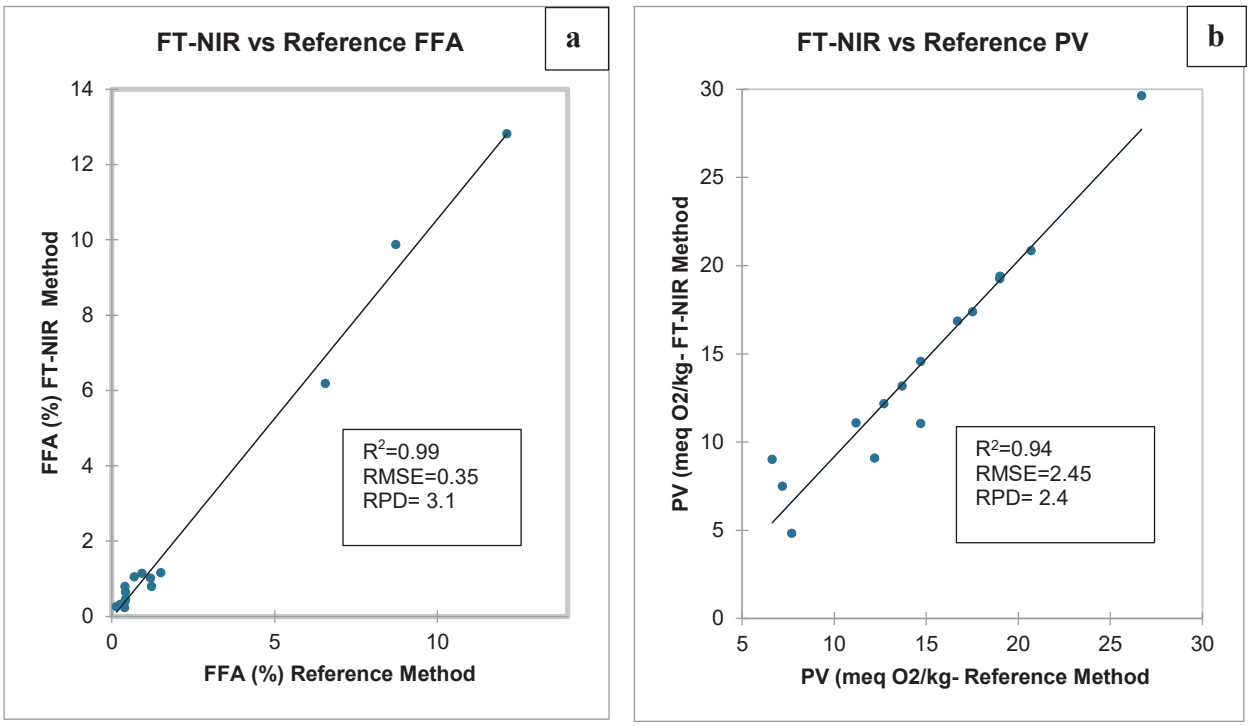

Figure 2 a-b - (a) Correlation plots and PLS-R modeling for Reference and-FT-NIR Method for (a): free fatty acidity-FFA (% as oleic acid), (b): peroxide value-PV (meqO2 kg^{-1} of oil) of edible oils and chicken fat.

can be used for screening quality, quality control, process control and high enough for reliability and prediction, RPD > 8 suitable for any application [18, 21, 25, 31, 32, 34] and RPD higher than 10 is considered equivalent to the reference method [35]. Thus, our results agree with previous studies, where the FT-NIR spectroscopy was used to predict edible oils quality parameters by using PLS-R models based on their R^2 and RPD values [14, 17, 33, 30]. This study showed that FFA and PV of edible oils and chicken fat were determined accurately by using the FT-NIR method. These quality indicators can be accurately predicted by utilising PLS-R methods. Because accuracy and repeatability were excellent, and the measurement time was only approximately 30 seconds per sample, the FT-NIR approach could be useful for determining FFA and PV of edible oils and chicken fat.

Similar results were also confirmed by correlation tests (data not shown). The correlation results clearly indicated that official reference methods and the corresponding FT-NIR methods confirms that FT-NIR is very strong tool to analyse edible oils and fat quality parameters. This method is a simple and convenient way to check quality, with the benefits of ease of use, quick sample turnover, and no sample pre-treatment. In terms of analytical performance, the results of multivariate aided FT-NIR analysis were statistically like those produced by official and traditional processes. Thus, this technique, could be applied for the quality control, safety evaluation and discrimination of different edible oils and fats, it reduces time, costs, and the possible chemical hazard of reference analysis. Successful prediction of ripening degree and phenolic compounds, chlorophyll content, essential oil of olives and olive oil, oregano oil and calila oil leaves [33, 36-38], FFA, PV, total phenolic content and fatty acids of oils [10, 18, 33] are a few examples of FT-NIR approaches being used in prediction research in literature.

3.3. DISCRIMINATION OF EDIBLE OILS AND CHICKEN FAT BY BOTH FT-NIR AND REFERENCE METHODS

The PCA score biplot of FFA and PV results of edible oils and chicken fat both by reference and FT-NIR method is shown in Figure 3a.

Various oils and chicken fat which were analysed with FT-NIR spectra were classified and clustering tendencies were determined by using the PCA method in literature [14, 24, 39].

PC1 was mainly correlated with the free fatty acids and PC2 was correlated with peroxide values determined by both methods. The first principal component (PC1) explains 64.3% of the total variance, and the second major component (PC2) represents 32.3% of the total variance. Edible oils show a negative contribution to PC1 and chicken fat that have the high free fatty acidity (9.6%) were positioned on the positive PC1 axis. The PCA graphs clearly demonstrated that chicken fat shows a significant difference than other edible oils (Fig. 3a). It was clear that PCA clustered oils and chicken fat into three groups of chicken fat highly and positively, sunflower oil and vir-

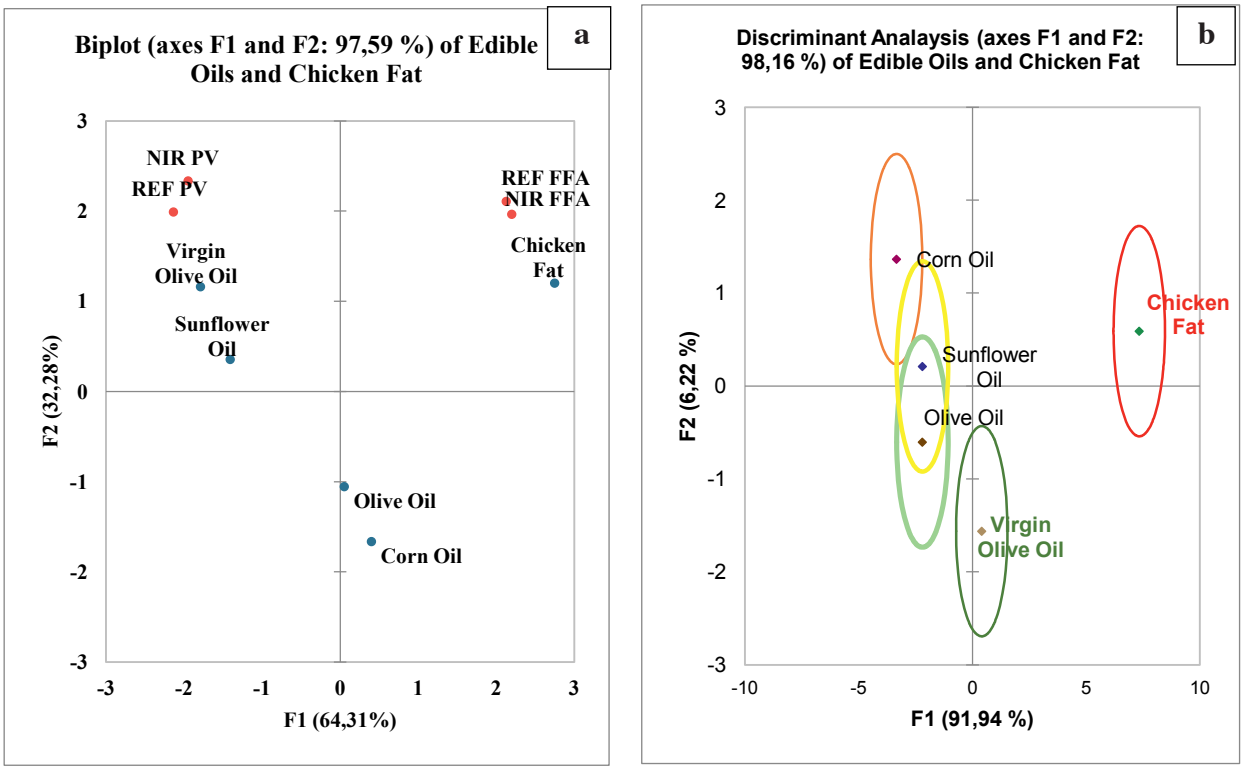

Figure 3 a-b - (a) Bioplot of first two principal components of PCA, (b) Discriminant Analysis (DA) of edible oils and chicken fat

gin olive oil are highly and negatively correlated with PC1 and corn oil, the olive oil are highly and negatively correlated with PC2 (Fig. 3a).
The discrimination of edible oils and chicken fat was achieved by DA analysis (Fig. 3b). The first two discriminant functions explained 98.16% of the total variance, according to the groups in the score plot for oils and chicken fat. Chicken fat and virgin olive oil were separated clearly from olive oil, sunflower oil and corn oil, and there are overlapping between sunflower and olive oil (Fig. 3b). The results clearly showed that good quality groupings were achieved by DA for edible oils and chicken fat. This study showed that using FFA and PV as quality criteria, FT-NIR with chemometric treatment could correctly distinguish virgin olive oil from olive oil, sunflower oil, corn oil by discriminant analysis.
This separation/discrimination of chicken fat could be due to its fatty acid composition and oxidative stability) and the highest concentration of FFA as compared to edible oils (Tab. I). Thus chicken fat and virgin olive oil could be discriminated from edible oils and fats based on FFA and PV values by using both reference method and FT-NIR methods with DA. Our results were consistent with the results of [16, 40] that could discriminate lard, butter from vegetable oils based on iodine values by using PCA, PLS-DA, DA and canonical variate analysis.
Our results are consistent with the results of [14, 24, 40] that managed to discriminate oils and [1, 26] chicken fat and animal fats based on quality parameters and fatty acid composition. [41] clustered the oils according to acidity and peroxide index manage to cluster olive oils from sunflower seed and corn oil and [14] separated olive oils depending on their grade as extra virgin, virgin, ordinary virgin and lampante oils [24] due to their own unique clustering trends linked with their storage durations, oils like soybean oil, rapeseed oil, corn oil, and sunflower seed oil were separated [40] clustered pure and adulterated palm oil samples according to PCA graph. Recently, [26] easily discriminated lard as animal fat from vegetable oils by using FAMEs as metabolomics with chemometric treatment.

4. CONCLUSIONS

The monitoring of acidity and peroxide value is very important for quality and the safety of edible oils and chicken fat. The official reference method for determining free fatty acidity and peroxide values in edible oils and chicken fat is tedious, time-consuming, arduous, tiresome, and damaging, and it is not suitable for online use. The results of study clearly showed that FT-NIR spectroscopy (4500 to 5600) was satisfactory to determine and predict free fatty acidity and peroxide contents of edible oils and chicken fat with a good correlation (94 to 99%) with reference analysis (R^2 = 0.99 and 0.94, RPD > 2) and to discriminate edible oils and chicken fat based on their acidity and peroxide value. Chicken fat and virgin olive oil was discriminated from other edible oils with a 98% accuracy, based on their FFA and PV. FT-NIR method with chemometric treatment could be used for quality control and prediction and discrimination purposes as a convenient, green, fast, and accurate alternative to reference titrimetric methods. It gives government authorities and stakeholders a useful tool for assessing the quality of culinary oils and chicken fat quickly. This method can be a time and solvent-saving option for routine analysis of a large number of oils and fats samples, particularly for high throughput results during industrial processing that allow in-process optimisation of technological parameters. Further research in this field with other quality and purity parameters for oils and fats is needed to confirm the possible application of FT-NIR with multivariate approaches for quality assurance and safety of the oils and fats.

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REFERENCES

[1] F. Gao, D. Ben-Amotz, S. Zhou, Z. Yang, L. Han, X. Liu, Comparison and chemical structure-related basis of species discrimination of animal fats by Raman spectroscopy using near-infrared and visible excitation lasers, LWT, 13. 110105 (2020).
[2] X. Li, J. Zhao, L.H. Liu, J. Tan, Optical properties of edible oils within spectral range from 300 to 2500 nm determined by double optical pathlength transmission method, Applied Optics 54, 3886-3893, (2015).
[3] L.M. Peña-Saldarriaga, J. Fernández-López, J.A. Pérez-Alvarez, Quality of Chicken Fat by-Products: Lipid Profile and Colour Properties, Foods 9, 1046 (2020).
[4] V.A.M. Selvan, N.K. Gurusala, A. Balan, S. Athreya, Optimization of Waste Chicken Fat Pre-Treatment Process for Biodiesel Production, Transactions of SAE, 2013 (2014).
[5] A.R. Putri, A. Rohman, W. Setyaningsih, S. Riyanto, Determination of acid, peroxide, and saponification value in patin fish oil by FTIR spectroscopy combined with chemometrics, Food Research 4, 1758-1766 (2020).
[6] I. Tarhan, A.A. Ismail, H. Kara, Quantitative determination of free fatty acids in extra virgin olive oils by multivariate methods and Fourier transform infrared spectroscopy considering different

absorption modes, International Journal of Food Properties 20, S790-S797 (2017).
[7] S.A. Mahesar, S.N. Shah, A.W. Mahesar, A.A. Kandhro, A.R. Khaskheli, P. Menghwar, S.T.H. Sherazi, A chemometric approach for the quantification of free fatty acids in cottonseed oil by Fourier transform infrared spectroscopy, International Journal of Food Properties 20, 1913-1920 (2017).
[8] A. Kiritsakis, T. M. Keceli, K. Kiritsakis, Olive Oil, in: F. Shahidi (Ed.) Bailey's Industrial Oil and Fat Products, 1-38, Wiley, Co. (2020).
[9] A.M. Marina, Quantitative Analysis of Peroxide Value in Virgin Coconut Oil by ATRFTIR Spectroscopy, The Open Conference Proceedings Journal 4, 53-56, (2013).
[10] W.E. Gilbraith, J.C. Carter, K.L. Adams, K.S. Booksh, J.M. Ottaway, Improving Prediction of Peroxide Value of Edible Oils Using Regularized Regression Models, Molecules 26, 7281 (2021).
[11] X.Yu, Q. Li, D. Sun, X. Dong, T. Wang, Determination of peroxide value of edible oils by FTIR spectroscopy using polyethylene film, Anal. Methods, 7, (2014).
[12] AOCS, AOCS Official methods and recommended practices of the American Oil Chemists' Society III. AOCS (Method Cd 8-53 and Ca 5a-40), in, AOCS, Campaign, IL, (2017).
[13] IOC, International Olive Council. Trade Standard Applying to Olive Oils and Olive Pomace Oils. COI/T.15/NC No 3/Rev. 16 June 2021, in: COI/T.15/NC No 3/Rev. 16 June 2021, International Olive Oil Council, Madrid, 1-18, (2021).
[14] N. Abu-Khalaf, M. Hmidat, Visible/Near Infrared (VIS/NIR) spectroscopy as an optical sensor for evaluating olive oil quality, Comput Electron Agr, 173 (2020).
[15] L. Dymińska, M. Calik, A.M.M. Albegar, A. Zając, K. Kostyń, J. Lorenc, J. Hanuza, Quantitative determination of the iodine values of unsaturated plant oils using infrared and Raman spectroscopy methods, International Journal of Food Properties 20, 2003-2015 (2017).
[16] P. Samyn, D. Nieuwkerke, G. Schoukens, L. Vonck, D. Stanssens, H. Aabbeele, Quality and Statistical Classification of Brazilian Vegetable Oils Using Mid-Infrared and Raman Spectroscopy, Appl Spectrosc 66, 552-565 (2012).
[17] H. Jiang, Y.C. He, Q.S. Chen, Determination of acid value during edible oil storage using a portable NIR spectroscopy system combined with variable selection algorithms based on an MPA-based strategy, J Sci Food Agr 101, 3328-3335 (2021).
[18] E.A. Seweh, Z. Xiaobo, F. Tao, S. Jiachen, H.E. Tahir, M. Arslan, Multivariate analysis of three chemometric algorithms on rapid prediction of some important quality parameters of crude shea butter using Fourier transform-near infrared spectroscopy, J near Infrared Spec 27, 220-231(2019).
[19] L.S. Vieira, C. Assis, M.E.L.R. de Queiroz, A.A. Neves, A.F. de Oliveira, Building robust models for identification of adulteration in olive oil using FT-NIR, PLS-DA and variable selection, Food Chem, 345 (2021).
[20] A. Rohman, M.A.I.B. Ghazali, A. Windarsih, I. Irnawati, S. Riyanto, F.M. Yusof, S. Mustafa, Comprehensive Review on Application of FTIR Spectroscopy Coupled with Chemometrics for Authentication Analysis of Fats and Oils in the Food Products, Molecules 25, 5485 (2020).
[21] P. Adewale, O. Mba, M.-J. Dumont, M. Ngadi, R. Cocciardi, Determination of the iodine value and the free fatty acid content of waste animal fat blends using FT-NIR, Vib Spectrosc 72, 72-78 (2014).
[22] I. Willenberg, B. Matthaus, C. Gertz, A New Statistical Approach to Describe the Quality of Extra Virgin Olive Oils Using Near Infrared Spectroscopy (NIR) and Traditional Analytical Parameters, Eur J Lipid Sci Tech, 121 (2019).
[23] K.C. Kaufmann, F.D. Favero, M.A.M. de Vasconcelos, H.T. Godoy, K.A. Sampaio, D.F. Barbin, Portable NIR Spectrometer for Prediction of Palm Oil Acidity, J Food Sci 84, 406-411(2019).
[24] Y.C. He, H. Jiang, Q.S. Chen, High-precision identification of the actual storage periods of edible oil by FT-NIR spectroscopy combined with chemometric methods, Anal Methods-Uk, 12 3722-3728 (2020).
[25] R.K.K. Raigar, S. Srivast, H.N. Mishra, Implementation of Non Destructive FTNIR Method for Quick Estimation of Peanut Quality Based on FFA and Peroxide Value, in, Research Square Platform LLC, (2021).
[26] M. Heidari, Z. Talebpour, Z. Abdollahpour, N. Adib, Z. Ghanavi, H.Y. Aboul-Enein, Discrimination between vegetable oil and animal fat by a metabolomics approach using gas chromatography–mass spectrometry combined with chemometrics, Journal of Food Science and Technology, 573415-3425 (2020).
[27] J. Mlček, K. Šustová, J. Simeonovová, Application of FT NIR spectroscopy in the determination of basic chemical composition of pork and beef, Czech Journal of Animal Science, 51361-368 (2011).
[28] R.M. Maggio, L. Cerretani, E. Chiavaro, T.S. Kaufman, A. Bendini, A novel chemometric strategy for the estimation of extra virgin olive oil adulteration with edible oils, Food Control 21, 890-895, (2010).
[29] P. Liang, H. Wang, C. Chen, F. Ge, D. Liu, S. Li, B. Han, X. Xiong, S. Zhao, The Use of Fourier Transform Infrared Spectroscopy for Quantification of Adulteration in Virgin Walnut Oil, Journal of Spectroscopy, 1-6, (2013).

[30] I.S. Ozdemir, O. Karaoglu, C. Dag, S. Bekiroglu, Assessment of sesame oil fatty acid and sterol composition with FT-NIR spectroscopy and chemometrics, Turk J Agric For 42, 444-452, (2018).
[31] I. Dogruer, H.H. Uyar, O. Uncu, B. Ozen, Prediction of chemical parameters and authentication of various cold pressed oils with fluorescence and mid-infrared spectroscopic methods, Food Chem 345, 128815 (2021).
[32] H. Jiang, Q.S. Chen, Determination of Adulteration Content in Extra Virgin Olive Oil Using FT-NIR Spectroscopy Combined with the BOSS-PLS Algorithm, Molecules 24, (2019).
[33] J.A.C. Sanchez, W. Moreda, J.M. Garcia, Rapid Determination of Olive Oil Oxidative Stability and Its Major Quality Parameters Using Vis/NIR Transmittance Spectroscopy, J Agr Food Chem, 618056-8062 (2013).
[34] N. Prieto, O. Pawluczyk, M.E.R. Dugan, J.L. Aalhus, A Review of the Principles and Applications of Near-Infrared Spectroscopy to Characterize Meat, Fat, and Meat Products, Appl Spectrosc 71, 1403-1426 (2017).
[35] R. Riovanto, M. De Marchi, M. Cassandro, M. Penasa, Use of near infrared transmittance spectroscopy to predict fatty acid composition of chicken meat, Food Chem 134, 2459-2464, (2012).
[36] C. Alamprese, S. Grassi, A. Tugnolo, E. Casiraghi, Prediction of olive ripening degree combining image analysis and FT-NIR spectroscopy for virgin olive oil optimisation, Food Control, 123, (2021).
[37] N. Wu, J.A. Liu, G.Y. Zhou, R.K. Yan, L. Zhang, Prediction of Chlorophyll Content of Leaves of Oil Camelliae after Being Infected with Anthracnose Based on Vis/NIR Spectroscopy, Spectrosc Spect Anal 32, 1221-1224 (2012).
[38] S.P. Zhu, G. Wang, F. Yang, J.Q. Kan, J. Guo, Q.M. Qiu, Effect of powder's particle size on the quantitative prediction of volatile oil content in zanthoxylum bungeagum by NIR technique, Spectrosc Spect Anal 28, 775-779, (2008).
[39] A.S. Luna, A.P. da Silva, J.S.A. Pinho, J. Ferre, R. Boque, Rapid characterization of transgenic and non-transgenic soybean oils by chemometric methods using NIR spectroscopy, Spectrochim Acta A, 100, 115-119, (2013).
[40] E. Teye, C. Elliott, L.K. Sam-Amoah, C. Mingle, Rapid and nondestructive fraud detection of palm oil adulteration with Sudan dyes using portable NIR spectroscopic techniques, Food Addit Contam A 36, 1589-1596, (2019).
[41] S. Armenta, S. Garrigues, M. de la Guardia, Determination of edible oil parameters by near infrared spectrometry, Anal Chim Acta 596, 330-337, (2007).

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- Determinazione del **triptofano**

Per informazioni:

Liliana Folegatti - Esperta Oli, Grassi e Derivati
liliana.folegatti@mi.camcom.it

Stefania De Cesarei - Esperta Analisi Sensoriali
stefania.decesarei@mi.camcom.it

Team Chimica, Tecnologia e Sicurezza Alimentare

Development and characterisation of olive oil based spreads containing different seasonings

Mustafa Öğütcü[1]✉
Elif (Dincer) Albayrak[2]
Hatice Çokay[1]
Emin Yakar[3]

[1]Department of Food Engineering
Faculty of Engineering
Çanakkale Onsekiz Mart University
Çanakkale, Turkey

[2] Bayramiç Vocational College
Çanakkale Onsekiz Mart University
Bayramiç, Çanakkale, Turkey

[3]Department of Material Science and Engineering
Faculty of Engineering
Çanakkale Onsekiz Mart University
Çanakkale, Turkey

✉ CORRESPONDING AUTHOR:
Mustafa Öğütcü
Department of Food Engineering
Faculty of Engineering
Çanakkale Onsekiz Mart University
17020, Çanakkale, Turkey
mogutcu@comu.edu.tr

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The main objective of the present study was to produce olive oil-based table spreads enriched with different spices by using the organogel technique and to investigate the effect of spices on the oleogel network formed by the self-assembly of natural waxes. For that reason, the oleogels were prepared with sunflower wax, beeswax and shellac wax, and enriched with poppy seeds, thyme, lemon peel, mint, and their physico-chemical, thermal, textural and structural properties were determined. In order to determine the storage stability, peroxide, acid and colour values were monitored during 90 days of storage at +4°C. The sunflower oleogels with and without additives had higher oil binding capacity, firmness and stickiness values than the beeswax and shellac wax oleogels. The sunflower and shellac wax oleogels had peaks around 3.70 and 4.10 Å that indicated presence of beta prime polymorphic form. Both enriched and plain oleogels prepared with sunflower wax remained stable even at the end of the storage period at 5% wax addition level. Similar results were observed in the beeswax and shellac wax oleogels without spices. Particularly, the oil-binding capacity, stability, firmness and stickiness values of the oleogels were influenced by spice addition. In conclusion, sunflower wax was found to be more suitable than beeswax and shellac wax for the formation of oleogels with additives, such as spices, at 5% wax addition level.
Keywords: Oleogels, Olive Oil, Natural waxes, X-ray diffraction, Particle size.

INTRODUCTION

Olive oil is one of the most beneficial oils and an indispensable item of the Mediterranean diet. Olive and olive tree are included in both mythology and the Holy Scriptures such as the Quran, the Bible, and the Torah. Olive oil is obtained from the olive that is the fruit of *Olea europaea* L., and its production dates to 4000 BC according to the archaeological data. Olive oil is obtained through a process which uses only mechanical processes; hence, it consists of fatty acids and triglycerides as well as minor ingredients such as hydrocarbons, sterols, phenolic compounds, volatile compounds, waxes, fatty alcohols, mono- and diglycerides, and pigments. Moreover, the major fatty acid is oleic acid (60-85%; C18:1n-9) which is the monounsaturated fatty acid and is known as omega-9 fatty acid. All these features make it superior, beneficial, healthier and more stable against oxidation compared to refined oils [1 - 3].

Olive oil is mostly consumed by adding seasonings in Aegean- and Mediterranean-type diets, and currently sold commercially in oils with seasonings. Gambacorta et al. [4] reported that the addition of some spices to olive oil was an ancient tradition that had an effect not only on flavour but also on shelf life and nutritional value. For this purpose, thyme, marjoram, rosemary, red pepper, basil, lavender, sage, mint, chili pepper, gooseberry and lemon were used as flavouring agents [5, 6]. Thyme is a good source of bioactive compounds, carvacrol, and thymol which are generally safe for consumption.

These compounds are used in dental applications, and the food and feed industry due to antiseptic, antibacterial and antiviral features [7]. Thyme is one of the most popular spices, particularly in the last decade, and some scientific studies in literature suggest that it could be used to reduce the symptoms of COVID-19 [8 - 10]. Poppy plant is grown for its seeds and opium. Poppy seed contains up to 50% oil as well as various bioactive compounds. Poppy seeds are widely used for confectionary, bakery, and extraction of oil [11, 12]. Dried mint leaves were used in ancient times and found in Egyptian pyramids in 1000 BCE. Mint was effective on the digestive system, and is widely used against the asthma, chest problems, and mouth ulcers [13]. Dried lemon peel contains polyphenols, dietary fibre, and volatile compounds due to its aromatic compounds used as a flavour enhancer in many kinds of food formulations [14].

Organogels have stood out as a popular technique in structuring liquid oils, especially in recent years. Organogels are defined as organic solvents entrapped in a three-dimensional network formed by organic gelators. Organogels are called oleogels when the liquid phase was oil. In recent years, many kinds of oils and gelators have been used for the preparation of oleogels. The most important advantages of oleogels are that they do not change the composition of fatty acids, have low saturated fat content, do not contain *trans*-fat, are spreadable and have plastic properties. These advantages not only meet consumer need but also increase the usage area of liquid oil [15, 16].

In literature, oleogels produced with natural waxes have been reported as being an alternative to spreadable margarine and butter [17 - 23]. However, spice-enriched oleogels (containing additives with different particle sizes and solid particles) were not used in the studies mentioned above, and their stability and structural form were not tested. Recently, Yılmaz and Demirci [24] reported common characteristic properties of virgin olive oil-sunflower oleogels enriched with thyme and cumin spices. Nevertheless, there were no detailed information on how different particle sizes (in the added additives) affected the gel structure and stability in previous studies.

The actual target of this study was to produce natural wax-olive oil oleogels enriched with spices and to determine the effects of spice addition on oleogel structure and stability. To the best of our knowledge, this study is the first report observing the effects of additives with different particle sizes on oleogel formation. In this context, we think that this study will be a guide for determining the different usage potential and purposes of oleogels.

MATERIALS AND METHODS

Olive oil was purchased from local producers in Ezine, Çanakkale, Turkey. Natural waxes (sunflower, shellac and beeswax) were purchased from KahlWax (Kahl GmbH & Co., Trittau, Germany). The producer provided the melting point for beeswax as 61-66°C, sunflower as 74-80°C, and shellac wax as 78-84°C. All the spices (mint, thyme, poppy seed and lemon peel powder) were purchased from a local herbalist (Çanakkale, Turkey). All other chemicals used in analysis were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, USA), and all chemicals were of an analytical grade.

PREPARATION OF THE OLEOGELS

All the prepared oleogels contained 5% waxes (sunflower, shellac and beeswax) and 1% (mint, thyme, lemon peel powder and poppy seeds) spices with 94% olive oil. The amounts of added waxes and spices were determined by preliminary trials. For the oleogel preparation, the waxes were completely melted at 85-90°C on water bath, and at the same time, the olive oil was heated at the same temperature. Then, the olive oil was added into the wax at isothermal conditions, and the mixture was stirred. After these procedures, spice was added into the mixture and stirred until the first crystal formation was observed. At the end of the process, the mixture was cooled at room temperature overnight and stored at 4°C until the planned analyses were performed. Formulations, sample codes of the oleogels, and particle size of used additives are given in Table I.

PHYSICOCHEMICAL ANALYSES

The crystal formation time (CFT), and oil-binding capacity (OBC) of all the oleogel samples were measured according to Öğütcü et al. [21]. The minimum gel formation (MGF) concentration represents the minimum wax addition level (%) that formed stable gel. The centrifuge stability test (CST) values of the samples were measured 1500 g centrifugation for 10 min at room temperature and results expressed +/- (+; no phase separation/ -; phase separation). Acid value of the samples were measured according to AOCS (Ca 5a-40) method [25]. Approximately 2.5 g samples dissolved in ethanol: diethyl ether (1:1) and titrating with ethanol-KOH solutions against the phenolphthalein indicator and results were expressed mgKOH/g. Peroxide value was measured by AOCS (Cd 8-53) method [25]. For the determination of PV, approximately 2.0 g sample dissolved in acetic acid: chloroform (3:2) and added potassium iodide in darkness, then titrating with a sodium thiosulfate solution for free iodine titration. The PV results were expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg). Iodine value (IV) was measured by AOCS (Cd 1-25) method [25] using with Wijs solutions and results were expressed as gI_2/100g.

The colour measurements of the samples were performed using a colorimeter (Konica, Minolta CR-400, Osaka, Japan) and L (lightness), a (+redness/-greenness) and b (+yellowness/-blueness) values were

Table I - Formulations of the produced oleogels and particle size distribution of seasonings used in oleogels

Samples	VOO (%)	SW (%)	BW (%)	SH (%)	Poppy Seeds (%)	Thyme (%)	Lemon Peel (%)	Mint (%)
SW	95	5.0	-	-	-	-	-	-
SWP	94	5.0	-	-	1.0	-	-	-
SWT	94	5.0	-	-	-	1.0	-	-
SWL	94	5.0	-	-	-	-	1.0	-
SWM	94	5.0	-	-	-	-	-	1.0
BW	95	-	5.0	-	-	-	-	-
BWP	94	-	5.0	-	1.0	-	-	-
BWT	94	-	5.0	-	-	1.0	-	-
BWL	94	-	5.0	-	-	-	1.0	-
BWM	94	-	5.0	-	-	-	-	1.0
SHW	95	-	-	5.0	-	-	-	-
SHP	94	-		5.0	1.0	-	-	-
SHT	94	-		5.0	-	1.0	-	-
SHL	94	-		5.0	-	-	1.0	-
SHM	94	-	-	5.0	-	-	-	1.0

Samples	5-2 mm (%)	2-1 mm (%)	1000-500 µm (%)	500-100 µm (%)	100-63 µm (%)	63-20 µm (%)	Sample Amount (g)
Mint	5.20	34.20	45.53	15.07	Nd	Nd	16.90
Thyme	21.21	64.57	13.64	0.58	Nd	Nd	16.01
Poppy Seeds	Nd	Nd	Nd	100.00	Nd	Nd	38.55
Lemon peel powder	Nd	Nd	1.89	56.02	16.73	25.37	19.69

VOO: virgin olive oil, SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint, Nd: not detected.

determined. The colour differences (delta E) of the samples were calculated from recorded CIE lab data. The acid, colour and peroxide values of the oleogels samples were measured once a month during the 90-day storage period at 4°C, while the other physicochemical analyses were conducted only with fresh samples. The measurement of the particle size distribution (PSD) of seasonings was performed by sieving (Retsch AS200 Retsch Technology, Haan, Germany).

STRUCTURAL ANALYSES

The textural properties of the oleogel samples were measured using texture analyser (TA-HD Plus, Stable Microsystems, UK), the texture analyser equipped with spreadability rig, and the results were calculated using the instrument software (Texture Exponent v.6.1.1.0, Stable Microsystems, UK). For the spreadability test, the oleogel samples were filled sufficiently into conic spreadability cup and waited for 15 min at room temperature, and the measurements were applied at this temperature. The spreadability test specifications were test mode compression, test speed at 3.0 mm/sec, post-test speed at 10 mm/sec and distance 23.00 mm. The XRD measurements of the oleogels samples were measured using a PANalytical empyrean XRD (PANalytical, Netherlands). The angular scans from 2.0° to 50° 2-theta range were performed by 2°/min scan rate. A Cu source x-ray tube (λ = 1.54056 Å, 45 kV and 40 mA) and an X'Pert Highscore Plus software were used for the data analysis. The macro images of the oleogels were taken with stereomicroscope (Zeiss, Stemi 305, Germany) equipped with a digital camera (Argenit, Kameram image processing system, Turkey).

THERMAL ANALYSES

The thermal properties of the enriched oleogels samples were evaluated using Differential Scanning Calorimeter (DSC7020, Hitachi High-tech Science Corporation, Japan), as explained in detail by Öğütcü et al. [21]. 5-7 mg oleogel samples were weighted into aluminium pan that was also used as standard (without sample). The samples were heated from 30°C to 120°C heating rate at 15°C/min and held at 1 min at this temperature for removal to water, and then the samples were cooled from 120 to -20°C cooling rate at 10°C/min and held at 3 min at this temperature for the crystallisation process of the oleogels to complete. After this section, the samples were heated again from -20°C to 100°C at 5°C/min heating rate, and the crystallisation and melting *onset*, *peak* temperatures and *enthalpy* values of the oleogels were calculated using the DSC software (TA7000 Measurement 10.5v, Hitachi High-Tech Science Corp.).

STATISTICAL ANALYSES

The oleogels prepared in the study were in two replications, and the planned analyses for these samples were performed at least three times. The data collected from the study were evaluated using

the MINITAB statistical software programme [26]. The results were presented as mean and standard deviation. The similarities and differences between the samples were evaluated using the Analysis of Variance (ANOVA) with Tukey multiple tests.

RESULTS AND DISCUSSIONS

The particle size distribution and average particle size influenced the rheology, viscosity, texture spreadability, stability, and mouthfeel properties of the emulsion and suspension [27]. The PSD of the seasonings in dry form is presented in Table I. Considering the particle size distribution of the mint, 45.53% consisted of 1000-500µm particles, while 34.20% contained 2-1 mm particles and 15.07% contained 500-100µm particles. The PSD of the thyme was formed mostly by 2-1 mm (64.57%), 5-2 mm (21.21%) and 1000-500 µm (13.64%) particles. Unlike thyme and mint, the PSD of the poppy seed was homogeneous and consisted of 500-100 µm particles. On the other hand, the PSD of the lemon powder was smaller than that of the others, and consisted of 500-100 µm (56.02%), 100-63 µm (16.73%) and 63-20 µm (25.37%) particles. The results showed that the PSD of the seasonings used in the prepared oleogels was quite different from one another.

The minimum gel concentrations (MGC), centrifuge stability test (CST), crystal formation time (CFT), oil-binding capacity (OBC) and iodine values (IV) of the oleogels are given in Table II. The MGC values of the sunflower (SW) beeswax (BW) and shellac waxes (SHW) were 1.0, 3.5 and 4.0%, respectively. In the literature, it was reported that, depending on the wax compositions, the critical gel concentrations of the SW, BW and SHW were 0.5, 2.0-3.0 and 5.0%, respectively [28, 29]. The CFT values of the plain SW, BW and SHW oleogels were 1.38, 7.42 and 16.50 min: s, respectively. Additionally, the plain oleogels had lower CFT values compared to the oleogels with additives (Table II). The CST results showed that the plain BW, SW and SHW oleogels were stable (no phase separation) at 5% wax addition level at room temperature. On the other hand, a phase separation was observed in the enriched oleogels prepared with BW and SHW (SHT and SHM) at 5% added wax concentration at room temperature, except the enriched SW-based oleogels. Öğütcü et al. [21] reported that the CFT values of the enriched and aromatised virgin olive oil prepared with 5% of SW and BW oleogels were 3.50 and 6.50 min:s, respectively. Fayaz et al. [30] reported that the gelling time of oleogels containing 5-15% of BW ranged between 3.3-4.8 min. The OBC values of the BW, SW and SHW oleogels enriched with spices ranged from 10.42 to 96.03%, while the OBC values of the oleogels without additives were > 97%. Like our findings, previous research reported that the OBC values of the 5% SW and BW oleogels prepared with olive oil were ≥ 97% [21, 22]. The enriched SW oleogels had higher OBC values compared to the enriched BW and SHW-based oleogels. These results showed that solid particles added to oleogels affected the OBC, CFT and CST values of the oleogels and decreased OBC and CST and increased CFT values (Table II). Furthermore, it also showed that enriched shellac and beeswax may have higher OBC and lower CFT value at higher concentration level than 5%. In other words, when it is desired

Table II - Physico-chemical parameters of oleogels prepared with different waxes and spices

Samples	MGC (%)	CST	CFT (min:s)	OBC (%)	IV (gI_2/100g)
SW	1.0	(+:+)	1:38	99.70±0.26Aa	46.58±3.75b
SWP	1.0	(+:+)	1:42	93.00±0.81b	
SWT	1.0	(+:+)	1:44	94.20±1.45b	
SWL	1.0	(+:+)	1:40	96.03±1.47ab	
SWM	1.0	(+:+)	1:40	93.05±1.44b	
BW	3.5	(+:+)	7:42	99.83±0.07Aa	58.15±3.74a
BWP	3.5	(-:-)	8:00	10.42±1.22b	
BWT	3.5	(-:-)	8:00	15.99±0.55b	
BWL	3.5	(-:-)	8:00	12.89±2.20b	
BWM	3.5	(-:-)	8:00	17.56±3.40b	
SHW	4.0	(+:+)	16:50	97.58±0.21Ba	60.87±6.82a
SHP	4.0	(+:+)	17:00	53.20±0.48c	
SHT	4.0	(+:-)	17:00	63.07±3.33b	
SHL	4.0	(+:+)	17:00	47.70±1.08c	
SHM	4.0	(-:-)	17:00	61.66±1.36b	

MGC: minimum gel concentration, CST: centrifuge stability test, CFT: crystal formation time, OBC: oil-binding capacity, IV: iodine value, SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

*The capital letters show differences among used waxes and the lower case letters show differences among the samples prepared with the same wax ($p \leq 0.05$).

Table III - Acid (AV), peroxide (PV), delta E values of the fresh and 90 days stored oleogels prepared with different waxes and spices.

Samples	AV (mgKOH/g)		PV (meqO_2/kg)		Delta E
	Fresh	Stored	Fresh	Stored	
SW	1.98±0.10a*	1.78±0.03ab	14.08±0.16cd	17.45±0.22a	3.83±0.04a
SWP	1.57±0.06ab	1.46±0.01b	15.44±0.26bc	16.13±0.16ab	2.92±0.02b
SWT	1.77±0.19ab	1.48±0.10b	13.86±1.16cd	15.01±0.07bc	3.05±0.03b
SWL	1.78±0.04ab	1.63±0.02ab	15.18±0.25bc	15.07±0.11bc	3.85±0.08a
SWM	1.40±0.26b	1.55±0.02ab	12.45±0.42d	17.42±0.14a	4.08±0.17a
BW	2.03±0.09a	1.93±0.01ab	17.51±0.34bc	17.36±0.49bc	2.44±0.01c
BWP	1.63±0.01c	1.67±0.01bc	14.92±0.27cd	17.04±0.05bcd	5.83±0.03a
BWT	1.66±0.08c	1.76±0.09bc	14.66±0.70cd	21.41±0.67a	5.16±0.02b
BWL	1.57±0.04c	1.67±0.02bc	14.08±0.58d	20.54±0.39a	5.10±0.07b
BWM	1.81±0.13abc	1.60±0.04c	14.73±1.98cd	18.78±0.02ab	2.00±0.08d
SHW	1.93±0.05a	1.72±0.13ab	19.74±1.11cde	21.43±0.56bc	6.37±0.06c
SHP	1.76±0.04ab	1.82±0.06ab	17.46±0.04def	19.80±0.37cd	6.18±0.01c
SHT	1.60±0.19b	1.82±0.03ab	17.20±1.06def	21.39±0.85bc	6.08±0.31c
SHL	1.65±0.08ab	1.85±0.01ab	17.08±0.55ef	23.07±0.15b	7.43±0.05b
SHM	1.59±0.03b	1.77±0.03ab	16.22±0.75f	26.03±0.39a	9.06±0.01a
OO**	1.97±0.14	1.94±0.01	18.67±0.17	21.82±0.13	Nd

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint. OO: Olive oil: Nd: Not detected.

* The lower case letters show differences among the samples prepared with same wax on the same column and show the differences between fresh and stored samples on the same line ($p \leq 0.05$).

** Free fatty acid values of the fresh and stored olive oil were 0.99 and 0.97% oleic acid, respectively.

to form a gel with particle-containing additives, more than 5% level of beeswax and shellac wax should be added. The results indicated that sunflower wax compared with beeswax and shellac wax could be effective to form oleogels with spices or like additives particularly at 5% addition level and even at lower concentrations. Like the CFT, CST and MGC values, the OBC values mostly changed depending on the composition, addition level and type of the waxes [29, 31]. Additionally, shear and cooling rate were effective on the OBC values of the oleogels, as similarly reported by Blake and Marangoni, [32, 33].

Iodine value (IV) is defined as a parameter indicating the degree of saturation/unsaturation of oils and fats [34]. The IV value of the olive oil was 84 gI_2/100g, and our findings were within legal limit (75-94 gI_2/100g) according to Codex Stan., 33 [34]. The IV values of the oleogels samples ranged from to 46.58-60.87 gI_2/100g. There were no specifications about iodine limit for butter and margarine in Codex Alimentarius, although in literature, the IV values of butter and margarine were reported as 29.70 and 57.85, respectively [23]. These results showed that the addition of 5% wax reduced the iodine values of the gels to the iodine values level of margarine and butter.

The acid (AV), peroxide (PV) and delta E values of the fresh and 90-day stored oleogels samples and olive oil are given in Table III. The FFA value is an important parameter, particularly for olive oil and olive oil-based products, because the FFA value is used in pricing and classification [35]. The FFA values of the fresh and stored olive oil were 0.99 and 0.97% oleic acid, respectively. The FFA values are given as 0.8, 2.0 and 3.3 for extra virgin olive oil, virgin olive oil and ordinary virgin olive oil in Codex Stan 33, respectively [34]. The olive oil used in this study was classified as virgin olive oil according to Codex Stan [34]. The limits of the acid value for the virgin and cold-pressed fats and oils were up to 4.0 mg KOH/g according to Codex Stan. [35]. The AV values of the fresh and stored samples ranged between 1.40-2.03 and 1.55-1.93 mg KOH/g, respectively. The acid values of the samples were found within legal limits according to Codex Alimentarius Standard [35]. The used additives affected the acid values of the samples (Table III). On the other hand, the statistical evaluations indicated that storage time and wax types were effective on the acid values of the samples ($p \leq 0.05$). The peroxide value is a parameter that shows the degree of oxidation in oils and is an indicator of whether the oil is stored under suitable conditions [36]. The PV values of the fresh samples ranged from 14.08 to 19.74 meqO_2/kg, while the stored samples ranged from 15.01 to 26.03 meqO_2/kg. The PV limits of the Codex Alimentarius Standard for olive oil (33-1981) and cold-pressed fats and oils subjected to modification and fractionation (19-1981) were up to 20 meqO_2/kg and 15 meqO_2/kg,

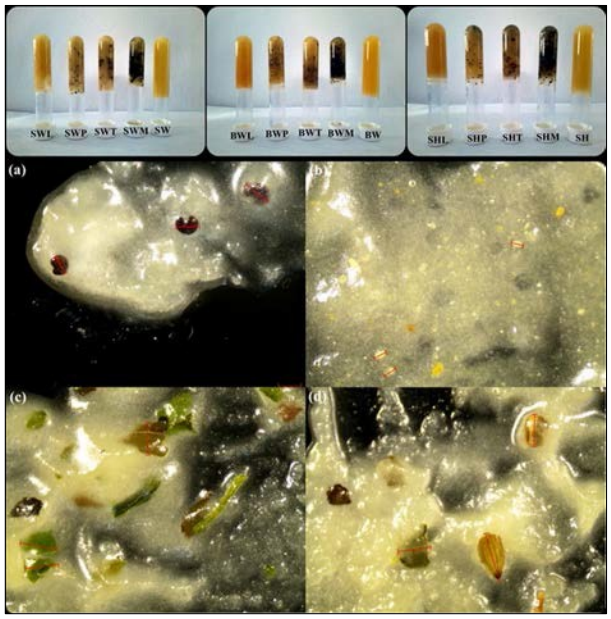

Figure 1 - Oleogels and stereomicroscope images (a) poppy seeds (b) lemon powder (c) mint and (d) thyme leaves in SW-based oleogels.

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

respectively [34, 35]. The fresh SW, SWT, SWM and the BW oleogels with spices were within legal limits according to Codex Stan., [35], and all the fresh oleogels were found within legal limits according to Codex Stan. [34]. On the other hand, all the stored oleogel samples were over legal limit as set by Codex Stan. [35], while the stored BW, BWP, BWM and the SW-based oleogels were within limits as determined by Codex Stan [34]. Additionally, the PV values of the oleogels increased depending on the storage time, and the SW-based oleogels had lower PV values than the BW and SHW oleogels at the end of the storage (Table III). The results indicated that storage time, used waxes and spices affected the PV and AV values of the oleogels. The differences among the PV and AV values of the oleogels may be explained with the differences among the PV and AV values of the used waxes. Delta E (colour differences) was used to determine the overall colour changes between the fresh and stored oleogels. The delta E values of the sunflower-, beeswax-, and shellac wax-based oleogels were 2.92-4.08, 2.00-5.83 and 6.08-9.06, respectively. The oleogels prepared with shellac wax had higher delta E values, while the oleogels with sunflower wax had lower delta E values at the end of the 90-day storage period.

The SW, BW and SHW based oleogels with different seasonings, and stereomicroscope images of SW-based oleogels are given in Figure 1. According to stereomicroscope images, the particle sizes of the lemon peel powder, poppy seeds, mint and thyme leaves used as additives were an average of 90.54, 162.16, 236.13 and 259.52 µm, respectively.

Similar to sieving results, the thyme leaves had the highest particle size, while the lemon peel had the lowest particle size than the other additives. Genovese et al. [37] indicated that food dispersion containing 10-100 µm solid particle size was classified as microscopic/non-colloidal, while food dispersions containing solid particle size over 100 µm were classified as macroscopic non-colloidal. The oleogels enriched with lemon peel were an example of microscopic dispersion, while the other oleogels with spices was an example of macroscopic dispersion, according to the definition of Genovese et al. [37]. The XRD patterns, firmness and stickiness values of the oleogels are shown in Table IV. The oleogels prepared with sunflower and shellac waxes had short spacing peaks around 3.70, 4.10 and 4.50 Å (Table IV). Additionally, the oleogels prepared with beeswax showed short spacing peaks around 4.50 and 4.10 Å. The oleogels prepared with beeswax had no peaks around 3.70 Å. The XRD peaks around 4.10 and 3.70 Å indicated the presence of β' prime polymorphic form [31, 38]. β' is characterised by melting at body temperature and providing a stable and smooth texture; hence, it provides to create the desired texture in margarines and various spreads [31]. On the other hand, the short spacing around 4.50 Å indicated the presence of β-form, while the peak around 4.10 Å indicated presence of α-form [38]. The XRD results of the shellac, sunflower and plain beeswax (without spices) oleogels showed that the oleogels had three polymorphic forms (α, β and β') characterized with presence of short spacing around 3.70, 4.10 and 4.50 Å. On the other hand, the spice-added beeswax oleogels were associated with α polymorphic form according to XRD patterns. The melting point, crystalline structure, and stability of polymorphic forms are different from each other, and unit cells of the alpha, beta and beta prime polymorphic forms have been associated with hexagonal, orthorhombic and triclinic packing, respectively [31, 38]. It has been pointed that α polymorphic form has a low melting point and is less stable than the β and β' forms. Additionally, the β-form has been reported to be an undesirable form for margarine and table spreads due to the rough and uneven texture [38]. Furthermore, like our findings, Da pieve et al. [39] reported that the broadness of the peak of the monoglyceride oleogels (around 24.20 and 4.50 Å) was due to amorphous scattering related to liquid-state triacylglycerol molecules that consisted of approximately 95% of the gel.

The firmness values of the plain oleogels prepared with BW, SW and SHW were 161.35, 885.20 and 70.22 g, respectively. The firmness values of the en-

riched oleogels formed with BW, SW and SHW were 34.82-78.02, 327.22-569.70 and 69.46-55.09 g, respectively. Furthermore, the stickiness values of the plain BW, SW and SHW gels were 214.03, 1100.30 and 73.43 g, respectively, while those of the enriched oleogels were 46.34-101.69, 363.83-575.60 and 60.98-66.10 g, respectively. Both plain and enriched SW gels had higher firmness and stickiness values than the oleogels formed with BW and SHW (Table IV). In terms of the firmness and stickiness values of the oleogels, there were statistically significant differences among the plain BW, SW and SHW-based oleogels, and similar results were observed in the enriched oleogels ($p \leq 0.05$). These results proved that addition of macroscopic and microscopic solid particles, such as spices, was effective on the textural features of the oleogels. It was concluded that 5% wax addition level was enough to form a stable plain oleogel structure for BW, although it was not enough to form SHW-gels with and without spices. As a result, SW was the most effective gel agent among the waxes to provide a stable gel structure with an additive containing macroscopic and microscopic solid particles at 5% addition level.

On the other hand, additive levels of 5% or more should be preferred for BW and SHW, particularly for the oleogels prepared with additives containing both macroscopic and microscopic solid particles. Similar to our findings, Fayaz et al. [30] reported that firmness of oleogels with 5% BW were 0.74 N. Similar results observed in the firmness and stickiness values of the enriched and aromatized hazelnut and olive oil oleogels prepared with 5% of beeswax and sunflower wax [21, 22]. Additionally, many of the previous research indicated that oleogels prepared with different oils and natural waxes may replace commercial breakfast margarine, spreads or hydrogenated fats in terms of firmness and stickiness values [18, 19, 23, 29].

Melting point is an important parameter, particularly together with spreadability for fat-based spreads. The desired and preferred melting points of fat-based spreads were body temperature (35-36°C). The melting point of these types of products is important for flavour release and consumer acceptance [40, 41]. The thermal properties of the oleogels prepared with BW, SW, SHW waxes and the addition of thyme, mint, lemon peel and poppy seeds are given in Table V. The crystallization point of the control group of the BW oleogel was 24.20°C, whereas that of the SW oleogel was 53.00°C and the SHW oleogel was 51.90°C. The crystallization points of the BW, SW and SHW oleogels prepared with spices were 18.20-31.50, 46.20-54.10 and 44.00-47.30°C, respectively. The SW and SHW oleogels had a higher crystallization point than the BW oleogels ($p \leq 0.05$). On the other hand, the melting point of the BW, SW, and SHW oleogels without spices was 28.80, 59.90 and 69.30°C, respectively. The melting points of the SW, BW and SHW oleogels enriched with seasonings ranged between 54.40-60.90°C, BW - 22.30-38.80°C and SHW - 62.00-66.30°C, respectively. Similar findings were reported by Patel et al. [42] for shellac wax oleogels and by Öğütcü et al. [21] for SW oleogels. Furthermore, the SHW oleogels had a higher melting point than the BW and SW oleogels at 5% added wax concentration. Previous research indicated that there was a linear relationship between the melting points of oleogels and the melt-

Table IV - XRD patterns and textural features of the oleogels.

Samples	2-theta	d (Å)	Firmness (g)	Stickiness (g)
SW	3.92:19.44:21.38:23.93	22.49:4.56:4.15:3.71	885.20±24.20*Aa	1100.30±86.00Ac
SWP	3.92: 19.23:21.58:23.72	22.49:4.61:4.11:3.74	327.22±12.92c	368.83±13.16a
SWT	4.12:19.64:21.98:24.13	21.38:4.51:4.09:3.68	500.30±28.00b	528.32±7.87ab
SWL	3.97:19.47:21.51:23.76	22.23:4.55:4.12:3.74	505.24±2.75b	530.54±12.33ab
SWM	4.74:19.23:21.27:23.62	18.62:4.61:4.17:3.76	569.70±25.30b	575.60±60.60b
BW	19.13:20.66	4.63:4.29	161.35±8.32Ba	214.03±9.53Bd
BWP	4.53:19.54:21.48	19.46:4.53:4.13	34.82±0.04d	46.34±0.31a
BWT	19.74:21:78	4.49:4.07	48.37±4.32cd	63.13±7.00ab
BWL	4.12:19.13:21.27	21.38:4.63:4.17	65.51±7.84bc	85.61±13.12bc
BWM	4.59:19.23:21.27	19.45:4.61:4.17	78.02±6.79b	101.69±8.76c
SHW	3.31:19.54:21.58:23.83	26.65:4.53:4.11:3.73	70.22±2.36Ba	73.43±13.16Ba
SHP	19.23:20.46:21.78:24.13	4.61:4.33:4.07:3.68	60.89±0.57a	66.10±1.44a
SHT	4.02:19.54:21.38:23.83	21.92:4.53:4.15:3.73	69.46±12.87a	68.74±3.70a
SHL	19.74:21.68:23.93	4.49:4.09:3.71	55.09±5.85a	60.98±6.15a
SHM	19.49:21.40:23.75	4.55:4.14:3.74	56.35±2.21a	62.67±3.14a

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

*The capital letters shows differences among the used waxes and the lower case letters shows differences among the samples prepared with same wax on the same column ($p \leq 0.05$).

Table V - Thermal properties of oleogels prepared with different waxes and spices

	Crystallization			Melting		
Samples	**Onset*c*** **(°C)**	**Peak *Tc*** **(°C)**	**Delta H*c*** **(j/g)**	**Onset*m*** **(°C)**	**Peak *Tm*** **(°C)**	**Delta H*m*** **(j/g)**
SW	58.60	53.00	-2.59	47.40	59.90	1.38
SWT	60.70	54.10	-7.44	46.50	60.90	2.51
SWP	58.00	51.90	-2.99	52.70	59.80	1.10
SWL	57.40	52.00	-1.73	48.90	59.70	0.61
SWM	51.90	46.20	-0.71	46.00	54.40	0.13
Mean±Sd	57.32±2.93a	51.44±2.74a	-3.09±2.31a	48.30±2.41b	58.94±2.31a	1.15±0.80a
BW	32.20	24.20	-0.30	19.60	28.80	0.15
BWT	31.90	18.20	-0.26	15.40	22.30	0.13
BWP	36.00	27.40	-0.39	20.00	31.70	0.04
BWL	34.60	30.50	-0.30	23.50	38.00	0.16
BWM	37.50	31.50	-0.34	25.20	38.80	0.25
Mean±Sd	34.44±2.16c	26.36±4.81b	-0.20±0.25b	20.74±3.40c	31.92±6.11b	0.15±0.07b
SHW	55.30	51.90	-1.91	60.20	69.30	1.22
SHP	52.50	46.50	-0.53	59.00	66.00	0.47
SHT	50.70	47.10	-0.63	67.40	62.00	0.48
SHL	49.30	44.00	-0.38	59.70	65.80	0.21
SHM	53.00	47.30	-0.48	59.20	66.30	0.27
Mean±Sd	52.16±2.05b	47.36±2.56a	-0.79±0.57ab	61.10±3.18a	65.88±2.32a	0.53±0.36ab

SW: oleogel with 5% sunflower wax, SWP: oleogels with 5% sunflower wax and 1% poppy seeds, SWT: with 1% thyme, SWL: with 1% lemon peel and SWM: with 1% mint. BW: oleogel with 5% beeswax, BWP: oleogels with 5% beeswax and 1% poppy seeds, BWT: with 1% thyme, BWL: with 1% lemon peel and BWM: with 1% mint. SH: oleogel with 5% shellac wax, SHP: oleogels with 5% shellac wax and 1% poppy seeds, SHT: with 1% thyme, SHL: with 1% lemon peel and SHM: with 1% mint.

*The small letters shows differences among the oleogels on the same column in terms of thermal properties ($p \leq 0.05$).

ing points of the waxes used [18, 29, 42]. Karabulut and Turan [43] showed that the slip melting point of margarines ranged between 31.2-34.9°C. It was determined that the oleogels produced with BW had the closest melting point to margarine, while the SW and SHW oleogels had higher melting points than margarine.

CONCLUSION

Different studies have been conducted on oil structuring, particularly on organogelation, in recent years. The present study focused on the effect of spices with different particle sizes on the structure and stability of oleogels. The results showed that both the used waxes and additives affected the oil-binding capacity, crystal formation time and centrifuge stability values of the oleogels and that the additives decreased oil binding capacity and stability and increased crystal formation time. Not only the additives and waxes but also the storage time affected the acid, peroxide, and colour values of the oleogels. The sunflower and shellac wax oleogels with and without additives had a beta prime polymorphic form according to the XRD patterns of the oleogels. The firmness and stickiness values of the prepared oleogels indicated that sunflower formed stable oleogels with spices at 5% addition level.

The thermal results showed that beeswax had a lower melting point than sunflower and shellac wax at the same wax concentration. As a result of the study, when it is desired to create a stable oleogel structure with additives containing flavouring substances and/or similar particles, 5% or less addition rates are sufficient for sunflower wax, while this ratio is recommended to be higher than 5% for BW and SHW waxes.

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Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES

[1] A. Roascio-Albistur, A. Gámbaro, C. Ivankovich, Consumers' perception of olive oil-based dressings evaluated by complementary techniques: Focus group and word association. International Journal of Gastronomy and Food Science 18, 100176 (2019)

[2] V. Uylaşer, G. Yildiz, The historical development and nutritional importance of olive and olive oil constituted an important part of the Mediterranean diet. Critical Reviews in Food Science and Nutrition 54(8), 1092-1101, (2014)

[3] D. Boskou, Olive oil: chemistry and technology. New York, USA: AOCS Publishing (2006)
[4] G. Gambacorta, M. Faccia, S. Pati, C. Lamacchia, A. Baiano, E. La Notte, Changes in the chemical and sensorial profile of extra virgin olive oils flavored with herbs and spices during storage. Journal of Food Lipids 14(2), 202-215, (2007)
[5] H. Akçar, A.S. Gümüşkesen, Determination of Chemical Properties of Flavored Extra Virgin Olive Oil. Gıda/The Journal of Food 37(6), 333-340, (2012)
[6] L. Dalgıç, G. Özkan, E. Karacabey, Optimization and Investigation of the influences of process conditions on quality parameters during production of olive oil flavoured with gooseberry. Süleyman Demirel University Journal of Agricultural Faculty 11(2), 21-34, (2016)
[7] K. Kachur, Z. Suntres, The antibacterial properties of phenolic isomers, carvacrol and thymol. Critical Reviews in Food Science and Nutrition 60(18), 3042-3053, (2020)
[8] S. Sardari, A. Mobaiend, L. Ghassemifard, K. Kamali, N. Khavasi, Therapeutic Effect of Thyme (Thymus Vulgaris) Essential Oil on Patients with COVID19: A Randomized Clinical Trial. Journal of Advances in Medical and Biomedical Research 29(133), 83-91, (2021)
[9] M.H. Sampangi-Ramaiah, R. Vishwakarma, R.U. Shaanker, Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. Current Science 118(7), 1087-1092, (2020)
[10] M.R. Basiri, Theory about treatments and morbidity prevention of corona virus disease (Covid-19). Journal of Pharmacy and Pharmacology 8(3), 89-90, (2020)
[11] M.M. Özcan, Ç. Atalay, Determination of seed and oil properties of some poppy (Papaver somniferum L.) varieties. Grasas y aceites 57(2), 169-174, (2006)
[12] B. Bozan, F. Temelli, Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. Bioresource Technology 99(14), 6354-6359, (2008)
[13] L.C. Tapsell, I. Hemphill, L. Cobiac, C.S. Patch, D.R. Sullivan, M. Fenech, S. Roodenrys, J.B Keogh, P.M. Clifton, P.G. Williams, V.A. Fazio, K.E. Inge, Health benefits of herbs and spices: the past, the present, the future. Med J Aust. 185, S4-S6, (2006)
[14] Y. Tekgül, T. Baysal, Comparative evaluation of quality properties and volatile profiles of lemon peels subjected to different drying techniques. Journal of Food Process Engineering 41(8), e12902 (2018)
[15] N.E. Hughes, A.G. Marangoni, A.J. Wright, M.A. Rogers, J.W. Rush, Potential food applications of edible oil organogels. Trends in Food Science & Technology 20(10), 470-480, (2009)
[16] A. Marangoni, N. Garti, Food oil gels: New strategies for structuring edible oils. Inform 22(5), 713-320, (2011)
[17] H.S Hwang, M. Singh, E.L. Bakota, J.K. Winkler-Moser, S. Kim, S.X Liu, Margarine from organogels of plant wax and soybean oil. Journal of the American Oil Chemists' Society 90(11), 1705-1712, (2013)
[18] M. Öğütcü, E. Yılmaz, Oleogels of virgin olive oil with carnauba wax and monoglyceride as spreadable products. Grasas y Aceites 65(3), 040 (2014)
[19] E. Yılmaz, M. Öğütcü, Comparative analysis of olive oil organogels containing beeswax and sunflower wax with breakfast margarine. Journal of Food Science 79(9), E1732-E1738, (2014)
[20] A.R. Patel, P.S. Rajarethinem, A. Gręd owska, O. Turhan, A. Lesaffer, W.H. De Vos, et al., Edible applications of shellac oleogels: spreads, chocolate paste and cakes. Food & Function 5(4), 645-652, (2014)
[21] M. Öğütcü, E. Yılmaz, O. Güneşer, Influence of storage on physicochemical and volatile features of enriched and aromatized wax organogels. Journal of the American Oil Chemists' Society 92(10), 1429-1443, (2015)
[22] E. Yılmaz, M. Öğütcü, Y.K. Yüceer, Physical properties, volatiles compositions and sensory descriptions of the aromatized hazelnut oil-wax organogels. Journal of Food Science 80(9), S2035-S2044, (2015)
[23] E. Yılmaz, M. Öğütcü, Oleogels as spreadable fat and butter alternatives: Sensory description and consumer perception. RSC Advances 5(62), 50259-50267, (2015)
[24] E. Yılmaz, Ş. Demirci, Preparation and evaluation of virgin olive oil oleogels including thyme and cumin spices with sunflower wax. Gels 7(3), 95 (2021)
[25] AOCS, AOCS, D. Firestone (Ed.), Official Methods and Recommended Practices of the American Oil Chemists' Society, Champaign, USA: AOCS Press (1998)
[26] Minitab, L. L. C. Minitab (2016)
[27] A. Shakerardekani, R. Karim, H.M. Ghazali, N.L. Chin, Textural, rheological and sensory properties and oxidative stability of nut spreads - a review. International Journal of Molecular Sciences 14(2), 4223-4241, (2013)
[28] A.R. Patel, M. Babaahmadi, A. Lesaffer, K. Dewettinck, Rheological profiling of organogels prepared at critical gelling concentrations of natural waxes in a triacylglycerol solvent. Journal of Agricultural and Food Chemistry 63(19), 4862-4869, (2015)
[29] H.S. Hwang, S. Kim, M. Singh, J.K. Winkler-Moser, S.X. Liu, Organogel formation of soybe-

an oil with waxes. Journal of the American Oil Chemists' Society 89(4), 639-647, (2012)
[30] G. Fayaz, S.A.H. Goli, M. Kadivar, F. Valoppi, L. Barba, C. Balducci, L. Conte, S. Calligaris, M.C. Nicoli, Pomegranate seed oil organogels structured by propolis wax, beeswax, and their mixture. European Journal of Lipid Science and Technology 119(10), 1700032 (2017)
[31] L.S.K. Dassanayake, D.R. Kodali, S. Ueno, K. Sato, Physical properties of rice bran wax in bulk and organogels. Journal of the American Oil Chemists' Society 86(12), 1163 (2009)
[32] A.I. Blake, A.G. Marangoni, The use of cooling rate to engineer the microstructure and oil-binding capacity of wax crystal networks. Food Biophysics 10(4), 456-465, (2015)
[33] A.I. Blake, A.G. Marangoni, The effect of shear on the microstructure and oil-binding capacity of wax crystal networks. Food Biophysics 10(4), 403-415, (2015)
[34] Codex Alimentarius (2017). Codex Standard for Olive Oil, Virgin and Refined and for Refined Olive-Pomace Oil (Codex Stan 33-1981, Rev. 2017)
[35] Codex Alimentarius (1999). Codex Standard for Edible Fats and Oils not Covered by Individual Standards (Codex Stan 19-1981, Rev. 2 - 1999)
[36] S. Okparanta, V. Daminabo, & L. Solomon, Assessment of rancidity and other physicochemical properties of edible oils (mustard and corn oils) stored at room temperature. Journal of Food and Nutrition Sciences 6(3), 70-5 (2018)
[37] D.B. Genovese, J.E. Lozano, M.A Rao, The rheology of colloidal and noncolloidal food dispersions. Journal of Food Science 72(2), R11-R20 (2007)
[38] A.G. Marangoni, L.H. Wesdorp, Structure and properties of fat crystal networks. Boca Raton, FL: CRC Press (2019)
[39] S. Da Pieve, S. Calligaris, M.C. Nicoli, A.G. Marangoni, Shear nanostructuring of monoglyceride organogels. Food Biophysics 5(3), 211-217, (2010)
[40] R.P. Borwankar, L.A. Frye, A.E. Blaurock, F.J. Sasevich, Rheological characterization of melting of margarines and tablespreads. In:. Borwankar R, Shoemaker CF editors. Rheology of foods. Elsevier, 55-74, (1992)
[41] V. Galindo-Cuspinera, J. Valença de Sousa, M. Knoop, Sensory and analytical characterization of the "cool-melting" perception of commercial spreads. Journal of Texture Studies 48(4), 302-312, (2017)
[42] A.R. Patel, D. Schatteman, W.H. De Vos, K. Dewettinck, Shellac as a natural material to structure a liquid oil-based thermo reversible soft matter system. RSC Advances 3(16), 5324-5327, (2013)
[43] I. Karabulut, S. Turan, Some properties of margarines and shortenings marketed in Turkey. Journal of Food Composition and Analysis 19(1), 55-58, (2006)

Optimization of leavened dough frying conditions using the response surface methodology

Semra Turan[1]✉
Sule Keskin[2]
Rukiye Solak[1]

[1]Department of Food Engineering,
Faculty of Engineering,
Bolu Abant Izzet Baysal University,
Golkoy Campus, Bolu, Turkey

[2]Department of Quality and Technology,
Central Research Institute for Field Crops,
Yenimahalle, Ankara, Turkey

✉ CORRESPONDING AUTHOR:
Semra Turan
Department of Food Engineering,
Faculty of Engineering,
Bolu Abant Izzet Baysal University,
Golkoy Campus, Bolu, Turkey
Tel: +90 0 374 254 10 00-48 30
Fax: +90 0374 253 45 58
E-mail: turan_s@ibu.edu.tr

Orcid numbers
Semra Turan: 0000-0002-1005-3590
Sule Keskin: 0000-0002-9957-3575
sule.keskin@tarimorman.gov.tr
Rukiye Solak: 0000-0001-5171-7587
islamoglusolakrukiye@gmail.com

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In this study, the optimum frying conditions of leavened doughs to minimise the oxidation products were investigated. Fifty repeated deep frying of leavened doughs with 0-2% salt content was performed for 1-5 min at 160-200°C. While K_{232}, K_{270}, *p*-anisidine and polymer triglycerides contents of fried dough oil (FDO) were noteworthy ($p < 0.05$) affected by the frying temperature and the frying time, the dough salt content did not affect these values significantly ($p > 0.05$). The combined effects of frying temperature and time on K_{270}, *p*-anisidine and polymer triglycerides contents were significant ($p < 0.05$). The effects of interaction of frying temperature and dough salt content on *p*-anisidine value were found to be significant ($p < 0.05$). The optimum frying conditions to minimize the K_{232}, K_{270}, *p*-anisidine values and polymer triglyceride content of FDO were observed where the frying time was 1 minute, the frying temperature was 160°C and the salt content was 2%.

Keywords Repeated frying; polymer triglycerides; K_{232} and K_{270} values, *p*-anisidine value

1. INTRODUCTION

Deep frying is a food cooking process which is commonly used for domestically and commercially. During this process, food is immersed in an oil bath at 175-190°C, which improves sensorial (flavour, taste) and textural (colour and crispness) properties of food products [1-5].

Frying oil serves as a heat transfer medium. Different types of fats/oils are used for frying purposes. The quality of fried foods is based on the frying conditions, the type of oil, and foods being fried [6, 7]. Lipid oxidation of fried foods varies depending on food and frying oil composition and water activity [8]. Water removes from capillary of foods and thus, oil is absorbed by food. The other changes in food are gelatinisation of starch, denaturation of proteins, and loss of some heat sensitive nutrients and the development of flavour [3]. In addition to these changes, chemical (formation of primary and secondary oxidation products), physical (such as density, viscosity and colour) and thermal (convective heat transfer coefficient) alterations occur in frying oil at high temperatures in the presence of air and moisture. The repeated and longer re-use of frying oil cause undesirable flavour, taste, colour, stability and texture in food. Furthermore, many harmful oxidation products are formed [7]. It was reported that fried food consumption and risk of developing chronic diseases in human is highly correlated [9].

Optimum frying conditions have been determined in several studies according to the chemical and textural characteristics, as well as sensory properties. Generally, response surface methodology (RSM) has been used to investigate the optimal frying conditions. RSM utilises the quantitative data from the suitable empirical designs to determine and solve the multivariate equations which is useful approach to study the effects of all the test variables on the responses [10]. Optimisation of process conditions for the preparation of puri,

a traditional product made from whole wheat flour, were examined by Vatsala et al. [11] using RSM according to sensorial and textural properties as well as oil uptake. Sobukola et al. [12] studied optimisation of pre-fry drying of yam (sweet potato) slices according to the colour, crispness, oil and moisture content. The optimisation details of pre-fry microwave dried French fries with respect to the moisture content, oil content, texture and color parameters explained by Hashemi Shahraki et al. [13]. During the preparation of sweet potato chips, optimization of processing variables was investigated by Singh et al. [14] to obtain the product with desired textural and sensorial properties. Characterisation of the fresh and blanched potato strips' frying process was carried out by Alvarez and Canet [1] according to similar quality attributes. In other respects, the influence of vacuum microwave pre-drying and vacuum frying conditions on the physical and chemical characteristics of potato chips were investigated by Song et al. [15]. Perez-Tinoco et al. [16], using central composite experimental design, prepared hybrid pineapple slices using vacuum frying. In other study, the effects of processing conditions on the quality of vacuum fried apple chips were studied [17]. Vacuum frying of kiwi slice was also optimised by Maadyrad et al. [18]. As it is seen, optimisation of processes conditions was done according to the textural and sensorial properties of the fried food products in literature.

Despite the many studies on the changes of oil properties during the frying process, little information has been compiled about the changes in oil, which is extracted from the fried substrate, especially fried dough. In our previous studies we determined the changes in sunflower oil during frying of leavened dough using response surface methodology (RSM) [19]. Thermal oxidation products transfer from oil to the frying material. Therefore, in this study we aimed to find the optimal frying conditions (frying time, frying temperature and dough salt content) to minimise specific absorbance values (K_{232} and K_{270}), *p*-anisidine value and polymer triglyceride contents of FDO.

2. MATERIALS AND METHODS

2.1 MATERIALS

Refined sunflower oil, wheat flour, yeast and salt were taken from a local market (Bolu, Turkey). *p*-anisidine (99%) and heptadecanoic acid were taken from Sigma-Aldrich (Buchs, Switzerland). Tetrahydrafuran, isopropyl alcohol, cyclohexane, iso-octane and glacial acetic acid were purchased from Merck (Darmstadt, Germany).

2.2 METHODS

2.2.1 Preparation of leavened dough

The dough was prepared according to Turan et al. [20] using refined flour, instant yeast, water, and salt (0, 1, 2%). The dough was kneaded (Kitchen Aid, Belgium) and fermented at 35°C and 80 ± 5% relative humidity for 45 min. Subsequently it was thinned and divided into small square pieces (3 cm × 3 cm).

2.2.2 Frying process

Frying of leavened doughs was carried out as described by Turan et al [20]. The leavened doughs were fried at different temperatures (160, 180 and 200°C) and times (1, 3 and 5 min) in a domestic fryer with a capacity of 1 L (Tefal Minuto, France). Fifty frying operations were conducted in sunflower oil per day. Number of the frying cycle was determined according to the polar material content of frying oil. The limit of polar material content of oil is 25% legally. Above this value, oil is discarded and not used in further frying processes in food industry. Oil replenishment was not performed during the repeated frying. At the 50th frying, fried dough samples were taken from the fryer, cooled to room temperature and put into plastic bag, labelled and kept frozen.

2.2.3 Extraction of oils from fried doughs

Oil was extracted from fried doughs according to Troncoso et al. [21] using Folch lipid extraction method. Briefly, the dough (approximately 5 g) was divided into pieces and oil was extracted from dough pieces using 20 mL of chloroform/methanol/water (1:1:0.8, v/v/v) mixture and then filtered through a filter paper. Extraction process was repeated twice. After the removing of upper methanol/water phase, the chloroform phase was evaporated using stream of nitrogen gas. The extracted oil was used for the analyses of K_{232}, K_{270}, *p*-anisidine values and polymer triglyceride content.

2.2.4 Analysis of oils extracted from the fried doughs (FDO)

K_{232}/K_{270} and *p*-anisidine values were determined according to of AOCS Official Methods [22] Ch 5-91 and Cd 18-90 by Shimadzu UV 1700 spectrophotometer, respectively. The polymer triglyceride content of FDOs were measured according to official method of AOCS [22] numbered Cd 22-91 and Gertz [23]. Two gel permeation columns (GPC, Agilent PL-Gel 100°A, 2 × 300 × 7.5 mm, 5 µm, UK) was used for the separation of polymer triglycerides in HPLC system (Shimadzu Prominence, Japan) equipped with refractive index detector. Tetrahydrofuran: isopropyl alcohol (99.5:0.5, v/v) was used as the mobile phase with 1 mL/min of flow rate. The column oven temperature was set 35°C. Percentages of polymer triglycerides (dimeric and oligomeric) in samples were calculated by dividing individual peak area to total areas.

2.3 EXPERIMENTAL DESIGN

A central composite design was carried out to find the effects of three factors (dough salt content, frying temperature and time) on response during deep

frying of leavened doughs. Design Expert version 10.0.5 program (Stat-Ease, Inc., Minneapolis, MN) was utilized for statistical evaluation, modelling and determination of the combined or individually effects of variables on the responses. The selected factors as independent parameters were frying temperature (A, 160-180°C), dough salt content (B, 0-2%) and frying time (C, 1-5 min) at three different levels (1, 0, +1). K_{232}, K_{270}, *p*-anisidine values and polymer triglyceride content of FDOs were designed as the response variables. Twenty frying experiments were conducted with six replicates at the central points. The details of the central composite design were explained in Turan et al [20].

3. RESULTS AND DISCUSSION

Scanning the content of primary or secondary oxidation products is commonly used to determine the rate at which the oxidation process is progressing [24]. In foods, the oxidation examination of fats and oils in food is important to protect against the deterioration of foods for human health [25].

3.1 K_{232} VALUES

K_{232} value shows primary oxidation products which is related to the content of hydroperoxides and conjugated dienes [26]. The K_{232} values of FDOs at the 50th frying operation is given in Table I. The K_{232} values of FDOs were in the range of 7.60 and 16.06. The highest K_{232} value was observed during frying of unsalted dough for 5 min at 200°C. We also determined the oxidation products of counterpart frying oil which were similar to oil extracted from leavened dough [19]. Bou et al. [27] confirmed the similarity of oxidation products of fried snacks from large scale producers and those obtained for their counterpart frying oil. Additionally, Wong et al. [28] reported high K_{232}, K_{268} and *p*-anisidine values of oil samples extracted from fried potatoes in repeated deep frying.
The effects of individual factors and their interactions on K_{232} values were well described by quadratic model with high R^2 values of 0.9665. High adequate R^2 (0.9364) and predicted R^2 (0.8304) values were also determined. According to the proposed models, the Eq. (1) is given for K_{232} values.

$$K_{232}=-55.59+0.63A+2.03B+1.99C-0.01AB+0.01AC-0.31BC-1.60A^2+0.20B^2-0.44C^2 \quad (1)$$

Where:
A is the frying temperature (°C)
B is the dough salt content (%)
C is the frying time (min).

The ANOVA results for K_{232} values are given in Table II. K_{232} values of FDOs were significantly affected by frying temperature and time ($p < 0.01$). As seen from *F* values for K_{232}, frying time was found to be more effective than frying temperature. The salt content of the dough did not lead significant effect on K_{232} values ($p > 0.05$). The influence of the dough salt content and frying time interaction (BC) on the K_{232} values of FODs-was determined significant ($p < 0.05$). K_{232} values increased with the prolonged frying time. This result was also confirmed by Wong et al. [28] in fried potatoes and by Lee et al. [29] in fried doughs containing carrot powder. Additionally, the K_{232} values of FODs decreased slightly as the dough salt content raised at 5 min frying times (Fig. 1). Chu and Luo [30] stated that adding sugar or salt in dough resulted in

Table I - Some properties of oil extracted from fried dough at the 50th frying

Run	Frying Temperature (°C)	Dough Salt content (%)	Frying time (min)	K_{232}	K_{270}	*p*-anisidine value	Polymer triglycerides (%)
1	160	0	5	11.91	5.26	71.12	6.13
2	160	1	3	11.24	5.27	81.05	5.03
3	180	0	3	14.16	3.60	95.70	6.70
4	180	1	3	13.25	4.60	103.66	7.93
5	180	1	3	13.75	4.73	111.80	7.64
6	200	2	5	14.12	4.88	144.50	10.89
7	180	1	3	12.93	4.78	93.82	6.82
8	200	0	5	16.06	4.98	121.65	9.39
9	200	2	1	10.70	4.36	71.51	6.19
10	160	2	1	8.98	3.27	58.32	4.24
11	200	1	3	13.98	6.02	99.50	7.88
12	180	1	3	14.41	4.49	104.00	8.94
13	180	1	3	13.52	5.04	116.44	8.86
14	160	0	1	7.60	3.45	84.90	3.97
15	180	1	5	12.64	4.32	111.11	8.46
16	200	0	1	10.63	4.44	62.99	5.80
17	180	1	3	13.57	4.75	99.35	7.69
18	180	1	1	10.32	3.80	77.00	5.30
19	180	2	3	12.75	3.39	103.69	8.04
20	160	2	5	10.30	4.50	64.22	5.83

Table II - Analysis of variance for response of K_{232} and K_{270}

Source	Sum of squares		Degrees of freedom	Mean square		F value		*p*-value, Prob > F	
	K_{232}	K_{270}	K_{232} /K_{270}	K_{232}	K_{270}	K_{232}	K_{270}	K_{232}	K_{270}
Model	79.88	9.02	9	8.88	1.00	32.06	19.09	< 0.0001**	< 0.0001**
A	23.89	0.86	1	23.89	0.86	86.29	16.42	< 0.0001**	0.0023**
B	1.23	0.18	1	1.23	0.18	4.45	3.34	0.0612	0.0974
C	28.21	2.13	1	28.21	2.13	101.92	40.64	< 0.0001**	< 0.0001**
AB	0.34	0.072	1	0.34	0.072	1.23	1.38	0.2936	0.2677
AC	1.31	0.49	1	1.31	0.49	4.72	9.31	0.0549	0.0122*
BC	3.13	0.045	1	3.13	0.045	11.32	0.85	0.0072**	0.3787
A^2	1.13	3.64	1	1.13	3.64	4.07	69.40	0.0713	< 0.0001**
B^2	0.12	2.74	1	0.12	2.74	0.42	52.14	0.5303	< 0.0001**
C^2	8.63	0.51	1	8.63	0.51	31.18	9.75	0.0002**	0.0108*
Residual	2.77	0.52	10	0.28	0.052				
Lack of Fit	1.52	0.35	5	0.30	0.070	1.21	1.99	0.4188	0.2335
Pure Error	1.25	0.18	5	0.25	0.035				
Cor Total	82.65	9.54	19						

[a]Letters A, B, C indicate frying temperature (°C), dough salt content (%) and frying time (min), respectively.
*p < 0.05, ** p< 0.01

a better quality of soybean oil since salt or sugar had the ability to absorb water. Also, quadratic effects of frying time (C^2) on K_{232} values was found significant (p < 0.01). When 1% salt containing dough was fried for 1 minute at 180°C (Run 18), the K_{232} values of the oil was 10.32. This value was found as 12.64 after frying 5 min (Run 15). In agreement with our results, Lee et al. [29] observed that as the number of the frying cycle increased, conjugated dienoic acids of fried dough increased. In another study, conjugated dienoic acid content of fried dough increased with storage time of the fried dough. Also, the lipid oxidation of the fried products made from longer time stored flour tended to be higher than that of those made from unstored or short time stored flour [8]. Additionally, Farmani et al. [31] observed a sharper conjugated diene formation in sunflower oil compared to canola oil by frying bamiyeh pastry due to its higher PUFA content. More intense oxidative degradation was determined in oils with high PUFA content. Besides, Farhoosh et al. [32] stated that the conjugated diene value showed linear raise with the frying time at 180°C for 7 min intervals for 8 hours interval and increased from 7.9 to 70.9 µmol/g after 32 hours. Further, they stated that the sunflower oil should be discarded after 20 hours of frying.

3.2 K_{270} VALUES

Ultraviolet (UV) absorption at 270 nm is mainly stems from the secondary oil oxidation products (conjugated dienals and ketodienes and ethylenic diketones) of oils [26]. During the deep-frying, hydroperoxides are unstable due to the applied high temperature and decompose to secondary oxidation products [2]. In this study, hydroperoxides formed for this reason were measured with K_{232} value, and secondary oxidation products were measured with K_{270} and *p*-anisidine values.

The K_{270} values of FDOs at the 50th frying operation are given in Table I. The K_{270} values of FDOs were in the range of 3.27 and 6.02. The highest value was found in dough sample where frying temperature, frying time and dough salt contents were 200°C, 3 min and 1% salt, respectively (Run 11). A quadratic model with R^2 of 0.9450, adequate R^2 of 0.8955 and predicted R^2 of 0.6944 well described the effects of factors (frying temperature, dough salt content and frying time) on K_{270} values of FDOs. Eq. (2) calculated for K_{270} values is shown as follows:

$$K_{270}=+90.07-1.00A+1.12B+2.03C+4.75AB-6.18AC-0.04BC+2.88A^2-0.10B^2-0.11C^2 \quad (2)$$

Where:
A is the frying temperature (°C)
B is the dough salt content (%)
C is the frying time (min).

In a similar manner with the K_{232} values, frying temperature (p < 0.01) and frying time (p < 0.01) significantly affected the K_{270} values of FDOs. However, salt

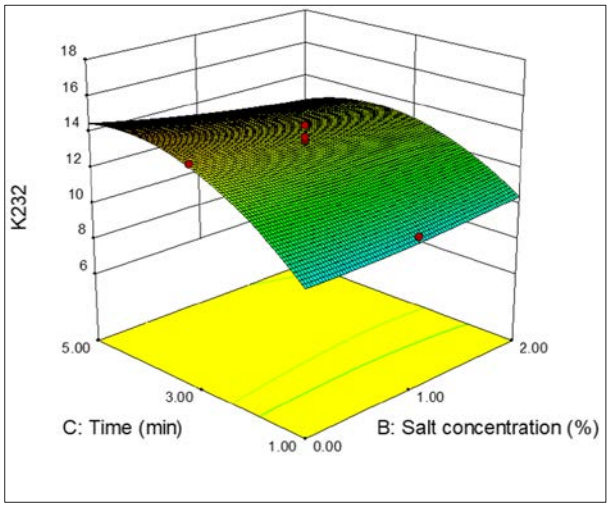


Figure 1 - Effects of dough salt content and frying time on K_{232} values of oils extracted from fried doughs at the 50th frying

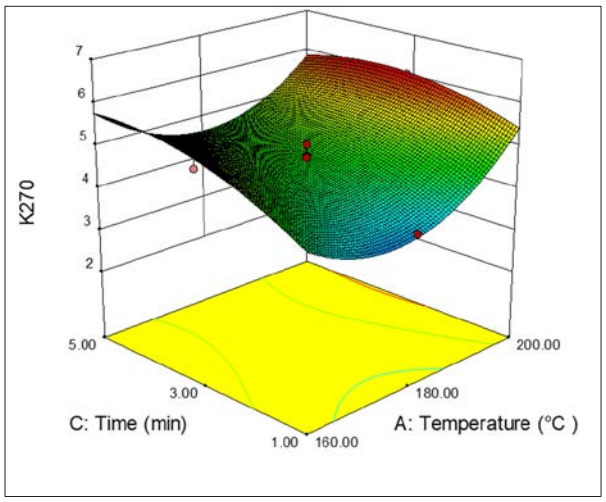
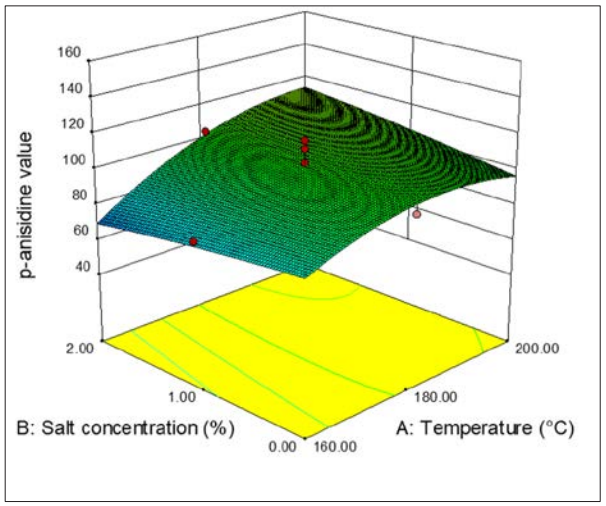
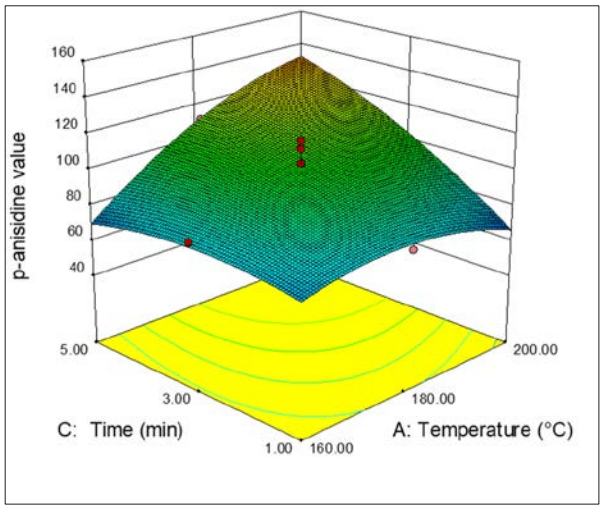


Figure 2 - Effects of frying temperature and frying time on K_{270} values of oils extracted from fried doughs at the 50th frying



a) - Frying temperature and dough salt content



b) - Frying temperature and frying time

Figure 3 - Effects of frying parameters on p-anisidine values of oils extracted from fried doughs at the 50th frying

content of the dough did not influence the K_{270} values remarkably ($p > 0.05$) (Tab. II). In contrast to our findings, Wong et al. [28] reported conjugated dienes and trienes increased as the amount of salt increased. In 1% salted doughs, K_{270} values of FDOs increased as a result of longer frying time and higher temperature (Fig. 2). Similarly, temperature increase, and prolonged frying duration increased conjugated trienes slightly in fried potatoes [28]. While the K_{270} value of FDO for 1% salted doughs and fried at 180°C for 1 min was 3.80 (Run 18), it was 4.32 (Run 15) fried at 180°C for 5 min with the same conditions. Since the frying oil is subjected to heat for longer periods with prolonged frying time, an increase in the K_{270} values were observed. Besides, the K_{270} value of FDOs increased from 5.27 (Run 2) to 6.02 (Run 11) when the frying temperature was raised from 160°C to 200°C for 1% salted doughs fried for 3 min.

3.3 P-ANISIDINE VALUES

p-Anisidine value shows the amount of the secondary oxidation products, 2-alkenals and 2,4-alkadienals [25, 26]. The *p*-anisidine values of FDOs at the 50th frying operation are given in Table I. The *p*-anisidine values of FDOs were in the range of 58.32 and 144.5. Bou et al. [27] reported lower p-anisidine values for oils extracted from potato chips or potato extruded snacks during five consecutive weeks frying at the large-scale producers. The effects of different factors on *p*-anisidine values of samples were described by a quadratic model with determination coefficient of 0.9493 ($p < 0.0001$). Adjusted R^2 and predicted R^2 were found as 0.9036 and 0.8491, respectively. The Eq. (3) is found from the proposed model as follows:

$$p\text{-anisidine} = -618.69 + 8.65A - 76.13B - 62.30C + 0.41AB + 0.44AC + 2.13BC - 0.03A^2 - 1.31B^2 - 1.74C^2 \quad (3)$$

Where:
A is the frying temperature (°C)
B is the dough salt content (%)
C is the frying time (min).

As in the K_{232} and K_{270} values, frying temperature and frying time had a significant effect on the *p*-anisidine values of FDOs ($p < 0.05$) whereas the effect of the dough salt content on the *p*-anisidine values was not significant ($p > 0.05$) (Tab. III). Inversely, Wong et al. [28] observed an increase in p-anisidine value as the amount of salt increased in fried potatoes.
The combination of the frying temperature and dough salt content resulted in significant increase ($p < 0.05$) in *p*-anisidine values of FDOs (Fig. 3a). The *p*-anisidine value of FDOs increased, as the temperature increased while the frying time was 3 min. Whilst the *p*-anisidine value of FDOs was 81.05 (run 2) at 160°C, it rose up 99.50 (run 11) at 200°C during frying of 1% salted doughs.
The combination of the frying time and temperature significantly affected the *p*-anisidine value of FDOs

Table III - Analysis of variance for response of *p*-anisidine and polymer triglyceride content

Source	Sum of squares		Degrees of freedom		Mean square		F value		*p*-value, Prob > F	
	p-anisidine	Polymer triglyceride	*p*-anisidine	Polymer triglyceride	*p*-anisidine	Polymer triglyceride	*p*-anisidine	Polymer triglyceride	*p*-anisidine	Polymer triglyceride
Model	8986.23	56.08	9	7	998.47	8.01	20.79	17.71	< 0.0001**	< 0.0001**
A	1975.27	22.32	1	1	1975.27	22.32	41.13	49.35	< 0.0001**	< 0.0001**
B	3.47	1.01	1	1	3.47	1.01	0.072	2.23	0.7935	0.1608
C	2492.61	23.05	1	1	2492.61	23.05	51.90	50.95	< 0.0001**	< 0.0001**
AB	525.55	0.46	1	1	525.55	0.46	10.94	1.01	0.0079**	0.3343
AC	2433.57	2.58	1	1	2433.57	2.58	50.67	5.71	< 0.0001**	0.0342*
BC	144.74	-	1	-	144.74	-	3.01	-	0.1132	-
A^2	316.58	2.31	1	1	316.58	2.31	6.59	5.10	0.0280*	0.0433*
B^2	4.69	-	1	-	4.69	-	0.098	-	0.7611	-
C^2	132.69	0.57	1	1	132.69	0.57	2.76	1.27	0.1275	0.2820
Residual	480.30	5.43	10	12	48.03	0.45				
Lack of Fit	143.56	2.17	5	7	28.71	0.31	0.43	0.47	0.8145	0.8207
Pure Error	336.74	3.26	5	5	67.35	0.65				
Cor Total	9466.54	61.51	19	19						

[a]Letters A, B, C indicate frying temperature (°C), dough salt content (%) and frying time (min), respectively.
*p < 0.05, **p< 0.01

(p < 0.05). The *p*-anisidine value ascended at the increased frying temperature or prolonged frying time (Fig. 3b). Similar results have been reported by Lee et al. [29] during frying of carrot powder containing doughs at 160°C for 1 min and by Wong et al. [28] during frying of potatoes. While the *p*-anisidine value of oils of the 1% salted doughs fried at 180°C was 77.00 (Run 18) when fried for 1 min, it was 111.11 (Run 15) when fried for 5 min. Besides, the *p*-anisidine value of oils of the 2% salted doughs raised from 58.32 (Run 10) to 71.51 (Run 9) for 1 min frying when the temperature was increased from 160°C to 200°C.

3.4 POLYMER TRIGLYCERIDE CONTENTS

The polymer triglyceride contents of FDOs at the 50th frying operation is given in Table I. The polymer triglyceride contents of FDOs in the range of 3.97 and 10.89. Lower polymer triglyceride contents were reported by Bou et al. [27] for oils extracted from fried potatoes and snacks. The determination coefficient (R^2) was found as 0.9117 for reduced model. Adjusted R^2 and predicted R^2 for the model were 0.8603 and 0.8006, respectively.

The equation (4) found from the proposed model as follows:

$$\text{Polymer triglycerides (\%)} = -68.24 + 0.78A - 1.83B - 1.16C + 0.01AB + 0.01AC - 2.12B^2 - 0.11C^2 \quad (4)$$

Where:

A is the frying temperature (°C)

B is the dough salt content (%)

C is the frying time (min).

According to the results obtained from central composite design, it was determined that both frying time and temperature affected the polymer triglyceride contents of FDOs significantly (p < 0.05), whereas the effect of the salt content of the dough on the polymer

Figure 4 - Effects of frying temperature and frying time on polymer triglyceride contents of oils extracted from fried doughs at the 50th frying

Table IV - Optimization of frying conditions according to physicochemical characteristics of frying oil

	Goal	Lower limit	Upper limit	Optimized values
Frying temperature (°C)	In the range	160	200	160.0
Dough salt content (%)	In the range	0	2	2.0
Frying time (min)	In the range	1	5	1.0
K_{232}	Minimize	7.60	16.06	8.89
K_{270}	Minimize	3.27	6.02	3.20
p-anisidine	Minimize	58.32	144.5	60.16
Polymer triglycerides (%)	Minimize	3.97	10.89	4.08

Desirability value: 0.950

triglyceride contents was not significant ($p > 0.05$) (Tab. III). The highest polymer triglyceride contents were found at high temperatures and prolonged frying where the dough salt content was kept constant (Fig. 4). Whilst the polymer triglyceride content of oils of 1% salted doughs fried at 180°C was 5.30% when fried for 1 min (Run 18), it increased to 8.46% when fried for 5 min (Run 15). On the other hand, the polymer triglyceride contents of oils of 1% salted doughs increased from 5.03% (Run 2) to 7.88% (Run 11), when the frying temperature was increased from 160°C to 200°C. Soriano et al. [33] reported that higher temperatures accelerate thermal and oxidative changes and raise the rate of decomposition products formation. They recommended a temperature range of 160-180°C for frying operations in the case of using sunflower oil for frying medium.

3.5 OPTIMISATION

Optimisation of frying conditions and monitoring the oxidation products during frying can help to minimize the formation of decomposition products [34]. The optimum frying conditions to minimize the oxidation products in FDOs were determined and shown in Table IV. Chemo-metric design allows us to determine the conditions that lead to the minimum formation of oxidation products during frying of leavened doughs in shortest time with the least number of experiments. During deep-frying, an increase in primary and secondary products, was observed as frying temperature and time increased. Optimum conditions were defined as the conditions that would reduce the K_{232}, K_{270}, p-anisidine and polymer triglyceride values. The desirability was found as 0.950 and the optimum conditions by numeric optimisation were: frying temperature of 160°C, frying time of 1 min and dough salt content of 2%. The calculated values at the optimum conditions for K_{232} value, K_{270} value, *p*-anisidine value and polymer triglyceride content were 8.89, 3.20, 60.16 and 4.08%, respectively. It was concluded that the R^2 value obtained with the quadratic equations (Tab. IV) represented at least more than 90% of the total regression model created, so the measured experimental parameters could be largely explained by the obtained data.

High temperature causes oxidative degradation, thermal decomposition and polymerisation of the oil, while the prolonged frying time triggers degradation caused by food-borne moisture such as hydrolysis in addition to these degradations. Volatile products and non-volatile monomeric and polymeric compounds are formed owing to the deep-frying. With continued heating and frying, these compounds form further breakdown products with potentially toxic effects and undesirable flavour making the oil unsuitable for frying [35]. The optimum conditions for frying depend on the oil type, the composition of the food (moisture, sugar, etc.), frying conditions (time, cycle and temperature). Optimisation of the food-specific frying conditions is of great importance.

In order to determine optimum operating conditions, in traditional products such as plantain chips [36, 37], kokoro [38], Chinese deep-fried dough [39], puri [11], or in fried potatoes such as orange-fleshed sweet potato [40], yam [12], pre-fry microwave dried French fries [13], the response surface method was used in the literature. Similar to our study, it was determined that frying temperature and frying time had significantly affected the quality parameters (moisture content, oil content, breaking force and colour) of plantain chips. Furthermore, the effects of the composition of fried food had also been studied [36].

When the food is fried, it absorbs oil from its environment. For this reason, changes in fried oil were also observed in fried food [41]. Kim et al. [42] stated that the free fatty acid content of potato chips obtained after 80th frying trial (each 4 minutes at 180°C) was like the oil used. Lee et al. [29] reported that the lipid oxidation taken place in the fried dough was very similar to that in the frying oil. They claimed that the extent of lipid oxidation in the fried dough can be calculated from oxidation rate of the frying oil.

4. CONCLUSION

Changes in oil and fried foods during the deep-frying process can be determined by different analysis methods. Fried foods and absorbed oil consumed after frying. Hence, the quality of the oil that penetrates the fried food is very important. Optimum conditions that can minimise the formation of undesirable compounds can be identified by the response surface methodology. In this study, the effects of frying time, frying temperature and dough salt content on the oxi-

dation products of FDOs were investigated. Based on the results, it was observed that both frying temperature and frying time had significant effects ($p < 0.05$) on the K_{232} and K_{270} values, *p*-anisidine value and polymer triglyceride content of fried dough samples while dough salt content was not significant effect ($p > 0.05$) on these parameters.

During deep frying, it was observed that oxidation products turned into dough from frying oil with increasing temperature and duration.

Optimum frying conditions to minimise the oxidation products in fried doughs were found as a frying temperature of 160°C, the frying duration of 1 min and the salinity of 2% by using desirability function.

In the later stages of the study, experiments will be conducted to determine the effects of other dough ingredients such as moisture, protein and starch content on the amount of absorbed oil oxidation products.

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Conflict of interest

Authors declare no conflict of interest.

REFERENCES

[1] D.M. Alvarez, W. Canet, Characterization of the frying process of fresh and blanched potato strips using response surface methodology. European Food Research and Technology *211*, 326-335 (2000).

[2] E. Choe, D.B. Min, Chemistry of deep-fat frying oils. Journal of Food Science *72*, R77-R86 (2007).

[3] K. Bordin, M. Tomihe Kunitake, K. Kazue Aracava, C. Silvia Favaro Trindade, Changes in food caused by deep fat frying - A review. Archivos latinoamericanos de nutricion *63*, 5-13 (2013).

[4] N. Aukkanit, Effect of Different Oils on Quality of Deep-fried Dough Stick. International Journal of Nutrition and Food Engineering *10*, 421-424 (2016).

[5] F. Salehi, Color changes kinetics during deep fat frying of carrot slice. Heat and Mass Transfer/Waerme- und Stoffuebertragung *54*, 3421-3426 (2018).

[6] Y. Chen, R.G. Moreira, Modelling of a batch deep-fat frying process for tortilla chips. Food and Bioproducts Processing: Transactions of the Institution of of Chemical Engineers Part C *75*, 181-190 (1997).

[7] S. Debnath, N.K. Rastogi, A.G. Gopala Krishna, B.R. Lokesh, Effect of frying cycles on physical, chemical and heat transfer quality of rice bran oil during deep-fat frying of poori: An Indian traditional fried food. Food and Bioproducts Processing *90*, 249-256 (2012).

[8] J. Lee, M. Kim, E. Choe, Effects of flour storage conditions on the lipid oxidation of fried products during storage in the dark. Korean Society of Food Science and Technology *15*, 399-403 (2006).

[9] T.V. Gadiraju, Y. Patel, J.M. Gaziano, L. Djoussé, Fried food consumption and cardiovascular health: A review of current evidence. Nutrients *7*, 8424-8430 (2015).

[10] M. Giovanni, Response surface methodology and product optimization. Food Technology *37*, 41-45 (1983).

[11] C.N. Vatsala, C.D. Saxena, P.H. Rao, Optimization of ingredients and process conditions for the preparation of puri using response surface methodology. International Journal of Food Science and Technology *36*, 407-414 (2001).

[12] O.P. Sobukola, S.O. Awonorin, S.L. Oladimeji, B.F. Olukayode, Optimization of pre-fry drying of yam slices using response surface methodology. Journal of Food Process Engineering *33*, 626-648 (2010).

[13] M. Hashemi Shahraki, A.M. Ziaiifar, S.M. Kashaninejad, M. Ghorbani, Optimization of pre-fry microwave drying of French fries using response surface methodology and genetic algorithms. Journal of Food Processing and Preservation *38*, 535-550 (2014).

[14] S. Singh, C.S. Raina, A.S. Bawa, D.C. Saxena, Optimisation of processing variables in the preparation of sweet potato chips using response surface methodology. European Food Research and Technology *217*, 374-381 (2003).

[15] X.J. Song, M. Zhang, A.S. Mujumdar, Optimization of vacuum microwave predrying and vacuum frying conditions to produce fried potato chips. Drying Technology *25*, 2027-2034 (2007).

[16] M.R. Perez-Tinoco, A. Perez, M. Salgado-Cervantes, M. Reynes, F. Vaillant, Effect of vacuum frying on main physicochemical and nutritional quality parameters of pineapple chips. Journal of the Science of Food and Agriculture *88*, 945-953 (2008).

[17] S.L. Shyu, L.S. Hwang, Effects of processing conditions on the quality of vacuum fried apple chips. Food Research International *34*, 133-142 (2001).

[18] A. Maadyrad, B. Ghiassi Tarzi, A. Bassiri, M.M. Bamenimoghadam Sc, Process Optimization in Vacuum Frying of Kiwi Slices Using Response

Surface Methodology. Journal of Food Biosciences and Technology 1, 33-40 (2011).
[19] S. Turan, S. Keskin, R. Solak, Determination of the changes in sunflower oil during frying of leavened doughs using response surface methodology. Journal of Food Science and Technology, 1-10 (2022).
[20] S. Turan, R. Solak, S. Keskin, Investigation of the Formation of Free and Bound 2- and 3-Monochloropropane-1,2-Diols During Deep Frying of Leavened Dough Using Response Surface Methodology. European Journal of Lipid Science and Technology 121, 1800019 (2019).
[21] E. Troncoso, F. Pedreschi, R.N. Zúñiga, Comparative study of physical and sensory properties of pre-treated potato slices during vacuum and atmospheric frying. LWT - Food Science and Technology *42*, 187-195 (2009).
[22] AOCS. Official Methods and Recommended Practices of the American Oil Chemists' Society. 17th edn. Champaign, IL: AOCS Press (2000).
[23] C. Gertz, Determination of polymerized triglycerides content in deep-frying fats and oils - Gertz - 2001 - European Journal of Lipid Science and Technology - Wiley Online Library. European Journal of Lipid Science and Technology *103*, 114-116 (2001).
[24] B. Saad, W.T. Wai, B.P. Lim, M.I. Saleh, Flow injection determination of anisidine value in palm oil samples using a triiodide potentiometric detector. Analytica Chimica Acta *591*(2), 248-254 (2007).
[25] M. Mohammadi, P. Hajeb, R. Seyyedian, G. Hossein Mohebbi, A. Barmak, Evaluation of oxidative quality parameters in imported edible oils in Iran. British Food Journal *115*, 789-795 (2013).
[26] J.A. Navas, A. Tres, R. Codony, F. Guardiola, Optimization of analytical methods for the assessment of the quality of fats and oils used in continuous deep fat frying. Grasas y Aceites *58*, 154-162 (2007).
[27] R. Bou, J.A. Navas, A. Tres, R. Codony, F. Guardiola, Quality assessment of frying fats and fried snacks during continuous deep-fat frying at different large-scale producers. Food Control *27*, 254-267 (2012).
[28] Y.H. Wong, K.M. Goh, K.L. Nyam, L.Z. Cheong, Y. Wang, I.-A. Nehdi, L. Mansour, C.P. Tan, Monitoring of heatinduced carcinogenic compounds (3monochloropropane1,2diol esters and glycidyl esters) in fries. Scientific Reports, *10*, 15110 (2020).
[29] J. Lee, M. Kim, K. Park, E. Choe, Lipid oxidation and carotenoids content in frying oil and fried dough containing carrot powder. Journal of Food Science *68*, 1248-1253 (2003).
[30] Y.H. Chu, S. Luo, Effects of sugar, salt and water on soybean oil quality during deep-frying. Journal of the American Oil Chemists' Society *71*, 897-900 (1994).
[31] J. Farmani, M. Rezaei, M. Mardani, M. Esmaeili, Effect of Bamiyeh Pastry Frying on Quality of Canola and Sunflower Oils. Advances in Food Sciences *37*, 8-16 (2015).
[32] R.Farhoosh, M.H. Tavassoli-Kafrani, Simultaneous monitoring of the conventional qualitative indicators during frying of sunflower oil. Food Chemistry *125*, 209-213 (2011).
[33] J.M., Soriano, J.C. Moltó, J. Mañes, Hazard analysis and critical control points in deep-fat frying. European Journal of Lipid Science and Technology *104*, 174-177 (2002).
[34] Ghidurus, M., M. Turtoi, G. Boskou, Hazards associated with fried fast food products. Romanian Biotechnological Letters (2013).
[35] J. Velasco, S. Marmesat, M.C. Dobarganes, Chemistry of Frying. In: Advances in Deep-Fat Frying of Foods (edited by S.S. Gulum Sumnu Servet), 33-54, CRC Press (2008).
[36] J.A., Adeyanju, J.O. Olajide, A.A. Adedeji, Optimisation of deep-fat frying of plantain chips (ipekere) using response surface methodology. Journal of Food Processing and Technology *7*, 584 (2016).
[37] N. Pambou-tobi, E. Arab-Tehrany, R.K. Niamayoua, M. Linder, RSM applied for optimization of deep-fat fried ripe plantain slices and study of oxidation kinetics of oil by a DSC and polar methods. Journal of Food Science and Technology 53, 269-280 (2016).
[38] A.O.Idowu, O.C. Aworh, Optimization of some processing conditions for Kokoro production using Response Surface Methodology. Agricultural Engineering International: CIGR Journal *16*, 187-195 (2014).
[39] C. Teangpook, U. Paosangtong, Y. Titatarn, S. Onhem, W. Puminat, Quality of repeated use of oil for frying Chinese deep fried dough (Pa-Tong-Kho). Current Applied Science and Technology, *18*, 1, 24-30 (2018)
[40] S. Nasir, G. Olatunde, A. Adebowale, I. Aiyelaagbe, Optimization of deep-fat frying conditions and quality attributes of orange-fleshed sweetpotato crisps by response surface methodology. Cogent Food & Agriculture *5*, 1658977 (2019).
[41] A.Martínez-Yusta, M.D. Guillén, Deep-frying. A study of the influence of the frying medium and the food nature, on the lipidic composition of the fried food, using 1H nuclear magnetic resonance. Food Research International *62*, 998-1007 (2014).
[42] N. Kim, K.S. Yu, J. Kim, T. Lim, K.T. Hwang, Chemical characteristics of potato chips fried in repeatedly used oils. Journal of Food Measurement and Characterization 12, 1863-1871 (2018)

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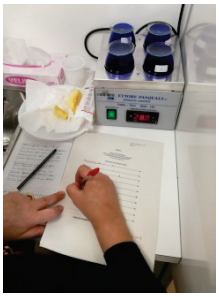
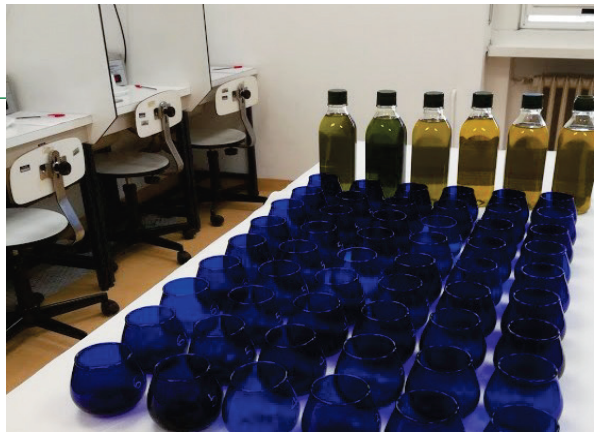
Virgin Olive Oil Organoleptic Assessment

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.





For further information:

Stefania De Cesarei
✉ **stefania.decesarei@mi.camcom.it**

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

Short note

Fatty acid composition of the seeds of two pepper varieties dried using different methods

Hasan Pinar[1], Beyza Ciftci[2]
Duran Simsek[3], Necati Cetin[4]
Kagan Kokten[5]✉
Muammer Ekmekci[6]
Mahmut Kaplan[2]

[1]Department of Horticulture
Faculty of Agriculture
Erciyes University
Kayseri, Turkey

[2]Department of Field Crops
Faculty of Agriculture
Erciyes University
Kayseri, Turkey

[3]Areo Seed Company
Antalya, Turkey

[4]Department of Agricultural Machinery and Technologies Engineering
Faculty of Agriculture
Ankara University
Ankara, Turkey

[5]Department of Plant Production and Technology
Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Turkey

✉ CORRESPONDING AUTHOR:
Department of Plant Production and Technology
Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Turkey
Fax: +90 3462191678
Tel: +90 5379352592
E-mail: kahafe1974@yahoo.com

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Optimum drying methods should be determined for seed longevity and preservation of the germination rate. Furthermore, while obtaining vigorous and healthy seedling by drying, the risk of genetic damage is minimised. In this study, fatty acid compositions of seeds of two pepper varieties dried by different methods were investigated. The fatty acid composition of the seeds of two pepper varieties dried by different methods had different saturated and unsaturated fatty acids. For saturated fatty acids, the seeds of two pepper varieties dried by different methods contained palmitic and stearic acids as the major component and contained small amount of myristic, margaric, arachidic and lignoceric acids. The major unsaturated fatty acids were identified as oleic and linoleic acids. Total saturated fatty acids contents varied between 13.41 and 21.56% and total unsaturated fatty acid contents varied between 78.44 and 86.59%. These findings may also help to better evaluate pepper seed oil in pharmaceutical and cosmetic commodities and confirm product authenticity.

Keywords: Pepper seeds, drying method, saturated fatty acid, unsaturated fatty acid

1. INTRODUCTION

Paprika is used in soups, stews, sausages, cheeses, snacks, salad dressings, sauces, pizzas, confectionery, drinks, etc. It is widely used as a food ingredient to change colour and flavour [1]. Drying is one of the most frequently used methods of processing and preserving red pepper [2]. Many drying methods are used for this purpose. Each drying method has negative and positive effects. After drying seeds are considered as by-products. Oil obtained from pepper is suitable for use as an alternative source of oil [3]. The chemical properties of pepper seed oil have been reported to be like safflower [4]. The potential of paprika seed oil for use in salads or cooking was recognised early [5].

The main reason of the differences in fatty acids composition of the cultivars could result from the genetic structure of the plant, irrigation, temperature and fertilisation [6, 7, 8, 9]. Drying methods significantly influence the fatty acid composition of the red peppers. Biochemical characteristics of the cultivars result in differences in drying methods. Not only the plant species, but also the cultivars should be taken into consideration in drying operations [10, 21].

Although there are some studies on the effect of the different drying steps on the fatty acid composition [11], it is not known whether there are differences air convective, microwave, freeze, open-sun, shade and greenhouse drying methods on fatty acid compositions of different pepper cultivars. The aim of this study is to examine the fatty acid composition of seed oil obtained from the seeds of two pepper varieties using different drying methods.

2. MATERIALS AND METHODS

2.1. MATERIALS

In the study, two pepper varieties named Pınar and Bozok, which are widely cultivated, were used. From the peppers harvested from the field, fruits that were not damaged and close in size and diameter were selected.

2.2. METHODS

2.2.1. Drying procedure

In this study, eight different drying processes (60 and 80°C air-convective, 300 and 600 W microwave, open-sun, shade, greenhouse and lyophilising) were performed on red pepper seeds. A hybrid oven with air-convective and microwave drying processes was used for drying seeds. Initial moistures of seeds were determined in an oven at 105°C for 24 h. In convective drying, drying processes were implemented 0.5 m s^{-1} air velocity. In open-sun, greenhouse, and shade drying, seeds were laid out on 50×50 drying papers. The seeds were dried under direct sunlight from 08:00 to 18:00 at temperatures between 25.8 and 42.5ºC in Kayseri, Turkey in August. The average relative humidity was 48.75%. The greenhouse is 72 m^2 (6 × 12 m) size which of 10 mm polycarbonate covered steel construction and has 4 ventilation and 1 circulation fan for homogeneous distribution of air. During the drying, the average temperature of the greenhouse was recorded as 34.55°C and relative humidity as 34.20%. Shade drying was carried out at room temperature. A lab-scale freeze dryer (Christ ALPHA 2-4 LSCplus, Germany) was used for freeze drying process at temperature of -55°C. The drying process was continued until the pepper varieties reached the equilibrium moisture value. After drying, the seeds of the peppers were separated and ground for analysis. All tests were conducted in 3 replications of factorial experimental design.

Moisture contents (wet basis) were determined with the use of followed equation [22].

$$M_c = \frac{W_i - W_f}{W_i} \times 100$$

Where:

M_c, moisture content (%, w.b),

W_i, initial weight of the product (g),

W_f, final weight of the product (g).

2.2.2. Oil Extraction and Preparation of Fatty Acid Methyl Esters (FAME)

Impurities were removed from the seeds, and the clean seeds were ground into powder using a ball mill. Lipids were extracted with hexane/isopropanol (3:2) [12]. The lipid extracts were centrifuged at 1 g for 10 min and filtered; then the solvent was removed on a rotary evaporator at 50°C.

2.2.3. Capillary GLC

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulfuric acid in methanol [13]. The fatty acid methyl esters were extracted with 2.5 ml hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionisation detection (Agilent brand 7890A model GC, 5975C model MS) coupled to a glass GC 10 software computing recorder. Chromatography was performed with a capillary column (100 m in length and 0.25 mm in diameter, BPX90: SGE 054596) using nitrogen as a carrier gas (flow rate 3 ml/min). The temperatures of the column, detector, and injector valve were 120-250°C and 230-270°C, respectively. Chromatographic conditions: starting at 50°C, then standing for 2 minutes and reaching 200°C at a rate of 20°C/min and then accelerating to 230°C at 5°C/min where it stood for 30 minutes. The total analysis time was 55.5 min. The identification of the individual method was performed by a frequent comparison with authentic standard mixtures that were analysed under the same conditions.

3. RESULTS AND DISCUSSION

Fatty acid composition of Bozok and Pinar seed varieties were dried using different methods presented in Table I and Table II, respectively. The seed oils of pepper varieties dried using different methods contain palmitic (12.62-14.46%) and stearic (4.41-8.08%) acids as the major component of fatty acids, among the saturated acids, with small amounts of myristic (0.26-0.27%), margaric (0.34-0.48%), arachidic (0.20-0.23%), and lignoceric (0.03-0.25%) acids. The major unsaturated fatty acids found in the seed oils were oleic (6.46-20.63%) and linoleic (64.65-73.91%) acids. Linolenic, palmitoleic and eicosapentaoic acids resulted to be lower than 1%. In this study, the total saturated fatty acids of pepper varieties dried using different methods were between 13.41 and 21.56%, while the amounts of total unsaturated fatty acids were between 78.44 and 86.59%.

Myristic acid was detected only in the Pinar variety seeds dried using 300 W and 600 W drying methods as 0.27% and 0.26%, respectively. Palmitoleic acid, on the other hand, was detected in seeds of the Bozok variety, dried in the shade, in addition to the applications in which myristic acid was detected. Margaric acid was detected only in the seeds of the Bozok variety dried in the greenhouse and 600 W drying applications. These results regarding myristic, palmitoleic and margaric acids do not agree with some researchers who reported that these acids were detected from different pepper seeds [14, 15, 16]. On the other hand, some scientists [17] reported that myristic and palmitoleic acids were found to be 0.17% and 0.29% in the pepper seed oil.

Palmitic acid was detected in all applications and varieties; it was found at the highest level (14.46%) in

Table I - Fatty acid composition of seeds of Bozok pepper varieties dried by different methods.

	14:0	16:0	16:1	17:0	18:0	18:1 (9)	18:2 (9,12)	18:3 (9,12,15)	20:0	20:2	24:0	∑SFA	∑TUSFA
B1	-	14.06	-	0.08	6.62	6.95	71.44	0.50	-	0.10	0.25	21.01	78.99
B2	-	14.46	0.34	-	6.72	6.58	71.07	0.49	-	0.14	0.20	21.38	78.62
B3	-	14.34	-	-	6.65	6.92	71.23	0.50	-	0.15	0.21	21.20	78.80
B4	-	12.62	-	-	7.02	7.48	71.95	0.54	-	0.17	0.22	19.86	80.14
B5	-	13.92	-	-	5.71	7.15	72.51	0.55	-	0.16	-	19.63	80.37
B6	-	12.84	-	-	4.94	8.21	73.26	0.54	0.21	-	-	17.99	82.01
B7	-	13.05	-	0.11	6.86	6.46	72.73	0.61	-	0.18	-	20.02	79.98
B8	-	12.96	-	-	4.41	8.29	73.91	0.43	-	-	-	17.37	82.63

Pepper varieties and drying methods: B1 Greenhouse-Bozok; B2 Shade-Bozok; B3 Open-Sun-Bozok; B4 60°C-Bozok; B5 80°C-Bozok; B6 300 W-Bozok; B7 600 W-Bozok; B8 Lyophilizer-Bozok; Fatty acids: C14:0 Myristic acid; C16:0 Palmitic acid; 16:1 Palmitoleic acid, 17:0: Margaric acid, 18:0: Stearic acid, C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; C20:0 Arachidic acid; C20:2 Eicosapentaoic acid, C24:0: Lignoceric acid; TSFA: Total saturated fatty acid; TUSFA: Total unsaturated fatty acid.

Table II - Fatty acid composition of seeds of Pinar pepper varieties dried by different methods.

	14:0	16:0	16:1	17:0	18:0	18:1 (9)	18:2 (9,12)	18:3 (9,12,15)	20:0	20:2	24:0	∑SFA	∑TUSFA
P1	-	13.64	-	-	5.51	14.17	64.65	0.58	-	1.45	-	19.15	80.85
P2	-	14.30	-	-	-	19.47	65.81	0.42	-	-	-	14.30	85.70
P3	-	12.77	-	-	5.19	11.90	68.85	0.70	0.21	0.21	0.17	18.34	81.66
P4	-	12.94	-	-	7.14	10.04	68.97	0.69	-	0.22	-	20.08	79.92
P5	-	13.18	-	-	-	20.63	65.45	0.51	0.23	-	-	13.41	86.59
P6	0.27	13.95	0.48	-	-	17.38	67.15	0.77	-	-	-	14.22	85.78
P7	0.26	13.98	0.38	-	7.29	10.44	67.00	0.45	-	0.17	0.03	21.56	78.44
P8	-	12.80	-	-	8.08	9.22	68.91	0.42	0.20	0.15	0.22	21.30	78.70

Pepper varieties and drying methods: P1 Greenhouse-Pinar; P2 Shade-Pinar; P3 Open-Sun-Pinar; P4 60°C-Pinar; P5 80°C-Pinar; P6 300 W-Pinar; P7 600 W-Pinar; P8 Lyophilizer-Pinar; Fatty acids: C14:0 Myristic acid; C16:0 Palmitic acid; 16:1 Palmitoleic acid, 17:0: Margaric acid, 18:0: Stearic acid, C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; C20:0 Arachidic acid; C20:2 Eicosapentaoic acid, C24:0: Lignoceric acid; TSFA: Total saturated fatty acid; TUSFA: Total unsaturated fatty acid.

the Bozok variety seeds dried in the shade, while the lowest level was detected in the Bozok variety seeds dried at 60°C (12.62%). While our findings on palmitic acid were consistent with the findings of some researchers [14, 16, 18, 19], it was higher than the findings of some researchers [11, 15, 17, 20]. Stearic acid was detected in all applications and varieties except the seeds of the Pınar variety, which were dried in shade, 80°C and 300 W drying methods. While the highest stearic acid was obtained from the seeds of the Pınar variety dried by the lyophilising method, the lowest stearic acid was found in the seeds of the Bozok variety, which were also dried using the lyophilising method. The findings we obtained on stearic acid were consistent with the findings of some researchers [18, 20], but higher than the findings of some researchers [11, 15, 16, 17, 19], and lower than the value of the researcher obtaining it as 11.69% [14].

The major unsaturated acids in the seed oils of all applications and varieties were oleic, linoleic, and linolenic acids. The oleic acid content was higher in the Pınar variety seeds dried at 80°C (20.63%), and lower in the Bozok variety seeds dried at 600 W (6.46%). The seed oils of all the applications and varieties were richer in linoleic than linolenic acid. The greatest proportion of Linoleic acid was found in the seed oil. The linoleic acid content was higher in the Bozok variety seeds dried using the lyophilising method (73.91%), and in the Bozok variety seeds dried at 300 W (73.26%) but lower in the Bozok variety seeds dried in the greenhouse (64.65%). Linolenic acid was detected in low levels in the Pınar variety seeds dried both using the lyophilising method and in the shade (0.42%) The linolenic acid content was higher in the Pinar variety seeds dried both at 300 W (0.77%) and at 60°C (0.69%). While the values we obtained for oleic and linoleic acids agreed with the values of many scientists [11, 15, 16, 17, 18, 19], the values we obtained for the linolenic acid was low compared to some investigators [16, 17] and high compared to other investigators [18, 19].

Arachidic acid was detected only in the Bozok variety seeds dried at 300 W, and in the Pinar variety seeds dried at Sun, 80°C and lyophilising drying methods as 0.21%, 0.21%, 0.23% and 0.20%, respectively. While the values we obtained for arachidic acid agreed with the values of many scientists [15, 17, 18], some investigators found low values [16], and some investigators found high values [14, 19]. Eicosapentaoic acid was detected in all applications except for 5 different applications. The highest eicosapentaoic acid was detected in the Pınar variety seeds dried in the greenhouse with 1.45%, while the lowest eicosapentaoic acid was detected in the seeds of the Bozok variety dried in the greenhouse with 0.10%. These results regarding eicosapentaoic acid was like some researchers who reported that eicosapentaoic acid were detected from Urfa pepper seeds as 0.18% [17]. On the other hand, some scientists [16] reported that eicosapentaoic acid ranged from 0.03% to 0.05% in the red pepper seed oils extracted using different methods. The lignoceric acid content of pepper seeds dried using different methods varied between 0.03% and 0.25%. The highest lignoceric acid was detected in the Bozok variety seeds dried in the greenhouse, while the lowest lignoceric acid was detected in the Pınar variety seeds dried at 600 W. While these results on lignoceric acid were consistent with the findings of some researchers [15, 16], it was lower than the lignoceric acid values that some scientists had found as 0.37% in the seeds of the world's hottest Naga king chili pepper [19].

The total saturated fatty acids (TSFA) of pepper seeds dried by different methods were between 13.41% and 21.56%. The Pınar variety seeds dried at 80°C had the lowest level of saturated acid, and the Pınar variety seeds dried at 600 W had the highest saturated fatty acid (SFA) concentration. The total unsaturated fatty acids (TUSFA) of the pepper seeds dried using different methods were between 78.44% and 86.59%. The Pınar variety seeds dried at 80°C had the highest level of unsaturated fatty acid (86.59%), along with the seeds of the Pınar variety dried at 300 W (85.78%), and the seeds of the Pınar variety dried at shade (85.70%) (Table II).

4. CONCLUSION

In this study, seeds of two different red pepper varieties were dried with the use of 8 different drying methods. Fatty acids of the varieties were presented in such differences in drying methods. Palmitic and stearic acids were determined as the major component fatty acids, among the saturated acids. Besides, oleic and linoleic acids were found as the major unsaturated fatty acids. The greatest results were obtained for both Bozok and Pinar cultivars in freeze, 300 W microwave and shade dried samples. In further studies, researchers can focus on different drying methods and conditions, considering the current findings.

Conflict of interest

The authors declare that they have no conflict of interest.

BIBLIOGRAPHY

[1] R. Arimboor, R.B. Natarajan, K.R. Menon, L.P. Chandrasekhar, V. Moorkoth, Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: analysis and stability-a review. Journal of Food Science and Technology 52(3), 1258-1271 (2015).

[2] L.Z. Deng, X.H. Yang, A.S. Mujumdar, J.H.

Zhao, D. Wang, Q. Zhang, Red pepper (*Capsicum annuum* L.) drying: effects of different drying methods on drying kinetics, physicochemical properties, antioxidant capacity, and microstructure. Drying Technology 36(8), 893-907 (2018).
[3] R.L. Jarret, I.J. Levy, T.L. Potter, S.C. Cermak, Seed oil and fatty acid composition in *Capsicum* spp. Journal of Food Composition and Analysis 30(2), 102-108 (2013).
[4] R. Sancho, C. Lucena, A. Macho, M.A. Calzado, M. Blanco-Molina, A. Minassi, G. Appendino, E. Muñoz, Immunosuppressive activity of capsaicinoids:capsiate derived from sweet peppers inhibits NF-kB activation and is a potentanti-inflammatory compound in vivo. European Journal of Immunology 32, 1753-1763 (2002).
[5] H.C. Ebert, H.S. Bailey, Pimento seed oil. Cotton Oil Press 7, 35-36 (1924).
[6] K. Kokten, A. Bakoglu, A. Kocak, E. Bagci, M. Akcura, M. Kaplan, Chemical composition of the seeds of some *Medicago* species. Chemistry of Natural Compounds 47(4), 619-621 (2011).
[7] M. Kaplan, K. Kokten, S. Uzun, Fatty acid and metal composition of the seeds of *Vicia ervilia* varieties from Turkey. Chemistry of Natural Compounds 50(1), 117-119 (2014).
[8] M. Kaplan, H. Kale, K. Karaman, A. Unlukara, Influence of different irrigation and nitrogen levels on crude oil and fatty acid composition of maize (*Zea mays* L.). Grasas Y Aceites 68(3), e207-e207 (2017).
[9] I.S. Varol, Y.M. Kardes, H.A. Irik, H. Kirnak, M. Kaplan, Supplementary irrigations at different physiological growth stages of chickpea (*Cicer arietinum* L.) change grain nutritional composition. Food Chemistry 303, 125402 (2020).
[10] H. Pinar, N. Çetin, B. Ciftci, K. Karaman, M. Kaplan, Biochemical composition, drying kinetics and chromatic parameters of red pepper as affected by cultivars and drying methods. Journal of Food Composition and Analysis 102, 103976 (2021).
[11] A. Pérez-Gálvez, J. Garrido-Fernández, M.I. Mínguez-Mosqueraa, M. Lozano-Ruiz, V. Montero-de-Espinosa, Fatty acid composition of two new pepper varieties (*Capsicum annuum* L. cv. Jaranda and Jariza) effect of drying process and nutritional aspects. Journal of the American Oil Chemists' Society 76(2), 205-208 (1999).
[12] A. Hara, N.S. Radin, Lipid extraction of tissues with a low toxicity solvent. Anal. Biochem. 90, 420-436 (1978).
[13] W.W. Christie, Gas Chromatography and Lipids: A practical guide. The Oily Press, Ayr. pp. 307 (1990).
[14] L.R. Silva, J. Azevedo, M.J. Pereira, P. Valentão, P.B. Andrade, Chemical assessment and antioxidant capacity of pepper (*Capsicum annuum* L.) seeds. Food and Chemical Toxicology 53, 240-248 (2013).
[15] L.R. Silva, J. Azevedo, M.J. Pereira, L. Carro, E. Velazquez, A. Peix, P. Valentão, P.B. Andrade, Inoculation of the nonlegume *Capsicum annuum* L. with *Rhizobium* strains. 2. Changes in sterols, triterpenes, fatty acids, and volatile compounds. J. Agric. Food Chem. 62, 565-573 (2014).
[16] M. Chouaibi, L. Rezig, S. Hamdi, G. Ferrari, Chemical characteristics and compositions of red pepper seed oils extracted by different methods, Industrial Crops & Products 128, 363-370 (2019).
[17] B. Basyigit, S. Daghan, M. Karaaslan, Biochemical, compositional, and spectral analyses of Isot (Urfa pepper) seed oil and evaluation of its functional characteristics. Grasas Y Aceites 71(4), e384 (2020).
[18] N. Zaki, A. Hasib, A. Hakmaoui, F. Dehbi, A. Ouatmane, Assessment of color, capsaicinoids, carotenoids and fatty acids composition of paprika produced from Moroccan pepper cultivars (*Capsicum annuum* L.). Journal of Natural Sciences Research 3(7), 111-118 (2013).
[19] R. Ananthan, K. Subhash, T. Longvah, Capsaicinoids, amino acid and fatty acid profiles in different fruit components of the world hottest Naga king chili (*Capsicum chinense* Jacq). Food Chemistry 238, 51-57 (2018).
[20] M. Ritota, F. Marini, P. Sequi, M. Valentini, Metabolomic characterization of Italian sweet pepper (*Capsicum annuum* L.) by means of HRMAS-NMR spectroscopy and multivariate analysis., J. Agric. Food Chem. 58, 9675-9684 (2010).
[21] N. Çetin, C. Sağlam, Rapid detection of total phenolics, antioxidant activity and ascorbic acid of dried apples by chemometric algorithms., Food Biosci. 101670 (2022).
[22] C. Sağlam, N. Çetin, Machine learning algorithms to estimate drying characteristics of apples slices dried with different methods. J. Food Process. Preserv. e16496 (2022).

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Olive oil proficiency tests
Chemical-physical parameters and contaminants

Edition 2023

Registration open until
30/04/2023

Since 2003, Innovhub SSI organizes every year an interlaboratory test on olive oil for different commercial categories among various olive oil laboratories.
The tests include all the chemical parameters.
Since 2016 the main contaminants are also considered.
Each participant will have the opportunity to compare his own test results with those obtained by the most accredited Italian and foreign laboratories.
The proficiency test has as main purpose, the ability to make corrections from deviation that might occur in the results, compared to the average value obtained by other laboratories.
At the end of the laboratory tests, the participants insert the results obtained directly in the web portal on the dedicated page:
https://proficiencytest.innovhub-ssi.it
The results will be statistically processed and delivered anonymously to each participant.

For the interlaboratory trial 2023 on the methods of analytical test of olive oils, each participant will receive 3 samples (Extra virgin, Refined, Lampante) for chemical-physical testing and 1 sample for the analysis of contaminants before the end of June 2023.

Deadline for sending results is October 31, 2023.

For organizational reasons, participation is limited to max. 50 laboratories.

For information and registration
please, contact Dr. Stefania De Cesarei
e-mail **pt.ssog@mi.camcom.it**
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Annunci di Ricerca Partner per Progetti di Ricerca

Enterprise Europe Network (EEN)

Anno 2023

(aggiornato al 30 marzo 2023)

Progetto BRHU20220513005

Hungarian SME is looking for producers/distributors of basic materials for the food industry

The Hungarian company owners started their business in 1991, so they have a long experience in wholesale of food ingredients. Its main goal is to make premium quality basic and auxiliary materials available for Hungarian food producers. Now the company offers premium materials for bakery, confectionery, meat processing, dairy and the pharmaceutical industry importing raw materials mainly from Europe. The main product portfolio of the company includes margarines, yeasts, soy products (flours, lecithin, etc.), gluten, starches, cocoa powders, coating mass, oils-fats (coconut, palm oil, sunflower seed oil, olive oils, pork fat), seeds, dried fruits, bakery flakes, milk and vegetable products, liquid and powdered egg, glycerine and dextrose. The Hungarian SME is searching for partners who are producers or distributors of the above mentioned products.
Dead-line for EOIs: 13/05/2023

Progetto BRPL20220516008

Polish producer of natural cosmetics and candles is looking for suppliers of raw materials

A Polish family-owned company, with 5 years of experience in the organic sector and hand-made cosmetics, specializes in the production of cosmetics and candles based on natural ingredients only, without fixatives, preservatives, or parabens. The production is based on traditional methods. Cosmetics are made from vegetable oils, some of which are produced by the manufacturer itself. The company is now looking for cooperation in the field of supply of raw materials for the production of candles such as rapeseed wax, essential oils and fragrance compositions under supplier agreement.
Dead-line for EOIs: 16/05/2023

Progetto BRBG20220801015

Bulgarian producer of organic cold-pressed oils is looking for distributors or producers of various organic seeds and nuts

Bulgarian family-owned company produces healthy organic high quality oils from seeds and nuts by the method of coldpressing, which allows to maintain all the beneficial properties of the substances they have been extracted from. The company can also produce customized oils with customers' raw materials from organically certified seeds and nuts. The Bulgarian company is looking for distributors or producers of organic seeds and nuts which they cannot source locally. The products must be suitable for the method of coldpressing, used in the manufacturing activity of the company, and have the relevant bio certification. Potential partners must be willing to supply small quantities at the beginning.
Dead-line for EOIs: 01/08/2023

Progetto TRNL20220815007

Sustainable cleaning technologies sought preventing disposal of cleaning cloths by Dutch-Belgian wholesaler

A Dutch-Belgian wholesaler supplying cleaning utilities to cleaning organizations in several European countries wants to make its cleaning solutions more circular. Therefore it is looking for technologies or solutions that prevent cleaning cloths to be disposed and incinerated. A pilot project showing the potential of the technology within the framework of a commercial agreement is aimed for. This technology request refers to an innovation challenge published on an open innovation platform.
Dead-line for EOIs: 18/08/2023

Progetto BRFR20221006004

An eco-friendly French SME is looking for foreign suppliers of cotton-lined cork, vegetable fibers or cellulose fabrics to produce high-end vegan bags

A French designer specialises in the production of leather goods adopting an eco-responsible approach. The collections are intended for men and women, the pieces are unique or in small series. As part of the manufacturing process, the designer pays the greatest attention to the choice of materials, assembly, stitching and finishes of each element. The French SME is now looking for alternatives to animal leather, which must also be resistant and eco-responsible, without petroleum products (no polyester) and biodegradable: cotton-lined cork, vegetable fibers, cellulose materials supplied in roll format and several colours. The company is seeking long-term partners under supplier agreements.
Dead-line for EOIs: 05/10/2023

Progetto BRPL20221010002

A Polish importer of Italian food specialties is looking for an Italian producer of *pesto genovese*

The Polish company business is focused on the import and distribution of Italian specialties and delicatessen. They operate on the Polish market since 2020 and work with individual clients, online sales, Horeca distribution and wholesale. The company imports various food products and soaps. Currently they are looking for a producer of *pesto genovese*. They want to import pesto in small, plastic, 40 g packages. The partner should be a medium or big producer of pesto able to supply around 10.000 packs monthly to the Polish company.
Dead-line for EOIs: 10/10/2023

Progetto BRBG20221016001

A Bulgarian manufacturer is looking for a supplier of sustainable packaging

A Bulgarian manufacturer of high quality marine salt is looking for a supplier of sustainable packaging for its high-end product - *Fleur de sel*. The company has more than 15 years of experience in producing salt and lay. It is now developing a new product - highest quality sea salt for the retail market. The company is seeking to match this high quality product with a sustainable packaging.

Dead-line for EOIs: 21/10/2023

Progetto BRRO20221128008

Romanian manufacturer of paper packaging solutions seeks international business partners able to supply kraft paper rolls as raw material under supplier agreements

The Romanian company is a paper packaging manufacturer with over 10 years' market experience. Their products are manufactured in kraft paper (natural/light brown or white) using sustainable sources of raw materials. To ensure excellent product quality, the company constantly strives to ensure a good supplier base. In this context it has established successful business relations with several partners across Europe. Aiming to further expand its network, the company is currently seeking new international business partners, manufacturers or stock resellers, able to supply kraft paper rolls as raw material. Cooperation will be based on supplier agreements.

Dead-line for EOIs: 28/11/2023

Progetto BRRO20230228012

Romanian manufacturer of natural cosmetic products seeks suppliers of olive oil (first press and pomace) under supplier agreements

The company, which was established by two young entrepreneurs with a keen interest in natural cosmetics, relies on their own recipes for their production. Its portfolio includes hand, face and body creams, lip balms, sunscreens and insect repellent lotions, natural soaps, cleansing and after-shave gels and lotions. All products are manufactured using natural ingredients, which include essential and vegetable oils, and active ingredients, such as hyaluronic acid and Q10 coenzyme obtained through plant synthesis. Ingredients used in the manufacturing process are sourced from suppliers in Romania and Germany. In order to diversify its network of suppliers, the Romanian company is now looking for EU producers of olive oil able to supply high quality virgin oil obtained via cold-pressing procedures, as well as pomace olive oil, to be used for cosmetic purposes. Cooperation will be based on supplier agreements.

Dead-line for EOIs: 01/03/2024

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.*

Enterprise Europe Network (EEN)

È la rete nata nel 2008 per volontà della Commissione Europea con l'obiettivo di supportare l'innovazione, il trasferimento tecnologico e l'internalizzazione di piccole e medie imprese ed enti di ricerca.
Si avvale di oltre 600 organizzazioni presenti in 60 paesi e offre un sistema integrato di servizi gratuiti per aiutare le imprese a individuare nuovi partner commerciali, produttivi e tecnologici all'estero; per promuovere la partecipazione ai programmi Europei per la ricerca, come Horizon Europe, e per fornire gli strumenti utili per essere più competitivi sui mercati internazionali, migliorando la conoscenza dei mercati e della legislazione europea.
In Lombardia i servizi di Enterprise Europe Network sono garantiti dal consorzio **Simpler** (Support Services to IMProve innovation and competitiveness of businesses in Lombardia and Emilia-Romagna), di cui Innovhub è partner.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

enterprise europe network
Business Support on Your Doorstep

I servizi della rete EEN sono gratuiti.
Per cercare il tuo partner in Europa, consulta il nostro database:
https://een.ec.europa.eu/partners

Per maggiori informazioni contattare:
Susy Longoni
susy.longoni@mi.camcom.it

Come ti può aiutare la rete EEN?

Far crescere l'azienda e sostenere l'internazionalizzazione:
- Informazioni sulla legislazione EU
- Informazioni e assistenza sul Regolmaneto REACH
- Ricerca di finanziamenti a supporto delle imprese
- Supporto per l'individuazione di opportunità commerciali all'estero
- Sostegno per lo sviluppo di nuovi prodotti o processi

Sviluppare partneriati:
- Supporto alla partecipazione a brokerage event e company mission e per la conclusione di accordi di trasferimento tecnologico
- Assistenza nella ricerca partner

Implementare processi di innovazione e trasferimento tecnologico:
- Servizio di analisi delle capacità di gestione e miglioramento dell'innovazione
- Supporto al trasferimento tecnologico/open innovation
- Informazione su bandi di finanziamento e supporto alla partecipazione a programmi di ricerca
- Pre-screening delle proposte progettuali EIC Accelerator

European Algae Industry Summit

19-20 April 2023 | Lisbon - Portugal

ACI is pleased to announce the 11th Annual European Algae Industry Summit will take place on the 19th-20th April 2023 in Lisbon, Portugal.

The event will bring together key players within the algae industry including leaders from cosmetics, food, feed, biomaterials, nutraceuticals and pharmaceuticals across the globe allowing attendees to gain a deeper understanding of recent industry developments and, most importantly, economically viable applications.

Key topics:

- Market Overview of Industry Trends & Drivers
- Algae Foods; an Industry Blooming
- Cutting-Edge Advancements in Algae Protein for Plant-Based Products
- Managing EU Regulations across the Industry
- Capitalising on the Success of Spirulina
- Astaxanthin the Oceanic Carotenoid
- Enhancing Algae's Reputation as a Sustainable Alternative
- Furthering the Evolution of Algae in Cosmetics and Personal Care
- Prospects for Algae bioplastics and Biofuel
- Adapting Approaches to Production in an Ever-Expanding Market

Who will attend?

- Algae producers & cultivation plant owners and operators
- Algae end market users in nutrition, cosmetics, pigments, animal feed, bioplastics, agriculture and many more
- Leading algae/biomass research institutes, technology providers for cultivation, harvesting, dewatering, drying, oil extraction and processing
- Plant engineers and constructors, green energy & biotech investors
- Green energy/biotechnology investors: venture capitalists, private equity firms etc. looking to invest in new & emerging markets
- Regulators & governments: organisations looking into the regulation of algae-based product
- Photobioreactor and Cultivation System Providers
- NGOs
- Academics

For updates:
https://www.wplgroup.com/aci/event/european-algae-industry-summit/

Euro Grain Hub Exchange & Forum

26-28 April 2023 | Bucharest

Agri business in the Central and Eastern European countries is confirming its leading position in the world grain and oilseeds trade as very important origination region. Black Sea countries were challenged as reliable suppliers under conditions of uncertainties and supply chain disruptions.

The war in Ukraine initiated great changes in the regional grain flows affecting the global commodity trade. Grain exports from the Central and East European countries - Romania, Bulgaria, Serbia, Hungary, Poland, Baltics, Moldova, Croatia and etc. - are in the spotlight to fill the gap after Ukrainian production and trade will suffer for years to come. The EuroGrainHub Exchange & Forum is addressing the most important developments and is delivering the best discussions and networking platform for all involved in the agricultural sector – from farmers, traders, input suppliers, animal and feed industry and stretching to logistics, storage, innovations, etc.

Curbing of global food inflation is still a major topic, but what's next?

EuroGrainHub is sending a powerful message to the world that grain trade is responding to the ongoing market extremes by rerouting of the trade flows and setting the global pricing. The key stakeholders of the global agri commodity supply chain will be in this April in Bucharest. Join the EuroGrainHub Exchange & Forum in April 26-28, 2023 as collaborator, partner and honorable guest. Be part of most important spring event in the grain industry in Europe!

Agenda and updates: https://eurograinevents.com/

2023 AOCS Annual Meeting & Expo

April 30-May 3, 2023 | Colorado Convention Center, Denver, Colorado, USA

Get ready for the premier international science and business forum on fats, oils, surfactants, proteins and related materials!

Connect with thousands of researchers, industry professionals, and government representatives and grow your network.

Discover the latest research and best practices from around the globe.

Share your ideas and research with fellow attendees to gain new perspectives and discuss innovations.

It's the premier global chemistry event by scientists for scientists. Join thousands of researchers, industry professionals, and government representatives in Denver to discuss challenges and find solutions you can take home. There is something for

everyone with 80+ sessions presented in-person at the Colorado Convention Center:

- *Meeting at a Glance* - provides an overview of what you can expect at this year's annual meeting, including technical sessions, Expo Hall hours, networking events, poster session details, and more!
- *Call for Papers*
- *Hot Topics and Featured Sessions* - focus on critical developments that impact industries related to edible oils and fats, surfactants and detergents, plant proteins and related sustainable materials.
- *Technical Sessions* - presentations in 10 interest areas. Browse the 80+ session topics featured at the 2023 annual meeting.
- *Student ePoster Pitch Competition*
- *Networking events*
- *Exhibition*

Register on https://annualmeeting.aocs.org/

13th ICIS World Surfactants Conference

4-5 May 2023 | The Hyatt Regency, Jersey City – USA

Bringing together industry professionals from all corners of the surfactants value chain for an informative and valuable two-day, the ICIS World Surfactants Conference is now in its thirteenth 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.

We have also listened to your feedback from the past and have planned the event to follow the New York Supplier's Day. This is so you can maximise your trip to the area and head back to the office well-equipped for the remainder of the year.

We are confident that this year's conference will once again be a rewarding and memorable experience for all attendees. We look forward to welcoming you and hope to see you at the 13th ICIS World Surfactants Conference!

Networking

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.

Partnership Opportunities

Take advantage of ICIS's strong market presence and extensive contact list, to increase your company's visibility and distinguish yourself from competitors. No matter if you are a well-established company or a newcomer seeking exposure, we tailor partnership packages to help you maximize your brand's exposure and meet your needs

The World Surfactants Conference attracts attendees from all sizes of companies, from large corporations to small start-ups and our partnerships provide the perfect backdrop to highlight thought leadership, introduce new products or drive sales.

For information visit:
https://events.icis.com/website/8544/home/

Globoil International

8-10 May 2023, Le Meridien Hotel & Convention Centre, Dubai

Globoil International is a lavish event in the month of May where the key players from the Edible Oil and Agri Trade will meet. The event consists of Interactive Discussions, Awards & Celebrations, Networking opportunities in the bucket for you. We will be taking Globoil International to Malaysia, Singapore, Thailand, Indonesia, and several other Edible Oil & Agri Tade hotspots around the globe in the coming years.

Further information:
https://www.globoilinternational.com/

Oleochemical Technical approach course

9-10 May 2023 | Louvain-la-Neuve, Belgium

A general introduction to vegetable oil and animal fats derivatives.

Broader your corporate scope and vision of Oleochemicals.

Dedicated to technical sales, purchasers, business and product managers, formulators, regulatory and applications managers, team managers.

For information:
info@triact.be
https://www.triact.be/courses/1

FENAGRA 2023
International Feed & Food Agroindustry Fair

10-11 May 2023 | Dom Pedro Expo - Campinas - SP

FENAGRA – International Agroindustry Fair is the junction of 6 major events in the same place and time. Inside the Expo Dom Pedro Pavilion (Campinas – SP) we will have the Animal Recycling, Expo

Pet Food, Expo Aqua Feed, Expo Animal Feed and Expo Oils & Fats segments.
In this way we have the main business fair in the Animal Nutrition and Human Nutrition sector together.
If your company is a manufacturer of machinery, equipment, inputs or raw materials, in the Feed and Food industry. You cannot fail to participate in the main fair in the sector.
6 thousand visitors from the Pet Food, Animal Nutrition, Refrigerators and Grease Shops, Human Nutrition, Vegetable Oils and Fats, Biodiesel, Grains and Derivatives Industry. You need to participate.

See commercial conditions that we have prepared for your company to expose all its products and services to a qualified audience at Fenagra 2023.
For updates please visit:
https://www.fenagra.com.br/

Biofuels International Conference & Expo

16-17 May | Brussels - BE

Decarbonisation, net zero and energy security are set to be hotly debated topics in 2023 with biofuels taking centre stage in helping companies and governments meet their environmental goals.
Following on from the European Parliament's recent target of 45% renewable energy consumption by 2030 and the European Union's REPowerEU plan for energy independence, the biofuels sector is presented with an excellent opportunity to scale-up and show the world that biofuels still have a crucial role to play in today's energy markets.
The event will bring together leading producers, suppliers, regulators and other engaged organisations over a two-day period. High-level speakers, experts in their field, will address a range of topical issues relating to the biofuels sector:

- European and global biofuels markets and drivers
- How the EU's Fit-for-55 package and REPower plan can benefit the biofuels sector
- Advanced biofuels – how far can the sector grow
- Updates on the latest policies and regulations affecting the industry
- How technology can improve performance and plant efficiencies
- Growth opportunities within the sustainable aviation and marine biofuels sectors
- How to choose the best feedstocks
- Unlocking the potential of biorefineries

Plus much, much more over the two days of the conference.
2023 conference will be held in Brussels, Belgium and co-located with the International Biogas Congress & Expo as well as the renowned Biomass Congress & Expo, making this series of bio events our largest gathering yet of bio related companies, giving participants unrivalled coverage.
To join as a sponsor, exhibitor or potential speaker please contact claire@woodcotemedia.com
For more information visit the conference website:
https://biofuels-news.com/conference/about/

RPFOODS'23

3rd International Conference on Raw Materials to Processed Foods

18-19 May 2023 | Istanbul

Dear Colleagues and Friends,

It is a great privilege for us to invite you to the 3rd International Conference on Raw Material to Processed Foods which will be held on 18-19 May 2023 in Istanbul, Turkey builds hugely successful preceding the past conferences.
One of the aims of this multi-track event is to bring together leading academic scientists, researchers, and scholars to present and discuss the most recent innovations and trends as well as to exchange and share their experiences and research results on all aspects of Food Science, Processing and Technology.

The Food and Agriculture industries effectuate the largest vital and economic sectors in the world. Therefore, the titles of Food Science, Processing, and Technology continue to evolve by gaining importance daily as within this food processing is already a crucial field since ancient times paving the way for humankind to lead a sedentary life and is also essential for supporting the civilization. Creating novel technologies and innovations in food science requires comprehensive knowledge about majors like biology, chemistry, physics, and engineering sciences forming a multidisciplinary field. Thus the International Conference on Raw Material to Processed Foods welcomes your ideas and research of all related disciplines including Food Science and Technology, Fisheries Science, Nutrition Science, Animal Science, Veterinary Science, Horticulture, Agricultural and Food Biotechnology.
Your valuable scientific contributions are welcomed as oral or poster presentations on the following main themes:

- Trends in new food products and technology development
- Quality and safety of raw and processed foods
- Nutrients and nutritional assessment of raw and processed foods
- Instrumental and sensory analysis strategies in raw and processed foods
- Process design and practices applied from raw material to processed foods
- Medicinal and aromatic plants: raw material to essential oils
- Meat and seafood processing technology, quality, and safety

- Postharvest applications and technologies for raw materials

This unique event will be an academically valuable Conference, with a scientific committee comprised of researchers from different universities and institutions all over the world serving as an opportunity to promote international novel research. Furthermore, participants will have the chance to meet relevant researchers and even to make new collaborations for future studies.

You are cordially invited to submit an abstract to the "3rd International Conference on Raw Material to Processed Foods". We look forward to meeting you for the conference in Istanbul, the heart of Turkey.

Conference Chairs: Prof. Dr. Serkan SELLI, Prof. Dr. Hasim KELEBEK

For more details please visit:
https://www.rpfoods.net/about

RSPO Inter-American Conference 2023

30-31 May 2023 | Miami, Florida

Key actors of the palm oil industry in Latin America and North America are set to gather in the city of Miami for the first RSPO Inter-American Conference. Focused on the theme, "From Emerging to Thriving: the role of the Americas as leaders in sustainable palm oil," the region's main conference advancing the sustainability of the palm oil sector anticipates around 300 leaders of companies that produce and use sustainable palm oil.

Registration on:
https://riac.cventevents.com/event/1f482f4c-8574-46db-80fc-2120e6100ada/summary?RefId=IAconference

International Conference "VegOil Trade 2023"

1-2 June 2023 | Rotterdam, Netherlands

VegOil Trade international conference will be dedicated to the EU vegetable oil market with an emphasis on the supply of oils and oilseeds from the Black Sea countries.

Exclusive information on production forecasts in 2023, prospects for the oilseed sector in the new season, further redistribution of trade flows as well as other most pressing issues of world trade in oilseeds and by-products will be discussed at VegOil Trade-2023 international conference.

Key Topics:

- Global trends of the world market of vegetable oils in 2022/23 MY
- Peculiarities of the European market of vegetable oils: higher production due to larger import of raw materials
- Global palm oil market: trade and trends, redistribution of trade flows
- Sunflower oil market: trends and features of 2022/23 MY, main players and prospects for 2023/24 MY
- Reduction in the supply of Ukrainian sunflower oil: interim results of processing in 2022/23 MY, energy crisis, price dynamics, sales markets
- Peculiarities of the logistics of vegetable oils and meal in Ukraine: the operation of the grain corridor and the development of alternative export routes
- Boom of oilseeds export from Ukraine: continuation of the trend or possible restrictions
- Prospects for oilseed production in the world, in particular in the EU and Ukraine in 2023/24 MY
- European meal market: suppliers and consumers
- Crude oil products and biofuels market trends as a key price driver

Target Audience:

Processors of oilseeds and traders, international trading companies, importers, agricultural holdings, industry organizations, leading domestic and international agricultural experts, equipment manufacturers, key exporters and consumers of oilseeds and by-products, representatives of scientific organizations, etc.

https://www.apk-inform.com/en/conferences/vegoil-trade-2023/about

4th International Congress on Mineral Oil Contaminants in Food

05 - 06 June 2023 | Berlin, Germany

The German Society for Fat Science as neutral platform for the exchange of knowledge between science and practise organises together with the Institut Kirchhoff Berlin GmbH - part of Mérieux NutriSciences, Berlin, as laboratory of the analytics of mineral oil components in food of the first hour the 4th International Congress on Mineral Oil Contaminants in Food - Toxicology - Risk Assessment - Analytics - Mitigation.

Contamination of foodstuffs and other products (cosmetics, packaging materials, etc.) with mineral oil components has been known since the early 1990s. Since then, the development of analytical techniques has considerably increased the data available for quantifying and identifying the sources of these undesirable substances. On the basis of these available data, toxicological evaluations were carried out, appropriate minimization measures were introduced and orientation values were jointly defined by official authorities and the food association. All these measures have led to a significant reduction of mineral oil components in all subsectors. However, the problem is still not solved. Mineral oil components in food are still a big challenge for producers. Toxicological and risk

assessment are still under discussion, consequently an European legal regulation is open. Today the LC-GC-FID method for the determination of MOSH and MOSH is the method of choice and a method standardized by the German Society for Fat Science is available but it is still challenging and not easily applicable for a wide range of matrices. Only on basis of validated analytics evidence-based toxicological and food law statements can be made and subsequently political decisions be taken. More and more, GCxGC-MS is used to identify sources for the input of mineral oil components in food, but several approaches are available and the method is not validated making the comparability of results difficult. For producers of food the progress in the identification of entry pathways and the development of mitigation strategies are important to ensure the high quality and safety of food. Much has already been implemented here in practice in recent years, but there are still open questions.

How is the current status of the toxicological assessment for mineral oil components? How does the European Food Safety Agency assess mineral oil components? Can we expect a legal regulation? What is the progress to improve the analytical method? Which further mitigation strategies can be expected and how does industry implement measures? And, and, and?

The congress will offer you the opportunity to discuss the latest findings on questions of toxicology, risk assessment, analytics and mitigation strategies with proven experts and thus bring yourself up to date on this important contamination. The presented information are aimed at all interested parties from the authorities, official and private laboratories, consumer protection and industry who are involved in quality control, analysis, evaluation and marketing of food and who have to make decisions on mineral oil components.

More information:
https://veranstaltungen.gdch.de/tms/frontend/index.cfm?l=11448&modus=

12th CESIO World Surfactant Congress

5-7 June 2023 | Rome, Italy

CESIO is the European association representing producers of surfactants and intermediates. Every 4 years, the association organizes the CESIO World Surfactant Congress and provides a unique opportunity for partners and contacts across the surfactants value chain to meet. 39 years after the first congress, the 12th World surfactant congress will be held in Rome from 5th to 7th June 2023. The theme for this edition will be: “Surfactants– High Performance Solutions for a Better World”. This event represents the perfect opportunity to learn about the latest developments in key areas such as Business & Market Trends, Safety & Regulatory affairs and Technical & Applications. Participants can take part in sessions covering scientific, technical and economic aspects of surfactants and their industrial and consumer applications.

Surfactants – High Performance Solutions for a Better World

Global warming, Environmental Legislation, COVID-19 the last years have clearly shown that “business as usual” will not be a viable option for the upcoming decades. To an extent global societies will need to adapt to minimise irreversible collateral damage. This will involve governments, regulators, citizens and industry and its supply chains. Some commentators think that new economies with new business models may be required for a better world – a threat to some, an opportunity for others.

With this background, what is the specific mission of surfactants and their producers? Surfactants play a significant role as part of high-performance solutions that are required to meet the global challenges. For example, surfactants add value by helping to keep the components within a complex product formulation compatible. They also provide performance features in their own right (e. g. cleaning, wetting, foaming, emulsification, etc).

Surfactants are thus one crucial component for consumer products that enable them to do what they are supposed to do, e. g. related to cosmetics, household applications, professional and industrial uses (e.g. I&I or agrochemicals and many others. With current trends surfactant producers are required to reduce the global warming potential of chemical processes and products. As a result, research and investment in several fields will be required for surfactants, including lower CO2, bio-based raw materials and circularity. The challenge will be to identify surfactants with lower Life Cycle Assessments (LCA) that can be produced at current mainstream surfactant scales, perform well (e. g. clean as well as current products), and be cost effective. On the retail consumer side, digitalization should help to speed up activities as in the COVID-19 era consumers bu more and more products online than off-the-shelf. COVID-19 has also resulted in a huge demand for products and surfactants for hygiene and sanitation.

An important role of our industry organization, CESIO, is to provide surfactant related knowledge and ensure that any industry regulations developed are based on facts, good science and recognised testing protocols. As regulations and raw material chains develop, there will be both restrictions and new opportunities opening up. This will affect surfactants as well as biocides, solvents, polymers and other ingredients. The surfactants

industry has a tremendous breadth and depth of expertise in bringing ingredients together in advanced formulations and is therefore well positioned to help downstream adapt to supplier changes and identify new business opportunities.
The conference has three clusters with the following topics:

Technical & Applications
- Environmental sustainability and circularity in surfactants
- Detergency, cleaning and sanitation
- Innovation in Household, Personal Care and I&I
- From structure to properties to applications
- Impact of digital, regulation and consumer behaviour
- Selected applications: household, I&I, personal care, agrochemicals, industrial use of surfactants including oil & gas, plastic additives, emulsion polymerization, etc.

Safety & Regulatory Affairs
- Sustainability Policies in USA, Europe and Japan
- REACH and CLP under the EU Chemicals Strategy
- How can the efficiency of sustainability be assessed?
- Relationship between biocides and surfactants

Business & Market Trends
- Legal and technical input into business models for sustainable products
- Global trends: Hygiene & sanitation beyond COVID-19
- Digitalisation and consumer behaviour
- Dinosaur meets Unicorn – what long-established companies can learn from start-ups

Learn more:
https://cesio-congress.eu/

European Fat Processors and Renderers Association (EFPRA) Congress 2023

7-10 June 2023 | Naples, Italy

We are pleased to invite you to the 21st Congress of EFPRA, the European Fat Processors and Renderers Association.
We look forward to welcoming you in Naples in June 2023.
Registration are now open!
https://efpra2023naples.eu/

International Conference on Algal Biomass, Biofuels and Bioproducts (AlgalBBB 2023)

11-14 June 2023 | Waikoloa Beach, Hawaii, USA

The International Conference on Algal Biomass, Biofuels and Bioproducts (AlgalBBB) will take place in-person, 11-14 June 2023, at the Waikoloa Beach Marriott Resort, Waikoloa Beach, Hawaii, USA. We are excited to be able to provide this much-awaited opportunity for the algae research community to meet in-person and discuss the very latest research and technologies and to interact with leaders in the field.

AlgalBBB places a major emphasis on the latest unpublished technical and scientific results, along with discussion and direct interactions with broad spectrum scientists and engineers, funding sponsors, and leaders in the field from all over the world.

The conference presents the work of the algae research community through a balanced set of oral presentations and posters selected from the best submissions to the conference. Our list of keynote and invited speakers includes funding agency sponsors, key industry players, and top scientists and engineers from the international community.

The conference will cover all areas of emerging technologies in all areas of algal research, including microalgae, macroalgae, and cyanobacteria: biology, biotechnology, biomass production, cultivation, harvesting, extraction, biorefinery, feedstock conversion into fuels, high value products, econometrics, and sustainability analyses.

In 2023, we look forward to hearing about the new emphasis on seaweed-based systems, engineering advances, molecular characterization technologies (e.g., genomics, proteomics, metabolomics), strain engineering technologies for biofuels and high value products and pharmaceuticals, biomaterials, photobioreactor design and control systems, and new technologies in characterization and analysis, among others.

Themes in algae research including microalgae, macroalgae and cyanobacteria:
- Algal Biotechnology - Molecular Engineering
- Algal Biology - Biodiversity and Bioprospecting of Algae for Biofuels and Bioproducts
- Algal Biotechnology - Metabolic Regulation of Algae for Biofuels and Bioproducts
- Algal Cultivation - Phototrophic Systems in Open Ponds
- Algal Cultivation - Phototrophic Systems in Photobioreactors
- Algal Cultivation - Heterotrophic Systems, including utilization of waste waters for algal production
- Bioreactor Design, Engineering and Control
- Algal Harvesting and Extraction Systems
- Engineering of Biorefinery Systems, Technologies, and End-to-end Integration
- New Technologies in Support of Algal Research - Areas of Separation, Refining, Detection, Characterization and Analysis
- Engineering Technologies for Algal Biofuels -

Thermal Catalytic and Non-Catalytic, and Enzymatic systems
- Bioproducts from Algae Including High-Value Products and Co-products
- Life Cycle, Technoeconomic, and Sustainability Modeling and Analysis of Algal Production and Fuel Cycle Systems
- Nutrient Recycling and Management
- Algal Biology – Improving photosynthetic growth and biomass productivity

To AlgalBBB 2023 we will provide a high-quality, peer-reviewed programme in which to showcase your research.

For more details please visit:
https://www.elsevier.com/events/conferences/international-conference-on-algal-biomass-biofuels-and-bioproducts

IGC Grains Conference 2023

12-13 June | London UK

Established in 1949, the International Grains Council (IGC) is an intergovernmental organisation whose objectives are to further international cooperation in trade, promote expansion, openness and fairness in the sector and to contribute to market stability and enhance world food security. Currently, IGC membership comprises 57 countries. Such objectives are sought by improving transparency through information-sharing, analysis and consultation on market and policy developments. Grains, rice, oilseeds and pulses market conditions are monitored on a daily basis, through the circulation of a daily report and the provision of web-based information services. Among other key services, weekly and monthly reports are provided. The flagship Grain Market Report, released monthly, provides comprehensive analysis and forecasts for global supply and demand across a range of commodities, spanning grains, rice, oilseeds, pulses and related agricultural products.
The IGC also actively collaborates with several international organisations, such as the International Sugar Organization (ISO), the United Nation's Food and Agricultural Organization (FAO), as part of the AMIS-G20 secretariat, the World Trade Organization (WTO) and the Organisation for Economic Cooperation and Development (OECD). Furthermore, since 2018, the IGC has developed collaborative relationships with regional organisations, such as Med-Amin, the Eurasian Economic Commission, AfricaRice and the Institute for the Development of Agricultural Cooperation in Asia (IDACA).
Being part of a series of related industry events under the banner "London Grains Week", the International Grains Conference is a truly global platform for dialogue between the policy makers and operators across the entire value chain. The event is held over two full days, the first of which is typically devoted to discussions surrounding the challenges, risks and opportunities in global trade. Day two of the event comprises a number of commodity-specific workshops, covering topical issues affecting markets for grains, rice, oilseeds, pulses and related sectors.

For more details visit
http://www.igc.int/en/conference/confhome.aspx

Oleofuels 2023

14-15 June | Seville, Spain

The European HVO market has seen substantial growth over the last 5 years, with more and more oil majors, such as Neste, Total, ENI and dedicated fuels producers alike continuing to invest heavily in HVO production units. With the continued focus on reducing GHG and CO2 emissions both in Europe, and across the globe, the demand for more sustainable fuels has never been higher. However with increasing competition from Asia and the US, the need for sustainable feedstocks, increasing regulatory pressures, price fluctuation, the need for more investment in R&D and adaptation to new technologies shows that the industry is still facing some significant challenges.
Already on its 14th edition of a very successful series, Oleofuels 2023 will bring together senior representatives from the biodiesel, renewable diesel and HVO industries to discuss the latest market advancements, developments & business opportunities.

Stay tuned for more information soon!
https://www.wplgroup.com/aci/event/oleofuels/

PALMEX Thailand 2023 Exhibition

17-18 August 2023 | Surratthani, Thailand

PALMEX Thailand 2023 is the only specialized Palm Oil event in Thailand that brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries gathered in South of Thailand, to showcase the latest developments in the palm oil industry.
Thailand, currently ranked #3 in the world for CPO is a potential and viable market for palm oil technology companies as the industry is currently honing new palm oil technologies and equipment to help spur its production further!
Fireworks Trade Media Group which have organized successful palm oil events such as PALMEX Indonesia and PALMEX Malaysia is the organizer of this event supported also by the Thai Oil Palm & Palm Oil Associations.

Highlights: Palm Oil Technology Seminars / Asia Palm Oil Conference (APOC) / Palm Oil Mill Visit / Business Matching.
For more detail visit: https://www.thaipalmoil.com/

Argus North American Biofuels, LCFS & Carbon Markets Summit

11 September - 13 September 2023 | Monterey, California, US & Online Access

Argus North American Biofuels, LCFS & Carbon Markets Summit returns to Monterey, California, September 11-13, 2023 to bring together 400+ regulators, key government, and industry participants across the entire biofuels supply chain for 3 days of networking and knowledge exchange.
Back in-person and with online access, do not miss this opportunity to reconnect with industry peers. Complete our form to register your interest in speaking, sponsoring or attending.
https://www.argusmedia.com/en/conferences-events-listing/biofuels-and-lcfs-markets

19th Euro Fed Lipid Congress and Expo: Fats, Oils and Lipids: from Raw Materials to Consumer Expectations

17-20 September 2023 | Poznań, Poland

On behalf of the Euro Fed Lipid, we cordially invite you to be a part of the 19th Euro Fed Lipid, which is scheduled to be held from the 17th to the 20th of September, 2023 in Poznań, Poland.
Euro Fed Lipid Congresses have been organized for more than 20 years in different locations all over Europe. After 11 years we have been trusted again and the 19th edition of the Congress will be held in Poland.
It will be an exciting time for all scientists and industry representatives, who work on fats and oils. We will start with advances in genetics and breeding of fats and oils sources, discuss the newest technologies and achievements, advances in analytical methods and usage of fats and oils by human. Special attention will be paid to consumer preferences and sensory quality.
I hope that we will be able to meet in person and together solve the emerging scientific problems.
The congress center is located on the grounds of the Poznań International Fair, which has been operating in the city since the 1920s.
The mission of this congress is to bring together world renowned experts with whom we will share experiences and increase the knowledge about fats, oils and lipids.
Congress Chairs:
Magdalena Rudzińska (Poznań University of Life Sciences, Poznan, Poland)
Dominik Kmiecik (Poznan University of Life Sciences, Poznan, Poland)
Dorota Klensporf-Pawlik (Poznan University of Economy, Poznan, Poland)
For updates and more details please visit:
https://veranstaltungen.gdch.de/tms/frontend/index.cfm?l=11215&sp_id=2

16th International Rapeseed Congress

24-27 September 2023 | Sydney, Australia

The 16th International Rapeseed Congress (IRC) to be held in Sydney in 2023 is organised jointly by GCIRC (Global Council for Innovation in Rapeseed and Canola) and AOF (Australian Oilseeds Federation).
IRC is held every four years and is the peak international conference for rapeseed R&D focused on advancement of global rapeseed production and utilisation. Since the 1960s the IRC has been helping rapeseed and canola professionals reach new markets and create enduring relationships in the extensive worldwide network of rapeseed experts.
It is a forum for ideas, innovation and networking and is highly respected among participants from industry, academia, and government, as well as sponsors and exhibitors.
IRC-2023 will bring together scientists and representatives from the global agricultural sector to showcase new developments in genetics, breeding, cultivation, plant protection, oil and meal product quality, compositional analysis, and utilisation of end products in the food, feed and energy sectors.
There will be a combination of field trips, presentations and discussions in plenary lectures, thematic sessions and working groups running over six days from 22 to 27 September 2023.
The Congress provides unique access to the world's largest gathering of rapeseed scientists, researchers and industry experts, creating outstanding business engagement opportunities for Sponsors and Exhibitors.
The program for IRC-2023 will focus around the theme Global Crop, Golden Opportunities.
Global crop
Rapeseed/canola is now well established as a major Global Crop with production spread across several geographical regions and products supplying important global markets for high quality oil and protein. Once known as the 'Cinderella crop' in the 1960's and 1970's it has expanded to become the second largest oilseed crop, now nudging 70 million tonnes of grain per year, and becoming the third largest source of food oils.
This spectacular rise has been driven by breakthrough R&D and continuous innovation from an expanding global network of crop scientists, agriculturalists, and processing sector experts. Rapeseed production continues to see growth in both area and productivity, and nowhere is this more

evident than in Australia. Australian canola production reached a record high of over 6 million tonnes in 2021/22, with the majority being exported, firmly establishing Australia as a major supplier for the rapidly expanding global market.
During this period, value adding of canola in Australia has also grown with substantial investment in expanding capacity of existing processing plants and constructing new facilities. IRC-2023 is a great opportunity to witness this expanding Australian industry and for Sponsors to showcase their contributions to the global rapeseed industry.

Golden opportunities
Off a strong global base, rapeseed is now poised to branch out into a new era of crop and product diversification, providing 'Golden Opportunities' to further expand production, improve profitability, and extend reach into new high-value and high-volume end use markets.
Breeders and agronomists are using the latest developments in biotechnology to create high-yielding and disease resistant varieties
Innovative rapeseed cropping systems are being developed, such as the dual-purpose grazing and grain systems being pioneered in Australia
Short-season Brassica crops (Carinata, Camelina, Cress) are being deployed as cover crops in the US to produce low-carbon intensity renewable diesel feedstocks
High-oleic rapeseed has become a preferred source of highly stable and nutritious oil in the food service sector.

Unique EPA/DHA canola oils have recently entered production and are finding applications in nutritional supplements and aquaculture feeds
Rapeseed protein is being developed as an additional high-quality protein source to supply the emerging plant-based meat replacement markets

Key themes of the event:
- Genetics, Genomics and Breeding
- Agronomy, Physiology and Management
- Diseases and Pests
- Quality and Products
- End Uses
- Economy and Markets

All information to the page:
https://www.irc2023sydney.com/

7th International conference on microbial diversity “Agrifood microbiota as a tool for a sustainable future”

26-29 September 2023 | Parma – Italy

The Conference is organized by University of Parma and the Italian Society of Food, Agricultural and Environmental Microbiology (SIMTREA).The MD is a prestigious international congress that has seen the number of participants grow over the years and the previous MD editions saw a multidisciplinary audience and included microbiologist working in the area of agriculture, food, environment, health industry members, researchers and basic scientists who came together from 36 countries to make for an exciting forum.
The theme of 7th Edition of MD is “Agrifood microbiota as a tool for a sustainable future” and includes the four sessions: FOOD microbiota as a tool for a sustainable future; HUMAN microbiota as a tool for a sustainable future; ENVIROMENT microbiota as a tool for a sustainable future; Sustainable future has come.
Sessions
- FOOD microbiota as a tool for a sustainable future
- HUMAN microbiota as a tool for a sustainable future
- ENVIRONMENT microbiota as a tool for a sustainable future
- Exploiting microbiomes for a sustainable future
- Sustainable future has come

All Abstracts accepted for oral or poster presentation will be eligible for the MD23 Awards Competition.
Abstract submission: March 20th - May 15th, 2023
For further information: www.md23.simtrea.org / secretariatMD23@simtrea.org

Globoil India

28-30 September 2023 | Mumbai

Increase your margins by connecting with competitive suppliers of cutting edge product innovations, as well as interacting the most popular international brands. Globoil India welcomes over 1,500 attendees from 50 countries.
As the global edible oil & agri trade community gears up for this principal opportunity to meet the key decision-makers of the industry, Globoil India is promising a wave of new services and innovations to reflect changes in consumer demand. The fully booked-out event will once again welcome more than 100 exhibitors showcasing products across various market sectors.
Join the 26th edition of Globoil, the world's leading edible oil & agri trade conference & exhibition, at the The Westin Mumbai Powai Lake, Mumbai.
See more information:
https://globoilindia.com/index.html

Palmex Indonesia 2023

4-6 October | Medan, Indonesia

The 13th PALMEX Indonesia 2023 brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries gathered in the capital city of

North Sumatera, Medan to showcase the latest developments in the palm oil industry.

North Sumatera, home to one of Indonesia's largest concentration of oil palm plantations and also the presence of many supporting facilities such as palm oil processing plants making its capital Medan the perfect venue for the show. This unique event seeks to educate the public on the importance of the palm oil industry in Indonesia and the future trends of palm oil in the region. More than 7,000 industry professionals from more than 10 countries would be expected to turn up at this event. The international character and regional audience of PALMEX Indonesia provides unparalleled marketing, education and networking opportunities.

For more info visit: https://palmoilexpo.com/

Argus Biofuels Europe Conference

11 - 13 October 2023 | London, UK & Online Access

The Argus Biofuels Europe Conference returns to London in-person and via online access, 11-13 October 2023. The event will bring together the biofuels industry for the industry's premier thought-leadership and networking event.

Do not miss your chance to join over 400 attendees at this flagship event! Complete our form to register your interest in attending, speaking at or sponsoring the event.

Feedstock focus. Gain insight on outlooks for 1st and 2nd generation feedstocks, what new technologies are emerging and how quickly can they be scaled up?

Regulatory updates. Hear from key regulators and industry stakeholders on their views on latest European Commission policy updates including Fit for 55 and RED III.

SAF Focus Day. Sign up to this pre-conference day for the latest insights on Argus' view for pricing mechanisms, key regulatory developments as well as key projects upcoming looking to unlock SAF production.

Argus expertise. Hear from Argus experts across sectors and geographies including agriculture, biofuels and Net Zero future fuels.

See more on:
https://www.argusmedia.com/en/conferences-events-listing/biofuels

Micronutrient Forum – 6th Global Conference

16-20 October | the Hague, the Netherlands

The Micronutrient Forum will be a hybrid event with a robust virtual program and in-person component at the World Forum in The Hague, Netherlands – a locus of international law and justice and well suited for a gathering centered on the human right to good nutrition.

By bringing together diverse stakeholders across sectors and disciplines, the Conference will help shape and establish a compelling and evidence-based agenda on the interdependence of nutrition and resilience – offering opportunities to advance integrated research, new policy priorities and investments for micronutrient interventions, and to accelerate progress towards global nutrition and development goals.

The conference will also embrace our traditional four tracks, exploring the latest science across the micronutrient program lifecycle from biology through effectiveness and implementation to the enabling environment.

The Scientific Program will include a mix of live, and on-demand sessions to allow both in-person and virtual delegates a chance to convene and engage directly with speakers and other attendees during the event, as well as having access to the recorded sessions after the in-person conference ends.

More information: https://mnforum2023.org/

Future of Biofuels 2023 – 5th European Conference

24-25 October | Copenhagen, Denmark

Along with the European Commission's REPowerEU plan, released in May 2022 in response to energy market disruptions from Russia's invasion on Ukraine, EU aims to rapidly reduce dependence on Russian fossil fuels by 2027. Also with EU's maritime fuel law to curtail shipping emissions and its sister regulation in the aviation sector, the EU sets the level of acceptable emissions and curtails them over time. Aiming to be serious drivers for biofuel market development.

This year we are focusing on production and implementation of biofuels and future fuels in maritime and aviation sectors to speed up their decarbonization.

Other points of focus are: development of new supply chains, latest trends and perspectives for low carbon fuels in fuels mix but also new production technologies, refineries case studies and more.

The event is set to bring industry stakeholders, unique content, workshop style discussions and networking. Gives an opportunity to showcase your products and services in the networking area and hold meetings with leaders from the industry.

For information: https://fortesmedia.com/future-of-biofuels-2023,4,en,2,1,27.html

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Editorial office:
Chiara Zigliani
risg@mi.camcom.it

Author instructions

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Via Giuseppe Colombo 79 - 21033 MILANO
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