RISG 2023

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

ISSN 2611-9013 RISG on-line 100(2) 79-144 (2023)
Finito di stampare nel mese di luglio 2023

LA RIVISTA ITALIANA DELLE SOSTANZE GRASSE

anniversario

100

N. 2 APRILE-GIUGNO 2023

La RIVISTA ITALIANA delle SOSTANZE GRASSE

La rivista edita da Innovhub Stazioni Sperimentali per l'Industria S.r.l. ha periodicità trimestrale e la scientificità dei contenuti è garantita da un Comitato Internazionale di Referee.
Pubblica lavori originali e sperimentali di autori italiani ed esteri riguardanti la chimica, la biochimica, l'analisi e la tecnologia nei settori: sostanze grasse e loro derivati, tensioattivi, detersivi, cosmetici, oli minerali. Pubblica inoltre un Notiziario con informazioni su congressi, notizie in breve e libri.
La Rivista viene consultata in Italia dalle industrie produttrici ed esportatrici di oli e grassi alimentari ed industriali, dalle industrie chimiche, da laboratori di enti statali, da istituti di ricerca e facoltà universitarie, da dove provengono diversi lavori scientifici. È inoltre consultata all'estero in vari Paesi come Spagna, Principato di Monaco, Canada, Paesi Bassi, Svizzera, Slovenia, Regno Unito, Turchia, Lussemburgo, Malaysia, Grecia, Francia, Germania, Tunisia, Nigeria, Congo, Polonia, Romania, Bulgaria, Russia, Stati Uniti, Brasile, Cina, Giappone.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

Via Giuseppe Colombo,79
20133 Milano

Biblioteca

La storica Biblioteca *Oli e Grassi*, attiva dai primi anni del '900, raccoglie circa 400 volumi e più di 100 titoli di riviste a partire dal 1849.
È specializzata in:

chimica generale
chimica organica
chimica analitica
scienze alimentari
sostanze grasse
aromi
prodotti petroliferi
oli minerali
lubrificanti
cosmetici e tensioattivi
vernici

Servizi Offerti

Ricerche bibliografiche
La Biblioteca offre la possibilità di commissionare ricerche bibliografiche che vengono prese in carico da esperti di settore

Fornitura di articoli
La Biblioteca offre il servizio di fornitura di materiale da riviste nazionali ed internazionali sia dalla propria dotazione che attraverso i circuiti nazionali interbibliotecari (a titolo gratuito) in osservanza della vigente normativa sul Diritto d'Autore
(Legge n. 633 del 22/4/1941 e successive modifiche e integrazioni)

2duemila**ventitre**
APRILE/GIUGNO 2023 - ANNO C

RISG
LA RIVISTA ITALIANA DELLE
SOSTANZE GRASSE
DI INNOVHUB
STAZIONI SPERIMENTALI PER L'INDUSTRIA S.r.l.

Indice

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

Autorizzazione del Tribunale di Milano 28.9.1948, N. 591

DIREZIONE E REDAZIONE
Via Giuseppe Colombo 79 – 20133 Milano

Sito web: www.innovhub-ssi.it

La **RIVISTA ITALIANA** delle **SOSTANZE GRASSE**

Impact Factor: 0.580
Subject Area: Organic Chemistry (Q4)

Indexed and Abstracted in:
- **Clarivate Analytics Company** (Philadelphia, USA)
- **Chemical Abstracts**
- **Elsevier Bibliographic Databases:** SCOPUS
- **FSTA – Food Science and Technology Abstract** (IFIS Publishing – UK)

A study of the fat component's quality and quantity and their effect on the oxidative stability of beef and chicken meat burgers and shawarma in Amman area

Sarra Ould Hocine
Basem Al-Abdullah Al-Sawalha✉
Khalid Al-Ismail

Department of Nutrition and Food Technology
Faculty of Agriculture
University of Jordan
Amman, Jordan

This study attempted to track the lipid oxidation in meat, on one hand, by giving a considerable attention to fatty acids (FAs) composition, cholesterol oxidation product (COPs) levels as well as fat and cholesterol content, and on the other hand, by measuring the peroxide value (PV), p-anisidine (PA), and TOTOX values. The investigation was carried out on one kilogram of meat from each of the products (beef burger (BB), chicken burger (CB), beef shawarma (BS) and chicken shawarma (CS). Cholesterol, COPs, and FAs composition were analysed by gas chromatography. Results showed that all of CB, CS, BB and BS were in an oxidised status and presented toxicologically significant levels of COPs.

Keywords: Burger, Shawarma, Beef, Chicken, Lipid oxidation, Fatty acids, Cholesterol oxidation products

1. INTRODUCTION

The rich nutritional composition of meat makes it very susceptible to chemical and bacterial deterioration. Lipid oxidation is the major cause of chemical deterioration in meat [1]. It is an inevitable and spontaneous process which depends mainly on the degree of unsaturation of fatty acids, level of oxygen, metals and on other factors such as storage conditions, processing methods, types of ingredients, as well as the presence and the concentrations of pro or antioxidants. The three major substrates for lipid oxidation in meat are triacylglycerols, phospholipids and cholesterol. The free radicals and cholesterol oxidation products (COPs) produced by lipid oxidation contribute to cell cytotoxicity, leading to several human pathologies, such as cancer [1].
Several scientific studies have demonstrated that meat-based fast foods, such as burgers and shawarma, are susceptible to lipid oxidation [1, 2, 3, 4, 5]. Therefore, this study aims at understanding what makes the meat in burgers and shawarmas susceptible to lipid oxidation, on one hand, by giving a considerable attention to fatty acid composition, COPs levels as well as fat and cholesterol content of the meats, and on the other hand, by tracking the degradation using peroxide value (PV), p-anisidine (PA) and TOTOX values.

2. MATERIALS AND METHODS

2.1. FOOD SAMPLE COLLECTION AND PREPARATION

One kilogram of meat from each of the products (beef burger, chicken burger, beef shawarma and chicken shawarma) was collected randomly from different restaurants in Amman. The collected samples were kept in an insulated ice box and were transported to the department of Nutrition and Food Technology laboratory to be analysed. Sampling was performed weekly over a period of 3 months from December to February 2021. The meat samples

✉ CORRESPONDING AUTHOR:
Tel. +96265355000
Fax +96265355577
e-mail: basem@ju.edu.jo

Received: April 5, 2022
Accepted: June 7, 2022

of both products were collected from the restaurants and immediately transferred to the laboratory of the department, prepared, and the fat was extracted and kept in glass vials and then the various analysis of fat were carried out immediately on the same or next day.

2.2. DETERMINATION OF FAT AND MOISTURE CONTENTS

Cold extraction of fat was carried out according to [6]. Briefly, 10 grams of meat sample were mixed with 150 ml of chloroform and 150 ml of methanol in a Mixer (Scovil, Hamilton Beach, Model NO. 936-1S, USA) and another 150 ml of chloroform was added during grinding after which the mixture was filtered using Whatman NO. 1 filter paper and transferred into a separatory funnel. Thereafter, potassium chloride (47 g in 500 ml water) was added to the mixture to facilitate phase separation. The lower layer (containing chloroform and fat) was withdrawn and sodium sulphate anhydrous was added to remove traces of water and then stored in closed vials at -18°C until further analysis. Moisture was determined following the method of [7]. Briefly, an aluminium empty plate was weighted (W1). Then, 10 grams of the meat samples were placed in the aluminium plate, weighted, and reported as (W2). The samples were then placed in an air-oven (Mermert, 854, West Germany), and dried at 105°C until a constant weight was obtained. Then, the samples were removed from the oven, cooled in a desiccator at room temperature and weighted (W3), and finally, the moisture content of the samples were calculated as follows:

Moisture content % = [Loss in moisture weight (W2 – W3) / weight of sample (W2 – W1)] × 100

2.3. FATTY ACID METHYL ESTER PREPARATION

Analysis of fatty acids involved 3 steps: extraction of lipids (as explained in the previous section); then the conversion of the extracted lipids to fatty acid methyl esters (FAMEs); which was carried out according to the procedure reported by [8]. In summary, 2 ml of hexane was added to 5 drops of the extracted fat (5 drops = approximately 100 mg of the sample), followed by the addition of 2 µl from KOH in methanol (2 N) with shaking for 1 minute. After that, 2 µl of acetic acid was added followed by shaking for 1 minute. One microliter (µl) was taken from the upper layer by a micro syringe and injected into the injection port of the gas chromatography (GC) (Shimadzu Corporation, Japan), at which fatty acids composition was analysed using a flame ionisation detector. Helium was used as a carrier gas. The fatty acids peaks were identified by comparing with the retention time of the reference standards. The quantification of the methyl ester fatty acids was then done by calculating: Area of the fatty acid% / total area of fatty acids.

2.4. DETERMINATION OF PEROXIDE VALUE

Peroxide value was determined according to the AOAC [9] method, where 5 g of the extracted fat was dissolved in 50 ml of chloroform-acetic acid solution (2:3) and agitated until the fat was dissolved after which 0.5 ml of freshly prepared and saturated potassium iodide (KI) was added. The mixture was agitated for 1 minute followed by addition of 50 ml of distilled water and few drops of starch indicator (1%). Afterwards, 0.01 N sodium thiosulfate was used to titrate the mixture with vigorous agitation until the blue colour vanished. The peroxide value was calculated using the following equation:

Peroxide value= V× N × 1000/ sample weight (g)

Where:
V = volume of the titrant,
N = normality of sodium thiosulfate.
Unit of peroxide value: milliequivalents (meq) of active oxygen per kilogram of oil.

2.5. DETERMINATION OF p-ANISIDINE VALUE

The determination of p-anisidine value was carried out according to [10]. Briefly, 0.5 g of extracted fat was dissolved in isooctane in a volumetric flask (25 ml capacity) and diluted up to the mark (solution 1). The optical density of solution I was measured at 350 nm using a spectrophotometer (Spectro, Model NO. 2000RS, USA) and isooctane as a blank (Ea). 5 ml of solution 1 was pipetted and transferred into a 10 ml test tube. Then 1 ml of p-anisidine was added, vigorously shaken, and allowed to stand for 10 minutes (solution2). The optical density of solution 2 was read at 350 mm using the same spectrophotometer and isooctane as a blank (Eb). P-anisidine index was calculated according to the following equation:

p-anisidine index = 25 (1.3 Eb-Ea)/ m

Where:
Ea= the optical density of solution 1,
Eb= optical density of solution 2,
M = weight of the sample in grams.

2.6. DETERMINATION OF TOTOX INDEX

TOTOX index was calculated using the following equation [42]:
TOTOX = 2 Peroxide Value + p-anisidine

2.7. DETERMINATION OF CHOLESTEROL OXIDATION PRODUCTS

2.7.1. Saponification of fat

Saponification of fat was conducted according to [11]. Briefly, 0.5 g of fat sample was mixed with 20 µl of α cholestane standard and 10 ml of KOH in ethanol (95% w/v), and then the mixture was stored in the dark for 24 hours after which it was placed in a water bath (Thermo Fischer Scientific, Model NO. H950D,

Massachusetts, USA) at 40°C for 1 hour to dissolve traces of unsaponifiable material. Afterwards, 5 ml of water was added to the mixture to dissolve the soap formed followed by addition of 10 ml of hexane to dissolve unsaponifiable materials. The mixture was shaken for 30 seconds, and the upper phase was separated followed by addition of sodium sulphate anhydrous to remove traces of water. Ten millilitres of hexane was added to the upper phase and the steps were repeated. Hexane was evaporated using a rotary evaporator (Heidolph, Heizbad WB, Model NO. 517-01000-00-0, Schwabach, Germany) and the dried sample was transferred into a test tube, flushed with nitrogen, and stored at -18°C.

2.7.2. Derivatisation of cholesterol and cholesterol oxides

Derivatization was carried out according to method described by [12]. In brief, the dried sample (non-saponified) was mixed with 0.5 g of trimethylsilyl derivatives (TMS) solution, prepared by mixing one volume of Trimethylchlorosilane, two volumes of hexamethyldisilazane and five volumes of pyridine, for 1 minute with shaking and then the mixture was placed in a water bath (Thermo Fischer Scientific, Model NO. H950D, Massachusetts, USA) at 40°C for 20 minutes followed by evaporating the TMS solution using nitrogen gas. Then, 100 µl of hexane was added to the dried sample followed by another 100 µl, mixed for 30 seconds and centrifuged (Hermle, Model NO. Z 326 K, Germany) for 5 minutes. After centrifugation, 10 µl was withdrawn from the supernatant using a micro syringe and injected into the injection port of the GC (Shimadzu Corporation, Japan). Cholesterol and COPs peaks were identified by comparing with the retention time of the reference standards. The quantification of COPs was done by using an internal standard: 5-alpha-Cholestane.

2.8. STATISTICAL ANALYSIS

Statistical analysis was carried out using statistical analysis system package (SAS Inc, 2000). Analysis of variance (ANOVA) was used following a Complete Randomised Design. Least Significant difference (LSD) test was used to test differences between the means. Data was presented as the mean ±standard deviation (±SD), and differences were considered significant at $P \leq 0.05$. All samples were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1. FAT AND MOISTURE CONTENT OF MEAT SAMPLES

The fat and moisture percentages of all chicken shawarma (CS), chicken burger (CB), beef shawarma (BS), and beef burger (BB) samples are presented in Table I. The chemical analysis of fat showed that the main significant difference was between the type of meat (chicken and beef) rather than the type of food (shawarma and burger). Both BB and BS contained the highest fat levels (41.34±7.03% and 36.39±11.73, respectively) compared to CB and CS (32.03±15.23% and 26.63±2.98%, respectively). This is probably due to the generally lower fat composition in chicken carcasses when compared to beef. Moreover, fat levels of chicken and beef shawarma were lower than that of chicken and beef burgers, this could be due to the higher fat loss, since shawarma grilling may last for as long as a day [13]. Moisture content of CB (61.12±3.53) was significantly higher than all of CS, BB, and BS. A similar moisture range of chicken burger was reported by [14]. Moreover, CB exhibited greater moisture and lower fat contents than BB. A similar trend was observed by [15], who reported that moisture content of meat is inversely related to its fat content. Data collected on fat content in this study agreed with [16] who reported an average fat content of beef sausages of 37.75%. However, fat contents were higher than those observed by [2,17,18,19, 20, 21]. These differences are expected and could be attributed to the effect of dietary factors, age, sex, and source of animals, as well as the proportion of water added mainly in beef and chicken burger, in addition to the animal skins used in preparation of different types of shawarma and burger. Moreover, cooking losses of meat products, which are mainly affected by the ability to retain fat and moisture throughout thermal treatment, vary according to the cooking procedure implemented and its characteristics such as heating rate [22].

3.2. FATTY ACID COMPOSITION OF MEAT SAMPLES

Twelve fatty acids were identified and quantified in CS, BS, CB, and BB samples. Individual percentages of each fatty acid are presented in Table II. The investigated shawarma and burgers were mainly rich in monounsaturated fatty acids (MUFAs) as well as saturated fatty acids (SFAs). This agrees with [15] who

Table I - Measured fat and moisture content* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples

Parameter	CS	BS	CB	BB
Fat (%)	26.63^{c} ±2.98	36.39^{ab} ±11.73	32.03^{bc} ±15.23	41.34^{a} ±7.03
Moisture (%)	49.30^{b} ±4.75	49.01^{b} ±7.20	61.12^{a} ±3.53	49.82^{b} ±6.29

*Values are means of triplicate determinations ±SD.
^{a,b,c} Superscripts within the same row indicate significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

Table II - Fatty acid composition* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

Fatty acid (g/100g total FA)	CS	BS	CB	BB
Myristic acid (C14:0)	0.53^{b} ±0.11	3.50^{a} ±2.27	1.58^{b} ±1.19	3.31±0.14^{a}
Palmitic acid (C16:0)	20.68^{b} ±0.93	27.27^{a} ±6.17	20.78^{b} ±4.35	24.79±1.68^{a}
Margaric acid (C17:0)	0.09^{c} ±0.10	2.50^{a} ±1.17	1.49^{b} ±1.51	1.69±0.69^{ab}
Stearic acid (C18:0)	6.90^{b} ±0.39	12.70^{a} ±4.68	9.40^{b} ±3.17	15.38^{a} ±4.15
Arachidic acid (C20:0)	2.69^{a} ±0.61	0.47^{b} ±0.28	2.06^{a} ±1.60	0.55^{b} ±0.12
Behenic acid (C22:0)	1.13^{ab} ±0.15	0.36^{bc} ±0.19	1.66^{a} ±1.80	0.25^{c} ±0.12
Palmitoleic acid (C16:1)	3.58^{a} ±0.50	3.14^{ab} ±1.33	2.21^{b} ±1.23	4.01^{a} ±0.86
10-cis-heptadecenoic acid (C17:1)	0.09^{c} ±0.10	1.57^{a} ±0.85	0.74^{b} ±0.73	1.34^{ab} ±0.77
Oleic acid (C18:1)	36.03^{bc} ±2.56	39.37^{ab} ±4.49	34.82^{c} ±6.76	41.53^{a} ±3.64
Linoleic acid (C18:2)	29.81^{a} ±2.72	6.62^{b} ±2.75	24.71^{a} ±16.44	4.59^{b} ±2.45
Linolenic acid (C18:3)	0.00^{d} ±0.00	0.39^{b} ±0.21	0.20^{c} ±0.25	0.55^{a} ±0.11
C18:1TRNS	0.05^{c} ±0.13	3.08^{a} ±1.00	1.08^{b} ±1.58	2.81^{a} ±0.84
Total saturated fatty acids (SFA)	32.04^{b} ±0.94	46.81^{a} ±6.94	37.00^{b} ±8.32	45.84^{a} ±5.15
Monounsaturated fatty acids (MUFA)	39.70^{bc} ±2.99	44.07^{ab} ±4.44	37.77^{c} ±8.36	46.89^{a} ±4.43
Polyunsaturated fatty acids (PUFA)	29.81^{a} ±2.72	7.00^{b} ±2.86	24.91^{a} ±16.29	5.14^{b} ±2.42
Trans fatty acids (TFA)	0.05^{b} ±0.12	3.08^{a} ±1.58	1.09^{b} ±0.98	2.80^{a} ±0.84

*Data are expressed as means of triplicate determinations ±SD.
^{a,b,c} Superscripts within the same row indicate statistically significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.
SFA is the sum of C14, C16, C17, C18, C20, and C22; MUFA is the sum of C16:1, C17:1 and C18:1; PUFA is the sum of C18:2 and C18:3; TFA is the amount of C18:1 TRNS.

reported that the fatty acid profile mainly demonstrate a dominance of SFAs and MUFAs. The most ubiquitous fatty acid in all of BB, BS, CB, and CS were oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. This was in accordance with previous studies [15,23,24,25]. The polyunsaturated fatty acids (PUFAs) detected represented the essential fatty acids, linoleic acid (C18:2) and linolenic acid (C18:3). Oleic acid (C18:1), was the most prevalent fatty acid in all the examined samples. This finding agrees with [22] and [26]. However, palmitic (C16:0), and stearic (C18:0) acids, were more abundant in BB and BS than in CB and CS. These results are supported by those reported by [27] who found that these fatty acids were the most predominant in the marbling of lean beef. Nevertheless, our results showed lower levels in CB (63.61%) and CS (65%) compared with those of [28] who have demonstrated that 76 to 82% of total fatty acids in hens came from palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). Both CS and CB contained somewhat higher proportion of unsaturated fatty acids (69.51% and 62.68%, respectively), than BS (51.07%) and BB (52.03%). These results were nearly like those previously reported in literature [15,29,30,31]. Saturated fatty acids were found to be greater in BS and BB than in CS and CB. The proportion of MUFAs was higher in BS and BB than in CS and CB. However, when comparing the fatty acid composition of meat origin, a notable amount of PUFAs was found in CS (29.81%) and CB (24.91%) compared to BS (7.00%) and BB (5.14%), with linoleic acid (C18:2) being the predominant PUFA. These results correlated with the findings of [15]. However, BS and BB showed higher levels of trans fatty acids (TFA) of 3.08 and 2.80, respectively, compared to CS and CB (0.05 and 1.09, respectively). Our results were slightly lower than those found by [32]. Nonetheless, both results exceeded the Danish legal limit of trans-fat (i.e., 2g/100 g fat). The differences in the degree of unsaturation could be explained by the differences in the metabolic process taking place in ruminant and non-ruminant animals, since hydrogenation of unsaturated fatty acids occurs extensively in ruminant animals. Hydrogenation occurs by two systems: 18:2 and 18:3 are hydrogenated to 18:1, and 18:1 to 18:0 [33].

3.3. CHOLESTEROL CONTENT AND COPS LEVELS

Table III shows the cholesterol content and cholesterol oxidation products (COPS) levels in CS, BS, CB, and BB samples. Among the tested samples, the highest cholesterol contents were 96.42 and 80.79 mg/100g, and were observed in CS and CB, respectively. The lowest cholesterol contents (74.35 and 65.22 mg/100g) were detected in BS and BB, respectively. However, no significant difference was found except between CS and BB. This could be attributed, on one hand, to the type of meat since the main fat source in poultry meat is the chicken skin, which is known to contain high levels of cholesterol. On the other hand, this difference could be due to the higher use of skin or animal fats during the preparation of shawarma than in burger. Our results agree with de [34], who also found that chicken meat presented higher cholesterol levels than beef. However, the cholesterol content found in our study was

Table III - Cholesterol and cholesterol oxidation products (COPs) content in the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

	CS	BS	CB	BB
Cholesterol (mg/100g food)	96.42^{a} ±28.95	74.35^{ab} ±38.55	80.79^{ab} ±25.37	65.22^{b} ±23.60
COPs (mg/100g food)	1.20^{b} ±0.52	2.48^{ab} ±2.19	4.69^{a} ±4.72	2.29^{ab} ±1.64

*Results are expressed as means of triplicate determinations ±SD.
^{a,b} Superscripts within the same row indicate statistically significant differences ($p<0.05$).
CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.
COPs: cholesterol oxidation products.

slightly higher compared to their results. Such differences could be attributed to variations in the parts of chicken and beef used for cholesterol determination. Nevertheless, our results were in accordance with those reported by [28], who found a cholesterol content of 75-98 mg/100g for broiler meat, as well as those previously reported in the literature which showed that the cholesterol content of meats varies between about 30 and 120 mg/100 g of food, being even higher in offals [23, 24, 25].

Table IV - Cholesterol and cholesterol oxidation products (COPs) content of the collected chicken shawarma, beef shawarma, chicken burger and beef burger samples.

Samples	Cholesterol (mg/ 100g food)	Cholesterol Oxidation Products (COPs) (mg/ 100g food)
CS1	84.48	0.95
CS2	142.02	2.16
CS3	53.25	0.55
CS4	116.51	1.49
CS5	81.59	0.92
CS6	100.71	1.16
MEAN	**96.42**	**1.21**
CB1	68.07	2.00
CB2	83.94	4.85
CB3	70.08	0.98
CB4	86.29	6.83
CB5	110.38	1.95
CB6	34.85	14.92
CB7	111.95	1.34
MEAN	**80.79**	**4.69**
BS1	33.84	0.65
BS2	47.81	2.26
BS3	117.64	1.46
BS4	30.55	1.33
BS5	65.13	7.06
BS6	93.93	0.71
BS7	131.63	3.94
MEAN	**74.36**	**2.48**
BB1	59.42	0.38
BB2	86.58	3.29
BB3	56.49	1.15
BB4	73.85	2.49
BB5	101.26	0.57
BB6	54.28	2.89
BB7	24.69	5.26
MEAN	**65.23**	**2.29**

CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

The results of COPs content in the investigated shawarma and burger showed that the significant difference was between CS and CB. In fact, CB exhibited a COPs level of nearly four times bigger than that of CS. These results lead us to the assumption that the COPs levels were influenced mainly by the type of food (shawarma and burger) rather than the type of meat (chicken and beef). This could be possibly due to the presence of high levels of hydroperoxides derived from the oxidation of unsaturated fatty acid during the preparation of CB which increased the oxidation levels of cholesterol. Since, [35], indicated that cholesterol oxidation may develop both directly, if oxygen is present, and indirectly, if other oxidisers are present in food, such as unsaturated fatty acids in the lipid fraction particularly rich in PUFA. Our results were nearly like those found by [36]. However, [37] found very low levels of cholesterol oxidation compared to our study. COPs in foods are considered potential health risk but there is no regulation limiting their levels in foods [38]. However, according to [15], our results concerning COPs are considered toxicologically significant, since they indicated that an amount of COPs above 0.5 mg represent a risk from the toxicological stand point. Our results showed mean values with a wide scatter, this could be related to the heterogeneity of the analysed samples in terms of composition, formulation, and processing between the different restaurants from where our samples were collected. In addition, and to explain the high values of the standard deviation of the results shown in Table III, all the individual values of the cholesterol and cholesterol oxidation products results were presented in Table IV.

3.4. THE OXIDATION STATUS OF MEAT SAMPLES EVALUATED BY PV, P-ANISIDINE AND TOTOX

Means of peroxide value (PV), para-anisidine value (PA) and total oxidation value (TOTOX), as indicators of lipid oxidation, are demonstrated in Table IV. PV is useful in evaluating the initial step of oxidation of edible fats and oils. The PV of samples obtained ranged from 1.30 to 2.51 mEq/Kg lipid for BB and CB, respectively. Our results were consistent with

[15]. Meanwhile, the level of lipid peroxides found in the present study is considered acceptable according to [39] and [40], if following the standard AOCS methods, a Peroxide value (mEq Active O2 /kg Lipid) <5 is expected for a weight of 5 g lipid sample [41]. However, no significant differences were detected regarding PV between BB, BS, CS and CB. Moreover, PV is notoriously an empirical assay [42], which could cause a difficulty in visually distinguishing the colour change marking the actual end point determination. [43] concluded that, due to the rapid decomposition of hydroperoxides at temperature ≥100°C, the determination of PV is not applicable for all types of cooking methods. Therefore, to get an overall picture of lipid oxidation, [44] required testing of secondary products.

Para-anisidine values, which show the secondary oxidation products, ranged from 13.09 for CS, to 47.01 for CB, whereas the TOTOX values, in like manner, ranged from 14.76 for CS, to 48.45 for CB. Although, CS and BS showed lower PA and TOTOX values compared to chicken burger and beef burger, no significant differences were found except with CB. This could be because shawarma slices could have been cut from a recently renewed surface which was less exposed to heat (short cooking time) or cut from the inner parts of the meat cone where perhaps the oxygen was low or absent. The highest PV, PA and TOTOX values were detected in CB. This may be elucidated by the relatively elevated levels of unsaturated fatty acids and low levels of natural tocopherols in poultry meat [20]. It is generally agreed that lipid oxidation increases significantly with the increase of unsaturated fatty acids [1,45,46]. Therefore, the level of PUFA in meat usually determines the susceptibility to lipid oxidation in different species. [47] found that the susceptibility to oxidation decreases in the order chicken>pork>beef>lamb. Poultry and poultry products are particularly prone to oxidative processes in lipids and proteins, by virtue of the fact that they contain unsaturated lipids. However, this was not the case in CS which showed lower oxidation levels than that in beef shawarma and beef burger. This could be attributed to the antioxidant effect of spices, that is mainly related to the presence of flavonoids and phenolic compounds which actively participate in neutralizing free radicals [1, 48]. The relatively high oxidation levels in BB could be explained by the presence of considerably larger amount of iron and myoglobin in bovine muscle [49,50]. Moreover, it was stated by [20] that fats of animal origin are less stable than equally saturated vegetable fats, because they lack natural antioxidants. The high levels of PA and TOTOX values found in our study showed that all of CB, CS, BB, and BS were in an oxidised status. These results reflected an unacceptable quality of the samples, since [42,51,52] indicated that for a high-lipid food to still be acceptable the TOTOX value should be less than 10. These findings could be attributed to bad storage conditions; since TOTOX value was considered to combine evidence about the history of fat (as reflected in p-anisidine value) with its present state (as evidenced by the pV). In addition to the lipid composition of the meat, many other factors were found to influence lipid oxidation, such as processing methods [53]. In fact, [54] reported that cooked meats are even more susceptible to lipid oxidation than raw meats, because higher temperatures lead to the release of oxygen and heme iron, thereby, inducing the production of free radicals. Furthermore, the mechanical processes employed in meat processing such as chopping, mixing, and grinding, massaging was also found to promote lipid oxidation by increasing surface-to-volume ratio in contact to oxygen [55]. Beside the latter, Sodium chloride which is considered one of the most important additives in meat industry, since it is used for enhancing preservation, flavour and softness [56], was reported as a pro-oxidant by several investigators [53, 57]. Regardless of the different factors influencing our results concerning lipid oxidation, PA value may not have reflected the accurate status of oxidation since, the fat extracted from our samples was observed to be intensely-coloured (orangish colour). This pigment might have contributed to an additional radiation absorbance in the 350nm wavelength range which could have led to an overestimation of the reported data. Furthermore, PA value is neither quantitative nor specific. Thus, all aldehydes react but unsaturated aldehydes have higher colour response than saturated, so results can give only relative, not absolute findings. Additionally, of all these difficulties, meat is a complex matrix that generally causes the appearance of compounds, affecting the obtained results. So, the analyte extraction and isolation process

Table V - Physicochemical properties* of the examined chicken shawarma (CS), beef shawarma (BS), chicken burger (CB) and beef burger (BB) samples.

Physicochemical property	CS	BS	CB	BB
PV	2.01^{a} ±1.17	1.40^{a} ±0.69	2.51^{a} ±2.55	1.30^{a} ±1.11
PA	13.09^{b} ±3.81	14.11^{b} ±10.86	47.01^{a} ±48.64	22.27^{b} ±15.03
TOTOX	14.76^{b} ±3.91	15.31^{b} ±10.62	48.45^{a} ±48.74	23.39^{b} ±15.13

*Results are expressed as means of triplicate determinations ±SD.

^{a,b} Superscripts within the same row indicate statistically significant differences ($p<0.05$). CS: Chicken shawarma; BS: Beef shawarma; CB: Chicken burger; BB: Beef burger.

PV: Peroxide value; PA: p-Anisidine value; TOTOX=2×PV+PA.

are the main challenge to ensure accurate results.

CONCLUSION

Our data demonstrated that all of CB, CS, BB and BS were in an oxidised status and presented toxicologically significant levels of COPs. CB was more susceptible to oxidation than the other samples, since it showed the highest level of PA and TOTOX. In addition, CB exhibited higher COPs levels compared to CS. These findings lead to the assumption that, on one hand, there is a strong relation between COPs and lipid oxidation and on the other hand, storage conditions, processing methods and the types of ingredients added, highly influences the lipid oxidation despite the type of meat.

BIBLIOGRAPHY

[1] A.B. Amaral, M.V. Silva, S.C. Lannes, Lipid oxidation in meat: mechanisms and protective factors – a review. Food Science and Technology 38(1), 1-15 (2018)

[2] M.T. Rodriguez-Estrada, G. Penazzi, M.F. Caboni, G. Bertacco, G. Lercker, Effect of Different Cooking Methods on Some Lipid and Protein Components of Hamburgers. Meat Science 45(3), 365-375 (1997)

[3] Y.A.M. Elkhateeb, M.R. Alshammary, Effects of Fast Foods in Relation to Free Radicals and Antioxidants. American Journal of Laboratory Medicine 2(6), 156-162 (2017)

[4] R. Domínguez, M. Pateiro, M. Gagaoua, F.J. Barba, W. Zhang, J.M. Lorenzo, A comprehensive review on lipid oxidation in meat and meat products. Antioxidants 8, 429 (2019)

[5] Y. Wang, R. Domínguez, J. M. Lorenzo, B. M. Bohrer, The Relationship between Lipid Content in Ground Beef Patties with Rate of Discoloration and Lipid Oxidation during Simulated Retail Display. Foods 10, 1982 (2021)

[6] AOAC (2011), Official methods of analysis of the Association of Official Analytical Chemists (18th ed.). Gaithersburg, MD: AOAC International

[7] Association of Official Analytical Chemists (AOAC). Official Methods of Analysis, 15th ed.; Helrich, K., Ed.; AOAC: Arlington, VA, USA, 2, (1990)

[8] W.N. Ratnayake, C. Galli, Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. Annals of Nutrition and Metabolism 55(1-3), 8-43 (2009)

[9] Official methods of analysis of the Association of Official Analytical Chemists (AOAC) (19th ed.). Gaithersburg, MD: AOAC International (2012)

[10] AOCS, Official Methods and Recommended Practices of the American Oil Chemists' Society, 4th edn., AOCS Press, Champaign, Additions and Revisions, Method Cd 18–90 (1992)

[11] Official Methods of Analysis 14th Ed., AOAC, Arlington, VA, sec. 43.283, (1984)

[12] K.M. Al-Mirazeeq, K.M. Al-Ismail, B.M. Al-Abdullah, Evaluation of some chemical properties of different burger formulations, Proceedings of the First International Conference of Food Industries and Biotechnology and Associated Fair. Al- Baath University, Syria (2008)

[13] M.H. Abdelhai, A.M.E. Sulieman, E.R.B. Babiker, Some Chemical and Microbiological Characteristics of Shawerma Meat Product. Journal of Food & Nutritional Disorders 4(2), (2015)

[14] K. Ramadhan, N. Huda, R. Ahmad, Physicochemical characteristics and sensory properties of selected Malaysian commercial chicken burgers. International Food Research Journal 18, 1349-1357 (2011)

[15] S.G. Karako, Y. Ozogul, M. Saler, F. Ozogul, Proximate analysis. Fatty acid profiles and mineral contents of meats: A comparative study. Journal of Muscle Foods 21(2), 210-223 (2010)

[16] M.M. Abul-Fadl, Nutritional and chemical evaluation of white cauliflower by-products flour and the effect of its addition on beef sausage quality. Journal of Applied Sciences Research 8(2) 693-704 (2012)

[17] B.M. Abdullah, Beef and Sheep Mortadella: Formulation, Processing and Quality Aspects. International Journal of Food Science and Technology 39, 177-182 (2004)

[18] S. Kayisoglu, I. Yilmaz, M. Demirci, H. Yetim, Chemical composition and microbiological quality of the doner kebabs sold in Tekirdag market. Food Control 14(7), 469-474 (2003)

[19] M. Nemati, H. Ghorbanpour, S.V.Razavieh, M. Hoseini, Chemical composition and microbiological quality of the Bonab kebabs sold in Tabriz market. Journal of food safety 28(3), 315-323 (2008)

[20] A.R. Alina, A.S. Babji, S.Affandi, Nutritional quality of palm fat substituted chicken nuggets. Nutrition & Food Science 39(2), 181-188 (2009)

[21] K.M. Al-Mrazeeq, B.M. Al-Abdullah, K.M. Al-Ismail, Evaluation of some sensory properties and cooking loss of different burger formulations. Italian Journal of Food Science 22(2), 134 (2010)

[22] L. Salcedo-Sandoval, S. Cofrades, C. Ruiz-Capillas, F. Jiménez-Colmenero, Effect of cooking method on the fatty acid content of reduced-fat and PUFA-enriched pork patties formulated with a konjac-based oil bulking system. Meat Science 98(4), 795-803 (2014)

[23] Ministry of Agriculture, Fisheries and Food, The composition of foods. London: Royal Society of Chemistry, 209 (1998)

[24] National Public Health Institute, Finnish food composition database, Fineli (2001)

[25] M.L. Ovaskainen, H. Reinivuo, T. Korhonen,

Elintarviketaulukko. Tiedotravintokoostumuksesta (in Finnish) (2001)
[26] T.T. Dinh, K.V. To, M.W. Schilling, Fatty Acid Composition of Meat Animals as Flavor Precursors. Meat and Muscle Biology 5(1) (2021)
[27] M. Kinney Sweeten, H.R. Cross, G.C. Smith, S.B. Smith, Subcellular Distribution and Composition of Lipids in Muscle and Adipose Tissues. Journal of Food Science 55, 43-45 (1990)
[28] A.O. Ajuyah, R.T. Hardin, K. Cheung, J.S. Sim, Yield, lipid, cholesterol and fatty acid composition of spent hens fed full-fat oil seeds and fish meal diets. Journal of Food Science 57, 338-341 (1992)
[29] K.T. Lee, T.A. Foglia, Synthesis, purification, and characterization of structured lipids produced from chicken fat. Journal of the American Oil Chemists' Society 77, 1027–1034 (2000)
[30] H.R. Mottram, Z.M. Crossman, R.P. Evershed, Regiospecificcharacterisation of the triacylglycerols in animal fats using high performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Analyst 126, 1018-1024 (2001)
[31] K.S.Sheu, T.C. Chen, Yield and quality characteristics of edible broiler skin fat as obtained from five rendering methods. Journal of Food Engineering 55, 263–269 (2002)
[32] M.A. Sadek, J.R. Daoud, H.Y. Ahmed, G.M. Mosaad, Nutritive value and trans fatty acid content of fast foods in Qena city, Egypt. Nutrition & Food Science 48(3), 498-509 (2018)
[33] T. Sanders, Functional Dietary Lipids: Food Formulation, Consumer Issues and Innovation for Health, Woodhead Publishing (2015)
[34] J.C. de Almeida, M.S. Perassolo, J.L. Camargo, N. Bragagnolo, J.L. Gross, Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. Brazilian Journal of Pharmaceutical Sciences 42(1), (2006)
[35] P. Zunin, R. Boggia, F. Evangelisti, Identification and Quantification of Cholesterol Oxidation Products in Canned Tuna. Journal of the American Oil Chemists' Society78(10) (2001)
[36] S. Barbieri, D. Mercatante, S. Balzan, S. Esposto, V. Cardenia M. Servili, E. Novelli, A. Taticchi, M.T. Rodriguez-Estrada. Improved Oxidative Stability and Sensory Quality of Beef Hamburgers Enriched with a Phenolic Extract from Olive Vegetation Water. Antioxidants 10(12), 1969 (2021)
[37] K. Al-Ismail, Effect of two methods of grilling on the oxidative rancidity and cholesterol oxidation in beef and chicken shawerma. Grasas y Aceites 53(3), 335-339 (2002)
[38] S.J.K.A.Ubhayasekera, A. Tres, R. Codony, P.C. Dutta, Effect of Feed Fat By-Products with Trans Fatty Acids and Heated Oil on Cholesterol and Oxycholesterols in Chicken. Journal of the American Oil Chemists' Society 87, 173–184 (2010)
[39] A.J.M. Sattar, J.C.A. Deman, Light-induced oxidation of edible oils and fats. Lebensmittel-Wissenschaft and Technologie, 9(3), 4 (1976)
[40] N. Huda-Faujan, A. Noriham, A.S. Norrakiah, A.S. Babji, Antioxidant effects of cosmos caudatus, polygonum minus and murrayakoenigil in spent hen burger. Malaysian Journal of Animal Science (1), 72-77 (2006)
[41] R.E. Wrolstad, T.E. Acree, M.H. Penner, D.S. Reid, S.J. Schwarts, C.F. Shoemaker, D.M. Smith, P. Sporns. Lipid oxidation/stability. In: Handbook of Food Analytical Chemistry, Water, Proteins, Lipids, and Carbohydrates. 1, 513-564 (2005)
[42] K.M. Schaich, CHAPTER 2 - Challenges in Analyzing Lipid Oxidation: Are One Product and One Sample Concentration Enough? Lipid Oxidation Challenges in Food Systems. 53-128 (2013)
[43] Y. Endo, Analytical Methods to Evaluate the Quality of Edible Fats and Oils: The Japan Oil Chemists' Society (JOCS) Standard Methods for Analysis of Fats, Oils and Related Materials (2013) and Advanced Methods. Journal of oleo science 67(1), 1-10 (2018)
[44] J.I. Gray, F.J. Monahan, Measurement of lipid oxidation in meat and meat products. Trends in Food Science & Technology 3, 5 (1992).
[45] A. Hogberg, J. Pickova, J. Babol, K. Anderson, P.C. Dutta, Muscle lipidvitamin E and A and lipids oxidation as affected by diet and RN genotype in female and castrated male Hampshire crossbreed pigs. Meat Science 60(4), 411-420 (2002)
[46] M. Lund, L. Skibsted, C. Claudi-Magnussen, M. Hviid, Effects of dietary soybean oil on lipid and protein oxidation in pork patties during chill storage. Meat Science 79, 727-733 (2008)
[47] K.S. Rhee, A.M. Anderson, A.R. Sams, Lipid oxidation potential of beef, chicken and pork. Journal of Food Science 61, 8-11 (1996)
[48] A. Patel, S. Tiwari, N. Pandey, D. Gupta, S.M. Prasad. Role of Spices Beyond a Flavouring Agent: The Antioxidant and Medicinal Properties. Research Anthology on Recent Advancements in Ethnopharmacology and Nutraceuticals, 616-648 (2022)
[49] B. Min, K. Nam, C.J. Cordray, D.U. Ahn, Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. Journal of Food Science, 73(6), 439-446 (2008)
[50] M. Estevez, Oxidative damage to poultry: from farm to fork. Poultry Science, 94(6), 1368-1378 (2015)
[51] F. Shahidi, Indicators for evaluation of lipid oxidation and off-flavor development in food. Developments in Food Science, 40, 55-68 (1998)

[52] G. Talbot, The Stability and Shelf Life of Fats and Oils. In The Stability and Shelf Life of Food. 461–503 (2016)
[53] B. Min, U. Ahn, Mechanism of lipid peroxidation in meat and meat products-a review. Food Science and Biotechnology 14(1), 152-163 (2005)
[54] D.V. Byrne, W.L. Bredie, D.S. Mottram, M. Martens, Sensory and chemical investigations on the effect of oven cooking on warmed-over flavour development in chicken meat. Meat Science 61(2), 127-139 (2002)
[55] J. Cheng, H.W. Ockerman, Effect of phosphate with tumbling on lipid oxidation of precooked roast beef. Meat Science 65, 1353-1359 (2003)
[56] J.H. Cheng, S.T. Wang, H.W. Ockerman, Lipid oxidation and color change of salted pork patties. Meat Science 75, 71-77 (2007)
[57] K.S. Rhee, Y.A.Ziprin, Pro-oxidant effects of NaCl in microbial growth-controlled and uncontrolled beef and chicken. Meat Science 57(1), 105-112 (2001)

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovation and research

Food Chemistry, Technology and Safety

Testing Services

- quality, authenticity and food safety
- targeted e untargeted screening
- compliance with regulations and commercial standards
- evaluation of organoleptic damage (odour and taint)
- organoleptic evaluation of virgin olive oil
- technological tests
- extractable and leacheables
- food contact materials

Consultancy

Research and Innovation

Technical Standardisation

Training

Contacts: pierangela.rovellini@mi.camcom.it | +39 02 8515.3571



www.innovhub-ssi.it | sales.innovhub@mi.camcom.it

Chemical composition and biological activity of lavandin and lavender essential oils

Dejan Pljevljakušić[a]
Sanja Kostadinović Veličkovska[b]✉
Ljupčo Mihajlov[b]
Aleksandar Čerepnalkovski[b]

[a]Institute for Medicinal Plants Research „Dr. Josif Pančić“, Belgrade, Republic of Serbia

[b]Faculty of Agriculture, University “Goce Delčev”, Republic of North Macedonia

✉ CORRESPONDING AUTHOR:
Email: sanja.kostadinovik@ugd.edu.mk
Faculty of Agriculture,
University “Goce Delčev”
Krste Misirkov bb, 2000 Štip,
Republic of North Macedonia
sanja.kostadinovik@ugd.edu.mk

Received: May 23, 2022
Accepted: July 20, 2022

The main object of our study was an investigation of the chemical composition, antioxidant, and antimicrobial activity of three *Lavandula* essential oils (two lavandin oils cv. Grosso and Budrovka, and one lavender cv. Hemus). The oils were produced by hydrodistillation from plant material organically planted in the region of North Macedonia. The gas chromatography-mass spectrometry technique was applied for the identification and quantification of 93 compounds. The main components in all three varieties were linalool and linalyl acetate. The highest amount of linalool was quantified in essential oils in lavandin cv. Budrovka (35.55%), while the smallest amount was measured in lavender cv. Hemus (21.20%). The highest amount of linalyl acetate was detected in the sample of lavandin cv. Grosso (30.49%). Although there is no relationship between antioxidant activity of lavandin and lavender essential oils measured by DPPH and ABTS radicals, results from antimicrobial activity showed statistically higher antibacterial activity of lavandin essential oils against *z aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). The strongest antifungal activity against *Candida albicans* (ATCC 1023) was measured by lavandin essential oil cv. Budrovka.

Keywords: *Lavandula* essential oil, lavandin, lavender, gas chromatography, chemical composition, antioxidant activity, total phenolic content (TPC), antimicrobial activity.

1. INTRODUCTION

The genus *Lavandula*, commonly known as lavender, is one of the economically important members of the Lamiaceae (*Labiatea*) family, comprising about 39 species and 400 varieties [1-3]. It is stated in some sources that etymologically the name “lavender” comes from the Latin verb “lavare” which means “to wash” or “to bathe”, as lavender was used for cleaning and disinfecting externally in addition to its internal use for disease treatment for many years [4]. This genus includes spontaneous and cultivated forms, widely distributed across the Mediterranean Basin, Canary Islands, Cape Verde Islands, North Africa, Southwest Asia, Arabian Peninsula, and tropical NE Africa and India [5-7]. Within this genus, many species are highly aromatic due to the presence of essential oils that are of important economic value for the perfume, cosmetic, flavouring, and pharmaceutical industries [8, 9]. Several lavender EOs are largely used in aromatherapy as antioxidant, antimicrobial, carminative, spasmolytic, sedative, antiseptic, anti-inflammatory, analgesic properties, antioxidant activity tonic, and anti-depressive agents [10-13]. Three Lavandula species are principally cultivated to produce essential oils: *L. angustifolia* (fine lavender), *L. latifolia* (spike lavender), and the sterile hybrid *L. intermedia* (lavandin). In the food industry *L. angustifolia*, well known as “true lavender” or “English lavender” is a small shrub that produces essential oil with a highly fragrant, refreshing, sweet, balsamic herbaceous odour imparting a sense of “clean” and woody undertones, which is frequently used as a flavouring agent for beverages, ice cream, candy, baked goods, chewing gum, etc [14-17]. In

general, the lavender essential oil is well known and the literature data state that the oil yield is up to 3.3%, but these data vary depending on the plant parts involved in the distillation process [18, 19]. Lavender doesn't contain only essential oils but also anthocyanins, phytosterols, sugars, minerals, coumaric acid, glycolic acid, valeric acid, and its esters, ursolic acid, herniarin, coumarin, and tannins [20].

The chemical composition of lavender oils depends largely on the species from which it was obtained. Several studies concerning the chemical composition of the essential oils of *L. stoechas* from some Mediterranean regions (e.g., Morocco, Corsica, Greece, and Turkey) highlight that the most common chemotype of the species is camphor–fenchone [24-27]. Some authors also report a fenchone–1,8-cineol chemotype and a pulegone chemotype [28-35]. The species *L. stoechas* presents a chemical composition quite different from other species like *L. dentata, L. angustifolia, L. latifolia* and *L. hybrida* [36-38]. The composition of essential oils is an important parameter for the qualitative evaluation of aromatic species. Essential oils are significantly influenced by abiotic (climatic, soil, topographic, agronomic, and post-harvest techniques) and biotic factors (plant age, stage of development, genetic characteristics) [39-42]. The main compounds in the *Lavandula* essential oils include a considerable number of bioactive constituents, such as monoterpenes, sesquiterpenes, diterpenes, triterpenes, polyphenols, and coumarins [42-46]. Cytotoxic activities have been attributed to the pre-eminence in many lavender oils of some monoterpenoids, including linalool, linalyl acetate, 1,8-cineole, β-ocimene, terpinen-4-ol, and camphor. According to some authors, the antimicrobial and antifungal activity of Lavandula essential oils is caused by the content of linalool. They have a strong antibacterial effect against *S. aureus*, *E. coli,* and *P. aeruginosa* [46-50].

The aim of this study was to estimate differences in biological activity between lavender and lavandin essential oils and to mark the most important components in the oils which contribute to the biological activity. Therefore, the determination of the chemical composition, antioxidant, and antimicrobial potential of *Lavandula* essential oils obtained from three cultivars was performed. The essential oils from two cultivars "Grosso" and "Budrovka" belong to the lavandin varieties and "Hemus" cultivar belongs to the lavender variety. The herbs were organically planted in the region of North Macedonia and this is the first report on the chemical composition, antioxidant and antimicrobial potential of *Lavandula* species growth in the region of North Macedonia.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Flowering stalks of lavandin and lavender were obtained from harvests conducted on 15th June 2021 at an organically cultivated trial located near Štip, Republic of North Macedonia (41°46'15.01"N, 22°5'41."E). In this experimental year, 175 L/m^2 rainfall amount was recorded. The samples were taken from three different *Lavandula* cultivars namely *L.* × *intermedia* cv. Grosso, *L. intermedia* cv. Budrovka and *L. angustifolia* cv. Hemus in the full flowering stage. At the time samples were taken the trial was in the fourth year of vegetation. The plant specimens were identified and authenticated by Dr. Ljupčo Mihajlov, from the Faculty of Agriculture, University "Goce Delčev"-Štip. The plant specimen vouchers of three observed cultivars are deposited in the herbarium of the Institute for Medicinal Plants Research "Dr. Josif Pančić" (Belgrade, Serbia) under numbers IPLB#210615_23-32.

2.2. SAMPLE PREPARATION

The collected plant samples were air-dried and grounded. The amount of 50 g of each variety separately was placed in the round-bottom flask (1 L) and subjected to hydrodistillation for 2.5h in the Clevenger-type apparatus according to the European Pharmacopoeia [51]. After the distillation process oil samples were dried through anhydrous sodium sulphate and collected for further analysis. For GC and GCMS analyses 20 µL of oil were dissolved in 2 mL of EtOH, while for the antioxidant and antimicrobial activities the essential oils were dissolved in hexane for DPPH analysis and other appropriate solvents for TPC and TEAC assay as described in sections 2.4. and 2.5.

2.3. GC AND GCMS ANALYSES

The chemical composition of the essential oils was analysed using the GC technique coupled with GCMS. Analyses were performed on a Shimadzu GCMS-QP2010 ultra mass spectrometer fitted with a flame ionic detector and coupled with a GC2010 gas chromatograph. The InertCap5 capillary column (60.0 m × 0.25 mm × 0.25 µm) was used for separation. Helium (He), at a split ratio of 1:5 and a linear velocity of 35.2 cm/s was used as carrier gas. Initially, the oven temperature was 60°C, which was held for 4 min, then increased to 280°C at a rate of 4°C/min, and held for 10 min. The injector and detector temperatures were 250°C and 300°C, respectively. The ion source temperature was 200°C. The identification of the constituents was performed by comparing their mass spectra and retention indices (RIs) with those obtained from authentic samples (homologous series of *n*-alkanes C8-C32) and/or listed in the NIST/Wiley mass-spectra libraries, using different types of searches (PBM/NIST/AMDIS) and available and available literature data [52, 53].

2.4. TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

The total phenolic content (TPC) of essential oils was determined with Folin–Ciocalteau reagent. For each

sample, 10 µL of diluted (1:100) oil were added to 750 µL water and 50 µL of Folin–Ciocalteu reagent. The solution with a total volume of 850 µL was incubated in the dark for 5 min. Then, 150 µL of 20% sodium carbonate solution was added and samples were incubated in the dark for 1 h. The reference solution was prepared with distilled water instead essential oils and treated with the Folin–Ciocalteu reagent in the same way as the assayed samples. The samples turned to a blue colour with different degrees, depending on the content of phenolic compounds in the samples. The absorbance at 750 nm was recorded against the absorbance of the reference solution. The measurements were performed in duplicate. The content of total phenolic compounds was calculated using a calibration curve of gallic acid (the linearity range: 5-50 mg/100 µL).

The Trolox equivalent antioxidant assay (TEAC) employed in this study gives a measure of the antioxidant activity. The chromophore ABTS (2,2'-azino-bis (3- ethylbenzothiazoline-6-sulphonic acid)) was dissolved in distilled water to 6.8 mM concentration and left the mixture to stand in dark at room temperature for 14 h before use. ABTS radical cation (ABTS•+) was produced by reacting with 5.2 mM potassium persulphate solution. After forming of ABTS radical cation, the solution was diluted with water in a 1:8 v/v ratio to 0.6 mM. The concentration of the resulting blue green ABTS radical solution was adjusted to an absorbance of 0.80 ± 0.020 at 735 nm. A 328 µL volume of reagent is pipetted into a quartz cuvette with the subsequent addition of 10 µL of essential oil. The decrease in absorbance at 735 nm was measured after 30 min and incubation at 37°C. The estimation of the antiradical activity with TEAC assay was calculated using a calibration curve of Trolox with different concentrations (1 - 20 mg/L) dissolved in methanol and was used as a standard for the preparation of the calibration curve. Trolox equivalent antiradical capacity (TEAC) was expressed as a percentage of decolorization of ABTS radical cation (ABTS•+).

For the DPPH assay, the antioxidant activities of the essential oils were expressed as a percentage of decolorisation of a solution of the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical) at 517 nm. DPPH reagent was dissolved in hexane and 0.25 mL of the stock solution with a concentration of 0.5M has used for the determination of the antioxidant activity. BHA (butylated hydroxyanisole) with different concentrations (10–100 mg/L) was dissolved in hexane and was used as a standard for the preparation of the calibration curve. The measurements for the oils were performed by direct incorporation of 5 mL of pure essential oil to 495 mL of DPPH reagent.

2.5. ANTIMICROBIAL ASSAYS

The samples of essential oils were investigated for their "*in vitro*" antibacterial and antifungal properties using a disk-diffusion method in Petri dishes. The essential oils were tested for antibacterial activity against one Gram-positive bacterial strain *S. aureus* (ATCC 25923), against one Gram-negative bacterial strain *E. coli* (ATCC 25922), and for antifungal activity using *C. albicans* (ATCC 1023). For this purpose, 5 µL of each essential oil or 4.42×10^3 µg (calculated by relative density 0.885 g/cm^3) was tested and compared by antimicrobial activity of commercial antibiotics.

In brief, each suspension of microorganisms was suspended in Mueller Hinton (MH) broth. Furthermore, the suspension of microorganisms is diluted by using the McFarland scale. An inoculum equivalent to the no. 1 of the McFarland scale was prepared and diluted approximately to 10^6 colonies forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and MH Dextrose Agar (MDA) and then dried. Six-millimetre diameter wells were cut from the agar using a sterile cork-borer, and 60 µL of each sample of *Lavandula* essential oils were delivered into the wells. The plates were incubated at 37 °C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (70 µg/well), nalidixic acid (80 µg/well), ciprofloxacin (15 µg/well), and erythromycin (30 µg/well) were used as a positive control. The controls were performed with only sterile broth and with only overnight culture and 10 µL of 70% ethanol.

The antibacterial activity is ranked from no activity (-, inhibition diameter < 10 mm), low (+, inhibition diameter between 10 and 15 mm), moderate (++, inhibition diameter between 15 and 20 mm), and high activity (+++, diameter inhibition ≥ 20 mm). All tests were performed in triplicate and clear halos greater than 10 mm were considered positive results. The antibacterial and antifungal activity tests of Lavandula essential oils from three varieties are shown in Table III.

2.6. STATISTICAL ANALYSES

All observations were done in triplicate. Differences among observed chemical composition, antioxidant, and antimicrobial activity of three essential oils (two lavandin oils and one lavender oil), were estimated by one-way ANOVA, followed by *post hoc* Tukey's test at $P < 0.05$ level of significance. Statistically significant differences between mean values were denoted by different row-wise letters (a-c). All observations of biological activities were done in triplicate. Statistical analysis was conducted using the R CRAN software package.

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION OF *LAVANDULA ESSENTIAL* OILS

The two major compounds in all three samples (two samples of lavandin and one sample of lavender) were linalool and linalyl acetate. According to the results in Table I, the highest amount of linalool was quantified in lavandin cv. Budrovka (35.55%), while the small-

Table I - Chemical composition of the essential oils of three *Lavandula* cultivars

			L. x intermedia		*L. angustifolia*
			cv. Grosso [b]	**cv. Budrovka**	**cv. Hemus**
#	Compound	RI [a]	%m/m	%m/m	%m/m
1	3-*cis*-hexenol	858.0	0.01±0.00a	-	-
2	*n*-hexanol	867.5	0.06±0.01b	0.33±0.03a	-
3	tricyclene	919.2	0.02±0.00b	0.03±0.00b	0.07±0.01a
4	α-thujene	922.2	0.11±0.03a	0.03±0.01b	0.18±0.02a
5	α-pinene	930.2	0.81±0.04a	0.40±0.04b	0.77±0.05a
6	camphene	947.2	0.41±0.02b	0.35±0.01b	0.76±0.02a
7	thuja-2,4(10)-diene	952.3	-	0.02±0.01a	-
8	sabinene	972.3	0.32±0.05a	0.08±0.01c	0.12±0.02b
9	1-octen-3-ol	975.7	-	0.36±0.07a	0.04±0.01b
10	β-pinene	977.6	1.14±0.04a	0.25±0.03b	0.29±0.04b
11	3-octanone	980.8	0.03±0.01c	0.08±0.02b	0.32±0.06a
12	myrcene	986.6	0.68±0.10a	0.13±0.02b	0.78±0.12a
13	butyl butanoate*	988.8	0.05±0.01b	0.07±0.02b	0.12±0.01a
14	dehydro-1,8-cineole	989.4	-	0.03±0.00a	-
15	3-octanol	991.4	0.02±0.00a	0.01±0.00b	-
16	α-phellandrene	1001.6	0.02±0.00b	0.01±0.00b	0.09±0.01a
17	hexyl acetate	1004.4	0.18±0.04b	0.12±0.02b	0.37±0.08a
18	δ-3-carene	1007.3	0.08±0.01b	0.03±0.00b	1.02±0.05a
19	α-terpinene	1012.4	0.01±0.00b	-	0.04±0.01a
20	*p*-cymene	1016.2	0.01±0.00b	0.03±0.01b	0.14±0.05a
21	*o*-cymene	1019.0	0.22±0.01b	0.44±0.02a	0.40±0.03a
22	limonene	1023.1	0.64±0.13b	0.60±0.13b	0.93±0.12a
23	1,8-cineole + *trans*-β-ocimene	1030.8	12.48±0.02b	12.11±0.07b	15.59±0.09a
24	*cis*-β-ocimene	1037.8	0.12±0.03b	0.04±0.01c	3.34±0.07a
25	γ-terpinene	1051.6	0.04±0.00b	0.02±0.00b	0.14±0.03a
26	*cis*-sabinene hydrate (IPP vs OH)	1061.4	0.19±0.02b	0.35±0.08a	0.06±0.00c
27	*cis*-linalool oxide (furanoid)	1065.6	0.12±0.02b	4.96±0.08a	0.12±0.01b
28	*p*-mentha-2,4(8)-diene	1079.2	-	-	0.07±0.00a
29	terpinolene	1081.9	0.21±0.04a	-	0.15±0.03b
30	*trans*-linalool oxide (furanoid)	1084.0	-	4.34±0.07a	0.05±0.00b
31	linalool	1102.0	30.19±2.04b	35.60±1.06a	21.11±3.5c
32	1-octen-3-yl acetate	1099.2	-	0.08±0.03b	0.55±0.11a
33	*trans*-*p*-mentha-2,8-dien-1-ol	1118.0	-	0.03±0.00a	-
34	*allo*-ocimene	1120.0	0.02±0.00c	0.08±0.01a	0.04±0.00b
35	α-campholenal	1123.5	0.04±0.00b	0.05±0.01b	0.20±0.04a
36	*cis*-*p*-mentha-2,8-dien-1-ol	1133.3	-	0.02±0.00b	0.04±0.01a
37	hexyl isobutanoate*	1136.6	0.14±0.02b	0.30±0.03a	0.08±0.01c
38	*trans*-pinocarveol	1137.5	-	-	0.03±0.00a
39	camphor	1146.2	5.64±1.12b	8.82±1.03a	0.59±0.09c
40	camphene hydrate	1149.4	0.02±0.00b	0.06±0.01a	-
41	isoborneol	1156.7	0.01±0.00b	0.05±0.01a	-
42	lavandulol	1159.8	0.39±0.10c	0.48±0.07b	0.52±1.12a
43	borneol	1164.8	-	-	1.81±0.03a
44	terpinen-4-ol	1180.1	3.03±0.09b	10.90±1.14a	3.90±0.05b
45	hexyl butanoate*	1181.8	2.80±0.09b	2.58±0.02b	0.33±0.03a
46	cryptone	1184.6	0.38±0.06b	1.02±0.03a	0.18±0.08c
47	*p*-cymen-8-ol	1191.5	0.03±0.00a	0.59±0.03a	-
48	α-terpineol	1191.5	0.71±0.11a	0.22±0.02b	0.95±0.08a
49	myrtenal	1194.8	-	0.11±0.02a	-
50	hexyl 2-methyl butanoate*	1229.1	0.01±0.00b	0.13±0.02a	-
51	nerol	1224.7	0.01±0.00b	0.04±0.00b	0.11±0.02a
52	isobornyl formate	1231.1	0.12±0.03b	0.44±0.08a	0.13±0.03b
53	hexyl isovalerate*	1233.6	0.11±0.08b	0.18±0.07a	-
54	cumin aldehyde	1242.4	0.02±0.01c	0.19±0.91a	0.06±0.01b
55	carvone	1245.6	-	0.12±0.02a	0.03±0.01b
56	linalool acetate	1253.4	30.97±2.34a	10.23±1.18c	26.62±4.52b
57	carvenone	1258.4	0.02±0.00b	0.07±0.02a	0.07±0.01a
58	lavandulyl acetate	1284.8	2.55±0.08b	1.09±0.08c	5.79±1.12a
59	bornyl acetate	1289.0	0.03±0.01c	0.11±0.01b	0.25±0.06a

Table I continue

			L. x intermedia		*L. angustifolia*
			cv. Grosso	**cv. Budrovka**	**cv. Hemus**
60	*p*-cymen-7-ol	1291.3	0.01±0.00b	-	0.03±0.00a
61	3-thujanol acetate	1293.1	0.01±0.00b	0.04±0.01a	0.03±0.00a
62	carvacrol	1295.3	-	0.04±0.01a	-
63	hexyl tiglate	1314.2	0.14±0.03a	0.02±0.00b	0.04±0.00b
64	*cis*-piperitol acetate	1334.9	0.03±0.00b	0.14±0.03a	-
65	linalool propanoate	1337.2	-	0.02±0.00a	0.03±0.00a
66	neryl acetate	1345.3	0.11±0.04b	0.04±0.01c	0.22±0.05a
67	geranyl acetate	1362.9	0.14±0.04b	0.03±0.00c	0.28±0.06a
68	hexyl hexanoate*	1364.8	-	0.14±0.02a	-
69	daucene	1373.2	0.09±0.01a	0.01±0.00b	-
70	2-epi-α-funebrene	1377.0	0.08±0.01a	0.02±0.00b	-
71	β-bourbonene	1380.8	0.04±0.00a	-	-
72	β-elemene	1383.0	0.01±0.00c	0.04±0.00b	0.08±0.02a
73	7-*epi*-sesquithujene	1390.3	0.08±0.01b	0.03±0.00a	-
74	sesquithujene	1402.3	0.06±0.00a	0.02±0.00b	0.07±0.02a
75	α-santalene	1408.1	0.16±0.41b	0.02±0.00c	0.48±0.12a
76	*trans*-caryophyllene	1414.8	1.18±0.06b	0.06±0.00c	4.23±0.09a
77	α-*trans*-bergamotene	1421.4	0.12±0.02b	0.11±0.01b	0.20±0.05a
78	β-copaene	1428.5	0.05±0.01a	0.03±0.00c	0.04±0.01b
79	*cis*-β-farnesene	1438.7	1.15±0.09b	0.02±0.00c	3.37±0.11a
80	*trans*-β-farnesene	1435.8	0.09±0.01b	0.15±0.03a	-
81	α-humulene	1452.9	0.01±0.00c	0.04±0.00b	0.09±0.01a
82	sesquisabinene	1457.6	0.04±0.01a	-	0.04±0.00a
83	*cis*-muurola-4(14),5-diene	1462.1	0.02±0.00b	-	0.13±0.03a
84	dauca-5,8-diene	1468.6	0.02±0.00b	-	0.04±0.00a
85	germacrene D	1483.8	0.40±0.09a	-	0.21±0.04b
86	neryl isobutanoate	1501.3	0.09±0.01b	0.12±0.04a	0.03±0.00c
87	bicyclogermacrene	1501.5	0.05±0.01a	0.01±0.00b	0.04±0.00a
88	γ-cadinene	1514.4	0.17±0.02b	-	0.39±0.10a
89	β-sesquiphellandrene	1516.9	0.08±0.01a	-	-
90	*trans*-calamenene	1521.4	0.01±0.00b	0.02±0.00a	-
91	caryophyllene oxide	1579.1	0.07±0.01b	0.04±0.00b	0.33±0.04a
92	τ-cadinol	1635.1	0.08±0.01a	-	-
93	*epi*-α-bisabolol	1674.1	0.15±0.04a	0.02±0.00b	0.18±0.07a
	Monoterpenes		54.67±4.04	72.53±3.80	54.01±5.73
	Sesquiterpenes		3.90±0.76	0.60±0.04	9.41±0.59
	Esters		37.48±2.80	15.80±1.64	34.32±5.97
	Alcohols		2.99±0.02	4.54±0.09	0.77±0.07
	Aldehydes		0.06±0.01	0.34±0.12	0.25±0.05
	Ketones		0.44±0.08	1.15±0.05	0.54±0.03
	Oxygenated compounds		0.40±0.02	4.60±0.07	0.63±0.08
	SUM of identified		99.94±7.73	99.56±11.8	99.93±12.52

[a] RI, retention indices as determined on HP-5 column using homologous series of C8-C30 alkanes
[b] Different letters next to mean values indicate statistical differences according to the post hoc Tukey's test at the level of P < 0.05 row-wise
* tentative identification

est amount was measured in lavender cv. Hemus (21.20%). On the other hand, the essential oil from cv. Budrovka had the lowest amount of linalyl acetate (10.31%), while the highest amount of this monoterpenoid was detected in the sample of lavandin cv. Grosso (30.49%). Different varieties of narrow-leaved lavender contained the same main compounds (linalool 15.9-23.9%, linalyl acetate 1.2-4.7%, *cis*-ocimene 1.1-2.4%, and lavandulol 3.4-4.6%), however, the compounds found in low concentrations were different, which could affect their biological properties [21-23]. Due to the same examined lavender cultivar, our results presented in Table I had very good similarity with the chemical composition of essential oil of *L. angustifolia* cultivated in Poland [27, 29]. According to their published results, the same components were quantified in the amounts of 30.6% and 14.2% respectively [27]. A statistically significant difference in amounts of 1,8-cineole and β-ocimene was detected in the essential oil of lavender cv. HEMUS (15.62%) while the other two samples of *Lavandula* oils had a similar amount of those compounds. The most important marker for lavandin essential oils was camphor which was quantified in the amounts of 5.70 and 8.77% in "Grosso" and "Budrovka" cultivars, respectively.

The highest abundance of total monoterpenes was identified and quantified for lavandin essential oil from "Budrovka" cultivar (70.24%), while lavandin essential oil from "Grosso" cultivar had the highest percentage of total esters (37.02%). The lavender essential oil from "Hemus" cultivar was the richest source of sesquiterpenes (9.53%) and alcohols (2.49%). Aldehydes, ketones, and other oxygenated compounds were presented in amounts of less than 2%. According to the ISO 3515:2002 standard as quality criteria [21], lavender essential oils should contain linalool (25-38%), linalyl acetate (25-45%), and camphor (0.5-1.0%), while lavandin essential oils should contain linalool (24-35%), linalyl acetate (28-38%), and camphor (6-8%) according to the ISO 8902:2009 [22].

In general, the chemical composition of essential oils from *Lavandula* varieties is affected by fertilizers and environmental and climatic conditions which vary during the harvesting year [4, 8, 12, 17, 24, 25, 31]. In addition, the fenchone chemotype of Sicilian biotypes of *L. stoechas* L. spp. *stoechas* had variations in the chemical composition of essential oils based on the areas of origin of the biotypes (major Sicily Island and minor Pantelleria island) [19]. Furthermore, the extraction techniques significantly influenced the yield and chemical composition of *Lavandula* essential oils. Some studies revealed that the distillation process should be continued for up to 2 hours to obtain optimum oil yields with better quality [24].

The sums of total alcohol and oxygenated compounds were significantly higher for the essential oil of lavandin cv. Budrovka since this oil had the highest amount of terpinene-4-ol and -*cis* and -*trans* linalool oxide (10.85%, 4.95%, and 4.35%, respectively). Although some authors referred to γ-terpinene as a major monoterpene in some varieties of *Lavandula* essential oils (26.8%), our study indicated a significant amount only in lavender cv. "Hemus" (0.14%) [1]. A research group by Wagner reported eucalyptol as a major terpene (34.33%) in the essential oil from *L. dentata* [2]. The main lactone in the essential oil of the aerial parts of *L. atriplicifolia* Benth was C-10 massoia lactone (46.45%) [7]. However, camphor was the main component (26.9%) in the *L. tenuisecta* essential oil [9]. Chemical compositions of essential oils obtained from our lavandin samples (cv. Budrovka and cv. Grosso) were in line with the results published by Rai et al. and Śmigielski et al. [12, 18]. According to their findings, the major component of essential oil from *L. angustifolia* was linalyl acetate and linalool with an amount of over 20% and 14.2%, respectively, and this oil provided a significant improvement in psoriatic conditions in experimental rats [12]. Other oxygenated compounds such as carvacrol were the most dominant in essential oils from wild and cultivated *L. mairei* [16]. One of the best reviews of the chemical composition of *Lavandula* essential oil was published by Eldeghedy et al., [33]. According to their findings, the main constituents in the different species were linalool (39.5%), linalyl acetate (26.7%), eucalyptol (43.08%), cadinol (28.63%), and linalyl acetate (46.41%) for lavender, Fren. lavender, lavandin, *L. angistifolia*, *L. latifolia*, and *L. asp*, respectively [33]. Results from gas chromatography analysis published by different authors showed that there is significant variation between species in the quality and quantity of *Lavandula* essential oil composition. In the work of Adaszynska-Skwirzynska et al., the main components of the essential oils of *L. angustifolia* 'Blue River' and 'Ellagance Purple' obtained from flowers were linalool, linalyl acetate, lavandulol acetate and α-terpineol [34]. Essential oils from leafy stalks contained mainly: borneol, *epi*-bicyclosesquiphellandrene, caryophyllene, eucalyptol, and linalool [34]. The working group of Kucukyumuk studied the effect of nitrogen fertiliser on the lavandin yield [35]. The conclusion was that nitrogen fertiliser has a significant effect, not only on plant growth but, also on lavandin yield. In addition, nitrogen fertilisation increased yields and some quality parameters such as plant height, branch height, length of flower, essential oil content, and affected essential oil components (such as linalool, linalyl acetate, and camphor) [35]. Our results presented in Table I were in good agreement with the chemical profile of *L*. x *intermedia* essential oil published by de Elguea-Culebras et al. [36]. Their results showed that the major compounds of *L*. x *intermedia* essential oil were linalool (38.5%), linalyl acetate (26.2%), and camphor (15.2%) [36, 37]. These three components were selected for a few studies because linalool and linalyl acetate contents are two major constituents, and the low content of camphor is very important because it gives lavender oil an undesirable odour. Usually, linalyl acetate is high in lavender oil while linalool is higher in lavandin oil [38]. Lavandulol and lavandulyl acetate are considered marker compounds for lavender essential oil [39]. In our samples, the lavandulyl acetate quantities were between 0.39 and 0.48% for lavandin and 0.52% for lavender essential oil. The highest amount of lavandulyl acetate was determined in lavender oil from "Hemus" cultivar (5.79%) while the other two examined lavandin essential oils from "Grosso" and "Budrovka" varieties had less than half of the amount (2.55 and 1.09 respectively). However, It is necessary to note that all three examined cultivars did meet the requirements of the Ph. Eur. for the lavandulol content (min. 0.1%).

3.2. ANTIOXIDANT ACTIVITY OF LAVANDULA ESSENTIAL OILS

Considering that essential oils are mixtures of organic compounds, it is difficult to distinguish compounds that play a crucial role in the antioxidant system. The ability of DPPH to neutralise a free radical is frequently consistent with the high level of a diverse group of phenolic compounds. The results from total phenolic content and antioxidant activity measured by two rad-

icals (ABTS and DPPH) indicated significantly higher antioxidant activity of essential oil from lavandin cv. *Budrovka* in comparison to the antioxidant activity of the other two samples (Table II). Statistical analysis from the total polyphenol content indicated no significant difference between the antioxidant activity of essential oils from lavandin cv. Grosso and lavender cv. Hemus (Table II). In the work of Badr et al., examined lavender oils displayed a remarkable antioxidant potential followed in descending order by α-terpinyl acetate, camphene, and α-terpinyl acetate [5]. Relatively low values for total phenolic compounds in our examined essential oils can be explained by the fact that phenolic compounds are concentrated mainly in the plant residue material [28]. New phenolic compounds such as lavandunat, lavandufurandiol, lavandufluoren, lavandupyrones A and B, lavandudiphenyls A and B were isolated by Yadikar et al. [40]. The amount of each of these constituents varies in different species and depends on the genotype, geographical origin, climatic conditions, growing conditions, harvest time, and extraction method. [41]. In the work of Lilia et al., the examined samples of essential oils of *L. stoechas* were very powerful in the case of the reference ascorbic acid which is a strong antioxidant [42]. Nurzyńska-Wierdak and Zawiślak explained high variability of the chemical composition of the examined lavender leaves, flower buds and flowers originate from ontogenetic variability which was related to the complicated transformations of these compounds, where the contents of phenolic compounds were less dependent upon developmental factors [43]. Interesting dependencies also result from the performed analysis of the correlation between the contents of bioactive substances and antioxidant activity against DPPH radical, indicating the significant share of phenolic compounds and essential oil in the antioxidant potential of lavender. A strong negative correlation between flavonoid compounds and antioxidant activity in turn most probably originates from the specific structure of these compounds, and mainly the position of OH radicles. It is well known that *Lavandula* essential oils are rich in oxygenated terpenoids mainly monoterpenes, which have a significant ability to neutralise free radicals. The antioxidant activity of examined raw material (lavender leaves and flowers) may result from the high content of linalool and linalyl acetate in the oil [43].

Table II - The total phenolic content and antioxidant activity results of essential oils from three *Lavandula* cultivars

Cultivars	Total phenolic content[a]	Antioxidant ABTS	Antioxidant DPPH
	[mg GAE/100 g DW]	[mg/L Trolox]	[mg/L BHA]
Grosso	45.2±1.5 b	15.2±1.1 b	50.9±2.3 b
Budrovka	58.9±2.8 a	19.1±2.0 a	59.8±4.9 a
Hemus	39.2±1.9 c	16.8±1.4 b	47.4±1.8 b

[a] Means followed by different letters differ significantly according to Tukey's *post hoc* test at P < 0.05 level

3.3. ANTIMICROBIAL ACTIVITY OF LAVANDULA ESSENTIAL OILS

Although results from antioxidant activity measured by two radicals (DPPH and ABTS) were not statistically linked by lavender and lavandin varieties, the antimicrobial activity was strongly determined. Both samples of essential oils of lavandin had significantly higher antibacterial activity against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) (Table III). Only antifungal activity against *C. albicans* (ATCC 1023) was the highest for lavandin variety cv. Budrovka. This might be explained by the fact that this oil had three times a higher amount of terpinene-4-oil and the highest amount of linalool in comparison to the other two samples. The Working group of D'Auria discovered that lavender oil (2%) killed 100% of the *C. albicans* ATCC 3153 cells within 15 min; linalool (0.5%) killed 100% of the cells within 30 s. According to their findings, essential oil inhibited germ tube formation as did the main components linalool and linalyl acetate. Both the essential oil and its main components inhibited hyphal elongation of *C. albicans* ATCC 3153 (about

Table III - Antimicrobial activity of essential oils from three *Lavandula* cultivars compared with commercial antibiotics as a positive control

Samples	Dosage	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (ATCC 25922)	*Candida albicans* (ATCC 1023)
	[µg]	[mm]	[mm]	[mm]
Cultivars				
Grosso	4.42x10^3 µg	92±3 a	74±2 a	53±1 b
Budrovka	4.42x10^3 µg	97±2 a	77±5 a	57±4 a
Hemus	4.42x10^3 µg	84±4 b	51±3 b	49±5 b
Positive control				
Gentamycin	70 µg	55	48	18
Nalidixic acid	80 µg	49	51	15
Ciprofloxacin	15 µg	32	32	22
Erythromycin	30 µg	79	17	13

[a] Means followed by different letters differ significantly according to Tukey's *post hoc* test at P < 0.05 level
[b] Conversion of 5 µL of oil in µg by their specific gravity (conversion factor 0.885 g/cm^3 at 25ºC)

50% inhibition at 0.016% with each substance). They conclude that linalool is responsible for the fungicidal activity, whereas linalyl acetate appears to be somewhat inhibiting germ tube formation and hyphal elongation [44]. Although our results showed that examined essential oils from *Lavandula* had lower antifungal activity against *C. albicans* (ATCC 1023), the working group of Zuzarte published impressive antifungal activity of essential oil from *L. luisieri* against three *C. albicans* strains (ATCC 10231, D5 and M1) [15]. The same variety of *L. intermedia Grosso* was studied by Moon et al., and they concluded that there is a strong correlation between linalool and linalyl acetate content and its antifungal activity. According to their findings, the lack of correlation between the major oil components and antifungal activity suggests that the different susceptibilities of the fungi may be related to either the minor components of the oil or differences in the cell wall/cell membrane of the fungi themselves [20]. Similar biological activity with Gentamicin as a positive control was obtained from the essential oil of *L. pubescens* [27]. Some authors stated that the vapor of lavender essential oil can exert an antibiotic activity in hospital contexts, especially against *S.* species and methicillin-resistant microorganisms [3, 13]. The latest research study indicated that nanosized *Lavandula* oil droplets of nanoemulsions can facilitate the interaction of the active components with the bacterial membranes and increase their antimicrobial efficacy [6]. The essential oil from *L. angustifolia* showed a significant effect against Gram-negative at concentrations of 5, 10, and 20 mg/mL, and against Gram-positive strains at concentrations of 10 and 20 mg/mL which was attributed to the chemical composition of L. *angustifolia* [14]. According to the finding of the research group of Rashed, the variation in inhibition may be attributed to the cell membrane constituents of bacteria. The cell membrane of Gram-positive bacteria contains an outer peptidoglycan layer which is an ineffective permeability barrier, while the cell membrane of Gram-negative bacteria contains a more effective permeability barrier in the outer membrane consisting of lipopolysaccharides and that is the reason why Gram-negative strains were more resistant than Gram-positive strains [14]. Some authors believe that essential oils affect the cell membrane of microorganisms [30]. That can be explained by the hydrophobicity of essential oils and their components which allows them to accumulate in cell membranes and disturb the structures. This causes impairment of microbial enzyme systems, due to a leakage of intracellular constituents, caused by the increase of membrane permeability [30]. It is interesting to notice that borneol exhibited antimicrobial activity against *B. cereus, E. coli* , and *S. aureus* (MIC ranging from 0.03 to 0.25 mg/mL) and *S. typhimurium* (MIC 0.12 to 800 mg/mL). Almost identical antibacterial activity was found for linalool (MIC 0.25 mg/mL), while 1,8-cineole was inactive against all tested strains [42]. Our results indicated a significant amount of borneol only in lavender essential oils from "Hemus" variety (1.81±0.03). The effect of the foliar application of 24-epibrassinolide (24-eBL), a brassinosteroid analogue, on the growth and secondary metabolite production of lavandin (*L.* x *intermedia* var. Super) was examined [46]. The published results unequivocally confirmed that 24-eBL may be a promising compound for use in lavandin cultivation because of its positive effects on plant growth, phenolic content, essential oil content, and oil quality [47]. The newest findings stated that *L. angustifolia* could be a promising choice for antidiabetic medication by inhibiting the carbohydrate metabolizing enzyme α-glucosidase [48]. Results initially showed promising *in vivo* toxicity of the essential oil on the tissue to achieve an anti-MRSA pharmaceutical use [48]. The latest research in natural insecticides proved that essential oils of some variety of *Lavandula* exhibited a strong fumigant and contact toxicity against *R. dominica* and *S. oryzae* adults. Published results from this field proved that the *Lavandula* essential oils flowering tops could be potentially exploited for the development of new antibacterial, antifungal as well as bio-insecticide products [49]. Promising results from the study of the working group of Miastkowska proved that lavender oils had a strong potential to enhance the local, tissue-derived proinflammatory and pro-regenerative response, while simultaneously limiting the inflammatory stimulation of the immune system cells, with in-house preparation performing significantly better in the in vitro cell models [50].

CONCLUSION

The results from our study showed that the chemical composition of three *Lavandula* essential oils (two lavandin oils cv. Grosso and Budrovka and one lavender cv. Hemus) organically planted in the North Macedonia region is strongly linked to the antioxidant and antimicrobial potential of the oils. Linalool and linalyl acetate were the most abundant compounds among the 93 identified. The difference in the chemical composition can be linked to the statistically higher antibacterial activity of lavandin essential oils against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). Camphor, β-pinene, and *cis*-linalool oxide are components that are predominant in lavandin oils and might be responsible for stronger antibacterial activity. Esters such hexyl hexanoate, hexyl isovalerate, neryl isobutanoate, terpenes such *trans*-β-farnesene and cumin aldehyde were the most abundant in the lavandin essential oil cv. Budrovka, in combination with linalool, might have a synergetic antifungal effect against *C. albicans* (ATCC 1023).

Acknowledgements

This work was supported by the bilateral Austri-

an-North Macedonian project called “Oxidomics-guided development of cold pressed oils rich in antioxidants by improving manufacturing processes” (2022-2023).

REFERENCES

[1] M.M. Al-Ansari, A.M.I. Andeejani, E. Alnahmi, R.H. AlMalki, A. Masood, P. Vijayaraghavan, A.A. Rahman, K.C. Choi. Insecticidal, antimicrobial and antioxidant activities of essential oil from *Lavandula latifolia* L. and its deterrent effects on *Euphoria leucographa*. Ind. Crops Prod. *170*, 113740 (2021).

[2] L.S. Wagner, C.J. Sequin, N. Foti, M.P. Campos-Soldini. Insecticidal, fungicidal, phytotoxic activity and chemical composition of *Lavandula dentata* essential oil. Biocatal. Agric. Biotachnol. *35*, 102092 (2021).

[3] A. Gismondi, G. Di Marco, E.L. Redi, L. Ferrucci, M. Cantonetti, A. Canini. The antimicrobial activity of *Lavandula angustifolia* Mill. essential oil against *Staphylococcus* species in a hospital environment. J. Herb. Med. 26, 100426 (2021).

[4] Z.H. Sharafabad, M. Abdipour, M. Hosseinifarahi, A. Kelidari, L. Rashidi. Integrated humic acid and vermicomposting changes essential oil quantity, and quality in field-grown *Lavandula angustifolia* L. intercropped with *Brassica nigra* L. Ind. Crops Prod. *178*, 114635 (2022).

[5] M.M. Badr, M.E.I. Badawy, N.E.M. Taktak. Characterization, antimicrobial activity, and antioxidant activity of the nanoemulsions of *Lavandula spica* essential oil and its main monoterpenes. J. Drug. Deliv. Sci. Technol. *65*, 102732 (2021).

[6] S. Garzolia, S. Petralito, E. Ovidi, G. Turchetti, V.L. Masci, A. Tiezzi, J. Trilli, S. Cesa, M.A. Casadei, P. Giacomello, P Paolicelli. *Lavandula* x *intermedia* essential oil and hydrolate: Evaluation of chemical composition and antibacterial activity before and after formulation in nanoemulsion. Ind. Crops Prod. *145*, 112068 (2020).

[7] M.H. Oueslati, N. Abutaha, F. Al-Ghamdi, I.A. Nehdi, F.A. Nasr, L. Mansour, M. AL-Zharani, A.H. Harrath. Analysis of the chemical composition and in vitro cytotoxic activities of the essential oil of the aerial parts of *Lavandula atriplicifolia* Benth. J. King Saud Univ. Sci. *32*, 1476-1481 (2020).

[8] E. Détár, É. Zámboriné Németh, B. Gosztola, I. Demján, Z. Pluhár. Effects of variety and growth year on the essential oil properties of lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula x intermedia* Emeric ex Loisel.). Biochem. Syst. Ecol. *90*, 104020 (2020)

[9] A. Sayout, A. Ouarhach, I. Dilagui, N. Soraa, A. Romane. Antibacterial activity and chemical composition of essential oil from *Lavandula tenuisecta* Coss.ex Ball. an endemic species from Morocco. Eur. J. Integr. Med. *33*, 101017 (2020).

[10] B. Soulaimani, A. Nafis, A. Kasrati, A. Rochdi, N.E. Mezrioui, A. Abbad, L. Hassani. Chemical composition, antimicrobial activity, and synergistic potential of essential oil from endemic *Lavandula maroccana* (Mill.). S. Afr. J. Bot. 125, 202-206 (2019).

[11] K.D. Giannoulisa, V. Evangelopoulos, N. Gougoulias, E. Wogiatzi. Could bio-stimulators affect flower, essential oil yield, and its composition in organic lavender (*Lavandula angustifolia*) cultivation? Ind. Crops Prod. *154*, 112611 (2020).

[12] V.K. Rai, P. Sinha, K.S. Yadav, A. Shukla, A. Saxena, D.U. Bawankule, S. Tandon, F. Khan, C.S. Chanotiya, N.P. Yadav. Anti-psoriatic effect of *Lavandula angustifolia* essential oil and its major components linalool and linalyl acetate. J. Ethnopharmacol. *261*, 113127 (2020).

[13] A. Bouyahya, A. Et-Touys, J. Abrini, A. Talbaoui, H. Fellah, Y/ Bakri, N. Dakka. *Lavandula stoechas* essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. Biocatal. Agric. Biotechnol. *12*, 179-184, (2017).

[14] M.M.A. Rashed, Q. Tong, A. Nagi, J.P. Li, N.U. Khane, L. Chen, Ashraf Rotail, A.M. Bakry. Isolation of essential oil from *Lavandula angustifolia* by using ultrasonic-microwave assisted method preceded by enzymolysis treatment, and assessment of its biological activities. Ind. Crops Prod. *100*, 236-245, (2017).

[15] M. Zuzarte, M.J. Gonçalves, M.T. Cruz, C. Cavaleiro, J. Canhoto, S. Vaz, E. Pinto, L. Salgueiro. *Lavandula luisieri* essential oil as a source of antifungal drugs. Food. Chem. *135*, 1505-1510 (2012).

[16] A. El. Hamdaoui, F. Msanda, H. Boubaker, D. Leach, I. Bombarda, P. Vanloot, N. El Aouada, A. Abbad, E.H. Boudyach, F. Achemchem, A. Elmoslihb, A.A. Ben Aoumar, A. El Mousadik. Essential oil composition, antioxidant and antibacterial activities of wild and cultivated *Lavandula mairei* Humbert. Biochem. Syst. Ecol. *76*, 1-7, (2018).

[17] S. Najafian. The effect of time and temperature on the shelf life of essential oils of *Lavandula officinalis*. J. Essent. Oil Res. *28*(5), 413-420, (2016).

[18] K.B. Śmigielski, R. Prusinowska, K. Krosowiak, M. Sikora. Comparison of qualitative and quantitative chemical composition of hydrolate and essential oils of lavender (*Lavandula angustifolia*). J. Essent. Oil Res. *25*(4), 291-299, (2013).

[19] T. Tuttolomondo, G. Dugo, G. Ruberto, C. Leto, E.M. Napoli, A.G. Potortì, M.R. Fede, G. Virga, R. Leone, E. D’ Anna, M. Licata, S. La Bella. Agronomical evaluation of Sicilian biotypes of *Lavandula stoechas* L. spp. *stoechas* and analysis

of the essential oils. J. Essent. Oil Res. *27*(2), 115-124, (2015).
[20] T. Moon, H.M.A. Cavanagh, J.M. Wilkinson. Antifungal Activity of Australian Grown Lavandula spp. Essential Oils Against *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*. J. Essent. Oil Res. *19*(2), 171-175, (2007).
[21] ISO 3515:2002/Cor 1:2004 . *Oil of Lavender (Lavandula angustifolia Mill.)—Technical Corrigendum 1.* ISO; Geneva, Switzerland: 2004. [(accessed on 15 March 2021)].
[22] ISO 8902:2009 Specifies Certain Characteristics of the Essential Oil of Lavandin Grosso [*Lavandula angustifolia* Mill. × *Lavandula latifolia* Medik.] French Type, Intended for Facilitating the Assessment of Its Quality. [(accessed on 15 March 2021)]
[23] Council of Europe. European Pharmacopoeia, 10th ed.; Council of Europe: Strasbourg, France, 2020.
[24] G.D. Kiran Babu, A. Sharma, Bikram Singh. Volatile composition of *Lavandula angustifolia* produced by different extraction techniques. J. Essent. Oil Res. *19*(2), 1-12, (2016).
[25] M.M. Barazandeh. Essential oil composition of *Lavandula lafifolia* Medik from Iran. J. Essent. Oil Res. *14*(2), 103-104, (2002).
[26] N. Bouzouita, F. Kachouri, M. Hamdi, M.M. Chaabouni. Volatile constituents and antimicrobial activity of *Lavandula stoechas* L. oil from Tunisia. J. Essent. Oil Res. *17*(5), 584-586, (2005).
[27] R.N. Al-Badani, J.K.R. da Silva, I. Mansi, B.A. Muharam, W.N. Setzer, N.A. Awadh Ali. Chemical composition and biological activity of *Lavandula pubescens* essential oil from Yemen. J. Essent. Oil-Bear Plants. *20*(2), 509-515, (2017).
[28] A. El Hassouni, A. El Bachiri, C. Belbachir. *Lavandula dentata* solid residue from essential oil industry. J. Essent. Oil-Bear Plants. *22*(6), 1601-1613, (2019).
[29] K. Smigielski, A. Raj, K. Krosowiak, R. Gruska. Chemical composition of the essential oil of *Lavandula angustifolia* cultivated in Poland. J. Essent. Oil-Bear Plants. *12*(3), 338-347, (2009).
[30] M.M. Imane, F. Houda, A.H.S. Amal, N. Kaotar, T. Mohammed, R. Imane, H. Farid. Phytochemical composition and antibacterial activity of Moroccan *Lavandula angustifolia* Mill. J. Essent. Oil-Bear Plants. *20*(4), 1074-1082, (2017).
[31] K. Smigielski, R. Prusinowska, A. Stobiecka, A. Kunicka-Styczyñska, R. Gruska. Biological properties and chemical composition of essential oils from flowers and aerial parts of lavender (*Lavandula angustifolia*). J. Essent. Oil-Bear Plants. 1-12, (2018).
[32] S. Gharby, A. Asdadi, M. Ibourki, A. Hamdouch, T. Ainane, L.A. Idrissi Hassani. Chemical characterization of the essential oil from aerial parts of *Lavandula rejdalii* Upson & Jury, a medicinal plant endemic to Morocco. J. Essent. Oil-Bear Plants. *23*(6), 1422-1427, (2021).
[33] H.I. Eldeghedy, A. El-Nasser G. El-Gendy, A.A. Nassrallah, A.M. Aboul-Enein, E.A. Omer. Comparative chemical profiles of *Lavandula* species essential oils grown in Egypt and others from France and Australia: evidence from chemometric analysis. J. Essent. Oil-Bear Plants. *25*(1), 52-63, (2022).
[34] M. Adaszynska-Skwirzynska, M. Dzieciol. Comparison of chemical composition and antimicrobial activity of essential oils obtained from different cultivars and morphological parts of *Lavandula angustifolia*. J. Essent. Oil-Bear Plants. *21*(6), 1532-1541, (2018).
[35] Z. Kucukyumuk, S. Erbas, I. Erdal, H. Baydar, F. Eraslan. Effect of Different Nitrogen Doses on plant growth, quality characteristics and nutrient concentrations of lavandin (*Lavandula* × *intermedia* Emeric ex Loisel. var. Super A). J. Essent. Oil-Bear Plants. *18*(1), 36-43, (2015).
[36] G.O. de Elguea-Culebras, M.I. Berruga, O. Santana-Méridas, D. Herraiz-Peñalver, R. Sánchez-Vioque. Chemical composition and antioxidant capacities of four Mediterranean industrial essential oils and their resultant distilled solid by-products. Eur. J. Lipid Sci. Technol. 119(12), 17000242, (2017).
[37] K. Tomi, M. Kitao, H. Murakami, Y. Matsumura, T. Hayashi. Classification of lavender essential oils: sedative effects of *Lavandula* oils. J. Essent. Oil Res. 1-13 (2017).
[38] F. Handan Giray. An analysis of world lavender oil markets and lessons for Turkey. J. Essent. Oil-Bear Plants. *21*(6), 1612-1623, (2018).
[39] K. Pokajewicz, M. Białoń , L. Svydenko, Roman Fedin, N. Hudz. Chemical composition of the essential oil of the new cultivars of *Lavandula angustifolia* Mill. Bred in Ukraine. Molecules, 26, 5681, (2021).
[40] N. Yadikar, K. Bobakulov, G. Li, H.A. Aisa. Seven new phenolic compounds from Lavandula angustifolia. Phytochem. Lett. 23, 149–154, (2018).
[41] N. Dobros, K. Zawada, K Paradowska. Phytochemical profile and antioxidant activity of *Lavandula angustifolia* and *Lavandula x intermedia* Cultivars extracted with different methods. Molecules, 11, 711, (2022).
[42] C. Lilia, A. Abdelkader, A.K. Aourahoun Karima, B. Tarek. The effect of ultrasound pre-treatment on the yield, chemical composition and antioxidant activity of essential oil from wild *Lavandula stoechas* L. J. Essent. Oil-Bear Plants. *21*(1), 253-263, (2018).
[43] R. Nurzyńska-Wierdak, G. Zawiślak. Chemical composition and antioxidant activity of lavender (Lavandula angustifolia Mill.) aboveground parts.

Acta Sci. Pol. Hortorum Cultus, 15(5), 225-241, (2016).
[44] F. Diodata D'Auria, M. Tecca, V. Strippoli, G. Salvatore, L. Battinelli, G. Mazzanti. Antifungal activity of Lavandula angustifolia essential oil against *Candida albicans* yeast and mycelial form Med. Mycol. 43(5), 391-6 (2005).
[45] C. Caprari, F. Fantasma, F. Divino, A. Bucci, M. Iorizzi, G. Naclerio, G. Ranalli, G. Saviano. Chemical Profile, In Vitro Biological Activity and Comparison of Essential Oils from Fresh and Dried Flowers of *Lavandula angustifolia* L. Molecules. 26(17), 5317, (2021).
[46] Ö.A. Asci, H. Deveci, A. Erdeger, K. Nur Özdemir, T. Demirci, N. Göktürk Baydar. Brassinosteroids promote growth and secondary metabolite production in lavandin (*Lavandula intermedia* Emeric ex Loisel.). J. Essent. Oil-Bear Plants. *22*(1), 254-263, (2019).
[47] S.N. Mir Najibullah, J. Ahamad, A.A. Aldahish, S. Sultana, S. Sultana. Chemical characterization and α-glucosidase inhibitory activity of essential oil of *Lavandula angustifolia* flowers. J. Essent. Oil-Bear Plants. *24*(3), 431-438, (2021).
[48] A. Khadir, M. Bendahou, F. Benbelaid, M.A. Abdoune, C. Bellahcene, F. Zenati, A. Muselli, J. Paolini, J. Costa. Chemical composition and anti-MRSA activity of essential oil and ethanol extract of *Lavandula multifida* L. from Algeria. J. Essent. Oil-Bear Plants. *19*(3), 712-718, (2016).
[49] D. Imen, H.H. Soumaya, C. Imed, M.B.J. Jouda, L. Ahmed, C. Rym. Essential oil from flowering tops of *Lavandula dentata* (L): chemical composition, antimicrobial, antioxidant and insecticidal activitie. J. Essent. Oil-Bear Plants. *24*(3), 632-647, (2021).
[50] M. Miastkowska, T. Kantyka, E. Bielecka, U. Kałucka, M. Kamińska, M. Kucharska, A. Kilanowicz, D. Cudzik, K. Cudzik. Enhanced Biological Activity of a Novel Preparation of *Lavandula angustifolia* Essential Oil. Molecules. 26(9), 2458, (2021).
[51] Ph.Eur. 10.0. (2019). European Pharmacopoeia 10.0., Council of Europe, Strasbourg Cedex, France
[52] R.P. Adams, (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, Allured Publishing Corporation.
[53] D. Hochmuth, (2006). Massfinder 3: Software for GC/MS interpretation and presentation, mass spectral library administration. Hamburg, Germany.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

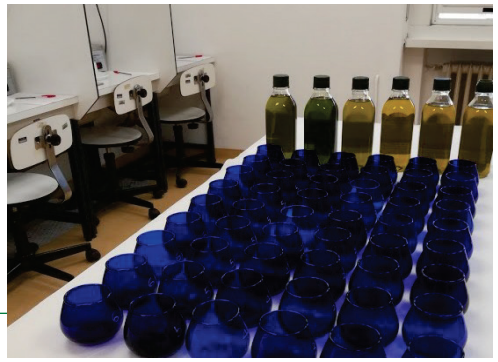
innovazione e ricerca

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

Fatty acid composition of stems, leaves, flowers, and seeds of some medicinal plants

Kagan Kokten[1]✉, Sam Mokhtarzadeh[2], Erdal Cacan[3], Mehmet Ali Kutlu[3], Selim Ozdemir[3], Ridvan Ucar[4], Muammer Ekmekci[5]

[1]Department of Plant Production and Technologies - Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Turkey
[2]Department of Field Crops
Faculty of Agriculture
Duzce University
Duzce, Turkey
[3]Department of Crop and Animal Production
Vocational School of Food, Agriculture and Livestock, Bingol University
Bingol, Turkey
[4]Department of Field Crops
Faculty of Agriculture
Pamukkale University
Denizli, Turkey
[5]Agricultural Research and Application Center Directorate
Bingol University, Bingol, Turkey

✉ CORRESPONDING AUTHOR:
Department of Plant Production and Technologies
Faculty of Agricultural Sciences and Technology
Sivas University of Science and Technology
Sivas, Turkey
Fax: +90 4262160030
Tel: +90 5379352592
E-mail: kahafe1974@yahoo.com

Received: July 21, 2022
Accepted: Sept 26, 2022

Medicinal plant species are economically important and have many uses. In this research, the stem, leaves, flowers, and seeds of some medicinal plant species such as *Lavandula intermedia, Satureja hortensis* and *Ocimum basilicum* were analysed. The fatty acid compositions of these three different species were determined by gas chromatography of methyl esters of fatty acids. The stem, leaf, flower, and seed oils of *L. intermedia* contain 44.72%, 45.11%, 29.89% and 8.55% palmitic acid and 16.18%, 23.21%, 34.76% and 39.76% linolenic acid, respectively, as the main component fatty acids, while the stem, leaf, flower and seed oils of *S. hortensis* contain 23.11%, 11.76%, 13.32% and 6.26% palmitic acid and 31.70%, 51.74%, 47.97% and 43.79% linolenic acid, respectively, as main component fatty acids. On the other hand, stem, leaf, flower, and seed oils of *O. basilicum* contain 44.72%, 45.11%, 29.89% and 8.55% palmitic acid and linolenic acid 16.18%, 23.21%, 34.76% and 39.76%, respectively, as main component fatty acids.

Keywords: *Satureja hortensis*, *Ocimum* basilicum, *Lavandula intermedia*, aerial parts, fatty acid

1. INTRODUCTION

The physical and chemical properties of the oils contained in the plants determine the composition of the fatty acids they contain. The fatty acid compositions of plants are constantly changing depending on many factors. The quality of the oil, its nutritional and processing values are largely dependent on its fatty acid composition. Knowing the fatty acid compositions of plants enables the production of oils according to their intended use [1]. The oils contained in the plants are not only a high energy source, but also have very important roles in nutrition and health depending on the properties of fatty acids. It is known that saturated fatty acids cause cardiovascular diseases and weight gain, while unsaturated fatty acids generally have positive effects on human health [2].

Medicinal and aromatic plants are widely used in these areas due to their potential to be a vegetable oil, functional food, drug active ingredient and cosmetic product. Among the medicinal and aromatic plants, lavender, thyme, and basil are from the Lamiaceae family and are among the plants used for these purposes [3]. Several studies have reported the fatty acids composition of some medicinal plant of Lamiaceae family such as: *Satureja*, *Thymus* and *Origanum* [4], *Salvia taxa* [5, 6, 7], *Ocimum basilicum* [8], *Lavandula* and *Salvia* [9]. The aim of this study was to determine the fatty acid composition of stems, leaves, flowers, and seeds of some medicinal plants in the eastern region of Anatolia. This is the first study to analyse four different parts of three different plants for fatty acids in Turkey, therefore it has an original value.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

L. intermedia was planted at a 100 cm distance between rows and a 40 cm distance between rows in 1 da on 2nd June 2020, *S. hortensis* was cultivated at a 40 cm distance row and 20 cm distance row in 1 da on 8th May 2020, and *O. basilicum* was planted at a 40 cm distance row and 40 cm distance row in 1 da on 22nd May 2021. In the flowering stage of each of the three mentioned plants the stems, leaves, flowers, and seeds were gathered collected from bee pasture in the Bingol province of Turkey between June and September 2021 after a sufficient maturity of plant seeds and prepared for analysis.

2.2. METHODS

2.2.1. Oil extraction and fatty acid methylation

Oil of the dried sample and derivatisation of fatty acids were extracted by revising the method of Hara and Radin (1978). At first, one g of plant samples was weighed. 5 ml of hexane/isopropanol (3:2) was added and vortexed. Then it was centrifuged at 4500 rpm for 10 minutes, and the upper part of the obtained material was filtered and transferred to test tubes and vortexed by adding 2.5 ml of 2% methanolic sulfuric acid. This mixture was kept at 50°C for 15 hours for methylation to occur, and after 15 hours, the tubes were removed and cooled at room temperature, and 2.5 ml of 5% NaCl was added and vortexed. In the next step, the fatty acid methyl esters formed in the tubes were extracted with 2.5 ml of hexane and the hexane phase was taken from the top with a Pasteur pipette and treated with 2.5 ml of 2% Na2CO3. After the above phase, the obtained material was taken and placed in test tubes, the mixture containing methyl esters was evaporated by nitrogen at 45°C. This procedure was carried out in three replications on all three plant materials. Finally, the fatty acids in test tubes were dissolved with 1 ml of hexane and taken into vials and stored in the GC-MS device.

2.2.2. Fatty acid and analysis

Extracted oil of the dried sample and derivatisation of fatty acids 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 in three replications. Chromatography was performed with a capillary column (60 m × 250 µm × 0.15 µm, J&W 122-7061) using nitrogen as a carrier gas (flow rate 34 ml/min). Chromatographic conditions: it started at 50°C, where it stood for 2 minutes and reached 200°C at a rate of 20°C/min and then accelerated to 230°C at 5°C /min where it stood for 30 minutes. Total analysis time was 55.5 min. MS results were determined by comparing the Whaley and NIST libraries in the device's memory.

3. RESULTS AND DISCUSSION

The stem, leaves, flowers, and seeds of some medicinal plants contain palmitic and stearic acids as the major component fatty acids, among the saturated acids, with small amounts of lauric, myristic, pentadecanoic, margaric, arachidic, heneicosanoic, behenic, tricosanoic, and lignoceric acids. The major unsaturated fatty acids found in the stem, leaves, flowers, and seeds of some medicinal plant oils were oleic, linoleic and liolenic acids. Pentadecenoic, hexadecatrienoic, margoleic, eicosenoic, eicosapentaoic, and docosaenoic acids were below 1%.

In this study, the average of total saturated fatty acids of the stem, leaves, flowers, and seeds of three medicinal plants were between 10.08 and 69.55% in three replications, while the average amount of total unsaturated fatty acids were between 30.45 and 89.92% in three replications. In all three plants, the highest saturated fatty acids were obtained from the plant stems, while the lowest saturated fatty acids were obtained from the plant seeds. Total percentage of saturated fatty acid of stem, leaf, flower, and seed of lavender were 69.55, 64.03, 45.77 and 10.08 respectively. Also, the total percentage of unsaturated fatty acid of lavender stem, leaf, flower, and seed were 30.45, 35.97, 54.23 and 89.92 respectively. Total percentage of saturated fatty acid of stem, leaf, flower, and seed of thyme were 41.71,16.24,18.22 and 11.68, respectively. Also, the total percentage of unsaturated fatty acid of thyme stem, leaf, flower, and seed were 58.29, 83.76, 81.78 and 88.34 respectively. Total percentage of saturated fatty acid of stem, leaf, flower, and seed of basil were 53.31, 38.12, 39.35 and 20.23 respectively. Also, the total percentage of unsaturated fatty acid of basil stem, leaf, flower, and seed were 46.69, 61.87, 60.65 and 79.79, respectively (Tab. I).

According to this study lauric acid was detected only in the leaves and stems of thyme and in the leaves of basil at a rate of 3.77%, 0.05% and 0.20%, respectively. Myristic acid obtained from stems, leaves, flowers, and seeds of some medicinal plants varied between 0.07% and 4.33%. The highest myristic acid was detected in lavender leaves, while the lowest myristic acid was detected in thyme seeds. Pentadecanoic acid was detected in other plant parts of thyme, lavender, and basil, except for the stem of thyme, stem, and leaves of basil, and varied between 0.03% and 0.53%. While some researchers have reported that lauric, myristic and pentadecanoic acids in Thymus capitatus were 0.703, 0.100 respectively [7], some researchers reported that lauric, myristic and pentadecanoic acids in some Lamiaceae taxa leaves were 0.05-0.14%, 0.23-1.46% and 0.17-0.59%, respectively [4]. In a study conducted in Pakistan, lauric acid was 0.85% and myristic acid was 0.36% in basil seeds [12]. In a study conducted in Tunisia, it was reported that myristic acid in basil leaves varied between 0.1-0.2% [8]. On the other hand, in a

Table I - Fatty acids of the stem, leaves, flowers and seeds of some medicinal plants

	Lavender					Thyme					Basil			
	Stem	**Leaf**	**Flower**	**Seed**		**Stem**	**Leaf**	**Flower**	**Seed**		**Stem**	**Leaf**	**Flower**	**Seed**
C 12:0	-	-	-	-		3.77	0.05	-	-		-	0.20	-	-
C 14:0	4.33	2.73	1.41	0.21		0.58	0.49	0.49	0.07		0.75	2.27	1.24	0.20
C 15:0	0.53	0.52	0.47	0.04		-	0.06	0.11	0.03		-	-	0.51	0.04
C 16:0	44.72	45.11	29.89	8.55		23.11	11.76	13.32	6.26		36.28	25.37	24.69	10.54
C 17:0	0.42	0.46	0.98	0.32		-	0.39	0.49	0.22		-	0.32	1.85	0.26
C 18:0	17.54	14.13	10.50	0.02		9.73	2.92	3.18	4.74		14.41	6.41	7.35	8.24
C 20:0	1.15	1.08	1.47	0.33		1.24	0.40	0.40	0.18		1.87	2.68	2.85	0.49
C 21:0	-	-	0.23	0.24		-	-	-	-		-	-	-	0.15
C 22:0	0.86	-	0.49	0.13		3.28	0.17	0.23	0.07		-	0.87	0.86	0.15
C 23:0	-	-	-	0.13		-	-	-	0.05		-	-	-	0.07
C 24:0	-	-	0.33	0.11		-	-	-	0.06		-	-	-	0.09
∑TSFA	**69.55**	**64.03**	**45.77**	**10.08**		**41.71**	**16.24**	**18.22**	**11.68**		**53.31**	**38.12**	**39.35**	**20.23**
C 15:1	-	-	-	-		-	-	-	0.02		-	-	-	-
C 16:1	1.35	1.41	0.74	0.61		-	0.36	0.46	0.17		-	1.88	0.38	0.65
C 16:3	-	-	-	0.02		-	-	-	-		-	-	-	-
C 17:1	-	-	-	0.04		-	-	-	0.02		-	-	-	0.02
C 18:1	7.35	6.12	8.72	24.41		7.49	8.37	7.98	11.46		6.84	6.41	6.21	14.07
C 18:2	5.57	5.23	10.01	18.76		19.10	23.04	22.60	27.40		15.11	9.91	19.92	23.02
C 18:3	16.18	23.21	34.76	39.76		31.70	51.74	47.97	43.79		24.74	43.68	33.74	36.43
C 20:1	-	-	-	0.56		-	0.10	-	0.22		-	-	0.40	0.15
C 20:2	-	-	-	-		-	0.05	-	0.14		-	-	-	-
C 20:3	-	-	-	5.70		-	0.10	2.77	5.08		-	-	-	5.41
C 22:1	-	-	-	0.06		-	-	-	0.02		-	-	-	0.02
∑TUSFA	**30.45**	**35.97**	**54.23**	**89.92**		**58.29**	**83.76**	**81.78**	**88.34**		**46.69**	**61.87**	**60.65**	**79.79**

C12:0 Lauric acid; C14:0 Myristic acid; C15:0 Pentadecanoic acid; C15:1 Pentadecenoic acid; C16:0 Palmitic acid; C16:1 Palmitoleic acid; C16:3 Hexadecatrienoic acid; C17:0: Margaric acid; C17:1 Margoleic acid; C18:0: Stearic acid; C18:1 Oleic acid; C18:2 Linoleic acid; C18:3 Linolenic acid; C20:0 Arachidic acid; C20:1 Eicosenoic acid; C20:2 Eicosapentaoic acid; C20:3 Eicosatrienoic acid; C21:0 Heneicosanoic acid; C22:0 Behenic acid; C22:1 Docosaenoic acid; C23:0 Tricosanoic acid; C24:0: Lignoceric acid; TSFA: Total saturated fatty acid; TUSFA: Total unsaturated fatty acid

study conducted in Sudan, myristic acid was found to be 0.11% and pentadecanoic acid was 0.04% in basil seeds [13].

Palmitic, stearic and arachidic acids, among the saturated fatty acids, were between 6.26-45.11%, 0.02-17.54% and 0.18-2.85%, respectively. The highest palmitic acid was obtained in the lavender leaf, the highest stearic acid was obtained in the lavender stem, and the highest arachidic acid was obtained in the basil flower. On the other hand, the lowest palmitic and arachidic acids were obtained in the thyme seeds, while the lowest stearic acid was detected in the lavender seeds. In a study on the chemical composition of some plants, palmitic and stearic acids of thyme were 2.91% and 0.77%, respectively, while palmitic and stearic acids of lavender were 5.83% and 1.53%, respectively [14]. In a study conducted to determine the fatty acid profiles of some *L.* species, it was reported that palmitic acid varied between 4.3-5.4%, stearic acid between 1.2-1.6% and arachidic acid between 0.1-0.2% [15]. While some researchers determined palmitic, stearic and archidic acids in basil leaves as 01.-0.2, 2.0-2.8 and 0.5-0.6%, respectively [8], some researchers found the same fatty acids in thyme as 1.196, 0.832 respectively [7]. On the other hand, in studies on basil seeds, palmitic, stearic and arachidic acids were reported as 6.8-8.8, 2.0-2.8 and 0.2% in Canada [16], 8.0-9.2, 3.6-3.8 and 0.2-0.3% in India [17], 1.6-7.5, 0.7-3.8 and 0.3% in Malaysia [18], 4.9, 2.5 and 0.25% in Iran [19]. Palmitic and stearic acids of basil were determined as 5-13% and 2-3% [20], and as 13.38% and 6.55% in Sudan [13], as 9.70% and 5.45% in Pakistan [12], and 6.23-10.16% and 2.97-4.88% in Iran [21]. It was reported that palmitic, stearic and arachidic acids of some Lamiaceae taxa were determined as 13.49-27.71%, 1.26-3.99% and 0.66-3.20%, respectively [4].

Margaric acid was detected in other plant parts of thyme, lavender, and basil except the stems of thyme and basil, while behenic acid was seen in other plant parts of thyme, lavender and basil except lavender leaves and basil stems. While the highest margaric acid observed in the flower of thyme with 1.85%, the highest behenic acid was detected in the leaf of basil with 0.87%, the lowest margaric and behenic acids were obtained in the seeds of thyme with 0.27% and 0.07%, respectively. Margaric and behenic acids were 0.015 and 0.002 g/kg of essential oil, respectively, in *Thymus capitatus* [7], while margaric and behenic acids were 0.65% and 0.17%, respectively, in *O. basilicum* seeds [13]. On the other hand, margaric and behenic acids of some Lamiaceae taxa were determined as 0.26-0.47% and 12.39-18.55%, respectively [4].

Heneicosanoic acid was detected only in lavender flowers and seeds and basil seeds, lignoceric acid in lavender, thyme and basil seeds and lavender flower, and tricosanoic acid only in lavender, thyme, and basil seeds. In the study examining the fatty acid profile of *Thymus capitatus*, lignoceric acid was 0.001 g/kg of essential oil [7], while in the study examining the fatty acid composition in *O. basilicum* seeds, heneicosanoic, tricosanoic and lignoceric acids it was 0.14%, 0.07% and 0.13%, respectively [13].

Pentadecenoic acid was found only in thyme seeds, while hexadecatrienoic acid was detected only in lavender seeds. On the other hand, margoleic acid exıstedonly in lavender, thyme, and basil seeds. It was reported that pentadecenoic acid of *Origanum* species was determined at 0.10-0.18% [4]. Palmitoleic acid was in other plant parts of lavender, thyme, and basil, except for the stems of thyme and basil, and varied between 0.17% and 1.88%. Palmitoleic acid was determined at 0.2-0.3% in basil seeds [16], 0.1-0.4% in some *L.* species [15], 0.4-0.5% in basil leaves [8], 0.2% in herb and seed of *O. basilicum* [17], 0.1% in seeds of *O. basilicum* [18], 0.30-1.70% in some Lamiaceae taxa [4], 0.07% in basil seeds [19], 0.022 g/kg of essential oil in *Thymus capitatus* [7] and 0.78% in *O. basilicum* seeds [13].

The main unsaturated acids in the oils of stems, leaves, flowers, and seeds of some medicinal plants are oleic, linoleic, and linolenic acids. The highest oleic acid content was in lavender seeds (24.41%) and the lowest in lavender leaves (6.12%). Linolenic acid was richer than linoleic acid in the oils of stems, leaves, flowers, and seeds of all medicinal plants. Linoleic acid was in the highest concentrations in the leaf, flower, and seed oil of thyme. The linoleic acid content was highest in the seeds of thyme (27.40%) but lowest in the leaves of lavender (5.23%). Linolenic acid was detected in low levels in the stems of lavender (16.18%). The linolenic acid content was highest in the leaves of thyme (51.74%).

According to a study on the fatty acid concentrations of the above-ground part of *Thymus capitatus*, fifteen fatty acids constituting 95.0% of the lipid content were identified and the two main fatty acid components reported, were linolenic (29.6%) and linoleic (15.1%) acids [22]. It has been reported that the main fatty acid composition of basil species is stearic acid, oleic acid, palmitic acid, linoleic acid, myristic acid, linolenic acid, carpic acid, lauric acid and arachidonic acid [23]. Oleic, linoleic and linolenic acids were determined as 8.7-11.6, 18.3-21.7 and 57.4-62.5% in basil seeds [16], as 4.37, 8.83 and 13.55% in lavender leaves, 4.33, 7.36 and 9.87% in thyme leaves [14], as 8.6-14.2, 9.5-16.5 and 64.9-73.0% in some *L.* species [15], as 6.0-10.0, 12.0-32.0 and 49.0-62.0% in fourteen basil accessions [20], as 13.33, 32.18 and 48.50% in basil seeds [12], as 1.4-2.0, 9.7-9.9 and 66.6-69.0% in *O. basilicum* leaves [8], as 10.3-12.3, 23.4-26.0 and 49.3-52.4% in herb and seed of *O. basilicum* [17], as 0.9-11.0, 1.8-19.1 and 6.1-50.1% in *O. basilicum* [18], as 2.50-8.29, 10.85-19.47 and 40.68-56.53% in some Lamiaceae taxa [4], as 7.55, 20.20 and 63.80% in basil seeds [19], as 6.22-19.92,

16.73-24.93 and 42.45%-61.85% in basil seeds [21], as 0.808, 0.231 and 0.094 g/kg of essential oil in *Thymus capitatus* [7], and as 0.01, 32.18 and 43.92% in *O. basilicum* seeds [13].

Eicosenoic acid was detected only in lavender seeds (0.56%) and flowers (0.40%) of and basil seeds (0.15%), thyme seeds (0.22%) and leaves (0.10%). On the other hand, eicosapentaoic acid was only obtained from thyme leaves and seeds. While eicosatrienoic acid was found at high levels in the lavender, thyme, and basil seeds (5.70, 5.08 and 5.41%, respectively), it was detected at a low rate in thyme leaves (0.10%) and flowers (2.77%). Docosaenoic acid was detected only at a very low rate in lavender (0.06%), basil (0.02%) and thyme (0.02%) seeds. While eicosenoic, eicosapentaoic and heneicosanoic acids were 0.036, 0.037 and 0.007 g/kg of essential oil in *Thymus capitatus*, respectively [7], eicosenoic acid was 0.2-0.4% in some *L.* species [13] and as 0.27% in basil seeds [13].

4. CONCLUSION

In this study, the fatty acid composition of stems, leaves, flowers, and seeds of three different medicinal plant species was investigated. Among the saturated acids, myristic, palmitic, stearic and arachidic acids were determined as the main component fatty acids. In addition, oleic, linoleic, and linolenic acids were found as the main unsaturated fatty acids. As a result, there are qualitative and quantitative differences in the fatty acid composition of stem, leaves, flowers, and seeds of medicinal plant species depending on collection time, environmental factors, climatic factors, genetics, season, analysed plant part, analytical methods.

Conflict of interest

The authors declare they have no conflict of interest.

Acknowledgment

This study was supported by Bingol University Pilot University Coordination Centre Unit (Project No: PIKOM-Bitki.2019.001). We would like to thank the Pilot University Coordination Central Unit for the support given.

BIBLIOGRAPHY

[1] E. Karaca, S. Aytac, The factors affecting on fatty acid composition of oil crops. Anadolu Journal of Agricultural Sciences 22(1), 123-131 (2007).

[2] S. Cakmakci, D. Kahyaoglu Tahmas, Effects of fatty acids on health and nutrition. Turkish Journal of Scientific Reviews 5(2), 133-137 (2012).

[3] M. Varli, H. Hanci, G. Kalafat, Production potential and bioavailability of medicinal and aromatic plants. Research Journal of Biomedical and Biotechnology 1, 24-32 (2020).

[4] E. Cacan, K. Kokten, O. Kilic, Leaf fatty acid composition of some Lamiaceae taxa from Turkey. Progress in Nutrition 20(1), 231-236 (2018).

[5] A. Bakoglu, O. Kilic, K. Kokten, Fatty acid composition of the leaves of some *Salvia* taxa from Turkey. Chemistry of Natural Compounds 52(4), 676-678 (2016).

[6] E. Tulukcu, Determination of fatty acid compositions of various plant parts of *Salvia sclarea*. European Journal of Science and Technology 20, 957-960 (2020).

[7] A. Zaïri, S. Nouir, A. Zarrouk, H. Haddad, A. Khélifa, L. Achour, F. Tangy, M. Chaouachi, M. Trabelsi, Chemical composition, fatty acids profile and biological properties of *Thymus capitatus* (L.) Hoffmanns, essential oil. Scientific Reports 9, 20134 (2019).

[8] I. Tarchoune, O. Baâtour, J. Harrathi, G. Hamdaoui, M. Lachaâl, Z. Ouerghi, B. Marzouk, Effects of two sodium salts on fatty acid and essential oil composition of basil (*O. basilicum* L.) leaves. Acta Physiologiae Plantarum 35, 2365-2372 (2013).

[9] E. Tulukcu, Herbal tea fatty acid contents of some medicinal plants grown in Konya, Turkey. Asian Journal of Chemistry 23(3), 1369-1372 (2011).

[10] A. Hara, N.S. Radin, Lipid extraction of tissues with a low toxicity solvent. Analytical Biochemistry 90, 420-436 (1978).

[11] W.W. Christie, Gas chromatography and lipids: A practical guide. The Oily Press. Ayr. pp. 307 (1990).

[12] A. Bilal, N. Jahan, A. Ahmed, S.N. Bilal, S. Habib, S. Hajra, Phytochemical and pharmacological studies on *O. basilicum* Linn-A review. International Journal of Current Research and Review 4, 73-83 (2012).

[13] A.A. Idris, A.H. Nour, M.M. Ali, I.Y. Erwa, O.A.O. Ishag, A.H. Nour, Physicochemical properties and fatty acid composition of *O. basilicum* L. seed oil. Asian Journal of Physical and Chemical Sciences 8(1), 1-12 (2020).

[14] M.H. Oh, H.J. Whang, Chemical composition of several herb plants. Korean Journal of Food Science and Technology 35(1), 1-6 (2003).

[15] N.A.R. Urwin, R.J. Mailer, Oil content and fatty acid profiles of seed oil from the genus *L.*. Journal of the American Oil Chemists' Society 85, 491-492 (2008).

[16] P. Angers, M.R. Morales, J.E. Simon, Basil seed oils. In: J. Janick (ed.), Progress in new crops. ASHS Press, Arlington, VA. 598-601 (1996).

[17] S.P. Kakaraparthi, S.K. Srinivas, K.J. Kumar, N.A. Kumar, A. Kumar, Composition of herb and seed oil and antimicrobial activity of the essen-

tial oil of two varieties of *O. basilicum* harvested at short time intervals. Journal of Plant Development 22, 59-76 (2015).
[18] Z. Amini, H.C. Ong, M.D. Harrison, F. Kusumo, H. Mazaheri, Z. Ilham, Biodiesel production by lipase-catalyzed trans esterification of *O. basilicum* L. (Sweet basil) seed oil. Energy Conversion and Management 132, 82-90 (2017).
[19] Z. Ghaleshahi, H. Ezzatpanah, G. Rajabzadeh, Comparison and analysis characteristics of flax, perilla and basil seed oils cultivated in Iran. Journal of Food Science and Technology 57, 1258-1268 (2019).
[20] A.H. Nour, S.A. Elhussein, N.A. Osman, A.H. Nour, Characterization and chemical composition of the fixed oil of fourteen basil (*O. basilicum* L.) accessions grown in Sudan. International Journal Technology 1(2), 52-58 (2009).
[21] S. Mostafavi, H.A. Asadi-Gharneh, M. Miransari, The phytochemical variability of fatty acids in basil seeds (*O. basilicum* L.) affected by genotype and geographical differences. Food Chemistry 276, 700-706 (2019).
[22] L. Tabti, M.E.A. Dib, N.G. Benyelles, N. Djabou, S.B. Alam, J. Paolini, J. Costa, A. Muselli, Fatty-acid composition and antifungal activity of extracts of *Thymus capitatus*. Journal of Herbs, Spices & Medicinal Plants 21(2), 203-210 (2015).
[23] M. H. Shahrajabian, W. Sun, Q. Cheng, Chemical components and pharmacological benefits of basil (*O. basilicum*): a review. International Journal of Food Properties 23(1), 1961-1970 (2020).

Essential oils composition and bioactivities of most prevalent species of genus *Piper* in Malaysia: a scope review

Nik Nur Asyiqin Nik Mohammed Ainul Azman[1]
Wan Mohd Nuzul Hakimi Wan Salleh[1]✉
Nurunajah Ab Ghani[2,3]

[1]Department of Chemistry
Faculty of Science and Mathematics
Universiti Pendidikan Sultan Idris
Tanjong Malim, Perak, Malaysia

[2]Atta-ur-Rahman Institute for Natural Product Discovery (AuRIns),
Universiti Teknologi MARA,
Puncak Alam Campus,
Bandar Puncak Alam,
Selangor, Malaysia

[3]Faculty of Applied Sciences,
Universiti Teknologi MARA,
Shah Alam, Selangor, Malaysia

✉ CORRESPONDING AUTHOR:
E-mail: wmnhakimi@fsmt.upsi.edu.my
Phone: +6015-48797123

Received: October 26, 2022
Accepted: December 8, 2022

The genus *Piper* is among the most important genera in the Piperaceae family, consisting of over 2000 species and widely distributed globally, mainly in the Southeast Asian region. It is known for several medicinally and economically important species that have been used throughout their native range. *Piper* species have great diversity in the world's tropical regions and are represented mainly by aromatic shrubs and trees with significant production of essential oils. Essential oils are largely consumed as they are beneficial to humans as natural remedies and have been known for years to find usefulness in foods, drugs, and cosmetics. In recent years, studies on the essential oils of the Malaysian *Piper* species have been progressing, and many of them have reported interesting pharmacological activities. This review attempts to summarize information on the essential oils from Malaysian *Piper* species concerning their medicinal uses, chemical composition, and bioactivities. Information on the *Piper* species was collected via electronic search (Pubmed, SciFinder, Scopus, Google Scholar, and Web of Science) and a library search for articles published in peer-reviewed journals. Throughout our literature review, seventeen *Piper* species have been studied for their essential oil compositions in Malaysia. They were found to contain mainly of β-phellandrene, safrole, β-caryophyllene, and aromadendrene. As for bioactivities, antimicrobial, antioxidant, and cytotoxicity activities were the most reported. This review is mainly meant to provide relevant information on the chemical components and features of Malaysian *Piper* species, with an emphasis on essential oils, providing guidance for the selection of accessions or species with the best chemical profiles.

Keywords: Piperaceae, *Piper*, essential oil, composition, caryophyllene, antimicrobial

1. INTRODUCTION

Plants are a versatile source of bioactive metabolites, including polysaccharides, phenolics, alkaloids, steroids, lignins, resins, tannins, as well as essential oils [1]. Among them, essential oils obtained from plants have various applications, especially in the health, agriculture, food, and cosmetic industries. Thus far, over 3000 essential oils have been isolated from about 2000 plant species, of which 300 have been commercially used for various purposes [2]. Previous scientific studies clearly revealed that essential oils possess various pharmacological properties such as antioxidant, antimicrobial, antiviral, antimutagenic, anticancer, anti-inflammatory, and immunomodulatory activities [3].

The genus *Piper* is one of the largest and most important aromatic and medicinal plants of the Piperaceae family, which comprises four genera and approximately 2000 species distributed in the tropical and subtropical regions [4]. *Piper* and *Peperomia* are the most representative of the Piperaceae family [5]. A common description for Piperaceae taxon includes spiral-leaves,

simple stipulate or not. The inflorescence is a spike or spadix with minute flowers uni- or bisexual, bracteate, perianth absent (flowers naked without sepals or petals). The flowers only have a single pistil and stamens [6-7].

Piper species are used all over the world in traditional remedies in the Indian Ayurvedic system of medicine and in folklore medicine of Latin America and the West Indies [8]. Economically, *Piper* known as a worldwide spice market as their leaves, stems, roots, and fruit have their own uses. In Malaysia, the leaves of *P. betle* (sireh) were used for mastication and also for relieving constipation in children and poulticing ulcerated noses. The leaves were often heated and applied to the chest to relieve coughs and asthma [9]. The decoction of *P. sarmentosum* (kadok) leaves was used as an embrocation to cure pains in bones and applied to the foreheads of children suffering from headaches [10]. The leaves of *P. nigrum* were used for stomachaches, treatment of coughs, and seasoning [11].

The chemistry of *Piper* species has been widely investigated, and the phytochemical investigations from all parts have led to the isolation of a number of pharmacologically active compounds such as alkaloids, amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, and flavonoids. They have been extensively investigated as a source of new natural products with potential antioxidant, antimicrobial, antifungal, anticholinesterase, anti-inflammatory, anti-tyrosinase, and insecticidal activities [12-17].

Recently, essential oils and other aromatic compounds sourced from plants and used as alternative medicine are gaining interest. Hence, the review regarding *Piper* essential oils has to be done to simplify and compile the information. The information was collected via electronic searches in databases such as Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. This review aims to give an overview of all published reports on the chemical composition, biological activities, and medicinal uses of Malaysian *Piper* essential oils.

2. SEARCH STRATEGY

The protocol for performing this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [18] (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied and (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again. Figure 1 shows the flow diagram of the identification and selection of articles. Following this step, we reached the articles chosen for the present study. This systematic review was conducted through searches using Scopus, PubMed, Science Direct, SciFinder, and Google Scholar. The keywords used were '*Piper*', 'essential oil', and 'biological activity' articles over the period from the beginning of the database until October 2022.

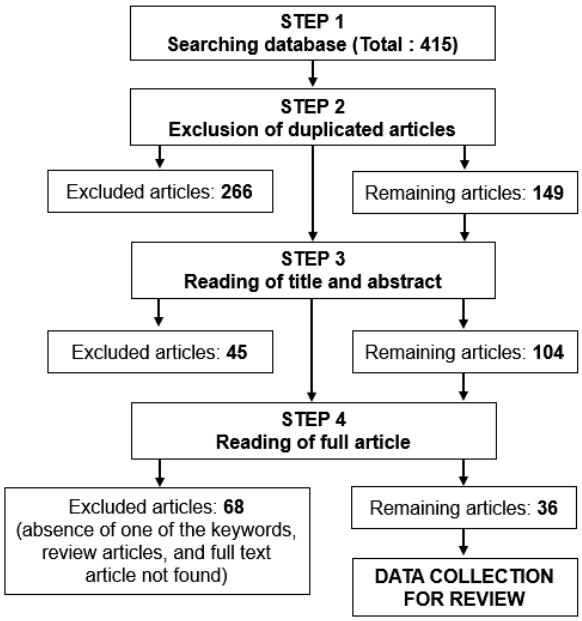


Figure 1 - PRISMA flow diagram of included studies

In addition, as a second search strategy, we included studies obtained by a manual search of the reference lists of the included studies. Articles on the genus of *Piper* that reported from Malaysia (traditional uses, essential oils, and bioactivities) were included. The inclusion of articles considered the following criteria: (1) type of publication - original research articles, (2) only articles in English, (3) articles must present the chemical composition of Malaysian *Piper* essential oils, (4) articles must discuss the bioactivity of the essential oils. As exclusion criteria, the following were used: (1) articles that did not present the search terms in the title and abstract; (2) review articles, (3) full-text articles not found, (4) articles without one of the keywords and (5) articles that did not present the composition of the essential oils.

3. MEDICINAL USES

Piper species have been known for their medicinal benefits for ages. People have been using the parts of the plant to extract essential oils for various reasons. The fruits, roots, leaves, and barks of these plant species are adopted for the therapy of diseases in different approaches, including (i) pharmaceutics (e.g. decoction, pill, powder, etc.) of signal medicine, or compound preparations with other traditional Chinese medicines; (ii) drunk as tea; (iii) eaten. The essential oils obtained are traditionally consumed as daily remedies to treat discomfort and maintain health both physically and mentally [19]. Table I shows the medicinal uses of Malaysian *Piper* species [20-32].

Table I - Medicinal uses of several Malaysian *Piper* species

Species	Part	Medicinal uses
P. abbreviatum	Leaf	Splenomegaly, stimulant, carminative, coughs & colds, flatulence [20]
P. aduncum	Leaf	Treating wounds, skin boils, infections, and diarrhea [21]
	Root	Bleeding control as antihemorrhagic [22]
P. arborescens	Leaf	Rheumatism, antiplatelet aggregation, cytotoxic [23]
P. betle	Leaf	Aromatic flavor and mouth freshness [24]
P. caninum	Leaf	Chewing, hoarseness, flavor, throat ache antiseptic [25]
P. cubeba	Leaf	Uses as diuretic and stimulant in cases of fever, gout andangina [26]
P. miniatum	Leaf	Spice, food flavour, food natural preservative [27]
P. nigrum	Leaf	To relief pain, atrophic arthritis, apathy,influenza, and febricity,and antibacterial agent,stimulant,digestive,and antitoxin [28]
P. officinarum	Fruit	As digestive, tonic, carminative in asthma, bronchitis, gastrointestinal ulsers, diarrhea or postpartum hemorrhage [29]
P. porphyrophyllum	Leaf	Leprosy, abdominal pain, skin disease, postpartum treatment, bone pain [30]
P. stylosum	Leaf	Vegetables, seasoning, poultice/ decoction, confinement [31]
P. ribesioides	Leaf	Asthma, diarrhoea, abdominal pain, flavour, alleviate chest congestion, treat urticarial [32]

4. CHEMICAL COMPOSITIONS OF THE ESSENTIAL OILS

In earlier reports, eighteen *Piper* species were described on the essential oil composition. These were *P. abbreviatum*, *P. erecticaule*, *P. lanatum*, *P. aduncum*, *P. arborescens*, *P. betle*, *P. caninum*, *P. pedicellosum*, *P. penangense*, *P. magnibaccum*, *P. maingayi*, *P. miniatum*, *P. nigrum*, *P. officinarum*, *P. porphyrophyllum*, *P. stylosum*, *P. ribesioides* and *P. muricatum.* Most of the species are reported from Sarawak, as well as from Kelantan, Perak, Johor, Selangor, and Terengganu. The extraction of the essential oils was done mostly from the leaf part, along with fruit and stem. The essential oil of *P. miniatum* has the highest total components, which was found to have sixty-four components, while the essential oil of *P. arborescens* has the highest percentage that contributed for about 97.5% of the total oils. Analysis of chemical components identified in the *Piper* essential oils shows that the oil consists of several groups of components, which are phenylpropanoids, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Table II shows the major components identified in *Piper* essential oils from various states of Malaysia [33-47].

The major component of *Piper* essential oils consists mainly of sesquiterpene hydrocarbons. β-Caryophyllene was identified as a major component in the leaf oil of *P. erecticaule* [33], leaf and stem oils of *P. maingayi* [40]*, P. officinarum* [44], and *P. ribesioides* [46]. Meanwhile, δ-cadinene was found dominantly in the fruit oil of *P. maingayi* [41]. In other studies, germacrene D was identified as a principal component from *P. magnibaccum* (leaf oil) [39], bicyclogermacrene from *P. porphyrophyllum* (leaf oil) [45], whereas aromadendrene from *P. stylosum* (leaf and stem oils) [46] and *P. muricatum* (whole plant) [47]. In the case of oxygenated sesquiterpenes, spathulenol, (E)-nerolidol, and caryophyllene oxide were identified by their richness in the leaf oil of *P. abbreviatum* [33], *P. penangense* [38], and *P. miniatum* [27], respectively.

Besides, monoterpene hydrocarbons were found in several *Piper* essential oils. β-Phellandrene was characterised as the main component in leaf and stem oils of *P. arborescens* [35] as well as the leaf oil of *P. pedicellosum* [38]. Meanwhile, sabinene and β-pinene was identified in the stem oil of *P. porphyrophyllum* [45] and the leaf oil of *P. nigrum* [42], respectively. On the other hand, boneol was the only oxygenated monoterpene present in *Piper* oil, which was detected from the leaf oil of *P. lanatum* [33]. Furthermore, phenylpropanoids were also found in Malaysian *Piper* oils. Safrole was detected mainly in the leaf and stem oils of *P. caninum* [37]. In addition, eugenol and apiole were characterised mainly in the leaf oil of *P. betle* [36] and *P. aduncum* [34], respectively.

Based on the above results, the chemical differences between species of *Piper* could be due to the stages of development, and distinct habitat in which the plant was collected. Besides, the chemical and biological diversity of aromatic, and medicinal plants depends on factors such as climatic conditions, phase of vegetation, and genetic modifications. Such variables affect the biosynthetic pathways of the plant and therefore, the relative proportion of the main characteristic compounds [33].

Furthermore, knowledge of the factors that determine the chemical variability and yield for each species are very important in particular for commercially important species, to optimise the conditions that may affect yields and quality of essential oils. In addition to the commercial importance of the variability in yield and composition, the possible changes are also important when the essential oils and volatile are used as chemo taxonomic tools [49, 50].

5. BIOACTIVITIES

The literature study reveals the need for a thorough investigation of the pharmacological characteristics of

Table II - Major components identified from the essential oils of Malaysian *Piper* species

Species	Locality	Part	Total (No. %)	Method (Yield)	Major components
P. miniatum	Selangor	Leaf	64; 89.2%	Hydrodistillation (0.45%)	Caryophyllene oxide (20.3%) and α-cubebene (10.4%) [27]
P. abbreviatum	Sarawak	Leaf	33; 70.5%	Hydrodistillation (0.22%)	Spathulenol (11.2%), (*E*)-nerolidol (8.5%), β-caryophyllene (7.8%) and ar-curcumene (5.8%) [33]
P. erecticaule	Sarawak	Leaf	35; 63.4%	Hydrodistillation (0.18%)	β-Caryophyllene (5.7%), spathulenol (5.1%), β-cadinene (3.8%) and α-amorphene (3.8%) [33]
P. lanatum	Sarawak	Leaf	39; 78.2%	Hydrodistillation (0.25%)	Borneol (7.5%), caryophyllene oxide (6.6%) and α-amorphene (5.6%) [33]
	Selangor	Leaf	38; 90.6%	Hydrodistillation (1.34%)	Chavibetol (42.7%), β-caryophyllene (6.8%), α-cadinene (6.6%), α-muurolene (6.2%), eugenol acetate (5.9%) and γ-elemene (5.4%) [38]
P. aduncum	Selangor	Leaf	35; 38.0%	Hydrodistillation (NS)	Apiole (38.0%), methyl isobutyl ketone (8.2%), piperitone (3.3%) and caryophyllene (2.4%) [34]
	Selangor	Leaf	32; 90.8	Hydrodistillation (1.30%)	Dillapiole (64.5%), β-selinene (5.2%) and β-caryophyllene (5.1%) [38]
P. arborescens	Sarawak	Leaf	36; 97.5%	Hydrodistillation (0.24%)	β-Phellandrene (24.3%), sabinene (16.3%) and α-pinene (10.4%) [35]
		Stem	46; 90.5%	Hydrodistillation (0.16%)	β-Phellandrene (20.4%), methyl eugenol (11.0%) and β-caryophyllene (9.0%) [35]
P. betle	Kelantan	Leaf	11; 98.9%	Hydrodistillation (NS)	Eugenol (15.6%) [36]
	Selangor	Leaf	15; 96.6%	Hydrodistillation (5.10%)	Chavibetol (69.0%), eugenol acetate (8.3%), chavicol (6.0%) and γ-muurolene (5.2%) [38]
P. caninum	Perak	Leaf	36; 77.9%	Hydrodistillation (0.46%)	Safrole (17.1%), β-pinene (8.9%), linalool (7.0%) and β-caryophyllene (6.7%) [37]
		Stem	37; 87.0%	Hydrodistillation (0.31%)	Safrole (25.5%), β-caryophyllene (9.8%), germacrene D (7.8%), β-pinene (4.9%) and δ-elemene (4.1%) [37]
P. pedicellosum	Selangor	Leaf	NS	Hydrodistillation (1.11%)	β-Phellandrene (21.9%) [38]
P. penangense	Selangor	Leaf	NS	Hydrodistillation (0.23%)	(*E*)-Nerolidol (17.5%) [38]
P. magnibaccum	Perak	Leaf	25; 93.5%	Hydrodistillation (0.02%)	Germacrene D (40.8%), α-caryophyllene (8.5%) and α-cadinol (6.1%) [39]
		Stem	33; 87.6%	Hydrodistillation (0.09%)	β-Caryophyllene (19.7%), germacrene D (10.7%) and α-cadinol (8.2 %) [39]
P. maingayi	Johor	Leaf	43; 91.2%	Hydrodistillation (0.21%)	β-Caryophyllene (39.6%) [40]
		Stem	34; 83.6%,	Hydrodistillation (0.09%)	β-Caryophyllene (26.2%), α-cedrene (8.4%), caryophyllene oxide (6.7%) and *cis*-calamenene (6.2%) [41]
		Fruit	18; 78.7%	Hydrodistillation (0.17%)	δ-Cadinene (22.6%), β-caryophyllene (18.8%), α-copaene (11.2%) and α-cadinol (7.1%) [41]
P. nigrum	Sarawak	Leaf	39; 64.0%	Hydrodistillation (0.80%)	β-Pinene (12.9%) and linalool (9.5%) [42]
		Seed	40; 99.8%	Hydrodistillation (3.34%)	β-Caryophyllene (24.3%), limonene (15.8%), sabinene (15.0%) [43]
P. officinarum	Sarawak	Leaf	41; 85.0%	Hydrodistillation (0.26%)	β-Caryophyllene (11.2%), α-pinene (9.3%), sabinene (7.6%), β-selinene (5.3%) and limonene (4.6%) [44]
		Stem	41; 93.0%	Hydrodistillation (0.22%)	β-Caryophyllene (10.9%), α-phellandrene (9.3%), linalool (6.9%), limonene (6.7%) and α-pinene (5.0%) [44]
P. porphyrophyllum	Sarawak	Leaf	34; 97.3%	Hydrodistillation (0.20%)	Bicyclogermacrene (14.7%), α-copaene (13.2%), β-phellandrene (9.5%), α-cubebene (7.4%) and β-caryophyllene (6.4%) [45]
		Stem	38; 95.5%	Hydrodistillation (0.18%)	Sabinene (15.5%), bicyclogermacrene (12.3%), α-copaene (8.1%), α-pinene (7.8%) and β-caryophyllene (7.1%) [45]
P. stylosum	Terengganu	Leaf	50; 89.2%	Hydrodistillation (0.08%)	Aromadendrene (26.6%), β-caryophyllene (11.5%) and sabinene (13.8%) [46]
		Stem	45; 88.8%	Hydrodistillation (0.07%)	Aromadendrene (18.8%), β-caryophyllene (17.9%) and sabinene (6.7%) [46]
P. ribesioides	Terengganu	Leaf	60; 87.0%	Hydrodistillation (0.03%)	β-Caryophyllene (20.0%), camphene (16.3%) and δ-cadinene (4.4%) [46]
		Stem	39; 82.9%	Hydrodistillation (0.04%)	β-Caryophyllene (14.4%), camphene (12.3%) and δ-cadinene (7.8%) [46]
P. muricatum	Terengganu	Whole plant	40; 90.8%	Hydrodistillation (0.46%)	Aromadendrene (16.2%), β-caryophyllene (8.8%), germacrene D (7.9%), γ-cadinene (7.9%), elemol (5.4%), γ-elemene (4.9%) and bicyclogermacrene (4.4%) [47]

*NS- not stated

the essential oils of *Piper* species. The biological activities including anti-microbial, antioxidant and cytotoxicity activities have been reported in some works. Indeed, the genus *Piper* has been exploited traditionally, thus revealing the medicinal variation it possesses. In addition, several species that have been used traditionally to treat some types of ailments have not been investigated for their bioactivities at all. Thus, this is an opportunity to find new pharmacological properties from this genus, and the information on the qualification of the essential oils is very important to be applied in functional food and pharmaceutical areas.

In the case of Malaysian *Piper* essential oils, antimicrobial [33,35,39,44-47] and antioxidant [37,39,41,44,46] activities have been reported and the details are described in Table III and Table IV, respectively.

For antimicrobial activity activity, four *Piper* essential oils have shown a good activity towards Gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) which are *P. abbreviatum* [33], *P. arborescens* [35], *P. stylosum* [46], and *P. muricatum* [47]. Meanwhile, the essential oils of *P. magnibacum* [39], *P. officinarum* [44], and *P. porphyrophyllum* [45] have shown significant activity towards Gram negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, and *Pseudomonas putida*, respectively. On the other hand, *P. erecticaule* and *P. lanatum* have shown antifungal activity towards *Aspergillus niger* [33]. β-Caryophyllene, germacrene D, bicyclogermacrene,

Table III - Antimicrobial activities of the essential oils of Malaysian *Piper* species

Species	Description
P. erecticaule	The leaf oil showed activity towards *Aspergillus niger* with MIC value of 31.3 µg/mL [33]
P. lanatum	The leaf oil showed activity towards *Aspergillus niger* with MIC value of 62.5 µg/mL [33]
P. abbreviatum	The leaf oil showed activity towards *Bacillus cereus, Staphylococcus aureus*, and *Enterococcus faecalis* with MIC value of 250 µg/mL each [33]
P. arborescens	The leaf oil showed activity towards *Bacillus subtilis*, *Staphylococcus aureus* and *Aspergillus niger* with MIC values of 500, 250 and 500 µg/mL, respectively [35]
	The stem oil showed activity towards *Pseudomonas putida* and *Apergillus niger* with MIC value of 500 µg/mL each [35]
P. magnibacum	The leaf oil showed activity towards *Pseudomonas aeruginosa* with MIC value of 250 µg/mL [39]
	The stem oil showed activity towards *Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli* with MIC value of 500 µg/mL [39]
P. officinarum	The leaf and stem oils showed activity towards *Escheric coli* and *Pseudomonas aeruginosa* with MIC value of 250 µg/mL each [44]
P. porphyrophyllum	The leaf and stem oils showed activity towards *Pseudomonas putida* with MBC values of 250 and 125 µg/mL, respectively [45]
	The leaf and stem oils showed activity towards *Pseudomonas putida* with MBC values of 500 and 250 µg/mL, respectively [45]
P. stylosum	The leaf and stem oils showed activity towards *Bacillus cereus* and *Staphylococcus aureus* with MIC value of 125 µg/mL [46]
P. muricatum	The leaf oil showed activity towards *Bacillus cereus, Streptococcus mutans* and *Pseudomonas aeruginosa* with MIC value of 250 µg/mL [47]

MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration

Table IV - Antioxidant activities of the essential oils of Malaysian *Piper* species

Species	Method	Description
P. caninum	DPPH	The leaf and stem oils showed activity with IC_{50} values of 187.6 and 452.4 mg/mL, respectively [37]
	β-carotene	The leaf and stem oils showed activity with percentage values of 103.5 and 114.9%, respectively [37]
P. magnibacum	DPPH	The leaf and stem oils showed activity with IC_{50} values of 20.5 and 17.5 µg/mL, respectively [39]
	ABTS	The leaf and stem oils showed activity with IC_{50} values of 11.7 and 12.9 µg/mL, respectively [39]
P. maingayi	ABTS	The stem and fruit oils showed activity with IC_{50} values of 12.6 and 13.9 µg/mL, respectively [41]
	DPPH	The stem and fruit oils showed activity with IC_{50} values of 14.9 and 20.8 µg/mL, respectively [41]
	TPC	The stem and fruit oils gave total phenolic content of 176.8 and 279.6 mg GA/g, respectively [41]
	β-carotene	The stem and fruit oils showed activity with percentage values of 91.8 and 83.6%, respectively [41]
P. officinarum	DPPH	The leaf and stem oils showed weak activity with IC_{50} values of 622.2 and 777.4 µg/mL, respectively [44]
P. stylosum	DPPH	The leaf and stem oils showed weak activity with IC_{50} values 605.8 and 623.2 µg/mL, respectively [46]
	TPC	The leaf and stem oils gave total phenolic content of 15.4 and 18.2 mg GA/g, respectively [46]
P. ribesiodes	DPPH	The leaf and stem oils showed activity with IC_{50} values of 831.5 and 692.4 µg/mL, respectively [46]
	TPC	The leaf and stem oils gave total phenolic content of 20.5 and 24.8 mg GA/g, respectively [46]

DPPH – 2,2-diphenyl-1-picrylhydrazyl; TPC – Total phenolic content; ABTS – 2,2'-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid)

and aromadendrene are among the major components identified from the above *Piper* essential oils which have shown potential against Gram positive bacteria, Gram negative bacteria, and fungus.
For antioxidant activity, six *Piper* essential oils have been reported using DPPH assay. However, most of the essential oils showed weak activity. It could be due to the presence of sesquiterpenes as major components. β-Caryophyllene has been found as a major component from *P. magnibacum* [39], *P. maingayi* [41], *P. officinarum* [44], and *P. ribesioides* [46], as well as germacrene D from *P. magnibacum* [39] and aromadendrene from *P. stylosum* [46] essential oils.
In addition, the cytotoxicity activity was also reported from the leaf and stem oils of *P. magnibacum* [39] which showed an activity against A-549 with inhibition values of 88.0% and 77.3%, as well as against MCF-7 with inhibition values of 88.7% and 91.3%, respectively [39]. In another study, the leaf oil of *P. aduncum* exhibited a high activity against *Aedes albopictus* with ED_{50} and ED_{90} values of 1.5 and 17.6 μg/cm^2, respectively [48].

6. CONCLUSION

This article aims to give the relevant literature on the medicinal uses, chemical compositions, and bioactivity information of the Malaysian *Piper* essential oils. The studies managed to declare that the essential oils of *Piper* species contain monoterpenes and sesquiterpenes, which potentially stimulate bioactivities such as antioxidant and antimicrobial properties. The diversity of quantitative and qualitative components observed could be due to genetic differences or to the environmental conditions of the plant material based on different geographic locations. More pharmacological investigations into other pharmacological activities should be performed to unravel the full therapeutic potential of Piper species. Furthermore, preclinical analyses as well as clinical trials, as conducted for essential oils from other species, are required to evaluate the potential of essential oils from *Piper* species for drug development. This information is also critical when selecting species that have an economic potential for the pharmaceutical and cosmetics industries.

REFERENCES

[1] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, R.M. Zulkifli. Essential oil compositions of Malaysian Lauraceae: A mini review. Pharm. Sci. 22, 60-67 (2016).

[2] J.S. Raut, S.M. Karuppayil. A status review on the medicinal properties of essential oils. Ind. Crops Prod. 62, 250-264 (2014).

[3] F. Bakkali, S. Averbeck, D. Averbeck, M. Idaomar. Biological effects of essential oils-A review. Food Chem. Toxicol. 46, 446-475 (2008).

[4] V.S. Parmar, S.C. Jain, K.S. Bisht, R. Jain, P. Taneja, A. Jha, O.D. Tyagi, A.K. Prasad, J. Wengel, C.E. Olsen, P.M. Boll. Phytochemistry of the genus *Piper*. Phytochemistry 46, 597-673 (1997).

[5] M.J. Kato, M. Furlan. Chemistry and Evolution of the Piperaceae. Pure Appl. Chem. 79, 529-538 (2007).

[6] W.S. Judd, C.S. Campbell, E.A. Kellogg, P.F. Stevens, M.J. Donoghue. Plant systematics, a phylogenetic approach, 3rd ed. Sinauer Associates, Inc. Publishers, Massachusetts (2008).

[7] D.J. Mabberley. The plant book, a portable dictionary of the vascular plants, 2nd ed. Cambridge University Press, Cambridge (1997).

[8] P.R.D. Dos Santos, D.L. Moreira, E.F. Guimaraes, M.A.C. Kaplan. Essential oil analysis of 10 Piperaceae species from the Brazilian Atlantic Forest. Phytochemistry 58, 547-551 (2001).

[9] N.I. Azahar, N.M. Mokhtar, M.A. Arifin. *Piper betle*: a review on its bioactive compounds, pharmacological properties, and extraction process. IOP Conf. Series: Mat. Sci. Eng. 991(1), 12044 (2020).

[10] A. Azelan, Z.M. Taher, S. Sasano, T. Ariga, A.A. Aziz. Chemical constituents and bioactivity of *Piper sarmentosum*: A mini review. Food Res. 4, 14-18 (2020).

[11] M. Rezvanian, Z.T. Ooi, J.A. Jamal, K. Husain, J. Jalil, Z. Yaacob, H.F. Mohamad, A.A. Ghani. Pharmacognostical and chromatographic analysis of Malaysian *Piper nigrum* Linn. fruits. Indian J. Pharm. Sci. 78(3), 334-343 (2016).

[12] A.A. Durant-Archibold, A.I. Santana, M.P. Gupta. Ethnomedical uses and pharmacological activities of most prevalent species of genus *Piper* in Panama: A review. J. Ethnopharmacol. 217, 63-82 (2018).

[13] J. Chahal, R. Ohlyan, A. Kandale, A. Walia, S. Puri. Introduction, phytochemistry, traditional uses and biological activity of genus *Piper*: A review. Int. J. Curr. Pharm. Rev. Res. 2(2), 130-144 (2011).

[14] W.M.N.H.W. Salleh. A systematic review of botany, phytochemicals and pharmacological properties of 'hoja santa' (*Piper auritum* Kunth). Z. Naturforsch., C, J. Biosci. 76(3-4), 93-102 (2020).

[15] W.M.N.H.W. Salleh, H. Kassim, A. Tawang. Traditional uses, chemical profile and biological activities of *Piper hispidum* Sw.: a review. Biointerface Res. Appl. Chem. 11(5), 13115-13129 (2021).

[16] W.M.N.H.W. Salleh, F. Ahmad. Phytopharmacological investigations of *Piper retrofractum* Vahl. – a review. Agric. Conspec. Sci. 85(3), 193-202 (2020).

[17] W.M.N.H.W. Salleh. Phytochemistry and anti-inflammatory activities of *Piper kadsura* (Choisy)

Ohwi – a review. B. Latinoam. Caribe. Pl. 19(3), 289-299 (2020).
[18] D. Moher, A. Liberati, J. Tetzlaff, G.A. Douglas, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. Ann. Intern. Med. 151, 264-269 (2009).
[19] Editorial Committee of Zhonghua Bencao National Traditional Chinese Herb Administration. Zhonghua Bencao. Shanghai Scientific and Technical Publishing House, Shanghai (1999).
[20] W.M.N.H.W. Salleh, M.A. Nafiah, K.H. Yen, H. Kassim, A. Tawang. Chemical constituents and acetylcholinesterase inhibitory activity of *Piper abbreviatum* Opiz. Bull. Chem. Soc. Ethiopia 34(3), 625-632 (2020).
[21] M. Taher, M.S. Amri, D. Susanti, M.B.A. Kudos, N.F.A.M. Nor, Y. Syukri. Medicinal uses, phytochemistry, and pharmacological properties of *Piper aduncum* L. Sains Malays. 49(8), 1829-1851 (2020).
[22] A. Durofil, M. Radice, J. Blanco-Salas, T.Ruiz-Téllez. *Piper aduncum* essential oil: A promising insecticide, acaricide and antiparasitic. A review. Parasite 28, 2021040 (2021).
[23] W.M.N.H.W. Salleh, N.A. Hashim, N.P. Fabarani, F. Ahmad. Antibacterial activity of constituents from *Piper retrofractum* Vahl. and *Piper arborescens* Roxb. Agric. Conspec. Sci. 85(3), 269-280 (2020).
[24] F. Fazal, P.P. Mane, M.P. Rai, K.R. Thilakchand, H.P. Bhat, P.S. Kamble, P.L. Platty, M.S. Baliga. The phytochemistry, traditional uses and pharmacology of *Piper betel.* Linn (Betel leaf); A panasiatic medicinal plan. Chin. J. Integr. Med. 1, 1-8 (2014).
[25] W.M.N.H.W. Salleh, N.A. Hashim, F. Ahmad, S. Khamis. Phytochemicals and tyrosinase inhibitory activity from *Piper caninum* and *Piper magnibaccum*. Pharm. Sci. 25(4), 358-363 (2019).
[26] T.Z. Abdul-Jalil, Z.A. Nasser. *Piper cubeba*: Phytochemical and pharmacological review of a routinely used spices. Int. J. Pharm. Res. 12, 761-768 (2020).
[27] W.M.N.H.W. Salleh, M.F. Kammil, F. Ahmad, H.M. Sirat. Antioxidant and anti-inflammatory activities of essential oil and extracts of *Piper miniatum*. Nat. Prod. Commun. 10(11), 2005-2008 (2015).
[28] H. Bagheri, M. Yazid, A. Manap, Z. Solati. Antioxidant activity of *Piper nigrum* L. essential oil extracted by supercritical CO_2 extraction and hydro-distillation. Talanta 121, 220-228 (2014).
[29] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen. Antioxidant and anti-tyrosinase activities from *Piper officinarum* C.DC (Piperaceae). J. Appl. Pharm. Sci. 4(5):87-91 (2014).
[30] E. Rajudin, F. Ahmad, H.M. Sirat, D. Arbain, H.Y. Aboul-Enein. Chemical constituents from tiger's betel, *Piper porphyrophyllum* N.E.Br. (Fam. Piperaceae). Nat. Prod. Res. 24(4), 387-390 (2010).
[31] W.M.N.H.W. Salleh, N.A. Hashim, S. Khamis. Chemical constituents and lipoxygenase inhibitory activity of *Piper stylosum* Miq. Bull. Chem. Soc. Ethiopia 33(3):587-592 (2019).
[32] W.M.N.H.W. Salleh, N.A. Hashim, S. Khamis. Chemical constituents of *Piper ribesioides*. Chem. Nat. Compd. 57(4), 795-797 (2021).
[33] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen. Chemical compositions and antimicrobial activity of the essential oils of *Piper abbreviatum, P. erecticaule and P. lanatum* (Piperaceae). Nat. Prod. Commun. 9(12), 1795-1798 (2014).
[34] S.N.H. Mahmood, O. Hidayatulfathi, S.B. Budin, G.A. Rohi, M.H. Zulfakar. The formulation of the essential oil of *Piper aduncum* Linnaeus (Piperales: Piperaceae) increases its efficacy as an insect repellent. Bull. Entomol. Res. 107(1), 45-57 (2016).
[35] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen. Essential oil compositions and antimicrobial activity of *Piper arborescens* roxb. Marmara Pharm. J. 20, 111-115 (2016).
[36] S.N.H.M. Latip, R. Ibrahim, N.K.C.M. Ramli. Clement M.U.; Molluscicidal activity of fresh leaves from *Curcuma longa* and *Piper betle* essential oil against *Pomacea canaliculata*. ASM Sci. J. 13(6), 82-89 (2019).
[37] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, H.M. Sirat. Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Piper caninum* blume. Int. J. Mol. Sci. 12, 7720-7731 (2011).
[38] I. Jantan, A.R. Ahmad, A.S. Ahmad, N.A.M. Ali. A comparative study of the essential oils of five *Piper* species from Peninsular Malaysia. Flav. Fragr. J. 9, 339-342 (1994).
[39] N.A. Hashim, F. Ahmad, N.A. Jani, D. Susanti. In vitro antioxidant, antityrosinase, antibacterial and cytotoxicity activities of the leaf and stem essential oil from *Piper magnibaccum* C.DC. J. Essent. Oil Bear. Pl. 20(1), 223-232 (2017).
[40] H.M. Sirat, O.B. Thai, F. Ahmad. Chemical composition of the essential oil of *Piper maingayi* Hk. F. J. Essent. Oil Res. 22, 323-324 (2010).
[41] N.A. Hashim, F. Ahmad, D. Susanti. Antioxidant and antityrosinase activities of *Piper maingayi* HK. essential oils. J. Teknol. 78(1), 169-174 (2016).
[42] L.S. Fan, R. Muhamad, D. Omar, M. Rahmani. Insecticidal properties of *Piper nigrum* fruit extracts and essential oils against *Spodoptera litura*. Int. J. Agric. Biol. 13, 517-522 (2011).
[43] H. Bagheri, M. Yazid, A. Manap, Z. Solati. Response surface methodology applied to supercritical carbon dioxide extraction of *Piper nigrum* L. essential oil. LWT Food Sci. Tech. 57, 149-155 (2014).

[44] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen, H.M. Sirat. Chemical compositions, antioxidant and antimicrobial activity of the essential oils of *Piper officinarum* (Piperaceae). Nat. Prod. Commun. 7(12), 1659-1662 (2012).
[45] W.M.N.H.W. Salleh, F. Ahmad, H.M. Sirat, K.H. Yen. Chemical compositions and antibacterial activity of the leaf and stem oils of *Piper porphyrophyllum* (LINDL.) N.E.Br. Excli J. 11, 399-406 (2012).
[46] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen. Chemical composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. essential oils, and their antioxidant, antimicrobial and tyrosinase inhibition activities. Bol. Latinoam. Caribe Plantas Med. Aromat. 13 (5), 488-497 (2014).
[47] W.M.N.H.W. Salleh, F. Ahmad, K.H. Yen. Chemical composition and antimicrobial activity of essential oil of *Piper muricatum* Blume (Piperaceae). J. Essent. Oil Bear. Pl. 17(6), 1329-1334 (2014).
[48] N. Misni, S. Sulaiman, H. Othaman, B. Omar. Repellency of essential oil of *Piper aduncum* against aedes *albopictus* in the laboratory. J. Amer. Mosquito Contr. Assoc. 25(4), 442-447 (2009).
[49] I. Almas, E. Innocent, F. Machumi, W. Kisinza. Effect of geographical location on yield and chemical composition of essential oils from three *Eucalyptus* species growing in Tanzania. Asian J. Trad. Med. 14(1), 1-12 (2019).
[50] H. Al-Hmadi, R. El Mokni, R.K. Joshi, M.L. Ashour, S. Hammami. The impact of geographical location on the chemical compositions of *Pimpinella lutea* Desf. growing in Tunisia. Appl. Sci. 11, 7739-7747 (2021).

Short note

Fatty acids, triglycerides, tocol, and sterol contents of oils of some Moringa seed varieties

Osman Kola[1]✉
Sümeyye Gökalp[1]
Erva Parıldı[1]
Neslihan Özkul[1]
Murat Reis Akkaya[1]
Ali Emrah Çetin[1]

[1]Department of Food Engineering
Faculty of Engineering
Adana Alparslan Türkeş Science
and Technology University
Sarıçam/Adana-Turkey

✉ CORRESPONDING AUTHOR:
E-mail: okola@atu.edu.tr
Phone: +905333953603

Received: September 18, 2022
Accepted: November 15, 2022

Three moringa oliefera varieties (MOMAX 3, ODC and PKM-1) seeds were used in this study. The oil was extracted from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties by cold press (CP) and the solvent extraction (SE) method.
Fatty acids, triglycerides, tocols (tocopherols and tocotrienols) and sterol contents of moringa seed oils obtained by two different methods were
determined and compared with one another. The crude oil yield was between 26.46 - 28.19% in solvent extraction and 24.20 - 26.30% in the cold press method. It was determined that the main fatty acid in moringa seed oils (ODC, PKM1 and MOMAX 3) was Oleic acid (63.31 - 70.04%). Other dominant fatty acids were determined to be palmitic acid (16:0) (5.59-7.26%), stearic acid (18:0) (5.37-5.89%), oleic acid (18:1n9) (%63.31-70.04), arachidic acid (20:0) (2.67 - 3.71%) and behenic acid (22:0) (3.82 - 5.73%). It was determined that the main triglyceride in moringa seed oils was triolein (OOO; 35.63 - 36.50%), the main sterol was β-Sitosterol (39.04 - 42.11%) and the main tocopherol was α-tocopherol (15.88 - 18.91 µg/g).
Keywords: moringa, seed oil, fatty acid, triglyceride, sterol, tocopherol

1. INTRODUCTION

It is known that medicinal and aromatic plants have been used in many sectors such as food, medicine, cosmetics and spices since the onset of human history. One of the most important features of medicinal and aromatic plants is their use for therapeutic purposes [1]. Herbal treatment has many advantages over chemical treatment, such as less side effects, lower cost and higher availability [2].
moringa is a versatile plant used for medicinal, aromatic, and therapeutic purposes. Moringa (*Moringa oleifera*-MO), a member of the *Moringaceae* family, is native to India. Moringa grows best in dry, sandy, or slightly alkaline soil. Various parts of the Moringa plant (leaf, seed, etc.) can also be consumed directly by eating them [3]. According to a report from the Bureau of the Plant Industry, moringa is reported to be an excellent food source. It has been stated that only leaves (according to the amount of dry matter) contain four times more calcium than milk, seven times more vitamin C than oranges, three times more potassium than bananas, three times more iron than spinach, four times more vitamin A than carrots and twice more protein than milk. In addition, the bark, and leaves of moringa contain high amounts of Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe [4]. Moringa is a great source of protein, and its protein content ranges from 7.12% to 39.17%.
In addition, it contains very low fat and carbohydrates. At the same time, moringa delays aging because it contains effective antioxidants and high levels of vitamins A, C and D. Antioxidants reduce the onset of wrinkles on the skin and fine lines on the face and help prevent and cure various chronic diseases such as arthritis, cancer, heart, and kidney diseases. The leaves can be con-

sumed directly by being dried and powdered. It has also been stated that moringa leaves have a prebiotic effect and regulate the intestinal flora [5].
Depending on the variety, moringa seeds contain about 30-60% oil. Moringa seed oil is commercially known as Behen oil or Ben oil [6, 7]. Moringa oil is classified as a nutraceutical food because of its chemical composition (tocopherols, triglycerides, fatty acids, sterols, and minor components), which includes high nutritional qualities linked to potential health advantages such as decreasing blood sugar levels and reducing the risk of cardiovascular disease. In addition to this, it can be used as an adjunct in the treatment of hyperthyroidism, rheumatism, gout, cramps, epilepsy and with these properties, it can function as antimicrobial and anti-inflammatory agents. Moringa esters might also be used in lubricants or as environmentally friendly additives [8]. *Moringa oleifera* oil is known to contain very high amounts of oleic acid (70%), palmitic acid, stearic acid, behenic acid, quercetin, luteolin and tocopherol [9].
The oxidative stability of *Moringa oleifera* oil is higher than that of canola oil, palm oil and soybean oil for use as frying oil [10, 11].
The fatty acid composition of moringa seed oil is equivalent to olive oil. This oil is also used in medical and mechanical industries [10]. Moringa oil has the potential to become a new source of oleic acid-type vegetable oil. Moringa oil, surprisingly, has a lower linoleic acid level (4.2%) than other vegetable oils (such as soybean, palm, and canola oils). Cold press (CP), solvent extraction (SE), enzyme-assisted aqueous extraction, and supercritical carbon dioxide extraction are some methods used to extract oil from *Moringa oleifera* seeds (SC-CO_2) [12].
In this study, oil was extracted from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties by cold pressing and solvent extraction method. Fatty acids, triglycerides, tocols (tocopherol and tocotrienols) and sterol contents of moringa seed oils obtained by two different methods (cold pressing and solvent extraction) were determined and compared with one another.

2. EXPERIMENTAL PROCEDURES

Three *Moringa oliefera* varieties (MOMAX 3, ODC and PKM-1) seeds were used in this study. Seeds of MOMAX 3, ODC and PKM1 moringa varieties were supplied by "MORINGANTEP Entrepreneurial Women Production and Development Cooperative", approximately 2 kg each from seeds imported directly from SVM Exports (No: 2F/1049 P&T Colony 6th Street West Tuticorin-628 008-Tamil Nadu, India). These seeds were packaged under vacuum in polyethylene bags and stored at room conditions until oil extraction. Moringa oils obtained by cold pressing and solvent extraction method were stored at -18°C until analysis.
Naturefuel 500 (NF 500) model cold press oil machine manufactured by Karaerler Machine (Ankara, Turkey) was used for the extraction of oil by cold pressing method.
Extraction of crude oil from moringa seeds with the Soxhlet method was carried out using the "Gerhardt Soxtherm brand SE-414" model oil extraction device.

2.1. EXTRACTION OF OILFROM MORINGA SEEDS

Seed oil was obtained from the seeds of MOMAX 3, ODC and PKM1 moringa varieties by cold pressing and solvent extraction methods in this study.

2.1.1. Extraction of Crude Oil from Moringa Seeds by Cold Pressing

Oil extraction from moringa seeds by cold pressing method was carried out with a cold press oil machine (Karaerler brand NF500 model, Turkey). Moringa seeds was pressed at a frequency of 15 Hz at 40 - 45°C in a cold press oil machine preheated to 100°C. It was ensured that the oil obtained was below 45°C. For this purpose, 300 g of seeds of MOMAX 3, ODC and PKM1 moringa varieties were weighed and fed through a cold-pressed oil machine to obtain moringa oil. The crude oil yield (%) was obtained by weighing the amount of cold pressed oil samples.

2.1.2. Extraction of Crude Oil from Moringa Seeds by Soxhlet Method

Extraction of crude oil from moringa seeds by Soxhlet method was carried out using a Gerhardt Soxtherm brand SE-414 model oil extraction device. A 10 g sample of each of the three moringa seeds was ground in a Waring brand blender, dried at 105°C for 2 hours and placed in a Soxhlet cartridge. Then, 150 mL of petroleum ether was added to the soxhlet flask which was placed in the extractor. At the end of the process, by reweighing the extraction container, the tare of which was determined before, the amount of crude oil in it was determined as a percentage based on dry substance with the help of the following formula:
Crude Oil (%, w/w) = $[100 \times (M_2 - M_1) / M_0]$
M_0 : Mass of the test sample (w)
M_1: Tare of extraction flask (w)
M_2: Mass of oil and balloon together after extraction (w)

2.2. DETERMINATION OF FATTY ACID COMPOSITION IN MORINGASEED OILS

For esterification in the fatty acids of oils of moringa varieties, 0.1 g of oil was taken into test tubes and 10 ml of n-heptane was added. Then 0.5 ml of methanolic KOH solution was added and the tubes were shaken vigorously for 30 seconds and centrifuged at 4000 rpm for 10 minutes. Then, the supernatant was placed into 2 ml vials and prepared for injection. Fatty acids methyl ester analyses were performed on a gas chromatography (Agilent 7820A GC) equipped with

flame ionization detector (FID). The fatty acid methyl esters (FAMEs) analysis was performed on an HP-88 Column (100 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 15mL/min. Detector temperature was set at 260°C and the column oven temperature at 230°C. The initial oven temperature was gradually increased starting from 50°C. It was increased at a ratio of 10°C/min to 175°C and preserved at 175°C for 10 min. Then the temperature was raised to a ratio of 2°C/min to 210°C and preserved at 210°C for 5 min. It was raised to a ratio of 10°C/min to 230°C and kept at 230°C for 2 min. A 1 µL of each of the diluted samples [n-heptane 1/100(v/v)] was injected automatically in split mode (1/100). The composition of fatty acids (%) was determined by defining individual fatty acids by checking with retention time of known standards and denoted as a percentage of the total fatty acids.

2.3. ANALYSIS OF TRIACYLGLYCEROLS BY HPLC

Triacylglycerol analysis was carried out by modifying the COI method (2017) and the method used by Essid et al. (2014) [13, 14]. Agilent Infinity II 1260 HPLC device with a reversed phase column and refractive index detector (RID) was used. Triacylglycerols were separated from other oil components by column chromatography. The oil sample dissolved in petroleum ether was placed on a previously conditioned chromatography column containing a silica gel absorber.

HPLC conditions: mobile phase acetonitrile/acetone (36.5:63.5), an ACE 5 C18 column (250 mm × 4.6 mm × 5 µm), column temperature 35°C, flow rate 1.0 mL/min, and injection volume of samples 20 µL. Triacylglycerols were identified by comparison with a reference chromatogram [14].

2.4. DETERMINATION OF TOCOLS COMPOSITION IN MORINGASEED OILS

Analysis of tocols (tocopherols and tocotrienols) in oils of moringa varieties was carried out using the Shimadzu Prominence-I LC 2030C 3D Plus model HPLC according to "TS ISO 9936 (2004)" method. Depending on the tocols concentration, approximately 1 g of oil sample was weighed into a 20 mL test tube. A 10 mL of hexane was added and the tube was vortexed for 2 minutes to dissolve the sample. It was then filtered through a 0.45 µm nylon filter and added to a 1.5 mL vials [15, 16].

The chromatographic column was an Inertsil NH2 5 µm 250 × 4.6 mm with 5 µm particle size. The mobile phase consisted of n-hexane/acetic acid/iso-propanol (IPA) (98.9:0.5:0.6 mL) in isocratic conditions at a flow rate of 1 mL/min. The tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) were detected by UV where the wavelengths were set up at 296 nm. The injection volume was set at 10 µL. The column temperature was set at room temperature (~ 20°C). Peak identification was carried out by comparing retention times of authentic standards of tocopherols and tocotrienols.

2.5. DETERMINATION OF STEROLCOMPOSITION IN MORINGASEED OILS

The sterol compositions of moringa oils were carried out according to TS EN ISO 12228-1. Moringa seed oils were saponified by boiling with ethanolic potassium hydroxide solution. The unsaponifiable matter was removed by solid phase extraction in an aluminium oxide column. The sterol was separated from the unsaponifiable matter by thin layer chromatography. Quantitative and qualitative compositions of the sterol, using betulin as internal standard, were determined with the help of gas chromatography device [17]. The sterols recovered from the plate were transformed into a mixture of ethanol and diethyl ether, and the mixture was analysed by GC using an Agilent 6850 GC equipped with FID detector and a HP-5 (30 m × 320 µm × 0.25 µm) column. The chromatographic conditions: injector at 280°C at 7.9 psi, HP-5 column at 260°C, and detector at 290°C. Injection volume was 1.0 µL and the split ratio was 10:1. Hydrogen as a carrier gas was used at a flow rate of 35 mL/min.

3. RESULTS AND DISCUSSION

3.1. CRUDE OIL YIELD OF MORINGA SEED OILS

It was determined that the total amount of crude oil of moringa varieties varied between 26.46 - 28.19% in solvent extraction and between 24.20 - 26.30% in cold pressing method. It was determined that the highest total crude oil amount was obtained from the seeds of PKM1 moringa variety by solvent extraction (28.19%), while the lowest total crude oil amount was obtained from the seeds of ODC moringa variety by cold pressing method (24.20%). According to these data, it was seen that the total crude oil amount of the seeds of moringa varieties was the highest in PKM1, MOMAX 3 and ODC, respectively.

Anwar and Bhanger (2003) determined in their study that the total amount of crude oil obtained by solvent extraction from moringa oleifera seeds using hexane varied between 38 - 42.00%. This result differs from the results of our study. It is thought that this difference is largely due to the differences between the moringa seed varieties used [18].

3.2. FATTYACID COMPOSITION OF MORINGA SEED OILS

The fatty acid composition of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table I and Figure 1. Major fatty acids in moringa seed oils are Palmitic acid (16:0), Stearic acid (18:0), Oleic acid (18:1n9), Arachidic acid (20:0) and Behenic acid (22:0). It was determined that these fatty acids varied between 5.59-7.26%, 5.37-5.89%, 63.31-70.04%, 2.67-3.71% and 3.82-5.73%, respectively, and the main

fatty acid was oleic acid (63.31 - 70.04%). The highest oleic acid content was obtained from the seeds of the MOMAX 3 moringa variety by solvent extraction (70.04%), while the lowest oleic acid content was obtained from the seeds of the PKM1 moringa variety by solvent extraction method (63.31%). In ODC and MOMAX3, the Behenic acid content is higher in CP than in SE, but in PKM1, the Behenic acid content is higher in SE than in CP.

It was determined that the seed oils of moringa varieties were rich in unsaturated fatty acids and the total unsaturated fatty acid (UFA) content varied between 67.86 - 74.66%. It was determined that the total saturated fatty acid (SFA) content was 10.90 - 16.82% and the main saturated fatty acid was palmitic acid (5.59 - 7.26%).

In a study by Lalas and Tsaknis (2002), similar to like our study, it was determined that the main unsaturated fatty acid was oleic acid (71.60%) [19]. Likewise, in another studies by Leone et al. (2016) and Özcan et al. (2019) it was determined that the main unsaturated fatty acid was oleic acid, and the ratio of these fatty acids were 73.59 and 75.49, respectively [20, 21]. These data were seen to be compatible with our study.

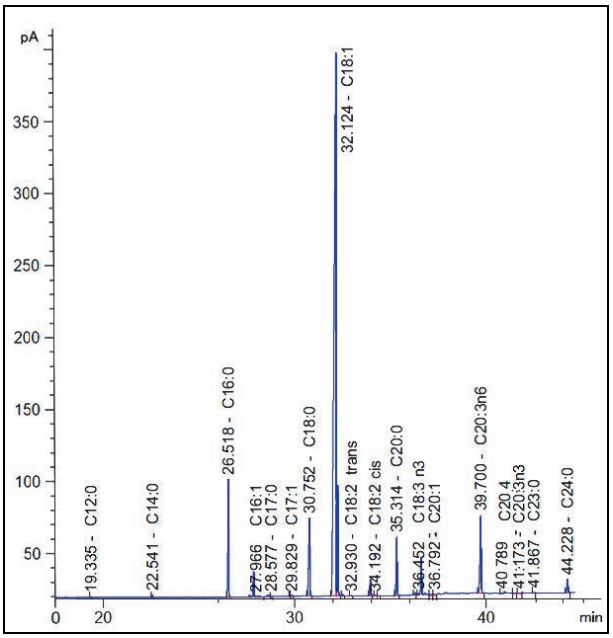


Figure 1 - The fatty acid composition of Moringa seed oils

3.3. GLYCERIDE COMPOSITION OF MORINGA SEED OILS

The glyceride compositions of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods were presented in Table II and Figure 2. It was observed that the main triglyceride structures in moringa seed oils were POL+SLL, OOO, SOL+POO, POP, SOO, and SOP. It was determined that these triglycerides varied between 0.80-2.48%,

Table I - Fatty acid composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Fatty Acid (%)		ODC		PKM1		MOMAX 3	
		SE	CP	SE	CP	SE	CP
Lauric acid	C12:0	0.01	0.02	0.01	0.02	0.01	0.02
Myristic acid	C14:0	0.10	0.13	0.11	0.13	0.11	0.13
Palmitic acid	C16:0	7.26	6.01	6.46	5.98	5.59	6.12
Palmitoleic acid	C16:1	0.03	0.08	0.07	0.02	0.06	0.08
Margaric acid	C17:0	0.08	0.11	0.09	0.10	0.07	0.09
Heptadecenoic acid	C17:1	0.04	0.05	0.04	0.05	0.04	0.04
Stearic acid	C18:0	5.70	5.89	5.58	5.71	5.37	5.79
Cis Oleic acid	C18:1	63.88	66.55	63.31	66.42	70.04	66.65
Trans Linoleic acid	C18:2	0.02	0.02	0.01	0.02	0.01	0.02
Cis Linoleic acid	C18:2	0.04	0.05	0.04	0.05	0.03	0.03
Linolenic acid	C18:3	0.06	0.08	0.07	0.08	0.06	0.08
Arachidic acid	C20:0	2.69	3.71	2.88	3.61	2.67	3.63
Eicosenoic acid	C20:1	0.07	0.10	0.08	0.10	0.07	0.10
Behenic acid	C20:3	3.82	5.73	4.24	4.11	4.36	5.72
Tricosylic acid	C23:0	0.05	0.06	0.05	0.06	0.05	0.06
Lignoceric acid	C24:0	0.67	0.98	0.76	0.98	0.76	0.98
Σ SFA		16.57	10.90	15.94	16.58	14.64	16.82
Σ MUFA		64.02	66.78	63.50	66.59	70.20	66.86
Σ PUFA		3.93	5.87	4.36	4.26	4.46	5.85
Σ UFA		7.96	72.66	67.86	70.85	74.66	72.71

SE: Solvent extraction, CP: Cold pressing

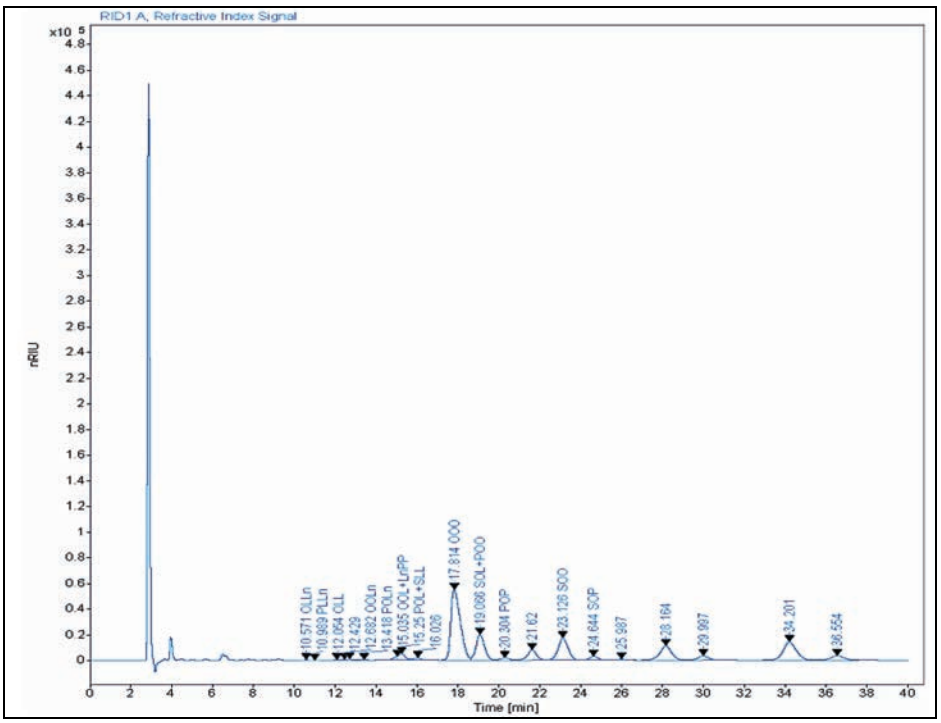


Figure 2 - The glyceride composition of Moringa seed oils

35.63%-36.50%, 11.49-12.06%, 1.08%-1.15%, 11.57-11.81% and 1.71-1.83%, respectively. In addition, it was determined that the main triglyceride structure was OOO (35.63 - 36.50%), followed by SOL + POO and SOO.

In a study, the triglyceride composition of the seed oil of moringa olifera was examined and the results showed that OOO, POO and SOO were the dominant triglycerides in the seed oil [22]. This data shows that our study agrees with previous studies.

3.4. TOCOLS COMPOSITION OF MORINGA SEED OILS

The tocols composition (tocopherols and tocotrienols) of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table III and Figure 3. It was observed that the main tocopherols and tocotrienols were α-tocopherol, α-tocotrienol and γ-tocopherol, varying between 15.88 - 18.91 µg/g, 1.27 - 1.51 µg/g and 5.93 - 7.38 µg/g, respectively. It was determined that the main

Table II - Glyceride composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Triglycerides Content (%)	ODC		PKM1		MOMAX 3	
	SE	CP	SE	SE	CP	SE
LLL	0.03	0.16	-	-	0.02	0.03
OLLn	0.10	0.12	0.07	0.10	0.09	0.11
PLLn	0.05	0.07	0.04	0.07	0.06	0.05
OLL	-	0.45	0.05	0.04	-	0.05
OOLn	0.37	0.34	0.39	0.36	0.62	0.57
POLn	0.16	0.08	0.14	0.12	0.17	0.15
OOL+LnPP	-	-	0.81	0.82	0.78	0.80
POL+SLL	2.25	2.32	2.11	2.16	2.48	0.80
PPL	0.20	0.13	-	-	0.20	0.20
OOO	35.69	35.63	36.25	36.50	36.37	36.10
SOL+POO	11.49	11.77	11.86	12.06	11.65	11.59
POP	1.14	1.09	1.09	1.08	1.15	1.13
SOO	11.79	11.57	11.80	11.81	11.62	11.72
SOP	1.83	1.76	1.71	1.80	1.78	1.80

SE: Solvent extraction, CP: Cold pressing

Table III - Tocols (tocopherols and tocotrienols) composition and distribution of oils obtained from seeds of different Moringa varieties (µg/g)

Tocols (µg/g)	ODC		PKM1		MOMAX 3	
	SE	CP	SE	CP	SE	CP
α-tocopherol	17.57	18.42	15.88	18.91	16.29	18.86
α -tocotrienol	1.30	1.34	1.27	1.30	1.51	1.50
β-tocopherol	0.00	0.00	0.00	0.00	0.00	0.00
β -tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00
γ-tocopherol	7.23	7.38	7.05	7.24	5.93	6.72
γ -tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00
δ-tocopherol	0.00	0.00	0.00	0.00	0.00	0.00
δ-tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00

SE: Solvent extraction, CP: Cold pressing

Table IV - Sterol composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Sterols (%)	ODC		MOMAX 3		PKM1	
	SE	CP	SE	CP	SE	CP
Kolesterol	0.20	0.20	0.21	0.23	0.88	0.90
Brassikasterol	0.10	0.09	0.13	0.14	0.46	0.44
24-Metilenkolesterol	1.12	1.14	1.17	1.16	1.18	1.19
Kampesterol	12.27	12.35	12.51	12.58	11.69	11.67
Kampestanol	0.13	0.11	0.12	0.10	0.15	0.13
Stigmasterol	21.87	21.93	21.84	21.89	21.03	20.98
Delta-7-Kampesterol	0.62	0.55	0.70	0.66	1.07	1.10
Delta-5-23-Stigmastadienol	0.33	0.32	0.22	0.23	0.50	0.48
Klerosterol	1.01	1.00	0.95	0.92	0.98	0.96
Beta-Sitosterol	40.91	40.86	42.07	42.11	39.08	39.04
Sitostanol	0.95	0.92	1.04	0.99	1.82	1.92
Delta-5-Avenasterol	12.93	13.00	12.23	12.27	12.53	12.61
Delta-5-24-Stigmastenol	2.99	3.03	2.74	2.72	4.37	4.41
Delta-7-Stigmastenol	1.74	1.71	1.66	1.61	1.52	1.47
Delta-7-Avenasterol	2.75	2.78	2.41	2.40	2.74	2.71

SE: Solvent extraction, CP: Cold pressing

tocopherol was α-tocopherol (15.88 - 18.91 µg/g), followed by γ-tocopherol and α-tocotrienol.
In previous research carried out by Leone et al. (2016) and Özcan et al. (2019), it was determined that the main tocopherols are α-, γ- and δ-tocopherols [20, 21]. This result is very close to the results of our study.

3.5. STEROL COMPOSITION OF MORINGA SEED OILS

The sterols composition of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table IV. It was determined that the main sterols in were campesterol, stigmasterol, β-Sitosterol and delta-5-Avenasterol and the sterol contents varied between 11.67-12.58%, 20.98%-21.93%, 39.04%-42.11% and 12.23-13.00%, respectively. It was determined that the main sterol was β-Sitosterol (39.04 - 42.11%), followed by stigmasterol, delta-5-Avenasterol and campesterol. In general, the sterol contents of oils obtained by cold pressing were higher than those obtained by solvent extraction.
In a previous study conducted by Lalas and Tsaknis (2002), it was determined that moringa oil contains high levels of β-sitosterol (up to 45.58%), stigmasterol (up to 23.10%) and campesterol (up to 15.81%) [19]. In another study, conducted by Leone et al. (2016), it was determined that the main sterols in the oils of moringa seeds were β-sitosterol, stigmasterol, delta-5-Avenasterol and campesterol [20]. These data were found to be compatible with our study.

CONCLUSION

In the light of the findings above, it was observed that there was no significant difference between the values of fatty acid composition, triglyceride composition, tocols (tocopherols and tocotrienols) and sterol contents of the oils obtained from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties, either by sol-

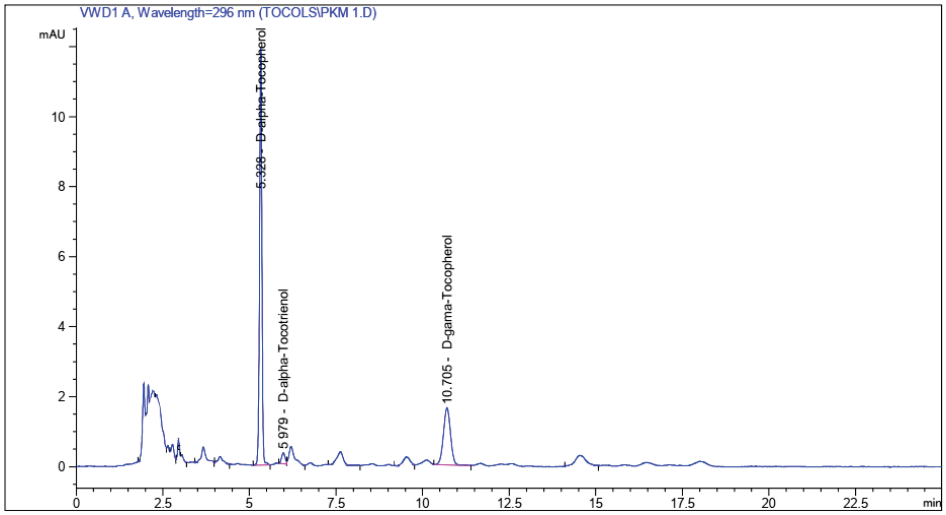


Figure 3 - The tocols composition (tocopherols and tocotrienols) of Moringa seed oils

vent extraction or by cold press method. It has been determined that the oils obtained from the seeds of moringa varieties are rich in oleic acid and have similar properties with olive oil (55 - 83%) and hazelnut oil (71 - 91%). Therefore, it seems that oils obtained from moringa seed can be considered as vegetable cooking oil. Moringa seeds and the oils obtained from the seeds can be easily used as a food supplement due to their nutritional properties.

The findings in our study show that moringa seed oil contains many components that are important in terms of both nutritional value and health.

Acknowledgment

This study was financially supported by Adana Alparslan Türkeş Science and Technology University Scientific Research
Projects Unit (Project number: 19103011).

REFERENCES

[1] P. Zubay, K. Ruttner, M. Ladányi, J. Deli, É. Németh, Zámboriné, K. Szabó, In the shade – Screening of medicinal and aromatic plants for temperate zone agroforestry cultivation. Industrial Crops and Products 170, (2021).

[2] L. Khedmat, S.Y. Mojtahedi, A. Moienafshar, Recent clinical evidence in the herbal therapy of neonatal jaundice in Iran: A review. Journal of Herbal Medicine 29, 100457, (2021).

[3] A. Hedhili, B.E. Akinyemi, G.A. Otunola, P.A. Ashie-Nikoi, M. Kulkarni, F. Husson, D. Valentin, Moringa oleifera Lam.: A comparative survey on consumer knowledge, usage, attitude and belief in Africa and India. South African Journal of Botany 147, 153-162, (2022).

[4] W. Nouman, S.M.A. Basra, M.T. Siddiqui, A. Yasmeen, T. Gull, M.A.C. Alcayde, Potential of Moringa oleifera L. as livestock fodder crop: A review. Turkish Journal of Agriculture and Forestry 38, 1-14, (2014).

[5] M. Mbikay, Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: A review. Frontiers in Pharmacology 3 MAR, 1-12, (2012).

[6] O.A. Fakayode, E.A. Ajav, Process optimization of mechanical oil expression from Moringa (Moringa oleifera) seeds. Industrial Crops and Products 90, 142-151, (2016).

[7] O.A. Valdés-Rodríguez, F. Giadrossich, A. Pérez-Vázquez, J.C. Moreno-Seceña, Above- and below-ground biomass and allometry of Moringa oleifera and Ricinus communis grown in a compacted clayey soil. Flora: Morphology, Distribution, Functional Ecology of Plants 241, 35-45, (2018).

[8] F.M.S. Wiltshire, A. de França Santos, L.K.B. Silva, L. Carvalho de Almeida, L. dos Santos Freitas, A.S. Lima, A.T. Fricks, C. Dariva, C.M.F. Soares, Influence Of Seasonality on the Physicochemical Properties of Moringa Oleifera Lam. Seed Oil and their Oleochemical Potential. Food Chemistry: Molecular Sciences 4, 100068, (2021).

[9] P.F. Martins, M.M.R. De Melo, C.M. Silva, Techno-economic optimization of the subcritical fluid extraction of oil from Moringa oleifera seeds and subsequent production of a purified sterols fraction. The Journal of Supercritical Fluids 107, 682-689, (2016).

[10] G.O. Omotoso, I.T. Gbadamosi, O.J. Olajide, S.O. Dada-Habeeb, T.T. Arogundade, E.O. Yawson, Moringa oleifera phytochemicals protect the brain against experimental nicotine-induced neurobehavioral disturbances and cere-

bellar degeneration. Pathophysiology 25, 57-62, (2018).
[11] J. Zhong, Y. Wang, R. Yang, X. Liu, Q. Yang, X. Qin, The application of ultrasound and microwave to increase oil extraction from Moringa oleifera seeds. Industrial Crops and Products 120, 1-10, (2018).
[12] COI. Determination of the difference between actual and theoretical content of triacyglycerols with ECN 42. International Olive Council 20, 1-22, (2017).
[13] K. Essid, W. Jahouach-rabai, M. Trabelsi, M.H. Frikha, Effects Of Neutralization And Bleaching Process On Fatty Acid And Triglyceride Compositions Of Pomace-Olive Oil. Journal Of Harmonized Research in Applied Sciences, 257-270, (2014).
[14] N.M. Han, C.Y. May, Chromatographic analyses of tocopherols and tocotrienols in palm oil. Journal of Chromatographic Science 50, 283-286, (2012).
[15] TSE. Hayvansal ve bitkisel katı ve sıvı yağlar tokoferol ve tokotrienol içeriklerinin tayini yüksek performanslı sıvı kromatografi yöntemi. TS ISO 9936, (2004).
[16] TSE. Determination of individual and total sterols contents – Gas chromatographic method - Part 1: Animal and vegetable fats and oils. TS EN ISO 12228-1, (2014).
[17] F. Anwar, M.I. Bhanger, Analytical Characterization of Moringa oleifera Seed Oil Grown in Temperate Regions of Pakistan. Journal of Agricultural and Food Chemistry 51, 6558-6563, (2003).
[18] S. Lalas, J. Tsaknis, Characterization of Moringa oleifera Seed Oil Variety “Periyakulam 1”. Journal of Food Composition and Analysis 15, 65-77, (2002).
[19] A. Leone, A. Spada, A. Battezzati, A. Schiraldi, J. Aristil, S. Bertoli, Moringa oleifera Seeds and Oil: Characteristics and Uses for Human Health. International Journal of Molecular Sciences 17, 2141, (2016).
[20] 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).
[21] A. Sengupta, C. Sengupta, P.K. Das, The triglyceride composition of Moringa concanensis seed fat. Lipids 6, 666-669, (1971).

Da Georgofili INFO, notiziario di informazione dell'Accademia dei Georgofili
www.georgofili.info

Il contributo degli insetti alla produzione di lipidi

di Paolo Bondioli, Elisabetta Rossi, Giuliano Mosca
29 marzo 2023

La crescita demografica prevista per questo secolo pone importanti interrogativi sulla disponibilità e sull'uso delle risorse alimentari e idriche: le stime indicano in più di 9 miliardi di persone, la consistenza della popolazione mondiale nel 2050. Sotto la spinta di queste previsioni, a partire dal 2013, la FAO ha iniziato un'attività di sensibilizzazione sul tema dell'uso degli insetti nell'alimentazione umana e degli animali di allevamento (FAO, 2013) come risposta alla sfida sulla crescente domanda di proteine. L'argomento ha dato origine ad un vasto dibattito nella comunità scientifica che prosegue tuttora: per quanto molti siano ancora gli aspetti tecnici e scientifici da chiarire, l'uso alimentare degli insetti è comunque un tema di grande attualità.

Ulteriore conseguenza dell'atteso aumento demografico è anche la crescente richiesta di sostanze grasse naturali per usi non alimentari. La quantità di lipidi naturali utilizzata a scopo alimentare copre attualmente circa il 75% di quella disponibile, mentre il restante 25% è usato per fini energetici, per la detergenza e la cosmetica, in prodotti vernicianti, nei biolubrificanti nonché in numerosissimi impieghi tecnici di nicchia.

La crescente domanda di sostanze grasse ha creato tensioni di mercato derivanti dalla competizione tra uso *food* e *non-food* di sostanze grasse naturali, causando accesi dibattiti sulla liceità di sottrarre risorse potenzialmente destinabili all'alimentazione, dirottandole verso la produzione di energia e prodotti per l'industria.

Negli ultimi anni si è quindi sviluppato un ampio ventaglio di studi per l'individuazione di sostanze grasse di origine naturale per utilizzi industriali, alternative alle fonti tradizionali. Così come nel caso delle fonti proteiche per uso alimentare, l'obiettivo è quello di ottenere grassi da produzioni sostenibili. Gli allevamenti massali di insetti sembrano rispondere perfettamente alle esigenze di sostenibilità, sia quando la produzione è destinata al diretto consumo da parte dell'uomo, sia quando, in genere sotto forma di farina, entra nella composizione dei mangimi per uso zootecnico. Gli allevamenti di insetti presentano infatti, rispetto a quelli zootecnici tradizionali, un impatto ambientale ridotto, con basse produzioni di gas serra, limitato consumo di suolo e di acqua, e producono spesso, come sottoprodotto dell'allevamento, del frass (escrementi di insetti allevati) utilizzabile come ammendante. Inoltre, quando le farine di insetto vanno a sostituire le fonti proteiche attualmente utilizzate nei mangimi zootecnici, quali le farine di soia e di pesce, si evita, da un lato, la competizione con una coltura utilizzata anche come food, dall'altro, si riduce l'impoverimento dell'ecosistema marino. Non va dimenticato che l'Unione Europea è deficitaria in materiale proteico ad uso zootecnico e ricorre ad importanti importazioni da Paesi terzi. Altro vantaggio degli allevamenti di insetti è quello legato all'utilizzo di specie saprofaghe come la mosca domestica (*Musca domestica*) o la mosca soldato nera (*Hermetia illucens*) che, sviluppandosi a spese di biomasse di scarto, consentono di abbassare i costi di produzione, con il duplice vantaggio di ottenere grandi quantità di produzione e, allo stesso tempo, consentendo la biotrasformazione del substrato, divenendo così un virtuoso esempio di applicazione

dei principi dell'economia circolare. Oltre a questo, gli insetti già costituiscono un elemento importante dell'alimentazione umana in diverse parti del mondo. In almeno 113 Paesi in Asia, Africa e America, infatti, l'entomofagia è una pratica diffusa, che riguarda circa 1500 specie di insetti e che coinvolge almeno 3000 gruppi etnici, per i quali l'uso alimentare degli insetti è parte integrante della dieta, costituendo una importante fonte proteica. In Occidente, il consumo alimentare degli insetti è raro e si rivolge solo ad alcune particolari specie (es. *Piophila casei* o mosca del formaggio), anche se è necessario ricordare che, sia pure in modo inconsapevole, possiamo ingerire insetti o loro frammenti attraverso molti prodotti di origine vegetale presenti nelle nostre diete.

Pensare agli insetti come fonte di sostanze grasse non rappresenta di per sé una novità assoluta. Infatti, nel notissimo compendio su "Tecnologia chimica industriale delle sostanze grasse e derivati" di Martinenghi (1963), una versione aggiornata ed ampliata dell'opera pubblicata nel 1948, la sostanza grassa preparata dalla larva del baco da seta (*Bombyx mori*) trovava ampia citazione in termini di sostanza grassa totale (25-27%), acidità libera (4-32 % in acido oleico), caratteristiche organolettiche (giallo rosso, talvolta bruno con odore disgustoso caratteristico), quasi fluido, indicazione della presenza di acidi oleico, linoleico e linolenico, numero di iodio (105-132), numero di saponificazione (190-194), insaponificabile (1,6-10 %). Con la scomparsa della lavorazione italiana della seta, anche la produzione di olio di crisalide è scomparsa. Restano alcune produzioni di nicchia non meglio identificate che commercializzano questo olio a scopi cosmetici sul web, vantandone proprietà emollienti ricordando la qualità delle mani delle operatrici che manipolavano i bachi da seta nelle bigattiere di un tempo.

L'interesse nei confronti delle sostanze grasse estratte da insetti è recentemente cresciuto per ragioni molto diverse da quelle di un tempo, andando a intercettare due diversi fenomeni: da un lato, la crescente richiesta del mercato di lipidi di origine naturale, attualmente attestata sul valore di circa 180 milioni di tonnellate ed in continuo aumento, dall'altro, il rinnovato interesse per gli insetti a scopo alimentare, che sta ovviamente incrementando la tecnologia e le conoscenze sugli allevamenti massali.

Gli insetti contengono i lipidi in massima parte all'interno del corpo grasso, un organo costituito da aggregati lassi di cellule rotonde o poliedriche (trofociti), generalmente dotate di vacuolo e avvolte da una membrana di connettivo, presenti a vario livello nella cavità emocelica, principalmente intorno al tubo digerente. La seconda riserva lipidica degli insetti è contenuta nell'emolinfa, il liquido circolante negli insetti destinato a trasportare sostanze nutritive e ormoni ai tessuti target e nel quale la frazione lipidica proviene dalla mobilizzazione delle riserve del corpo grasso e dalla digestione degli alimenti che avviene nel mesenterio. La componente lipidica di un insetto è quantitativamente variabile in funzione di diverse caratteristiche, a iniziare dalla specie.

Inoltre, il contenuto lipidico può subire forti variazioni in funzione di parametri biologici e genetici: le larve e le pupe contengono più grassi rispetto agli adulti e le femmine sono normalmente più ricche di lipidi rispetto ai maschi. Anche particolari condizioni fisiologiche come la diapausa o situazioni ambientali diverse (es. differenti temperature di sviluppo) possono influenzare la qualità e quantità della componente lipidica degli insetti. La stessa dieta può determinare variazioni nella composizione della componente lipidica dell'insetto, così da poterlo rendere, entro certi limiti, adattabile alle esigenze produttive. Molto, tuttavia, rimane da chiarire su questo aspetto, soprattutto in vista dello sviluppo di allevamenti di insetti destinati alla produzione di sostanze grasse.

Dal punto di vista chimico, la sostanza grassa contenuta negli insetti appare di tipo convenzionale, vale a dire costituita sostanzialmente da esteri degli acidi grassi con glicerolo (triacilgliceroli o trigliceridi), come un qualsiasi grasso di natura animale o vegetale convenzionale e quindi contenente in piccola quantità anche acidi grassi liberi e costituenti dell'insaponificabile.

Come in tutte le sostanze grasse, ciò che le differenzia è la composizione in acidi grassi, che ne influenza le caratteristiche fisiche, quali punto di fusione, viscosità, stabilità all'ossidazione, etc. Le composizioni acidiche rilevate vengono considerate comparabili a quelle dei comuni oli vegetali, ad

esempio essendo l'olio di cimice del melone principalmente costituito da acido palmitico, Palmitoleico, e Oleico, mentre nell'olio di cimice del sorgo si rilevano principalmente Acido Palmitico, oleico e linoleico. Per quanto riguarda la presenza di tocoferoli negli oli ottenuti da insetti non sono state rilevate concentrazioni interessanti. Nell'olio di *Hermetia illucens* si ritrovano quantità importanti di acido laurico, di scarso appeal dal punto di vista nutrizionale, ma molto interessante per il mercato della detergenza. Nella composizione sterolica nell'olio di larva di *Sarcophaga carnaria* è stato principalmente riscontrato colesterolo, mentre nell'olio estratto da adulti di cimice del melone e del sorgo, sono stati riscontrati fitosteroli in miscela, con una preponderanza di β sitosterolo.

Scarsi sono al momento i dati disponibili sull'influenza del substrato utilizzato per l'allevamento degli insetti sulla sostanza grassa prodotta, in termini di composizione in acidi grassi. Si tratta di un argomento che richiede di essere al più presto approfondito, in considerazione del fatto che la quantità e qualità della sostanza grassa influenzano pesantemente la valorizzazione del prodotto e la fattibilità economica delle iniziative industriali. Altro aspetto che dovrà essere tenuto in considerazione è quello relativo alla costanza delle produzioni, soprattutto in termini di composizioni. Infatti, la maggior parte delle applicazioni industriali richiede un elevato livello di standardizzazione del materiale in entrata per garantire la stabilità dei processi di trasformazione e la qualità costante dei prodotti finiti.

Per quanto riguarda la tecnologia di preparazione, questa può essere mutuata da quella delle sostanze grasse di origine vegetale o animale. Le larve di insetto contengono elevate quantità di acqua (60-80%) essendo la restante parte costituita da sostanza grassa e proteine, in rapporto di circa 1:3. La presenza di importanti quantità di acqua rappresenta un ostacolo per l'estrazione della sostanza grassa sia con solvente che mediante spremitura. L'estrazione con solvente non deve essere considerata come opzione possibile, in quanto questa tecnologia presenta numerosi problemi dal punto di vista energetico e dell'impatto ambientale. Volendo quindi procedere all'estrazione mediante pressa si renderà necessaria una preliminare riduzione, peraltro necessaria anche nel caso di estrazione con solvente, del contenuto in umidità del materiale fino a raggiungere un'umidità residua inferiore a l'8% circa. L'essiccazione del materiale, oltre a renderlo idoneo alla successiva spremitura presenta effetti accessori non trascurabili, quali ad esempio quello relativo alla bonifica microbiologica del materiale trattato termicamente. Questo presenta aspetti assolutamente rilevanti, in considerazione del fatto che il residuo proteico, per essere utilizzato in alimentazione animale, deve presentare livelli di contaminazione microbica assai ridotti. La spremitura del materiale essiccato consente la preparazione di sostanza grassa con buona resa, anche se non quantitativa. Il prodotto liquido che esce dalla pressatura continua si presenta torbido per la presenza di solidi derivanti dalle strutture cellulari del materiale in ingresso, che devono essere prontamente separate prima di inviare la sostanza grassa a stoccaggio. Anche l'eliminazione dell'umidità disciolta o dispersa nel prodotto rappresenta una tappa fondamentale per conferire la necessaria stabilità al grasso/olio estratto. In alternativa potrebbe essere possibile ottenere sostanza grassa dalla biomassa mediante il processo di rendering o fusione, tecnologia normalmente in uso per la preparazione dei grassi animali e che non richiede la preliminare eliminazione dell'acqua. Il materiale in ingresso, previa eventuale macinazione viene trasferito in un cuocitore, costituito da una caldaia aperta o chiusa, all'interno della quale, eventualmente in presenza di acqua il materiale viene portato ad elevata temperatura. Il trattamento consente la denaturazione delle proteine, la sanitizzazione microbiologica del materiale e l'affioramento della sostanza grassa che viene quindi separata mediante centrifugazione. Il materiale solido risultante, parzialmente disoleato viene quindi trasferito ad una pressa a coclea, mediante la quale si recupera una ulteriore quantità di sostanza grassa unitamente ad una farina proteica pronta all'impiego o allo stoccaggio.

L'estrazione della frazione lipidica con anidride carbonica in campioni di larve di *Tenebrio molitor*, ha portato all'ottenimento di olio con caratteristiche differenti in funzione delle condizioni operative (pressione e temperatura) utilizzate nel corso dell'estrazione. L'esperienza descritta è sicuramente interessante in quanto illustra le possibilità di ottenere prodotti diversi da una sola materia prima

anche se i costi fissi ed energetici connessi alla tecnologia descritta quasi sicuramente impediranno l'industrializzazione del processo.

In una prospettiva futura di utilizzo degli insetti per fini alimentari (umani e/o animali) e per la produzione di oli, sicuramente gli aspetti tecnologici legati alla produzione massale dovranno essere supportati da un adeguato quadro normativo che garantisca produzioni di qualità, tutelando nel contempo il personale coinvolto negli allevamenti e nelle successive fasi di trasformazione e i consumatori. Fino a qualche anno fa, infatti, gli insetti, ad eccezione di api e baco da seta, non erano contemplati nelle normative che disciplinavano gli allevamenti animali ed erano considerati, dal punto di vista alimentare, un *novel food* in ambito europeo (Regolamento (UE) 2015/2283).

Un parere espresso dall'EFSA (2015) segnalava l'esistenza di un'oggettiva carenza conoscitiva sui possibili rischi biologici e chimici connessi all'uso degli insetti nell'alimentazione umana e animale, soprattutto in relazione ai substrati utilizzati per il loro allevamento, che non consentirebbe di aprire senza riserve agli allevamenti massali con finalità alimentari. Nel settore del *petfood* al contrario l'impiego è già consentito ma i prodotti formulati con farina di insetti sono scarsamente diffusi a causa della diffidenza dei proprietari degli animali. Diverso potrebbe invece essere il destino commerciale degli oli da insetto che, in quanto derivati da estrazione, potrebbero essere addizionati ai mangimi come integratori. Oggi dopo le larve della farina e la locusta migratoria la Commissione UE ha autorizzato la commercializzazione dei grilli domestici: ecco le novità in materia di insetti che potranno anche essere utilizzati in una serie di alimenti (23 gennaio 2023).

(Il testo riprende fedelmente i principali concetti già pubblicati in "Oli e grassi" (2019) pag.259-263, da Edagricole – Ed. agricole di New Business Media).

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

Analisi dei contaminanti

ACROLEINA

ANIDRIDE MALEICA

AMIGDALINA

BENZENE E DERIVATI

BISFENOLO A

COLORANTI

FORBOLI

FTALATI e PLASTICIZZANTI

FITOFARMACI

2, 3 MCPD, GLICIDOLO E DERIVATI

GOSSIPOLO

IDROCARBURI POLICICLICI AROMATICI

METALLI PESANTI

MICOTOSSINE

NICOTINA

OCTILFENOLO, NONILFENOLO ED ETOSSILATI

PIROFEOFITINA A RAMEICA

SALI DI AMMONIO QUATERNARIO

SOLVENTI ALOGENATI

Pierangela Rovellini
Responsabile
Team Chimica, Tecnologia e
Sicurezza Alimentare
pierangela.rovellini@mi.camcom.it

MATERIALI A CONTATTO CON ALIMENTI

TEST e ANALISI

CONFORMITÀ BfR XXXVI Carta fibra vergine e riciclata e DGCCRF *MCDA n°4(V02-01/01/2019)*

- Determinazione della ***formaldeide*** in estratto acquoso - (UNI EN 1541:2002)
- Determinazione del contenuto di ***gliossale*** - (DIN 54603:2008)
- ***Imbiancanti ottici*** migrabili - (UNI EN 648:2019)
- Migrazione specifica della somma delle ***ammine aromatiche primarie*** (UNI EN 13130-1:2005+BVL LFGB §64 L 00.00-6:1995/Cor:2002)
- Determinazione e quantificazione degli ***ftalati*** - (metodo interno)
- ***Bisfenolo A*** - (UNI EN 17497:2020)
- Determinazione di ***diisopropilnaftalene*** (DIPN) mediante estrazione con solvente - (UNI EN 14719:2005)
- ***Cadmio***, ***piombo*** e ***alluminio*** in estratto acquoso - (UNI EN 12498:2019 + metodo interno)

RICERCA e SVILUPPO

Sviluppo di ***nuove metodiche analitiche*** per la determinazione e quantificazione di contaminanti o molecole di interesse

ENTE ITALIANO DI NORMAZIONE · DG CCRF · BfR Bundesinstitut für Risikobewertung · cen EUROPEAN COMMITTEE FOR STANDARDIZATION

NIAS *Non Intentionally Added Substances*

"impurità presente nelle sostanze utilizzate, intermedio di reazione formatosi durante il processo produttivo o prodotto di reazione o di decomposizione"
Reg. UE N. 10/2011 Consideranda 18-20, articolo 3

VALUTAZIONE

della conformità ai requisiti riportati nell'**articolo 3 del Regolamento CE N. 1935/2004** sui materiali e gli oggetti destinati a venire a contatto con gli alimenti (**MOCA**)

UNTARGETED ANALYSIS

HPLC-PDA-HRMS
Thermo Scientific™ *Orbitrap Exploris 120 Mass Spectrometer*
Thermo Scientific™ *Compound Discoverer*™ Software

INNOVHUB
STAZIONI SPERIMENTALI PER L'INDUSTRIA

www.innovhub-ssi.it

Riferimenti:
PIERANGELA ROVELLINI
Responsabile Team Chimica e Sicurezza alimentare
pierangela.rovellini@mi.camcom.it

Annunci di Ricerca Partner per Progetti di Ricerca Enterprise Europe Network (EEN)

Anno 2023
(aggiornato al 30 giugno 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 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 BRGR20221031003

A Greek SME active in the printing and packaging sector is in search for a partner who will act as an intermediary in Italy in order to facilitate its entry into the Italian market

A Greek SME active in the field of printing and packaging since 1898, located in Thessaly, Central Greece is interested in growing their business through international cooperations in Italy. The company produces wet glue paper labels, cardboard boxes, offset welle trays and welle boxes, possesses quality certifications such as EN ISO 9001:2008 and EN ISO 22000:2005, and is already engaged in international collaborations. The company is willing to get involved in an outsourcing agreement with a potential partner who will work on commission basis in order to introduce the company to new clients in Italy. The partner is not expected to sell the company's products but they should be able to properly present the company, its production capacities, certificates, etc. and in addition should have the experience to evaluate the credibility of business offers and bring them to an agreement readiness stage. Once the initial intercession has taken place, the partner is expected to bring the possible clients in further contact with the sales department of the Greek company..
Dead-line for EOIs: 14/11/2023

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

Progetto BRRO20230420001

Romanian manufacturer of natural cosmetic products seeks European suppliers, including resellers, of eco-friendly cardboard lip balm tubes under supplier agreements

The Romanian company specialises in the production of natural cosmetics for women, men,

and children and offers a wide range of products based on the firm's original recipes, such as lip balms, face and body creams, natural soaps, face tonics etc. To ensure a high level of sustainability for its products, the company seeks new European suppliers able to provide quality push-up lip balm tubes made of cardboard/kraft paper, with a capacity of 5-6 ml. Tubes should be suitable for storing grease, as lip balms contain oils and butters. Cooperation will be based on supplier agreements.
Dead-line for EOIs: 19/04/2024

Progetto BRPT20230525025

Portuguese company seeks new products in energy reduction/energy efficiency for commercial agreement

The Portuguese SME has more than 12 years of experience in energy efficiency projects. In order to expand its portfolio, the company is looking for disruptive technological solutions focused on decreasing energy and therefore carbon emissions or generating savings. The existing clients from all over the country include manufacturing industries, hospitals, hotels, restaurants, business offices and retail. The company seeks private partners offering innovative technical solutions for energy consumption minimization, with special interest in motors, refrigeration and HVAC. Envisaged type of partnership: commercial agreement.
Dead-line for EOIs: 24/05/2024

Progetto BRPL20230523006

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

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

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

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.

Discover new opportunities for business networking, participants may come from a range of industries and functions, the program will benefit Senior Professionals.

2023 speakers:

Mr.Asanee Mallamphut, Chairman of the Palm Oil Industry Club, The Federation of Thai Industries (F.T.I); Chairman of Thai Palm Oil Refinery Association; Managing Director of Sime Darby Oils Morakot Public Company Limited

Dr.Ahmad Parveez Ghulam Kadir, Director General of Malaysian Palm Oil Board (MPOB)

Mr.Sanin Triyanond, Chairman of Thai Biodiesel Producer Association; Director of Patum Vegetable Oil Co., Ltd.

Dr.Fadhil Hasan, Head of Foreign Affairs Department of Indonesian Palm Oil Association (IPOA)

Mr.Mohamad Hafiz Sa'adun, Industry Technology Specialist of Novozymes Malaysia Sdn Bhd

Ms.Ang Yonny Setiady, Commercial Head VOP SEA & Country Manager Indonesia of PT Novozymes Indonesia Biotechnology

Dr.Mohd Azinuddin Bin Ahmad Mokhtar, Head of Plant Breeding Unit of FGV Research and Development Sdn Bhd

Dr. Sukganah Apparow, Manager, Molecular Breeding Laboratory of Sime Darby Plantation Technology Centre Sdn Bhd

Mr.Samir Bhandarkar, Business Unit Head – CODEL of Forbes Marshall Private Limited

For more details visit: https://www.thaipalmoil.com/

North American SAF Conference & Expo

29 - 30 August 2023 | Minneapolis Convention Center, Minnesota, USA

Taking place August 29-30, 2023 in Minneapolis, MN, the North American SAF Conference & Expo, produced by SAF Magazine, in collaboration with the Commercial Aviation Alternative Fuels Initiative (CAAFI) will showcase the latest strategies for aviation fuel decarbonization, solutions for key industry challenges, and highlight the current opportunities for airlines, corporations and fuel producers.

The North American SAF Conference & Expo is designed to promote the development and adoption of practical solutions to produce SAF and decarbonize the aviation sector. Exhibitors will connect with attendees and showcase the latest technologies and services currently offered within the industry. During two days of live sessions, attendees will learn from industry experts and gain knowledge to become better informed to guide business decisions as the SAF industry continues to expand.

Foreseen topics for speeches and round tables:

Setting the Scene: Why Sustainable Aviation Fuels Are Critically Important for Producers and Airlines;

Grabbing the Next Rung: A Conversation with Technology Developers about SAF Innovation and the Coming Build-Out;

The Keys to Successfully Deploying SAF Production in Corn Country;

Eyeing the Potential for Ethanol as a Precursor to SAF Production;

Reviewing Cutting Edge Technologies Aimed at Commercial-Scale SAF Production;

Understanding and Capitalizing on the Low Carbon Attributes of SAF;

Strategies for Ensuring Feedstock Availability and Alignment with Federal Production Credit Requirements;

The Work Underway to Build Ample Inventories of Sustainable Feedstock for SAF Production

Perfecting the Interplay of Policy, Incentives and Financing to Increase the Chances of Project Success

For updates and more details please visit:
https://saf.bbiconferences.com/ema/DisplayPage.aspx?pageId=Home

FOSFA Introductory Course + FOSFA Advanced Course

3 - 8 September 2023 | University of Greenwich, London, UK

Two globally recognised, week-long residential training courses which are open to members and non-members involved in all aspects of the global supply chain.

The Introductory course will be held from Sunday 3 to Friday 8 September 2023 and is ideal for newcomers to the industry with minimal trade knowledge or experience.

The Advanced course will also take place in parallel with the Introductory course in early September in 2023 and is ideal for individuals with three or more years of trade experience.

Each course covers key subjects, case studies and group work.

Keynote speakers from the trade present the subjects and share their extensive knowledge and experience with delegates.

Courses takes place at The University of Greenwich situated on a UNESCO World Heritage Site on the banks of the River Thames near to the centre of London. Lectures take place at the Old Royal Naval College campus, which has a lively atmosphere and is surrounded by historical landmarks and near to the centre of London.

Delegates will enjoy the university's excellent facilities, including accommodation, gym and student union and the attractive town of Greenwich.

All meals are provided including a drinks reception and gala dinner.

Lectures will take place on campus at the Old Royal Naval College (pictured) which has a lively atmosphere and is surrounded by historical landmarks with the centre of London just a short distance upriver.

The Advanced Course is designed to update delegates on current trade practices and regulations, and introduce several specialist managerial subjects, case studies and group work. It is ideal for individuals with more than three years' experience in the trade.

The course covers:

- Trade practices and regulations update
- Case studies
- Introduction to specialist managerial subjects

The Introductory Course covers elementary aspects of the international trade in oilseeds, oils and fats. It is ideal for members of the trade and industry with little knowledge or experience in a trading environment.

The course covers:

- FOSFA contracts
- Contract documents
- Arbitration and dispute resolution
- Shipping
- Trade finance
- Technical matters
- Mock Arbitration

To register your interest in attending either course(s), please send an email to fosfa.education@fosfa.org stating which course you are interested in.

More information: https://www.fosfa.org/education/

Biofuels and Feedstocks Conference in Rotterdam

5 - 6 September 2023 | Rotterdam, Netherlands

As the European Union develops new directives and fine tunes existing legislation to achieve sustainable transportation and mobility, what is the outlook for the European biofuels industry in the greater context of the continental energy transition?

Following the success of Fastmarket Biofuels and Feedstocks USA and Global Grain events, the inaugural Biofuels and Feedstocks Europe 2023 conference aims to address market issues affecting the biofuels sector, from the European feedstocks trading dynamics, to the continental forecast for traditional and advanced biofuels production, to the analyses of how EU directives are shaping the market and how individual governments incentivise domestic certified trading, etc.

Biofuels and Feedstock Europe 2023 is your chance to connect and actively participate in today's most relevant conversation between agriculture and energy sectors. We look forward to seeing you in Rotterdam.

Foreseen Talks:

- EU Energy Market Outlook, Correlation with Biofuels Markets, US Dollar Strength and Impact on Europe (Marc Ostwald)
- European Biofuels Market Outlook (Scott Wellcome, Tim Worledge, Marc Ostwald, John Cusick)
- EU Vegoils Trading Dynamics and EU Market Fundamentals (Jose Angel Olivero)
- Managing Risks and Volatility in the Biofuels Sector
- Biofuels international trade: logistics aspects

Key Speakers:

Marc Ostwald, Chief Economist & Global Strategist ADM Investor Services International;

John Cusick, President Ash Creek Renewables;

Ryan Standard, Managing Editor Fastmarkets;

Jose Angel Olivero, Sales Director Lipidos Santiga (Lipsa);

Tim Worledge, Managing Editor Fastmarkets;

Francesco Morici, Sogestran\Navquim;

Patrick Lynch, Founder Bioledger.

For updates and more details please visit: https://www.fastmarkets.com/agriculture/biofuels-feedstocks-europe/*

Argus North American Biofuels, LCFS & Carbon Markets Summit

11 - 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.

Topics include:

*Carbon markets and regulation in North Americ*a: Including an opening with a keynote from the California Air Resources Board, a case study on the development of nature based off-sets, a panel with corporates and standards about the development of the voluntary market, and more!

Biofuels— LCFS program developments: Including a panel with California, Oregon, and Washington on LCFs updates and a Canadian LCFs and decarbonization afternoon

Focus on biogas, renewable diesel, SAF and feedstocks: The program will focus exclusively on biofuels… from sessions dedicated to SAF, to biogas/RNG, renewable diesel, and the role of EVs and ZEVs in state decarbonization plans.

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

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.
Experts 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 it will be possible to 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)

Programme Topics:

- Processing and Sustainability
- Bioscience, Biocatalysis, Biochemistry
- Lipids in Pharmaceutics and Cosmetics
- Olive Oil and Olive Products
- Oleochemistry, Molecule and Polymer Sciences
- By-Products in Fat Technology
- Oilseeds, Plant Lipids and Alternative Resources
- Lipid Oxidation and Antioxidants - "Oxidation Mechanisims"
- Marine and Algae Lipids
- Lipid Oxidation and Antioxidants - "Stabilization by Processing"
- Physical Chemistry - "Oleogels"
- Physical Chemistry - "Product Structure"
- Physical chemisty - "Crystalization"
- Insect and Invertebrate Lipids
- Lipidomics
- Health, Nutrition and Disease
- Quality and Consumer Expectations
- Lipids in Animal Science, Milk and Dairy Products
- Contaminants and Adulteration - "Mineral Oil Contamination"
- Analytics and Authenticity
- Frying Processes

For updates and more details please visit:
https://veranstaltungen.gdch.de/tms/frontend/index.cfm?l=11215&sp_id=2

30th Oilseed and Oil Processing short course

20 - 21 September 2023 | Poznań, Poland

Smart Short Courses is a joint operation of ID&A Ignace Debruyne & Associates and Filtration and Membrane World, represented by Ignace Debruyne, PhD and Sefa Koseoglu, PhD. Smart Short Courses offers crash course programs for marketing, technical and plant personnel.

Short courses offer the opportunity for those who are experienced to meet experts in the field to discuss their current problems and enhance their product innovation or plant operation. The course materials serve as useful reference for processors, product formulators, chemists and technicians, as well as marketers and business managers familiar with the topic.
Smart Short Courses offers extensive programs in Europe, Asia and the Americas covering a wide range of topics with specific practical aspects:
Aquaculture feed; CBD Oil Processing, Food Texture; Emulsion Technology; Functional Lipids; Functional Beverages; BioActives; Advanced Oil Processing; Fish Oil; Palm Oil; Oil Methods; Industrial Oil Uses; Algae Technology; Snackfoods; Breakfast Cereals; Protein Extrusion; Soyfoods
Target Group: Decision makers such as product technicians, R & D engineers, engineering supervisors, QA technicians, project engineers, process improvement engineers, business development managers, sales and marketing specialists, equipment manufacturers, product formulators, plant engineers, processors, chemists, and technicians.
On September 20 & 21, 2023, the 30th course will be held: *Fundamentals & New Techniques in Oilseed and Edible Oil - Processing and Application*, in annex to the 19th EuroFedLipid Congress
The technical program covers different topics:

- Oilseed ectraction and protein processing
- Fundamentals of edible oil processing & modifocation
- New techniques in oil processing and refinery optimization

For more information and registration visit:
http://www.smartshortcourses.com/oilprocess30/program.html

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/

POWTECH - International Processing Trade Fair for Powder, Bulk Solids, Fluids and Liquids

26-26 September 2023 | Nuremberg, Germany

Take advantage of the overall dynamics offered by POWTECH, the leading exhibition for experts in powder and bulk solids technology! This event provides a presentation platform for innovations and advancements in processes for the manufacture of quality products made and processed from powder, granules, bulk solids and liquids – also for the environmental and recycling sector.

Expert Forum stagetalks: the mastermind of the processing technology practical solutions and discuss with you future-oriented strategies for your processes. Expand your know-how with valuable knowledge and impulses from our specialists on the topics: New food, Process optimisation and Industry 4.0, Perfection in the supply chain, Fluids meet solids, Sustainability & safety, Future energies. Participation in the short presentations and discussion rounds is free of charge and registration is not required.

Expert Forum stagetalks Pharma: the forum offers a broad variety on talks on all aspects of drug development, manufacturing and characterization. This years edition has a special focus on lyophilization and fill&finish of liquid dosage forms to empower the new range of POWTECH with liquids and fluids.

POWTECH Campus: universities & colleges present themselves. Students will benefit from networking with industry, getting to know future employers and finding inspiration for bachelor's and master's theses, as well as experiencing the professional trade show action up close.

Live demonstrations on explosion protection: demonstration of the effects of the Phenomen of Dust Explosions.

International meeting of bulk solids associations: a networking event to know the international markets.
And much more.
For all information about the fair and the conferences program, please visit
https://www.powtech.de/en.

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

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.

Network with leading experts and influential decision-makers from across the international market.
Develop new contacts during round table discussions, networking lunches, and evening cocktail receptions.
Join international companies from over 40 countries and build strategic partnerships with your global peers.
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.

Main topic & key points
Europe's oil/gas independence & green transition

- European and world markets outlook
- Biofuels market development – perspective for next 10 years
- Overview of European and national policies – FuelEU, Fit for 55, REPowerEU
- Global acceleration of biofuels production – new wave
- Best practice examples of meeting emission neutrality goals
- Production and use of alternative fuels in aviation and marine sector
- Digitization in biofuel industry
- Overview of production technologies of cellulosic ethanol, ammonia and hydrogen
- Bunkering, storage & handling technologies news

During this 2-day event reach in presentations from industry end-users and running projects case studies will give you a comprehensive overview of the biofuels industry. You can hear experts focusing on new regulations, changing market situation and more. Get a possibility to network and exchange ideas with industry leaders.

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

Malaysian Palm Oil Board (MPOB) International Palm Oil Congress and Exhibition (PIPOC 2023)

7 - 9 November 2023 | Kuala Lumpur Convention Centre, Malaysia

The congress features four concurrent conferences covering upstream, midstream, downstream & value addition; processing, food safety & nutrition & global economics & marketing.
A keynote address will be delivered by distinguished expert in the palm oil industry. Four plenary lectures will be held in the afternoon of 7 November 2023. The lectures will cover various topics of interest in line with the theme of the congress.

Objectives:

- To discuss strategic R&D findings in all aspects of the oil palm and palm oil industry
- worldwide;
- To reveal recent technological findings that will improve the oil palm/palm oil industry;
- and
- To formulate strategies towards enhanced sustainability, competitiveness, automation
- and policies on trade, market trends and trade challenges.

PIPOC 2023 caters for those involved in the oil palm, palm oil and other oils and fats industry including R&D personnel, scientists, planters, millers, traders, processors, manufacturers, economists, policy makers and academicians. The Congress will be especially useful for networking with your peers in the oils and fats industry while exchanging views and sharing new ideas.
Technical tours are foreseen to oil palm plantation, palm oil mill, refinery and R&D facilities.

Concurrent programme:
Agriculture, biotechnology & sustainability conference (abs)

- Session 1: yield performance
- Session 2: sustainable development
- Session 3: mechanisation and automation in oil palm plantation
- Session 4: oil palm farmer's empowerment

Downstream and value addition (dva)

- Session 1 & 2: oleo & specialty chemicals
- Session 3 : biomass utilisation
- Session 4 : feed innovation & food valorisation
- Session 5 : renewable energy

Processing, food safety and nutrition (PFSN)

- Session 1: milling & processing technology
- Session 2: environmental management technology
- Session 3: food safety & quality
- Session 4: lipid research

- Session 5: diet & lifestyle
- Session 6: phytonutrients

Global economics and marketing (GEM)

- Session 1: transcending the challenges of the oil palm industry
- Session 2: ensuring business sustainability through palm oil
- Session 3: building resilience through innovations
- Session 4: the new paradigm for malaysian oil palm industry

For more details: http://pipoc.mpob.gov.my

FOFSA Annual Dinner 2023

9 November 2023 | Geneva, Switzerland

The Annual Dinner returns to Geneva in 2023 and will be hosted at the Hilton Geneva Hotel and Conference Centre on Thursday 9 November 2023.
The venue near the city centre, just across the street from the airport and one mile from Lake Geneva.
You can book up to a maximum of four tables of ten if you want to come as a large group, or book as a smaller group or as an individual and we will designate you a table. Reserve your table(s) early to avoid disappointment.
For further information please visit:
https://www.fosfa.org/news/events/fosfa-annual-dinner-2023/

2023 AAOCS lipid conference Australia

13 - 15 November 2023 | Newcastle, Australia

The next conference will be held at Noah's on the Beach in Newcastle Australia Nov 13-15th 2023
The theme of this year's event is Future of lipids: health and sustainability and aims to look at the future of fats and oils in research and industry.
The program will present the latest science and industry updates relating to edible oil and ingredient supply, biotechnology, manufacture of fat-based products, nutritional research, oil analysis, latest developments with omega-3 oils, dairy lipids and other activities that support the industry. Furthermore, planned workshops led by international experts, are in planning to occur prior to the conference.

AAOCS2023 aims to provide an opportunity to learn and share science and industry progress related to all aspects of fats and oils. We aim to continue to provide a social setting where the latest in the oil and fats industry, science and health can be discussed and connections made or renewed.
The range of topics covered in this meeting include:

- Food industry and processing
- New analytical methods
- Agriculture
- Aquaculture
- Lipidomics
- Lipid oxidation and antioxidants
- Omega-3 fatty acids
- Fatty acids, lipids and health
- Nutrition and health
- Lipids and metabolic syndrome
- Lipids and cognitive function
- Novel foods and supplements
- Olive and other vegetable oils
- Biotechnology
- Omega-3 index

The AAOCS are hosting two workshops prior to the meeting.
These will both be held on Monday 13th November 2023 at Noah's on the beach Newcastle. These workshops will run concurrently.
The two workshops are:
Sustainable ingredients for food and feeds (focus of novel lipids and protein products entering our food systems)
The program will present the latest science and industry details about alternative sustainable sources of lipid and protein in the food and feed sectors. Topics covered include:

- novel lipids sources (eg Chiuri Oil, High Oleic Safflower)
- alternative lipid products (flavour enablers, Shea butter, lipids for plant based foods)
- fermentation products (omega 3, carotenoids)
- alternative proteins
- microalgal products
- insect oils/proteins.

Lipids in the health of our futures (focus on nutrition)
The program will present the latest nutritional science and industry details about the use of lipids in nutrition. Fats traditionally have some negative connotations when it comes to nutrition. With the rise of healthy fats (such as omega 3 oils/ olive oils etc) this program aims to update attendees with the latest information and to dispel popular misconceptions.
Topics covered include the roles of fats/lipids at various stages of life:

- Heathy infants/babies
- Sports nutrition
- Healthy women
- Healthy aging

The aim of the workshop is to provide nutritionists, dietitians, students and academics information on the latest knowledge on the role lipids play in nutrition. The work will be presented by a number of industry leaders and scientists.

The following speakers are foreseen:
Tom Brenna, Professor of Pediatrics, of Human Nutrition, and of Chemistry at the University of Texas at Austin.

Silvana Martini, BSc in Biochemistry and PhD in Chemistry from the University of La Plata, Buenos Aires, Argentina; Postdoctoral Researcher in the Department of Food Science at the University of Guelph, Canada, and Assistant Professor in the Department of Nutrition, Dietetics, and Food Sciences in Utah State University.
Ellen Schutt, Managing Director of GOED, the Global Organization for EPA and DHA Omega-3s.
Laurence Eyres, from Chief Chemist to Technical and Operations Director in several multinational food companies, operating in fats and oils, snack foods, dairy products, and process engineering.

Further information and program:
https://aaocs2023.wordpress.com/

Roundtable on Responsible Palm Oil (RSPO) c/o RT2023

20 - 22 November 2023 | Hotel Mulia Senayan Jakarta, Indonesia

Save the date for the RSPO Annual Roundtable Conference on Sustainable Palm Oil (RT2023), which will be held at the Hotel Mulia Senayan Jakarta in Jakarta, Indonesia, with virtual access available, from 20 – 22 November 2023.
Registration details will be made available in due course. In the meantime, please save the date and stay tuned for more information.

Please contact rt@rspo.org if you have any questions. https://rspo.org/event/save-the-date-rt2023-in-jakarta-20-22-november-2023/

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

Secondo le vigenti normative Comunitarie dal 2007 tutte le sostanze prodotte ed immesse sul mercato devono essere sottoposte a test di Biodegradabilità.

TEST DI BIODEGRADABILITA'

Ready Biodegradability:

OECD 301B,
Reg CE 440/2008 30/05/2008 GU L142
31/05/2008 Met C.4-C
(CO2 evolution test: modified Sturm test)

Servizi per
Cosmetica e Detergenza

Davide Mariani
Esperto Home and Personal Care
Team Prestazioni Dei Materiali
E-mail: **davide.mariani@mi.camcom.it**

La **BIODEGRADAZIONE COMPLETA** consiste nella totale degradazione (mineralizzazione), ad opera di microorganismi, di un composto organico in composti inorganici.

Il **metodo OECD 301B**, applicabile per testare la **biodegradabilità completa (ready) di qualunque sostanza organica non volatile nell'arco di 28 giorni,** è inserito nell'elenco **prove accreditate** ed è pertanto eseguito in conformità a quanto prescritto dalla norma **UNI CEI EN ISO/IEC 17025**

La **BIODEGRADAZIONE PRIMARIA** consiste nella perdita del gruppo funzionale caratteristico della molecola di tensioattivo mediante reazioni di ossidazione o altre alterazioni a carico di microorganismi.

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

www.innovhub-ssi.it

La
Rivista Italiana
delle
Sostanze Grasse

Via Giuseppe Colombo, 79
20133 MILANO

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

Author instructions

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

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

The Authors publishing their manuscripts on our scientific Journal are authorized and encouraged to upload the full text in .pdf format on their page of **ResearchGate**, increasing the diffusion and visibility of the results.

From 2020 papers are available as **Open access** on our website for free download.

In our website you can also find the *guide-lines* for manuscript preparation and submission as well as *Privacy information* notice pursuant to Regulation (EU) 2016/679 and current legislation on personal data processing.

Visit our website:
https://www.innovhub-ssi.it/media/pubblicazioni/risg-rivista-italiana-sostanze-grasse.kl

INNOVHUB - STAZIONI SPERIMENTALI PER L'INDUSTRIA S.r.l.
Sede Legale: Via Meravigli 9/b, 20123 Milano
Capitale sociale: Euro 10.000.000,00 i.v.
C.F. e n. iscr. al R.I. di Milano, Monza Brianza, Lodi: 97425580152 - P. IVA: 05121060965
REA: MI -1798570 - Socio Unico: Camera di Commercio di Milano, Monza-Brianza, Lodi
Via Giuseppe Colombo 79 - 21033 MILANO
www.innovhub-ssi.it