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Validation of a time saving method for saponification value estimation using microwaves technologies

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The use of microwave technology is increasing in routine laboratories especially for synthesis reaction and sample preparation procedure in the last decades. This technology strongly affects the reaction rate reducing analysis time and side reactions and increasing percentage yield and reproducibility. Nevertheless, microwaves are mainly used in sample preparation for chromatographic analysis while only few works have been published regarding bromatological determinations.
The aim of this work is to improve the determination of the saponification number avoiding the use of laboratory heaters, reducing the space required in the lab and taking advantage of microwave technologies to reduce sample preparation times improving reaction rate. For this purpose, the method developed with the use of microwaves was compared with the official ISO 3657:2020 method for animal and vegetable fats and oils and European Pharmacopoeia ones for cosmetic raw materials.
Keywords: Saponification, Microwaves Technologies, Validation, Vegetable Oils, Cosmetic Raw Materials

INTRODUCTION

One of the most common indices to evaluate oils and fats quality is the saponification value, that is the measurement of free and esterified acids in lipid-based products. As described in the ISO 3657:2020 [1] method, the analysis is based on saponification of a known amount of sample with excess of KOH ethanolic solution; the remaining alkali solution is then back titrated with HCl acid solution in presence of phenolphtalein as an indicator. Furthermore, the number of moles of fatty acids in the sample, reacting stoichiometrically one to one with KOH, are strictly related to the difference between the total KOH number of moles in the early solution and the titrant ones needed to reach the indicator colour turning from purple to colourless/white. Thus, saponification value shows changes inversely proportional to the length of fatty acyl chains constituting triacylglycerols.
However, despite the historicity of the analysis, only few works are available in literature on this topic. Some authors demonstrated how this parameter can be used to highlight the adulteration of cow and buffalo milk with coconut oil [2, 3]. In fact, the typical saponification value of coconut oil ranges from 243 to 262 mg KOH/g, due to its amount of lauric and myristic fatty acids [4, 5], that is significantly higher than milk value, usually varying from 213 to 227 mg KOH/g fat due to the abundance of short (C4–C6) and medium chain (C8–C12) fatty acids [6, 3]. However, except for producer countries, coconut oil can result to be an expensive product for the adulteration of dairy products so, on the other side the saponification value allows to detect adulterations with cheaper vegetable oils or fats rich in long chain fatty acids (C16 and 18), characterised by a saponification value of 168-196 mg KOH/g oil and for

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this reason bringing to an overall saponification value reduction of the hypothetical mixture.
Although the ISO 3657:2020 reference method is easy and accurate, it is time consuming (the saponification must be complete before titration and this step takes approximately one hour) and adequate laboratory spaces are needed especially if a large number of samples has to be processed.
Therefore, like many other authors in recent years [7,8,9], in order to optimise the analysis, reduce time and costs, a new reliable and time-saving method for determining the saponification value would be preferable.
To our knowledge only one attempt to improve this method has been made by Umarani et al. [10] using a domestic microwave oven and few ice pieces introduced on the saponification solution to avoid excessive solvent evaporation.
In the last years, the use of microwave technology has become widely diffused through the scientific community and laboratory microwave ovens able to process several samples together (up to 24 per run) are now easily available. Furthermore, microwave appliances can resist really high pressure and temperature conditions improving yields and speeding up reactions.
Finally, this technology can be termed as 'e-chemistry' because it is easy, effective, economic, and ecofriendly [11], improves reproducibility and reduces both side reactions and operation times [12,13,14] also helping in minimising environmental pollution.
In this work, we have developed a simple, accurate and faster procedure for the determination of the saponification value, reducing the saponification time and space needed to perform the analyses using microwaves technology.
The ISO 3657:2020 method and the microwave-assisted one were compared firstly using some reference material vegetable oils and then with the most diffused vegetable oils on the market. Furthermore, the microwave method (MW) is also applied to some widely diffused cosmetic raw materials obtaining comparable results to the official method.

MATERIALS AND METHODS

The reference materials used in this study were vegetable oils bought at B.I.P.E.A. (Proficiency testing programs Paris – FRANCE) while vegetable oils analysed as commercial samples were taken from the market or supplied directly by Associazione Granaria - Milan (Italy); some laboratory samples with certified value were used for cosmetic raw materials.
Potassium hydroxide (KOH) 0.5 mol/l solution in ethanol and hydrochloric acid (HCl) 0.5 mol/l standard volumetric solutions were used for saponification and titration respectively with phenolphthalein solution (0.1 g/100 ml of 96% ethanol) as indicator. All chemicals and solvents used with analytical purity were purchased from Sigma-Aldrich (Milan, Italy).
The reference method used was "Determination of saponification value EN ISO 3657:2020 applied to animal and vegetable fats and oils" [1], applicable to crude and refined vegetable fats and Pharmacopeia 01/2008:20506 [15] for cosmetic raw materials.

MICROWAVE-ASSISTED SAPONIFICATION

The saponification of vegetable oils was done with an ETHOS X microwave system (MW) equipped with FastEX rotor of 12 vessels in PTFE with disposable glass vial from Milestone Srl (Milan, Italy).

SAMPLE PREPARATION

The sample to be saponified was added to a 100 ml microwave vessel in a different amount depending on the expected saponification value suggested respectively by the ISO 3657:2020 method for vegetable oils reported on Table I and by Pharmacopeia 01/2008:20506 for cosmetic raw materials reported on Table II. Then, the stir bar and 25.0 ml of ethanolic KOH 0.5 M solution were added to the glass vial with a two-mark bulb 25 ml pipette. The glass vial is then transferred inside the PTFE vessel for subsequent microwave saponification carried out in an ETHOS X microwave system equipped with the FastEX rotor from Milestone Srl (Milan, Italy). The treatment temperature (120°C) was reached within 5 min at 800 W and maintained for 15 min, under constant magnetic stirring. After cooling, the exceeding amount of KOH solution is directly titrated with HCl 0.5 M solution into the glass vial, using from 0.5 to 1 ml of the colour indicator solution (Phenolphthalein) until the colour of the indicator changes at the equivalence point (from pink/purple to white or colourless depending on the analysed sample). While most of the vegetable oils can be titrated at room temperature, coconut oil, palm oil and the cosmetic raw material should be titrated

Table I - Oil sample amount based on expected saponification value

Expected Saponification Value	Sample Amount (g)
150 to 200	2.2 to 1.8
200 to 250	1.7 to 1.4
250 to 300	1.3 to 1.2
> 300	1.1 to 1.0

Table II - Cosmetic sample amount based on expected saponification value

Expected Saponification Value	Sample Amount (g)
< 3	20
3 to 10	15 to 12
10 to 40	12 to 8
40 to 60	8 to 5
60 to 100	5 to 3
100 to 200	3 to 2.5
200 to 300	2 to 1
300 to 400	1 to 0.5

Table III - Results, mean, Standard Deviation, % Standard Deviation reference value and repeatability limit (r) for eight different reference material oils using the ISO 3657:2013 standard procedure

	ISO Method							
Sample	A	B	C	Mean mg KOH/g	SD mg KOH/g	% RSD	Reference Value mg KOH/g	r*
Conventional Sunflower Oil	189.7	190.2	189.9	190.0	0.3	0.1	190.5	1.5
Sesame Oil	188.9	188.7	189.2	188.9	0.2	0.1	189.2	1.5
Mix Refined Oils	189.2	189.8	190.0	189.7	0.4	0.2	189.2	2.5
Fish Oil	189.2	189.1	188.0	188,8	0.6	0.3	188.6	4.0
Grapeseed Oil	192.3	190.9	190.3	191.2	1.0	0.5	191.3	6.2
Crude Rapeseed Oil	190.7	190.3	190.1	190.4	0.3	0.2	190.7	1.9
Palm Oil	200.1	200.6	200.8	200.5	0.4	0.2	197.6	2.2
Coconut Oil	257.1	256.9	257.4	257.1	0.3	0.1	255.3	1.5

Table IV - Results, mean and reference value for eight different reference material oils using the microwaves saponification

	MW Saponification							
Sample	A	B	C	D	E	F	Mean mg KOH/g	Reference Value mg KOH/g
Conventional Sunflower Oil	189.9	190.6	190.9	190.9	190.0	190.6	190.5	190.5
Sesame Oil	189.0	188.8	190.2	188.5	189.5	189.2	189.2	189.2
Mix Refined Oils	189.6	189.8	190.1	189.5	189.1	189.7	189.6	189.2
Fish Oil	188.6	189.0	189.4	189.6	188.5	189.9	189.2	188.6
Grapeseed Oil	192.4	192.1	192.7	192.4	191.5	192.8	192.2	191.3
Crude Rapeseed Oil	191.0	191.4	191.1	191.4	190.3	191.4	191.1	190.7
Palm Oil	201.5	201.6	201.0	202.9	201.7	202.5	201.9	197.6
Coconut Oil	258.2	259.5	259.1	260.0	259.5	259.9	259.4	255.3

while still warm to avoid sample solidification that can affect the final result of the analysis.

STATISTICS

The experiments were made at least in triplicate for both the MW and ISO 3657:2020 method. The blank tests were carried out following the procedure specified using 25.0 ml of ethanolic potassium hydroxide solution but omitting the test portion. The results were expressed in mg KOH/g fat as the mean values, standard deviation (SD) and relative standard deviation (% RSD). Furthermore, saponification number values were subjected to analysis of variance (ANOVA) to calculate the precision under conditions of repeatability, intermediate reproducibility, and accuracy [16].

RESULT AND DISCUSSION

The ISO 3657:2020 method was first applied in triplicate to eight different reference materials: seven vegetable oils (conventional sunflower oil, sesame oil, mix of refined oils, grapeseed oil, crude rapeseed oil, palm oil and coconut oil) and a fish oil. The results obtained were comparable with the assigned value for all samples, with a % RSD reaching a maximum value of 0.5 as reported on Table III. The repeatability limit generally showed good results except for fish oil and grapeseed oil that have a quite high value.

Then the saponification with MW was applied on the same eight matrixes, six times for each sample and the results are reported on Table IV. Also, in this case results were comparable with the assigned value and showed a great repeatability of the analyses with % RSD values really close to those obtained with the ISO 3657:2020 official method and even better in the case of fish oil and grapeseed oil (Table V). Furthermore, compared to the ISO method, lower values of repeatability limit were observed.

For the sample processed by microwave saponification then the accuracy was also calculated.

The accuracy was evaluated comparing the average

Table V - Standard Deviation, % Standard deviation, repeatability limit and trueness for eight different reference material oils using the microwaves saponification

Sample	SD mg KOH/g	% RSD	r*	Trueness
Conventional Sunflower Oil	0.4	0.2	1.6	0.014
Sesame Oil	0.6	0.3	2.2	0.001
Mix Refined Oils	0.3	0.2	1.1	0.143
Fish Oil	0.6	0.3	2.0	0.186
Grapeseed Oil	0.7	0.4	1.7	0.297
Crude Rapeseed Oil	0.5	0.2	1.6	0.141
Palm Oil	0.7	0.3	2.5	1.421
Coconut Oil	0.7	0.3	2.4	0.813

*Repeatability limit

Table VI - Mean, Standard Deviation and % Standard Deviation of intermediate reproducibility for three different reference material oils using the microwaves saponification repeated in six different days

	MW Saponification									
Sample	A	B	C	D	E	F	Mean mg KOH/g	SD mg KOH/g	% RSD	Reference Value mg KOH/g
Crude Rapeseed Oil	191.4	191.2	191.1	191.5	190.4	191.4	191.2	0.4	0.2	190.7
Palm Oil	199.4	200.8	200.3	201.1	200.0	200.7	200.4	0.6	0.3	200.4
Coconut Oil	259.7	259.5	259.5	259.3	259.7	259.8	259.6	0.2	0.1	259.6

All data for the six days (A, B, C, D, E, F) are reported as mean of three analyses

Table VII - Mean, Standard Deviation, % Standard Deviation, % Horwitz Standard Deviation and HORRAT value for three different reference material oils considered

Parameter	Rapeseed Oil	Palm Oil	Coconut Oil	Rapeseed Oil (ISO)
Mean (means of six different days)	191.2	200.4	259.6	190.2
SD	0.4	0.6	0.2	1.8
RSD	0.2	0.3	0.1	0.9
RSD % Horwitz	0.49	0.51	0.64	-
HORRAT Value	0.38	0.55	0.10	-

of six measurements with the declared value of a certified reference material with a composition very similar to the matrixes under examination.
To verify the result reliability, a Student t-test was performed (with a significance value of 95%). Based on the positive results the test gave, it was possible to declare that the method provides accurate results at the chosen significance level.
All results obtained showed values lower than 1 exception made for the palm oil with an accuracy of 1.420, nevertheless, all samples' trueness agreed with the difference between the calculated and theorical t-Student (Table V).
The microwave method described in this study was in-house validated by assessing the precision, expressed in terms of standard deviation for repeatability and intermediate reproducibility calculated by Horwitz equation; correctness was then calculated with the reference value. Finally, our values were compared with those of the ISO 3657:2020 method for rapeseed oil.

PRECISION

The precision of the method was determined by carrying out six analyses under repeatability conditions on reference materials, in which the tests were performed on the same day and by the same technician. The value below the absolute difference between two single test results, is expected to be found with a 95% of probability. In the intermediate reproducibility conditions, the experiments were carried out over six different days in triplicate and results are reported on table VI.
Experimental intermediate reproducibility values (RSD_R%) were used to calculate an acceptable predictive value obtained by applying the Horwitz equation, an empirical relationship between the acceptable precision and analyte concentration.
The results of the precision study are illustrated in Table VII for the three reference materials used.
The ratio between the relative standard deviation % (RSD%) under intermediate precision and the RSD% calculated by Horwitz equation is an indicator of the precision of the analysis and it is known as HORRAT value (Table VII).
Usually, HORRAT is used to indicate the presence of analytical problems that compromise the precision of the analysis: values lower than 1 indicate a good analytical precision, values between 1 and 1.5 are acceptable results while values above 2 highlight analytical issues.
Once the new method showed to be effective, it was applied, together with the ISO one, to several samples representing the main vegetable oils available on the market and used by industries in food production. For each sample the analyses were performed in triplicate for both methods and the results are reported on Table VIII.
Both methods showed results with a good repeatability, but a general lower RSD was obtained with the MW one. The better result of MW is due to the more homogeneous saponification process compared to the traditional heating processes and the constant agitation thanks to the magnetic stir bar obtaining a complete and constant homogenization of the sample during heating process.
The method using microwave saponification was also applied in triplicate on cosmetic products. The values are reported in Table IX with reference value.
As can be seen, both saponification techniques provide excellent analytical results for determining the saponification number. Microwave extraction with

Table VIII - Results, mean, Standard Deviation and % Standard Deviation for the main categories of vegetable oils available in the market

	ISO Method						MW Saponification					
Sample	A*	B*	C*	Mean	SD	% RSD	A*	B*	C*	Mean	SD	% RSD
Cocoa Butter	189.2	192.1	195.6	192.3	2.6	1.4	192.3	192.5	193.0	192.6	0.3	0.2
Extra Virgin Olive Oil	190.4	190.2	193.9	191.5	1.7	0.9	193.4	193.7	193.7	193.6	0.1	0.1
Conventional Soybean Oil	182.8	192.8	193.2	189.6	4.8	2.5	190.4	190.8	191.1	190.8	0.3	0.2
Mais Oil	190.4	190.3	191.5	190.7	0.5	0.3	191.2	191.9	190.7	191.2	0.5	0.3
Conventional Sunflower Oil	190.5	189.2	184.2	188.0	2.7	1.5	189.0	189.9	190.7	189.9	0.7	0.4
Olive Oil	191.1	193.6	190.8	191.8	1.3	0.7	191.6	191.4	190.8	191.3	0.4	0.2
Peanut Oil	183.2	188.0	187.6	186.2	2.2	1.2	188.4	189.3	190.1	189.3	0.7	0.4
Coconut Oil	256.0	257.3	261.4	258.2	2.3	0.9	259.4	259.7	260.1	259.7	0.3	0.1
Palm Oil	190.7	199.4	197.5	195.9	3.8	1.9	197.5	197.3	197.4	197.4	0.1	0.0
HO Sunflower Oil	182.9	188.8	190.4	187.4	3.2	1.7	194.1	193.6	193.8	193.8	0.2	0.1
Avocado Oil	187.1	194.0	190.4	190.5	2.8	1.5	191.0	191.0	190.5	190.8	0.2	0.1
HO Soybean Oil	187.6	191.0	190.2	189.6	1.4	0.8	191.0	191.0	190.5	190.8	0.2	0.1
HO Rapeseed Oil	186.9	184.2	189.8	187.0	2.3	1.2	187.5	188.4	188.6	188.2	0.5	0.3
Safflower Oil	190.7	185.8	190.8	189.1	2.3	1.2	192.1	191.9	191.9	192.0	0.1	0.1
Conventional Rapeseed Oil	188.0	187.9	189.3	188.4	0.6	0.3	191.0	190.5	191.1	190.9	0.2	0.1
Sesame Oil	187.5	188.2	187.3	187.6	0.4	0.2	187.9	187.7	188.6	188.1	0.4	0.2
Linseed Oil	188.6	188.9	190.4	189.3	0.8	0.4	190.6	190.6	191.3	190.8	0.3	0.2

***** mg KOH/g

Table IX - Results, mean, Standard Deviation and % Standard Deviation for some cosmetics ingredients frequently used on cosmetic products formulation

Sample	A	B	C	Mean mg KOH/g	SD mg KOH/g	% RSD	Reference Value mg KOH/g
Trioctyldodecyl citrate	142.4	142.2	142.9	142.5	0.4	0.3	145.5
Hydrogenated castor oil dimer dilinoleate	185.3	188.6	186.2	186.7	1.7	0.9	188.2
Vegetal Stearine	206.3	203.7	208.2	206.0	2.3	1.1	207.3
Blend of Mono-, Di- and Triglycerides	277.5	277.7	277.1	277.4	0.3	0.1	284.0
Isostearyl isostearate	103.8	102.8	102.7	103.1	0.6	0.6	103.0
Dipentaerythrityl tetrabehenate/polyhydroxy stearate	183.8	183.0	183.7	183.5	0.4	0.2	184.0
Glyceryl Undecilenate	209.9	209.5	208.9	209.5	0.5	0.2	207.3
Polyglyceryl-10Pentahydroxystearate	127.3	127.0	126.4	126.9	0.5	0.4	130.0

Milestone ETHOS X is performed in a few minutes and multiple samples can be analysed simultaneously, in safe and constantly monitored conditions.
Microwave-assisted saponification uses closed vessels allowing to reach higher pressures and consequently temperatures above sample solution atmospheric boiling point; the increased solubility of the analytes and lower viscosity of the solvent speed up the reaction with the matrix reducing analysis time.

CONCLUSION

The method developed seems reliable to be used for saponification number evaluation as a possible alternative to the ISO 3657:2020 official one, in terms of repeatability, intermediate reproducibility and accuracy. Advantages are the use of MW as heating source that saponify the samples in less time (20 min compared to 1 h of the official method), the better results of this analysis compared to ISO method is due also to the constant agitation allowed by the magnetic stir bar during both saponification and titration processes. Furthermore, the rotor able to host up to 24 samples reduce the necessary laboratory space and times needed to analyse several samples.
Compared to the conventional saponification techniques for the SV determination (ISO 3657:2020 and European Pharmacopoeia 01/2008:20506), the obtained microwaves-based saponification values suggest the new method as a more sustainable and rapid alternative approach.

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Conflict of interest

Authors declare no conflict of interest.

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Replacement of meat fat with olive oil and its effect on mortadella properties

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The use of healthy ingredients in the formulation of meat-based products has gained a growing interest. The effect of replacing meat fat with olive oil on mortadella properties was investigated. Mortadella was produced by the addition of the normal level of meat (control sample), low levels of meat fat with or without the addition of the replacements (olive oil 0%, 3%, 5%, and 8%), and the lipid reformulation effects on mortadella properties were studied. The results revealed that the control mortadella had the highest cholesterol content compared to the experimental treatments. Mortadella samples with 8% olive oil were less acceptable to the panellists. Juiciness and soft texture parameters of mortadella were not significantly different among the control and samples with 8% and 5% olive oil, nevertheless, they were significantly higher than lean and low-fat mortadella (0% and 3% olive oil, respectively). Lean meat samples (without any fat addition) received lower scores in terms of overall acceptability but had the lowest TBA value. The control sample had the highest saturated fatty acid percentage (52%) which was higher ($p<0.05$) when compared to samples incorporated with olive oil, however, it did not statistically differ when compared to lean meat mortadella. Mortadella sample produced with 3% olive oil exhibited the highest monounsaturated fatty acid percentage. This study demonstrated the importance of fat in mortadella products. The results indicated that the complete replacement of animal fat in mortadella by olive oil is not sensorially possible, however, a partial replacement of low levels of olive oil (3%-5%) can be successfully achieved.
Keywords: Mortadella, Fat replacement, Cholesterol, Sensory characteristics.

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1. INTRODUCTION

Fats and oils play vital functional and sensory roles in food products, since they carry, enhance, and release the flavour of other ingredients. Fats also aid in developing texture, mouth feel, and the overall sensation of lubricity and moistness in the mouth [1]. Besides their importance in the food industry, fats have positive nutritional aspects, since they are essential for the human body's process of cell building and repairing. Moreover, fats carry and aid the absorption of fat-soluble vitamins A, D, E, and K [2-3].

However, there are several health concerns associated with fat consumption that can promote obesity among other serious non-communicable diseases, including diabetes, cardiovascular disease (CVD), coronary heart disease, and hypertension [4]. The world health organisation has recently declared that it is mandatory to reduce the total fat intake (particularly saturated) to maintain a healthy diet since, according to previous studies, high-fat diets are linked to an increased risk of colon cancer [5-7].

In recent times, healthy eating has become an ongoing topic around the globe [5]. Thus, to meet consumers' demand for healthier foods, food technologists have put an emphasis on developing reduced-fat products without any adverse effect on their organoleptic properties, texture, and flavour [8,

9]. To succeed in developing low-fat palatable products, other ingredients have to be chosen to replace fat, since for instance, 20-30 g of fat per 100 g is necessary to make burgers avoiding compromising its quality traits [9]. These ingredients must impart the flavour and mouth feel that would normally be derived from fat [10]. Additionally, fat replacers must not only mimic the natural fat in delivering similar structural configuration and quality characteristics but should be healthier from a nutritional perspective than that of animal fat [11]. For example, [6] investigated the influence of partially and completely replacing pork backfat with soybean oil in mortadella. The authors declared that there were no differences ($p>0.05$) in any of the technological and physicochemical parameters evaluated, whereas mortadella incorporated with vegetable oil showed a higher unsaturated fatty acid content comparable to products made with pork backfat. However, treatments made with vegetable oil showed lower ($p\leq0.05$) sensorial perception than those made with pork fat on all the tested attributes. Similar findings were reported by [12] to substitute animal fat with vegetal fat.

In this study, olive oil was chosen to replace animal fat in mortadella. Olive oil, the primary source of fat in the Mediterranean diet, contains a high percentage (77%) of the monounsaturated oleic acid [13, 14]. This particular fatty acid reduces low-density lipoprotein (LDL) cholesterol and increases high-density lipoprotein (HDL) cholesterol [6]. Moreover, the mixture of oleic acid and polyphenolic compounds (which exist naturally in olive oil) imparts antioxidant and anti-inflammatory properties to olive oil, which tend to promote protection against the development of certain diseases such as CVD, diabetes (type II), and cancer [15].

To the best of our knowledge, there are no sufficient studies on a partial or total substitution of meat fat with olive oil in beef mortadella, with different fat levels and blends, particularly in Jordan. In this context, this study aimed to evaluate the possibility of optimising the fatty acid profile of mortadella by partially or completely replacing meat fat with olive oil, determine the properties of the developed products including sensory characteristics and lipid profile, and evaluate the effect of meat fat replacement with olive oil on cholesterol level of the product.

2. MATERIALS AND METHODS

2.1 MORTADELLA MANUFACTURE

Five mortadella treatments were formulated as shown in Table I. The formula of one of the local meat factories was used. One treatment (sample 1) was produced by the addition of the normal level of beef meat fat commercially used (i.e., the control group). The remaining four treatments were prepared by using lean beef with lower levels of meat fat with or without the addition of fat replacers (olive oil). Replacement levels used were 0%, 3%, 5%, and 8%, and were according to work done by many researchers [16-19].

2.2 RAW BATTER PREPARATION

Batches of mortadella were made using the formulations shown in Table I. Commercial olive oil and beef meat were purchased from a local market in Amman (Jordan). Tthe other ingredients used were obtained from a local meat factory (Siniora Factory, Amman, Jordan) where mortadella processing took place. Three treatments contained the replacer, olive oil (acidity 1.5%) at three levels (3%, 5%, and 8%) in the form of emulsion with soybean concentrate and water. Other ingredients were added in order, sodium tripolyphosphate, sodium nitrite, sodium ascorbate, garlic, and water (ice). The mixture was chopped for 2 minutes, then spices, soybean, and finally starch were added and chopped for 5 additional minutes. All ingredients added, except the beef fat amount and source, were constant for all treatments. The mixture was chopped for a total of 10 minutes; the

Table I - Formulations of reduced-fat mortadella samples prepared with the addition of olive oil

		Treatments				
Ingredients		**1**	**2**	**3**	**4**	**5**
Meat (%)	Normal fat level meat	81.3	-	-	-	-
	Reduced fat level meat (Lean)	-	81.3	73.3	76.3	78.3
Sodium nitrite (ppm)		120	120	120	120	120
Sodium tripolyphosphate (g)		60	60	60	60	60
Starch (%)		4.0	4.0	4.0	4.0	4.0
Soybean (%)		1.0	1.0	1.0	1.0	1.0
Salt (%)		2.0	2.0	2.0	2.0	2.0
Spices (%)		1.5	1.5	1.5	1.5	1.5
Sodium ascorbate (ppm)		500	500	500	500	500
Water (ice) (%)		10	10	10	10	10
Garlic (g)		20	20	20	20	20
Olive oil (%)		-	-	8.0	5.0	3.0
Total (Kg)		20	20	20	20	20

Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat + 8% olive oil, 4 = lean meat + 5% olive oil, 5 = lean meat + 3% olive oil.

final meat blend temperature was 15°C. Immediately after chopping, the batter was stuffed in polyethylene-based casings (a roll of 90mm diameter, 500g weight) using a stuffer (Handtman, Germany). The method of mortadella preparation and cooking used is according to an internal factory procedure.

2.3 OIL EMULSION PREPARATION

Olive oil was heated to 60°C and mixed with soybean and warmed water (80°C) at the ratio of (1:3:4) soybean concentrate (powder): olive oil: water, respectively in a bowel chopper (Alpina, Switzerland) for 5 minutes. The mixture was then frozen for one week prior to using. In treatments 3 and 4 (8% and 5% olive oil, respectively), the soybean concentrate incorporated was less than 1% to achieve a similar quantity of soybean concentrate to that in the other treatments.

2.4 COOKING AND STORAGE

Mortadella batches were thermally processed in an oven (Franco-Mat) with the following temperature/humidity cycles:

- Heating: 30 minutes at 55°C and 10% relative humidity (RH).
- Cooking at 80°C to reach 72°C internal temperature and 100% (RH).

Internal temperatures were measured by inserting the thermometer in the centre of the mortadella samples before cooling. After cooling the mortadella, samples were stored at 4°C until analysed. This procedure was followed for both the first and second experiments.

2.5 CHEMICAL ANALYSES

2.5.1 PH

Mortadella samples were homogenized in distilled water in a ratio of 1:10 sample/water, the pH was measured using a pH meter (model WPA, Cambridge) after proper calibration [20]. The measurements were performed in duplicate.

2.5.2 Proximate analyses

Samples were analysed for moisture, ash, fat, and protein following AOAC [21] procedures. The oven drying method was used to determine the moisture content at which 5g sample was heated at an oven temperature of 105°C for 3 hours (AOAC Method 950.46). The ash contents were assessed by sample incineration in a Muffle Furnace at 550°C for 12 hours (AOAC method 940.26).

Protein was determined by the micro-Kjeldahl method, a 0.5 g of the sample was first digested in a 10 ml sulfuric acid (98% w/w) with the addition of a digestive mixture of K_2SO_4+$CuSO_4$×$5H_2O$ to break down organic matter and reduce nitrogenous compounds to ammonium salts. Ammonia was liberated by boiling with sodium hydroxide (50% w/v) aqueous solution and steam distilled into boric acid to form ammonium borate. Ammonia was then titrated with 0.1 N HCl and screened methyl red was used as an indicator solution. Protein nitrogen content was obtained by multiplying the result by the factor 6.25.

For fat determination, the Soxhlet method (AOAC Method 963.15) was applied. In brief, ether was continuously volatilised then condensed and allowed to pass through the 5 g moisture-free sample, prepared into the extraction thimble and covered with cotton wool. The extract was collected in a beaker after extraction for 16 hours, the ether was afterward distilled and collected in another container and the remaining crude fat was dried and weighed. All determinations were performed in duplicate.

2.5.3 Thiobarbituric acid number (TBA)

Ten-gram portions of mortadella were combined with each of 25 ml of 20% trichloroacetic acid (TCA) and 20 ml of warmed distilled water, and homogenised in a stomacher (Model AES, Labprat) for 30 seconds. The homogenate was filtered through a Whatman #1 filter paper and then in a test tube, 2 ml of the filtrate was combined with a 2 ml of 0.02 M aqueous 2- thiobarbituric acid (TBA). The tubes were incubated at 22°C in the dark for 20 hours. At the end of that time, the absorbance of the resulting solution was measured at 532 nm according to [22] using a UV-visible spectrophotometer (Spectro 2000RS, Labomed, Inc., USA).

The thiobarbituric number (TBA) mg of malondialdehyde/kg sample (ppm) was calculated by multiplying the measured absorbance of pink-coloured chromagen of the TBA-reactive substances at 532 nm by a factor of 7.8 [23]. The TBA was carried out in triplicate at weeks 1, 2, 3, 4, and 5 of refrigeration storage.

2.5.4 Cholesterol content

Cholesterol content was determined in triplicate using the Colorimetric method directly in mortadella samples according to the test kit instructions (Cat. No.139,050, Boehringer Mannheim, Germany) [24]. First, cholesterol is oxidised by cholesterol oxidase to cholestenone. Then, in the presence of catalase, the hydrogen peroxide produced in this reaction oxidises methanol to formaldehyde. The latter reacts with acetylacetone forming a yellow lutidine dye in the presence of NH_4 ions.

The concentration of the lutidine-dye (3,5-diacetyl-1,4-dihydrolutidine) formed is stoichiometric to the amount of cholesterol and is measured by the increase of light absorbance in the visible range using a spectrophotometer at 405 nm. Subsequently, 10 ml of a freshly prepared methanolic potassium hydroxide solution (1mol/L) was added to a 2.5 g mortadella placed in a 50 ml round-bottomed flask. The flask was heated under a reflux condenser for 25 minutes, and then the supernatant solution was transferred into a 25ml volumetric flask using a pipette and allowed to cool. Afterward, the contents were diluted up to the mark with isopropanol and fil-

tered. The clear solution was used for the assay in which 5 ml of cholesterol reagent mixture was mixed with 0.4 ml of the sample solution. Then, 2.5 ml of this mixture was pipetted into a test tube, followed by the addition of 0.02 ml of cholesterol oxidase. After covering the test tube, it was incubated in a water bath at 39 °C for 60 minutes. The absorbance readings of both the blank and the sample were measured using a UV spectrophotometer (Spectro 2000RS, Labomed, Inc., USA) at 405nm. Cholesterol concentrations (g/L sample solution) were measured by multiplying the absorbance difference by 0.711. Cholesterol content was calculated according to the following equation and expressed as mg × 100 g^{-1} product:

Cholesterol content (mg/100 g) =

= [C cholesterol (g/L sample solution)/ Weight sample (g)] × 100 × 25

2.5.5 Fatty acid profile determination

For the extraction of lipids, a 10 g mortadella sample was homogenised in chloroform: methanol (2:1 v/v) according to [25, 26], then filtered. The filtrate was then placed in a separatory funnel; the lipid phase of the chloroform fractions was collected.

A rotary evaporator was used to get rid of chloroform; 3 ml of petroleum ether was added to 0.5 g of the extracted fat, then 0.15 ml of potassium hydroxide solution (2N) was added. It is worth noting that sodium hydroxide was substituted in this procedure with potassium hydroxide because it was very difficult to prepare sodium methoxide. Potassium hydroxide, however, gave good results for the fatty acid esterification. The mixture was left for 5 min and then injected into a gas chromatograph (Shimadzu GC-2010). The employed working conditions were as follows: column (Restek, Rtx-225, USA, cross bond 50%-cyanopropylmethyl 50%-phenylmethyl polysiloxane, 60 m, 0.25 mm/D, 0.25 µm df) carrier gas, helium; injector temperature, 250°C; detector temperature, 280°C; temperature program, 175°C at first, then 220°C at a rate of 2°C/min for 20 minutes. The fatty methyl esters were identified and quantified using the concept of internal standardisation by comparing their retention periods to those of established standards. Each measurement was performed in triplicate.

2.6 SENSORY EVALUATION

A 9-point hedonic scale test was carried out as described by [27] to investigate the degree of preference of the mortadella treated with different levels of olive oil. Twenty panellists were chosen from the staff of the Department of Nutrition and Food Technology of the University of Jordan for the sensory evaluation. These panellists were from both sexes and different age groups and were requested to test each sample separately without considering the other samples. Each panellist was instructed to express their evaluation of colour, flavour, juiciness, texture, and overall acceptability by filling out a copy questionnaire. Samples were evaluated in triplicate in separate sessions.

2.7 STATISTICAL ANALYSIS

All statistical analyses were performed by the analysis of variance (ANOVA) using JMP (release 10, SAS institute, Cary, NC) to determine any significant differences among the parameters associated with the study. The significant differences of means were determined at $p \leq 0.05$ using least significant differences (LSD) method [26].

3. RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

Table II shows the proximate composition and pH values of the developed mortadella treatments. Moisture content ranged from 57.4 to 62.5%, fat content from 2.8-14.8% protein content from 19.3-26.4%, and ash content from 2.84-3.86%. The lipid reformulation did not influence the pH values of all mortadella samples ($p>0.05$) stored at 4°C, which ranged from 6.2- 6.3. Moisture, fat, protein, and ash contents were within the range specified in the Jordanian Standard for meat and meat products – sausage products (JS: 816/2008), which is 65% maximum for moisture, 25% maximum for fat, and 12% minimum for protein content.

Regarding the moisture content, treatment 1 (i.e., the control) had a significantly lower value when compared with the other treatments. This was probably due to the higher fat content of this sample, since water i.e., the most important component of meat quantitatively, comprising up to 75% of its weight, is inversely related to fat content [28]. These results agreed well with the findings of [29, 30] who worked on canned luncheon meat formulations as affected by different raw meat sources. [29] reported that the moisture content of the different formulations reflected the amount of water, where formulation 1 (F1), which had the highest amount of added water and no beef fat (0%) added to its formula during preparation, showed a significantly higher moisture content (63.5%) than the remaining samples. Moreover, the sample that showed the lowest moisture content (61.0%) was the one with the lowest amount of added water and the highest amount of added beef fat (11.2%) to its formulation, and reportedly, that could have been the cause of its reduced moisture content. The authors also concluded that the type of meat used in the product making is more important than the chemical composition. Similarly, [30] found the low-fat product had a significantly higher moisture content than the high-fat version subjected to the same conditions. However, in the current study, there were no significant ($p>0.05$) differences in moisture % among all other experimental treatments ($p>0.05$). Treatment 2 had the highest protein percentage, which was significantly higher when compared with treatments 1, 3, and 4. However, treatment 2 had a

comparable protein% to treatment 5, probably because these two treatments had the lowest fat content among all treatments. Since treatment 1 had significantly lower moisture content compared with the other treatments, it expectedly showed the highest fat content. In addition, protein content did not differ (p>0.05) between the control and treatments 3, 4, and 5.

Despite having no significant differences in ash contents among treatments, treatments 2 and 5 showed the highest ash contents (3.86% and 3.7%, respectively). This is probably due to the higher meat percentages (81.3% and 78.3%) incorporated in the formulation of these two treatments. However, even though a high meat percentage was used in preparing treatment 1 as well, the reason it showed a lower ash content may be attributed to its elevated fat level (14.8%) when compared with treatments 2 and 5, which contained 2.83% and 5.10% fat, respectively.

3.2 CHOLESTEROL CONTENT

The values for the cholesterol content of mortadella are shown in Table III. Treatment 1 (the control mortadella) had the highest cholesterol content of 117 mg/100 g, which was significantly higher (>225%; p<0.05) when compared with all other treatments. This was attributed to the high animal fat percentage in the control sample. [31] reported a significant decrease in the cholesterol level in meat burgers when ground poppy seed was used as a fat replacer. Similar results were observed by [32-38]. Overall, authors reported a cholesterol reduction in meat products when animal fat was replaced by a healthier substitution.

Treatments 3, 4, and 5 did not differ (p>0.05) in cholesterol content, and this can be related to the cholesterol-free olive oil that was added to these treatments. Cholesterol content in these treatments came from the low animal fat content of the lean meat that was used in mortadella preparation because it is a well-known fact that fatty meats contain higher cholesterol levels than lean ones [39, 40].

Although treatment 2 had higher cholesterol content (52 mg/100 g) than treatments 3, 4, and 5, no significant differences were observed among these four experimental treatments. This is due to the notably low animal fat percentage (2.83%) of treatment 2. This value was lower when compared with other studies (13), in which extra-lean ground beef (Total fat is 17.1%) was used. [41] reported that it is important to remove external fat to reduce the total fat intake and total cholesterol intake. Consistent data were also provided by [42].

A wide range of cholesterol is found in separable lean meat of beef and pork, which is influenced by many factors such as age, breed, gender, kind of muscle (type of cut), animal diet, and degree of marbling [43-46]. Previous literature also demonstrated that greater cholesterol content is usually found in cooked or processed meat products than that of raw, which is due to moisture loss while cholesterol is retained in the tissues [44, 47].

3.3 FATTY ACID PROFILE

Percentages of major fatty acids of the prepared mortadella are displayed in Table IV. Stearic acid, which is found in many animal fats in relatively large amounts, was significantly lower in treatments 3, 4, and 5 when

Table II - Proximate analysis and pH values of the reduced-fat mortadella samples formulated with meat fat replacement with olive oil.

Treatments	Moisture%**	Fat%	Protein%	Ash%	pH
1	57.37^{b} ±0.02	14.80^{a} ±0.05	19.30^{a} ±0.02	2.84^{a} ±0.01	6.3^{a} ±0.02
2	62.51^{a} ±0.01	2.83^{c} ±0.00	26.39^{b} ±0.04	3.86^{a} ±0.01	6.2^{a} ±0.01
3	61.85^{a} ±0.01	11.30^{a} ±0.02	19.26^{a} ±0.01	2.85^{a} ±0.00	6.3^{a} ±0.01
4	62.30^{a} ±0.05	8.10^{b} ±0.01	21.47^{a} ±0.01	3.10^{a} ±0.00	6.2^{a} ±0.01
5	62.32^{a} ±0.02	5.10^{b} ±0.01	23.40^{ab} ±0.02	3.70^{a} ±0.01	6.2^{a} ±0.01

Each value is the average of two determinations, with coefficient of variability less than 5%.

^{a,b} Superscripts within the same column indicate statistically significant differences ($p<0.05$).

*Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat+8% olive oil, 4 = lean meat+5% olive oil, 5 = lean meat+3% olive oil.

Table III - Cholesterol content (mg/100g) of the mortadella samples formulated with the substitution of meat fat by olive oil.

Treatment*	1	2	3	4	5
Cholesterol (mg/100g food)	117^{b} ±0.36	52^{a} ±0.28	48.2^{a} ±0.23	48.1^{a} ±0.18	47.6^{a} ±0.19

*Results are expressed as means of triplicate determinations.

^{a,b} Superscripts indicate statistically significant differences ($p<0.05$).

*Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat+8% olive oil, 4 = lean meat+5% olive oil, 5 = lean meat+3% olive oil.

Table IV - Means of the major fatty acids, and the saturated, monounsaturated, polyunsaturated fatty acids (expressed as percentage by weight of the total fatty acids detected), and "saturated to unsaturated fatty acids" ratio (SFA/UFA) of the mortadella samples prepared with the replacement of meat fat by olive oil.

Fatty acids	Treatment				
	1	2	3	4	5
Stearic (C18:0)	16.5^{a} ±0.01	15.4^{ab} ±0.01	8.2^{d} ±0.01	11.1^{c} ±0.01	13.3^{bc} ±0.01
Palmitic (C16:0)	27.5^{a} ±0.01	27.2^{ab} ±0.01	23.1^{c} ±0.01	24.6^{bc} ±0.01	25.4^{abc} ±0.01
Oleic (C18 :1)	48.0^{a} ±0.01	50.0^{ca} ±0.02	60.0^{d} ±0.02	53.9^{b} ±0.01	53.3^{bc} ±0.02
Linoleic (C18:2)	-	-	8.0^{a} ±0.01	10.3^{b} ±0.01	7.8^{a} ±0.01
Myristic (C14:0)	8.0^{a} ±0.01	7.4^{a} ±0.01	-	-	-
ΣSFA	52.0^{a}	50.0^{a}	31.3^{b}	35.7^{b}	38.7^{b}
ΣMUFA	48.0^{a}	50.0^{ca}	60.0^{b}	53.9^{b}	53.3^{bc}
ΣPUFA	-	-	8.0^{a}	10.3^{b}	7.8^{a}
SFA/UFA	1.1	1.0	0.41	0.64	0.72

Data are expressed as means of triplicate determinations.

^{a,b,c,d} Superscripts within the same column indicate statistically significant differences ($p<0.05$).

*Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat+8% olive oil, 4 = lean meat+5% olive oil, 5 = lean meat+3% olive oil.

Table V - Sensory evaluation scores of the reduced-fat mortadella samples formulated with the substitution of meat fat by olive oil

Treatments	Color	Flavor	Juiciness	Texture	Overall acceptability
1	7.7^{a} ±0.00	7.5^{a} ±0.00	7.6^{a} ±0.01	7.8^{a} ±0.00	7.5^{a} ±0.02
2	7.9^{a} ±0.00	6.6^{b} ±0.01	6.0^{b}±0.02	6.0^{b} ±0.01	6.3^{ab} ±0.01
3	7.5^{a} ±0.01	6.4^{b} ±0.01	7.7^{a} ±0.01	7.5^{a} ±0.00	6.1^{b} ±0.02
4	7.4^{a} ±0.01	7.3^{a} ±0.00	7.5^{a} ±0.00	7.4^{a} ±0.01	7.3^{ab} ±0.01
5	7.5^{a} ±0.00	7.3^{a} ±0.01	6.5^{b} ±0.01	6.6^{b} ±0.01	6.9^{ab} ±0.01

Data are expressed as means of triplicate determinations.

^{a,b} Superscripts within the same column indicate statistically significant differences ($p<0.05$).

*Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat+8% olive oil, 4 = lean meat+5% olive oil, 5 = lean meat+3% olive oil.

compared to the control. The lowest stearic acid percentage was reported for treatment 3.

Stearic acid and palmitic acid, which were, in quantitative terms, the major saturated fatty acid (SFA) found in the samples' lipid fraction, were significantly lower in olive oil-containing treatments (i.e., 3, 4, and 5) than the rest of the treatments. This finding was attributed to the reduction of the total SFA (Table IV) in the lipid profile of these modified samples compared to the control, as demonstrated by [38]. Oleic acid (C18:1) was the main fatty acid found in the lipid fraction of the mortadella, with significantly higher values for the modified olive oil-incorporated treatments. This is possibly due to the elevated amount of oleic acid in olive oil, which represents 70-80% of its composition [48]. Other studies have yielded similar findings [38, 49, 50]. These results indicate that the use of olive oil as a fat replacer in mortadella has positively impacted its health properties. Myristic acid (C14:0) was only detected in treatments 1 and 2, while not detected in the rest of the treatments (i.e., olive oil included). [38] studied the effect of the partial and total replacement of pork backfat by oleogel from high-oleic sunflower oil gel on the fatty acid profile of bologna-type sausages, which is a product similar to the mortadella sausage, the subject of this study. The authors found that when the replacement percentage increased from 25 to 100%, the myristic acid content reduced from 2.37% in the control bologna-type down to 1.56% in the same product with 100% replacement. This, in addition to our results, can be mainly attributed to the very low content of myristic acid (0.03 g/100 g total fatty acid) in olive oil compared to 3.54 g and 1.19 g/100 g total fatty acid in the beef fat and pork backfat, respectively [51].

[52] Reported that in contrast to palmitic acid, olive oil elicits a beneficial influence on insulin sensitivity. Moreover, the authors added that oleic acid inhibits palmitic acid-induced inflammation and insulin resistance. Health studies have shed light on increasing oleic acid intake, and if possible, lowering the palmitic acid content of meat fats by using oleic acid-rich oils such as high oleic sunflower and olive oils [52-55]. It was stated by [56] that the most consumed saturated long-chain fatty acids in the American diet are palmitic acid, myristic acid, and stearic acid.

Percentages of the SFA, monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids, in addition to the saturated to unsaturated fatty acid (UFA) ratio of the prepared mortadella treatments, are shown

in Table IV. Treatment 1 (the control, whichcontained normal fat level without olive oil) had, as expected, the highest level of SFA as palmitic acid (C16:0), stearic acid (C18:0), and myristic acid (C14:0), the lowest MUFA content, and thus the highest SFA/UFA ratio. A similar trend was observed by [50] who reported that the inclusion of olive oil had modified the total fatty acid profile of dry-ripened sausages. Additionally, both treatments 1 and 2, which were prepared without the addition of olive oil, had a significantly higher SFA content and subsequently higher SFA/UFA ratio when compared with treatments 3, 4, and 5.

Treatment 3 (highest olive oil content) had the highest MUFA fatty acid percentage as oleic acid (C18:1), followed by treatments 4 and 5. This can be attributed to the abundantly high oleic acid content in olive oil, which was added to these three treatments. [57] reported that when MUFAs are used as a substitute for certain SFAs, they tend to reduce plasma LDL (undesirable) cholesterol without decreasing plasma HDL (desirable) cholesterol, whereas PUFAs may decrease both. According to [58], lean roasted beef or lean roasted pork contains about 15 g total fat /100 g meat, from which 6 g are SFA in beef and 4 g in pork, with equal amounts of MUFA (6 g), and with 1.5 g PUFA in pork and a trace in beef. The author reported that the degree of saturation of fatty acids in beef is more than that of pork. [59] reported that subjects consumed higher meat fat when they ate beef and pork than when they ate poultry or fish, and more SFA when they ate beef than other meats, and yet the mean values for serum total cholesterol did not differ significantly.

Polyunsaturated fatty acids were only detected in treatments 3, 4, and 5 as linoleic acid (C18:2), with significantly higher linoleic acid content in treatment 4 than in treatments 3 and 5.

It is worthy to highlight that, according to the food labelling guide established by the US food and drug administration [58], treatments 3, 4, and 5 can be nutritionally labelled as SFA-reduced since they contained 39.8%, 31.3%, and 25.5% less SFA, respectively than the control (must achieve at least 25% less SFA).

3.4 OXIDATIVE RANCIDITY TEST

The estimated TBA values, a measure of oxidative rancidity, are shown in Figure 1. Treatment 2 (lean without any fat addition) showed the lowest TBA value of 0.172, which was significantly lower when compared with the other treatments. This is probably due to the diminished fat level in treatment 2 (2.83%). The TBA values of all mortadella treatments increased with time, indicating that the oxidation process had taken place during storage. A similar trend was reported in previous studies done with cured meat products [34, 38]. Moreover, an increase in TBA values was observed with increasing the amount of olive oil included in the formulation. These results are in accordance with [34] who reported higher TBA values in samples containing olive oil. This may be related to the increased level of UFA in olive oil, which is more susceptible to oxidation reactions as demonstrated by [50]. On the other hand, in a study of fat replacement by high-oleic oleogel in bologna-type sausages, [38] found that TBA values of the experimental samples were lower than the controls. All treatments, in our study, had acceptable TBA values for rancidity (<1.0) after 5 weeks of storage, which was consistent with other studies [38, 49].

Olive oil-incorporated treatments (3, 4, and 5) had the highest TBA values. This fact could be related to the greater susceptibility of UFA to lipid oxidation, in addition to PUFAs that are present in olive oil such as linoleic acid. [61] and [62] reported that the presence

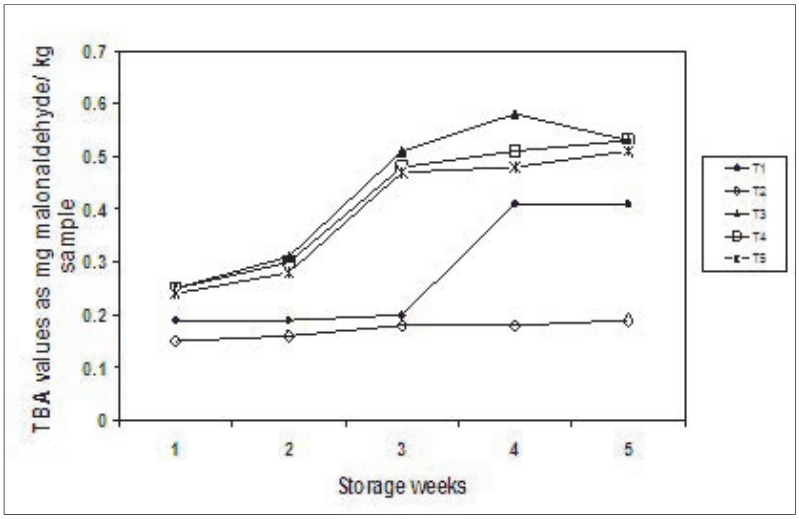

Figure 1: Thiobarbituric acid (TBA) values of the mortadella samples prepared with the substitution of meat fat by olive oil. (Treatments: 1 = control mortadella (normal fat level), 2 = lean mortadella (reduced fat level), 3 = lean meat+8% olive oil, 4 = lean meat+5% olive oil, 5 = lean meat+3% olive oil).

of PUFAs in meats is a major factor in lipid oxidation and shelf-life shortening.
However, the modified treatments; 3, 4, and 5 did not differ (p>0.05) in TBA values when compared to the control. Suggestively, this may be due to the presence of natural antioxidants in olive oil, such as tocopherols which might have reduced lipid oxidation in these treatments. This finding agrees with previous studies [12, 63] which declared that TBA values were not influenced by the fat replacement despite the high UFA content. Additionally, olive oil contains an elevated level of MUFA in the form of oleic acid (77%), which is less susceptible to lipid oxidation.

3.5 SENSORY EVALUATION

Table V displays the sensory evaluation scores of the developed mortadella using a 9-point hedonic scale. Colour, flavour, juiciness, texture, and overall acceptability scores of all samples were relatively within acceptable ranges, with mean scores above 6 ("like"). No significant differences were observed between all treatments for the attribute colour (p>0.05). This result suggested that the inclusion of olive oil did not alter visual sensory features. Several studies have reported that low-fat products were darker, darker red, or rather more intensive in colour than high-fat products [64-69]. The authors have further added that the increase in the red colour intensity presumably resulted from both the increased lean meat content and the lack of fat which possesses a whitening property [68]. This might justify the fact that, in the present study, treatment 2, which had the lowest fat content, received the highest colour-liking scores. Moreover, [70] found that burger patties treated with olive oil presented a darker colour, whilst like our study, [71] and [34] reported no significant differences in terms of salami and Turkish soudjouk colour, respectively, between oil-containing samples and the basic formulations.
The estimated scores for mortadella flavour are shown in Table V. No statistical differences (p>0.05) were observed between control and treatments 4 and 5. However, treatment 2 was less preferred than samples with higher fat content, probably due to its reduced fat level which is known to affect meat flavour and palatability [72]. Treatment 3, which had 8% olive oil, achieved the least consumer acceptance, possibly due to the distinguished olive oil flavour. Thus, these results imply that olive oil can be used as a fat replacer in mortadella at levels below 8% without negatively affecting its acceptance. The juiciness and texture acceptability scores of treatments 1, 3, and 4 were comparable (p>0.05), but they were significantly higher when compared with treatments 2 and 5. This is probably due to differences in the fat content, since fat is known to affect the juiciness of meat products, and a decrease in meat juiciness was reported [68, 73, 74] as a result of fat reduction. Also, low-fat products were further reported to easily become dry, firm, and rubbery [75, 76] and higher tenderness and juiciness values were related to higher fat contents [16, 74, 77]. These results were consistent with those of other researchers [63, 78, 79]. They generally reported that low-fat meat products formulated with healthier substitutions were relatively similar in juiciness and/or texture to high-fat products.
Overall acceptability scores were not significantly different (p>0.05) among treatments 1, 2, 4, and 5. Treatment 5, which contained 3% olive oil, achieved a lower degree of consumer acceptance than treatments 1 and 4. A possible explanation is that the latter two treatments were softer than treatment 5. Moreover, treatment 3 received the lowest acceptance scores among all treatments (significantly lower than the control). This was probably attributed to the influence of olive oil taste in this treatment which contained 8% olive oil in its formulation. [80] declared that fat replacers can affect meat flavour by adding flavours of their own.
Treatment 2, which contained only lean meat without any fat addition, was less acceptable by consumers, possibly due to the extremely low-fat content. This agreed with previous studies [18, 76, 81] which reported that fat acts as a reservoir for flavour compounds and contributes to the texture of the product. The authors added that reducing the fat content could alter product quality. Additionally, [80] and [82] reported that the decrease in fat level leads to a reduction of the flavour intensity, juiciness, tenderness, and thus overall acceptability of meat products. [16] and [83] reported that a low-fat product can be made from a lean (greater than 90% fat-reduction) all-meat formulation, but the sensory characteristics would not be acceptable to consumers.

CONCLUSIONS

In this study, the effects of meat fat substitution with olive oil on mortadella properties were evaluated. Results showed that treatments prepared without the addition of olive oil had a significantly higher SFA content than olive oil-incorporated treatments. The lipid reformulation did not only reduce the fat and cholesterol levels, but also enhanced the fatty acid profile. Treatments that contained olive oil could be nutritionally labelled, according to the USFDA guidelines, as "SFA-reduced". Results suggest that it is possible to substitute animal fat with low levels of olive oil (3% - 5%) in mortadella without jeopardising its quality. However, the manufacture of lean mortadella without the addition of any fat replacers has yielded a less desirable product from a sensory standpoint.

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Chemical composition, anti-tyrosinase, and molecular docking studies of *Knema furfuracea* Warb. essential oil

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Knema furfuracea Warb., a tree species belonging to the family Myristicaceae and indigenous to Southeast Asia, has been traditionally used by the Dai people for the treatment of inflammation and other illnesses. This work investigates the chemical composition of the essential oil of *K. furfuracea*, antityrosinase activity, and molecular docking studies. The essential oil was obtained through hydrodistillation and characterised through gas chromatography (GC/FID) and gas chromatography mass spectrometry (GC/MS). The anti-tyrosinase activity was determined using mushroom tyrosinase enzyme. The major components of the essential oil were studied for their potential interactions with tyrosinase using Autodock vina. A total of thirty-one components (96.0%) were identified from the leaf oil and composed mainly of bicyclogermacrene (23.1%), δ-cadinene (17.2%), (*E*,*E*)-α-farnesene (9.1%), and β-caryophyllene (7.7%). The essential oil demonstrated moderate activity towards tyrosinase with an IC_{50} value of 80.3 µg/mL. Regarding the molecular docking study, β-caryophyllene indicated a strong inhibitory activity (–6.7 Kcal/mol). These findings suggest that *K. furfuracea* essential oil has the potential as a natural source of tyrosinase inhibitors.

Key words: β-caryophyllene, essential oil, *Knema furfuracea,* Myristicaceae, tyrosinase

1. INTRODUCTION

Essential oils are a type of secondary metabolite produced by plants. Essential oils are concentrated, natural combinations of bioactive compounds with a wide range of organic structures [1]. Essential oils are a valuable natural resource used in a wide variety of industries, including cosmetics, food and beverage, home and personal care, and perfume and chemical industries [2]. Since antiquity, it has been understood that the essential oils extracted from aromatic and therapeutic plants contain biological activities, including, most notably, antibacterial, antifungal, and antioxidant characteristics [3-6].

The genus *Knema* Lour. (Myristicaceae) is commonly found in tropical countries such as Asia, Africa, and Australia. Southeast Asia has about 60 species, locally known as '*pala hutan*' or '*penarahan'* [7]. The seeds and bark of several *Knema* species are utilized in traditional medicine to treat diseases, including cancer, skin disorders, and ulcers in the mouth and throat [8]. The phytochemical composition of the genus *Knema* has been a topic of interest in previous studies. Despite this, only a limited number of species have been investigated. Several compounds have been identified, such as alkyl and acyl resorcinol derivatives of phenylalkylphenol, flavonoids, anarcadic acid, lignans, and stilbenes [9-14]. Moreover, various species of *Knema* have demonstrated significant properties such as acetylcholinesterase, cytotoxicity, anti-inflammatory, nematicidal, and antibacterial activities [15,16]. Against NCI-H187 and Vero cells, for instance, the IC_{50} values for the hexane extract of *K. globularia* were 47.5 and 29.0 µg/mL, respectively [17]. Additionally, IC_{50}

values for knecorticosanone B and malabarocone, both isolated from *K. globularia*, ranged from 8.76 to 18.74 μM against Hep-G2, MCF-7, and SK-LU-1 cell lines [18]. Acetylcholinesterase was inhibited strongly by 2-hydroxy-6-(10′(Z)-heptadecenyl) benzoic acid, also found in *K. laurina* stem barks (IC_{50} value 0.57 μM) [19]. Knepachycarpanone A which was isolated from the fruit extract of *K. pachycarpa* showed considerable inhibitory action (IC_{50} value 26.92 μM) against the Hela cancer cell line [20]. The acetone extract of *K. furfuracea* included three compounds that actively inhibited nitric oxide (NO) generation in LPS-activated RAW264.7 macrophages (IC_{50} values of 3.79, 9.28, and 15.14 μM, respectively). These compounds were 7,4′ - dihdroxy-4′-methoxyflavanol, fisetinidol, and virolanol C [21].

Knema furfuracea Warb. is a species of tree in the family Myristicaceae. It is native to Southeast Asia and can be found in Thailand, Malaysia, Indonesia, and the Philippines. The tree can grow up to 25 meters of height. The twigs at the top are about 4-10 mm in diameter, with dense hairs that eventually fall off. The leaves are oblong to lanceolate, coriaceous, and about 10-50 cm long by 3-21 cm wide. The male and female flowers are small and have hairs about 0.5-1 mm long. The tree produces small, yellowish-green flowers that are grouped in clusters. The fruit is a brownish-red, pear-shaped nut that contains a single seed. The phytochemicals of *K. furfuracea* have been studied. It has been found to contain compounds such as phenolic acids, flavonoids, and lignans, some of which have shown promising biological activities [21,22].

Tyrosinase, an enzyme that inhibits melanogenesis and contains copper, has been extensively studied for its potential as a cosmetic agent. While human and mammalian tyrosinases are glycosylated monomeric enzymes anchored to the melanosome membrane, mushroom tyrosinase is a soluble tetrameric enzyme found within the cytoplasm [23]. Due to its low price and commercial availability, mushroom tyrosinase has been widely used as the foundation for developing tyrosinase inhibitors of melanogenesis. However, despite some promising compounds, only some have been used in clinical settings due to a lack of efficacy or undesirable side effects such as potential carcinogenicity. Natural derivatives such as azelaic acid, hydroquinone, kojic acid, and arbutin have been utilised as skin lightening products for scientific and beauty purposes. However, concerns have been raised about their safety and potential toxicity to various systems [24]. Therefore, there is a need for new and effective tyrosinase inhibitors to address these side effects.

As we continue our investigation into bioactive volatile components from Malaysian species, this study focuses on the chemical composition of the essential oil of *K. furfuracea* and its potential anti-tyrosinase activity through molecular docking studies. This research is motivated by the fact that previous studies on the essential oils of the *Knema* genus are limited, and there is a need to further explore their chemical constitution and potential bioactivity.

2. MATERIAL AND METHODS

2.1 PLANT MATERIAL

The plant material used in the study was obtained by collecting leaves from *K. furfuracea* in Behrang, Perak, (3° 44’ 51.612” N 101° 27’ 19.9008” E) during October 2019. The identification of the plant was carried out by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM) and voucher specimens (SK376/19) were placed in the UKMB Herbarium at UKM.

2.2. ISOLATION OF ESSENTIAL OIL

The 300 g of *K. furfuracea* leaves were cut up and hydrodistilled in a Clevenger apparatus for 4 hours. After collecting the essential oil, it was dried with anhydrous magnesium sulphate, filtered, and stored in brown glass vials at 0°C until further examination could be performed. The average moisture content was between 85% and 89%, and the oil yield was 0.21% based on the weight of the fresh leaves [25].

2.3 ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) analyses were conducted using Agilent Technologies 7890B and 7890A GC systems, respectively. GC-FID and GC-MS were equipped with HP-5MS capillary columns of 30 m × 0.25 mm × 0.25 μm film thickness. Helium was used as the carrier gas with specific flow rates for each analysis. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was programmed to increase from 50°C to 280°C at a rate of 5°C/min and held isothermally for 15 min. Diluted samples were manually injected with split ratios and volumes as mentioned. Peak area percentage was calculated using the GC HP Chemstation GC software. GC-MS detection was performed with electron ionisation at 70 eV, using a scan rate of 0.5 s (cycle time: 0.2 s) and covering a mass range of 40-400 amu. Identification of essential oil components was done using co-injections with selected standards, retention index, and mass spectra comparisons with libraries and literature [26-27]. Semi-quantification was performed by normalising peak areas using the same response factor for all volatile components. Standards used were obtained from Sigma-Aldrich.

2.4 ANTI-TYROSINASE ACTIVITY

A modified version of the previously reported method [29] was used to conduct the tyrosinase inhibition assay. Essential oils and kojic acid were dissolved in dimethyl sulfoxide (DMSO) at concentrations ranging from 20 to 100 μg/mL. The reaction was carried out

Table I - Chemical components identified from *K. furfuracea* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identifications[c]
1.	α-Pinene	932	930	0.5	RI, MS
2.	Camphene	946	944	0.2	RI, MS
3.	Limonene	1033	1032	1.1	RI, MS, Std
4.	Linalool	1092	1090	0.2	RI, MS, Std
5.	Borneol	1165	1165	0.8	RI, MS
6.	Bornyl acetate	1283	1285	0.2	RI, MS
7.	α-Cubebene	1345	1345	2.4	RI, MS
8.	(*E*)-α-Damascone	1385	1383	1.9	RI, MS
9.	β-Cubebene	1388	1387	0.6	RI, MS
10.	β-Elemene	1390	1389	4.9	RI, MS
11.	Longifolene	1407	1407	1.4	RI, MS
12.	β-Caryophyllene	1415	1417	7.7	RI, MS, Std
13.	α-Humulene	1435	1436	1.8	RI, MS
14.	Aromadendrene	1439	1439	1.6	RI, MS
15.	(*E*)-β-Farnesene	1455	1454	0.7	RI, MS
16.	Germacrene B	1460	1559	1.4	RI, MS, Std
17.	γ-Muurolene	1476	1478	1.0	RI, MS
18.	β-Selinene	1486	1489	4.2	RI, MS
19.	α-Selinene	1495	1498	0.7	RI, MS
20.	Cadine-1,4-diene	1495	1495	1.6	RI, MS
21.	Bicyclogermacrene	1500	1500	23.1	RI, MS, Std
22.	α-Muurolene	1502	1501	2.1	RI, MS
23.	(*E*,*E*)-α-Farnesene	1504	1505	9.1	RI, MS
24.	γ-Cadinene	1516	1513	1.7	RI, MS
25.	δ-Cadinene	1520	1522	17.2	RI, MS, Std
26.	(Z)-Nerolidol	1530	1531	1.8	RI, MS
27.	α-Calacorene	1544	1545	1.1	RI, MS
28.	γ-Selinene	1598	1598	2.8	RI, MS
29.	1-*epi*-Cubenol	1625	1627	1.2	RI, MS
30.	τ-Muurolol	1645	1644	2.3	RI, MS, Std
31.	α-Cadinol	1650	1652	1.7	RI, MS
	Monoterpene hydrocarbons			**1.8**	
	Oxygenated monoterpenes			**1.2**	
	Sesquiterpene hydrocarbons			**89.0**	
	Oxygenated sesquiterpenes			**7.0**	
	Identified components			**99.0**	

[a] Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes
[b] Linear retention index taken from Adams and literatures
[c] Identification methods: Std, based on comparison with authentic compounds; RI, MS - based on comparison with Wiley, Adams, FFNSC2 and NIST08 MS databases

in a 96-well microplate, and the absorbance at 475 nm was measured using an ELISA microplate reader (VersaMax, Molecular Devices, USA). Each well contained 40 µL of essential oil dissolved in DMSO, 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase enzyme, and 40 µL of L-dopa. A control sample containing all components except L-dopa was included for each sample. Kojic acid was used as a reference inhibitor. The percentage of tyrosinase inhibition was calculated using the formula: $I\% = [A_{control} - A_{sample} / A_{control}] \times 100$,
where $A_{control}$ represents the absorbance of the control reaction and A_{sample} represents the absorbance of the essential oil/reference. The concentration of the sample causing 50% inhibition (IC_{50}) was determined by plotting the percentages of inhibition against the sample concentrations. The results were expressed as means ± standard deviation (SD) of triplicate analyses [29].

2.5. MOLECULAR DOCKING

The crystal structure of tyrosinase (PDB ID code 2Y9X) was obtained from the Protein Data Bank (PDB) website (http://www.rcsb.org). The retrieved protein structure was subjected to energy minimisation using the conjugate gradient algorithm and AMBER force field with UCSF Chimera 1.10.1. The major components, namely bicyclogermacrene, (E,E)-α-farnesene, δ-cadinene, and β-caryophyllene, were obtained from PubChem in sdf format. Each ligand was individually energy minimised using the OpenBabel tool embedded in PyRx, with default parameters including steepest descent steps of 100 with a step size of 0.02 Å, conjugate gradient steps of 100 with a step size of 0.02 Å, and an update interval fixed at 10 [30]. Molecular docking was performed using the PyRx virtual screening tool with the AutoDock VINA Wizard approach. The grid box centre values for X, Y, and Z were adjusted to -9.8702, -27.3402, and -40.6008, respectively, to ensure sufficient coverage

of the binding pocket and allow for ligand mobility in the search space. The exhaustiveness value was set to 8 to maximise binding conformational analysis. Docked compounds were evaluated based on the lowest binding energy (Kcal/mol) among all docked complexes. 2D and 3D visualisations of the docking complexes were generated using Discovery Studio 2021 [32]

3. RESULTS AND DISCUSSION

The fresh leaves of *K. furfuracea* were hydrodistillated to produce yellow oil with a yield of 0.21%. The volatile components have been successfully identified by GC-FID and GC-MS analysis, and their percentages are shown in Table 1 in relation to their Kovats index in the HP-5 column. The essential oil of *K. furfuracea* revealed the presence of thirty-one components with a percentage of 96.0%. The essential oil was characterised by the high concentration of sesquiterpene hydrocarbons (89.0%) and dominated by its richness in bicyclogermacrene (23.1%), δ-cadinene (17.2%), (*E*,*E*)-α-farnesene (9.1%), β-caryophyllene (7.7%), and β-elemene (4.9%). The other minor components detected in the essential oil in more than 2% were β-selinene (4.2%), γ-selinene (2.8%), α-cubebene (2.4%), τ-muurolol (2.3%), and α-muurolene (2.1%).
According to the available literature, only four studies have been conducted on the essential oils belonging to the genus *Knema* [33-36]. The chemical composition of Malaysian *K. kunstleri* and *K. hookeriana* and *K. intermedia* leaf oil has been recently described by us [33,35,36]. The essential oil of *K. kunstleri* leaf contained 77.3% of sesquiterpene hydrocarbons, with β-caryophyllene (23.2%) as the major component [33], and that of *K. hookeriana* consisted majorly

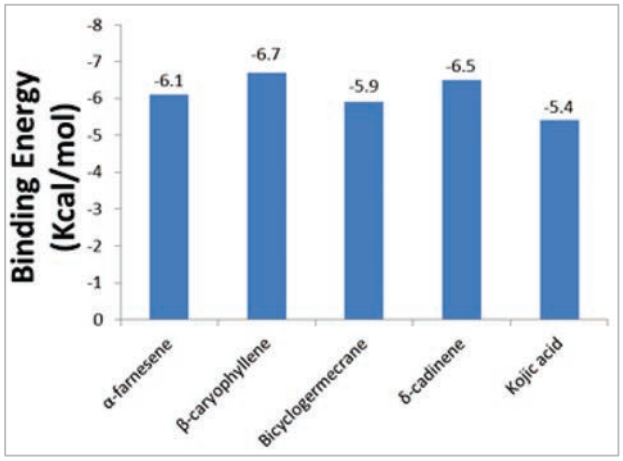

Figure 1 - Docking energies of the major components and control

of β-caryophyllene (26.2%), germacrene D (12.5%), δ-cadinene (9.2%), germacrene B (8.8%) and bicyclogermacrene (5.5%) [35], while *K.intermedia* contains mainly of τ-muurolol (20.1%), α-copaene (14.4%), δ-cadinene (13.9%), germacrene B (9.5%), and δ-selinene (7.0%) [36]. Meanwhile, another study on the leaf oil of *K. globularia* from Vietnam gave β-elemene (25.48%) as the major component [34]. The components' differences between *Knema* species may be influenced by the method of extraction, genetic factors, the season, stage of development, chemotype, distinct habitat in which the plant was collected, and the nutritional status of the plants, which influence plant biosynthetic pathways and consequently, the relative proportion of the main characteristic compounds [33].
Essential oil was tested for its anti-tyrosinase activi-

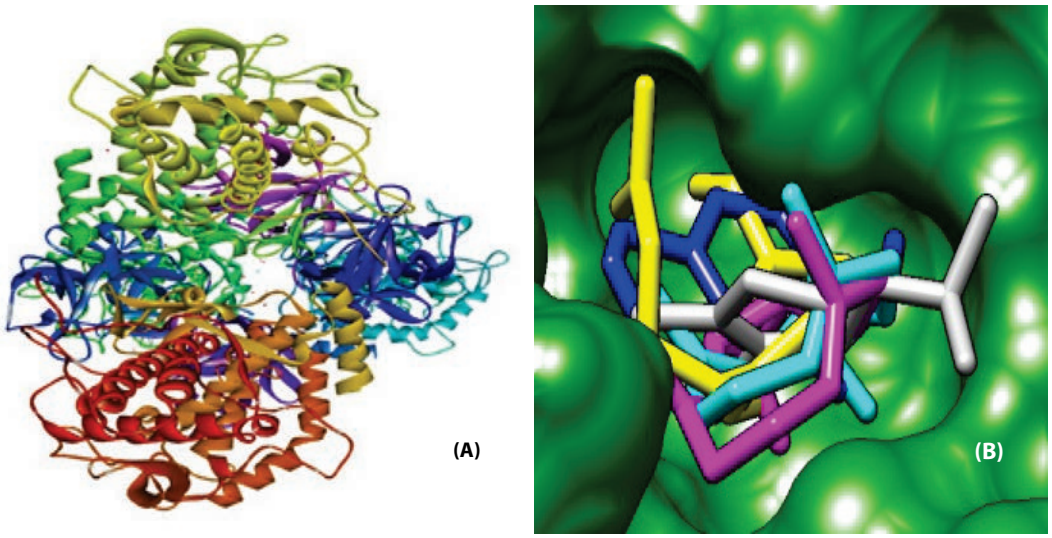

Figure 2 - (**A**) Structure of Tyrosinase; PDB ID: 2Y9X (**B**) The binding pocket of target protein in surface format is represented in dark green color with conformational position of ligands.

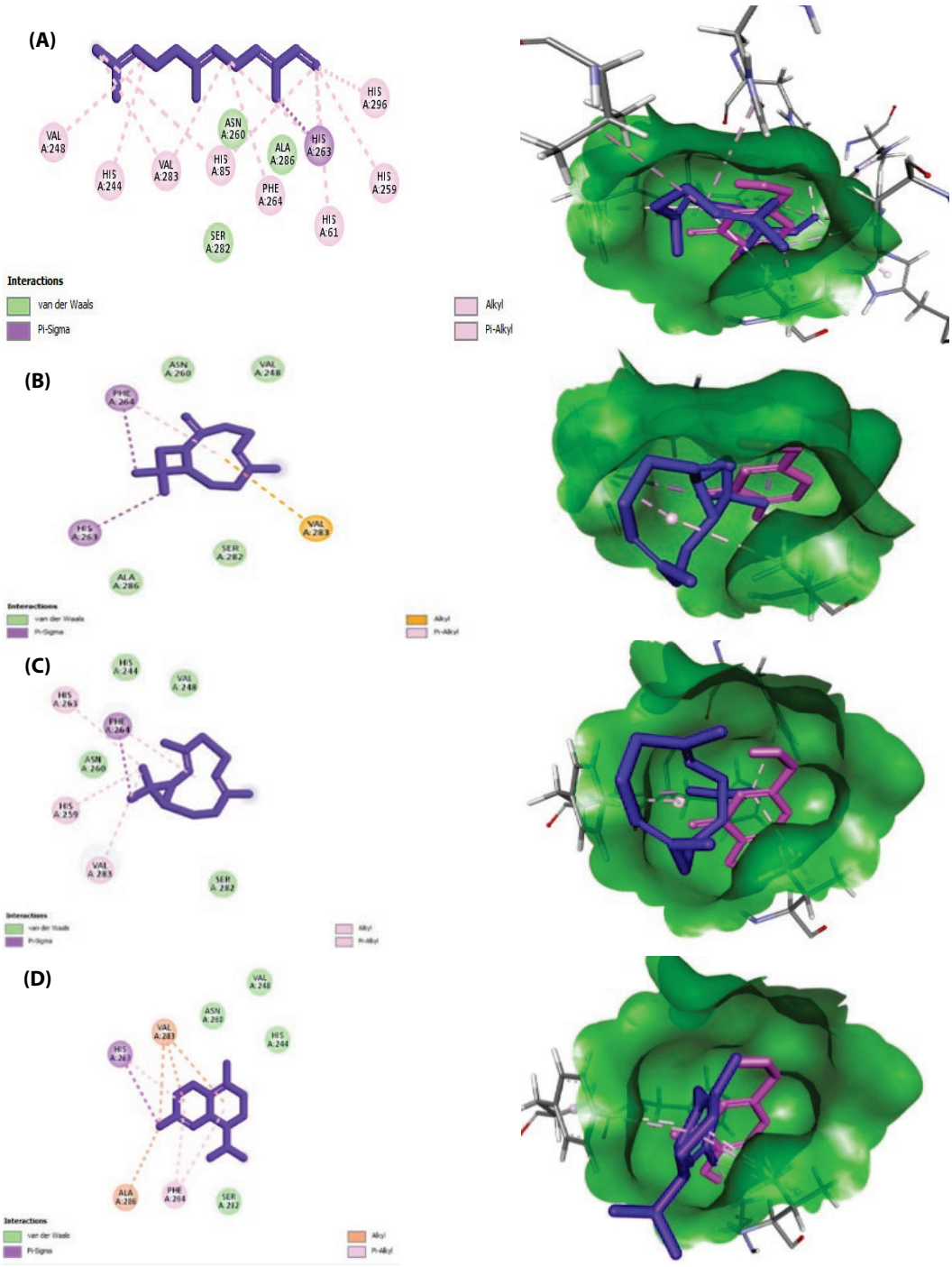

Figure 3 - 2D and 3D interactions view of ligands inside tyrosinase receptor; the representation of (**A**) α-farnesene (**B**) β-caryophyllene (**C**) bicyclogermacrane (**D**) δ-cadinene, superimposed unto the 2Y9X-kojic acid complex

ty using L-DOPA as a substrate and kojic acid as a positive control. The t-test was used for statistical analysis, and a significance level of $p<0.05$ was used. In this experiment, the essential oil's enzyme-inhibiting ability was measured by looking at the doses at which 50% inhibition (IC_{50}) occurred. This investigation found modest success with the essential oil, with an IC_{50} value of 80.3 µg/mL compared to the standard's IC_{50} value of µ10.5 µg/mL for kojic acid. Previous studies have revealed that the essential oil's phenolic components may have served as tyrosinase examples, resulting in conformational or steric alterations. However, because of its low proportion of oxygenated components that may chelate or link with the copper metal [37,38], the enzyme displays less enzymatic behaviour.

Molecular docking is an excellent method for studying the binding configuration of ligands at the active site of target proteins. The binding affinity and active amino acid residues of the selected components in the target protein were investigated using molecular docking. The major components (bicyclogermacrene,

(*E*,*E*)-α-farnesene, δ-cadinene, and β-caryophyllene, since they constitute the highest percentage) were docked into the binding site of the mushroom tyrosinase, and their affinity was tested. Figure 1 shows the binding affinity values of the components and kojic acid (control). The lowest binding energy values (kcal/mol) and the interaction pattern were used to examine the docked complexes of the components with tyrosinase receptor. All ligands had comparable docking energies with conventional kojic acid (–5.4 kcal/mol) and interacted with the active site residues of the tyrosinase receptor.

Analysing the binding pocket, all ligands were found in the target protein's active region. Superimposing the docked complexes allowed us to verify the binding conformation of each ligand in the active domain of the target protein. Furthermore, the ligands bound in the binding pocket showed a comparable conformational pattern. All ligands are attached to the target protein with minor rotational deflection. The fact that most of the ligands were bound in the same area validated our docking results, as shown in Figure 2. The significant components had a binding energy range from –5.9 to –6.7 Kcal/mol, with β-caryophyllene having the highest (–6.7 Kcal/mol). All components showed better docking scores than kojic acid (–5.4 kcal/mol). The low activity observed might be due to many components' presence since the essential oil was tested and not the individual components.

Binding analysis showed that β-caryophyllene forms a two hydrophobic π-σ bond with the imidazole side chain of His263 at a distance of 3.89 Å and the benzyl side chain of Phe264 at a distance of 3.93 Å. With a binding distance of 4.81 Å, β-caryophyllene rings also form a π -alkyl bond with the aromatic portion of Phe264 and an alkyl bond with Val283 with a binding distance of 5.38 Å. Additionally, it displayed van der Waals interactions with Ala286, Ser282, Asn260, and Val248, as shown in Figure 3. According to molecular docking, compared with the reference drug kojic acid, the prepared ligand with the lowest binding energy might be a suitable inhibitor of tyrosinase receptors. The theoretical investigation suggested excellent outcomes when compared to the experimental study.

4. CONCLUSIONS

In conclusion, this article presents a study on the chemical composition and biological activities of the essential oil extracted from *K. furfuracea*, a plant species found in Southeast Asia. The chemical composition of the essential oil was analysed using GC/FID and GC/MS, which identified 31 compounds, with bicyclogermacrene, (*E*,*E*)-α-farnesene, δ-cadinene, and β-caryophyllene being the major components. The essential oil was also evaluated for its anti-tyrosinase activity, which is vital for its potential use in skin whitening products. Results showed that the essential oil exhibited a moderate anti-tyrosinase activity, indicating its potential as a natural skin whitening agent. Furthermore, molecular docking studies were performed to predict the interactions between the essential oil compounds and the tyrosinase enzyme. In addition, the major components obtained promising scores for docking in the active sites of the examined target enzymes. The results also provide insight into the molecular interactions between the essential oil major components and the tyrosinase enzyme. β-caryophyllene had the highest binding affinity with the tyrosinase enzyme, suggesting its potential as an active ingredient in anti-tyrosinase products. Overall, this study highlights the potential of *K. furfuracea* as a natural source of anti-tyrosinase agents, this could open ways for further research on the use of *K. furfuracea* essential oil in the development of tyrosinase inhibitors for commercial and medicinal applications.

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The effect of pan frying on 3-monochloropropane-1,2-diol and glycidyl ester contents of vegetable oils

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Abstract: The aim of this work was to determine the effect of the pan-frying process on 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl ester (GE) amounts of vegetable oils. For this purpose, potatoes were pan-fried with refined sunflower, corn, hazelnut oils, virgin olive oil and margarine for 5, 10 and 15 minutes at 160, 180 and 200°C. The fried oil samples were analysed for their 3-MCPD and glycidyl ester levels. Results have shown that 3-MCPD ester concentrations were higher than the GEs' for all types of oils. Virgin olive oil was found to be devoid of the contaminants and no endogenous formation was observed throughout frying cycles for any of the matrices tested. Margarines were determined to have the highest content of both esters. The GE amounts of margarines were found to increase by the intensity of the frying process. The 3-MCPD-E levels of hazelnut oil did not vary significantly, whereas slight differences were observed for sunflower and corn oils by varying the process parameters.
Keywords: 3-monochloropropane-1,2-diol ester; Glycidyl ester; Margarine; Pan-frying

1. INTRODUCTION

Frying is used as a traditional technique for food preparation worldwide. Both deep-fried and pan-fried foods are preferred by the consumers for the palatable taste, unique flavor, and texture of the product. During frying, food is in contact with hot oil (150-190°C) and both the heat and mass transfer occur simultaneously [1]. Besides, a series of chemical, physical and thermal changes including hydrolysis, oxidation and polymerisation take place during frying [2]. As the reactions progress, the nutritional and sensorial quality of the frying medium, the oil, degenerates continuously, accompanying with a formation of free fatty acids, the darkening in colour and the off flavoring [3]. The quality and the stability of the frying oil is notably important since it is absorbed by the fried product.

The 3-monochloropropane-1,2-diol (3-MCPD) and glycidyl esters are heat-induced chemical contaminants that occur in refined vegetable oils and oil containing foods. These contaminants have gained increased interest in recent years for their potential toxicity due to the release of 3-MCPD and glycidol, the hydrolysates of their parent esters (3-MCPD esters and glycidyl esters) during digestion [4]. 3-MCPD has been classified as "possible human carcinogens" (group 2B) and glycidol has been grouped as "probably carcinogenic to humans" (group 2A) by the International Agency for Research on Cancer [5]. A critical tolerable daily intake for the protection of human health of 2 $\mu g \cdot kg^{-1}$ body weight for 3-MCPD and its fatty acid esters was suggested by CONTAM Panel [6]. Moreover, the European Commission defined a maximum limit of 1 ppm for glycidyl fatty acid esters in vegetable oils and fats, fish oils and oils from other marine organisms placed on the market for the final consumer or for use as an ingredient in food. Additionally, the maximum level of 1.25 ppm was established for the sum of 3-monochloropropanediol (3-

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MCPD) and 3-MCPD fatty acid esters for vegetable oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils (composed of refined olive oil and virgin olive oil) and mixtures of oils and fats. In addition, the maximum tolerable limit of 3-MCPD and fatty acid esters was determined as 2.5 ppm for other vegetable oils (including olive pomace olive oil), fish oils and oils obtained from other marine organisms [7].

The formation of 3-MCPD esters (3-MCPD-Es) and glycidyl esters (GEs) occur mainly during vegetable oil refining process. The high temperatures achieved at the deodorisation step encourage the formation of the esters in the presence of precursors such as organic and inorganic chlorinated compounds and partial acylglycerols [8]. Certain food preparation techniques such as frying, grilling, and baking with high temperatures may also cause the formation of these undesired process contaminants. The temperatures used for deep-fat frying vary in 160-190°C and fall within the temperature range of refining. In addition, fried foods are sometimes salted or coated before frying which causes the inclusion of chloride that may promote the formation of 3-MCPD in the frying medium. Hence, the effect of frying on 3-MCPD and glycidyl ester levels has received attraction from researchers and some reports have been published recently on the behaviors of 3-MCPD-E and GEs when exposed to different frying parameters. Although factors such as frying oil, temperature, time, chlorine source, moisture, the composition of the food were reported to influence the 3-MCPD and glycidyl ester content of fried product [9], no consistent conclusion has been drawn yet [10]. The majority of the earlier works on the topic mainly focused on the changes during deep-fat frying; however, there is a lack of research on the variation of these process contaminants during pan-frying. To the authors' knowledge, the unique study was published by Raczyk et al. [11], who reported remarkable formation of 3-MCPD esters and moderate increases of glycidyl esters in margarine samples pan-fried over 15 minutes. The objective of this work was to study the formation of 3-MCPD and glycidyl esters during the pan-frying process conducted under varying conditions of temperature and time.

2. MATERIALS AND METHODS

2.1. MATERIALS AND CHEMICALS

Frying oils (refined sunflower oil, refined corn oil, refined hazelnut oil, virgin olive oil, margarine) and potatoes were purchased from local markets in Aydın. Chemicals used during analyses, namely, 3-monochloropropane-1,2-diol-d5, diethyl ether, ethyl acetate, glycidol, *n*-hexane, isooctane, methanol, phenyl boronic acid, sodium hydroxide, sodium methoxide, *tert*-butyl methyl ether and toluene were purchased from Sigma-Aldrich (St-Louis, ABD). Sodium bromide, sodium chloride and sulfuric acid were purchased from Merck (Darmstadt, Germany).

2.2. METHODS

Preparation and pan-frying of potatoes

In the first step, the potatoes were washed and peeled. Each potato was sliced to equal dimensions (0.5 cm × 2 cm × 5 cm). The frying process was carried out in the Teflon pan (Tefal-24 cm, France) and 100 g of potatoes were fried in 100 ml oil. Samples were fried in five different edible oils (sunflower oil, corn oil, hazelnut oil, virgin olive oil and margarine) for 5, 10 and 15 minutes at 160, 180 and 200°C. The frying was performed using a conventional electric kitchen cooker. Each frying process was performed in duplicate. There was a total of 18 (3 times × 3 temperatures × 2 replicates) different frying processes for each oil. Some of the frying oil was absorbed by the potatoes and the remaining oil was transferred to 100 ml dark colored glass bottles and kept at +4°C until analysis. Margarine samples were melted at 50°C before analysis.

Determination of 3-MCPD and glycidyl esters contents

3-MCPD and glycidyl ester content analysis was carried out according to the DGF C-VI 18 (10) standard method [12] which is an indirect analysis method and based on alkaline transesterification. 3-MCPD-d5 was used as the reference standard. Quantification was performed according to the method of Cheng et al. [13] and quantitative analysis of 3-MCPD and glycidyl esters were performed in gas chromatography (Shimadzu QP2020 system-Shimadzu, Kyoto, Japan) with mass spectrometry (GC-MS). Separation of the target components was performed with HP-5MS capillary column (30 m length, 0.32 mm inner diameter and 0.25 µm film thickness, Agilent Technologies, USA). The oven temperature was programmed from 80°C to 155°C at a rate of 5°C/min then temperature was increased to 300°C at a rate of 60°C/min and held for 5 min. Helium was used as carrier gas at a flow rate of 1.18 ml/min. 1 µl of oil sample was injected in splitless mode for each analysis. The mass spectrometer detector was operated in selected ion monitoring mode (SIM mode) with positive electron ionisation (EI+) at a 70 eV ionisation voltage. The temperature of the ion source and interface in the mass spectrometer was 200 and 280°C, respectively. The quantitative analysis of the derivatised 3-MCPD compound was examined by monitoring characteristic ions. The ion traces m/z 147 was selected for 3-MCPD and m/z 150 for 3-MCPD-d5. The limit of detection of the method was 0.07 mg/kg.

Statistical analysis

Each frying process was performed in duplicate and the measurements were replicated twice. The results were expressed as mean ± standard deviation of four measurements for the analytical determination. Statistical analysis was carried out using SPSS 15.0 statistical software (SPSS Inc., Chicago, USA). Data

were evaluated by the single factor analysis of variance (one-way ANOVA) procedure using the Duncan multiple range test (p<0.05).

3. RESULTS AND DISCUSSION

3.1. 3-MCPD ESTER CONTENT OF FRYING OILS

The effects of frying time and temperature on 3-MCPD-E content of various vegetable oils is given in Table I. 3-MCPD esters of fried and non-fried virgin olive oil samples were below the detectable limit, proposing that the virgin olive oil has none or negligible amount of these contaminants. 3-MCPD and glycidyl esters are known to be formed during refining, especially at the deodorisation step of the process. Virgin olive oils are obtained only by mechanical techniques and can be consumed without further refining. Like current findings, virgin olive oil has been mainly reported to be devoid of these contaminants in previous literature [14]. Thus, it can be hypothesised that pan-fried potatoes in virgin olive oil are more likely to include lower 3-MCPD-E amounts.

The 3-MCPD ester content of fresh sunflower oil was 0.18 mg·kg^{-1}. European Food Safety Authority (EFSA) determined the mean concentration of 3-MCPD esters in fresh sunflower oils (refined-non fried) as 0.521 mg·kg^{-1} in 2016 [15]. The frying time was found to be ineffective at three frying temperatures. Merkle et al. [16] emphasised that pre-frying temperature and heating time were the most important parameters regarding the MCPD-E contents during deep-fat frying of frozen fish products with sunflower oil. Dingel and Matissek [17] reported non-formation of these undesirable contaminants in high oleic sunflower oil during deep-fat frying of potato crisps at large scale production.

Corn oil was the other linoleic rich seed oil in the current work and had 0.43 mg·kg^{-1} 3-MCPD-E in fresh form, higher than both sunflower and hazelnut oils. The pan-frying process at 200°C caused slight decreases in 3-MCPD-E content of corn oil by the increase in process time. The frying processes at 180°C caused significant increases in 3-MCPD-E contents in comparison with fresh oil. Arisseto et al. [18] reported no endogenous formation of bound 3-MCPD during frying, when corn oil containing non-significant levels of the contaminant at the beginning of the process was used.

The 3-MCPD-E content of non-fried hazelnut oil was found to be 0.19 mg·kg^{-1}. The frying time and temperature were found to be ineffective on bound 3-MCPD content of the hazelnut oils during frying. Arslan et al. [19] detected 0.44 mg·kg^{-1} of 3-MCPD-E in fresh hazelnut oil and reported a reduction in the concentration of the contaminant with frying temperature and time. Similarly, Wong et al. [20] described a decrease in bound 3-MCPD with an increase in frying time.

The highest contents of 3-MCPD-Es were detected in margarine samples that ranged in 1.50 and 1.65 mg·kg^{-1}, which may be attributed to palm oil and chlorinated compounds present in the formulation. Palm oil has been reported to be a critical source of 3-MCPD and glycidyl esters [21, 22], whereas chlorinated components have been defined as principal precursors of the contaminants [17]. Several works, investigating the 3-MCPD-E content of margarines purchased from various markets, have been published. Goh et al. [23] described up to 3.83 mg·kg^{-1} 3-MCPD-E in five different margarine samples, Jedrkiewicz et al. [22] found a range of 1.3-7.3 mg·kg^{-1} of 3-MCPD-E in the lipid fraction of five margarines for different brands, Şirinyıldız et al. [24] reported a range of 0.57-4.54 mg·kg^{-1} for 26 different margarines sold in market and Custodio-Mendoza et al. [14] determined up to 8.09 mg·kg^{-1} of the contaminant in lipid part of margarines. The results indicate that margarines are important contributors to dietary exposure of 3-MCPD-Es. In the current work, the pan-frying process caused a slight increase in 3-MCPD-E content of the samples fried at 160°C for 10 minutes.

Table I. The effect of time and temperature on 3-MCPD ester content of frying oils (mg·kg^{-1})

Frying temperature (°C)	Frying time (min)	Sunflower oil	Corn oil	Hazelnut oil	Virgin olive oil	Margarine
Fresh oil		0.18±0.00^{a}	0.43±0.00^{ab}	0.19±0.00	ND	1.50±0.03^{a}
160	5	0.18±0.00^{ab}	0.44±0.01^{abc}	0.19±0.01	ND	1.56±0.02^{ab}
	10	0.20±0.02^{b}	0.43±0.02^{ab}	0.19±0.01	ND	1.65±0.12^{b}
	15	0.20±0.01^{ab}	0.46±0.02^{bcd}	0.19±0.01	ND	1.54±0.08^{a}
180	5	0.18±0.01^{a}	0.49±0.03^{e}	0.19±0.00	ND	1.59±0.04^{ab}
	10	0.19±0.00^{ab}	0.47±0.04^{cde}	0.19±0.00	ND	1.59±0.06^{ab}
	15	0.18±0.01^{a}	0.48±0.01^{de}	0.19±0.01	ND	1.52±0.05^{a}
200	5	0.18±0.01^{a}	0.48±0.02^{de}	0.19±0.00	ND	1.54±0.01^{a}
	10	0.18±0.02^{ab}	0.42±0.01^{a}	0.19±0.01	ND	1.56±0.06^{ab}
	15	0.19±0.01^{ab}	0.43±0.01^{abc}	0.20±0.01	ND	1.50±0.07^{a}

Mean values followed by the same letter in each column are not significant different at p<0.05 by ANOVA and Duncan's test.
ND: Not detected.

Raczyk et al. [11] reported significant increases by 15 minutes of pan-frying in 3-MCPD-E content of margarine samples by pan-frying.

3.2. GLYCIDYL ESTER CONTENT OF FRYING OILS

The effects of frying time and temperature on glycidyl ester contents of different vegetable oils is given in Table II. The GE content of the oils were found to be lower than 3-MCPD esters' and no significant correlation was detected between the two contaminants due to different formation mechanisms [25]. Virgin olive oil samples had no glycidyl ester contents in fresh forms and no formation of GE was determined throughout pan-frying cycles. Unrefined virgin vegetable oils have been reported to contain undetectable contents of 3-MCPD and glycidyl esters [26].

The glycidyl ester content of non-fried sunflower oil was 0.09 mg·kg^{-1} and the level of glycidyl esters increased with frying at 180° for 10 minutes and 200°C for 10 and 15 minutes of processes. Yıldırım and Yorulmaz [27] reported GE contents that vary between 0.19 and 0.48 mg·kg^{-1} for sunflower oils used in deep-fat frying of potatoes. Xu et al. [28] determined 0.58 mg·kg^{-1} of GE in fresh high oleic sunflower oil and reported a degradation rate for GEs by deep-fat frying process, decreasing to 0.05 mg·kg^{-1} at the end of the frying. Kalkan et al. [29] investigated the formation of GEs in sunflower oil by frying using central composite design. The process parameters were temperature, duration, salinity, and the results have shown that the formation of GEs was observed in hardest process conditions (180°C, 40 minutes, 300 mg NaCl/100 ml).

The glycidyl ester content of fresh corn oil was 0.10 mg·kg^{-1}. Xu et al. [30] reported 0.25 mg·kg^{-1} and MacMahon et al. [26] reported 0.68 mg·kg^{-1} of GE for corn oils. The corn oil samples of the current work that were pan-fried at 160 for 15 minutes, at 180°C for 10 and 15 minutes and at 200°C for 5 and 15 minutes were found to be devoid of the glycidyl esters.

The GE levels of hazelnut oils used in pan-frying trials of the current work ranged in 0.09-0.14 mg·kg^{-1}. Arslan et al. [18] previously described 0.04-0.14 mg·kg^{-1} levels of GE for hazelnut oils. The oil sample used for pan frying at 160°C for 10 minutes had slightly lower GE level than other samples.

Margarine was detected to be the richest fat in terms of GEs in the present study. The fresh margarine sample contained a mean of 0.54 mg·kg^{-1} of GE lower than the findings of Goh et al. [26] and higher than the results of Hidalgo-Ruiz et al. [31]. The GE levels of margarine samples were found to increase as the intensity of the process was increased. Raczyk et al. [11] reported a moderate increase for margarines pan-fried over 15 minutes.

4. CONCLUSION

The study reports the changes in 3-MCPD and GE contents of different vegetable oils by frying time and temperature during pan-frying process. 3-MCPD ester contents of the margarine samples were detected to be over the regulatory limit of 1.25 ppm in both fresh and fried samples, whereas corn, sunflower and hazelnut oils' concentrations were within the established value. The GE levels of all frying oil samples were within the maximum regulatory limit of 1 ppm. Margarines are possible important contributors to dietary exposure of 3-MCPD-Es and GEs. Fresh virgin olive oil was found to be free of the contaminants and no endogenous formation was observed during pan-frying. Hence, potatoes pan-fried in virgin olive oil sound to be safer in terms of bound 3-MCPD and glycidol amounts. The 3-MCPD and GE content of the initial fresh oil seem to be more effective on the content of final oil and consequently the fried product than the formation or reduction of the contaminants throughout the frying cycles. A number of works have been published on the effect of frying on the formation of these undesired heat-induced contaminants.

Table II. The effect of time and temperature on glycidyl ester content of frying oils (mg·kg^{-1})

Frying temperature (°C)	Frying time (min)	Sunflower oil	Corn oil	Hazelnut oil	Virgin olive oil	Margarine
Fresh oil		0.09±0.01^{a}	0.10±0.03^{a}	0.14±0.01^{a}	ND	0.54±0.02^{a}
160	5	0.11±0.02^{ab}	0.09±0.02^{a}	0.12±0.03^{ab}	ND	0.66±0.09^{c}
	10	0.10±0.02^{ab}	0.10±0.01^{a}	0.09±0.02^{b}	ND	0.65±0.04^{c}
	15	0.11±0.01^{ab}	ND	0.14±0.01^{a}	ND	0.67±0.01^{c}
180	5	0.12±0.01^{ab}	0.08±0.03^{a}	0.14±0.02^{a}	ND	0.56±0.01^{ab}
	10	0.11±0.01^{b}	ND	0.14±0.01^{a}	ND	0.67±0.05^{c}
	15	0.10±0.00^{ab}	ND	0.13±0.02^{a}	ND	0.76±0.06^{d}
200	5	0.11±0.01^{ab}	ND	0.14±0.03^{a}	ND	0.63±0.03^{bc}
	10	0.12±0.01^{b}	0.08±0.02^{a}	0.14±0.02^{a}	ND	0.65±0.02^{c}
	15	0.12±0.01^{b}	ND	0.13±0.02^{a}	ND	0.66±0.05^{c}

Mean values followed by the same letter in each column are not significant different at $p<0.05$ by ANOVA and Duncan's test.
ND: Not detected.

Yet, the present work is the first one investigating the effect of frying temperature and time on 3-MCPD and glycidyl ester levels of different vegetable oils during pan-frying. More studies should be conducted to better understand the contamination routes of the fried foods to reduce dietary human exposure.

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Conflict of interest

The authors declare they have no conflict of interest.

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Biochemical and Pomological Variability of Several Autochthonous Olive Cultivars Grown in Algeria

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This work aimed to characterise the morphological traits of olives and physico-chemical of oil issued from 10 endemic cultivars harvested in the north-east of Algeria. The pomological parameters of the fruits showed a very significant difference ($p < 0.0001$) between the studied cultivars. Quality indices indicated that olive oil varieties belong to the extra virgin and virgin categories. Highly significant differences ($p < 0.0001$) were noted between the 10 varieties studied for the analytical parameters examined (fatty acid composition, total phenol, chlorophyll, and carotenoid content). *Boughenfous* showed the highest values in oleic acid with (75.15%) and in total phenols with 435.88 (mg of Gallic acid/kg of olive oil). Phenolic compounds (hydroxytyrosol, tyrosol, oleocanthal, total phenolic compounds) have shown important differences between varieties. The principal component analysis carried out on the profile of total fatty acids distinguishes 5 groups, group 2 and 3 proved to be the most interesting in terms of nutritional characteristics, they include three cultivars, characterised by the highest levels of oleic acid and MUFA and the MUFA/PUFA ratio. These results confirm the importance of the varietal character in determining the chemical characteristics of the oil. To enhance the olive sector, this characterisation must be extended to other secondary olive cultivars that are unexplored.
Keywords: Algerian cultivars, characterisation, fatty acid profile, chemical composition, HPLC-UV, fruit characteristics.

1. INTRODUCTION

Olive cultivation (*Olea europaea* L.) is one of the oldest crops in the world [1]**.** Olive tree is grown mainly in the Mediterranean region (Spain, Italy, Greece, Tunisia, Turkey, Morocco, and Algeria) for climatic reasons [2], and for the high nutritional value of its products (oil and table olives) and its economic importance [3]. Most of the olive production is intended for oil extraction and the other part to produce table olives [1]. It has many varieties with a significant phenotypic diversity [5]. The work of Hauville [6] in Algeria distinguished more than 150 varieties of olive trees. Although the Algerian olive growing heritage is rich in varieties, our traditional olive growing is dominated by two main varieties, *Chemlal* and Sigoise. *Chemlal* olives are used only for oil extraction; this cultivar is grown mainly in Kabylie (central Algeria) and represents 40% of the national olive area. The *Sigoise* variety that represents 20% is cultivated in the west of the country for its dual use (the quality of its oil and table olives). The other Algerian cultivars have a limited implantation and distribution. The olive sector has experienced significant growth in recent years, from 200,000 ha in 2011 to 401,181 ha in 2015 [7]. Several studies have focused on the nutritional and organoleptic properties of olive oil [8], which is a key factor in the healthy aspects attributed to the Mediterranean diet [9]. The benefits of virgin olive oil are mainly attributed both to its high oleic acid content which contributes to the balance of the amount of polyunsaturated fatty acids and

its richness in phenolic compounds, which act as natural antioxidants and can contribute to the prevention of several human diseases [10]. These beneficial traits, usually associated with the genotype of the cultivar, highlight the need to identify characteristics of olive oil that will ensure its authenticity [11]. The chemical composition of extra virgin olive oil is influenced by the olive cultivar, pedoclimatic conditions, geographical site, and stage of maturity [12]. In terms of genetic diversity, monovarietal olive oils, produced from a specific cultivar, have particular physical and biochemical characteristics and attributes that result in distinctive composition and performance [11]. Additionally, many pre-harvest and post-harvest factors are involved in the quality of olive fruit and its oil compositions [13].
In this context, the purpose of this study concerns the physicochemical and pomological characterisation of 10 Algerian endemic cultivars of olive tree (*Olea* L.) maintained at the national collection of the Takerietz demonstration farm (municipality of Souk Oufella, wilaya of Bejaïa).

2. MATERIAL AND METHODS

2.1. SAMPLING

This study includes 10 cultivated olive varieties of local population (Table I). The olives of the different cultivars were harvested during 2018-2019 at the experimental station of the Technical Institute of Fruit Trees and Vines, Sidi-Aich, Bejaia (Algeria). The geographical coordinates of the station area are as follows: latitude: 36°58'19" North, longitude: 4°66'69" East. Approximately 3 to 5 kg of olives were harvested manually from three trees for each variety. The sampling was done at man's height around the tree canopy. The extraction of olive oil was carried out using an oleodosor, a discontinuous two-phase cold centrifugation system. The extracted oil was decanted and stored in labelled opaque glass bottles, at a cold temperature (4°C) until analysis.

Table I - Characteristics of the plant material

Cultivars	Use
Bouchouk Lafayette	Dual purpose
Souidi	Oil
Aghchren d'El Ousseur	Dual purpose
Azeradj	Dual purpose
Boughenfous	Oil
Aguenaou	Dual purpose
Aghchren de Titest	Dual purpose
Aberkane	Dual purpose
Limli	Oil
Sigoise	Dual purpose

2.2. MORPHOLOGICAL CHARACTERS

The characters were evaluated according to the method of the International Olive Council [14]. The maturity index method consists of evaluating the colour of the skin and the pulp of the fruit. 100 olives were chosen randomly, ordered into seven groups from 0 to 7. The average fruit and stone weight (g) were determined on 40 fruits for each variety. The other characteristics such as flesh weight and flesh percentage were estimated by subtraction and ratios between the measured characteristics. To determine the percentage of humidity, about 50 g of olive samples were weighed and dried at 105°C in an oven, until a constant mass was reached. The oil content of the olives was determined by the Soxhlet method using a quantity of 20 to 30 g of crushed and dried olive paste for each cultivar.

2.3. QUALITY INDICES

Free acidity, peroxide value, UV spectrophotometric indices (K232, K270), evaluated according to the official methods described in Regulation EEC 2568/91 of the Commission of the European Union [15].

2.4. CHLOROPHYLL AND CAROTENOID

The pigment content was evaluated by measuring the absorbance at 670 nm for chlorophylls and 470 nm for carotenoids [16].

2.5. TOTAL PHENOLS

The total phenol contents of the oils were determined by the Folin-ciocalteu reagent [17]. The absorbance was measured at 725 nm. Results were expressed as mg Gallic acid equivalent/kg of oil using a calibration curve ($R^2 = 0.9947$).

2.6. CHROMATOGRAPHIC ANALYSIS OF PHENOLS BY HPLC-UV

To isolate the phenolic fraction of olive oils, we used the method described by the IOC [18]. 2 g of olive oil were weighed from each sample and added to1 ml of the internal standard solution (using syringic acid as an internal standard). 5 ml of methanol/water (80/20), the mixture was shacked for 1 minute, then centrifuged at 4000 rpm/min for 15 minutes. The methanol/water layer was separated, and the extraction repeated twice. After that, the extracts were evaporated to dryness under vacuum in the rotavapor instrument at a low temperature (less than 35°C). The residue was dissolved in 1 mL of methanol/water (1/1, v/v) and subjected to filtration with a filter for syringe. Then, analysed in a Knauer HPLC system with a column C18 (4,6 mm I.D. × 250 mm length, particle size 5 µm), and Spectrophotometric UV detector at 280 nm. The mobile phase consisted of a mixture of Solvent (A): water 0.2% H_3PO_4 (V/V) and Solvent (B): methanol /acetonitrile (50/50, V/V). The chromatograms were recorded at 280 nm using syringic acid as internal standard and identified by comparison with relative retention times of pure compounds.

Table II - Morphological characters of different varieties studied

Cultivars	Morphological characters						
	Maturity index	Fruit moisture (%)	Fruit weight (g)	Stone weight (g)	Flesh weight (g)	Flesh percentage (%)	Oil content %
Bouchouk Lafayette	3,54± 0,08 e	0,54 ±0,01d	4,08±1.12 a	0,66±0,19 a	3,42±1,19 b	82,37±7.60 b	33,27±0,04 d
Souidi	6,04± 0,02 a	0,54±0,01 d	0,59±0,12 e	0,18±0,03 f	0,41±0.12 f	67,45±10.52 d	15,49±0,01 j
Aghchren d'El Ousseur	5,18±0,03 b	0,61±0,00 b	3,34±0.98 b	0,46± 0,11 c	2,87±0.98 c	84,91±5.92 ab	46,57±0,06 a
Azeradj	2,26± 0,04 i	0,46 ±0,01 e	4,50±0,93 a	0,51±0,13bc	3,99±0.96 a	88,15±3.81 a	25,39±0,01 i
Boughenfous	2,62± 0,06 h	0,47 ±0,00 e	0,85±0,16 de	0,28±0,04 de	0,57±0.16 f	65,51±9.5 d	27,93±0,03 h
Aguenaou	3,42± 0,03 f	0,66±0,01 a	0,85±0,16 de	0,22±0,05ef	0,63±0.17 f	72,99±8.3 c	33,51±0,02 c
Aghchren de Titest	4,33 ±0,03 c	0,67±0,01 a	2,60±0,72 c	0,36±0,09 d	2,24±0,76 d	84,57±6.63 ab	33,08±0,04 e
Aberkane	3,25 ±0,07 g	0,61 ±0,00 b	4,01±1.11 a	0,56±0,11 b	3,45±1.14 b	85,13±6.56 ab	30,88±0,06 g
Limli	4,02±0,02 d	0,48 ±0,01 e	1,18±0,27 d	0,27±0,04 e	0,92±0,27 f	76,04±7.95 c	33,91±0,02 b
Sigoise	2,53± 0,04 h	0,57 ±0,00 c	2,16±0,28 c	0,49±0,10bc	1,66±0,30 e	76,75±5.52 c	31,67±0,04 e

Values are shown as Mean Standard deviation (n = 3). The results are statistically analyzed by ANOVA followed Turkey's: The mean in each column with different letters indicates a significant difference (P < 0.05)

2.7. FATTY ACIDS

The composition of fatty acids was determined by gas chromatography (GC). The fatty acids of the different samples were prepared according to the method described by the European Union Commission Regulation EEC No. 2568/91 (EEC, 2015) [19]. Methyl esters were formed by cold transesterification in a methanolic solution of potassium hydroxide. The fatty acid esters obtained were analysed using a Chrompack CP 9002 device, with detector FID (T = 250°C). The column used was a capillary column, with a length of 30 m and an internal diameter of 0.32 mm × 0.25 µm. The carrier gas was nitrogen at a flow rate of 1ml/min and the oven temperature was 200°C, and injection temperature at 250°C, a split splitless injection at 250°C.

2.8. STATISTICAL ANALYSES

All experiments were performed in triplicate; all data are expressed as the mean ± standard deviation. The statistical analysis was performed with the XLSTAT statistical software, version 2016. An analysis of variance (ANOVA) with one factor (cultivar) followed by the Tukey method at the level of 5% significance, to determine the different homogeneous groups was carried out. A Principal Component Analysis (PCA) of fatty acids was performed, with the aim of revealing associations and differences between the different cultivars.

3. RESULTS AND DISCUSSION

3.1. MORPHOLOGICAL CHARACTERS

Table II presents some agronomic parameters of the10 cultivars studied. The maturity index ranged widely from 2.26 to 6.04. The lowest maturity index was marked by the *Azeradj* variety with 2.26, while the highest maturity index was 6.04 for the *Souidi* variety. The fruits weight of different varieties (Table 2) ranged from 0.59 g (*Souidi*) to 4.50 g (*Azeradj*). *Boughenfous, Limli, Souidi, Aguenaou* have a weight fruit lower than 2 g. *Aghchren d'ElOusseur, Aghchren de Titest, Sigoise,* have intermediate mass olives ranged from 2-4 g. The highest fruit weight was recorded for the following cultivars *Azeradj* (4.50 g), *Aberkane* (4.01 g), *Bouchouk Lafayette* (4.08 g). These three cultivars are used for table olives or for a double purpose due to their size. The analysis of the variance showed a very highly significant difference ($p \leq 0.0001$) for fruit weight between cultivars. The study carried out in the centre and the east of Algeria [20] has shown lower values to our results, especially for the varieties *Bouchouk*, *Azeradj* and *Boughenfous.* Abdessemed et *al*. [21] reported close results for the *Sigoise* variety, *Aghchren de Titest*, on the other hand, very low results for *Limli*.

The water content of the different olive varieties varies between 46% (*Azeradj*) to 67% (*Aghchren de Titest*). Our results fit into the range given by Ravetti [22] that vary between 40 to 75%. It was reported [23] that a decrease in fruit moisture was proportional to an increase in oil concentration. high moisture content indicates both a lower oil and dry flesh content [24]. Regarding the oil content (table 2), the Souidi variety contains the lowest value (15.50%), while the Aghchren of el-Ousseur has the highest value (46.5709%). The analysis of the variance indicates a very highly significant difference between the cultivars tested ($p < 0.0001$). Our results are similar for those reported for Algerian varieties [25].

Table III - Physico-chemical indices of the oil of different Algerian olive varieties

Cultivars	Analytical oil parameters			
	Free acidity (% of oleic acid/kg)	Peroxide value (meq O_2/kg of oil)	Specific extinction K_{232}	Specific extinction K_{270}
Bouchouk Lafayette	0,35±0,02 cd	10,53±0,04 g	3,00±0,00 a	0,16±0,00 b
Souidi	0,38±0,02 cd	15,51±0,02 b	2,25±0,00 c	0,13±0,00 d
Aghchren d'El Ousseur	0,27±0,03 f	13,02±0,02 e	1,98±0,00 f	0,11±0,00 e
Azeradj	1,58±0,00 a	11,52±0,02 f	1,94±0,01 g	0,17±0,00 a
Boughenfous	0,41±0,00 c	6,02±0,03 i	1,42±0,01 i	0,13±0,00 d
Aguenaou	0,35±0,04 cd	14,51±0,02 d	2,05±0,01 e	0,13±0,00 d
Aghchren de Titest	0,48±0,01 b	15,01±0,01 c	2,37±0,00 b	0,13±0,00 d
Aberkane	0,31±0,01 ef	23,01±0,02 a	2,08±0,00 d	0,14±0,00 c
Limli	0,33±0,00 de	4,02±0,03 j	1,76±0,01 h	0,13±0,00 d
Sigoise	0,35±0,00 cd	7,00±0,01 h	1,17±0,01 j	0,11±0,00 e

Values are reported as Mean (n = 3). The results are statistically analyzed by ANOVA followed by Turkey's: The mean in each column with different letters indicates a significant difference (P < 0.05)

3.2. QUALITY PARAMETERS

The quality indices of different cultivars studied were presented in Table III. The free acidity of the studied samples has shown average values that oscillate from 0.31% (*Aberkane*) to 1.58% (*Azeradj*), which distinguished two distinct categories of oil (Extra virgin and virgin olive oil), according to the standards of the International Olive Council [26]. Almost all the oil samples were classified as extra virgin oils (acidity ≤ 0.8%), except for the *Azeradj* cultivar, which corresponded to the category of virgin oils (acidity ≤ 2%) with value 1.58%.Our results are very low for the oil obtained from *Limli* (0.33%) variety, compared to those reported [27], where the free acidity is 2.84% for the olive oils produced in the East of Algeria region. The analysis of the peroxide index revealed average values vary from 4.01 to 23.0 expressed in Table 3. All samples studied of olive oil have a peroxide index less than 20 meq O_2/ kg, except for the oil obtained from *Aberkane* variety with a peroxide value of 23.0 meq O_2/ kg of olive oil, which exceeds the limit (20 meq O_2/ kg) established by the IOC [26]. The minimum value was recorded in the *Limli* cultivar with a peroxide content of 4.01 meq O_2/kg. Our results are close to those found [28]; peroxide index values varied between 5.19 and 18.76 meq of O_2 / kg for Algerian and Italian varieties. Values of specific extinctions in the ultraviolet at 232 nm and 270 nm are presented on table 3. The values for the specific extinction K_{232} of the varieties studied show values that vary between 1.17 and 3.00. The nine samples studied indicate that they do not exceed the limit established by IOC [26] (≤2.5) except for *Bouchouk Lafayette* cultivar with 3.00. For the specific extinction K_{270}, the values vary from 0.11 to 0.17. However, all samples do not exceed the limit established by the IOC (≤0.22). Our results are close to those reported [29, 30].

3.3. PIGMENT CONTENT

As shown in Table 4, the pigment content is strongly influenced by the cultivar, showing very highly significant differences between samples (p < 0.0001). For chlorophylls, the lowest value was observed in the *Bouchouk Lafayette* variety with 1.71mg/kg. The highest value is recorded in the oil of the *Azeradj* variety with a value of 7.03 mg/kg.

Concerning carotenoids, the analysis of variance revealed a highly significant difference in carotenoid content between the different cultivars studied (P < 0.001), which varies between 1.22 mg/kg for *Bouchouk Lafayette* variety to 3.20 mg/kg for *Boughenfous* variety. β-carotene is a major carotenoid, which

Table IV: Pigments and total phenols content of olive oil varieties

Cultivars	Chlorophyll (mg/kg)	Caroténoïds (mg/kg)	Total phenols (mg /kg of oil)
Bouchouk Lafayette	1,71±0,23 g	1,22±0,03 h	168,83±0,01 g
Souidi	2,64±0,19 f	1,45±0,03 g	198,32±0,01 f
Aghchren d'El Ousseur	2,06±0,17 g	1,30±0,02 h	92,17±0,01 i
Azeradj	7,03±0,13 a	2,82±0,01 b	258,97 ±0,01 c
Boughenfous	4,48±0,08 c	3,20±0,06 a	435,88±0,01 a
Aguenaou	3,67±0,06 d	2,11±0,07 d	258,12±0,01 d
Aghchren de Titest	3,08±0,12 f	1,80±0,01 e	122,50±0,01 h
Aberkane	3,24±0,13 e	1,60±0,03 f	56,79± 0,01 j
Limli	5,75±0,08 b	2,61±0,05 c	205,90±0,01 e
Sigoise	2,88±0,01ef	2,56±0,04 c	357,53±0,01 b

Values are reported as Mean (n = 3). The results are statistically analyzed by ANOVA followed by Turkey's: The mean in each column with different letters indicates a significant difference (P < 0.05)

gives carotenoids a protective effect against degradation [31]. It has been noted that the content of chlorophyll and carotenoid pigments varies significantly with the variety [30]. Lower levels of chlorophylls and carotenoids was noted for Algerian varieties [32].

3.4. TOTAL PHENOLS

The results obtained (table IV) showed a very highly significant difference ($p < 0.0001$) between the studied varieties. The richest varieties are *Boughenfous and Sigoise,* with contents of 435.88 and 357.53 mg/kg, respectively. The poorest are *Aberkane and Aghchren d'El Ousseur* varieties, with 56.79 and 92.17 (mg of gallic acid/kg) respectively. Phenolic compounds contribute to the taste characteristics and high stability of virgin olive oil against oxidation [10]. Our results agree with those reported [33] for the Algerian varieties and those for varieties from Argentina [28]. On the other hand, our results are very high compared to those reported [28]; lower levels of polyphenols (from 27 to 184 mg/kg) have been noted for Algerian and Italian olive oils.

3.5. PHENOLIC PROFILES

Table V shows the quantitative composition (mg/kg) of the phenols determined by HPLC analysis. 3 phenolic compounds were identified and quantified: Hydroxytyrosol, Tyrosol and Oleocanthal (fig.1). Hydroxytyrosol and tyrosol, represent hydrolysis products of secoridoid compounds, like oleuropein and ligstroside aglycons [28]. The hydroxytyrosol content was higher in *Sigoise*, *Azeradj* and *Souidi* cultivars, while this compound was lower in other cultivars. Hydroxytyrosol is one of the major phenolic

Table V - HPLC phenolic composition of olive oil varieties.

Cultivars	Hydroxytyrosol (mg/kg VOO)	Tyrosol (mg/kg VOO)	Oleocanthal (mg/kg VOO)	Phenols (mg T/kg VOO)
Bouchouk Lafayette	4,98±0,11 c	27,98±0,06 d	nd	173,25±0,35g
Souidi	16,71±0,17 b	48,58±0,12 a	22,93±0,08 c	218,15±0,21c
Aghchren d'El Ousseur	nd	3,7±0,28 i	nd	105,17±0,23j
Azeradj	16,31±0,13 b	24,68±0,13 f	40,63±0,09 b	216,13±0,18d
Boughenfous	3,31±0,27 e	37,77±0,08 b	67,93±0,03 a	263,01±0,01a
Aguenaou	5,01±0,06 c	25,83±0,19 e	nd	205,01±0,01e
Aghchren de Titest	nd	8,62±0,11 g	nd	112,03±0,04i
Aberkane	nd	6,84±0,12 h	nd	148,01±0,01h
Limli	4,31±0,03 d	36,27±0,38 c	nd	181,05±0,07f
Sigoise	33,74±0,05 a	35,96±0,01 c	nd	253,02±0,03b

Values are reported as Mean (n = 2). The results are statistically analyzed by ANOVA followed by Tukey's: The mean in each column with different letters indicates a significant difference ($P < 0.05$), nd: not detected, T: Tyrosol.

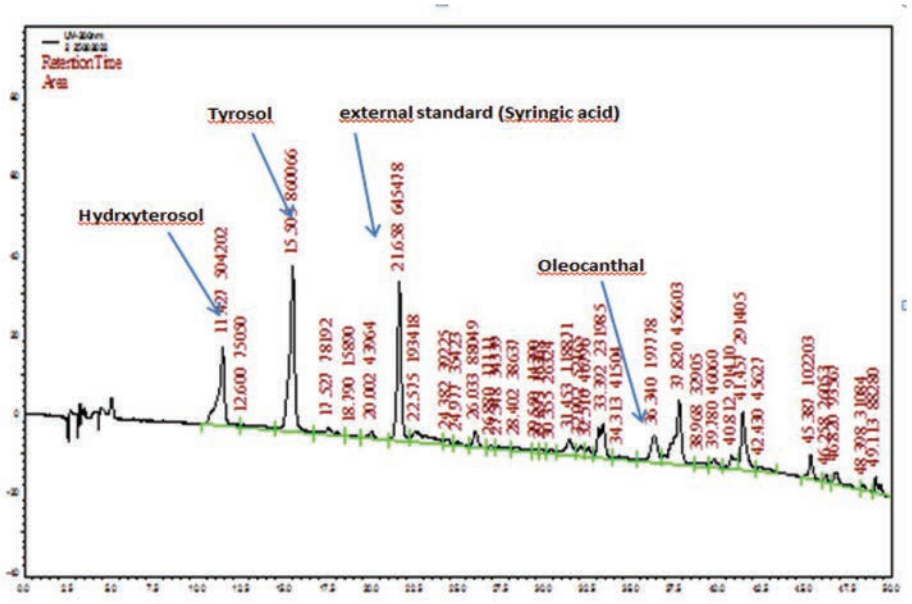

Figure 1 - HPLC chromatograms at 280 nm of phenolic extracts from olive oil and external standard peaks

Table VI - Profile of fatty acids of olive oil different Algerian varieties

Cultivars/ Fatty acids (%)	C16:0	C16:1ω 7	C17:0	C18:0	C18:1ω 9	C18:2ω 6	C18:3ω 3	C20:0	C20:1ω 9	C22:0	C22:1	C24:0	∑SFA	∑MUFA	∑PUFA	Oleic/ Linoleic	MUFA/ PUFA
Bouchouk lafayette	13,31 f	0,89 f	0,15 cd	2,60 f	61,86 j	17,70 a	0,63 g	0,48 f	0,33 ab	0,14 d	1,01 b	0,08 a	16,76 g	64,09 f	18,32 a	3,50 j	3,50 i
Souidi	13,93 e	1,04 e	0,21 a	2,70 e	66,26 g	13,79 b	0,78 e	0,47 f	0,20 f	0,14 d	0,25 h	0,00 b	17,44 d	67,75 e	14,57 b	4,80 i	4,64 h
Aghchren d'el ousseur	11,73 i	0,68 h	0,17bc	2,08 i	70,91 d	9,95 d	0,71 f	0,65 a	0,35 a	0,17bc	1,16 a	0,00 b	14,81 i	73,10bc	10,66 e	7,13 f	6,86 d
Azeradj	14,31 c	1,35 b	0,14 d	3,06 a	65,14 i	9,18 f	0,69 f	0,56 c	0,30 cd	0,15 cd	0,63 d	0,00 b	18,22 c	67,42 e	9,87 g	7,10 g	6,83 e
Boughenfous	14,17 d	1,17 c	0,17bc	2,26 g	75,14 a	4,82 j	0,75 e	0,52	0,32bc	0,21 a	0,31 g	0,00 b	17,32 e	78,44 a	5,57 j	15,59 a	13,81 a
Aguenaou	13,12 g	0,82 g	0,16bc	2,81 d	70,67 e	8,79 h	1,01 c	0,60 b	0,35 a	0,18 b	0,04 i	0,00 b	16,87 f	71,88 c	9,80 h	8,04 c	7,33 c
Aghchren de tistest	15,08 b	1,14 d	0,17bc	2,90 c	68,14 f	9,44 g	1,04 b	0,53 de	0,28 d	0,16bc	0,95 c	0,00 b	18,84 b	70,51 cd	10,48 f	7,22 e	6,73 f
Aberkane	12,41 h	0,88 f	0,18 b	2,97 b	73,37 c	7,33 i	0,98 d	0,51 e	0,29 d	0,15 cd	0,62 d	0,00 b	16,22 h	75,16 b	8,31 i	10,01 b	9,05 b
Limli	15,79 a	1,71 a	0,00 e	2,91 c	65,65 h	11,72 c	0,75 e	0,54 cd	0,25 e	0,11 e	0,40 f	0,00 b	19,34 a	68,01 de	12,47 c	5,60 h	5,45 g
Sigoise	11,18 j	0,70 h	0,00 e	2,13 h	73,47 b	9,87 e	1,11 a	0,35 g	0,33 ab	0,09 e	0,55 e	0,00 b	13,75 j	75,05 b	10,98 d	7,44 d	6,84 de

Values are reported as Mean (n=3). The results are statistically analyzed by ANOVA followed by Turkey's: The mean in each column with different letters indicates a significant difference (P <0.05), SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

compounds in olive oil that exerts antioxidant, anti-inflammatory, anti-platelet aggregation and anti-atherogenic activities in in vitro and animal models [34]. Recently, the European Food Safety Authority (EFSA) has recognised protective effects of the olive oil phenolic compounds on LDL oxidation, in particular of HT (Commission Regulation, 2012) [35]. The claim may be used only for olive oil, containing at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil.

The concentration of tyrosol was significantly high in all oils which are in accordance with the recent results on Algerian varieties. Similar results were noted for these two compounds in the Algerian varieties [32]. The oleocanthal content was found higher in *Boughenfous, Azeradj and Souidi cultivars,* for other varieties has not been detected. Besides, very significant differences were observed in the concentration of phenolic compounds ($p \leq 0.0001$) between the varieties studied. It was reported [36] that the phenolic profile differs between varieties of plants of the same species. Phenols are recognised as important antioxidant compounds that protect the oil against auto-oxidation, at the cellular level, against oxygen radicals [37], and contribute to its pungent and bitter taste. Hydroxytyrosol (HTyr), tyrosol (Tyr), oleacein and oleocanthal are the main compounds responsible for the beneficial effects of EVOO as part of the Mediterranean diet [38, 39].

3.6. FATTY ACIDS

The fatty acids identified and quantified are shown in table VI. In all cultivars, the main fatty acids were oleic, linoleic, palmitic, and stearic acids. Oleic acid has always been the most abundant compound, accounting for more than 60% of the total fatty acids. We note that the level of oleic acid, the major fatty acid of olive oil, is very low in *Bouchouk Lafayette* cultivar with concentration of 61.86%, although *Boughenfous* proved to be the best performing variety with the highest rate (75.15%); followed by *Sigoise* (73.48%) and *Aberkane* (73.38%).The analysis of variance showed a very highly significant difference for oleic acid between the different varieties studied ($p < 0.0001$).The fatty acid composition varies relatively due to genetic and environmental factors [40]. Our results were close to those obtained [41] on six olive oils from six cultivars of Tunisian origin. Similar results were reported for different Algerian varieties namely *Azeradj*, *Blanquette*, *Bouricha*, *Chemlal*, *Limli, Sigoise* [42].

Concerning the linoleic fatty acid (C18:2ω6), *Bouchouk Lafayette* cultivar has maximum with 17.70% against 4-15% for the other cultivars, hence the difference is very highly significant between the varieties ($p < 0.0001$). Palmitic acid was the major saturated fatty acids; it varies between 11.18% (*Sigoise*) to 15.79% (*Limli*). The analysis of the variance showed a very highly significant difference between the cultivars

tested ($p < 0.0001$). Another saturated acid is stearic acid that varies between 2.08% (*Aghchren d'ElOusseur*) to 3.06% (*Azeradj*).

The two ratios between (MUFA/PUFA) and Oleic/Linoleic are strongly influenced by the cultivar ($p < 0.0001$). *Boughenfous* recorded the highest ratio with 13.82 and followed by *Aberkane* with 9.06 against the other cultivars which vary between 3.50-8%. Furthermore, the Oleic/Linoleic ratio varies between 3.50% (*Bouchouk Lafayette*) to 15.59% (*Boughenfous*). The content of this ratio is five times more for the cultivar *Boughenfous* compared to *Bouchouk and Lafayette*. According to [29, 33] the MUFA/PUFA ratio is of great importance because of the nutritional properties and oxidative stability of olive oils. The high proportions of monounsaturated fatty acids (∑MUFA) represent one of the most important technological and nutritional characteristics of olive oils [44]. Our results are higher than those reported [45] for Tunisian varieties at different altitudes. Authors [43, 46] noted that varietal character strongly influenced fatty acid composition. The variability in our study could be explained by the genetic characters of cultivars since the culture conditions and oil extraction conditions were the same.

3.7. PRINCIPAL COMPONENTS ANALYSIS (PCA)

The principal component analysis was applied to the fatty acid profile of the different varieties, to evaluate their correlation (Figure 2a 2b, Table 6). The projection of the parameters on the factor plane F1-F2 of the PCA (Figure 2) evaluates the variability between the parameters, by their dispersion on the two axes which explain about 64.59% of the total variance. The axes 1 and 2 explain 40.33 and 24.27% of the variance, respectively.

The projection of the points on the circle indicates a good dispersion of the variables on the two axes. All the variables are well represented in this factorial plane since their correlation with the axes are relatively important, which means that the samples studied show a great chemical diversity. The projection is relatively far from the centre for some parameters. The axis 1 in the positive direction associates the following fatty acids: 22:0, C18: 1, C20: 0, C18: 3w3, C18: 1w9, Oleic acid / Linoleic acid ratio, ∑MUFA, MUFA / PUFA ratio, C20: 1w9, while the negative direction of the same axis is associates the following fatty acids: C16: 1w7, ∑SFA, C18: 0, C16: 0, ∑PUFA, C18: 2w6, C17: 0, C22:1. However, the axis 2 on the positive side is defined by saturated fatty acids (∑SFA), C16: 0, C18: 0, C16: 1w7, C20:0, C17:0, C22:0, Oleic/Linoleic, MUFA / PUFA and in the negative sense it is defined by the following fatty acids: C20: 1w9, C18: 2w6, C22:1, C18:3w3, C18:1w9, ∑MUFA, ∑PUFA.

The projection of the individual elements showed a good dispersion on the factorial plane and reveals the grouping of individual elements (figure 3). According to the F1 axis we can distinguish 5 groups or each group's varieties that have similar coordinates. In comparison between figure 2 and 3, axis 1 shows that the group1 associates the following cultivars: *Sigoise*, *Aghchren d'el- Ousseur* are on the positive side of the F1 axis indicating that they have the highest percentages in C18:1, and approximate contents in Oleic acid / Linoleic acid ratio, ∑MUFA, MUFA / PUFA ratio. According to the axis F2, group1 is located on the negative side which indicates that they have high values in C20:1w9, C18: 3w3. Group 2 represented by two varieties: *Aberkane*, *Aguenaou* according to F1 axis are located on the positive side and close to the centre of the axis and are characterised by a more homogeneous grouping according to their high oleic acid content: (C18:1w9) and the ratio (C18:1/C18:2) and MUFA/PUFA ratio and ∑MUFA, C20:0. G3 is rep-

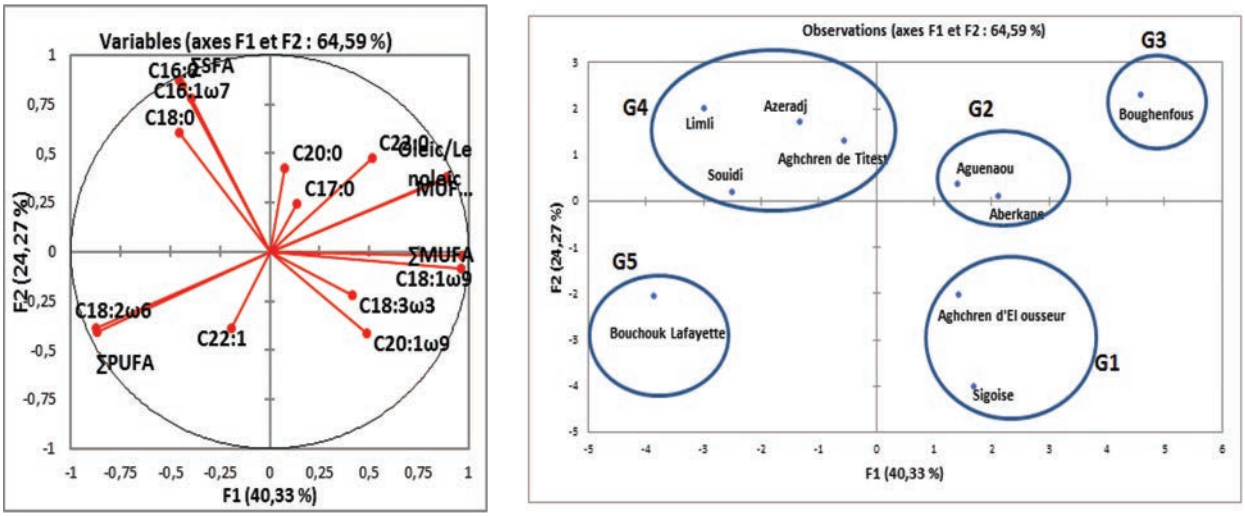

Figure 2 - a) Correlation circle of fatty acid composition and the F1 and F2 axes of the PCA

b) Representation of the results of the principal component analysis carried out for the different varieties studied.

resented by a single variety *Boughenfous* on the end of the positive portion of the F1 axis; it has the best characteristics with high value in the ∑MUFA, MUFA / PUFA ratio and oleic acid. Group 4 contains *Limli*, *Aghchren de Titest*, *Azeradj*, *Souidi*, located on the negative side of the F1 axis to their high-grade graders in saturated fatty acids (∑SFA) and C16:0, C18:0, C16:1. G5 is represented by Bouchouk Lafayette which is located on the negative side of the F2 axis having high levels of polyunsaturated fatty acids and C18: 2w6. This variety is more sensitive to oxidation. G5 is represented by Bouchouk Lafayette which is located on the negative side of the F2 axis having high levels of polyunsaturated fatty acids and C18: 2w6. This variety is more sensitive to oxidation.

4. CONCLUSION

The olive tree is one of the most important crops in Algeria. The local genetic resources of olive trees have features and performances that deserve to be valued. According to the results obtained, the varietal character influences significantly the physico-chemical parameters of virgin olive oil, including the total phenol content and fatty acids. Thus, the major fatty acids of olive oil in particular oleic acid, palmitic, stearic, and linoleic, were significantly influenced by the cultivar ($p < 0.0001$). The best result in terms of total polyphenols and oleic acid of olive oil was obtained with the *Boughenfous* variety that presented a low maturity index 2.623. The varieties with a maturity index between 2.5 and 3.5 presented the best features in minor compounds of olive oil. Globally, the results showed that the cultivar plays an important role in the quantitative and qualitative characteristics of olive oils. This characterisation must be extended to other secondary olive cultivars that remain unexplored.

Olive growers must be encouraged to promote the local olive-growing heritage by cultivating varieties approved by the National Centre for seeds and plants Control and Certification (CNCC).

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Data availability The datasets generated and analysed for this study are available from the corresponding author upon reasonable request.

Declaration

Conflict of interest The authors declare that they have no conflicts of interest.

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Short Note

Chemical composition and cytotoxicity of *Garcinia urophylla* Scort. ex King essential oil

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Essential oils, a volatile mixture derived from plants, have shown a wide biological activity, and have been used as ancient remedies for the treatment of various diseases. The objective of this study was to investigate the chemical composition and cytotoxicity of the essential oil obtained from *Garcinia urophylla* leaves collected from Malaysia. Eighteen components were identified using gas chromatography-flame ionisation detection (GC-FID) and gas chromatography/mass spectrometry (GC-MS), which represent 99.9% of the essential oil. The major components were β-caryophyllene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%). The cytotoxicity of essential oil was evaluated using an MTT assay. The essential oil exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7, and A549 with the IC50 values of 71.5, 56.2, and 68.5 µg/mL, respectively. To the best of our knowledge, this is the first report on the essential oil composition obtained from *Garcinia urophylla*, which may have implications on the pharmaceutical and therapeutic applications of Garcinia genus essential oils.
Keywords: Essential oil; *Garcinia urophylla*; β-caryophyllene; cytotoxicity

1. INTRODUCTION

Essential oils have been widely studied as anticancer drugs in recent years. They are secondary metabolites with a key role in plant protection, consisting primarily of terpenes with a volatile nature and a diverse array of chemical structures. Essential oils exhibit a wide range of bioactivities, most notably antibacterial, antifungal, and antioxidant properties and have long been utilised for treating various human ailments and diseases [1-5]. In recent years, essential oils have been introduced as alternatives to the well-known side effects caused by synthetic chemotherapeutic drugs. In addition, chemical compounds from plants have been reported to prevent carcinogenic processes by cell arrest, induce both inner and outer apoptosis pathways, inhibit the mutagen in cells, and reduce oxidative stress in cells [6]. Therefore, the cytotoxicity of essential oils against different cell lines is necessary.

Clusiaceae are a tropical family mainly composed of latex with about 40 genera. In Malaysia, four genera and 121 species of the Clusiaceae are found namely, *Garcinia* (49 sp.), *Calophyllum* (45 sp.), *Mesua* (23 sp.) and *Mammea* (4 sp.) in different habitats [7]. *Garcinia* is an economically important genus of Clusiaceae consisting of about 400 species within palaeotropical regions concentrated mainly in Southeast Asia and secondarily in India and West Africa. Species of this genus are typically small to medium dioecious evergreen fruit trees, occasionally shrubs, usually with hard timber and abundant latex. The fruits, latex (gum and resin), timber, leaves, and roots of several species are of economic and medicinal value [8]. The major classes of biomolecules reported to be present in these plants include xanthones, benzophenones, flavonoids, phloroglucinols, fatty acids, and terpenoids [9]. Additionally, important pharmacological properties such as antioxidant, anticancer, anti-di-

abetic, anti-inflammatory, cardioprotective, neuroprotective, antimicrobial, and hepatoprotective activities along with the toxicological studies of the *Garcinia* have been documented [10].

G. urophylla, locally known as 'kandis hutan' in Malaysia, which are native to Peninsular Malaysia, Thailand, and India. It is a shrub and grows primarily in the wet tropical biome. The fruits are traditionally used to treat stomach-ache and the leaves are used to treat fever [11]. Previously, the stem extract of *G. urophylla* was found active against human tumour cell lines, representing tumours of the breast (MCF-7), lung (NCI-H460) and prostate (DU-145) with IC_{50} values of 8.0, 37.0, and 32.0 µg/mL, respectively [12]. Meanwhile, the leaf extract of *G. urophylla* showed a strong nitric oxide inhibitory activity in RAW 264.7 macrophage cell line with IC_{50} value 22.0 µg/mL [12]. Another study revealed the isolation of xanthones in the phytochemical investigation of *G. urophylla* leaves [13].

As part of a systematic evaluation of Malaysian *Garcinia* species [14-17], we present the chemical composition and cytotoxicity of the essential oil extracted from the leaves of *G. urophylla*. Literature searches have revealed no reports on the composition of leaf oil in this species.

2. MATERIALS AND METHODS

2.1 PLANT MATERIAL

The samples of *G. urophylla* were collected from Fraser Hill, Pahang (January 2023) and were identified by Shamsul Khamis from Universiti Kebangsaan (UKM). The voucher specimen (SA30-39) was deposited at Herbarium of UKM.

2.2 ISOLATION OF ESSENTIAL OIL

The fresh leaves of *G. urophylla* (400 g) were subjected to hydro distillation for 4 hours in a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous magnesium sulphate and stored at 4-6°C.

2.3 ANALYSIS OF ESSENTIAL OIL

Gas chromatography-flame ionisation detection (GC-FID) analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with HP-5 column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually increased to 280°C at 5°C/min rate and finally held isothermally for 15 min. Diluted samples (1.0 µL, 1/100 v/v in diethyl ether) were injected manually (split ratio 50:1). Injection was repeated three times and peak area percentages were reported as means ±SD of triplicate. Peak area percentages were calculated from flame ionisation detection (FID) using GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a gas chromatograph Hewlett Packard Model 5890A and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with HP-5 column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system with an ionisation energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50–400 amu.

2.4 IDENTIFICATION OF ESSENTIAL OIL COMPONENTS

The essential oil components were identified by co-injection with standards (major components: β-caryophyllene, α-humulene, and α-gurjunene) and their comparison with reported retention indices and mass spectra found in Adams, NIST 08 and FFNSC2 libraries [18]. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components.

2.5 CYTOTOXICITY

Cytotoxic examination of the essential oil was carried out using the MTT assay [19]. Briefly, the cells were diluted in a 96-well microplate (5×10^4 cells per well of 200 µL mixture). The samples (1-100 µg/mL) and the positive control, doxorubicin (0.05-1.56 µg/mL), were added to the cells and incubated at 37°C for 48 h with 5% CO_2. MTT (20 µL) was added to the wells and incubation was continued at 37°C for 4 h. Absorbance was recorded at 540/720 nm using a Spark multimode reader (Tecan). Each experiment was repeated in triplicate. The inhibitory percentage (%) = $[1 - OD_{sample}/OD_{conc}] \times 100\%$; where OD_{sample} and OD_{conc} were the optical densities of the samples and the control, respectively. Data obtained from the cytotoxicity are expressed as mean values. Statistical analyses were carried out by employing one-way ANOVA ($p > 0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

3. RESULTS AND DISCUSSION

Hydrodistillation by fresh *G. urophylla* leaf produced (w/w) 0.29% of essential oil. A summary of the chemical components identified in the essential oil is shown in Table I. GC and GC-MS analysis (Figure 1) of the essential oil successfully revealed the existence of 18 chemical components, representing 99.9% of the total essential oil composition. Sesquiterpene hydrocarbons were the most dominant group in the essential oil components, accounting for 96.1% of the total composition, respectively. The essential oil composition was demonstrated by its richness in β-caryophyl-

Table I - Chemical components identified from *Garcinia urophylla* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identification[c]
1	α-Pinene	934	935	0.2	RI, MS
2	*o*-Cymene	1022	1020	0.4	RI, MS
3	γ-Terpinene	1052	1055	0.2	RI, MS
4	Terpinen-4-ol	1169	1166	1.9	RI, MS
5	α-Copaene	1374	1372	0.8	RI, MS
6	β-Elemene	1389	1390	0.6	RI, MS
7	**α-Gurjunene**	1405	1405	**6.3**	RI, MS, Std
8	**β-Caryophyllene**	1420	1420	**56.2**	RI, MS, Std
9	**α-Humulene**	1436	1435	**26.3**	RI, MS, Std
10	Alloaromadendrene	1460	1462	2.2	RI, MS
11	γ-Gurjunene	1470	1470	0.7	RI, MS
12	α-Selinene	1490	1491	0.6	RI, MS
13	Viridiflorene	1505	1504	1.0	RI, MS
14	(*E*,*E*)-α-Farnesene	1505	1506	0.3	RI, MS
15	δ-Cadinene	1524	1522	1.1	RI, MS
16	Palustrol	1567	1565	0.3	RI, MS
17	Caryophyllene oxide	1582	1580	0.5	RI, MS
18	*neo*-Intermedeol	1658	1658	0.3	RI, MS
	Monoterpene hydrocarbons			**0.8**	
	Oxygenated monoterpenes			**1.9**	
	Sesquiterpene hydrocarbons			**96.1**	
	Oxygenated sesquiterpenes			**1.1**	
	Total identified			**99.9**	

RI: based on comparison of calculated RI with those reported in Adams
MS: based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases
Std: based on comparison with standard compounds
[a] Linear retention index experimentally determined using homologous series of C6-C30 alkanes
[b] Linear retention index taken from Adams, Wiley or NIST08 and literature
[c] Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

lene (56.2%), α-humulene (26.3%), and α-gurjunene (6.3%).

In comparison to the previous studies, β-caryophyllene has also been reported as a major component in Malaysian *Garcinia* species such as from the leaf oils of *G. nigrolineata* (25.2%) [20], *G. gummi-gutta* (53.82%) [21], and *G. celebica* (5.85%) [22]. In addition, Indian *Garcinia* species were also reported the high content of β-caryophyllene which are from the leaf oil of *G. morella* (69.6%), *G. assamica* (31.0%), *G. lanceifolia* (15.9%), *G. xanthochymus* (15.7%), *G. pedunculata* (9.8%), *G. dulcis* (9.2%) [23], *G. imberti* (38.1%), *G. rubro-echinata* (37.9%), *G. talbotii* (30.4%), *G. wightii* (19.0%), *G. indica* (18.6%), and *G. pushpangadaniana* (11.4%) [24]. Furthermore, it was also reported from *G. quaesita* (Sri Lanka: leaf oil 6.7%), *G. zeylanica* (Sri Lanka: leaf oil 12.9%) [25], *G. huillensis* (Zimbabwe: fruit oil 12.6%) [26], and *G.*

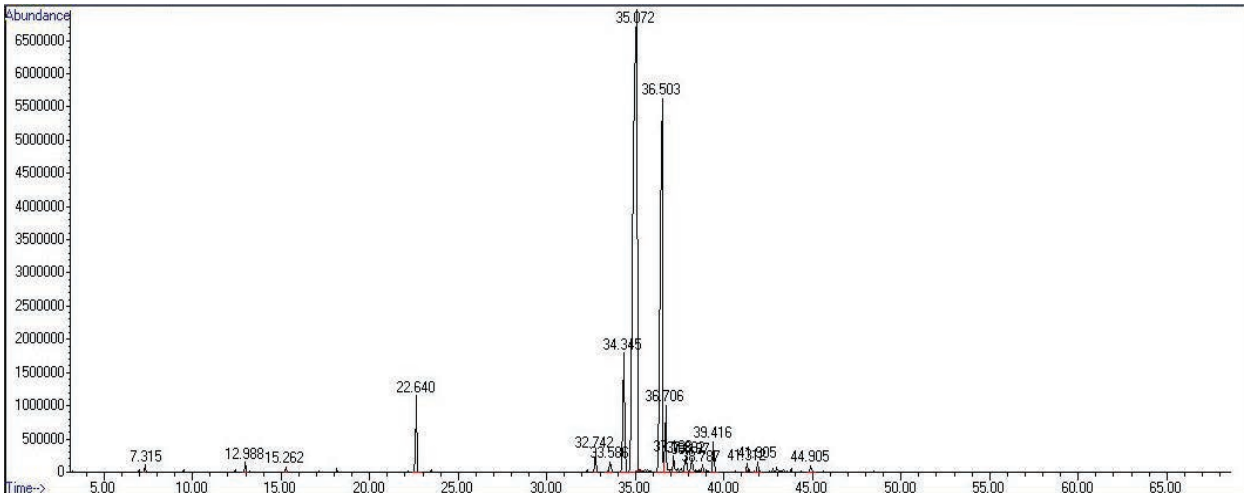

Figure - 1 Chromatogram of *Garcinia urophylla* essential oil

Table II – Cytotoxicity of *Garcinia urophylla* essential oil

Cell lines	IC_{50} value (µg/mL)	
	Essential oil	Doxorubicin
Human liver cancer HepG2	71.5	0.76
Human breast cancer MCF7	56.2	0.20
Human lung carcinoma cancer A549	68.5	0.95

mangostana (Nigeria: leaf oil 17.3%, stem-bark oil 21.1%) [27]. These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotype, and nutritional status of the plants, which may influence their oil composition [28].

β-Caryophyllene is found in numerous edible plants that are ingested daily, and it is approved as a food additive by the Food and Drug Administration. This compound can change the inflammatory processes in humans through the endocannabinoid system [29]. Furthermore, this compound could increase the intracellular accumulation of anticancer agents, thereby potentiating their cytotoxicity due to the absorption of 5-fluorouracil across human skin. β-Caryophyllene facilitates the passage of paclitaxel through membranes and thus potentiates its anticancer activity [30].

Following a similar line of thought, the essential oil was subjected to a preliminary test to verify the cytotoxicity effect using the MTT assay. The results are shown in Table II. Doxorubicin remarkably inhibited the growth of the studied cancer cell lines with lower IC_{50} values compared to the essential oil. The best inhibitory result of the essential oil with IC_{50} value of 56.2 µg/mL reported against the MCF7 cell line. The cytotoxicity of the essential oil may be attributed to the presence of the major component in the sample. Previous studies have revealed that sesquiterpenes exhibited cytotoxic effects in several cancer cell lines [31]. Previously, β-caryophyllene was reported to show cytotoxicity on breast carcinoma cells [32]. Besides, the treatment of β-caryophyllene alone with human tumour cell lines has stimulated apoptosis and suppressed tumour growth [30]. Furthermore, in studies on *G. atroviridis* [33] and *G. celebica* [22] essential oils, the high content of sesquiterpenes was found in the leaf oil and exhibited potent cytotoxicity on human breast cancer cells. According to the results, the potent cytotoxicity of *G. urophylla* essential oil could be due to the major occurrence of sesquiterpenes, which accounted for 96.1% of the total oil.

4. CONCLUSION

Essential oils from aromatic plants are recognised as a perfect source of food additives and pharmaceuticals. This study of *G. urophylla* essential oil has revealed the existence of sesquiterpene hydrocarbons as the main component of the group, dominated by β-caryophyllene, α-humulene, and α-gurjunene. The essential oil also revealed a significant cytotoxic effect, mainly against MCF7 MCF-7 human breast cancer cell line with IC_{50} value 56.2 µg/mL. Hence, the species could be a source of natural products for further research into the development of chemopreventive or cosmetic agents.

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RESOCONTO ATTIVITÀ UNI - ANNUALITÀ 2023

GRUPPO LAVORO 18

"OLI, GRASSI ANIMALI E VEGETALI E LORO SOTTOPRODOTTI, SEMI E FRUTTI OLEAGINOSI"

DELLA COMMISSIONE AGROALIMENTARE CT/003

Le Riunioni si sono svolte nelle seguenti date: 14 febbraio, 17 aprile, 13 giugno, 21 luglio, 24 ottobre e gli esperti del gruppo di lavoro hanno contribuito nel corso dell'anno alla pubblicazione delle seguenti norme:

- **UNI 11923:2023** Oli e grassi vegetali ed animali e derivati - Olio di grano duro (Triticum durum) raffinato - Caratteristiche e metodi di analisi
- **EC 1-2023 UNI 22620:2022** Semi e frutti oleaginosi e derivati - Sostanze proteiche vegetali - Determinazione del contenuto di amminoacido triptofano mediante cromatografia a scambio ionico
- **UNI 11929:2023** Determinazione dei biofenoli degli oli d'oliva mediante HPLC
- **UNI ISO 23942:2023** Determinazione del contenuto di idrossitirosolo e tirosolo negli oli extra vergini di oliva - Metodo di cromatografia liquida ad alta prestazione in fase inversa (RP-HPLC)

oltre a occuparsi alla revisione di tutte le norme degli oli di semi di pressione per migliorare la descrizione del processo della loro produzione.

Inoltre, è in corso di stesura la seguente nuova Norma UNI: *Determinazione dello stato di ossidazione degli oli vergini di oliva mediante HPLC (ex NGD C88-07)*.

Al **meeting della ISO/TC 34/SC 2 n 1070 E ISO/TC 34 SC 11**, tenutosi a Bruz (France) il 23-24-25 maggio, la delegazione italiana del GL18 UNI ha partecipato da remoto contribuendo con le proprie competenze ai punti dell'agenda dei diversi giorni. In tale occasione è stato proposto di organizzare la **prossima riunione del 2024 in Italia a Milano** e, dopo ballottaggio internazionale, tale invito è stato accolto favorevolmente.

Durante le riunioni si è inoltre commentato insieme e formulata la **posizione italiana dei documenti in votazione** all'ISO riguardanti oli e grassi animali e vegetali, semi e analizzato i trial collaborativi in corso.

Per quanto riguarda i lavori futuri, è stato dimostrato interesse nella futura possibile pubblicazione di norme che riguardino il grasso degli insetti ad uso alimentare, per il quale è in corso un confronto l'Università degli Studi di Torino e con l'International Platform of Insects for Food and Feed.

Sono stati condotti degli studi collaborativi che hanno riguardato i seguenti metodi:

- Determinazione degli steroli negli oli vegetali, eritrodiolo e uvaolo, con lo scopo di sviluppare un nuovo progetto di metodo, valido per tutti i tipi di oli vegetali, revisionando il metodo ISO.
- Estrazione del grasso totale attraverso un sistema a microonde – proposto da Milestone. È stato eseguito lo studio collaborativo su 7 matrici applicando a scelta i metodi ufficiali AOAC, ISO e il metodo proposto da Milestone. L'elaborazione statistica dei dati ha permesso di preparare una bozza del metodo per la sua futura pubblicazione come metodo UNI e intraprendere anche il percorso alla ISO.

Alcuni esperti del GL18 collaborano anche ai seguenti gruppi di lavoro:

- **GL23** Autenticità degli alimenti
- **GL25** Nuove tecniche analitiche sostenibili

Per quanto riguarda **nuove linea guida**, ne è stata elaborata una relativa alle caratteristiche tecniche dei tappi antirabbocco, destinati agli olii alimentari, sottoposta al momento a convalida ministeriale.

Pierangela Rovellini
Coordinatore del GL18

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Enterprise Europe Network (EEN)

Anno 2024
(aggiornato al 31 marzo 2024)

Progetto TRIT20230512014

Smart packaging solutions for food freshness monitoring: new partners are sought for tailored development and industrial scale-up

A highly scientific Italian start up and academic spin off devoted to transfer chemical sensing knowhow into smart packaging solutions for freshness monitoring of perishable foods, developed and patended several lab-tested prototypes. New partners are sought for tailored development and industrial scale-up under Commercial agreement with technical assistance or Research and development cooperation agreement.
Dead-line for EOIs: 11 May 2024

Progetto BRAT20230801027

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

The Austrian SME was founded in Innsbruck in 2019 and specialises in trading hemp derivates for the food, feed and cosmetic industry. In recent years, they also added oilseeds, oils and nuts to their portfolio. In sum, they offer a vast portfolio of oilseeds, nuts, cereals and oils to satisfy their clients demands. The company is currently seeking to expand its network of producers of.oilseeds, cereals, dried and dehydrated fruits. They are also looking for new suppliers of refined oils such as sunflower, rapeseed, soya, palm, grape seed, hemp. The company requires raw materials that comply with EU food and feed regulations. As a trading company, the sole purpose of their purchases is resale to their network of customers across Europe. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 17 Sept 2024

Progetto BOGR20230921006

Highly-beneficial olive oil and olive oil supplements from Greece offered for commercial cooperation

The company's extra virgin olive oil is made from 3 varieties of olive: koroneiki, ladolia and organic (properly preserved in specially-certified tanks). It is specifically offering the following products from its range: - extra virgin olive oil in 500ml, 1l & 5l packaging for retail as well as 1l & 5l for restaurants; - it can also offer 10l sunflower oil; - polyphenolic extra virgin olive oil food supplement in 150ml.
Dead-line for EOIs: 20 Sep 2024

Progetto BOIT20220831002

Italian company producer of high-quality eco-friendly coffee products seeks distributors in Europe, preferably in the hotel, restaurant and catering (HoReCa) sector

Italian producer offers a wide range of high-quality coffee products including coffee beans, ground coffee, 100% compostable pods and capsules – and is looking for retailers and/or distributors in Europe, mainly - but not exclusively - in the hotels, restaurants, and catering (i.e. HoReCa) sector.
Dead-line for EOIs: 19 Sep 2024

Progetto BOGR20230920011

Greek company - producer of Premium Extra Virgin Olive Oil seeks importers, distributors and wholesalers, as well as commercial agents

The company is a Premium Extra Virgin Olive Oil producer in Greece and is involved in the production chain from the beginning of the cultivation of the trees to the end of the packaging. They offer Extra virgin olive oil (EVOO), table olives in brine and olive paste.
They have won various international quality awards and are eager to expand their business internationally. They search for business partners who will help them expand and export to different countries.
Dead-line for EOIs: 01 Oct 2024

Progetto TOES20231110025

Basque SME offers technology combining photonics and IoT sensors to monitor quality in food production industrial processes

A fast-growing innovative Basque startup founded to achieve more efficient and sustainable production by applying photonic solutions to industrial processes is looking for partners interested in in either adopting the technology for their quality monitoring processes or integrating it into their portfolio as an added-value feature (joint venture). A commercial agreement with technical assistance is envisaged.
Dead-line for EOIs: 09 Nov 2024

Progetto BRSE20231201006

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

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

Progetto TOES20231204004

Accelerated shelf-life studies in food products

A Spanish technological center offers accelerated shelf-life studies in food products. This method allows to predict the behaviour of the products and anticipate their evolution under the usual storage and distribution conditions. To achieve this, an es-

timate is made using predictive models in which the parameters that most influence its deterioration are modified, such as temperature, humidity, and light, among others.
They seek commercial agreement and/or R&D agreement.
Dead-line for EOIs: 03 Dec 2024

Progetto BOCZ20221208018
Czech innovative company and producer of vertical hydroponic growing systems for growing herbs, fruits, vegetables and microgreens is looking for distributos for international cooperation
The company, engaged in research, development and production of indoor vertical systems, technologies and elements for growing plants under artificial lighting, is looking for trade intermediaries (distributors/agents/representatives) or franchise partners to distribute, represent or offer its products on external markets.
Dead-line for EOIs: 07 Dec 2024

Progetto BOES20231229002
Spanish company specialized in food products packaging is offering outsourcing services.
A Spanish raw materials supplier specializing in packaging solutions for businesses is actively seeking commercial brands interested in outsourcing their packaging services
The company is able to provide a diverse range of both organic and conventional raw materials, including superfoods, proteins, seeds, grains, sugars, sweeteners, dried fruits, flours, butters, cups, rice, legumes, vegetables, and puffed cereals sourced from various regions worldwide.
Additionally, the company specializes in transforming bulk quantities of both organic and non-organic products into private label finished.
Dead-line for EOIs: 28 Dec 2024

Progetto TOTR20240105014
Turkish Agricultural Research Institute looking for partnerships for applying under HORIZON-CL6-2024-FARM2FORK-01-2: New healthy and sustainable food products and processes project call
Turkish Agricultural Research Institute has been expertised mainly on the breeding of field crops and horticulture as well as seed production and conservation of these genetic resources, protection of these plants from stressors in soil to provide food safety and nutrition.
Dead-line for EOIs: 04 Jan 2025

Progetto RDRCO20231221024
Colombian foodtech is in search of partners to collaborate in the creation of research, development, and innovation (R&D&I) projects, as well as to identify financing opportunities in the agri-food sector.
The foodtech is an entity with over 21 years of experience in the agro-industrial sector. Seeking to contribute through a comprehensive portfolio of services that encompass research in globally relevant thematic areas, laboratory testing services, knowledge transfer activities, design and development of food products, specialized consulting, as well as the formulation and execution of research, technological development, and innovation (R&D&I) projects.
Dead-line for EOIs: 12 Jan 2025

Progetto BRAT20240305003
Producers of bottles and lids for pumpkin seed oil needed
The firm is a trading company mainly working in e-commerce and local retailing business (B2C) and is located in Austria. The searching company is looking for a producer of glass bottles and lids for pumpkin seed oil. The company is looking for a production company in Europe. The producing company should meet the following requirements: high quality producer, prototype before first order, fast implementation and delivery, long time partnership, on-site contact person.
Dead-line for EOIs: 05 Mar 2025

Progetto BOES20240311022
Spanish organic extra virgin olive oil producer is looking for distributors in EU and EEN countries
Spanish company specialized in the production of organic extra virgin olive and olive oil related gourmet products seeks distributors for their products in EU and EEN countries. The extra virgin olive oil produced is of single-varietal and native regional varieties of the Mediterranean coast and comes in different sizes and packages.
The aim for the distributors is to on-sell to specialized gourmet/organic retail shops and Spanish products retailers.
Dead-line for EOIs: 11 Mar 2025

Per ricevere ulteriori informazioni e per entrare in contatto con i soggetti titolari degli annunci si prega di inviare una mail al seguente indirizzo: **federico.agostini@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'internazionalizzazione 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 da **Simpler** (Support Services to IMProve innovation and competitiveness of businesses in Lombardia and Emilia-Romagna), di cui Innovhub SSI è partner.

Come ti può aiutare la rete EEN?

Far crescere l'azienda e sostenere l'internazionalizzazione:
- Informazioni sulla legislazione EU
- Informazioni e assistenza sul Regolamento 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

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:
Federico Agostini
federico.agostini@mi.camcom.it

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

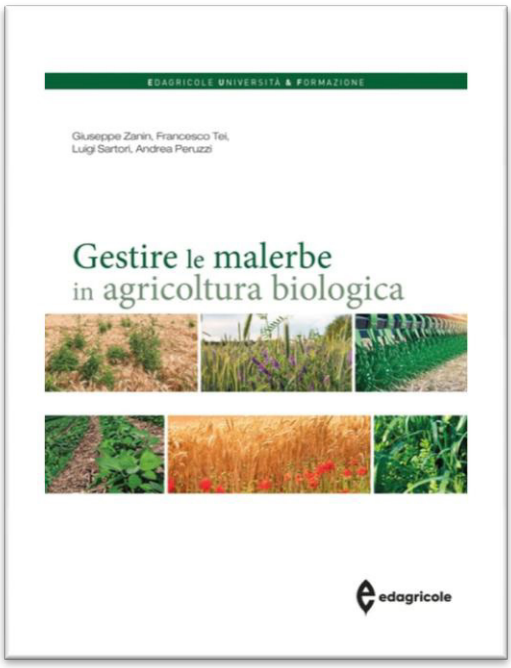
innovazione e ricerca

enterprise europe network

RECENSIONI DI LIBRI

GESTIRE LE MALERBE

AUTORI:
GIUSEPPE ZANIN
FRANCESCO TEI
LUIGI SARTORI
ANDREA PERUZZI



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www.edagricole.it

Il volume illustra le strategie agronomiche, meccaniche e fisiche di gestione delle malerbe in un'ottica di agricoltura biologica ma pienamente utilizzabili anche in agricoltura convenzionale.
Partendo all'inquadramento bio-ecologico, i diversi capitoli espongono le azioni proattive che rendono il terreno meno adatto alle malerbe, le misure preventive per il contrasto alle infestanti, prima e durante la semina, e gli interventi diretti (meccanici e fisici) il cui timing e frequenza devono essere decisi in base all'infestazione, allo sviluppo della coltura e ai corrispondenti parametri competitivi temporali.
La descrizione delle diverse attrezzature meccaniche per gli interventi diretti valorizza in particolare le condizioni che ottimizzano l'abbinamento attrezzatura-utensili-infestazione-coltura-terreno.

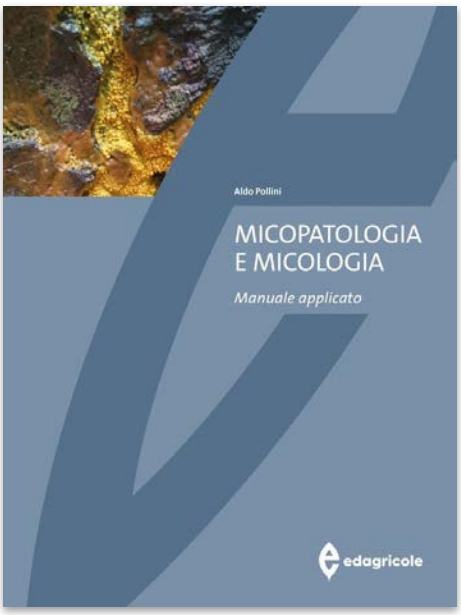
INDICE:
Biologia ed ecologia delle malerbe - Strategie proattive - Strategie preventive - Interventi di controllo diretti dopo l'emergenza della coltura - Biologia delle malerbe perenni: conoscerle per gestirle - Gestione delle malerbe perenni - Conclusioni - Appendici.

GLI AUTORI:
Giuseppe Zanin, Professore Emerito dell'Università degli Studi di Padova. Si è occupato da sempre di gestione delle malerbe, della loro biologia, della risposta agli interventi agronomici e in particolare della competizione sviluppando sistemi di supporto alle decisioni (SSD) in grado di prevedere gli esiti competitivi di infestazioni miste. È stato per lunghi anni responsabile del "Centro di Studio sulla Biologia ed il controllo delle piante infestanti" del CNR. Autore di numerose pubblicazioni e di libri e capitoli di libri, è coautore del testo didattico Malerbologia. Un suo lavoro è stato premiato dalla Società Americana di Weed Science.
Francesco Tei, Professore Ordinario di Agronomia e Coltivazioni erbacee dell'Università degli Studi di Perugia. Autore di più di 250 pubblicazioni scientifiche in campo agronomico. Nel settore della malerbologia si è occupato dei metodi di gestione integrata delle malerbe sia nelle colture orticole sia in quelle estensive. Dal 1998 è membro dell'Editorial Board della rivista scientifica Weed Research. Dal 1996 al 2010 è stato coordinatore del gruppo di lavoro "Weed Management Systems in Vegetables" della European Weed Research Society (EWRS).
Luigi Sartori, Professore Ordinario di Meccanizzazione agricola presso il Dipartimento Territorio e Sistemi Agroforestali dell'Università degli Studi di Padova e attualmente Presidente della Sezione "Meccanizzazione e tecnologie per le produzioni agricole" dell'Associazione Italiana di Ingegneria Agraria (AIIA). La sua attività di ricerca copre tematiche inerenti le tecnologie meccaniche e digitali utilizzate nell'agricoltura conservativa e nell'agricoltura di precisione. Ha partecipato ed è stato responsabile scientifico di progetti europei, nazionali e regionali su argomenti di sua pertinenza ed è autore di numerose pubblicazioni scientifiche e di articoli e monografie a carattere divulgativo.
Andrea Peruzzi, Professore Ordinario di Meccanica Agraria presso il Dipartimento di Scienze Agrarie, Alimentari e Agroambientali (DiSAAA-a) dell'Università di Pisa, svolge da poco meno di 40 anni attività di ricerca e attività didattica sulla meccanizzazione in agricoltura biologica con particolare riguardo alla gestione fisica della flora spontanea e, in tempi più recenti alla definizione di stra-

tegie agronomiche e alla realizzazione di macchine innovative per l'agricoltura biologica e conservativa. È autore e/o coautore di circa 600 pubblicazioni scientifiche, libri e capitoli di libri ed è titolare del brevetto di una macchina per il controllo meccanico delle malerbe.

MICOPATOLOGIA E MICOLOGIA MANUALE APPLICATO

AUTORE: ALDO POLLINI



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Al regno dei funghi appartengono organismi viventi come parassiti, saprofiti o associati in simbiosi con altri organismi, come accade per i licheni e per le micorrize e le piante superiori.
Il volume prende in esame i più significativi agenti micotici che risultano patogeni nei confronti delle piante di importanza agraria, forestale ed ornamentale, nonché una modestissima parte dei funghi con cappello o di altra forma (commestibili e non) che vivono sulle piante, che hanno rapporti micorrizici con le loro radici (p. es. i tartufi), che crescono nei prati o che possono essere oggetto di coltivazioni su substrati artificiali.
Delle diverse specie prese in esame sono indicate le caratteristiche morfologiche, le sintomatologie prodotte dagli agenti, gli aspetti bio-epidemiologici e sono sinteticamente esposti i principali provvedimenti di prevenzione e di difesa.

INDICE:
Generalità - REGNO PROTISTA (Classe Myxomycetes - Classe Phytomyxea Engler & Prantl em. Cavalier-Smith) - REGNO CHROMISTA (Classe Peronosporea) - REGNO FUNGI (Classe Chytridiomycetes T. Cavalier-Smith (1998) - Classe Zygomycetes - Classe Taphrinomycetes O.E. Eriksson & Winka (1997) - Classe Saccharomycetes O.E. Eriksson & Winka (1997) - Classe Eurotiomycetes O.E. Eriksson & Winka (1997) - Classe Dothideomycetes O.E. Eriksson & Winka (1997) - Classe Leotiomycetes O.E. Eriksson & Winka (1997) - Classe Pezizomycetes O.E. Eriksson & Winka (1997) - Classe Sordariomycetes O.E. Eriksson & Winka (1997) - Classe Coniocybomycetes M. Prieto & Wedin (2013) - Classe Agaricomycetes Doweld (2001) - Classe Pucciniomycetes R. Bauer, Begerow, J.P. Sampaio, M. Weiss & Oberwinkler (2006) - Classe Ustilaginomycetes R. Bauer, Oberwinker & Vánky (1997) - Classe Microbotryomycetes R. Bauer, Begerow, J.P. Sampai, M. Weiss & Oberwinkler (2006)) - FUNGHI ANAMORFICI O ANAMORPHIC FUNGI (Funghi anamorfici o Anamorphic fungi).

L'AUTORE:
Aldo Pollini ha svolto attività di ricerca e sperimentazione per quasi tre decenni presso l'Osservatorio Malattie Piante di Bologna (ora Servizio Fitosanitario Regionale dell'Emilia-Romagna). Dal 1999 esercita la libera professione nel settore della consulenza fitopatologica e dell'estimo legale.

CONGRESSI

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

22 - 24 April | Turin, Italy

The aims of the IEEE International Workshop on Measurements and Applications in Veterinary and Animal Sciences (MeAVeAS) are to comprehensively explore the various aspects of interactions among the realms of instrumentation and measurement, bio-engineering, material science, chemical and biological measurements, and the field of veterinary medicine and animal and food science.
Metrology applied to medical and biological disciplines is an ancient and multidisciplinary science of which there is evidence already from ancient Egypt, when a measure called "cubit", which included the distance between the hand and elbow of individuals, was used to classify morphologically

the people, referring to them the reference measure represented by the "cubit" of the pharaoh.
Metrology studies measurements of magnitude in order to have official references (units of measurement) to refer to, to identify measurement systems, means and methods for following measurements of various kinds.
The Workshop provides a platform for researchers, veterinarians, and animal scientists to engage in the exchange of ideas and information, establish connections and collaborations, and stay updated on innovations in the fields of veterinary medicine and animal science.
MeAVeAS is dedicated to bringing together professionals working on the development of instrumentation and measurement methods, not only for veterinary medicine but also for ecology and for food and animal science. Our focus extends to various aspects, including cutting-edge technologies for monitoring the health of animals, metrology-assisted production in the food industry, sensors and associated signal conditioning techniques tailored for veterinary medicine and animal science, as well as calibration methods for electronic test and measurement applications in these fields.
A Call for papers and a Call for Special sessions are foreseen. Special Sessions have the primary objective of establishing mini-workshops dedicated to specific topics. Within these sessions, researchers with shared interests can collectively expand their knowledge, engage in fruitful discussions, exchange ideas, and foster collaborative endeavors.
Discover more on the web site:
https://www.meaveas.org/home

4th Oils & Fats Exhibition And Conference Bangladesh - 2024

25 - 27 April 2024 | Dhaka, Bangladesh

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

2024 AOCS Annual Meeting & Expo

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

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

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

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

Short courses:

- *Edible Fats and Oils Refining | 27-28 April*
- *Lipid Oxidation in Foods | 28 April*

For more information visit:
https://annualmeeting.aocs.org/

Globoil International 2024

6 - 8 May 2024|Dubai

Navigating the Future of Edible Oils: Innovation, Sustainability, and Market Dynamics.
Globoil International is the leading global forum for the edible oil and agri-trade industry, set to unfold in the dynamic city of Dubai. An unparalleled gathering of industry leaders, innovators, and experts from around the world. Dive into the latest trends, technologies, and strategies shaping the future of

edible oils, agriculture, and global trade. Engage in valuable networking, explore business opportunities, and gain insights from the forefront of industry innovation.
See more information and the program at:
https://www.globoilinternational.com/

14th ICIS World Surfactants Conference

9 - 10 May 2024 | Jersey City, NJ

Bringing together industry professionals from all corners of the surfactants value chain for an informative and valuable two days, the ICIS World Surfactants Conference is now in its fourteenth year.With the demand for surfactants continuing to grow, it is more important than ever for industry professionals to stay informed about developments in the field. This year's conference will once again provide a trusted and valuable platform for sharing knowledge, connecting peers, and helping leaders stay up to date on the latest trends and innovations in the surfactants industry.
The conference is aimed at those looking to gain critical insight into the surfactants industry and the pain points stakeholders are having. With the addition of speakers from across the value chain industry groups:

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

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

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

Netwoking. We understand that the surfactants industry is complex and involves a range of professionals from different parts of the value chain. That's why we focus on bringing together a diverse community across the industry, to ensure that our conference provides a valuable and meaningful experience for all in attendance.
By gathering a diverse group of experts and providing ample opportunities for connection and collaboration, we strive to create a dynamic and productive environment that supports the success of everyone who attends.
Collaborate. Foster collaboration with industrial peers and work towards achieving sustainability targets and overcoming market challenges, through new insight gained at the conference and the networking opportunities available to those in attendance.
More info: https://events.icis.com/website/8544/

5th MS-Wine Day

22 - 24 May 2024 | Asti, Italy

Thanks to its application versatility and its innovative potential, mass spectrometry offers advanced tools for the compositional characterization of oenological matrices and the traceability of wine products: it allows the accurate evaluation of quality with the analysis of aromatic volatile compounds, polyphenols and antioxidant compounds present in wines and grapes, proves to be fundamental for the identification of contaminants, such as pesticides and mycotoxins, and represents an indispensable tool for the monitoring and optimization of production techniques, the study of vine diseases and the monitoring of aging and fermentation processes.
The Mass Spectrometry Division of the Italian Chemical Society has established the series of "MS-Wine Day" conferences every two years with the aim of creating a stable scientific reference point of synergy between Public Bodies, Private Bodies and Companies operating in the analytical sector -oenological.
These events have the aim to connect experts, researchers and professionals, promoting discussions focused on the potential and benefits of mass spectrometry in the wine industry.
During the event the applications of mass spectrometry will be explored in the fermented beverage sector in general, thus broadening the horizon of discussion and research.
Main topics

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

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

Future of Surfactants Summit

22 – 23 May 2024 | Seville, Spain

The aim is to find solutions to pressing issues such as the potential restrictions on 1,4-Dioxane, alternative feedstocks, becoming more biodegradable and becoming a circular economy. Participants also have the chance to discuss new technologies and solutions that are constantly evolving in the industry and increasing efficiency in chemical manufacturing. The event will give important updates and revisions from the past year while foreseeing what is next to come for the industry and

encouraging the use of more biodegradable and bio-based surfactants without the overuse of greenwashing marketing techniques often found in these type of industries. The event will bring together key industry stakeholders from feedstocks suppliers, raw material producers and suppliers, chemical distributors, surfactant manufacturers, consumer product manufacturers, private labels, retailers, industrial cleaning product manufacturers, lab equipment suppliers, technology suppliers.
Key Topics:

- Designing A Circular Economy Model for the Surfactants Industry
- Updates within the European and Global Market
- Moving Towards Oleochemical Feedstocks
- Gaining a Better Understanding of Consumer Needs
- Adopting More Sustainable Practices within the Industry
- Emerging Trends and future Directions in Surfactant Research
- Advances of AI Technology and its Involvement in Surfactants

More information on:
https://www.wplgroup.com/aci/event/surfactants-summit/

12th Workshop on Fats and Oils as Renewable Feedstock for the Chemical Industry

3 – 5 June | Dusseldorf, Germany

The environmentally sound and sustainable use of natural resources is an important worldwide challenge. At present, fats and oils are the most widely used renewable raw materials in the chemical industry, since they offer widespread possibilities for different applications. The Workshop has evolved to a major global oleochemical meeting, attracting participants from all over the world. Important contributions have been and will be documented in special issues of European Journal of Lipid Science and Technology. The Workshop will discuss new developments as well as future perspectives of the chemical usage of fats and oils including terpenes. Presentations from industry representatives will show the progress in introducing renewable materials to the market.
The workshop will cover many topics, such as:

- Chemistry of fats and oils & other renewable resources
- Synthesis of fine chemicals
- Monomer / Polymer synthesis
- Catalysis
- Sustainability
- Introducing renewable materials to the market

See more on: http://www.abiosus.org/workshop-2024.html

The 32nd Nordic Lipidforum Symposium

9–12 June 2024 | Turku, Finland

The main heading for the symposium is: *Lipids for the future - contribution of lipids in nutrition, food products and pharmaceuticals.*
The symposium will cover a variety of topics relevant for new science of lipids including:
• health and nutritional aspects of lipids
• pharmaceutical aspects of lipids
• lipids in personalised nutrition
• lipids in skin care
• lipids in aquaculture and pet nutrition
• novel lipids and fatty acids
• lipid oxidation and antioxidants
• novel processing and technology
• advanced lipid analysis and food safety
The symposium is an important meeting place for scientists, engineers, sales and marketing personnel and all others working within academia, health care and industry. The event is a unique possibility to exchange science and technology issues related to lipids, fats, and oils.
More information and Program at:
https://eurofedlipid.org/the-32nd-nordic-lipidforum-symposium/

IGC Grains Conference 2024

11 - 12 June 2024 | London, UK

The broad theme of the IGC Grains Conference 2024 will be centred on the role of global trade in the context of volatile markets and food security. The event will provide the perfect platform for policymakers and industry leaders to engage in meaningful discussions surrounding key challenges in relation to climate change, protectionism, technological advances, as well as trade finance. A special geographical focus will assess emerging trends and business opportunities in the Eurasian grains and oilseeds sectors.
The International Grains Conference is a truly global platform for dialogue between policymakers and operators across the entire value chain. The event is held over two full days, the first of which is typically devoted to discussions surrounding the challenges, risks and opportunities in global trade. Day two of the event comprises a number of commodity-specific workshops, covering topical issues affecting markets for grains, rice, oilseeds, pulses and related sectors.
More information and program at the event page:
https://www.igc.int/en/conference/confhome.aspx

Oleofuels 2024

12 - 13 June 2024 | Milan, Italy

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

for networking and knowledge exchange.
In this two-day conference, industry leaders, manufactures, researchers, policymakers, and market experts will come together to discuss the latest advancements, challenges, and innovations in the field of oleofuels. The event will feature informative presentations, interactive panel discussions, and engaging networking sessions.
The conference offers a valuable opportunity to gain insights into the current market trends, learn about the most recent technological developments, and explore potential collaborations within the industry. It will provide participants with an in-depth understanding of the global oleofuels market, its future prospects, and the regulatory framework shaping the industry.
Key topics:

- Oleofuel Global Outlook, Trends & Divers
- Exploring Growing Mandates and Legislations in Europe: What Impact Will This Have on the European Market?
- Overcoming Feedstock Challenges: Sourcing & Supply
- Moving Towards a More Cohesive Future
- Supply Chain Management
- Case Studies: FAME vs HVO
- Optimising New Technologies
- Decarbonising the Transport Sector: Road
- Decarbonising the Transport Sector: Aviation
- Decarbonising the Transport Sector: Maritime

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

EFPRA Congress 2024

12 – 15 June | Amsterdam

This will be the 22nd EFPRA Congress which has become one of the main events for the European and global rendering industry to network and learn about the latest developments.
Info: https://efpra2024amsterdam.com/home.php

Vegoils&Meals Trade

June 14 in Seville, Spain

The international conference will be dedicated to the EU oilseeds and by-products market with a focus on premium vegetable oils.
Key subjects:

- Prospects for oilseed production in the world, in particular in the EU and the Black Sea region in 2024/25 MY
- Global trends of the global vegetable oils market: results of 2023/24 MY and forecasts for 2024/25 MY
- Logistics as one of the main challenges for the agrarian market, which is gaining global scale
- European vegetable oil market: main players, production, consumption
- Sunflower oil market: trends and features of 2023/24 MY, competition from soybean oil
- European market of premium vegetable oils: features of the olive and high oleic oil segment, development vector, import of raw materials
- Latin America as a key supplier of oilseeds
- The meal market: consumption patterns, increase in imports
- Biofuel market: key trends, key players, segment prospects
- Quality requirements for key oilseed products supplied to the EU market: expert opinion
- Green Deal in agriculture: trends and prospects
- European integration of Ukraine as a key player in the global agricultural market

Target audience: oilseed processors; traders; agricultural holdings; industry organisations; leading domestic and international agricultural experts; equipment manufacturers; major exporters and consumers of oilseeds and processed products; representatives of scientific organisations, etc.
For more information visit:
https://www.apk-inform.com/en/conferences/Veg-Oils-Meals-Trade-2024/about

5th International Symposium on Lipid Oxidation and Antioxidants

08 - 10 July 2024 | Bologna, Italy

Euro Fed Lipid organise the upcoming 5th ISLOA, which will take place in the fascinating city of Bologna.
Lipid oxidation is a critical area of research with far-reaching implications in various fields, including food science, nutrition, pharmaceuticals, and health. The role of antioxidants in mitigating lipid oxidation is equally significant.
The congress aims to bring together experts, scholars, researchers, and professionals from around the world to exchange knowledge, share their latest findings, and foster collaboration in this important domain.
The meeting will cover, among others, the following topics:
1. Innovative methods for lipid oxidation and antioxidant evaluation (e.g. fluorescent probes, mass spectrometry, lipidomics, NMR);
2. Elucidation of lipid oxidation and antioxidant mechanisms (e.g. free radical chemistry, multiphasic systems);
3. Lipid oxidation and antioxidant effects in real systems (e.g. bulk oil, emulsions, oleogels, food, recycled oils);
4. Protein oxidation in lipid-containing model systems and food: oxidative interactions and antioxidant effects;
5. Nutritional and physiological effects of oxidized lipids and antioxidants.
For more information and update visit:
https://veranstaltungen.gdch.de/microsite/index.cfm?l=11650&modus=

PALMEX Thailand 2024

1-2 August 2024 | Suratthani, Thailand

This 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, 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.
See more: https://www.palmoil-conference.com/

21st International Sunflower Conference

20-24 August 2024 | Bayannur, China

The 21st ISC will be a 5-day conference consisting of plenary talks, scientific and industry workshops, and poster sessions.Topics of interest include Genetics and Breeding, Genomics and Biotechnology, Biotic Stress and Abiotic Stress, Crop Production and other fields of the entire sunflower industry chain. We believe that this event will strengthen the global scientific sunflower community, as well as demonstrate the dynamic changes and significant accomplishments of the confection sunflower industry in China.
The aim of the event is to strengthen the global scientific sunflower community, as well as demonstrate the dynamic changes and significant accomplishments of the confection sunflower industry in China.
See the event page: http://www.esanrui.com/isc

PALMEX Malaysia 2024

28 – 29 August | Kuala Lumpur

The PALMEX Malaysia 2024 is the specialized Palm Oil event in Asia that brings together an international congregation of both upstream and downstream palm oil companies and also gathers its supporting industries to showcase the latest developments in the palm oil industry.
Currently ranked as the world's 2nd largest palm oil producer, this event will be supported by the local Malaysian Palm Oil Community ensuring major players in the industry would be represented at this event.
The event is addressed to Palm Oil Estate Owners, Producers, Policy & Decision Makers, Scientists, Engineers & Technologists, Importers, Exporters & Traders, Processing Planters, Refiners, Regulatory Bodies, Agents/distributors/traders, Agriculturist, Biotechnologist, Builders & Construction Workers, Buyers/Purchasers, Chemists, Design & Consultancy Experts, IOT Associatied Parties, Engineers & Contractors, Environmentalist, Government Agencies/Academia, Manufacturers Representative, Material Testing & Inspector.
Event updates on: https://asiapalmoil.com/

Sustainable Aviation Futures North America Congress

2 - 4 October 2024 | Houston, USA

With the USA positioned as a global leader for announced SAF projects, and exciting offtake agreements being announced with increasing frequency, the path to meeting the SAF Grand Challenge's ambitious targets appears promising. Over the three days, our expert speakers will:
Dissect regulatory frameworks across North America and how national Book and Claim standardization may be implemented
Dive into the challenges of financing the scaling of SAF and what role sustainable finance should play
Examine the latest permitted feedstocks and share insights on lifecycle analysis
Discuss exciting developments in hydrogen, hybrid, and electric aircraft
Hear from some of the most influential experts working within the aviation and energy industry, including airlines, fuel producers, policymakers, hydrogen and energy developers and OEMs. Sustainable Aviation Futures North America Congress will feature 40+ hours of industry-leading content in panels, keynotes, and interactive workshops, covering the A-Z of SAF, feedstocks, policy, sustainable, aerospace technologies and eFuels and hydrogen.
More information: https://www.safcongressna.com/

8th MS Food Day

16 - 18 October 2024 | Brindisi, Italy

High quality, healthy and safe food, with good nutritional and sensory characteristics, are necessary for a satisfactory quality of life.
For this reason, authentication and traceability of foodstuffs, characterization of food components, quality control, identification and quantification of additives, allergens, chemical and microbiological contaminants, preservation of food components during storage and processing, packaging technology, determination of nutritional and sensory properties, are essential for citizens and consumers with widespread consequences on health, and on agriculture, industry and economy.
In all these aspects mass spectrometry plays a key role. The impressive evolution of its applications, methods, instrumentation and technology yielded highly sensitive, specific, fast, robust and validated methods that are fundamental tools in food science and technology.
The 8th MS Food DAY is a biannual conference focused on all topics related to the use, methods and applications of mass spectrometry in food.
It represents an excellent occasion for presenting the state-of-the-art of mass spectrometry in food chemistry & technology, along with the latest innovations and novelty in instrumentation and applications.

As already testified by the successful previous editions, this will also create the optimum opportunity to meet the needs and opportunities of academic institutions, research and control institutions, private enterprises and food companies.
The conference will include plenary lectures, oral and poster communications.

Topics include:

Innovations in food science applications of MS

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

Methodological and instrumental developments

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

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

NOVITÀ IN OCL

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www.ocl-journal.org

OCL - Oilseeds and fats, Crops and Lipids is a peer-reviewed full Open-Access scientific journal devoted to fats, lipids and oil- and protein-crops.
Summary of OCL 2023 issues (vol. 30):

NEWS

Contribution of Chevreul to lipid chemistry
Claude Leray
OCL, 30 (2023) 9
DOI: https://doi.org/10.1051/ocl/2023006

From fats to lipids: 80 years of shared knowledge AFECG-SFEL (1943–2023)
Emile Choné
OCL, 30 (2023) E1
DOI: https://doi.org/10.1051/ocl/2023010

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Impact of deficit irrigation on the physiological and agronomic traits of 24 safflower (*Carthamus tinctorius* L.) genotypes grown in Iran
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Changing cropping pattern of oilseed crops and its diversification: The case of Thar Desert, Rajasthan (1985–1986 to 2015–2016)
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Correlation and sequential path analysis of oil yield and related characteristics in camelina under seasonal variations
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Geospatial assessment of potential land suitability for oil palm (*Elaeis guineensis Jacq*) cultivation in the western parts of Ethiopia
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