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Determination of Mineral Oil Aromatic Hydrocarbons (MOAH) in vegetable oils and fats - Reverse Phase High-Performance Liquid Chromatography Fluorimetric (RP-HPLC-FLUO) method

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Mineral oil hydrocarbons (MOH) or mineral oil products are constituted by hydrocarbons containing from 10 to about 50 carbon atoms. Due to their extensive use, mineral oils could come into contact with food. The source of contamination is difficult to be predicted and can occur at any stage of food production. The first successful method was designed for the MOSH fraction detection by the Grob lab [1]. EN-ISO-16995 [7] is today referred to a method of choice for detecting MOSH and MOAH mineral oils in routine analysis. It has some critical points concerning mainly the preparation of the sample for analysis: automated purification procedures may be necessary to avoid or minimise the risk of interferences and the need to dedicate a specific system to this determination, with the consequent cost to be incurred [3]. This work suggests a simple and easily applicable method to carry out a pre-assessment of the MOAH content in vegetable oils and fat samples using an instrumentation commonly present in analytical laboratories (RP-HPLC-FLUO) and a simple sample preparation. The article reports the validation parameters: linearity, limit of quantification, recovery, and repeatability. To test the accuracy of the method, the laboratory has joined several international correlation circuits in the period 2020-2022. The method was applied mainly to vegetable fats and oils refined or crude and may be used also for animal fats.

Keywords: Mineral Oil Aromatics Hydrocarbons, MOAH, RP-HPLC-FLUO, Vegetable oils and fats.

1. INTRODUCTION

1.1 CHEMISTRY, SOURCE OF CONTAMINATION AND ANALYSIS HISTORY

Mineral oil hydrocarbons (MOH) or mineral oil products are constituted by hydrocarbons containing 10 to about 50 carbon atoms and are alkylated more than 98% [2]. It is a fraction obtained from petroleum refining and can be destined for various uses: cosmetic products, release agents (bakery industries), packaging materials (wax paper) or others such as technical products (lubricant oils). MOH comprises complex mixtures, principally of straight and branched open-chain alkanes (paraffins), largely alkylated cycloalkanes (aliphatic or aromatics such as naphthene), collectively classified as mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH).

MOAH, in particular contain at least one aromatic ring. They are considered as total content and/or in different fractions for their different biological effects. Recently, particular attention has been paid to MOAH due to the biological effects toward human health. Compounds such as benzene hydrocarbons derivatives may be of potential health hazard. Among the possible risks there is also that of being carcinogenic. In fact, it has also been proven that MOAH with 3-7 ring cyclic aromatic moiety are a main concern for the genotoxic and carcinogenic potential effect [2;4]. Recent studies have shown that mutagenicity decreases with the increase in the length of the alkyl chain that can be in these compounds. The reason is to be found in the greater or lower possibility of binding to the CYP450 coenzyme responsible for initiating oxidative reactions with the intercalation of activated metabolites in DNA [4]. The associated cellular damage could cause mutations, malformations, tumour and cancer.

Due to their extensive use, mineral oils can come into contact also with food. The source of contamination is difficult to be predicted and can occur at any stage of food production. Among the most common sources there is environmental contamination (e.g., from particulate matter originating from incomplete diesel combustion), and farming practices (phytosanitary and fertilising treatments). A part of contamination could be attributed to the biosynthetic mechanism intrinsic to the plant), to the food transformation process (lubricant and release agents) and to migration from packaging materials (recycled paperboard and mineral oil-based inks). Good harvesting practices are suggested to minimise the risk [5;15].

Historically, the method for the analysis of mineral oil and their components, was a standardised method based on a gravimetrical determination of the dimethyl sulfoxide extract residue [6]. The method suitable for pure mineral oil was not able to detect contamination in trace. Mineral oil products could migrate into food due to the wide application in technical products including food contact materials (FCM). The first successful method was designed for detection of the MOSH fraction by the lab of Grob in [1]; later also the MOAH fraction was investigated with a LC-GC-FID system; using this procedure it was not possible to resolve mineral oils into single components because the system resolved 2 fractions: MOSH and MOAH. This method today refers to a method of choice for detecting mineral oils in routine analysis and is known as EN-ISO-16995 [7]. Recently a new analytical method has been pointed out in ISO TC34 SC 11 Committee Standard to reach lower LOQ (Limit of Quantification) [11]. This new international standard, according to the results obtained, has been proven suitable for MOSH mass concentrations above 3 mg/kg and MOAH mass concentrations above 2 mg/kg. For this, an alternative method for the epoxidation of the MOAH fraction (with performic acid) and partially modified processing steps are improved. GCxGC-TOF-FID technique could be used for confirmation and characterisation of contaminants [8;10;13;14]. The revision of the UNI EN 16995:2017 standard is expected in 2024 (Draft ISO-FprEN 17517)[11].

1.2 LEGISLATION ASPECTS

Currently there is no law in force in the European Union that regulates the presence of MOAH in food products, but in some Member State there are national directives to prevent this type of contamination. In food products only national benchmark levels have been set: they are not safety levels but indicate that need to further investigation, to conduct a correct risk analysis. In 2021 German food industry established benchmark levels [9] in different food categories: for vegetable oils (excluding those of tropical origin) the MOAH level should be lower than LOQ (Limit of Quantification) of 2 mg/kg for each C-fraction (according to JRC guideline) [10]. The JRC technical report points out that analytical method used must be sufficiently sensible to ensure that food is not contaminated with potentially carcinogenic MOAH. The use of the most advanced methods of laboratory analysis is suggested; the on-line LC-GC-FID method and GC x GC-TOF-FID are recognised as reference methods, but any other detention technique is acceptable only if it provides equivalent results to the LC-GC-FID [7]. During 2023, the JRC updated the Technical Report introducing substantial changes: the quantification of MOH will no longer be carried out for the subfractions of MOSH and MOAH, it has also modified the performance requirements [13]. In 2022 the Member States of standing committee on plants, animals, food, and feed of European Commission agreed to withdraw or if necessary to recall products from the market when total concentration of MOAH is above the maximum LOQ of 2 mg/kg for fats and oils [19]. A recent update of the risk assessment of mineral oil hydrocarbons (MOH) in food has been published by EFSA in 2023. It suggests to pay attention for MOAH with 3-or more aromatic rings due to their human toxicity [20].

1.3 OFFICIAL METHOD EN ISO 16995

The official reference method is: EN ISO 16995 [7] Foodstuffs. “Vegetable oils and foodstuff on basis of vegetable oils. Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis”. It is an international standard method for the determination of saturated and aromatic hydrocarbons from 10 to 50 carbonic atoms in vegetable fats and oil and foodstuffs, interlaboratory validated with online LC-GC-FID. According to the results of interlaboratory studies, the method was deemed suitable for MOSH and MOAH in vegetable oils for a concentration above 10 mg/kg [7].

Although the LC-GC-FID method is nowadays used in several laboratories it has some critical points concerning mainly the preparation of the sample for analysis: automated purification procedures may be necessary to avoid or minimise the risk of interferences and the need to dedicate a specific system to this determination, with the consequent cost to be incurred.

2. AIM OF THE WORK

The purpose of this work is to suggest a simple and easily applicable method to carry out a pre-assessment of the MOAH content in vegetable oil and fat samples using instrumentation commonly present in analytical laboratories (RP-HPLC-FLUO) and a simple sample preparation. This work also reports the validation results [17;18].

Vegetable oils and fats are dissolved in an appropriate volume of isopropyl alcohol or tetrahydrofuran, filtered and analysed with RP-HPLC-FLUO system. The proposed method is highly specific.

Fluorescence is the light emission by a molecule that has absorbed light or magnetic radiation.

This technique has already been used successfully in the analysis of polycyclic aromatic compounds; due to its high sensitivity, quickness, selectivity, and relative low cost we think it could also be applied to the MOAH analysis. This technique is also very sensitive due to the direct measurement of the emitted light intensity, has low background noise and is slightly affected by interferences species [12;16].

Because MOAH compounds contain at least one aromatic ring they are well suited for fluorescence technique because they have a molecular resonance spectrum due to their aromatic and polyaromatic structure. The analysis is carried out on two acquisition channels set at 254 nm in excitation, 280 nm in emission for the determination of mono-aromatic compounds and 254 nm in excitation, 430 nm in emission for the determination of polyaromatic compounds. The amount of MOAH is calculated with the use of two external standards (one for each specific acquisition channel).

3. EXPERIMENTAL

3.1 EQUIPMENT AND SUPPLIES

Standard Marker solutions for qualitative analysis

Naphthalene (Marker C10) $C_{10}H_8$ CAS Number 91-20-3 was purchased from Sigma-Aldrich (Merck Life Science srl Milano - Italy).

The stock standard solution was prepared at 0,5 mg/ml weighting accurately, to the nearest 0,1 mg, about 5 mg of naphthalene in a graduated 10 ml class A flask and filling to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v. The solution was diluted with acetonitrile/isopropyl alcohol solution 1/4 v/v to obtain a final concentration of 0,5 ug/ml.

Pyrene (Marker C16) $C_{16}H_{10}$ CAS Number 129-00-0 was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy).

The stock standard solution was prepared weighting accurately, to the nearest 0,1 mg, about 4 mg of pyrene in a graduated 10 ml class A flask and filling to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v. This solution was diluted up to a final concentration of 6 ng/ml.

Coronene (Marker C24) $C_{24}H_{12}$ CAS Number 191-07-1 was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy).

A coronene stock standard solution (concentration of 10 ng/µl) was diluted until a concentration of 2 ng/ml with acetonitrile/isopropyl alcohol solution 1/4 v/v.

Standard calibration solutions for quantitative analysis

4-Ethyl-Toluene standard (CAS 99 620-14-4) was purchased from Sigma-Aldrich (Merck Life Science srl Milano- Italy). This standard was used for the determination of mono-arylated-aromatic compounds at 254 nm channel in excitation and at 280 nm in emission (Channel 1).

A stock standard solution was prepared weighing, to the nearest 0,1 mg, about 10 mg of 4-Ethyl-Toluene in a graduated 10 ml class A flask and filled to volume with acetonitrile/isopropyl alcohol solution 1/4 v/v.

The standard working solutions for calibration curve were prepared in acetonitrile/isopropyl alcohol solution 1/4 v/v at different concentrations of 4-Ethyl-Toluene in the range of 0,1-1,0 µg/ml. Two injections for each level were performed.

The Chiron AS Stock Standard solution is a mixture of 20 compounds (C10-C15) dissolved in isooctane at a concentration of 100-500 µg /ml each, was purchased from Chiron AS (Stiklestadvn. 1 N-7041 Trondheim Norway). List of Chiron compounds solution is: 1-Methylnaphtalene (CAS 90-12-0), 2-Methylnaphtalene (CAS 91-57-6), 1,3-Dimethylnaphtalene (CAS 575-41-7), 1,4-Dimethylnaphtalene (CAS 571-58-4), 1,5-Dimethylnaphtalene (CAS 571-61-9), 1,6-Dimethylnaphtalene (CAS 575-43-9), 1,7-Dimethylnaphtalene (CAS 575-37-1), 2,6-Dimethylnaphtalene (CAS 581-42-0), 2,7-Dimethylnaphtalene (CAS 582-16-1), Biphenyl (CAS 92-52-4), Phenanthrene (CAS 85-01-8), 1-Methylphenantrene (CAS 832-69-9), 2-Methylphenantrene (CAS 2531-84-2), 3-Methylphenantrene (CAS 832-71-3), 9-Methylphenantrene (CAS 883-20-5), Dibenzotiophene (CAS 132-65-0), 1-Methyldibenzotiophene (CAS 31317-07-4), 2-Methyldibenzotiophene (CAS 20928-02-3), 3-Methyldibenzotiophene (CAS 16587-52-3), 4-Methyldibenzotiophene (CAS 7372-88-5). The total concentration of all compounds of Chiron AS Stock Standard solution was about 7000 µg/ml. The standard working solutions for calibration curve were prepared in acetonitrile/isopropyl alcohol solution 1/4 v/v at the different concentrations in the range 0,07-7 µg /ml as sum of all compounds. Two injections for each level were performed.

All the solvents used were HPLC grade.

To avoid contamination due to glassware washing residues it is recommended, prior use, to perform at least two rinses with acetone and n-hexane for HPLC and drying in an oven at 200°C for two hours.

4. SAMPLE PREPARATION

0,5 g of well-homogenized vegetable oil or fat was weighted in a 5 ml volumetric class A flask. The sample was dissolved into a volume of isopropyl alcohol and the sample solution was mixed with vortex for 30 seconds. When necessary, to facilitate dissolution, an ultrasonic bath was used for about 5 minutes at room temperature. An aliquot solution (1 ml) for HPLC analysis was filtered through a 13 mm Nylon 0,45 µm membrane syringe filter directly into a 2 ml HPLC vial. Three independent replicates were conducted for all samples and the result was the average of all the determinations.

For samples with a MOAH concentration level over 5 mg/kg and additional dilution is need to obtain a fluorimetric response less than 10% for channel 1, and 20% for channel 2 (of the full scan).

For fat solid samples, after the addition of solvent, it was necessary to heat the solution for a complete dissolution. The sample was left in the fridge at + 4°C for 5 hours to bring down triacylglycerides. Then, the supernatant was filtered first and then injected.

5. HPLC ANALYSIS

The HPLC system used was a HPLC quaternary pump with a degassing system (Shimadzu LC-30AD pump) equipped, with a C18 Reverse Phase column. The following column was selected for the determination: Repro-Sil 80 ODS-2 (250 mm x 4,0 mm, 3 µm - dr. Maisch GmbH). The analysis was carried out at 40°C column compartment and autosampler. The Fluorescence detector (Shimadzu RF-20A XS) was set up for the simultaneous acquisition on two channels: at 254 nm in excitation, 280 nm in emission for channel 1 and 254 nm in excitation, 430 nm in emission for channel 2. An integration system was needed. Photodiode Array Detector (PDA-Shimadzu SPD-M20A) for spectra recording (from 200 nm to 600 nm) could be used coupled with fluorescence detection when the analyte amount allows its detection also in PDA (spectra qualitative analysis).

5.1 ANALYSIS CONDITION

The elution was achieved with a linear gradient for 50 minutes starting from 60% of (A) HPLC grade water and 40% (B) HPLC grade Acetonitrile to 2% of (A) and 98% of (B). This condition was then maintained for 40 minutes. The flow rate was 0.4 ml/min. The total acquisition time was 90 minutes.

In the HPLC-FLUO system 20 µl of each sample solutions were injected.

The first sample injected as part of a series of analysis must always be a “blank” constituted by sample dissolution solvent (isopropyl alcohol or tetrahydrofuran) filtered through a 13 mm Nylon 0,45 µm membrane syringe filter. Pay attention to interfering signals present in the chromatographic run at the same retention time of calibration standards in the C-range considered (C10-C50). The blank content must be considered to be subtracted to the sample content.

5.2 CALIBRATION SOLUTIONS

Starting from calibration solutions, two calibration curves were obtained: one for 4-Ethyl-Toluene and one for Chiron AS Stock Standard Solution and the relative linear correlation equation forcing through the axis origin was calculated (Y = peak area, X = concentration in ng injected, Y = m x X, the respective slopes corresponded to unitary response for 1 ng of 4-Ethyl-Toluene and 1 ng Chiron AS Solution).

5.3 EXPRESSION OF RESULTS

Data processing was performed with Lab Solution software (Version 5.73 Shimadzu Corporation).

Quantification was performed and expressed in mg/kg with 4-EthylToluene as external standard on channel 1 and Chiron AS external Standard solution on Channel 2.

The total content of MOAH (C10-C50) expressed in mg of MOAH /kg of sample was the sum of the two contents.

5.4 SUB-FRACTION (C-FRACTION) DEFINITION

In the 2019 JRC Technical Report, sub-fractions of MOAH (so-called C-fractions) in the chromatograms are defined by the position of the elution signals of n-alkanes from the GC column. Each C-fraction starts at the retention time of the peak end of the first n-alkane of the range and stops at the retention time of the peak end of the second n-alkane of the range. Only the C-fraction ≥ n-C10 to ≤ n-C16 starts at the retention time of the peak start of n-C10 and stops at the retention time of the peak end of n-C16 [10].

They are:

Table I - C-fraction definition according to JRC Technical Report [10]

MOAH
MOAH ≥ n-C_{10} to ≤ n-C_{16}
MOAH > n-C_{16} to ≤ n-C_{25}
MOAH > n-C_{25} to ≤ n-C_{35}
MOAH > n-C_{35} to ≤ n-C_{50}

Also in HPLC elution it was possible to separate approximately some of those fractions but on the basis of the the retention times of the Naphthalene, Pyrene and Coronene markers. In fact, the retention time of those polyaromatic compounds, which are detectable in channel 2, were used to divide the different fractions range also on channel 1.

Each C-fraction starts at the retention time of the peak of the first polyaromatic reference compound and end at the retention time of the second polyaromatic reference compound, both in the first and second channel.

The HPLC C-fractions are not fully corresponding to JRC C-fractions.

Nowadays the JRC technical report 2023 [13] has eliminated the C-fraction in expression of results.

6. METHOD VALIDATION

The method was validated for linearity, detection limit, quantification limit (LOQ), recovery and repeatability; the method was compared with other methods participating in different collaborative studies between 2020-2022 for the accuracy evaluation (Table VIII).

6.1 CALIBRATION AND CORRELATION COEFFICIENT (LINEARITY)

Different calibration solutions for both types of external standards were prepared and analysed for the definition of the calibration curve and the relative slopes corresponding to the response factor of 1 ng di 4-Ethyl-Toluene on channel 1 and of 1 ng Chiron AS Solution on Channel 2. The correlation coefficient was ≥ 0,99 for both calibration standard curves. The slopes showed a repeatability CV% value of 7% for 4-Ethyl-Toluene and 11% for Chiron AS Solution obtained from statistical elaboration data from 7 different calibration curves. For both calibration standard curves the linearity residuals showed a value < 20% [17].

6.2 ACCURACY EVALUATION

To test the accuracy, the method was applied to several international proficiency tests in the period 2020-2022. Table III shows the results obtained after the statistical evaluation processing carried out by each of the organising committees.

6.3 HPLC-FLUO METHOD COMPARISON WITH EN 16995 METHOD

To test the possibility of using the HPLC-FLUO method as a screening method, some samples were compared to the results obtained by the LC-GC-FID method (EN 16995). Table IV shows the samples submitted to both analyses and the results obtained. The method was in agreement with the official method (LC-GC-FID), in some cases the content was above those quantified with the reference justifying proceeding, in this case, with a more accurate analysis.

Table II - C-fraction definition in HPLC FLUO method [10]

	MOAH
1	MOAH ≥n-C_{10} Naphthalene to < n-C_{16} Pyrene
2	MOAH ≥n-C_{16} Pyrene to < n-C_{24} Coronene
3	MOAH ≥n-C_{24} Coronene

6.4 RECOVERY

The determination of the recovery value was performed by analysing a refined sunflower oil sample suitably spiked with MOAH from mineral oil at six different concentration levels between 0,5 and 20,0 mg/kg.

Table V shows the results obtained for each level of concentration.

6.5 REPEATABILITY

To test the repeatability of the method, 10 independent replicates were performed on an olive oil sample obtained from a proficiency test with an assigned value of MOAH content. The data obtained were processed with a software program for the statistical analysis of the data provided by ARPAT (ARPA Tuscany region). On all the data obtained, the presence of a normal distribution was verified according to the Shapiro-Wilks test at the 95% confidence level. Subsequently single Dixon, single and pair Grubbs and Huber tests were performed, always with a 95% confidence level.

Table III - Accuracy evaluation through participation to proficiency tests

Year	Sample	PT Commitee Organizative	Assigned Value mg/kg (C10-C50)	HPLC-PDA- FLUO Value mg/kg	Z score
2020	Contaminated vegetable oil	Innovhub	13,3	8,1	2,47
2021	Vegetable oils blend	27th DGF *	3,91	3,61	-0,1
	Olive oils blend	27th DGF *	4,39	5,21	0,32
	Contaminated vegetable oil	Innovhub	21,6	22,2	0,83
	Contaminated olive oil	IOC **	40,9	51,92	1,08
	Certified diatermic oil	Certified Material	<1	0,9	-
2022	Olive oil	JRC ***	43,54±2,41	10,78	-2,85
	Olive oil	JRC ***	2,8±0,5	3,95	1,31
	Contaminated olive oil	Innovhub	5,5	5,7	0,30

(*) DGF = Deutsche Gesellschaft für Fettwissenschaft (German Society for Fat Science).
(**) IOC = International Olive Council.
(***) JRC = Join Research Centre (European Commission).
For one sample the Z score is not present as due to the low content and the organizer decided that it was not possible to proceed with the statistical processing.

Table IV - Comparison between data obtained from LC-GC-FID method (EN 16995) and HPLC-FLUO method

Sample	Matrix	Total MOAH Content LC-GC-FID Method (EN 16995) mg/kg	Total MOAH Content HPLC-PDA-FLUO Method mg/kg
1	EXTRA VIRGIN OLIVE OIL	<2,0	0,9
2	EXTRA VIRGIN OLIVE OIL	2,7	2,2
3	EXTRA VIRGIN OLIVE OIL	<2,0	0,9
3	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
4	EXTRA VIRGIN OLIVE OIL	<2,0	0,2
5	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
6	EXTRA VIRGIN OLIVE OIL	2,0	0,5
7	EXTRA VIRGIN OLIVE OIL	<2,0	0,2
8	EXTRA VIRGIN OLIVE OIL	<2,0	0,1
9	EXTRA VIRGIN OLIVE OIL	<2,0	0,3
10	OLIVE OIL	2,0	1,1
11	FRYING OIL	<2,0	0,2
12	GRAPESEED OIL	7,4	8,6
13	GRAPESEED OIL	8,0	15
14	GRAPESEED OIL	4,4	5,3
15	GRAPESEED OIL	<2,0	0,8
16	GRAPESEED OIL	<2,0	2,5
17	GRAPESEED RAW OIL	4,9	11,5
18	GRAPESEED RAW OIL	4,8	13,1
19	GRAPESEED RAW OIL	6,1	13,2
20	GRAPESEED RAW OIL	5,0	15,7
21	PEANUTS OIL	<2,0	0,2
22	PEANUTS OIL	4,4	2,1
24	CORN OIL	<2,0	1,6
25	CORN OIL	<2,0	2,0
26	CORN OIL	<2,0	2,1
27	CORN OIL	<2,0	1,0
28	CORN OIL	4,2	2,4
29	CORN OIL	<2,0	1,7
30	SUNFLOWER OIL	<2,0	0,2
31	SUNFLOWER OIL	<2,0	2,0
32	SUNFLOWER OIL	<2,0	1,9
33	SUNFLOWER OIL	<2,0	2,5
34	SUNFLOWER OIL	<2,0	2,0
35	SUNFLOWER OIL	<2,0	0,5
36	SUNFLOWER OIL	<2,0	1,2
37	SUNFLOWER OIL	<2,0	1,8
38	SUNFLOWER OIL	<2,0	1,9
39	SUNFLOWER OIL	<2,0	1,0
40	SUNFLOWER OIL	<2,0	0,2
41	SUNFLOWER OIL	<2,0	0,7
42	SUNFLOWER OIL	<2,0	1,8
43	SUNFLOWER OIL	<2,0	1,2
44	SUNFLOWER OIL	<2,0	0,6
45	SUNFLOWER OIL	<2,0	1,3
46	SUNFLOWER OIL	<2,0	2,1
47	SUNFLOWER OIL	<2,0	1,4
48	SUNFLOWER OIL	<2,0	0,1
49	SUNFLOWER OIL	<2,0	1,4
50	SUNFLOWER OIL	<2,0	1,3

Table V - Recovery data on six different concentration level

Sample	Theoretical MOAH Content (mg/kg)	MOAH Content HPLC-FLUO method (mg/kg)	Recovery (%)
1	0,5	0,5	106
2	1,0	1,7	106
3	2,0	1,7	83
4	5,0	4,4	88
5	10,0	8,9	86
6	20,0	17,5	88

Table VI - Sample repeatability data

N° of Replication	Total MOAH content mg/kg
1	5,9
2	5,6
3	5,6
4	5,4
5	5,5
6	5,6
7	5,5
8	5,6
9	5,4
10	5,5
Average	5,5
Standard Deviation	0,1
Repeatability	0,3
Variation Coefficient %	1,4
Expanded Uncertainty	0,3

Table VI reports the results obtained from the repeatability test and the related statistical indices.

7. SAMPLES OF VEGETABLE OILS AND FATS ANALYSED

Several vegetable oil samples (109 samples) were analysed for screening purposes by our laboratory in the period 2020-2022. These were samples of olive oils, extra virgin olive oils, seed oils and vegetable oils and fats.

Before the analysis they were stored in the refrigerator at a temperature of +4°C, in glass bottles.

Table VII shows the list of analysed samples sorted by matrix and relative numerosity.

Table VII - Analysed sample numerosity, MOAH content (C_{10}-C_{50})

Sample	Number of sample analysed	Range of MOAH Content (C_{10}-C_{50}) mg/kg
Extra Virgin Olive oil	51	<0,1-6,8
Refined Olive oil	6	2,3-9,2
Lampante Olive oil	2	9,3-9,6
Refined Sunflower oil	17	0,2-20,1
Refined Almond oil	6	0,9-4,2
Refined/Raw Grapeseed oil	8	2,5-29.8
Refined Palm oil	2	9,3-24,2
Refined Coconut oil	2	0,6-8,2
Refined Sesame seed oil	2	0,6-6,1
Refined Lineseed oil	2	6,0-7,4
Pressed Hemp seed oil	2	7,5-15,3
Refined Corn seed oil	2	1,7-2,4
Refined Peanuts seed oil	2	0,2-2,1
Animal oil (chicken, salmon)	4	0,8-4,6
Anydrous milk	1	<0,1-0,4

8. COMPARISON OF PRECISION DATA BETWEEN JRC REQUEST

Table VIII shows values of LOQ, recovery and intermediate precision obtained with the HPLC-FLUO method in comparison with those suggested by JRC guidelines [10; 13]

9. CONCLUSIONS

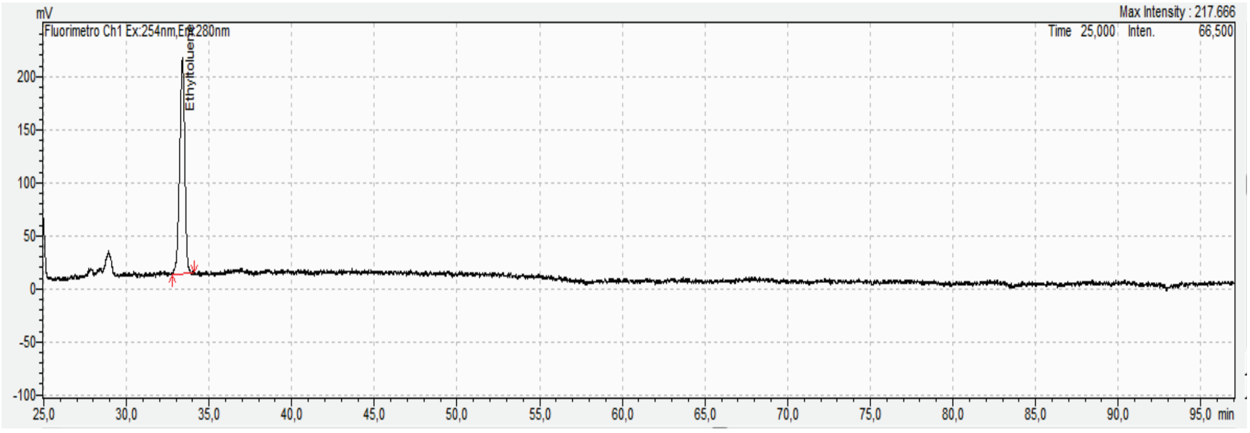
A method for the analysis of MOAH was developed using the HPLC-FLUORIMETRIC system. The method was fully validated for vegetable fats and oils and the LOQ was 0,5 mg/kg for both matrices. The validation of the method also showed suitable accuracy

Table VIII – Precision values obtained with the HPLC-FLUO method

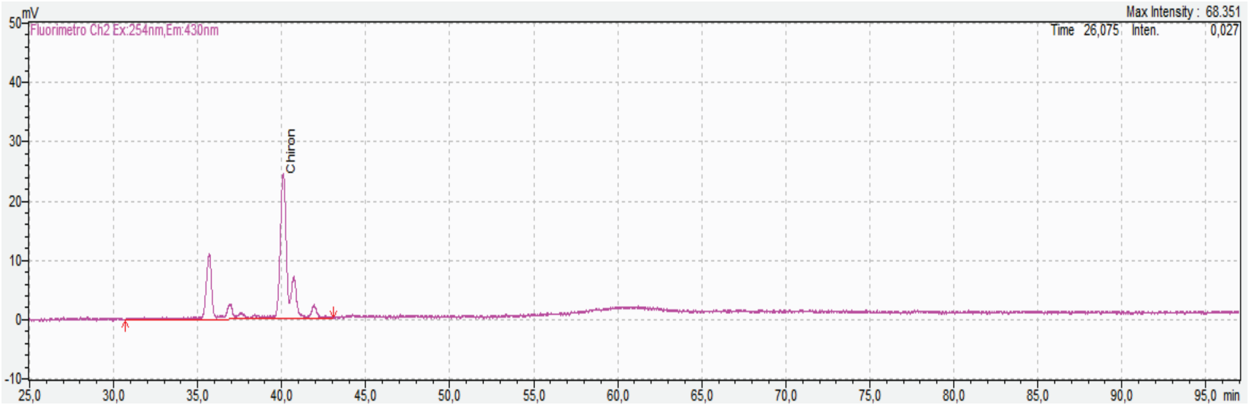
	Range	LOQ max (mg/kg)	LOQ-t (mg/kg)	Recovery (%)	Intermediate precision (%)	BIAS (%)
HPLC-FLUO	C10-C50	0,5	0,1	83-106*	3**	8-19
JRC 2019	C10-C50	2	0,5	70-120	20	-
JRC 2023	C10-C50	2	-	80-110	20	-

**Referred to a spiked range of 0,5-20 mg/kg MOAH*
*** referred to a PT sample with an assigned value of 4 mg/kg MOAH*

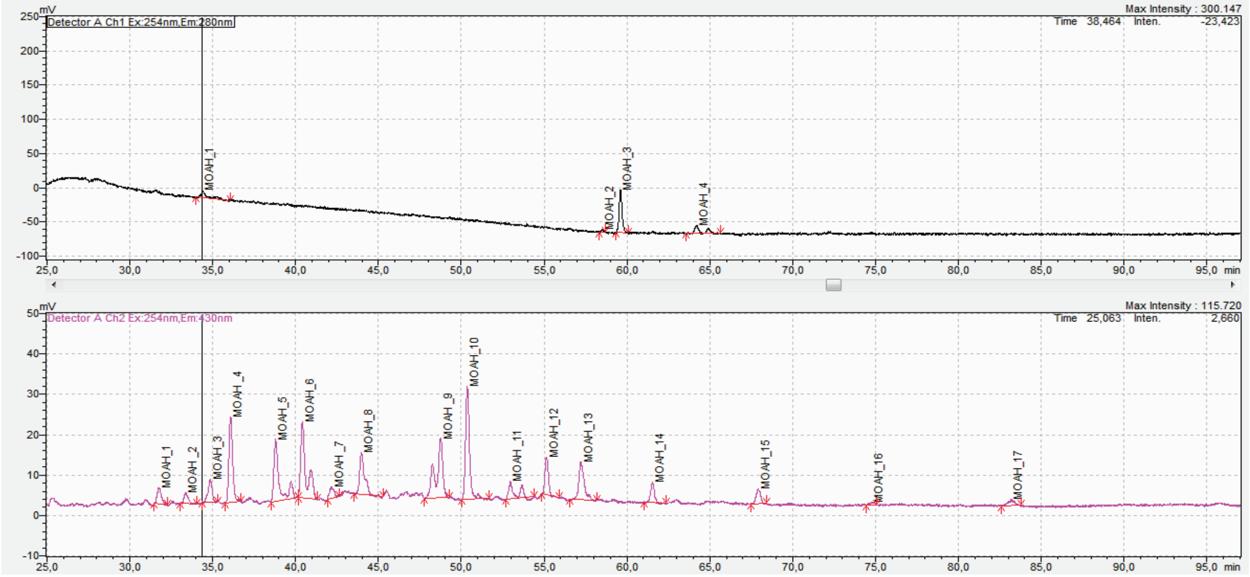
Chromatogram traces obtained by HPLC- FLUO method



Chromatogram 4-Ethyl-Toluene Standard Channel 1



Chromatogram Chiron AS Standard Channel 2



Chromatogram Sample (grapeseed oil) content of 12 mg/kg MOAH acquired on Channel 1 and 2 and precision values in accordance with the JRC prescription, reaching the LOQ of 0,5 mg/kg. The accuracy was evaluated obtaining good Z scores with international and national proficiency test participation. The validated method was applied to a total of 109 samples, detecting MOAH in most of them.

The higher MOAH content was found in refined grapeseed, palm, and sunflower oil. Moreover, a comparison between samples analysed with on-line LC-GC-FID and HPLC-FLUO was performed showing that the method is in agreement with the official one. The method is simple and easily applicable to carry out a pre-assessment in the routine control of the MOAH content in vegetable oil and fat samples using instrumentation commonly present in analytical laboratories (HPLC-FLUO) and a simple sample preparation.

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Composizione biochimica e proprietá antiossidanti dell’olio di semi di dattero (*Phoenix dactylifera* L.) *

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Biochemical composition and antioxidant properties of palm seed oil (Phoenix dactylifera L.)*

Date palm (*Phoenix dactylifera* L.) trees are largely cultivated across the Algerian oases; they are main sources of remuneration and the economic basis for residents of these areas. Date palm fruits are rich sources of essential nutrients, vitamins, minerals, and dietary fibres, with many potential health benefits, yet there are few studies on the chemical composition and biological properties of date palm seed oil. In this study, we present an in-depth characterisation of the biochemical composition and antioxidant properties of date palm seed oil (DPSO) produced in Algeria. DPSOs of eight Algerian cultivars, Arechti, Degla-Baida, Deglet-Nour, Ghars, Haloua, Itima, Mech-Degla, and Tentbouchet, were investigated to determine their biochemical compositions and antioxidant properties. The results highlight the potential of DPSO as an alternative food and a natural resource, thanks to several important compounds having high antioxidant capacity. In particular, fatty acids analyses showed that oleic (42.74-50.19%), lauric (18.40-22.2%), and myristic (8.83-10.17%) were the major fatty acids. Biophenols and tocopherols analyses revealed the presence of important compounds, such as catechin (22.04-24.92 mg/kg), vanillin (10.67-23.98 mg/kg), and α-tocopherol (443.59 mg/kg), at highly remarkable levels. Therefore, a comparison with the literature data concerning other seed oils, including olive oil, confirms that DPSO can be considered a high-quality oil, from a biochemical and biological point of view.

INTRODUZIONE

La palma da dattero è anche nota, nella sua accezione scientifica, come *Phoenix Dactylifera*. La palma da dattero è uno degli alberi più vetusti di cui l’uomo abbia beneficiato e viene coltivata fin dall’antichità [1] principalmente in Medio Oriente e in Nord Africa, con una produzione globale annua di 9,24 milioni di tonnellate, produzione che è aumentata notevolmente negli ultimi 30 anni [2]. In queste aree sono state censite più di 13 milioni di palme da dattero e 940 varietà, con una produzione totale di circa 1,13 milioni di tonnellate [3, 4]. Grazie alla sua tolleranza alle alte temperature, alla siccità e alla salinità rispetto ad altre specie da frutto, essa è considerata un simbolo di vita in diverse aree aride del pianeta. Il frutto della palma da dattero è considerato un alimento ideale in quanto fornisce un’ampia gamma di nutrienti essenziali correlati a notevoli benefici per la salute: è una ricca fonte di fibre alimentari, vitamine essenziali, minerali e metaboliti secondari [5-9]. Tra questi ultimi, i principali composti fenolici presenti nel dattero sono gli acidi gallico, protocatecuico, p-cumarico e ferulico, oltre ad alcuni derivati dell’acido cinnamico [8, 9]. Questi composti bioattivi, in grado di annichilire i radicali liberi reattivi, come il radicale superossido, il radicale idrossile o il perossido di idrogeno, possono inibire l’ossidazione di proteine e lipidi [10]. Inoltre, gli antiossidanti svolgono un ruolo importante per la salute umana, in quanto riducono il rischio di potenziali problemi di salute cronici, come diversi tipi di cancro e malattie cardiovascolari e neurologiche [11]. Concentrazioni eccezionalmente elevate di metaboliti secondari si ritrovano nei semi [12-16]. È interessante notare come i semi, in generale, dal punto di vista della sopravvivenza di una pianta, siano i componenti più importanti dei frutti, il che potrebbe spiegare l'accumulo di questi composti fenolici proprio a livello del nocciolo. Dai dati disponibili, si è potuto apprendere come l'area di coltivazione, le pratiche colturali e il genotipo influiscano molto sul patrimonio fenolico dei semi [9, 13, 17-19] e che i semi di dattero rappresentano una buona fonte di olio (dal 5 al 13%) ricco in composti fenolici, tocoferoli e fitosteroli. Il profilo chimico, in termini di composizione di vitamine, minerali e acidi grassi, è stata studiata da diversi ricercatori che hanno dimostrato la preziosità dell'olio di semi di dattero (OSD) per l'utilizzo in formulazioni alimentari [16, 20-33]. Tuttavia, pochi sono i lavori ad oggi presenti in letteratura e il presente studio ha proprio lo scopo di contribuire all'arricchimento di questa conoscenza. In particolare, otto tipologie di oli di semi di dattero provenienti dalle cultivar *Arechti*, *Degla-Baida*, *Deglet-Nour*, *Ghars*, *Haloua*, *Itima*, *MechDegla* e *Tentbouchet,* diffuse localmente in tutta la provincia di Biskra, in Algeria (Ziban oasi), sono state studiate in termini di composizione dei tocoferoli, acidi grassi, fenoli singoli e totali. Lo studio condotto ha dimostrato che i semi di dattero, producendo un olio ricco di composti bioattivi, sono ottimi ingredienti per l'industria nutraceutica, farmaceutica e cosmetica e non dovrebbero essere trattati come "semplici rifiuti", ma come materie prime. La produzione dell'olio di semi dovrebbe essere considerata una nuova risorsa economica che potrebbe contribuire allo smaltimento dei sottoprodotti derivanti dalla lavorazione dei datteri.

PARTE SPERIMENTALE

MATERIALE VEGETALE E PREPARAZIONE DEI CAMPIONI

I semi di dattero oggetto di studio sono stati collezionati da diverse varietà di palme nell'annata 2019-2020. Nello specifico, le varietà selezionate sono state scelte sulla base di un'indagine preliminare avuta con gli agricoltori locali, preziosi conoscitori del patrimonio di palme da dattero. Sono state, quindi, selezionate tre tipologie di dattero (secco, morbido o semi-morbido) da palmeti situati nella provincia di Biskra (Algeria sud-orientale, regione arida). Seguendo questi criteri, le varietà di dattero collezionate, tra le più rappresentative della Regione, sono state otto: quattro datteri morbidi (*Deglet-Nour*, *Ghars*, *Itima e Tentbouchet),* tre datteri secchi (*Degla-Baida*, *Haloua* e *Mech-Degla*) e un dattero semi-morbido (*Arechti*) (Fig. 1).

Il metodo di campionamento, per ogni varietà, è stato condotto suddividendo il palmeto in diverse parcelle e selezionando dieci alberi. I datteri sono stati raccolti in maniera casuale, da diverse posizioni e altezze durante l'ultimo stadio di maturazione. Dopo la raccolta, avvenuta manualmente, i frutti sono stati selezionati e snocciolati. I semi così ottenuti sono stati, quindi, lavati per allontanarne eventuali residui di polpa e sottoposti ad un processo di essiccamento a 60°C per 24 ore. Dopo l'essiccamento è seguita la macinazione con successiva liofilizzazione.

ESTRAZIONE DELL'OLIO

L'estrazione dell'olio dai semi di *Phoenix dactylifera* L. è stata eseguita secondo la procedura che prevede l'impiego dell'estrattore Soxhlet utilizzando n-esano come solvente di estrazione. Brevemente, l'estrazione dell'olio è stata condotta mettendo a rifluire per 3 ore 30 g di semi macinati con 400 mL di n-esano. La temperatura della piastra è stata regolata in modo tale da permettere una costante e fluida ebollizione del solvente. Dopo l'estrazione, una distillazione sottovuoto a 40-50°C ha permesso la rimozione del solvente. L'olio ottenuto è stato filtrato, conservato in un contenitore scuro e mantenuto alla temperatura di 4°C fino al momento delle analisi.

ESTRAZIONE DELLA COMPONENTE FENOLICA

L'estrazione dei composti minori polari di natura fenolica dai campioni sottoposti ad esame è stata effettuata secondo le direttive descritte dal Consiglio Oleicolo Internazionale (COI) [52] apportando opportune modifiche al protocollo estrattivo [53-55]. In generale, l'estrazione dei composti fenolici è stata la seguente: 2,5 g di campione sono stati trattati con una soluzione idroalcolica in un bagno ad ultrasuoni. Una successiva centrifugazione ha permesso il recupero del surnatante che, previa filtrazione, è stato utilizzato per l'analisi dei fenoli totali e dei fenoli singoli.

ANALISI DEI FENOLI TOTALI

I fenoli totali (FT) sono stati misurati mediante spettrofotometria a 756 nm secondo il metodo di Folin-Ciocalteu [34]. In particolare, 2,5 mL di reagente, op-

Figura 1 - Semi di diverse cultivar di palma da dattero (Phoenix dactylifera L.). (a) Arechti, (b) Degla-Baida, (c) Deglet-Nour, (d) Ghars, (e) Haloua, (f) Itima, (g) Mech-Degla, (h) Tentbouchet.

portunamente diluito, sono stati aggiunti all'estratto fenolico (0,5 mL) e agitato vigorosamente. Successivamente, la soluzione è stata lasciata a riposare per 2 ore a temperatura ambiente con 2 mL di Na_2CO_3 al 7,5%. I risultati ottenuti sono stati espressi come mg di acido gallico equivalente per 100 g di olio (mg AGE/100 g di olio). Ogni analisi è stata condotta in duplice.

ANALISI DEI FENOLI SINGOLI

La determinazione e l'analisi quantitativa dei fenoli singoli è stata ottenuta con l›ausilio di curve di calibrazione esterne costruite tramite un'analisi di regressione attraverso il metodo dei minimi quadrati. Per ogni analita di interesse (tirosolo, idrossitirosolo, oleuropeina, acido vanillico, vanillina, luteolina, luteolina-7-O-glucoside, luteolina-4-O-glucoside, catechina, acidi caffeico, ferulico e omovanillico) sono state preparate soluzioni standard stock da cui, con opportune diluizioni, sono state ottenute soluzioni a concentrazione decrescente. Tutte le curve di calibrazione sono risultate lineari nel range di concentrazione considerato (1-2000 ng/ml) con coefficiente di correlazione compreso tra 0.9990 e 0.9997. L'analisi HPLC è stata eseguita tramite un sistema Agilent Technologies 1200. Gli analiti sono stati separati tramite una colonna HPLC Eclipse XDB-C8-A [granulometria 5 μm, lunghezza 150 mm e diametro interno 4,6 mm (Agilent Technologies, Santa Clara, California)] ad un flusso 250 μl/min ed un volume di iniezione di 10 μl. L'eluizione degli analiti è stata eseguita tramite il seguente gradiente di fase mobile (A: H2O, 0,1% acido formico; B: Metanolo): da 10% a 100% B in 15 minuti e tenuto 2 minuti e da 100% a 10% B in 8 minuti. Il tempo di eluizione totale per ogni iniezione è di 25 minuti. Le analisi ESI-MS/MS sono state eseguite tramite uno spettrometro di massa MSD Sciex Applied Biosystem API 4000 Q-Trap in modalità ionica negativa utilizzando il monitoraggio delle reazioni multiple (MRM). Le condizioni sperimentali sono state le seguenti: ionspray voltage (IS) -4500 V; curtain gas 20 psi; temperature 400°C; ion source gas (1) 35 psi; ion source gas (2) 45 psi; collision gas thickness (CAD) medium. I risultati sono la media di due analisi indipendenti.

ANALISI DEGLI ESTERI METILICI DEGLI ACIDI GRASSI

L'identificazione e conseguente quantificazione degli acidi grassi presenti nell'olio di semi di dattero (OSD) è stata eseguita sugli esteri metilici degli stessi ottenuti tramite reazione di transesterificazione durante il trattamento dei campioni secondo la metodica descritta da Moss *et al.*[35]. L'analisi degli esteri metilici degli acidi grassi, noti anche con l'acronimo FAME, è stata condotta mediante gascromatografia associata a rilevatore a ionizzazione di fiamma (GC-FID). Le temperature dell'iniettore e del rivelatore sono state mantenute a 250 e 280°C, rispettivamente. Il flusso del gas vettore, l'idrogeno, e il rapporto di splittaggio sono stati impostati a 40 mL/min e a 1/50, rispettivamente. L'identificazione dei FAMEs è stata condotta confrontando il tempo di ritenzione di ciascun FAME con i FAMEs standard di riferimento. Ogni campione è stato trattato in duplice.

ANALISI DEI TOCOFEROLI

La composizione dei tocoferoli è stata determinata secondo il metodo descritto da Nehdi *et al*. [16]. In generale, 0,2 g di olio sono stati trattati con 2 mL di esano. Un'aliquota di soluzione (20 μl) opportunamente filtrata è stata iniettata in un sistema HPLC (Agilent 1100) dotato di una colonna Zorbax NH_2 (25 cm × 4,6 mm d.i., granulometria 5 μm, Agilent) utilizzando una fase mobile isocratica di esano:acetato di etile (80:20). Il sistema di rivelazione a fluorescenza è stato settato alle lunghezze d'onda di 295 e 325 nm. L'analisi quantitativa dei singoli tocoferoli è stata ottenuta con l'ausilio di curve di calibrazione esterne costruite tramite un'analisi di regressione attraverso il metodo dei minimi quadrati. I risultati, espressi in mg di tocoferolo α, β, γ e δ per kg di olio, sono la media di due analisi indipendenti.

ANALISI STATISTICA

Tutti i dati ottenuti sono stati trattati statisticamente utilizzando il software SPSS versione 25. È stata quindi condotta l'analisi della varianza (ANOVA) e il test di Duncan ($p \leq 0{,}05$).

RISULTATI E DISCUSSIONE

RESA IN OLIO

Le diverse cultivar hanno presentato variazioni significative in termini di contenuto in olio (Tab. I). La cultivar risultata più ricca è stata *Arechti* (5,30 g/100g), valore che è risultato essere inferiore rispetto a quello evidenziato da Nehdi *et al*. [28] (7,83 g/100g). *Degla-Baida* è risultata, invece, la più povera in olio, con una

Tabella I - Resa in olio e fenoli totali (FT) degli oli di semi di dattero in esame. Lettere diverse nella stessa riga indicano differenze significative per p <0,05.

	Arechti	**Degla-Baida**	**Deglet-Nour**	**Ghars**	**Haloua**	**Itima**	**Mech-Degla**	**Tent-bouchet**
Resa in olio (g/100g)	5,30±0,31^{d}	3,41± 0,27^{a}	4,93±0,39^{c}	4,54±0,53^{b,c}	4,52±0,04^{b,c}	4,61±0,10^{b,c}	4,68±0,55^{b,c}	4,53±0,46^{b,c}
FT (mg AGE/100g)	156,09	193,35	154,59	173,19	166,43	170,05	157,04	177,66

resa totale di 3,41 g/100g. Per le restanti cultivar le rese in olio sono risultate abbastanza paragonabili fra loro e comprese tra 4,52 e 4,93 g/100g. Questi valori sembrano essere simili a quelli indicati da Ali *et al*. [36]. Come confermato da diversi ricercatori, però, i solventi e i metodi di estrazione utilizzati per l'ottenimento dell'OSD potrebbero influire direttamente sulla resa di estrazione e, conseguentemente, sulla qualità dell'OSD stesso [36-38].

CONTENUTO FENOLICO TOTALE

Il contenuto dei fenoli totali (FT) negli oli analizzati (Tab. I) è compreso in un range di 154,59 (*Deglet-Nour*) e 193,35 mg AGE/100g (*Degla-Baida*). I dati ottenuti e quelli disponibili in letteratura hanno evidenziato che gli oli di semi di dattero prodotti in Algeria hanno un valore di FT più alto rispetto a quelli prodotti in Marocco (181,03 mg AGE/100g), [32] ma più basso rispetto a quelli prodotti in Iran (1952,93 mg AGE/100g) [23, 33]. Il processo di estrazione dell'olio e il solvente utilizzato potrebbero influenzare, come per la resa in olio, anche questo valore e, quindi, tutta l'attività antiossidante degli oli [26].

CONTENUTO IN FENOLI SINGOLI

Per le otto cultivar, i profili qualitativi fenolici sono risultati simili (Tab. II), ma le loro concentrazioni hanno mostrato notevoli variazioni. In totale sono state determinati e quantificati 12 composti fenolici. Il principale fenolo trovato in tutti i campioni di OSD è la catechina (da 22,04 a 24,92 mg/kg) seguita dalla vanillina (da 10,67 a 23,98 mg/kg), dall'acido vanillico (da 2,04 a 4,94 mg/kg), dalla luteolina (da 2,76 a 3,45 mg/kg), dal tirosolo (da 1,23 a 2,39 mg/kg) e dall'oleuropeina (da 0,37 a 1,38 mg/kg). Non è stato osservato l'acido omovanillico se non nell'olio della cultivar *Itima* (5,26 mg/kg), mentre l'acido caffeico, l'acido ferulico, l'idrossitirosolo, la luteolina-7-O-glucoside e la luteolina-4-O-glucoside sono stati riscontrati, pur se a concentrazioni molto basse, in tutti gli oli analizzati. Tuttavia, nonostante i valori bassi, essi sono considerati comunque sufficienti ai fini di una attività biologica. Dal confronto tra le cultivar è emerso che *Haloua* vanta la quantità più elevata di catechina, acido vanillico, luteolina e tirosolo mentre la cultivar *Mech-Degla* ne presenta i valori più bassi. La determinazione di fenoli come la catechina e la luteolina in questi oli è stato un dato rilevante e sorprendente sia perché la loro presenza non era stata ancora registrata prima d'ora [25, 33], sia per il valore nutraceutico che può derivare proprio dalla loro presenza, dato che questi fenoli risultano utili nella prevenzione di patologie neoplastiche e cardiovascolari [40-42].

CONTENUTO IN ACIDI GRASSI

Il profilo qualitativo degli acidi grassi (Tab. III) è risultato essere abbastanza simile per tutte le cultivar tranne che per qualche acido grasso presente solo in alcune delle cultivar studiate (acidi palmitoleico, eptadecenoico, eicosanoico). In particolare, si è evidenziata la presenza di dieci acidi grassi saturi e sei insaturi. I principali acidi grassi risultano essere: l'acido l'oleico (C18:1-ω9) in quantità variabili, dal 42,74 al 50,19%; l'acido laurico (C12:0), dal 18,4% al 22,26%; l'acido miristico (C14:0), dall'8,83% al 10,17%; l'acido palmitico (C16:0), dal 9,11% al 10,37%; l'acido linoleico (C18:2ω6), dal 6,58% all'8,12% e l'acido stearico (C18:0), dal 3,07% al 3,64%. Gli altri acidi grassi (beenico, lignocerico, linolenico, arachidico, caprico, caprilico, margarico, palmitoleico, eptadecenoico ed eicosenoico) sono stati trovati in piccole quanti-

Tabella II - Contenuto in fenoli singoli degli oli di semi di dattero in esame. Lettere diverse nella stessa riga indicano differenze significative per p <0,05. ND: non determinabile.

Fenoli (mg/kg)	***Arechti***	***Degla-Baida***	***Deglet-Nour***	***Ghars***	***Haloua***	***Itima***	***Mech-Degla***	***Tent-bouchet***
Vanillina	20,13±0,0^{g}	15,41±0,01^{d}	14,89±0,04^{c}	11,77±0,01^{b}	18,75±0,01^{f}	17,13±0,0^{e}	10,67±0,01^{a}	23,98±0,03^{h}
Acido vanillico	3,60±0,03^{e}	3,49±0,0^{d}	3,73±0,0^{f}	4,56±0,01^{g}	4,94±0,02^{h}	2,75±0,01^{b}	2,04±0,02^{a}	2,96±0,01^{c}
Acido caffeico	0,27±0,01^{d}	0,24±0,01^{c}	0,39±0,02^{f}	0,19±0,01^{b}	0,22±0,01^{b,c}	0,14±0,01^{a}	0,35±0,01^{e}	0,14±0,01^{a}
Acido ferulico	0,05±0,01^{d}	0,04±0,01^{c,d}	0,03±0,01^{b,c}	0,31±0,01^{g}	0,01±0,01^{a,b}	0,09±0,02^{e}	ND	0,19±0,01^{f}
Catechina	23.91±0,0^{e}	22,04±0,04^{d}	24,21±0,03^{a}	20,31±0,04^{a}	24,92±0,04^{c,d}	24,34±0,04^{e}	22,91±0,02^{b}	24,07±0,03^{b,c}
Acido omovanillico	ND	ND	ND	ND	ND	5,26±0,11^{a}	ND	ND
Idrossitirosolo	0,45±0,03$_{d}$	0,34±0,04^{c}	0,18±0,02^{b}	0,07±0,02^{a}	ND	ND	ND	ND
Tirosolo	1,81±0,02$_{e}$	1,24±0,01^{a}	1,7±0,01^{d}	1,64±0,02^{c}	2,39±0,01^{g}	1,5±0,01^{b}	1,23±0,01^{a}	2,17±0,01^{f}
Luteolina	3,06±0,01$_{c}$	3,33±0,03^{e}	3,23±0,01^{e}	3,14±0,01^{d}	3,45±0,01^{g}	2,76±0,01^{a}	3,35±0,01^{f}	2,93±0,01^{b}
Luteolina-7-O-glucoside	0,25±0,01$_{b}$	0,17±0,1	ND	ND	ND	ND	ND	ND
Luteolina-4-O-glucoside	0,09±0,01^{c,d}	0,04±0,02^{a}	0,1±0,02^{d}	0,05±0,02^{a,b}	0,06±0,01^{a,b}	0,04±0,01^{a}	0,07±0,01^{b,c}	0,06±0,01^{a,b}
Oleuropeina	1,38±0,02^{h}	1,05±0,01^{f}	1,09±0,01^{g}	0,88±0,01^{e}	0,52±0,01^{b}	0,84±0,01^{d}	0,65±0,01^{c}	0,37±0,01^{a}

Tabella III – Contenuto degli esteri metilici degli acidi grassi (FAMEs) degli oli di semi di dattero in esame. Lettere diverse nella stessa riga indicano differenze significative per p <0,05. ND: non determinabile.

Acidi grassi (%)	Arechti	Degla-Baida	Deglet-Nour	Ghars	Haloua	Itima	Mech-Degla	Tent-bouchet
Caprilico (C8:0)	0,26±0,02^{b,c}	0,37±0,01^{f}	0,42±0,02^{g}	0,31±0,01^{d}	0,24±0,02^{b}	0,28±0,02^{c,d}	0,16±0,01^{a}	0,35±0,01^{e}
Caprico (C10:0)	0,36±0,01^{a}	0,45±0,01^{a}	0,45±0,0^{a}	0,45±0,01^{a}	0,33±0,33^{a}	0,38±0,04^{a}	0,25±0,25^{a}	0,61±0,0^{a}
Laurico (C12:0)	19,76±1,01^{a,b}	22,26±1,77^{b}	22,03±0,99^{b}	20,51±1,19^{a,b}	20,12±0,86^{a,b}	21,02±0,85^{a,b}	18,4±18,4^{a}	22,19±0,97^{b}
Miristico (C14:0)	9,94±0,66^{a}	10,17±0,97^{a}	8,83±1,02^{a}	9,57±0,93^{a}	10,07±0,72^{a}	9,84±0,04^{a}	9,87±0,63^{a}	10,12±1,19^{a}
Palmitico (C16:0)	9,38±0,39^{a,b}	10,37±0,22^{b}	9,11±0,33^{a}	9,58±0,80^{a,b}	9,17±0,70^{a}	9,82±0,08^{a,b}	9,74±0,51^{a,b}	9,78±0,61^{a,b}
Palmitoleico (C16:1ω7)	0,16±0,01^{b,c}	ND	ND	0,13±0,0^{a,b}	ND	0,18±0,04^{c}	0,13±0,0^{a,b}	0,12±0,0^{a}
Margarico (C17:0)	0,19±0,01^{e}	0,14±0,01^{d}	0,06±0,01^{a}	0,06±0,01^{a}	0,13±0,01^{c,d}	0,2±0,01^{e}	0,11±0,01^{b}	0,12±0,01^{b,c}
Eptadecenoico (C17:1ω7)	0,13±0,01^{b}	0,07±0,0^{a}	0,06±0,01^{a}	0,11±0,01^{b}	0,12±0,02^{b}	0,19±0,03^{c}	0,12±0,01^{b}	ND
Stearico (C18:0)	3,52±0,05^{d,e}	3,59±0,01^{e}	3,36±0,09^{c,d}	3,49±0,01^{c,d,e}	3,64±0,13^{e}	3,19±0,09^{a,b}	3,07±0,08^{a}	3,34±0,06^{b,c}
Oleico (C18:ω9)	42,74±1,47^{a}	43,81±0,04^{a,b}	46,18±1,49^{a,b,c}	48,14±1,74^{c,d}	44,66±0,64^{a,b}	46,54±1,67^{b,c}	50,19±1,59^{d}	44,2±2,06^{a,b}
Linoleico (C18:2ω6)	6,82±1,01^{a}	7,45±0,87^{a}	7,15±0,95^{a}	6,58±1,12^{a}	8,12±1,11^{a}	6,87±1,09^{a}	6,78±0,87^{a}	7,89±0,73^{a}
Linolenico (C18:3ω3)	0,66±0,03^{d}	0,32±0,01^{a}	0,69±0,03^{d}	0,47±0,01^{b,c}	0,54±0,08^{c}	0,82±0,10^{e}	0,47±0,03^{b,c}	0,41±0,03^{a,b}
Arachidico (C20:0)	0,59±0,03^{c,d}	0,33±0,02^{d}	0,66±0,04^{d}	0,52±0,03^{b,c}	0,48±0,02^{b}	0,77±0,09^{e}	0,5±0,07^{b,c}	0,5±0,02^{b,c}
Eicosanoico (C20:1ω9	0,13±0,01^{b}	0,06±0,01^{a}	ND	ND	ND	ND	ND	ND
Beenico (C22:0)	1,88±0,02^{e}	0,22±0,02^{a}	0,59±0,03^{d}	0,41±0,03^{c}	0,31±0,01^{b}	0,43±0,01^{c}	0,32±0,02^{b}	0,25±0,01^{a}
Lignocerico (C24:0)	1,59±0,02^{e}	0,17±0,0^{b}	0,29±0,02^{c}	0,25±0,03^{b}	0,33±0,0^{d}	0,28±0,02^{c}	0,24±0,01^{b}	0,22±0,02^{b}

Table IV - Cholesterol content of soft white cheeses fortified with flaxseed oil

Samples	Cholesterol mg/100g ± SD	Fat g/100g ± SD	Cholesterol/fat
SCN	133.7^{a} ± 0.10	20,06^{a} ± 0.08	6.66
SCO	131.5^{b} ± 0.14	17,96^{b} ± 0.08	7.32
RSCO	123.8^{c} ± 0.02	16,46^{c} ± 0.08	7.52

Values are means of triplicate determinations ±SD
a,b,c lower case letters within each column indicate statistically significant differences (P <0.05)
SCN: Regular soft white cheese, SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil

tà e in meno dell'1,88%. L'analisi degli acidi grassi ha mostrato che l'OSD è un'importante fonte di acidi grassi saturi (dal 42,97 al 48,39%), insaturi (51,39-57,22%), monoinsaturi (43,16-50,44%) e polinsaturi (7,05-8,3%). Per la valutazione della stabilità ossidativa degli oli, sono stati considerati due interessanti descrittori: il rapporto fra gli acidi grassi insaturi e quelli saturi (UFA/SFA) ed il rapporto fra acido oleico e linoleico (O/L) (Tab. IV) [46]. I rapporti oleico/linoleico (O/L) e UFA/SFA variavano rispettivamente da 5,5 (*Haloua*) a 7,41 (*Mech-Degla*) e da 1,06 (*Degla-Baida*) a 1,33 (*Mech-Degla*). I rapporti UFA/SFA osservati sono risultati simili a quelli riportati nell'OSD saudita (da 1,23 a 1,48) [31]. Il rapporto O/L è risultato molto più basso rispetto all'olio d'oliva, nel quale può variare da 3 a 25. Anche i valori UFA/SFA si presentano molto inferiori rispetto quelli dell'olio di oliva (4,8) e di girasole (6,75) [30,46]. Rispetto agli OSD prodotti da altre varietà coltivate in altri Paesi, il profilo degli acidi grassi è risultato relativamente simile a quello degli OSD prodotti in Tunisia, Marocco, Arabia Saudita, Emirati Arabi Uniti e Sudan [22, 26, 31, 39, 47]. Tuttavia, gli OSD delle varietà coltivate in Iran hanno prodotto livelli inferiori di acido oleico (37,60%) e linoleico (6,93%) [48]. Una possibile spiegazione per la variazione dei risultati potrebbe essere attribuita ai fattori pedoclimatici che influenzano la composizione quantitativa e qualitativa degli acidi grassi.

Tabella V - Contenuto in tocoferoli singoli e totali (TT) degli oli di semi di dattero in esame. Lettere diverse nella stessa riga indicano differenze significative per p <0,05.

Tocoferolo	***Arechti***	***Degla-Baida***	***Deglet-Nour***	***Ghars***	***Haloua***	***Itima***	***Mech-Degla***	***Tent-bouchet***
Alpha	310,51±05,65^{d}	260,95±05,13^{e}	543,95±11,11^{a}	432,91±14,78^{b}	310,86±07,05^{d}	295,6±10,35^{b}	443,59±9,64^{b}	379,86±3,00^{c}
Beta	89,27±09,46^{a}	51,6±06,86^{d}	32,04±7,59^{e}	54,64±3,69^{d}	69,18±1,56^{b,c}	35,82±0,77^{e}	60,48±08,7^{c,d,e}	71,68±01,57^{b}
Gamma	55,36±04,30^{d,e}	54,45±05,5^{d,e}	45,49±3,6^{d}	95,6±13,26^{b}	52,85±5,72^{d}	64,25±01,46^{c}	113,19±1,51^{a}	86,54±04,48^{b}
TT	716,44^{b}	560,12^{a}	878,85^{e}	769,16^{c}	946,26^{f}	809,82^{d}	942,35^{f}	746,02^{b,c}

CONTENUTO IN TOCOFEROLI

Nell'analisi della frazione insaponificabile dell'OSD sono emersi quattro isomeri della vitamina E (Tab. V); di questi, il più abbondante è risultato essere l'α-tocoferolo in quantità variabile da 260,95 a 543,95 mg/kg. I valori di β-tocoferolo e γ-tocoferolo riscontrati possono essere compresi in un range da 32,04 a 89,27 mg/kg e 45,49 a 113,19 mg/kg, rispettivamente. Per quanto riguarda il δ-tocoferolo il valore più alto è stato registrato nell'olio di semi di *Haloua* (513,37 mg/kg). Il contenuto minimo e massimo di tocoferolo totale (TT) (560,12 e 946,26 mg/kg) è stato osservato nelle varietà *Degla-Baida* e *Haloua*, rispettivamente. Dai risultati ottenuti, possiamo considerare l'olio di semi di dattero una buona fonte di vitamine E; infatti, in termini di contenuto di tocoferolo l'OSD si colloca al quarto posto tra gli oli di semi, segue il melograno (3483,4 mg/kg), gli oli di semi di germe di grano (3117,5 mg/kg) e l'olio di fico (1400,2 mg/kg) [50]. La vitamina E svolge un ruolo importante per la salute, proteggendo gli acidi grassi dalla dall'ossidazione e, nel nostro organismo, questo permette di promuovere la stabilità delle membrane lipidiche [49]. Data la facilità di estrazione dell'olio di semi di dattero rispetto ai semi delle piante sopra menzionate, questo può essere tranquillamente considerato un'importante fonte naturale di vitamina E la quale, grazie alle sue caratteristiche antiossidanti, è attivamente coinvolta in molte funzioni biologiche, dalla stimolazione immunitaria, alle attività di protezione renale, cardiaca ed epatica [51].

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Characterisation of soft white cheese fortified with flaxseed oil to enhance its quality, lipid profile and health benefits

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This study aimed to investigate the influence of flaxseed oil addition on the chemical composition and fatty acid profile of soft white cheese. The physicochemical characteristics (moisture, ash, salt, fat, protein, carbohydrate, and energy), pH, TBARS, fatty acid profile and sensory properties of soft white cheese samples were studied during 7 weeks of storage. Results showed that cheese moisture ranged from 58.16-65.37%, ash 1.54%-2.7%, protein 10.41%-12.97%, and fat 16.46%-20.06%. Fortification of White soft cheese with flaxseed oil have led to changes in the values of health lipid indices associated with a significant decrease in the values of Atherogenic index, Thrombogenic index, Lipid Preventive Score and n6/n3 ratio, in addition to a significant increase in Health Promoting Index, Desirable fatty acids, and polyunsaturated/saturated fatty acids ratio which were within the range of the optimal values for healthy nutrition. There was a clear difference in the chemical composition and fatty acid profile between the control and the fortified cheeses. The conclusion was that omega-3, which is an excellent nutrient, can be easily added to cheese with desirable changes.

Keywords: white brined cheese, Flaxseed oil, Fatty acid profile, health indices.

INTRODUCTION

In the East Mediterranean and neighbouring countries, such as Serbia, white brined cheeses are the most popular type of cheese. They are typically consumed locally, but the demand for this type of cheese is increasing in markets around the world [1].

Soft white cheese, which is described as the fresh or ripened product formed after coagulation and whey separation of milk, cream or partly skimmed milk, buttermilk, or a blend of these products, is the most common cheese type made in Jordan [2]. It can also be made from ovine, buffalo, bovine and/or caprine milk or from mixtures of these milks [3]. The production procedure can be characterised by the rennet coagulating the milk for 40-60 minutes after heating to around 35°C and pressing in cheese cloth. The cheese is frequently consumed immediately after manufacturing or used to make Arabian confectioneries like kunafeh [4]. Cheese has a long history in human diet as a source of critical nutrients, since it is a rich source of protein and nutritional elements (such as calcium and phosphorus) and is also necessary for the development of healthy bones and teeth, as well as providing essential fatty acids to the brain [5, 6].

As a result, individuals are increasingly aware of the importance of including such items in their diet to preserve and improve their quality of life [7]. In recent years, consumers have become more aware of the significance of maintaining an appropriate nutrition. In cheese manufacture, vegetable fats and oils replaced saturated milk fat, resulting in food formulations with elements that help minimise health hazards. The nutritional profile of cheese is improved by using high-quality vegetable fat as a substitute for milk fat, resulting in a reduction in cholesterol and a shift in saturated and unsaturated fatty acid content [8].

Food engineers and nutritionists are working hard to create nutritious and healthful foods. Incorporating nuts, fruits, vegetables, oils, herbs, and spices into the formulations of processed foods is one of the approaches [9]. Flaxseed oil (FO) is naturally low in saturated fat, high in monounsaturated fat, and notably high in alpha-linolenic acid (ALA), an omega-3 fatty acid; it contains less linoleic acid (LA), an omega-6 fatty acid. Flaxseed oil has a 1:3 omega-6/omega-3 fatty acid ratio due to its high ALA content [10].

Producers developed low-fat food items in response to the consumer's desire to reduce fat intake; making reduced-fat goods with the same qualities and functions as full-fat products, on the other hand, is a difficult task. Low-fat and reduced-fat cheeses are commonly described as soft, hard, rubbery, and discoloured. Fewer fat globules are integrated into the protein matrix in low-fat cheeses, and these globules are typically smaller than in full-fat cheese. As a result, using fat substitutes to improve the quality of low-fat cheeses is a viable option [11].

The link between nutrition and health is becoming more widely recognised among consumers. Many people believe milk fat to be "bad", and scientists are being pressed to explain the role of nutrition in chronic diseases. As a result, numerous markers - lipid indices - have been developed to assess the preventative characteristics of meals [12].

Due to the potential negative health effects of saturated fatty acids and cholesterol in high-fat cheese, the primary goals of this study were to examine the physicochemical, fatty acid composition and sensory properties of soft white cheese fortified with flaxseed oil, which is rich in unsaturated fatty acids, particularly omega-3, to benefit from the advantageous health effects of these acids in lowering the incidence of heart disease, as well as health-related lipid indices, like the Atherogenic and Thrombogenic indices (AI and TI, respectively).

MATERIALS AND METHODS

PREPARATION OF WHITE SOFT CHEESE FORTIFIED WITH FLAXSEED OIL

Cheese production was carried out at the dairy pilot plant, Mazraa dairy factory located in Amman, Jordan according to Gurdian et al. [13] with some modification. Ten kilograms of raw milk were pasteurised at 72°C for 15 seconds. Approximately 1 g Flaxseed Oil (FO) was added per 1 litre of milk at the homogenisation processes. Rennet was dissolved in a small amount of distilled water and added to milk and afterwards incubated at room temperature for 30 min until the milk was coagulated. The curd was cut using a knife and pressed in a cheese mould containing cheese cloth. Afterwards, it was recreated and shaped well and soaked overnight in a cold brine solution consisting of 10% salt. Three soft white cheese formulations were produced: Control (SCN) was manufactured from fresh whole cow milk, soft white cheese fortified with flaxseed oil (SCO) (whole cow's milk + 1% flaxseed oil), and reduced fat soft white cheese fortified with flaxseed oil (RSCO) (skim milk + 1% flaxseed oil). Cheese samples were packed in transparent plastic containers and kept in the refrigerator at 4°C until the time of analysis.

ANALYTICAL METHODS

Cheese samples were analysed for moisture, fat, protein, salt, ash, and pH according to AOAC [14]. Carbohydrate amounts were determined according to Cebeci et al. [15] by determining the moisture, ash, protein and fat amounts of cheese and subtracting them from 100

$$\%CHO = 100 - (\%Moisture + \%Protein + \%Fat + \%Ash)$$

Total energy content of samples was calculated using a conversion factor for each energy yielding substrate of each food sample according to Nunoo [16].

LIPID OXIDATIVE STABILITY OF STORED MANUFACTURED WHITE CHEESES (TBARS)

White soft cheeses were analysed for lipid oxidation by determining thiobarbituric acid reactive substances (TBARS) according to the method described by Faustman et al. [17] and Johns [18]. The absorbance of the resulting solution was measured at 532 nm using a UV-vis spectrophotometer (spectro 2000 spectrophotometer, LaboMed.Inc). The thiobarbituric numbers (TBN) in mg of malondialdehyde/kg sample were calculated by multiplying the measured absorbance by a factor of 7.8.

DETRMINATION OF FATTY ACIDS PROFILES

Fatty acid methyl esters of the fat of the soft white cheese samples were prepared according to the method described by Chritopherson and Glass [19]. The prepared methyl esters were analysed using capillary GLC column (Restek, Rtx-225, USA, crossbond 100%-cyanopropylmethylpolysiloxane, 100 m, 0.25 µm df) immediately after esterification by injection 1 µl of the hexane layer through the injection port of the GLC (model GC-2010, shimadzu Inc., Kyoto, Japan). The fatty acids methyl esters (FAMEs) were injected after adjusting the GLC condition; column oven temperature was 70°C, increased to 165°C for 10 min., kept at 185°C for 1 min, then increased to 220°C for 15 min. Injector temperature was 240°C flame ionisation detector temperature was 250°C, flow rate 1 ml / min He, and split ratio used was 80. The fatty acids peaks were identified by comparing with the retention time of the reference standards. The quantification of the methyl ester fatty acids was then done by: Area of the fatty acid% / total area of fatty acids [20]. The areas of the peaks were corrected by the theoretical correction factors (TCF).

The TCF for the fatty acids less than 16 carbon atoms: (C4:0), (C6:0), (C8:0), (C10:0), (C12:0), (C14:0), (C14:1), (C15:0), (C15:1), (C16:0) were 1.540, 1.308, 1.193, 1.123, 1.077, 1.067, 1.058, 1.054, 1.045, 1.042 respectively.

HEALTH NUTRITIONAL INDICES

The Atherogenic index (AI) and Thrombogenic index (TI) were calculated according to Ulbricht and Southgate [21].

$$AI = 12{:}0+4\times14{:}0+16{:}0/[\Sigma MUFAs+PUFAn6+PUFAn3]$$

$$TI = (14{:}0+16{:}0+18{:}0)/[0.5\times\Sigma MUFAs+0.5\times PUFAn6+3\times PUFAn{-}3+PUFAn3/PUFAn6]$$

Desired fatty acid (DFA), Lipid preventive score (LPS) were determined according to Lima et al. [22]

$$DFA = \sum MUFA + \sum PUFA + C18{:}0$$

$$LPS = FAT+2\times SFA\text{-}MUFA\text{-}0.5PUFA$$

Health-promoting index (HPI) was calculated according to Chen et al. [23] to assess the nutritional value of dietary fat, which focuses on the effect of FA composition on CVD. The formula is:

$$HPI = \Sigma UFA/[C12{:}0 + (4 \times C14{:}0) + C16{:}0]$$

DETERMINATION OF CHOLESTEROL CONTENT

Cholesterol was extracted by enzymatic hydrolysis and oxidation according to Boehringer [24]. In which Cholesterol is oxidised by Cholesterol oxidase to cholestenone. In the presence of catalase, the hydrogen peroxide produced by this reaction oxidises methanol to formaldehyde. The latter, in turn, reacts with acetyl acetone, forming a yellow lutidine dye with ammonium ions. The concentration of the lutidine dye formed is stoichiometric with the amount of CHOL and is measured at 405 nm.
Calculation:

$$c=0.711\times\Delta A(\text{g/lsample solution})$$

$$\text{Content cholesterol} = \frac{(\text{ c cholesterol g/l solution})}{(\text{weight of sample in g/l sample solution})}\times100(\text{g/100g})$$

ΔA (subtract absorbance of the blank from the absorbance of the sample)

SENSORY EVALUATION

Sensory testing was conducted in the sensory evaluation lab at the University of Jordan. Sensory evaluation of cheese samples was performed by a group of twenty specially trained panellists belonging to the staff and students of the Faculty of Agriculture at the University of Jordan who were recruited to evaluate samples using a 9-point hedonic scale (where 1 = dislike extremely, and 9 = like extremely). Samples were given random numbers so the panellists gave their opinion without knowing the sample type. Parameters of appearance, colour, aroma, flavour, softness, texture, and overall acceptability were assessed through the test. Data collected from the panellists were subjected to a statistical analysis [4].

STATISTICAL ANALYSIS

All measurements were performed in triplicate and mean values were reported. Analysis of variance (ANOVA) using JMP (release 10, SAS institute, Cary, NC) was carried out to determine any significant differences among the treatment parameters associated with the developed cheese properties. Least significant difference (LSD) at 5% level of probability was determined to separate differences in the properties among treatments.

RESULTS AND DISCUSSION

PROXIMATE ANALYSIS

Table I shows the proximate analysis of the control and FO-fortified soft cheeses. Moisture contents of cheeses ranged from 58.16% for soft white cheese fortified with FO (SCO), to 65.37% for reduced soft white cheese sample fortified with FO (RSCO). Moisture level of RSCO was found to be significantly higher ($p < 0.05$) than both the control (SCN) and SCO; which were insignificantly different. This indicates that the addition of FO has reduced the moisture content of soft white cheese. Similar results were reported by Aguirre and Canovas [25], which could be linked to the cheese's water-holding capacity (WHC) as a possible explanation for the decrease in moisture.

Haddad and Yamani [4] reported that the moisture content of soft cheese in major governorates of Jordan ranged between 39.5 and 74.5% with an average of 56.5%. Al-Manhal [26] demonstrated that the

Table I - Proximate analysis* of soft white cheese samples fortified with flaxseed oil.

Samples	Moisture (g/100g)	Ash (g/100g)	Protein (g/100g)	Fat (g/100g)	NaCl (g/100g)	CHO (g/100g)	Energy (Kcal)
SCN	$58.52^b \pm 0.46$	$1.79^a \pm 0.57$	$12.04^b \pm 0.03$	$20.06^a \pm 0.08$	$1.30^a \pm 0.1$	$7.57^b \pm 0.28$	$259.00^a \pm 0.53$
SCO	$58.16^b \pm 0.55$	$2.17^a \pm 0.002$	$12.97^a \pm 0.08$	$17.96^b \pm 0.08$	$1.34^a \pm 0.09$	$8.72^a \pm 0.54$	$248.43^b \pm 2.34$
RSCO	$65.37^a \pm 0.46$	$1.54^a \pm 0.15$	$10.41^c \pm 0.05$	$16.46^c \pm 0.08$	$1.05^b \pm 0.04$	$6.21^c \pm 0.74$	$214.64^c \pm 2.06$

*Values are means of triplicate determinations ±SD

a,b,c lower case letters within each column indicate statistically significant differences ($P < 0.05$).

SCN: Regular soft white cheese (i.e., control), SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil moisture content of soft cheese ranged between 55.33-69.85%. Our results agreed with the results indicated above.

The ash content ranged from 1.54% for RSCO, to 2.17% for SCO. According to the performed statistical analysis, there were no significant differences ($p > 0.05$) in ash contents among different samples. The low ash content of pasteurized milk cheese could be explained by the diffusion of salts from the curd into the pickling solution because of high moisture content in cheese. The results obtained were lower than those of Haddad and Yamani [4] and Mahrous [27] who found that the ash percentage of soft cheese in major governorates of Jordan ranged between 3.3 and 17.3 with an average of 9.5%, and in white cheese manufacturing processes using FO and skim milk, it ranged from 3.3% to 3.60%, respectively.

Significant differences in fat content ($p < 0.05$) were found between cheese samples, where values ranged from 16.46% for RSCO, to 20.06% of total cheese weight for SCN. The fat content decreased in reduced white soft cheese fortified with FO, whereas the moisture increased. These results agreed with those of Mahrous [27], Akan and Kinik [28] and Manuelian et al. [29].

Regarding protein content, significant differences in ($p < 0.05$) were detected between cheese samples, where values varied from 10.41% for RSCO, to 12.04% for SCN and 12.97% for SCO. In this study, protein content was like that reported by Salwa and Galal [30] who found a value of 13.8%, and lower than the findings of Haddad and Yamani [4] and Manuelian et al. [29] who reported a protein content of 16.4% and 15.66 to 19.73%, respectively.

As shown in Table I, salt (i.e., NaCl) contents ranged from 1.05% in RSCO, to 1.34% in SCO. Values of the control and SCO were statistically similar, whereas RSCO showed a significantly ($p < 0.05$) lower value. Salt helps in controlling microbial growth and activity, slowing down various enzyme activities, reducing moisture content, and preventing physical changes in proteins; all of which can affect the cheese texture and flavour [31]. The results agreed with those reported by Gomes et al. [32].

As seen in Table I, there were significant differences regarding carbohydrate (CHO) content among the control and the fortified treatments. Values varied between 6.21, 7.57 and 8.72 for RSCO, SCN and SCO, respectively. The lowest CHO value was in RSCO, whereas the highest was in SCO cheese.

Mean caloric values of the cheese samples are also presented in Table I. The lowest calorie content (214.64 kcal/100g) was, as expected, determined in the RSCO, whereas the highest value detected (259.00 kcal/100g) was in the regular soft white cheese. It is a well-known fact that fat content is an important factor in calculating the energy value of foods [33]. Consequently, values were consistent with each sample's fat level; since the lowest fat content was detected in RSCO, and the highest was found in the control.

OXIDATIVE STABILITY OF STORED MANUFACTURED WHITE CHEESES (TBARS)

Figure 1 demonstrates the weekly-measured TBARS values of the FO-fortified soft white cheeses during a storage period of 2 months. Values were significantly ($p < 0.05$) greater in the control as compared with fortified white soft cheeses; at which it (i.e., SCN) showed a significant increase from week 0 to week 4,

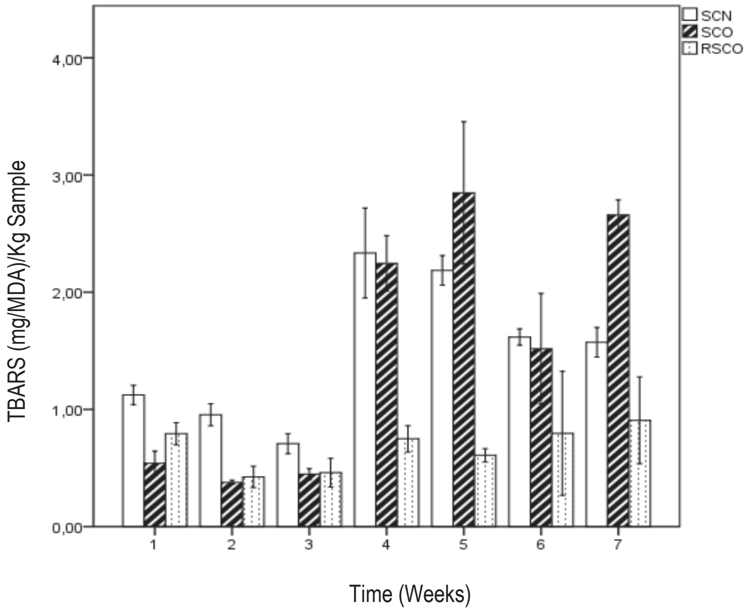

Figure 1 - TBARS of soft white cheeses incorporated with flaxseed oil during a storage period of 7 weeks at 4°C.
SCN: Regular soft white cheese (i.e., control), SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil. TBARS = Thiobarbituric-acid-reactive-substances, MDA = Malondialdehyde.

followed by a drop from week 5 to week 7.

SCO showed a significant increase ($p < 0.05$) in TBARS values from week 0 to week 7. It had significantly higher TBARS than the other treatments. This indicates that less secondary oxidative products were found in the control and RSCO when FO was added. RSCO had the lowest TBARS values during the entire storage period, which can be linked to the lower amount of fat in the cheese. Interestingly, the development of rancid off-flavour in FO-based skimmed milk cheese products was quite stable against oxidation, intense lighting, and is predicted to be very low during storage at 4°C [27]. Chen et al. [22] and Simbalista et al. [34] reported that ALA is a component in oil which is susceptible to auto-oxidation and polymerisation when exposing to air, light or high temperatures.

These results agreed with Abd el-aziz et al. [35] who found that TBA values of Egyptian white brined cheese increased during storage until 2 months. According to Sallam [36], the maximum level of TBARS is 5 mg malondialdehyde/kg sample for good quality food. Meanwhile, TBARS values obtained in the present study for SCN, SCO, and RSCO were 1.57, 2.65, and 0.9 mg malondialdehye/kg sample, respectively.

PH

Figure 2 shows the weekly-measured pH values of FO-incorporated soft white cheeses during storage for 2 months at 4°C. There was a significant difference ($p < 0.05$) between all samples. The lowest pH detected was in RSCO, whereas the greatest was in SCO. During the ripening period, pH values among cheese samples fluctuated between 6.7-6.47 for the control, 6.92-6.36 for SCO and 6.49-6.08 for RSCO. The pH values generally decreased throughout maturation for all samples, which is a sign of over-fermentation [37]. This decreasing trend in pH of white cheeses was observed till up to 2 months of storage. Similar values of pH were reported by Gurdian et al. [13] and Haddad and Yamani [4].

According to Ismail et al. [38], cheese containing vegetable oils showed slightly higher pH values than the control cheese. These results reflect the low amount of lactose content in the control cheese. In addition, increasing the level of milk fat substitution leads to a slight decrease in pH value. Additionally, Effat et al. [39] reported that the short chain fatty acids, which are produced in varied amounts as a metabolic end product of probiotic bacteria, may be responsible for the reduction in pH values.

FATTY ACID PROFILE

Table II presents the fatty acid profile (i.e., saturated, monounsaturated, polyunsaturated and trans fatty acids) of FO-fortified soft white cheeses, as well as FO. Additionally, the sum of lipid (SFA, MUFA, PUFA, Trans FA, n-6 and n-3) composition, and nutritional quality indices (Atherogenic index (AI); Thrombogenic index (TI); desirable fatty acids (DFA); lipid preventive score (LPS); health-promoting index (HPI); and n6/n3 ratio) of fortified soft white cheeses and FO are listed in Table III.

Analysis of fatty acid profile in the control and fortified soft white cheeses identified a total of 23 fatty acids where the control had the greatest SFA percentage, and both SCO and RSCO cheeses had the highest PUFA, ALA, and n-3 and n-6 percentages (Table III).

A significant difference was detected between the control and other experimental treatments; noting that there was a significant decrease in the concentration

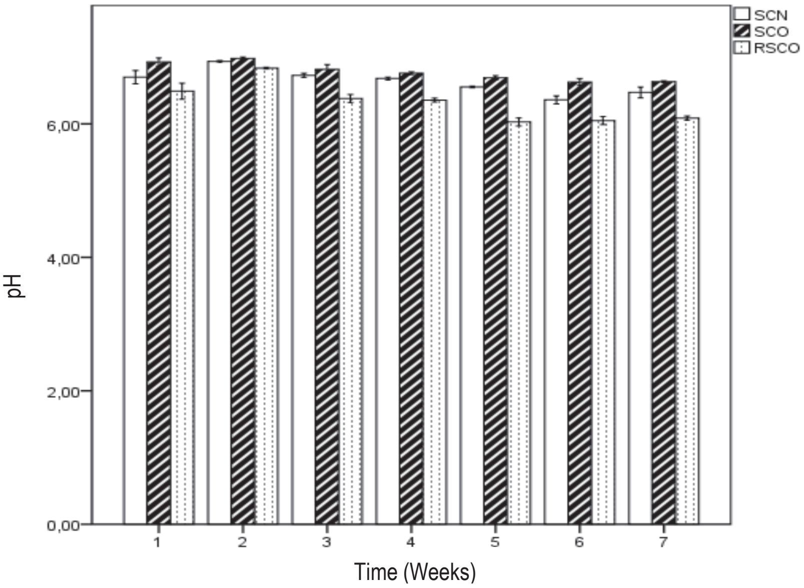

Figure 2 -The pH of soft white cheeses fortified with flaxseed oil during a storage period of 7 weeks at 4°C.
SCN: Regular soft white cheese (i.e., control), SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil.

Table II - Saturated, monounsaturated, polyunsaturated and trans fatty acids (g/100g total FA) of fortified soft white cheeses and flaxseed oil.

Item	Samples			
Saturated fatty acids	**SCN**	**SCO**	**RSCO**	**FO**
C4:0 Butyric acid	$1.80^{a} \pm 0.04$	$1.36^{c} \pm 0.03$	$1.52^{b} \pm 0.03$	ND^{d}
C6:0 Caproic acid	$0.60^{a} \pm 0.01$	$0.28^{c} \pm 0.007$	$0.44^{b} \pm 0.01$	ND^{d}
C8:0 Caprylic acid	$1.17^{a} \pm 0.02$	$0.79^{c} \pm 0.01$	$0.97^{b} \pm 0.02$	ND^{d}
C10:0 Capric acid	$3.06^{a} \pm 0.07$	$2.09^{c} \pm 0.05$	$2.42^{b} \pm 0.05$	ND^{d}
C12:0 Lauric acid	$3.93^{a} \pm 0.09$	$2.74^{c} \pm 0.06$	$3.02^{b} \pm 0.07$	$0.03^{d} \pm 0.0008$
C14:0 Myristic acid	$12.94^{a} \pm 0.31$	$9.11^{c} \pm 0.22$	$9.61^{b} \pm 0.23$	$5.30^{d} \pm 0.12$
C15:0 Pentadecylic acid	$1.55^{a} \pm 0.03$	$1.10^{b} \pm 0.02$	$0.94^{c} \pm 0.02$	ND^{d}
C16:0 Palmitic acid	$39.15^{a} \pm 0.95$	$29.40^{b} \pm 0.71$	$29.85^{b} \pm 0.72$	ND^{d}
C17:0 Heptadecanoic acid	$0.76^{a} \pm 0.01$	$0.53^{b} \pm 0.01$	$0.55^{b} \pm 0.01$	ND^{d}
C18:0 Stearic acid	$9.18^{a} \pm 0.22$	$7.98^{c} \pm 0.19$	$8.64^{b} \pm 0.21$	$4.19^{d} \pm 0.1$
C20:0 Arachidic acid	$0.34^{a} \pm 0.008$	$0.26^{b} \pm 0.006$	$0.26^{b} \pm 0.06$	$0.15^{c} \pm 0.003$
C22:0 Behenic acid	$0.15^{a} \pm 0.003$	$0.1^{b} \pm 0.002$	$0.1^{c} \pm 0.002$	$0.02^{d} \pm 0.0005$
C24:0 Lignoceric acid	$0.2^{a} \pm 0.004$	$0.12^{c} \pm 0.002$	$0.25^{d} \pm 0.0006$	$0.19^{b} \pm 0.004$
Monounsaturated fatty acids				
C14:1 Myristoleic acid	$0.007^{d} \pm 0.0001$	$0.06^{a} \pm 0.001$	$0.012^{c} \pm 0.0003$	$0.05^{b} \pm 0.001$
C15:1 cis-10-Pentadecenoic acid	$0.31^{a} \pm 0.007$	$0.22^{b} \pm 0.005$	$0.22^{b} \pm 0.005$	$0.03^{c} \pm 0.0009$
C16:1 Palmitoleic acid	$2.39^{a} \pm 0.58$	$1.71^{b} \pm 0.04$	$0.15^{c} \pm 0.003$	ND^{d}
C17:1 cis-Heptadecenoic acid	$0.23^{a} \pm 0.005$	$0.17^{b} \pm 0.004$	$0.17^{b} \pm 0.004$	ND^{d}
C18:1 (n-9)-Oleic acid	$21.69^{ab} \pm 0.52$	$21.18^{bc} \pm 0.51$	$22.21^{a} \pm 0.54$	$20.34^{c} \pm 0.49$
Polyunsaturated and Trans fatty acids				
C18:2 (n-6)-Linoleic acid	$3.57^{c} \pm 0.08$	$6.6^{b} \pm 0.16$	$6.81^{b} \pm 0.16$	$15.51^{a} \pm 0.37$
C18:3 α-Linolenic acid	$0.51^{d} \pm 0.01$	$17.67^{b} \pm 0.43$	$16.17^{c} \pm 0.39$	$55.05^{a} \pm 0.34$
C18:1 trans trans-9-Elaidic acid	$1.52^{b} \pm 0.03$	$0.71^{c} \pm 0.01$	$0.49^{d} \pm 0.01$	$1.59^{a} \pm 0.03$
C18:2 trans-Linolelaidic acid	$1.11^{a} \pm 0.02$	$0.92^{b} \pm 0.02$	$0.74^{c} \pm 0.01$	$0.05^{d} \pm 0.001$
C18:3 trans	$0.28^{a} \pm 0.006$	$0.22^{b} \pm 0.005$	$0.21^{b} \pm 0.005$	$0.28^{a} \pm 0.006$

*Values are means of triplicate determinations ±SD

^{a,b,c,d} Different letters within the same row differ significantly at $p < 0.05$.

SCN: Regular soft white cheese, SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil, FO: Flaxseed oil.

of short-chain fatty acids and saturated fatty acids in cheeses incorporated with FO (i.e., SCO and RSCO), and an increase in C18:2 and C18:3 compared to the control sample. Similar findings were reported by Veena et al. [40]. This has a positive impact on reducing cholesterol and the risk of heart disease [41].

In the control sample, palmitic acid (C16:0) was the most abundant fatty acid, followed by myristic (C14:0), and stearic (C18:0) acids. In cheeses containing FO, oleic acid (C18:1) was the most abundant fatty acid, followed by palmitic (C16:0), linolineic (C18:3), myristic (C14:0) and stearic (C18:0) acids. The fatty acids C18:1, C18:2 and C18:3 are some of the most vital fatty acids that are necessary to maintain human health [9]. Oleic, linoleic, and stearic acids have a higher perception threshold and are thought to play a less important role in cheese flavour [42].

A significant ($P < 0.05$) increase in mono-poly unsaturated fatty acid (MUFA- PUFA) in cheese supplemented with FO compared to the control cheese sample. These results are attributable to the addition of flaxseed, which is high in linolenic, oleic, linoleic, and conjugated linoleic acid (CLA), and low in palmitic acid.

Saturated fatty acids ranged from 74.89% in control, 55.92% in SCO and 58.39% in RSCO. Our findings, regarding SFA contents, were like those reported by Donmez et al. [43]. Specific SFA are involved in cell regulation and gene expression, n-3 FA produce anti-inflammatory eicosanoids [29].

Goyal et al. [44] also reported that dahi (Indian yoghurt) fortified with microencapsulated FO powder at 2% level showed an increased ALA content (10.62% of total fatty acids) compared to control dahi (1.92% ALA).

As demonstrated in Table III, trans fatty acids varied from 2.91% in SCN, to 1.86% in SCO and 1.46% in RSCO, while were 1.93% in FO. Our samples had considerably less trans fatty acids in total fatty acids. Trans fatty acids are found in ruminant fats (dairy products, beef, lamb) because of bacterial action in the rumen, as well as in shortening and spreads as a result of industrial hydrogenation of oils [45].

The control showed ALA content of 0.51%, LA content of 3.57% and oleic acid of 21.69%. The FO incorporated in cheese had oleic acid level of 20.34%, LA around 15.51%, and ALA around 55.05%. Thus, it is evident that supplementation of FO has significantly

Table III - The sum of lipid (SFA, MUFA, PUFA, Trans FA and n-6, n-3) composition, and nutritional quality indices; Atherogenic index (AI), Thrombogenic index (TI), desirable fatty acids (DFA), lipid preventive score (LPS), health-promoting index (HPI),PUFA/SAF, and n6/n3 ratio of fortified soft white cheeses and flaxseed oil.

Sample	ΣSFA	ΣMUFA	ΣPUFA	ΣTrans	n6	n3	AI	TI	DFA	LPS	HPI	n6/n3	PUFA/SFA
SCN	74.89^{a} ± 1.83	25.15^{a} ± 0.45	4.08^{c} ± 0.09	2.91^{a} ± 0.07	3.57^{c} ± 0.08	0.51^{d} ± 0.01	3.24^{a} ± 0.09	3.81^{a} ± 0.09	38.42^{c} ± 0.46	142.66^{a} ± 3.70	0.26^{d} ± 0.009	6.91^{a} ± 0	0,05^{d} ± 0.000004
SCO	55.92^{b} ± 1.36	23.36^{b} ± 0.56	24.27^{b} ± 0.59	1.86^{b} ± 0.04	6.60^{b} ± 0.16	17.67^{b} ± 0.43	1.43^{b} ± 0	0.65^{c} ± 0.0005	55.62^{b} ±1.35	94.31^{b} ± 1.78	0.34^{b} ± 0	0.37^{c} ± 0	0,43^{b} ± 0
RSCO	58.39^{b} ± 1.42	22.79^{b} ± 0.55	22.99^{b} ± 0.56	1.46^{c} ± 0.03	6.81^{b} ± 0.16	16.17^{c} ± 0.39	1.55^{b} ± 0	0.73^{b} ± 0.0005	54.42^{b} ± 1.32	98.97^{b} ± 1.93	0.31^{c} ± 0	0.42^{b} ± 0	0,39^{c} ± 0
FO	9.79^{c} ± 0.41	20.43^{c} ± 0.49	70.56^{a} ± 1.72	1.93^{b} ± 0.04	15.51^{a} ± 0.37	55.05^{a} ± 1.34	0.23^{c} ± 0	0.05^{d} ± 0.00002	95.19^{a} ± 2.32	63.86^{c} ± 0.58	0.96^{a} ± 0.13	0.28^{d} ± 0	7,20^{a} ± 0.14

Values are means of triplicate determinations ±SD.

The results are shown as a percentage of total FA

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

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, n-6: omega 6 fatty acid series, n-3: omega 3 fatty acid series, AI: atherogenic index, TI: thrombogenic index, DFA: desirable fatty acids, LPS: lipid preventive score, HPI: health-promoting index.

SCN: Regular soft white cheese, SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil, FO: Flaxseed oil.

($P < 0.05$) increased the level of omega-3 fatty acids in the final product.

The most important fatty acids for human health are: C18:1, C18:2 and C18:3. In the obtained data, there was a significant difference in the content of the polyunsaturated fatty acid C18:3 between the control and cheeses containing FO. Total amount of long-chain fatty acids (above C18) in the cheese samples were significantly ($P < 0.05$) higher compared to the control.

To evaluate the nutritional value of lipids, the different sums of fatty acids and lipid health indices are presented in Table III. Atherogenicity index (AI), thrombogenicity index (TI), lipid preventive score (LPS) and ratio of n6/n3 significantly decreased with the incorporation of FO, while the amount of desirable fatty acids (DFA) and health-promoting index (HPI) increased significantly in fortified cheeses.

According to Lima et al. [22], there are currently no criteria for the AI and TI of dairy products, although lower indices are regarded to be better for human health. Lower AI and TI are thought to translate into more anti-atherogenic fatty acids and better disease prevention profiles. Our results agreed with Caroprese et al. [46].

In SCO and RSCO, the Atherogenic and Thrombogenic indices, as well as the ratio of n-6 to n-3, were lower; indicating that the nutritional characteristics of cheeses made with FO had improved. The balance of n-6 and n-3 PUFA in the diet, in particular, is important in the prevention of numerous ailments, including coronary artery disease [47].

The polysaturated fatty acid/Monounsaturated fatty acid (PUFA/SFA) ratio was found to be 0.05, 0.43, 0.39 in the control, SCO and RSCO respectively. Short-chain fatty acids (i.e., C4-C10) in ester form play a vital role in milk fat, giving it its distinctive flavour and aroma. Short-chain fatty acids in free form, on the other hand, are responsible for the rancid flavour [48]. According to Abbas et al. [9], Nutritional guidelines recommend a PUFA/SFA ratio between 0.4-1.0 and ω6/ω3 PUFAs less than 4 to prevent cardiovascular disease.

Dietary recommendations for omega-3 fatty acids can be obtained from the diet by the consumption of foods rich in these fatty acids. According to Joint WHO/FAO 2010, the daily recommended intake of omega-3 fatty acids should be at least 250 mg/day for a healthy life [49].

CHOLESTEROL CONTENT

Significant differences in cholesterol content ($p < 0.05$) were found between cheese samples. From the data presented in Table IV, it is apparent that the cholesterol content decreased with the decreasing fat content. The highest cholesterol content among all cheeses analysed was that found in SCN (133.7 mg/100g) cheese due to their high fat content and as expected, the cholesterol level in regular soft white

Table IV - Cholesterol content of soft white cheeses fortified with flaxseed oil

Samples	Cholesterol mg/100g ± SD	Fat g/100g ± SD	Cholesterol/fat
SCN	133.7^a ± 0.10	20,06^a ± 0.08	6.66
SCO	131.5^b ± 0.14	17,96^b ± 0.08	7.32
RSCO	123.8^c ± 0.02	16,46^c ± 0.08	7.52

Values are means of triplicate determinations ±SD
a,b,c lower case letters within each column indicate statistically significant differences ($P < 0.05$)
SCN: Regular soft white cheese, SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil

Table V - Sensory characteristics of regular and supplemented soft white cheeses fortified with flaxseed oil.

Samples	Appearance	Color	Aroma	Flavor	Softness	Texture	Overall Acceptability
SCN	8.05^a ± 1.27	8.1^a ± 1.11	7.4^a ± 1.46	6.75^a ± 2.02	7.25^a ± 1.2	7.3^a ± 1.38	7.55^a ± 1.19
SCO	7.4^a ± 1.23	7.4^a ± 1.18	7.00^a ± 2.00	5.85^a ± 1.72	6.3^a ± 1.62	6.4^a ± 1.60	6.45^b ± 1.57
RSCO	7.75^a ± 1.40	7.8^a ± 1.5	6.2^a ± 2.52	5.2^a ± 2.19	6.6^a ± 2.30	6.5^a ± 1.96	5.3^c ± 2.08

Values are means of triplicate determinations ±SD.
^{a,b,c} lower case letters within each column indicate statistically significant differences ($P < 0.05$)
SCN: Regular soft white cheese, SCO: Soft white cheese fortified with flaxseed oil, RSCO: Reduced soft white cheese fortified with flaxseed oil

cheese containing milk fat was significantly higher because its main source of cholesterol is animal fat. The cholesterol observed in fortified samples, which was almost 131.5 mg/100g in SCO and 123.8 mg/100g in RSCO, could be originated from milk serum [41]. The cholesterol contents were highly correlated with the fat levels (r = 0.919). Namely, the cholesterol/fat ratio was the lowest in the regular soft white cheese (6.66) in contrast to RSCO cheese (7.52). In addition, a trend of increasing cholesterol/fat ratio with decreasing fat content suggests that the addition of FO may reduce the cholesterol content. Our finding was in line with Donmez et al. [43] and Abou Jaoudeet al. [50] and disagreed with Andrikopoulos et al. [51] and Arslan et al. [48]. According to Ullah et al. [52], the addition of chia oil to fortified cheddar cheese resulted in a drop in cholesterol and an increase in ALA fatty acid.

Arslan et al. [48] found that Turkish white cheese supplemented with corn oil had a better fatty acid profile than the control cheese, indicating a rise in the ratio of PUFA/SFA and a decrease in cholesterol level. The consumption of dairy products with lower AI values leads to a decrease of the total cholesterol and LDL-cholesterol in human blood plasma [53].

SENSORY EVALUATION

Sensory profile of FO-incorporated white soft cheeses is presented in Table V. No significant difference ($p > 0.05$) was observed in mean scores for appearance, colour, aroma, flavour, softness and texture between the control and FO-fortified cheese samples.

The control sample exhibited the highest scores in terms of all sensory attributes among the examined cheeses samples. This was possibly because fat plays an effective role in the colour, taste, flavour and texture [54]. Similar trend was also observed for aroma and flavour scores which were higher in SCO than RSCO. Moreover, RSCO received higher scores in terms of appearance, colour, softness and texture as compared to SCO.

Flavour ratings were lower in FO-incorporated samples than in the control. This could be due to the breakdown of carbohydrates into lactic acid and the release of taste components like acetaldehyde, or the breakdown of fat into volatile fatty acid [55].

All cheese samples were characterised with white colour, milk odour, salty taste, soft body and grittiness during mastication. Nevertheless, all cheese samples were judged to be acceptable products by the panellists. During storage, no off-flavours or bitterness were observed in any of the cheeses. As a result, PUFA enrichment has no negative impact on customer acceptability. Similar results were obtained by Hassan et al. [56].

There were significant ($P \leq 0.05$) differences in the overall acceptability between control and fortified white cheese samples. According to the panellists, full-fat cheese (SCN) showed a significantly higher overall acceptability compared to SCO and RSCO. Our results are in harmony with Sulejmani et al. [8], who investigated the effect of vegetable fat on the texture, colour and sensory properties of Macedonian white brined cheese.

Low-fat cheeses fortified with FO (RSCO) were characterised by a lower overall score compared to all other cheese samples. This could be due to the significantly higher salty taste and lower fat content in the low-fat cheese. Our results disagree with those reported by Mahrous [27] and Felfoul et al. [57].

Hassan et al. [56] reported that the effects of FO in Egyptian lactating buffalo and cow diets on milk and soft cheese quality were minimal, with no significant changes in soft cheese sensory and textural attributes.

CONCLUSION

This study was carried out to characterise the changes that occur in cheese formulations containing FO and compare them to control cheeses (made with full fat milk) and skim milk cheeses, as well as to improve the nutritional and therapeutic value of processed cheeses using FO as a functional element due to its high nutritional and health value. Based on these findings, FO could be recommended as a useful ingredient in cheese production due to its high content of ALA. The Dietary recommendations for omega-3 fatty acids can be obtained from the diet by the consumption of foods rich in these fatty acids. According to Joint WHO/FAO 2010, the daily recommended intake of omega-3 fatty acids should be at least 250 mg/day for a healthy life. The fortified soft white cheese with FO showed an increase of ALA content (17.67% of total fatty acids) compared to control (0.51% ALA). It is recommended to take foods that supply omega-6 and omega-3 in an ideal ratio of 4:1 to prevent the development of chronic diseases such as cardiovascular disease. Furthermore, by combining dairy products with vegetable oils, it is possible to boost the nutritional value of fat in the diet. Indeed, today's market includes cheeses with fat content as low as 3%. As a result, reduced-fat cheeses have a good chance of becoming as enticing and popular as full-fat cheeses. To decrease lipid oxidation of FO during homogenisation, a future study could focus on microencapsulation or on the use of edible films and coatings.

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

according to the intensity of the defects and of the fruitness perceived, as determined by a group of tasters selected, trained and monitored as a panel, using statistical techniques for data processing.

It also provides information on the organoleptic characteristics for optional labeling.

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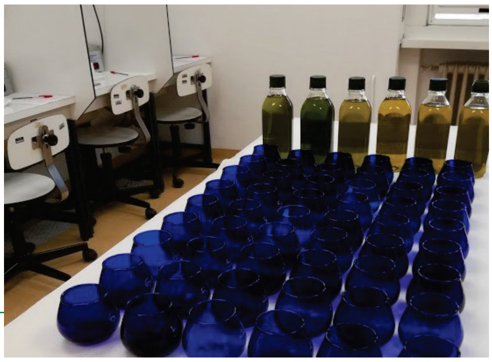

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Phenolic constituents, antioxidant and anti-inflammatory effects of *Heliotropium europaeum* (Boraginaceae)

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Heliotropium europaeum L. (Boraginaceae) has been traditionally used by Mediterranean peoples as folk medicine against several types of disease to treat diverse illnesses. In this study, the antioxidant, and anti-inflammatory activities of *H. europaeum* extracts were investigated for the first time. Additionally, the methanol extract was analysed for phenolic acids and flavonoids by HPLC. The antioxidant potencies of the extracts (ethanol, methanol, chloroform, water) were carried out via six different antioxidant assays (radical quenching (ABTS and DPPH), metal chelating, reducing power (FRAP), phosphomolybdenum and β-carotene/linoleic acid assay) and the methanol extract showed the highest antioxidant activity. To determine the anti-inflammatory effects of *H. europaeum* extract in LPS-stimulated RAW 264.7 macrophage cells, basically two methods were used. While Griess assay was applied to detect the nitrite level as an index of nitric oxide (NO) production, enzyme-linked immunosorbent assay (ELISA) was used for determining the levels of pro-inflammatory cytokines (TNF-α, IL-6 and IL-12) generated in the culture. *H. europaeum* inhibited not only NO production but also the levels of proinflammatory cytokines, without any cytotoxicity. HPLC analysis showed that the major phenolic compounds in the extract were caffeic acid (15971.41 µg/g), 2,5-dihydroxybenzoic acid (11469.11 µg/g) and epicatechin (7243.33 µg/g). These results indicate that *H. europaeum* possesses potent antioxidant and inflammatory effects associated with its bioactive phenolic constituents.

Keywords: *Heliotropium europaeum*, pro-inflammatory cytokines, antioxidant, phenolic compounds

INTRODUCTION

A great number of medicinal plants in Turkey have widely been used in many fields such as food, tea, spices, ornaments, smell, taste industry, perfumes and cosmetics by the people for many years [1]. Plants contain thousands of constituents and are a valuable source of new and biologically active molecules. Identifying plants with therapeutic properties is a great purpose of novel research. In recent years, the requisition for medicinal plants and their individual bioactive components have dramatically enhanced due to the comprehensive growth of nutraceutical, pharmaceutical, cosmeceutical, or food industries [2].

Heliotropium is a genus of flowering plants in the Boraginaceae family, which is distributed through temperate regions but more abundantly in the Mediterranean region [3]. There are 270 to 330 species in this genus, which are commonly known as heliotropes. The word “heliotrope” in Greek, has been remarked for plants that have the characteristic of turning their leaves in the orientation of the sun [4, 5]. In Turkey, this genus is represented by 16 species, 4 of which are endemic [6]. *Heliotropium europaeum* L. (called ‘akrep otu’ in Turkish) possesses a wide variety of biological activities, including antitumor, anti-inflammatory, antimicrobial, insecticidal and antispasmodic effects and is used as antipyretic, cholagogue, emmenagogue, cardiotonic and anthelmintic, in the treatment of gastrointestinal disorders, headache and gout. Moreover, it is applied topically for snake bites and scorpion stings and for promoting wounds [7-9]. Besides recent studies have revealed the antibacterial and antifungal activities of *H. europaeum* essential oil [7]. Twenty-six compounds were identified in the essential oil of *H. europaeum* represented 91.4% of the total essential oil [10]. The essential oils of some *Heliotropium* species can be used in cosmetics, aromatherapy, massage, perfumery, soap and candle making.

Inflammation is a body's natural defense system triggered by a variety of harmful stimuli such as damaged cells, pathogens, lipopolysaccharides, or irritants. Immune cells, especially macrophages, release biochemical mediators, such as pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha (TNF-α) interleukin-6 (IL-6) and IL-12), nitric oxide (NO) and chemokines to coordinate the immune reaction that will eliminate the inflammatory triggers and promote tissue repair and recovery [11]. A prolonged inflammatory response is often associated with chronic diseases such as cancer, arthritis and autoimmune disorders. In ancient times, inflammation and its related disorders treated with some plants or plant-based treatments including herbs, weeds, fruits and vegetable parts. It has been demonstrated by experimental studies that different plant-based extracts and their chemical constituents possess the anti-inflammatory activity [12, 13]. Macrophages are essential for host immunity and by detecting pathogenic substances, they initiate and regulate inflammatory responses. Lipopolysaccharide (LPS), an endotoxin derived from Gram-negative bacteria, is a powerful activator of macrophage cells, and activated macrophages are known to produce inflammatory mediators [14].

Plant extracts have been used as natural therapeutic agents against inflammation, characterised by an overproduction of inflammatory mediators such as reactive oxygen species (ROS) and pro-inflammatory cytokines [15]. Recently, there has been considerable interest in finding natural antioxidants from plant materials as alternatives to synthetic ones. Antioxidants are substances that neutralise both free radicals and their negative effects. The data from both scientific reports and laboratory studies show that plants contain a wide range of substances that possess antioxidant activities [16]. Phytochemical analysis of *H. europaeum* indicated the presence of alkaloids, terpenoids, steroids, saponins, flavonoids, phenols, and tannins [7]. Also, the fatty acid composition of the seed oils of *H. europaeum* was reported in a previous study [17]. Most of the previous reports were focused on the alkaloid content and the associated toxic effects, while other active metabolites and their beneficial biological activities were mostly neglected. In this respect, the present study for the first time aimed to investigate the effects of *H. europaeum* on LPS-induced inflammatory responses of murine RAW 264.7 macrophages. Additionally, the antioxidant activities and phenolic and flavonoid contents of *H. europaeum* were also determined.

MATERIALS AND METHODS

CHEMICALS

The chemicals and reagents for the HPLC, antioxidant and anti-inflammatory assays were purchased from Sigma-Aldrich (Germany), Merck (USA) and Boster Biologicals (USA).

PLANT MATERIAL AND EXTRACTION

The individuals of *H. europaeum* were collected in July 2014 from Denizli-Turkey (Pamukkale University Kınıklı Campus, maquis shrubland, 430 m) and identified (Voucher No: C. Ozay 3002). The aerial parts of the plant were air-dried and coarsely ground. The extracts were prepared using ethanol, methanol, chloroform, and water in a shaker water bath for 12h. Subsequently, the extracts were concentrated at reduced temperature and pressure and lyophilised. The crude extracts were kept at -20°C until needed. The dried extracts were weighed to determine the percent of yield [18]. The percentage yield was obtained using this formula W1/W0 × 100. Where W1 is the final weight of the extract and W0 is the initial weight of sample.

DETERMINATION OF TOTAL SECONDARY METABOLITES AMOUNT

To obtain total levels of phenolic, flavonoid and saponin contents in the extracts, colorimetric assays were used as described in the previous paper [19]. These contents were expressed as gallic acid (mg GAEs/g), quercetin (mg QEs/g) and quillaja (mg QAEs/g) equivalents, respectively.

PHENOLIC COMPOUND CHARACTERİZATION BY HPLC

Phenolic compounds of *H. europaeum* were analysed by RP-HPLC (Shimadzu, Japan) as described by Caponio et al. [20] with some modifications. Separation was performed at 30°C by using a reversed phase column (Agilent Eclipse XDB C-18, 250 mm × 4.6 mm, 5 µm) using the mixture of two solvents (A: the acetic acid solution 3% and B: methanol) as a mobile phase. Gradient conditions were particularised at a flow rate of 0.8 mL/min as follows: 93% A + 7% B for 0-20 min, 72% A + 28% B in 20-28 min, 75% A + 25% B in 28-35 min, 70% A + 30% B in 35-60 min, 67% A + 33% B in 60-62 min, 58% A + 42% B in 62-70 min, 50% A + 50% B in 70-73 min, 30% A + 70% B in 73-75 min, 20% A + 80% B in 75-80 min, 0% A + 100% B in 80-81 min, 93% A + 7% B in 81-90 min. Phenolic compounds in the methanolic extract of *H. europaeum* were expressed as µg/g extract, which were analysed with diode array dedector (DAD) at 280 nm (for the phenolic acids) and 320 and 360 nm (for the flavones, flavonoles). The identification of each target compound was based on a combination of retention time and spectral matching.

ANTIOXIDANT ACTIVITY ASSAYS

- DPPH radical scavenging activity

When the 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical gets an electron from the antioxidant compound, the color changes from violet to yellow which is detected colorimetrically. The impact of the extracts on DPPH radical was determined with the method described by Ozay and Mammadov [18] with slight modifications. Various concentrations of the extracts were added to DPPH metanolic solution, and the mixture was incubated for 30 min in the dark at room temperature. After incubation, the absorbances were measured at 517 nm. The synthetic antioxidant, butylated hydroxytoluene (BHT) was used as positive control. The results were indicated as the half-maximal inhibitory concentration (IC_{50}) values.

- ABTS radical cation activity

The ABTS assay is based on the generation of a blue/green 2,2' azino-bis (3-ethylbenzothiazloine-6 sulfonic acid) (ABTS) radical cation that can be reduced by antioxidants. ABTS radical removal activity was analyzed as described by Re et al. [21] with some modifications. Freshly prepared and diluted ABTS solution were mixed with the various solvent extracts of *H. europaeum* and the absorbances were read after 30 min at 734 nm. BHT was used as positive control. The results were indicated as IC_{50}.

- β-Carotene/linoleic acid assay

The ability of the extracts regarding the inhibition of linoleic acid oxidation was investigated using β-carotene test system according to Ozay and Mammadov [18]. This method is based on the monitoring of the color opening of β-carotene by alkyl peroxides formed by free radical chain reaction by heat and air oxidation of linoleic acid. The results were calculated with the formula using the initial and final absorbances of samples and control group.

$[1 - (A_C - A_S / A_C^o - A_S^o)] \times 100$

where A_C and A_C^o were absorbance values initial and final measurement (120 min) of contol group; A_S and A_S^o were absorbance values of samples or standard, respectively. BHT was used as standard antioxidant.

- Phosphomolybdenum (PM) assay

This assay is based on the reduction of Mo (IV) to Mo (V) with antioxidant agents. The green color resulting from the reduction is measured at 695 nm. PM assay of *H. europaeum* extracts carried out Prieto et al. [22]. Results are given as ascorbic acid equivalents (mg AEs/g extract).

- Ferric reducing antioxidant power (FRAP) assay

The principle of this method is based on the reduction of a Fe(III)-tripyridyltriazine (TPTZ) complex to Fe(II)-TPTZ in the presence of antioxidants. The results measured at 593 nm are given as equivalent to trolox (mg TEs/g extract). This assay was carried out according to Apak et al. [23].

- Metal chelating activity

The ferrous chealating capacity of *H. europaeum* extracts was determined with Dinis et al. [24]'s method with slight modification. According to this method, extracts inhibit ferrozine complexing with Fe^{2+} and color expansion is determined spectrophotometrically (562 nm). The results were provided as ethylenediaminetetraacetic acid (EDTA) equivalents (mg EDTAEs/g extract).

- Cell viability assay

The extract used in all cell culture assays was diluted in the growth media of the RAW 264.7 cell line. The vehicle for the initial stock of the extract was 0.1% dimethyl sulfoxide (DMSO). The effect of *H. europaeum* on cell viability was determined by MTT assay [41]. RAW 264.7 cells were mechanically scraped and plated at 5×10^3 cells/well in 96-well plates containing 100 μl of DMEM medium with 10% heat-inactivated FBS and incubated for 24 h (37°C and 5% CO_2). After incubation, the cells were treated with different concentrations of the extract (50-400 mg/mL) for 24 h. Then, MTT solution was added to the wells and incubated for 4h at 37°C. Finally, DMSO was added to dissolve the formazan crystals and absorbance was measured at 570 nm. Cell viability (%) was calculated as follows:

Cell Viability (%) = $(Abs_{sample} / Abs_{control}) \times 100$

- Griess nitrite assay

The NO production was measured as described by Han et al. [25]. RAW 264.7 cells at 5×10^3 cells/well were cultured in 96-well plates in triplicate for 24 h and preincubated with different concentrations of the extract (50-200 μg/mL) for 1 h and were then stimulated with LPS (1 μg/mL). The culture supernatants were collected 24 h after the LPS stimulation and the concentrations of NO were measured according to the Griess reaction. 50 μL of supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine in 5% phosphoric acid) and incubated for 10 min at room temperature, then the absorbance was measured at 550 nm. Finally, the concentration of nitrite was calculated from a standard curve ($y = 0.0123x + 0.0185$, $R^2 = 0.9995$) drawn with known concentrations (1-100 μM) of sodium nitrite dissolved in DMEM.

- Pro-inflammatory cytokine determination

RAW 264.7 cells were pretreated with different concentrations of the extract (50-200 μg/mL) for 1 h and

Table I - Extract yield and total secondary metabolites amount of *H. europaeum* according to different solvents (mean ± SD)

	Chloroform	Ethanol	Methanol	Water
Extraction yield (%)	5.13±0.11^{a}	11.48±0.17^{a}	16.51±0.20^{b}	20.12±0.14^{b}
TPA (mg GAEs/g)	13.04±0.20^{a}	27.35±0.28^{b}	42.01±0.43^{c}	33.11±0.34^{b}
TFA (mg QEs/g)	5.01±0.09^{a}	20.64±0.27^{b}	38. 14±0.45^{c}	27.45±0.32^{b}
TSA (mg QAEs/g)	nd	11.09±0.15^{a}	18.22±0.22^{a}	30.35±0.46^{b}

TPA: total phenolic amount; *TFA:* total flavonoid amount; *TSA:* total saponin amount; *GAEs:* gallic acid equivalents; *QEs:* quercetin equivalents; *QAEs:* quillaja equivalents, *nd:* not detected. In each row, different letters indicate significant difference ($P \leq 0.05$)

then LPS (1 µg/mL) was added to the treatment cells to activate the macrophages. After 24 h of cell incubation, the supernatants of cell cultures were collected and used to measure the levels of TNF-α, IL-6, and IL-12 proteins using ELISA kit (Boster, USA) according to the manufacturer's instructions. ELISA results were recorded using a microplate spectrophotometer at 450 nm and the corrected absorbance was noted as per manufacturer's instruction. Results were expressed in pg/mL. Each sample was measured in triplicate and values were derived from the standard curve.

- Statistical analysis

The results obtained in this study are expressed as mean ± standard deviation (SD). Statistical analysis and data processing were performed by using SPSS 22.0. Comparisons of the treatments among groups were analysed by one-way ANOVA with post-hoc Tukey's test. Significance was accepted as $P \leq 0.05$.

RESULTS AND DISCUSSION

This contemporary age is extensively involved in herbal medicine investigation as witnessed by plenty of peer-reviewed research on herbs published by the research groups. A vast number of novel insights have been asserted, and many conventional beliefs have been confirmed, refused, or adopted again. However, even by conceding the abundance of research being performed, the search for novel drugs from plants stand before us as an ongoing necessity [26, 27].

Heliotropium is one of the most significant plant genera to have conventional folklore significance, hence is a potential source of bioactive compounds [28]. In this study, total phenolic content of *H. europaeum* extracts was calculated equivalent to gallic acid and the highest content was observed in methanol extract (42.01 ± 0.43 mgGAEs/g). Our results showed that methanol extract have the highest total flavonoid amount (38.14 ± 0.45 mgQEs/g), while water extract have the highest total saponin amount (30.35 ± 0.46 mgQAEs/g). Total saponin content could not be determined in the chloroform extract, because when the extract was mixed with the highly polar vanillin-sulfuric acid solution, a cloudy emulsion was formed. Therefore, the saponin content could not be measured spectrophotometrically in the chloroform extract. The yield of extracts from *H. europaeum* is listed in Table I. The efficiency of extracts prepared with four solvents with different polarities was calculated. The highest extract amount obtained from water extract (% 20.12). This result can be related to that water have the highest polarity. However, methanol, ethanol and chloroform present the lower extraction yield with a percentage of 16.51%, 11.48% and 5.13%, respectively.

Although there are many studies on alkaloids of the genus *Heliotropium*, few studies have described its phenolic compounds [29, 30]. Because methanol extract has a higher antioxidant capacity and total secondary metabolites amount than others, methanol extract was used in HPLC analysis and anti-inflammatory activity assays. To identify the phenolic compounds in the methanolic extracts of the aerial parts of *H. europaeum*, 15 standard compounds (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, chlorogenic acid, vanillic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, ellagic acid, naringin, cinnamic acid, quercetin) were used in the HPLC analysis. The phenolic compounds were detected in the extract with varying amounts and were listed in Table II. According to these results, caffeic acid (15971.41 µg/g), 2,5-dihydroxybenzoic acid (11469.11 µg/g), epicatechin (7243.33 µg/g) and quercetin (4465.59 µg/g) are most common phenolic compounds of methanol extract of *H. europaeum.* It has been reported that these phenolic acids possess anti-inflammatory and anti-oxidative activities [31-33]. The results of the present study are in accordance with the results of Jasim and Hamad [30]. Six phenolic compounds (syringic acid, silybin, kaempferol, apigenin, caffeic acid and genistein) were isolated and identified from Iraqi *H. europaeum* plant. According to their result, one of the most abundant phenolic compounds in *H. europaeum* extract was caffeic acid. Al-Saleem et al. [28] reported that eight phenolic compounds were isolated from the aerial parts of *H. europaeum,* which was collected from Saudi Arabia. The isolated compounds were identified as kaempferol (1), luteolin (2), quercetin (3), kaempferol-3-O-glucoside (4), and luteolin-7-O-glucoside (5), in addition to caffeic acid (6), rosmarinic acid (7), and methyl rosmarinate (8). These phenolic metabolites from *H. europaeum* exhibited antioxidant, angiotensin-converting enzyme

(ACE) and lipoxygenase (LOX) inhibitory activities.

It is known that phenolic compounds are molecules with antioxidant activity due to their hydroxyl groups and phenolic rings [34]. To determine the antioxidant activity of *H. europaeum* extracts with different solvents, we preferred six diverse methods to compare the results with each other and provide more reliable data. The antioxidant activity of the extracts is presented in Table III. β-carotene/linoleic acid and phosphomolybdenum assay were used to evaluate total antioxidant capacity and the water extracts (%75.06 ± 1.46) have the highest antioxidant activity according to β-carotene/linoleic acid assay, but methanol extracts (85.24 ± 0.62 mgAEs/g) possess most effective activity in phosphomolybdenum assay. DPPH and ABTS radical scavenging assays are commonly carried out for fast evaluation of antioxidant activity because of their stability in the radical form and simplicity of the assay. In both DPPH (IC_{50}: 28.02 ± 0.37 µg/mL) and ABTS (IC_{50}: 37.55 ± 0.06 µg/mL) tests, methanol extracts showed the highest radical scavenging activity. In a previous study, the highest DPPH radical scavenging activity of *Heliotropium strigosum* was observed in ethyl acetate (94.5%) and aqueous (94.7%) fractions [3]. When the DPPH assay was applied to the *Heliotropium samolifolium* subsp. *erzurumicum*, the highest activity was found in above-ground ethanol+aqueous, chloroform and below-ground ethanol extracts [1]. FRAP assay was carried out to determine the reduction power of the extracts. Results which are equivalent to trolox standard, show that aqueous extracts have high reducing power activity (97.08 ± 1.67 mgTEs/g). And also, ferrious metal chelating capacity of the extracts were evaluated, and results were given as the standard equivalent of EDTA. According to this test, methanolic extract had highest chelating capacity (32.07 ± 0.65 mgTEs/g) ($P \leq 0.05$). Due to the different antioxidant potentials of compounds with different polarity antioxidant activity of the extracts are strongly dependent on the solvent type [19].

The total phenolic and flavonoid amount of *H. crispum* methanol extract were reported earlier as 24.84 mg GAE/g and 19.73 mg REs/g, respectively [35]. According to these results, *H. europaeum* methanolic extract used in the current study had more abundant (42.01 mg GAEs/g) total phenolic amounts than *H. crispum.* Savran et al. [36] have demonstrated that methanol extracts from different plant species remarkable bioactive components, which is in agreement with the present results. In present study, some phytochemical analysis and biological activity of *H.*

Table II - Phenolic compounds characterization of methanolic *H. europaeum* extract by HPLC (mean ± SD).

No	Identified phenolic compounds	RT (min)	UV_{max} (nm)	LOD (µg/mL)	µg/g extract (mean ± SD)
1	Gallic acid	6.8	280	0.015	47.83 ± 0.42
2	3,4-dihydroxybenzoic acid	10.7	280	0.031	68.95 ± 0.61
3	4-hydroxybenzoic acid	15.7	280	0.014	70.64 ± 0.65
4	2,5-dihydroxybenzoic acid	17.2	320	0.753	11469.11 ± 228.2
5	Chlorogenic acid	18.2	320	0.011	307.34 ± 3.11
6	Vanillic acid	19.2	320	0.112	847.95 ± 6.47
7	Epicatechin	21.3	260	0.433	7243.33 ± 120.1
8	Caffeic acid	22.7	280	0.018	15971.41 ± 250.3
9	*p*-coumaric acid	26.1	320	0.020	468.76 ± 4.24
10	Ferulic acid	30.1	320	0.012	241.47 ± 2.54
11	Rutin	45.6	360	0.576	217.76 ± 2.15
12	Ellagic acid	47.7	240	0.455	1345.09 ± 15.10
13	Naringin	49.7	280	0.404	109.95 ± 1.17
14	Cinnamic acid	67.8	280	0.016	265.02 ± 2.71
15	Quercetin	71.1	360	0.578	4465.59 ± 74.20

RT: retention time, LOD: limit of detection

Table III - Antioxidant activity of *H. europaeum* extracts (mean ± SD).

	Chloroform	Ethanol	Methanol	Water	BHT
β-carotene/linoleic acid assay (% inhibition)	50.01±1.10^{a}	62.11±1.35^{a}	70.13±1.40^{b}	75.06±1.46^{b}	95.02±1.66^{c}
Phosphomolybdenum assay (mg AEs/g)	61.76±1.33^{a}	73.08±1.46^{a}	85.24±0.62^{b}	78.44±0.48^{a}	nd
DPPH assay (IC_{50} value, µg/mL)	125.06±1.84^{d}	35.82±0.68^{b}	28.02±0.37^{b}	84.15±0.51^{c}	9.40±0.05^{a}
ABTS assay (IC_{50} value, µg/mL)	120.54±1.80^{d}	45.16±1.13^{b}	37.55±0.06^{b}	71.26±1.42^{c}	20.15±0.30^{a}
FRAP assay (mg TEs/g)	32.27±0.01^{a}	86.03±0.67^{b}	92.00±1.72^{b}	97.08±1.67^{b}	nd
Metal chelating activity (mg EDTAEs/g)	11.04±1.07^{a}	15.71±0.27^{a}	32.07±0.65^{b}	26.48±0.30^{b}	nd

AEs: ascorbic acid equivalents, *TEs:* trolox equivalents, *EDTAEs:* EDTA equivalents, *nd:* not detected. In each row, different letters indicate significant difference ($P \leq 0.05$)

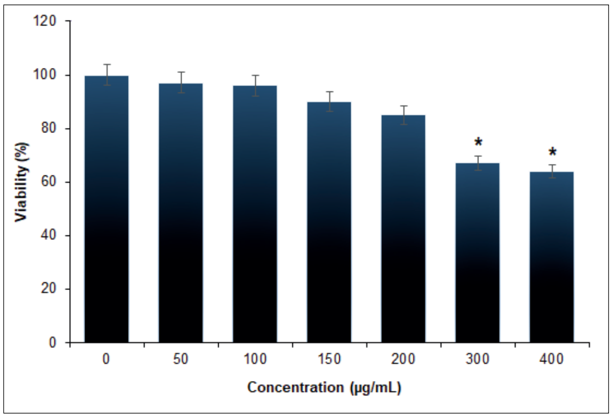

Figure 1 - Effect of *H. europaeum* extract on cell viability of RAW 264.7 cell line. Data are presented as mean ± SD. **P* ≤0.05

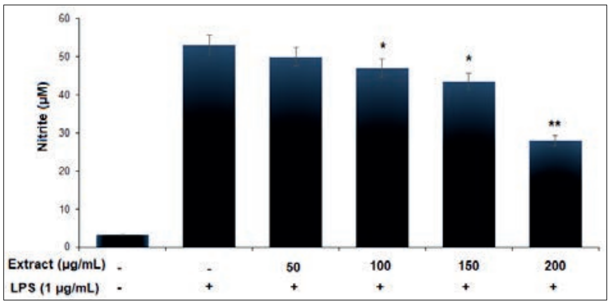

Figure 2 - Effect of *H. europaeum* extract on nitric oxide production by LPS-induced macrophage RAW 264.7 cells. Data are presented as mean ± SD. **P* ≤0.05, ***P* ≤0.01; compared to the LPS-treated group.

europaeum extracts was determined. Our studies were revealed that *H. europaeum* methanolic extracts possess the high total phenolic, flavonoid amount and also total antioxidant capacity, radical scavenging activity and chealating power. The results on the total phenolic content in this study displayed a similar propensity to those of the antioxidant abilities. Accordingly, the high content of total phenolics in the extracts might explain the antioxidant properties of the extracts. These results were consistent with other results in literature which demonstrated a strong relationship between antioxidant activities and total phenolic contents [18, 37].

Drugs for inflammation treatment are effective but have serious side effects when used for a prolonged time. For this reason, it is crucial to search for new and safe anti-inflammatory agents. Medicinal plants are a valuable source of novel molecules and efficient alternative strategy for newer therapeutics development [38]. The potential anti-inflammatory effects of *H. europaeum* were examined and RAW 264.7 murine macrophage cell line, which is frequently used as an in vitro model in studies of inflammation, was selected in this study. Detection of suitable concentration ranges, which are not toxic, can be used for further *in vitro* anti-inflammatory screening assays of the extracts. Cytotoxicity assay was performed for methanol extracts at different concentrations (50-400 µg/mL) and the results were shown in Figure 1. The viability of RAW 264.7 cells decreased below 70% after 24 hours exposure to extracts at 300 and 400 µg/mL. Other concentrations of the extracts, that resulted in a cell viability > 70%, were used for the nitrite assay. Therefore, the possibility that the observed NO production inhibitory activities were due to the cytotoxicity was excluded.

Because NO is considered as an important parameter of inflammation and NO production is related with many inflammatory diseases, we investigated the effect of *H. europaeum* on LPS-induced NO production in RAW 264.7 macrophages by using Griess assay. The quantity of nitrite in the culture medium was measured as an indicator of NO production. Amount of nitrite (µM), a stable metabolite of NO, significantly decreased due to the rising extract concentration (Fig. 2). A 200 µg/mL *H. europaeum* methanol extract demonstrated the highest nitrite inhibitory activity. As for the pro-inflammatory cytokines (TNF-α,

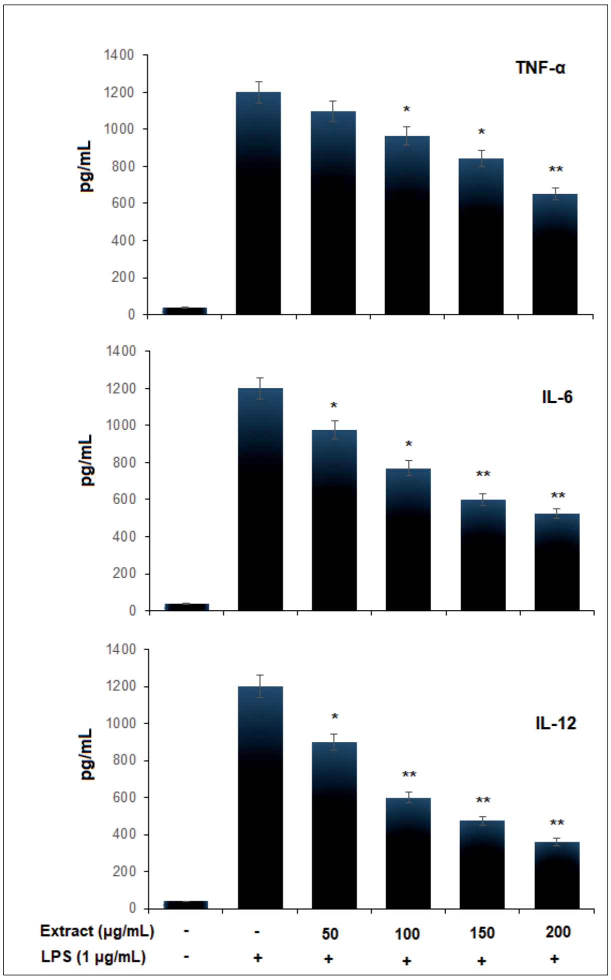

Figure 3 - Effects of *H. europaeum* extract on TNF-α, (a) and IL-6 (b) and IL-12 (c) generation by LPS-induced RAW 264.7 macrophage cells. Data are presented as mean ± SD. **P* ≤0.05, ***P* ≤0.01; compared to the LPS-treated group.

IL-6 and IL-12) levels, *H. europaeum* has a greater effect on decreasing IL-6 and IL-12 levels than TNF-α level. TNF-α, IL-6 and IL-12 are three major macrophage-derived mediators of inflammatory responses in mammals. In this respect, it is important to investigate their quantities. As shown in Figure 3, there was a 3-fold reduction in LPS induced IL-12 production in RAW 264.7 cells pre-treated with 200 µg/mL of extract. Two major cytokines; TNF-a and IL-6 are implicated in the pathogenesis of rheumatoid arthritis, ulcerative colitis and several other inflammatory disorders [39]. A previous study showed that isolating two compounds (4,7,8-trimethoxy-naphthalene-2-carboxylic acid and 6-hydroxy-5,7-dimethoxy-naphthalene-2-carbaldehyde) from *Heliotropium ovalifolium* inhibited the release of IL-6 and TNF-a by LPS-stimulated THP-1 (human monocytic cell line) cells [40]. In another study, the whole-plant butanol fraction of *Heliotropium europaeum* showed anti-inflammatory activity on ROS [41].

CONCLUSIONS

Phenolic compounds possess a remarkable anti-inflammatory capacity due to their multiple inhibitory activities of pro-inflammatory mediators. In this study, we first demonstrated the anti-inflammatory effect of *H. europaeum* using RAW 264.7 macrophage cells. *H. europaeum* inhibited not only NO production but also the levels of proinflammatory cytokines. The antioxidant and anti-inflammatory activities observed could be related to the polyphenolic compounds in the extract. However, the isolation of bioactive compounds and analysis of the molecular mechanisms responsible for the potential anti-inflammatory effect should be performed.

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Gli amminoacidi analizzati includono sia i 20 standard che quelli fisiologici (fino a 40 composti diversi), presenti nel campione in forma libera o dopo idrolisi delle proteine. L'analisi è effettuata mediante un analizzatore automatico che impiega la cromatografia a scambio cationico e la derivatizzazione post-colonna con ninidrina per la separazione e la quantificazione.

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- Determinazione degli amminoacidi **solforati** (metionina e cist(e)ina)
- Determinazione del **triptofano**

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Short note

Chemical components and cytotoxicity of *Syzygium filiforme* var. *filiforme* (Myrtaceae)

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Syzygium filiforme var. *filiforme* Chantar. & J.Parn. is a plant variety from the dicotyledonous plant family (Myrtaceae). This work is designed to examine the chemical composition and cytotoxicity of *Syzygium filiforme* essential oil. The essential oil was obtained through hydrodistillation and the volatile components were analysed using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The cytotoxicity of essential oil was evaluated using an MTT assay. A total of 34 chemical components, which constitute 96.1% of oil content, were successfully identified. The most abundant components were α-cadinol (23.3%), t-muurolol (9.1%), geraniol (6.6%), germacrene D (6.3%), and γ-gurjunene (5.0%). The essential oil exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7, and A549 with the IC_{50} values ranging from 70.2-88.1 µg/mL. The current study highlights the potential of the use of essential oils as an alternative to the development of pharmaceutical anti-chemopreventive or cosmetics.

Keywords: *Syzygium filiforme*, Myrtaceae, essential oil, GC-MS, cytotoxicity, α-cadinol

1. INTRODUCTION

Essential oils can be extracted from a variety of plant parts, including leaves, flowers, roots, seeds, fruits, and wood. They are made up of volatile aromatic compounds that come from the secondary metabolism of plants. Mono- and sesquiterpenes, alcohols, aldehydes, ketones, and ethers are only a few of the compounds that make up essential oils. In plants, essential oils play a protective role against herbivores and solar radiation and in attracting pollinating insects. Essential oils have been the focus of several studies because they have several bioactive chemical substances with a great potential for use, and several biological activities have been demonstrated, such as antioxidant, antimicrobial, anti-inflammatory and antifungal activities. Their uses range from agriculture to therapies to treat diseases. Many plants produce essential oils, including species of the Myrtaceae family [1-5].

The Myrtaceae family has nearly about 55,000 species classified into two subfamilies, 17 tribes, and 142 genera. The species are evergreen shrubs or woody trees and are mainly found in North Africa and South America along the Mediterranean [6]. *Syzygium* is the largest genus in the Myrtaceae family, with a wide range of species throughout Asia's tropical regions. The genus consists of about 1800 species and can be found mainly in Southeast Asia, Southern China, Australia, New Caledonia, East Africa, Madagascar, the Mascarenhas Islands, the Southwest Pacific Islands, Taiwan, and Southern Japan [7].

Syzygium species have a long history of use in traditional medicinal systems. For instance, *S. aromaticum* has promising inhibitory activities on fatty acid synthase, which in turn helps reduce food intake and subsequently induces weight loss [8]. It is also well-known for its anticancer effects and has been the focus of much research [9]. Besides, *Syzygium* species is also renowned for having a high concentration of volatile oils, especially in the parts of the fruit [10]. Meanwhile, the species have been reported previously to treat diabetes, diarrhoea, stomach-aches, colds, and ulcers [11].

Syzygium filiforme var. *filiforme*, locally known as *kelat merah* or *kelat manik* in Malaysia, is mainly distributed in Southwest Thailand, Peninsular Malaysia, and Singapore. It is a tree that can grow up to 42 m tall, and attain 210 cm in trunk girth size, with a dense crown, drooping branches, and slender twigs. It is mainly found in terrestrial (primary rainforest, secondary rainforest, freshwater swamp forest) and tropical forests. It is harvested for timber and used for house posts [12]. According to the literature study, phytochemical studies of *S. filiforme* extracts produced triterpenoid compounds [13], which showed antioxidant, antimicrobial, and antiglucoside activity [14].

In continuation of our search for bioactive components from Malaysian species [15-20], we have investigated the chemical composition present in the leaves oil of *S. filiforme.* To the best of our knowledge, this is the first report on the essential oil of this species and its cytotoxicity.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

The leaves of *Syzygium filiforme* were collected from Fraser Hill, Pahang (N 3°44’43.8756”, E 101°26’59.1864”) (January 2019) and identified by Shamsul Khamis. The voucher specimen (SK359/19) was deposited at UKMB Herbarium.

2.2. EXTRACTION OF ESSENTIAL OIL

The fresh leaves (300 g) were subjected to hydrodistillation in Clevenger-type apparatus for 4 hours. The essential oil obtained was dried over anhydrous magnesium sulphate and stored at 4-6°C. The oil yield (w/w) was 0.12% based on a fresh weight basis.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) analysis was performed on an Agilent Technologies 7890B equipped with a DB-5 capillary column (30 m long, 0.25 µm thickness, and 0.25 mm inner diameter). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. The injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min, and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times, and the peak area percentage was reported as means ± SD of triplicates. Calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies). Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using an Agilent Technologies 7890A/5975C MSD equipped with HP-5MS fused silica capillary column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as 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 ionisation system, with an ionisation energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu.

2.4. IDENTIFICATION OF OIL COMPONENTS

For the identification of essential oil components, co-injection with the standards was used, together with correspondence of their retention indices and mass spectra as reported in Adams [21], NIST 08, and FFNSC2 libraries. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components.

2.5. CYTOTOXICITY ASSAY

Cytotoxic examination of the essential oil was carried out using the MTT assay [22]. 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.

Inhibitory percentage (I%) = $(1 - OD_{sample}/OD_{conc}) \times 100\%$; where OD_{sample} and OD_{conc} stand for 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

The essential oil components identified with their percentages are listed in Table 1 in order of their elution from the HP-5 column. The GC-FID and GC-MS analysis of the essential oil revealed the presence of thirty-four chemical components with a constitution of 96.1%. The identified components were oxygenated sesquiterpenes (10 components) (48.2%), sesquiterpene hydrocarbons (23 components) (41.3%), and oxygenated monoterpene (1 component) (6.6%). The most abundant components were α-cadinol (23.3%), t-muurolol (9.1%), geraniol (6.6%), germacrene D (6.3%), and γ-gurjunene (5.0%). Meanwhile, other minor components which exceeded >2%, were

Table I - Chemical components identified in *Syzygium filiforme* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)[c]	Identifications[d]
1	Geraniol	1250	1249	6.6 ± 0.2	RI, MS, Std
2	δ-Elemene	1335	1335	0.8 ± 0.1	RI, MS
3	α-Copaene	1375	1374	2.5 ± 0.1	RI, MS
4	β-Bourbonene	1385	1386	0.3 ± 0.2	RI, MS
5	β-Elemene	1390	1390	1.7 ± 0.1	RI, MS
6	α-Cedrene	1408	1410	0.3 ± 0.1	RI, MS
7	β-Caryophyllene	1415	1416	3.7 ± 0.2	RI, MS
8	γ-Elemene	1434	1435	3.3 ± 0.1	RI, MS
9	Aromadendrene	1440	1440	0.4 ± 0.2	RI, MS
10	α-Humulene	1450	1452	0.9 ± 0.2	RI, MS
11	Alloaromadendrene	1456	1458	0.2 ± 0.1	RI, MS
12	γ-Gurjunene	1470	1470	5.0 ± 0.2	RI, MS
13	γ-Muurolene	1476	1478	2.4 ± 0.2	RI, MS
14	Amorpha-4,7(11)-diene	1480	1479	0.6 ± 0.1	RI, MS
15	δ-Amorphene	1483	1485	0.5 ± 0.2	RI, MS
16	Germacrene D	1484	1485	6.3 ± 0.1	RI, MS, Std
17	β-Selinene	1490	1489	0.9 ± 0.2	RI, MS
18	γ-Selinene	1492	1492	0.5 ± 0.2	RI, MS
19	Bicyclogermacrene	1500	1500	2.5 ± 0.1	RI, MS
20	α-Muurolene	1501	1500	1.3 ± 0.1	RI, MS
21	γ-Cadinene	1513	1512	0.7 ± 0.1	RI, MS
22	δ-Cadinene	1522	1523	3.6 ± 0.2	RI, MS
23	Zonarene	1528	1530	0.5 ± 0.2	RI, MS
24	α-Cadinene	1537	1537	2.4 ± 0.1	RI, MS
25	Spathulenol	1577	1578	3.0 ± 0.2	RI, MS
26	Caryophyllene oxide	1582	1584	1.2 ± 0.1	RI, MS
27	Globulol	1590	1590	4.3 ± 0.2	RI, MS
28	Rosifoliol	1600	1600	1.2 ± 0.2	RI, MS
29	Ledol	1602	1602	0.7 ± 0.1	RI, MS
30	Junenol	1618	1618	2.7 ± 0.2	RI, MS
31	t-Muurolol	1645	1644	9.1 ± 0.1	RI, MS, Std
32	α-Cadinol	1653	1652	23.3 ± 0.1	RI, MS, Std
33	Selin-11-en-4α-ol	1685	1658	2.4 ± 0.2	RI, MS
34	β-Acoradienol	1762	1762	0.3 ± 0.1	RI, MS
Group components					
	Oxygenated monoterpenes			6.6	
	Sesquiterpene hydrocarbons			41.3	
	Oxygenated sesquiterpenes			48.2	
Identified components (%)				96.1	

[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

[d] RI, based on comparison of calculated RI with those reported in Adams; MS, based on comparison with Wiley; Std, based on comparison with standard compounds

globulol (4.3%), β-caryophyllene (3.7%), δ-cadinene (3.6%), spathulenol (3.0%), γ-elemene (3.3%), junenol (2.7%), α-copaene (2.5%), bicyclogermacrene (2.5%), γ-muurolene (2.4%), α-cadinene (2.4%), and selin-11-en-4α-ol (2.4%).

There have been no reports on the leaves part in this study. These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotypes, and nutritional status of the plants, which may influence their oil composition [23]. α-Cadinol has been identified as the major and most common sesquiterpene present in some *Syzygium* species. Among them are *S. cumini* (fruit oil, 25.8%) [18], *S. caryophyllatum* (leaf oil, 18.3%) [24], *S. samarangense* (leaf oil, 12.7%) [25], and *S. zeylanicum* (leaf oil, 12.2%) [26]. In addition, α-cadinol is also reported as the major component in other plants of the genus *Horsfieldia* [27], *Goniothala-*

mus [28], and *Beilschmiedia* [29]. Recently, the in-silico drug-likeness analysis showed that α-cadinol is appropriate for the human system with no predicted hepatotoxicity or mutagenicity (AMES toxicity) [30].

The essential oil was subjected to cytotoxic examination using an MTT assay. The essential oil showed activity against three cancer cell lines HepG2, MCF7, and A549 with the respective IC_{50} values of 88.1, 70.2, and 75.5 μg/mL, as compared with those of the positive control doxorubicin (IC_{50} 0.76 μg/mL for HepG2, IC_{50} 0.20 μg/mL for MCF7, and IC_{50} 0.95 μg/mL for A549). At the highest concentration of 100 μg/mL, the essential oil responses for inhibitory 69.5% at least. As the previous study showed that the α-cadinol was found to exhibit selective potent cytotoxicity in breast adenocarcinoma cells (MCF7) with IC_{50} value of 18.0 μg/mL [31]. The present result suggests that the occurrence of this compound as the major component (23.3%) could be associated to the cytotoxic activity detected in the essential oil. In addition, essential oils are complex mixtures of different volatile components, where their synergistic and antagonistic interactions could affect the effectiveness of oil samples as inhibitors [32]. Further studies must be carried out to understand better the basic mechanism involved in the activity of this essential oil.

4. CONCLUSION

This study is the first report on the composition of essential oil of *S. filiforme* growing in Malaysia. In the present study, the analysis of GC-FID and GC-MS of essential oil gave α-cadinol as the most abundant component and reveals significant cytotoxicity. This species may therefore become a source of natural products and is intended to further explore the development of chemotherapy or cosmetics. In addition, future studies are needed to assess the side effects, safety, and effectiveness of the *Syzygium* essential oil to facilitate its clinical application as modern medicine for human health.

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ANALISI PRODOTTI FINITI

- dosaggio di conservanti, antiossidanti, disinfettanti, filtri solari, metalli
- punto di infiammabilità, densità, viscosità, Ph
- stabilità all'ossidazione tramite Rancimat e Petroxy

CARATTERIZZAZIONE DI MATERIE PRIME

- oli e grassi vegetali, quali ad esempio, olio di Mandorle dolci, Rosa Mosqueta, Argan, Baobab, Avocado, Borragine, Enotera, Cartamo, Fico d'India, Oliva, burro di Cacao, burro di Karitè, lipidi dell'Amazzonia
- alcoli grassi, acidi grassi, esteri, cere di origine naturale (ad es. olio di jojoba)
- microalghe
- antiossidanti, conservanti, battericidi, filtri solari, siliconi volatili
- purezza e identificazione delle impurezze

PROFUMI PER PERSONA E PROFUMATORI PER AMBIENTE

∞ contenuto in etanolo
∞ composizione dell'essenza
∞ impurezze dell'alcool etilico
∞ sostanze allergeniche volatili
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GLI OLI MINERALI NELLA FILIERA AGROALIMENTARE

Paolo Bondioli, Igor Calderari, Giuliano Mosca

Le materie prime rappresentano il fattore della produzione biologica conosciuto e studiato ormai da tempo. Un approfondimento della loro conoscenza è possibile considerando la composizione immediata del vivente, visto come rapporto quantitativo fra costituenti caratterizzati da analogia di struttura e comportamento chimico-fisico. Tra i costituenti immediati, oltre all'acqua, ai carboidrati, alle proteine e ai sali minerali, vi sono anche i lipidi. È il gruppo di composti più eterogeneo sotto il profilo chimico-fisico, sono solubili in solventi organici e insolubili in acqua. I lipidi hanno la funzione biologica di riserva energetica e di veicolo di ormoni e vitamine, ma la loro funzione più importante resta tuttavia quella di natura strutturale. La cellula e le membrane, infatti, senza i lipidi non esisterebbero sotto forma differenziata, bensì indifferenziata. Il gruppo dei lipidi include oli e grassi, fosfolipidi, glicolipidi ed esteri. Il binomio coltura-fabbrica è oggi più che mai imprescindibile per poter parlare di sostenibilità. Il processo produttivo in una fabbrica è sostenuto da materie prime, energia, tecnologia e organizzazione. Il ruolo di ciascuno di questi fattori è ben evidente e stabilire il peso relativo di ciascun fattore in relazione all'efficienza produttiva di questa particolare "bio-fabbrica" appare dunque molto interessante. Accanto agli oli naturali si collocano quelli minerali di derivazione fossile molto usati nella lubrificazione ad esempio.

L'impatto sulla catena alimentare della contaminazione da lubrificanti a base di oli minerali, carburanti e prodotti tecnici vari diventa ogni giorno più evidente. In letteratura sono disponibili numerosi studi sulla contaminazione di numerose filiere agro alimentari.

La tossicologia correlata a queste sostanze non è omogenea: sostanzialmente gli oli minerali (MOH – Mineral Oil Hydrocarbons) possono essere suddivisi in due sottocategorie aventi tossicità estremamente differenziata: idrocarburi saturi alifatici e ciclici (MOSH – Mineral Oil Saturated Hydrocarbons) e idrocarburi aromatici (MOAH – Mineral Oil Aromatic Hydrocarbons). Sulla base delle più recenti pubblicazioni, anche dell'EFSA, gli appartenenti alla prima categoria non pare possano dar luogo a particolari problemi, mentre per i secondi sono in corso di valutazione le potenziali proprietà carcinogeniche per numerosi costituenti della famiglia.

In attesa del Parere EFSA, il Comitato Permanente per la Sicurezza Alimentare della Commissione Europea (cd Scopaff) nel 2022, dal punto di vista regolatorio, ha pubblicato uno Statement in cui gli Stati membri dell'UE hanno concordato dei limiti massimi per i MOAH per differenti prodotti alimentari e che, di fatto, ha valore cogente. La Comunità Europea, alcuni anni fa, ha dato mandato all'EFSA di realizzare un'indagine relativa all'attuale presenza di oli minerali negli alimenti e di valutarne, conseguentemente, la tossicità. Nello scorso mese di aprile, EFSA ha pubblicato una bozza di Parere Scientifico la cui versione finale è attesa per il prossimo mese di luglio.

Questa indagine dovrebbe consentire un'analisi dell'esposizione potenziale, cui farà seguito una normativa Europea recante limiti specifici per le varie tipologie di prodotto alimentare.

La contaminazione può derivare da gas di scarico di automobili, fuoriuscite di olio da dispositivi idraulici di macchine agricole, aerosol prodotti da motori a due tempi in uso durante l'attività in campo (soffianti ad aria, tagliaerba, scuotitori rami, ecc.). Può verificarsi contaminazione anche lungo la parte di filiera relativa al trasporto, stoccaggio e trasformazione dei prodotti alimentari.

Nel settore primario una delle dispersioni di olio più impattanti è stata identificata nei sistemi di lubrificazione a perdere (lost lubrication) legati all'uso di motoseghe in silvicoltura e nella pratica agricola per le fasi di manutenzione e potatura. L'uso di un lubrificante a perdere si rende necessario per garantire la corretta lubrificazione e il raffreddamento della catena e di altri dispositivi durante l'uso. L'olio lubrificante entra nell'ambiente sotto forma di aerosol generato, olii adsorbiti su residui di legno e segatura e direttamente sul terreno sottoforma di gocce.

Una possibile soluzione a questo problema può essere l’introduzione nella catena produttiva di fluidi definiti come biolubrificanti. I biolubrificanti sono prodotti organici che contengono almeno un legame estere tra uno o più acidi carbossilici di origine naturale ed un alcol, sia esso mono-, di- o polifunzionale, di origine naturale o sintetica. In ogni caso questi lubrificanti sono caratterizzati da biodegradabilità, ridotta volatilità, assenza di tossicità e di idrocarburi di origine minerale. e Sono in toto o in parte di natura rinnovabile e possono trovare altri impieghi a fine vita.

Per sua natura l'agricoltura svolge un ruolo centrale come fonte di materie prime e per l’attuazione di un continuo sviluppo di pratiche agricole sostenibili alla base di un'economia circolare. Tra le numerose coltivazioni le oleaginose (1) sono una fonte rinnovabile di bioprodotti come oli vegetali e biomasse ricche di molecole bioattive e proteine. Il potenziale utilizzo degli oli vegetali in diversi settori della chimica verde assume oggi particolare importanza in termini di sostenibilità ambientale, in linea con gli obiettivi del Green Deal europeo, grazie alla loro bassa tossicità ed eco-compatibilità. Gli oli vegetali sono noti per le loro potenziali applicazioni come fluidi idraulici, grazie alla loro elevata capacità di lubrificazione, ridotte perdite per evaporazione, elevati indici di viscosità e punto di infiammabilità, che ne consentono un uso, trasporto e stoccaggio in condizioni di maggiore sicurezza. Non va dimenticato anche il contributo fornito alla sicurezza degli operatori i quali, utilizzando biolubrificanti non saranno più esposti al contatto con vapori ed aerosol di discussa salubrità.

Di conseguenza, dopo un lungo periodo di ricerca e sperimentazione, sono state poste in essere una serie di iniziative aventi come oggetto i biolubrificanti, con lo scopo di:

- Promuovere l'impiego di oli vegetali quali possibili sostituti degli oli minerali, quando possibile con l’uso di additivi anch’essi biodegradabili o idonei all’uso alimentare in concentrazione la più bassa possibile, come lubrificanti lungo la catena alimentare;

- Contribuire alla riduzione della contaminazione da olio minerale negli alimenti e nei campi coltivati.

Nel corso di questi ultimi anni è stato sviluppato un solido background mediante diversi progetti di ricerca avviati sin dalla fine del secolo scorso, l'ultimo dei quali è stato il progetto AGROENER, promosso dal Ministero dell'Agricoltura Italiano e che ha portato alla progettazione e al test con fluidi idraulici a base di oli di Crambe abyssinica (2) e Carthamus tinctorius (3) minimamente raffinati e coltivati in regime di basso input agronomico. In un’ulteriore ricerca, quasi contemporanea, è stato preparato un fluido idraulico a base di olio di sansa di oliva raffinato. Anche per questo fluido sono stati realizzati test finali di valutazione (4).

La ridotta stabilità ossidativa rappresenta la principale barriera all’impiego degli oli vegetali ed è correlata alla loro natura chimica. Tuttavia è possibile ovviare a questo problema con una appropriata additivazione o al limite aumentando il turnover dei fluidi impiegati. Ovviamente questa caratteristica non ha impatto sulle applicazioni a perdere, ove vengono premiati altri fattori, quali la capacità di trasferimento del calore, la riduzione dell’attrito tra due parti in movimento, il consumo specifico delle macchine utilizzate ad esempio per il taglio.

Per quanto riguarda i problemi legati all’industria di trasformazione degli alimenti esistono lubrificanti di natura idrocarburica e di bassa tossicità, che negli Stati Uniti sono da tempo classificati secondo FDA/NSF, classificazione normalmente recepita anche in Europa.

Le diverse categorie catalogate da FDA/NSF per i lubrificanti idonei all’impiego alimentare sono:

- H1 - lubrificanti autorizzati per il contatto accidentale con gli alimenti
- H2 - lubrificanti che non devono entrare in contatto con gli alimenti
- H3 - coadiuvanti tecnologici utilizzati a diretto contatto con gli alimenti: lubrificanti per alluminio, agenti distaccanti per prodotti da forno, ecc.

Tutte le categorie elencate possono in qualche modo contribuire alla contaminazione degli alimenti da parte degli idrocarburi, anche se si tratta di oli bianchi esenti da idrocarburi aromatici o di oli di sintesi (polialfaolefine, polialchilenglicoli) per i quali è stata ipotizzata la possibilità di accumulo nei tessuti epatici.

Anche per la sostituzione di questa categoria di prodotti è possibile ipotizzare l’impiego di biolubrificanti a base di oli vegetali. Deve essere infine ricordato che oltre agli oli vegetali raffinati è possibile utilizzare una serie di derivati a base di acidi grassi e sostituenti alcolici diversi dal glicerolo, mediante i quali è possibile andare a soddisfare un’ampia gamma di applicazioni per le quali le caratteristiche di viscosità e stabilità al freddo degli oli vegetali non sono sufficienti a soddisfare le prestazioni richieste.

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INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

Analisi dei contaminanti

ACROLEINA

ANIDRIDE MALEICA

AMIGDALINA

BENZENE E DERIVATI

BISFENOLO A

COLORANTI

FORBOLI

FTALATI e PLASTICIZZANTI

FITOFARMACI

2, 3 MCPD, GLICIDOLO E DERIVATI

GOSSIPOLO

IDROCARBURI POLICICLICI AROMATICI

METALLI PESANTI

MICOTOSSINE

NICOTINA

OCTILFENOLO, NONILFENOLO ED ETOSSILATI

PIROFEOFITINA A RAMEICA

SALI DI AMMONIO QUATERNARIO

SOLVENTI ALOGENATI

Pierangela Rovellini
Responsabile
Team Chimica, Tecnologia e
Sicurezza Alimentare
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"Filiera Agroalimentare e Oli Minerali"

Giornata di studio Accademia dei Georgofili

28 giugno 2023

L'impatto sulla catena alimentare della contaminazione da lubrificanti a base di oli minerali, carburanti e prodotti tecnici vari diventa ogni giorno più evidente. In letteratura sono disponibili numerosi studi sulla contaminazione di numerose filiere agro-alimentari.

La giornata di studio ha voluto fornire elementi per una corretta valutazione di un problema che coinvolge tutti gli attori della filiera agro-alimentare, dal campo fino alla tavola. Sono stati passati in rassegna la natura chimica dei contaminanti, le tecniche analitiche disponibili, la situazione normativa e le sue prossime evoluzioni, la possibilità di sostituire gli oli minerali per molte applicazioni con biolubrificanti. Alla conclusione una descrizione dello stato attuale della contaminazione in alcune filiere e prodotti finiti e i risultati di una ricerca condotta nell'ambito di un progetto italiano volto a valutare le performances di alcuni biolubrificanti nel settore agricolo.

Relazioni

- Liliana Folegatti - Innovhub Stazioni Sperimentali Industria
  Idrocarburi negli alimenti: classificazione e metodi di analisi
- Igor Calderari - Associazione Italiana Industria Olearia
  Aspetti normativi e regolatori
- Lanfranco Conte - Accademia dei Georgofili, Società Italiana Studio Sostanze Grasse
  Sabrina Moret, Luca Menegoz-Ursol, Laura Barp - Università degli Studi di Udine
  Alimenti e contaminazione da oli minerali
- Paolo Bondioli - Accademia dei Georgofili, Società Italiana Studio Sostanze Grasse
  Possibilità di sostituire gli oli minerali nella filiera agro-alimentare. I biolubrificanti
- Roberto Fanigliulo, Luisa Ugolini, Daniele Pochi, Roberto Matteo - CREA
  Esempi pratici di impiego di oli vegetali come fluidi tecnici in agricoltura. Il Progetto AGROENER

È possibile rivedere la giornata sul canale youtube dell'Accademia dei Georgofili,
all'indirizzo www.youtube.com/watch?v=-CN0zYOpsp8

IDROCARBURI NEGLI ALIMENTI: CLASSIFICAZIONE E METODI DI ANALISI

Liliana Folegatti ***(INNOVHUB - Stazioni Sperimentali Industria)***

Gli oli minerali che possono essere ritrovati a livello di tracce in numerosi prodotti alimentari (MOH – Mineral Oil Hydrocarbons) rappresentano un insieme eterogeneo di sostanze, tutte appartenenti alla famiglia degli idrocarburi, ma con importanti differenze in termini di caratteristiche chimiche e tossicità. EFSA ha identificato due diverse categorie di MOH: MOSH (Mineral Oil Saturated Hydrocarbons) costituita da idrocarburi saturi lineari, ramificati o ciclici e MOAH (Mineral Oil Aromatic Hydrocarbons). I costituenti la prima famiglia presentano caratteristiche di pericolosità ancora non bene evidenziate, ma comunque di basso livello, al contrario per alcuni costituenti della famiglia dei MOAH certe sono le proprietà cancerogene e genotossiche. I MOH possono entrare nella catena alimentare attraverso la contaminazione ambientale o da contatto con i prodotti lubrificanti utilizzati per le pratiche agronomiche, per la logistica, per la trasformazione nelle industrie alimentari o attraverso i materiali a contatto con gli alimenti, quali ad esempio gli imballaggi. La presentazione prosegue illustrando le differenti tecniche analitiche disponibili per identificazione e dosaggio dei MOH, a partire dal metodo UNI EN 16995-2017 che prevede un sistema on-line HPLC-GC per il frazionamento delle frazioni MOSH e MOAH accoppiato alla tecnica GC×GC-MS/FID quale metodologia di conferma ed infine una tecnica che consente la determinazione dei soli MOAH ma in tempi estremamente ristretti e utilizzando un'apparecchiatura HPLC-FLUO relativamente economica. Viene infine illustrato un progetto di ricerca ancora in fase di realizzazione, che prevede la valutazione della contaminazione da MOH lungo l'intera filiera, dal ricevimento dei semi in azienda alla bottiglia per oli di girasole, vinacciolo e germe di mais.

HYDROCARBONS IN FOODS: CLASSIFICATION AND METHODS OF ANALYSIS

Liliana Folegatti ***(INNOVHUB - Stazioni Sperimentali Industria)***

Mineral oils (MOH - Mineral Oil Hydrocarbons) that can be found at trace levels in numerous food products represent a heterogeneous set of substances, all belonging to the hydrocarbon family, but with important differences in terms of chemical characteristics and toxicity. EFSA has identified two different categories of MOH: MOSH (Mineral Oil Saturated Hydrocarbons) consisting of linear, branched or cyclic saturated hydrocarbons and MOAH (Mineral Oil Aromatic Hydrocarbons). The constituents of the first family have harmful properties that are not yet well highlighted, but still of a low level. On the contrary for some constituents of the MOAH family the carcinogenic and genotoxic properties are certain. MOHs can enter the food chain through environmental contamination or by contact with lubricant products used for agronomic practices, for logistics, for transformation in the food industries or through materials in contact with food, such as packaging. The presentation continues by illustrating the different analytical techniques available for the identification and dosage of MOH, starting from the UNI EN 16995-2017 method, which provides an on-line HPLC-GC system for the fractionation of the MOSH and MOAH fractions coupled to the GC×GC-MS/FID technique as a confirmation methodology and finally a technique that allows the determination of only MOAH, but in a short time and using a largely available HPLC-FLUO equipment.

Finally, a research project still under construction is illustrated, which involves the evaluation of MOH contamination along the entire supply chain, from the receipt of the seeds on the farm to the bottle for sunflower, grape seed and corn germ oils.

ASPETTI NORMATIVI E REGOLATORI

Igor Calderari ***(ASSITOL-Associazione Italiana Industria Olearia)***

Il tema della potenziale contaminazione da oli minerali nel settore alimentare verrà affrontato dal punto di vista regolatorio, ripercorrendo, in particolare, le varie fasi che hanno caratterizzato il panorama delle norme europee in tale ambito negli ultimi anni.

Verrà posta particolare attenzione all'attività del Comitato Permanente per la Sicurezza Alimentare della Commissione Europea (cd Scopaff) che nel 2022 ha pubblicato uno Statement in cui gli Stati membri dell'UE hanno concordato, in attesa del Parere EFSA, in corso di finalizzazione, dei limiti massimi per i MOAH per differenti prodotti alimentari e che, di fatto, ha valore cogente.

La pubblicazione del Parere scientifico definitivo dell'EFSA dovrebbe, poi, consentire un'analisi della esposizione potenziale, a cui farà seguito la discussione e possibile pubblicazione di un Regolamento europeo con limiti specifici di MOAH per le varie tipologie di prodotto alimentare.

LEGISLATIVE AND REGULATORY ASPECTS

Igor Calderari ***(ASSITOL-Associazione Italiana Industria Olearia)***

The issue of potential contamination by mineral oils in the food sector will be addressed from a regulatory point of view, in particular by retracing the various phases that have characterized the panorama of European standards in this area in recent years.

Attention will be paid to the activity of the Standing Committee for Food Safety of the European Commission (so-called Scopaff) which in 2022 published a Statement in which the EU Member States agreed, pending the EFSA Opinion, currently being finalised, of the maximum limits for the MOAH for different food products and which, in fact, has a binding value.

The publication of the definitive scientific opinion of EFSA should then allow an analysis of the potential exposure, which will be followed by the discussion and possible publication of a European Regulation with specific MOAH limits for the various types of food products.

ALIMENTI E CONTAMINAZIONE DA OLI MINERALI

Lanfranco Conte ***(SISSG - Società Italiana Studio Sostanze Grasse, Accademico dei Georgofili)***
Sabrina Moret, Luca Menegoz-Ursol, Laura Barp ***(Università degli Studi di Udine)***

La presenza di contaminanti di natura idrocarburica riconducibili alla vasta ed eterogenea categoria degli "oli minerali" (MOH) negli alimenti venne messa in evidenza da diversi ricercatori a partire dai primi anni '90, successivamente informazioni sempre più dettagliate vennero acquisite grazie allo sviluppo delle tecniche analitiche sino a giungere ad un metodo UNI/EN del 2017 validato, basato su tecniche cromatografiche accoppiate.

A parte la separazione dei singoli componenti, particolarmente critica per la frazione poliaromatica, un altro punto di peculiare importanza è la preparazione del campione, che deve necessariamente tenere conto della composizione della matrice alimentare sottoposta ad analisi.

L'origine fossile della frazione aromatica (MOAH) di questi contaminanti venne comprovata da Populin et Al. nel 2004, mediante la separazione, identificazione e dosaggio degli opani.

Molte sono le possibili vie di contaminazione degli alimenti con gli oli minerali, ma essenzialmente possono essere ricondotte a tre principali: migrazione dal packaging, contaminazione involontaria (ambientale, contatto accidentale con lubrificanti non food grade ecc.), usi (legalmente ammessi) di oli minerali raffinati nella produzione di alimenti. La contaminazione può quindi avvenire a differenti livelli della catena produttiva, a partire dalla produzione primaria come ad esempio l'uso di alcuni pesticidi e lubrificanti delle macchine agricole. La relazione presenterà alcuni esempi di contaminazione in oli vergini di oliva, in polvere di cacao, e derivante da cessione di imballaggi.

FOOD CONTAMINATION BY MINERAL OILS

Lanfranco Conte ***(SISSG - Società Italiana Studio Sostanze Grasse, Accademico dei Georgofili)***
Sabrina Moret, Luca Menegoz-Ursol, Laura Barp ***(Università degli Studi di Udine)***

The presence of contaminants belonging to the chemical class of hydrocarbons dealing with the widespread and heterogeneous category named "mineral oils" (MOH) was highlighted by several researchers since early '90th, then more detailed information were acquired thanks to the development of analytical techniques that in 2017 lead to a validated UNI/EN method, based on coupled chromatographic techniques.

Main critical points are the chromatographic separation of single compounds, mainly for what concerns the polyaromatic fraction and the sample preparation that must be tuned according to the composition of each food.

The fossil origin of the aromatic fraction (MOAH) of these contaminants was established by Populin et al through the determination of hopanes.

Main routes of contamination by MOH are migration from packaging, accidental contamination (e.g. depending on environment, contact with not food grade lubricants), legal use of refined mineral oils in the food production chain.

Contamination can occur at different points of the production chain as depending on the use of pesticides or agricultural devices lubrication. This contribute will present some examples of contamination e.g. in virgin olive oils, in cocoa powder and as packaging migration.

POSSIBILITÀ DI SOSTITUIRE GLI OLI MINERALI NELLA FILIERA AGRO-ALIMENTARE I BIOLUBRIFICANTI

Paolo Bondioli (SISSG – Società Italiana per lo Studio delle Sostanze Grasse – Accademico dei Georgofili)

Possiamo definire i biolubrificanti come fluidi costituiti da molecole di acidi grassi, legati mediante un legame estere ad un sostituente alcolico o polialcolico. Tre sono le caratteristiche fondamentali dei biolubrificanti: biodegradabilità, versatilità e rinnovabilità. Nel corso degli ultimi venti anni numerose sono state le iniziative di ricerca che hanno consentito di costruire un solido background per introdurre i biolubrificanti come sostituti dei lubrificanti convenzionali per alcune selezionate applicazioni, dove maggiore è il rischio di dispersione anche accidentale nell’ambiente.
Vengono prese in esame le dimensioni del mercato mondiale dei lubrificanti tradizionali, le quantità recuperabili e quelle che invece, a causa di applicazioni a perdere, entrano in modo incontrollato nell'ambiente. Sono questi ultimi i settori nei quali è auspicabile la sostituzione con biolubrificanti.

Non è possibile ipotizzare una sostituzione *tout court* di tutti i lubrificanti tradizionali con biolubrificanti, per questioni di disponibilità e di idoneità all’impiego. Tuttavia per le applicazioni per le quali la sicurezza dell’ambiente e dei lavoratori costituisce il fattore trainante, la sostituzione è sicuramente possibile.

Nella presentazione vengono illustrati per sommi capi le diverse categorie di biolubrificanti, a partire dagli oli nativi fino a prodotti più sofisticati che consentono di coprire tutti i campi di applicazione che sono preclusi, per caratteristiche di viscosità o di stabilità termica, idrolitica o ossidativa, agli oli nativi.

ABOUT THE POSSIBILITY OF REPLACING MINERAL OILS IN THE AGRI-FOOD CHAIN THE BIOLUBRICANTS

Paolo Bondioli – SISSG – Società Italiana per lo Studio delle Sostanze Grasse – Accademico dei Georgofili

The biolubricants can be defined as fluids constituted of molecules of fatty acids, linked by an ester bond to an alcoholic or polyalcoholic substituent. There are three fundamental properties of biolubricants: biodegradability, versatility and renewability. Over the last twenty years there have been numerous research initiatives allowing us to build up a solid background for use of biolubricants as substitutes for conventional lubricants for some selected applications, where the risk of dispersion, even accidental, into the environment is greater.
The dimensions of the classic lubricant world market are discussed, as well as the recoverable quantities and those which instead, due to disposable applications, enter the environment in an uncontrolled way.

It is not possible to hypothesize a *tout court* replacement of all traditional lubricants with bio-lubricants, due to availability and suitability for use issues. However, for applications where environmental and worker safety is the driving factor, substitution is certainly possible.

The presentation briefly discusses the different categories of biolubricants, starting from native oils up to more sophisticated products allowing to cover all application fields which are precluded, due to viscosity characteristics or thermal, hydrolytic or oxidative stability, by native oils.

ESEMPI PRATICI DI IMPIEGO DI OLI VEGETALI COME FLUIDI TECNICI IN AGRICOLTURA. IL PROGETTO AGROENER

Roberto Fanigliulo, Daniele Pochi, Luisa Ugolini, Roberto Matteo ***(CREA – Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria)***

L'intervento dei ricercatori del CREA verterà sulle sperimentazioni condotte nell'ambito del Progetto di ricerca AgroEner "Energia dall'agricoltura: innovazioni sostenibili per la bioeconomia", finanziato dal MiPAAF, e del Progetto di ricerca "Impiego dell'olio di sansa quale fluido tecnico da utilizzare in macchine agricole – Fasi 1 e 2", finanziato da INNOVHUB, Milano. L'obiettivo è stato quello di verificare l'utilizzo di prodotti "Bio-based" come fluidi idraulici in sostituzione degli oli minerali e sintetici utilizzati in applicazioni agricole. Nel progetto Agroener, sono state prese in considerazione due colture oleaginose, Crambe abyssinica e Carthamus tinctorium, per lo sviluppo di una possibile filiera aziendale legata alla produzione di olio vegetale dai semi. Gli oli ottenuti sono stati caratterizzati e parzialmente modificati e infine valutati per le prestazioni tecniche e proprietà lubrificanti. Nel progetto INNOVHUB è stato valutato l'impiego di olio raffinato di sansa di oliva come fluido idraulico e come lubrificante di scorrimento per lame di motoseghe. I fluidi sono stati testati in un banco prova sperimentale in grado di applicare severi cicli di lavoro idraulico caratterizzati da sollecitazioni sull'olio tipiche dell'impianto idraulico di un trattore agricolo, ma più intensive in termini di pressioni e temperature di esercizio per accelerarne l'invecchiamento, dimostrando di possedere e mantenere prestazioni e proprietà comparabili con le controparti minerali.

PRACTICAL EXAMPLES OF THE USE OF VEGETABLE OILS AS TECHNICAL FLUIDS IN AGRICULTURE. THE AGROENER PROJECT

Roberto Fanigliulo, Daniele Pochi, Luisa Ugolini, Roberto Matteo (CREA – Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria)

CREA presentation will focus on the experimental studies carried out within the research project AgroEner "Energy from agriculture: sustainable innovations for bioeconomy", financed by MiPAAF and the research project "Use of Olive-pomace oil as technical fluid in agricultural machines – part 1 and 2", financed by INNOVHUB, Milan. The aim of the projects was to evaluate the use of "Bio-based" fluids as alternatives to mineral and synthetic oils in agricultural applications. Within the AgroEner project, two oilseed crops of interest were considered to develop a possible biolubricants supply-chain based on the vegetable oils obtained from their seeds: Crambe abyssinica Hochst. EX R.E.Fr. (Abyssinian kale) e Carthamus tinctorium L. (Safflower). The oils were characterized, partially modified, and finally tested for their technical performance as hydraulic fluids. The INNOVHUB project concerned the use of refined olive-pomace oil as hydraulic fluid and chainsaw lubricant. The performances of these oils were studied by means of an experimental test rig able to apply severe hydraulic work cycles capable to emphasize the working conditions typical of hydraulic agricultural machinery in terms of operating pressure and temperature, to strongly accelerating their aging. Eventually, the olive pomace oil was also tested in a series of chainsaws as possible chain lubricant to replace the conventional ones. In all cases, the tested fluids showed good performances and properties, comparable to conventional oils.

Annunci di Ricerca Partner per Progetti di Ricerca

Enterprise Europe Network (EEN)

Anno 2023
(aggiornato al 30 settembre 2023)

Progetto BRRO20221128008

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

The Romanian company is a paper packaging manufacturer with over 10 years' market experience. Their products are manufactured in kraft paper (natural/light brown or white) using sustainable sources of raw materials. To ensure excellent product quality, the company constantly strives to ensure a good supplier base. In this context it has established successful business relations with several partners across Europe. Aiming to further expand its network, the company is currently seeking new international business partners, manufacturers or stock resellers, able to supply kraft paper rolls as raw material. Cooperation will be based on supplier agreements.
Dead-line for EOIs: 28/11/2023

Progetto BRRO20230228012

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

The company, which was established by two young entrepreneurs with a keen interest in natural cosmetics, relies on their own recipes for their production. Its portfolio includes hand, face and body creams, lip balms, sunscreens and insect repellent lotions, natural soaps, cleansing and after-shave gels and lotions. All products are manufactured using natural ingredients, which include essential and vegetable oils, and active ingredients, such as hyaluronic acid and Q10 coenzyme obtained through plant synthesis. Ingredients used in the manufacturing process are sourced from suppliers in Romania and Germany. In order to diversify its network of suppliers, the Romanian company is now looking for EU producers of olive oil able to supply high quality virgin oil obtained via cold-pressing procedures, as well as pomace olive oil, to be used for cosmetic purposes. Cooperation will be based on supplier agreements.
Dead-line for EOIs: 01/03/2024

Progetto BRRO20230420001

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

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

Progetto BRPT20230525025

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

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

Progetto BRPL20230523006

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

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

Progetto BRPT20230809030

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

Aware of the critical role that packaging plays in the process of conveying the value of food products to consumers, this Portuguese-based company has developed a line of portable food boxes with a revolutionary design that builds the bridge between sustainability and cost effectiveness, allowing users to eat without compromising their freedom. The Portuguese company has recently innovated a new line of portable food boxes that

facilitate the handling and transport of the packaging. They are seeking experts/manufacturers with the capacity to develop products with new shares, sizes and materials. It will be necessary to carry out prototyping on emerging materials, including compostable, biodegradable and recycled solutions. The ideal partner will be able to offer small order quantities to start with and possibly have in-house R&D capability or connection with certified laboratories to test new materials. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 8/08/2024

Progetto BRFR20230828003

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

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

Progetto BRAT20230801027

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

The Austrian SME was founded in Innsbruck in 2019 and specialises in trading hemp derivates for the food, feed and cosmetic industry. In recent years, they also added oilseeds, oils and nuts to their portfolio. In sum, they offer a vast portfolio of oilseeds, nuts, cereals and oils to satisfy their clients demands. The company is currently seeking to expand its network of producers of.oilseeds, cereals, dried and dehydrated fruits. They are also looking for new suppliers of refined oils such as sunflower, rapeseed, soya, palm, grape seed, hemp. The company requires raw materials that comply with EU food and feed regulations. As a trading company, the sole purpose of their purchases is resale to their network of customers across Europe. Envisaged type of partnership: supplier agreement.
Dead-line for EOIs: 17/09/2024

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

Enterprise Europe Network (EEN)

È la rete nata nel 2008 per volontà della Commissione Europea con l'obiettivo di supportare l'innovazione, il trasferimento tecnologico e l'internalizzazione di piccole e medie imprese ed enti di ricerca.
Si avvale di oltre 600 organizzazioni presenti in 60 paesi e offre un sistema integrato di servizi gratuiti per aiutare le imprese a individuare nuovi partner commerciali, produttivi e tecnologici all'estero; per promuovere la partecipazione ai programmi Europei per la ricerca, come Horizon Europe, e per fornire gli strumenti utili per essere più competitivi sui mercati internazionali, migliorando la conoscenza dei mercati e della legislazione europea.
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Susy Longoni
susy.longoni@mi.camcom.it

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- Ricerca di finanziamenti a supporto delle imprese
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- Sostegno per lo sviluppo di nuovi prodotti o processi

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- 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

MATERIALI A CONTATTO CON ALIMENTI

TEST e ANALISI

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

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

RICERCA e SVILUPPO

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

ENTE ITALIANO DI NORMAZIONE

DG CCRF

BfR Bundesinstitut für Risikobewertung

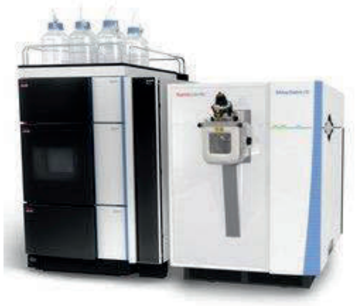
cen EUROPEAN COMMITTEE FOR STANDARDIZATION

NIAS *Non Intentionally Added Substances*

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

VALUTAZIONE

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



UNTARGETED ANALYSIS

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

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

MATERIALI A CONTATTO CON ALIMENTI

Innovhub Stazioni Sperimentali per le Industrie S.r.l. offre un pacchetto analitico consolidato utile ad ottenere la conformità dei prodotti destinati al contatto con alimenti a base carta alla raccomandazione tedesca **BfR XXXVI** e alla nota informativa francese **DGCCRF MCDA n°4 (V02-01/01/2019)**. Alcune delle determinazioni effettuate riguardano i seguenti parametri:

- *Determinazione della formaldeide in estratto acquoso - (UNI EN 1541:2002)*
- *Determinazione del contenuto di gliossale - (DIN 54603:2008)*
- *Imbiancanti ottici migrabili - (UNI EN 648:2019)*
- *Migrazione specifica della somma delle ammine aromatiche primarie (UNI EN 13130-1:2005+EN17163)*
- *Determinazione e quantificazione degli ftalati - (UNI EN 16453:2014 o metodo interno in HPLC)*
- *Bisfenolo A - (UNI EN 17497:2020)*
- *Determinazione di diisopropilnaftalene (DIPN) mediante estrazione con solvent (UNI EN 14719:2005)*
- *Cadmio, piombo e alluminio in estratto acquoso - (UNI EN 12498:2019 + metodo interno)*
- *Trasferimento dei costituenti microbici – (UNI EN 1104:2018)*
- *Determinazione della solidità del colore della carta e del cartone colorati (UNI EN 646:2019)*
- *Contenuto in estratto acquoso 1,3-Dicolor-2-propanolo (metodo interno)*
- *Contenuto in estratto acquoso 3-monocloro-1,2-propandiolo (metodo interno)*
- *Benzofenone + 4-metilbenzofenone + 4,4'-bis(dimetilamminio)-benzofenone (BVL B 80.56-2 Correzione 2004-06)*

Le determinazioni avvengono seguendo metodi ufficiali UNI, CEN e DIN, e metodi interni sviluppati nei laboratori analitici di INNOVHUB.

Da oggi, l'offerta analitica di INNOVHUB si arricchisce grazie all'acquisto del nuovo sistema Orbitrap Exploris 120 Mass Spectrometer (Thermo Scientific™) e del pacchetto software Compound Discoverer™ (Thermo Scientific™) focalizzati all'esecuzione di analisi untergeted

L'implementazione del parco strumentale, e la consolidata esperienza in materia, permetterà di ottemperare alla sempre più stringente normative in materia che richiede di valutare in modo accurato e specifico anche la presenza di NIAS (Non Intentionally Added Substances) "impurità presente nelle sostanze utilizzate, intermedio di reazione formatosi durante il processo produttivo o prodotto di reazione o di decomposizione" all'interno dei prodotti cartari.

Riferimenti:

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RECENSIONI DI LIBRI

IL CARRUBO
Caratteristiche, coltivazione e utilizzi
A CURA DI:
STEFANO LA MALFA
MASSIMILIANO BRUGALETTA

I Edizione
€ 20,00 - Edagricole di New Business Media srl
ISBN: 978-88-506-5647-9
Pagine 176 - formato 14 x 21 cm
Tel.051.65751
e-mail: libri.edagricole@newbusinessmedia.it
www.edagricole.it

La coltivazione del carrubo, specie rustica e aridoresistente tipica del Mediterraneo, ha origini antiche che affondano nello sfruttamento dei baccelli per il consumo umano e per l’alimentazione zootecnica. Nel tempo sono cresciuti i suoi usi anche a scopo ornamentale e di valorizzazione del paesaggio.

Se attualmente l’interesse principale è rivolto alla farina dei semi, utilizzata in campo alimentare in una varietà di impieghi, ma principalmente come addensante o stabilizzante, stanno emergendo spunti interessanti in campo nutraceutico e cosmetico per altre parti del frutto.

Dopo i capitoli su inquadramento botanico e risorse genetiche, il libro affronta le tematiche legate alla propagazione, alle tecniche colturali e all’utilizzo del prodotto senza trascurare la descrizione di patogeni e parassiti. In conclusione, un’analisi del mercato mondiale e italiano delinea le potenzialità produttive del carrubo.

Indice: Introduzione - Inquadramento della specie - Caratteristiche morfologiche e fisiologiche - Risorse genetiche e patrimonio varietale - Propagazione e vivaismo - Tecniche colturali - Principali avversità biotiche e strategie di controllo - Caratteristiche, gestione e utilizzo del prodotto - Importanza economica del carrubo.

Stefano La Malfa è Ordinario di Arboricoltura generale e Coltivazioni arboree presso il Dipartimento di Agricoltura, Alimentazione e Ambiente dell’Università degli Studi di Catania. Componente del Consiglio direttivo della Società di Ortoflorofrutticoltura Italiana (SOI), è attualmente Presidente della sezione Frutticoltura e Coordinatore del gruppo di lavoro Didattica e Ricerca nel settore dei Sistemi Arborei.

Massimiliano Brugaletta Phd in Scienze e Tecnologie Agrarie Tropicali e Subtropicali, è agronomo. Dopo varie esperienze di ricerca all’estero lavora in diversi ambiti legati al settore del carrubo e dei prodotti derivati ed è attualmente vicepresidente del Distretto della Frutta Secca di Sicilia e componente del Coordinamento nazionale della frutta in guscio.

Volume realizzato in collaborazione con SOI (Società di Ortofrutticoltura Italiana).

CONGRESSI

IPOC 2023 Indonesian Palm Oil Conference & Price Outlook

1-3 November 2023 | Bali

The Indonesian Palm Oil Conference & Price Outlook is an annual forum for palm oil industry stakeholders, organized by the Indonesian Palm Oil Association (better known as GAPKI--Gabungan Pengusaha Kelapa Sawit Indonesia). This event has become a benchmark for palm oil stakeholders in keeping themselves updated with the palm oil market trends and price outlook. Latest issues, regulations and policies of the industry are discussed and shared by prominent and authoritative speakers.
With more than one thousand participants from various background as well as experts in the industry, this conference serves as a very excellent opportunity to build up and expand your business network as well promoting your company brand awareness.
IPOC website: www.gapkiconference.org

EUCARPIA International Congress on Oil and Protein Crops

2-4 November 2023 | Megasaray Westbeach Hotel, Antalya, Turkey

The International Congress Oil and Prtein Crops Section Conference of EUCARPIA is organized by Trakya University and the International Researchers Association in cooperation with the European Association for Research on Plant Breeding (EUCARPIA). The congress will be held with the support of several national and international partners.

The Congress topics will cover Oil and Protein Crops:

- Plant Breeding and Genetics
- Molecular Genetics and Biotechnology
- Biology and Physiology
- Genetic Resources,
- Plant Protection,
- Agronomy, Economy,
- Animal feeding
- Food Science and Nutrients
- Fats, lipids, and Protein studies

Oil crops are rich sources of oils, proteins, minerals, vitamins, and dietary fibers for both human and animal feeding and provide the raw material for the production of biodiesel. Oil crops are soybean, cottonseed, sunflower, canola, rapeseed, peanut, safflower, flax, sesame, coconut, castor, copra, and other minor crops.

Almost 50% of the global food protein supply comes from cereal seeds. Soybean, peanut, common bean, pea, lupine, chickpea, faba bean, lentil, grass pea, cowpea, pigeon pea, etc. are currently the most important legumes for human consumption and animal feed. Because of the protein content of their seeds; Grain legumes, Cereals, industrial crops, and other minor crops such as amaranth, quinoa, hemp, caraway, etc. are protein crops growing for plant protein for plant protein food and feed.

The Congress is intended that the subjects in mentioned crops above to be kept broad in order to provide an opportunity for the science and research community to present their works as oral or poster presentations. The Congress language will be English.

Researchers, breeders, and others with an interest in the genetics and breeding of oil and protein crops are invited to participate. Among the topics to be discussed are directions of breeding for resistance to abiotic and biotic stresses, improved industrial use, and conventional versus organic production.

As there have been many different scientific meetings around the world, the organizers aim to bring three different communities together, namely science, research and private investment groups considering practical information sharing that will be of value for breeders, seed enterprises, researchers and scientists, in a friendly environment of Antalya, Turkey to share their knowledge and experience and benefit from each other.

The congress will gather scientists from around the world, and present their recent achievements. The organizers will also invite relevant stakeholders to provide a view on the current situation around the world as well as prospects to overcome the limitation for sustainable crop production to feed the world.

For further information, visit www.protoil.org

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

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

The congress features four concurrent conferences covering upstream, midstream, downstream & value addition; processing, food safety & nutrition & global economics & marketing.

A keynote address will be delivered by distinguished expert in the palm oil industry. Four plenary lectures will be held in the afternoon of 7 November 2023. The lectures will cover various topics of interest in line with the theme of the congress.

Objectives:

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

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

Technical tours are foreseen to oil palm plantation, palm oil mill, refinery and R&D facilities.

Concurrent programme:

Agriculture, biotechnology & sustainability conference (abs)

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

Downstream and value addition (dva)

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

Processing, food safety and nutrition (PFSN)

- Session 1: milling & processing technology
- Session 2: environmental management technology
- Session 3: food safety & quality
- Session 4: lipid research
- Session 5: diet & lifestyle
- Session 6: phytonutrients

Global economics and marketing (GEM)

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

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

FOFSA Annual Dinner 2023

9 November 2023 | Geneva, Switzerland

The Annual Dinner returns to Geneva in 2023 and will be hosted at the Hilton Geneva Hotel and Conference Centre on Thursday 9 November 2023.

The venue near the city centre, just across the street from the airport and one mile from Lake Geneva.

You can book up to a maximum of four tables of ten if you want to come as a large group, or book as a smaller group or as an individual and we will designate you a table. Reserve your table(s) early to avoid disappointment.

For further information please visit:
https://www.fosfa.org/news/events/fosfa-annual-dinner-2023/

2023 AAOCS lipid conference Australia

13 - 15 November 2023 | Newcastle, Australia

The next conference will be held at Noah's on the Beach in Newcastle Australia Nov 13-15th 2023

The theme of this year's event is Future of lipids: health and sustainability and aims to look at the future of fats and oils in research and industry.

The program will present the latest science and industry updates relating to edible oil and ingredient supply, biotechnology, manufacture of fat-based products, nutritional research, oil analysis, latest developments with omega-3 oils, dairy lipids and other activities that support the industry. Furthermore, planned workshops led by international experts, are in planning to occur prior to the conference.

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

The range of topics covered in this meeting include:

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

The AAOCS are hosting two workshops prior to the meeting.

These will both be held on Monday 13th November 2023 at Noah's on the beach Newcastle. These workshops will run concurrently.

The two workshops are:

Sustainable ingredients for food and feeds (focus of novel lipids and protein products entering our food systems)

The program will present the latest science and industry details about alternative sustainable sources of lipid and protein in the food and feed sectors. Topics covered include:

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

Lipids in the health of our futures (focus on nutrition)

The program will present the latest nutritional science and industry details about the use of lipids in nutrition. Fats traditionally have some negative connotations when it comes to nutrition. With the rise of healthy fats (such as omega 3 oils/ olive oils etc) this program aims to update attendees with the latest information and to dispel popular misconceptions.

Topics covered include the roles of fats/lipids at various stages of life:

- Heathy infants/babies

- Sports nutrition
- Healthy women
- Healthy aging

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

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

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

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

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

The Roundtable on Sustainable Palm Oil (RSPO) is pleased to announce that the Annual Roundtable Conference on Sustainable Palm Oil (RT2023) will be held from 20 - 22 November 2023 at the Hotel Mulia Senayan in Jakarta, Indonesia.
The first physical RT after a long pandemic period was held last year in Kuala Lumpur, Malaysia, becoming the most successful RT to date with more than 800 delegates from over 40 countries.
The theme of RT2023 is “Partners for the Next 20”, presenting an opportunity to collaboratively take concrete action on the core challenges facing the sustainable palm oil industry. RT2023 in Jakarta, Indonesia will open up more opportunities for RSPO and their partners to achieve a shared vision of making palm oil sustainable as they head towards RSPO’s 20th anniversary in April 2024.

RT2023 will be an excellent platform to network, explore new opportunities for cross-sector collaboration, meet potential business partners, and increase awareness of your brand and its role in promoting certified sustainable palm oil.

Program overview - 21 November

Plenary session: Scaling Climate Action: Advancing Collaboration Between Financial Institutions and Downstream Actors

Breakout sessions:
- Labour Unions as Vital Partners in the Next 20: Strategies to Prevent and Resolve Worker Grievances
- Solutions versus Sanctions: Meeting the Evolving Demands of Social Assurance in the Next 20
- Jurisdictional Certification: Catalysing the Future of Sustainable Palm Oil
- Living Wage in the Palm Oil Supply Chain: Achievable or mere Utopia?
- The Future of Certification, Trade and Traceability in the Next 20
- Shared Responsibility in Future Supply Chains: Increasing Uptake and Smallholder Inclusion

*Program overview - 22 Novembe*r

Plenary sessions:
- Shaping the Next 20: Synergising Policies and Strategies for Sustainable Palm Oil
- Bridging the Gap - Empowering Smallholders to Succeed Amidst Tightening International Regulations

Breakout sessions:
- Envisioning the Future of Palm Oil - What Role Does it Play in Food Security?
- Future-proof Supply Chains: Unlocking the Role of Oleochemicals
- Solutions for Worker Health and Safety in a Heating Climate
- Sustainability in the Eyes of the Global South
- Navigating the Corporate Sustainability Reporting Directive: The Future of Doing Business in Europe

For more information:
https://rt.rspo.org/event/679b9d86-ab0f-405e-831f-8d868646a64f/summary

Palm Oil Economic Review & Outlook Seminar 2024

11th January 2024, Pullman Kuala Lumpur City Centre, Malaysia

Palm Oil Economic Review and Outlook Seminar (R&O) is an annual event organised by MPOB with the main objective to disseminate information on the performance of the Malaysian oil palm industry and to forecast its performance for the current year. This seminar will also focus on the Government initiatives to help the industry to continuously maintain their competitiveness and sustainability. This seminar provides the platform to discuss and exchange views in further strengthening the role of palm oil in the global oils and fats.
Objectives:

- To disseminate information regarding the performance of the Malaysia oil palm industry in 2023 and prospects for 2024.
- To deliberate issues and challenges in 2023 and its impact to the global palm oil supply and demand.
- To highlight the latest market developments and opportunities for palm oil.
- To encourage sharing of knowledge.

Focus Areas:

- The Malaysian oil palm industry performance 2023 and prospects for 2024
- Mechanisation and automation in oil palm plantation.
- Commitment on sustainable development while fulfilling global needs.
- Impact of global challenges on palm oil supply and demand.

For further information visit:
https://mpob.gov.my/conferences-courses/palm-oil-economic-review-outlook-seminar-2024

21st International Conference on Renewable Mobility "Fuels of the Future 2024"

22 - 23 January 2024 | Berlin

The conference will host more than 60 speakers & more than 700 national and international participants from various industries, including: Biofuels, Agriculture, Recycling/waste Industry, Mineral oil, Trade, Automotive, E-Mobility, Shipping, Aviation, Chemical Industry, Science, Consulting, Energy, Certification companies, Politics, Associations.

Program 23 January

Session 1 - Energy transition in transport between the challenges of security of supply and climate change mitigation
Session 2 - Panel discussion: consequences of the war in Ukraine: is security of supply sidelining climate change mitigation?
Session 3 - Implementation of RED III: how will quota regulations in the EU look in future?
18:15 Young scientists award
Presentation of a research project in 160 seconds using the dynamic.

Program 24 January

Session 4a: E-fuels | the journey is the reward – progress in R&D projects
Session 4b: Bioethanol
Session 4c: Biofuels and renewable fuels in agriculture and forestry
Session 4d: Advanced alternative fuels – from research to practice
Session 5a: Fuel research
Session 5b: Biomethane as a fuel
Session 5c: Biofuels and renewable fuels in shipping
Session 5d: Biofuels from waste and residues
Session 6a: Energy industry – green hydrogen
Session 6b: Biomethane international
Session 6c: Biofuels and renewable fuels in aviation
Session 6d: International biofuel trade

Full program and registration (early bird within 30.11.23): https://www.fuels-of-the-future.com/en

RISG

LA
RISTA ITALIANA
DELLE
SOSTANZE GRASSE

Anniversario
100

15 novembre 2023
ore 9:30 – 17:00

Sostanze grasse: ricerca, innovazione e scenari futuri

In occasione del centenario de La Rivista Italiana delle Sostanze Grasse, Innovhub SSI organizza una giornata per festeggiare 100 anni di ricerca, innovazione e passione.

L'evento rappresenta un momento di aggiornamento sui temi emergenti e attuali del settore delle sostanze grasse, con un focus in ambito alimentare: nuove prospettive per il food, sicurezza alimentare, nuove tecnologie produttive, aspetti nutrizionali, novel food, problematiche attuali dell'agricoltura, materiali a contatto con gli alimenti, autenticità, normativa, ricerca e innovazione.

La giornata si rivolge a specialisti e studiosi che operano all'interno delle industrie produttrici di oli e grassi alimentari e industriali, delle industrie chimiche, di laboratori di enti statali, istituti di ricerca e facoltà universitarie, così come alle Associazioni di settore e alla Pubblica Amministrazione.

Evento organizzato da:

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

Palazzo Turati
Via Meravigli 9/b, Milano

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La
Rivista Italiana
delle
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Author instructions

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

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

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Con il contributo di

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L'Europa alla portata della vostra impresa.