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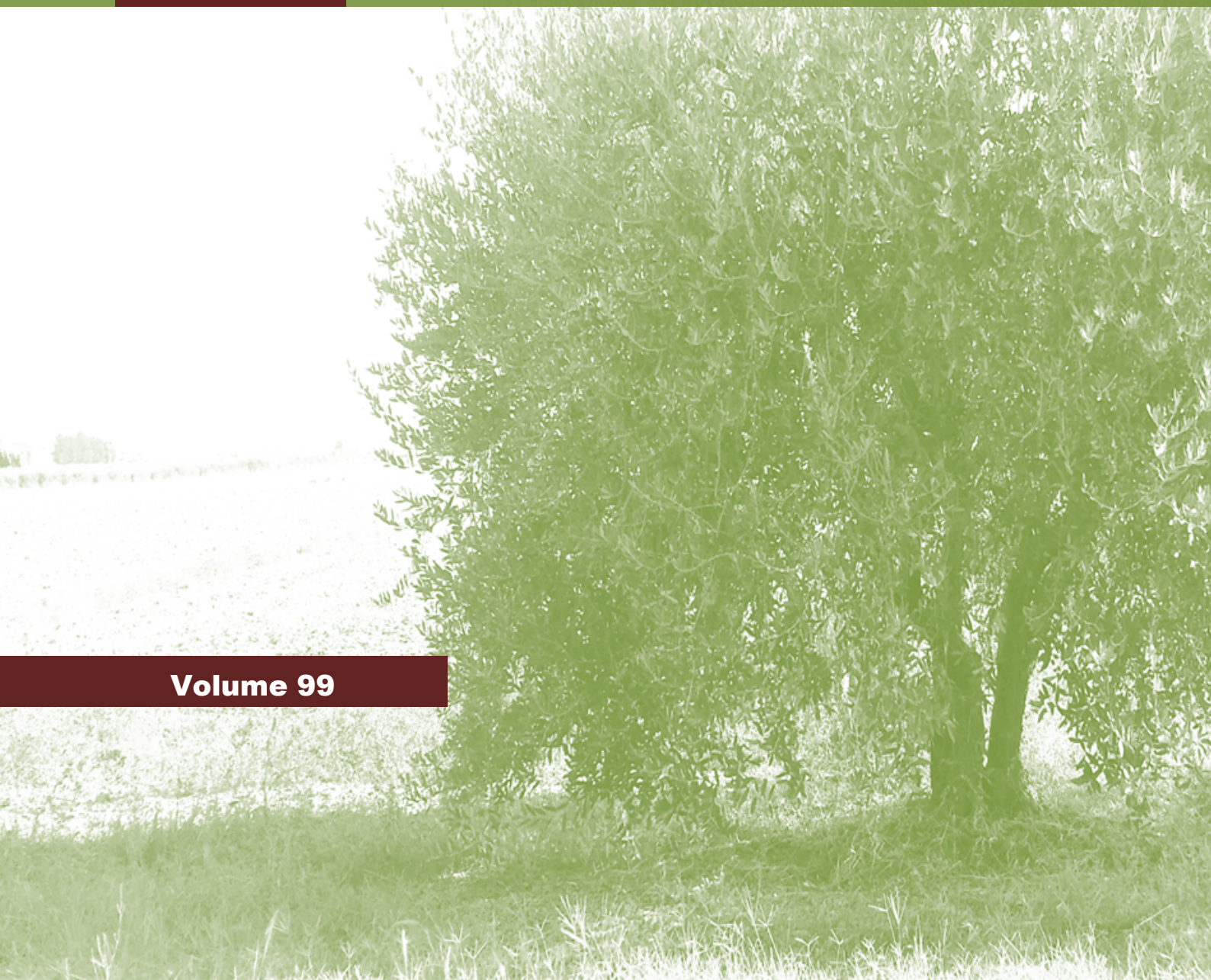

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La RIVISTA ITALIANA delle SOSTANZE GRASSE

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Impact of several physical treatments on the improvement of some quality parameters of crude olive oil

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In this study, fresh olive oil was exposed to oxidation followed by elution at room temperature through an adsorbent packed in the glass column (10 × 75 cm) loaded with one of the following beds: activated charcoal, calcium chloride ($CaCl_2$), alumina (Al_2O_3), bentonite, Arabic gum, and silica gel to investigate the effect of using adsorbents on the quality improvement of oxidised olive oil. The used ratio of adsorbent on oxidised olive oil was 1:5. The results show that the peroxide value (PV) of oxidised olive oil eluted through silica gel improved significantly by 22.6% (from 32.39 to 25.06 meq/kg) and for free fatty acids (FFA %) the improvement was 45.37% (from 1.675 to 0.915 %). The PV of eluted oxidised olive oil through Arabic gum and $CaCl_2$ was significantly improved by 45.08% and 25.65%, respectively and for FFAs (%) were 40.0% and 31.64%, respectively. Elution of oxidised olive oil through other adsorbents showed different results on FFA (%) and PV. Elution of oxidised olive oil through tested adsorbents exhibited a negative impact on the total phenolics and vitamin E contents. Different responses on specific absorption coefficient at 232, and 270 nm were observed for the eluted olive oil through used adsorbents.

Keywords: Adsorbent, Free fatty acids, Olive oil, Phenolic contents, Peroxide value, Vitamin E.

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INTRODUCTION

Virgin olive oil is the oil obtained from the olive fruit through manual and mechanical procedures without refining or being mixed with other oils or any substances. Olive (*Olea europaea*) oil is a fundamental component of the Jordanian and Mediterranean diet and there has been a significant increase in the consumption of such oil due to its nutritional and health-promoting effects and its association with the prevention of several diseases like cancer, heart disease, and aging by inhibiting the oxidative stress [1-3].

Olive oil has higher stability than other edible oils, due to its low content of polyunsaturated fatty acids (PUFA`s) and a higher content of monounsaturated fatty acids and is obtained solely through physical means by mechanical or direct pressing of the olives and not subjected to any treatment other than washing, decantation, centrifugation, and filtration, and may be consumed without refining [4, 5]. The International Olive Council [6] and Jordan Institution for Standards and Metrology [7] have designated the quality characteristics for each type of olive oil (Extra-virgin, virgin, regular…etc). However, some of these ascertained figures may increase and become incorrect during normal storage, due to hydrolysis, oxidation, polymerisation, and further oxidation reactions resulted from hydrolysed fatty acids and formation of primary and secondary oxidative products that cause quality deterioration of olive oil that could adversely affect human health and the quality of olive oil [8].

Although the refining process of crude olive oil can remove undesirable sub-

stances that affect the oil quality, the problem of seeping for some of the refined olive oil bioactive substances that contribute to the oil healthy and sensory properties may arise. Re-refining of used cooking seeds oil by different techniques like, supercritical extraction, membranes filtration and adsorbent treatments to improve its quality and safety have been studied and found to be the cheapest, efficient, and easiest methods using magnesite, silica gel, alumina, and different type of resins [9-10].

The aim of the current study is to investigate the effect of elution of oxidised olive oil through different adsorbents (silica gel, aluminium oxide, calcium chloride, activated bentonite, activated charcoal granular and Arabic gum) in the improvement of some of the quality characteristics of olives oil. The efficiency of partial refining of oxidised olive oil through elution from each adsorbent is judged by analysing free fatty acids, peroxide value, extinction coefficient at 232 and 270 nm, total phenolic contents, and vitamin E contents.

2. MATERIALS AND METHODS

2.1 CHEMICALS

Silica gel powder (60-200 mesh), Aluminium oxide (70-290 mesh), activated charcoal granular, calcium chloride, activated bentonite were purchased from LABCHEM chemicals (Zelienople, USA). Folin–Ciocalteu reagent and α-tocopherol acetate were purchased from AppliChem GmbH (Darmstadt, Germany). Methanol, Hexane, and diethyl ether (HPLC-grade) were purchased from ASTM Co., (USA). Potassium iodide (KI), chloroform, sodium hydroxide (NaOH) was purchased from SD Fine-Chem limited (UK). Sodium thiosulfate ($Na_2S_2O_{3)}$ was purchased from Brix worth (Northhants, UK). Arabic gum and other chemicals of reagent grade were purchased from local companies.

2.2 OLIVE OIL SAMPLE

Olive oil sample (30 Kg) was purchased from an olive oil refinery in the northern part of Jordan (Irbid-Jordan). The olive oil sample was obtained after a mechanical extraction of Nabali olive harvested from one of the farms after 5 days of olive harvesting in November 2020. Chemical quality tests for fresh olive oil were determined in triplicate.

2.3 OXIDATION INDUCTION OF OLIVE OIL

Ten kilograms of the freshly produced olive oil was placed in an open glass container with large surface area. The oil was left in open air at room temperature to expose it for the oxidation process using sunlight and oxygen for around one month. The olive oil free fatty acid (%) and peroxide value (PV) were measured routinely to check the oil oxidation every 5 days. After the oil becomes rancid (PV = 31.8 meq O_2/Kg) and free fatty acids increased (1.67%), a 500 gram was eluted from the oxidised olive oil at room temperature through an open glass column (10 × 75 cm) loaded with a matrix (~100 g) of one of the following adsorbents: silica gel, bentonite, Arabic gum, aluminium oxide, calcium chloride and activated granular charcoal. The elution time consumed for each adsorbent was recorded, followed by centrifugation (3000 rpm × 5 min) using Heraus Sepatech Megafuge 1.0 (Germany) and kept refrigerated in brown glass bottles for further analysis.

2.4 ALKALINITY OF ADSORBENTS AND IMPROVEMENT EFFECIENCY (%)

The alkalinity of each adsorbent was determined by direct titration with HCl using a phenolphthalein indicator. In brief, 5.0 g from each adsorbent was accurately weighted into a 250 ml Erlenmeyer flask, followed by addition of 100 ml of distilled water and the solution was swirled to dissolve the adsorbent. A few drops of phenolphthalein indicator 1% were added. Solution showed pink colour was titrated with 0.1N HCl solution until the colour turned from pink to being colourless. Alkalinity for pink positive colour adsorbent was calculated according to the following formula and expressed as NaOH (%):

$$\text{Alkalinity as NaOH (\%)} = \frac{(V1 - V2) \times N \times 40 \times 10}{\text{Adsorbent weight}}$$

Where:
V1 = Volume of HCl consumed by each adsorbent in ml
V2 = Volume of HCl consumed by the blank in ml
N = Normality of HCl
40 = The equivalent weight of Sodium hydroxide

The following formula was used to determine the efficiency of adsorbents in the improvement of measured quality parameters (%):

$$\text{Improvement efficiency (\%)} = \frac{\text{Value (before elution)} - \text{Value (after elution)}}{\text{Value (before elution)}} \times 100$$

2.5 DETERMINATION OF FREE FATTY ACIDS (%)

The acidity of olive oil was determined by the AOAC method [11]. In brief, 5.0 g of olive oil was accurately weighted into 250 Erlenmeyer flask, followed by an addition of 50 ml of equal mixture solution from ethanol: diethyl ether, the solution was swirled to dissolve the oil in the solvent. A few drops of phenolphthalein indicator 1% were added, and then the solution was titrated with 0.1N sodium hydroxide solution until the colour turned to a faint pink colour. Acidity was calculated according to the following formula and expressed as oleic acid (%):

$$\text{Acidity \%} = \frac{(V1 - V2) \times N \times 282}{10 \times \text{Sample weight}}$$

Where:
V1 = Volume of Sodium hydroxide consumed by each sample in ml
V2 = Volume of Sodium hydroxide consumed by the blank in ml
N = Normality of alkali
282 = The equivalent weight of oleic acid

2.6 DETERMINATION OF PEROXIDE VALUE

The peroxide value of olive oil was determined by AOAC method [11]. The PV was expressed in milliequivalents of oxygen per kg of oil (meq of O_2/kg). In brief, 5 grams of olive oil were taken into Erlenmeyer flask 250 ml and the sample was dissolved in a 30 ml of 3:2 acetic acid - chloroform solution and shaken for few second. Then 0.5 ml of freshly prepared saturated KI was added, and shaken again for 1 min, followed by an addition of 30 ml distilled water to stop the reaction. The mixture was slowly treated with 0.01 ($Na_2S_2O_3$) with vigorous shaking until the solution with starch indicator become colourless.
The peroxide value was calculated according to the following formula and the results were expressed as milliequivalents of oxygen per kilogram of oil (meq O_2/kg oil):

$$PV = \frac{(Vs\text{-}Vb) \times (N) \times 1000}{\text{Sample weight}}$$

Where:
Vs = Volume of sodium thiosulfate consumed by sample in ml
Vb = Volume of sodium thiosulfate consumed by the blank in ml
N = Normality of sodium thiosulfate.

2.7 DETERMINATION OF TOTAL PHENOLIC CONTENTS

The total phenol contents (TPC) of the fresh, oxidised and eluted olive oil were determined separately, by the Folin–Ciocalteau spectrophotometrically at 725 nm using Capannesi et al. [12]. A sample of olive oil 10 grams was weighted into a 250 ml Erlenmeyer flask followed by addition of 50 ml of hexane and was mixed vigorously, then the sample was transferred to separatory funnel and extracted with 80 ml methanol (80%) several times. One ml from the collected methanolic phase layers was placed into a 10 ml volumetric flask followed by addition of 5 ml of distilled water and 0.25 (2 N) Folin Ciocalteau and the solution was then mixed well for 3 min. After that 2 ml of Na_2CO_3 (17%) added and the flask was then filled with distilled water up to the mark. The absorbance for each sample was measured at 765 nm using a spectrophotometer (model UVD-2900, Labomed, USA). The total phenolic compound contents were expressed as a Gallic acid equivalent (mg GAE/100g) and determined from the following regression equation based on the established calibration curve of gallic acid: $Y = 0.0742X$, $r^2 = 0.9963$.
Where Y is the absorbance and X the Gallic acid concentration in mg/l. All measurements were done in triplicate.

2.8 DETERMINATION OF SPECIFIC EXTINCTION COEFFICIENT AT 232 AND 270 nm (K_{232} AND K_{270})

European Official Method of Analysis (Commission Regulation EEC N-2568/91 (1991)) was used for the determination of specific extinction coefficients of the olive oil samples [13]. In brief: 250 mg of olive oil was weighed into a 25 mL volumetric flask and diluted to 25 mL with hexane. The sample was homogenised using vortex for 30 seconds and then the resulting solution was taken into a quartz cuvette. Absorbance at 232 and 270 nm was determined in a spectrophotometer (model UVD-2900, Labomed, USA) using the hexane as the blank.

2.9 DETERMINATION OF VITAMIN E

Vitamin E content in fresh, oxidised, and eluted olive oil was determined according to Gimeno et al. [14] method with slight modification using RP-HPLC. In brief, 1 gram of olive was weighed into a 10 ml volumetric flask and diluted to 10 ml with hexane (1:10), thereafter, 200 µL of sample and hexane mixture was transferred to a screw-capped tube. Then 600 µL of methanol and 200 µL of the internal standard solution (300 µL/ml of α-tocopherol acetate in ethanol) were added. After that, they were mixed by vortex, and centrifuged (3000 rpm × 5 min) using heraus sepatech megafuge 1.0. model. Samples were then filtered through a 0.45 mm pore size filter and an aliquot of the overlay was directly injected into Knauer High Performance Liquid Chromatography (HPLC) system, equipped with ACE 5, C18, 250 × 4.6 mm column (Advanced Chromatography Technologies-Scotland), the injection volume was 50 µL. The mobile phase with methanol and elution was performed at a flow rate of 1.5 ml/min. The analytical column was kept at 30°C and detection was performed using UV detector at 280 nm. To determine the compounds in the samples, the working standard solutions were analysed together with the samples and peak-area ratios were used for calculations following the internal standard.

2.10 STATISTICAL ANALYSIS

Statistical calculations were performed using statistical analysis system, SAS program, 2000 (SAS Institute Inc., Cary, NC, USA) [15]. Significant and non-significant differences among means of treatments were determined using LSD test. Differences at $P<0.05$ were considered significant and $P>0.05$ were considered non-significant. All treatments were conducted in triplicate.

3. RESULTS AND DISCUSSION

3.1 FREE FATTY ACIDS (FFA %) AND ELUTION TIME

Olive oil has some elementary criterions that distinguish it from other oils. Olive fruits should be picked and processed directly to preserve the produced oil quality. The free fatty acid (%) is a measure of the

quality of the oil, and reflects the care taken in producing the oil and the quality of the in-coming fruit [16]. The fresh olive oil sample used in our experiment meets the criteria of virgin olive oil grade (acidity was 1.24%). During the intentional exposure of investigated olive oil to light, heat and air, free fatty acids were increased (1.24-1.67%), due to the presence of lipase enzymes that hydrolysis triacylglycerols which continue to occur in the oil. The presence of fatty acids also leads to the formation of more fatty acids in the oil; that act as a catalyst for the further production of free fatty acids. In general, hydrolysis resulted from the olive fruit damage, fruit quality, time, and temperature of the oil extraction from the fruit. This damage occurs prior to the oil being separated from the water and solid portions of the fruit [17]. Although the olive oil from chemical point of view was oxidised and its acidity increased, but the oxidised olive oil still considered as virgin oil according to IOC (FFA ≤ 2%) [6]. Thus, we can conclude that the oxidation of olive oil may not affect the grade of oil in terms of acidity in comparison with its peroxide value.

Table I shows the time consumed by each oxidised olive oil (500 g) to elute through each adsorbent from the open glass column and the effect of each adsorbent on the efficiency of the improvement in FFA (%). The elution time varied from several minutes to 7 hours. For example, the elution of oxidised olive oil through granular charcoal lasted 20 minutes, while for silica gel it lasted 7 hours. This variation in time consumed for elution may be due to the differences in the surface area of each adsorbent, pore structure, form and texture of the adsorbents used in this study. Also, the impurities in the eluted oil may be trapped in the pores of adsorbents due to different affinities resulting in different elution times [9].

All the used adsorbents were significantly effective in lowering FFA (%), except for activated charcoal (Tab. I). Silica gel achieved the highest efficiency in the reduction of FFA (%) from oxidised oil when compared to other adsorbents and could lower the FFA contents to about 45.4% due to its high polarity that may aid in the attraction of the polar contaminants, which attribute to the reduction of eluted olive oil acidity. This indicated that the use of silica gel as adsorbents potentially improved the oil quality and its application as active adsorbents in oil treatment showed less accumulation of FFA compared to the control. Our results are in agreement with previous report findings on using silica gel as an effective adsorbent in reducing the FFA content of re-refined cooking oils [9, 18].

The effectiveness of the elution of oxidised olive oils through several tested adsorbents in reducing FFA contents were in the following increasing order:

Silica gel > Arabic gum > Bentonite > Aluminium oxide > Calcium chloride > Charcoal.

The alkalinity of each adsorbent was measured to eliminate the possibility of free fatty acid neutralisation from adsorbents. Table I, also shows the alkalinity of used adsorbents. Alkalinity was observed only in bentonite (0.028%) and Aluminium oxide (0.25%). However, the found alkalinity percentage were insignificant for the neutralisation of the fatty acids in the eluted and oxidised olive oil.

The ability of Arabic gum to adsorb FFA from olive oil may be related to its ability to form hydrogen bonding with the FFA and it forms a hydrophobic interaction with hydrophobic group of these CHO products. The efficiency of bentonite in the reduction of FFA (%) was about 38% in comparison with the control sample. Bentonite usually used in vegetable oil production as bleaching agent. The improvement in FFA (%) reduction after elution of oxidised olive oil through bentonite may be due to its sorption capability which serves as a filter for the removal of FFA [15]. Calcium chloride ($CaCl_2$) reduces the FFA content by 31.6%, and this may be related to the reaction of FFA with calcium chloride to form calcium based saponified solids [20]. Alumina was efficient in the reduction of FFA content in oxidised olive oil by 32.2%. Alumina is useful for the separation of aldehydes, ketones, quinones, esters, lactones, and glucosides and effective in reducing the acid value of used cooking oil [9]. The FFA content did not change, significantly, from the control after elution through activated charcoal, thus charcoal is expected not to adsorb any of FFA from oxidised oil sample and could not improve the efficiency of the free fatty acid removal after elution.

Table I - Free fatty acid contents of oxidized olive oil after elution through several adsorbents, improvement efficiency (%), elution time and alkalinity (% NaOH) of adsorbents[a]

Treatment (Adsorbents)	FFA (%) after elution	Improvement efficiency (%)	Elution time	Alkalinity of adsorbents (NaOH %)
Control	1.675 ± 0.007 [a]	0.00	0.00	ND[b]
Charcoal	1.664 ± 0.014[a]	0.66	20 min	ND
Bentonite	1.025 ± 0.035[c]	38.80	6 hours	0.028
Silica gel	0.915 ± 0.007[d]	45.37	7 hours	ND
Arabic gum	1.005 ± 0.035[c]	40.00	50 min	ND
Aluminum oxide	1.135 ± 0.007[b]	32.24	3 hours	0.25
Calcium chloride	1.145 ± 0.014[b]	31.64	5 hours	ND

[a] Results are means of triplicate ± SD and results with the same letter are not significantly different. [b]ND: Not detected.

3.2 PEROXIDE VALUE

Peroxide value (PV) is used as an indicator of the early oxidation of oils (primary oxidation products) and measures the value of peroxides and hydroperoxides formed in the early phases of lipid oxidation. The Initial PVs of the fresh olive oil samples used in this experiment, before oxidation was 7.76 meq O_2/kg and within the permitted limit values established by IOC standards (≤ 20.0 meq O_2/kg). However, after the intentional oxidation induction of fresh olive oil, the level was increased above the permitted level (32.39 meq O_2/kg). After the elution of the oxidised olive oil through several adsorbents used in this experiment, the PV`s were varied and the efficiency of the used adsorbents in reducing PV is shown in Table II. Arabic gum, calcium chloride and silica gel, were shown to be the most effective in the reduction of peroxide levels by 45.08, 25.65 and 22.63%, respectively. The samples eluted through Arabic gum adsorbent resulted in the greatest improvement of PV reduction (45.08%) from 32.39 to 17.79 meq O_2/kg and this may suggest the application of Arabic gum as an adsorbent and filters for the removal of peroxide products in oxidised olive oil. Silica has excellent adsorption capacities at low relative humidity conditions, which explain its capability in decreasing PV in our experiment. In addition, silica can remove polar contaminants. Silica offers the greatest potential for the edible oil refining industry [21, 9]. McNeill et al. [18] studied the effect of different mixtures between activated carbon and silica to improve the quality of canola oil and found that the canola oil treated with mixed adsorbents were effective in lowering acid values, peroxide value, saturated and unsaturated carbonyl contents polar compounds and photometric colour than the control.

The PV content of oxidised olive oil after elution through activated charcoal or aluminium oxide did not change significantly from the control. The elution of oxidised oil through bentonite showed a negative impact on PV improvement.

3.3 TOTAL PHENOLIC CONTENTS

Table III shows the impact of elution of the oxidised olive oil through studied adsorbents on the total phenolic contents. Significant reduction in phenolic content was observed when silica gel and aluminium oxide used as adsorbent (55.87 and 50.64%, respectively). The effect of oxidised olive oil elution through studied adsorbents on phenolic content reduction was in the following decreasing order: Bentonite > Charcoal > Arabic Gum > Calcium Chloride > Aluminium Oxide > Silica Gel. The reduction in phenolic contents after treatments may be due to the bound of phenolic compounds in olive oil to the surface of adsorbent by Van der Waal's forces and the adsorption capacity resulted is directly related to the pore structure, contact time and surface area of the adsorbents. Our results indicated that using adsorbents resulted in reduction of total phenolic contents, which may negatively affect the shelf-life stability of olive oil. However, the use of Arabic gum or calcium chloride as adsorbents had a minor effect on the total phenolic content reduction and a higher effect on PV and FFA % improvement, thus suggesting their uses as effective adsorbents. Zogorski et al. [22] studied the kinetics of the adsorp-

Table II - Peroxide value (PV) (meq O_2/kg) of fresh olive oil (control) and oxidized olive oil after elution through several adsorbents and improvement efficiency (%)[a]

Treatment (Adsorbents)	PV (meq O_2/kg) (after elution)	Improvement efficiency in PV (%)
Control	32.39 ± 0.86^{bc}	0.00
Charcoal	32.37 ± 0.56 ^{ab}	0.06
Bentonite	33.55 ± 0.35 ^{a}	- 3.58
Silica gel	25.06 ± 0.16^{d}	22.63
Arabic gum	17.79 ± 0.41^{g}	45.08
Aluminum oxide	32.35 ± 0.38 ^{ab}	0.12
Calcium chloride	24.11 ± 0.48^{d}	25.65

[a] Results are means of triplicate ± SD and results with the same letter are not significantly different

Table III - Total phenolic content (TPC) (mg GAE/Kg) of fresh olive oil (control) and oxidized olive oil after elution through several adsorbents and TPC reduction (%)[a]

Treatment (Adsorbents)	TPC (mg GAE/Kg) after elution	Reduction in TPC (%)
Control	101.40 ± 0.28^{a}	0.00
Charcoal	89.60 ± 0.56^{b}	11.64
Bentonite	91.10 ± 1.69^{b}	10.16
Silica gel	44.75 ± 0.77^{e}	55.87
Arabic gum	89.40 ± 0.56^{b}	11.83
Aluminum oxide	50.05 ± 0.77^{d}	50.64
Calcium chloride	86.90 ± 0.56^{c}	14.30

[a] Results are means of triplicate ± SD and results with the same letter are not significantly different

tion of phenols on granular activated carbon. They observed that 60% to 80% of the adsorption occurs within the first hour of contact followed by a very slow approach to the final maximum equilibrium concentration.
The main phenolic compound of olive fruit is oleuropein and polyphenols correlate with key sensory oil properties: bitterness and pungency that are associated with olive oil style [23]. Olive oil classification as mild, medium, or robust can be associated to the total phenol content. The total phenolic content of virgin olive oil expressed as Gallic acid equivalent (GAE) ranges from 50 to 800 mg/kg [24].
In this study, the total concentration of polyphenols for the fresh sample of olive oil was 101.4 mg Gallic acid equivalent /kg, which are within the range stated by IOC of virgin olive oil. Thus, the elution through used adsorbent may negatively affected the sensory properties of the resulting olive oil, but the level of phenolic after elution is still within the range of accepted figures for virgin olive oil, except for the silica gel adsorbent (44.8 mg GAE/Kg).

3.4 VITAMIN E

The concentrations of α-tocopherols in olive oil varied from traces to 25 ppm [25]. Results in Table IV show that vitamin E content in control olive sample was 34.45 ppm and decreased significantly after the elution of oxidised olive oil through several adsorbents due to the adsorption of tocopherols in the used adsorbents. The vitamin E loss were in the following increasing order: Control (0.0%) > Charcoal (36.6%) > Silica Gel (49.6%) > Arabic Gum (64.4%) > Calcium Chloride (66.7%) > Bentonite = Aluminium Oxide (69.9%). Four different types of tocopherols, namely α-, β-, γ- and δ-tocopherol have been reported in olive oil. Tocopherols are sensitive to light and heat; thus, we performed the experiment in a very protective environment to prevent its partial degradation; however, losses of tocopherols even in protective olive oil, such as darkness and high nitrogen during saponification, may have resulted [26]. Tocopherols are the most important lipid soluble natural antioxidants, which prevent lipid peroxidation by scavenging radicals in membranes and lipoprotein particles [27]. Results indicates a huge loss of this vitamin upon the use of any adsorbents for the partial refining of olive oil due to its adsorption in used filters.

3.5 SPECIFIC ABSORPTION COEFFICIENTS (K_{232} AND K_{270})

The absorbance at K_{232} nm, and K_{270} nm may correlate with the state of oxidation alteration (primary and secondary oxidation), adulteration of crude olive oil with refined oils and reflects the stage of oxidation for olive oil during storage by the increase in the K_{232} absorption coefficient. More specifically, in 232 nm primary oxidation products show absorption (conjugated peroxides) and in 270 nm secondary oxidation products show absorption (aldehydes and ketones) [28].
In this study, the extinction coefficient K_{232} of oxidised olive oil after elution through adsorbents increased significantly from that of the control (1.41), except with silica gel, bentonite, and aluminium oxide (Tab. V). Elution through silica gel was the best and could improve the oxidised oil quality by 15.36%, while elution through charcoal decreased the oxidised oil quality (7.21%). Different responses were recorded for the extinction coefficient measured at 232 nm and 270 nm (K_{232} and K_{270}) after elution of oxidised olive oil through adsorbents. Significant reduction in secondary products at K_{270} was the most after elution of oxidised oil through silica gel (73%). Our results agree with previous reports showing that synthetic silica compounds have greater selectivity for the adsorption of secondary oxidation compounds and reduce the conjugated diene [9, 29] However, aluminium oxide, bentonite and calcium chloride also showed pronounced improvement in K_{270} (Tab V).
Increase in K_{232} and K_{270} values is very common between the extraction and consumption of olive oil. These values are also affected by storage time and conditions. Such an increase is due to the degradation of primary oxidation products (peroxides) to form secondary oxidation products such as aldehyde and ketone. K_{232} representing the number of conjugated dienes of the primary oxidation products and are transformed to triene measured by K_{270} [30].

Table IV - Vitamin E content (mg/Kg) of fresh olive oil (control) and oxidized olive oil after elution through several adsorbents and reduction in vitamin E (%)[a]

Treatment (Adsorbents)	Vitamin E (mg/Kg) (after elution)	Reduction in Vitamin E (%)
Control	34.45 ± 0.49[a]	0.00
Charcoal	21.82 ± 0.38[b]	36.66
Bentonite	10.36 ± 0.41[f]	69.93
Silica gel	17.36 ± 0.79[c]	49.61
Arabic gum	12.26 ± 0.64[d]	64.41
Aluminum oxide	10.36 ± 0.13[f]	69.93
Calcium chloride	11.38 ± 0.60[e]	66.69

[a] Results are means of triplicate ± SD and results with the same letter are not significantly different.

Table V - Extinction Coefficient at 232 and 270 nm of fresh olive oil (control) and oxidized olive oil after elution through several adsorbents and improvement efficiency (%)[a]

Treatment (Adsorbents)	K_{232} (after elution)	Improvement efficiency (%) in K_{232}	K_{270} (after elution)	Improvement efficiency (%) in K_{270}
Control	1.595 ± 0.024[d]	0.00	0.260 ± 0.007[b]	0.00
Charcoal	1.710 ± 0.002[a]	- 7.21	0.240 ± 0.002[d]	7.70
Bentonite	1.580 ± 0.004[e]	0.94	0.220 ± 0.003[e]	15.38
Silica gel	1.350 ± 0.001[f]	15.36	0.070 ± 0.001[g]	73.08
Arabic gum	1.630 ± 0.005[c]	- 2.19	0.280 ± 0.009 [a]	- 7.70
Aluminum oxide	1.580 ± 0.008[e]	0.94	0.230 ± 0.004[d]	11.54
Calcium chloride	1.660 ± 0.019[b]	- 4.08	0.180 ± 0.002[f]	30.77

[a] Results are means of triplicate ± SD and results with the same letter are not significantly different

CONCLUSIONS

In this work, the specific impact of using several natural adsorbents to improve some of the oxidised olive oil quality characteristic like PV, FFA, K_{232} and K_{270} was comprehensively investigated to improve the shelf life and stability of olive oil. The results may suggest the use of the granular form of Arabic gum or silica gel or calcium chloride during the malaxation stage or after the final centrifugation step in olive oil production to improve some quality parameters of produced olive oil. Despite the loss of some of the active compounds in oil (vitamin E and phenolic compounds) due to the use of adsorbents, the impact of adsorbent usage during olive oil production still has an advantage. The effect of coating adsorbents on immobilised glass beads to improve some of negative results obtained from this research and how they interact with the olive oil elution time, phenolics and vitamin E contents, smoking points and GC-MS analysis of volatiles are under investigation.

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TEST e ANALISI

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- 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)

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- *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.

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Effect of the enrichment with natural antioxidants obtained by maceration or ultrasound-assisted extraction from olive leaves on organic extra virgin olive oil

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Improvement of nutritional quality and oxidative stability of Tunisian organic extra virgin olive oil (OEVOO) from "Chetoui" variety were studied after the enrichment with natural antioxidants obtained by maceration and ultrasound-assisted extraction of phenols from organic olive leaves. Results showed that the enrichment conserved the organic criteria and did not affect the OEVOO quality. The ultrasound assisted extraction of phenols in OEVOO induced a significantly higher oleic acid content (67.75) and lower linoleic acid (18.67). This sample showed the highest biophenols (269 mg/kg), chlorophylls (4.67ppm) and carotenoids (1.67ppm) contents and the lowest PV (14.52 meq-O2/kg-oil) and K_{232} (22.2) values, at the end of storage. However, Tocopherols content increased by maceration during storage. These findings explained the significant anti-radical activity of macerated and ultrasound enriched samples (29.82% and 35.5%) at the end of storage compared to control (8.03%). Thus, enrichment by ultrasonic extracts was more reliable against oxidation compared to macerated oil and control. Moreover, ultrasound assisted extraction improved the nutritional quality and sensorial properties of olive oil which was devoid of defects with a slight bitterness when compared to macerated oil having an unacceptable taste.

Keywords: Organic extra virgin olive oil, organic olive leaves, ultrasound-assisted extraction, maceration, stabilisation, natural antioxidants.

INTRODUCTION

In recent years, the global economy has shifted from mass production to quality production. This leads to new perceptions encouraging demand for" functional foods". It is a relatively new term used to describe food products that have been enriched with natural substances improving their quality and resistance to some phenomena such as the oxidation. To avoid or delay this phenomenon, recent studies have focused on the valorisation of natural plants extracts such as, tocopherols and polyphenols**.** These are known, by their fight against the oxidation of foods, their ability to reduce the risk of cancer, optimisation of infertility treatments [1] heart disease and diabetes [2], as well as their antibacterial, antioxidant, antiviral, anti-inflammatory and anti-allergenic activities [3]. Some fats such as olive oil are partially protected against oxidation by their natural antioxidant content but remain sensitive to photo-oxidation. In this approach, the stabilisation of vegetable oils (olive oil, sunflower, soybean, etc.) has been the subject of a great number of research, having opted for the enrichment of these oils with vegetable matrices. Among the matrices found abundantly in nature and little exploited olive leaves, which present 10% of the total mass of harvested olives [4]. It contains between 15 and 70 mg of phenolic compounds per gram of fresh mass, from which they can be exploited for food, pharmaceutical and cosmetic purposes [5]. Olive leaves phenols have a strong antioxidant capacity [6], broad antimicrobial

activity and several health benefits such as anti-inflammatory, cardio-protective and anti-diabetic effect [7]. Therapeutic interest of olive leaves was correlated also to its richness in Oleuropein whose concentration can reach 6.8% [8]. Several studies have proven antioxidant [6], antimicrobial [9], and anti-tumour [10] activities of oleuropein. Classical methods of extracting secondary metabolites from plants have been used such as maceration but innovative techniques such as supercritical fluid extraction, microwave assisted extraction and ultrasound assisted extraction, may increase production efficiency, and contribute to environmental preservation by reducing the use of water and solvents [11]. Although global demand for organic products has recently increased, studies on organic olive oil are still limited.

In this study, the stability of Tunisian organic extra virgin olive oil of the "Chétoui" variety enriched by olive leaves extracts, was studied. The enrichment assisted by ultrasound was tested and compared to the control and to the macerated oil. The quality criteria, tocopherols and polyphenols contents, antioxidant activity and sensorial properties were performed, during six months of storage at room temperature.

1. MATERIALS AND METHODS

1.1 PREPARATION OF RAW MATERIALS

The basic product used for this study was organic extra virgin olive oil from the "Chétoui" variety, extracted using a continuous three-phase system in the Al-jazzira-Morneg oil mill located in the north of Tunisia during 2019/2020 drop. Organic olive leaves (*Olea Europaea*) of the same variety were collected from an organic farm located in the same area. After washing the harvested leaves, they were dried in the open air until a constant mass was obtained, which makes possible the preservation of thermo-labile substances such as polyphenols and vitamins.

1.2 PREPARATION OF ENRICHED OLIVE OIL

1.2.1 Maceration

The olive leaves have been incorporated into the organic extra virgin olive oil in whole form at raison of 2% [12]. Enriched olive oil was stored in black glass bottles at room temperature and sampled during six months of storage.

1.2.2 Ultrasound assisted extraction

According to Achat et al. [13], the ultrasound-assisted extraction method was used to incorporate phenolic extracts directly from olive leaves into olive oil without solvents.

Twenty grams of dried and crushed organic olive leaves were added to 1L of organic extra virgin olive oil, which was used as a solvent. The whole was transmitted in a 3 L ultrasound tank. The optimal parameters used for more efficient extraction in terms of concentration of total phenols, oleuropein, tyrosol and hydroxytyrosol were power (60W), temperature (16°C) and time (45 min) [13]. The resulting mixture was filtered. All control and enriched samples were stored in black glass bottles in the dark at room temperature for six months.

1.3. HUMIDITY, PESTICIDES, AND IMPURITIES CONTENTS

For the characterization of the organic olive oil subject of this study, impurities contents and humidity were carried out, respectively according to ISO 663 [14] and ISO 662 [15]. The level of pesticides was analysed at the beginning and at the end of storage using the HPLC method to verify if the flavoring affects the biological criterion of the studied oil. The described analytical procedure was validated according to the SANCO/10684/2009 [16] validation protocol for analytical techniques for pesticide residues analysis in food and feed. This procedure fulfils the European Decision 2002/657/EC requirements [17]. The molecules sought for this analysis were: Dimethoate, Malation, ChlopyrifosEthyl, Methidathion, Phosmet, Féniquazin, Lamdacyhalothin, Acrinathin, Permethrin, Cypermethrin, Definiconazol, Deltamthrin.

1.4. QUALITY INDEXES

The acidity was determined according to ISO 660 [18] amending the regulations EEC (EEC n° 2568/91) [19], the peroxide value (PV) following the international standard ISO 3960 [20] and extinction coefficients (K_{232}, K_{270}) were performed according to the standard ISO 3656 [21].

1.5. FATTY ACIDS COMPOSITION

The determination of the total fatty acids composition of all studied olive oil samples was carried out at the beginning of storage by preparing the methyl esters according to the international standard ISO 5509 [22]. These esters were, then, analysed by gas chromatography (GC) according to the ISO 5508 [23].

1.6. PIGMENTS CONTENTS MEASUREMENTS

The pigments contents were performed using spectrophotometric method as described by Haddada et al. [24] using the following formulas:

Chlorophyll (mg/kg): $(A670 \times 10^6) / (E1 \times 100 \times d)$;
Caroténoïd (mg/kg): $(A470 \times 10^6) + (E2 \times 100 \times d)$
Where:
d: optical path = 1 cm;
A 670: absorbance at 670 nm;
A 470: absorbance at 470 nm;
E1: coefficient linked to the spectrophotometer = 613;
E2: coefficient linked to the spectrophotometer = 2000.

1.7. BIOPHENOL CONTENT

The determination of biophenols content was carried out by referring to the standard recommended by the

International Olive Council (IOC) [25]. The method was based on an extraction of minor polar compounds of biophenolic nature directly from olive oil using methanolic solution, followed by their assay by HPLC using an UV developer at 280 nm. The internal standard consists of syringic acid. The content corresponding to natural and oxidized derivatives of oleuropein and ligstroside, lignans, flavonoids and phenolic acids was expressed in mg / kg of tyrosol.

1.8. DETERMINATION OF TOCOPHEROLS

Tocopherols composition was determined according to the ISO 9936 [26]. The compounds were identified by chromatographic comparisons with authentic standards by co-elution and by their UV spectra.

1.9 SENSORY EVALUATION

The sensorial evaluation of the studied olive oils was carried out according to the IOC [27] by 8 expert panellists of the Tunisian National Oil Office. The test conditions were chosen according to the same standard. Panellists smelled and tasted each oil and carried out on the profile sheet made available to him the intensity at which they perceived each of the negative (fusty/muddy, musty, winey, metallic, rancid, Frost-bitten olives (wet wood)) and positive (fruity, bitter, pungent) attributes. The head of the jury, after having collected the profile sheets completed by each of the tasters, checked the assigned intensities and calculated the medians of the various attributes.

1.10 STATISTICAL ANALYSIS

Results of different parameters were expressed as the mean ± standard deviation. An analysis of variance (ANOVA) was performed at a significance level of 5%. Duncan multiple range test (DMRT) is a multiple comparison method in which group means were ranked in ascending order. This method was performed using IBM SPSS Statistics version 23. All analytical determinations were performed at least in triplicate.

2. RESULTS AND DISCUSSION

2.1 CHARACTERISATION OF ORGANIC EXTRA VIRGIN OLIVE OIL

According to the standard IOC [28], obtained results on raw material shown in Table I, proved that the studied olive oil was of good quality and under the nomination “extra virgin”. Initial values of peroxide value (PV), free fatty acids (FFA), K_{232} and K_{270} were respectively 7.39 meqO_2/Kg±0.45; 0.24±0.03; 1.87±0.04 and 0.17±0.01 which correlate with those found by Ben Tkaya et al. [29] for an extra virgin olive oil (EVOO) from the same studied variety. Compared to the results found by Oueslati et al. [30] for various varieties of Tunisian EVOO, the initial chlorophylls and biophenols contents noted on the studied extra virgin olive oil were lower (4.51±0.05 et 255±0.05 respectively). However, it was observed that carotenoids and α-tocopherol contents were higher (1.55±0.06 et 417.32±5.6, respectively).

Table I - Characterization of organic extra virgin olive oil

Paramètre	Value
FFA (%)	0.24±0.03
K_{232}	1.87±0.04
K_{270}	0.17±0.01
PV (meq O_2/Kg)	7.39±0.45
Carotenoid content (ppm)	1.55±0.06
Chlorophyll content (ppm)	4.51±0.05
Humidity (%)	0.067
Impurities content (%)	0.0063
Biophénols content (mg/kg)	255±0. 05
α-tocopherols (ppm)	417.32±5.6
β-tocopherols (ppm)	n.d
Ɣ-tocopherols (ppm)	22.81±1.5
δ-tocopherols (ppm)	5.58±0.2

Table II - Initial fatty acids composition (%) of control and flavored oils

	Control	MOO	UOO	Limit (IOC, 2019)
$C_{16:0}$	10.78±0.28^a	10.78±0.32^a	11.47±0.42^a	7.20-20.00%
$C_{16:1}$	0.48±0.05^a	0.46±0.05^a	0.49±0.05^a	0.30-3.50%
$C_{17:0}$	0.05±0.00^a	0.05±0.01^a	0.05±0.00^a	≤0.40
$C_{17:1}$	0.05±0.01^a	0.05±0.00^a	0.06±0.02^a	≤0.60
$C_{18:0}$	3.06±0.0^a	3.05±0.01^a	3.12±0.00^a	0.5-5.00%
$C_{18:1}$	63.06±0.39^a	63.34±0.76^a	67.75±0.42^b	55.00-83.00%
$C_{18:2}$	20.75±0.63^b	20.67±0.37^b	18.67±0.24^a	2.50-21.00%
$C_{18:3}$	0.71±0.03^a	0.7±0.00^a	0.7±0.04^a	≤1.00
$C_{20:0}$	0.43±0.01^a	0.44±0.00^a	0.45±0.09^a	≤ 0.60%
$C_{20:1}$	0.36±0.08^a	0.36±0.01^a	0.39±0.03^a	≤0.50

Control: Unenriched olive oil; MOO: enriched olive oil by maceration UOO: Ultrasound enriched olive oil.
Data are mean ± standard deviation, n=3. Means with different superscripts are significantly different ($p < 0.05$). Letters represent the statistical difference between samples.

2.2 ASSESSMENT OF PESTICIDE LEVELS

The results related to the levels of pesticides analysed at the beginning and at the end of the storage period showed that the enrichment of EVOO by natural antioxidants using maceration or ultrasound-assisted extraction maintained the biological criterion of studied olive oil. In fact, all pesticides were absent in control and enriched EVOO samples during the whole of storage period.

2.3 CHANGES IN FATTY ACIDS COMPOSITION

The initial fatty acids composition of the studied olive oil is shown in Table II. The results demonstrated a predominance essentially of oleic acid (63.06%) and a richness in palmitic acid (10.78%) and linoleic acid (20.75%) in the control olive oil, compared to the Spanish, Italian and Greek olive oils as reported before by Boudiche et al. [31]. It was noted that the enrichment by maceration of organic olive leaves had no effect on the fatty acids composition. However, applying ultrasound-assisted extraction leaded to an EVOO with a significant ($p<0.05$) high amount in oleic acid (67.75%) and low level in linoleic acid (18.67%) compared to the control. This finding was attributed, first, to the richness of olive leaves from Chetoui variety in oleic acid (25.07%) [32]. Besides, according to Hang et al. [33] the amount of unsaturated fatty acids decreased in various olive oils after 1h of ultrasonic processing at 80°C that explain the decrease of linoleic acid level. Similar results were found by Jaber et al. [34] reporting that the enrichment of a refined olive oil by the chlorophyll extract of olive leaves increased the oleic acid content and decreased that of the linoleic acid.

2.4 CHANGES IN QUALITY INDICES

Acidity (FFA)

From the Table III, no significant differences ($p>0.05$) in terms of acidity were observed between control and enriched EVOO initially and during the six-month storage. However, a significant increase ($p<0.05$) of

Table III - Changes in quality parameters and pigments contents of control and enriched olive oils during six months of storage

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
	Acidity (%)						
Control	$0.24\pm0.03^{a,1}$	$0.27\pm0.01^{a,1,2}$	$0.29\pm0.01^{a,1,2}$	$0.3\pm0.6^{a,1,2}$	$0.31\pm0.06^{a,1,2}$	$0.33\pm0.04^{a,1,2}$	$0.35\pm0.05^{a,2}$
MOO	$0.24\pm0.03^{a,1}$	$0.27\pm0.00^{a,1,2}$	$0.29\pm0.02^{a,1,2,3}$	$0.31\pm0.3^{a,1,2,3,4}$	$0.33\pm0.04^{a,2,3,4}$	$0.36\pm0.07^{a,3,4}$	$0.39\pm0.03^{a,4}$
UOO	$0.24\pm0.02^{a,1}$	$0.26\pm0,05^{a,1,2}$	$0.29\pm0.01^{a,1,2,3}$	$0.3\pm0.4^{a,1,2,3}$	$0.32\pm0.05^{a,2,3}$	$0.33\pm0.04^{a,2,3}$	$0.35\pm0.04^{a,3}$
	PV ($meqO_2/Kg$)						
Control	$7.39\pm0.45^{a,1}$	$8.82\pm0.49^{a,2}$	$9.76\pm0.43^{b,3}$	$10.77\pm0.46^{b,4}$	$12.89\pm0.43^{b,5}$	$14.58\pm0.50^{b,6}$	$16.84\pm0.52^{b,7}$
MOO	$6.69\pm0.39^{a,1}$	$8.51\pm0.48^{a,2}$	$9.16\pm0.41^{b,2,3}$	$9.65\pm0.24^{a,3}$	$10.81\pm0.35^{a,4}$	$12.72\pm0.53^{a,5}$	$14.85\pm0.54^{a,6}$
UOO	$7.24\pm0.35^{a,1}$	$8.52\pm0.48^{a,1,2}$	$9.77\pm0.26^{a,2}$	$9.24\pm0.25^{a,3}$	$11.41\pm0.12^{a,4}$	$12.27\pm0.47^{a,5}$	$14.52\pm0.48^{a,6}$
	K_{232}						
Control	$1.89\pm0.04^{a,1}$	$2.01\pm0.04^{a,2}$	$2.15\pm0.04^{b,3}$	$2.25\pm0.04^{c,4}$	$2.35\pm0.05^{c,5}$	$2.43\pm0.05^{c,6}$	$2.5\pm0.06^{c,6}$
MOO	$1.87\pm0.03^{a,1}$	$1.92\pm0.07^{a,1}$	$2.03\pm0.05^{a,2}$	$2.13\pm0.03^{b,3}$	$2.23\pm0.04^{b,4}$	$2.28\pm0.06^{b,4,5}$	$2.35\pm0.05^{b,5}$
UOO	$1.87\pm0.02^{4,a,1}$	$1.93\pm0.03^{a,1,2}$	$1.97\pm0.03^{a,2,3}$	$2.03\pm0.05^{a,3,4}$	$2.08\pm0.06^{a,4}$	$2.17\pm0.04^{a,5}$	$2.22\pm0.04^{a,5}$
	K_{270}						
Control	$0.17\pm0.01^{a,1}$	$0.18\pm0.02^{a,1}$	$0.18\pm0.02^{a,1}$	$0.19\pm0.03^{a,1}$	$0.2\pm0.03^{a,1}$	$0.2\pm0.03^{a,1}$	$0.21\pm0.03^{a,1}$
MOO	$0.17\pm0.01^{a,1}$	$0.19\pm0.04^{a,1}$	$0.18\pm0.04^{a,1}$	$0.20\pm0.02^{a,1}$	$0.20\pm0.04^{a,1}$	$0.2\pm0.03^{a,1}$	$0.22\pm0.05^{a,1}$
UOO	$0.17\pm0.03^{a,1}$	$0.18\pm0.02^{a,1}$	$0.19\pm0.04^{a,1}$	$0.19\pm0.04^{a,1}$	$0.19\pm0.03^{a,1}$	$0.21\pm0.04^{a,1}$	$0.21\pm0.04^{a,1}$
	Chlorophyll contents (ppm)						
Control	$4.5\pm0.05^{a,7}$	$4.27\pm0.06^{a,6}$	$4.09\pm0.05^{a,5}$	$3.84\pm0.06^{a,4}$	$3.48\pm0.06^{a,3}$	$2.90\pm0.06^{a,2}$	$1.95\pm0.06^{a,1}$
MOO	$4.5\pm0.08^{a,5}$	$4.44\pm0.05^{b,5,4}$	$4.32\pm0.06^{a,b,4,3}$	$4.18\pm0.03^{b,3,2}$	$4.07\pm0.04^{b,2}$	$3.4\pm0.05^{b,1}$	$3.26\pm0.05^{b,1}$
UOO	$4.67\pm0.03^{b,4}$	$4.57\pm0.03^{b,4}$	$4.43\pm0.09^{b3,}$	$4.32\pm0.02^{b,3,2}$	$4.25\pm0.03^{c,2}$	$4.00\pm0.03^{c,1}$	$3,97\pm0,04^{c,1}$
	Carotenoids contents (ppm)						
Control	$1.55\pm0.06^{a,5}$	$1.45\pm0.02^{a,5,4}$	$1.36\pm0.05^{a,4}$	$1.17\pm0.09^{a,3}$	$1.09\pm0.00^{a,3,2}$	$0.96\pm0.04^{a,2}$	$0,54\pm0.04^{a,1}$
MOO	$1.56\pm0.03^{a,5}$	$1.47\pm0.04^{a,b,5}$	$1.39\pm0.04^{a,b,4}$	$1.25\pm0.04^{a,b,4,3}$	$1.18\pm0.04^{a,b,3,2}$	$1.07\pm0.03^{b,2}$	$0,86\pm0.06^{b,1}$
UOO	$1.67\pm0.03^{b,5}$	$1.55\pm0.02^{b,4}$	$1.5\pm0.02^{b,4}$	$1.34\pm0.04^{b,3}$	$1.25\pm0.04^{b,2}$	$1.17\pm0.05^{c,2}$	$1,04\pm0.05^{c,1}$

PV: Peroxide value; K_{232} and K_{270} : specific extinctions Control: Unenriched olive oil; MOO: enriched olive oil by maceration UOO: Ultrasound enriched olive oil. Data are mean ± standard deviation, n=3. Means with different superscripts are significantly different ($p < 0.05$). Lowercase letters (a, b, c) represent the statistical difference between samples; Numbers (1-7) represent the statistical difference between the same sample during storage period.

acidity was noted for all studied olive oils that can be explained by the enzymatic activity caused by lipolytic reactions in olive oil [35]. This evolution was faster especially in macerated olive oil (MOO). Acidity increased to reach values of about 0.35±0.05, 0.39±0.04 and 0.35±0.03, respectively for the control, MOO, and ultrasound enriched olive oil (UOO). These results agreed with those of several studies showing that adding extracts from aromatic and medicinal plants to olive oil leads to an increase in acidity. Indeed, the addition of the aqueous extract of olive leaf during the mixing step resulted in an increase in acidity [36]. Similar results were found by Sousa et al. [35] reporting that adding garlic significantly increased acidity values compared to the control.

Peroxide value

Obtained initial peroxide values (PV) showed that studied EVOO were of good quality (Tab. III). These values increased significantly ($p<0.05$) during storage for all analysed olive oils particularly from the third month of storage. This result could be assigned to the increase of storage temperature as described by Ben Tekaya et al. [29] suggesting that an increase of temperature by about 10 degrees could accelerate oil oxidation leading to high PV values. Besides, it was confirmed in the literature that once auto-oxidation is started, it does not stop until all the free radicals that are formed are inactivated [37]. Results showed that enriched EVOOs were more stable than control over time favouring olive oil enriched by ultrasonic extracts having the lowest PV (14.52±0.48 meqO_2/kg), at the end of the sixth month of storage, when compared to MOO (14.85±0.54 meqO_2/kg) and the control (16.84±0.52 meqO_2/kg) (Tab. III). These findings confirmed that the enrichment with olive leaves rich in antioxidants and oleuropein reduced the formation of lipid hydroperoxides and the oxidation of the olive oil compared to the control. These results agreed with those of Jaber et al. [34] reporting that the addition of chlorophylls extracted from Chemlali olive leaves resulted in an appreciable resistance to oxidation. Likewise, Shahin et al. [38] found that olive oil enriched with oleuropein has a lower PV (7.07) than that of pure oil (9.09).

Specific extinction coefficients

In this study, the obtained values of the K_{232} coefficient were in line with those of PV previously reported. Indeed, from the second month of storage, this coefficient noted for the control (2.15±0.4) became significantly higher ($p<0.05$) than those of enriched samples (Tab. III). Similar results were found in the literature [34, 36]. K_{232} values increased significantly ($p<0.05$), during storage, proving that flavouring by ultrasound-assisted extraction is more reliable against oxidation than that by maceration. The decrease in the quality of the oil during the maceration of the olive leaves may be due to oxidative enzyme (lipoxygenase in olive leaves).

Concerning the extinction coefficient K_{270}, no significant differences ($p>0.05$) were revealed between all analysed EVOOs from the beginning and until the end of storage period.

2.5 CHANGES IN PIGMENTS CONTENTS

From the first day of storage, initial chlorophylls contents were equal to 4.5±0.05 ppm; 4.5±0.08 ppm and 4.67±0.03 ppm; respectively for control, MOO and UOO showing a significant ($p<0.05$) higher level for the olive oil enriched by the ultrasonic extracts (Tab. III). Later, this also had a higher initial carotenoids content (1.67±0.03 ppm). These findings can be explained by the high time of contact (45 min) between EVOO and olive leaves, when using ultrasound-assisted extraction. Indeed, ultrasonic extraction is a very simple method that relies on the mechanical effect caused by the implosion of micro-bubbles that cause a rapid breakdown of the tissues allowing the release of compounds in the solvent [39] representing, in our study, the olive oil itself. During the six months of storage, the pigment contents decreased significantly ($p<0.05$) in all studied EVOO samples. At the end of storage, the highest chlorophylls (3.97±0.04) and carotenoids (1.04±0.05) contents were noted for ultrasonic enriched EVOO. These results were in accordance with those of Wang et al. [40] suggesting that the yellow pigment yield could be improved by an ultrasound-assisted extraction and will lead to antioxidant activity in treated olive oil.

2.6. CHANGES IN BIOPHENOL AND TOCOPHEROLS CONTENTS

2.6.1 Variations in tocopherols contents

Tocopherols are important components of olive oil because they have interesting properties due to their vitamin function and valuable antioxidant power, which makes their characterisation essential. As expected, the α-tocopherols were dominant in all studied EVOOs. As shown in Table IV, their levels varied between 416±8.1 ppm (MOO) and 418±5.3 ppm (UOO), at the beginning of storage without significant difference ($p>0.05$) between all analysed samples. The studied olive oils did not contain β-tocopherols. After six months of storage, α-tocopherol contents decreased significantly ($p<0.05$) to reach 383.57±3.2ppm and 381.57±8.2 ppm, respectively in UOO and control. However, the maceration leaded to a slight and significant increase of α-tocopherol content reaching a value of about 425.5 ppm, at the end of storage. For γ-tocopherols and δ-tocopherols contents evolution, it followed the same trends, during storage. The increase of tocopherols in macerated olive oil can be attributed to the migration of vitamin E from organic olive leaves, rich in tocopherols [41] to olive oil which played the role of a 'green' solvent. In fact, extracts from olive leaves had shown

Table IV - Changes in tocopherols and biophenols contents (ppm) of control and enriched olive oils during six months of storage

	Tocopherols contents (ppm)						
	Day 0			Day 180			
	α-tocopherols	β-tocopherols	Y-tocopherols	δ-tocopherols(mg/kg)	α-tocopherols	β-tocopherols	Y-tocopherols	δ-tocopherols(mg/kg)
Control	417.32±5.6^{a,2}	n.d	22.81±1.5^{a,2}	5.58±0.2^{a,2}	381.57±8.2^{a,1}	n.d	19,62±0.5^{a,1}	4,35±0.2^{a,1}
MOO	416.55±8.1^{a,1}	n.d	21.53±0.8^{a,1}	5.44±0.7^{a,1}	425.5±1.5^{b,2}	n.d	24,5±1.9^{b,2}	7,28±0.5^{b,2}
UOO	418.12±5.3^{a,2}	n.d	21.22±1.9^{a,2}	5.46±0.5^{a,2}	383.57±3.2^{a,1}	n.d	18,17±0.7^{a,1}	4,84^{a,1}
	Biophenols contents (mg/Kg)							
	Day 0			**Day 90**			**Day 180**	
Control	255±0.05^{b,3}			128±0.02^{a,2}			61±0.03^{a,1}	
MOO	233±0.06^{a,3}			138±0.06^{b,2}			84±0.04^{b,1}	
UOO	269±0.06^{c,3}			154±0.04^{c,2}			101±0.05^{c,1}	

Control: Unenriched olive oil; MOO: enriched olive oil by maceration UOO: Ultrasound enriched olive oil; n.d: None detected; Data are mean ± standard deviation, n=3. Means with different superscripts are significantly different ($p < 0.05$). Lowercase letters (a, b, c) represent the statistical difference between samples; Numbers (1-3) represent the statistical difference between the same sample during storage period.

their ability to improve the quality of olive oil regarding tocopherol contents in several studies [38].

2.6.2. Variations in biophenols contents

The initial values of biophenols contents registered for all studied extra virgin olive oils are shown in Table IV. The higher content was observed for UOO (269±0.06 mg/kg) followed by control (255±0.05 mg/kg) then macerated oil (233±0.06 mg/kg). This result can be explained by the absence of oleuropein in the control and the stability of natural phenols like tyrosol and hydroxytyrosol in EVOO, under sonication. As expected, a reduction of phenolic contents in all organic extra-virgin olive oils was registered, throughout the six months of storage (Tab. IV). At the end of storage, the lowest phenolic content was detected for control (61±0.03 mg/kg) and the highest contents were observed in enriched oils by ultrasonic extracts (101±0.05 mg/kg) and maceration (84±0.04 mg/kg). These findings agreed with the results registered in previous studies. Indeed, Jaber et al. [34] reported that the total phenol content decreased considerably by over 2% in refined olive oil enriched with chlorophyll pigments extracted from Chemlali olive leaves after 2 months of storage. The same authors showed that, the phenolic content decreased by about 5% compared to the initial content after 6 months of storage.

Contrastingly, these results disagree with those of Sousa et al. [35], who reported that the incorporation of different flavouring dried agents (garlic, laurel, oregano) did not show any protective effect against the oxidation of olive oil stored in the same conditions. This finding suggested a higher efficiency of ultrasound-assisted extraction compared to maceration explained by the fact that ultrasound waves after interaction with plant material alter its physical and chemical properties and that cavitation facilitates the release of extractable compounds and enhances the mass transport by disrupting the plant cell walls [12].

2.6.3 Variations in antioxidant activity

The antioxidant activity of various studied olive oils decreased significantly ($p<0.05$), during storage, to reach 8.03±0.02, 29.82±0.00 and 35.5±0.02, respectively for control, MOO and UOO (Tab. V). This result could be attributed to the fact that natural antioxidants such as tocopherols, chlorophylls, sterols, and polyphenols undergo auto-oxidation, leading to their degradation and a decrease in anti-radical activity [42]. It was noted that the antioxidant activity of enriched oils was higher than that of the control (Tab. V). Although the macerated olive oil exhibited the highest α-tocopherol content at the end of storage, UOO appeared to be more effective in scavenging the DPPH radical. This result can be attributed to the

Table V - Evolution of the antioxidant activity (RSA(%)) of control and enriched olive oils during six months of storage

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
	DPPH Essay						
Control	57.82±0.07^{a,4}	36.55±0.04^{a,3}	18.04±0.03^{a,2}	13.17±0.02a,2,1	11.87±0.01^{a,2,1}	9.19±0.02^{a,1}	8.03±0.02^{a,1}
MOO	64.75±0.09^{a,6}	52.98±0.04^{b,5}	48.94±0.04^{b,5,4}	45.05±0.02^{b,4,3}	40.70±0.01^{b,3,2}	33.90±0.02^{b,2,1}	29.82±0.00^{b,1}
UOO	60.60±0.03^{a,6}	58.74±0.02^{b,5}	51.44±0.01^{b,4}	47.75±0.03^{b,4,3}	44.21±0.05^{b,3,2}	39.00±0.04^{b2,1}	35.50±0.02^{c,1}

Control: Unenriched olive oil; MOO: enriched olive oil by maceration UOO: Ultrasound enriched olive oil;
Data are mean ± standard deviation, n=3. Means with different superscripts are significantly different ($p < 0.05$). Lowercase letters (a, b, c) represent the statistical difference between samples; Numbers (1-6) represent the statistical difference between the same sample during storage period.

Table VI - Sensory evaluation values of control and enriched olive oils during storage

	Day 0			Day 180		
Attributes	**Control**	**MOO**	**UOO**	**Control**	**MOO**	**UOO**
Fusty	0	0	0	0,5^{b}	0^{a}	0^{a}
Musty	0	0	0	0^{a}	0,4^{b}	0^{a}
Winey	0	0	0	0^{a}	1,4^{b}	0^{a}
Wet wood	0	0	0	n.d	n.d	n.d
Metallic	0	0	0	n.d	n.d	n.d
Rancid	0	0	0	n.d	n.d	n.d
Fruity	3^{a,2}	3.5^{b,2}	4^{c,2}	1.5^{a,1}	2^{b,1}	3.2^{c,1}
Pungent	3^{a,2}	3.25^{b,2}	3.8^{c,2}	2.2^{b,1}	0.4^{a,1}	2.6^{c,1}
Bitter	3^{a,2}	3.25^{b,2}	3.5^{c,2}	2.2^{b,1}	1.8^{a,1}	2.8^{c,1}

Control: Unenriched olive oil; MOO: enriched olive oil by maceration UOO: Ultrasound enriched olive oil; n.d: None detected; Data are mean ± standard deviation, n=3. Means with different superscripts are significantly different ($p < 0.05$). Lowercase letters (a, b, c) represent the statistical difference between samples; Numbers (1-2) represent the statistical difference between the same sample during storage period.

richness of UOO sample with phenolic antioxidants (mainly oleuropein) and pigments, which confirmed the usual correlation between the antioxidant activity and total phenolic content. Therefore, Interesse et al. [43] reported that pigments had pro-oxidising power in oil samples exposed to light and an antioxidant power in the dark.

2.7 CHANGES IN SENSORIAL PROPERTIES

At the beginning of storage, the sensory profiles of all studied EVOOs were devoid of defects. Moreover, the difference was significant ($p<0.05$) between all studied samples in terms of positive attributes. In fact, enriched oils were more acceptable, especially ultrasound enriched one, with stronger fruity smell (4), bitter (3.8) and pungent taste (3.5) (Tab. VI). Similar results were found by [33] who proved that sensory attributes were enhanced with the addition of olive leaves, in term of green colour and fruity attributes.
At the end of storage, the fusty defect was identified for the control which had also the lowest fruity intensity (1.5). Macerated organic EVOO was musty (0.4), with an unacceptable taste. More, MOO showed a marked decrease in the bitterness probably explained by the reduction of phenolic content particularly oleuropein during storage. In fact, it was reported by Betran et al. [44] the significant positive correlation between bitterness intensity and the level of phenols. These findings showed the deterioration of the quality of whole fresh olive leaves in MOO, during storage, which leaded also, to the appearance of the 'winey' attribute (1.4) usually caused by the formation of acetic acid during storage. However, at the end of storage, the UOO sample had no defects and presented a slight increase in bitterness compared to maceration and unenriched oil due to the presence of the highest amount of phenols. This result was in line with those of Achat et al. [13]

CONCLUSION

During storage of organic extra virgin olive oils, at room temperature and in darkness, results revealed that enrichment using ultrasound-assisted extraction from olive leaves increased oleic acid content and reduced linoleic acid one compared to control and macerated oil. The enrichment of organic olive leaves extracts using the two methods improved the oxidation stability of virgin olive oil by reducing the PV and extinction coefficients. The antioxidant activity was higher when the ultrasound-assisted extraction was applied which can be explained by their highest content of pigment and biophenols, during the storage period. Sensorial analysis showed an improvement in taste and odour of olive oil enriched with ultrasonic extracts with the highest overall acceptability after six months compared to control and macerated olive oil. These results encourage the use of organic olive leaves as a source of natural antioxidants for improvement of quality and oxidative stability of olive oil

and mainly the application of an ultrasound-assisted extraction, which is a potential emerging technology that can accelerate heat and mass transfer with shorter processing times and reduced operating and maintenance costs leading to better quality. Thus, it should be mentioned on the label that it is an "enriched olive oil" and not "extra virgin olive oil" from a legislation point of view.

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I PROFUMI

Il **laboratorio Cosmetica e Detergenza** presenta un'**offerta analitica per profumi per persona, profumatori per ambiente, fragranze**

Le principali **analisi** eseguite sono:

- Colore
- Contenuto in alcol etilico
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- Quantificazione delle fragranze allergeniche volatili per GC-MS (UNI EN 16274:2013)
- Determinazione degli ftalati per GC-MS (UNI EN 16521:2014)
- Indice di rifrazione

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I **profumi** e i **profumatori** sono prodotti di consumo largamente diffusi e usati quotidianamente da uomini e donne. Le **fragranze** entrano a far parte della **formulazione** di numerosi prodotti cosmetici come **bagnoschiuma, creme, shampoo**.

La **legislazione europea** prevede l'obbligo di **informare il consumatore** sulla presenza di fragranze **potenzialmente allergeniche** nei prodotti cosmetici e quindi ha stabilito che tali sostanze siano indicate in **etichetta** quando presenti.

Le industrie del settore quindi devono controllare le materie prime e i prodotti finiti per redigere un'**etichetta corretta a tutela dei consumatori**: sono indispensabili le **determinazioni delle fragranze potenzialmente allergeniche e degli ftalati**.

I prodotti possono inoltre presentare problemi di **odori anomali** la cui fonte è spesso da ricercare nella **purezza delle materie prime**, quali ad esempio l'alcool etilico.

Improvement of Tunisian 'Chemlali' extra virgin olive oil stability with rosemary and laurel herbs and essential oils

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This study was carried out to investigate the effect of different flavourings (laurel and rosemary), commonly used in the Mediterranean diet, on the quality of Tunisian extra virgin olive oil derived from the variety "Chemlali". The maceration of the two herbs or the incorporation of their associated essential oils were applied.
The resistance to oxidation of flavoured and enriched olive oils was determined by measuring quality index values. During three months of storage, an increase of these indexes was recorded for all analysed olive oils. However, this increase was less pronounced in flavoured virgin olive oils when compared to control. Also, results showed more stability of total polyphenols as well as chlorophylls and carotenoids pigments essentially for macerated enriched olive oils which were characterised by a high antioxidant capacity. Finally, based on the sensory evaluation, flavoured olive oils with essential oils were more appreciated by consumers than olive oils incorporated with rosemary essential oil.
Keywords: Extra virgin olive oil, Rosemary, Laurel, Maceration, Essential oil, Stability.

1. INTRODUCTION

Since the early 1990s, the dynamics of the global olive oil market have been marked by the increase in demand and the appearance of new markets such as Canada, the United States of America, Brazil, Japan, and China. These mutations have offered Tunisian exporters opportunities to increase exports and diversify markets. However, this country has been faced with competition from European countries which are constantly increasing their market shares in these new markets. In addition, the emergence of new producer countries such as Turkey, Syria, Morocco, Jordan and recently some Latin American countries have led Tunisia to face several challenges such as the differentiation of its product to maintain its competitiveness in the world olive oil markets.
Extra virgin olive oil is a key ingredient widely produced and consumed in Mediterranean diet. It is appreciated for its nutritional properties, pleasant aroma, and delicious taste [1] and for having the most restrictive quality criteria among olive oils categories [2]. Virgin olive oil is characterised by high contents of monounsaturated fatty acids (oleic acid) and natural antioxidants known to show protective effects against many modern life-style diseases [2, 3, 4]. In recent years, the olive oil consumption is increasing due to its sensorial characteristics and health claims [4]. However, in the olive sector, face to consumer increasing demand for top quality, healthy, and innovative products, it has been shown that packaging and aromatisation of olive oils has immerging as interesting innovation practice in new olive oil markets [1, 2]. In 2010, the launch of the "Bio Tunisia" label to create AOCs in Tunisia, increased international demand for standard quality and orientation towards stabilization and prevention from oxidation of olive oil by the addition of appropriate natural antioxidants [2] could be a solution to widen the destina-

tions. In fact, Tunisian olive oil is very appreciated and 90% of its exports is in conditioned form that offer to the Tunisian olive oil its own identity [5].
Furthermore, according to Farras et al. [6], the consumption of antioxidant-rich or functional virgin olive oil promotes high-density lipoprotein (HDL) and prevent against cardiovascular diseases. They reported that bioactive compounds and essential oils can decrease low-density lipoprotein cholesterol concentrations. In this regard, several kinds of flavourings in olive oils were used particularly: essential oils, fruits (apple, orange and lemon), aromatic plants (basil, fennel, laurel, oregano, rosemary, and thyme), mushrooms, nuts, spices and vegetables (dried tomatoes, hot chili peppers, onions, pepper) [4]. Among these flavourings, the common traditional practice was the aromatization with aromatic plants and spices well known for containing essential oils with antioxidant and antimicrobial properties by using different methods [2, 7]. In fact, these flavourings could be added to the olive oil after its extraction by infusion or maceration or can be mixed directly with the olive paste fruits during the oil-productive process [1, 4, 8]. The efficiency of these bioactive flavourings, particularly rosemary and laurel, was proved in some studies on olive oils stability by protecting the oils from thermal oxidation with improvement of their sensorial properties (aroma, taste and colour) due to their health benefits and organoleptic and antioxidant characteristics [2, 7]. Also, flavouring could add further value to this precious agricultural product when increasing its use among non-traditional consumers.
In this connection, with the present study we intend to compare the influence of two enrichment methods: the maceration of two common herbal plants (rosemary and laurel) and the adjunction of their essential oils on chemical and sensory quality of flavoured olive oil as well as his stability during 84 days of storage. The effect of these aromatic plants on the quality parameters (free acidity, peroxide value and K_{232}, K_{270}), fatty acids profile, total phenols content, antiradical scavenging activity and oxidative stability were investigated.

2. MATERIALS AND METHODS

2.1. MARKET STUDY

The objective of this part is to carry out a market study for flavoured olive oil to understand the behaviour and preferences of Tunisian consumers towards this product. Studying consumer behaviour is an essential tool, especially for innovative products, because it helps guide a company's business decision and reduces uncertainty about the choice of target consumers [9].
In this study, the consumer survey was disseminated online (through a questionnaire posted on a social network) and face to face. The online questionnaire has the advantage of being able to be self-administered and does not require the presence of the interviewer. In Tunisia there are 6 million active users of the social network. This approach therefore makes it possible to reach a wide spectrum of consumers who are geographically dispersed and who use the Internet at very different frequencies.
The studied sample consists of 200 people. The mentioned data in the questionnaire such as sex, age, socio-professional category and geographical area allowed us to classify the people questioned. The processing of the survey data was carried out using two methods.

2.1.1. The one-dimensional method (flat sorting)

It represents the distributions with a single variable giving the frequencies relating to each variable and constituting the simplest examples of statistical tables. These tables are of great importance for reading quantitative data [10].

2.1.2. The two-dimensional method (cross sorting)

It represents the two-variable distributions and consists of crossing the results of the variables two by two (cross sorting, or two-dimensional cross tables or even double entry tables) to determine whether there is a significant correlation between two well-defined variables [10].

2.2. RAW MATERIAL

Tunisian olive oil used in this study derived from the variety "Chemlali". The preliminary analysis on the obtained olive oil showed low level of oxidative degradation and then the good results of the panel test allowed classification of the oil as extra virgin. To produce flavoured and enriched olive oil, two aromatic and medicinal plants that grow in abundance in Tunisia were used. Rosemary and laurel were collected from North of Tunisia, identified and authenticated by a plant taxonomist. Essential oils were purchased from Orient Laboratory, Tunisia.

2.3. PREPARATION OF ENRICHED OLIVE OIL

Fresh aromatic plants were washed, gently dried at 40°C and then, added to olive oil at a rate of 2% (w/w) [3, 4]. Their correspondent essential oils were added at 0.2% w/w to olive oil. Before being tested, the mixtures were stored at constant temperature and humidity in hermetically sealed dark glass bottles. After that, all flavoured olive oils were sampled each 21 days during 84 days of storage at room temperature.
Three independent trials were carried out for the flavouring maceration, starting from the same olive lot. On the whole, five different flavoured and enriched olive oils were produced: Unenriched olive oil (Control), enriched olive oil using maceration of rosemary (MR), enriched olive oil using maceration of laurel (ML), enriched olive oil with rosemary essential oil (REO) and enriched olive oil with laurel essential oil (LEO).

2.4. FATTY ACIDS COMPOSITION

Fatty acids were evaluated as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution and extraction with n-heptane [11]. The initial fatty acid profiles of different olive oils were determined as described by Limon et al. [12].

2.5. DPPH ANTIOXIDANT ASSAY

The antioxidant activity of the phenolic extracts of olive oil with different flavourings was evaluated on the basis of the scavenging activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical. Briefly, olive oil was diluted in ethyl acetate (100 mL/mL of ethyl acetate) and mixed with a DPPH solution with a concentration of 1 10^4 mol/L in ethyl acetate. The mixture was then homogenised and kept in the dark for 30 min for reaction. After that the absorbance was registered at 515 nm against a blank solution. These assays are based on the abilities of the antioxidants present into the extracts to scavenge the radical in comparison with that of a standard antioxidant (trolox, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid). The inhibition percentage obtained for the samples was interpolated on the calibration curve to calculate the concentration in trolox equivalents (mmol/L TE) [1, 4].

2.6. QUALITY PARAMETERS DETERMINATION

Free fatty acid (FAA), peroxide value (PV), and spectrophotometric indexes (K_{232} and K_{270}) were determined following the European Union standard methods [11] and analytical methods described by Ayadi et al. [3].

2.7. TOTAL PHENOLIC CONTENT MEASUREMENTS

The total phenolic content of enriched olive oils was measured using the Folin–Ciocalteau as described by Yang et al. [13]. A 100 mg aliquot of each oil sample was mixed with the Folin–Ciocalteau reagent (0.5 mL) and methanol (2 mL). The mixture was shaken before adding 1.5 mL of 15% Na_2CO_3. After 30s of homogenization, distilled water was added to make a final volume of 7 mL. Then, the mixture was incubated at 50°C for 20 min and centrifuged (MPW Med. Instruments, MPW-350R Centrifuge, Poland) at 2000 g for 10 min. The absorbance of the obtained supernatant was measured at 750 nm. A standard curve was prepared using diluted solutions of gallic acid. The total phenolic content of the olive oil samples was expressed as milligrams of gallic acid equivalents per kg.

2.8. CHLOROPHYLLS AND CAROTENOIDS CONTENTS MEASUREMENTS

Each sample of enriched olive oil (7.5 g) was placed in a falcon tube and filled until 25 mL with cyclohexane. The chlorophyll fraction was measured in a UV spectrophotometer (Jenway 6352 spectrophotometer) at 670 nm and the carotenoids fraction at 470 nm. The concentrations of pigments were expressed following the equations described by Ayadi et al. [3].

2.9. PANEL AND CONSUMERS TESTS

Sixty trained panellists (food engineering students at the Higher Institute of Food Industries) and 8 expert panellists (National Olive Oil Center of Tunis, Tunisia) performed the sensory analysis on flavoured olive oils. The expert panellists were asked to evaluate positive sensory attributes and the defects (musty, smells of fusty, winey-vinegary, metallic, and rancid) of virgin olive oil samples, immediately after their elaboration date by using the profile sheet for virgin olive oil with a continuous unstructured line scale of 10 cm, ranging from low to high intensity [1]. The trained panellist tested by both olfactory and gustatory assessments olive oil samples for odour, taste, colour, after taste, bitterness, flavouring intensity, and overall acceptability. The various flavoured olive oils kept in the dark, at room temperature, were served to panellists in a randomised order codified by a 3-digit number and submitted to both panels. Fresh bread was used as a carrier and water as a palate cleanser between tastings [3]. The panellists were asked to rank the intensity of different attributes on a 5-point scale (1: “very weak”; 5: “very strong”). The mean sensory scores for various attributes of the flavoured oils were calculated [1].

2.10. STATISTICAL ANALYSIS

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation. An analysis of variance (ANOVA) was performed at a 5% significance level.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF THE MARKET STUDY FOR ENRICHED OLIVE OIL

3.1.1. One-dimensional method

The results showed that consumers interested in this new product are generally women aged between 20-45 years. It was shown that 97.3% of the questioned panellists consumed olive oil. Among them, 81% bought a food product, particularly olive oil, for their good quality, and taste and price were classified at the second and third place. However, 2.7% of surveyed said that they do not consume olive oil because of its strong taste. In the Tunisian market, this is an opportunity for flavoured and enriched olive oil that could be used to attract this category of consumers due to its new taste and richness in natural antioxidants. Besides, 63% of current consumers of olive oil justified their choice for this product by their nu-

tritional value and health benefits. However, 47% go towards extra virgin olive oil. Indeed, this new health concern is a favourable advantage for the consumption of flavoured olive oil.
The results showed that the attitude of consumers to try a new olive oil on the market was in progress with 67% of consumers that accepted to try new olive oil flavours against 33% who seemed attached to their habits regarding the purchased category of olive oil. This showed that the decision to bring new categories of olive oil to the market was appreciated by more than 2/3 of consumers. Moreover, 55% of respondents had a very positive attitude and 31% were in favour of the proposal to consume a new extra virgin olive oil enriched with aromatic plants or their extracts, richer in natural antioxidants and more stable against the oxidation. Also, when choosing one plant to flavour olive oil, the results of the questionnaire showed that garlic and rosemary are the two most requested plants by consumers, representing respectively 27% and 24% of choices, followed by olive leaves (16%).

3.1.2. Two-dimensional method

This method has been adapted to determine if there is a significant correlation between two variables. In this study, the calculated coefficient is the Pearson coefficient which is an index reflecting a linear relationship between two continuous variables taken in pairs. A negative value (negative correlation) means that when one of the variables increases, the other decreases. A significance level less than 0.05 reflects a significant relationship between these two variables (Data not shown).
From the results relatives to Pearson correlation coefficient, between a few variables, no statistically significant link was observed between the geographic origin of the questioned person and the plant chosen for flavouring olive oil with a significance level (Sig) (0.155) greater than 0.05. Besides, results showed a negative Pearson correlation coefficient (-0.14) between gender and attitude towards the consumption of flavoured olive oil. This result allows us to deduce that women have a positive attitude for the new offered product compared to men. On the other hand, a strong relation between the favourable attitude of consumers towards this new product and age was observed through the estimation of the correlation coefficient with a positive significant level less than 0.05 (0.046).

3.2. FATTY ACIDS PROFILES

The fatty acids profiles were assessed in the unenriched olive oil and olive oils enriched with two aromatic and medicinal herbs: rosemary and laurel. The initial composition of different olive oils samples is reported in Table I. In this study, in all analysed samples, oleic acid (C18:1) was the most abundant (57.99%) monounsaturated fatty acid (MUFA), followed by linoleic acid (C18:2) (18.18%) and palmitic acid (C16:0) which was the main (18.00%) saturated fatty acid (SFA) in olive oil. These results were partially in agreement with those reported by Limon et al. [12] and Sousa et al. [4]. In fact, they noted that C18:1 was the prominent fatty acid while, they reported higher contents of C18:1 (78.09% and 74.47%) but lower amounts of C18:2 and C16:0. However, Ollivier et al. [14] showed levels of C18:1 ranging from 59.93% to 80.97%.
Oleic acid content increased significantly ($p<0.05$) with the addition of rosemary and laurel herbs. The C18:1 content varied from 57.99% in the control to 59.15% and 59.05% respectively in olive oil added with laurel and rosemary herbs (ML and MR). Similarly, the C18:2 content (18.00%) increased in all treated olive oils. For C16:0, its content decreased for the two flavoured olive oils when compared to the

Table I - Fatty acids composition (g/100g fatty acids) of enriched and unenriched olive oils

Fatty acids (%)	*Control*	*REO*	*LEO*	*MR*	*ML*
Palmitic acid: $C_{16:0}$	18.18	17.27	17.88	17.23	17.02
Palmitoleic acid: $C_{16:1}$	2.47	2.45	2.40	2.33	2.33
Margaric acid: $C_{17:0}$	0.03	0.03	0.06	0.05	0.05
Heptadecenoic acid: $C_{17:1}$	0.08	0.07	0.10	0.08	0.09
Stearic acid: $C_{18:0}$	2.25	2.20	2.20	2.19	2.23
Oleic acid: $C_{18:1}$	57.99	59.08	58.71	59.05	59.15
Linoleic acid: $C_{18:2}$	18.00	18.01	17.95	18.20	18.12
Linolenic acid: $C_{18:3}$	0.59	0.54	0.49	0.53	0.64
Arachidic acid: $C_{20:0}$	0.27	0.24	0.13	0.23	0.24
Eicosenoic acid: $C_{20:1}$	0.13	0.10	0.07	0.10	0.12
SAFA	20.73	19.74	20.27	19.70	19.54
MUFA	60.67	61.7	61.28	61.59	61.69
PUFA	18.59	18.55	18.44	18.73	18.76

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Values are means of three replicates, standard deviation are in the range [±0.00 to ±2.06]. Means with different superscripts are significantly different ($p < 0.05$).

Table II - Radical scavenging activity (DPPH) of enriched and unenriched olive oils at the beginning of storage

Day	*Control*	*REO*	*LEO*	*MR*	*ML*
0	70.285±0.00^{aA}	67.082±0.00^{aA}	67.438±0.00^{aA}	70.641±0.00^{aA}	70.818±0.00^{aA}
4	62.149±1.7$^{b\ A}$	61.74±0.00^{bB}	59.11±0.04^{aB}	69.039±0.00^{cA}	69.395±0.10^{cB}
8	57.167±1.89^{aB}	60.158±0.00^{aB}	58.756±0.30^{aB}	67.182±0.00^{bB}	67.616±0.01^{bC}
12	54.156±0.04^{aB}	57.957±0.00^{cB}	56.534±0.28^{bC}	67.013±0.01^{dB}	67.275±0.00^{dC}
16	52.775±0.01^{aC}	57.438±0.02^{cC}	55.174±0.15^{bC}	65.836±0.28^{dB}	65.48±0.09^{dD}

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Data are mean ± standard deviation, n=6. Means with different superscripts are significantly different ($p < 0.05$).

control (18.18%) with significantly higher values when olive oils were incorporated with EOs. These findings were in line with the standard set by the European Community Regulation [11] which requires C16:0 and C16:1 levels ranged respectively from 7.5% to 20% and from 0.3 to 3.5% for an extra virgin olive oil. In fact, it has been proved that C16:1 is a minor fatty acid since its content is generally low in a good quality olive oil [14]. On the other hand, all enriched olive oils presented equal or inferior values to the control for different fatty acids fractions that compose the studied olive oils (Tab. I) which mean that herbal incorporation didn't influence significantly ($p>0.05$) the contents of C17:0, C17:1, C20:0 and C20:1 as described before by Sousa et al. [4].

Furthermore, the obtained results revealed that MUFA were the most abundant with values ranging from 60.67% (Control) to 61.7% (REO) and 61.69% (ML), followed by SFA and polyunsaturated fatty acids (PUFA). In this connection, the addition of both rosemary and laurel herbs decreased significantly SFA contents and increased MUFA contents. Otherwise, olive oils incorporated with EOs contained higher SFA amounts than oils added with respective herbs. Also, MUFA amounts in flavoured olive oils were always higher than the untreated control. Concerning PUFA, the addition of rosemary and laurel herbs significantly influenced their contents (18.73% and 18.76%, respectively) which were higher than control. These findings were in accordance with the maximum levels to be considered as extra-virgin olive oils as recommended by the European Community Regulation [11] and the results reported by Sousa et al. [4].

3.3. ANTIOXIDANT ACTIVITY

The results of the evolution of the antioxidant activity of control and treated samples during the first two week of storage are illustrated in Table II. A significant decrease ($p>0.05$) of this parameter was observed, during storage.

At production, the results did not show significant difference ($p>0.05$) among control and tested enriched olive oils. The highest antioxidant activity was reported for ML (70.82%) and MR (70.64%) oils when compared to oils incorporated with their respective EOs and the control.

During storage, the antioxidant capacity of control and enriched olive oils decreased significantly ($p<0.05$). These findings were in line with those of Taoudiat et al. [2] reporting that antiradical activity decreased in virgin olive oils with laurel EO. However, Sousa et al. [4] showed that enrichment of olive oil by dried laurel, oregano and pepper did not protect olive oil against oxidation. Moreover, the results partially agreed with those of Baiano et al. [8] who found that the antioxidant potential of olive oils enriched with herbs like oregano and rosemary decreased significantly during 9 months of storage but more slowly in control olive oil.

After 16 days of storage, results showed that the antiradical activity of virgin olive oils enriched with herbs was greater with no significant difference ($p<0.05$) observed between incorporation of rosemary and laurel. This finding was in accordance with that of Yang et al. [13] reporting that the major active compound in rosemary extract known as carnosic acid had an important antioxidant activity.

Also, after two weeks of storage, a significant difference ($p>0.05$) was observed between the control, the macerated virgin olive oils with herbs and aromatized virgin olive oils with essential oils. Contrastingly, Taoudiat et al. [2] and Ben Rached et al. [15] suggested an improvement of antioxidant activity with EOs addition and thus, their efficiency in virgin olive oils compared to fresh and dried herbs.

3.4. QUALITY PARAMETERS EVOLUTION DURING STORAGE

An extra-virgin olive oil is a liquid fat free of defects and compliant with a serial of chemical parameters with maximum levels permitted by the International Olive Council IOC [16] (Free fatty acid percentage ≤0.8 g oleic acid/kg oil, peroxide value ≤20 meq O_2/kg, K_{232}≤2.50, K_{270}≤0.22, median of fruity >0) [3].

3.4.1. Free Acidity

Free acidity changes of unenriched control and enriched olive oils during storage are presented in Table III. Values of free acidity expressed in oleic acid showed that enrichment increased slightly this acidity. Thus, initial free acidity increased significantly ($p<0.05$) in unenriched and enriched olive oils with rosemary and laurel plants to reach about 0.26±0.0%, 0.32±0.07%

Table III - Evolution of quality parameters and pigments of enriched and unenriched olive oils during storage

Day	Analyses	*Control*	*REO*	*LEO*	*MR*	*ML*
0						
	FA(%)	0.23±0.00^{aA}	0.24±0.00^{aA}	0.25±0.00^{aA}	0.25±0.00^{aA}	0.24±0.00^{aA}
	PV (mg.O_2)	10.02±0,00^{aA}	10.02±0,00^{aA}	10,02±0,00^{aA}	10,02±0,00^{aA}	10,02±0,00^{aA}
	K_{232}	2.001±0,00^{aA}	2.001±0,00^{aA}	2,001±0,00^{aA}	2,001±0,00^{aA}	2,001±0,00^{aA}
	K_{270}	0.117±0,00^{aA}	0.117±0,00^{aA}	0,117±0,00^{aA}	0,117±0,00^{aA}	0,117±0,00^{aA}
	Carotenoids (ppm)	1.03±0,00^{aA}	1.03±0,00^{aA}	1,03±0,00^{aA}	1,03±0,00^{aA}	1,03±0,00^{aA}
	Chlorophylls (ppm)	1.21±0.00^{aA}	1.21±0.00^{aA}	1,21±0.00^{aA}	1,21±0.00^{aA}	1,21±0.00^{aA}
	Total phenols (mg GAE/kg)	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}	1510±56,57^{aA}
21						
	FA(%)	0,25±0,07^{aA}	0,27±0,14^{aB}	0,28±0,007^{bB}	0,28±0,14^{bB}	0,27±0,07^{bB}
	PV (mg.O_2)	13,90±0,04^{cB}	11,74±0,08^{bAB}	11,12±0,05^{Ab}	11,78±0,08^{bB}	12,58±0,14^{cB}
	K_{232}	2,448±0,03^{dB}	2,141±0,01^{bB}	2,174±0,02^{bB}	2,031±0,04^{aA}	2,316±0,05^{aB}
	K_{270}	0,127±0,001^{Ab}	0,125±0,004^{aA}	0,126±0,004^{aB}	0,121±0,007^{aB}	0,122±0,005^{aB}
	Carotenoids (ppm)	0,98±0,01^{aA}	0,99±0,04^{aA}	1,01±0,028^{aA}	1,07±0,02^{aA}	1,05±0,05^{aA}
	Chlorophylls (ppm)	1,16±0,02^{aA}	1,17±0,02^{aA}	1,19±0,04^{aA}	1,27±0,04^{bA}	1,29±0,02^{bA}
	Total phenols (mg GAE/kg)	1400±28,28^{aA}	1430±25,45^{aB}	1421±12,71^{aB}	1550±7,07^{bA}	1590±28,07^{bA}
42						
	FA(%)	0,25±0^{aA}	0,27±0,14^{aB}	0,28±0,14^{bB}	0,29±0,14^{bB}	0,27±0^{aB}
	PV (mg.O_2)	17,22±0,02^{eC}	16,17±0,14^{cC}	16,75±0,08^{dC}	15.36±0,02^{aC}	15,90±0,04^{bC}
	K_{232}	2,503±0,02^{dC}	2,257±0,008^{aC}	2,396±0,01^{cC}	2,239±0,01^{aB}	2,319±0,009^{bB}
	K_{270}	0,131±0,004^{aB}	0,129±0,004^{aB}	0,128±0,005^{aB}	0,123±0,007^{aB}	0,124±0,007^{aB}
	Carotenoids (ppm)	0,97±0,02^{aA}	0,98±0,05^{aA}	0,99±0,02^{aA}	1,06±0,04^{aA}	1,01±0,02^{aA}
	Chlorophylls (ppm)	1,04±0,01^{aB}	1,09±0.04^{aB}	1,12±0,02^{bA}	1,11±0,02^{aB}	1,21±0,02^{cA}
	Total phenols (mg GAE/kg)	1318±29,02^{aA}	1377±29,69^{bC}	1377±12,66^{bC}	1452±9,89^{cA}	1483±28,80^{cA}
63						
	FA(%)	0,26±0,14^{aA}	0,28±0.00^{aB}	0,28±0,07^{aB}	0,31±0.00^{bC}	0,28±0,01^{aB}
	PV (mg.O_2)	18,21±0,02^{Dd}	17,19±0,04^{aD}	17,61±0,02^{bD}	17,93±0,08^{cD}	17,71±0,12^{bD}
	K_{232}	2,511±0,00^{eC}	2,348±0,00^{aD}	2,486±0,01^{dD}	2,386±0.00^{Bc}	2,408±0,009^{cC}
	K_{270}	0,134±0,005^{aB}	0,132±0,00^{aC}	0,131±0,00^{aB}	0,126±0,00^{aB}	0,127±0,00^{aB}
	Carotenoids (ppm)	0,93±0,02^{aA}	0,95±0,04^{aA}	0,94±0,02^{aA}	0,98±0,04^{aA}	0,97±0,01^{aA}
	Chlorophylls (ppm)	1,02±0,01^{aB}	1,04±0,02^{aB}	1,06±0,04^{aB}	1,07±0,00^{aB}	1,19±0,04^{bA}
	Total phenols (mg GAE/kg)	1273±28,12^{aA}	1359±14,14^{bD}	1341±14,01^{bC}	1395±16,97^{cA}	1410±12,73^{cA}
84						
	FA(%)	0,26±0^{aA}	0,28±0,01^{bB}	0,29±0,00^{bB}	0,32±0,07^{cC}	0,3±0.00^{dC}
	PV (mg.O_2)	20,55±0,01^{cE}	19,39±0,04^{aE}	19,46±0,05^{aE}	19,90±0,07^{bE}	19,82±0,04^{bE}
	K_{232}	2,823±0,00^{eD}	2,512±0,01^{cE}	2,613±0,009^{dE}	2,446±0,01^{aD}	2,486±0,007^{aC}
	K_{270}	0,15±0,004^{aC}	0,149±0,008^{aD}	0,145±0,005^{aC}	0,141±0,004^{aC}	0,142±0,004^{aC}
	Carotenoids (ppm)	0,91±0,01^{aA}	0,92±0,01^{aA}	0,92±0,01^{aA}	0,96±0,02^{aA}	0,94±0,02^{aA}
	Chlorophylls (ppm)	1,01±0,04^{aB}	1,03±0,00^{aB}	1,04±0,02^{aB}	1,06±0,05^{aB}	1,12±0,01^{bB}
	Total phenols (mg GAE/kg)	1218±43,84^{aB}	1333±14,04^{bD}	1327±16,17^{bD}	1363±11,13^{cB}	1374±16,01^{cB}

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
FA: Free Acidity; PV: Peroxyde value; Specific extinction (K_{232} and K_{270}).
Data are mean ± standard deviation, n=6. Means with different superscripts are significantly different ($p < 0.05$).

and 0.29±0.0%, respectively for the control, MR and ML, at the end of storage. Indeed, the enrichment with natural antioxidants using maceration of plants recorded the highest free acidity content particularly when rosemary aromatic plant was used.
In all tested samples, all values of acidity were lower than the limits set by EEC [11] for extra virgin olive oil. Also, acidity values in this study were in line with those found by Limon et al. [12] and lower than those reported in previous studies [1, 2, 3].

3.4.2. Peroxide value

The results related to the evolution of the peroxide value (PV) of the control and enriched olive oils during about three months of storage at room temperature are illustrated in the Table III. The PV indicates the formation of primary compounds of oxidation [4]. In this study, initial PV was about 10.02±0.0 meq O_2/kg showing a low rate of oxidation as described by other authors [3, 12]. This result disagreed with the results found by Sousa et al. [4] and Taoudiat et al. [2] re-

porting lower PV (4.8 meq O_2/kg and 3 meq O_2/kg, respectively) after addition of laurel herb and EO in virgin olive oil.
During storage at room temperature, the initial peroxide values of all analysed samples increased significantly ($p>0.05$) to reach, after 84 days of storage, values of about 20.55±0.01 meq O_2/kg; 19.9±0.07 meq O_2/kg; 19.82±0.04 meq O_2/kg; 19.39±0.04 meq O_2/kg and 19.46±0.05 meq O_2/kg, respectively, for the control, MR, ML, REO and LEO. It should be noted that the difference was significant ($p>0.05$) between the control and the flavoured samples and that the enrichment using incorporation of essential oils was remarkably more accentuated than that by maceration in term of peroxide value. Nevertheless, the difference was not significant ($p<0.05$) between both MR and ML as well as between REO and LEO. As a result, all the samples analysed underwent a slight increase in peroxide value without exceeding 20 meq O_2/kg, value overcoming the maximum permitted limit except in the untreated control that consequently lost the classification of virgin olive oil category after 84 days of storage [8].

3.4.3. Specific extinctions

The values of the PV<20 meq O_2/kg of olive oil does not always mean the absence of the oxidation phenomenon. The use of ultraviolet absorbance coefficients (K_{232}, K_{270}) provides information on the presence or absence of secondary oxidation products in the oil. The hydro-peroxides of the early stages of oxidation absorb at 232 nm, while the secondary oxidation products such as ketones absorb near 270 nm [17] and their presence is indicative of an extensive oxidation [4].
The evolution of specific extinction parameters for the different samples are reported in Table III. Initial values were lower than registered values in other studies [8]. During the storage period, all analysed olive oils underwent a significant increase ($p<0.05$) of these parameters until the 84th day. Referring to the table, this increase was significantly different ($p<0.05$) over time for all analysed samples with maximum attributed to unflavoured control confirming the protective effect of natural herbs and their essential oils. As expected, this increase was attributed to the primary oxidation product's evolution into secondary oxidation products such as hydroperoxides as described by Taoudiat et al. [2].
Regarding the obtained results in the present study, unflavoured olive oils reported the highest K232 values and could not be considered as extra virgin olive oil, after 63 days of storage, according to the European legislation [11]. In contrast, flavoured olive oils with EOs exceeded the recommended limit (2.5), after 84 days. It was observed that maceration using natural herbs gave more stability to the olive oil than enrichment with essential oils.
Indeed, the secondary oxidation content of control and those relative to enriched olive oils with EOs appeared slightly higher than those of enriched olive oils using herbal maceration. By comparing the obtained pairs of values (MR and ML or REO and LEO), no significant difference ($p<0.05$) was observed when adding herbs contrary to flavouring with EOs. All tested oils did not exceed the maximum legal value for K_{270} values (0.22). Therefore, particular attention must be given to these two quality parameters to avoid the declassification of the olive oil from the extra virgin or virgin categories.

3.5. PIGMENTS AND POLYPHENOLS CONTENTS EVOLUTION DURING STORAGE

Olive oil contains minor compounds that give it its organoleptic and nutritional quality. Among these compounds are pigments known for their antioxidant nature in the dark and pro-oxidising in the light. They play an important role in the oxidative stability of the oil during its storage [2, 18] and in the preservation of its quality [18, 19].

3.5.1. Beta carotene content

The main carotenoids present in olive oil are lutein and β-carotene. The presence of carotenoids in olive oil is closely related to that of green pigments and influenced by the same factors. Numerous studies have shown the anti-carcinogenic activity of β-carotene and other carotenoids and their role in the prevention of cardiovascular diseases and eye diseases [20]. The results related to the evolution of the β-carotene level during 84 days of storage at room temperature are registered in the Table III.
During storage at room temperature, the initial value (1.03±0.00 ppm) of β-carotene content of all analysed samples decreased to reach, after 21 days of storage, values of the order of 1.07 ppm and 1.05 ppm, respectively for MR and ML. This result was explained by the richness of plants with β-carotene and the instantaneous migration of pigments from plants to olive oil. Then, β-carotene content decreased and reached, after 84 days, about 0.91 ppm; 0.92 ppm; 0.92 ppm; 0.96 ppm and 0.94 ppm, respectively for control, REO, LEO, MR and ML without any significant differences ($p>0.05$).
This could be due to β-carotene degradation following the presence of oxygen, as described by Criado et al. [21]. They reported that a significant loss of β-carotene could occur even at a low oxygen concentration and that the existence of free radicals can also accelerate the rate of degradation of carotenoids. In this connection, rosemary oil proved to be very rich in β-carotene with a maximum content compared to other oils.

3.5.2. Chlorophyll content

The colour of olive oil is the result of green and yellow hues due to the presence, respectively, of chlorophylls and carotenoids [2]. During the first 3 weeks,

the analysis of chlorophyll content showed that the enrichment of olive oils with rosemary and laurel maceration increased this content slightly but significantly of about 0.06 ppm and 0.08 ppm, respectively (Tab. III). This can be explained by the instantaneous migration of pigments from plants to olive oil during maceration.
After 84 days of storage, the behaviour of these pigments showed a slight decrease for the control and the other enriched olive oils. It should be noted that the difference was not significant ($p>0.05$) between all samples and macerated olive oil with laurel.
Thus, olive oil enrichment using rosemary or laurel maceration ensured more pigment stability for olive oil than other studied oils. In fact, the addition of aromatic plants helped strengthen the antioxidant activity of olive oil by increasing its levels of β-carotene and chlorophyll contributing to olive oil stability.

3.5.3. Polyphenol content

The initial polyphenols level was about 1510±56.57 mg GAE/Kg (Tab. III). This content was higher than that (1036.72 mg GAE/Kg) found by Taoudiat et al. [2] confirming that this content is influenced by several factors like the extraction system and olive variety.
Total polyphenols in MR and ML registered an increase, during the first three weeks. However, the other analysed enriched samples with EOs showed a slight decrease ($p>0.05$) during the same period. Thus, virgin olive oils with rosemary and laurel herbs exhibited significantly ($p<0.05$) higher total phenolic content than control and treated oils with EOs. This rise in polyphenols is probably due to the migration of phenolic compounds, which are very abundant in laurel and rosemary plants, to olive oil during its storage.
After that and as expected, phenols content decreased significantly ($p>0.05$), during storage period, for all analysed oils with a significant difference ($p>0.05$) between the control and treated oils. In fact, the phenols contents of enriched and unenriched olive oils decreased with increasing storage time. This decrease in total phenols content may be caused by the decomposition and oxidation of phenolic compounds in oils which undergo qualitative and quantitative modifications during storage [22]. In addition, the total antioxidant activity agreed with the phenolic levels, with higher values in the enriched oils obtained by herbal maceration confirming their effective and protective role in virgin olive oil [2]. In fact, the phenolic compounds in oils may act as an antioxidant by donating H-atom(s) to free radicals which contributes to its decrease [13]. Nevertheless, oils added with rosemary and laurel herbs still showed the highest total phenolic content until the end of storage. These findings disagreed with those of Sousa et al. [4] and Taoudiat et al. [2] suggesting that flavouring olive oil with essential oils was more efficient compared to dried herbs.

3.6. SENSORY ANALYSIS

A descriptive sensory study was carried out on olive oils flavoured by the maceration or addition of essential oils of rosemary and laurel to determine which of these flavoured oils was most appreciated by consumers. The results of a panel test carried out on the studied oils showed, from the sensory profiles of control and flavoured olive oils obtained by using the two different flavouring methods, that all of them were devoid of defects.
From Table IV, it was concluded that the addition of flavourings to olive oil influenced several properties and improved their sensorial characteristics. As reported by Sousa et al. [4], Consumer's acceptability of olive oil-aromatic plants is very important for the introduction of these products to the market. Thus, several descriptors evaluated this acceptability. Regarding colour, MR and ML oils presented a dark yellowish green colour while the colour of REO and LEO oils was light green. The panellists preferred the colour of oils flavoured with rosemary and laurel essential oils and appreciated the taste of olive oils flavoured with rosemary essential oil more. In fact, olive oils incorporated with essential oils were less bitter than macerated ones.
It was also noticed that panellists preferred olive oil flavoured with rosemary essential oil considering the colour, smell, taste, texture, and overall acceptability. Moreover, macerated oils with tested herbs proved to be the less appreciated. Thus, the flavoured olive oils can be classified by ascending order based on the consumer's preference as follow; REO>LEO>MR>

Table IV - Sensory evaluation of enriched and unenriched olive oils

Descriptor	Odor	Color	Taste	Aftertaste	Bitterness	Flavoring intensity	Global appreciation
Control	3.4±0.15a	1.8±0.04a	3.67±0.18b	2.9±0.1b	2.2±0.2a	3±0.14a,b	3.6±0.2a
REO	3.8±0.25a	2±0.02a,b	4.62±0.32b	2.9±0.18b	2.7±0.2b	3.6±0.22a,b	3.95±0.1b
LEO	3.7±0.16a	2.24±0.03b	4.61±0.28b	2.6±0.16a	2.5±0.1a,b	3.8±0.25b	3.7±0.2a,b
MR	3.52±0.26a	2.25±0.02b	3.85±0.35a	2.5±0.2a	2.48±0.15a,b	3.2±0.18a,b	3.55±0.25a
ML	3.5 ±0.2a	2.26±0.02b	3.9±0.3a	2.45±0.22a	2.3±0.09a,b	2.8±0.24a	3.5±0.2a

Control: Unenriched olive oil; REO: Enriched olive oil with rosemary essential oil; LEO: Enriched olive oil with laurel essential oil; MR: Enriched olive oil using maceration of rosemary; ML: Enriched olive oil using maceration of laurel.
Means with different superscripts are significantly different ($p < 0.05$).

ML>C. These findings were partially in agreement with other studies reporting that the inclusion of essential oils at low or moderate concentrations avoided over-aromatisation, improved their sensorial characteristics and acceptability by consumers [4]. Also, according to Ayadi et al. [3], flavoured olive oils prepared with the maceration of aromatic plants should not only satisfy the sensory requirements of consumers, but also other qualities needed in the food market when compared to standard olive oils.

4. CONCLUSION

In this study, the enrichment of olive oil using maceration or EOs incorporation improved the chemical and sensory qualities as well as oxidative stability of analysed olive oils. This treatment added further value to this precious product due to the abundance of natural antioxidants which were transferred into olive oils following the maceration of rosemary and laurel herbs or the addition of their EOs. The results showed that rosemary and laurel herbs and EOs exhibited good antioxidant properties that control lipid oxidation during storage. Besides, flavoured olive oils had desired aromatic characteristics when compared to the control. These results may be an opportunity for Tunisia to improve its competitiveness in the world's olive sector, precisely on the import markets where conditioned Tunisian olive oil is highly appreciated.

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Impact of the hybrid on the fatty acid composition and thermal stability of cold-pressed sunflower oils produced from 17 newly cultivated hybrids from the region of North Macedonia

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Cold-pressed and refined sunflower oils rich in linolenic acid, are particularly susceptible to undesirable changes during deep-frying. The main object of this study was determination of physicochemical parameters such free fatty acids, peroxide value, saponification value, iodine number, density, oxidative stability, and fatty acid composition on cold-pressed oils from 17 newly cultivated hybrids from sunflower from the region of North Macedonia. Results from fatty acid methyl esters indicated Experto hybrid as the high oleic sunflower hybrid with 86.2% of oleic acid. Moreover, iodine number for sunflower oil from this hybrid was 87.5 g I_2 per 100 g oil, which was expected due to the high level of monosaturated fatty acid. Negative correlation confirmed inverse relationship between the amounts of oleic acid and values of iodine number ($r = -0.896$). Opposite, positive correlation between iodine number and amount of linoleic acid ($r = 0.892$) means that sunflower oils with higher value of iodine number will be thermally unstable and not suitable for deep-frying. Furthermore, the highest value for oxidative stability was measured for cold-pressed sunflower oils obtained from Talento, BG Fil and "Dijamantis hybrids (over 8, 9 and 5 h, respectively). This can be explained by the fact that the oils from those three hybrids had the highest level of oleic acid (83.1, 82.3 and 79.4%, respectively). Monounsaturated fatty acids are more stable then polyunsaturated, which makes sunflower oil suitable for deep-frying. The positive linear correlation between the amount of oleic acid and oxidation stability ($r = 0.687$) confirmed our statement that a higher amount of monounsaturated fatty acids (as oleic acid) can improve thermal stability and makes the sunflower oils from Talento, Fila BG and Dijamantis hybrids suitable for cooking and deep-frying. The oxidative stability of other examined hybrids at around 3 h can be explained by a dominance of polyunsaturated linoleic acid with levels between 43% and 57.1%. Statistical analysis confirmed our findings due to the negative linear correlation between oxidative stability and amount of polyunsaturated linoleic acid ($r = -0.698$). We expected higher value for oxidative stability (over 6 h) for cold-pressed sunflower oil from Experto hybrid due to its fatty acid composition. More precisely, the level of oleic acid for this oil was 86.2% and only 4.6% of polyunsaturated linoleic acid. Surprisingly, the oxidative stability of these oils was only 2.64% which can be explained as oxidation of the oil during production. According to the results from our study, we recommended the gap of oleic/linoleic acid as the most important for the determination of the thermal stability of sunflower oils. Finally, physicochemical parameters iodine number and oxidation stability can be a significant parameter for the prediction of the dominance of fatty acids in sunflower oil.

Keywords: cold-pressed sunflower oils, 17 hybrids, free fatty acids, fatty acid composition, iodine number, saponification value, refractive index, oxidative stability

1. INTRODUCTION

Virgin oils are very popular as natural products with a deep colour, typical taste, and smell, produced only with cold pressing and without any step of refining. Cold pressed oils occupy a very popular place in human nutrition

due to the high level of polyunsaturated fatty acids (PUFA) and tocopherols [1]. A positive correlation between PUFA-rich vegetable oil consumption and a reduced risk of coronary heart diseases level of LDL, degenerative diseases, and cancer is very well known [2, 3]. However, PUFA-containing edible oils are highly prone to lipid oxidation leading to the formation of potentially toxic compounds, rancid flavour and a reduced shelf life [4]. Vegetable oils high in PUFA, such as sunflower oil and flaxseed oil, might be converted to oils with detrimental health effects. To overcome the adverse effects of lipid oxidation, food manufacturers add antioxidants to vegetable oils thereby enhancing their oxidative stability. While synthetic antioxidants were shown to be very effective, consumers demand natural products since synthetic antioxidants were also associated to the progression of cancer development [5]. Stability of cold-pressed sunflower oil depends not only on the fatty acids composition but also on the content of antioxidants, primary and secondary oxidation products, metals and other contaminants which might accelerate or inhibit oxidation process [6].

Classical sunflower oil fatty acid composition is saturated acids 11% (stearic, palmitic), oleic 20% and linoleic acid 69% [7].

Cold-pressed and refined sunflower oils rich in linolenic acid, are particularly susceptible to undesirable changes during deep-frying [8, 9]. Frying oils made from sunflower have lower stability because of their high polyunsaturated fatty acids and low γ-tocopherol content [10-13].

The working group of Orsavova et al., studied fatty acid composition of some vegetable oils, energy contribution of saturated and polyunsaturated fatty acids, n-3 PUFAs and n-6 PUFAs of analysed oils to recommended dietary intakes for total fat [14]. The research studies of new sunflower oils with modified tocopherol and fatty acid composition developed as feedback for the food industry requirements to offer healthier products, and the two commodity oils available nowadays (normal and high-oleic sunflower oils) can cover the requirements of the food industry without chemical manipulation with the aim of increasing the consumers' quality of life [15]. According to the findings of Cao et al., (2021) compound sodium nitrophenolate can be effective modulator for sunflower seeds growth by boosted the triacylglycerol hydrolysis, promoted the conversion of fatty acids to sugars, and decreased the abscisic acid content during imbibition of aged sunflower seed [16]. Sunflower (*Helianthus annuus* L.) is an important oilseed crop in the world; however, no comprehensive study on exploring the role of FAD family in relation to stress tolerance in sunflower has been studied by Xu et al., (2021) [17]. Blended sunflower (SO) (50-80%) and sesame oils (SEO) (20-50%) were evaluated for thermo-oxidative stability (induction period, IP), oxidation kinetics (rate constant, *k*), synergy and shelf-life (25°C) (IP_{25}) using Rancimat (100, 110, 120, and 130°C) [18, 19]. The aim of the work of research group of Aguirrezábal was to assess the response of oil fatty acid composition of the new high oleic mutation to MNT compared to traditional and Pervenets genotypes [20-22].

The aim of this study is to cultivate hybrids from which we can produce stable high-oleic sunflower oil, which can be used as cooking oil for deep-frying and the thermal processing of food. Furthermore, the goal of this study was to find difference in the composition of fatty acids among the hybrids as well as the overall quality of cold-pressed sunflower oils from 2016 and 2017 harvesting years.

2. MATERIALS AND METHODS

2.1 FIELD TRIALS

Field trials were carried out at the experimental research area in Ovče Pole valley, near Sveti Nikole municipality, in the east-central part of the Republic of North Macedonia. Ovče Pole is a plain situated around the flow of Sveti Nikole's River, which is a tributary to the Bregalnica River, within the following geographic coordinates N: 41°49′21.9″ and E: 21°59′03.9″. The climate in Ovče Pole valley is characterised by hot and dry summers (the temperature is in the range from 31°C to 40°C) and temperate cold winters (the temperature is in the range from -10°C till 7°C), with occasional sharp drops. The experimental area belongs to the continental sub-Mediterranean area. The field experiments were set up during two consecutive growing seasons (2016 and 2017). The investigated area on which the research was conducted is owed by the Faculty of Agriculture, "Goce Delchev" University - Štip, the Republic of North Macedonia. A randomised block system, with three replications for each sunflower hybrid, was used for field research and the plot size was 5 m^2. The distance between the row was 70 cm and 1 metre between the hybrids. Sunflower hybrids were sown by a seed sower in April in both testing years. Pre-sowing soil preparation was conducted in suitable timing, in both years and in accordance with weather conditions. During the vegetative period, standard agronomic practices were applied. The amount of rainfall was also observed, during both years of research. In the first experimental year (2016) 295 l/m^2 rainfall amount was recorded during vegetative period (refer to Hydrometeorological Institute, Skopje). In the second testing year, the rainfall amount was by half less (167 l/m^2) compared to the amount of precipitation in 2016. No additional irrigation was conducted during the field experiment. The sunflower hybrids were harvested by hand in September in both years.

2.2 PLANT MATERIAL

In this research, seventeen sunflower hybrids were used as an experimental material (Experto, Armoni,

Fortimi, Adagio, Neoma, Torino, Arisona, Bacardi, Feliks, Neostar, Kondi, Talento, Subaru, Edison, BG Fila, Sumiko and Dijamantis). Feliks hybrids have Serbian origin; BG Fila is Bulgarian hybrid and the rest belong to Syngenta Seed Company. All sunflower hybrids were new varieties and were introduced to our country to be tested. During the vegetative period, in both years of testing, resistance of diseases, drought and lodging were recorded. All seventeen hybrids were included, for the first time, in this type of research to evaluate their potential, qualitative and quantitative properties under environmental conditions in the region of North Macedonia. The samples of 17 hybrids from sunflower oil were not mixed and oils obtained from every hybrid was examined separately from each harvested year.

2.3 APPLIED METHODS

A few parameters were examined to determine the overall quality of 17 hybrids of cold-pressed edible oils from the region of North Macedonia.

2.3.1 Determination of free fatty acid

Determination of free fatty acid was done by a cold solvent method using potentiometric titration (ISO 660:2010). In 10 g mass of test portion 50 ml of the neutralised solvent mixture (ethanol and diethyl ether) was added and the sample dissolved.

2.3.2 Determination of peroxide value

Peroxide value is a measure of the peroxides contained in the oil. Peroxide value is determined by measuring iodine released from potassium iodide. Furthermore, this value is a dynamic value, dependent upon the history of the test sample. The determination of peroxide value is a highly empirical procedure and the value obtained depends on the mass of the test portion. Actually, the determination of peroxide value was done by ISO 27107:2011.

2.3.3 Determination of density of oils

Density was determined by using a pycnometer at 20°C that performed according to ISO 6683:2014 [20].

2.3.4 Determination of iodine value

The determination of the iodine value was performed by ISO 3961:2013. The iodine number is determined by the mass of halogen, expressed as iodine, absorbed by the test portion following the specified procedure, divided by the mass of the examined sample of sunflower oil or more precisely, the iodine number equals the number of mg of iodine required to saturate the fatty acids present in 100 mg of the oil. Usually, iodine number is used to determine the amount of unsaturation in sunflower oils. In fatty acids, unsaturation occurs mainly as a double bond, very reactive towards halogens, iodine in this case. Thus, the higher the iodine value, the more unsaturation are present in the sunflower oil. The iodine number for high-oleic sunflower oil is usually in the range from 78-90 while for high linoleic sunflower oil is normally in the range from 118-141.

2.3.5 Determination of saponification value

The saponification value is determinate by taking 2.0 g mass of test portion in a conical flask to which 25 ml 0.5 mol/L ethanolic potassium hydroxide solution is added. Determination of saponification value was done by ISO 3657:2013.

2.3.6 Determination of oxidative stability of oils

Oxidative stability of oil was evaluated by the Rancimat method. Stability was expressed as the oxidation induction time (h) measured with the Rancimat 743 (Metrohm Co., herisau, Switzerland), using 3 g oil sample heated to 120°C with an air flow of 10 l/h. In general, sunflower oil will be kept at temperature of 120°C by Rancimat instrument. This temperature for our experiments is determined by several reasons. Firstly, frying food at a temperature which is too low results in an increased fat uptake. Water, which is contributed by the foods that are fried in an oil enhances the breakdown of fatty acids which occurs during heating. In addition, hydrolysis results in a poor-quality oil that has a reduced smoke point, a darker colour and altered flavour. During heating, oils also polymerise, creating a viscous oil that is readily absorbed by foods and that produces a greasy product. The more saturated (solid) the oil, the more stable it is to oxidative and hydrolytic breakdown, and the less likely it is to polymerise.

2.3.7 Determination of fatty acid methyl esters

In brief, 2 drops of oil were dissolved in 1 ml of heptane. Furthermore, 50 µL of sodium methylate (2 mol/L) was added and the samples were vigorously mixed for 1 min. Afterwards 100 µL of distilled water was added to each sample. After centrifugation of the samples, the lower phase was removed while the upper phase was mixed with 50 µL of 1 M HCl (with methyl orange for acidification control). After centrifugation at 4500 g for 10 min, the n-heptane phase was transferred to a new vial and the fatty acid methyl esters were analysed using an Agilent 6890 GC-chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a CP7420 Select FAME column (Agilent Technologies, Santa Clara, CA) (100 m 9 0.25 mm i.d. with 0.25 lm film thickness) and FID detector following the ISO standard ISO 5509:2000. The oven temperature was programmed to increase from 150 to 240°C with rate of 1.5°C/min and maintained isotherm at 240°C for 20 min. Pentadecanoic acid was used as an internal standard for quantitative analysis. The injector and detector temperature were both 260°C. Hydrogen was used as the carrier gas at an average velocity of 2 ml/min.

2.4 STATISTICAL DATA PROCESSING

Data collected from both years of the experiment were subjected to variance analysis using JMP statistical software. Fit analysis was performed to obtain the least significant differences (LSD) for tested hybrids by different properties. Based on the LSD data, the sunflower hybrids were statistically processed and grouped.

The statistical one-way ANOVA analysis was applied to see the level of every particular minor and major compound by consideration of the hybrid of oil with the significance level of 0.05. The level of significance of differences between the percentages of free fatty acids, fatty acid methyl esters, peroxide value, iodine value, saponification value, density and oxidative stability mean values was determined at 5% by a one-way ANOVA using the Tukey's test. This treatment was performed by SPSS v.16.0 software (IBM Corporation, USA). The ANOVA results were classified using letters (different letters mean significant differences among results). The letters are a, b, c, d, e and f according to the decrease of the result values.

At the end, a linear correlation between tested physicochemical properties was applied by SPSS statistical software.

3. RESULTS AND DISCUSSION

Results from fatty acid methyl esters presented in table I and values for oxidative stability presented in Tables II and III indicated strong relationship between percentage of oleic acid, iodine number and thermal stability of cold-pressed edible oils produced from 17 newly cultivated hybrids. The percentage of fatty acid composition was determined by every harvested year (by three replications by each sample) and statistically analysed. Statistically significant differences were detected between tested sunflower varieties. Results from the Table I, unequivocally indicated a strong relationship between the fatty acid composition and a variety of sunflower oil. The highest amount of monounsaturated oleic acid was related to 4 varieties: Experto, Talento, BG Fila and Dijamnatis. On the other hand, the next 4 varieties Fortimi, Torino, Felix and Neostar were related with the amount of polyunsaturated linoleic acid with over 50% of all fatty acids. As we can see from Table I, the percentage of oleic acid in cold-pressed edible oils from Experto hybrid was 86.2% and the value for iodine number was 87.5 g I_2/100 g which was expected due to the high level of monosaturated fatty acid. The same tendency was observed for cold-pressed edible oils produced from BG Fila and Dijamantis hybrids. The free fatty acid value of the tested oils varied between 0.10 and 0.60% oleic. Negative correlation confirmed inverse relationship between the amounts of oleic acid and values of iodine number (r = –0.896). Opposite, positive correlation between iodine number and amount of linoleic acid (r = 0.892) means that sunflower oils with higher value of iodine number will be thermally unstable and not suitable for deep-frying (Tab. IV). Furthermore, the highest value for oxidative stability was measured for cold-pressed sunflower oils obtained from Talento, BG Fila and Dijamantis hybrids (over 9 and 5 h, respectively). This can be explained by the fact that the oils from those three hybrids had the highest level of oleic acid (83.1, 82.3 and 79.4% respectively) (Tab. III).

Monounsaturated fatty acids are more stable then polyunsaturated which makes sunflower oil suitable for deep-frying. A positive linear correlation between the amount of oleic acid and oxidation stability (r = 0.687) confirmed our statement that higher amount of monounsaturated fatty acids (as oleic acid) can improve thermal stability and makes the sunflower oils from Fila BG and Dijamantis hybrids suitable for cooking and deep-frying. The oxidative stability of other examined hybrids at around 3 h can be explained by dominance of polyunsaturated linoleic acid with levels between 43% and 57.1% (Tab. I). Statistical analysis confirmed our findings due to the negative linear correlation between oxidative stability and the amount of polyunsaturated linoleic acid (r = –0.698) (Tab. IV). Due to its fatty acid profile, we expected higher value for oxidative stability (over 6 h) for cold-pressed sunflower oil from Experto hybrid. More precisely, the

Table I - Fatty acid methyl esters of 17 hybrids of sunflower oils (%)

	Experto	Armoni	Fortimi	Adagio	Neoma	Torino	Arisona	Bacardi	Felix
C16:0	4.9±0.3^{d}	5.5±0.1^{c}	6.5±0.4^{b}	5.9±0.2^{b}	5.1±0.1^{c}	7.1±0.3^{a}	5.5±0.1^{c}	6.1±0.4^{b}	5.8±0.1^{b}
C18:0	2.2±0.4^{c}	2.6±0.2^{b}	2.6±0.0^{b}	3.1±0.4^{a}	1.9±0.0^{d}	2.5±0.5^{b}	3.7±0.7^{a}	3.5±0.3^{a}	2.6±0.3^{b}
C18:1	86.2±3.2^{a}	41.8±4.8^{d}	32.0±4.5^{e}	40.8±2.3^{d}	57.7±9.2^{c}	34.0±7.1^{e}	45.8±6.3^{d}	40.4±5.4^{d}	33.7±4.9^{e}
C18:2	4.6±0.2^{f}	49.3±0.3^{b}	57.1±7.1^{a}	48.3±3.0^{b}	32.4±4.1^{d}	53.4±9.8^{a}	42.1±4.1^{c}	48.8±3.9^{b}	55.8±7.7^{a}
C18:3	1.9±0.0^{c}	0.4±0.0^{f}	0.9±0.1^{e}	1.3±0.0^{d}	2.9±0.0^{a}	1.5±0.1^{d}	2.6±0.0^{b}	1.2±0.0^{d}	1.1±0.0^{e}
	Neostar	**Kondi**	**Talento**	**Subaru**	**Edison**	**BG Fila**	**Sumiko**	**Dijamantis**	
C16:0	6.4±0.1^{b}	5.5±0.1^{c}	4.8±0.6^{d}	5.6±0.3^{c}	3.8±0.1^{e}	4.5±0.2^{d}	6.8±0.5^{b}	4.3±0.4^{d}	
C18:0	2.9±0.1^{a}	3.1±0.3^{a}	2.2±0.2^{b}	1.8±0.1^{c}	2.1±0.0^{b}	2.8±0.0^{a}	2.8±0.1^{a}	1.7±0.2^{c}	
C18:1	35.3±4.5^{e}	42.5±2.7^{d}	83.1±5.7^{a}	43.9±5.1^{d}	50.1±7.2^{c}	82.3±9.9^{a}	39.3±5.1^{d}	74.9±6.7^{b}	
C18:2	50.7±4.4^{b}	45.5±2.3^{c}	6.7±0.8^{f}	48.3±4.7^{b}	43.0±6.8^{c}	7.6±1.1^{f}	48.7±6.0^{b}	18.2±3.4^{e}	
C18:3	2.3±0.0^{b}	2.1±0.0^{c}	3.6±0.0^{a}	0.2±0.0^{f}	0.1±0.0^{f}	2.8±0.1^{b}	2.3±0.2^{b}	0.7±0.1^{e}	

Table II - Mean values of free fatty acid, peroxide value and density of different sunflower oils

Hybrids/ Properties	Free fatty acid (%)			Peroxide value (O_2/kg)			Density (mg/cm^3)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Experto	0.42^{c}	0.15^{bcd}	0.29	0.69^{m}	0.78^{i}	0.74	0.912^{h}	0.913^{f}	0.913
Armoni	0.47^{b}	0.16^{b}	0.32	1.21^{de}	0.89^{h}	1.05	0.919^{bcd}	0.919^{a}	0.919
Fortimi	0.22^{j}	0.12^{ef}	0.17	1.14^{f}	1.03^{f}	1.09	0.917^{abc}	0.918^{b}	0.918
Adagio	0.60^{a}	0.12^{ef}	0.36	1.28^{b}	1.14^{d}	1.21	0.915^{ef}	0.918^{b}	0.917
Neoma	0.29^{h}	0.14^{bcde}	0.22	1.07^{h}	0.98^{g}	1.03	0.918^{abc}	0.919^{a}	0.919
Torino	0.25^{i}	0.13^{cde}	0.19	1.14^{f}	0.56^{l}	0.85	0.916^{def}	0.915^{d}	0.916
Arisona	0.36^{de}	0.15^{b}c	0.26	0.99^{j}	0.72^{j}	0.86	0.916^{def}	0.919^{a}	0.918
Bacardi	0.32^{g}	0.14^{bcde}	0.23	0.84^{l}	0.78^{i}	0.81	0.918^{ab}	0.919^{a}	0.919
Feliks	0.33fg	0.12^{ef}	0.23	0.63^{n}	0.65^{k}	0.64	0.916^{def}	0.919^{a}	0.918
Neostar	0.37^{d}	0.14^{bcde}	0.26	1.35^{a}	0.78^{i}	1.07	0.919^{a}	0.919^{a}	0.919
Kondi	0.46^{b}	0.16^{b}	0.31	1.19^{e}	0.77^{i}	0.98	0.916^{def}	0.918^{b}	0.917
Talento	0.30^{h}	0.15^{bcd}	0.23	0.86^{k}	0.78^{i}	0.82	0.913^{gh}	0.914^{e}	0.914
Subaru	0.14^{k}	0.13^{cde}	0.14	1.21^{d}	1.30^{b}	1.26	0.916^{def}	0.916^{c}	0.916
Edison	0.13^{kl}	0.13^{cde}	0.13	1.10^{g}	1.13^{d}	1.12	0.916^{cde}	0.916^{c}	0.916
BG Fila	0.35^{ef}	0.33^{a}	0.34	1.36^{a}	1.34^{a}	1.35	0.912^{h}	0.912^{f}	0.912
Sumiko	0.14^{k}	0.13^{de}	0.14	1.25^{c}	1.23^{c}	1.24	0.918^{ab}	0.918^{b}	0.918
Diamantis	0.11^{l}	0.10^{f}	0.11	1.05^{i}	1.09^{e}	1.07	0.914^{fg}	0.914^{de}	0.914
Average	**0.31**	**0.15**	**0.23**	**1.08**	**0.94**	**1.01**	**0.916**	**0.917**	**0.916**
$LSD_{0.05}$	0.02	0.02		0.02	0.02		0.001	0.001	
CV (%)	3.82	9.90		0.93	1.37		0.12	0.06	

level of oleic acid for this oil was 86.2% and only 4.6% of polyunsaturated linoleic acid. Surprisingly, the oxidative stability of the oils was only 2.64% which can be explained as an oxidation of the oil during production (Tab. III).

Fatty acid composition affects oil oxidative stability [23, 24]. Oils with a high content of polyunsaturated fatty acid are more easily oxidised. Symoiniuk et al. (2018) used 27 different cold press oil samples (linseed, rapeseed, camelina, black cumin, prim-

Table III - Mean values of iodine number, saponification measurement and oxidative stability of different sunflower oils

Hybrids/ Properties	Iodine value (g I_2/100 g)			Saponification value (mg KOH/g)			Oxidative stability (h)		
	2016	2017	Mean	2016	2017	Mean	2016	2017	Mean
Experto	85^{jk}	90^{l}	87.5	188^{d}	188^{e}	188.0	1.80^{n}	3.48^{f}	2.64
Armoni	123^{c}	138^{b}	130.5	191^{abc}	189de	190.0	2.86^{j}	2.92^{l}	2.89
Fortimi	129^{a}	140^{a}	134.5	190^{abc}	189de	189.5	2.68^{m}	2.86^{m}	2.77
Adagio	120^{def}	131^{e}	125.5	190^{c}	189de	189.5	2.96^{i}	3.17^{h}	3.07
Neoma	127^{b}	135^{cd}	131.0	190^{c}	189de	189.5	2.81^{k}	2.97^{k}	2.89
Torino	109^{h}	121^{g}	115.0	190^{c}	190^{cd}	190.0	3.66^{d}	3.76^{d}	3.71
Arisona	121^{cde}	125^{f}	123.0	191^{abc}	190^{cd}	190.5	2.73^{l}	3.10^{i}	2.92
Bacardi	120^{ef}	136^{c}	128.0	190^{bc}	190^{bc}	190.0	3.59^{f}	2.99^{k}	3.29
Feliks	122^{cd}	136^{bc}	129.0	190^{abc}	190^{cd}	190.0	2.66^{m}	2.98^{k}	2.82
Neostar	122^{cd}	136^{c}	129.0	191^{abc}	189de	190.0	2.66^{m}	2.93^{l}	2.80
Kondi	121^{cde}	134^{d}	127.5	190^{c}	190^{c}	190.0	2.72^{l}	2.88^{m}	2.80
Talento	87^{j}	90^{l}	88.5	191^{ab}	190^{bc}	190.5	8.68^{b}	10.16^{a}	9.42
Subaru	115^{g}	116^{i}	115.5	191^{abc}	191^{ab}	191.0	3.16^{g}	3.20^{g}	3.18
Edison	111^{h}	111^{j}	111.0	190^{abc}	190^{bc}	190.0	3.64^{e}	3.71^{e}	3.68
BG Fila	83^{k}	85^{m}	84.0	191^{abc}	190^{c}	190.5	9.04^{a}	9.10^{b}	9.07
Sumiko	118^{f}	119^{h}	118.5	192^{a}	192^{a}	192.0	3.04^{h}	3.06^{j}	3.05
Diamantis	95^{i}	94^{k}	94.5	191^{ab}	190^{bc}	190.5	5.24^{c}	5.28^{c}	5.26
Average	**112.2**	**119.8**	**116.0**	**190.4**	**189.8**	**190.1**	**3.76**	**4.03**	**3.90**
$LSD_{0.05}$	2.03	1.80		1.52	1.21		0.02	0.03	
CV (%)	1.09	0.90		0.48	0.38		0.36	0.44	

Table IV - Linear correlation between some physicochemical properties and Fatty acid methyl esters of 17 hybrids of sunflower oils

Traits	Free fatty acid	Peroxide value	Density	Iodine value	Sapunification value	Oxidative stability	C16:0	C18:0	C18:1	C18:2	C18:3
Free fatty acid	**1**	-0.058	-0.034	0.024	-0.410	0.089	0.000	**0.524***	0.119	-0.164	0.354
Peroxide value		**1**	-0.111	-0.051	0.435	0.161	-0.091	-0.138)	-0.009	0.029	0.135)
Density			**1**	**0.951****	0.104	**-0.689****	**0.562***	0.400	**-0.822****	**0.814****	-0.162
Iodine value				**1**	0.013	**-0.742****	**0.585***	0.395	**-0.896****	**0.892****	-0.271
Sapunification value					**1**	0.260	0.161	0.068	-0.197	0.199	0.063
Oxidative stability						**1**	-0.453	-0.186	**0.687****	**-0.698****	0.458
C16:0							**1**	0.433	**-0.725****	**0.676****	0.023
C18:0								**1**	-0.412	0.356	0.245
C18:1									**1**	**-0.995****	0.403
C18:2										**1**	-0.479
C18:3											**1**

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

rose, hempseed, milk thistle, poppy, pumpkin, and sunflower) to determine the fatty acid composition. Their results showed that sunflower oil was characterised by the smallest proportion of saturated fatty acid (linoleic acid was 5.49%). Our results showed the highest abundance of palmitic acid for Torino hybrid (7.1%) while the highest amount of stearic acid was detected for Bacardi hybrid. Oleic acid, one of the monounsaturated fatty acids, was most dominant in sunflower oil (86.52%) compared to the other analysed oils. Also, sunflower oil has the highest value for oxidative stability (102.84 min) [25].

From the results in this paper (Tab. II), Diamantis showed the lowest value for free fatty acid in both years of testing, but, at the same time, had high oxidative stability (5.26 h, Tab. III) and presented a high resistance of diseases and drought. Edison, Sumiko and Subaru also showed lower values for free fatty acid, compared to the other tested varieties. They had a good oxidative stability, around 3 h and a good resistance to diseases. Fortimi showed 0.17% free fatty acid and during the vegetation presented tolerance to drought, lodging and diseases.

On the other hand, the lowest peroxide value was obtained by Feliks, followed by Experto, Bacardi, Talento, Torino and Arisona (Tab. II). Also, Talento showed the highest oxidative stability (9.42 h, Tab. III) and was resistant to lodging and diseases. Torino and Bacardi also had high value for oxidative value (3.71 h and 3.29 h, consequently).

Aşkin (2018) analysed six sunflower hybrids in his research. Major fatty acid composition in tested hybrids agreed with contents mentioned among literatures and registration reports of the food ministry in Turkey. The oleic acid ranges from 36.6% to 87.23%, while linoleic fatty acid from 5.72% to 52.93% [26].

The Mediterranean diet is well-known as a diet with a high consumption of olive oil and a minimal amount of saturated fatty acids. Red meat, whole fat milk products, nuts, and high fat fruits, such as olives and avocados are among the natural sources of monounsaturated fatty acids [27]. For the proper utilisation of sunflower oils in food and other industries, oil, moisture and protein contents, fatty acid compositions and quality characteristics of sunflowers should be quickly and reliably evaluated by analytical tools upon harvesting, marketing, and processing [28]. The fatty acid composition of sunflower, rapeseed, mustard, peanut, and olive oils were subject to examination in Konuskan et al. (2019) research. According to those authors sunflower oils have the highest oleic acid (68.88%) followed by olive oil. Our examinations indicated Experto as the richest hybrid of oleic acid (86.2%) [30]. Kefale et al. (2017) also investigated fatty acid composition in different oils from sunflower and safflower. We agree with their conclusion that sunflower hybrids with low levels of saturated fatty acids were suitable for edible oil processing for edible purposes [29]. The results from the examination

of Konuskan et al. (2019) showed that the free fatty acid value of the tested oils varied between 0.43 and 1.36% which is significantly higher than the free fatty acids of all 17 hybrids examined in our study (Tab. II) [30]. Generally speaking, results from Tables II and III indicated better properties of samples of sunflower oils produced from 2017. In the second testing year, the average percentage of free fatty acids was 0.15 compared to the harvesting year 2016 when the average percentage of free fatty acids was double. Furthermore, the average value of peroxide value for 2017 harvesting year was 0.94 O_2/kg while for 2016 was 1.08 O_2/kg. The difference between varieties produced from both harvesting years and values for free fatty acids and peroxide value was the most significant for the Torino and Neostar varieties (Tab II).

A total of 320 edible oils on Indian market were included in research of the working group of Dorni et al (2018). We agree with their statement that every variety of edible oil showed its own unique fatty acid profile with a significant variation within each individual fatty acid [31]. Our findings stated that few hybrids of sunflower oil such as Experto and BG Fila had their unique fatty acid profile with over 80% oleic acid (Tab. I) [32, 33]. Furthermore, the fatty acid composition was studied considering the general groups saturated, unsaturated, monounsaturated, and polyunsaturated. Saturated and unsaturated are influenced by environmental conditions such as temperature, rainfall, and genotypes [34]. Remarkable studies demonstrated that the health beneficial effects of vegetable oils have been often attributed to their antioxidant properties and abilities to increase cellular antioxidant defence system and thereby scavenge free radicals, inhibit lipid peroxidation, and augment anti-inflammatory potential [35]. We assumed that the results of the oxidative stability of examined oils depends on the level of unsaturated fatty acids and the level of natural antioxidants which include α-tocopherol as the most abundant Vitamin-E-active compound in sunflower oil [12, 13, 36]

The results presented by different authors in the last decade have shown that it is possible to obtain sunflower oils of very different qualities combining the genetic variability in the response of the fatty acid composition to temperature and the climatic diversity under which the sunflower is cultivated [23, 37, 38].

Cold-pressed sunflower oil is a rich source of β-sitosterol, campesterol and total phytosterol and might be a better choice for patients with high cholesterol and cardiovascular diseases [39, 40].

4. CONCLUSIONS

Generally speaking, results from free fatty acids and peroxide value indicted better properties of samples of sunflower oils produced from 2017 in comparison to 2016. Furthermore, results from our study showed a strong relationship between the fatty acid composition and the variety of sunflower oil. The highest amount of monounsaturated oleic acid was related to Experto, Talento, BG Fila and Dijamnatis while Fortimi, Torino, Felix and Neostar hybrids were related to the amount of polyunsaturated linoleic acid with over 50%. Due to the fatty acid profile, we recommend the cold-pressed sunflower oils from three hybrids Experto, BG Fila and Dijamnatis as cooking oils suitable for the thermal processing of food. Furthermore, we recommended the gap of oleic/linoleic acid as the most important for the determination of the thermal stability of sunflower oils. Lastly, physicochemical parameters, iodine number and oxidation stability can be significant parameters understand the dominance of fatty acids in sunflower oil.

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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
- ftalati

Optimisation of low-fat high-protein cookie formulation: effects of using butter and composite flour on nutritional, physical and sensory properties

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Consumers demand in food products have changed significantly in the last century with lifestyle changes related to new eating habits. Based on the consumers' demand, the food industry and scientists focus on low fat and calorie functional foods that avoid causing nutrition-related illnesses. Therefore, the aim of this study is to develop a low fat-high calorie functional cookie fortified with composite flour (chickpea 50%, whole grain wheat 25% and oat 25%), butter, almond, dried mulberry, egg powder and whey powder. The statistical analyses were carried out by using response surface methodology (RSM). The nutritional (moisture, ash, fat, protein, carbohydrate, and energy content), physical (diameter and thickness measurement, spread ratio) and sensory properties (colour, appearance, taste-odour, texture, overall acceptability, and affordability) of produced cookies were evaluated. The results indicate that the protein content of cookies increased from 13 g/100 g to 24.38 g/100 g with a 9% reduction in fat and calories for the cookies. A cookie containing 15% butter and 15% composite flour has the highest score for overall acceptability and affordability among the cookie samples. The research showed that low fat-high protein cookies fortified by composite flour, with a highly acceptable and nutrition composition can be produced.

Keywords: Butter, Low Fat Cookie, High Protein, Reduced Calorie, Response Surface Methodology

INTRODUCTION

Obesity and overweight have been increasing in many parts of the world [1, 2]. These increases are related with many chronic diseases such as high blood pressure, type 2 diabetes, cardiovascular disease [3-6]. Many studies show that a prevalence of obesity and overweight is associated with eating habits like increasing fat and caloric intake [7-9]. Fat is a necessary nutrient for humans and one of the essential food substances. However, a high fat diet may cause an increasing risk of many health problems [7, 8, 10, 11]. Consumer's awareness of eating healthy increased during the last century in parallel with the increase in diseases related to nutrition [12]. This increasing awareness of consumers has led to understanding that their food choices may have consequences on their health. Moreover, consumers pay more attention to the health benefits of food to maintain a healthy lifestyle. In general, readymade foods consumed by most consumers have a high fat content and a low content of minerals, vitamins, dietary fibres, and proteins. For these reasons, consumers have been tending towards prefering low fat, reduced fat, fat free and functional foods during the last decade [13-15]. One of the biggest challenges today is to improve cheap foods, which have a high nutritional value, and are mostly acceptable by the average customers [16]. Functional food promotes health benefits above normal nutrition. The functional food sector is one of the fastest increasing markets of the food sector

worldwide. This situation can lead to different areas of science providing factories with opportunities to improve various new functional productions [17].
Bakery productions are one of the excellent tools for fortification, value addition and food intake on a mass scale. Currently, the fortification of cookies has evolved to develop its functional and nutritional properties because of the healthy eating awareness of consumers [17-19]. Cookies are consumed ready-made from a high fat bakery production due to the cheapness, acceptable taste, availability, long shelf life and quick release of energy [20]. Fat is a basic ingredient in cookies because it is responsible for flavour, mouthfeel, texture, nutritional and sensory properties [21]. However, cookies are low in, proteins, vitamins, minerals, fibre and rich in undesirable fats, carbohydrates, and calories. Therefore, it is necessary to improve low fat and high nutritional value cookies. In the last decade, researchers and the food industry have focused on the development of the fortification of cookies. It was reported that extruded bean flour was used in cookies to reduce fat, improve nutritional value and sensory properties in the final product [22]. Fortified cookies with vitamins, prebiotic fibres and reduced fat have been accepted by the consumers in terms of colour, flavour and eating qualities [23-25]. Moreover, cookies can be readily fortified with legume flour to increase protein and fibre. The use of mixed flour to improve cookies to develop nutritional values has been reported by several studies [26-31].
Recently, oat has gained the attention of researchers because of a high amount of beta glucan content composites of antioxidant activity and lipid fraction that has a significant impact on the nutritional and technological quality [31-34]. Oats are a good source of beta glucan which reduce the risk of some diseases like diabetes, hypertension, cardiovascular diseases, and obesity [35-41]. Compared to other cereals, oats involve much more fat that is rich in polyunsaturated fatty acids. This fat is unstable, because of the rapid oxidation process so oat productions, for example, oat flakes, oat flour can be used in bakery productions like cookies to improve shelf-life [42, 43]. The addition of oat flour in the cookies can lead to improving protein quality, dietary fibre, shelf life and sensory properties [44].
Chickpea, characterised by the highest nutritional value among all legumes consists of 50% carbohydrates, 17-20% protein, 5-6% of fat, and 3-4% crude fibre [45]. Moreover, chickpea seed is a good source of carotenoids, folic acid, sterols, tocopherols, B-group vitamins, microelements, magnesium, potassium, selenium, zinc, phosphorous. In addition, chickpea has a great digestible plant protein, a complex carbohydrate with a low glycemic index and dietary fibre that can protect against cardiovascular diseases. Using chickpea flour to make bakery products not only raises the mineral and protein content but also contributes to lowering glycemic response in consumers [46, 47]. Studies on the use of chickpea in bakery production have increased in recent years [48-54].
Wheat (*triticum aestivum*) is a nutritionally substantial cereal and a staple food for humans all over the world [55, 56]. It is widely used in bakery productions like pasta, cookies, snacks and crackers due to the valuable properties of the protein (gluten) that combine elasticity and strength to obtain a desirable flavour and texture [55, 57-59]. Consumption of whole grain wheat productions is known to have a beneficial impact on the human body related with their high substance of bioactive phytochemicals, minerals, vitamins, protein, and dietary fibre [60]. It is reported that the consumption of whole grain wheat has positive effects on type-2 diabetes, cancer, obesity, and cardiovascular diseases [61-63]. For this reason, whole grain wheat has attracted attention of customers and of the food industry in recent years [64, 65]. Using whole grain or refined varieties can contribute significantly to a nutritional and functional variation [66-70]. Considering all the above, the purpose of this study is to improve functional cookies that increase protein quality and quantity, dietary fibre, reduce fat and calorie intake with composite flour, oat, chickpea, and whole grain wheat flour.

2. MATERIAL-METHOD

2.1 MATERIALS

Pre-cooked chickpea flour, whole grain wheat flour, oat flour, corn starch, rice flour, butter, almond, dried mulberry, egg powder, whey powder, salt, guar gum, sodium bicarbonate, and ammonium bicarbonate were purchased commercially. All chemicals and reagents were graded analytically.

2.2 METHODS

2.2.1. Determination of the Suitable Mixture and Parameter Ranges for Cookie Production

It is planned to obtain a biscuit formulation with an increased protein quality and quantity by preparing a mixture consisting of pre-cooked chickpea flour, whole wheat flour, oat flour, corn starch and rice flour. As a result of preliminary studies, the mixing ratio of chickpeas, whole wheat grain and oat flour (CWO) was determined as 2:1:1. As a result of preliminary trials, the CWO ratio was determined as 25-100 g/100 g flour and 10-25 g butter as independent variables. Corn starch: rice flour was used at a 1:1 ratio in the productions with a CWO mixture ratio of less than 100 g.

2.2.2. Experimental Design for Cookies Formulation

Designed according to the Response Surface Method, which is an experimental design method. For the optimisation of rich protein and low-fat cookies, experiments were conducted according to a central composite design containing two independent vari-

ables that dictated 13 experimental sessions. Independent variables used to determine optimum cookie formulations were CWO and butter content.
The range of upper and lower values of independent variables were 25-100 g/100 g for CWO and 10-25 g for butter was used to optimise and evaluate the impact of independent variables on the dependant variables (Table I).

2.2.3. Cookie Preparation

The production of cookies was carried out by making some changes to the method specified in AACC Method No: 10-54. Cookies were produced by adding rice flour and corn starch (1:1) in the ratio of 0%, 37.5% and 75% to the CWO flour mixture. First, flours and all powder components were mixed to obtain a homogeneous element using a mixer (Kitchenaid, U.S.) for 2 minutes. Then the specified amount of butter was added and stripped every 30 seconds and mixed for 3 more minutes. After that, different amounts of water were added according to the amount of butter and flour specified in the experimental design and the kneading process was completed by mixing for 2 more minutes by stripping every 30 seconds. The obtained dough was placed to rest for 20 minutes and then shaped into discs of a diameter of approximately 50 mm and a thickness of 5 mm. The cookies were transferred to the oven (M4256, Simfer, Kayseri, Turkey) and baked at 180°C for 20 minutes. After baking, the cookies were cooled at room temperature for nearly 30 minutes and then the necessary measurements were made, and the rest of the cookies were ground in a grinder (Premier PRG 259, Istanbul Turkey) and stored in polyethylene containers for further analyses.

2.2.4. Proximate and Nutritional Composition

Crude protein, crude fat, total ash, moisture was determined by employing a standard AOAC analysis method, 1990 [71]. The total carbohydrate and energy content of cookies was calculated by using the following formula [72]:

$$\% \text{ Carbonhydrate} = 100 - (\text{misture } \% + \% \text{ protein} + \% \text{ fat} + \% \text{ ash})$$

$$\text{Energy} = 4\ (\% \text{ Protein content of cookies} + \% \text{ carbonhydrate content of cookies}) + (\% \text{ fat content of cookies}) \times 9$$

2.2.5. Physical Analyses

2.2.5.1. Diameter, Thickness and Spread ratio

The diameter (D) and thickness (T) values of the cookie samples were measured using a vernier calliper as specified in the AACC Standard Method No: 10-54 [73]. After the diameter (mm) and thickness (mm) values of the biscuits, the spread ratio is determined. The spread ratio of the cookies was determined by calculating the ratio of the diameter to the thickness for each sample.

2.2.5.2. Colour Analyses

Colour parameters of cookie samples were measured with a Hunterlab MiniScan EZ (Reston, Virginia, USA), and the values were expressed based on the CIAL-AB measurement system. White and black calibration tiles were used to standardise the device before analysis. In HunterLab colour scale, *L** (lightness factor 0=black, 100 white); a* (*–a* green, *+a* red); *b** (*-b* blue, *+b** yellow) values were recorded at the daylight (D65/10°) setting.

2.2.6. Sensory Analyses

The sensory evaluation of the cookies was conducted using 10 trained panelists from Hatay Mustafa Kemal University Food Engineering Department. Cookies were coded with three-digit numbers and positioned randomly. The sensory evaluation sheet was provided to the panelist who assessed the colour, appearance, flavour, texture, overall acceptability, and affordability according to their preferences on a 1-5 hedonic scale. According to the scale; 1: bad, 2: not enough,

Table I - Experiment design of independent variables of cookie samples

Independent Variables	Code	-1	0	+1
Butter content (g/100g flour)	X_1	10	17.50	25
CWO (g/100g flour)	X_2	25	62.50	100

Production	X_1	X_2
1	-1.00	-1.00
2	1.00	1.00
3	-1.00	1.00
4	0.00	0.00
5	0.00	-1.00
6	0.00	0.00
7	0.00	0.00
8	-1.00	0.00
9	1.00	-1.00
10	0.00	0.00
11	0.00	0.00
12	0.00	1.00
13	1.00	0.00

3: acceptable, 4: good, 5: very good. All sensory evaluations were conducted at room temperature and water was served to the panellists for mouth cleaning between the sample evaluations [74, 75].

2.2.7. Statistical Analyses

In determining the effects of independent variables on dependent variables, the Central Composite Design of the Response Surface Method was used for the variance analysis. As a result of the variance analysis, significant differences between group means were determined using the SPSS package program. Chemical and physical analyses were performed in triplicate and two replications for the sensory evaluation.

3. RESULT AND DISCUSSION

3.1. PROXIMATE AND NUTRITIONAL COMPOSITION

The result of moisture, ash, fat, protein, carbohydrate, and energy content of cookies are summarised in Table II.

3.1.1. Moisture Content

The result of moisture content of cookies ranged between 10.11% and 13.30%. The moisture content of a production effect on the quality of foods [76]. As it can be seen in Figure 1, the increasing of the ratio of CWO in cookies leads to an increase in moisture content, and this is significant $p<0.01$.

3.1.2. Ash Content

The value of the ash content of cookies ranged from 2.18% to 3.07%. While sample number 3 has the highest ash value, sample number 9 has the lowest ash content. The response surface plot (Fig. 2) showed that the ash values of cookies increased significantly with the rising of the CWO ratio in the cookies $p<0.01$. A similar result showed that using composite flour increases the ash content of cookies [77].

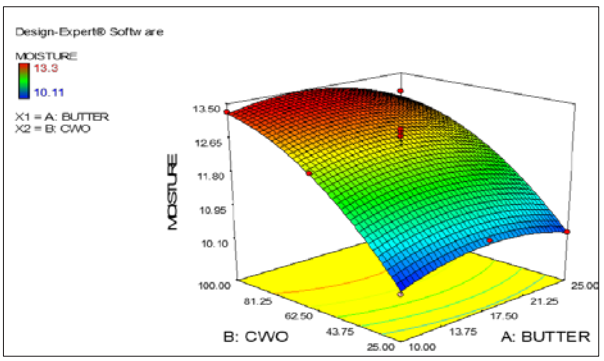

Figure 1 - Response surfaces for the effect of CWO concentration and butter on moisture of cookie samples

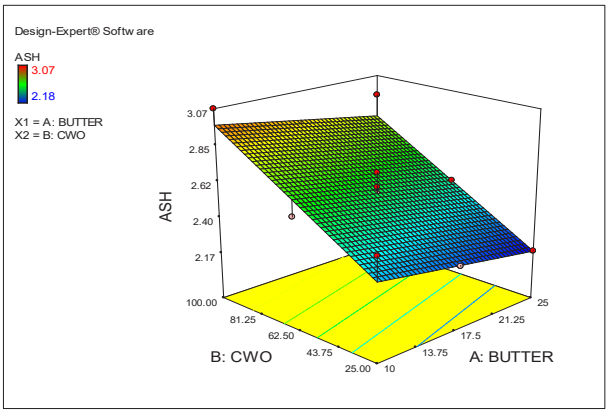

Figure 2 - Response surfaces for the effect of CWO concentration and butter on ash of cookie samples

3.1.3. Fat Content

Sample number 2 has the highest fat value (19.23%) and sample number 1 has the lowest fat value (11.22%). The response surface plot (Fig. 3) showed that an increased ratio of CWO and butter led to an increase in the fat content in cookies. The fat values of cookies increased slightly with the increasing of the CWO ratio in the cookies $p<0.05$. The fat content of mixed flour was similar, 15.75% [19], 14.1% [78],

Table II - Nutritional properties of low fat high protein cookies

Sample Number	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Energy (kcal/100g)
1	10.11±0.01^{a}	2.50±0.00^{d}	11.22±0.07^{a}	13.93±0.10^{b}	62.24±0.18^{g}	406^{b}
2	13.00±0.01^{i}	2.94±0.00^{i}	19.23±0.07^{i}	24.17±0.71^{f}	40.66±0.78^{a}	432^{h}
3	13.30±0.11^{j}	3.07±0.02^{j}	12.70±0.02^{c}	24.38±0.96^{f}	46.55±1.02^{c}	398^{a}
4	12.83±0.01^{h}	2.59±0.00^{f}	14.26±0.02^{d}	18.40±0.21^{d}	51.92±0.17^{e}	410^{d}
5	10.70±0.04^{c}	2.26±0.01^{b}	14.25±0.08^{d}	15.01±0.78^{c}	57.77±0.83^{f}	419^{g}
6	12.23±0.07^{e}	2.55±0.00^{e}	14.85±0.07^{e}	18.35±0.10^{d}	52.02±0.10^{e}	415^{e}
7	12.45±0.05^{f}	2.68±0.02^{g}	15.55±0.11^{f}	18.28±0.76^{d}	51.04±0.91^{d}	417^{f}
8	12.32±0.10^{e}	2.56±0.03^{e}	11.51±0.14^{b}	15.60±0.16^{c}	57.99±0.37^{f}	398^{a}
9	10.26±0.01^{b}	2.18±0.00^{a}	17.02±0.10^{g}	13.00±0.70^{a}	57.55±0.80^{f}	435^{j}
10	12.89±0.04^{h}	2.43±0.01^{c}	14.20±0.00^{d}	18.09±0.66^{d}	52.40±0.70^{e}	410^{d}
11	12.71±0.00^{g}	2.43±0.00^{c}	14.20±0.01^{d}	18.60±0.36^{d}	52.05±0.35^{e}	410^{d}
12	13.29±0.11^{j}	2.79±0.01^{h}	14.20±0.05^{d}	24.27±0.05^{f}	45.45±0.03^{b}	407^{c}
13	12.00±0.18^{d}	2.49±0.02^{d}	18.28±0.05^{h}	20.23±0.54^{e}	47.00±0.69^{c}	433^{i}

$^{a\text{-}j}$ For each parameter. different superscript letters indicate a significant difference ($p<0.01$) among cookie samples

18-22% [79]. Moreover, increasing the butter ratio increases significantly the fat content of cookies p<0.01.

3.1.4. Protein Content

The protein content values varied from 13 to 24.38 g/100 g. The high protein content may be attributed to the presence of chickpea flour. Protein is a significant component that improves the nutrient properties of composite flours [80]. As it can be seen in Figure 4, the increasing in the percentage of CWO in cookies can lead to increasing significantly the protein content in cookies p<0.01. The results of protein content obtained in this study is in close agreement with to rise in protein content using composite flour reported by several studies [77, 81-86].

3.1.5. Carbohydrate Content

The results of the carbohydrate content in cookies ranged from 40.66% to 62.24%. The response surface plot (Fig. 5) shows that the rising percentage of using CWO and butter leads to a significant decrease in carbohydrate content in cookies p<0.01. Similarly, a study on fortified cookies with chickpea and wheat flour reports that the carbohydrate content of cookies ranged from 47.30%-50.03% [77]. Another study reported that increasing chickpea flour can decrease the carbohydrate content [53]. The comparable outcome of the study that used wheat flour in cookies shows that the content of carbohydrates was 44-46% [82]

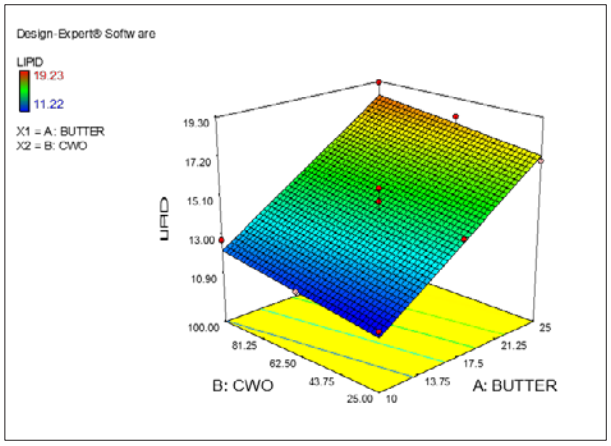

Figure 3 - Response surfaces for the effect of CWO concentration and butter on lipid of cookie samples

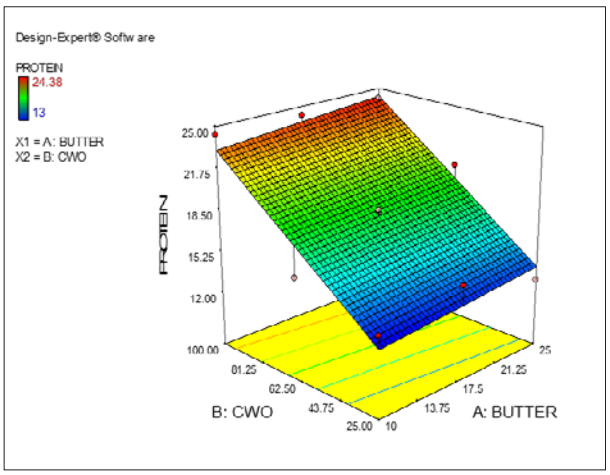

Figure 4 - Response surfaces for the effect of CWO concentration and butter on protein of cookie samples

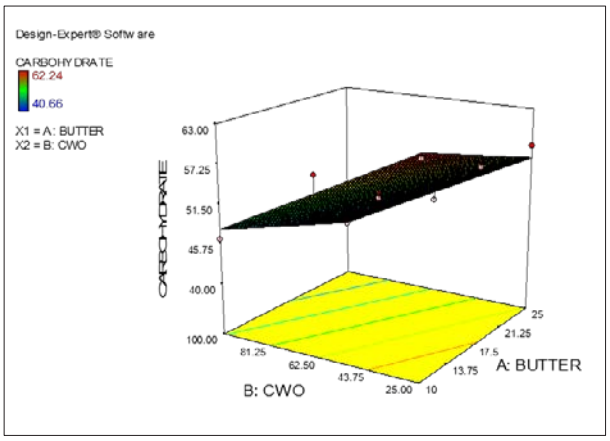

Figure 5 - Response surfaces for the effect of CWO concentration and butter on carbohydrate of cookie samples

Table III – Diameter, thickness and spread ratio values of low fat high protein cookie samples

Sample Number	Diameter	Thickness	Spread Ratio
1	4.74±0.05	0.82±0.04^{h}	5.78±0.33^{a}
2	4.4±0.00	0.52±0.04^{a}	8.46±0.66^{f}
3	4.48±0.04	0.62±0.04$^{b.c}$	7.23±0.54$^{d.e}$
4	4.64±0.09	0.7±0.07$^{d.e.f}$	6.63±0.62$^{b.c.d}$
5	4.58±0.04	0.78±0.04$^{g.h}$	5.87±0.30^{a}
6	4.48±0.04	0.6±0.00^{b}	7.47±0.07^{e}
7	4.7±0.07	0.68±0.04$^{c.d.e}$	6.91±0.51$^{c.d.e}$
8	4.64±0.05	0.74±0.05$^{e.f.g}$	6.27±0.45$^{a.b.c}$
9	4.6±0.00	0.78±0.04$^{g.h}$	5.90±0.37^{a}
10	4.62±0.08	0.66±0.05$^{b.c.d}$	7.00±0.59$^{d.e}$
11	4.66±0.05	0.76±0.05$^{f.g.h}$	6.13±0.51$^{a.b}$
12	4.7±0.07	0.68±0.04$^{c.d.e}$	6.91±0.59$^{c.d.e}$
13	4.6±0.07	0.78±0.04$^{g.h}$	5.90±0.45^{a}

$^{a-h}$ For each parameter. different superscript letters indicate a significant difference (p<0.01) among cookie samples

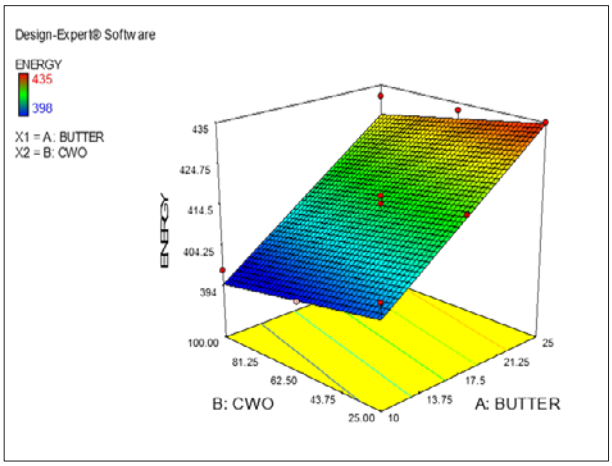

Figure 6 - Response surfaces for the effect of CWO and butter concentration on energy content of cookie samples

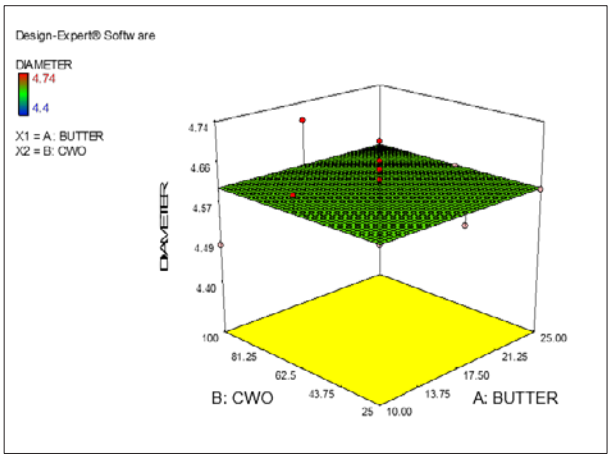

Figure 7 - Response surfaces for the effect of CWO concentration and butter on the diameter of cookie samples

3.1.6. Energy

As it can be seen in Table II the energy content of cookies ranged between 398 and 435 kcal. The response surface plot (Fig. 6) shows that, as the percent of WCO increases in cookies, there was a significant decrease in energy content $p<0.05$. However, the raising of butter ratio in cookies can lead to increase the energy content significantly in cookies $p<0.01$. This finding corresponded to previous studies showing that mixing flours lead to an increase in the energy content [52, 77, 87].

3.2. PHYSICAL ANALYSES

The result of diameter, thickness, spread ratio, and colour of the cookie samples are shown in Table III and Table IV respectively.

3.2.1. Diameter, Thickness and Spread Ratio

The results of the diameter of the cookie samples are given in Table III. Diameter values of cookie samples ranged from 4.40 to 4.70. It can be seen in Figure 7 that the diameters of cookie samples are not affected significantly by the ratio of CWO and butter. While the percentage of CWO in cookie samples affect the thickness of cookie samples significantly, there is no relationship between the percentage of butter in cookie samples and the thickness of cookie samples. As it can be seen in Figure 8, the increasing in the percentage of the CWO in cookie samples can lead to a significant decrease of the thickness $p<0.05$. The value in thickness of the cookie samples shows a significant decrease from 0.82 to 0.52 with an increasing CWO percentage in cookie samples. Spread ratio values of cookie samples ranged from 5.78 to 8.46. The response surface pilot Figure 9 shows that there is a significant effect of the percentage of using CWO on spread ratio in cookies. Nevertheless, the percentage of using butter in cookies does not affect the spread ratio statistically. Spread ratio results showed that, as the concentration of incorporated treatments of whole grain wheat increased, the spread ratio increased significantly $p<0.05$. Higher protein content impacts negatively on the spread ratio in cookies [88]. However, cookies developed by a high percentage of chickpea flour, despite having high protein content, showed a higher spread ratio. This anomalous be-

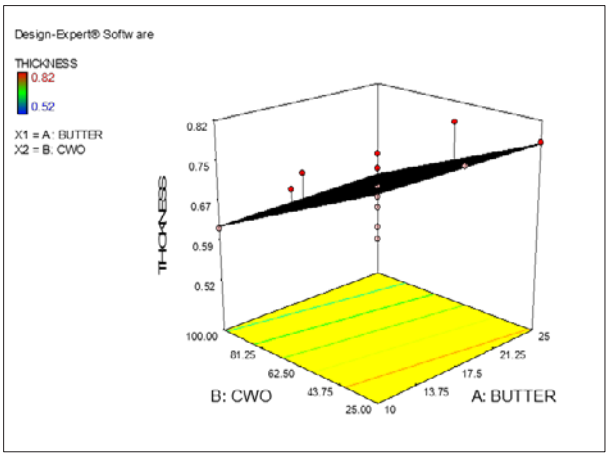

Figure 8 - Response surfaces for the effect of CWO concentration and butter on the thickness of cookie samples

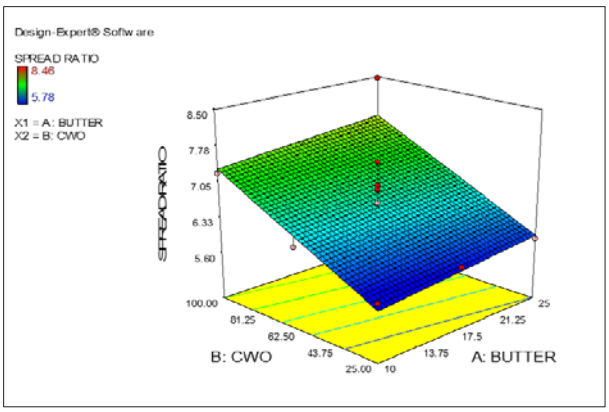

Figure 9 - Response surfaces for the effect of CWO concentration and butter on spread ratio of cookie samples

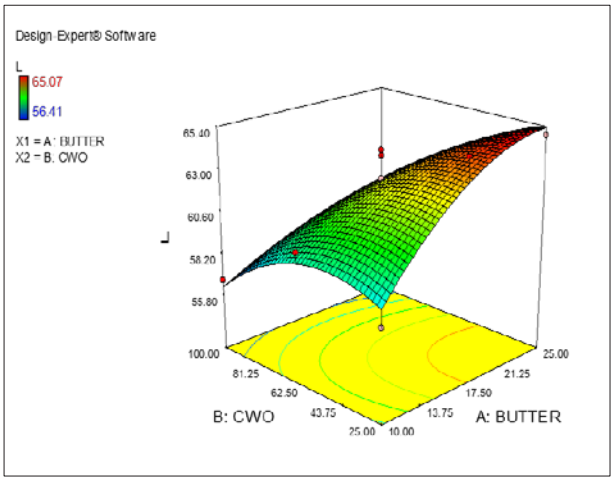

Figure 10 - Response surfaces for the effect of CWO concentration and butter on L value of cookie samples

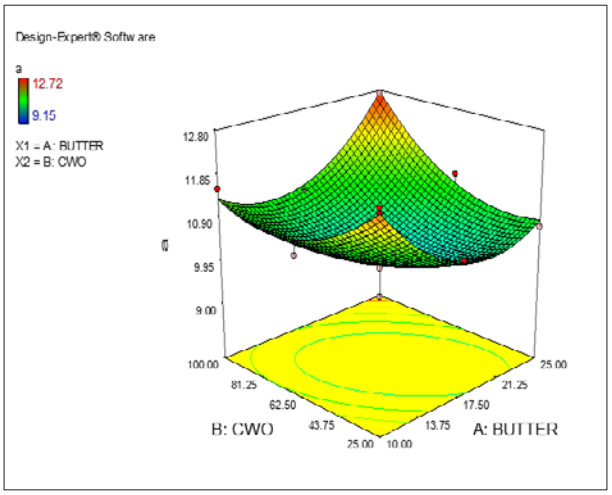

Figure 11 - Response surfaces for the effect of CWO concentration and butter on a value of cookie samples

haviour could be attributed to the reduced viscosity of chickpea dough, and it causes a higher spread ratio. A previous study indicates a decrease in the viscosity of the dough with the addition of chickpea flour [89]. A similar result showed that the lower the viscosity of dough, the faster the spreading rate of cookies [90].

3.2.2. Colour

As it can be seen in Figure 10, the L values of cookies are affected significanlty by the CWO and butter concentration in cookies $p<0.01$, $p<0.05$ respectively. Moreover, the interaction between butter and CWO concentration can impact the L values of cookies statistically $p<0.05$. Increasing the percentage of butter leads to an increase in L values of cookie samples. While increasing CWO concentration up to 65.50 g/100 g in cookie samples leads to an increase in L values, using a CWO concentration above 65.5 g/100g causes a decrease in L values in the cookie samples. As it can be seen from Figure 11, interaction of the butter concentration affects significantly the a values of cookies samples $p<0.05$. Increasing the butter concentration up to 17.50 g/100g gram in the cookie samples leads to a decrease in values of the cookie samples. However, using butter concentrations above 17.50 g/100g causes an increase of b values of cookie samples. The response surface plot Figure 12 shows that increasing the ratio of CWO in cookies led to a significant decrease in the b values of cookie samples $p<0.01$. However, the b values of cookie samples increase significantly by increasing the butter percentage in cookie samples $p<0.01$.

3.3. SENSORY EVALUATION OF COOKIES

Sensory evaluation of cookie samples is summarised in Table V. The scores for colour, appearance, flavour, texture, overall acceptability, and affordability ranged from 2.89 to 4.33, 3.22 to 4.22, 2.33 to 4.33, 2.00 to 4.56, 2.44 to 4.33 and 2.22 to 4.22 respectively based on the panellists assigned for each parameter using a 5- point hedonic scale. There were significant differences between the treatment of fortification of the CWO and butter ratio in cookie samples in terms of colour, flavour,

Table IV - L, a, b values of cookie samples

Sample Number	L	a	b
1	57.83 ± 0.08^{c}	12.32 ± 0.04^{j}	$28.03\pm0.11^{c,d}$
2	56.78 ± 0.06^{b}	12.72 ± 0.01^{k}	28.22 ± 0.06^{e}
3	56.67 ± 0.03^{b}	11.55 ± 0.09^{i}	26.68 ± 0.04^{a}
4	64.11 ± 0.11^{j}	9.15 ± 0.00^{a}	27.42 ± 0.02^{b}
5	65.07 ± 0.04^{l}	10.64 ± 0.06^{d}	29.95 ± 0.08^{i}
6	63.81 ± 0.03^{i}	9.80 ± 0.01^{b}	28.31 ± 0.06^{e}
7	62.52 ± 0.01^{h}	9.96 ± 0.24^{c}	28.55 ± 0.13^{f}
8	59.97 ± 0.24^{d}	10.75 ± 0.01^{e}	$28.17\pm0.33^{d,e}$
9	64.93 ± 0.06^{k}	$10.73\pm0.02^{d,e}$	30.85 ± 0.08^{j}
10	61.13 ± 0.13^{f}	11.03 ± 0.01^{g}	28.91 ± 0.01^{g}
11	60.95 ± 0.06^{e}	$10.94\pm0.01^{f,g}$	29.06 ± 0.06^{h}
12	56.41 ± 0.14^{a}	10.92 ± 0.02^{f}	28.01 ± 0.01^{c}
13	62.01 ± 0.06^{g}	11.33 ± 0.06^{h}	30.04 ± 0.09^{i}

[a-j] For each parameter, different superscript letters indicate a significant difference ($p<0.05$) among cookie samples

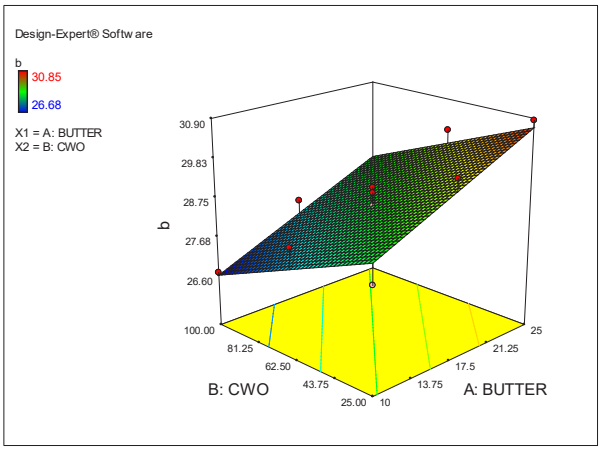

Figure 12 - Response surfaces for the effect of CWO concentration and butter on b value of cookie samples

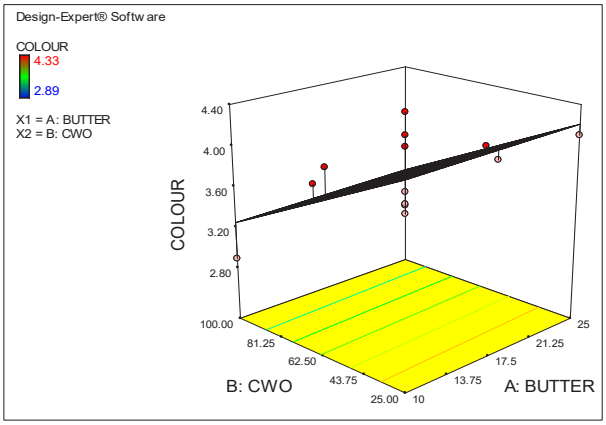

Figure 13 - Response surfaces for the effect of CWO concentration and butter on the score given to the colour of cookie samples

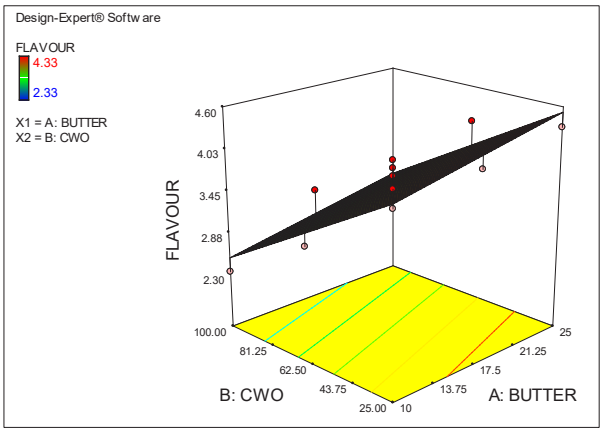

Figure 14 - Response surfaces for the effect of CWO concentration and butter on the score given to the flavour of cookie samples

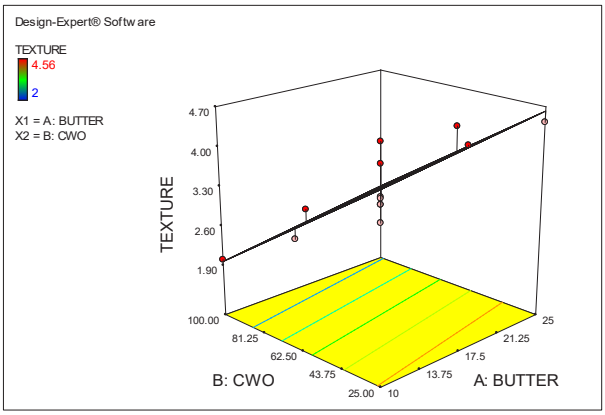

Figure 15 - Response surfaces for the effect of CWO concentration and butter on the score given to the texture of cookie samples

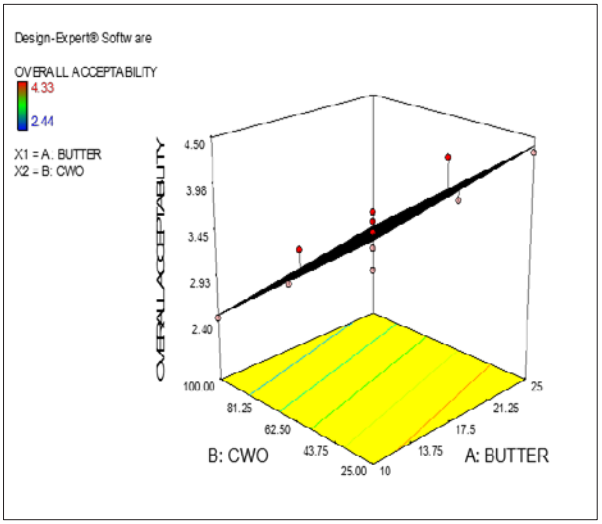

Figure 16 - Response surfaces for the effect of CWO concentration and butter on the score given to the overall acceptability of cookie samples

texture, overall acceptability, and affordability. However, there the statistical results indicate that no differences in appearance were found between cookie samples in terms of the ratio of CWO and butter. As it can be seen from Figure 13, the CWO ratio increased, the score given to colour in the sensory evaluation decreased and this ratio was noticed as statistically significant $p < 0.05$. The response surface plot Figure 14 shows that the increase in the percentage of CWO in cookie samples can lead to a significant decrease in the value of the score given to flavour $p<0.01$. It can be seen in Figure 15 that the scores given to the texture of the cookie samples decreased significantly as the CWO ratio increased. Similarly, as it can be seen in Figure 16, the increase in the CWO ratio in cookie samples affect negatively the score given to overall acceptability and this ratio was noticed as statistically significant $p<0.01$. This effect was noticed as statistically significant. The response surface plot Figure 17 shows that, as the CWO ratio increased, the score given to the affordability by the panellists decreased $p<0.01$. Sample number 9, the highest score for overall acceptability and affordability.

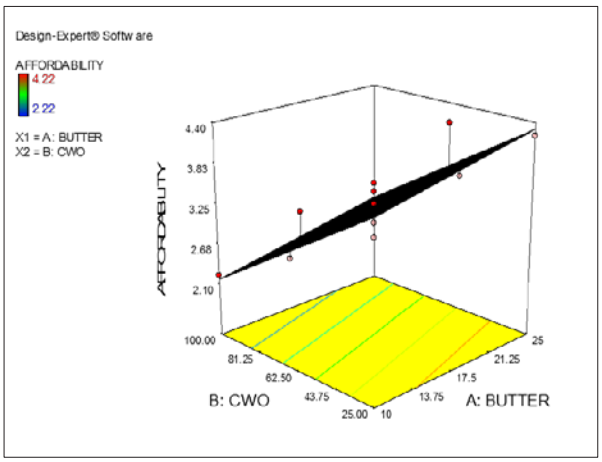

Figure 17 - Response surfaces for the effect of CWO concentration and butter on the score given to the affordability of cookie samples

4. CONCLUSION

The above research shows that composite flour consisting of 32% chickpea, 16% whole grain wheat, and 16% oat flours can be used successfully to replace 100% of the refined wheat flour to formulate healthy low fat high protein cookies having the additional benefit of daily nutrition. Thus, from the results, it may be concluded that cookies high in proteins (nearly 100% increase) and low in calories (nearly 9% decrease) could be made with composite flour. Also from the physical analyses, it may be concluded that cookies are acceptable for their sensory quality. The optimised cookie production chosen by the software was 25% chickpea flour, 12.9% whole grain wheat flour, 12.9% oat flour and 15% butter that give a protein value of 20%. The formulated functional cookies had higher protein content than cookies in literature. The research demonstrated that highly acceptable reduced calorie and high protein cookies, fortified by composite flour, almond, butter, dried mulberry can be produced with a highly acceptable and nutrition composition.

Acknowledgment

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Notes on Contribute

Neslihan Özbuldu is a doctoral student who get sponsorship from higher education institution (100/2000). She researches on healthy nutrition, healthy life, and food additives.

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Table V - Sensory scores for low-fat high-protein cookie samples

Sample Number	Colour	Appearance	Flavour	Texture	Overall Acceptability	Affordability
1	3.89±0.60$^{a.b}$	4.00±1.22	4.22±0.97$^{e.f}$	4.56±0.73^{d}	4.11±0.78$^{d.e}$	4.00±1.00$^{c.d}$
2	2.89±1.05^{a}	3.78±0.83	2.67±1.12$^{a.b}$	2.22±0.83$^{a.b}$	2.56±0.53^{a}	2.22±0.83^{a}
3	2.89±1.17^{a}	3.22±0.97	2.33±0.71^{a}	2.00±0.87^{a}	2.44±0.73^{a}	2.22±0.83^{a}
4	4.33±0.87^{b}	4.33±0.71	3.33±0.71$^{b.c.d.e}$	3.00±0.71$^{b.c}$	3.44±0.88$^{b.c.d}$	2.78±1.20$^{a.b}$
5	4.11±1.36^{b}	3.89±1.36	4.11±0.78$^{d.e.f}$	4.44±0.73^{d}	4.11±0.78$^{d.e}$	4.00±1.00$^{c.d}$
6	4.00±0.71^{b}	4.22±0.67	3.67±0.87$^{c.d.e.f}$	3.00±0.71$^{b.c}$	3.44±0.73$^{b.c.d}$	3.11±0.78$^{a.b.c}$
7	3.56±0.88$^{a.b}$	3.44±0.88	3.78±0.97$^{c.d.e.f}$	4.11±0.78^{d}	3.56±0.53$^{b.c.d.e}$	3.44±0.73$^{b.c.d}$
8	3.89±0.93$^{a.b}$	3.89±1.27	3.11±1.17$^{a.b.c}$	2.89±0.93$^{b.c}$	3.22±0.97$^{a.b.c}$	2.89±1.27$^{a.b}$
9	4.11±0.93^{b}	4.11±0.93	4.33±0.71^{f}	4.44±0.88^{d}	4.33±0.87^{e}	4.22±1.09^{d}
10	3.44±1.13$^{a.b}$	3.44±0.73	3.22±0.44$^{a.b.c.d}$	2.67±0.50$^{a.b.c}$	3.00±0.71$^{a.b}$	2.78±0.67$^{a.b}$
11	4.11±0.60^{b}	3.56±0.88	3.89±1.05$^{c.d.e.f}$	3.11±1.05^{c}	3.67±0.71$^{b.c.d.e}$	3.56±0.88$^{b.c.d}$
12	3.56±1.13$^{a.b}$	3.56±1.01	3.11±0.78$^{a.b.c}$	2.44±0.73$^{a.b.c}$	2.89±0.78$^{a.b}$	2.78±1.09$^{a.b}$
13	3.78±1.09$^{a.b}$	3.56±1.24	4.11±0.78$^{d.e.f}$	4.00±0.87^{d}	4.00±0.87$^{c.d.e}$	4.11±0.78$^{c.d}$

$^{a-f}$ For each parameter, different superscript letters indicate a significant difference ($p<0.05$) among cookie samples

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INNOVHUB
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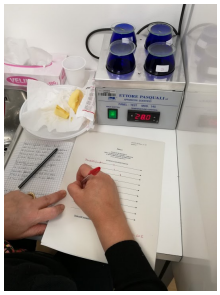
Analisi Sensoriale dell'Olio di Oliva vergine

Il Reg. UE 2019/1604 (modifica del Reg. CE 2568/1991) stabilisce i parametri chimico-fisici e i metodi per il controllo di qualità dell'olio di oliva.
La valutazione organolettica (Panel test) concorre alla definizione della qualità dell'olio e alla classificazione merceologica di appartenenza.
Il Regolamento classifica l'olio di oliva vergine nelle categorie:

OLIO EXTRA VERGINE DI OLIVA
OLIO DI OLIVA VERGINE
OLIO DI OLIVA LAMPANTE

in funzione dell'intensità del fruttato, della presenza e dell'intensità di eventuali difetti. Fornisce inoltre indicazioni sulle caratteristiche organolettiche per l'etichettatura facoltativa.
La valutazione organolettica è qualificata da un livello di affidabilità paragonabile a quello delle prove analitiche e viene eseguita da un panel di assaggiatori selezionati e addestrati avvalendosi di tecniche statistiche per il trattamento dei dati.
Il nostro Panel è riconosciuto dal MiPAAF (Ministero delle Politiche Agricole Alimentari e Forestali) come comitato di assaggio incaricato del controllo ufficiale delle caratteristiche degli oli di oliva vergini e degli oli DOP e IGP e dal COI (Consiglio Oleicolo Internazionale).
La valutazione organolettica è accreditata da ACCREDIA (Ente Italiano di Accreditamento). Il Panel è al servizio dell'industria, di consorzi di produzione, di enti certificatori e della grande distribuzione.



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Review

A systematic review on essential oils and biological activities of the genus *Syzygium* (Myrtaceae)

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Syzygium belongs to the myrtle family, Myrtaceae with about 1800 species and located in the tropical region of Asia. The species have economic, medicinal, and pharmacological properties as well as being a source for pharmacochemistry studies. The local populations often use this species for different medicinal purposes, like to treat diabetes, dysentery, stomach-ache, cold, and ulcer. The objective of this study was to review the essential oils of the genus *Syzygium* and their biological activities. The data were collected from the scientific electronic databases including SciFinder, Scopus, Elsevier, PubMed, and Google Scholar. A total of twenty-six *Syzygium* species have been reported for their essential oils and biological activities. Sesquiterpenes were identified as the major group components for *Syzygium* species with the presence of α,β-caryophyllene, caryophyllene oxide, α-cadinol, germacrene D, viridiflorol, nerolidol, together with monoterpenes, α-pinene, β-pinene, o-cymene, β-ocimene, and limonene. The essential oils also presented remarkable bioactivities such as antioxidant, antibacterial, antifungal, antimalarial, acetylcholinesterase, anti leishmanicidal, cytotoxicity, larvicidal, oviposition deterrent, toxicity, genotoxicity, antimicrobial, α-amylase, anti-inflammatory, and molluscicidal properties. Hence, these studies may contribute to the rational and economic exploration of *Syzygium* species since it has been identified as potent natural and alternative sources to the production of new herbal medicines.

Keywords: Essential oil, *Syzygium*, caryophyllene, pinene, antioxidant, antimicrobial

1. INTRODUCTION

The Myrtaceae family includes around 55,000 species, classified into two subfamilies, 17 tribes, and 142 genera [1]. The name derives from the shrub '*Myrtus*' which is located near the Mediterranean in North Africa and South America. The plant contains both trees and shrubs and was an ecologically important angiosperm family [2]. They are generally found in an environment that is a waterlogged and humid rain forest. Most of them are bisexual with polysemous and actinomorphic, the flowers have inferior ovaries that are partially or fully developed, while the fruits are generally fresh or dry [1]. Many of these members of the family have paramount uses in history as a traditional medicine in divergent ethnobotanical practices throughout the tropical and subtropical world [2].

Syzygium is the largest genus in the Myrtaceae family located in a tropical region with high diversity in Asia [1]. About 1800 species of *Syzygium* were recorded and mainly found in Southern and Southeast Asia, Southern China, Australia, New Caledonia, and some in East Africa, Madagascar, Mascarenhas Islands, Southwest Pacific Islands, Taiwan, and Southern Japan [3]. *Syzygium* species present economic and medicinal consequentiality and pharmacological proprieties being a potential source for pharmacochemistry studies. Meanwhile, traditional communities utilise the infusions and de-

coction leaves of *Syzygium cumini* and *S. aqueum* to treat diabetes, stomach pains, and dysentery [3]. *Syzygium* species can be trees with thick, granular bark, twigs usually glabrous, the leaves are opposite, entire, penninerved, usually gland-dotted; lateral nerves united, forming a clear or faint intramarginal vein. The flowers are bisexual, in terminal or axillary corymbose cymes or panicles; calyx tube hemispherical, globose, or turbinate, tube produced above the summit of the ovary, lobes four or five, ovate to suborbicular, imbricate; petals four or five, orbicular, pellucid-glandular; stamens numerous, filaments inflexed in bud; staminal disc broad or absent; anthers globose; ovary inferior, two-celled; ovules few to several in each cell; style 1, subulate, stigma simple. The fruit is a berry, one-celled, with a few seeds [3]. *Syzygium* is one of the most common tree genera in the forest ecosystem, presenting nectariferous flowers, often in mass and typically fleshy fruits; it is used as food by birds, insects, and small and large mammals [3].

Essential oils, which serve as secondary metabolites, involve complex mixtures of natural compounds and versatile organic structures [4]. They are an involute cumulation of terpenic compounds, especially monoterpenes, sesquiterpenes, alcohols, aldehydes, ethers, esters, ketones, and phenols that are mainly responsible for aroma and odour. The oils are mainly responsible for the fragrance in spice and condiments, as well as used as a repellent agent in insecticides and herbicides [5]. Essential oils from aromatic and medicinal plants have been known to possess biological activity since antiquity, most notably antibacterial, antifungal and antioxidant properties [6]. Nowadays, researchers around the world produce medicines from the essential oils of natural products like plants.

Hence, the review regarding *Syzygium* essential oils must be done to simplify and compile the information. The information available on the *Syzygium* essential oils of various species was searched thoroughly via electronic search (SciFinder, Scopus, Elsevier, Pubmed, Google Scholar, and Web of Science) and the articles published in peer-reviewed journals were collected via a library search. However, *S. aromaticum* essential oils have been subject to several reviews, which will not be repeated here [7-9]. The review aims to compile the information regarding their medicinal uses, chemical composition, and bioactivities of the essential oils from the genus *Syzygium*.

2. SEARCH STRATEGY

The protocol to perform this study was developed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [10] (a) the first step was to exclude duplicate articles, (b) titles and abstracts were then read and the inclusion and exclusion criteria were applied and (c) all articles resulting from this stage were read in full, and the inclusion and exclusion criteria were applied again. Figure 1 shows the flow diagram of the identification and selection of the articles. Following this step, we reached the articles chosen for this study. This systematic review was conducted through searches using Scopus, Medline, Scielo, ScienceDirect, SciFinder, and Google Scholar. The keywords used were '*Syzygium*', 'essential oil', and 'biological activity' articles over the period from the beginning of the database until May 2021. In addition, as a second search strategy, we included studies obtained by a manual search of the reference lists of the included studies. Articles on the genus of *Syzygium* that reported traditional uses, essential oils, and their biological activities were included (except for *Syzygium aromaticum*). The inclusion of articles considered the following criteria: (1) type of publication - original journal articles, (2) only articles in English, Portuguese, and Spanish, (3) articles must present the chemical composition of essential oils, (4) articles must discuss the biological activity of the essential oils. The following were used as the exclusion criteria: (1) articles that did not show the search terms in the title and in the abstract; (2) articles reported on essential oils of *Syzygium aromaticum* (3) review articles, (4) full-text articles not found, and (5) articles that did not show the composition of the essential oils.

3. MEDICINAL USES OF THE GENUS *SYZYGIUM*

Since antiquity, medicinal plants have been commonly used amongst rural inhabitants worldwide. Herbal medicines are widely used for the treatment of differ-

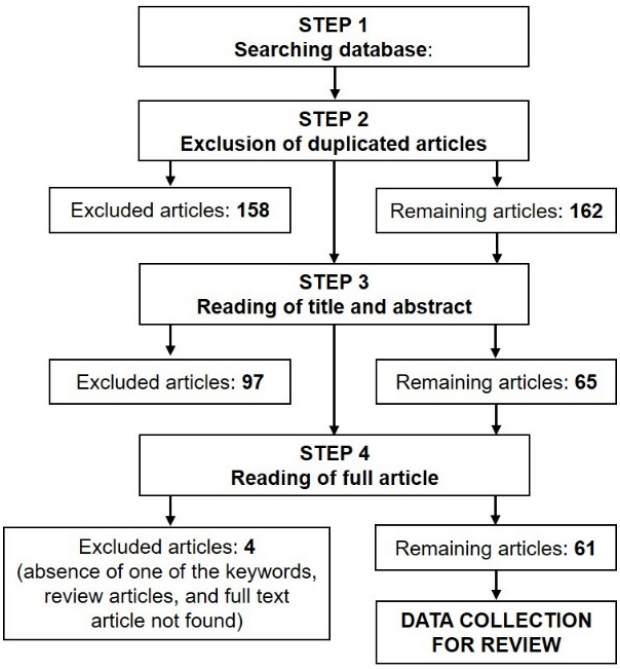

Figure 1 - PRISMA flow diagram of included studies

ent types of diseases such as skin and throat infections and other diseases in developing countries [11]. Natural products have been an integral part of the archaic traditional medicine systems such as Ayurveda, Chinese, and Egyptian [12]. People have been using various parts of the plant such as the leaf, stems, roots, flowers, and seeds extracted for the benefit of humans and used as traditional medicine [6]. *Syzygium* is known to be rich in volatile oils, mainly the part of the fruit used is edible and used for traditional medicines in divergent ethnobotanical practices [13]. Recently, *Syzygium* species have been used to treat diabetes, dysentery, stomach-ache, cold, and ulcer [11]. *Syzygium aromaticum* and *S. cumini* are, arguably, the most useful, with their fruits and leaves used as components of multiple traditional therapies [14]. In India, *Syzygium cumini* is one of the best-known species and it is very often cultivated locally where it is known as jambolan [13]. Table I shows several *Syzygium* species and their medicinal uses [11-23].

4. CHEMICAL COMPOSITIONS OF *SYZYGIUM* ESSENTIAL OILS

In earlier reports, there are twenty-six *Syzygium* species described on the essential oil composition [24-58]. Most of the species were identified in India where fifteen species were reported. Besides, six species were reported in Vietnam, and two species in Mauritius, Thailand, Germany, Pakistan, Nigeria, Egypt, and

Table I - Medicinal uses of several *Syzygium* species

Species	Part	Traditional uses
S. cumini	Leaf	Used to treat diabetes, opium poisoning, centipede bites, renal problems, dysentery, inflammation, leucorrhoea, stomachache, fever, dermopathy, constipation, inhibition of blood discharge in the faces, and reduce radiation-induced DNA damage, prescribed for nausea, vomiting, bleeding disorders, and metrorrhagia [11]
	Fruit	Helps to convert starch into energy which helps in regulating blood sugar levels and possesses a low glycemic index, gastric problems, anorexia, stomachic, astringent, antiscorbutic, diuretic, diabetic, reduced splenomegaly, prevents chronic diarrhea, excellent food remedy for hemorrhoids and liver disorders, abdominal diseases such as loss of appetite, abdominal pain, dysentery, and irritable bowel syndrome [15]
	Bark	To treat dysentery, repeated abortion, and headache [16]
	Seed	To treat sores and ulcers, cold, cough, fever, skin diseases like rashes, the genitourinary tract ulcers, stoppage of urinary discharge, mouth, throat, and intestine infections [16]
S. aqueum	Leaf	Used for stomach pains and dysentery [3]
S. cordatum	Bark	To treat amenorrhea, anemia, burn, chest complaints, emetics, gonorrhea, respiratory ailments, sexually transmitted infections (STIs), sores, and tuberculosis [17]
	Leaf	Used to treat colds, fever, gastro-intestinal complications, herpes simplex, herpes zoster, pre-hepatic jaundice, skin rash, and stomach problems [17]
	Root	Used as treat cough, diarrhea, dysentery, malaria, malaria, wounds, and headache [17]
	Fruit	To treat wound in the mouth [17]
S. polyanthum	Leaf	Used for antiulcer, antidiabetes, anti-inflammatory, and antidiarrhea treatment [18]
S. caryophyllatum	Fruit	Treating diabetes [19]
S. aromaticum	Clove	Used to relieve the stomach pain, the pain of muscle cramps and some nerve conditions, nausea, vomiting, diarrhea, hernia, bad breath, toothache, skin counterirritant, and mouth and throat inflammation [20]
S. samarangense	NM	For the treatment of fever and diarrhea [21]
S. malaccense	Bark	Used for stringent, treat cracked tongue, itching and diuretic, blood pressure, respiration, alleviate edema dysentery, antibiotic, mouth ulcer and used by women with irregular menstruation [22]
S. curanii	Leaf	Cure for high blood sugar [22]
S. jambos	Flower	Tonic for the brain and liver, diuretic, reduce fever, diarrhea, dysentery, diabetics, anesthetic property and catarrh. The leaf decoction is applied to eyes sore, emetic and cathartic, relieve asthma, bronchitis and hoarseness [22]
S. lineare	Leaf	Astringent, refrigerant, diuretic, to cool the body, and increase stamina [22]
S. polycephalum	Fruit	Used for curing high blood sugar [22]
S. suboriculare	NM	Used to treat coughs and colds, diarrhea, and dysentery [23]
S. moorei	NM	Antiseptic properties [23]
S. francisii	NM	Antiseptic properties [23]
S. grande	NM	Antiseptic properties [23]
S. forte	NM	Antiseptic properties [23]
S. guineense	NM	Antihypertensive properties [23]

Table II - Major components identified from several *Syzygium* essential oils

Species	Locality	Part	Extraction	Method	Total components %	Major groups %	Major components %
S. samarangense	Mauritius	Leaf	Hydro-distillation	GC-MS (HP-ınnowax FSC)	-	-	β-Pinene (21.3%), α-pinene (8.9%), γ-terpinene (7.9%), limonene (7.7%), *p*-cymene (5.9%) [25]
	Thailand	Leaf	Hydro-distillation	GC-MS (BPX-5)	11 (77.03%)	-	*o*-Cymene (54.33%), α-pinene (7.14%) [26]
	Germany	Leaf	Hydro-distillation	GC-MS (ZB-5)	91 (86.30%)	Sesquiterpene hydrocarbons (47.44%)	Germacrene D (21.62%), cuminyl aldehyde (10.56%), β-caryophyllene (5.93%), β-cadinene (5.25%) [27]
	India	Leaf	Hydro-distillation	GC-MS (CP-Sil-5 CB)	25 (99.24%)	Monoterpene hydrocarbons (30.27%)	Viridiflorol (15.05%), β-pinene (11.64%), 1-naphthalenol (11.07%) α-pinene (9.61%), α-cubebene (7.71%) [28]
	Nigeria	Leaf	Hydro-distillation	GC-MS (CP-Sil-5 CB)	40 (90.3%)	-	α-Cadinol (12.7%), juniper camphor (12.5%), caryophyllene oxide (8.2%), δ-cadinene (5.7%) [29]
S. coriaceum	Mauritus	Leaf	Hydro-distillation	GC-MS (HP-ınnowax FSC)	-	-	(*E*)-β-Ocimene (24.4%), (*Z*)-β-ocimene (10.7%), α-guaiene (12.6%), β-selinene (9.7%), myrcene (7.8%), δ-guaiene (7.2%) [25]
S. cumini	Pakistan	Leaf	Hydro-distillation	GC-MS (HP-5)	73 (93.82%)	Monoterpene hydrocarbons (27.00%)	Fenchol (4.22%), 5-methyl-1,3,6-heptatriene (4.90%), *cis*-β-ocimene (4.40%), γ-cadinene (4.09%) [11]
	India	Leaf	Hydro-distillation	GC-MS (HP-5)	25 (95.66%)	Oxygenated sesquiterpenes (51.43%)	τ-Cadinol (21.44%), τ-muurolol (12.01%), globulol (7.98%), caryophyllene (6.69%), δ-cadinene (6.56 %), α-pinene (6.32 %) [15]
		Leaf	Hydro-distillation	GC-MS (Elite-5)	- (93.6%)	Monoterpene hydrocarbons (62.5%)	α-Pinene (17.2%), (*Z*)-β-ocimene (10.9%), (*E*)-β-ocimene (9.6%), β-pinene (8.6%), δ-cadinene (7.5%), β-myrcene (5.4%) [30]
		Leaf	Hydro-distillation	GC-MS (Elite-5)	66 (95.3%)	Monoterpene hydrocarbons (38.8%)	α-Pinene (21.5%), α-terpinene (9.5%), δ-cadinene (8.3%), *trans*-□-ocimene (6.8%) [31]
		Fruit	Hydro-distillation	GC-MS (Elite-5)	34 (99.3%)	Monoterpenes hydrocarbons (41.9%)	α-Cadinol (25.8%), α-pinene (12.4%), β-pinene (8.0%), myrcene (8.4%), δ-cadinene (7.7%), α-terpinene (7.4%) [31]
		Fruit	Hydro-distillation	GC-MS (HP-5)	32 (90.35%)	-	α-Gurjunene (38.35%), α-caryophyllene (7.15%), guaiol (7.00%), β-caryophyllene (6.96%), aromadendrene (6.62%), α-selinene (5.20%) [32]

Continua Tabella II

Species	Locality	Part	Extraction	Method	Total components %	Major groups %	Major components %
	Thailand	Leaf	Hydro-distillation	GC-MS (BPX-5)	15 (87.50%)	-	Terpinolene (19.08%), γ-terpinene (16.63%), (*E*)-caryophyllene (12.25%), platyphyllos (9.69%), *o*-cymene (7.34%), α-humulene (5.58%) [26]
	Brazil	Leaf	Hydro-distillation	GC-MS (HP-5)	38 (99..0%)	Monoterpene hydrocarbons (34.48%)	α-Pinene (21.20%), globulol (15.30%), eugenol (11.20%), α-terpineol (8.88%), aromadendrene (6.79%), limonene (6.08%) [33]
		Leaf	Hydro-distillation	GC-MS (HP-5)	38 (95.50%)	Monoterpene hydrocarbons (57.00%)	α-Pinene (22.20%), limonene (7.30%), *cis*-β-ocimene (10.20%), *trans*-β-ocimene (5.88%), α-terpineol (7.00%), β-caryophyllene (9.45%), α-humulene (5.50%) [34]
		Leaf	Hydro-distillation	GC-MS (DB-5)	11 (99.98%)	Monoterpene hydrocarbons (87.12%)	α-Pinene (31.85%), (*Z*)-β-ocimene (28.98%), (*E*)-β-ocimene (11.71%), (*E*)-β-caryophyllene (5.02%), β-pinene (5.57%) [35]
		Leaf	Hydro-distillation	GC-MS (Restex RTX-5)	12 (75.68%)	-	α-Caryophyllene (25.24%), β-caryophyllene (16.00%), α-terpineol (9.08%), *epi*-globulol (5.23%) [16]
	Egypt	Leaf	Hydro-distillation	GC-MS (HP-5)	-	-	α-Pinene (17.26%), α-terpineol (13.88%), β-pinene (11.28%), *cis*-ocimene (11.27%), *trans*-caryophyllene (6.96%) [36]
		Leaf	Hydro-distillation	GC-MS (HP-5)	-	-	α-Pinene (17.26%), α-terpineol (13.88%), β-pinene (11.28%) [37]
		Leaf	Hydro-distillation	GC-MS (HP-5)	49 (98.30%)	-	α-Pinene (32.32%), β-pinene (12.44%), *trans*-caryophyllene (11.19%), 1,3,6-octatriene (8.41%) [38]
S. nervosum	Vietnam	Leaf	Hydro-distillation	GC-MS (ZB-5)	61 (90.2%)	Monoterpene hydrocarbons (31.7%)	(*Z*)-β-Ocimene (20.3%), caryophyllene oxide (13.2%), (*E*)-caryophyllene (12.1%), α-pinene (5.2%) [39]
S. hancei	Vietnam	Leaf	Hydro-distillation	GC-MS (HP-5)	50 (97.30%)	Sesquiterpene hydrocarbons (80.87%)	γ-Guaiene (11.07%), β-caryophyllene (9.11%), *cis*-calamenene (7.46%), α-copaene (6.97%), *trans*-cadina-1,4-diene (5.09%) [40]
S. caryophyllatum	Vietnam	Leaf	Hydro-distillation	GC-MS (HP-5)	40 (97.66%)	Sesquiterpene hydrocarbons (72.73%)	β-Caryophyllene (42.53%), (*E*)-β-ocimene (19.38%), α-humulene (5.37%)
	India	Leaf	Hydro-distillation	GC-MS (HP-5)	24 (89.7%)	Oxygenated sesquiterpene (59.5%)	δ-Cadinol (42.0%), 9-*epi*-β-caryophyllene (13.4%), selin-11-en-4α-ol (5.4%) [41]

Continua Tabella II

Species	Locality	Part	Extraction	Method	Total components %	Major groups %	Major components %
		Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	8 (80.2%)	Sesquiterpene hydrocarbons (57.5%)	β-Caryophyllene (32.4%), 1-*epi*-cubenol (11.8%), δ-cadinene (10.0%) [42]
		Leaf	Hydro-distillation	GC-MS (Omega Wax-250)	129 (99.98%)	-	α-Cadinol (18.30%), myristicin (12.02%), caryophyllene oxide (10.72%), α-pinene (10.55%), 4,8,13-duvatriene-1,3-diol (10.44%) [43]
	Pakistan	Leaf	Steam distillation	GC-MS (DB-5)	8 (99.99%)	-	Caryophyllene (96.42%) [44]
S. lanceolatum	India	Leaf	Hydro-distillation	GC-MS (HP-5)	18 (96.3%)	-	Phenyl propanal (18.3%), β-caryophyllene (12.8%), α-humulene (14.5%), caryophyllene oxide (10.7%) [45]
		Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	21 (91.5%)	Sesquiterpene hydrocarbons (55.8%)	α-Humulene (23.1%), β-caryophyllene (16.1%), phenyl propanal (13.5%) [42]
S. lineatum	Vietnam	Leaf	Hydro-distillation	GC-MS (HP-5)	32 (96.91%)	Sesquiterpene hydrocarbons (88.26%)	β-Caryophyllene (64.53%), α-pinene (6.14%) [40]
S. kanarense	India	Aerial	Hydro-distillation	GC-MS (BP-1)	52 (91.9%)	Sesquiterpene hydrocarbons (49.5%)	Seychellene (7.3%), α-muurolol (5.4%), *cis*-cadinene ether (5.3%), β-vetivenene (5.1%),10 *epi*-γ-eudesmol (4.8%), guaiol (4.5%) [46]
S. polyanthum	Malaysia	Leaf	Hydro-distillation	GC-MS (DB-1)	18 (61.69%)	Oxygenated sesquiterpene (53.91%)	*trans*-Nerolidol (30.87%), farnesol (6.23%), α-cadinol (5.58%) [47]
		Stem	Hydro-distillation	GC-MS (DB-1)	23 (56.78%)	Oxygenated sesquiterpene (42.80%)	Cubenol (14.15%), *n*-hexadecanoic acid (11.20%), α-cadinol (6.92%) [47]
	Indonesia	Leaf	Hydro-distillation	GC-MS (ZBHP-5)	27 (99.99%)	-	*cis*-4-Decanal (43.48%), 1-decyl aldehyde (19.75%), capryl aldehyde (14.09%) [18]
S. grande	Vietnam	Leaf	Hydro-distillation	GC-MS (HP-5)	22 (91.4%)	Sesquiterpene hydrocarbons (52.9%)	β-Caryophyllene (25.6%), sabinene (16.8%), (*E*)-β-ocimene (11.9%), α-copaene (5.0%) [48]
		Stem	Hydro-distillation	GC-MS (HP-5)	44 (88.4%)	Sesquiterpene hydrocarbons (54.0%)	β-Caryophyllene (29.3%), sabinene (10.2%), (*E*)-β-ocimene (9.5%), δ-cadinene (6.6%) [48]
	India	Leaf	Hydro-distillation	GC-MS (DB-5)	30 (99.0%)	Sesquiterpene hydrocarbons (69.24%)	β-Caryophyllene (18.38%), 10s,11s-himachala-3(12),4-diene (12.06%), aromadendrene (10.51%), α-caryophyllene (10.22%), α-selinene (8.94%), δ-cadinene (6.49%) [49]

Continua Tabella II

Species	Locality	Part	Extraction	Method	Total components %	Major groups %	Major components %
S. sterrophyllum	Vietnam	Leaf	Hydro-distillation	GC-MS (HP-5)	38 (97.1%)	Monoterpene hydrocarbons (42.0%)	α-Pinene (35.4%), (*E*)-nerolidol (30.4%) [48]
S. jambos	Egypt	Leaf	Hydro-distillation	GC-MS (TGS-MS)	24 (92.00%)	-	δ-Cadinene (10.85%), cumaldehyde (10.75%), β-himachalene (6.40%), isocaryophyllene (6.39%), β-cedrene (5.63%) [50]
S. aqueum	Germany	Leaf	Hydro-distillation	GC-MS (ZB-5)	84 (90.53%)	Sesquiterpene hydrocarbons (53.13%)	α-Selinene (13.85%), β-caryophyllene (12.72%), β-selinene (11.94%), cuminyl aldehyde (9.82%) [27]
S. zeylanicum	India	Leaf	Hydro-distillation	GC-MS (HP-5)	18 (93.90%)	-	α-Humulene (37.85%), β-elemene (10.70%) [51]
		Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	12 (97.7%)	Oxygenated sesquiterpenes (59.5%)	β-Caryophyllene (11.1%), α-cadinol (12.2%), humulene epoxide II (17.6%), caryophyllene oxide (18.9%), α-humulene (14.0%) [42]
S. arnottianum	India	Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	21 (82.8%)	Oxygenated sesquiterpenes (58.2%)	Caryophyllene oxide (15.4%), selina-11-en-4α-ol (13.0%) [42]
S. hemisphericum	India	Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	21 (98.7%)	Sesquiterpene hydrocarbons (84.2%)	β-Caryophyllene (40.5%), α-humulene (39.7%) [42]
S. laetum	India	Leaf	Hydro-distillation	GC-MS (CP-Sil-8CB)	10 (72.2%)	Sesquiterpene hydrocarbons (42.6%)	(*Z,E*)-α-Farnesene (21.5%), γ-amorphene (12.1%), *epi*-α-cadinol (10.2%) [42]
S. alternifolium	India	Leaf	Hydro-distillation	GC-MS (CP-Sil-5 CB)	25 (99.0%)	Monoterpene hydrocarbons (53.53%)	β-Myrcene (24.04%), β-pinene (9.23%), *trans*-β-ocimene (9.20%), cyclofenchene (7.21%) [28]
S. malaccense	Nigeria	Leaf	Hydro-distillation	GC-MS (CP-Sil-5 CB)	23 (97.0%)	-	Limonene (48.8%), γ-terpinene (26.2%) [29]
		Leaf	Hydro-distillation	GC-MS (HP-5, HP-Innowax, Cydex-B)	37 (91.8%)	Monoterpene hydrocarbons (41.6%)	*p*-Cymene (13.5%), caryophyllene oxide (8.8%), β-pinene (8.0%), α-terpineol (7.5%), α-pinene (7.3%), terpinen-4-ol (5.5%), γ-terpinene (5.0%) [52]
S. benthamianum	India	Leaf	Hydro-distillation	GC-MS (DB-5)	63 (99.63%)	-	Sitosteryl acetate (11.83%), stigmastan-3,5,22-trien (7.00%), 2,6-dimethyl-2-octene (6.99%), estra-1,3,5(10)-trien-17-β-ol (6.30%) [53]
S. densiflorum	India	Leaf	Hydro-distillation	GC-MS (DB-5)	84 (99.0%)	Sesquiterpene hydrocarbons (28.37%)	β-Maaliene (17.43%), isoledene (12.46%), α-gurjunene (10.44%), β-elemene (9.90%), β-vatirenene (8.50%), α-panasinsen (5.95%), 8,9-dehydrocycloisolongifolene (5.59%) [54]

Continua Tabella II

Species	Locality	Part	Extraction	Method	Total components %	Major groups %	Major components %
S. cordatum	South Africa	Leaf	Hydro-distillation	GC-MS (DB-5)	60 (79.0%)	-	6,10,14-Trimethylpentadecane-2-one (14.4%), 2,3-butanediol diacetate (13.3%), *n*-hexadeconic acid (7.2%), 2-chloro-1,1-*bis*(2-chloroethoxy)ethane (6.2%) [55]
S. gardneri	India	Leaf	Hydro-distillation	GC-MS (SE-30)	20 (88.8%)	Oxygenated sesquiterpenes (65.6%)	Caryophyllene oxide (49.6%), β-caryophyllene (5.3%), humulene epoxide (5.6%) [56]
S. travancoricum	India	Leaf	Steam distillation	GC-MS (HP-5)	-	-	*trans*-β-Ocimene (44.7%), *trans*-β-caryophyllene (32.9%), α-humulene (6.7%), nerolidol (8.1%) [57]
S. paniculatum	South Africa	Aerial	Hydro-distillation	GC-MS (HP-5)	75 (96.73%)	-	α-Pinene (33.13%), *n*-hexadecanoic acid (19.14%), limonene (14.26%), farnesol (14.21%), β-ocimene (13.04%), citronellol (12.67%), linoleic acid (11.50%), octahydro-1,4-dimethyl azulene (11.57%), citral (9.91%) [58]

South Africa. The extraction of the essential oils was done mostly from the leaf part. And also, the fruit, stem, and aerial part were also investigated. *Syzygium cumini* gave the highest percentage that contributed to about 100% of total oil [33]. Meanwhile, *S. caryophyllatum* showed the highest total components that comprise 129 chemical components [43]. The analysis of the chemical components identified in *Syzygium* essential oils shows that the oil consists of several groups of components, which are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Besides, oxygenated diterpenes, aldehydes, alcohols, ketones, and alkanes were also identified. Table II shows the major components identified in several *Syzygium* essential oils from various origins.

The major components of *Syzygium* essential oils mainly consist of sesquiterpene hydrocarbons. Ten *Syzygium* species identified sesquiterpene hydrocarbons as their major group components. Caryophyllene was identified as the major component of six *Syzygium* species, which are *S. cumini* (Brazil) [16], *S. caryophyllatum* (Vietnam) [40, 42], *S. lineatum* (Vietnam) [40], *S. grande* (Vietnam) [48], *S. zeylanicum* (India) [42], and *S. hemisphericum* (India) [42]. Other sesquiterpene hydrocarbons were also identified which are germacrene D [27], α-gurjunene (Singh et al., 2014), γ-guaiene [40], α-humulene [42, 51], seychellene [46], δ-cadinene [50], α-selinene [27], and β-maaliene [54]. Oxygenated sesquiterpenes were also characterised in several *Syzygium* essential oils. Besides, seven oxygenated sesquiterpenes were identified as the major contributors to the oil with the presence of viridiflorol (*S. samarangense*) [28], α-cadinol (*S. samarangense*, *S. cumini, S. caryophyllatum*) [29, 31, 43], fenchol (*S. cumini*) [11], t-cadinol (*S. cumini*) [15], δ-cadinol (*S. caryophyllatum*) [41], *trans*-nerolidol (*S. polyanthum*) [47], caryophyllene oxide (*S. arnottianum* and *S. gardneri*) [42, 56], and cubenol (*S. polyanthum*) [47]. Meanwhile, monoterpene hydrocarbons were characterised by the presence of high concentrations of a- and β-pinene. a-Pinene was detected in *S. cumini* [30, 31, 33-38], *S. sterrophyllum* [48], and *S. paniculatum* [58], whereas β-pinene was identified from *S. samarangense* [24, 25]. Other monoterpenes were also characterised by *Syzygium* essential oils in a large proportion, which are o-cymene [26], ocimene [24, 25], terpinolene [26], β-myrcene [28], limonene [29], and (*E*,*Z*)-α-farnesene [42].

5. BIOLOGICAL ACTIVITIES OF *SYZYGIUM* ESSENTIAL OILS

The literature study reveals that *Syzygium* essential oils have been reported in various biological activities mainly for antioxidant [11, 28, 31, 32, 38, 43, 58, 59, 60], antibacterial [11, 33, 38, 43, 59, 61] and antimicrobial [18, 28, 44, 49] activities. In antioxidant

Table III - Biological activities of *Syzygium* essential oils

Bioactivities	Essential oils	Description
Antioxidant	*S. cumini*	The leaf oil showed DPPH radical scavenging with IC_{50} value 1.2 mg/mL [11]
		The fruit oil showed high DPPH radical scavenging with IC_{50} value 219 µg/mL [31]
		The leaf oil showed DPPH radical scavenging with IC_{50} value 357 µg/mL [31]
		The fruit oil showed strong activity against FRAP and DPPH assay with IC_{50} values 8.1 µmol/g and 236 g/mL, respectively [32]
		The leaf oil showed DPPH radical scavenging with IC_{50} value 76.40 µg/mL [59]
		The leaf oil showed weak activity against FRAP with 0.47 µg/100 mg, while DPPH assay with inhibition percentage of 55.87% [38]
	S. alternifolium	The leaf oil showed significant DPPH scavenging activity with percentage inhibition 90% [28]
	S. samarangense	The leaf oil exhibited strong concentration dependent DPPH scavenging activity with percentage inhibition 90% [28]
	S. caryophyllatum	The leaf oil in winter and summer seasons showed high DPPH radical scavenging with percentage inhibition 85.83% and 83.36%, respectively [43]
	S. densiflorum	The leaf oil showed moderate activity against DPPH radical scavenging with percentage inhibition 32.09-49.74% [60]
	S. paniculatum	The summer stem-bark oil showed weak DPPH radical scavenging with IC_{50} value 0.11 µg/mL [58]
Antibacterial	*S. cumini*	The leaf oil showed activity against *Streptococcus pyogenes* and *Escherichia coli* with MIC values 1.1 and 2.1 mg/mL, respectively [11]
		The leaf oil showed activity *Streptococcus mutans, S. mitis, S. sanguinis, S. sobrinus, S. salivarius, Actinomyces naeslundii*, *Bacillus fragilis, B. thetaiotaomicron, P. nigrescens* and *P. gingivalis*, each with MIC values lower than 10 µg/mL [33]
		The leaf oil showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Klebsiella pneumonia* with inhibition zone of 28-48 mm [59]
		The leaf oil showed moderate inhibition zones against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Bacillus subtilis, Staphylococcus aureus* and *Enterococcus faecalis* with a range of 12–14 mm [38]
		The leaf oil showed good activity against *Samonella typhimurium* with zone of inhibition 20 mm [61]
	S. caryophyllatum	The leaf oil collected from winter and summer seasons showed MIC value against *Aerococcus viridans* (5.6 and 22.5 µg/mL), *Bacillus cereus* (22.5 and 5.6 µg/mL) and *Staphylococcus aureus* (11.2 and 22.5 µg/mL) [43]
	S. travancoricum	The leaf oil showed moderate activity against *Staphylococcus aureus* and *Samonella typhimurium* with zone of inhibition of 20 mm [61]
Antifungal	*S. cumini*	The leaf oil showed strong activity against *Aspergillus flavus* with MIC value 0.083 mg/mL [11]
		The leaf oil showed pronounced activity against *Alternaria alternate* with EC_{50} value 115 mg/L [37]
Antimalarial	*S. cumini*	The leaf oil showed weak activity against Heme Biocyrstallization assay with IC_{50} value 15.25 mg/mL [11]
Acetylcholinesterase	*S. cumini*	The leaf oil showed weak activity against AChE enzyme with percentage inhibition 19.97 mg/mL [26]
	S. samarangense	The leaf oil showed weak activity against AChE enzyme with percentage inhibition 13.78 mg/mL [26]
Antileishmanicidal	*S. cumini*	The leaf oil showed affected promastigote growth against *Leishmania amazonensis* with MIC value 8.78 µg/mL [33]
		The leaf oil showed minimal activity against *Leishmania amazonensis* with IC_{50} value 60 mg/L [35]

Continua Tabella III

Bioactivities	**Essential oils**	**Description**
Cytotoxicity	*S. cumini*	The leaf oil showed highest value against lung fibroblasts cell line with MIC value 679 µg/mL [33]
	S. polyanthum	The oil did not showed any significant cell injury [47]
Larvicidal	*S. lanceolatum*	The leaf oil showed high acute toxicity activity against *Anopheles stephensi* (LC_{50} 51.20 µg/mL), *Aedes aegypti* (LC_{50} 55.11 µg/mL), *Culex quinquefasciatus* (LC_{50} 60.01 µg/mL), *Anopheles subpictus* (LC_{50} 61.34 µg/mL), *Aedes albopictus* (LC_{50} 66.71 µg/mL), and *Culex tritaeniorhynchus* (LC_{50} 72.24 µg/mL) larvae [45]
	S. zeylanicum	The leaf oil exhibited significant activity against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* with LC_{50} values 83.11, 90.45, and 97.96 µg/mL, respectively [51]
Oviposition deterrent	*S. lanceolatum*	The leaf oil showed effective activity as oviposition deterrent against *Culex tritaeniorhynchus* with percentage effective repellency 92.97% [45]
Toxicity	*S. lanceolatum*	The leaf oil showed low activity against *Anisops bouvieri*, *Diplonychus indicus*, *Gambusia affinis* and *Poecilia reticulate* with LC_{50} ranging between 4148 and 15,762 µg/mL [45]
	S. cumini	The leaf oil showed weak activity against *Sitophilus oryzae* and *Tribolium castaneum* with LC_{50} value greater than 50 mg/L [36]
		The leaf oil showed low contact against *Sitophilus oryzae* with LC_{50} value higher than 0.6 mg/cm^2, while against *Tribolium castaneum* with LC_{50} value 0.41 mg/cm^2 [36]
Genotoxicity	*S. polyanthum*	The leaf and stem oils had no detachable or comet tail [47]
Antimicrobial	*S. polyanthum*	The leaf oil showed strongly inhibited activity against *Bacillus subtilis* growth with MIC value 31.25 µg/mL [18]
	S. alternifolium	The leaf oil showed strongly inhibited against *Candida rugosa*, *Bacillus subtilis* and *Staphylococcus aureus* with lowest value of MIC (0-2 mg/mL) [28]
	S. samarangense	The leaf oil showed strongly inhibited towards *Candida rugosa* and *Escherichia coli* with lowest value of MIC (0-2 mg/mL) [28]
	S. caryophyllatum	The leaf oil showed good activity against *Aspergillus niger, Aspergillus fumigatus* and *Pencillium digitatum* with inhibition zones were 20 mm [44]
	S. grande	The leaf oil showed high activity against *Escherishia coli, Pseudomonas aeroginosa, Klebsiella pneumonia, Proteus vulgaris, Staphylococcus aureus* and *Bacillus subtilis* with MIC values 0.5, 0.25, 0.25, 0.5, 0.75, and 0.5 mg/mL, respectively [49]
α-Amylase	*S. cumini*	The fruit oil showed mild activity with IC_{50} value more than 1000 µg/mL [31]
		The leaf oil showed mild activity with IC_{50} value more than 1000 µg/mL [31]
Anti-inflammatory	*S. cumini*	The leaf oil showed effectively activity on inhibited eosinophil migration with percentage inhibition of 67% [34]
Molluscicidal	*S. cumini*	The leaf oil showed highest activity against *Biomphalaria glabrata* with LC_{50} value of 90 mg/L [35]

activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH radical scavenging capacity assay), ferric reducing (FRAP) assay, Trolox equivalent antioxidant capacity (TEAC or ABTS) assay, copper reduction (CUPRAC) assay, and reducing power assay (RP) have been reported. In DPPH radical scavenging capacity, the leaf oil of *S. cumini* [11] and stem bark oils of *S. paniculatum* [58] gave the strong activity with IC_{50} values of 1.2 mg/mL and 0.11 µg/mL, respectively. İn addition, the leaf oils of *S. alternifolium* and *S. samarangense* gave the highest percentage inhibition with 90% [28]. Meanwhile, the leaf oil of *S. cumini* indicated a strong activity against *Streptococcus pyogenes* and *Escherichia coli* with MIC values of 1.1 and 2.1 mg/mL, respectively [11]. İn another study, the leaf oil of *S. polyanthum* showed strong activity against *Bacillus subtilis* with MIC value 31.25 µg/mL [18]. Other bioactivity studies were also reported and are antifungal [11, 37], antimalarial [11], acetylcholinesterase [26], antileishmanicidal [33, 35], cytotoxicity [33, 47], larvicidal [45, 51], oviposition deterrent [45], toxicity [45, 36], genotoxicity [47], α-amylase [31], anti-inflammatory [34], and molluscicidal [35] activities. Table III describes the details of these activities.

6. CONCLUSION

In this article, we reviewed the relevant literature to congregate the medicinal uses, chemical composition, and bioactivities information on the *Syzygium* essential oils. According to the study, analysis of the essential oil of *Syzygium* species revealed a high content of α-pinene, β-caryophyllene, and α-cadinol. There are variations between different species and between the same species with a different origin. Further pharmacological investigations into other pharmacological activities should be performed to unravel the full therapeutic potential of the *Syzygium* species. Furthermore, preclinical analyses, as well as clinical trials as conducted for essential oils from other species, are required to evaluate the potential of essential oils from the *Syzygium* species for drug development.

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Short note

Expired bakery products as a promising alternative source for biodiesel production

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Expanding the use of biofuels has led to attract attention to focus on the alternative sources of fatty systems. In the current investigation, the oil extracted from expired croissant samples has been investigated as a potential source for biodiesel production. Fatty substrates have been extracted from expired croissant samples. The physic-chemical properties of extracted oil were determined. The fatty acid composition of extracted oil was performed, then the calculated oxidation stability value (COX) of the extracted oil was calculated. Produced biodiesel was evaluated for density, moisture content, acid value, peroxide value, iodine index and oxidation stability 110°C. The oil yield from croissant samples was 27.8%, wt./wt. The predominant fatty acids in the extracted oil were oleic (34.50%), palmitic (27.02%), linoleic acid (25.42%), stearic (8.90%) and linolenic-acid (2.43%). Biodiesel yield (%) of the obtained biodiesel was 86.45%. The density, moisture content, acid value, iodine index and oxidation stability of obtained biodiesel complied with specifications established by ASTM D6751and EN 14214 standards for this type of fuel.

Keywords: Biodiesel, bakery products, croissant, extracted oil, Transesterification, COX value.

1. INTRODUCTION

Recently, energy demand has been increased due to modernisation and industrial growth. It is also expected that the global energy demand will increase by 49% in 2030 [1]. On the other hand, petroleum liquid reserves are depleting at an alarming rate [2] and these reservations could be drained within the coming 45 years [3].

Biodiesel is becoming increasingly important as a promising alternative fuel due to its renewability, biodegradability, nontoxicity, and environment-friendly nature [4]. Biodiesel is one of the sustainable energy sources derived from different sources such as vegetable oils, animal fat, and algae oil [5].

Shortenings, margarine, butter, and vegetable fats are the major sources of fat in confectionery and bakery products [6]. The amounts of fat in some bakery products from different countries varied from 9.4 to 31% [7]. These findings imply that some of bakery products are potential and significant sources of lipids. It was found that, household food waste has increased more than twice in the last five years [8]. Recently, it is estimated that 1.2-2 billion tons (approximately one-third of the food produced in the world for human consumption) is wasted [9].

Croissants are high fat bakery products, with flaky structure formed by repeated rolling and folding of butter or margarine in laminated doughs [10]. A croissant contains approximately 30-40% fat by weight [11]. Puff pastry is a popular confectionery product with a unique texture. Puff pastry is processed from a dough consisting of enriched with fat and other ingredients and the

finished puff product may contain about 30% fat on a dry matter basis [12], and thus, it is considered a high-fat food. Danish pastries are also produced from laminated dough with high levels of fats [13]. The Recent studies focus on low-cost alternatives, such as inedible resources as well as animal fat waste [14]. Thus, the use of oil waste and animal fats is increasing for industrial-scale biodiesel production, this makes the production process more sustainable [15]. Therefore, the oil extracted from expired croissant samples has been investigated in this investigation as a potential source for biodiesel production.

2. MATERIAL AND METHODS

2.1. COLLECTION OF EXPIRED CROISSANT SAMPLES

In total, 500 croissant-type) 85 g) samples were collected from a private factory in Buraidah, Qassim, Saudi Arabia. Samples were collected in 2020.

2.2. LIPID EXTRACTION OF EXPIRED CROISSANT SAMPLES

The croissant samples were cut into a slice having an even thickness of approximately 1.5 cm. Croissant slices were dried in an electric oven (Model: Leicester, LE67 5FT, England) at 60°C for 24 h. The dried croissant samples were ground in an electric grinder (Braun Model 1021) and passed through a 0.6 mm sieve. Croissant powder was extracted for 18 h. with n-hexane in a Soxhlet apparatus [16]. Solvent was removed at 50°C under reduced pressure using a rotary evaporator. The extracted oil was stirred with the anhydrous sodium sulphate for 5 min, and then filtered (vacuum filtration through a Whatman No 1 paper (Whatman International Ltd, Maidstone, UK). The obtained oil was packed in stainless steel vessels.

2.3. DETERMINATION OF OIL YIELD

The oil yield of expired croissant samples was calculated using the following equation:

$$\text{Oil yield (\%)} = W_{EO} / W_{EC} \times 100$$

Where:

W_{EO} = the weight of extracted oil (g),

W_{EC}= the weight of expired croissant samples (g).

2.4. DETERMINATION OF PHYSIC-CHEMICAL PROPERTIES OF EXTRACTED OIL

Extracted oil was analysed for water content (expressed as g/100g), AOAC n. 930.15 [17], acid value (expressed as mg KOH/g oil.), AOAC n. 940.28 [18], peroxide value (expressed as meq O2/kg oil) AOAC n. 965.33 [18], Saponification value (mg KOH/g oil), (AOAC n.920.160 [18] and Iodine value (g I_2/100 g oil) AOAC n. 993.20 [18]. The density (gr/ml) of oil was measured by a mass over volume measurement at 40°C [19]. Brookfield viscometer equipped with a thermo-container (Brookfield Engineering Laboratories, Inc.; Middleboro MA, USA) was used to determine the viscosity of the extracted oil samples at 45°C, according to the method described by Ismail et al., [20].

2.5. FATTY ACID PROFILE DETERMINATION

The fatty acid composition of extracted oil was carried out using gas chromatography (HP 6890), following the previous procedures described by Ali and El-Anany [16].

Oxidation value (COX).

The calculated oxidation stability value (COX) of extracted oil was calculated by applying the formula proposed by Fatemi and Hammond [21] as follow:

$$COX = \frac{[1\,(16{:}1\% + 18{:}1\% + 20{:}1\% + 22{:}1\%) + 10.3\,(18{:}2\%) + 21.6\,(18{:}3\%)]}{100}$$

2.6. BIODIESEL PRODUCTION

One thousand grams of extracted oil were placed into a 2000 mL round-bottom flask equipped with a condenser. After the oil was heated to 65°C, the solution of sodium hydroxide (5.0 g) in methanol (144.82 ml, 6:1 molar ratio of methanol to oil) was slowly added into the reaction. The reaction temperature of the transesterification treatment was set at 60°C. The reaction continued for 110 min. At the end of the incubation stage, the mixture was transferred into a reparatory funnel, left for 24 h to separate the glycerine and impurities from the biodiesel. The produced biodiesel was washed with deionised hot water (50°C) several times to remove the catalyst, glycerol, and impurities [22].

2.7. BIODIESEL YIELD

Biodiesel yield was calculated using the following equation [23]

$$\%\ \text{Yield} = \frac{\text{grams of biodiesel}}{\text{grams of oil}} \times 100$$

2.8. QUALITY PARAMETERS FOR BIODIESEL

The produced biodiesel was evaluated for density at 40°C (ASTM D 6751), moisture content, acid value, peroxide value, Iodine index and oxidation stability 110°C based on the previous procedures described by Ali and El-Anany, [24].

3. RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL PROPERTIES OF EXTRACTED OIL

Table I shows the physicochemical properties of extracted oil. No water content of extracted oil was detected. The oil yield (%, wt/wt) from croissant samples was 27.8%. Recovery of oil is dependent on the

extraction technique, moisture content, type of solvent and extraction period [25]. The acid value of raw materials used for biodiesel production has an influential and effective role in the percentage of fatty acid methyl esters of the final product [26]. The acid value measures the content free fatty acids formed after the hydrolytic degradation of lipid molecules [20]. The acid value of extracted oil under investigation was 1.96 mg KOH/g oil (Tab. I). This result suggests that the triacylglycerol molecules were subjected to hydrolytic degradation process. Free fatty acids content significantly increased with the increase in the storage duration in all the food products [27]. Peroxide value of the fat extracted from croissant samples was 7.03 ± 0.07 meq peroxide/kg (Tab. I). Codex specification suggests that the peroxide value limit might reach 10 meq/kg when the products were at the end of their shelf life [28]. Saponification value measures the mean of the molecular weight of the glycerides expressed in milligrams of potassium hydroxide (mg KOH/g oil). Saponification value of the extracted oil was 196.0±4.7 mg KOH/g. This finding indicates that the saponification value of extracted oil was within the recommended values of 180-199 mg KOH/g oil [29]. The iodine value measures the degree of fatty acid unsaturation in oil components. Iodine values of oil extracted from croissant samples was 65.30±2.08 g I_2/100 g oil, this finding indicates that the obtained oil is considered non-drying oil.

The density is a physical measure of adulteration of vegetable oils. Each oil has a specified density. Density of vegetable affected by fatty acid composition, minor components as well as temperature degree [30]. Density is an important factor which influences the design of unit operations such as distillation, heat exchangers, tubes, and reactors [31]. The density of oil extracted from croissant samples was 0.889 g/cm3 at 40°C. The absolute viscosity is one of the most important physical parameters of fatty systems. viscosity measures the resistance of oil to flow. It is necessary for pumping process, flow measurement, and heat transfer unit operations [32]. The viscosity value of oil extracted from croissant samples was 54.6 centipoises at 40°C.

Table I - Physicochemical properties of extracted oil

Parameters	Units	Value
Water content	g /100g	ND
Acid value	mg KOH/g Oil	1.96
peroxide value	meq/kg	7.03±0.07
Saponification value	mg KOH/g oil	196.0±4.7
Iodine value	(g I_2/100 g oil	65.30±2.08
Density at 40°C	g/cm3	0.889
Viscosity at 40°C	centipoises	54.6
Oil yield on dry weight basis	(%, w/w)	27.8

Table II - Fatty acid compositions of oil extracted from croissant samples

Fatty acid	CC:DB*	Extracted oil sample
Butyric	C 4:0	ND
Caproic	C 6:0	ND
Caprylic	C 8:0	ND
Capric	C 10:0	ND
Lauric	C 12:0	0.24
Myristic	C 14:0	0.60
Myristoleic	C 14:1	ND
Palmitic	C 16:0	27.02
Stearic	C 18:0	8.90
Oleic	C 18:1	34.50
Linoleic	C 18:2	25.42
Linolenic	C 18:3	2.43
Arachidic	C 20:0	0.89
Eicosenoic	C 20:1	ND
SFA		37.65
MUFA		34.50
PUFA		27.85
Calculated oxidation stability value (COX)		3.5

* Carbon content (CC) per double bonds (DB)
SFA, refers to Saturated Fatty Acids; MUFA, Monounsaturated Fatty Acids; PUFA, Polyunsaturated Fatty Acids.

3.2 FATTY ACID COMPOSITIONS OF OIL EXTRACTED FROM CROISSANT SAMPLES

Fatty acid composition of extracted oil is shown in Table II.

The oil extracted from croissant samples mainly composed of 34.50 wt.% oleic acid (18:1), 27.02 wt.% palmitic acid, 25.42 wt.% linoleic (C18:2), 8.90 wt.% stearic acid (18:0), 2.43 wt.% linolenic-ω3 acid (18:3), 0.89 wt.% arachidic acids (C20:0), 0.60 wt.% myristic acid (C14:0) and 0.24 wt.% lauric acid (C12:0). These findings indicate that the oil extracted contains 37.65, 34.50 and 27.85% saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), respectively. These findings are in good agreement with those reported by Anwar et al., [33]. The viscosity of a fatty acid ester usually increases as chain length and saturation increases. On other side, cis double bonds cause a remarkable reduction of viscosity as esters [34]. In the current investigation, the calculated oxidisability COX value of oil extracted from croissant samples was 3.5. COX value is a useful element usually used for evaluation tendency of oil to undergo autoxidation [21]. This finding indicates that the oil extracted from croissant samples is almost stable which provides a particular resistance to oxidation.

3.3 PHYSICOCHEMICAL CHARACTERISTICS OF THE OBTAINED BIODIESEL COMPARED TO ASTM D6751 AND EN 14214 STANDARDS

Table III shows the Physicochemical characteristics of the obtained biodiesel compared to ASTM D6751and EN 14214 standards. Moisture content has not been detected in the obtained biodiesel

samples. The presence of water in biodiesel causes microbial growth in storage tanks. This process could lead to the corrosion of metals, formation of sludge and slime, thereby causing blockage of fuel filters and fuel lines, which could, in turn, damage the vehicle fuel injection system [35]. The maximum amount of allowable water content in biodiesel as specified in the ASTM standard D6751 is 0.050% vol. [36]. Density is one of the most important physical properties of fuel products, because it is concerned with the mass of fuel that is injected into the combustion chamber and thus air-fuel ratio [37]. Density of the obtained biodiesel was 0.879 g cm-3. This value conforms to the density value recommended by the American Society for Testing and Materials [36] and European standard [38]. Viscosity parameter is one of the most important physical properties, required to identify the quality properties of biodiesel [19]. The dynamic viscosity at 40°C of the produced biodiesel was 23.61 centipoises at 40°C. Although viscosity is not compliant with biodiesel specifications, work is in progress to solve this problem by applying purification systems. The acid value is considered as an indicator of biodiesel deterioration. The high levels of free fatty acids have an influence on the engine fuel injection system and cause the corrosion of engine components [39]. The acid value of the obtained biodiesel was 0.48 mg KOH/g. This value was less than the maximum allowed acid value recommended by the American Society for Testing and Materials [36] and European standard [38]. Hydroperoxides are the initial products of oxidation process, these oxidations products increase the viscosity of biodiesel products [24]. Peroxide value of obtained biodiesel was 2.62 milliequivalent peroxides/Kg (Tab. III). Oxidation rancidity of biodiesel fuel is linked to its iodine value, which, in turn, is a measure of the unsaturation degree. Iodine value of obtained biodiesel was 61.17 g I_2/100 g. The maximum limit of iodine value was defined as 120 by biodiesel standard EN 14214. Oxidation stability is one of the most important properties of biodiesel fuel and primarily affects the stability of biodiesel during the storage period. The induction period of obtained biodiesel was 6.2 h. This value complies with international standards for biodiesel (six hours minimum to the European Standard EN 14214 and three hours minimum to the American Standard ASTM D6751. Biodiesel yield (%) of the obtained biodiesel was 86.45%. In this regard, Meneghetti et al. [40] reported that the maximum yield of biodiesel production using methanol was 90% with 1 h of reaction time at 60°C. The above-mentioned results indicate that the moisture content, density, acid value, peroxide value iodine number, and oxidation stability at 110°C of the obtained biodiesel meet the requirements of international standards for biodiesel.

4. CONCLUSIONS

The results indicate that the oil yield (%, wt./wt.) from croissant samples was 27.8%. The predominant fatty acids in extracted oil were oleic acid, palmitic acid, linoleic, stearic acid and linolenic-ω3 acid. The physico-chemical properties of the obtained biodiesel meet the requirements of international standards for biodiesel.

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Declarations Competing interests

The authors declare they have no competing interests.

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Table III - Physicochemical characteristics of the obtained biodiesel compared to ASTM and EN 14214 standards.

Characteristics	Values	ASTM specification	EN 14214
Moisture content %	ND	0.05 maxmum	–
Density (g cm^{-3})	0.879	0.87 to 0.90	0.86 to 0.90
Dynamic viscosity at 40°C (centipoises)	23.61		
Acid value mg KOH/g	0.48	0.8 maxmum	0.50 maxmum
peroxide value (milliequivalent peroxides/kg)	2.62	–	–
Iodine value(g I2/100 g)	61.17	–	120 maxmum
Oxidation Stability 110°C (hours)	6.2	3 minimum	6 minimum
Yields (%)	86.45	–	–

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

Anno 2022
(aggiornato al 01 giugno 2022)

Progetto TRUA20210622001

Express diagnostics of pesticides' presence in fruits and vegetables

A Ukrainian company from the agro-food and food service activities field seeks technology for express diagnostics of pesticides' presence in fruits and vegetables, under technical cooperation or license agreement.

Dead-line for EOIs: 30/06/2022

Progetto RDRES20220530002

Erasmus plus project to create the "rural innovator profile".

A Spanish regional public body is preparing an Erasmus Plus project aimed to define training resources to create a new professional profile, the rural innovator, to address economic, social and environmental challenges of the rural environment. It is searching for partners to collaborate to identify skills and training needs, define new courses and extracurricular activities and test them through pilots.

Dead-line for EOIs: 04/07/2022

Progetto RDRFR20220425017

Eurostars – End-user or manufacturer of aluminium parts sought for a Eurostars project aiming at improving material performances thanks to clean micro-arc oxidation processes

A French SME specialised in micro-arc oxidation (MAO) coating allowing industrial aeronautical parts to reach high mechanical and chemical properties intends to adjust this REACH-compliant process to other sectors. The company is looking for end-user partner to submit an Eurostars proposals aiming at adjusting the process to parts dedicated to other applications: Transportation, medical, hydraulics

Dead-line for EOIs: 31/07/2022

Progetto TRNL20211008001

Dutch wastewater treatment company is looking for expertise and solutions to sustainably extract mineral oil from pre-pressed sludge cake.

A Dutch SME has extensive knowledge of unblocking, inspecting, installing, maintaining and repairing sewers. They also inspect and empty oil and grease separators. The waste products released are recycled in an environmentally responsible way in their own wastewater treatment plants. The SME is looking for technical cooperation partners / SMEs that helps them to extract mineral oil from pre-pressed sludge cake. The SME is willing to conclude a technical cooperation. agreement.

Dead-line for EOIs: 17/11/2022

Progetto TRKR20211123001

A Korean SME seeks partner companies with expertise of vertical farming systems and AI technologies under technical cooperation, license, and joint venture agreements

A Korean SME specializing in manufacturing agricultural machinery is looking for overseas partners with knowledge of vertical farming systems using 'aeroponics' and 'hydroponics' (water farming) for applying to agricultural machines. The company is open to cooperation under technical cooperation, license, and joint venture agreements.

Dead-line for EOIs: 07/12/2022

Progetto TRKR20211201001

A Korean SME is looking for partner companies with expertise in surge protective devices(SPD) and non-combustible panels

A Korean manufacturer of surge protective devices (SPD) and non-combustible panels for multiple usages is looking for partners with expertise in SPDs and panels for enhancing the performance of their products. They are open to technical cooperation, license, and also joint venture agreements.

Dead-line for EOIs: 18/12/2022

Progetto TRKR20210824001

A South Korean company is looking for partners with crude protein extraction technology under licensing agreements

A Korean Company is currently looking for partners companies possessing technologies to extract crude protein from 'hydrolysates of pigs' membrane for high filtration rates to meet local standards of sewage treatment. The firm is seeking for partner companies that can cooperate under license agreements.

Dead-line for EOIs: 23/12/2022

Progetto RDDE20211201001

HORIZON-CL4-2022-TWIN TRANSITION-01-07: Digital tools to support the engineering of a Circular Economy: European consortium is looking for a European recycling company, who is dismantling and recycling end-of-life vehicles

A European consortium led by a German based engineering company specialized in Computer Aided Engineering is applying for the call TWIN-TRANSITION-01-07: Digital tools to support the engineering of a Circular Economy, with the aim to develop a digital twin for the recycling industry. The consortium is looking for a partner from the European vehicle recycling industry to join the

project to integrate the prototype of the autonomous system in a side line of the real industrial recycling process.
Dead-line for EOIs: 31/12/2022

Progetto DAMRC

The Danish tech organization DAMRC is looking for technological research institutions to start common research projects on all fields related to advanced manufacturing

The Danish technological organization DAMRC is a research and development center for advanced production technologies that develops and transforms the latest knowledge into practical solutions in close collaboration with the universities.
The current focus for DAMRC is in the metalworking and composite industry. DAMRC has more than 80 members gathered in a group consisting of advanced companies and organizations.
DAMRC continuously screens new technologies and evaluates whether these technologies can benefit production companies.
DAMRC already cooperates with a number of universities and institutions all over the world (e.g. Fraunhofer), but DAMRC search for more partners.
DAMRC is searching for technological research institutions and organizations, as well as universities.
Dead-line for EOIs 31/12/2022

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

Enterprise Europe Network (EEN)

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

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

enterprise europe network
Business Support on Your Doorstep

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

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

Come ti può aiutare la rete EEN?

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

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

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

NOTIZIE IN BREVE

I grandi oli a Tokyo per la cerimonia di premiazione di Japan Olive Oil Prize (JOOP) e JOOP Design Award

13 maggio 2022 | Tokyo

La Camera di Commercio Italiana in Giappone (ICCJ) ha annunciato oggi i vincitori di Japan Olive Oil Prize (JOOP) e JOOP Design Award. Riuscito a crescere e ad affermarsi come una delle competizioni più importanti in Asia, con l'obiettivo di promuovere le eccellenze olearie mondiali, JOOP celebra oggi i suoi 10 anni e vede la partecipazione al concorso di 500 etichette provenienti da 21 paesi. "*Nei suoi primi 10 anni JOOP è diventato un'istituzione, sta crescendo lentamente ma con carattere, tranquillità e qualità. Le persone che organizzano questo concorso e la giuria JOOP, composta da professionisti riconosciuti a livello internazionale, si dedicano e cercano di raggiungere il miglior risultato possibile per i produttori*" dichiara Konstantinos Liris, panel leader della giuria.

"*Il Giappone si colloca come primo consumatore di olio EVOO tra i Paesi asiatici importatori e all'ottavo posto nel mondo. JOOP si pone come obiettivo principale quello di promuovere ed educare i consumatori sugli effetti benefici di questo prodotto. Stiamo coordinando attività di promozione per dare visibilità agli oli premiati nella grande distribuzione come nelle due catene Hankyu e Isetan, le più importanti del Giappone*" ha commentato Davide Fantoni, Segretario Generale della Camera di Commercio Italiana in Giappone.

Negli ultimi 5 anni le importazioni da parte del Giappone sono cresciute del 33% in volume per un valore totale di 206 milioni nel 2020. Le importazioni di olio extra-vergine italiano rappresentano il 39% del valore importato complessivamente dal Giappone, per un valore di 81 milioni di euro nel 2020 con volumi pari a 16 mila tonnellate. Il Giappone continua a mostrare buone prospettive di ampliamento con un consumo di olio d'oliva in crescita.

Nell'ambito di JOOP, da tre anni il concorso JOOP Design Award premia i produttori che si sono distinti nel comunicare l'identità del loro prodotto, attraverso il logo, l'etichettatura e il design della bottiglia. Anche quest'anno il concorso vede la partecipazione di una giuria di creativi di fama internazionale: Nini Andrade Silva (Portogallo), Piero Lissoni (Italia), Sibel Kutlusoy (Turchia), Adrián Pierini (Argentina) e Giovanna Talocci (Italia).

JOOP conta una giuria di 9 giudici internazionali certificati, supervisionati da 3 panel leader: Konstantinos Liris (Grecia), Antonio G. Lauro (Italia), Yamada (Giappone). La rigorosa selezione ha proclamato i vincitori nelle categorie I.G.P., D.O.P., Biologico, Monocultivar, Blend e Aromatizzato. A seconda del punteggio ottenuto, agli oli sono stati attribuiti i premi Best in Class, Gold e Silver.

A seguire, durante la cerimonia di premiazione, sono stati annunciati i 3 vincitori del **JOOP Design Award:**

1. Vallillo / Monocultivar Peranzana - Agrideavallillo Srl (Italy)
2. Mimi' - Denocciolato Coratina - Azienda Agricola Donato Conserva (Italy)
3. Ootopia Organic Single Estate Iliokastro - Mb Eleon (Greece)

Vincitori JOOP 2022 Best in Country

- BEST OF ARGENTINA
- Establecimiento Olivum (Picual) – Establecimiento Olivum Sa
- BEST OF CROATIA Opg Rheos – Rheos Premium (Blend) BEST OF FRANCE 1ère Récolte – Parcelle 26(hdmp)
- BEST OF GREECE Iliada Kalamata Pdo Extra Virgin Olive Oil – Agrovim S.A.
- BEST OF ITALY Crux – Fattoria Ambrosio
- BEST OF PORTUGAL Gallo Azeite (Bio) – Gallo Worldwide
- BEST OF SPAIN Knolive Epicure – Knolive Oils, S.l.
- BEST OF TUNISIA Picholine High Polyphenols – Adonis Olive Oil
- UNITED STATES Truly - Corto Olive Co.
- TURKEY Hermus Memecik - Hermus Ltd
- BEST OF FLAVORED (Greece) Oleoastron Gourmet Evoo (Flavored Evoo With Fennel, Bay Leaves, Rosemary And Oregano) – Sakellaropoulos Organic Farms
- BEST OF POLYPHENOLS (Italy) Oro Di Rufolo (Elite) – AZ. AGR. ORTOPLANT SS

CONGRESSI

WCOS 2022

2nd World Congress on Oleo Science

23 August - 3 September, 2022 | Kushiro, Japan

In the summer of 2022, the Japan Oil Chemists' Society, JOCS, will hold the 2nd World Congress on Oleo Science, WCOS 2022, in the eastern Hokkaido city of Kushiro to commemorate its 70th anniversary. Oleo science has played an important role in making life clean, healthy, and beautiful, and its future is bright with innovation.

WCOS 2022 will offer following three sessions:

Dear Colleagues and Friends,

You are warmly invited to the 14th European Pesticide Residue Workshop in person!

On behalf of the Scientific and Organizing Committee, I have the honor to announce the next EPRW which returns to Italy after 20 years.

We are looking forward to meeting all of you again from the 19th to the 23rd of September 2022 at Palazzo dei Congressi, in Bologna, Italy.

Do not miss Europe's leading meeting for the latest concepts and developments in the field of pesticide residues in food and drink. The EPRW is an excellent opportunity to exchange information and experience among colleagues and connect experts from all over the world, coming from govermental and private food control laboratories, public authorities, regulatory bodies, universities and research institutes, food producers and distributors, agrochemical manufactures and other interested parties. We expect proficous interactions between the experts and all important vendors of analytical equipment and consumables that will present their latest equipment for pesticide residue analysis in a large exhibition area.

The EPRW is hosted in Bologna, a UNESCO city with the largest medieval city area in the world, ancient porticoes and towers, home to one of the oldest universities, founded in 1088. Bologna is a charming but also a testing destination for the quality and variety of food. Last but not the least, Bologna is easily connected to international airports and offers a variety of accommodation.

We are looking forward to welcoming all of you in Bologna, Palazzo dei Congressi, 19-23 September 2022!

Patrizia Pelosi
EPRW 2022 Chair

Contact: eprw2022@fullday.com
Web site in progress: eprw2022.com

- *Science on Lipids, Oils, Fats, and their related industrial technologies*
- *Oleo Materials & Nano-Technologies*
- *Surfactant, Detergent, and Interface Science*

The congress will also include the JOCS-AOCS Joint Meeting, ISF Plenary Lecture, and Kaufmann Memorial Lecture. Let's share our cutting-edge research with each other.
We have established a fund to bring select speakers from overseas. In addition, excellent oral and poster presentations will be awarded the prize, and their papers will be published in our journal, Journal of Oleo Science. We are looking forward to welcoming you to Kushiro, the coolest city in Japan, in the summer of 2022.
For informations and uptdates:
https://jocs.jp/en/conference-meeting/

World Congress on Oleo Science, hosted by the Japan Oil Chemists' Society (JOCS)

August 27 - September 1, 2022 | Kushiro, Japan

In 2022, we will host the 2nd World Congress on Oleo Science (WCOS 2022), where we can discuss the potential of oleo science.
We encourage you to attend WCOS 2022 and take full advantage of the emerging oleo science.
The 2nd World Congress on Oleo Science will take place in the summer of 2022 to commemorate JOCS's 70th anniversary. Oleo science has played an important role in making life clean, healthy, and beautiful, and the future is expected to be exciting and full of innovation.
WCOS 2022 will have three sessions:
1) Science on lipids, oils, fats, and their related industrial technologies
2) Oleo materials and nanotechnologies
3) Surfactant, detergent, and interface science. Let's share our cutting-edge research with each other.
For updates
https://confit.atlas.jp/guide/event/wcos2022/static/

High Oleic Congress 2022

8-9 September 2022 | Madrid, Spain

The 9th High Oleic Oils Congress (HOC2022) will be held from the 8th to the 9th of September 2022, in Spain, City of Madrid.
The High Oleic market deeply reacted to the Covid-19 pandemic: the demand slowed down in Western Countries due to several lock downs and restaurants closure while the Asiatic countries continuously ramped up their imports following prices competitiveness.
A new market mutation is observed: will China be the next engine of growth in the HO oilseeds & oils industry? Will China start locally producing HO oilseeds? This will be the pivot discussion during our 2022 con-gress.
The HOC congress is a fast growing event leaving with already 30+ different countries and 200+ guests atten-ding every year.
Compared to the previous years, we will reinforce its business focus, by facilitating the networking opportunities with larger breaks, and informing you about the last market updates and trends with lecture and panel opinion!
Do not miss this unique event dedicated to the High Oleic Oils market FAT & Associés team
All lectures will be in English.
The themes of the sessions will be the following:
Thursday, September 8th
- Session 1 - *Agricultural landscape & production analysis*
- Session 2 - *Changes in HO oils demand*

Friday, September 9th
- Session 3 - Innovation & investment potential

Learn more:
http://higholeicmarket.com/hoc-congress-2/

4th Edition of Euro-Global Conference on Food Science and Technology

12-13 September 2022 | Paris, France and Online (hybrid event)

After prodigious success of the previous editions of this annual conference series on food science and technology, Magnus Group is gratified to invite you to its prestigious hybrid event **"**4th Edition of Euro-Global Conference on Food Science and Technology**"** FAT 2022**.** The congress will be taking place in the month of September 12-13, 2022 at Paris, France and Virtually with the theme of **"***Harnessing the Latest Innovations and Laying Foreground for Future of Food Science and Technology*."
The aim of this colloquium is to highlight key research and applications, as well as emerging technology in all fields relating to food science and technology. It will feature oral and poster presentations, as well as debates and information exchange on a variety of Food Science and Technology related topics.
Though productivity gains and technological advancements have contributed to more effective usages of natural resources and enhanced food security, the sustainability of agricultural productivity and food security is threatened by climate change, the intensification of natural disasters, and an increase in the movement of pests and diseases across national borders. The food industry offers the greatest potential for research and de-

velopment. Sensor fusion, CPS design, HMI, robot learning and training software, vision systems, and robot structural re-configurability are all possibilities. Integrating multiple types of technological sectors is crucial for achieving competitive and creative solutions. Experts from academia and industry should join up to improve the food sector, which is long overdue.
The global summit is a two-day hybrid event that will host some of the most influential figures in the food and beverage sector including researchers, scientists, food technologists, nutritionists, botanists, healthcare professionals, policymakers, government representatives and industry key players to stimulate debate, foster collaborations, and expand knowledge base. FAT 2022 is composed of high achievers and professionals from the food, beverage, nutrition, and wellness industries. We attempt to provide a forum for networking and professional development among a select group of well-established individuals and businesses.
We hope this one-of-a-kind conference aims to transform the entire ecosystem by sparking new food industry talks.
Scientific sessions:
- Food Science and Technology
- Food Microbiology and Enzymology
- Bio Active Constituents of Food
- Food Toxicology
- Food, Nutrition and Health
- Current Trends in Food Technology
- Chemical Process: Biological and Non-Biological
- Advanced Research and Trends in Food Sciences
- Food - Quality Control and Quality Assurance
- Dairy Science & Technology
- Agronomy and Agricultural Research
- Food Substitution and Adulteration
- Food Legumes Research
- Food Chemistry and Biochemistry
- Food Nanotechnology
- Food Saftey and Standards

For updates:
https://food-chemistry-technology-conferences.magnusgroup.org/

Oils+fats Munich 2022

12-16 September 2022 | Munich, Germany

Oils+fats 2022 is regarded as one of the top international trade fairs for business, technology and innovation in the fat and oils industry in the world. With each edition, the event has developed into a top trend-and-order platform in the sector for concluding new contracts and establishing companies in the market. In 2022 the event will take place at Messe München from the 12th to the 16th of September and more than 50 exhibitors and many trade visitors will participate.
Oils+fats 2022 will cover a wide range of brand new developments. The exhibitors will present a vast amount of products in the following sectors:
- Economy
- Production technology
- Vegetable and animal oils and fats and others.

The expo will also be complemented by numerous networking workshops and special events. For example, the Food Safety Forum - a platform about food oil contaminants - will be the pivot point of the educational course of the trade show.
For more informations and updates:
https://www.oils-and-fats.com/en/

EPRW 2022 - 14th European Pesticide Residue Workshop pesticides in food and drink - in person

19-23 September 2022 | Bologna, Italy

On behalf of the Scientific and Organizing Committee, you are warmly invited to the 14th European Pesticide Residue Workshop, which will take place in person.
We are looking forward to meeting all of you again from the 19th to the 23rd of September 2022 at Palazzo dei Congressi, in Bologna, Italy.
The European Pesticide Residue Workshop (EPRW) – hosted every second year in a different European city – has become, after thirteen successful editions, the leading meeting for the presentation and discussion of latest concepts and developments in the field of pesticide residues in food and drink. The EPRW events put together more than 500 experts from all over the world, coming from govermental and private food control laboratories, public authorities, regulatory bodies, universities and research institutes, food producers and distributors, agrochemical manufactures and other interested parties. The EPRW is an excellent opportunity for international experts to connect and exchange information and experience in all the fields related to the evaluation and control of pesticides residues. It is the occasion for proficous interactions between the experts and all important vendors of analytical equipment and consumables that will present their latest equipment for pesticide residue analysis in a large exhibition area.
Main topics:
- Advanced analytical techniques and methods

- Qualitative/quantitative methods using High Resolution Mass Spectrometry
- Single residue methods
- Guidelines for analytical quality control and validation procedures
- Quality assurance and requirements for laboratory accreditation
- Toxicology and risk assessment
- Trends in pesticide registration and use
- Monitoring programmes
- Regulatory issues
- Green analytical chemistry
- Analysis of "new food"

Congress Information
The conference will be held in Bologna at the Palazzo dei Congressi, from Tuesday (morning) to Friday (afternoon). On Monday afternoon we will have a pre-workshop course and in the late afternoon the registration and the welcome reception for all the attendees at the EPRW 2022. As in the previous editions the scientific programme of EPRW 2022 will cover plenary lectures, oral presentations, poster sessions, roundtable discussions, young scientists oral presentations, vendor sessions, and poster awards. Likewise, during the workshop we will have a large exhibition area as an integral part of the meeting.
For information and updates, visit our website which will be implemented soon:
https://www.eprw2022.com
We are looking forward to welcoming all of you in Bologna, save the date!

Palmex Malaysia 2022

20-21 September 2022 | Kuala Lumpur, Malaysia

PALMEX Malaysia 2022, a specialized Palm Oil event in Asia that brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries to showcase the latest developments in the palm oil industry.
Currently ranked as the world's 2nd largest palm oil producer, this event will be supported by the local Malaysian Palm Oil Community ensuring major players in the industry would be represented at this event.

Top 3 objectives of exhibiting
- *Opportunities to meet new & existing clients*
- *Launching & demonstration on new products & service*
- *Gaining new contracts & obtaining new leads and more*

Who should Exhibit?
- Air Compressors
- Agriculture Equipment
- Argo Chemical
  - Fertilizer
  - Pesticide
  - Insecticide
- Boilers
- Biomass Processing Technologies
- Contract Manufacturing & Turnkey Projects
- Certification
- Design & Consultancy Services
- Electrical & Electronics Industries
- Manufacturing Waste and Water Management & Recycling
- Material Handling
- Material Testing & Inspection
- Metal Components & Products
- Oil & Fats Producers & Processors
- Laboratory Equipment
- Palm Oil Processing Plants
- Palm Oil Processing Equipment
- Palm Oil Refineries
- Processing & Packaging Machinery Suppliers
- Storage Companies Tools, Dies & Moulds
- Transportation & Heavy Equipment

For information email us at:
http://asiapalmoil.com/

Plant Protein Science and Technology Forum

4-6 October 2022 | Chicago, Illinois, USA - Online

We look forward to seeing you virtually at the third annual Plant Protein Science and Technology Forum! Join us fully online to discover the latest breakthroughs in plant protein science and technology. As an attendee, you will gain insights from thought leaders in nutrition, edible applications, processing and manufacturing of plant-based foods.

Why Attend?
This one-of-a-kind online conference brings together leaders from all components of the food system, from production and processing to distribution and consumption. As an attendee, you will be able to:
- Conveniently Connect virtually with colleagues from around the globe to explore the future of plant-based food systems.
- Learn the latest information on the analysis, nutrition and applications of plant proteins.
- Participate in online live, interactive sessions. The meeting is designed to provide a convenient, inclusive and safe experience to meet all attendees' needs.

- Make sure your voice is heard as we shape the future of the plant protein environment together.

Explore Technical Sessions

Immerse yourself in the latest research and innovations with a program. Watch more than 30 livestream invited and keynote presentations and interactive discussion panels featuring thought-leaders from around the world.

- Nutritional Functionality and Quality of Plant Based Proteins: Linking Form to Function
- New Frontiers on Extraction and Separation Technologies for Plant Proteins
- Overview of Structure-Function Relationships of Plant Proteins
- Global Challenges in Food Security and the Technological Innovations Needed to Overcome Them

Join us for the 2022 Sustainable Protein Forum Join us at the Millennium Knickerbocker Hotel, Chicago, USA, or participate fully online at the 2022 Sustainable Protein Forum, October 4-6, 2022. Live presentations, interactive discussion panels and networking events will provide the latest information from industrial, academic and government laboratories around the world.

Make your connection into the growing field of sustainable protein science and technology.

For update and information to the page:
https://plantprotein.aocs.org/

26th International Conference on Food Technology & Processing

5-6 October, 2022 | Zurich

Food Technology 2022 scientific committee feels esteemed delight to invite participants from around the world to join us at 26th International Conference on Food Technology and Processing schedule to be held on October 05-06, 2022 in Zurich, Switzerland. The Conference will primarily emphasize on the various topics related to Food Technology, Food Industry, Food Engineering etc. It is a worldwide dais that combines different spheres, stimulate the exchange of ideas and enable participants to grasp the latest developments and ideas in different areas of Food Technology and Processing. It will serve as a great platform to improve your knowledge and skills in this field through the various research experience and presentations. It also helps gaining a view about the career development and job search.

The Conference contains Keynote Forum, Oral and Poster presentations, Young Research Forum and Exhibitions. The ultimate aim is to gather prominent academic scientists, researchers, specialists, industrialists and research scholars to discussed and share their know-hows and research works on all aspects of Food Technology, Food Processing and Food Industry. Conference Series LLC Ltd organizes 3000+Global Events comprises of 600+ Conferences, 1200+ Workshops and 1200+ Symposiums every year in USA, Europe & Asia with support from 1000 more scientific societies.

Target Audience:

- Food technologist
- Microbiologist
- Food safety officers
- Nutritionists
- Dietician
- Quality control officers
- Quality assurance officers
- Scientists
- Researchers
- Biotechnologists
- Industrialists
- Food Engineers

Why to attend?

With members from around the world focused on learning about Food Technology and its advancement so this is your best opportunity to reach the largest assembly of participants of the Food Technology and Processing community. This conference seek to bring all such scientists, Noble Laureates, researchers, research scholars, students and people together who are involved in Food Technology and Processing ground and provide them to discuss about their unique innovation, sharing ideas and interaction with each other. World-renowned speakers, the most recent development and advancement in the field of food technology are the limelight of the conference.

https://foodtechnology.insightconferences.com/

FCT 2022

About Food Chemistry & Technology

12-14 October 2022 | Rome

It is our great pleasure to invite you to participate in the 8th International Conference on Food Chemistry & Technology, that will be held on October 12-14, 2022, in the amazing city of Rome, Italy. This eighth conference in the series comes after the prodigious success of the previous meetings, held in San Francisco in 2015, Las Vegas in 2016, Baltimore in 2017, Berlin in 2018, Los Angeles in 2019, Virtual in 2020 & 2021. All editions were a great success. We are, therefore, committed to match these standards and are preparing what we are sure will be a unique event.

FCT-2022 provides a unique opportunity to network with your peers and it consists of highly proficient series of talks, poster presentations, workshops, discussions, and networking events that

will keep our fellow participants engaged in learning and making new connections.
This 3-day conference features the world-class renowned food scientists and experts, and we will bring together experts, young researchers, education scientists, technologists, and food industry representatives to debate on the latest scientific developments in the field of food chemistry and technology that helps to improve the current and future challenges in food research.
FCT-2022 is a part of successful series of the conference started from FCT-2015, San Francisco, and held annually since then. This is going to be a global event with participants from more than 40 countries and ample opportunities for networking and learning from Senior Investigators, Scientists, and Experts from elite organizations & institutes that work on food chemistry.
Make sure to block October 12-14, 2022 on your calendar, to join world-renowned researchers, scientists, policy makers, professionals, and students from multidisciplinary food-related fields to share the ongoing research and create new partnerships & collaborations.

About Organizer

United Scientific Group (USG), a non-profit organization, an expert-driven initiative led by the editor's association and the advisory board which includes academicians, researchers, and industry leaders across various fields of research. USG provides broad range of services in the fields of science and technology including publishing, conducting world class scientific events, and holding highly interactive and proficient world forums. For more information, please visit: https://unitedscientificgroup.org/
We look forward to welcoming you all at FCT-2022.

Scientific Sessions

Disruptive technology intervention
- Cold-Plasma
- Ultrasounds
- Food digital twins
- Reversed engineering
- Cooking precision

3D food printing
- Printing meat products
- Digital design, full control printing movements
- Printability of food formula

Food sensory evaluation and novel experience for a better health and sustainability
- Tools for sensory evaluation
- Mastication work and sensory experience
- Food design and inhomogeneous food
- Stratification of tastants and modulated intake
- Improved sensory acceptance for reducing food waste
- Multi-sensory experiences
- Augmented food perceptions

Food customization and health
- Hydrocolloids and biofunctionalities
- Probiotic and prebitiocs
- Double emulsions and nutrients bioavailability
- Bioaccessability
- Nutrient security
- Food Personalized Manufacturing
- Delivery functionality

Sustainability in food production and technology
- Alternatives source of proteins from vegetable/marine environment
- On-demand food manufacturing
- Extraction and valorization of the sidestream
- Food packaging materials
- Prolonging and modeling shelf life

Food chemistry
- Food chemistry to improve nutrition and health
- Food chemistry and functionalities
- Novel sources of nutrients and bioactives
- Effect of nutritional, technological, and sensory properties
- Chemical analyses for food quality and functionality

Physical properties in food
- Food texture
- Mechanical properties, inhomogeneues food and novel sensory experiences
- Food microstructure
- Bioaccessability

Traditional process for food scale up and adaptation in other localities
- Traditional fermented food
- Local food products and food security
- Fermentation as bio-preservation methods
- Traditional food process
- Food technology in developing countries

Open science culture, data mining and visualization in food science
- The impact of open data
- Tools for data sharing and collaborations
- Alternative tools and methods for data visualization
- Open Science
- Machine learning
- Computational tools

Consumer's trust and awareness in food science and technology
- Social acceptance of radical innovation in the food sector
- Consumer-centric approach for practical innovation
- Communication and better awareness of the food science and technology
- The above topics may include, but are not limited.

For upadates:
https://foodchemconference.com/about-fct-2022

Palmex Indonesia 2022

25-27 October 2022 | Medan, Indonesia

The 12th PALMEX Indonesia 2022 is the only specialized Palm Oil event in Asia that brings together an international congregation of both upstream and downstream palm oil companies and also its supporting industries gathered in the capital city of North Sumatera, Medan to showcase the latest developments in the palm oil industry. North Sumatera, home to one of Indonesia's largest concentration of oil palm plantations and also the presence of many supporting facilities such as palm oil processing plants making its capital Medan the perfect venue for the show. This unique event seeks to educate the public on the importance of the palm oil industry in Indonesia and the future trends of palm oil in the region. More than 7,000 industry professionals from more than 10 countries would be expected to turn up at this event. The international character and regional audience of PALMEX Indonesia provides unparalleled marketing, education and networking opportunities.

Learn more:
http://palmoilexpo.com/

6th International Conference on Food Chemistry, Nutrition and Safety 2022

10-11 November 2022 | Vancouver Canada

We are delighted to invite you to attend the upcoming 6th International Conference on Food Chemistry, Nutrition and Safety which is going to be held during November 10-11, 2022 in Vancouver, Canada.

For more details please visit:
https://foodchemistry.conferenceseries.com/

15th International Conference & Expo on Chromatography Techniques

January 30-31, 2023 | Barcelona, Spain

Advanced Chromatography 2023 organizing committee invites analytical expertise, researchers, professors, scientifica communities, delegates, students, business professionals and executives to attend the *"15th International Conference & Expo on Chromatography Techniques"* which is to be held during January 30-31, 2023

In the light of this theme, the conference series aims to provide a forum for international researchers from various areas of analytical chemistry, pharmacy, pharmacology, bioinformatics and other life science groups by providing a platform for critical analysis of new data, and to share latest cutting-edge research findings and results about all aspects regarding advances in HPLC and Chromatography techniques.

The conference provides a platform to detail the research works of analytical expertise from various scientific backgrounds and the same can be perceived by young researchers and students. The conference mainly aims to promulgate knowledge on chromatography and unveil the advances in HPLC techniques.

Both Life Science and Chemical Sciences need analytical techniques in course of research work and therefore Advanced Chromatography 2022 would be a perfect venue to share and develop knowledge on key analytical tools.

Target Audience:
- Analytical experts in chromatography
- Research Heads from Pharmacy and Chemical Industries
- Industrial expertise working with various novel solid & liquid columns
- Marketing teams of Industries with novel products to show case at the conference
- Directors and Professors from Universities and Institutions
- Post-doctoral & PhD student working on analytical & Bio-analytical method development
- Theoretical scientists working on deriving analytical hypotheses
- Relevant Graduate and Post graduate students

Sessions/Tracks:
- Major Chromatographic Techniques
- Advances in Chromatography-HPLC Instrumentations
- Chromatography & Mass Spectrometry
- Chromatography in Pharmacy & Pharmaceutical
- Chromatography in Food Science Technology
- Biochemical Applications of Chromatografy-HPLC
- HPLC Fingerprinting in Bioinformatics & Computational Biology
- Analytical & Bio-analytical Applications of Chromatography
- Chromatography-HPLC in Bio-Medical Research
- Hyphenated Chromatography Methods
- Chromatography-HPLC as Separation Techniques
- High Efficiency & High Resolution Techniques
- Method Development & Validation
- Recent Advances in Chromatography-HPLC
- Chip Based Chromatography Separations
- Market Growth of Chromatography- HPLC

For more details please visit:
https://chromatography.pharmaceuticalconferences.com/

12th CESIO World Surfactant Congress

5-7 June 2023 | Rome, Italy

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

The conference has three clusters with the following topics:

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

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

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

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

16th International Rapeseed Congress

24-27 September 2023 | Sydney, Australia

The GCIRC Executive board and General Assembly approved the proposition to organize the next 16th International Rapeseed Congress, in 2023, in Sydney. CMS Australasia is the Organiser who will managing the 16th International Rapeseed Congress 2023. Save the date!
All information to the page:
https://www.irc2023sydney.com/

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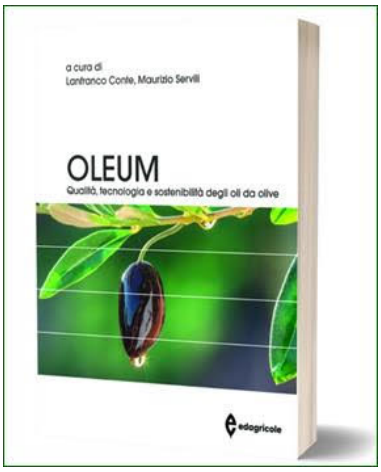

OLEUM

QUALITÀ, TECNOLOGIA E SOSTENIBILITÀ DEGLI OLI DA OLIVE

A CURA DI:
LANFRANCO CONTE, MAURIZIO SERVILI

Il volume copre l'intera filiera olivicolo-olearia: dalle caratteristiche compositive alla qualità dell'olio vista in relazione alle variabili agronomiche e tecnologiche di produzione, dalle nuove tecnologie volte alla valorizzazione dei co-prodotti ottenuti dal processo estrattivo, in un'ottica di economia circolare, fino agli aspetti normativi aggiornati relativi alla commercializzazione del prodotto.

Nel testo vengono messe in luce le più moderne metodiche analitiche sia per indagare la caratterizzazione e l'origine geografica degli oli sia per approfondire gli aspetti relativi alle fonti di contaminazione e di sicurezza alimentare.

Largo spazio trovano inoltre le innovazioni di processo nel settore dell'estrazione meccanica degli oli vergini di oliva: una vera e propria "rivoluzione tecnologica" volta al miglioramento della qualità del prodotto e dell'efficienza estrattiva.

Un capitolo è completamente dedicato al rapporto tra consumo di olio d'oliva, principale fonte di grassi della Dieta mediterranea, e salute, relativamente ai principali gruppi di malattie cronico-degenerative.

Indice: Composizione chimica degli oli vergini di oliva rispetto alle altre fonti vegetali - L'impianto

per l'estrazione dell'olio vergine - Raffinazione dell'olio d'oliva vergine e lampante ed estrazione e raffinazione dell'olio di sansa - Estrazione, conservazione, packaging e qualità - Uso e valorizzazione dei sottoprodotti dell'estrazione meccanica - Nuovi approcci alla valutazione di qualità e autenticità - Metodi analitici per la determinazione della origine geografica - Ossidazione e stabilità dell'olio - Contaminanti e residui di trattamenti - Olio d'oliva e salute - La normativa sugli oli d'oliva e di sansa di oliva.

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Lanfranco Conte

INNOVHUB
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innovazione e ricerca

Olive oil proficiency tests

Chemical-physical parameters and contaminants

Since 2003, the Oils and Fats Area, organizes every year an interlaboratory test on olive oil for different commercial categories among various olive oil laboratories.

The tests include all the chemical parameters.
Since 2016 the main contaminants are also considered.

Each participant will have the opportunity to compare his own test results with those obtained by the most accredited Italian and foreign laboratories.

The proficiency test has as main purpose, the ability to make corrections from deviation that might occur in the results, compared to the average value obtained by other laboratories.

At the end of the laboratory tests, the participants insert the results obtained directly in the web portal on the dedicated page: https://proficiencytest.innovhub-ssi.it

The results will be statistically processed and delivered anonymously to each participant.

For information:
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www.innovhub-ssi.it

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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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