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Biodiesel production from waste cooking oil

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Abstract: Biodiesel production from waste cooking oil was obtained using *Thermomyces lanuginosus* (TL) lipase (E.C.3.1.1.3) anchored on Fe_3O_4/Au nanoparticles through physical interactions. A remarkable biodiesel yield of ~90% was obtained without any pre-treatment and at a lipase concentration of 20%, 45°C reaction temperature, 1:6 oil/methanol molar ratio, after 24 h. The immobilized enzyme showed fast kinetic (the biodiesel yield was already of 34.6% after only 3 h) and activity slightly dependent on the length of the acid chains. The effect of the Au NPs sizes was monitored, to study the role of Au conduction centres in facilitating enzymes favourable orientation. The immobilized lipase activity stays above 74% after the first 3 cycles of use. In particular, the produced biodiesel presents an ester content of 97.8% ± 0.21 and a linolenic methyl ester content of 0.53% ± 0.03, in agreement with EN14214 requirements.

Keywords: biodiesel production; waste cooking oil; enzyme activity and stability; effect of cooking; compliance with European Standard

1 Introduction

In the last decades, energy consumption has increased significantly because of the change in lifestyles and growth of population. The increase in the energy demand was typically provided by fossil fuel resources, which, otherwise, are limited and cause of serious environmental concerns. Therefore, there is an urgent need for alternative and renewable fuel, such as biodiesel. Biodiesel is a renewable, clean and environmentally friendly fuel derived from vegetable oils and animal fats [1]. However, it has been reported that the cost of the raw materials is about 80% of the total biodiesel production cost [2]. In this context, the cheaper waste cooking oils (WCOs) are potential substitutes for vegetable oils in the production of biodiesel. The use of WCOs, as a secondary raw material, provides the additional advantage to reduce the disposal concerns [3]. The amount of cooking oil produced every year is immense, over 15 million of tons, which, if converted, can satisfy to a large extent the world demand of biodiesel [4]. The production of biodiesel from WCOs allows for a 21% in crude oil saving and 96% in fossil energy saving [5]. Each kilogram of WCOs can be converted into biodiesel with very high yields.

On the other hand, the use of WCOs is challenging, as they basically contain free fatty acid (FFA) and water which make them unsuitable for homogeneous alkaline transesterification catalysis. The typical alkaline catalysts, such as sodium hydroxide (NaOH) and potassium methoxide ($KOCH_3$) [6], incur in saponification reaction in the presence of water [7-9]. More in general, conventional chemical processes, either from acid or base catalysts, suffer for difficulties in glycerol recovering and removal of inorganic salts, high temperatures, undesirable side reactions [10], and require the use of pretreatments.

Enzymes catalyzed processes have great potential compared to chemical methods [11,12]: intrinsic low environmental impact, no need of pretreatments to remove water, FFAs, etc., high efficiency and conversion of free fatty acids, lower energy consumption, mild conditions during the reactions, product purity and facilitated separation of glycerol are the main advantages of the enzymatic processes [13]. The process of transesterification is affected by different variables. The enzymatic production of biodiesel can be influenced by many aspects such as activity, stability and specificity of the enzyme, alcohol-oil ratio, temperature and water content [11,14,15]. On the other hand, the enzymes can give high biodiesel yields and can exhibit reaction times comparable to those of base catalysts, requiring, in general, higher amounts of catalyst but lower of alcohol [16]. Three major drawbacks still persist the enzyme availability at commercial scale; the enzyme costs; and, when they are surpassed, i.e., in the case of enzyme immobilization, the difficulties to reproduce laboratory experiments in large (i.e. design and up-scaling of the reactor).

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Immobilization permits to increase the enzyme time of usage [17,18]. For example, immobilized *Thermomyces lanuginosus* (TL) lipase on hydrotalcite was found able to catalyze transesterification of WCOs [17]. In particular, the authors observed, after seven cycles, activity retention of 36% at 45°C and of 14% at 55°C. Although, in the paper of Wang et al. [18], MAS1, a laboratory prepared enzyme, showed higher stability and activity, immobilized *Thermomyces lanuginosus* (TL) lipase on a XAD1180 resin provided similar results to those observed by Yagiz et al. [17]. More recently, magnetic nanomaterials offer in this context a new opportunity [13,19-21], i.e., higher activities of immobilized enzymes, providing: a high surface area support to anchor high payload of enzymes; a facile separation between the product and the catalyst; reduced diffusion limitation, i.e. easy transition from laboratory to industrial scale and reactor scale-up [14,18,22]. Moreover, physical absorption allows support reusability, whereas maintaining activity [23]. Activity retention, after 5 cycles, of about 58 %, and biodiesel yield of about 20%, after 3 h, were obtained in [19], through the use of *Pseudomonas cepacia*, immobilized on Fe_3O_4 NPs. Similar results were shown by *Km 12* lipase and *Burkholderia sp.* enzyme immobilized on Fe_3O_4 NPs [20,21]. Lipase from *Candida antarctica*, covalently immobilized on magnetic NPs, in the presence of molecular sieves, achieves an activity of 100%, after 96 h [13]. On the other hand, although support plays a key role, it has been little or no studied. Typically, after support choice, the papers focus on the evaluation of the operation parameters effects or enzymes choice.

Here we report, the production of biodiesel from WCOs, using a commercial *Thermomyces lanuginosus* (TL) lipase directly linked on citric acid and residual oleic acid modified Fe_3O_4/Au nanoparticles [24], consisting of magnetite NPs supporting smaller Au NPs, through physical adsorption including interfacial activation [23].

Our nanocatalyst, tested on non-pretreated WCOs [25] and without other additives, due to support specificity and favourable interaction with the support, shows a very remarkable combination of properties: excellent activity, also in the presence of high amount of water; stability; recyclability, also of the support because of the physical interaction. It ensures an almost homogeneous catalysis of waste oil. Furthermore, considering the key role of the support, particular attention was devoted to elucidate the role of Au conduction centres in facilitating transfer of electrons and enzymes favourable orientation, through the evaluation of the Au NPs sizes effect.

Moreover, an accurate analysis, which is rarely shown, of the different phases and of the catalyst activity, and a characterization according to EN14214 were performed and reported.

2 Feedstock and experimental

2.1 Materials

All chemicals were analytical grade and acquired from Aldrich Chemical Co.

WCOs were obtained from olive oil (Sigma Aldrich-O1514, analytical grade), after a simulated cooking (temperature of 180°C for 5 h) [25].

2.2 Physicochemical characterization of WCOs

Prior to the biodiesel production, the properties of the WCOs were determined, including: (i) saponification value, (ii) water content, (iii) iodine value and (iv) acid value. The oil properties were analysed in accordance with EN14214. All experiments were run in triplicate and mean values were reported. The psychical properties of WCOs used were collected in Table 1.

2.3 Synthesis of Fe_3O_4/Au NPs, ligand exchange to obtain hydrophilic NPs, lipase immobilization

The Fe_3O_4/Au@OA nanoparticles were prepared as previously reported [24]. In particular, two diefferent samples were prepared increasing the amount of Au NPs precursor ($HAuCl_4$) from 42 mg to 62 mg.

For the immobilization, optimized conditions [24] were chosen. In particular, modified NPs were mixed [24] with 10 mL of buffer solution (phosphate buffer 0.1 M, to give at pH = 3.0) with 2 mg of TL (solution ≥ 100,000 U/g from Sigma Aldrich) and shaken at 200 rpm, T = 4°C, for 180 min, obtaining TL immobilized lipase, named NPs@TL in the following. pH 3, lower than isoelectric point of

Table 1: Physicochemical properties of WCO. GC-MS configuration. Each value represents the mean of three replicates ± SD.

Physicochemical properties		
Property	**Value**	**Unit**
Acid value	1.85 ± 0.08	(mg KOH/g)
Free fatty acid content	0.93 ± 0.06	(%)
Moisture	0.08 ± 0.003	(%)
Saponification Index	182.32 ± 0.55	(mg KOH/g)
Iodine value	71.67	(g I_2/100g oil)

lipase, was chosen for immobilization, resulting in a more stable enzyme.

2.4 Synthesis of methyl ester

A vessel reactor (25 mL in volume) continuously stirred (200 rpm speed) was used for the methyl ester productions at temperatures of 45°C and 55°C. Three different experiments were performed starting from 1 g of WCOs, in the presence of free or immobilized lipase (5%, 10%, 20% *g of enzyme/g of WCO*), oil/methanol ratio 1:6. Furthermore, three additional experiments were performed in presence of 10% of enzyme at different oil/methanol ratio, obtained following the procedure described in [24] to give 1:3, 1:6 and 1:18 molar ratio. At an oil/methanol ratio of 6:1 two new experiments were performed in presence of water 10 wt% and 15 wt%. The effect of the cycles number was finally examined at 10% of enzyme (grams of enzyme per grams of WCO) also in presence of water 15 wt%.

Immobilized enzyme was recovered at the end of the transesterification processes under a magnetic field. After few minutes of centrifugation the upper layer of the sample was washed with hot water at a temperature of 60°C and finally dried with anhydrous sodium sulphate to obtain biodiesel.

The oil conversion to methyl ester, i.e. biodiesel yield, was determined as in the following:

$$yield\ (\%) = \frac{m_{ester}}{m_{oil}}\ \% \tag{1}$$

The analysis of the fatty acids methyl esters (FAME) produced was carried out by a GC-MS (Thermo Fischer Scientific) equipped with a TraceGOLD™ TG-Polar capillary column (0.25 µm × 0.25 mm × 60 m). GC-MS configuration: initial temperature 120°C for 4 min, rate 1, 6.5°C/min to 170°C, rate 2, 2.75°C/min to 250°C for 9 min. Injector and detector temperatures were set 250°C and 230°C, respectively. Helium was used as the carrier gas. Methanol:BF3 method [26] was used for WCOs derivatization the to obtain composition and times of retention of FAMEs, that were compared with a known concentration FAME mixture and the biodiesel produced. It has been observed that the retention times of the produced biodiesel were almost similar.

EN14214 was used for the methyl ester content evaluation in the produced biodiesel [24]. Measurements were performed in triplicate.

3 Results and discussion

The effect of the oil/methanol molar ratio on the biodiesel yield, obtained by using immobilized lipase at 45°C, and after 24 h of reaction, is shown in Figure 1. Biodiesel yield at an oil methanol ratio of 1:3 results equal to 81.8%. It increases, up to a maximum of 84.5%, for an oil methanol ratio of 1:6. However, further increase in oil/methanol molar ratio results in a lower biodiesel yield, likely due to the inactivation of the lipase exposed to higher concentrations of methanol [22]. Therefore, although the difference in the biodiesel yield obtained at 1:3 and 1:6 oil/methanol molar ratio are limited, a molar ratio of 1:6 was chosen for the further experiments.

Figure 2 shows the effect of enzyme loading on biodiesel yield. Biodiesel yield, after 24 h, increased from 65.42% to 89.53% when enzyme loading ranged from 5 to 20 *(g of enzyme/g of WCO)*. Moreover, after 3 h and 6 h, at 20% enzyme loading, the biodiesel yields was already 34.6% and 70.1%, indicating a fast kinetics for our immobilized TL. Although this is due to the enzyme specificity, the comparison with other literature results [17,18], obtained in similar conditions, suggests a key role of the enzyme interaction with the support, while the quasi-homogeneous catalysis cannot be neglected as well. Enzyme activity is typically slightly affected by water contents. The activity of the immobilized lipase, after 24 h at a methanol/oil molar ratio of 6:1 and at a temperature of 45°C, was also evaluated mixing additional water in the reaction medium. A minor decrease of the enzyme activity was observed, the

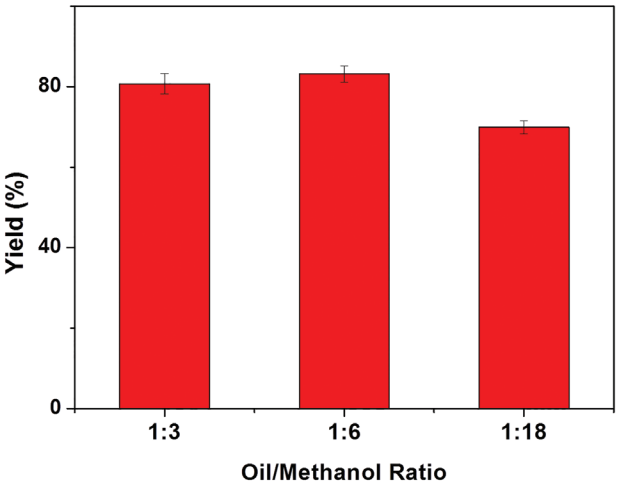


Figure 1: Effect of methanol to oil molar ratio on the free and immobilized lipase for biodiesel production. Immobilization conditions – coupling temperature: 4°C; coupling pH: 3; coupling time: 3 h; lipase amount: 2 mg. Reaction conditions – reaction time: 24 h; reaction temperature: 45°C; lipase concentration: 10%.

biodiesel yield reached value up to 76% and 72% at a water content of 10 wt% and 15 wt%, respectively. Moreover, the activity of the immobilized TL, after 24 h methanol/oil = 6:1 M and T = 55°C, results equal to 67%, in agreement with previous observation [23,24], which indicates improved stability of the immobilized enzyme with temperature.

The reusability of immobilized lipase was shown in Figure 3. Relative activity of the immobilized lipase, that is the activity of the immobilized enzyme respect to its best result, after the third reaction was 77.51%. After five cycles, the relative activity in biodiesel production still is higher than 55% and 32% in presence of 15% of water, showing an excellent reusability in the experimental conditions chosen, likely due to the enzyme interaction with the support inducing stability.

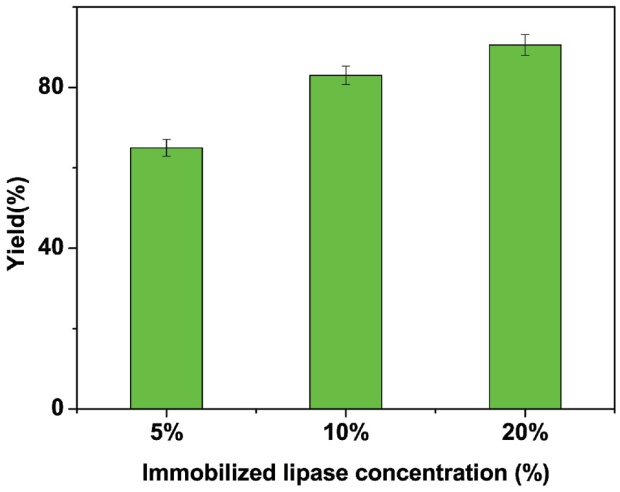


Figure 2: Effect of amount of immobilized lipase used for biodiesel production. Immobilization conditions – coupling temperature: 4°C; coupling pH: 3; coupling time: 3 h; lipase amount: 2 mg. Reaction conditions – reaction time: 24 h; reaction temperature: 45°C; methanol/oil ratio: 6:1 M.

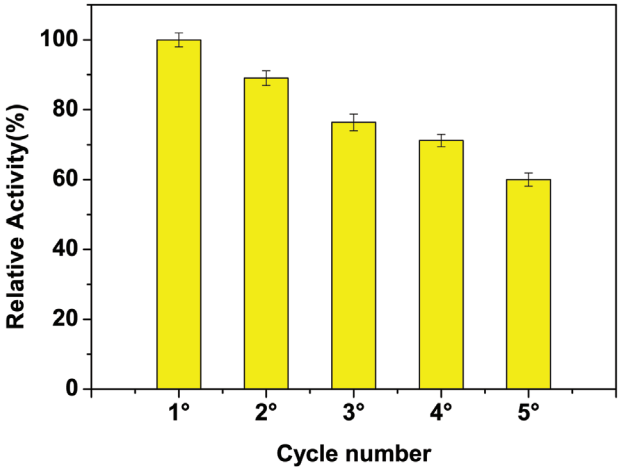


Figure 3: Effect of cycle number on biodiesel production for immobilized lipase. Methanol/oil ratio 6:1 M. Immobilization conditions – coupling temperature: 4°C; coupling pH: 3; coupling time: 3 h; lipase amount: 2 mg. Reaction conditions – reaction time: 24 h; reaction temperature: 45°C; lipase concentration: 10%.

As far as the biodiesel production by WCOs is concerned, in Table 2 the main relevant results obtained in the last years are summarized. It is evident that immobilization permits to increase the enzyme time of usage [17,18]. Moreover, nanomaterials offer in this context a new opportunity to obtain higher activities for immobilized enzymes [13,19-21]. On the other hand, the very remarkable combination of properties showed by our nanocatalyst can be ascribed to a favourable enzyme orientation on the support, and support surface functionality. In particular, the enzyme is anchored thanks to the interaction with citric acid, while the presence of residual oleic acid, which exposes its a-polar tail, not only favours interfacial activation, but also probably protects the enzyme from moisture. Moreover, the heterojunction between magnetite and gold, which have different work functions [27,28], inducing a Fe_3O_4 surface polarity modification determines an enhanced affinity with citric acid, favouring an increased stability, e.g. strong ionic interaction [29]. To better elucidate the role of Au conduction centres in facilitating electrons transfer [24], the effect of the Au NPs sizes was also monitored. In Table 3, the results obtained by using nanoparticles with different Au NPs sizes were reported. The comparison highlights the role of Au, helping enzymes to assume a favourable orientation and thus an increased enzyme loading and activity.

The efficiency of the biodiesel syntheses was checked by employing GC-MS analyses of derivatized olive oil, derivatized waste cooking oil and biodiesel. The spectra were reported in Figures S1, S2 and 4, respectively.

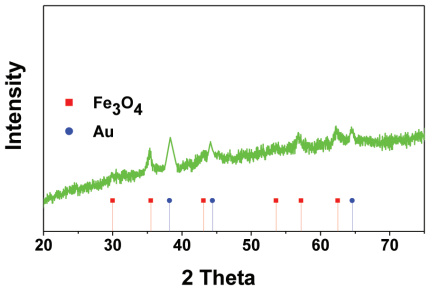
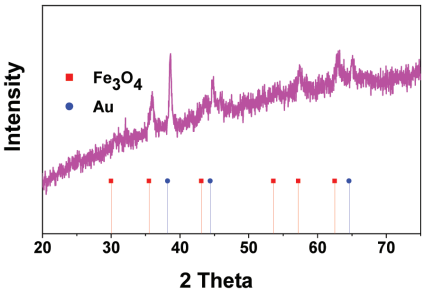
Fatty acid methyl esters (FAMEs) composition of the biodiesel was identified by comparing with the retention times of a pure FAME standard (C14-C22 standard, Sigma Aldrich) and of derivatized WCOs. The olive oil before cooking simulation consisted of 13 fatty acids, in particular: tetradecanoic acid, palmitic acid, palmitoleate acid, heptadecanoic acid, cis-10-heptadecanoic acid, stearic acid, oleic acid, linoleic acid, eicosanoic acid, linolenic acid, cis-11-eicosanoic acid, behinic acid, and lignoceric acid. The relative composition is reported in Table 4 Column 4°. After cooking simulation (see Table 4 column 5°), new compounds, originated by the cooking process (caprylic acid, octadecanoic acid

Table 2: Comparison of the catalytic abilities of different enzymes for biodiesel production from WCOs.

Support	Immobilization	Lipase	Activity maintenance (%)		Biodiesel conversion (%)		Maximum biodiesel conversion		Enzyme/oil	MeOH/oil	Ref.
					at 3 h	at 6 h	(%)	(h)			
Hydrocalc.	Physical ads.	*Thermomyces lanuginosus*	~72 after 3 cycles	36 after 7 cycles	< 10[c]	< 10[c]	92.8	105	0.04 g/g	4:1	[17]
XAD1180 resin	-	*Thermomyces lanuginosus*	-	-	~5	~7	~12	12	80 U/g	3:1	[18]
XAD1180 resin	-	*S. sp. strain W007(MAS 1)*	~82 after 3 cycles	~66 after 4 cycles	~42	~71	~90	12	80 U/g	3:1	[18]
Fe_3O_4 NPs	-	*Pseudomonas cepacia*	~80 after 3 cycles	58 after 5 cycles	< 20	< 20	80	72	0.4 g/g	6.6:1	[19]
Fe_3O_4 NPs	Covalent bond	*Km 12 lipase*	~100 after 3 cycles	80 after 9 cycles	-	~18	71	36	0.03 g/g	3:1	[20]
Amino-Fe_3O_4-SiO_2 NPs	Physical ads.	*Burkholderia sp.*	~75 after 3 cycles	54 after 5 cycles	< 20[$]	< 20[$]	91[$]	35	0.25 g/g	6:1	[21]
Fe_3O_4-silica NPs[§]	GPTMS-covalent bond	*Candida Antarc. B*	~100[§] after 3 cycles	~62[§] after 10 cycles	~3[§]	~10[§]	100[§]	96	0.045 g/g	9:1	[13]
Fe_3O_4/Au	Physical interaction	*Thermomyces lanuginosus*	~75 after 3 cycles	~61 after 5 cycles	34.6	70.1	~90	24	0.2 g/g	6:1	*This paper*
Fe_3O_4/Au	Physical interaction	*Thermomyces lanuginosus*	~77 after 3 cycles	~60 after 5 cycles	-	-	~80	24	0.1 g/g	6:1	*This paper*

[§] In presence of 70% molecular sieve; [c] 10 % yield at 24 h; [$] with n-hexane content of 10 wt%.

Table 3: Effect on size of Au on Fe_3O_4 for enzyme immobilization and biodiesel production.

Au NPs (nm)	< 2	2÷4
XRD spectra		
Immobilization efficiency (%)	~85	~90
Biodiesel yield (%)°	~90	~98.5
Re-use*	~61	~66

° Biodiesel reaction conditions: time, 24 h; temperature, 45°C; methanol/oil ratio, 6:1 M; Lipase concentration, 20%. * Activity maintenance after 5 cycles.

9.10 epoxy, stearic acid allyl, 14-methylhexadecanoic acid, octadecanoic acid 15.16 epoxy) were detected. In particular, the palmitic acid increase and the appearance of 14-methylhexadecanoic acid account for the palmitoleate acid decrease. Methylation of stearic acid probably leads to the formation of stearic acid allyl. The appearance of caprylic acid is most likely due to oxidation occurring on the methyl in α position to the double bond [30] in linoleic acid [31], through the formation of a peroxide [32] and decomposition in short-chain products. Octadecanoic acid

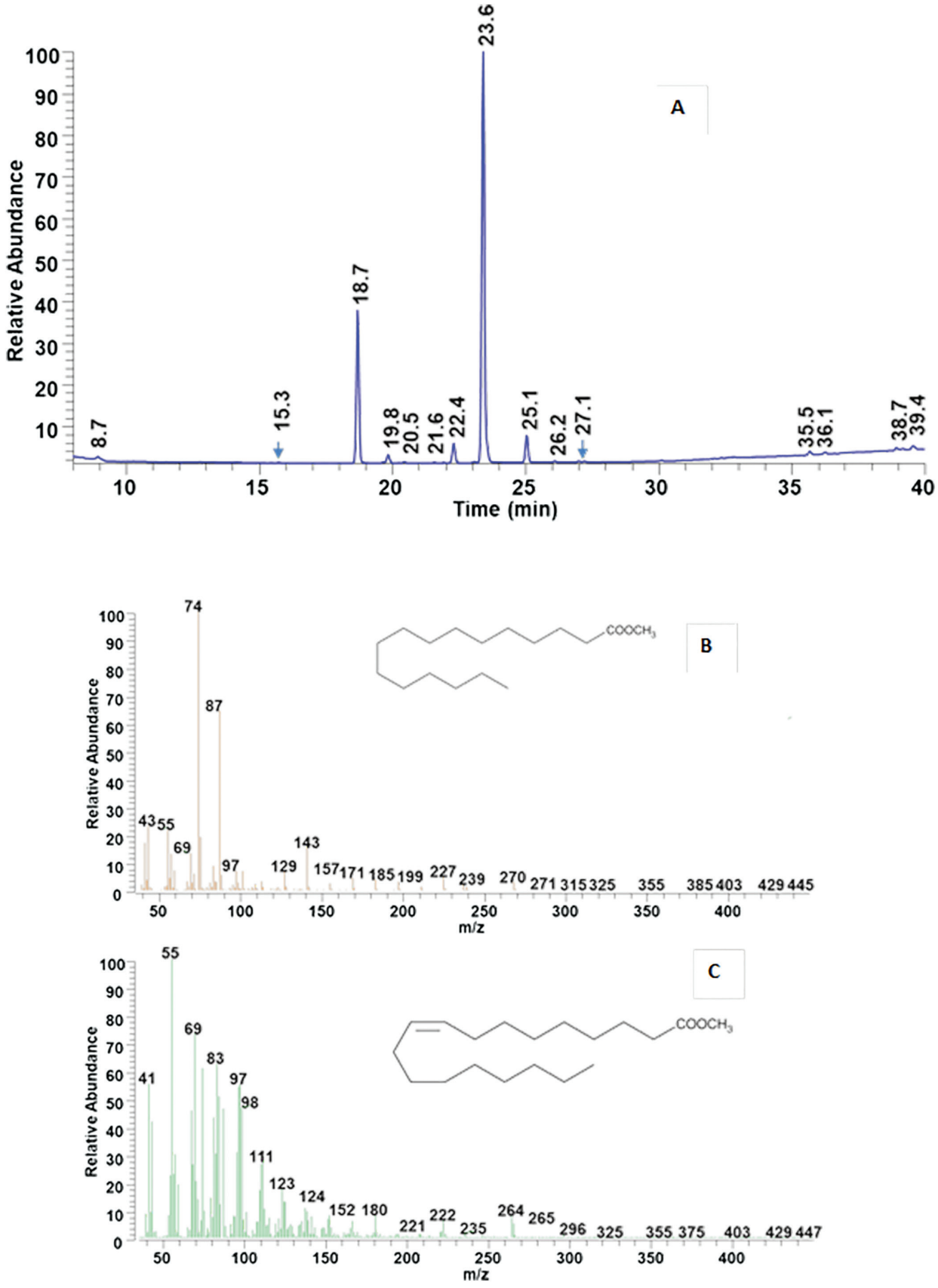


Figure 4: GC spectrum of biodiesel (a). Mass spectra of palmitic acid, methyl ester (b) and oleic acid, methyl ester (c).

Table 4: Retention times (RT) and area (%) of each fatty acid of olive oil from Sigma Aldrichof waste cooking oil, of biodiesel with of 20% of immobilized lipase concentration.

Fatty Acid		Time (min)	Area (%) of olive oil from Sigma Aldrich	Area (%) of waste cooking oil	Area (%) of biodiesel with of 20% of immobilized lipase concentration
Caprylic Acid	C8:0	8.7	-	0.20 ± 0.1	0.30 ± 0.1
Tetradecanoic Acid	C14:0	15.3	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02
Palmitic Acid	C16:0	18.7	16.60 ± 0.06	18.76 ± 0.07	22.43 ± 0.08
Palmitoleate Acid	C16:1	19.8	2.47 ± 0.03	1.60 ± 0.03	1.79 ± 0.03
Heptadecanoic Acid	C17:0	20.5	0.24 ± 0.02	0.14 ± 0.02	0.16 ± 0.04
Cis-10-Heptadecanoic Acid	C17:1	21.6	0.31 ± 0.02	0.19 ± 0.02	0.25 ± 0.03
Stearic Acid	C18:0	22.4	4.85 ± 0.2	4.39 ± 0.3	3.11 ± 0.4
Oleic Acid	C18:1	23.6	63.60 ± 0.2	63.32 ± 0.2	64.31 ± 0.2
Linoleic Acid	C18:2	25.1	9.60 ± 0.2	7.21 ± 0.1	4.19 ± 0.05
Eicosanoic Acid	C20:0	26.2	0.63 ± 0.05	0.55 ± 0.06	0.20 ± 0.08
Linolenic Acid	C18:3	27.1	0.91 ± 0.05	0.54 ± 0.03	0.37 ± 0.03
Cis-11-Eicosanoic Acid	C20:1	27.3	0.50 ± 0.02	0.59 ± 0.02	-
Behinic Acid	C22:0	30.0	0.18 ± 0.03	0.27 ± 0.03	-
Lignoceric Acid	C24:0	33.7	0.08 ± 0.01	0.06 ± 0.01	-
Octadecanoic Acid 9.10 Epoxy		35.5	-	0.59 ± 0.02	0.81 ± 0.03
Stearic Acid Allyl	C18:1	36.1	-	0.47 ± 0.04	0.63 ± 0.04
14-Methylhexadecanoic acid	C16:0	38.7	-	0.47 ± 0.05	0.65 ± 0.05
Octadecanoic Acid 15.16 Epoxy		39.4	-	0.62 ± 0.02	0.77 ± 0.02

9.10 epoxy and octadecanoic acid 15.16 epoxy likely come from linoleic and linolenic acid epoxydation occurring during cooking.

The comparison between the GC-MS of derivatized WCOs and biodiesel (see Table 4 Column 6°) evidences the ability of the nanocatalyst to convert the oil fatty acids into methyl esters. On the other hand, as the length of fatty acid chains increases, a reduction of the catalytic activity was observed. The amounts of octadecanoic acid 9.10 epoxy and octadecanoic acid 15.16 epoxy increases only apparently, as they now weigh on an esterified smaller fraction of the starting oil. In conclusion, the difference between the calculated biodiesel yields and 100%, after 24 h, is due to the presence of unconverted acids, which amount is more pronounced the longer the chains to be converted are.

Biodiesel from waste cooking oil, obtained through the use of our immobilized lipase, after 24 h of synthesis, presents a linolenic methyl ester amount equal to 0.54% ± 0.03, which is in agreement with the EN14214. Ester content, calculated according with the modified method of EN14214, equal to 97.8 ± 0.21, in agreement with the EN14214. The iodine value, calculated according with EN 14214 Annex B, results equal to 66.75 (g iodine/100 g) in agreement with the European standard. The validation through the standard, that is typically a key aspect, is more necessary in this case, given the origin, complexity and variety of the raw material. Although the enzyme activity is very high, also if compared with other literature results [13,17,18], it is lower than that shown on different oils [23,24], likely due to the FFA, water and degraded product content [10,32]. It is worth noticing, that for more degraded oils a pre-treatment may be needed in order to respect the standard. Conversions to biodiesel still higher than 90% can be obtained for longer times, also because of the progressive enrichment in shorter fractions along the cooking. In particular, in Table 5 the results of biodiesel characterization are reported, demonstrating the feasibility of WCOs oil biodiesel as fuel.

4 Conclusion

Biodiesel was obtained from WCOs without any pretreatment. In particular, a very high conversion yield, e.g. up to about 90%, was achieved at a lipase loading of

Table 5: Characterization of the Biodiesel from WCOs.

Fuel properties	unit	value	Biodiesel Standard [33]
Density at 15°C	Kg/m^3	790	878
Viscosity at 40°C	mm^2/s	2.9	1.9-6.0
Flash point	°C	> 130	100 to 170
Moisture content	ppm	Trace	0.05% max
Cetane number	-		48-65
Acid value	mg KOH/g	0.5	0.5
Polyunsaturated (≥4 double) methyl esters	% m/m	0.0	0.0
Methanol content	% m/m	0.12	0.2

20%, after 24 h. The immobilized enzyme shows a fast kinetic and high activity to form methyl esters (the biodiesel yield was already of 34.6% after only 3 h and of 70.1% after 6 h, at the same operating conditions). The very remarkable combination of properties showed by our nanocatalyst can be ascribed to a favorable enzyme orientation on the support, and support surface functionality. In particular, the enzyme is anchored thanks to the interaction with citric acid, and the presence of residual oleic acid, which exposes its a-polar tail to the medium, not only favors interfacial activation, but also helps enzyme to work in the presence of water. Moreover, the heterojunction between magnetite and gold, inducing a Fe_3O_4 surface polarity modification, determines an enhanced affinity with citric acid, favoring increased stability, e.g. strong ionic interaction. The comparison between different Au NPs sizes containing catalysts highlights the role of Au, helping enzymes to assume a favourable orientation and thus an increased enzyme loading and activity.

The olive oil before cooking simulation consisted of 13 fatty acids, in particular: tetradecanoic acid, palmitic acid, palmitoleate acid, heptadecanoic acid, cis-10-heptadecanoic acid, stearic acid, oleic acid, linoleic acid, eicosanoic acid, linolenic acid, cis-11-eicosanoic acid, behinic acid, and lignoceric acid. After cooking simulation, new compounds, originated by the cooking process (caprylic acid, octadecanoic acid 9.10 epoxy, stearic acid allyl, 14-methylhexadecanoic acid, octadecanoic acid 15.16 epoxy) were detected. The GC-MS characterization evidences the slight different activity of the enzyme as the length of the chains to be converted increases. The significant activity and stability of the bio-catalyst (activity retention ~60% and above 32% in presence of water, after 5 cycles) can be ascribed to support size and the support enzyme interactions.

Biodiesel from waste cooking oil, obtained through the use of our immobilized lipase, was analysed according to the European Standard, which is, here, a fundamental step because of the origin, complexity and variety of the raw material. In particular, the biodiesel presents an ester content equal to 97.8 ± 0.21 in agreement with the EN14214, all over the characterization demonstrate the feasibility of WCOs oil biodiesel as fuel.

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