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Effect of γ-Irradiation in the Fatty Acid Profile and Physicochemical Properties of Biodiesel Obtained from *Chlorella vulgaris*

Mohammed Hasan Mohammed[1], Maha Elttayef Jasim[1], Fathy A. El-Saied[2], Moussa H.R.[3*], Abd El-Fatah M. Ahmed[3], Mona I. Mohamed[2], Mai S. Maize[2]

[1]Al-Dour Technical Institute, Northern Technical University, Iraq
[2]Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Koom, Egypt
[3]Radioisotope Department, Nuclear Research Center, Atomic Energy Authority, Egypt

[*]Corresponding Author: helal_moussa@hotmail.com

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ABSTRACT

Biodiesel derived from microalgae is a sustainable, renewable, and readily biodegradable alternative fuel. The use of γ-irradiation to promote biological processes in microalgae has significantly increased in recent years. The purpose of the study was to evaluate the effects of γ-irradiation in the characterization and production of microalgae biodiesel from *Chlorella vulgaris* grown in BG-11 media utilizing many analytical methods, including elemental analysis (CHNSO), fourier-transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS). The data proved that γ-irradiation treatments (200 Gy) can be used for enhancing the amount of particular fatty acids in *C. vulgaris* that influence the quality of biodiesel in order to meet international biodiesel standards. A total of twelve different fatty acids were found. Furthermore, the majority of the unsaturated and saturated fatty acids in fatty acid methyl esters (FAMEs) have carbon chains that range from C12 to C24. *C. vulgaris* is the most promising source of biodiesel according to the data since it contains a cetane number (88.6), a low iodine value (103.73), a high content of saturated fatty acids (87.2%), and numerous other biodiesel characteristics that are within the acceptable range. γ-irradiated *C. vulgaris* has higher elemental levels of produced blended biodiesel (CHNO), as compared to the control. Sulphur was not present in the produced biodiesel from *C. vulgaris.* The increased oxygen level of bio-oil makes it desirable for use in transportation fuel manufacturing. The proportion of carbon increases gradually, improving combustion efficiency and encouraging full combustion of fuels based on carbon.

INTRODUCTION

There has been a growing interest in applying γ-irradiation in recent years to enhance biological mechanisms in microalgae **(Tale *et al.*, 2018; Abdel-Alim *et al.*, 2023a&b; Helal *et al.*, 2023)**. γ-irradiation treatment increased the biochemical composition and growth of microalgae **(Cheng *et al.*, 2013; Gomes *et al.*, 2017; Golz & Bradshaw, 2019)**. Stress generally causes microalgae to change their metabolism, accumulating carbohydrates and lipids rather than synthesizing new cell components **(Hu, 2004)**. Recent studies have looked at extracting biodiesel derived from microalgae as a potential substitute for fossil fuels as a practical and cost-effective method **(Chisti, 2007; Senousy *et al.*, 2023)**. The increase in the world population density and the great development in all aspects of life have led to the consumption of very large and unacceptable amounts of energy. Fossil fuels are unsustainable and non-renewable energy sources and cause the depletion of natural environmental resources, instability of fuel prices, and also cause an increase in the occurrence of the global warming phenomenon **(Schenk *et al.*, 2008)**.

More recently, the world has become increasingly interested in using renewable and sustainable energy sources as an alternative to fossil fuels **(Ameen *et al.*, 2019)**. The fast growth rates, mass productivity, and high fat content, which exceed most oil crops of microalgae make them a promising natural source for producing biofuel needed in all areas of life **(Chisti, 2007; Rittmann, 2008)**. Under exposure to many environmental and nutrient stresses, all algae change their metabolism in order to generate hydrocarbons (carbohydrates and fats). This mechanism may have been used in the production of biodiesel **(Yu *et al.*, 2016)**.

Using conventional energy sources like natural gas, coal and oil has increased atmospheric carbon levels, causing severe damage to the environment and thus causing global warming and acid rain **(Bekirogullari *et al.*, 2017)**. Currently, the use of biodiesel as an alternative energy source is given priority due to its distinct environmental characteristics, which include clean combustion behavior, rapid biodegradability, and renewable capacity **(Dong *et al.*, 2016)**. There are many sources of biodiesel production from plant seed oil, waste oil, oil crops, vegetable waste, and fruits **(Wagutu *et al.*, 2009; Ejigu *et al.*, 2010; Moser, 2010; Tang *et al.*, 2011; Liu *et al.*, 2013; Khan *et al.*, 2015)**.

Biofuel extracted from algae is the renewable and most promising alternative source for producing biodiesel and is classified as a third generation due to its many benefits, including minimal costs for both production and harvesting and its rapid growth rates accompanied by high rates of biomass and oil production, as algae oils consist of triglycerides (fatty acid esters of glycerol), which can be converted into glycerol and methyl esters (biodiesel) through the process of transesterification **(Brennan & Owende, 2010; Mata *et al.*, 2010; Arun & Singh, 2012; Chen *et al.*, 2018; Dawit *et al.*, 2020)**. Additionally, using *C. vulgaris* biomass as a feedstock for biofuel production is a sustainable alternative to fossil fuels **(Khan *et al.*, 2018)**.

The purpose of this study was to investigate the impact of γ-irradiation treatment at a dose of 200 Gy in *C. vulgaris* on the total fatty acid content as a promising raw material for biodiesel production and to study the physical and chemical properties of this fuel.

MATERIALS AND METHODS

Growth medium, growth conditions, and preparation of biodiesel

The algae used in this study, *C. vulgaris*, were obtained from the National Institute of Oceanography and Fisheries, hydrobiology laboratory, Qanater branch, Egypt, and cultured in BG-11 media **(Al-Habeeb *et al.*, 2024)**. The cultural medium was autoclaved at 120°C for 20 minutes before inoculation, and the required illumination was provided by sunlight. The solution was continually mixed by an aerator at a temperature of 30±2°C, pH was 7.5, along with the photoperiod being 16/8h of a day/night cycle. The harvested biomass was allowed to precipitate before being filtered using 0.45-mm pore-size Whatman GF/C filter paper to get a concentrated algae paste **(Hamid *et al.*, 2016)**. To eliminate the moisture content, wet microalgae biomass was collected and exposed to direct sunshine for 6–8 days. Oil was extracted from the biomass after it had been dried and ground into a powder.

γ-irradiation treatment of *C. vulgaris*

Volumes of 500mL of *C. vulgaris* from a four-day-old culture were subjected to the optimum dose of 200 Gy of γ-irradiation **(Al-Habeeb *et al.*, 2024; Helal *et al.*, 2025a, b)**. The γ-irradiation is produced using a Co^{60} source in Nasr City, Egypt, at the Egyptian Atomic Energy Authority. A 0.84 Gy min^{-1} exposure rate was used.

Metabolomics of γ-irradiated *C. vulgaris*

Oil extraction and preparation of fatty acid methyl esters (FAMEs)

Following the removal of impurities from *C. vulgaris* extracts, the cleaned samples were processed in a ball mill to a powder. The lipids were extracted using a 3v/v ratio of hexane /isopropanol **(Hara & Radin, 1978)**. After centrifuging the lipid extracts for five minutes at 11g and filtering them, the solvent was removed using a rotary evaporator at 38°C.

Capillary gas-liquid chromatography (GLC)

Using 3% sulphuric acid in methanol, the fatty acids in the lipid extracts were transformed into methyl esters **(Christie, 1990)**. N-hexane was used to extract the fatty acid methyl esters. Then, using gas chromatography and flame ionisation detection (Schimadzu G C, 17 Ver.3) connected to GC 10 software computer recorder, the methyl esters were separated and measured. Chromatography was carried out using a capillary column (30m in length and 0.26mm in diameter, Permabound 25, Germany) with nitrogen as the carrier gas (flow rate of 0.8ml/ min). The injector valve, detector, and column had respective temperatures of 230–290 and 120–230°C. The individual fatty acids were identified through regular comparisons with authentic standards under the same conditions **(Bakoğlu *et al.*, 2017)**.

Transesterification and production of biodiesel

The lipid extract were mixed with NaOH catalyst (0.7 wt%) and methanol (1:5 w/v) and centrifuged at 400 rpm for three hours. In order to achieve phase separation, the solution was let to settle for sixteen hours. A separating funnel was used to separate the top layer of biodiesel, and any remaining methanol, NaOH, or glycerol was cleaned off three times using water that was 7% of the ester phase. The crude glycerol and the yield of the generated biodiesel were then measured by gravimetric analysis after it was dried over anhydrous sodium sulphate **(Alvarez & Tonetto, 2019)**.

Estimation of physico–chemical characterization of produced biodiesel

At the Egyptian Petroleum Research Institute in Nasr City, Cairo, Egypt, the refined product of oil esterification was evaluated in order to estimate and assess its fuel qualities. The findings were contrasted with the biodiesel standards set by the American and European standards of biodiesel (ASTM D6751-08 and EN14214, respectively). Three duplicates of each property were examined, and the average values were the final results.

Fourier transform infrared spectroscopy (FTIR).

After 20 days of growth, the functional group of *C. vulgaris* and/or γ-irradiation (200 Gy) was investigated using Fourier transform infrared spectroscopy, Shimadzu, Prestige 21 (Shimadzu Europa GmbH), and spectra within the range of 500–4000cm^{-1}. The scan outputs from the integrated computer system were obtained as spectra **(Sangiliyandi *et al.*, 2014)**.

Elemental analysis

Elemental analysis was done by using a Fisons-EA-1108 CHNS-O-Element Analyzer (Thermo Scientific, Waltham, USA) to analyze the common organic elements, including carbon, hydrogen, oxygen, nitrogen, and sulfur (CHONS). The constituent elements of C, N, H, O, and S were proportionally evaluated by heating a sample of 2mg in a tin boat array at 970°C, while it was exposed to a steady and continuous flow of helium-enriched oxygen gas **(Verma *et al.*, 2024)**.

Statistical analysis

The statistical software program (SPSS version 17, SPSS Incorporated Company, Illinois, USA) was used to conduct analytical statistics. Results were expressed as means ± SD **(Moussa & Hassen, 2017; Abdel-Alim *et al.*, 2023)**.

RESULTS AND DISCUSSION

Fatty acid composition in *C. vulgaris* and/or γ-irradiation (200 Gy) after 20 days of growth

The profile of fatty acids in *C. vulgaris* was described in Fig. (1). γ-irradiation application at a dose of 200 Gy increased fatty acid composition as compared to the control samples. Fatty acid analysis is an essential criterion for obtaining high quality biodiesel fuel.

The findings showed that twelve different fatty acids were present. Furthermore, the majority of the unsaturated and saturated fatty acids in fatty acid methyl esters (FAMEs) have carbon chains that range from C12 to C24 in *C. vulgaris*. This finding is in agreement with **El-Sheekh and Alaa (2009)**. Exposure of microalgae to γ-irradiation increases the levels of saturated and monounsaturated fatty acids, with a significant decrease in polyunsaturated fatty acids **(Abo-State *et al.*, 2019; Oliver *et al.*, 2020)**. Exposure to gamma radiation causes accumulation of ROS and marked increases in lipid levels. This could be a possible mechanism leading to increased lipid biosynthesis **(Tale *et al.*, 2018)**. **Tale *et al.* (2018)** demonstrated that after γ-irradiation, the expression of several key genes for lipid metabolism, including diacylglycerol acyl transferase and acetyl-CoA carboxylase are activated supporting a potential mechanism by which γ-irradiation could cause *Chlorella sorokiniana* to produce high levels of lipids. γ-irradiation increases the lipid accumulation of *C. vulgaris* **(Abo-State *et al.*, 2019)**.

The profiling of fatty acids demonstrated the existence of palmitoleic, myristic, palmitic, linoleic, oleic, and stearic acids. These fatty acids have a high cetane number with good combustion properties for an ideal biodiesel **(Adhoni & Shivasharana, 2016)**.

FTIR spectroscopy

The FTIR spectra of biodiesel in *C. vulgaris* and/or γ-irradiation (200 Gy) were carried out within the range of 500–4000cm^{-1} to define the distinct functional groups existent in the oil (Fig. 2). The distinctive regions of absorption for the C-H vibrations, around 2922.1 and 2852.7cm^{-1} in accordance with the symmetric and asymmetric vibration modes of methyl groups, respectively, indicate the existence of a strong appearance of alkane in the biodiesel **(Carrillo *et al.*, 2004)**. The peaks at 1650 and 1770.86cm^{-1} (ketone/Aldehyde) were characteristic for C=O group verifying the presence of ester groups in the biodiesel **(Mahamuni & Adewuyi, 2009)**. The transmittance peaks at 966.64 and 1063cm^{-1} reflect alcohol, which is a carbohydrate functional group. According to the interpretation of the absorbance peaks at 719.4 and 1465.2cm^{-1}, the existence of methylene groups (CH_2) is indicated by a C-H group. A higher carbon content in mixed biodiesel is shown by the noticeable peak at 1465.2cm^{-1}. Moreover, aromatic and C-X stretching lead to peaks below 1,000cm^{-1}, which are caused by C = C stretching. Peaks in the 1,000–1,300cm^{-1} range are identified as O–CH_2–C and -CH_2–OH stretching **(Wahab *et al.*, 2010)**. Biodiesel spectra exhibit complete transesterification by the lack of a peak larger than 3000cm^{-1}, which is indicative of the -OH of carboxylic acid **(Mostafa & El-Gendy, 2013)**. Given that the main sharp transmittance peaks in FTIR spectrum are alkanes, biodiesel is a saturated hydrocarbon with potential for fuel application **(Nabi *et al.*, 2013)**.

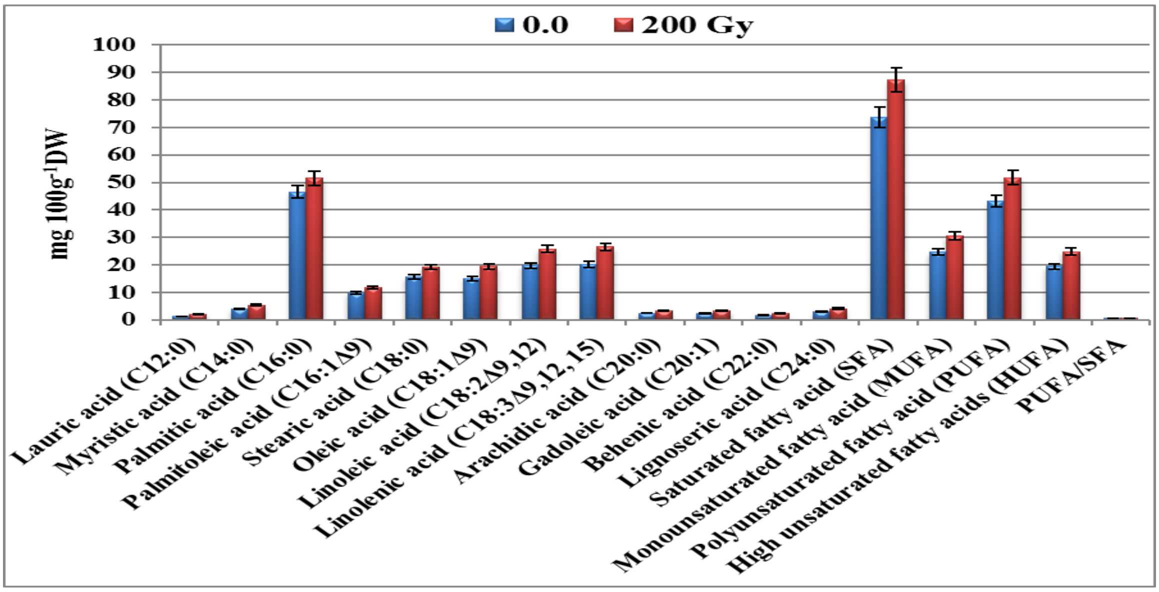

Fig. 1. Fatty acid compositions in *C. vulgaris* and/or γ-irradiation (200 Gy) after 20 days of growth. The values are the means of at least three replicates ± standard deviation (SD)

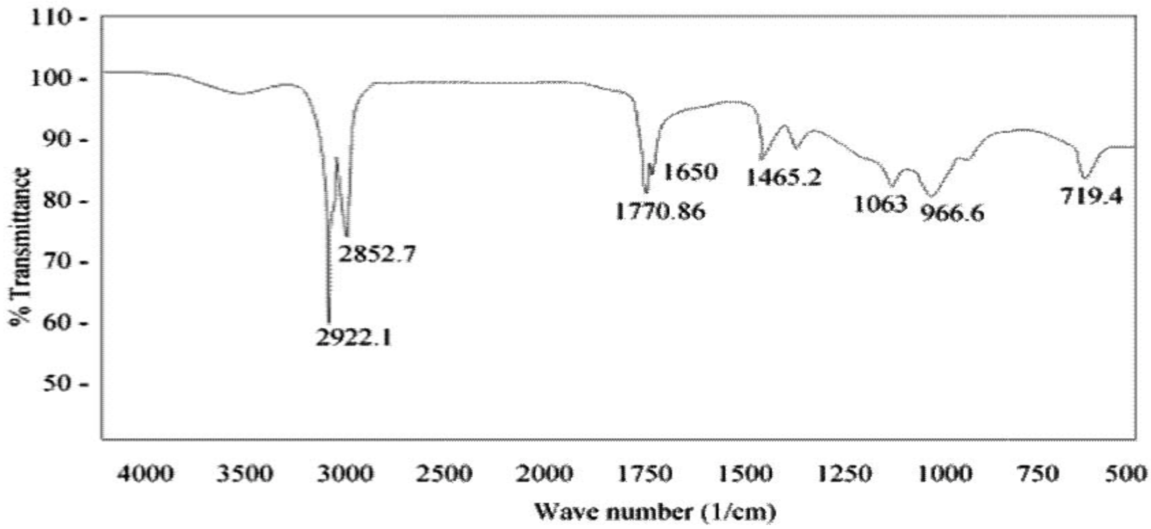

Fig. 2. FTIR spectra of biodiesel in γ-irradiated *C. vulgaris*

Estimation of physico-chemical characterization of produced biodiesel derived from *C. vulgaris* and/or γ-irradiation (200 Gy) after 20 days of growth

The biodiesel produced has been evaluated by international standards based on distillation characteristics as well as fuel properties, as shown in Table (1). The most frequent sources of biodiesel are algal esters, vegetable or animal fats, and waste cooking oils. The viscosity differs from petroleum diesel, but other than that, the biodiesel's qualities are all perfectly acceptable and satisfy the majority of requirements. As a result, it can be categorized as a practical substitute fuel for petroleum diesel. Biodiesel is a safe and appealing substitute fuel for diesel engines because of its advantages in terms of the environment, technology, and strategy.

The results of fatty acid contents showed the existence of twelve known fatty acids. Furthermore, fatty acid methyl esters mostly contain unsaturated and saturated fatty acids with carbon chain lengths ranging from C12 to C24. As stated by the biodiesel characteristics, the fatty acid profiles of the *C. vulgaris* verified their appropriateness for biodiesel generation within the limits of international requirements. According to the data, *C. vulgaris* is the most promising possible source for biodiesel production due to its high concentration of saturated fatty acids (87.2%), cetane number (88.6), low iodine value (103.73), and numerous other biodiesel characteristics that fall within the acceptable range.

Algae are classified as a third generation of renewable and environmentally friendly bioenergy sources, which can be directly converted into biodiesel, biomethanol, and bioethanol **(Mata *et al.*, 2010; Najafi *et al.*, 2011; El-Sheekh *et al.*, 2018; Shanab *et al.*, 2021)**. Carbohydrate saccharification to ethanol production **(Matsumoto *et al.*, 2003; Abou El-Souod *et al.*, 2021)**, converting biomass into syngas through gasification **(Matsumoto *et al.*, 2003)** and lipid transesterification into biodiesel **(Chisti, 2007; El-Sheekh *et al.*, 2013, 2017)**, represent examples of algal biomass-based renewable, carbon-neutral fuel applications. Biofuel is viewed as a way to minimize CO_2 emissions that contribute to global warming and replace petroleum fuel **(Mostafa, 2021)**.

The lipid production of many microalgae species is much higher than that of most oil crops **(Chisti, 2007)**. Microalgae are considered one of the most promising raw materials for biofuel production due to their rapid growth rates and high fatty acid content **(Rittmann, 2008)**. After extracting the oil from the algae, the residual biomass fraction can serve as a high-protein diet for fish and livestock **(Adeniyi *et al.*, 2018)**.

Complete combustion and a cleaner burn are characteristics of biodiesel **(El-Sheekh *et al.*, 2018)**. A major benefit for the nutraceutical production and biofuels is the identification of oily microalgae with high lipid production. The most useful parts of microalgae biomass for the generation of biodiesel are lipids. Saturated fatty acids (SFAs) can serve two purposes: they can store PUFAs needed for phospholipid synthesis in different membrane structures or to be incorporated into many metabolic pathways (**Goswami & Kalita, 2011)**.

The components of the cell are affected biologically by γ-irradiation, particularly the water molecules. When γ-rays interact with growth media, they produce reactive oxygen species (free radicals) that can change the cell composition **(Kovacs & Keresztes, 2002)**. However, upon exposure to elevated levels of γ-irradiation, the microalgal cells broke down or disintegrated, losing their capacity for self-healing and failing to completely recover **(Kovacs & Keresztes, 2002; Agarwal *et al.*, 2008)**. In contrast, when exposed to low doses of γ-irradiation, however, cells still suffered minor damage, but they quickly returned to their original state **(Fuma *et al.*, 2009)**. Gamma irradiation of *Chlorella* sp. increases biomass and lipids needed for biofuel production **(Abo-State *et al.*, 2019)**. γ-irradiation treatments increased the lipid content of microalgal biomass, which is one of the crucial factors for assessing the potential of microalgae for biodiesel production **(Manisha *et al.*, 2018)**.

Additionally, it was shown that gamma irradiation immediately increased the expression of the two most crucial genes in the lipid biosynthesis pathway: diacylglycerol acyl transferase and acetyl-CoA carboxylase **(Manisha *et al.*, 2018)**. When microalgae are exposed to γ-irradiation, they induce oxidative stress and the lipid biosynthetic pathway is upregulated, which ultimately results in high lipid accumulation **(Manisha *et al.*, 2018)**. According to **Tale *et al.* (2018)**, γ-irradiation seems to have enormous promise for the commercial synthesis of biodiesel since it produces more lipids and has a larger percentage of short-chain fatty acids that are useful for the process. The lipids that microalgae produce can also be utilized to produce biodiesel and as a source of important fatty acids for nutrition and medicine, like omega-3 and omega-6 **(Chen *et al.*, 2023)**. The increased content of carbohydrates (more than 47% by dry weight) in *C. vulgaris* made them viable feedstocks for bioethanol production **(Ho *et al.*, 2013)**.

According to the literature, *C. vulgaris* also has a significant amount of starch, which may make it a valuable feedstock for the synthesis of bioethanol **(Monjed *et al.*, 2021)**. However, because of higher production costs, biofuel made from microalgae is less competitive than traditional fossil fuels **(Hariram *et al.*, 2022)**. The fatty acid profile of microalgae, which are used to produce biodiesel from *C. vulgaris*, is comparable to that of vegetable oils; however, it mostly comprises C16 and C18 fatty acids, which are acceptable to biodiesel **(Converti *et al.*, 2009; Francisco *et al.*, 2010)**. Thus, C16–18 fatty acids are preferred for biodiesel since longer chains often result in viscosity, higher heat of combustion, and cetane number **(Francisco *et al.*, 2010)**. Furthermore, the data indicated that C16–18 fatty acids made up 80.78% of the fatty acids in *C. vulgaris*, possibly having the best correlation between stability against oxidation and cold flow characteristics **(Knothe, 2009)**. MUFAs are typically made up of the most prevalent and appropriate fractions for the synthesis of biodiesel, oleic acid (18:1) and palmitoleic acid (16:1) **(Knothe, 2011)**.

The primary components of the generated lipid are saturated fatty acids and unsaturated fatty acids. The cloud point and lubricating qualities of the biodiesel produced will be enhanced by the high USFA content in microalgal lipids **(Knothe, 2005)**. Moreover, **Song *et al.* (2014)** discovered a greater percentage of fatty acids (MUFAs: 48.79% and SFAs: 50.16%). According to these results, *C. vulgaris* lipid may have a low iodine value and a high cetane number that satisfy the specifications of both US (ASTM D6751) and European (EN 14214) standards **(Hoekman *et al.*, 2012)**. Furthermore, a significant percentage of polyunsaturated fatty acids can be found in the FAMEs profile. According to the European standard for biodiesel, 1% of the fuel should contain polyunsaturated fatty acids (≥3 double bonds), which might have an impact on the fuel's characteristics **(Branco-Vieira *et al.*, 2017)**. The predominant components of the total fatty acids were palmitic acid (C16:0) and linoleic acid (C18:2), which clearly demonstrated that *C. vulgaris* is a promising source for the production of biodiesel **(Abo-State *et al.*, 2019)**. Significant amounts of C18:2 and C18:3 were shown by *C. vulgaris*, resulting in low melting points that are also suitable for the low biodiesel temperature **(Knothe, 2005)**. The majority of most biodiesel is composed of fatty acids with carbon numbers ranging from 16 to 18 fatty acid esters, which include oleic, linoleic, linolenic, stearic, and palmitic acids **(Senousy *et al.*, 2023)**.

Table 1. Elemental contents uof produced biodiesel obtained from *C. vulgaris* and/or γ-irradiation

Properties	**Biodiesel of *C. vulgaris***		**Biodiesel (ASTM D6751-08)**	**Biodiesel (EN14214)**
	0.0 Gy	**200 Gy**		
ADU	1.14	1.73	–	–
Kinematic viscosity ($mm^2\ s^{-1}$)	4.32	5.44	1.9 – 6.0	3.5 – 5.0
Specific gravity (Kg^{-1})	0.86	0.89	0.85 – 0.90	–
Cloud point (ºC)	2.45	2.57	–	–
Cetane number	55.56	88.62	Minimum 47	51 – 120
Iodine value (g I_2/100 g)	112.40	103.73	–	Maximum 120
HHV (MJ Kg^{-1})	41.92	44.89	–	–
Db (≥ 4 (wt %)	0.7	0.9	≤ 1	–

HHV : higher heating value
ADU : average degree of unsaturation
Db : double bond

Table 2. Elemental contents of produced biodiesel obtained from *C. vulgaris* and/or γ-irradiation

Element (%wt)	Biodiesel of *C. vulgaris*		Diesel	Biodiesel (ASTM D6751-08)
	0.0 Gy	200 Gy		
C (Carbon)	62.4	68.1	87.13	n.a.
H (Hydrogen)	9.1	11.3	14.1	n.a.
O (Oxygen)	15.8	20.4	0.01 max.	n.a.
N (Nitrogen)	1.7	2.3	-	n.a.
S (Sulfur)	-	-	0.040	n.a.

n.a.= Not available

Elemental composition of the produced biodiesel obtained from *C. vulgaris* and/or γ-irradiation after 20 days of growth

The elemental composition of the biodiesel generated by γ-irradiation and/or *C. vulgaris* is reported in Table (2). γ-irradiated *C. vulgaris* (200 Gy) increased the elemental contents of produced biodiesel. In comparison to the control, which has a carbon content of 62.4 %wt. and an oxygen content of 15.8 %wt., the biodiesel produced from the treatment with γ-irradiation (200 Gy) has a higher carbon concentration (68.1 %wt.) and oxygen level (20.4%wt.). The increased oxygen level of bio-oil makes it appealing for use in transportation fuel manufacturing. Sulfur was absent in the biodiesel of *C. vulgaris*. The carbon proportion increases steadily. This effect could promote full combustion of carbon-based fuels by increasing combustion efficiency **(Rahman *et al.*, 2017)**.

CONCLUSION

Compared to diesel, the biodiesel produced from γ-irradiated *C. vulgaris* shows that sulphur is absent, indicating better combustion efficiency and reduced emissions. FTIR examination verifies that biodiesel contains functional groups, such as esters, alkanes, alcohols, and alkynes, as well as other substances, demonstrating a higher level of carbon in the biodiesel sample. The production of ester groups is confirmed by a noticeable peak at 1,465.2cm^{-1} and absorption peaks at 1770.8cm^{-1}. The chemical composition is clarified by GC-MS analysis, which also highlights the presence of fatty acid, indicating favourable fuel characteristics. Therefore, it was determined that the biofuel derived from γ-irradiated *C. vulgaris* biomass has great properties that make it appropriate for the transport industry and can be suggested as an economically feasible renewable energy source. The use of *C. vulgaris* as a natural and safe feedstock for the manufacturing of biodiesel as a potential fuel in diesel engine applications is a sustainable substitute for fossil fuels, providing valuable information about its characteristics and composition**.**

Conflicts of interest

We have no conflicts of interest to disclose.

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