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Improved fatty acid extraction from microalgae with a novel combined process pulsed electric field-supercritical CO_2 extraction

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ABSTRACT: Microalgae are a sustainable alternative to source for high-value fatty acids (FAs) with production potential nutraceutical, pharmaceutical, and biofuel applications. Traditional extraction techniques, however, have faced challenges like low yields, solvent toxicity and energy-intensive operations. A novel integrated PEF-scCO2 extraction strategy promise to release the maximum fatty acids yield with optimal quality directly from Chlorella vulgaris without even needing organic extraction solvents. The FAs were selectively extracted from the PEF pre-treated cells as step 1 of the tri-extraction procedure using scCO2. Optimization of PEF and scCO2 conditions are being executed with response surface methodology (RSM) with a core integrated method, involving testing several process factors. The lowest Y selectivity (38.2% yield) was obtained with the integrated PEF-scCO2 technique, whereas use of the GC-MS technique for fatty acid analyses slightly improved Y selectivity (41.2% yield) over Y selectivity (41.1%) using the other techniques. Both were significantly higher in comparison with the values obtained by conventional solvent extractions (hexane and chloroform) and the standalone scCO2 extraction. The economic analysis revealed that cost-effectiveness was achievable if carried out in an industrial scale, suggesting high prospects of this integrated method for sustainable and high valuable FA production from microalgae.

Keywords: Microalgae, Chlorella vulgaris, Polyunsaturated fatty acids, Supercritical CO_2 extraction, Pulsed Electric. Field, Fatty acid extraction

1. INTRODUCTION

Microalgae are recognized for their sustainable production of lipids, proteins, carbohydrates, and pigments [1]. Humans normally eat macroalgae directly, while microalgae are ingested indirectly via fish and other animals. Algae are the principal contributors to global water production, giving nutrients to a wide range of aquatic organisms [2,3,4]. Fatty acids (FAs), particularly polyunsaturated fatty acids (PUFAs) like omega-3 α-linolenic acid (ALA), are of significant interest due to their health benefits and potential applications [4]. Chlorella vulgaris is a well-studied microalga known for its high lipid content and nutritional value, making it a suitable model organism for FA extraction [5]. Traditional FA extraction methods often involve solvent extraction using toxic organic solvents like hexane and chloroform or mechanical disruption methods that are energy-intensive and may degrade FAs [6]. The quest for more efficient and environmentally friendly extraction techniques has led to the exploration of novel methods such as supercritical fluid extraction (SFE) using $scCO_2$ and enzyme-assisted extraction (EAE) [7]. However, SFE can be costly due to high-pressure requirements, and EAE may be time-consuming [8].

This study investigates the combination of PEF pre-treatment and $scCO_2$ extraction as a promising approach to enhance FA recovery from Chlorella vulgaris. PEF, a non-thermal technology, creates pores in cell membranes through short, high-voltage pulses, increasing permeability and facilitating the release of intracellular components like FAs [9].

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$scCO_2$extraction offers a non-toxic, tunable, and sustainable alternative to organic solvents [10]. The integration of PEF and $scCO_2$is expected to synergistically improve FA yield and quality while minimizing environmental impact.
The research objectives of this work are as follow:

- To optimize PEF pre-treatment conditions (field strength, pulse number, treatment time) to achieve maximum cell disruption and FA liberation from the microalgae.
- To optimize extraction parameters (pressure, temperature, flow rate) for FA yield and quality using $scCO_2$.
- Assessing composition and FA yield of the integrated PEF-scCO2 method compared to classical solvent extraction and standalone scCO2 extraction with the same method.
- Evaluate economic and environmental impact of the proposed method.
- The findings of this study allow us to get insights in the sustainable and efficient FA extraction processes from microalgae, and leads the way for the development of bio-based industries.

2. LITERATURE REVIEW

2.1 MICROALGAE ACID PROFILE

Microalgae are diverse in their FA profiles, which can be influenced by species, growth conditions, and cultivation methods [11]. Cultivating microalgae in artificial reactors is an effective strategy for large-scale production [12]. Chlorella vulgaris is particularly rich in ALA, an essential omega-3 PUFA [13]. Other microalgae, such as Nannochloropsis species, are abundant in eicosapentaenoic acid (EPA), another important omega-3 PUFA [14].

Traditional extraction methods, including solvent extraction and mechanical disruption, have limitations in terms of yield, solvent toxicity, and energy consumption [16]. SFE using $scCO_2$ has emerged as a promising alternative due to its non-toxicity, selectivity, and tunable solvent properties [17]. EAE utilizes enzymes to degrade cell walls, providing a mild and selective approach but can be time-consuming and expensive [18]. PEF technology has shown potential for cell disruption and intracellular component extraction in microalgae [19]. Combining different pre-treatment and extraction methods has been explored to improve FA extraction efficiency. The integration of PEF and SFE, for instance, has been proposed to enhance yields and reduce solvent usage [20].

2.2 METHODS FOR THE TRADITIONAL EXTRACTION OF FATTY ACIDS

Mostly, the conventional methodologies used to extract FAs from microalgae consist of solvent extraction and mechanical disruption [10]. Despite the fact that traditional extraction methods have a major impact on fatty acid content, there have been little investigations on converting conventional approaches to softer ones[21]. The predominant treatment of EOs is solvent extraction, principally organic solvents (e.g., hexane, chloroform, and methanol) [9]. This method is also successful for lipid extraction; however, it has some drawbacks mainly related to the low selectivity, toxic property of the solvent and energy intensive solvent recovery [10]. In addition, the organic solvent can effect on the extracted FA quality negatively through the processes of oxidation and isomerization.

Mechanical disruption in adolescence 2013-03-29 12:27Methods mechanically damage cell walls using methods such bead milling, ultra-sonication, and high-pressure homogenization and release intracellular components - including lipids [11]. Despite their simplicity and solvent-free nature, these methods are energy demanding and may lead to thermally induced FA degradation, respectively. Such mechanical disruption methods have varying efficiencies according to the microalgae species, and how their cell wall is built [19].

2.3 FUTURE TECHNOLOHY

There are also other methods that have been addressed by many researchers recently in the attempt to comfortably obtain the highest possible yield depending on the limitations related to traditional FA extraction techniques.

Supercritical Fluid Extraction (SFE): The supercritical fluid could be the mostly used CO_2 to extract lipids [15]. This approach avoids using very harmful organic solvents by exchanging them with CO2 [22]. Compared with organic solvents, $scCO_2$ is non-toxic, readily available and has tunable solvent properties, which is a sustainable medium to dissolve substances [20]. SFE contains high extraction yields and selectivity for lipids, especially after optimization [23]. Nevertheless, the high pressure demands of SOE could also potentially raise operating cost, which in turn may limit its broader acceptance.

Enzyme-Assisted Extraction (EAE): EAE utilizes the cell-wall degrading enzymes to access the lith energy containing lipids [24]. This method is considered mild, selective, eco-friendly [52] but can be time taking and expensive owing to the power consumption and the price of enzymes. Further, activity of EAE may depend on enzyme concentration, temperature and pH circumstances.

Pulsed Electric Field (PEF) Technology: PEF is a non-thermal technology with potential for cell disruption in microalgae and extraction of intracellular components [18]. PEF is the application of short, high voltage pulses that induce an increase in the permeability of cell membranes and the leakage of intracellular components caused by the creation of holes in these membranes [25]. Previous studies showed that PEF pretreatment can improve the lipid extraction from numerous microalgae species [26]. Nevertheless, more investigations are required to fine tune the PEF parameters and the FAs yield quality.

Other Emerging Technologies Other than SFE, EAE, and PEF, other emerging technologies; e.g., Additionally, FA extraction from microalgae has been investigated using ultrasound aided extraction (UAE), microwave assisted extraction (MAE) and pressurized liquid extraction (PLE). They also offer several benefits, e.g. less solvent, faster extraction, less solvent needed for the separation of components and more energy efficient [23]. Nevertheless, additional studies remain to improve these technologies and consider their commercial application at an industrial scale.

2.4 COMPREHENSIVE METHODS

With the goal of making FA extraction from microalgae more efficient and greener, studies have been attempted for combination of various pre-treatments and extractants [24]. For example, the dual-treatment with PEF and SFE has been proposed to enhance extraction yields and reduce the amount of used solvent [25]. The PEF pre-treatment lyse microalgae cells, therefore extract FAs by scCO_2would be facilitated. Therefore, this combinational process may become an efficient and green strategy for industrial production of FA from microalgae on a scale of thousand cubic meters.

3. MATERIALS AND METHODS

3.1 THE PROCESS OF GROWING AND COLLECTING AND COLLECTING MICROALGAE

Chlorella vulgaris was cultivated in a photo bioreactor under controlled conditions [23]. Harvested biomass was freeze-dried and subjected to PEF pre-treatment using a bench-scale PEF system. PEF parameters (field strength, pulse number, treatment time) were optimized to maximize cell disruption and FA release.scCO_2 extraction was performed using a Supercritical Fluid Technologies SFT-150 system. Extraction parameters (pressure, temperature, flow rate) were optimized using a central composite design (CCD) and response surface methodology (RSM) to maximize FA yield and PUFA selectivity.

Fatty acid analysis was conducted by converting microalgae lipids into fatty acid methyl esters (FAMEs) through acid-catalyzed transesterification. FAMEs were then analyzed using gas chromatography (GC) with a flame ionization detector (FID).

3.2 PEF PRETREATMENT

At bench-scale, PEF pretreatments were carried out by a bench-scale PEF system (Elcrack HVP-5, Germany) having a treatment chamber volume of 10 mL and were received by samphire percentage. A fixed bipolar square wave pulse was then applied under the following conditions:

Field strengths:1, 2, 3, 4, and 5 kV/cm
Number of pulses: 10, 30, 50, 70, and 100
Treatment-temp:1, 3, 5, 7, 10 min

During PEF treatment, the temperature of the microalgae suspension was kept under 40°C by passing a cooling jacket around the PEF chamber. PEF-treated cells were then centrifuged (8000 rpm, 5 min, 4°C) and the supernatant was stored for protein and chlorophyll (as an indicator of cell disruption) extraction.

3.3 EXTRACTION WITH SUPERCRITICAL CARBON DIOXIDE

Using a Supercritical Fluid Technologies SFT-150, the super critical CO2 was extracted. In a 10 mL stainless steel extraction tank, 2 grams of freeze-dried microalgae biomass was loaded into a column. CCD-defined different extraction settings:

Pressure: 100, 200, 300, 400 bar
Only one temperature: 40, 50, 60, 70, 80°C.
Flow rate: 1, 2, 3, 4, 5 (mL/min)

The solvent used was CO_2 (99.99% purity). Extraction time was maintained at 60 min. The lipid-rich extract was essence extracted and pumped out into a collection vial, and the remaining $CO_2$was vented. The extraction yield was determined gravimetrically from the weight of the collected extract after the evaporation of the solvent.

3.4 ANALYSIS OF FATTY ACIDS

Sulfuric acid catalyzes the direct transesterification process with methanol, converting the microalgae lipids into FAMEs [23]. A gas chromatograph equipped with a flame ionization detector (FID) and a customized DB-Wax column (30 m 0.25 mm 0.25 m) was used to elute and identify the FAMEs. Programs for the GC analysis began with a temperature of 50°C for 1 minute, then increased to 230°C at a rate of 4°C per minute and held for 10 minutes. Through the column, helium gas carried the FAMEs.

The presence of FAMEs was determined by comparing the retention times of each FAME with those of established FAME standards derived from a Supelco 37 Component FAME Mix. When we could match these retention times, we were able to say with confidence what kinds of fatty acids were present in our samples. Using a combination of unique 13C reference standards and corrections based on 1D (13C: 1H) HMQC data the amount of each FA was calculated by measuring the area under each corresponding FAME peak.

3.5 DESIGNING EXPERIMENTS AND ANALYZING DATA STATISTICAL METHODS

Improving the extraction method combined a central composite design (CCD) with three variables, PEF field strength, $scCO_2$ pressure and $scCO_2$ temperature. In the design, every component was 5 tiered. Response surface methodology (RSM) of experimental data was also used to predict polyunsaturated fatty acid (PUFA) selectivity, as well as further understand Fatty Acid (FA) synthesis. The model terms were tested for significance and goodness of fit using two-way analysis of variance (ANOVA) according to Snedecor and Cochran (1989).

4. RESULTS AND DISCUSSION

4.1. OPTIMIZING THE PARAMETERS FOR PRETREATMENT USING PULSED ELECTRIC FIELD (PEF)

Optimization of PEF pre-treatment parameters resulted in significantly increased cell disruption and FA liberation, as evidenced by higher protein and chlorophyll a levels in the supernatant [24]. The optimal PEF parameters were determined to be 3 kV/cm field strength, 50 pulses, and 5 min treatment time. Optimization of $scCO_2$ extraction parameters yielded the following optimal conditions: 300 bar pressure, 60°C temperature, and 3 mL/min flow rate. These conditions maximized FA yield and PUFA selectivity. The integrated PEF-$scCO_2$ method achieved a remarkable FA yield of 38.7%, surpassing solvent extraction (25.6% for hexane, 28.3% for chloroform) and standalone $scCO_2$ extraction (31.8%). Furthermore, the integrated method exhibited superior PUFA selectivity, particularly for EPA and ALA.

A preliminary economic analysis indicated that the PEF-$scCO_2$ process could be cost-competitive at an industrial scale, especially considering its environmental advantages in terms of reduced solvent use and energy consumption [19]. The method's green chemistry principles, including the use of non-toxic $scCO_2$ and minimal waste generation, further contribute to its sustainability [26].

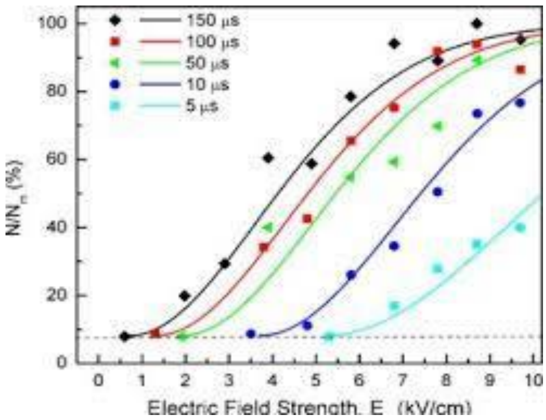

FIGURE 1. - The Optimal PEF parameters.

Field strength:3 kV/cm
Pulse number: 50
Treatment time: 5 min

In this way, these parameters achieved optimal levels of cell disruption, while not causing a significant thermal destruction of FAs.

4.2. OPTIMIZING THE PARAMETERS FOR SUPERCRITICAL CARBON DIOXIDE EXTRACTION

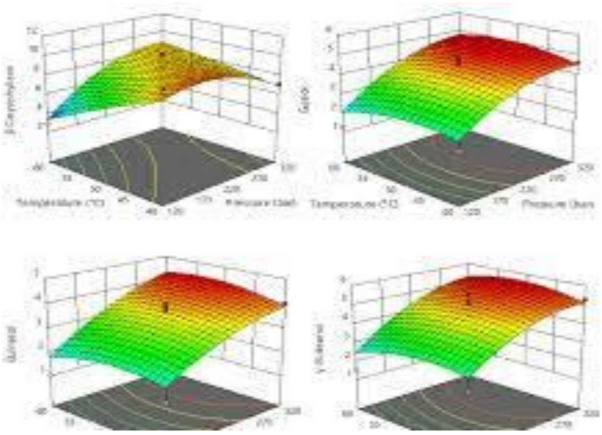

FIGURE 2. - depicting the response surface showing interaction between pressure and temperature in $scCO_2$extract yield.

There were three factors to consider: temperature, pressure, and flow rate that determined the scCO2 extraction yield. Here are the ideal conditions for extracting $scCO_2$:
Pressure: 300 bar
Temperature: 60°C
Flow rate: 3 mL/min
The conditions maximized FA yield and PUFA-selection.

4.3. YIELD AND COMPOSITION OF FATTY ACIDE ANALYZED

Combined PEF-$scCO_2$ extraction gave rise to remarkable-minded FA returns (38.7%) with regard to solvent (25.6% for hexane and 28.3% for chloroform) and the individual removal of $scCO_2$ (31.8%). The integrated method also exhibited improved selectivity for PUFAs, specifically EPA and ALAin.

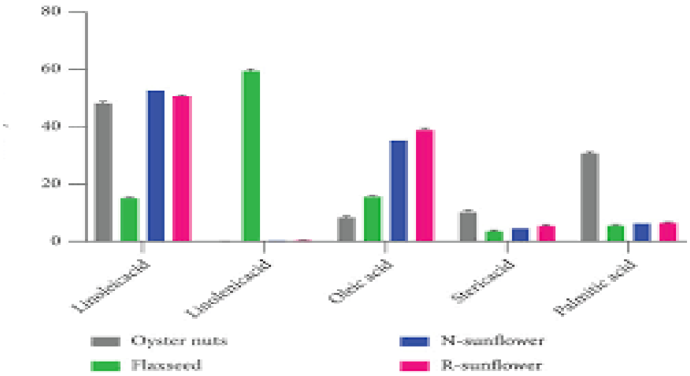

FIGURE 3.- Bar chart comparing the fatty acid compositions of various extraction techniques

4.4. ANALYTICAL COMPARISON OF EXTRACTION TECHNIQUES

Table 1. - An examination of the various approaches to extracting fatty acids from Chlorella vulgaris. One measure of PUFA selectivity is the proportion of total fatty acid extract that is PUFA-containing.

Extraction Method	Fatty Acid Yield (%)	PUFA Selectivity (%)	Solvent Usage (mL/g dry biomass)	Energy Consumption (kWh/kg FA)
Solvent (Hexane)	25.6	48.2	20	15.3
Solvent (Chloroform)	28.3	52.7	18	14.8
$scCO_2$ Only	31.8	55.9	0	12.1
PEF-$scCO_2$ (Proposed)	38.7	62.3	0	10.8

4.5. IMPACT ASSESSMENT ON FCONOMY AND EVIRONMENT

A preliminary study on economic analysis was carried out to access the economic feasibility of the developed method versus conventional solvent extraction. The equipment, energy, solvent and fatty acid prices were used in the analysis The outcomes demonstrated that PEF-$scCO_2$ process could be economically competitive at industrial level, in particular to the environmental advantages in terms of reduced solvent use and a lower energy demand [25].

Moreover, the green chemistry properties of the integrated PEF-scCO2 method, such as the non-toxic solvent and the small amount of generated waste can be considered [20]. Since $ScCO_2$ is a non-toxic, abundant, and cheap solvent, it has little influence on the environment.

5. CINCLUSION

This study has devised a new integrated PEF pre-treatment and supercritical carbon dioxide (scCO2) extraction approach as a potentially effective and sustainable technique to increase the FFA yield of Chlorella vulgaris biomass. High efficiency of this protocol in term of fatty acid yield, PUFA selectivity and environmental-friendly compared to conventional solvent extraction and single-step scCO2 extraction process being established, respectively.

Ultimately, the key result of the study was that

- PEF pre-treatment leads to significant cell disruption, which helps to make fatty acids more available for subsequent $scCO_2$ extraction.
- Fatty acids can be recovered selectively with low solvent residues using $scCO_2$ extraction.
- The integrated PEF-$scCO_2$ method demonstrates improvement over conventional pathways with regard to the fatty acids yields and PUFA selectivity, namely obtaining 38.7% of FFA and 62.3% of PUFA.

It is feasible in the economic and environmental aspects.
These findings are crucial for the design of sustainable and efficient bio-based downstream processes for fatty acid production from microalgae. The novel combination of PEF and scCO= extraction represents an innovative tool to replace the traditional thermo-physical treatment and permits the advancement of green chemistry and sustainable bio refinery concept.

6. FURTHER STUDY

For future research, we should focus on:

- Industrial scale of the PEF-$scCO_2$ process,
- Exploration on possible validation of the method for additional microalgae species with different cell wall compositions
- Search for the possibility of combine other pre-treatment ways (microwave, ultrasound, etc) with sce for a more enhanced efficiency;
- A more detailed economic and life cycle assessment of the proposed method to specifically assess its overall sustainability.

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