



Microalgae lipid extraction: a novel lab-scale method within a biorefinery approach (fractioning)

Extracción de lípidos de microalgas: un método novedoso a escala de laboratorio dentro de un enfoque de biorrefinería (fraccionamiento)

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Resumen

La extracción y fraccionamiento de lípidos de microalgas ha sido objeto de estudio recurrente debido al valor de mercado de ciertas familias de lípidos (carotenoides y xantofilas) y la oportunidad que representan los aceites de microalgas para la obtención de biocombustibles. La mayoría de los métodos desarrollados para la extracción total de lípidos de microalgas requieren una posterior separación de las diferentes familias (lípidos neutros, lípidos polares) a cuantificar. Además, la mayoría de ellos utilizan disolventes tóxicos y caros.

En el presente estudio, se probaron solventes menos tóxicos que son ampliamente utilizados en la industria, como hexano, acetona y etanol, en diferentes órdenes para la extracción de biomasa de microalgas de distinta composición y contenido de lípidos (es decir, tres niveles diferentes de estrés nutricional). También se presenta una comparación de los métodos propuestos por Bligh y Dyer, Folch y Hara y Radin. El nuevo método logra la extracción de lípidos totales de microalgas y la separación de las familias de lípidos simultáneamente.

Palabras claves: Microalga, Lípido, Fraccionamiento, Biorrefinería.

Abstract

Extraction and fractioning of microalgal lipids have been recurrently studied because of the market value of certain lipid families (i.e. carotenoids and xanthophylls) and the opportunity that microalgal oils represent for obtaining biofuels. Most of the methods developed for the total extraction of microalgal lipids require a subsequent separation for the different families (i.e neutral lipids, polar lipids) to be quantified. Moreover, most of them use toxic and expensive solvents.

In the present study less toxic solvents that are widely used in the industry, such as hexane, acetone and, ethanol were tested in different orders for the extraction of microalgal biomass of different lipid composition and content (i.e. three different levels of nutritional stress). A comparison of the methods proposed by Bligh and Dyer, Folch and, Hara and Radin is also presented. The new method achieves microalgal total lipid extraction and separation of the lipid families simultaneously.

Keywords: Microalgae, Lipid, Fractioning, Biorefinery

1. Introduction

Cultivation of microalgae under nutritional stress conditions induces changes in the metabolism of these organisms as a response to adversity. These changes cause variations in their composition (Hu, 2004; Markou & Nerantzis 2013). Under unfavorable growth conditions, microalgae synthesize secondary metabolites, such as compounds that act as antioxidants, hormones, antibiotics, allelochemicals and toxins, which are used by microalgae as a form of energy storage (Carmichael, 1992; Skjånes et al., 2012) and thus increase their possibility of surviving. Many of these secondary metabolites are of high value because they have industrial applications (Markou & Nerantzis, 2013). In addition, under nutritional stress conditions, microalgae can produce lipids of remarkable importance, such as oils of paramount importance (i.e.. eicosapentaenoic acid and docosahexaenoic acid), or lipids of high commercial value, such as carotenoids and xanthophylls, both free and esterified with fatty acids (Yuan & Chen, 2000). Since these

lipids have different polarity, it is possible to think of a fractional extraction based on the polarity of solvents. Of all the fractions, the lipid fraction is the one with greatest importance because this fraction has multiple uses at the industrial level, either for the production of biodiesel or to obtain natural dyes of high commercial value. The microalgae industry is thus working on improving the fractionation of these families of components for different purposes.

The reference methods currently used to extract lipids from complex matrices are those developed by Folch (1957), Bligh & Dyer (1959) and Hara & Radin (1978). Folch's method was developed to extract lipids from brain samples by using a mixture of organic solvents (chloroform/methanol, 2:1) followed by washing the chloroform fraction with weak saline solutions to retain the acid fractions of lipids (Folch et al., 1957). Later, Bligh & Dyer developed their method in animal tissue by using the same solvent system as Folch but varying the chloroform/methanol ratio to 1:2. Since subsequent investigations determined the possible carcinogenic power of chloroform and the damage to the visual system caused by methanol, Hara & Radin (1978) developed a new method of lipid extraction with less toxic solvents: hexane:isopropanol, followed by a wash to eliminate possible non-lipid contaminants. Although all these methods were developed to determine lipids in animal tissue, they were validated not only in samples of low lipid content, but also in vegetable samples, such as microalgae (Singh et al., 2014).

Since the three aforementioned methods perform a total extraction of lipids from the samples, it is then necessary to purify the different fractions such as to separate neutral lipids to obtain methyl esters of fatty acids or to separate carotenoids and xanthophylls (Molina Grima et al., 2003). Many authors have studied and optimized the process of purification of the extracted lipid fraction (Folch et al., 1957; Bligh & Dyer, 1959; Hara & Radin, 1978; Zhukov & Vereshchagin, 1981; Kates, 1986; Molina Grima et al., 1994, among others).

Iverson et al. (2001) compared the results obtained by extracting lipids by the Folch and Bligh & Dyer methods from samples with different lipid contents and found that, in samples with low lipid content (> 2%), the Bligh & Dyer method slightly underestimates the lipid content, but also that, as the amount of total lipids increases, this method leads to considerable errors due to a high underestimation of the total value (50% of the lipid content).

The solvent mixtures used by Folch and Bligh & Dyer, i.e. chloroform/methanol, have a slightly high polarity and therefore, oils are not fully extracted. Further, this mixture of solvents poses multiple health hazards. Chloroform and its vapors are corrosive to the skin, eyes, and respiratory system. In addition, repeated and prolonged contact with this solvent has effects on the liver (including hepatitis and jaundice), the central nervous system, and the kidneys (Eschenbrenner, 1945; Torkelson et al., 1976). Similarly, methanol and its vapors are corrosive to the eyes and can cause loss of vision or even blindness. In addition, chronic exposure to this solvent can cause liver damage (Gutierrez, 2011). As mentioned above, due to these drawbacks, Hara & Radin (1978) developed a new method by using less toxic solvent mixtures, but, according to Gunnlaugsdottir & Ackman (1992), this method is able to extract a significantly smaller amount of lipids, probably due to the less efficient extraction of the polar lipid classes. An alternative would thus be fractionating each lipid family by using a sequence of solvents of different polarities (Molina Grima et al., 2003). Solvents such as hexane, acetone and ethanol, which are cheaper and less toxic and have been used for a long time for the extraction of

phytometabolites, are able to dissolve many components. Hexane, which is a non-polar solvent, dissolves the neutral lipids present in the sample, whereas acetone and ethanol, which are widely used in the industry, dissolve the polar lipids present.

| Polarity ↓ | Solvents | Formula | Density (g mL ⁻¹) | Relative polarity* |
|---------------|----------|---|----------------------------------|-----------------------|
| | Hexane | CH ₃ -(CH ₂) ₄ -CH ₃ | 0.659 | 0.009 |
| | Acetone | CH ₃ -C(=O)-CH ₃ | 0.791 | 0.355 |
| | Ethanol | CH ₃ -CH ₂ -OH | 0.785 | 0.654 |

Table 1. Relative polarity of solvents

*The values of relative polarity are normalized from measurements of solvent shifts of absorption spectra and were extracted from Christian Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Publishers, 3rd ed., 2003.

Based on the above, the aim of this work was to introduce a new method of lipid extraction by using a mixture of solvents of different polarity (hexane, acetone and ethanol) to replace the hazardous solvents currently used by other methods and to achieve the separation of the different lipid fractions present in algal biomass (at three different levels of nutritional stress), pointing to the possible industrial use of each fraction.

2. Materials and Methods

2.1. Culture

An isolated strain of the microalga *Scenedesmus dimorphus* from a local urban effluent was cultured in a tubular photobioreactor in Bold's Basal medium (Nichols & Bold, 1965). The culture was carried out in 34°35'37"S 58°29'03"O (Buenos Aires, Argentina) during June-August 2015, with the temperatures and light conditions imposed by the environment, in a 70-L vertical tubular photobioreactor, of translucent plastic, with constant aeration and without carbon dioxide injection.

During the period of exponential growth, determined by Neuberger's camera count, a fraction of the microalgal culture was harvested and flocculated by increasing the pH value to 13 by adding NaOH, and later stirred for 10 minutes at 30-80 rpm. Then, the wet cell mass was filtered and dried at 60°C under vacuum, to obtain the control algal biomass for its extraction.

The non-harvested fraction was separated in two fractions, which remained inside two different photobioreactors and were subjected to nutritional stress by dilution (by adding

water) without addition of macronutrients or micronutrients. In one case, the microalgae were diluted by adding 1/3 of water, while in the other, the microalgae were diluted by adding 2/3 of water. Thus, two stress levels were obtained: moderate and high stress. Cells were counted daily for 10 days under a microscope (Moheimani et al., 2013). After this 10-day period, the number of cells began to decrease. Finally, cells were harvested following the procedure described above.

2.2. Lipid extractions

2.2.1. Novel lipid extraction method

Lipids were extracted from the algal biomass by using a batch method extraction with n-hexane, acetone and ethanol as solvents, in different order. This extraction consisted in adding 12 mL of the first solvent (separated into five fractions) to 1 g algal biomass, and vortexing for 5 minutes between each addition. The extractive obtained with this first solvent was separated from the biomass and evaporated by rotavapor at 40°C and 0.6 atm of vacuum. The solvent-free extract obtained was weighed and the extractable content in each solvent expressed as a percentage on a dry basis. The procedure was repeated on the residual mass with the second solvent and finally with the third one. Three different sequences were tested. The total extracted lipids were calculated by adding the extracts obtained by using the three solvents. All extraction were made by triplicated.

2.2.2. Folch's method (1957)

Algal biomass (1 g) was contacted with 20 mL of solvent mixture (chloroform-methanol, 2/1), and then stirred for 20 min at room temperature. After centrifuging the homogenate, the liquid phase was separated, washed with a dilute NaCl solution and centrifuged to separate the phases. The lower phase (chloroform) was evaporated in a rotary evaporator.

2.2.3. Bligh & Dyer's method (1959)

Total lipids were extracted with 20 mL of chloroform-methanol and 1g algal biomass in a 1:2 ratio. The homogenate was stirred for 5 min and the chloroform fraction obtained after decanting the mixture was evaporated in a rotary evaporator.

2.2.4. Hara & Radin method (1978)

In this method, 1 g of microalgal biomass was added with 18 mL of hexane:isopropanol (3:2), and then the mixture was homogenized for 30 s, and the suspension was filtered. The homogenizer residue was washed three times with 2-mL portions of hexane:isopropanol, by resuspending the residue each time and allowing the solvent soak for 2 min before applying air pressure. The extracted lipid material was freed of solvent at 60°C under vacuum by using a rotary evaporator.

2.3. Determination of extractive percentage

The components extracted by various solvents (extracts) were quantitatively evaluated by gravimetry. The extractive was obtained from the difference between the weight of the empty test tube and that after solvent evaporation. Every extraction was carried out in triplicate.

2.4. Characterization and quantification of lipids

The different groups of lipids present in the extractives were characterized by thin-layer

chromatography (TLC), by comparison with known standard samples and published TLCs (Jaime et al., 2010).

Plate chromatography was used to qualitatively evaluate the presence of carotenoids and xanthophylls as well as the presence of saponifiable lipids and elution order. The extracted biomass was resuspended in 50 µL of extraction solvent. Each extract was then spotted on a 10 x 10 cm silica gel 60 F254 TLC plate (Merck, Germany). Plates were placed in a closed chamber and eluted with a solvent mixture of n-hexane:acetone (7:3) as mobile phase and potassium permanganate 1% acidified as developer.

Standards were obtained from their natural sources frequently mentioned in the bibliography and by comparison with publications of processes in which they were obtained under the same conditions as those used in this study (Jaime et al., 2010). B-carotene was obtained from carrot extract, lycopene from tomato, lutein from egg, and chlorophyll and pheophytin from spinach. The extraction methodology was the same as that mentioned above, but, in this case, the standards were purified by column chromatography.

2.5. Quantification of proteins

To achieve an accurate determination of proteins, it is convenient to perform a method of acid hydrolysis and subsequent analysis of amino acids. Since the method developed by Lowry (1951) has been widely used for the determination of proteins with sufficiently reproducible and sensitive results, it can be used in both simple and complex samples (Waterborg, 2002). In the method proposed by Lowry, two reactions are used: the Biuret reaction, with reduction of Cu^{2+} to Cu^+ and subsequent reaction with the Folin reagent, and the Folin-Ciocalteu reaction, in which the aromatic amino acids reduce phosphomolibdotungstate with ion Cu^{2+} as catalyst. The method is used in solutions with a concentration range of 0.01-1.0 mg mL⁻¹ of protein.

2.6. Quantification of carbohydrates

One of the easiest and cheapest methods still in use today to determine and quantify carbohydrates is the colorimetric method (Albalasmeh et al., 2013). To this end, 10 mg of microalgal biomass was hydrolyzed with 5 mL of sulfuric acid solution 1M, boiled for 1 h and centrifuged. An aliquot of the hydrolyzed carbohydrate solution was mixed with concentrated sulfuric acid and vortexed for 30 seconds. After addition of sulfuric acid, the temperature of the mixture increased rapidly within 10–15 s. Finally, the solution was cooled to room temperature to perform the measurement by UV spectrophotometer at 315 nm.

2.7. Determination of ash

Using a porcelain marker, mark an appropriate number of crucibles with identifiers, and place them in the muffle furnace at 575 ± 25 °C for a minimum of four hours. (Marking crucibles with a porcelain marker will permanently mark them, so a generic identifier is recommended.) Remove the crucibles from the furnace directly into a desiccator. If using a furnace set to 575 ± 25 °C, cool for a specific period of time, one hour is recommended. Record the cool time. Weigh the crucibles to the nearest 0.1 mg and record this weight. Place the sample back into the muffle furnace at 575 ± 25 °C and dry to constant weight.

To calculate the percentage of ash in samples, formula 1 was used.

$$\% \text{Ash} = \frac{\text{Weight}_{\text{ash plus cr}} - \text{Weight}_{\text{crucible}}}{\text{oven dry weigh}} \times 100 \quad (1)$$

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using STATISTICA Software (version 7.0) from StatSoft Inc. [27]. A P value of <0.05 was considered statistically significant, and if significant differences were observed, treatment means were pairwise compared with the Tukey test.

3. Results and Discussion

3.1. Lipid extractions

The amounts of lipid extracted from microalgal biomass by the three different extraction procedures used are shown in Table 2. In all the extraction procedures tested, the control sample (non-stressed biomass) gave lower values of extracted lipids, followed by the moderately stressed biomass. The severely stressed biomass was the one that showed the highest values of extractive. In accordance with the results reported by Hu (2004), under conditions of optimal growth or favorable environmental conditions, algae produce only small amounts of lipids. In contrast, under nutritional stress, algae not only synthesize and accumulate large amounts of lipids but also alter their intracellular composition (Hu et al., 2008).

| Method | Extractive percentages (%) \pm SD | | |
|-------------------------|-------------------------------------|-----------------------------------|------------------------------------|
| | Control | Moderate nutritional stress | Severe nutritional stress |
| Bligh & Dyer | 5.52 \pm 0.71 | 7.52 \pm 1.02 | 9.74 \pm 0.93 |
| Folch | 5.96 \pm 0.55 | 8.04 \pm 1.14 | 17.78 \pm 2.31 |
| Hara & Radin | 2.91 \pm 0.22 | 3.22 \pm 0.32 | 6.20 \pm 0.67 |

Table 2. Comparison of extractive percentages obtained in lipid extraction from the three samples of algal biomass using reference methods. Results are express on dry basis weight /weight (w/w)

In agreement with results of Gunnlaugsdottir & Ackman (1993), comparison of the amounts of lipids recovered by the three different extraction procedures showed that these were significantly lower for all the samples when the Hara & Radin method was used (Figure 1, Table 2). As mentioned, this method uses hexane/isopropanol as the extraction solvent system. Methods using only hexane as the extraction solvent have been previously shown to give lower lipid recoveries than the chloroform-based Bligh & Dyer method (de Koning et al., 1985; de Koning & Mol, 1989; Gunnlaugsdottir & Ackman, 1993). Besides, the hexane/isopropanol extraction solvent system allows extracting only neutral non-polar lipids and, in this method, the contact time between the biomass and the solvents is only 30 s, preventing a good contact between the phases. Folch's chloroform-based method gave the highest lipid recovery for all the samples studied (Figure 1, Table 2) (de Koning et al., 1985; de Koning & Mol, 1989).

Both chloroform-based methods showed similar extraction percentages with low amounts

of lipids (Table 2). According to several authors, increasing the microalgal lipid contents reduces the efficiency of the Bligh and Dyer method (Smedes & Thomasen, 1996; Iverson et al., 2001). This might have been due to the solubility of the lipids in the solvents used because both methods use a mixture of relatively polar solvents, while the samples contain significant contents of non-polar lipids (Sharma et al., 2012). Another important cause may have been the dry biomass/solvent ratio, because comparing the results obtained between the chloroform-based methods even at low amounts of lipids (<2%) the extracted amount was underestimated (Iverson et al., 2001). These results suggest that Folch's method is the best to compare with the new lipid extraction method here proposed.

Figure 1 shows the chromatogram obtained for the lipids extracted from microalgal biomass under severe nutritional stress by the Bligh & Dyer, Folch and Hara & Radin methods. The isolated lipids subjected to thin-layer chromatography (TLC) showed that although all methods extracted the same families of lipids, the Folch method extracted all in greater proportion.

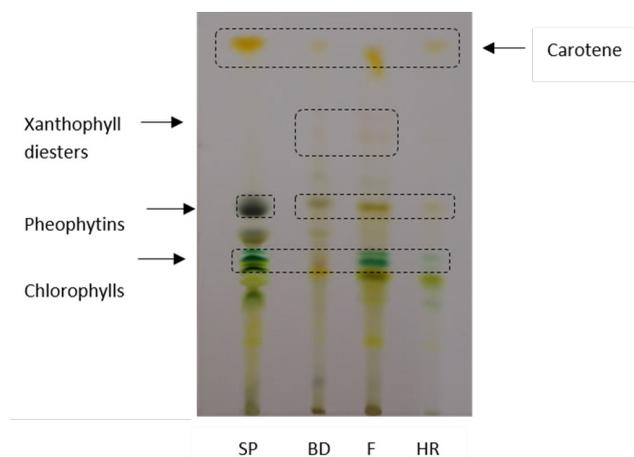


Figure 1. Fractions separated by thin layer chromatography (TLC) from microalgal biomass under severe nutritional stress obtained by the Bligh & Dyer (BD), Folch (F) and Hara & Radin (HR) methods. The elution solvent used was hexane-acetone (7+3). The spinach (SP) pattern obtained by Khalyfa et al., method (1992) is shown for comparative purposes.

To replace the hazardous chloroform/methanol/water system used by Folch and Bligh & Dyer's methods and to separate the polar fraction from the neutral saponifiable lipids (Knothe, 2008; Ríos, 2013), an alternative method was here developed with solvents widely used in the industry (hexane, acetone and ethanol), which in addition have low cost and low toxicity. To develop a technique in view of a biorefinery approach, it is necessary to consider that the extraction method not only separates the different lipid fractions but also preserves these fractions during the extraction (Serive et al., 2012).

As our interest was focused on the removal of all lipids present (polar and non-polar ones), the new extraction method was developed using hexane as a non-polar solvent, which dissolves mainly the neutral lipids and carotenes. In contrast, acetone and ethanol dissolve the polar lipids present in the algal biomass, like xanthophyll, chlorophyll, phospholipid and glycolipid. Three different sequences were tested and the results obtained are presented in table 3.

| Method | Solvent | Extractive percentages (%) | | |
|---------------------|---------|----------------------------|-----------------------------|---------------------------|
| | | Control | Moderate nutritional stress | Severe nutritional stress |
| First | Hexane | 2.2 | 3.8 | 6.1 |
| | Acetone | 3.7 | 4.4 | 5.4 |
| | Ethanol | 2.8 | 5.5 | 7.6 |
| Total Lipids | | 8.7 | 13.7 | 19.1 |
| Second | Hexane | 1.8 | 3.8 | 5.9 |
| | Ethanol | 5.8 | 7.8 | 9.3 |
| | Acetone | 1.6 | 2.5 | 4.1 |
| Total Lipids | | 9.2 | 14.1 | 19.3 |
| Third | Ethanol | 4.9 | 7.1 | 14.4 |
| | Hexane | 2.0 | 2.5 | 2.6 |
| | Acetone | 1.4 | 2.9 | 3.4 |
| Total Lipids | | 8.3 | 12.5 | 20.4 |

Table 3. Comparison of extractive percentages obtained in lipid extraction from the three samples of algal biomass using different sequences of extraction solvents. Results are express on dry basis (w/w)

The main difference between the sequences was the polarity of the solvents used. First, a sequence of increasing polarity was used. Then, to evaluate a possible increase in cell disruption and a consequent improvement in extraction, two more sequences were studied, varying the order of polarity of the solvents. One of them was initiated with the least polar solvent, whereas the other was initiated with the most polar solvent.

The results shown in table 3 clearly show that changing the order of the solvents practically did not modify the total percentage of extraction. However, the extractive percentages obtained with each solvent varied considerably. A more detailed analysis of table 3 allows us to conclude that if the aim is to achieve a first separation of the lipids according to their polarity, as the first step of a biorefinery, it is better to use the first sequence – i.e. solvents of increasing polarity – because this sequence yields the best results. These results are consistent with those of Lewis et al., (2000), who demonstrated that the lipid extraction was significantly more efficient when solvents were added in an order of increasing polarity.

In the first sequence of total lipid extraction from severely stressed sample, 32% corresponded to non-polar lipids (hexane fraction), 28% corresponded to medium polar lipids (acetone fraction) and 40% corresponded to polar lipids (ethanol fraction). In the following sequences, it can be observed that varying the order of the solvents did not modify the total lipids extracted from the sample but did modify the amount extracted by each solvent. Mainly in the third

sequence, the fraction of lipids extracted by the solvent of greater polarity corresponded to 71% of the total lipids, 13% to non-polar lipids and 16% to medium polar lipids.

Due both to the complexity of the samples and to the nature of the products, the most difficult step in the biorefinery of natural products is the correct fractioning and separation of compounds from a complex sample, since it is difficult to separate them without damaging the other components (Markou & Nerantzis, 2013; Vanthoor-Koopmans et al., 2013). A closer look at Table 3 reveals that if the aim is the biorefinery of algal biomass, the first extraction is the one that sheds a better distribution of the lipid family extracted in each case (Figure 2). This minimizes the mixing of different families of lipids, facilitating the subsequent separation. By comparing results of Table 2 and Table 3, the new method presents a bigger percentage of extractive.

Figure 2 shows the TLC obtained for the new lipid extraction method. These results correspond to the lipids extracted from microalgal biomass under severe nutritional stress by the first sequence of solvents tested, i.e. solvents of increasing polarity. The lipids extracted by the Folch method were analyzed on the same TLC to compare the results obtained by the fractional extraction method with the reference method. Although the two methods extracted similar compounds, the proposed new method achieves a first fractioning step of the lipid families in three different extracts.

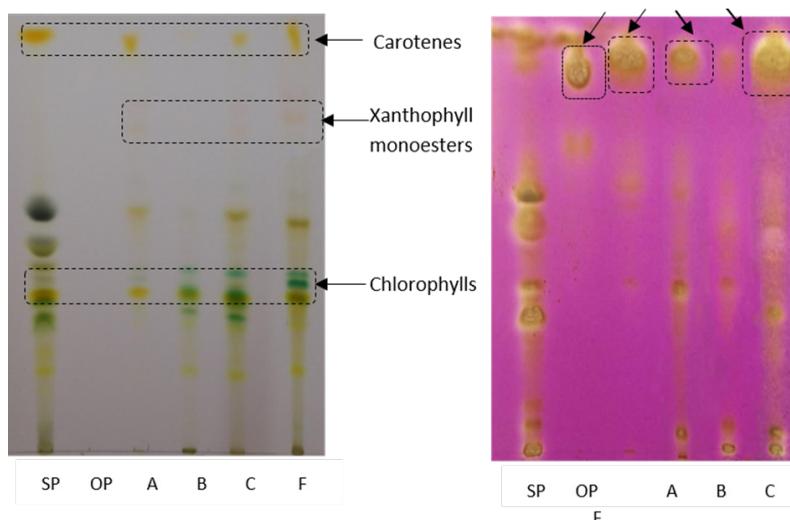


Figure 2. Fractions separated by thin layer chromatography (TLC) from severely stressed microalgal biomass obtained by the new lipid extraction method (A, B, C) and Folch's method (F). Sequence using hexane (A), acetone (B) and ethanol (C). Elution solvent: hexane-acetone (7:3). The TLCs were revealed with acid potassium permanganate (pink plates), which allows observing the colorless fraction of neutral lipids (fats and oils) present in the samples. The spinach (SP) pattern obtained by Khalyfa et al., method (1992) and neutral lipids pattern (NP) are shown for comparative purposes.

The fraction extracted with hexane in the first place allowed separating mainly neutral lipids and carotenes (beta-carotene) from the fraction of polar carotenoids (astaxanthin), a product of great value in the market. On the other hand, this would improve the production of biodiesel by separation of the saponifiable fractions of lipids (Knothe, 2008; Ríos, 2013).

3.2. Characterization of microalgal biomass

The bioavailability of nutrients has a direct influence on the growth of microalgae and an

indirect influence on the composition of the microalgal biomass. Nutritional stress in microalgae affects not only the metabolism of lipids but also the composition of carbohydrates and proteins (Sharma et al., 2012).

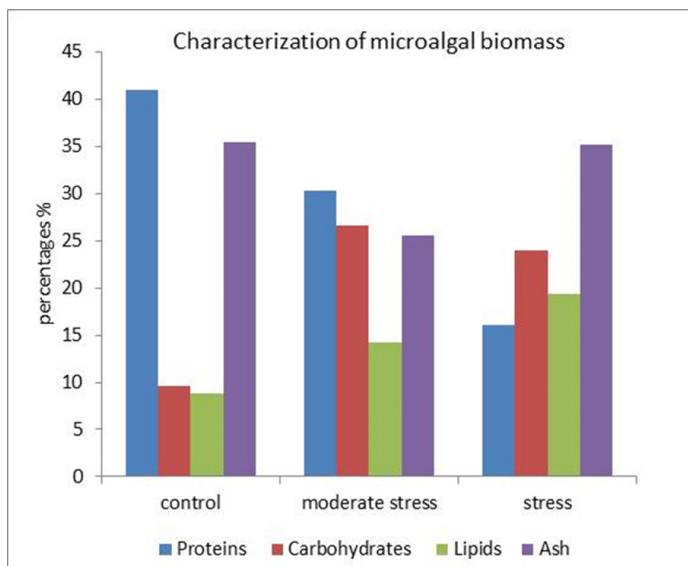


Figure 3. Composition of microalgal biomass expressed in percentages. 1- control; 2- moderate stress and 3- severe stress. Lipid composition determined by the new method, carbohydrates by the colorimetric Sulfuric Acid-UV method (Albalasmeh et al., 2013), proteins by Lowry et al., method (1951) and ashes by the ASTM E1755-01 Standard Test Method for Ash in Biomass.

As already mentioned, when microalgae are grown in an unfavorable nutritional environment, they change their metabolism to synthesize energy-rich compounds such as lipids and carbohydrates and this leads to competition in the metabolism of both metabolites (Siaut et al., 2011; Pancha et al., 2014). Lipids and carbohydrates are the main energy storage products because they can be easily packed and used in adverse conditions, thus ensuring cell survival (Courchesne et al., 2009; Pancha et al., 2014).

Figure 3 shows that increasing the nutritional stress in the growth medium significantly enhanced the lipid and carbohydrate content in samples. The results also indicate that, under moderate nutritional stress, the carbohydrate content increased in a greater proportion, whereas when the stress was severe, both carbohydrates and lipids were in similar proportions in the algal biomass. The increase in carbohydrates and lipids may have been due to a decrease in protein content. On the other side, ash content decreases under moderate nutritional stress. A similar behavior of lipid accumulation has been observed by several authors (Giordano et al., 2001; Dean et al., 2010; Siaut et al., 2011; Ördög et al., 2012; Pancha et al., 2014).

4. Conclusion

In summary, the results of the present study show that using solvents of lower cost and toxicity than those used in traditional chloroform-based methods, it is possible to extract

polar and non-polar lipids in similar quantities from algal biomass produced under different levels of nutritional stress and control. In addition, using the solvents in increasing polarity allows achieving a first fractionation that would facilitate the subsequent separation of the components of commercial interest. Our findings are important not only to use safer and cheaper solvents, but also to improve the separation of saponifiable neutral lipids from polar lipids and xanthophylls.

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