10.18605/2175-7275/cereus.v14n4p251-260

ORIGINAL ARTICLE

<< Recebido em: 08/11/2022 Aceito em: 29/11/2022. >>

REVISTA CEREUS ISSN: 2175-7275

Optimization of lipid extraction from aquatic plant *Salvinia molesta* D. S. Mitchell (Salviniaceae) with alternative solvents

Otimização da extração de lipídios da planta aquática Salvinia molesta D. S. Mitchell (Salviniaceae) com solventes alternativos

João Paulo Aquino Correia[1], Leila Cristina Konradt Moraes[2], Thiago Luis Aguayo de Castro[3], Aricely Aparecida Silva Leite[4], Bianca Ferreira Duarte[5], Carmem Cícera Maria Silva[6], Marina Rodrigues Maestre[7]

ABSTRACT

Salvinia molesta D. S. Mitchell (Salviniaceae) is an invasive aquatic plant that causes environmental and economic damage, but its rapid growth in eutrophic media can be used for the biotechnological use of its biomass. Thus, the aim of this work was to determine the best technique for lipid extraction from *S. molesta*, testing the solvents hexane:methanol (2:1 v/v), ethanol:water (10% v/v) and ethanol:water (4 % v/v), in association with auxiliary techniques: ultrasound with and without glass beads and the use of magnetic preparation at 17 and 34 ºC. The best yields obtained were the ones that used hexane:methanol 2:1 with the aid of ultrasound (8.30 ± 0.05%). The mixture of ethanol:water 10% with magnetic agitation at 34 ºC was also potential, as it uses less toxic solvents and presents a yield of 6.90 ± 0.21%.

Palavras-chave: Green chemistry. Bioresources. Macrophyte.

RESUMO

Salvinia molesta D. S. Mitchell (Salviniaceae) é uma planta aquática invasora que causa problemas ambientais e econômicos, mas seu rápido crescimento em meio eutrofizado pode ser utilizado para o aproveitamento biotecnológico desta biomassa. Assim, o objetivo deste trabalho foi determinar a melhor técnica de extração de lipídios de *S. molesta*, testando os solventes hexano:metanol (2:1 v/v), etanol:água (10% v/v) e etanol:água (4 % v/v), em associação com técnicas auxiliares: ultrassom com e sem esferas de vidro e uso de preparação magnética a 17 e 34 ºC. Os melhores rendimentos obtidos foram os que utilizaram hexano:metanol 2:1 com auxílio de ultrassom (8,30 ± 0,05%). A mistura de etanol:água 10% com agitação magnética a 34°C também se mostrou potencial, pois utiliza solventes menos tóxicos e apresenta um rendimento de 6,90 ± 0,21%.

Keywords: Química verde. Biorecursos. Macrófita.

[1] Bachelor Environmental and Sanitary Engineering (UEMS). Email: joaoaqicor@gmail.com https://orcid.org/0000-0002-1283-8930

[2] PhD in Chemical Engineering, Environmental Engineering Professor (UEMS). Email: leilackm@uems.br https://orcid.org/0000-0001-7574-8978

[3] Bachelor of Industrial Chemistry, Postgraduate Program in Natural Resources (UEMS). Email: thiagoaguayo@gmail.com https://orcid.org/0000-0002-8127-1990

[4] Bachelor Environmental and Sanitary Engineering (UEMS). Email: Aricellysilva79@gmail.com https://orcid.org/0000-0002-9018-6595

[5] Master in Natural Resources, Postgraduate Program in Natural Resources (UEMS). Email: biancaduarte.bf@gmail.com https://orcid.org/0000-0003-2803-9620

[6] PhD in Environmental Science and Technology, Postgraduate Program in Chemistry (UFES). Email: carmemcenos@gmail.com https://orcid.org/0000-0001-6986-4905

[7] Doctor of Science, Computer Science Professor (UEMS). Email: marina.maestre@uems.br https://orcid.org/0000-0002-4740-1333

1. INTRODUCTION

Lipid extraction must be carried out with care and precision, choosing methods that preserve the properties of the extracted oil and that are environmentally friendly. Thus, hot procedures at high temperatures such as the ones presented by Soxhlet in 1879 are not recommended for this purpose, as they promote peroxidation and hydrolysis, in addition to increasing the energy expenditure (MARTINS et al., 2013).

As a result, there are established methods that provide excellent results, in terms of quality and quantity. The methodology described by Bligh and Dyer (1959) is one of the most indicated and used for extracting lipids. This method enables the extraction of all lipid categories without the need for significant heating and expensive equipment (UNDELAND et al., 1998).

According to the literature, the methodology of Bligh and Dyer has already been used for the lipids extraction from *Salvinia molesta* D. S. Mitchell (Salviniaceae), using the methanol:chloroform (2:1) mixture, however, the use of toxic solvents is one of the main disadvantages of this technique (MUBARAK et al., 2016).

To overcome this problem, alternative solvents with lower toxicity from renewable sources were used. In addition to being a simple alternative that allows adaptations (D'OCA et al., 2011), it is also possible to use other mechanical methods together to extract intracellular lipids as a means to increase yields (LEE et al., 2012).

The ultrasound is an alternative to increase the yield of lipid extraction, among its characteristics stands out its easy handling, reduced level of impurities in the waste water, fast execution, high reproducibility, in addition to being able to be operated at low temperatures, being considered an economic and ecological process with low energy expenditure (KUMAR et al., 2015). Whereas the magnetic stirring promotes better dispersion and homogenization of the solvent inside the cell for lipid extraction, which can also be performed at ambient or moderate temperature ranges (VIÊGAS et al., 2010; KUSS et al., 2020).

In this context, it makes necessary the study of the efficiency of solvent mixtures that can cause a lower environmental impact for the lipids extraction from the aquatic plant *S. molesta*, making its biotechnological exploration more sustainable.

2. MATERIAL AND METHODS

COLLECTION AND PLANT MATERIAL

The aquatic plants were identified and collected in the Antenor Martins Municipal Park, in the city of Dourados, state of Mato Grosso do Sul, Brazil (22°13'44.0"S, 54°49'55.0"W).

The region has a high population density, as it is widely used by surrounding residents for recreation and leisure. The park has a central lake that is inserted in the watershed of the Água Boa stream, which flows into the Dourados River. In a north/south direction, the area was fenced and reforested with native vegetation. The Água Boa stream basin, part of the Dourados River hydrographic basin, is formed by the Água Boa, Rego D'água and Paragem streams.

The chosen location is a eutrophicated stretch fed by rainwater and a spring that is located inside the vegetation remnant.

The collection was carried out with the aid of a plastic container with a capacity of 15L. The aquatic plants were separated and stored in another container that contained some of the local water to preserve the structure of the plants. The collect was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge – SisGen (number A77F799).

After collection, the aquatic plants were manually washed in running water, dried in an oven at 60 ºC until constant mass and ground to a powder.

LIPID EXTRACTION

For the extraction of lipids, the Bligh and Dyer (1959) method was used, with adaptations regarding the solvents used and complementary methods. 0.100 g of the biomass was weighed and 3 mL of the hexane:methanol (2:1) (H:M) solution was added, then the sample was centrifuged with KASVI brand bench equipment, model K14 - 4000 in rotation 2000 rpm for 3 minutes. The supernatant was then separated and transferred to another dry test tube of known mass. The addition of solvent and other extraction steps with the same biomass were repeated three times. The solvent was evaporated in an oven at 60 °C until constant mass.

The extraction was also performed using an ultrasound (LOGEN Scientific equipment, 2668 series, model LS 1400-A) at 25 KHz for 20 minutes, with and without glass beads, to determine if there would be an increase in yield.

Another auxiliary technique used in the extraction was magnetic stirring at temperatures of 17 and 34 °C. This procedure was performed with the same period of time as the ultrasound method.

To evaluate the efficiency of the other mixtures, the same methodology described for the mixture of H:M (2:1) was used, however changing the solvent for ethanol:water (4%) (E:W 4%) and ethanol:water (10%) (E:W 10%). All previous procedures were performed in triplicate.

STATISTICAL ANALYSIS

Statistical analysis was performed using R software, version 4.0.3. The normality of the replicas was analyzed using the Shapiro Wilk and Bartlett test, in order to verify if the data are parametric and if there is a correlation between the studied variables. ANOVA was performed in order to verify if there is a significant difference between the treatments of the analyzes used and the Tukey test was used with a significance of 5% for the samples with a significant difference.

3. RESULTS

Analyzing the Table 1, it is possible to observe that there were significant differences ($p > 0.05$) in yields according to the different solvents and methods.

Table 1. Yields obtained using different solvents and auxiliary extraction techniques.

Auxiliary technique used	H:M (M ±SD)	E:W 10% (M ±SD)	E:W 4% (M ±SD)
No ultrasound (17 ± 1°C)	4.43 ± 0.42 Bc	5.40 ± 0.52 Ab	4.80 ± 0.42 ABc
Ultrasound (17 ± 1°C)	8.30 ± 0.05 Aa	5.69 ± 0.72 Cb	6.82 ± 0.06 Ba
Ultrasound and glass beads (17 ± 1°C)	4.82 ± 0.40 Bbc	5.72 ± 0.43 Ab	4.51 ± 0.16 Bbc
Magnetic agitation (17 ± 1°C)	4.56 ± 0.15 Bc	5.83 ± 0.15 Ab	3.80 ± 0.20 Cc
Magnetic agitation (34 ± 1°C)	5.42 ± 0.24 Bb	6.90 ± 0.21 Aa	4.89 ± 0.28 Bb

Legenda: H:M = Hexane:methanol (2:1); E:W 4% = Ethanol:water (4%); E:W 10% = Ethanol:water (10%); M = Mean; SD = Standard deviation. Means followed by different uppercase letters in the column and lowercase letters in the rows differ from each other by the Tukey test with a level ($p < 0.05$) of significance.

The highest means obtained were with the aid of ultrasound in association with hexane:methanol, and magnetic stirring (34 ± 1°C) together with the solvents ethanol:water 10%, with no significant difference between them. On the other hand, the lowest mean found

was at the junction of magnetic stirring (17 ± 1°C) with the solvents ethanol:water (4%), being significantly lower than the other methods.

Regarding the hexane:methanol solvents and the means of extraction, it was possible to verify that there was a 3.87% increase in yield when using ultrasound (Table 1), with a significant difference in yield compared to the same solvent without ultrasound assistance ($p < 0.05$), obtaining the highest average among all the methods performed.

The procedure involving magnetic stirring (34 ± 1°C) with hexane:methanol presented the second best average among the methods used. However, there was no significant difference between the methods without ultrasound and the magnetic stirring (17 ± 1°C) for the same temperature range and solvents.

The methods that associated the E:W 10% solvents, without ultrasound (17 ± 1°C), with ultrasound and glass beads (17 ± 1°C) and magnetic stirring (17 ± 1°C) did not show significant differences, although magnetic stirring (17 ± 1°C) is more cost effective as it has the highest average.

.

4. DISCUSSION

The increase in the efficiency using ultrasound is associated with the affinity of the solvent and the extracted lipid and other factors such as the vapor pressure that the solvent exerts and the surface tension (LI et al., 2004).

The ultrasound bath causes cell disruption through cavitation by forming microbubbles that generate pressure on plant cells, in addition to helping the contact between the biomass and the solvent used through the acoustic transmission of ultrasonic waves (KUMAR et al., 2015). In this case, the ultrasound proved to be an efficient auxiliary technique, as it was probably capable of disrupting a large number of cells, influencing the increase in the yield of extracted lipids from *S. molesta*.

Considering the results obtained (table 1), the extraction performed only with solvent is more economically favorable to be used compared to magnetic stirring (17 ± 1° C), since it needs a stirrer equipment that involves energy costs for its operation, and the one with solvents dispenses with this step.

Concerning the ethanol:water (10%) solvents, it is possible to verify that magnetic stirring (34 ± 1°C) expressed the highest average among the methodologies used. This mixture of solvents also showed no significant difference when compared to the mixture of

hexane and methanol, both using ultrasound. Although the use of ultrasound has higher averages, the positive effects of using solvents that are environmentally friendly should be highlighted, considering their origins and properties.

Ethanol and water are advantageous in the current context, due to the non-toxic character of water and less toxic of ethanol, in addition to having a renewable source and being of low cost (MORAES; BACCHI, 2014; MENEGUETTI et al., 2012). The use of ethanol instead of methanol for extractions is recommended, as it strengthens the sustainable objective of this research and encourages support for the Brazilian market in the economic and social scenario with the generation of jobs, considering that Brazil is the world's second largest producer of ethanol (VIDAL, 2020).

Regarding the extraction procedures that used the mixture of ethanol:water (4%) solvents, it appears that the best yield was the one that used ultrasound, followed by the second highest average with magnetic stirring (34 ± 1°C), at 5% significance.

When comparing the procedures with and without ultrasound, in the 3 solvent mixtures used, it was observed that the use of ultrasound promoted an increase in the yield of extracted lipids.

In all tested methodologies, the use of glass beads associated with ultrasound did not show a significant increase compared to the procedure using only ultrasound (Table 1) ($p > 0.05$). The addition of glass beads to ultrasound is performed to increase the collision force, promoting cell disruption and releasing cell constituents to the extraction solvent exposure (LEE et al., 2012), however Halim et al. (2012) argue that the efficiency of glass beads cannot be universalized, since different biomasses have different propensities to cell disruption by this technique. However, the absence of increased yield may be associated with insufficient abrasion to generate cell disruption

The use of magnetic stirring showed a significant difference in the three solvents mixture, at temperatures of 17 ± 1°C and 34 ± 1°C. The temperature of 34 ± 1°C showed the highest yield. As with the cavitation process, heating may be related to cell disruption (BARBA et al., 2014). In this sense, it is coherent that heating has resulted in an increase in the yields associated with magnetic stirring. It is important to emphasize that the temperature used cannot be elevated to avoid degradation of extracted lipids and promote a significant increase in energy costs.

The polarity of the solvents used in these experiments must also be critically analyzed. Based on the results in Table 1, when comparing the E:W 10% and H:M solvents, the highest

averages obtained were those using the E:W 10% solvents, with the exception of the method using ultrasound. The same dynamics is observed when with the solvents E:W 10% with E:W 4%, higher means are observed according to the higher percentage of water, except for the method with ultrasound.

The hexane:methanol (2:1) solvents contains a polar solvent (methanol) and a non-polar solvent (hexane), culminating in a more comprehensive mixture, however the ethanol:water mixtures have only polar solvents. According to Tir et al. (2012), the increase in solvent polarity accelerates the process of destruction of the association between the lipoprotein and the cell membrane and reduces surface tension.

Thus, it is possible to see that the addition of a greater amount of water for extraction may have increased the polarity of the medium and contributed to the dissociation of cell wall components, resulting in increased yields of lipids extracted with the E:W 10% solvents, for most of the methods studied, except for the one that used only ultrasound.

Analyzing the levels obtained with those reported in the literature for other aquatic plants (Table 2), it is possible to see that those presented in this study for all treatments (Table 1) are higher than the levels reported for most species (Table 2). Salvinia auriculata (Table 2) presented levels similar to those obtained for *S. molesta* (Table 1).

Table 2. Lipid content of different types of aquatic plants according to the literature consulted.

Species	Lipid (%)	Reference
Salvinia natans (L.) All.	13.50–27.10	(ROZENTSVET ET AL., 2005)
Salvinia auriculata Aubl.	3.28–8.71	(OLIVEIRA JUNIOR ET AL., 2021)
Azolla filiculoides Lam.	15.00	(SALEHZADEH ET AL., 2014)
Azolla filiculoides Lam.	6.5–13.5	(MIRANDA ET AL., 2018)
Porphyra sp.	3.34	(PATARRA ET AL., 2013)
Chaetomorpha pachynema (Montagne) Kützing	3.54	(PATARRA ET AL., 2013)

Legenda: H:M = Hexane:methanol (2:1); E:W 4% = Ethanol:water (4%); E:W 10% = Ethanol:water (10%); M = Mean; SD = Standard deviation. Means followed by different uppercase letters in the column and lowercase letters in the rows differ from each other by the Tukey test with a level ($p < 0.05$) of significance.

Pai et al. (2020) used hexane to extract lipids from *S. molesta*, obtaining a 4.2% yield. Comparing with the results obtained for hexane:methanol (2:1) (Table 1), it can be seen that the mixture obtained a similar value when ultrasound was not used and twice as much with

ultrasound. This reinforces the hypothesis that ultrasound helped to extract polar compounds from the interior of cells that were not available without its use.

In the research by Mubarak et al. (2016) it was also possible to observe the increase in yield when using ultrasound in the extraction of lipids from *S. molesta* with the solvent mixture methanol:chloroform (2:1), obtaining 19.97%, with this method was more efficient than microwave (16.60%), grinding with glass spheres (16.46%), grinding with sand (16.26%) and autoclaving (15.36%).

It is also possible to verify that the Bligh and Dyer method using methanol:chloroform (2:1) studied by Mubarak et al. (2016) showed greater efficiency compared to those obtained in this work (Table 1). The formation of a biphasic mixture helps in the extraction process, however the toxicity of this mixture is not advantageous (TANAMATI et al., 2010). In this sense, the solvent mixtures studied in this research are promising, due to their lower environmental impact.

It can be seen that *S. molesta* has interesting levels of lipids compared to other aquatic plants and that it is feasible to explore alternative solvents in the extraction process.

5. CONSIDERAÇÕES FINAIS

Due to the problems arising from the rapid growth of *S. molesta*, studies are needed for the biotechnological application of this abundant biomass. This research demonstrated alternative methods and solvent combinations to obtain lipids from *S. molesta* with significant yield. In addition, it brought important information about the best combination of solvents and auxiliary methods in the sustainable context.

The hexane and methanol solvents had a positive prominence, since in association with ultrasound, they had the highest average among all the analyzed methods. Regarding the ethanol and water solvents, the most advantageous mixture was the one with 10% water in ethanol, responsible for relatively higher yields in most extraction media, compared to the 4% and hexane:methanol mixture.

The promising results of ethanol and water solvents are important as they allow the exploitation of this biomass with less environmental impact. However, research is needed on the possible biotechnological applications of these lipids.

REFERÊNCIAS

ARNIKAR, H. J.; RAO, T. S.; BODNE, A. A gas chromatographic study of the kinetics of the

uncatalysed esterification of acetic acid by ethanol. **Journal of Chromatography**, v. 47, e265268, 1970.

HOLLAND, C. D. **Fundamentals of Multicomponent Distillation**. McGraw-Hill, New York, 1981.

KOMATSUE, L. Application of the relaxation method for solving reacting distillation problems. **Journal of Chemical Engineering of Japan**, v. 10, p. 200-205, 1977a.

KOMATSUE, L. A new method of convergence for solving reacting distillation problems. **Journal of Chemical Engineering of Japan**, v. 10, p. 292-297, 1977b.

KOMATSUE, L.; SIMAND, J. Simulation of reactive distillation by the insideoutside method. **Proceeding 37th Chemical Engineer Conferece**, p. 365-367, 1987.

BARBA, F. J.; GRIMI, N.; VOROBIEV, E. New Approaches for the use of non-conventional cell disruption technologies to extract potential food additives and nutraceuticals from microalgae. **Food Engineering Reviews**, v. 7, p. 45-62, 2014.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. Canadian **Journal of Biochemistry and Physiology**, v. 37, 911-917, 1959.

D'OCA, M. G. M.; VIÊGAS, C. V.; LEMÕES, J. S.; MIYASAKI, E. K.; MORÓN–VILLARREYES, J. A.; PRIMEL, E. G.; ABREU, P. C. Production of FAMEs from several microalgal lipidic extracts and direct transesterification of the *Chlorella pyrenoidosa*. **Biomass and Bioenergy**, v. 35, p. 1533-1538, 2011.

HALIM, R.; HARUN, R.; DANQUAH, M. K.; WEBLEY, P. A. Microalgal cell disruption for biofuel development. **Applied Energy**, v. 91, p. 116-121, 2012.

KUMAR, R. R.; RAO, P. H.; ARUMUGAM, M. Lipid extraction methods from microalgae: A comprehensive review. **Frontiers in Energy Research**, v. 2, e61, 2015.

KUSS, V. V.; CARLIZ, R. G.; DÍAZ, G. C.; VIÊGAS, C. V.; ARANDA, D. A. G.; CRUZ, Y. R. Evaluation of lipid yield for biodiesel production extracted from microalgae *Scenedesmus sp.* submitted to different homogenization times and physicochemical changes. **Brazilian Journal of Development**, v. 6, n. 4, p. 22066-22081, 2020.

LEE, A. K.; LEWI, D. M.; ASHMAN, P. J. Disruption of microalgal cells for the extraction of lipids for biofuels: Processes and specific energy requirements. **Biomass and Bioenergy**, v. 46, p. 89-101, 2012.

LI, H.; PORDESIMO, L.; WEISS, J. HIGH intensity ultrasound-assisted extraction of oil from soybeans. **Food Research International**, v. 37, p. 731-738, 2004.

MARTINS, G. B. C.; MELLO, V. M.; SUAREZ, P. A. Z. Processos térmicos em óleos e gorduras. **Revista Virtual de Química**, v. 5, p. 16–25, 2013.

MENEGUETTI, N. F. S.; ZAN, R. A.; MENEGUETTI, D. U. O. Potencial do sebo bovino rondoniense como matéria-prima para produção de biodiesel em Ariquemes – RO. **Revista Eletrônica de Gestão e Tecnologias Ambientais**, v. 8, p. 1889-1899, 2012.

MORAES, M. L.; BACCHI, M. R. P. Etanol: Do início às fases atuais de produção. **Revista**

de Política Agrícola, v. 23, p. 1-22, 2014.

UNDELAND, I.; HÄRRÖD, M.; LINGNERT, H. Comparison between methods using low-toxicity solvents for the extraction of lipids from herring (*Clupea havengus*). **Food Chemistry**, v. 61, p. 355-365, 1998.

Mubarak, M.; Shaija, A.; Suchithra, T. V. Ultrasonication: An effective pre-treatment method for extracting lipid from *Salvinia molesta* for biodiesel production. **Resource-Efficient Technologies**, v. 2, n. 3, p. 126-132, 2016.

MIRANDA, A. F.; LIU, Z.; ROCHFORT, S.; MOURADOV, A. Lipid production in aquatic plant Azolla at vegetative and reproductive stages and in response to abiotic stress. **Plant Physiology and Biochemistry**, v. 124, p. 117-125, 2018.

OLIVEIRA JUNIOR, J. P.; KONRADT-MORAES, L. C.; CASTRO, T. L. A.; MASCARENHAS-SANTOS, M. S. **Produção de biodiesel com *Salvinia auriculata* cultivada com biossólidos ou vinhaça**. In: Maziero, R. (Org). Engenharias: Tendências e Inovações. Editora Synapse, p. 117-127, 2021.

PAI, A.; PAUL, P.; KINI, C.; YADAV, A.; MALLADI, A. A Brief study on biodiesel production using oleaginous hydrophytes and waste cooking oil with physico-chemical characterization. **Journal of Advanced Research in Fluid Mechanics and Thermal Sciences**, v. 67, (1), 43-51, 2020.

PATARRA, R. F.; LEITE, J.; PEREIRA, R.; BAPTISTA, J.; NETO, A. I. Fatty acid composition of selected macrophytes. **Natural Product Research**, v. 27, n. 7, p. 665-669, 2013.

ROZENTSVET, O. A.; REZANKA, T.; BOSENKO, E. S.; UZHAMETSKAYA, E. A.; DEMBITSKII, V. M. Fatty acids, phospholipids, and the betaine lipid DGTS from the aquatic fern *Salvinia natans*. **Chemistry of Natural Compounds**, v. 41, n. 5, p. 487-490, 2005.

SALEHZADEH, A.; NAEEMI, A. S.; ARASTEH, A. Biodiesel production from *Azolla filiculoides* (Water Fern). **Tropical Journal of Pharmaceutical Research**, v. 13, n. 6, p. 957-960, 2014.

TANAMATI, A. A. C.; TANAMATI, A.; GANZAROLI, J. F.; SANCHEZ, L.; SILVA, M. V. Estudo comparativo de métodos de extração de lipídios totais em amostras de origem animal e vegetal. **Brazilian Journal of Food Research**, v. 1, n. 2, p. 73-77, 2010.

TIR, R.; DUTTA, P.C.; BADJAH-HADJ-AHMED, Y. Effect of the extraction solvent polarity on the sesame seeds oil composition. **European Journal of Lipid Science and Technology**, v. 114, n. 12, p. 1427-1438, 2012.

VIDAL, F. Produção e mercado de etanol. **Caderno Setorial ETENE**, v. 5, p. 1-10, 2020.

VIÊGAS, C. V.; MENDES, L. B. B.; JOÃO, R. R.; DÍAZ, G. C.; ARANDA, D. A. G.; CRUZ, Y. R. Quantification of lipid content and identification of the main lipid classes present in microalgae extracts *Scenedesmus sp.* for obtaining fatty compounds for biofuel production. **Energy and Power Engineering**, v. 12, n. 6, p. 273-287, 2010.