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RECOVERY OF PHENOLIC COMPOUNDS FROM *Arthrospira (Spirulina) platensis*: AN APPROACH ON GREEN EXTRACTION THROUGH INNOVATIVE ALTERNATIVE TECHNIQUES FOR FOOD APPLICATION

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ABSTRACT

Arthrospira (Spirulina) platensis has attracted growing interest due to its ability to produce bioactive compounds such as polyphenols. However, their profitable recovery is currently a hot investigation topic, which has been remained a challenge due to the inherent limitations of various conventional extraction methods. The aim of this study was to select the most suitable extraction method to recover phenolic compounds and antiradical power (ARP) from A. platensis biomass. For this purpose, green extraction techniques, namely ultrasound-assisted, microwave-assisted and high pressure/temperature extractions, were compared with classic solid–liquid extraction. The extracts were characterized in terms of total polyphenols yield (TP) and the ARP. This study demonstrated that HPTE is the most efficient method to recover high-ARP phenolic-rich extracts from A. platensis biomass using green solvent (ethanol); therefore, may be used as a potential source of natural antioxidants to formulate functional foods or prepare dietary supplements, being a safe alternative to synthetic compounds.

1. INTRODUCTION

Arthrospira (Spirulina) platensis has attracted growing interest due to its ability to produce high-added value compounds such as proteins, vitamins, minerals, essential fatty acids, pigments and polyphenols (Silva et al., 2016; Esquivel-Hernández et al., 2017a). These latter compounds are an important class of secondary metabolites that can be used to enhance the nutritional value of foods by addition of natural substances with important beneficial attributes that confer health-promoting properties, including anti-inflammatory, antiviral, anticancer, antibacterial and antioxidant activities. However, their profitable recovery is currently a hot investigation topic, which has been remained a challenge due to the inherent limitations of various conventional extraction methods (Esquivel-Hernández et al., 2017b; Esquivel-Hernández et al., 2017a).

Innovating "green" extraction approaches are currently investigated by several research groups to improve the recovery of bioactive metabolites for further application (Paini et al., 2016; Esquivel-Hernández et al., 2017a). Among them, pressurized liquid (PLE), microwave-assisted (MAE) and ultrasound-assisted (UAE) extractions have been reported to enable faster heat and mass transfer, reduction in solvent consumption, savings in working time, yield increase and higher extract/product quality (Esquivel-Hernández et al., 2017b). High-pressure/temperature extraction (HPTE) in stirred reactor is one of the emerging technologies investigated and compared in this study with conventional solid-liquid extraction (SLE), MAE and UAE. Recently, HPTE has been successfully applied to the extraction of phenolic compounds from different matrices such as grape marc and olive pomace (Paini et al., 2016). To the best of our knowledge, there was no previous report on the production of phenolic-rich ethanolic extracts of *A. platensis* with high antioxidant activity by HPTE. Based on such a background, the aim of this study was to select the most efficient extraction method to recover phenolic compounds and antiradical power (ARP) from *A. platensis* biomass.

2. MATERIAL AND METHODS

2.1. Microorganism and Culture Conditions

Arthrospira (Spirulina) platensis UTEX 1926 (University of Texas Culture Collection, Austin, TX, USA) was grown in a 3.5 L-horizontal tubular photobioreactor at 100 ± 5 µmol photons m^{-2} s^{-1} in the Schlösser medium (Schlösser, 1982). Cultivations were carried out as previously described (Silva et al., 2016), and at the end of the exponential phase (about 9 days of cultivation), the cells were centrifuged (ALC 4226, Milan, Italy) at 6000×*g* for 10 min, and then lyophilized for later analysis.

2.2. Extraction Process

Polyphenols were extracted from dried biomass of *A. platensis* (2.0 g ± 0.1 g, except those at high pressure and temperature [3.0 ± 0.1 g]) using green solvent (ethanol) with a constant solid/liquid ratio of 1:10 (w/v). Extraction tests were performed with the following methods: *Conventional solid–liquid extraction* (SLE). The extraction was performed for 19 h in dark conditions on a magnetic stirrer (model Mr. 3001; Heidolph, Kelheim, Germany) at room temperature; *Microwave-assisted extraction* (MAE). A microwave multimode oven operating at 2.45 GHz (MicroSYNTH Milestone, Sirisole, Italy) was used. The extraction was conducted at 110 °C under nitrogen atmosphere and microwave irradiation (60 W) for 60 min; *Ultrasound-assisted extraction* (UAE). This extraction was carried out at 25 °C, 21.5 kHz and 60 W for 15 min in an ultrasonic bath (model UTA 90; FALC, Treviglio, Italy); *High pressure/temperature extraction* (HPTE) in stirred reactor (model 4560; PARR Instrument, Moline, IL, USA). The extraction was performed at 90 °C for 90 min under nitrogen atmosphere (Aliakbarian et al., 2011). After the extraction, all samples were then centrifuged as described above, and the supernatant was stored at –20 °C for further analyses.

2.3. Analytical Methods

A. platensis extracts obtained by SLE, MAE, UAE and HPTE were analyzed in terms of total polyphenol yield (TP) using the Folin–Ciocalteu colorimetric assay (Swain & Hillis et al., 1959) as well as the antiradical power (ARP), which was measured according to their ability to scavenge $ABTS^{\bullet+}$ radical cation, as described by Re et al. (1999). All the analysis were carried out in triplicate (n = 3).

3. RESULTS AND DISCUSSION

The TP in *A. platensis* ethanolic extracts was the highest (3.32 ± 0.08 $mg_{GAE}\ g_{DB}^{-1}$) when using HPTE, followed by MAE (3.04 ± 0.08 $mg_{GAE}\ g_{DB}^{-1}$) and UAE (2.07 ± 0.01 $mg_{GAE}\ g_{DB}^{-1}$) (Fig. 1). On the other hand, the conventional SLE showed the worst performance (1.61 ± 0.07 $mg_{GAE}\ g_{DB}^{-1}$) when compared with all the selected techniques, likely due to degradation caused by exposition for prolonged time (19 h) at room temperature (Casazza et al., 2012). As expected by the well-known antioxidant activity of many polyphenols, the ARP showed a qualitatively similar trend as TP, in that the *A. platensis* extract obtained by HPTE exhibited the highest value (58.30 ± 0.12 $\mu mol_{Trolox}\ g_{DB}^{-1}$), followed by MAE (39.22 ± 0.75 $\mu mol_{Trolox}\ g_{DB}^{-1}$), UAE (29.85 ± 0.39 ($\mu mol_{Trolox}\ g_{DB}^{-1}$) and SLE (22.37 ± 0.42 $\mu mol_{Trolox}\ g_{DB}^{-1}$). These results suggest that the optimal stirring conditions and inert atmosphere (Casazza et al., 2012) as well as the higher pressure in the reactor chamber (Paini et al., 2016), which can enhance cell matrix disruption, may have been the main reasons of the best HPTE performance, compared with the other extraction methods. In conclusion, this study demonstrates the potential of this emerging technology as a promising alternative technique to obtain high-ARP

phenolic-rich ethanolic extract from *A. platensis*. Therefore, it may serve as a potential source of natural antioxidants to formulate functional foods or prepare dietary supplements. This work may be considered a useful starting basis for future advance in the field of recovery of high-added value compounds from cyanobacterial biomass.

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