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# A NEW APPROACH FOR THE RECOVERY OF POLYPHENOLS FROM *Arthrospira platensis*: ADVANCEMENT OF GREEN PROCESS THROUGH HIGH PRESSURE/TEMPERATURE EXTRACTION

**Milena F. da Silva[1], Alessandro A. Casazza[2], Pier F. Ferrari[2], Bahar Aliakbarian[2], Attilio Converti[2], Raquel P. Bezerra [3], Ana Lúcia F. Porto[3], and Patrizia Perego[2]**

[1] Federal University of Pernambuco, Bioscience Center
[2] University of Genoa, Department of Civil, Chemical and Environmental Engineering (DICCA)
[3] Federal Rural University of Pernambuco, Department of Morphology and Animal Physiology

E-mail: milen_sil@hotmail.com

**ABSTRACT**

*Arthrospira platensis has attracted attention due to its ability to produce valuable compounds such as polyphenols. However, the setting up of efficient methods for their profitable recovery are currently hot investigation topics, which has been recognized as a big challenge. This work was aimed to study the recovery of phenolic compounds from A. platensis biomass in hydroalcoholic solutions and the antiradical power (ARP) by high pressure/temperature extraction (HPTE) in stirred reactor. This study demonstrated that HPTE is an efficient green extraction process to obtain high-ARP phenolic-rich hydroalcoholic extracts from A. platensis biomass; therefore, may be used as a potential natural source of bioactive compounds to formulate new functional foods or prepare dietary supplements.*

## 1. INTRODUCTION

*Arthrospira platensis* has attracted worldwide attention due to its ability to produce valuable compounds such as proteins, vitamins, minerals, essential fatty acids, pigments and polyphenols (Ferrari et al., 2015; Silva et al., 2016; Esquivel-Hernández et al., 2017). These latter compounds, are an important class of secondary metabolites that can, in fact, 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 (Rodríguez De Marco et al., 2014; Ferrari et al., 2015). However, the development new green extraction processes for their profitable recovery is currently a hot

investigation topic, which has been recognized as a big challenge (Esquivel-Hernández et al., 2016; Esquivel-Hernández et al., 2017).

Innovating extraction approaches are currently investigated by several research groups (Shabana et al., 2017; Esquivel-Hernández et al., 2017) to improve the recovery of bioactive metabolites for further application. Among them, high-pressure/temperature extraction (HPTE) in stirred reactor is one of the emerging technologies most effective. Recently, HPTE has been successfully applied to the extraction of phenolic compounds from different matrices such as barley grains (Bucić-Kojić et al., 2015), grape marc and olive pomace (Paini et al., 2016). To the best of our knowledge, this is the first study where HPTE was used to recover phenolic compound with high antioxidant activity from *A. platensis* hydroalcoholic extracts.

Based on such a background, the aim of this study was to select the most suitable conditions to recover phenolic compounds and antiradical power from *A. platensis* biomass in hydroalcoholic solutions by HPTE using RSM.

## 2. MATERIAL AND METHODS

### 2.1. Microorganism and culture conditions

*Arthrospira 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 7500 rpm for 10 min, then lyophilized for later analysis.

### 2.2. Extraction Process

Phenolic compounds were extracted from dried biomass of *A. platensis* (3.0 ± 0.1 g) using different extraction temperature ($90 \leq T \leq 180$ °C) and ethanol concentration in hydroalcoholic solution (20, 60 and 100% v/v) with a constant solid/liquid ratio of 1:10 (w/v). Extraction tests were performed with High pressure/temperature extraction (HPTE) using an agitated reactor at fixed time (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* extract obtained by HPTE was analyzed in terms of total polyphenol yield (TP) using the Folin–Ciocalteu colorimetric assay (Swain & Hillis et al., 1959). TP was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry biomass ($mg_{GAE}\, g_{DB}^{-1}$). The antiradical power (ARP) of the extracts was measured according to their ability to scavenge $ABTS^{\bullet+}$ radical cation, as described by Re et al. (1999). The results were expressed as micromoles of Trolox equivalent per gram of dry biomass ($\mu mol_{Trolox}\, g_{DB}^{-1}$). All analyses were performed in triplicate.

### 2.4. Experimental design and statistical analysis

A $3^2$-full factorial experimental design combined with Response Surface Methodology (RSM) was applied to evaluate the effects of the independent variables, namely extraction temperature and

solvent (ethanol) concentration (Sc) in the hydroalcoholic solution on the responses, namely TP, TF and ARP. The Statistica v. 8.0 software (StatSoft, Tulsa, OK, USA) was used for data analysis. The results were considered statistically significant at $p$ values < 0.05.

## 3. RESULTS AND DISCUSSION

The experimental results of HPTE tests combined with RSM in hydroalcoholic extracts obtained from *A. platensis* biomass revealed that both T and Sc significantly influenced ($p < 0.05$) TP and ARP. The most suitable condition to efficiently recover TP (26.00-28.04 $mg_{GAE}\ g_{DB}^{-1}$) was achieved at the highest temperature (180 °C) and Sc in the range 20-60%, respectively, while a different condition was observed for ARP (69.02 $\mu mol_{Trolox}\ g_{DB}^{-1}$), which was favored by simultaneous decreases of both independent variables as well as kept always high whole tested temperature range 90 ≤ T ≤ 135 ºC, almost irrespective of Sc. As previously suggested (Aliakbarian et al., 2011; Paini et al., 2016), these optimal results at high temperature in HPTE may have improved the extraction efficiency due to decreases in solvent viscosity and surface tension, leading to rapid solvent penetration and enhancement of the disruption of the strong solute–matrix interactions (hydrogen bonds, dipole attractions and van der Waals forces).

It is noteworthy that, the presence of water in the hydroalcoholic solution led to higher TP values, when compared with pure ethanol as an extraction solvent. This behavior may be explained not only by the high solubility of some phenolic classes in polar solvents, due to their generally polar nature (Goiris et al., 2012), but also by the high pressure in the reactor chamber that likely prevented solvent boiling at the extraction temperature, thus enhancing cell matrix disruption. In addition, when Ferrari et al. (2015) used HPTE at 180 °C for 90 min and 100% water as solvent to recover polyphenols from *A. platensis* biomass, TP was lower (13.51 $mg_{GAE}\ g_{DB}^{-1}$) than that obtained in this work at the same temperature and extraction time. These findings suggest that, although some water is necessary to perform highly effective HPTE, it is unavoidable also the presence of a polar organic solvent to this purpose.

## 4. CONCLUSIONS

The HPTE proved to be an efficient method to recover total polyphenols and antiradical power from *Arthrospira platensis* biomass using binary mixtures of green solvents (ethanol/water). This study demonstrates the potential of HPTE as a promising green extraction process to obtain high-ARP phenolic-rich hydroalcoholic extracts from *A. platensis*, therefore it may be used, after additional purification steps, as natural source of compounds to formulate functional foods or prepare dietary supplements. Furthermore, this work opens new avenues in the use of this emerging technology, for the recovery of high-added value compounds from cyanobacterial biomass.

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