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Microwave-Assisted Alginate Extraction from Portuguese *Saccorhiza polyschides* – Influence of Acid Pretreatment

Mário Silva, Filipa Gomes, Filipa Oliveira, Simone Morais, Cristina Delerue-Matos

***Abstract*—**Brown seaweeds are abundant in Portuguese coastline and represent an almost unexploited marine economic resource. One of the most common species, easily available for harvesting in the northwest coast, is *Saccorhiza polyschides* grows in the lowest shore and costal rocky reefs. It is almost exclusively used by local farmers as natural fertilizer, but contains a substantial amount of valuable compounds, particularly alginates, natural biopolymers of high interest for many industrial applications.

Alginates are natural polysaccharides present in cell walls of brown seaweed, highly biocompatible, with particular properties that make them of high interest for the food, biotechnology, cosmetics and pharmaceutical industries. Conventional extraction processes are based on thermal treatment. They are lengthy and consume high amounts of energy and solvents. In recent years, microwave-assisted extraction (MAE) has shown enormous potential to overcome major drawbacks that outcome from conventional plant material extraction (thermal and/or solvent based) techniques, being also successfully applied to the extraction of agar, fucoidans and alginates. In the present study, acid pretreatment of brown seaweed *Saccorhiza polyschides* for subsequent microwave-assisted extraction (MAE) of alginate was optimized. Seaweeds were collected in Northwest Portuguese coastal waters of the Atlantic Ocean between May and August, 2014. Experimental design was used to assess the effect of temperature and acid pretreatment time in alginate extraction. Response surface methodology allowed the determination of the optimum MAE conditions: 40 mL of HCl 0.1 M per g of dried seaweed with constant stirring at 20ºC during 14h. Optimal acid pretreatment conditions have enhanced significantly MAE of alginates from *Saccorhiza polyschides*, thus contributing for the development of a viable, more environmental friendly alternative to conventional processes.

***Keywords*—**Acid pretreatment, Alginate, Brown seaweed, Microwave-assisted extraction, Response surface methodology.

I. Introduction

PORTUGUESE coastline has an extension of over 800 km, representing one of the most important and underexploited richness of the country. Marine economic activities represent a little over 2% of the gross domestic product (GDP) and incomes are almost entirely originated by the fishing industry. In recent years, strategic guidelines and policies have been developed to invert this situation but the advances are yet insufficient. Seaweeds are abundant in the Portuguese coastline, where Green (Chlorophyta), red (Rhodophyta) and brown (Phaeophyta) macroalgae offer the opportunity to find a wide range of natural compounds with remarkable properties and high interest for many applications. Portugal algal flora is characterized by around 250 species of Rhodophyta, 100 of Phaeophyta and 60 of Chlorophyta [1]. Seaweeds are potentially good sources of minerals, polysaccharides, lipids, amino acids, antioxidants and certain vitamins, thus representing a bio-resource of high interest. Brown algae are one of the groups of seaweed with higher importance. They are rich in polysaccharides, primarily alginates, which may reach up to 40% of the seaweed's dry weight [2]. *Saccorhiza polyschides* is a large brown seaweed commonly found in European coastlines in the lowest shore or subtidal rocky reefs, and one of the most abundant in Portuguese northwest coastal waters. It has an annual life cycle, with sporophytes growing very quickly during spring and summer and a strong decay in autumn, before being detached by winter storms [3].

Alginates are polysaccharides composed mainly of linear polymers of β-(1-4)-D-mannuronic (M) and α-L-guluronic (G) acids. The sequence of monomers and the M:G ratio differs widely between algae species giving them different properties. Because of their colloidal properties alginates are widely used in food processing, biotechnology industry as well as in numerous applications in the pharmaceutical industry [4]. The alginate of greatest industrial importance is the sodium salt. The chemistry of the processes used to make sodium alginate from brown seaweeds is relatively simple. The difficulties of the processes arise from the physical separations which are required, such as the need to filter slimy residues from viscous solutions or to separate gelatinous precipitates which hold large amounts of liquid within their structure [5]. Alginates exist in brown seaweed mainly in the calcium salt form and smaller amounts of magnesium, potassium and sodium salts [5], [6]. Typically, sodium alginate production processes from brown seaweeds fall into two different categories that differ from each other essentially in the intermediates formed during the extraction process: in one, the principal intermediates are calcium alginate and alginic acid. In the other, no calcium alginate is formed, only alginic acid [5]. The first process is usually preferred as the obtained calcium alginate is more easily processed through industrial filtering systems while alginic acid forms an insoluble gel that simply plugs any type of filter [7]. Regardless of the used process, it usually contains

Mário Silva, Filipa Gomes, Simone Morais and Cristina Delerue-Matos are with REQUIMTE, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072 Porto, Portugal (corresponding author Simone Morais, phone: +351228340500, e-mail: sbm@isep.ipp.pt).

Filipa Oliveira is with WeDoTech, Companhia de Ideias e Tecnologias, Lda., CiDEB, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal.

the following 5 steps: size reduction of raw material to make processing easier, acid pretreatment to break cell walls and convert and convert alginate salts into alginic acid, alkaline extraction that converts alginic acid into sodium alginate and solubilizes it, separation of seaweed residues and precipitation and drying of solubilized sodium alginate, step where intermediates may differ, as original extract might be precipitated as calcium alginate or alginic acid [5]. While alkaline extraction is usually carried out under very similar conditions, 2% $CaCO_3$ (pH 10) at 80ºC, independently of the species of seaweed used as raw material and author, acid pretreatment varies greatly both with used seaweed species and author [6]-[10].

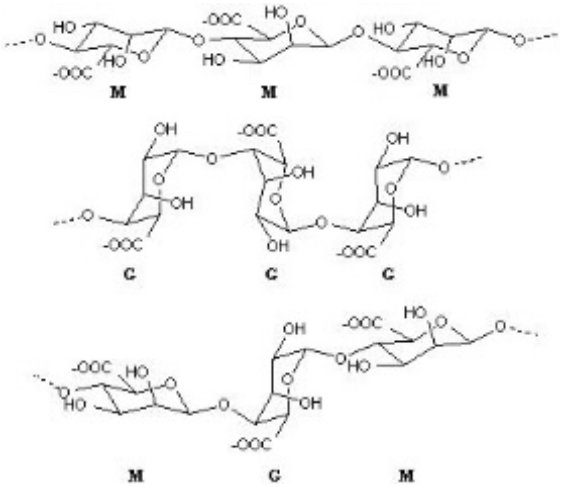

Fig. 1 Structures of Alginate Chains in Regard to Their Linked Monomers [11]

In recent years, the use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Conventional techniques for the extraction of active constituents are time and solvent consuming, and the effective extraction of numerous interest constituents in plant material is limited by the extraction step. High and fast extraction performance ability with less solvent consumption, and protection offered to thermal unstable constituents are some of the attractive features of this new promising microwave assisted extraction (MAE) technique [12]. MAE has been applied successfully to isolate plant material. Although being an important extraction technique, only few works reported the use of microwave technology for extraction of polysaccharides (i.e. agar and fucoidans) from seaweeds [13], [14] and, to the best of our knowledge, only a previous work from our research group [15] focuses on the extraction of alginates from the brown seaweed species *Saccorhiza polyschides*. In this study microwave operating conditions for alginate extraction were optimized and yield of extraction was reported to be of 11.3% referred to dry mass. Typical alginate content of *Saccorhiza polyschides* has been reported to be in a range of 22%-25% [16]. Thus the aim of this study was to optimize the acidic pretreatment (prior to MAE) for the extraction of alginate from the brown algae species *Saccorhiza polyschides* and evaluate the influence of the seaweed's age in its alginate content. Acid concentration, pretreatment time, and temperature were the operational parameters evaluated to maximize the alginate extraction.

II. Materials and Methods

A. Reagents

Analytic grade reagents were employed in this study. Hydrochloridric acid (35%-37%, Panreac, Barcelona, Spain), anhydrous calcium carbonate (assay > 99.5%, Merck, Darmstadt, Germany) and calcium chloride.$2H_2O$ (Panreac, Barcelona, Spain) were used. Ultra-pure water (18.2 MΩ cm) was produced by a Simplicity 185 apparatus (Millipore, Molsheim, France).

B. Seaweed Sampling and Processing

Saccorhiza polyschides was collected in coastal waters of the Atlantic Ocean, at beach of Angeiras in the NW region of Portugal (41º16'06.16''N, 08º43'39.33''W), from May 2014 until August 2014. Seaweeds were subject to a thorough visual observation in search of peeling or fractures and damaged seaweeds were rejected in sight. Live specimens plucked from rocky reefs were preferred. Seaweeds were then transported to the laboratory, washed abundantly with tap water to remove sand and incrustations, and then with deionized water and their holdfasts were rejected. They were then dried until constant weight (approximately 96 h) at 60°C, chopped into small pieces, grinded to dimensions of < 2 mm and kept in dry and dark environment until processing.

C. Conventional Acid Pretreatment

This procedure was based on the work of H. Andriamanantoanina et al. [10]. 1 g of dry seaweed was mixed with 40 mL of deionized water and pH was adjusted to 4.0 with hydrochloric acid 1 M with constant stirring for 15 minutes. Seaweeds were then filtered and washed with deionized water. The procedure was repeated once more and afterwards the same acid conditions were imposed and the mixture was left overnight with constant stirring. Subsequently the liquid was removed by filtration and the seaweed was rinsed with deionized water. Finally, deionized water was added (25 mL) to the acidified seaweed and the pH was adjusted to 10 with Na_2CO_3 10% (w/v).

D. Optimization of the Acid Pretreatment

Conventional acid pretreatment was employed to every harvest of seaweeds and preliminary tests were carried out with different values of temperature and time for the acid pretreatment, varying each parameter at a time. The optimization of the sodium alginate extraction acid pretreatment was performed using response surface methodology (RSM) according to Montgomery et al. [17]. 1 g of dry seaweed from July's 2014 harvest was mixed with 40 mL of hydrochloric acid 0.1 M. With constant stirring, different tests were carried out at maximum, minimal and center positions of time and temperature (-1; +1; 0), which correspond to times of 0.5 h, 14 h and 7.25 h and temperatures

of 20 ºC, 40 ºC and 30 ºC. MAE was performed afterwards as described in "*Microwave-Assisted Extraction"*. Operating conditions and limits for the optimization were selected based on preliminary tests results and literature information.

RSM procedure was applied to obtained data. RSM is a powerful and useful tool for modeling and analyzing a response of interest, which is influenced by several variables, allowing also to assess, not only individual variable influences, but also the effect of their interactions. The objective is to optimize this response or determine the region that satisfies the operating specifications [17]. This procedure implies the development of a mathematical model fitting experimental data with subsequent employment of numerical optimization techniques to obtain optimum values for the variables with statistically significant influence on the measured response, usually tested experimentally. In most cases, the real relation between the response and the independent variables is unknown [18]. Therefore obtained models should be used with caution and best adjusted functions, linear or polynomial should never be used outside studied ranges.

E. Microwave-Assisted Extraction

Microwave-assisted extractions were performed with a MARS-X 1500 W (Microwave Accelerated Reaction System for Extraction and Digestion, CEM, Mathews, NC, USA) configured with a 14 position carousel. The operational parameters of the MAE apparatus applied were the following: temperature of 100 ºC for 20 minutes, magnetron power 100%, time to reach settings 10 min and medium stirring speed. During operation, both temperature and pressure were monitored in a single vessel (control vessel). Magnetic stirring in each extraction vessel and a sensor registering the solvent leaks in the interior of the microwave oven were also utilized.

F. Sodium Alginate Isolation and Processing

The alginate soluble fraction was precipitated with CaCl2 10% (w/v) and collected by filtration with gauze.

The conversion of calcium alginate into alginic acid was performed by several washes with HCl 1 mol/L. The fibers of alginic acid were treated with ethanol/H2O (1:1, v/v) and the pH was adjusted to 8 with Na2CO3 10% (w/v) in order to obtain sodium alginate. This procedure intended to replicate as closely as possible the most common industrial process for alginate production, where the calcium alginate "path" is preferred for separation, isolation and production of sodium alginate [5], [7].

The collected sodium alginate was dried by lyophilization at -50 ºC and the yield (mass of alginate/mass of algae, %) was calculated on a dry weight basis.

III. Results and Discussion

A. Influence of Sample Storage

In order to characterize the influence of the time of algae storage (0-97 days for May's samples, 0-68 days for June's samples, 0-40 days for July's samples and 0-33 days for August's samples) in the alginate yield and reproducibility, extractions were performed after conventional acid pretreatment. Obtained yields of alginate ranged between 9.0%-10.7% for May's seaweeds, 9.3%-11.2% for June's seaweeds, 9.5%-10.2% for July's seaweeds and 10.9%-11.2% for August's seaweeds. Storage conditions (dry and dark environment at room temperature) did not influence significantly the extraction performance. It seems that the obtained results are mainly dependent on the extraction conditions.

B. Optimization of Acid Pretreatment Conditions

Preliminary tests were conducted. Seaweed harvested in May 2014, June 2014 and July 2014 were submitted to the conventional acid pretreatment and to 40 mL of HCl 0.1 M per g of dry seaweed at 20ºC for 2 h. Increased yields of extraction were obtained for all seaweed samples with the second treatment, with values of 22.8% vs 17.8% to May's harvest, 23.4% vs 10.5% for June's harvest and 23.8% vs 7.5% for July's harvest.

In the preliminary tests, highest yields of extraction were obtained from *Saccorhiza polyschides* harvested in July and so this sample of seaweed was selected to conduct the optimization study. A 2^2 experimental design with temperature (T) and time (t) as input variables was built, with center point (0) tests with 3 replicates. The main objective of RSM is to assess the influence of inlet variables and their interactions in a measured response, thus allowing its optimization or determine the region that satisfies the operating specifications. The model was developed considering the factors -1, 0 and +1 for the two parameters. Alginate yield ranged from 20.8% to 26.4%, with the maximum results being obtained at point t=1; T=-1. At center point (0,0) the mean yield of extraction was 24.6% with a standard deviation of 0.4%. The equation that describes the model for the considered parameters in their studied range is the following:

$$\eta\,(\%) = 24.6 + 1.88 \times t - 0.93 \times t \times T \quad (1)$$

Statistical analysis of the model showed that the studied range of temperature has no significant effect on the extraction yield. The model also suggests the combined effect of time and temperature of the acid pretreatment to have a negative impact on yield of extraction. High temperatures, usually indicated of over 60ºC, have been described to dramatically degrade alginates, which corroborates the observed negative impact of interaction between time and temperature on extraction yield. Although maximum operating temperature was 40ºC, it is a logical assumption that this value combined with long lasting treatments, might degrade some amount of alginate. On the other hand, the model suggests a positive effect of time on the extraction yield. This might be due to a more effective break of seaweed's cell walls that facilitates the extraction of alginates. Optimal operating conditions for acid pretreatment were determined to be a temperature of 20ºC, time of 14 h and 40 mL of HCl 0.1 M / g of dried seaweed. With these conditions alginate yields obtained from *Saccorhiza polyschides* are of the same magnitude as those

described by Perez et al .[16] as expected for this species.

C.Acid Pretreatment Method Comparison and Seasonality

MAE of alginate was then carried out in all harvested samples submitting them to the previously described conventional acid pretreatment and to optimized conditions. This comparison aimed to obtain data between both procedures performed with short time difference and to evaluate if significant yield of alginate variations were observed in *Saccorhiza polyschides* that might outcome from its natural life cycle.

An increase in Yield of alginate extraction from Saccorhiza polyschides submitted to optimal acid pretreatment conditions was observed in every seaweed sample tested. Increases ranged from 100% in August's harvest to 143% in July's harvest, against values obtained with conventional acid pretreatment.

Results clearly show a strong influence of acid pretreatment on MAE of alginates. Extractions from every harvested seaweed submitted to optimal acid pretreatment conditions, produced yields of over the double of the ones obtained with conventional acid treatment.

It is not possible to observe a trend in yield of extraction as to the harvest date. Seaweeds used in this study cover a period of time of approximately four month and, although significant morphological differences were observed between different harvests, their alginate content appears not to vary significantly.

IV. Conclusions

Optimal acid pretreatment conditions for MAE of alginates from brown seaweed *Saccorhiza polyschides* were investigated. In the studied range of values, optimal treatment was determined to be a mixture of 40 mL of 0.1 M HCl with 1 g of dried seaweed under constant stirring at a temperature of 20ºC during 14h. The extraction yields obtained under these conditions are clearly better than the obtained under the "conventional" acid pretreatment and reach the values described in literature as expected for the tested species of brown seaweed, showing that the MAE alginate extraction is greatly influenced by the conditions of acid treatment of raw materials. During the period of four months covered by the present study, no significant modification was observed in alginate content of *Saccorhiza polyschides*.

Higher processing speeds and lower solvent consumption are the main advantages of microwave-assisted alginate extraction. In the present study, the acid pretreatment of brown seaweed *Saccorhiza polyschides* for posterior MAE if alginates was optimized and will hopefully contribute for the implementation in large scale of this process.

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