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Chemical composition and functional properties of dietary fibre extracted by Englyst and Prosky methods from the alga *Ulva lactuca* collected in Tunisia

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ABSTRACT

Nowadays there is a growing trend to find new sources of dietary fibre (DF), such as marine algae by-products that have traditionally been undervalued. In this respect, the aim of the present investigation was firstly to compare two methods of dietary fibre quantification (Englyst and Prosky) and secondly to determine the chemical composition and some of the functional properties of total and insoluble fibres extracted in accordance with the Englyst method. The dietary fibres of dried *Ulva lactuca* collected from the Tunisian littoral were determined by the Prosky (gravimetric method) and Englyst (enzymatic-chemical method) methods. The two extraction methods (Englyst–Prosky) provided approximately the same values in total fibres (~54%). However, they had different insoluble and soluble fibre contents. *U. lactuca* contained 20.53% and 31.55% of soluble fibres and 34.37% and 21.54% of insoluble fibres using the Prosky and Englyst methods, respectively. The fractionation of the insoluble dietary fibre concentrate revealed that hemicellulose was the most abundant fraction (32.49%), followed by cellulose (16.59%) and "lignin-like" compounds (1.53%). For both fibre concentrates, the main neutral sugar was glucose (20.70%–27.59%), which corresponded to hemicellulose and cellulose. The water holding capacity of insoluble fibre concentrate was relatively high. It varied between 9.32 g and 10.3 g of water/g of dry fibre at 25 °C and 80 °C, respectively. Nevertheless, the oil holding capacity of the insoluble fibre concentrate was not affected by temperature. It was about 1.08 and 1.01 g of oil/g of dry fibre at 25 °C and 80 °C. Despite the significant functional properties of fibre concentrate, the presence of lead limits its use as an ingredient in the food industry.

1. Introduction

Ulva lactuca is a marine green alga. It represents an important biomass in eutrophic areas and can cause economic and ecological problems. The biomass of this alga is generally gathered and disposed of despite its utility in making compost [1].

This algal species has traditionally been consumed in Asia and recently approved for human consumption in European countries as a vegetable. Among the nutritional benefits of this edible alga is its high percentage of dietary fibre composed of polysaccharides which are resistant to endogenous human digestive enzyme. These food polymers could reduce the incidence of diseases such as obesity, diabetes, cancers and cardio-vascular diseases [2,3]. These fibres are composed of soluble and insoluble fibres.

Soluble fibres such as ulvan showed several physicochemical and biological properties [4,5]. Notably, soluble fibres in *Ulva* could form unusual soft gels in the presence of borate and calcium ions [1,6].

The insoluble polysaccharides derived from *U. lactuca* can form up to 33% (dry weight) of this alga. It has been used for various applications such as paper making [1].

A noticeable disadvantage is that some algae exhibit a high affinity for metals [7]. Indeed, in a recent research, Yaich et al. [8] have shown that *U. lactuca* collected from the littoral between Taboulba and Sayada had high content of metals especially manganese (Mn), lead (Pb), copper (Cu) and cadmium (Cd) compared to the algae used for human consumption in the European Union. For this reason, the alga can be valorised through the extraction of the specific compounds, particularly the total, soluble and insoluble dietary fibres. However, the polysaccharide has to be purified to avoid the presence of high content of inorganic material in the final product. In fact, the dietary fibres with high purity levels can have a very high added-value as functional ingredients.

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In light of the literature, only the insoluble fibres, which were extracted either through the gravimetric method of Prosky et al. [9], or after its submission to some modifications [2,10–12], were used in order to determine their chemical and functional properties. However, the characterisation of fibre extracted in accordance with the Englyst procedure [13] (enzymatic–chemical method) has not been studied. According to the authors, the chemical composition of algae varies with species, habitats, maturity and environmental conditions [3,14]. Furthermore, it was important to compare the content and the characteristics (chemical composition, functional properties) of algal fibre from the Tunisian littoral to those of algal polysaccharide from different areas in the world.

In the present study, the fibre content of *Ulva* was quantified and compared using two different methods. They were the Englyst procedure (enzymatic–chemical method) and the Prosky method (gravimetric method). In addition, the chemical composition (uronic acids, neutral sugars, amino acids, fatty acids, ash) of the total and the insoluble dietary fibre extracted according to the Englyst method was investigated. Finally, the effect of temperature on functional properties such as water holding capacity, swelling capacity and oil holding capacity of insoluble fibre was studied.

2. Materials and methods

2.1. Sample collection

A total of 60 kg of wet weight of *U. lactuca* was collected by hand from the beach in the littoral zone between the areas of Taboulba and Sayada, Monastir-Tunisia (35°40 08.8 N, 10°54 30.3 E), in July 2007. This site is characterised by shallow depths (0.5 m) and high nutrient levels. The algae were exhaustively rinsed immediately with seawater to remove sand and shell debris. Upon arrival at the laboratory, the algal samples were washed again with distilled water and manually sorted to remove epiphytes. The total and homogeneous samples were dried in continuous air flow (35 °C, 72 h). The dried alga was then milled in a mechanical grinder for 5 min to obtain a fine and homogeneous powder. The powder was stored in hermetic bags in a dry and dark area at room temperature (25 °C) until use.

2.2. Extraction and quantification of dietary fibre

2.2.1. Englyst method

The total and insoluble dietary fibres were quantified and extracted from the *U. lactuca* algal powder according to the method of Englyst et al. [13].

Two portions, A and B, of each sample (500 mg of algal powder) were needed to obtain separate values for total, insoluble and soluble dietary fibres. Portion A was used to measure the total dietary fibre. Portion B was used to measure the insoluble dietary fibre. The soluble fibre represented the difference between total and insoluble fibres. The extraction was carried out as shown in Fig. 1 [13].

2.2.1.1. Preparation of reagents

2.2.1.1.1. Enzyme solution 1. 2.5 ml of Termamyl (Novo Nordisk Bioindustries, Farnham, Surrey, UK) was diluted to 200 ml with sodium acetate buffer (0.1 mol/ml, pH 5.2).

2.2.1.1.2. Enzyme solution 2. The quantity of 1.2 g of protease (Paynes and Byrne, Greenford, Middlesex, UK) was dissolved in 12 ml of water. The solution was mixed for 10 min with a magnetic stirrer. The obtained mixture was centrifuged for 10 min at 1500 g. After its centrifugation, 2.5 ml of pullulanase (Promozyme, Novo Nordisk, Bioindustries) was added to 10 ml of the supernatant. After that the mixture was stirred [13].

2.2.1.2. Dispersion and enzymatic hydrolysis. 500 mg of algal powder was dispersed in 2 ml of dimethyl sulphoxide (DMSO). The obtained mixture was left in a boiling water bath for 30 min. The enzymatic treatment was performed by adding 8 ml of enzyme solution 1 and placing the mixture in a boiling water bath for 10 min. After cooling, 0.5 ml of enzyme solution 2 was added to each tube. The tubes were kept at 50 °C for 30 min. Next, they were put in a boiling water bath for 10 min [13]. After being enzymatically treated, portions A and B were handled identically throughout the existing procedure, unless otherwise stated.

2.2.1.3. Precipitation and washing of the residue for measurement of the total fibre. After the enzymatic treatment, only sample A was cooled by placing it in a room temperature water. The precipitation of fibres was completed by adding 40 ml of absolute ethanol and then by leaving it in ice-water for 30 min. After its centrifugation (1500 g, 10 min), the supernatant was removed and 50 ml of 85% v/v ethanol was added to the precipitate. This mixture was then centrifuged. Afterwards the precipitate was washed twice by 50 ml of absolute ethanol. The residue was washed with 50 ml of acetone and then dried at 40 °C after centrifugation [13].

2.2.1.4. Extraction and washing of the residue for measurement of insoluble fibre. After the enzymatic treatment and cooling sample B to room temperature, 40 ml of the sodium phosphate buffer (0.2 mol/dm^3; pH 7) was added. The mixture was placed in a boiling water bath for 30 min in order to release the soluble fibre. The tubes were placed in water and left at room temperature for 10 min. They were centrifuged at 1500 g for 10 min. The supernatant was collected and 50 ml of water were added to the precipitate. The suspension was centrifuged and the supernatant liquid was removed. This step was repeated twice by using 50 ml of absolute ethanol. The residue was washed with 50 ml of acetone and then dried at 40 °C after centrifugation [13].

2.2.2. Prosky method

The insoluble and soluble dietary fibre contents were determined according to the AOAC enzymatic–gravimetric method of Prosky et al. [9]. The algal powder samples were gelatinized with a heat stable α-amylase (A-3306, Sigma Chemical Co., St. Louis, MO, USA) for 15 min in a boiling water bath. Then, they were enzymatically digested with protease (P-5380, Sigma Chemical Co., St. Louis, MO) (60 °C, pH 7.5, 30 min) in order to solubilize protein. This step was followed by incubation with amyloglucosidase (A-9268, Sigma Chemical Co., Poole, Dorset, UK) (60 °C, pH 4.5, 30 min) in order to remove starch. After that, they were filtered through sintered glass (porosity N.2), washed (with water, 95% ethanol and acetone), dried and weighed to determine insoluble fibre. Four volumes of absolute ethanol were added to the filtrate as well as to the water washings. Then, the precipitates were filtered and washed twice with 80% ethanol and acetone. Next, the residues (soluble fibre) were dried and weighed. The obtained values were corrected for ash and protein. The total dietary fibre was determined by summing the amounts of insoluble and soluble dietary fibre.

2.3. Chemical methods

The chemical analyses were carried out only on the extracts of the total fibre and the insoluble fibre obtained according to the method of Englyst et al. [13].

2.3.1. Dry matter

The dry matter was ascertained according to the Association of Official Analytical Chemists [15].

2.3.2. Protein content

The total protein content was determined by the Kjeldahl method. The protein content was calculated by using a nitrogen conversion factor of 6.25 [3]. The findings were expressed in percent of dry weight.