Algal Research 32 (2018) 150–161

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall related polysaccharides

Tom M.M. Bernaerts[a], Lore Gheysen[b], Clare Kyomugasho[a], Zahra Jamsazzadeh Kermani[a], Stéphanie Vandionant[a], Imogen Foubert[b], Marc E. Hendrickx[a], Ann M. Van Loey[a,*]

[a] Laboratory of Food Technology (Member of Leuven Food Science and Nutrition Research Center, LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven, Kasteelpark Arenberg 22, Box 2457, 3001 Heverlee, Belgium
[b] Laboratory Food and Lipids (Member of Leuven Food Science and Nutrition Research Center, LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven Kulak, E. Sabbelaan 53, 8500 Kortrijk, Belgium

ARTICLE INFO

Keywords:
Biomass composition
Cell wall polysaccharides
Exopolysaccharides
Monosaccharide
Uronic acid
Sulfate

ABSTRACT

Microalgae are rich in several nutritional and health-beneficial components, showing great potential as functional food ingredients. To this extent, knowledge of the biomass composition is essential in the selection of suitable microalgae species for specific food applications. Surprisingly, although cell wall polysaccharides are generally reported to play a role in functionality, limited attention has been given to the cell wall related polysaccharides of microalgae so far. Therefore, this study aimed to characterize dry biomasses of ten microalgae species with potential as functional food ingredients, with a particular focus on the composition of cell wall related polysaccharides. The investigated species were *Arthrospira platensis*, *Chlorella vulgaris*, *Diacronema lutheri*, *Tisochrysis lutea*, *Nannochloropsis* sp., *Odontella aurita*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Schizochytrium* sp. and *Tetraselmis chuii*. Lipids, proteins and ash made up a large fraction of the biomasses, except for the freshwater algae *C. vulgaris* and *A. platensis* which were mainly composed of proteins and polysaccharides. Generally, low amounts of storage polysaccharides (2–8%) were observed in the investigated microalgae species, while extracellular polymeric substances were only present in *P. cruentum*, *O. aurita*, *C. vulgaris* and *A. platensis*. Cell wall polysaccharides contributed approximately 10% of the biomass and were composed of heteropolysaccharides, showing at least five different monosaccharides. Moreover, the presence of uronic acids and sulfate groups provides anionic characteristics to the cell wall related polysaccharides of several microalgae. As a result, these polysaccharides show potential to display interesting functionalities as bioactive or technological substances.

1. Introduction

Microalgae are a promising source of several nutritional and health-beneficial components, including omega-3 long chain polyunsaturated fatty acids (ω3-LC-PUFA), proteins, minerals and antioxidants. In recent decades, research has been exploring their potential as a functional food ingredient, to enhance the nutritional value of food products [1–3]. Since there is a large number of microalgae species available and the composition of microalgal biomasses largely varies among different species, knowledge on the biochemical composition is required for the selection of suitable microalgae towards specific food applications. However, even for a specific microalga species, variable biomass profiles are reported in different studies. Part of this variability is attributed to differences in cultivation conditions, since many environmental factors such as temperature, salinity and nutrient availability can strongly affect the chemical composition of microalgae [4]. While this allows the optimization of cultivation conditions to maximize the production of specific biomolecules, it also results in an increased complexity in comparing different microalgal biomass profiles. On the other hand, the diverse biomass compositions found in literature can also be attributed to distinct analytical approaches used in different studies. For instance, protein contents can be determined by colorimetric assays or elemental analysis of nitrogen. While the former methods are sensitive to interferences and require pretreatments to completely release intracellular proteins, the latter relies on the use of nitrogen-to-protein conversion factors, but different conversion factors

Abbreviations: ω3-LC-PUFA, omega-3 long chain polyunsaturated fatty acids; CWPS, cell wall polysaccharides; EPMS, extracellular polymeric substances; EPS, extracellular polysaccharides; SPS, storage polysaccharides; UHPH, ultra high pressure homogenization

* Corresponding author.
E-mail address: ann.vanloey@kuleuven.be (A.M. Van Loey).

https://doi.org/10.1016/j.algal.2018.03.017
Received 30 October 2017; Received in revised form 22 March 2018; Accepted 27 March 2018

have been used by different authors (ranging from 4.44 to 6.25) [5,6]. As a consequence, there is still a demand for studies comparing microalgal biomass profiles using standardized protocols.

The nutritional valuable components of microalgae are stored inside the microalgal cell, which is protected by a cell wall (except for a few species). As a consequence, the cell wall plays an important role as a natural barrier, limiting extraction yields of high-value products or resulting in a low bioavailability of intracellular components [7,8]. In this context, extensive research has been performed on the disruption of microalgal cells, including chemical modifications and mechanical, thermal or ultrasonication processes [9]. Although several treatments proved successful for many microalgae, optimization is still required for species possessing a very rigid cell wall. In recent decades, the use of cell wall degrading enzymes has gained interest as this shows some advantages, such as a minimal impact on the desired nutrients and low energy requirements [7,10,11]. However, this approach requires the precise knowledge of the cell wall composition, for the appropriate selection of specific cell wall degrading enzymes.

Insight into the composition of the cell wall is not only desired in terms of process optimization, but also because distinct cell wall related polysaccharides might show potential for several biotechnological purposes. To date, commercialization of high value products from microalgae is mainly targeted to ω3-LC-PUFA, antioxidants or pigments, while microalgal polysaccharides are receiving limited attention. This might be due to the lack of knowledge on the composition and structure of cell wall related polysaccharides, with only few studies suggesting the potential of cell wall related polysaccharides for several applications. According to de Jesus Raposo et al. [12], sulfated polysaccharides of microalgae display various bioactivities, such as antiviral, antioxidant and anti-inflammatory activities. Moreover, exopolysaccharides of the red microalga *Porphyridium* sp. show unique rheological properties and might therefore be used as thickening agents in food products [13]. Thus, establishing the composition of cell wall related polysaccharides, such as the monosaccharide profile or the degree of sulfation, could increase the functionality of microalgal sources towards several applications.

Cell wall related polysaccharides comprise different types of polymers, including cell wall polysaccharides (CWPS) and extracellular polymers. The latter are generally described as polymers that can be secreted into the surrounding environment, such as the cultivation medium. Since the amount of secreted material depends on the growth conditions and time of harvesting, the extracellular polymers can be both found as solubilized polymers in the aqueous phase as well as an external layer still surrounding the microalgal cell. Although many microalgae species and cyanobacteria secrete extracellular polymers into the cultivation medium, the type of secreted material is often unclear in literature, primarily due to distinct terminology. The secreted material is often called EPS, referring to either extracellular polymeric substances, extracellular polysaccharides or exopolysaccharides, although terms as released polysaccharides (RPS), extracellular organic matter (EOM) or algogenic organic matter (AOM) are also commonly used [14,15]. Depending on the definition, different classes of organic macromolecules are included, such as polysaccharides, proteins, nucleic acids, phospholipids and smaller molecules [15]. In this study, a distinction will be made between all polymeric material that can be secreted into the environment (referred to as extracellular polymeric substances, EPMS) and polysaccharides that can be secreted (referred to as extracellular polysaccharides, EPS).

To date, information in literature on the amount of cell wall related polysaccharides in microalgae is scarce. In fact, quantification of microalgal polysaccharides is usually done by analyzing the total carbohydrate content, thus including both storage polysaccharides (SPS) and cell wall related polysaccharides. However, these two types of polysaccharides exhibit different functions in the microalgal cell. The main function of SPS is the storage of energy, providing substrates for metabolic processes and allowing survival of the organism during dark periods. In contrast, cell wall related polysaccharides, comprising CWPS and EPMS, play an important structural role in the microalgal cell. Whereas CWPS provide resistance to turgor pressure, interactions between EPMS of different cells allow the creation of multicellular structures [16]. As a consequence, both types of polysaccharides can contribute to different functional properties of the biomass, depending on their structure and composition. Total carbohydrate contents (in fact expressed as glucose-equivalents due to the use of non-specific colorimetric assays) do therefore not allow the prediction of the potential of microalgal polysaccharides.

Although several authors have reported monosaccharide profiles of microalgae, the composition of the cell wall related polysaccharides of many microalgae is still unknown. On the one hand, some studies presented monosaccharide profiles after hydrolysis of the total biomass [17,18]. However, due to possible interference of other components, such as SPS and glycolipids, these results provide only limited information on the cell wall composition. On the other hand, some authors have described the composition of cell wall related polysaccharides, but the results were mostly concerning specific polysaccharide fractions obtained by a selective extraction procedure [19,20]. Studies focusing on the polysaccharide composition of the whole cell wall are therefore very limited. Moreover, large variability in cell wall composition has been reported within a genus, a species and even within a strain, which can be due to differences in cultivation conditions or depending on the life stage of the cell [7], further limiting the comparison among the studies available. Therefore, the aim of this study is to apply a universal procedure for extraction of the total cell wall related polysaccharides, including CWPS and EPMS, of commercially available microalgae species followed by a detailed characterization.

The microalgae species used in this study were selected for their potential as functional food ingredients: *Arthrospira platensis*, *Chlorella vulgaris*, *Diacronema lutheri*, *Tisochrysis lutea* (formerly listed as *Isochrysis galbana*), *Nannochloropsis* sp., *Odontella aurita*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Schizochytrium* sp. and *Tetraselmis chuii*. Most of them show interesting nutritional profiles, e.g. containing ω3-LC-PUFA, proteins rich in essential amino acids and antioxidants. In addition, some of these biomasses have been accepted or authorized under the European novel food regulation, or applications are ongoing. Finally, by selecting these microalgae a diverse taxonomic spectrum was obtained, composed of photoautotrophic eukaryotic species classified as Chlorophyta (*C. vulgaris*, *T. chuii*), Rhodophyta (*P. cruentum*), Haptophyta (*D. lutheri*, *T. lutea*), Eustigmatophyta (*Nannochloropsis* sp.), Bacillariophyta or diatoms (*O. aurita*, *P. tricornutum*), one heterotrophic species belonging to Labyrinthulomyceta (*Schizochytrium* sp.) and one prokaryotic cyanobacterium (*A. platensis*) [21].

The objective of this study is to provide a fair comparison of ten microalgae species that are of interest for use as functional food ingredients. On the one hand, the microalgae were characterized in terms of biomass composition. On the other hand, the composition of the cell wall related polysaccharides CWPS and EPMS were established, by determining the monosaccharide profile, uronic acid content and sulfate content. All analyses were performed on commercially available dry biomasses, with regard to the application of dried microalgal biomass as a functional ingredient in food products, as food ingredients are generally delivered in a dry form to guarantee long term storage stability. The insights provided by this study can facilitate an appropriate selection of microalgae species for enhancing the nutritional value of food products, as well as revealing their potential as bioactive or biotechnological substances.

2. Materials and methods

2.1. Microalgal biomass

Commercially available microalgal biomass was obtained from