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The potential of microalgae and their biopolymers as structuring ingredients in food: a review

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Abstract

Microalgae are considered promising functional food ingredients due to their balanced composition, containing multiple nutritional and health-beneficial components. However, their functionality in food products is not limited to health aspects, since microalgae can also play a structuring role in food, for instance as a texturizing ingredient. Photoautotrophic microalgae are actually rich in structural biopolymers such as proteins, storage polysaccharides, and cell wall related polysaccharides, and their presence might possibly alter the rheological properties of the enriched food product. A first approach to benefit from these structural biopolymers consists of isolating the cell wall related polysaccharides for use as food hydrocolloids. The potential of extracted cell wall polysaccharides as food hydrocolloids has only been shown for a few microalgae species, mainly due to an enormous diversity in molecular structure and composition. Nevertheless, with intrinsic viscosities comparable or higher than those of commercial thickening agents, extracellular polysaccharides of red microalgae and cyanobacteria could be a promising source of novel food hydrocolloids. A more sustainable approach would be to incorporate the whole microalgal biomass into food products, to combine health benefits with potential structuring benefits, i.e. providing desired rheological properties of the enriched food product. If microalgal biomass would act as a thickening agent, this would actually reduce the need for additional texturizing ingredients. Even though only limitedly studied so far, food processing operations have been proven successful in establishing desired microstructural and rheological properties. In fact, the use of cell disruption techniques allows the release of intracellular compounds, which become available to create strong particle aggregates resulting in an improved viscosity and network structure. Food processing operations might not only be favorable in terms of rheological properties, but also for enhancing the bioaccessibility of several bioactive compounds. However, this research area is only very scarcely explored, and there is a demand for more standardized research studies to draw conclusions on the effect of processing on the nutritional quality of food products enriched with microalgae. Even though considered as promising food ingredients, some major scientific challenges have been pointed out throughout this review paper for the successful design of microalgal based food products.

Keywords

Microalgae, cyanobacteria, cell wall, polysaccharides, exopolysaccharides, rheology, microstructure, processing, disruption, bioaccessibility

Abbreviations

CWPS	cell wall bound polysaccharides
EPS	extracellular polysaccharides
TLS	trilaminar sheath

1 Introduction

In the context of developing food products with improved nutritional, structural, and sensory characteristics, food technologists are continuously exploring the potential of new ingredient sources. Although many of these novel ingredients are often referred to as functional ingredients, this term can only be used if the ingredients provide scientifically proven benefits in the human body beyond their nutritional value, thereby improving the health and well-being of the consumer or reducing the risk of disease (Siró et al., 2008). In this context, microalgae are generally considered as one of the promising sources of functional food ingredients (Buono et al., 2014; Matos et al., 2017). Microalgae are a rich source of numerous nutrients and health-beneficial components, including vitamins, minerals, proteins with essential amino acids, polyunsaturated fatty acids, antioxidants, and dietary fiber. As a consequence, the literature on microalgae as healthy ingredients is extensively reviewed by many authors, including Buono et al. (2014), Chacón-Lee and González-Mariño (2010), Gouveia et al. (2010), Matos et al. (2017), and Plaza et al. (2009).

Beside these nutritional components, microalgae generally contain large amounts of structural biopolymers, including proteins and carbohydrates. These structural biopolymers might possibly display interesting technological functionalities in food products, e.g. showing a potential role as texturizer, stabilizer, or emulsifier. Rheological properties are one of the most important quality aspects of food products, not only for their physical appearance (e.g. creating a desired texture or preventing phase separation phenomena during storage), but also in terms of sensory perception (such as the mouthfeel of a food during consumption) (Steffe, 1996). In fact, many processed food products require characteristics of a structured system, including a high viscosity, which is often achieved by the addition of thickening agents. In this context, microalgae could possibly be a sustainable source of food thickeners. However, apart from a limited number of fragmentary studies, this research area is still unexploited. The lack of knowledge on the functionality of microalgal cell wall related polysaccharides in particular is surprising, since cell wall polysaccharides of many taxonomically related macroalgae are among the common thickening and gelling agents used in food applications, such as carrageenans, agars, and alginates (Saha and Bhattacharya, 2010). The lack of reports on the functionality of microalgal polysaccharides might be due to the diversity and complexity of molecular structures of the cell wall related polysaccharides.

The potential of biopolymers from microalgae as structuring food ingredients can generally be explored using two different strategies. The first strategy consists of a biorefinery based approach, in which the microalgal biopolymers are isolated as high-value co-products of the side streams of biofuel production for application as food hydrocolloids. Downstream processing is obviously required to accomplish this strategy, particularly in terms of extraction, separation, and purification of the polysaccharides and/or proteins. As a consequence, microalgal biopolymers should display rather unique functionalities in order to be able to compete with conventional hydrocolloids from plants or macroalgae. The lack of studies reported on the functionality of microalgal biopolymers is related to the limited knowledge of the molecular structure of these polymers, in particular for the cell wall related polysaccharides. Compilation of the molecular characteristics of microalgal cell wall related polysaccharides in relation to their functional properties (rheological properties in particular) is therefore required as a first step in evaluating their potential as food hydrocolloids. The second strategy for introducing microalgal biopolymers in food is the incorporation of the whole microalgal biomass into food products. This might actually be a more efficient and more sustainable strategy to combine health-beneficial components with possible structuring benefits from microalgal polymers. However, the use of food processing operations might be required to fully exploit the structuring potential of the different biopolymers. On the one hand, thermal processes might be advantageous to induce temperature-dependent reactions, such as denaturation of proteins and heat-induced gelation of polysaccharides. On the other hand, cell disruption techniques might be of interest to release intracellular biopolymers and to promote polymer interactions. Moreover, cell disruption might possibly be

beneficial to create food products with enhanced nutritional value in terms of nutrient bioaccessibility and bioavailability. However, this research area is only very limitedly explored so far.

In this review paper, a general overview will be provided on the current knowledge in this research domain, as shown in the schematic representation in **Fig. 1**. The aim of this work is twofold: (i) to evaluate the potential of cell wall related polysaccharides of microalgae as food hydrocolloids, and (ii) to evaluate the structuring potential of whole microalgal biomass in food products and the implications for the bioaccessibility of the health-beneficial compounds.

2 Structural biopolymers in microalgae

Due to the enormous evolutionary diversity of microalgae, a large diversity in the biochemical composition of these organisms has been observed. In addition, biomass profiles can be drastically changed by adapting different cultivation conditions, including light intensity, temperature, and nutrient availability (Hu, 2004). As a consequence, largely variable literature data can be found on the composition of microalgae, with ranges of 9 – 77% proteins, 6 – 54% carbohydrates, and 4 – 74% lipids being reported. Biomass profiles of several microalgae species relevant for use as a food ingredient are compiled in **Table 1**. While some microalgae possess a low lipid content (< 20%), such as *Arthrospira platensis* (*Spirulina*), *Dunaliella* sp., and *Porphyridium cruentum*, most microalgae species have a substantial fraction of lipids (~15 – 40%), including *Haematococcus pluvialis*, *Isochrysis galbana*, and *Phaeodactylum tricornutum*. Examples of lipid-rich microalgae (> 40%) are *Schizochytrium* sp. and some strains of *Nannochloropsis* species. Generally, the higher the lipid content of microalgal biomass, the lower the amount of structural biopolymers, i.e. proteins and carbohydrates.

2.1 Proteins

Proteins can be found in high amounts for all microalgae species listed in **Table 1**, except for the obligate heterotroph *Schizochytrium*. In fact, apart from the water soluble proteins in the cytoplasm, proteins are mainly associated with the chloroplast, i.e. the photosynthetic organelle of the microalgal cell (**Fig. 2**). Examples are the Rubisco enzyme in the pyrenoid of eukaryotic microalgae and phycobiliproteins as photosynthetic accessory pigments in thylakoids of red microalgae and cyanobacteria (Arad and Yaron, 1992; Safi et al., 2014a). Even though many microalgae species can possess high protein contents, *Chlorella vulgaris* and *Arthrospira platensis* are most commonly produced as protein sources (Pulz and Gross, 2004). Their nutritional value is extensively studied, for *Arthrospira* sp. in particular, showing favorable amino acid profiles and a good digestibility (Becker, 2004). Furthermore, several reports have recently been published on their promising techno-functionalities. On the one hand, microalgal proteins could possibly display beneficial protein-surface related properties, such as foaming and emulsifying properties. For instance, Ursu et al. (2014) investigated the emulsifying capacity of *Chlorella vulgaris* proteins, and concluded them to be competitive with commercial emulsifying ingredients. The same conclusion was drawn for the emulsifying and foaming properties of protein isolates from *Tetraselmis* sp. by Schwenzfeier et al. (2013a, 2013b). On the other hand, microalgal proteins might be relevant in the food industry because of their hydrodynamic properties, such as thickening or gelling properties. In this context, Chronakis (2001) studied the rheological properties of a protein isolate from *Arthrospira platensis*. An increased viscosity above 50 °C was related to the denaturation of the proteins, which resulted in elastic gels upon heating to 90 °C. Subsequent cooling resulted in an increased network elasticity. The authors concluded that *Arthrospira platensis* protein isolates showed good gelling properties at concentrations between 1.5% and 2.5% (Chronakis, 2001). Similar gelling behavior was observed for proteins extracted from *Tetraselmis suecica*, resulting in a superior gelation behavior compared to whey protein isolate (Suarez Garcia et al., 2018a).

2.2 Carbohydrates

Carbohydrates make up another important fraction of microalgal biomass. Even though they have been mainly determined as the total carbohydrate content, it is important to distinguish storage carbohydrates from structural carbohydrates (i.e. cell wall related polysaccharides). These two types of carbohydrates do not only exhibit different functions in the microalgal cell, they might also display different functionalities in food products.

2.2.1 Storage polysaccharides

Five types of storage polysaccharides (starch, floridean starch, glycogen, chrysolaminarin, and paramylon) can be found in microalgae and cyanobacteria, with the type of storage polysaccharide being species specific. The former three are polyglucans consisting of α-1,4 and α-1,6-linkages in different ratios, whereas chrysolaminarin polymers are composed of β-1,3 and β-1,6-linked glucose residues. Paramylon polymers are only composed of β-1,3-linked glucose residues (De Philippis et al., 1992; Myklestad, 1988; Percival, 1979; Suzuki and Suzuki, 2013). As a consequence, the different types of storage polysaccharides are identified by different degrees of branching, as shown in **Fig. 3**. Whereas amylopectin possesses 5 – 6% of α-1,6-linkages, which is about one branch point for every 20 glucose residues (α-1,4/α-1,6 ≈ 20:1), a higher degree of branching is found in chrysolaminarin (β-1,3/β-1,6 ≈ 11:1) and in glycogen (α-1,4/α-1,6 ≈ 10:1) (Beattie et al., 1961; Buléon et al., 1998; Calder, 1991). Since floridean starch, sometimes referred to as amylopectin-rich starch, typically contains little or no amylose (0 – 5%), its granules are composed of more branched polymers compared to starch (McCracken and Cain, 1981; Percival, 1979). Moreover, the location of these types of storage polysaccharides in the microalgal cell differs (**Fig. 2**). While starch granules are typically stored in the chloroplasts, chrysolaminarin is accumulated in the vacuoles of the cells. The other three types (floridean starch, paramylon, and glycogen) are located as granules in the cytosol (Suzuki and Suzuki, 2013). Although starch isolated from plant sources is a commonly used thickening agent in the food industry, the diversity of microalgal storage polysaccharides does not allow the prediction of the functionality of these other types of storage carbohydrates in food products. To the best of our knowledge, no studies have been reported on the functionality of microalgal starch or the four other types of storage polysaccharides as a thickening agent.

2.2.2 Cell wall related polysaccharides

Except for a few microalgae species, the microalgal cell is surrounded by a cell wall (**Fig. 2**). Microalgal cell walls are mainly composed of polysaccharides, which are referred to as cell wall bound polysaccharides, although several other components have been identified, including proteins, resistant biopolymers, silicified or calcified structures, and so on (de Leeuw et al., 2006; Tomaselli, 2004). In addition, the cell wall of several microalgae is surrounded by an external layer of polymers. Depending on several factors such as the growth stage of the microalgae, these extracellular polymers can either remain associated to the cell surface or be released into the surrounding environment (Delattre et al., 2016). The status and the nature of these polymers is often unclear in literature, partly due to the large variety in terminology, including exopolysaccharides or extracellular polysaccharides (EPS), extracellular polymeric substances (EPS or EPMS), released polysaccharides (RPS), capsular polysaccharides (CPS), colloidal exopolysaccharides (cEPS), extracellular organic matter (EOM), dissolved organic matter (DOM), and algogenic organic matter (AOM) (Abdullahi et al., 2006; Bernaerts et al., 2018a; Delattre et al., 2016; Henderson et al., 2008; Kuhnhenn et al., 2006). For cyanobacteria in particular, terms as sheaths, capsule, and slime are often used to distinguish between extracellular layers with different affinity to be released into the medium (De Philippis and Vincenzini, 1998). In this review paper, the term extracellular polysaccharides will be used to indicate the polysaccharides in the external layer.

Reports on the quantification of cell wall polysaccharides are very scarce, which might be due to the difficult extractability of the total cell wall polysaccharides. Furthermore, while glucose is the dominant sugar in storage polysaccharides, structural polysaccharides are generally composed of multiple monosaccharide residues. Rapid colorimetric assays are therefore not suitable for accurate quantification of cell wall related polysaccharides and should be replaced by more advanced chromatographic techniques. Bernaerts et al. (2018a) determined the amount of cell wall related polysaccharides for 10 microalgae species by use of anion exchange chromatography and concluded that they generally account for approximately 10% of the dry biomass. Some microalgae species displayed lower amounts of cell wall polysaccharides (3.8 – 7.4%), but the authors attributed this to the presence of non-polysaccharide substances in their cell wall, such as algaenan polymers in *Nannochloropsis* sp. and a silica frustule in *Odontella aurita* (Bernaerts et al., 2018a). As a consequence, the majority of carbohydrates as shown in **Table 1** is probably attributed to storage polysaccharides, which are generally more sensitive to variable cultivation conditions (González-Fernández and Ballesteros, 2012; Markou et al., 2012).

2.3 Influence of cultivation parameters on structural biopolymers in microalgae

One of the advantages of microalgae is that their biomass composition can be accurately regulated by controlling the cultivation conditions, at least when grown in closed systems such as photobioreactors or fermenter tanks (Brennan and Owende, 2010). The optimal cultivation conditions are generally considered as those resulting in the highest biomass productivity. However, a common strategy to alter the biomass composition is the use of more unfavorable environmental conditions to enrich the biomass in a specific component. Environmental factors such as light, temperature, and nutrient availability influence the activity and pathways of the cellular metabolism, resulting in a dynamic cell composition (Hu, 2004). A drawback of adapted cultivation parameters is the decrease in biomass productivity, and it could even lead to culture collapse because of potential contamination or culture instability. This might however be avoided by applying a two-stage (or two-phase) culture mode, ensuring a maximum biomass production under optimum conditions in a first stage, followed by the accumulation of the desired products under unfavorable conditions in the second stage (Markou et al., 2012). The strategy of adapted cultivation parameters for accumulation of macronutrients is generally applied for carbohydrates and lipids, which function as storage compounds to provide energy for the metabolic processes (Bellou et al., 2014; Hu, 2004). In contrast, unfavorable cultivation conditions mostly result in a decreased protein content, since proteins rather play a structural role than a storage role in the microalgal cell, with exceptions of cyanophycin and phycocyanin in cyanobacteria (Boussiba and Richmond, 1980; Simon, 1971). Proteins are actually primarily generated under optimal growth conditions (Safi et al., 2014b).

Nutrient limitation is a common strategy to steer the biomass composition of microalgae. If a nutrient is limited or omitted from the cultivation medium, microalgae will change their metabolic pathways for cell survival (Hu, 2004). As many nutrients (such as nitrogen, phosphorous, and sulfur) are essential in the photosynthesis of autotrophic microalgae, nutrient starvation usually leads to a decreased synthesis of proteins, together with accumulation of either carbohydrates or lipids (Bellou et al., 2014; Bellou and Aggelis, 2012; Markou et al., 2012). As such, deprivation of nitrogen, phosphorous, or sulfur are common strategies to enhance accumulation of carbohydrates (or lipids), at the expense of protein synthesis (Brányiková et al., 2011; Dragone et al., 2011; Zhu et al., 2014). Even though it is generally assumed that the increase in carbohydrates is mainly related with storage polysaccharides rather than structural carbohydrates (González-Fernández and Ballesteros, 2012; Markou et al., 2012), recent studies also indicated changes in cell wall related polysaccharides under adapted cultivation parameters. For instance, an increased cell wall thickness of up to 70% was reported for different microalgae grown in nitrogen-deplete and hypersaline culture conditions (Beacham et al., 2014; Yap et al., 2016a). Furthermore, the synthesis of extracellular polysaccharides can also be regulated by the cultivation parameters, as has been reviewed by Delattre et al. (2016).

Apart from nutrient limitation, changes in light also affect the biomass composition. For instance, Friedman et al. (1991) reported a threefold increase in polysaccharides when grown under higher light intensities, and showed that the carbohydrate increase was not only related to the storage polysaccharides but also to the cell wall related polysaccharides. In contrast, the effect of temperature on the carbohydrate content is less clear. Even though some authors reported some carbohydrate accumulation at elevated temperatures, other authors did not observe clear changes in carbohydrates for different microalgae species grown at different temperatures (de Castro Araújo and Tavano Garcia, 2005; Ogbonda et al., 2007; Renaud et al., 2002).

3 Potential of microalgal cell wall polysaccharides as food hydrocolloids

3.1 The microalgal cell wall

An overview on the presence of a polysaccharidic cell wall and/or an extracellular layer of polysaccharides in some microalgae species is provided in **Table 2**. The absence of a cell wall is rather unusual in microalgae. One of the few microalgae species lacking a cell wall is *Dunaliella*. The cells of this microalga do possess a mucilaginous cell coat, but this is typically considered as an extracellular layer which can be visualized with Indian ink, similar to the capsule in cyanobacteria (Ben-Amotz and Avron, 1992; De Philippis and Vincenzini, 1998). Furthermore, no consensus has been reached on the presence of a cell wall in *Isochrysis* species. The lack of a cell wall in *Isochrysis galbana* was suggested by Zhu and Lee (1997) and was related to the fragility of the cells. However, Pales Espinosa et al. (2010) concluded the presence of mannose and glucose on the surface of *Isochrysis* sp. based on strong binding of fluorescently labeled lectins. Glucose and mannose were also identified as the principal monosaccharides by Bernaerts et al. (2018a) in the cell wall fraction of *Tisochrysis lutea*. As a consequence, it is believed that a cell wall is present in *Isochrysis* sp. and that the fragility of the cells is related to the cell wall composition, probably lacking fibrillar or resistant polymers responsible for the robustness of the cell wall (Bernaerts et al., 2018a).

The ability of microalgae to produce extracellular polysaccharides seems to be less conserved. While extracellular polysaccharides are typically (but not necessarily) synthesized by cyanobacteria, diatoms, green microalgae, and red microalgae, they have been less studied in other classes of microalgae (**Table 2**). To date, the physiological function of microalgal extracellular polysaccharides is poorly understood, but they are thought to protect the algal cells from fluctuations in environmental conditions and/or predators. As a result, production of extracellular polysaccharides is not only dependent on the microalga species or strain, but also on cultivation conditions such as nutrient availability and other culture parameters (Delattre et al., 2016; Xiao and Zheng, 2016). Moreover, correct identification of microalgal extracellular polysaccharides is challenging, since polysaccharides isolated from microalgal cultures might also originate from extracellular polysaccharide producing bacteria which adhere to microalgal cells (Nichols et al., 2005). It was also shown that not all morphotypes within one microalgae species have the ability to synthesize extracellular polysaccharides. For instance, while they are produced by the ovoid type of *Phaeodactylum tricornutum*, the fusiform and triradiate forms do not produce extracellular polysaccharides (Desbois et al., 2010). Finally, it should be noted that extracellular polysaccharides are not always easily differentiated from cell wall bound polysaccharides, as is the case for red microalgae. In fact, red microalgae are often described as cells encapsulated within a polysaccharide complex in the form of a gel, comprised of soluble and bound polysaccharides (Arad and Levy-Ontman, 2010). Nevertheless, Bernaerts et al. (2018b) attributed differences in molecular weight of these fractions in *Porphyridium cruentum* to a distinct molecular organization, confirming that a structural distinction can be made between cell wall bound polysaccharides and extracellular polysaccharides (Bernaerts et al., 2018b).

3.2 Molecular composition of cell wall polymers

Despite the importance of the microalgal cell wall properties towards several biotechnological applications, microalgal cell walls are poorly understood. Due to their evolutionary diversity, microalgal cell walls differ in molecular components, intra- and intermolecular linkages, and overall structure (Scholz et al., 2014). Besides, drastic variability in the composition and structure of microalgal cell walls has been reported within a genus, a species, or within a strain (Baudelet et al., 2017). Furthermore, comparison of the work in this domain that was reported in the past half-century has been challenged by recent taxonomic revisions, for instance in the organization of Chlorophyta. An extensive review on this topic was provided by Baudelet et al. (2017), clearly illustrating the diversity in the cell wall composition of green microalgae.

In general, microalgal cell walls are often described as fibrillar layers of rigid wall components that are embedded in a more plastic polymeric matrix (Gerken et al., 2013; Passos et al., 2014). While this description might be valid for the majority of microalgal cell walls, it must be noted that several species lack rigid polymers in their cell wall, and some are known for a glycoprotein-based cell wall such as *Chlamydomonas reinhardtii* and *Euglena gracilis* (Mussgnug et al., 2010). The polymeric matrix is generally defined as the fraction that is hydrolyzed under mild acid conditions such as 2 M trifluoroacetic acid, liberating different monosaccharides and uronic acids, including glucose, galactose, rhamnose, arabinose, mannose, xylose, fucose, ribose, glucuronic acid, and galacturonic acid (Gerken et al., 2013). In contrast, the rigid or fibrillar polymers are (partially) resistant to mild acids and require harsh conditions for complete hydrolysis, such as 72% sulphuric acid or 6 M hydrochloric acid. Various fibrillar polymers have been found in microalgal cell walls, such as cellulose and chitin (Baudelet et al., 2017). Finally, some microalgal cell walls contain non-hydrolyzable biopolymers that are resistant to any acid and alkaline treatment, often defined as resistant biopolymers or algaenans (de Leeuw et al., 2006).

3.2.1 Cell wall bound polysaccharides

Microalgal cell wall polysaccharides are generally heteropolymers consisting of different monosaccharides, uronic acids, and amino sugars. **Table 3** gives an overview of the cell wall composition of different microalgae species, in which predominant monosaccharides are indicated in bold. Furthermore, microalgal cell wall polysaccharides are often sulfated, but quantitative data on the amounts of sulfate esters are lacking in literature.

Cell walls of the prokaryotic cyanobacteria are usually composed of peptidoglycan (also called murein), polymers of alternated N-acetylglucosamine and N-acetylmuramic acid residues connected by β-1,4-linkages. Together with cross-linked oligopeptides a three-dimensional network is formed, providing the (limited) rigidity of the cyanobacterial cell wall (Van Eykelenburg et al., 1980). Peptidoglycan is also present in *Arthrospira platensis*, despite the fact that glucosamine is not always detected as the predominant monosaccharide. Peptidoglycan actually requires harsh acid conditions for complete hydrolysis to glucosamine (Templeton et al., 2012). The structural peptidoglycan polymers are embedded in a polysaccharide matrix, mainly composed of glucose and mannose (Bernaerts et al., 2018a; Bertocchi et al., 1990). It is suggested that peptidoglycan and matrix polysaccharides are present in equal amounts in cyanobacteria, as was previously observed for some species (Bertocchi et al., 1990). Despite the presence of a structural peptidoglycan network, *Arthrospira platensis* has a relatively fragile cell wall that is easily disrupted by mechanical forces (Bernaerts et al., 2017; Safi et al., 2014a).

The cell wall composition of eukaryotic microalgae is very diverse, even within a phylum, class, or species (**Table 3**). This is illustrated by the complex cell wall composition of *Chlorella* species. Three types of cell walls have been reported for *Chlorella* sp. based on their composition: (i) glucosamine-rich cell walls, (ii) cell walls with mannose and glucose as main constituents, and (iii) cell walls rich in galactose, glucose, and rhamnose (Blumreisinger et al., 1983). Glucosamine originates from a chitin-like polysaccharide which serves as the rigid cell wall polymer in the first type of *Chlorella* species. Up to

66% of the total cell wall in *Chlorella* sp. can be made up from this rigid polymer (Baudelet et al., 2017). The other part of the cell wall corresponds to hemicellulosic material, mainly constituting rhamnose, arabinose, and galactose (Baudelet et al., 2017; Blumreisinger et al., 1983; Kapaun et al., 1992). In contrast, the occurrence of rigid cell walls is not clear for the other two types of *Chlorella* sp. cell walls. Some authors suggest the presence of cellulose microfibrils to be responsible for the rigidity of these *Chlorella vulgaris* cell walls (Bernaerts et al., 2018a; Gille et al., 2016). Whereas Safi et al. (2015) successfully used calcofluor white for fluorescent staining of beta-glucans in the cell wall of *Chlorella vulgaris* suggesting the presence of cellulosic polymers, this could not be demonstrated by other authors, although the latter did not verify which wall-type of *Chlorella* sp. was used (Baudelet et al., 2017). Little similarity has been observed for other species of the Chlorophyta listed in **Table 3**. No glucosamine was detected in *Scenedesmus* sp. and *Tetraselmis* sp. cell walls, indicating the absence of a chitin-like rigid wall. Major amounts of glucose in the cell wall of *Scenedesmus* sp. (up to 85%) suggest the presence of cellulose (Blumreisinger et al., 1983; Takeda, 1996), however this was only verified for one strain (Bisalputra and Weier, 1963). In contrast, the rigidity of *Tetraselmis* sp. is created by mineralized scales or theca, mainly composed of acidic polysaccharides such as 3-deoxy-manno-2-octulosonic acid (Kdo), galacturonic acid (GalA), and 3-deoxy-lyxo-2-heptulosaric acid (Dha) (Becker et al., 1998).

Glucose is also the predominant monosaccharide in *Nannochloropsis* sp. (Eustigmatophyta) and in the Haptophyta *Isochrysis* sp. and *Pavlova lutheri*, although it is not attributed to the same polymers. While the majority of glucose in *Nannochloropsis* sp. and *Pavlova lutheri* was ascribed to cellulose polymers, it was suggested that no cellulose is present in *Isochrysis* (Arnold et al., 2015; Bernaerts et al., 2018a; Scholz et al., 2014). In addition, it was proven that the cellulose polymers in *Nannochloropsis* sp. exist as microfibrils resulting in a rigid cell wall layer, while *Pavlova lutheri* cells are protected by small cellulose scales (Arnold et al., 2015; Scholz et al., 2014). As a consequence, not only the presence but also the state (crystalline or amorphous) and the configuration (fibrils or scales) of cellulose polymers determine the rigidity of the cell wall layers. Scales have also been reported in the cell walls of Labyrinthulomyceta like *Schizochytrium* sp., although they are made up of non-cellulosic material (Bahnweg and Jackle, 1986; Honda et al., 1999).

The cell wall of diatoms is generally described as silica valves (called frustule) covered with hemicellulosic polymers (Gügi et al., 2015). The siliceous skeleton was visualized in *Odontella aurita* by Bernaerts et al. (2017), and the matrix polysaccharides mainly consisted of galactose and mannose (Bernaerts et al., 2018a). Even though *Phaeodactylum tricornutum* is also classified as a diatom, the frustule is only synthetized by the ovoid form, while the cell walls of fusiform and triradiate morphotypes contain almost exclusively organic components (Francius et al., 2008; Tesson et al., 2009). In fact, a sulfated glucuronomannan was described as the prominent polysaccharide in the cell wall of *Phaeodactylum tricornutum* (Ford and Percival, 1965). It comprises a backbone of α-1,3-linked mannose residues with side chains of mannose and glucuronic acid, resulting in a molar ratio of mannose to glucuronic acid of approximately 4:1 (Ford and Percival, 1965; Le Costaouëc et al., 2017). Because of the absence of the silicified frustule in the fusiform and triradiate forms, these morphotypes are about five times softer and will presumably be less resistant to mechanical disruption techniques than the ovoid form (Francius et al., 2008). This was evidenced by Gille et al. (2019), as the authors observed intact ovoid cells of *Phaeodactylum tricornutum* after sonication, while the fusiform and triradiate morphotypes were destroyed.

In general, knowledge on the molecular characteristics of cell wall bound polysaccharides is restricted to the monosaccharide composition investigated by a limited number of researchers. To date, detailed structural analyses to explore the architecture of microalgal cell wall polysaccharides are lacking in literature, except for the sulphated glucuronomannan of one *Phaeodactylum tricornutum* morphotype and the cellulose microfibrils in *Nannochloropsis* sp. (Le Costaouëc et al., 2017; Scholz et al., 2014). Hence,

it is not surprising that almost no studies have been found on the rheological characterization of cell wall bound polysaccharides of microalgae, since detailed knowledge on the molecular structure is at the scientific basis of understanding and/or engineering specific rheological properties of polysaccharide solutions.

3.1.2 Resistant biopolymers

In many microalgae species, the rigidity of the cell wall is caused by the presence of resistant biopolymers. These are typically defined as algaenans, aliphatic macromolecules that are resistant to any acid or alkaline chemical. However, their structure might be somewhat modified under harsh acid or alkaline conditions due to hydrolysis of associated compounds (de Leeuw et al., 2006). It is worth noting that they have been often referred to as sporopollenin, cutin- or cutan-like polymers due to their similar function and resistance to biodegradation as those polymers in spores, pollen, and plants (Tegelaar et al., 1989). However, due to essential differences in chemical structure of the microalgal biopolymers compared to the above-mentioned polymers, it is suggested to use the term algaenans for all resistant biopolymers originating from (micro)algae (Burczyk and Dworzanski, 1988; de Leeuw et al., 2006). The occurrence of algaenan polymers in several microalgae species is presented in **Table 4**. Whereas algaenans appear in various members of Chlorophyta and Eustigmatophyta, they are less common in other classes of microalgae. The resistant polymers are absent in *Arthrospira platensis*, *Porphyridium cruentum*, and *Schizochytrium* sp., and have not been detected in Haptophyta and diatoms in general (de Leeuw et al., 2006; Gelin et al., 1999; Kodner et al., 2009).

As algaenans represent a series of polymers based on their physicochemical characteristics, different types of algaenan structures have been identified. Most Chlorophyta contain algaenan structures constructed by building blocks of linear even-numbered carbon chains of 22 to 34 carbon atoms, cross-linked by ether and ester bonds. However, larger building blocks have been observed for *Botryococcus braunii* (A-Race in particular), showing an average length of 40 carbons (Baudelet et al., 2017; de Leeuw et al., 2006). In contrast, algaenans produced by Eustigmatophyta typically contain building blocks of 25 to 36 carbons, odd- and even-numbered, cross-linked by ether bonds (de Leeuw et al., 2006).

The presence of algaenans in microalgal cell walls is sometimes confused with the occurrence of a trilaminar sheath (TLS). Microalgal TLS are characterized by two electron-dense sublayers enclosing a third sublayer with low electron density when examined by transmission electron microscopy. Algaenans are presumably located in this inner electron-lucent sublayer. However, Allard and Templier (2000) showed that there is no direct relation between the presence of TLS and algaenan polymers, as proven for two marine microalgae. Moreover, condensation reactions initiated by certain isolation procedures might lead to artifactual non-hydrolyzable polymers, which could mistakenly be identified as algaenans. Hence, even though a TLS often creates rigidity of the cell wall, it is not necessarily composed of non-hydrolyzable algaenan polymers (Allard and Templier, 2000).

The presence of algaenan polymers in the cell wall is often related with a high rigidity and resistance to mechanical disruption techniques, such as bead-milling and high pressure homogenization (Montalescot et al., 2015; E M Spiden et al., 2013). However, cell disruption is required in case of extraction of intracellular components of the microalgae. Even though the algaenan cell walls must contain pores for exchanging compounds with the outer environment, algaenans proved to form an effective barrier for extracellular enzymes (de Leeuw et al., 2006). As a consequence, the extractability of intracellular components and cell wall bound polysaccharides might be hampered by these resistant biopolymers in the outer cell wall layer.

3.1.3 Extracellular polysaccharides

Extracellular polysaccharides from microalgae have received more attention than the cell wall bound polysaccharides, especially for Cyanobacteria and Rhodophyta, and to a lesser extent for Chlorophyta

and diatoms. To the best of our knowledge, no extracellular polysaccharides have been reported for Haptophyta or Eustigmatophyta. The molecular structure of microalgal extracellular polysaccharides has been previously reviewed by several authors, including De Philippis and Vincenzini (1998), De Philippis et al. (2001), Delattre et al. (2016), and Xiao and Zheng (2016). In the present work, we focus on the extracellular polysaccharides of microalgae with high relevance for food applications, in relation to their rheological properties. The monosaccharide and uronic acid composition of these extracellular polysaccharides are shown in **Table 5**.

Extracellular polysaccharides of Cyanobacteria are typically composed of heteropolymers of six to ten different monosaccharides. They usually possess anionic characteristics because of substantial amounts of uronic acid residues and charged substituents such as sulphate and pyruvate esters (De Philippis et al., 2001). Even though glucose has been reported to be the most abundant monosaccharide in cyanobacterial extracellular polysaccharides, this could not be stated for *Arthrospira platensis* and *Cyanospira capsulata*. In fact, a large diversity has been found for these extracellular polysaccharides, principally composed of rhamnose (Depraetere et al., 2015), rhamnose and glucose (Roussel et al., 2015), rhamnose and ribose (Bernaerts et al., 2018a), galactose (Filali Mouhim et al., 1993), or similar ratios of five monosaccharides (Cesàro et al., 1990; Trabelsi et al., 2009; Vincenzini et al., 1993). This diversity might possibly result from variation in cultivation conditions, since extracellular polysaccharides are typically sensitive to changes in cultivation parameters (Delattre et al., 2016; Xiao and Zheng, 2016).

Large diversity has also been reported for extracellular polysaccharides of Chlorophyta, despite the low number of reports available. Extracellular polysaccharides of *Chlorella* sp. were characterized by large amounts of glucose, galactose, and mannose (Bernaerts et al., 2018a), or arabinose and glucuronic acid (Yalcin et al., 1994). In contrast, mannose was the predominant monosaccharide in extracellular polysaccharides of *Scenedesmus* sp. (Lombardi et al., 2005). While cell wall bound polysaccharides of *Chlorella* sp. have been distinguished in three major types based on their composition, it is unclear whether such classification might also be valid for extracellular polysaccharides of this microalga species. Furthermore, diversity in composition of extracellular polysaccharides is plausible given the taxonomic complexity of the Chlorophyta (Baudelet et al., 2017).

Even for diatoms, a class based on a common silicified cell wall structure, little analogies have been found in the composition of extracellular polysaccharides (Delattre et al., 2016). Galactose was the predominant monosaccharide in extracellular polysaccharides of *Odontella aurita*, but only one report was found in literature (Bernaerts et al., 2018a). In contrast, the ovoid form of *Phaeodactylum tricornutum* produced extracellular polysaccharides consisting of five different monosaccharides (Willis et al., 2013). Abdullahi et al. (2006) reported mannose and glucose as predominant monosaccharides in *Phaeodactylum tricornutum*, however, it was unclear whether these polysaccharide fractions were isolated from ovoid or fusiform morphotypes. In fact, it is generally presumed that extracellular polysaccharides are not synthetized by the fusiform types (Desbois et al., 2010).

The molecular composition and structure of extracellular polysaccharides of the red microalga *Porphyridium* sp. are well studied, mainly due to intensive research by the research group of Arad and colleagues (Arad and Levy-Ontman, 2010). Extracellular polysaccharides of *Porphyridium* sp. are composed of xylose, glucose, galactose, and glucuronic acid, although different ratios have been reported by several authors. While xylose was the predominant monosaccharide according to most authors (Bernaerts et al., 2018b; Geresh et al., 2002; Geresh and Arad, 1991; Heaney-Kieras and Chapman, 1976; Percival and Foyle, 1979; Soanen et al., 2015), some studies reported galactose to be the principal monosaccharide (Patel et al., 2013; Roussel et al., 2015). Nevertheless, *Porphyridium* sp. extracellular polysaccharides have always been described as high molecular weight polymers, showing a weight average molar mass between 2.4×10^5 g/mol and 4×10^6 g/mol (Bernaerts et al., 2018b; Geresh et al., 2002; Patel et al., 2013). The polysaccharides have anionic characteristics, not only due to the presence

of glucuronic acid residues, but also because of sulfate groups esterified to glucose or galactose residues in the 6- or 3-positions (Geresh and Arad, 1991). However, large diversity has been found in the percentage of ester sulfates, ranging from 1 to 15.1% (Bernaerts et al., 2018a; de Jesus Raposo et al., 2014; Geresh and Arad, 1991; Heaney-Kieras and Chapman, 1976; Patel et al., 2013; Percival and Foyle, 1979). The use of more advanced techniques, such as linkage analyses and NMR spectroscopy, allowed to obtain more insight in the molecular structure of the polysaccharides. Geresh and Arad (1991) established a disaccharide as a basic building block in *Porphyridium* sp. extracellular polysaccharides, which was an aldobiouronic acid composed of glucuronic acid and galactose. This aldobiouronic acid was later shown to be part of a larger linear building block, constituting (1 → 2 or 1 → 4)-linked xylopyranosyl, (1 → 3)-linked glucopyranosyl, (1 → 3)-linked glucopyranosyluronic acid, and (1 → 3)-linked galactopyranosyl residues (Geresh et al., 2009). The use of uronic degradation of *Porphyridium* sp. extracellular polysaccharides resulted in two other oligosaccharide fragments, also characterized by (1 → 3)-linked glucopyranosyl and galactopyranosyl residues, and (1 → 2)- as well as (1 → 4)-linked xylopyranosyl residues (Gloaguen et al., 2004). Differences in the oligosaccharide structures observed in both studies might be related with differences of microalgae strains or isolation procedures of the polysaccharides (so-called soluble polysaccharide versus bound polysaccharide) (Geresh et al., 2009; Gloaguen et al., 2004). Nevertheless, extracellular polysaccharides of *Porphyridium* sp. are characterized by a unique molecular structure, resulting in interesting rheological properties as discussed in next paragraphs.

3.2 Rheological properties of extracted microalgal polysaccharides

To evaluate the potential of microalgal polysaccharides as structuring agents for food products, two types of rheological properties are of interest. On the one hand, thickening agents are used to steer the flow behavior of the products, resulting in a desired viscosity and mouth feel. On the other hand, the use of gelling agents is more related to the texture of a food product, typically analyzed as the linear viscoelastic behavior. While most hydrocolloids display thickening properties, gel formation is restricted to a limited number of hydrocolloids with a specific molecular structure (Saha and Bhattacharya, 2010). To date, only a limited number of microalgal polysaccharides have been investigated for their rheological properties, with main focus on the flow behavior or the viscosity. Apart from some fragmentary studies of cyanobacterial extracellular polysaccharides, the majority of the studies focused on the rheological properties of extracellular polysaccharides of *Porphyridium*.

Geresh and Arad (1991) were among the first ones to investigate the viscosity of *Porphyridium* sp. polysaccharide solutions. They observed similar viscosities for *Porphyridium* sp. polysaccharide solutions as for xanthan gum. However, the solutions were prepared in relatively low concentrations (0.25% w/w), resulting in rather low viscosity values between 32 and 85 mPa s at a shear rate of ~40 s^{-1} (Geresh and Arad, 1991). Nevertheless, this concentration was shown to be above the critical overlap concentration (C*) of 0.6 g/L, i.e. approximately 0.06%, as determined by Patel et al. (2013). Above C*, the solution is in the semi-dilute regime in which polysaccharide entanglements take place. These entanglements are disrupted at higher shear rates, resulting in shear-thinning flow behavior, as observed by several authors for *Porphyridium* sp. extracellular polysaccharide solutions (Eteshola et al., 1998; Geresh et al., 2002; Patel et al., 2013). Slight Newtonian plateaus were observed at very low ($\dot{\gamma} < 0.01\ s^{-1}$) and very high ($\dot{\gamma} > 500\ s^{-1}$) shear rates (Badel et al., 2011; Patel et al., 2013). In the shear-thinning region, i.e. at intermediate shear rates, viscosity curves frequently displayed a slope of approximately –1 in a $\log(\eta) - \log(\dot{\gamma})$ plot for polysaccharide concentrations between 0.125% and 2% (de Jesus Raposo et al., 2014; Eteshola et al., 1998; Patel et al., 2013). This was confirmed by Liberman et al. (2016), reporting low values for the flow behavior index n (below 0.2). Hence, *Porphyridium* sp. extracellular polysaccharide solutions are characterized by a strong shear-thinning behavior, inferring the presence of multiple interactions between polysaccharide chains that break down under shear, typical of a (weak) structured

medium. Some authors actually suggested that the shear-thinning behavior resulted from dissociation of hydrogen bonds under high shear forces, similar to xanthan solutions (Eteshola et al., 1998; Ginzberg et al., 2008).

The weak gel character has been confirmed by small deformation oscillatory measurements. It was shown that *Porphyridium* sp. extracellular polysaccharide solutions displayed a large linear viscoelastic region, with critical strains of 50% or higher (Bernaerts et al., 2018b; Eteshola et al., 1998). In contrast with strong gels (also called true gels), the storage modulus (G') of *Porphyridium* sp. extracellular polysaccharide solutions was not constant, but slightly dependent on the angular frequency. While this frequency dependence was obvious at a concentration of 0.5%, it became less pronounced at concentrations of 1% and 2%, between $G' \propto \omega^{0.1}$ and $G' \propto \omega^{0.2}$ (Bernaerts et al., 2018b; Eteshola et al., 1998; Ginzberg et al., 2008). The gel behavior of *Porphyridium* sp. extracellular polysaccharides is similar to or somewhat stronger than that of xanthan gum (Rochefort and Middleman, 1987).

However, gelation of food hydrocolloids typically occurs in the presence of cations or under specific temperature conditions. Generally, three main mechanisms have been described for gelation of food hydrocolloids: (i) ionotropic gelation, typically cation-mediated gelation, (ii) cold-set gelation, and (iii) heat-set gelation (Saha and Bhattacharya, 2010). The first is common for several anionic hydrocolloids such as carrageenans, alginates, and pectins, which require either monovalent or divalent cations for successful gelation (Imeson, 1997, 2011). Even though *Porphyridium* sp. extracellular polysaccharides have been described as anionic polymers due to the presence of glucuronic acid residues and sulfate groups, no successful gelation has been observed in the presence of different cations (Na^+, K^+, Ca^{2+}, or Zn^{2+}) (Bernaerts et al., 2018b; Eteshola et al., 1998; Liberman et al., 2016). X-ray diffraction studies had previously revealed that *Porphyridium* sp. polysaccharides appear as a single two-fold helical structure (Eteshola et al., 1998). It was shown by Liberman et al. (2016) that a low concentration of Zn^{2+} (250 ppm) was sufficient for screening of electrostatic repulsions of the negatively charged polysaccharide chains, but that higher concentrations did not lead to any network formation (Liberman et al., 2016). In addition, most attempts to induce gelation by using temperature were unsuccessful. Even though Eteshola et al. (1998) reported *Porphyridium* sp. extracellular polysaccharides to have unique thermoreversible properties resulting in a strong gel upon heating, this observation could not be confirmed by other authors. In fact, different studies reported no drastic changes in storage modulus or viscosity when applying temperatures up to 80 °C, not during heating nor during cooling (Bernaerts et al., 2018b; de Jesus Raposo et al., 2014; Patel et al., 2013). Hence, it is likely that no three-dimensional networks are formed by cold-set gelation or heat-set gelation, and that *Porphyridium* sp. extracellular polysaccharides only show potential as a thickening agent, not as a gelling agent.

Despite the promising application of *Porphyridium* sp. extracellular polysaccharides for use as thickening agents in food products, one of the drawbacks is that traditional extraction techniques for cell wall polysaccharides result in low purified polymers with relatively poor solubility. In fact, polysaccharide purities below 33% and 39% have been reported for alcohol precipitation and dialysis, respectively, mainly due to high salt contents and co-extraction of proteins (Bernaerts et al., 2018b; Marcati et al., 2014; Patel et al., 2013). As a consequence, the storage moduli and viscosity values reported in these studies might be an underestimation of the potential of purified *Porphyridium* sp. extracellular polysaccharides. This is illustrated in **Fig. 4**, by comparing the intrinsic viscosities of *Porphyridium* sp. extracellular polysaccharides to those of currently used thickening and gelling agents, since this property is independent from the purity of the polysaccharide sample. In spite of the large variability, it is obvious that *Porphyridium* sp. extracellular polysaccharides show substantially higher intrinsic viscosities than most other hydrocolloids, except for xanthan gum. The high intrinsic viscosity results from the high molecular weight and hydrodynamic volume, which is probably related to a high chain rigidity, a high electrostatic charge density, and a relatively low degree of branching (Saha and Bhattacharya, 2010). It should be noted

that intrinsic viscosity is not related to the ability to form gels. In fact, some of the hydrocolloids shown in **Fig. 4** are rather used as gelling agents than as thickening agents, like pectins and guar gum. In spite of their lower intrinsic viscosity, the use of temperature and/or cation addition results in the formation of strong gels, a property which could not be appointed to *Porphyridium* sp. extracellular polysaccharides. Nevertheless, it is obvious that extracellular polysaccharides of *Porphyridium* sp. are a promising source of thickening agents for food applications.

Beside *Porphyridium* sp., other extracellular polysaccharides investigated for their rheological properties are mostly originating from cyanobacteria. Several authors observed relatively high viscosities for extracellular polysaccharides of *Cyanospira capsulata* in low concentrations (0.05 – 1.1%), comparable to *Porphyridium* sp. extracellular polysaccharide solutions and to xanthan gum (Navarini et al., 1992, 1990; Vincenzini et al., 1990). However, in comparison with extracellular polysaccharides of *Porphyridium* sp., C* is expected to be somewhat higher, since a Newtonian-like behavior was still observed for solutions in a concentration of 0.25% w/v (Navarini et al., 1992). Similarly, the intrinsic viscosity of *Cyanospira capsulata* extracellular polysaccharides was lower than for *Porphyridium* sp., between 20 and 30 dL/g (**Fig. 4**). This is presumably related to the slightly lower average molecular weight compared to extracellular polysaccharides of *Porphyridium* sp. (Cesàro et al., 1990). In spite of the high viscosity values, dynamic oscillatory measurements showed that *Cyanospira capsulata* polysaccharide solutions displayed a liquid-like behavior up to concentrations of 1.1% w/v, indicated by a higher frequency dependence between $G' \propto \omega^{0.6}$ and $G' \propto \omega^{0.8}$. It is therefore suggested that next to polysaccharide entanglements, occasional cross-interactions between polysaccharide chains might be present, but a too high chain flexibility prevents it from forming a solid-like gel structure (Navarini et al., 1992). Similar viscosities were obtained for extracellular polysaccharide solutions of other cyanobacteria, including *Anabaena* sp., *Nostoc* sp., and *Arthrospira platensis* (Badel et al., 2011; Bhatnagar et al., 2012; Han et al., 2014; Moreno et al., 2000).

Some fragmentary studies are available on the rheological properties of extracellular polysaccharides of Chlorophyta. Yalcin et al. (1994) reported shear-thinning behavior for 0.5% solutions of *Chlorella* sp. extracellular polysaccharides, but viscosities were low (below 25 mPa s). Somewhat higher values were observed for *Neochloris oleoabundans*, however still limited to 65 mPa s for a 0.5% concentration. Even though the addition of NaCl resulted in a drastic viscosity increase up to 375 mPa s, these extracellular polysaccharides cannot compete with those of *Porphyridium* sp. or cyanobacteria, partly due to the lower average molecular weight of the polysaccharides (Wu et al., 2011). Nevertheless, no general conclusions should be drawn from this, since to date the rheological properties of extracellular polysaccharides isolated from Chlorophyta are underexplored.

4 Potential of microalgal biomass as food structuring agent

Even though microalgal polysaccharides have potential to be used as thickening agents in the food industry, the incorporation of the whole microalgal biomass into food products might be a more attractive strategy to modify the food structure, since the nutritional value of the food product is also improved due to the introduction of multiple nutritional and health-beneficial components. Moreover, the structuring potential of microalgal biomass might be extended because of the presence of other structural biopolymers, including storage polysaccharides and proteins. Finally, this approach is also considered more sustainable, since no extraction solvents are required and waste streams are avoided.

4.1 Rheological properties of microalgal biomass in aqueous systems

To understand the intrinsic structuring potential of microalgal biomass, the rheological behavior of microalgal biomasses of different species can be compared in simple model systems such as aqueous

suspensions. Rheological properties have only been studied for a limited number of microalgae, with most studies focusing on the viscosity, whereas the linear viscoelastic behavior has been very poorly investigated. In fact, most of the experiments reported were established in the context of optimization of cultivation and down-stream processing of microalgae, resulting in a broad range of biomass concentrations. On the one hand, very dilute systems (0.004 – 0.5%) have been analyzed to simulate concentrations in raceway ponds (typically up to 0.5 g/L) or photobioreactors (typically up to 5 g/L). On the other hand, concentrations up to 25% have been studied to determine the rheological properties of algal slurries during downstream processing, which are obtained after dewatering of microalgal cultures (Yap et al., 2016b; Zhang et al., 2013). Microalgal suspensions exhibit Newtonian flow behavior at low concentrations, often associated with low viscosities, whereas non-Newtonian behavior is observed above a critical concentration (Wileman et al., 2012). The value of this critical concentration is depending on the microalga species and might be an indication of the structuring capacities of the microalgal biomass. Furthermore, a wide range of shear rates (0.01 – 1000 s^{-1}) has been applied in analyzing the viscosity of microalgal suspensions. Nevertheless, most analyses were performed between 5 and 200 s^{-1}, which are relevant shear rates for food processing operations such as mixing, stirring, pumping, and swallowing of food products (Rao, 2013).

Different strains of *Chlorella* have been investigated for their rheological properties. Wileman et al. (2012) reported Newtonian flow behavior for *Chlorella vulgaris* up to concentrations of ~4%, while substantial shear-thinning behavior was observed at higher concentrations, indicated by flow behavior indices between 0.62 and 0.78. A more pronounced shear-thinning degree was shown for a concentration of 8% w/w, with flow behavior indices of 0.35 – 0.41 at different pH values (Bernaerts et al., 2017). Shear-thinning behavior was also observed for *Chlorella pyrenoidosa* at higher concentrations up to 20% w/w. However, above shear rates of 215 s^{-1} the viscosity was no longer dependent on the shear rate, reaching the infinite viscosity as predicted by the Cross model (Chen et al., 2018). The critical concentration between 4% and 6%, as concluded from the work of Wileman et al. (2012), coincides with a volume fraction of approximately 0.115. At higher volume fractions, the formation of cell aggregates occurs, which are elongated to the flow at higher shear rates, resulting in shear-thinning behavior (Cagney et al., 2017; Souliès et al., 2013). In addition, an apparent yield stress arises above a volume fraction of ~0.25 (Souliès et al., 2013). Even though some authors have observed a yield stress behavior for *Chlorella* sp. suspensions, they emphasized that the values were rather low (Bernaerts et al., 2017; Wu and Shi, 2008).

Shear-thinning flow behavior has been reported for several other microalgae species. For suspensions of *Arthrospira platensis*, deviation from Newtonian behavior was even observed at biomass concentrations of 0.5%. This was related to the filamentous morphology of *Arthrospira platensis* cells, which have stronger interactions with the medium at rest compared to spherical cells, but align to the flow when shearing forces are applied (Buchmann et al., 2018; Torzillo, 1997). Even though the critical concentration is much lower compared to *Chlorella* sp., i.e. a lower biomass concentration of *Arthrospira platensis* is required to achieve shear-thinning flow behavior, viscosities at higher concentrations (8 – 10%) were approximately ten times lower than those of *Chlorella* sp. suspensions (Bernaerts et al., 2017; Buchmann et al., 2018). In contrast, the critical concentration observed for suspensions of *Nannochloropsis* sp. was drastically higher. Schneider et al. (2016) proved that suspensions up to 15.8% behaved as a Newtonian fluid, while shear-thinning flow behavior was only reported above 17.7%. Hence, *Nannochloropsis* sp. cells do not tend to interact and require higher amounts to induce aggregation, simply based on increased volume fractions (Schneider et al., 2016). In addition, the shear-thinning region was shown to be very wide for concentrated suspensions of 24 – 25%, ranging from 0.001 s^{-1} to 1000 s^{-1} without observing the zero-shear or infinite viscosity plateaus, and no yield stress was observed (Schneider and Gerber, 2014; Yap et al., 2016b). As a consequence, the occurrence of strong interactions between *Nannochloropsis* sp. cells is unlikely, possibly

related to their rigid and uncharged outer cell wall, and to the absence of extracellular polysaccharides in this microalga species.

The importance of extracellular polysaccharides in the rheological behavior of biomass suspensions was demonstrated for *Porphyridium cruentum*. Bernaerts et al. (2017) reported high viscosities and a pronounced shear-thinning behavior for suspensions of 8% w/w. Flow behavior indices of 0.20 – 0.23 actually imply a very strong dependence of the shear rate. On the one hand, high viscosities were ascribed to the presence of extracellular polysaccharides that cause intercellular interactions, resulting in large and stable cell clusters. On the other hand, the shear-thinning behavior was also caused by solubilization of the extracellular polysaccharides into the continuous phase, inducing shear-thinning behavior of the serum phase (Bernaerts et al., 2017, 2018c). Consequently, viscosities up to 100 Pa s (i.e. 100 000 mPa s) were created, resulting in a sample that hardly pours and almost appears as a solid to the human eye (Steffe, 1996).

Relatively low viscosity values were observed for *Phaeodactylum tricornutum* and *Tetraselmis chuii*. In fact, only little deviation from Newtonian behavior was reported for *Phaeodactylum tricornutum* suspensions up to 8%, indicated by flow behavior indices between 0.83 and 1 (Bernaerts et al., 2017; Wileman et al., 2012). Despite the fact that the fusiform morphotypes of *Phaeodactylum tricornutum* have a higher aspect ratio than most spherical microalgae cells, and that non-spherical particles are orienting themselves to the flow, no pronounced shear-thinning behavior has been observed (Cagney et al., 2017). It can be assumed that little intercellular interactions occur in *Phaeodactylum tricornutum*, possibly due to the absence of extracellular polysaccharides in the fusiform morphotypes. Cagney et al. (2017) reported similar flow curves for *Phaeodactylum tricornutum* and motile *Tetraselmis chuii* in the same volume fractions.

Finally, the cultivation mode might influence the rheological properties of microalgal cultures. Chen et al. (1997) showed substantial differences in viscosity between heterotrophic and mixotrophic cultures of *Haematococcus lacustris* for concentrations between ~1% and ~4%. The authors reported unusual plastic flow behavior for both cultures, i.e. viscosity independent of the shear rate but dominated by a yield stress. However, it should be noted that the latter was obtained by extrapolation, since only few data points were collected at low shear rates (Chen et al., 1997). In contrast, the heterotrophically grown *Schizochytrium* sp. displayed shear-thinning behavior in aqueous suspensions. However, substantial deviation from Newtonian behavior was only observed for concentrations above 8%, and relatively low viscosities were obtained (Bernaerts et al., 2017; Chang et al., 2014).

While the flow behavior or viscosity has been studied for several microalgae species, little information is available on their linear viscoelastic behavior. However, the use of small (oscillatory) deformations within the linear viscoelastic region allows to characterize the native structure of the sample, while viscosity measurements somehow disturb this structure by applying larger deformations. Bernaerts et al. (2017) concluded an enormous variability in the linear viscoelastic behavior of seven microalgae species. As a matter of fact, three microalgae were characterized as weak gels, while no network structure was detected for the other four microalgae species (Bernaerts et al., 2017). The former microalgae (*Porphyridium cruentum*, *Chlorella vulgaris*, and *Odontella aurita*) were actually the extracellular polysaccharides producing strains, as shown in a subsequent study (Bernaerts et al., 2018a). Even though a low frequency dependence of approximately $G' \propto \omega^{0.1}$ was observed for the three microalgae, a stronger network structure was observed for *Porphyridium cruentum* as concluded from the higher absolute values of G' (Bernaerts et al., 2017). In contrast, a strong frequency dependence was shown for suspensions of *Scenedesmus obliquus* in concentrations between 3% w/v and 9% w/v. The linear relationship between storage modulus and angular frequency indicates a liquid-like behavior and consequently a low network structure in *Scenedesmus obliquus* suspensions (Adesanya et al., 2012). There is an obvious need for more research studies characterizing the linear viscoelastic properties of different microalgae species.

4.2 Food processing operations as a tool to functionalize microalgal biomass

The use of microalgal biomass in food products requires knowledge on their behavior upon processing. Many food products actually require processing, as a functionalization step to improve the quality of the product and/or as a preservation step to guarantee the safety of the product for consumption. While thermal processing is commonly used as a conventional preservation technique, it often leads to altered rheological properties of the food product. In addition, mechanical processes are often used as functionalization techniques, e.g. for physical stabilization of milk products or for creating desired structural properties of vegetable based products. These modified physical characteristics, including the rheological properties, are generally related to microstructural changes. A functionalization technique that is often applied in this context is high pressure homogenization.

The rheological properties of microalgal systems upon processing are very limitedly investigated. Bernaerts et al. (2017) studied the effect of high pressure homogenization and subsequent thermal processing on the rheological properties of seven microalgae species in aqueous suspensions (8% w/w). High pressure homogenization for 1 pass at 100 MPa variously affected the rheological properties for different microalgae. An improved viscosity was for instance observed after high pressure homogenization for *Arthrospira platensis* and *Chlorella vulgaris* due to the release of intracellular components as a result of cell disruption. A mechanical pretreatment might actually be desired to liberate intracellular components for further functionalization in a subsequent processing step (Bernaerts et al., 2017). In that context, high pressure homogenization has been proved one of the most effective disruption treatments for most microalgae. Safi et al. (2014a) concluded high pressure homogenization to be more efficient than manual grinding, ultrasonication, and chemical treatments for rigid cell walled species (*Nannochloropsis oculata*, *Haematococcus pluvialis*, and *Chlorella vulgaris*) as well as for microalgae with fragile cell walls (*Arthrospira platensis* and *Porphyridium cruentum*). But despite the very harsh homogenization conditions that were applied in that study (2 passes at 270 MPa), the recovery yield of released proteins was limited to 41 – 53% for the microalgae with rigid cell walls (Safi et al., 2014a). However, the use of protein release as an indicator for cell disruption generally underestimates the degree of cell rupture at high disruption levels due to degradation of the metabolites (E M Spiden et al., 2013). Nevertheless, *Nannochloropsis* sp. have been shown to be among the most resistant microalgae by several authors, requiring multiple passes at ultra high pressures above 250 MPa to achieve full cell disruption (Bernaerts et al., 2019; Erin M Spiden et al., 2013). As a consequence, the use of moderate high pressure homogenization conditions that are generally used in the food industry (typically below 100 MPa) limits the obtained cell disruption for this microalga. Indeed, no increase in viscosity was observed for *Nannochloropsis* sp. suspensions after high pressure homogenization at 100 MPa due to minimal cell disruption (Bernaerts et al., 2017).

Although a substantial degree of cell disruption was obtained for *Porphyridium cruentum*, the use of high pressure homogenization at 100 MPa resulted in a decreased viscosity and gel strength for this microalga (Bernaerts et al., 2017). It was shown that high shear forces disrupt the interactions and the network structure of microalgal extracellular polysaccharides (Ramus and Kenney, 1989). As a consequence, the stable network due to the cell clusters in *Porphyridium cruentum* suspensions, presumably created by the extracellular polysaccharides, was destroyed upon high pressure homogenization (Bernaerts et al., 2017). The effect of the pressure level was shown in a following study, as the use of a lower homogenization pressure of 20 MPa on thermally pretreated suspensions did not result in a decreased viscosity, while a drastic viscosity decrease was observed at 100 MPa (Bernaerts et al., 2018c). Hence, the presence of viscosity-increasing extracellular polysaccharides in *Porphyridium cruentum* dominates the structuring capacities of other (intracellular) components.

The use of thermal processing has been shown successful for several microalgae species in the creation of structured systems. The gel strength and viscosity of *Porphyridium cruentum* and *Chlorella vulgaris* increased up to 7 times by performing a thermal treatment of 15 min at 95 °C, which was in both cases

attributed to the formation of strong aggregates. However, whereas gelation of extracellular polysaccharides was identified as the mechanism for *Porphyridium cruentum*, protein solubilization and/or denaturation contributed to the formation of larger aggregates in *Chlorella vulgaris* (Bernaerts et al., 2018c). Although protein solubilization was also reported for *Nannochloropsis* sp. suspensions, Schneider and Gerber (2014) even observed a decreased viscosity for thermally pretreated suspensions at higher concentrations (10 - 22%). The authors attributed this to a smaller size and altered shape of the thermally treated *Nannochloropsis* sp. cells (Schneider and Gerber, 2014; Schwede et al., 2013). It is obvious that the intensity of the thermal treatment drastically affects the rheological properties. In fact, an intense sterilization process generally resulted in a significantly higher viscosity and gel strength than a milder pasteurization treatment (Bernaerts et al., 2017). Gonzalez-Fernandez et al. (2012) reported an intact cell wall when biomass of *Scenedesmus* sp. was treated at 70 °C, while cell disruption took place when heated at 90 °C. Consequently, the release of intracellular material resulting in the formation of aggregates only occurred when heated at 90 °C (González-Fernández et al., 2012).

Even though no studies were found concerning the rheological behavior of microalgal suspensions as affected by other food processing techniques, knowledge on the microstructural changes might be indicative for possible rheological changes. Microwave treatments have been shown to disrupt cell membranes and/or cell walls of microalgae, due to the heat and pressure build-up inside the cells (Günerken et al., 2015). As a result, cells are ruptured allowing intracellular material to be released, however to a lesser extent than high pressure homogenization (Cho et al., 2012; Heo et al., 2017). In contrast to the previously mentioned techniques, pulsed electric field caused permeation of the cell membrane rather than disruption of the cell wall. Carullo et al. (2018) clearly showed the shrinkage of *Chlorella vulgaris* cells after pulsed electric field treatment, indicating the partial release of intracellular compounds through the electroporated cell membranes (Carullo et al., 2018). However, since high molecular weight polymers are assumed to retain inside the cell wall, the formation of aggregates is less expected compared to high pressure homogenization and possible viscosity increases are therefore supposed to be less pronounced. Finally, extrusion was shown to result in a higher lipid extractability due to cell rupture, although the microstructure was changed to a lesser extent than for high pressure homogenized samples (Wang et al., 2018).

A combination of processing techniques might however be the best strategy to maximally exploit the structuring potential of the microalgae. A thermal treatment was actually proven more effective for *Chlorella vulgaris* suspensions when preceded by a high pressure homogenization step, since more released components were available for heat-induced interactions during the subsequent thermal treatment. Furthermore, it was shown that different processing sequences for successfully improving rheological properties of *Porphyridium cruentum* and *Chlorella vulgaris* also led to distinct microstructures (e.g. the presence of intact versus disrupted cells) (Bernaerts et al., 2018c). This was also previously reported by Spiden et al. (2015), who proved that a thermal pretreatment of *Chlorella* sp. resulted in the solidification of the protoplast. Although subsequent high pressure homogenization resulted in the removal of the cell wall, the protoplasts remained intact, in contrast to untreated cells where the intracellular material was released after high pressure homogenization (Spiden et al., 2015). Since the microstructure might be important for the digestibility and bioavailability of the nutritional compounds (as discussed in section **4.4**), processing sequences should be optimized to combine structural and nutritional benefits of the microalgae as functional food ingredients.

4.3 Microalgal biomass in real food matrices: impact on structure and texture

Throughout the past decade, many reports have been published on the addition of microalgal biomass to real food products, as presented in **Table 6**. A remarkable number of studies dealt with the use of *Arthrospira* sp. and *Chlorella vulgaris* biomass, although not surprising since these two microalgae are yet commercialized as healthy ingredients for human food. In contrast, not more than five reports have been

found for other microalgae species. Most studies are related to cereal based products, with main interest for enrichment of bread, cookies, and pasta, or to dairy products. Furthermore, different quality aspects have been evaluated, in particular nutritional value, color, texture, and sensory properties. In contrast, the rheological properties of fluid and semi-solid enriched food products are only limitedly studied. The next paragraphs will address the impact of microalgal biomass on the rheological properties of semi-solid food products, i.e. viscosity and/or viscoelastic behavior, based on the available studies summarized in **Table 7**.

García-Segovia et al. (2017) reported an increased viscosity of sourdoughs enriched with different microalgae species in a concentration of 1.5% w/w. However, while the viscosity was almost doubled by the addition of *Isochrysis galbana*, viscosity increases for *Nannochloropsis gaditana*, *Tetraselmis suecica*, and *Scenedesmus almeriensis* were rather limited (García-Segovia et al., 2017), which was in agreement with their rheological behavior in model systems (as discussed in section **4.1**). The effect of *Chlorella vulgaris* enrichment on the network structure of bread dough was shown to be dependent on the added concentration. In fact, the storage moduli only increased after addition of *Chlorella vulgaris* in the wheat flour up to 3% (i.e. resulting in a final concentration in the bread dough of approximately 1.8%), due to the reinforcement of the viscoelastic protein network by the microalgae. At higher concentrations, a decreased network structure was observed due to phase separation phenomena and disruption of the gluten matrix, as shown for concentrations of 4% to 5% (i.e. final concentrations of ~2.4% and ~3.0%, respectively). Nevertheless, the rheological properties of the dough were not representative for the texture of the final bread, since the firmness was not substantially affected by any microalga concentration (Graça et al., 2018). The hardness of several other cereal based products such as cookies and pasta generally increased by the addition of various microalgae (Babuskin et al., 2014; Batista et al., 2017; De Marco et al., 2014, 2018; Fradique et al., 2010; Gouveia et al., 2007, 2008b). The structuring potential of *Chlorella vulgaris* has also been proved in o/w-emulsions, representing mayonnaises and salad dressings. The presence of high amounts of proteins and carbohydrates in *Chlorella vulgaris* was evidenced to strengthen the emulsion structure through the formation of physical entanglements, resulting in an increased storage modulus and zero shear viscosity (Raymundo et al., 2005). In contrast, the addition of *Arthrospira platensis* did not result in increased viscosities of yogurt (Sengupta and Bhowal, 2017). A slight decrease in viscosity was even observed in an ice cream mix, but biomass of *Arthrospira platensis* was used to replace the commercial stabilizer sodium alginate. The authors therefore concluded that *Arthrospira platensis* can only be used to partially replace sodium alginate in ice cream, due to a lower water holding capacity of the cyanobacterium (Malik et al., 2013). The type of food product might also be a determining factor in the structuring capacity of microalgae. Even though *Arthrospira platensis* did not improve the rheological properties of dairy products, addition of the cyanobacterium to a gelatin-maltodextrin gel resulted in a strong reinforcement of the gels, even more pronounced than addition of *Chlorella* sp. (Firoozmand and Rousseau, 2014). In contrast, the efficiency of structuring gels with *Arthrospira maxima* was dependent on the type of biopolymer gel, while *Haematococcus pluvialis* resulted in stronger gel systems for all tested biopolymers (Batista et al., 2011, 2012).

It should be noted that food processing in the above-mentioned studies was restricted to incubation or fermentation at relatively low temperatures (**Table 7**). To maximally exploit the structuring potential of the microalgae, the use of other food processing operations might be an efficient strategy. However, no information is available on the functionalization of microalgae in a food matrix. In fact, the question arises whether microstructural changes (e.g. cell disruption, polymer interactions,…) will occur to the same extent in more complex food matrices as demonstrated in aqueous systems (section **4.2**), and whether drying techniques impact the rheological properties in the context of adding functionalized microalgae to food products. Despite the promising outcome of some exploratory studies, more research is required to evaluate the general potential of microalgal biomass as structuring agent in food products.

In spite of the increasing number of scientific reports investigating the addition of microalgal biomass to food products, to date only a limited number of microalgae-enriched food products are commercialized (Caporgno and Mathys, 2018; Lafarga, 2019). In fact, today's low marketability of microalgae based products is mainly attributed to challenges in scaling up microalgal biomass cultivation and in legislative approval of these products by regulatory authorities (Caporgno and Mathys, 2018; Sidari and Tofalo, 2019). Moreover, in most microalgae-containing food products available on the market, microalgae were not used for their techno-functional role, and were generally added in rather low concentrations. Lafarga (2019) actually concluded that to date microalgal biomass is either used as a coloring agent, as a nutritional ingredient, or as a marketing strategy (Lafarga, 2019). Hence, if the technological functionalities of microalgae in food products would be proven (e.g. microalgae functioning as thickening agent, resulting in a reduced need for additional food texturizers), the economic feasibility of this application would obviously be favored.

4.4 Implications of the use of whole biomass for nutrient bioaccessibility

The use of whole microalgal biomass in food products might have some implications for the nutritional efficacy of microalgal compounds in the human body. In this context, three important concepts should be introduced: bioaccessibility, bioavailability, and bioactivity. Bioaccessibility represents the fraction of the compound that has been released from the food matrix and has become available for absorption, and is usually evaluated by *in vitro* experiments. Bioavailability refers to the compounds that have been absorbed and have reached the systemic circulation, implying that they are available for the human body, and can be both determined by *in vitro* and *in vivo* tests. Only when a physiological response of the compound has been proved, it can be considered a bioactive compound (Carbonell-Capella et al., 2014). The extent to which a bioactive compound is released upon digestion in the gastrointestinal tract depends on several factors, including the physical entrapment in the food matrix, the physiological conditions and the digestive enzymes in the gastrointestinal tract. The latter consist of starch-degrading enzymes (α-amylase), proteases (pepsin, trypsin, chymotrypsin), and lipases (gastric and pancreatic lipase) (Minekus et al., 2014). However, apart from hydrolysis of α-1,4-linked glucans like starch, humans lack the ability to digest polysaccharides. Since most microalgal cell walls are composed of heteropolysaccharides or β-linked glucans (e.g. cellulose), it is assumed that microalgal cell walls will not be digested in the stomach and small intestine. In that case, the resistant cell wall acts as a physical barrier for the release of nutritional and health-beneficial components that are stored inside the cells. In this context, the use of processing techniques and cell disruption steps in particular might be desired to enhance the bioaccessibility of the health-beneficial components. However, very limited research has been performed on this topic so far.

The bioaccessibility of (intracellular) components of untreated microalgae after *in vitro* digestion seems to be species-dependent (**Table 8**). Generally, low bioaccessibility values (0 – 7%) have been reported for carotenoids in *Chlorella* sp. and *Scenedesmus almeriensis*, with the exception of 26% lutein bioaccessibility in *Chlorella vulgaris* (Cha et al., 2011, 2012; Gille et al., 2016; Granado-Lorencio et al., 2009). In contrast, higher values were observed for carotenoids in *Chlamydomonas reinhardtii* (10 – 20%) and *Phaeodactylum tricornutum* (27 – 52%) (Gille et al., 2016, 2018a; Kim et al., 2016). A possible explanation might be the distinct composition of the cell walls of these microalgae species. While *Scenedesmus almeriensis* and (some strains of) *Chlorella* sp. contain cellulose polymers in their cell wall, these are absent in *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*. The cell wall of *Chlamydomonas reinhardtii* is actually composed of glycoproteins, which might be sensitive to enzymatic degradation by the proteases in the gastrointestinal tract (Gille et al., 2016). However, more research is required to conclude a direct relation between the cell wall composition and carotenoid bioaccessibility. As a matter of fact, while the fragile cell wall of *Isochrysis galbana* (even considered absent by some authors) would suggest a high bioaccessibility of intracellular compounds, low values for fatty acid bioaccessibility

(8 – 13%) have been reported in this microalga. However, the authors also reported a very low degree of lipid hydrolysis after *in vitro* digestion, but it was unclear whether these low values could be attributed to the role of the cell wall as a physical barrier (Bonfanti et al., 2018).

Some fragmentary studies have been reported on the impact of processing on the carotenoid bioaccessibility of microalgal biomass, including different processing techniques such as sonication, bead and ball milling, microfluidization, and pulsed electric field treatment (**Table 8**). Sonication for 15 min at 20 kHz to increase the bioaccessibility of carotenoids was species-dependent. While an increased bioaccessibility of lutein and β-carotene was observed for *Chlorella vulgaris*, no increase was observed for *Chlamydomonas reinhardtii* after sonication. The latter already showed substantial bioaccessibility values before the sonication treatment, indicating that the cell wall of this microalga had little impact on the carotenoid bioaccessibility (Gille et al., 2016). Similarly, the effect of sonication on the carotenoid bioaccessibility in *Phaeodactylum tricornutum* was rather limited, with only slight increases for fucoxanthin and zeaxanthin. The average β-carotene bioaccessibility was largely increased to 76%, but a large standard error was observed from different repetitions of the experiments (Gille et al., 2019). Microfluidization was also considered a successful technique for improvement of the carotenoid bioaccessibility in *Chlorella* sp., but depending on the applied pressure level (Cha et al., 2011, 2012). In contrast, no improved carotenoid bioaccessibility was observed by ball milling of *Scenedesmus almeriensis*. In fact, below 1% of the initial lutein and zeaxanthin was recovered in the micellar phase. However, details on the pretreatment conditions were lacking, as well as on the cell wall integrity after ball milling (Granado-Lorencio et al., 2009). Higher values for carotenoid bioaccessibility were observed by Gille et al. (2018b) for other ball milled microalgae, between 5% and 26% for *Chlorella vulgaris* and between 17% and 20% for *Phaeodactylum tricornutum*. However, as no values were available for the untreated biomasses, no conclusions can be drawn on the effect of ball milling (Gille et al., 2018). The effectiveness of bead milling has however been proven by Cavonius et al. (2016) for the rigid microalga *Nannochloropsis oculata*, as the protein digestibility increased from 3% to 32% and the free fatty acid release from 0% to 34% (Cavonius et al., 2016). Finally, the use of pulsed electric field for increasing nutrient bioaccessibility has not been successfully proven yet. Rego et al. (2015) observed no increased chlorophyll bioaccessibility for *Chlorella* sp., but high values were yet observed for untreated biomass, indicating that the cell wall does not seem to be a barrier for chlorophylls. Moreover, since pulsed electric fields generally have little impact on the cell wall integrity, but mainly cause permeation of the cell membrane, no direct expectations can be established about the effect on the bioaccessibility.

It should be noted that various *in vitro* digestion models have been used in these studies. As a consequence, comparison of results obtained in distinct research studies is limited because of different simulated conditions, including sample volume, pH, ionic strength, enzyme activities, bile concentrations, and digestion times. For instance, incorporation of lipophilic compounds into mixed micelles is related to the amount of bile salts present. Under- or overestimation of the bile salts in the simulated digestion model might mistakenly lead to conclusions on the bioaccessibility of lipophilic nutrients, such as carotenoids and poly-unsaturated fatty acids. The use of a standardized *in vitro* digestion protocol, like the static digestion model proposed by Minekus et al. (2014), would aid the production of more comparable data in the future. In addition, in order to verify the hypothesis of the microalgal cell wall as a barrier for nutrient bioaccessibility, design of experiments should include microstructural analyses to obtain clear evidence on the cell wall integrity of the microalgal cells.

5 Conclusions and future outlook

Microalgae are considered one of the promising sources of functional food ingredients because of their attractive composition in terms of nutritional and health-beneficial components. Besides, structural

biopolymers such as proteins and carbohydrates generally represent a large fraction of the microalgal biomass. In spite of their potential as a technological food ingredient, e.g. functioning as a texturizer or stabilizer in food products, little knowledge has been gathered so far in this research domain. It is obvious that the evolutionary diversity of microalgae involves an enormous complexity in terms of presence, composition, and molecular organization of structural biopolymers. In addition, the impact of cultivation conditions on the biomass composition implicates a wide variability for microalgae, complicating the comparison of different research studies. At the same time, it creates the opportunity to modify cultivation parameters as an efficient strategy for enriching biomass in structural biopolymers. However, more clarity is required in the context of carbohydrate accumulation to clearly differentiate enrichment in storage polysaccharides from cell wall bound and extracellular polysaccharides.

Although several attempts have been made to characterize the molecular structure or composition of cell wall related polysaccharides, these microalgal polymers are poorly understood. Not only because of the limited number of microalgae species that have been investigated, but also because of a high variability in polysaccharide composition, even within a genus, a species, or a strain. However, since the functionality of cell wall polysaccharides is related to the precise molecular structure of the polymers, there is an urgent need for more insights into cell wall related polysaccharides of microalgae. In fact, to successfully introduce microalgal polysaccharides as novel food hydrocolloids, characterization and targeted functionalization should be performed for polysaccharides from individual strains. Fundamental knowledge on the polysaccharide composition might however aid to predict the functionality of specific polysaccharide extracts. Although very limitedly studied, the rheological properties of extracellular polysaccharides of *Porphyridium* sp. and *Cyanospira capsulata* reveal the promising outlook for microalgal polysaccharides in general. Their potential was obvious from intrinsic viscosities being higher than current thickening agents, but challenges with regard to extraction and purification of the polysaccharides need to be tackled. Furthermore, the question arises whether microalgal polysaccharides will be able to compete with conventional polysaccharides from plants or macroalgae, since the high production costs and complex regulatory issues (e.g. European novel food regulation) are limiting the industrial exploitation of microalgal polysaccharides.

A more economic and sustainable strategy is the incorporation of the whole microalgal biomass into food products, to overcome extraction protocols and waste streams. As such, the simultaneous introduction of nutritional and health-beneficial compounds allows the creation of high-quality food products, often accompanied by quality labels and/or nutrition claims. However, the structuring capacity of intact microalgal biomass is rather low, except for a few extracellular polysaccharide producing species. Therefore, food processing operations need to be considered and investigated in more detail to maximally exploit the structuring capacity of microalgal biomass, as indicated by some exploratory studies. The use of processing might not only be favorable for steering the rheological properties, there are indications that the use of cell disruption techniques also results in an improved bioaccessibility of intracellular components such as carotenoids. However, there is a need for more harmonized studies investigating the effect of processing on the bioaccessibility of microalgal compounds, for instance by using standardized *in vitro* digestion models. In addition, knowledge on the impact of processing should be extended, by considering other types of food processing operations and by optimizing process conditions such as pressure levels and time-temperature combinations for high pressure homogenization and thermal processing, respectively. Furthermore, it should be investigated whether this functionalization, e.g. cell disruption and improvement of rheological properties, can be performed to the same extent in a complex food matrix. The latter strategy would actually be preferred over a preceding functionalization of microalgal slurries that would later on be added to the food product, especially since the obtained functionalities might get lost during inevitable drying steps of the microalgal suspensions.

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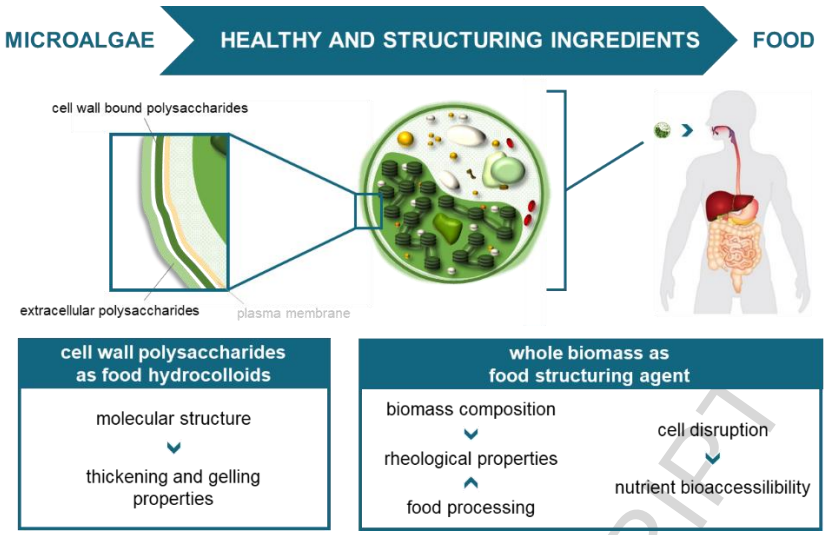

Fig. 1 Schematic representation of the different approaches to introduce microalgal biopolymers as structuring agents in food products.

Table 1 Proximate biomass composition of different microalgae species, expressed as percentage of dry matter (%).

Microalga species	Proteins (%)	Carbohydrates (%)	Lipids (%)	References
Arthrospira platensis (Spirulina)	43 - 77	8 - 22	4 - 14	Becker (2004); Bernaerts et al. (2018a); Ciferri (1983); Gouveia and Oliveira (2009); Kent et al. (2015); Matos et al. (2016); Parages et al. (2012); Safi et al. (2013); Tibbetts et al. (2015); Tokusoglu and Unal (2003); Wild et al. (2018)
Chlorella vulgaris	38 - 53	8 - 27	5 - 28	Batista et al. (2013); Bernaerts et al. (2018a, 2018c); Gouveia and Oliveira (2009); Kent et al. (2015); Matos et al. (2016); Rodolfi et al. (2009); Safi et al. (2013); Templeton et al. (2012); Tibbetts et al. (2015); Tokusoglu and Unal (2003); Wild et al. (2018)
Diacronema vlkianum	24 - 39	15 - 31	18 - 39	Batista et al. (2013); Fradique et al. (2013); Ponis et al. (2006)
Dunaliella sp.	27 - 57	14 - 41	6 - 22	Becker (2004); Gouveia and Oliveira (2009); Kent et al. (2015); Kim et al. (2015)
Haematococcus pluvialis	10 - 52	34	15 - 40	Batista et al. (2013); Damiani et al. (2010); Safi et al. (2013)
Isochrysis galbana	12 - 40	13 - 48	17 - 36	Batista et al. (2013); Fernández-Reiriz et al. (1989); Fradique et al. (2013); Ryckebosch et al. (2014); Sánchez et al. (2000); Tokusoglu and Unal (2003)
Nannochloropsis sp.	18 - 47	7 - 40	7 - 48	Bernaerts et al. (2018a); Gouveia and Oliveira (2009); Guil-Guerrero et al. (2004); Hu and Gao (2003); Huerlimann et al. (2010); Kent et al. (2015); Matos et al. (2016); Rebolloso-Fuentes et al. (2001a); Rodolfi et al. (2009); Ryckebosch et al. (2014); Safi et al. (2013); Templeton et al. (2012); Tibbetts et al. (2015); Wang and Wang (2012); Wild et al. (2018)
Odontella aurita	9 - 28	30 - 54	13 - 20	Bernaerts et al. (2018a); Xia et al. (2013)
Pavlova lutheri (*)	16 - 43	15 - 53	6 - 36	Bernaerts et al. (2018a); Fernández-Reiriz et al. (1989); Rodolfi et al. (2009)
Phaeodactylum tricornutum	13 - 40	6 - 35	14 - 39	Bernaerts et al. (2018a); Fernández-Reiriz et al. (1989); Guil-Guerrero et al. (2004); Matos et al. (2016); Rebolloso-Fuentes et al. (2001b); Rodolfi et al. (2009); Ryckebosch et al. (2014); Templeton et al. (2012); Tibbetts et al. (2015); Wild et al. (2018)
Porphyridium cruentum	27 - 57	12 - 39	5 - 13	Bernaerts et al. (2018a, 2018c); Matos et al. (2016); Rebolloso Fuentes et al. (2000); Rodolfi et al. (2009); Safi et al. (2013)
Scenedesmus sp.	31 - 56	6 - 28	8 - 21	Becker (2004); Gouveia and Oliveira (2009); Ho et al. (2012); Kent et al. (2015); Rodolfi et al. (2009)
Schizochytrium sp.	10 - 14	12 - 24	46 - 74	Bernaerts et al. (2018a); Chen et al. (2016); Johnson and Wen (2009); Liang et al. (2010); Qu et al. (2013); Wang and Wang (2012)
Tetraselmis sp.	14 - 58	12 - 43	8 - 33	Bernaerts et al. (2018a); Eboibi et al. (2015); Fernández-Reiriz et al. (1989); Huerlimann et al. (2010); Rodolfi et al. (2009); Schwenzfeier et al. (2011); Suarez Garcia et al. (2018b); Tibbetts et al. (2015)

(*) taxonomically revised to Diacronema lutheri

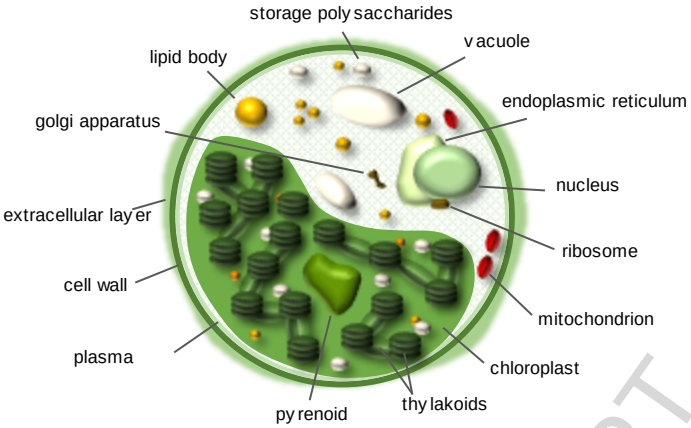

Fig. 2 Schematic representation of a eukaryotic microalgal cell, based on Pignolet et al. (2013), Baudelet et al. (2017), and Tomaselli (2004). Some organelles might be absent or differently organized in certain microalgae species. For a schematic representation of the ultrastructure of prokaryotic cyanobacteria in comparison to a eukaryotic microalgal cell, the authors recommend the review paper published by Pignolet et al. (2013).

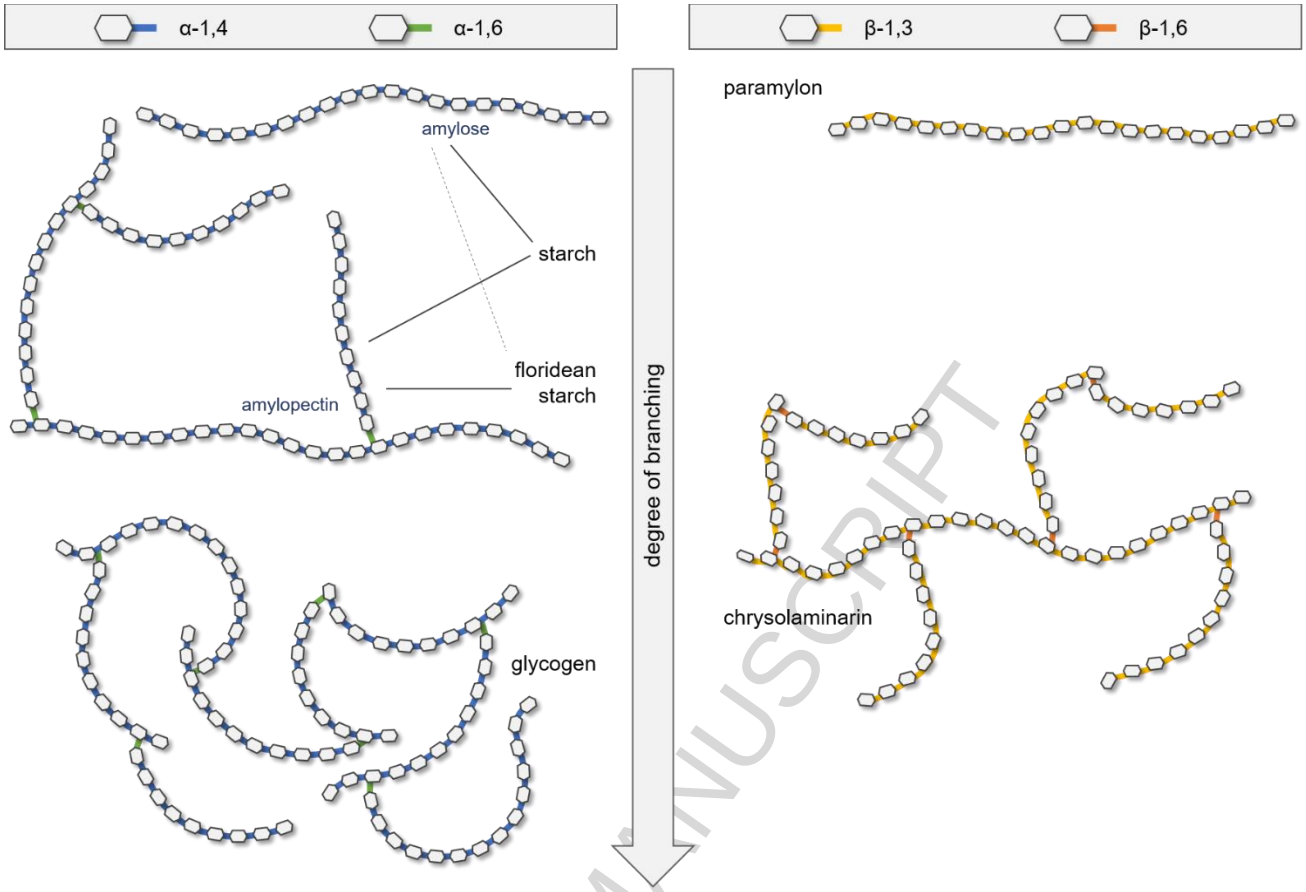

Fig. 3 Schematic representation of the five types of storage polysaccharides in microalgae and cyanobacteria.

Table 2 Presence of cell wall bound polysaccharides (CWPS) and extracellular polysaccharides (EPS) in some microalgae species (+ present; – absent; +/– only present in some subspecies or morphotypes; ? no consensus on the presence of a cell wall).

	Microalga species	CWPS	EPS	References
Cyanobacteria	*Arthrospira platensis (Spirulina)*	+	+	Bernaerts et al. (2018a); Bertocchi et al. (1990); Depraetere et al. (2015); Filali Mouhim et al. (1993); Roussel et al. (2015); Trabelsi et al. (2009)
Chlorophyta	*Chlorella* sp.	+	+	Bernaerts et al. (2018a, 2018c); Chen et al. (2015); Cheng et al. (2011, 2015); Kaplan et al. (1987); Yalcin et al. (1994); Yang et al. (2010)
	Dunaliella salina	–	+	Ben-Amotz and Avron (1992); Mishra et al. (2011); Mishra and Jha (2009)
	Scenedesmus sp.	+	+	Halaj et al. (2018); Lombardi et al. (2005); Takeda (1996)
	Tetraselmis chuii	+	–	Becker et al. (1995); Bernaerts et al. (2018a); Kermanshahi-pour et al. (2014)
Haptophyta	*Isochrysis* sp.	?	–	Bernaerts et al. (2018a); Pales Espinosa et al. (2010); Zhu and Lee (1997)
	Pavlova lutheri (*)	+	–	Arnold et al. (2015); Bernaerts et al. (2018a)
Eustigmatophyta	*Nannochloropsis* sp.	+	–	Arnold et al. (2015); Bernaerts et al. (2018a); Scholz et al. (2014)
Diatoms	*Odontella aurita*	+	+	Bernaerts et al. (2018a)
	Phaeodactylum tricornutum	+	+/–	Abdullahi et al. (2006); Bernaerts et al. (2018a); Guzmán-Murillo et al. (2007); Willis et al. (2013)
Rhodophyta	*Porphyridium* sp.	+	+	Bernaerts et al. (2018a, 2018b, 2018c); de Jesus Raposo et al. (2014); Geresh et al. (1992, 2002); Geresh and Arad (1991); Gloaguen et al. (2004); Patel et al. (2013); Percival and Foyle (1979); Roussel et al. (2015); Soanen et al. (2015)
Labyrinthulomyceta	*Schizochytrium* sp.	+	+/–	Bahnweg and Jackle (1986); Bernaerts et al. (2018a); Darley et al. (1973); Lee Chang et al. (2014)

(*) taxonomically revised to Diacronema lutheri

Table 3 Monosaccharide and uronic acid composition of cell wall bound polysaccharides of some microalgae species (data are not exhaustive). Predominant monosaccharides and uronic acids are indicated in bold. (Glc glucose; Gal galactose; Xyl xylose; Man mannose; Rha rhamnose; Ara arabinose; Fuc fucose; Rib ribose; GlcN glucosamine; Kdo 3-deoxy-manno-2-octulosonic acid; Dha 3-deoxy-lyxo-2-heptulosaric acid; GalA galacturonic acid; GlcA glucuronic acid; UA uronic acids; / unknown).

	Microalga species	**Monosaccharides and uronic acids**	**Sulfate esters (%)**	**References**
Cyanobacteria	*Arthrospira platensis (Spirulina)*	**Glc**, Gal, **Man**, Rha, **GlcN**, GalA, GlcA	0.6	Bernaerts et al. (2018a); Bertocchi et al. (1990)
Chlorophyta	*Chlorella* sp. (GlcN-rich type)	**Glc**, Gal, Xyl, Man, **Rha**, Ara, Fuc, **GlcN**, UA	/	Blumreisinger et al. (1983); Cheng et al. (2011, 2015); Kapaun et al. (1992)
	Chlorella sp. (Glc-Man-rich type)	**Glc**, Gal, **Man**, Rha, Rib, GlcN, GalA, GlcA	1.1	Bernaerts et al. (2018a); Loos and Meindl (1982); Shi et al. (2007)
	Chlorella sp. (Glc-Gal-Rha-rich type)	**Glc**, **Gal**, Xyl, Man, **Rha**, Ara, Fuc, Rib, GlcN, GalA, GlcA	/	Templeton et al. (2012)
	Scenedesmus sp.	**Glc**, Gal, Man, Fuc	/	Blumreisinger et al. (1983); Takeda (1996)
	Tetraselmis sp.	**Glc**, **Gal**, Xyl, **Man**, Rha, Ara, Fuc, **Kdo**, Dha, **GalA**, GlcA	1.2 - 6	Becker et al. (1995); Bernaerts et al. (2018a); Kermanshahi-pour et al. (2014); Schwenzfeier et al. (2011); Whyte (1987)
Haptophyta	*Isochrysis* sp.	**Glc**, Gal, Xyl, Man, Rha, **Ara**, Fuc, Rib, GalA, GlcA	1.1 - 5.5	Bernaerts et al. (2018a); Sun et al. (2014); Whyte (1987)
	Pavlova lutheri (*)	**Glc**, Xyl, Man, Ara, Fuc, Rib, GlcA	6	Arnold et al. (2015); Bernaerts et al. (2018a)
Eustigmatophyta	*Nannochloropsis* sp.	**Glc**, Gal, Xyl, Man, Rha, Fuc, Rib	6.4	Arnold et al. (2015); Bernaerts et al. (2018a); Scholz et al. (2014); Templeton et al. (2012); Vieler et al. (2012)
Diatoms	*Odontella aurita*	Glc, **Gal**, Xyl, **Man**, Rha, Fuc, Rib, GalA, GlcA	10.9	Bernaerts et al. (2018a)
	Phaeodactylum tricornutum	Glc, Gal, Xyl, **Man**, Rha, Fuc, Rib, GalA, **GlcA**	6.9 - 13.3	Bernaerts et al. (2018a); Ford and Percival (1965); Guzmán et al. (2003); Templeton et al. (2012)
Rhodophyta	*Porphyridium* sp.	**Glc**, **Gal**, **Xyl**, Man, Rha, GlcN, GalA, **GlcA**	4.2	Bernaerts et al. (2018a, 2018b)
Labyrinthulomyceta	*Schizochytrium* sp.	**Glc**, **Gal**, Xyl, Man, Rha, GlcN	2.7	Bahnweg and Jackle (1986); Bernaerts et al. (2018a); Darley et al. (1973)

(*) taxonomically revised to Diacronema lutheri

Table 4 Presence of algaenan in the cell wall of some microalgae species (+ present; – absent; +/– only present in some subspecies; (–) not investigated for these particular species, but typically not found in Haptophyta and diatoms).

	Microalga species	**Algaenan**	**References**
Cyanobacteria	*Arthrospira platensis (Spirulina)*	–	Ometto et al. (2014)
Chlorophyta	*Chlorella* sp.	+/–	de Leeuw et al. (2006)
	Dunaliella salina	–	Ben-Amotz and Avron (1992)
	Haematococcus pluvialis	+	Montsant et al. (2001)
	Scenedesmus sp.	+	Allard and Templier (2000); Burczyk and Dworzanski (1988)
	Tetraselmis chuii	–	Gelin et al. (1999)
Haptophyta	*Diacronema vlkianum*	(–)	de Leeuw et al. (2006)
	Isochrysis sp.	(–)	de Leeuw et al. (2006)
	Pavlova lutheri (*)	(–)	de Leeuw et al. (2006)
Eustigmatophyta	*Nannochloropsis* sp.	+	Gelin et al. (1999)
Diatoms	*Odontella aurita*	(–)	de Leeuw et al. (2006)
	Phaeodactylum tricornutum	(–)	de Leeuw et al. (2006)
Rhodophyta	*Porphyridium* sp.	–	Bold and Wynne (1985)
Labyrinthulomyceta	*Schizochytrium* sp.	–	Bahnweg and Jackle (1986)

(*) taxonomically revised to Diacronema lutheri

Table 5 Monosaccharide and uronic acid composition of extracellular polysaccharides of some microalgae species (data are not exhaustive). Predominant monosaccharides and uronic acids are indicated in bold. (Glc glucose; Gal galactose; Xyl xylose; Man mannose; Rha rhamnose; Ara arabinose; Fuc fucose; Rib ribose; GalA galacturonic acid; GlcA glucuronic acid; / unknown).

	Microalga species	**Monosaccharides and uronic acids**	**Sulfate esters (%)**	**References**
Cyanobacteria	*Arthrospira platensis (Spirulina)*	Glc, Gal, Xyl, Man, Rha, Ara, Fuc, Rib, GalA, GlcA	1.3 - 5.0	Bernaerts et al. (2018a); Depraetere et al. (2015); Filali Mouhim et al. (1993); Roussel et al. (2015); Trabelsi et al. (2009)
	Cyanospira capsulata	Glc, Man, Ara, Fuc, GalA	/	Cesàro et al. (1990); Vincenzini et al. (1993)
Chlorophyta	*Chlorella* sp.	Glc, Gal, Xyl, Man, Rha, Ara, Fuc, GalA, GlcA	1.7	Bernaerts et al. (2018a); Yalcin et al. (1994)
	Scenedesmus sp.	Glc, Gal, Xyl, **Man**, Rha, Ara, **Fuc**, GalA, GlcA	/	Halaj et al. (2018); Lombardi et al. (2005)
Diatoms	*Odontella aurita*	Glc, **Gal**, Xyl, Man, Rha, Fuc, GalA, GlcA	13.9	Bernaerts et al. (2018a)
	Phaeodactylum tricornutum	Glc, Gal, Xyl, Man, Rha, Ara, Fuc, Rib	/	Abdullahi et al. (2006); Willis et al. (2013)
Rhodophyta	*Porphyridium* sp.	**Glc**, **Gal**, **Xyl**, Man, Rha, Ara, Fuc, **GlcA**	1.0 - 15.1	Bernaerts et al. (2018a, 2018b); de Jesus Raposo et al. (2014); Geresh et al. (1992, 2002, 2009); Geresh and Arad (1991); Heaney-Kieras and Chapman (1976); Patel et al. (2013); Percival and Foyle (1979); Roussel et al. (2015); Soanen et al. (2015)

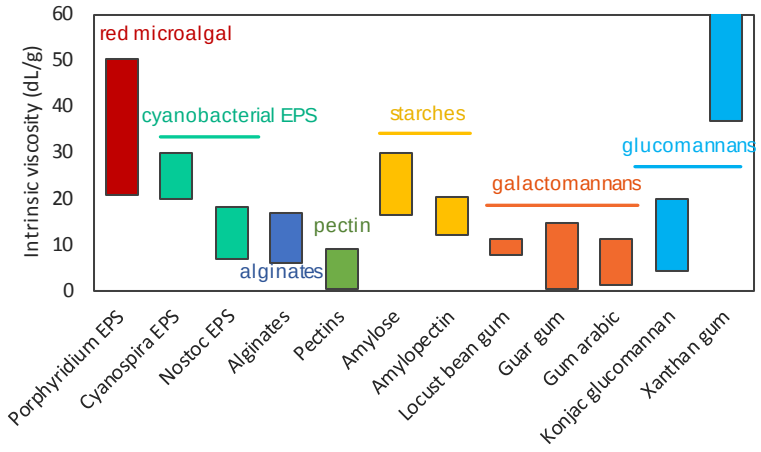

Fig. 4 Intrinsic viscosity of microalgal and cyanobacterial extracellular polysaccharides (EPS) in comparison with common thickening and gelling agents. Intrinsic viscosities of xanthan gum are not completely shown in the graph, but values up to 183 dL/g have been reported. References: BeMiller and Whistler (2009); Bernaerts et al. (2018b); Cesàro et al. (1990); Eteshola et al. (1996, 1998); Heaney-Kieras and Chapman (1976); Imeson (1997, 2011); Kishida et al. (1978); Parikh and Madamwar (2006); Rao (2010, 2013).

Table 6 Research studies on the incorporation of microalgal biomass in food products.

Microalga species	Food products		References
Arthrospira sp. *(Spirulina)*	Cereal based products	Bread, cassava cake, cookies, croissants, doughnuts, extruded snacks, noodles, pasta, strudel	Abd El Baky et al. (2015); Agustini et al. (2017); Batista et al. (2017); Bolanho et al. (2014); De Marco et al. (2014); Fradique et al. (2010); Joshi et al. (2014); Khosravi-Darani et al. (2017); Lemes et al. (2012); Lucas et al. (2017, 2018); Massoud et al. (2016); Navacchi et al. (2012); Onacik-Gür et al. (2018); Pagnussatt et al. (2014); Rabelo et al. (2013); Selmo and Salas-Mellado (2014); Shahbazizadeh et al. (2015); Singh et al. (2015); Tańska et al. (2017); Zouari et al. (2011)
	Dairy products	Fermented dairy product, feta cheese, yogurt	Barkallah et al. (2017); Beheshtipour et al. (2012); Golmakani et al. (2019); Guldas and Irkin (2010); Mazinani et al. (2016); Sengupta and Bhowal (2017); Varga et al. (2002)
	Fruit and vegetable products	Broccoli soup, smoothies	Castillejo et al. (2018); Lafarga et al. (2019)
	Meat products	Burgers, pâté, sausages, turkey breasts	Marti-Quijal et al. (2019a, 2019b); Thirumdas et al. (2018); Zamuz et al. (2019)
	Others	Gelled desserts, kiwifruit pastilles, shakes	Gouveia et al. (2008a); Khazaei Pool et al. (2016); Santos et al. (2016)
Chlorella vulgaris	Cereal based products	Bread, cookies, croissants, pasta	Batista et al. (2017); Fradique et al. (2010); Gouveia et al. (2007); Graça et al. (2018); Sahni et al. (2019); Shalaby and Yasin (2013)
	Dairy products	Cheese analogues, yogurt	Beheshtipour et al. (2012); Mohamed et al. (2013); Shalaby and Yasin (2013)
	Fruit and vegetable products	Broccoli soup, smoothies	Castillejo et al. (2018); Lafarga et al. (2019)
	Meat products	Burgers, pâté, sausages, turkey breasts	Marti-Quijal et al. (2019a, 2019b); Thirumdas et al. (2018); Zamuz et al. (2019)
	Others	Mayonnaise, o/w-emulsions	Abd El-Razik and Mohamed (2013); Gouveia et al. (2006); Raymundo et al. (2005)
Diacronema vlkianum	Cereal based products	Pasta	Fradique et al. (2013)
	Others	Gelled desserts	Gouveia et al. (2008a)
Dunaliella salina	Cereal based products	Pasta	El-Baz et al. (2017)
Haematococcus pluvialis	Cereal based products	Cookies	Mofasser Hossain et al. (2017)
	Others	o/w-emulsions	Gouveia et al. (2006)
Isochrysis galbana	Cereal based products	Bread, chikki, cookies, pasta	Babuskin et al. (2015); Fradique et al. (2013); García-Segovia et al. (2017); Gouveia et al. (2008b)
	Fruit and vegetable products	Tomato puree	Gheysen et al. (2019a)
	Others	Chewing gum	Palabiyik et al. (2018)
Nannochloropsis sp.	Cereal based products	Bread, chikki, cookies, pasta	Babuskin et al. (2014, 2015); De Marco et al. (2018); García-Segovia et al. (2017)
	Fruit and vegetable products	Tomato puree	Gheysen et al. (2019a, 2019b)
	Others	Chewing gum	Palabiyik et al. (2018)
Phaeodactylum tricornutum	Cereal based products	Cookies	Batista et al. (2017)
	Fruit and vegetable products	Tomato puree	Gheysen et al. (2019a)
Scenedesmus almeriensis	Cereal based products	Bread	García-Segovia et al. (2017)
Schizochytrium sp.	Fruit and vegetable products	Tomato puree	Gheysen et al. (2019a)
Tetraselmis suecica	Cereal based products	Bread, cookies	Batista et al. (2017); García-Segovia et al. (2017)
	Fruit and vegetable products	Broccoli soup	Lafarga et al. (2019)

Table 7 Research studies evaluating the rheological properties of food products as affected by the addition of microalgal biomass.

Microalga species	Food product	Biomass concentration (%)	Food processing	Rheology	References
Arthrospira platensis (Spirulina)	Ice cream mix	0.075 - 0.3	/	Viscosity (↘)	Malik et al. (2013)
	Yogurt	0.1 - 1.5	Incubation (24 h, 37 °C)	Viscosity (=)	Sengupta and Bhowal (2017)
Chlorella vulgaris	Bread dough	~0.6, ~1.2, ~1.8	Fermentation (1 h, 37 °C)	Viscoelastic (↗)	Graça et al. (2018)
	Bread dough	~2.4, ~3.0	Fermentation (1 h, 37 °C)	Viscoelastic (↘)	Graça et al. (2018)
	Mayonnaise	~0.5, ~1.5, ~2.5	/	Viscosity (=)	Abd El-Razik and Mohamed (2013)
	o/w-emulsion	2	/	Viscosity (↗) Viscoelastic (↗)	Raymundo et al. (2005)
Isochrysis galbana	Bread sourdough	1.5	Fermentation (2x 24 h, 8 °C)	Viscosity (↗)	García-Segovia et al. (2017)
Nannochloropsis gaditana	Bread sourdough	1.5	Fermentation (2x 24 h, 8 °C)	Viscosity (↗)	García-Segovia et al. (2017)
Scenedesmus almeriensis	Bread sourdough	1.5	Fermentation (2x 24 h, 8 °C)	Viscosity (↗)	García-Segovia et al. (2017)
Tetraselmis suecica	Bread sourdough	1.5	Fermentation (2x 24 h, 8 °C)	Viscosity (↗)	García-Segovia et al. (2017)

Microalga species	Bioactive compound	Processing technique	Bioaccessibility without processing (%)	Bioaccessibility after processing (%)	References
Chlamydomonas reinhardtii	β-carotene	Sonication	10	= ~10	Gille et al. (2016)
	Lutein	Sonication	20	= ~20	Gille et al. (2016)
Chlorella sp.	Antheraxanthin	Microfluidization	0	= 0	Cha et al. (2012)
	β-carotene	Ball milling	/	traces	Gille et al. (2018)
		Sonication	0	↗ 13	Gille et al. (2016)
		Microfluidization	2.6	↗ 7 - 32	Cha et al. (2012)
	Chlorophylls	Pulsed electric field	77 - 84	= 73 - 80	Rego et al. (2015)
	Lutein	Ball milling	/	5 - 26	Gille et al. (2018)
		Sonication	7	↗ 18	Gille et al. (2016)
		Microfluidization	26	↗ 57 - 73	Cha et al. (2011)
	Zeaxanthin	Microfluidization	1.7	↗ 3 - 18	Cha et al. (2012)
Isochrysis galbana	Fatty acids	/	8 - 13	/	Bonfanti et al. (2018)
Phaeodactylum tricornutum	β-carotene	Ball milling	/	19	Gille et al. (2018)
		Sonication	27	↗ 76	Gille et al. (2019)
	Fucoxanthin	/	40 - 44	/	Kim et al. (2016)
		Ball milling	/	20	Gille et al. (2018)
		Sonication	52	↗ 62	Gille et al. (2019)
	Zeaxanthin	Ball milling	/	17	Gille et al. (2018)
		Sonication	29	= ~30	Gille et al. (2019)
Scenedesmus almeriensis	Lutein	Bead milling	<1	= <1	Granado-Lorencio et al. (2009)
	Zeaxanthin	Bead milling	<1	= <1	Granado-Lorencio et al. (2009)

Table 8 In vitro bioaccessibility of different bioactive compounds (carotenoids, chlorophylls, and fatty acids) in microalgae, with and without different processing techniques (= unchanged by processing; ↗ increased after processing). Bioaccessibility of lipophilic components is defined as the ratio between the amount that is incorporated in mixed micelles and the initial amount present before the in vitro digestion procedure.