**Microalgal biomass as a (multi)functional ingredient in food products:
rheological properties of microalgal suspensions as affected by mechanical and
thermal processing**

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Abstract

Microalgae show great potential for use as novel ingredients in food products, as they are rich in several nutritional and health-beneficial components. However, addition of total microalgal biomass might alter the structural properties of the food system. Therefore, information is required about their rheological characteristics towards selection of microalgae species for specific food products. This study comprises the rheological characterization of seven commercially available microalgae species in aqueous suspensions, before and after mechanical and thermal processing. Substantial differences in rheological properties were observed between the investigated microalgal suspensions. Among the untreated suspensions, *Porphyridium cruentum*, *Chlorella vulgaris* and *Odontella aurita* showed the largest structural properties and could be described as weak gels. All suspensions showed shear-thinning flow behavior at the examined concentration of 8% w/w, except for *Nannochloropsis* species. Shear-thinning behavior was also observed for the separated serum phase of *P. cruentum*, which might be attributed to the presence of sulfated exopolysaccharides. During processing, rheological properties were significantly altered. High pressure homogenization was used as a mechanical treatment, followed by a pasteurization or sterilization process. Whereas suspensions of *Arthrospira platensis* and *C. vulgaris* showed an increased storage modulus and viscosity after processing, the opposite was observed for *P. cruentum* and *O. aurita*. No clear effect of processing was observed for suspensions of *Nannochloropsis* sp., *Schizochytrium* sp. and *Phaeodactylum tricornutum*. Investigation of the microstructure revealed differences in degree of cell disruption by high pressure homogenization, with *Nannochloropsis* sp. being the most resistant. Subsequent thermal processing resulted in aggregation of released cell material and/or intact cells. In conclusion, the obtained results provide the scientific knowledge base for the selection of microalgae species towards food applications. Whereas some microalgae species hardly affect the structural properties of the food product, other microalgae species show large potential for use as a structuring agent in food applications.

Keywords

Microalgae, aqueous suspensions, rheology, microstructure, high pressure homogenization, thermal processing

Highlights

- Rheological characteristics are largely depending on the microalga species
- *Porphyridium cruentum* possesses the largest structuring potential
- Most microalgae display shear-thinning flow behavior in 8% w/w suspensions
- HPH affects rheological properties by disrupting cells and releasing cell material
- Thermal processing increases viscosity by aggregation of (released) cell material

Abbreviations

ω 3-PUFA omega-3 polyunsaturated fatty acids

HPH high pressure homogenization

1. Introduction

Microalgae are considered an interesting biomass source towards various applications, including biofuels, nutraceuticals and pharmaceuticals. Moreover, they show great potential for use as functional ingredients in food products, as they are rich in several nutritional and health-beneficial components such as omega-3 polyunsaturated fatty acids (ω 3-PUFA), proteins and antioxidants [1]. In addition, microalgae offer many advantages over conventional crops, such as high photosynthetic efficiency and no competition for arable lands [2]. In recent decades, research has mainly focused on the extraction of specific components from microalgae, such as antioxidants, pigments and ω 3-PUFA enriched oil [3]. However, a more interesting approach would be the incorporation of the total dried biomass into food products. In this way, multiple nutritional and health-beneficial components are introduced in the food product and waste streams are avoided. However, this approach can affect the rheological properties of the enriched food product, an important quality aspect of the food product.

Processed food products present a large diversity in rheological properties, depending on the type of product. As a consequence, the appropriate rheological characteristics should be considered when selecting microalgae species for application in a specific food product. Low viscosities are typically desired for fluid food products, such as milk and fruit juices. In contrast, semi-solid products such as soups and sauces present characteristics of a more structured system, including a higher viscosity which is often achieved by addition of thickening agents. In this context, several studies investigated the rheological behavior of sulfated exopolysaccharides of *Porphyridium* sp., showing viscous solutions at low concentrations and gelling properties to some extent [4,5]. As a consequence, addition of biomass of certain microalgae species to food products might create the opportunity to increase structural properties of the enriched product, allowing their use as a multifunctional ingredient (i.e. combining health-related and structural properties). In fact, addition of *Isochrysis galbana* biomass (up to 3% w/w) in sweet biscuits resulted not only in ω 3-PUFA enrichment, but also improved textural properties of the biscuits [6]. However, information about the structuring potential of microalgal biomass in food products is limited to a few case studies, in which only a small number of microalgae species were studied in particular food systems [7–9].

A more fundamental approach, characterizing the rheological properties of several microalgae species in model systems such as aqueous suspensions, will provide the necessary scientific knowledge base towards these applications. In this context, microalgal suspensions can be described as complex systems consisting of algal cells and cell debris that are dispersed in a continuous phase containing water, dissolved salts and polymeric substances (e.g. extracellular polysaccharides). The rheological properties of microalgal suspensions can be attributed to both the particle characteristics, such as biomass concentration and physical properties of the algal cells, as well as the rheological properties of the continuous phase [10]. Moreover, previous studies have shown that most microalgal suspensions display Newtonian flow behavior at low biomass concentrations, while above a critical concentration, depending on the microalga species, microalgal suspensions exhibit non-Newtonian behavior [11–13].

With the aim of using microalgal biomass as a (multi)functional ingredient in food applications, fundamental knowledge is also required about their rheological behavior after processing. Mechanical processing is often applied for functionalization of food products, e.g. creating desired structural properties of vegetable based products [14]. On the other hand, thermal processing is a conventional preservation technique in food processing to create shelf-stable food products. Safety as well as quality of the product should be guaranteed, targeting both pathogenic and spoilage microorganisms, respectively. The presence and/or growth of these microorganisms is determined by the intrinsic properties of the product, such as pH. Generally, low-acid foods ($\text{pH} > 4.5$) require a sterilization process, targeting a 12 log reduction of the spore forming microorganism *Clostridium botulinum*. High-acid foods ($\text{pH} < 4.5$) on the contrary are preserved by a less intense pasteurization process, usually targeting the spoilage organism *Alicyclobacillus acidoterrestris* [15]. As a consequence, these differences in intensity of thermal processing might result in altered rheological characteristics.

As a mechanical process, high pressure homogenization (HPH) has proven an effective technique for disruption of microalgal cells [16,17]. Furthermore, thermal processing of microalgal suspensions might damage cell walls and result in solubilization of components and agglomeration of cell debris [18]. These changes in particle size and morphology as well as the release of intracellular components into the continuous phase might affect the rheological properties of the suspension. Moreover, the pH of the

suspensions might also influence the rheological properties of microalgal suspensions, since pH-dependent interactions between several biopolymers could be present.

To date, rheological properties of microalgal suspensions are very limitedly described in literature. In addition, comparison between different studies is difficult due to variation in biomass concentrations (ranging from 0.05 to 25% w/w), composition of the medium and applied rheological analyses (e.g. the range of shear rates during steady-shear measurements). Furthermore, to the best of our knowledge no studies were performed about the effect of mechanical and thermal processing on the rheological properties of microalgal suspensions.

The objective of this study is to characterize the rheological behavior of several commercially interesting microalgae species in aqueous suspensions. The microalgae species used in this study were selected based on their potential as a (multi)functional food ingredient, as most of them are rich in ω 3-PUFA, proteins and/or antioxidants [1,3]. On the one hand, acid food systems such as fruit juices and acidified food products were simulated by adjusting the pH of the suspensions to 4. On the other hand, suspensions at pH 6 were included to mimic low-acid to neutral food systems, such as vegetable based foods and dairy products. Both viscoelastic behavior (under small deformations) and flow behavior (under larger deformations) were studied. In addition, the effect of HPH and thermal processing on the rheological properties and particle characteristics of the microalgal suspensions was investigated. The intensity of the thermal treatments was based on industrial thermal processes used in the food industry. The understanding of the rheological behavior before and after processing provides the scientific knowledge base for the selection of appropriate microalgae species for food (and non-food) applications.

2. Materials and Methods

2.1. Microalgal biomass

Commercially available microalgal biomass was purchased from different companies. Lyophilized biomass of *Nannochloropsis* sp. was obtained from Proviron (Hemiksem, Belgium), *Odontella aurita* from Innovalg (Bouin, France) and the cyanobacterium *Arthrospira platensis* from Earthrise (Irvine,

CA, USA). Lyophilized biomass of *Schizochytrium* sp. was kindly donated by Mara Renewables Corporation (Dartmouth, Canada). Spray-dried biomass of *Chlorella vulgaris* was obtained from Allma (Lisbon, Portugal). Biomass of *Phaeodactylum tricornutum* and *Porphyridium cruentum* was obtained as a wet paste from Necton (Olhão, Portugal) and lyophilized before use. All biomasses were stored in closed containers at -80°C until use.

2.2. Preparation and processing of microalgal aqueous suspensions

A schematic overview of the preparation and processing of the microalgal aqueous suspensions is presented in **Fig. 1**.

2.2.1. Preparation of microalgal aqueous suspensions

Dry microalgal biomasses were suspended overnight in demineralized water by gentle stirring and pH of the suspension was adjusted to pH 4 or 6 using HCl. Demineralized water was subsequently added to obtain a final concentration of 8 g biomass per 100 g suspension (8% w/w). The systems were then mixed for 10 min at 3500 rpm (except for *P. cruentum* 10 min at 5500 rpm) with a lab mixer (Silverson L5M-A, East Longmeadow, MA, USA) to obtain a homogeneous suspension. The obtained suspensions at respective pH were considered as control suspensions. All suspensions were prepared in duplicate.

2.2.2. Mechanical treatment

Microalgal aqueous suspensions were mechanically treated by HPH (Panda 2K, Gea Niro Soavi, Parma, Italy) at 100 MPa for a single pass. The inlet of the homogenizer was thermostated at 4 °C using a cryostat (Haake, Karlsruhe, Germany) and homogenized samples were collected in an ice bath.

2.2.3. Thermal treatment

Homogenized suspensions were thermally treated by either a pasteurization (suspensions at pH 4) or a sterilization process (suspensions at pH 6). Thermal treatments were performed in a pilot-scale water-cascading retort (Barriquand Steriflow, Paris, France). Glass jars (95 mm height, 45 mm diameter) were filled with 80 mL of suspension and closed with metal lids. Temperature profiles were recorded both in the retort and in the coldest point of the jar using type T thermocouples and registered by the Ellab Valsuite Plus software (Ellab, Hillerød, Denmark).

Intensity of the thermal treatments was chosen based on industrially relevant thermal processing for shelf-stable food products. Suspensions at pH 4, simulating acid food matrices, were pasteurized at 90°C to obtain a process value of $^{10^\circ\text{C}}\text{PU}_{90^\circ\text{C}} = 3 \text{ min}$. For suspensions at pH 6, representing low-acid food products, a sterilization process at 121°C was selected with a process value of $^{10^\circ\text{C}}\text{F}_{121.1^\circ\text{C}} = 5 \text{ min}$. Due to differences in heat transfer characteristics of the investigated microalgae species, the length of the thermal processes varied from 44 to 84 min for the pasteurization process, and between 70 and 94 min for the sterilization process.

Fig. 1 Schematic overview of the preparation and processing of microalgal aqueous suspensions. Boxes with 'Control', 'HPH' and 'HPH+TT' indicate the moment of collecting respective samples.

2.3. Rheological measurements

2.3.1. Rheological characterization of microalgal suspensions

Prior to rheological analyses, stability of the suspensions against creaming or sedimentation was checked for 30 min, i.e. the longest time of a rheological measurement, by use of a Turbiscan MA 2000 (Formulation, L'Union, France). None of the suspensions showed significant creaming or sedimentation within this time frame.

The rheological properties of the microalgal suspensions were then analyzed with a stress-controlled rheometer (MCR 302, Anton Paar, Graz, Austria) at 25 °C. The concentric cylinder Couette (CC27, diameter 26.6 mm and measuring height 40.0 mm) was used as measuring system. A standardized volume of 22 mL suspension was loaded into the cup (diameter 28.9 mm) for each measurement. To avoid effects of loading on the structure, samples were presheared for 30 s at a shear rate of 20 s⁻¹

followed by 120 s of rest, i.e. shear rate of 0 s^{-1} , before each measurement. A minimum torque limit was fixed at $0.1 \text{ }\mu\text{N}\cdot\text{m}$ to ensure reliability of the obtained data.

The viscoelastic behavior of the suspensions was studied by oscillatory shear measurements. First, the linear viscoelastic region was determined by performing a strain sweep test at a constant angular frequency of 10 rad/s and a logarithmically increasing shear strain from 0.01 to 1000%. A constant shear strain of 1% within the linear viscoelastic region was then selected for the frequency sweep, with the exception of control suspensions of *O. aurita* for which a constant shear strain of 0.139% was used. The frequency sweep test was performed to determine the frequency dependence of storage modulus (G') and loss modulus (G''), by decreasing the angular frequency logarithmically from 10 to 0.1 rad/s . The flow behavior of the suspensions was studied by steady-shear measurements, with logarithmically increasing shear rate from 0.1 to 100 s^{-1} . During this test, 50 measuring points were recorded and each shear rate was applied for 10 s.

All measurements were performed in duplicate. A fresh sample was loaded into the cup for each measurement.

2.3.2. Viscosity measurements of the serum phase

Microalgal suspensions were centrifuged for 30 min at 10000 g and $25 \text{ }^{\circ}\text{C}$ (J2-HS centrifuge, Beckman, CA, USA) in order to separate particles from the continuous phase. The supernatant was collected and denoted as serum phase.

Viscosity of the serum phase was measured with a stress-controlled rheometer (MCR 302, Anton Paar, Graz, Austria) using the double wall Couette geometry (DG26.7, internal diameter 12.3 mm, external diameter 13.3 mm and measuring height 40 mm) to maximize the accessible range of torques. A standardized volume of 6.5 mL serum was loaded into the cup (internal diameter 11.9 mm, external diameter 13.8 mm) for each measurement. Shear rate was increased logarithmically from 0.1 to 100 s^{-1} and each shear rate was applied for 10 s. Preshear conditions as described in section 2.3.1 were used.

2.4. Characterization of the microstructure

2.4.1. Particle size distribution

Particle size distribution was analyzed by laser diffraction (Beckman Coulter LS 13 320, Miami, FL, USA). Sample was added to a stirring tank filled with deionized water and was pumped through the measuring cell at 30% pump speed, where particles were scattered by the laser light (wavelength of main illumination source: 750 nm; wavelengths of halogen light for polarization intensity differential scattering: 450 nm, 600 nm, 900 nm). Volumetric particle size distributions were calculated with the Fraunhofer optical model by use of instrument software. All measurements were carried out in duplicate.

2.4.2. Differential interference contrast microscopy

The microstructure of the samples was visualized by microscopic images, using an Olympus BX-51 light microscope equipped with a XC-50 digital camera (Olympus, Optical Co.Ltd., Tokyo, Japan) and visualized using Cell^F software. Objectives of 10×, 40× or 100× were used and at least ten images were obtained for each sample.

2.5. Statistical analysis

All treatments of the microalgal suspensions were performed in duplicate and for each individual sample, analyses were done in duplicate. As a result, the obtained data are presented as the mean of four measurements $\pm$ standard error.

The flow curves of the control suspensions were fitted to rheological models using linear and non-linear regression procedures with SAS statistical software (SAS 9.4, Cary, NC, USA). The estimated model parameters of different samples were statistically compared by using 95% confidence intervals. Differences in mean values for viscosity of mechanically and/or thermally treated suspensions were statistically analyzed using one-way ANOVA combined with Tukey's test for multiple comparison ($P < 0.05$) with JMP statistical software (JMP Pro 12, Cary, NC, USA).

3. Results and discussion

3.1. Rheological characterization of control suspensions

The rheological behavior of microalgal suspensions was characterized on two levels. On the one hand, small amplitude oscillatory measurements were performed to study the linear viscoelastic behavior of the

suspensions. In this case, small deformations were applied to gain insight into the network structure, without disturbing the structural properties of the system. By applying deformations within the linear viscoelastic region, the original structure of the suspensions could be characterized. On the other hand, the flow behavior was studied under larger deformations. This non-linear flow behavior can be related to food operations, including unit operations like mixing and pumping, as well as chewing and swallowing during consumption of a food product [19]. The combination of both linear viscoelastic and flow behavior provided a better understanding of the rheological properties of the microalgal suspensions.

3.1.1. Linear viscoelastic behavior

Small amplitude oscillatory measurements were performed to gain insight into the network structure of the microalgal suspensions. First, a strain sweep test was executed to determine the linear viscoelastic region. Strain sweep curves of *A. platensis*, *C. vulgaris*, *O. aurita* and *P. cruentum* are presented in **Fig. 2**, as reliable torque values ($> 0.1 \mu\text{N}\cdot\text{m}$) were only observed for these microalgae species. In these graphs, a zone of linear viscoelasticity could be observed as a plateau at low shear strains, with storage modulus (G') and loss modulus (G'') independent of the shear strain. In this linear viscoelastic region, suspensions of *C. vulgaris*, *O. aurita* and *P. cruentum* at both pH values showed $G' > G''$, indicating predominantly elastic behavior. In contrast, control suspensions of *A. platensis* at pH 6 displayed predominantly viscous behavior, since $G' < G''$ in the linear viscoelastic region. Further increase of the shear strain led to a decrease of both G' and G'' for *A. platensis*, *C. vulgaris* and *O. aurita*, showing that at these deformations the network structure was destroyed, typical for polymeric systems. In contrast, *P. cruentum* showed an increase in G'' together with a decrease of G' at the end of the linear viscoelastic region. The latter is typical suspension behavior. The main goal of the strain sweep was to select a constant shear strain for the frequency sweep test. A shear strain of 1% was found to be in the linear viscoelastic region for suspensions of *A. platensis*, *C. vulgaris* and *P. cruentum*. On the other hand, a shear strain of 0.139% was selected for control suspensions of *O. aurita*.

Fig. 2 Strain sweeps at a constant angular frequency of 10 rad/s of control suspensions at pH 4 (A) and pH 6 (B), showing storage modulus (G' , filled symbols) and loss modulus (G'' , empty symbols) as a function of shear strain (γ), for *A. platensis* ($\blacklozenge, \lozenge$), *C. vulgaris* ($\blacksquare, \square$), *P. cruentum* ($\bullet, \circ$) and *O. aurita* ($\blacktriangle, \triangle$).

Secondly, frequency sweep measurements were performed to characterize the network structure of the microalgal suspensions. Frequency sweeps of *C. vulgaris*, *P. cruentum* and *O. aurita* suspensions at both pH values are presented in **Fig. 3**. These suspensions showed an elastic-like behavior with $G' > G''$ over the complete range of angular frequencies. Since the other microalgae species did not display reliable torque values over the whole range of angular frequencies, they will not be further discussed in this paragraph.

Fig. 3 Frequency sweeps of control suspensions at pH 4 (A) and pH 6 (B), showing storage modulus (G' , filled symbols) and loss modulus (G'' , empty symbols) as a function of the angular frequency (ω), for *C. vulgaris* ($\blacksquare, \square$), *P. cruentum* ($\bullet, \circ$) and *O. aurita* ($\blacktriangle, \triangle$).

To determine the frequency dependence of G' and G'' , data of the frequency sweep were fitted by a power law model:

$$G' = a \cdot \omega^c \quad (\text{Eq. 1})$$

$$G'' = b \cdot \omega^d \quad (\text{Eq. 2})$$

with ω the angular frequency (rad/s) and a , b , c and d model parameters. Estimates for these parameters are presented in **Table 1**. The discussion will mainly focus on the frequency dependence of G' , since G'' values are more sensitive to inertia phenomena of the measuring system when analyzing samples with limited structural properties.

All suspensions showed similar values of parameter c and thus a similar frequency dependence of G' . With these values ranging between 0.11 and 0.17 and with $G' > G''$ over the whole range of angular frequencies, the rheological behavior of these suspensions coincides with that of weak gels. Indeed, whereas viscoelastic fluids exhibit high frequency dependence with $G' \propto \omega^2$ and $G'' \propto \omega^1$ at low angular frequencies, parameter $c < 0.5$ indicates the transition towards solid-like behavior [20,21]. According to Kavanagh and Ross-Murphy (1998), a weak gel consists of chains which are physically crosslinked into networks, but the crosslinks are of small finite energy and/or of finite lifetime [20]. Next to possible covalent crosslinks, also non-covalent crosslinks might be present in these microalgal suspensions. It is clear that none of these microalgal suspensions can be described as a ‘true gel’ of covalently crosslinked networks, which would show a clear independence of the angular frequency with $G' \propto G'' \propto \omega^0$. The ratio of G''/G' at low angular frequency ($\omega = 0.1$ rad/s) was between 0.19 and 0.49, comparable to that of weak gels (order of 10^{-1}) [22].

Higher values of parameters a and b were obtained for *P. cruentum* suspensions compared to those of *O. aurita* and *C. vulgaris*. This indicates that a stronger network structure is formed in *P. cruentum* suspensions. The presence of sulfated exopolysaccharides in *P. cruentum*, which have been shown to form a weak gel structure [23], might be one of the reasons for the stiffer network structure compared to the other microalgae species. Values of parameters a and b for *O. aurita* suspensions were of the same order

of magnitude as *C. vulgaris*. While suspensions of *P. cruentum* and *O. aurita* at pH 6 showed higher values of parameter *a* compared to pH 4 (and thus a stiffer network structure), the opposite was found for *C. vulgaris*. This might indicate the importance of ionic crosslinks between particles in network formation in these microalgal suspensions.

Table 1 Parameters *a*, *b*, *c* and *d* ($\pm$ standard error) of the power law models ($G' = a \cdot \omega^c$ and $G'' = b \cdot \omega^d$) fitted to the storage modulus (G') and loss modulus (G'') as a function of the angular frequency (ω) of the control suspensions of *P. cruentum*, *O. aurita* and *C. vulgaris*.

		a	b	c	d
<i>P. cruentum</i>	pH 4	41.64 $\pm$ 1.31	11.53 $\pm$ 0.26	0.15 $\pm$ 0.02	0.13 $\pm$ 0.02
	pH 6	48.62 $\pm$ 0.20	13.73 $\pm$ 0.05	0.17 $\pm$ 0.01	0.08 $\pm$ 0.01
<i>O. aurita</i>	pH 4	4.28 $\pm$ 0.15	1.25 $\pm$ 0.03	0.13 $\pm$ 0.02	0.29 $\pm$ 0.01
	pH 6	5.61 $\pm$ 0.31	1.35 $\pm$ 0.05	0.13 $\pm$ 0.04	0.31 $\pm$ 0.02
<i>C. vulgaris</i>	pH 4	6.77 $\pm$ 0.18	2.68 $\pm$ 0.06	0.11 $\pm$ 0.02	0.09 $\pm$ 0.02
	pH 6	6.00 $\pm$ 0.04	1.82 $\pm$ 0.02	0.13 $\pm$ 0.01	0.29 $\pm$ 0.01

3.1.2. Flow behavior

The flow behavior of all microalgal suspensions is visualized in **Fig. 4**, presented as the viscosity (η) as a function of the shear rate ($\dot{\gamma}$). With both axes in logarithmic scale, deviations from the Newtonian behavior can be easily observed. Among all microalgal suspensions, only *Nannochloropsis* sp. at both pH values exhibited Newtonian flow behavior (i.e. viscosity independent of the applied shear rate). In case of all other suspensions, viscosity decreased with increasing shear rate, indicating shear-thinning flow behavior. Since microalgal suspensions are dispersions of microalgal cells and cell debris in a liquid phase containing water, polymeric substances (e.g. exopolysaccharides) and dissolved salts, non-Newtonian flow behavior could be expected [11]. From **Fig. 4** it can be observed that *P. cruentum* suspensions were the most viscous over the whole range of shear rates, followed by suspensions of *C. vulgaris* and *O. aurita*.

Fig. 4 Flow curves of control suspensions at pH 4 (**A**) and pH 6 (**B**) showing viscosity (η) as a function of shear rate ($\dot{\gamma}$) for seven microalgae species: *P. cruentum* (●), *C. vulgaris* (●), *O. aurita* (●), *A. platensis* (●), *Schizochytrium* sp. (●), *P. tricornutum* (●) and *Nannochloropsis* sp. (●). Only data points with reliable torque values ($> 0.1 \mu\text{N}\cdot\text{m}$) are shown. For interpretation of color mentioned in this figure legend, the reader is referred to the web version of this article.

To describe the rheological characteristics of the control suspensions quantitatively, flow curves were fitted to rheological models. Newtonian, Power Law and Herschel-Bulkley models are frequently used to describe Newtonian or shear-thinning flow behavior, which are presented in **Eq. 3**, **4** and **5** respectively:

$$\sigma = \mu \cdot \dot{\gamma} \quad (\text{Eq. 3})$$

$$\sigma = K \cdot \dot{\gamma}^n \quad (\text{Eq. 4})$$

$$\sigma = \sigma_0 + K \cdot \dot{\gamma}^n \quad (\text{Eq. 5})$$

with σ representing the shear stress (Pa), $\dot{\gamma}$ the shear rate (s^{-1}), μ the dynamic viscosity ($\text{Pa}\cdot\text{s}$), K the consistency coefficient ($\text{Pa}\cdot\text{s}^n$), n the flow behavior index (-) and σ_0 the dynamic yield stress (Pa). The former two models can be considered as special cases of the Herschel-Bulkley model, with yield stress $\sigma_0 = 0$ and flow behavior index $n = 1$ or $n < 1$ for Newtonian and Power Law model respectively [19].

To select the appropriate model for each suspension, shear stress was plotted logarithmically as a function of logarithmically increasing shear rate. Since no constant values of $\log(\sigma)$ were reached at low shear rates and thus no clear yield stress was observed, all shear-thinning suspensions were fitted to the Power Law model. The used model and the parameter estimates are presented in **Table 2**.

As also observed visually from **Fig. 4**, *Nannochloropsis* sp. was the only microalga species presenting Newtonian flow behavior at the examined concentration. Its dynamic viscosity (i.e. consistency coefficient parameter in the Newtonian model) was low at both pH 4 and 6, approaching the viscosity of pure water (1 mPa·s). Thus, it can be inferred that no interactions between particles and only limited solubilization of cell components occurred in *Nannochloropsis* sp. suspensions. All other microalgal suspensions showed shear-thinning behavior with a flow behavior index $n < 1$. The smaller the value of n , the larger the deviation from Newtonian behavior. In this regard, the investigated microalgae showed a large variation in rheological properties with n ranging from 0.86 to 0.20. The smallest estimates of n were found for suspensions of *C. vulgaris*, *O. aurita* and especially *P. cruentum*, the viscosity thus showing the highest dependency on the applied shear rate. No consistent effect of pH on the flow behavior index n was observed among the different microalgal suspensions. Whereas *C. vulgaris* showed smaller values of n for suspensions at pH 6 compared to pH 4, the opposite was observed for *A. platensis* and *P. cruentum* suspensions. In contrast, no effect of pH on n was observed for the other microalgae species.

Considering the values of the consistency coefficient (K), the microalgae species can be divided into two groups. Control suspensions of *Nannochloropsis* sp., *P. tricornutum*, *Schizochytrium* sp. and *A. platensis* presented only limited consistency at both pH values. On the other hand, estimated values for K were about 10 to 100 times higher for suspensions of *C. vulgaris*, *O. aurita* and *P. cruentum*. Though, care should be taken when comparing the estimated values for K . In fact, these values are expressed in mPa·s ^{n} . Due to large variations in (non-)Newtonian behavior among the examined microalgae species, large differences were found in n which resulted in slightly different units of the consistency coefficient between different microalgae species. Nevertheless, the obtained shear stress at any shear rate can be described as a combination of an elastic and a hydrodynamic component [24]. When considering K as the hydrodynamic contribution to the shear stress at $\dot{\gamma} = 1 \text{ s}^{-1}$, parameter K is expressed in mPa, irrespective of the used (non-)linear regression model. The larger K , the larger the required shear stress to obtain a certain shear rate and thus the more consistent the fluid, leading to the same conclusions. Next to differences in flow behavior between the investigated microalgae species, significant differences were observed between suspensions at pH 4 and pH 6 for each microalga species. Higher values of K were obtained for suspensions

at pH 6 (with the exception of *Schizochytrium* sp.), suggesting the presence of ionic interactions between particles or polymers in the microalgal suspensions.

Although the Herschel-Bulkley model was not selected to fit the data, for some microalgae species a plateau of constant $\log(\sigma)$ values might be present at lower shear rates than the experimentally tested 0.1 s^{-1} . Extrapolation of the curve in the $\log(\sigma) - \log(\dot{\gamma})$ plot would result in estimated yield stresses around 4 Pa for *P. cruentum* at both pH values and well below 1 Pa for *C. vulgaris* and *O. aurita* suspensions. Nevertheless, since these are low yield stress values, their contribution will be very limited in food products.

Table 2 Consistency coefficient (K) and flow behavior index (n) ($\pm$ standard error) of the control suspensions estimated by Newtonian (N) or Power Law (PL) model. Goodness of fit of linear or non-linear regression is presented as R^2_{adj} . The estimated values of each parameter were compared statistically by use of 95% confidence intervals. Significant differences are indicated with different letters.

		Model	Consistency coefficient ($\text{mPa}\cdot\text{s}^n$)	Flow behavior index (-)	R^2_{adj}
<i>Nannochloropsis</i> sp.	pH 4	N	2.30 ± 0.01 (*)	1 (**)	0.99
	pH 6	N	2.40 ± 0.01 (*)	1 (**)	0.99
<i>P. tricornutum</i>	pH 4	PL	10.86 ± 0.80 j	0.86 ± 0.02 a	0.98
	pH 6	PL	16.40 ± 1.61 i	0.83 ± 0.02 a	0.96
<i>A. platensis</i>	pH 4	PL	50.83 ± 3.23 gh	0.55 ± 0.02 c	0.96
	pH 6	PL	58.17 ± 0.36 f	0.72 ± 0.01 b	0.99
<i>Schizochytrium</i> sp.	pH 4	PL	63.30 ± 5.39 fg	0.43 ± 0.02 d	0.89
	pH 6	PL	45.21 ± 1.89 h	0.43 ± 0.01 d	0.97
<i>C. vulgaris</i>	pH 4	PL	675.96 ± 21.66 e	0.41 ± 0.01 d	0.98
	pH 6	PL	1666.04 ± 22.36 c	0.35 ± 0.01 e	0.99
<i>O. aurita</i>	pH 4	PL	1393.29 ± 47.95 d	0.32 ± 0.01 e	0.97
	pH 6	PL	1687.11 ± 53.22 c	0.34 ± 0.01 e	0.98
<i>P. cruentum</i>	pH 4	PL	7735.59 ± 76.79 b	0.20 ± 0.01 g	0.99
	pH 6	PL	8227.23 ± 55.84 a	0.23 ± 0.01 f	0.99

(*) Represented as the dynamic viscosity ($\text{mPa}\cdot\text{s}$) in the Newtonian model

(**) Flow behavior index fixed at 1 by Newtonian model

Only few studies were found in literature about the rheological characteristics of microalgal aqueous suspensions. Moreover, results were difficult to compare due to differences in biomass concentration, suspending medium and analytical methods (e.g. range of shear stress used for fitting rheological models). As mentioned before, microalgal suspensions behave as Newtonian fluids below a critical concentration of suspended solids [11,13]. From the obtained results it can be concluded that the applied concentration of

8% w/w was above this critical concentration, resulting in shear-thinning flow behavior for all microalgae species except for *Nannochloropsis* sp. The latter has been reported to have smaller and more rigid cells compared to other species, hence being less sensitive to elastic deformations under shear [11,25]. However, at higher concentrations up to 25% w/w, shear-thinning flow behavior has been reported for *Nannochloropsis* sp. suspensions [12,13].

Shear-thinning flow characteristics have also been reported for cultures of *A. platensis* [26], in agreement with the obtained results. In contrast, Wileman, Ozkan and Berberoglu (2012) reported no deviation from the Newtonian behavior ($n = 1$) for suspensions of *P. tricornutum* at a concentration (80 kg/m^3) similar to the one used in this study [11]. Slurries of *C. vulgaris* were described as pseudoplastic materials by the same authors ($n = 0.62$), however they reported lower values for K compared to our findings. Variations in flow behavior might be explained by differences in suspending media. Differences in pH can alter the flow behavior of microalgal suspensions, as proven in this study. Moreover, the presence of dissolved salts in culture media might enhance possible interactions between microalgal polymers, resulting in altered rheological characteristics. Lastly, it might be possible that rheological properties were influenced during harvesting or drying steps of the microalgal biomass, resulting in differences when comparing the obtained results with rheological characteristics of growing cultures as mostly reported in literature.

3.2. Effect of mechanical and thermal processing on the rheological properties of microalgal suspensions

Next to the rheological characterization of the untreated microalgal suspensions, the effect of processing on the rheological properties was investigated. Processes were selected based on their industrial relevance in food processing. On the one hand, the impact of a mechanical treatment was examined, which is often applied for tailoring structural properties of food products [14]. In addition, HPH has been described in literature as a successful technique for disruption of microalgal cells [16,17]. On the other hand, the combined effect of mechanical and thermal treatments on the rheological properties of microalgal suspensions was investigated. Hereto, high pressure homogenized suspensions underwent either a pasteurization process (suspensions at pH 4) or a sterilization process (suspensions at pH 6), to simulate the creation of shelf stable food products. As a consequence, the intensity of the thermal treatment in this study

was always linked to the pH of the suspensions, an important fact to keep in mind during the interpretation of the obtained results.

3.2.1. Effect of processing on the linear viscoelastic behavior

To study the viscoelastic behavior of the processed suspensions, strain sweep and frequency sweep measurements were performed. A shear strain of 1% was found to be in the linear viscoelastic region for all treated suspensions. In order to provide a clear presentation of all data, the G' value at a certain angular frequency (namely $\omega = 10$ rad/s) was chosen for each sample to represent the rheological behavior. The results are shown in **Fig. 5**. Since frequency sweep measurements were performed at a constant shear strain within the linear viscoelastic region and with G' showing a limited dependence of ω , this approach was assumed to give an adequate representation of the results. It was validated that no major differences were observed when comparing values of G' at a lower angular frequency ($\omega = 1$ rad/s) (data not shown).

Oscillatory measurements on *P. tricornutum* and *Schizochytrium* sp. suspensions indicated that no network structure was created in suspensions of these two microalgae species, neither before nor after processing. Furthermore, no detectable G' was observed for control and HPH-treated suspensions of *Nannochloropsis* sp., while the combination of HPH and sterilization at pH 6 resulted in a measurable G' . Nevertheless, compared to (un)processed suspensions of other microalgae species, this increase in G' was rather limited. For the other microalgae species, different effects of processing were observed. Whereas HPH and/or thermal processing of *A. platensis* and *C. vulgaris* suspensions resulted in an increase of G' , the opposite was observed for suspensions of *P. cruentum* and *O. aurita*.

Control suspensions of *A. platensis* showed a negligible network structure at both pH values. While no reliable value of G' was observed upon HPH at pH 6, HPH at pH 4 significantly increased G' . No further increase was observed due to the pasteurization process. In contrast, sterilization of the suspension at pH 6 resulted in a large increase of G' , implying that a stiff network structure is created by an intense thermal treatment of *A. platensis* suspensions at pH 6. The same behavior was noticed for suspensions of *C. vulgaris*. An increase in G' upon HPH was only observed at pH 4 and a subsequent pasteurization

treatment did not result in a significant increase of G' , whereas the sterilization process at pH 6 led to the largest value of G' .

On the other hand, HPH resulted in a significant decrease of G' for suspensions of *P. cruentum* and *O. aurita* at both pH values. Thus, the network structure that was present in the control suspensions of these microalgae species was destroyed by the mechanical treatment under the investigated conditions. Ramus and Kenney (1989) reported a loss in bulk rheological properties of microalgal exopolysaccharides due to shear stress. Shear forces are suggested to disrupt the interactions between copolymers, thereby destroying the network structure of these exopolysaccharides [27]. Furthermore, Yap, Martin and Scales (2016) showed HPH causing breakage of cell aggregate networks in microalgal slurries, resulting in a reduction of the rheological properties [12]. From our results pH did not seem to play a significant role during the mechanical treatment of *P. cruentum* and *O. aurita*. During subsequent thermal processing, the network structure was somewhat recovered depending on the intensity of the thermal treatment (in combination with pH). However, although a significant increase in G' was observed after sterilization at pH 6 compared to the HPH-treated suspension, no significant differences were found in G' between the pasteurized and sterilized suspensions. It is clear that the slight regeneration of the network structure after thermal treatment did not restore to the initial value of G' for *P. cruentum* suspensions.

Fig. 5 Effect of processing on the storage modulus (G') at $\omega=10$ rad/s ($\pm$ standard error) of untreated microalgal aqueous suspensions (Control) and suspensions treated by high pressure homogenization (HPH) and a combination of high pressure

homogenization and thermal treatment (HPH+TT). Suspensions at pH 4 are represented by hatched bars, suspensions at pH 6 by filled bars. The obtained data were compared statistically (one-way ANOVA). Significant differences (Tukey test, $P < 0.05$) within one microalga species are indicated with different letters. Data below the reliable torque limit ($0.1 \mu\text{N}\cdot\text{m}$) are indicated as not detected (n.d.).

3.2.2. Effect of processing on the flow behavior

The flow behavior of the processed suspensions was studied under larger deformations. Results are presented in **Fig. 6** as the viscosity at a certain shear rate ($\dot{\gamma} = 10.5 \text{ s}^{-1}$) for each suspension. Comparing the values of η at a lower shear rate ($\dot{\gamma} = 1.1 \text{ s}^{-1}$) led to the same conclusions (data not shown).

Suspensions of *Nannochloropsis* sp., *P. tricornutum* and *Schizochytrium* sp. presented low viscosities in comparison with the other microalgae species. In case of *Nannochloropsis* sp., only the sterilization treatment at pH 6 caused a significant increase in η . Since the same was observed for G' (section 3.2.1), it can be concluded that the sterilization process mainly influenced the particle characteristics resulting in an increase of both G' and η . Although no significant differences were found between control suspensions and suspensions after HPH and/or pasteurization, it must be noted that both mechanical and thermal treatments changed the flow behavior of *Nannochloropsis* sp. suspensions from Newtonian to shear-thinning behavior (data not shown). No clear effects of processing were noticed for suspensions of *Schizochytrium* sp. and *P. tricornutum*.

For the other microalgae species, conclusions were consistent with those discussed in paragraph 3.2.1. Whereas mechanical and/or thermal processing (mainly sterilization) resulted in an increased viscosity of suspensions of *A. platensis* and *C. vulgaris*, a decrease in viscosity was observed after HPH in case of *P. cruentum* and *O. aurita*. Hence, the network structure formed by particle interactions throughout processing has a major contribution to the viscosity and flow characteristics of the microalgal suspensions. However, comparison of viscoelastic properties and flow properties indicate the importance of the continuous phase for some suspensions. Whereas control suspensions of *C. vulgaris* at pH 4 and pH 6 showed a similar value of G' , a significantly higher viscosity was observed for the suspension at pH 6. In other words, as the contribution of the network structure to the rheological properties was similar for both suspensions, the viscosity values suggest that additional structure build-up is brought by the liquid serum

phase. The same reasoning stands for HPH-treated suspensions of *C. vulgaris*, since a larger viscosity was obtained at pH 6 while a lower G' was observed compared to pH 4. Similarly, for *P. cruentum* suspensions it was also suggested that pH was an important factor determining the viscosity throughout the processing.

Fig. 6 Effect of processing on the viscosity (η) at $\dot{\gamma}=10.5 \text{ s}^{-1}$ ($\pm$ standard error) of untreated microalgal aqueous suspensions (Control) and suspensions treated by high pressure homogenization (HPH) and a combination of high pressure homogenization and thermal treatment (HPH+TT). Suspensions at pH 4 are represented by hatched bars, suspensions at pH 6 by filled bars. The obtained data were compared statistically (one-way ANOVA). Significant differences (Tukey test, $P < 0.05$) within one microalga species are indicated with different letters.

The contribution of the serum phase to the overall viscosity of the suspensions was further investigated by determining the viscosity of the separated serum phase. Indeed, for diluted suspensions comprising rigid particles, Einstein's relation (**Eq. 6**) shows that the viscosity is determined both by the properties of the dispersed particles and by the characteristics of the continuous serum phase:

$$\eta = \eta_s \cdot (1 + [\eta] \cdot \varphi) \quad (\text{Eq. 6})$$

with η the viscosity of the suspension (Pa·s), η_s the viscosity of the suspending medium i.e. the serum phase (Pa·s), $[\eta]$ the intrinsic viscosity (dL/g) and φ the phase volume of the suspended particles (g/dL) [21]. To study the contribution of the suspending medium to the overall viscosity of the suspension, steady-shear experiments were performed on the separated serum phase. Due to the high lipid content of

Schizochytrium sp., no perfect separation of serum and particle phase could be achieved by the applied centrifugation conditions for this microalga species.

The flow curves of the serum phase of the control suspensions are visualized in **Fig. 7**. Both graphs clearly show the non-Newtonian behavior of the serum phase of *P. cruentum* suspensions, while all other serum phases behaved as Newtonian fluids. The flow curves of the serum phase of *P. cruentum* suspensions were fitted by the power law model (**Eq. 4**). Low estimated values of the flow behavior index n (0.38 ± 0.01 and 0.32 ± 0.01 for pH 4 and pH 6, respectively) indicate the strong shear-thinning behavior. This shear-thinning behavior might be attributed to the presence of exopolysaccharides in the serum phase. In literature, dispersions of exopolysaccharides of *P. cruentum* have been reported to exhibit shear-thinning flow properties in a large range of shear rates, due to orientation and disentanglement of the polymer chains under shear [23,28]. The cells are assumed to be intact in the control suspensions, implying that the shear-thinning behavior and viscosity of the serum phase could be mainly attributed to the exopolysaccharides that easily dissolve in the continuous serum phase. In contrast, the serum phases of the other microalgae species were characterized by Newtonian flow behavior, but with different viscosity values. This might be due to the type and amount of salts and/or polymeric substances dissolved in the serum phase of the different microalgal biomasses. The occurrence of exopolysaccharides in these serum phases cannot be excluded, however it can be inferred that their contribution to the flow behavior was negligible.

Fig. 7 (A) Serum viscosity (η_s) and **(B)** shear stress (σ_s) as a function of shear rate ($\dot{\gamma}$) for control suspensions of six microalgae species. The serum phase of *P. cruentum* (●,▲) shows shear-thinning behavior, in contrast to the Newtonian behavior of the other microalgae species, *C. vulgaris* (●,▲), *O. aurita* (●,▲), *A. platensis* (●,▲), *P. tricornutum* (●,▲) and *Nannochloropsis* sp. (●,▲).

Suspensions at pH 6 are presented as solid circles, suspensions at pH 4 as empty triangles. For interpretation of color mentioned in this figure legend, the reader is referred to the web version of this article.

The properties of particles and serum phase might be altered after processing, possibly leading to changes in serum viscosity. It was observed that the serum phases of all microalgae species (except for *P. cruentum*) also exhibited Newtonian behavior after mechanical and thermal processing. Therefore, the serum viscosity of each (un)processed suspension was estimated by fitting the Newtonian model (Eq. 3) for these five microalgae species. These data are compared with the value of η_s at $\dot{\gamma} = 10.5 \text{ s}^{-1}$ for the (non-Newtonian) serum phase of *P. cruentum* in Fig. 8. At this selected shear rate, the serum viscosity of *P. cruentum* is more than ten times higher compared to the other microalgae species, before and after processing. Furthermore, the serum phase of *P. cruentum* is largely affected by processing. After HPH, a decrease in η_s was observed. Hence, these results support the hypothesis that HPH destroyed the exopolysaccharide network in *P. cruentum* suspensions. The subsequent thermal treatment seemed to induce interactions between polymers in the serum phase. It is known that thermal processing leads to solubilization of water soluble polysaccharides or particulate biomass [29,30], possibly resulting in an altered viscosity of the serum phase.

Different effects of processing on the serum viscosity were observed for the other microalgae species. Serum phases of *Nannochloropsis* sp. and *P. tricornutum* showed a constant viscosity around 1 mPa·s before and after processing, indicating that no viscosity-increasing components were present in the serum phase of these microalgae species. In contrast, control suspensions of *A. platensis*, *C. vulgaris* and *O. aurita* showed a higher serum viscosity, with significantly higher values at pH 6 compared to pH 4. This leads to the conclusion that pH-dependent interactions were present in the continuous phase of these suspensions. After processing, serum viscosity decreased for the three above-mentioned microalgae species. Lower serum viscosities upon processing might indicate the transfer of viscosity-increasing components out of the serum phase, possibly related to changes and interactions with particles during mechanical and thermal processing.

Fig. 8 (A) Serum viscosity (η_s) at $\dot{\gamma} = 10.5 \text{ s}^{-1}$ ($\pm$ standard error) and **(B)** serum viscosity (μ_s) estimated by the Newtonian model ($\pm$ standard error) of untreated microalgal aqueous suspensions (Control) and suspensions treated by high pressure homogenization (HPH) and a combination of high pressure homogenization and thermal treatment (HPH+TT). Suspensions at pH 4 are represented by hatched bars, suspensions at pH 6 by filled bars. The viscosity values at $\dot{\gamma} = 10.5 \text{ s}^{-1}$ were analyzed statistically by one-way ANOVA followed by Tukey test ($P < 0.05$). The estimated values of each parameter by the Newtonian model were compared statistically by use of 95% confidence intervals. Significant differences are indicated with different letters within each microalga species.

3.3. Relation between the rheological properties and microstructural characteristics of particles and serum phase during processing

The effect of mechanical and/or thermal processing on the microstructure was evaluated by studying the particle size distribution and was visualized by microscopic images. As different effects of processing on the rheological characteristics were observed in paragraph 3.2, the effect on the microstructure was likewise depending on the microalgal species and on the pH. Volumetric particle size distributions are presented for a selection of microalgae species in **Fig. 9** and representative micrographs (100 $\times$ magnification) are shown in **Fig. 10**.

The particle size of *A. platensis* was mainly affected by processing in suspensions at pH 6, while minimal changes were observed for suspensions at pH 4 (**Fig. 9A**). At pH 6, HPH resulted in a complete disruption of the microalgal cells, resulting in a narrow particle size distribution below 1 μm . The complete disruption might be ascribed to the cell wall characteristics of *A. platensis*. This species, belonging to the prokaryotic cyanobacteria, contains a thin cell wall mainly composed of peptidoglycan layers, which is easily disrupted by mechanical forces [31]. The subsequent sterilization process led to aggregation into larger particles up

to 100 μm . This phenomenon is assumed to be aggregation of denatured proteins, since the protein content can account up to 63% of the total biomass of *A. platensis* [32–34]. Since the contribution of the serum viscosity was very limited, the large increase in rheological parameters G' and η after sterilization of *A. platensis* suspensions can be attributed to the formation of these aggregates. Similar observations were made for suspensions of *O. aurita* (**Fig. 9B**). HPH resulted in a decreased particle size by the complete disruption of the microalgal cells, since particle sizes below 50 μm (the average length of an intact cell of *O. aurita* [35]) were observed. This was also visually confirmed by the micrographs presented in **Fig. 10**. Thermal treatment resulted in an increased particle size due to the formation of aggregates. The changes in rheological properties G' and η upon processing of *O. aurita* suspensions could be attributed to these changes in particle size. For this microalga species, no effect of pH was observed, neither on rheological characteristics nor on particle size distribution.

HPH under the applied conditions proved a successful technique for cell disruption of *A. platensis* and *O. aurita*. However, *Nannochloropsis* sp. cells showed to be more resistant to these pressure conditions, as no differences in particle size distribution were observed before and after HPH at both pH values (**Fig. 9C**) and intact cells were visualized in the micrographs after HPH (**Fig. 10**). *Nannochloropsis* sp. is known to be very resistant to several cell disruption techniques [17], which might be ascribed to the composition of the cell wall, a bilayer structure of cellulosic and algaenan polymers [25]. Spiden *et al.* (2013) reported that still 80% of *Nannochloropsis* sp. cells were intact after HPH for 1 pass at 90 – 102 MPa based on cell count analyses [16]. This low degree of cell disruption is in agreement with the results obtained in the present study. However, an increase in particle size was observed after thermal processing, especially for sterilized suspensions at pH 6. The micrograph shows aggregates of intact cells which seem to be embedded in solubilized cell material. Agglomeration of cell debris after a thermal treatment of microalgal biomass has been previously reported in literature without a preceding HPH treatment [18]. Since no changes in serum viscosity were observed for *Nannochloropsis* sp. suspensions, it can be concluded that the increase in rheological parameters G' and η after sterilization was caused by the formation of these larger aggregates.

Fig. 9 Volumetric particle size distribution of suspensions of *A. platensis* (A), *O. aurita* (B), *Nannochloropsis* sp. (C), *P. cruentum* (D), and *C. vulgaris* (E). Untreated control suspensions (—, —) and suspensions treated by high pressure homogenization (•••, •••) and a combination of high pressure homogenization and thermal treatment (—, —) are presented. Suspensions at pH 4 are shown in gray, suspensions at pH 6 in black.

While HPH and thermal processing largely affected the rheological properties of *P. cruentum* suspensions (Fig. 5 and Fig. 6), only small changes in particle size of this microalga species were observed (Fig. 9D). Bimodal particle size distributions were observed in the control suspensions at both pH values, in which one peak (1 – 10 μm) accounts for single cells and the other peak (10 – 100 μm) for clusters of intact cells, as observed visually in the micrographs (Fig. 10). Exopolysaccharides in *P. cruentum* might act as a glue between intact cells, in that way enhancing cell clustering. Subsequent HPH resulted in a decreased particle size for suspensions at pH 6, while larger particles were formed in homogenized suspensions at pH 4. It

was observed from microscopic images (not shown) that the degree of cell disruption seemed to be pH-dependent, since more intact cells were found in HPH-treated suspensions at pH 4 compared to pH 6. The larger aggregates at pH 4 consisted of intact cells embedded in a matrix of cell material, while at pH 6 smaller aggregates of mostly released material were observed. The effect of pH on the particle size is consistent with a larger G' for pH 4 compared to pH 6 (however, not significant). These aggregates might be composed of several components, such as denatured proteins and cell wall related (exo)polysaccharides, since equal amounts of proteins and carbohydrates are found in *P. cruentum* biomass [36]. Moreover, differences in aggregate formation between pH 4 and pH 6 suggest the presence of pH-dependent interactions between above-mentioned components. Whereas the pasteurization at pH 4 did not lead to significant changes in particle size, the sterilization process at pH 6 resulted in an increased size of the particles. However, these aggregates were still smaller compared to the particles in the pasteurized suspension. The larger suspension viscosity of the sterilized sample of *P. cruentum* can thus not only be ascribed to the size of the particles, but also to the contribution of other particle properties and the somewhat higher viscosity of the serum phase.

Similarly to *P. cruentum*, bimodal particle size distributions were observed for control suspensions of *C. vulgaris* (**Fig. 9E**). Larger aggregates of intact cells suggest the presence of a gel of extracellular polysaccharides in this microalga species. However, from literature it is not clear whether exopolysaccharides are formed by *Chlorella* species. Whereas some authors have described the presence of sulfated extracellular polysaccharides of *Chlorella* sp. [37,38], other studies did not report any cell envelope in this microalga [39]. When suspensions of *C. vulgaris* were treated by HPH, larger aggregates were formed, especially at pH 6. From the micrographs it can be observed that the aggregates were made of released cell material, indicating that cell disruption has taken place. This observation corresponds with a study of Spiden *et al.* (2013), where HPH under similar conditions (1 pass at 86 – 96 MPa) was found to disrupt around 50% of *Chlorella* sp. cells [16]. This might be even an underestimation of the degree of cell disruption, as breakdown of the cell wall might result in intact protoplasts. These protoplasts showed similar size and shape to undamaged cells, complicating it to distinguish them from intact cells during cell counting [18]. Indeed, Yap *et al.* (2014) observed only 25% intact cells after a single pass of HPH at 100

MPa [40]. Subsequent thermal processing resulted in an increased particle size, especially for sterilized suspensions at pH 6. As *C. vulgaris* biomass contains around 49 – 57% proteins [34,41], it is very likely that the increased particle size can be ascribed to aggregation of denatured proteins. Based on the observed changes in microstructure, the rheological behavior of *C. vulgaris* suspensions during processing can be explained. A combination of intact cells and cell clusters resulted in a large G' of the control suspensions, with a higher value of η at pH 6 compared to pH 4 due to a higher serum viscosity. After mechanical and/or thermal processing, the suspension viscosity increased due to formation of larger particles. In contrast, the viscosity of the serum phase decreased. As observed from the micrographs (Fig. 10), released cell material was found in the aggregates in HPH- and HPH+TT-treated samples. It is hypothesized that suspended metabolites are also involved in the formation of aggregates, leading to less viscosity-increasing components in the serum phase and thus resulting in a lower serum viscosity.

Fig. 10 Microscopic images of untreated microalgal suspensions (Control) and suspensions treated by high pressure homogenization (HPH) and a combination of high pressure homogenization and thermal treatment (HPH+TT). All micrographs represent suspensions at pH 6, implying that samples of HPH+TT were sterilized.

666

667 **4. Conclusions**

668 This study compared the rheological properties of seven commercially available microalgae species in
669 aqueous suspensions. Rheological characteristics were largely dependent on the microalga species.
670 Suspensions of *P. cruentum*, *C. vulgaris* and *O. aurita* presented the largest structural properties, whereas
671 other suspensions could not be analyzed under small deformations. This suggests differences in biopolymer
672 composition among the investigated microalgae species. As the composition of microalgal biomass is
673 depending on cultivation and harvesting conditions, characterization of the biopolymer composition of the
674 investigated microalgal biomasses would provide useful additional insights into their rheological behavior.
675 Furthermore, it was observed that rheological properties of the control suspensions were pH-dependent,
676 since larger consistency coefficient values were observed for suspensions at pH 6 compared to pH 4. The
677 interactions of several biopolymers, like cell wall polysaccharides and proteins, are influenced by
678 environmental conditions such as pH. Therefore, detailed investigation of the structure of the cell wall
679 polysaccharides would provide a better understanding of the structuring potential of microalgae,
680 information that is at the moment very scarce in literature.

681 The obtained results also proved that rheological properties of microalgal suspensions can be altered during
682 mechanical and/or thermal processing. Differences in cell rupture by HPH were found between the
683 investigated microalgae species, which can be related to size and rigidity of the cells. The effect of
684 subsequent thermal processing on the microstructure and rheological properties is affected by the previous
685 mechanical treatment, as thermal processing was shown to enhance interactions between released cell
686 material. On the other hand, it was observed that a sterilization process might also lead to solubilization of
687 components of intact cells, as was the case for *Nannochloropsis* sp. suspensions. Therefore, applying
688 different sequences of mechanical and thermal processing might provide interesting additional information
689 in tailoring the structural properties of microalgal suspensions.

690 The present study allows the selection of microalgae species towards desired applications. For instance,
691 biomass of *Nannochloropsis* sp., *P. tricornutum* and *Schizochytrium* sp. could be of interest for enriching

food products in ω 3-PUFA, without disturbing the structural properties of the product. This can be desired in fluid food systems, such as fruit juices and smoothies. On the other hand, biomass of *P. cruentum* showed large structuring potential and could thus be used as a multifunctional ingredient in food products, providing improved nutritional value as well as thickening effects to the food product. Similarly, biomasses of *C. vulgaris* and *A. platensis* find applications as a multifunctional ingredient in food products which are processed mechanically and/or thermally, such as vegetable based soups or several dairy products. Although large potential is shown for the application of microalgal biomass in food products based on structuring properties, additional research is required on the nutritional stability of microalgal components during processing as well as the effect on other food quality aspects, such as color and flavor of the enriched food product.

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