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Integrity of the microalgal cell plays a major role in the lipolytic stability during wet storage

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ABSTRACT

Microalgae are promising sources of lipids for applications in food, fuel, pharmaceutics and cosmetics. However, these lipids are also subject to lipolysis reactions during processing and storage. Especially during wet biomass storage, substantial amounts of free fatty acids (FFA) are formed, which has negative effects on flavor and nutritional value and can cause downstream processing problems in biodiesel production. This study contributes to a better understanding of the underlying processes leading to lipolysis by focusing on cell wall differences between species. Therefore, in a first phase, a comparison was made between the lipolytic stability of *Nannochloropsis oculata* and *T-Isochrysis lutea*, two species reported to have substantial differences in cell wall structure and strength. *Nannochloropsis* appeared to be stable during the first days of wet storage, while in *T-Isochrysis*, lipolysis started immediately after harvest. In a second experiment, the influence of cell disruption by high pressure homogenization (HPH) on lipolytic stability during wet storage of *Nannochloropsis* was investigated. It was observed that the HPH treatment induced lipolysis, as the FFA content was rapidly increasing immediately after the treatment, in contrast to the control sample, in which the FFA content was constant during the first days of storage. The integrity of the microalgal cell was thus found to play a crucial role in lipid stability during short-term post-harvest wet storage.

1. Introduction

Microalgae are an extremely diverse group of micro-organisms, living in marine, brackish or freshwater environments. Between 6 and 50% of the biomass is made up of lipids, depending on the species and the cultivation conditions [1,2]. These lipids are promising for several **applications**. Many autotrophic microalgae contain extensive percentages of health-promoting long chain omega-3 polyunsaturated fatty acids (ω-3 LC-PUFA), especially docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) [3] and can therefore be used in food applications, pharmaceuticals and cosmetics [4,5,6]. Furthermore, lipids from microalgae can possibly be used in biodiesel production [7,8].

However, for these different applications, there are some stability problems that have to be overcome, but are nowadays often underestimated. During processing and storage, **lipolysis**, mostly caused by endogenous enzymes (lipases), can take place, a process during which fatty acids are released from lipids. The formed free fatty acids (FFA) are deleterious for lipid quality, for fuel and food applications, as well as for pharmaceuticals and cosmetics. Firstly, FFA cause several rancid off-flavors, which are disadvantageous for food applications [9]. Furthermore, in oils containing more than 0.5% FFA, FFA can react in a saponification reaction with alkali catalysts used in biodiesel industry, leading to a reduced efficiency and an increased need for downstream processing [10]. In addition, FFA are known to have a pro-oxidative effect [11,12] and to be more readily oxidized by lipoxygenase enzymes [13,14]. This secondary effect of the presence of FFA also has detrimental effects on oil quality, as oxidation products of lipids can have a negative impact on flavor and nutritional value, can cause bleaching [15] and formation of deposits in biodiesel production systems [16].

Storage of microalgal biomass can be conducted either as a paste after harvesting (dry weight 5–25%, “wet storage”) or as a powder after drying the paste (dry weight 90–95%, “dry storage”) [8,17]. The difference in moisture content between these two storage stages has an

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important impact on the stability of the biomass and the lipids in particular. A higher moisture content during wet storage implies higher reaction rates and thus faster lipolysis processes. Dry storage is thus preferred to obtain products of good quality [18,19]. However, a (short) period of wet storage is often necessary, either for practical reasons, or to avoid the energy consuming drying step [20]. The latter implies lipid extraction from wet biomass or direct transesterification reactions in wet biomass [21,22].

In previous research, Balduyck et al. [23] already showed that wet storage has detrimental effects on lipolytic stability. To the best of our knowledge, that was the first detailed study focusing on the impact of wet storage on lipolysis in microalgae. In that study, the temperature dependence of lipolysis during wet storage of *T-Isochrysis lutea* was investigated. A very fast increase of the FFA content was observed during storage at 20 °C and 4 °C, while at − 20 °C, almost no changes were observed. After 7 days at 20 °C and after 14 days at 4 °C, the FFA content was about 30% of the lipids and was found to stabilize at this value. The presence of fatty acyl esters suggested esterification reactions between alcohols and FFA, causing the FFA content not to increase further.

It was suggested that the low lipolytic stability in *T-Isochrysis* was due to the weak cell wall of this microalgal species, consisting mainly of mannose [24,25]. The cell (either the cell wall, cell membrane and/or organelle membranes) was possibly already disrupted during the harvesting process, leading to an increased contact between enzymes and substrates and possibly to the activation of lipase enzymes, as was already observed in some studies on diatoms [26,27,28]. In these studies, the resulting FFA were further degraded by lipoxygenase and hydroperoxide lyase enzymes to among others oxylipins (e.g. aldehydes), reactions that were attributed to a defense mechanism against grazers [26,27,28].

This study investigates the species dependency of the lipolysis process during wet storage and the consequences for lipid quality. As the strength and structure of the cell were hypothesized to play a major role in the cell integrity and consequently in the lipolytic stability, two species that are strongly differing in this characteristic, namely *T-Isochrysis lutea* and *Nannochloropsis oculata*, were selected. In contrast to the weak cell wall of *T-Isochrysis*, consisting of mannose [24], the tough cell wall of *Nannochloropsis* consists of cellulose and algaenan [29]. Firstly, the temperature dependence of lipolysis in *Nannochloropsis* was studied and compared with the observations made for *T-Isochrysis* in Balduyck et al. [23]. The observed big difference in lipid stability between these two species was hypothesized to originate from the difference in cell structure and strength, causing a different cell integrity after harvesting. Therefore, the tough cell wall of *Nannochloropsis* was disrupted by high pressure homogenization (HPH). Subsequently, both the disrupted and non-disrupted biomass was stored as a wet paste at 4 °C for 4 weeks to investigate the influence of cell disruption on lipid stability. This could elucidate the role of cell integrity, as determined by among others the cell wall structure and strength, in lipid stability during wet storage.

2. Materials and methods

2.1. Cultivation and harvesting

Cultivation of *T-Isochrysis lutea*, formerly named *Isochrysis sp.* (CCAP 927/14; Culture Collection of Algae and Protozoa, Oban, United Kingdom), and *Nannochloropsis oculata* (SAG 38.85; University of Goettingen, Germany) was performed as described in Balduyck et al. [23]. Briefly, microalgae were cultured in Wright's Cryptophyte (WC) medium [30] to which artificial sea salt (Homarsel, Zoutman Industries, Roeselare, Belgium) was added. The cultivation was conducted in 30 L photobioreactors with controlled temperature (20 °C), pH (8.5 ± 0.2) and light-dark cycles (16 light hours and 8 dark hours).

Harvesting was carried out in the early stationary phase using a lamella centrifuge (4000 g), followed by a further concentration by centrifugation (9500 g, Sorvall RC-5B, Du Pont Instruments). Cultivation and harvesting was performed once for each part of the experiment (the storage experiment described in Section 2.2.1 was performed separately from the experiment described in Section 2.2.2).

2.2. Wet storage of microalgal paste

2.2.1. Influence of storage temperature on lipid stability during wet storage of Nannochloropsis

The harvested, wet microalgal paste of *Nannochloropsis* (approximately 5% dry matter) was stored in falcon tubes in the dark at 20 °C, 4 °C and − 20 °C. After different time intervals (0, 0.5, 1, 2, 3, 7, 14, 21 and 28 days), the microalgal paste was freeze dried and subsequently stored at − 80 °C until analyses. This storage experiment was executed once.

2.2.2. Influence of cell disruption on lipid stability during wet storage of Nannochloropsis

The harvested, wet microalgal paste of *Nannochloropsis* (approximately 5% dry matter) was stored at 4 °C for one day (for practical reasons). Thereafter, a part of this batch was disrupted by **HPH** (4 passes at 1000 bar, Panda 2 k high pressure homogenizer, GEA Niro Soavi, Parma, Italy). These conditions have been shown to be sufficient to disrupt the cell wall of *Nannochloropsis* [31]. Although cooling at 4 °C was conducted by a cryowaterbath (Haake, Karlsruhe, Germany) during passes through the HPH system, the sample had warmed up during the treatment and was subsequently cooled in an ice bath. The other part of the batch was used as a control and was not treated by HPH.

The disrupted and non-disrupted microalgal paste was stored in falcon tubes in dark at 4 °C, during a period of 4 weeks. Samples were collected at 6 different time points (0, 1, 3, 7, 14 and 28 days), immediately freeze dried and subsequently stored at − 80 °C until analyses.

The stability of the disrupted and non-disrupted *Nannochloropsis* paste was compared to that of (non-disrupted) *T-Isochrysis* paste (approximately 4% dry matter) by storing under the same conditions and during the same time periods. This storage experiment was executed once.

2.3. Lipid extraction from microalgal biomass

The total lipid content (on dry basis) of all collected samples was determined gravimetrically by extraction with chloroform/methanol (1:1 v/v) according to the method described in Ryckebosch et al. [32]. All extractions were done in triplicate.

2.4. Determination of FFA content

The FFA content in the lipid fraction was determined by derivatization of the FFA in the lipid extract to diethylamide derivatives and subsequently analysis by gas chromatography as described in Balduyck et al. [23], based on Kangani et al. [33]. Before extraction, 5 mg of lauric acid (C12:0) (Nu-Check Prep, Elysian, USA), dissolved in chloroform, was added as an internal standard. The peak area of the internal standard was then compared to the area of the other peaks to obtain the FFA content. Determination of the FFA content was performed in triplicate on each sample.

2.5. Determination of the degree of cell disruption

The degree of cell disruption obtained by high pressure homogenization was determined in two ways. Next to a visual comparison by means of **microscopy**, the **extraction efficiency with hexane/isopropanol** (3:2) was determined as the ratio of the extraction yield with hexane/isopropanol (HI) compared to the extraction yield with