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# Biochemical composition and amino acid profiles of *Nannochloropsis granulata* algal biomass before and after supercritical fluid $CO_2$ extraction at two processing temperatures

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ABSTRACT

To assess the effect of supercritical fluid $CO_2$ ($SCFCO_2$) extraction on the biochemical composition of algal biomass, samples of untreated *Nannochloropsis granulata* biomass (Initial) and residual biomass after $SCFCO_2$ extraction at 70 and 90 °C (C70 and C90) were analyzed. $SCFCO_2$ extraction significantly reduced crude lipid content from 285.5 g/kg to 256.2–256.3 g/kg (P=0.032) and caloric content from 23.4 MJ/kg to 23.0–23.1 MJ/kg (P=0.004). Concomitantly, significant increases in ash content from 77.8 g/kg to 83.8–86.2 g/kg (P=0.002) and carbohydrate content from 149.1 g/kg to 155.9–165.9 g/kg (P=0.033) were observed and no significant differences between C70 and C90. Small, but significantly lower levels of crude protein (CP, N × 6.25 or N × 4.78) and non-protein N (NPN) were observed for C70 with no differences between Initial and C90. However, sum of amino acid (AA) residues (ΣAA), a more direct estimate of true protein content, indicated no significant difference in protein content (P=0.536) between treatments (average, 362.0 g/kg). Only minor differences in total and free AA profiles were observed between Initial, C70 and C90 and the free AA content as percentage of total AA was insignificant (P=0.564) between treatments (average, 4.9%). However, several free essential AAs (EAAs) were significantly higher in C70 and C90 than Initial resulting in $SCFCO_2$-extracted biomass having significantly higher (P=0.007) total free EAA content (2438.0–2697.0 μg/g of DW) than Initial (2100.4 μg/g of DW) indicating some protein damage and free EAA liberation. While there was a trend toward most AAs slightly increasing in C70 and C90, the EAA lysine; particularly sensitive to high-temperature processing, was significantly lower (P=0.003) in C90 (6.0 g lysine/100 g protein) than Initial (6.9 g lysine/100 g protein) while C70 was intermediate (6.4 g lysine/100 g protein) providing evidence that lysine damage was more severe at 90 °C than 70 °C. The essential AA index (EAAI) of 0.9 for *N. granulata* biomass is highly comparable to reference proteins such as egg albumin, soy, *Chlorella* and *Spirulina* (0.9–1.0) indicating very good potential for use in animal and fish feeds. Further investigations involving species-specific *in vitro* protein quality and *in vivo* biological performance of target animals fed diets supplemented with these products are warranted.



*Abbreviations:* $SCFCO_2$, supercritical fluid $CO_2$; DW, dry weight; N, nitrogen; CP, crude protein; NPN, non-protein nitrogen; AA, amino acid; ΣAA, sum of amino acid residues; EAA, essential amino acid; EAAI, essential amino acid index; NEAA, non-essential amino acid; PBR, photobioreactor.

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## 1. Introduction

Commodity oils for biodiesel production will likely have low economic value (<$2 $kg^{-1}$ USD; FAO, 2009). Using the equation of Chisti (2007, pg. 301), a reasonable assumption of crude oil prices in the near future ($100/bbl USD) and a density conversion of 0.9 kg/L, algal oil for biofuels production will likely have a market value of ~$0.7 $kg^{-1}$. This value is similar to other conventional plant-based oil sources presently used such as soybean, palm, canola and sunflower at $0.6–1.0 $kg^{-1}$ (Indexmundi, 2015). In fact, Subhadra and Grinson (2011) and Demirbas and Demirbas (2011) have estimated that feedstock algal oil must be priced at $0.40–0.48 $L^{-1}$ in order to be competitive with conventional crude oil. Despite technological advances, however, the estimated cost of production of algae-based oil remains extraordinarily high ($450–2300/bbl USD; Kightlinger et al., 2014) relative to non-renewable fossil-based crude oil ($80–100/bbl USD). As such, it is important to make attempts to optimize the value proposition of algae production, especially when costly oil extraction methods are used. A growing number of experts believe that in the absence of very high value (>$20 $kg^{-1}$ USD) products (*e.g.* biopharmaceuticals, essential fatty acids, pigments, *etc.*), full utilization of the entire microalgal crop, through a balanced microalgae 'biorefinery' approach that can sustainably satisfy demands for biofuels, animal feeds and aquaculture is the only feasible strategy to increase the price competitiveness of a microalgae industry (Pulz and Gross, 2004; Chisti, 2007; Brune et al., 2009; FAO, 2009; Stephens et al., 2010; Subhadra and Grinson, 2011; Ahmed et al., 2012; Bryant et al., 2012; Shields and Lupatsch, 2012). A current and relevant example of this scenario is the rising production of biofuels from corn (and other grains) in North America. While production facilities are designed to ferment carbohydrates into bio-based ethanol, the resultant fuel has a relatively low commodity value of $2–3/US gallon (~$0.5–0.8 $kg^{-1}$). As such, the residual biorefinery co-products (*e.g.*, distillers dried grains with solubles [DDGS] and high protein distillers dried grains [HPDDG]) with a market value of $0.2–0.3 $kg^{-1}$ are marketed predominantly for animal and fish feeds and represent a critical cost-recovery revenue stream for these biofuel and bioproducts companies (Renewable Fuels Association, 2013). Given the high protein productivity of microalgae, the developing algal biofuels sector would surely benefit by adopting this biorefinery strategy. Since the high protein algal meal after oil-extraction may have significant economic value, both directly for animal feeds and indirectly as algal biofuel co-product credits (Bryant et al., 2012), this lipid extracted, solvent-free biomass warrants significant investigation.

We recently demonstrated that microalgae cultured in our patented PBRs (Brite-Boxes) produced biomass with moderate to high total protein yields of 132–465 g/kg (Tibbetts et al., 2015a,b), even when the culture protocols were designed for high accumulation of other non-protein nutrients (*e.g.* lipids). We further demonstrated that the total protein content was increased to 173–521 g/kg after crude lipid extraction, which falls into the mid-value 'protein-rich' ingredient commodity sector presently dominated by terrestrial crops (*e.g.* soy, canola, corn, *etc.*). Perhaps of greater importance than 'total' protein yield is the protein 'quality' with respect to EAA profile. Our recent work with algal cultures produced at our facility (Tibbetts et al., 2015a,b) indicate that the EAA profiles of some microalgae are better balanced than other terrestrial crops having EAAI scores of 0.9–1.2, which approach or exceed that of an ideally-balanced egg albumin protein (1.0), further demonstrating that microalgae are highly attractive sources of protein for animal feed applications (Becker, 2013).

$SCFCO_2$ extraction has been used extensively in bioprocessing of feed ingredients, nutritional supplements and in other areas of biotechnology (Khosravi-Darani and Vasheghani-Farahani, 2005; Khosravi-Darani and Mozafari, 2011). In particular, $SCFCO_2$ extraction has been used to isolate components from microalgae such as essential fatty acids, carotenoids, biopharmaceuticals and triglycerides (Herrero et al., 2006; Yen et al., 2015). Under most circumstances, the targeted extraction products have very high economic value (Egardt et al., 2012) and therefore justify the relatively high costs associated with $SCFCO_2$ extraction. Recently, Bjornsson et al. (2012) compared the extraction efficiency of triglycerides (TAG) from freeze-dried *Nannochloropsis granulata* biomass under various $SCFCO_2$ conditions. However, since the focus of this study, and those of the other aforementioned purposes, was on the extracted oil-soluble components for various purposes (biodiesel production in this case), the residual algal biomass remaining in the vessel post-extraction has rarely been characterized for its nutritional composition as a feed ingredient; which could demand a relatively high market value (Egardt et al., 2012). As such, we collected the residual biomass after $SCFCO_2$ extraction and characterized it in terms of its biochemical composition and AA profiles.

Bjornsson et al. (2012) found that the highest TAG yield was with $SCFCO_2$ extraction conditions of 35 MPa pressure at 70 °C for 270 min. It is well known that protein quality, which ultimately impacts the nutritional value of feed ingredients, is highly affected by processing temperature (Bender, 1972) but may be only minimally affected under $SCFCO_2$ extraction processing conditions (Zagrobelny and Bright, 1992). In view of this, we compared the biochemical composition and AA profile of the base un-extracted freeze-dried algal biomass with those extracted by $SCFCO_2$ at 70 and 90 °C. Early work by Cowey et al. (1971) reported that freeze-drying is a particularly mild form of processing and prevents or minimizes chemical changes in feed ingredients. Higher temperatures (above 70 °C), however, may result in heat damage which can reduce protein digestibility and biological availability of AAs when consumed by rats, fish and poultry (Tarr et al., 1954; Miller and Bender, 1955; Cowey et al., 1971; Opstvedt et al., 1984; March et al., 1985). The protein damage caused by high temperature processing is attributed to denaturation, the formation of disulfide bonds between some AAs, liberation of polypeptides and free AAs, Maillard browning reactions between AAs and carbohydrates and the direct destruction of AAs by oxidation. Hedenskog and Morgen (1973) reported that processing of single-celled organisms such as algae at high temperatures can improve digestibility by cracking the rigid cell walls, permitting more efficient proteolytic enzyme activity in the gut, but also found that excessive heating can negate this benefit, due to effects of temperature described above. Many studies involving biochemical characterization, *in vitro* enzymatic experiments and *in vivo* bioavailability trials with rodents and farm animals