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Review

Understanding differences in protein fractionation from conventional crops, and herbaceous and aquatic biomass - Consequences for industrial use

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ABSTRACT

Background: Alternative protein sources are constantly explored to secure the future food and protein demand. Among these sources, biomasses originating from algae, seaweed or leaves receive lots of attention. However, when the yields and purities of protein extracted from these sources are compared to the corresponding data for protein crops such as soy, lupine and pulses, much lower yields are reported for alternative biomasses.
Scope and approach: In this overview paper, we analyse whether this difference is due to lack of scientific insight and technology or that more fundamental reasons are behind the variations in the extraction behaviour. For this purpose, we prepared a description of herbaceous and aquatic biomasses (denoted as *green* biomass/sources) and their protein extraction practices, final products, and common trends and challenges. The discussion continues with a general comparison to protein crops and the implications for future research.
Key findings and conclusions: Overviewing the state of the art, we tend to conclude that physiological and biochemical factors hinder efficient fractionation of *green* sources. Such factors include cell architecture and high interconnection between cell components; and biochemical differences, in particular the type of proteins present. These fundamental differences imply that *green* sources should be explored in a different manner, with higher emphasis on the interesting functional properties of enriched fractions and less on their purity. This approach is further encouraged by highlighting examples where the intricate structures found in *green* biomass can give rise to positive effects (e.g. health, food structure) when integrally applied in food products.

1. Introduction

Novel protein sources are nowadays explored to secure the future food and protein demand. Herbaceous and aquatic biomasses are among these sources together with insects, single cells, and waste/side streams from several industries (e.g. spent grains). Herbaceous biomass comprises grass, Lucerne, leaves from agro-industrial crops (e.g. sugar beet, cassava, etc.) and trees (e.g. *Moringa oleifera*); whereas aquatic biomass comprises micro and macro algae, and aquatic plants like duckweed. Herbaceous and aquatic biomasses are explored as food sources due to their high protein contents and/or their large availability. Further advantages of these biomasses are their protein yield per hectare, and their higher protein conversion efficiency. Advantages in particular for aquatic biomass are their lack of competition for arable land and water, and their high growth rates (Leng, Stambolie, & Bell, 1995; van Krimpen, Bikker, van der Meer, van der Peet-Schwering, & Vereijken, 2013).

The protein content in leaves and duckweed ranges between 16 and 29% of the dry matter (Fig. 1), while for microalgae the protein content is atypical high (~50%), and can go as high as 71% depending on the species (Becker, 2007). Broadly spoken, these protein contents are comparable to those of soybean (35–40%) (Day, 2013) and lupine (39–55%) (Bähr, Fechner, Hasenkopf, Mittermaier, & Jahreis, 2014). In contrast, macroalgae have lower protein content (9–22%), which is balanced out by their large availability. Besides proteins, those biomasses are rich in dietary fibres, antioxidants, vitamins, minerals, pigments that are suitable as colorants, and other bioactive compounds that can increase their value.

The rich and variable composition of herbaceous and aquatic biomasses leads to different ways for valorisation and challenges during processing. For example, leaves and duckweed have been processed as direct protein source due to their protein contents (Edwards et al., 1975; Fasakin, 1999), whereas microalgae have been considered as raw materials for the production of biofuels due to their high lipid content,

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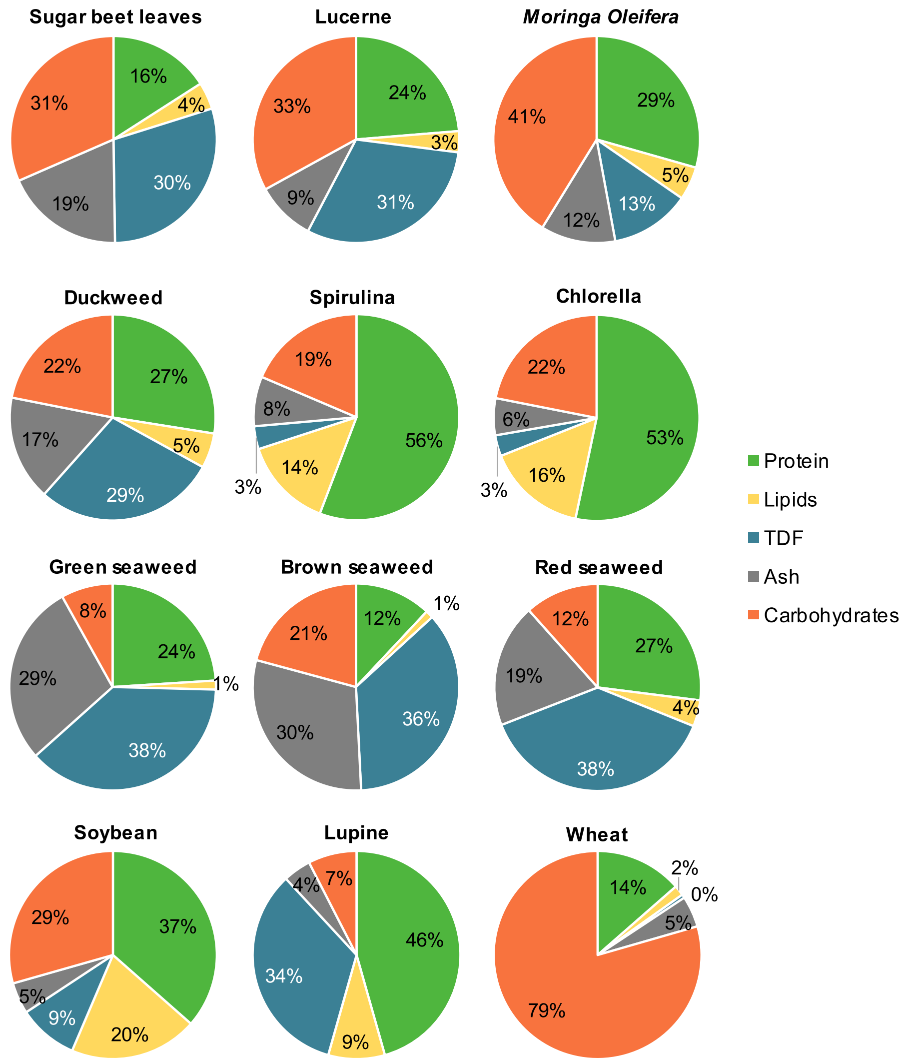

Fig. 1. Average dry matter composition of biomass, TDF = total dietary fibre. Herbaceous biomass: sugar beet leaves (Tamayo Tenorio et al., 2016); Lucerne (Digman, Runge, Shinners, & Hatfield, 2013); and *Moringa oleifera* (Gopalakrishnan, Doriya, & Kumar, 2016). Aquatic biomass: duckweed (Appenroth et al., 2017); microalgae *Chlorella* (Tibbetts, Milley, & Lall, 2015) and *Spirulina* (Tibbetts et al., 2015; USDA, 2016a); and macroalgae green, brown and red seaweeds (Dumay & Morançais, 2016, pp. 275–318; van den Burg et al., 2013). Protein crops: soybean (USDA, 2016b), Lupine (Bähr et al., 2014); and wheat (Multari et al., 2016).

rendering proteins as a co-product (Eppink et al., 2017). Similarly, the strong cell wall of microalgae sets the cell wall disruption process as the most determinant step for the recovery of all cell contents (Safi, Zebib, Merah, Pontalier, & Vaca-Garcia, 2014c). Another example of processing challenges is found in seaweeds. Their high content of neutral polysaccharides implies a high dispersion viscosity during processing, which hinders protein extraction and requires additional adaptations of the fractionation steps (Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995).

In this review, herbaceous and aquatic biomass are referred to as *green* biomass, due to their common traits of high chlorophyll content (i.e. green pigment) and primary photosynthetic function. Despite the current technological developments and the clear advantages, large scale production of protein from *green* biomass has presented limited success. Therefore, we examine the state of the art of these novel protein sources, through describing the crops studied, and reporting protein extraction practices, final products, common trends and challenges. Besides revising their potential as food sources, we prepared a general comparison with traditional protein crops, recognising a large difference on protein yield and protein purity of their products. The discussion outlines the technologies available and highlights fundamental differences between plant tissues that can serve as an explanation for the limited industrial application of these *green* biomasses. Finally, new approaches are proposed to facilitate the use of *green* biomass, motivating the production of less refined material, while exploring novel functionalities. Obtaining more products out of a single biomass is part of the holistic approach necessary for successful biorefineries. In addition, each biomass has particular traits that broaden its potential uses when given sufficient research attention.

2. *Green* biomass as a food source

The sections below give an overview of the *green* biomasses investigated for potential application in foods, highlighting main compositional traits and current practices. Additionally, protein extraction methods are described for the biomass groups.

2.1. Green leaves

Plant leaves are recognised as a potential protein source for food applications based on their nutritional profile and their large availability in agricultural waste streams. For most industrialised crops, only specific parts of the plants (e.g. root, flowers and fruits) are harvested and processed, while the leaves, accounting for numerous tonnes of biomass per year, are left unused. Several crops have been studied for leaf protein extraction, including alfalfa (Lamsal, Koegel, & Boettcher, 2003; Wang & Kinsella, 1976), spinach (Barbeau & Kinsella, 1988), tobacco (Fu et al., 2010), cassava (Coldebella et al., 2013), *Moringa oleifera* leaves (Teixeira, Carvalho, Neves, Silva, & Arantes-Pereira, 2014), soybean leaves (Betschart & Kinsella, 1973), among many local crops in different countries. The protein content ranges between 16 and 29% in dry basis (Fig. 1.), and the variations depend on the crop, plant