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How biomass composition determines protein extractability

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ABSTRACT

Biomass consists of a complex mixture of different components, of which protein potentially has a high added value for biorefinery. In this study, protein extractability of different types of biomass, mostly by-products, was analyzed. Protein yield obtained from a three step extraction using alkali was correlated to biomass chemical composition through Partial Least Square (PLS) regression. The results showed that protein extractability depended crucially on the type of biomass used. Protein from cereals and legumes were highly extracted, compared to other materials. High protein extractability coincides with the biological function of protein as a storage protein, as opposed to functional protein. Protein extraction was furthermore correlated to the composition of the biomass. Especially cellulose and oil hamper extractability of protein whereas lignin has no significant influence, suggesting that alkaline treatment removed lignin sufficiently.

1. Introduction

Agriculture provides food for human consumption and energy for the new bio-based society. However, it also generates side-streams that are currently only re-used in low economic value applications such as animal feed or as fuel for power generation. Examples are grain residues (Klopfenstein, 1996) and meal residues (Rutkowski, 1971) from biodiesel production. However, in an efficient and ethically sound bio-based economy all parts of the biomass will have to be used at their highest value without competing for food and feed.

Due to the complex nature of biomass a wide variety of products can be extracted from it. Protein will be the focus point for this article, agricultural side streams can increase value in overall plant production chains. Proteins are also of interest because of the presence of functional side groups that makes protein and amino acids interesting for chemistry (Scott et al., 2007). In addition, valorisation of other components present in biomass is needed to increase overall sustainability and economic feasibility of bio-based processes. The use of cellulose from wheat straw instead of starch from sweet corn as a sugar feedstock may, e.g., improve its valorisation but also the sustainability of bioethanol farming (Kootstra et al., 2009). Pulp industries already try to re-use their lignin containing waste stream for technical applications such as binders instead of just burning it for energy (Gosselink et al., 2010).

Selection of type of biomass for protein extractability may be based on technical, economic, environment or social aspects (Wang et al., 2009). Here, we only consider the first two aspects. Technically speaking some biomass is easier to extract than others, as is also demonstrated in this article. Leafy biomass is, e.g., less easily extracted than oil seed meals and still needs further technical optimization to get to higher extraction yields (Bals and Dale, 2011; Zhang et al., 2014). From an economic point of view biomass availability and prices are important. Oil seed meals may be one of the types of biomass to select, with their high protein content before and after de-oiling. However, because of their already high value for feed there is less added economic value in protein extraction. In addition to oilseed/legume meals and leaves, dry biomass such as hull, stover and stalks are also interesting resources due to their abundance. The biomass sources that were chosen for our experiment are all commonly used in agro-industries such as beer and vegetable oil production. These biomass sources contain different levels of the components and fall into different categories of biomass: legume, leafy, tuber or cereal.

When extracting biomass, different conditions can be used, with or without the addition of enzymes. pH is an important parameter, and it has already been shown that under alkaline condition

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more protein is extracted than at lower pH (Sari et al., 2012). Alkali effectively extracts proteins, either by breakdown of, e.g., cell wall components (Knill and Kennedy, 2003; Krall and McFeeters, 1998) or by breakdown of the protein itself (Hamada, 1997). Addition of proteases can further increase yields (Jung, 2009; Sari et al., 2012).

The efficiency of alkali in extracting biomass protein is influenced by several factors including the type of biomass and temperature. Soybean meal is one of the most easy types of biomass to extract protein by alkali (Sari et al., 2012). Under similar conditions less protein was extracted from rapeseed meal (Sari et al., 2012), microalgae meal (Sari et al., 2012), rice bran (Yadav et al., 2011), and dwarf elephant grass (Urribarrí et al., 2005). However, still little is known about the underlying mechanisms in alkaline protein extraction and the interactions that occur between protein and the other components present under these conditions. Therefore, protein extraction was performed under alkaline conditions on sixteen biomass. Three sequential increasing temperatures were chosen. The lowest extraction temperature, 25 °C, was chosen to avoid thermal energy input into the process. The second temperature, 60 °C, was tested to see if an increase in temperature under alkaline conditions could increase protein extraction for some types of biomass. After that the residue was extracted at 120 °C, without adding more alkaline. This was to see the influence of temperature at its most extreme. High temperature and pH combination is expected to severely damage protein and may therefore not be recommendable. However, it does give further insight the temperature dependence of protein extraction from biomass. The results were used to study the correlation between protein extraction and the chemical composition of the biomass at different temperatures.

Biomass sources with different chemical composition were selected and tested on protein extractability. Aiming for valorisation of agricultural by-products, twelve out of the sixteen chosen biomass are by-products. In addition to these, barley grain, soybean, and microalgae were tested to compare extraction yields with their corresponding by-products; ryegrass was tested to represent leafy biomass. To get an insight of chemical composition on protein extractability, the selection of biomass comprised of biomass that was rich in protein (wheat gluten) and/or oil (untreated soybean), and/or cellulose (soybean hull) and/or lignin (palm kernel meal), and/or starch (barley grain). Also biomass with a more balanced chemical composition were selected, such as barley rootlets (balanced in protein and hemicellulose), microalgae (balanced in protein, oil, and cellulose), and palm kernel meal (balanced in protein and cellulose). The results from the extraction experiments are discussed in this paper and protein extractability was correlated to biomass chemical composition through regression analysis. In our case, the protein yield is identified as a function of eight chemical components. Thus, multiple linear regression is used in this study. For this, a Partial Least Square (PLS) method is selected. The Variable Importance Plot (VIP) scores obtained by PLS in this study are used as an importance measure of each explanatory variable (Chong and Jun, 2005). The knowledge generated from this research is expected to aid in selecting biomass in biorefinery for protein.

2. Materials and methods

2.1. Materials

Rapeseed meal, soybean hull, soybean meal, sunflower meal, and palm kernel meal were obtained from Schouten Ceralco (The Netherlands). Microalgae and microalgae meal (*Chlorella* sp.) were obtained from Ingrepro (The Netherlands). Wheat gluten, barley grain, barley mill run, barley rootlets, and malt by-products were obtained from Cargill (The Netherlands). Wheat middling was obtained from Meneba (The Netherlands). Ryegrass was obtained from a local farmer in Wageningen (The Netherlands). Sugar beet pulp was obtained from the sugar mill in Dinteloord (The Netherlands). For soybean without oil removal, we used the commercially available one (Heuschen & Schrouff, The Netherlands).

2.2. Protein content analysis

Protein content was determined as nitrogen content using DUMAS analysis (FlashEA 1112 series, Thermo Scientific, Interscience) using a nitrogen to protein conversion factor of 6.25. Methionine was used as a standard for the calibration. Protein extraction yield was determined as the ratio of nitrogen content in the supernatant to the nitrogen content of the raw material.

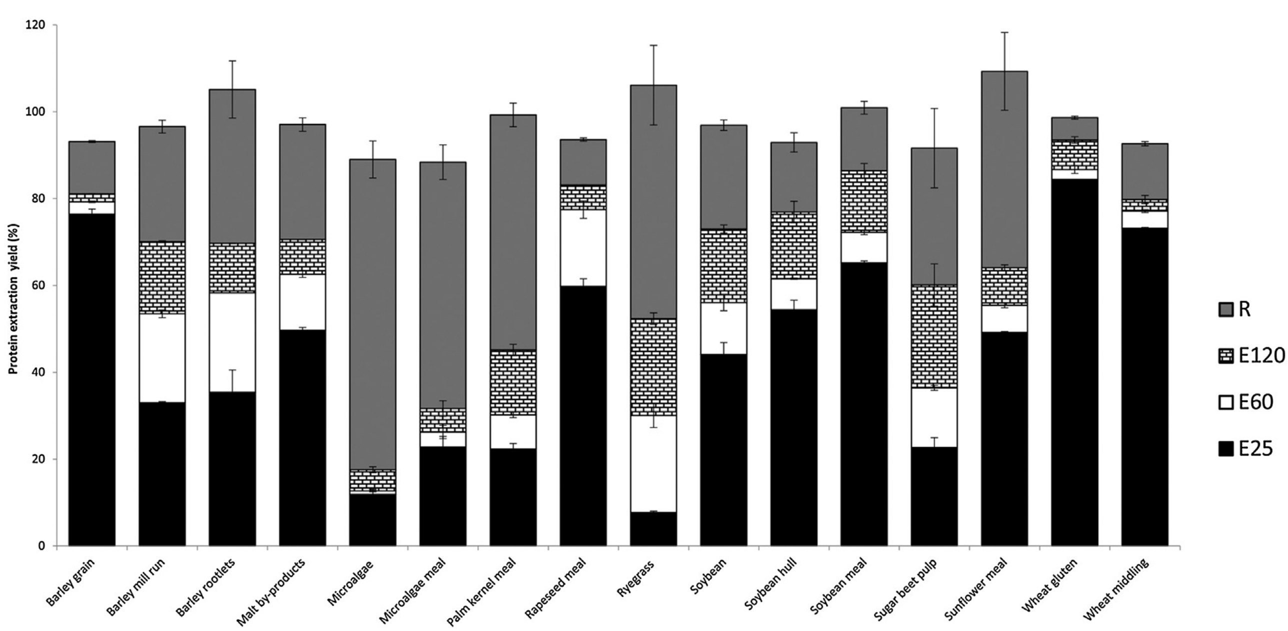

Fig. 1. Protein extraction yield.