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Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks

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ABSTRACT

Production of a broad spectrum of products from biomass is of key importance for an economically viable and sustainable biorefinery sector. The aim of this study was to determine the total crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks optimized for supplying biomass to biorefineries. Field experiments during 2013–2014 with perennial crops (pure grasses: cocksfoot, festulolium, reed canary, tall fescue, two miscanthus species and two grass-legume mixtures) and annual crops in optimized rotations (winter rye, sugar beet, maize, triticale, hemp and grass-clover) were compared to traditional crops common in Danish agriculture (maize, barley, wheat and triticale). Theoretical extractable true protein was determined according to the Cornell Net Carbohydrate and Protein System, which fractionates the crude protein based on solubilities. The easily extractable fraction of the true protein was denoted as neutral-extractable. Concentration of crude protein was on average 164–191 g kg^{-1} DM per cut of pure grasses and grass-legume mixtures, with the summer cuts having the lowest values. Pure grasses produced the highest crude protein yield per hectare annually, ranging from 2595 to 3693 kg ha^{-1} irrespective of the year, of which 920–1640 kg ha^{-1} was neutral-extractable protein. Whilst the neutral-extractable true protein per hectare of festulolium and tall fescue was superior to those of all other crops, the neutral extractable true protein per hectare of grass-legume mixtures and of winter rye and maize double crop was similar to those of reed canary and cocksfoot. On a mass basis, 34–46% of crude protein in pure grasses was neutral-extractable, depending on the year. The potential extractability of crude protein may be increased by 14–35% if the cell wall-bound protein can be extracted too.

1. Introduction

Most conventional annual crops are highly reliant on agricultural inputs such as chemical fertilizers and pesticides (Gabrielle et al., 2014). Perennial crops, on the other hand, can produce high amounts of biomass per hectare (in non-water-limited areas) with low environmental impacts compared to annual crops (Cadoux et al., 2010). The use of perennial grass instead of annual crops decreases nitrate leaching in nitrate sensitive areas, reduces pesticide use, accumulates soil organic matter, and therefore, seems to be a more sustainable choice for the delivery of biomass (Pugesgaard et al., 2015; Manevski et al., 2018). One of the main reasons for the higher yield potential of grass is the higher annual interception of solar radiation (e.g., Manevski et al., 2017). In summary, grass biomass is considered a highly sustainable raw material, which is available in substantial amounts throughout Europe (Mandl, 2010). However, the current market for grass is almost limited to ruminant feed.

Soya bean meal is currently the main source of protein for the feed sector in Europe. Of the 30 Mt total soya bean meal consumption in the EU-27, only 1 Mt (2.5%) is produced in the EU while the rest is imported (Schreuder and de Visser, 2014). Concurrently, the world faces an increasing demand for animal products including meat and milk (WHO, 2009), which calls for alternative sustainable protein sources for livestock production. The use of land resources for dedicated bioenergy crop production competes with its use for production of food and feed, while recovering protein from biomass crops can sustain the food production. Consequently, co-production of protein with biofuels in a biorefinery would result in a more efficient land use (Dale, 2008), especially if more biomass productive crops are grown (Manevski et al., 2017). When biomass and especially grasses that contain considerable amounts of protein are used for production of ethanol, nitrogenous waste from biorefinery system is generated (Kammes et al., 2011),

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which can be converted into feed protein if extracted early on during the processing of biomass. Protein has a very high value per unit weight that can contribute to the overall economics of the biorefinery system producing fuels and chemicals (Dale, 1983) and it can be recovered using a variety of techniques in an integrated biorefinery system (Dale et al., 2009). Furthermore, protein has a very high synthesis energy cost (approx. four times that of carbohydrates) in plants, which is not reflected in a similarly high combustion value (Bentsen and Møller, 2017), and protein should thus rather be used for high-value feeding or chemical conversion purposes, than be converted into energy.

Biorefineries operate in accordance with the chemical nature of the biomass and are intensely based on sustainability principles (sustainable raw materials, sustainable land use etc.; Kamm et al., 2016). Moreover, they have the potential to enhance biomass conversion efficiency, improve feed quality by eliminating harmful constituents of biomass, produce building blocks of other biobased materials (Teekens et al., 2016) and provide the opportunity for manufacture of highly concentrated and purified proteins (Dale et al., 2009). Processing of biomass in the biorefinery consists of a primary mechanical fractionation step where press juice and fibrous materials are generated. The juice consists of cell contents including proteins, water soluable carbohydarates, minerals, organic acids, etc. (Kamm and Kamm, 2004), while the fiber mainly consists of plant cell wall that encompasses cellulose, hemicellulose and lignin (Waldron, 2014). Recovered proteins from the juice can be utilized for feeding monogastrics such as poultry and pigs, whereas the fibrous material can be converted into pellets and used for feeding of cattles. According to Pirie (1969), the juice fraction produced by mechanical fractionation contains between 50 and 70% of the total protein.

Numerous studies have described the protein extraction procedure from different types of plant materials and using different extraction conditions and techniques (Bals et al., 2007; Sari et al., 2013,2015a,b). Solati et al. (2017) have estimated extractable true protein (ETP) using the Cornell Net Carbohydrate and Protein System (CNCPS), which fractionates the crude protein (CP) into three fractions of A, B and C (Licitra et al., 1996) and is extensively used in relation to characterizing ruminant feed (Sniffen et al., 1992). They assumed that protein extractability based on CNCPS correlates with actual extractability of protein in the biorefinery. Fraction A is non-protein nitrogen (N) and consists of nitrate, ammonia, amines and free amino acids, which can be easily extracted into the juice after mechanical pressing. Fraction B is the true protein and is further sub-fractioned into B_1, B_2 and B_3 based on decreasing solubility from B_1 to B_3. B_1 and B_2 are neutral soluble protein fractions assumed to be extractable into the juice by mechanical fractionation. Fraction B_3 is acid soluble protein fraction, which is associated with plant cell wall and therefore its recovery may depend on extraction conditions (Dale et al., 2009; Zhang et al., 2014) or on using pre-treatment techniques (De La Rosa et al., 1994; Dotsenko and Lange, 2017). Fraction C is acid insoluble protein, which is bound to lignin, hence unextractable. True protein generally makes up 75–85% of CP in grasses (Buxton and O'Kiely, 2003).

Plant proteins can generally be classified into leaf protein and seed protein. In the leaf, there are two main groups of soluable proteins namely, chloroplastic and cytoplasmic. The chloroplastic protein mainly consists of the enzyme Rubisco, which makes up approximately 50% of the protein extracted from the leaf. In contrast, cytoplasmic proteins are heterogeneous combination of proteins (structural proteins or enzymes) from both the chloroplast and cytoplasm, accounting for about 25% of total protein (Rooke and Hatfield, 2003). According to Pheloung and Brady (1979) there is no precise definition for soluble protein as it depends on the protein extraction conditions (i.e. likely to increase with pH). They indicated that between 33 and 48% of the extracted protein from C3 grass leaf is neutral soluble, out of which 29–43% is the major soluble protein in chloroplast. According to Lyttleton (1973) about 50% of protein in herbage species is insoluble. In seeds, fraction B_1 consists of salt soluable globulins and water soluable albumins. Fraction B_2 consists mostly of albumins and glutelins (soluble in acid or alkalai). Fraction B_3 consists of prolamines (soluble in alcohol), extensin proteins and heat denatured proteins (Phillips, 2010). Prolamines are known to be the main storage proteins in maize (Wu, 1994). Identifying the protein production potential of lignocellulosic biomass is essential in order to maximize its utilization value. Consequently, the knowledge of protein composition in biomass is crucial for development of an energy-efficient biorefinery process and conversion technologies (Maity, 2015) and for estimation of conversion yields and process economics (Sluiter et al., 2010). Moreover, studies have shown that plants such as grasses and legumes have high protein content and a balanced amino acid composition (e.g. high amounts of lysine and methionine) comparable to that of soy (Dale et al., 2009; Edmunds et al., 2013), which is an important parameter for economic potentials of green plant biorefinery. Prior detailed amino-acid profiling, however, protein screening information is necessary for further optimizing the enzymatic hydrolysis and pre-treatment processes (Lynd et al., 1991).

This paper focuses on protein yield per hectare and potential ETP revenue of crops grown in novel systems for biomass production and supply to biorefinery. The systems have previously been evaluated for crops light interception, radiation use efficiency and biomass yield (Manevski et al., 2017). They included 1) annual crops grown in rotation optimized for biomass production (maize, beet, hemp, triticale, grass-clover and winter rye), 2) perennial crops (festulolium, reed canary, cocksfoot, tall fescue, miscanthus and grass-legume mixtures), and 3) traditional crops (maize, wheat and winter/spring barley). The main objective was to determine the theoretical ETP using CNCPS in different cropping systems that were designed to increase efficiency in biomass production.

2. Materials and methods

2.1. Study site and experimental design

We studied samples taken in 2013 and 2014 from a field experiment established in 2012 in Denmark at Foulum (56°30″ N, 9°35″ E) on a sandy loam soil (Typic Hapludult). The average percentage of clay, silt, fine and coarse sand in the top 25 cm were 8, 11, 42 and 36, respectively. The climate is temperate and wet, characterized by mild summers and cool to cold winters with moderate seasonal temperature variation. Table 1 summarizes the most important weather characteristics for the study years.

A range of alternative cropping systems including either perennial grasses or annual crops in a new innovative crop rotation optimized for biomass yield were compared with three different traditional cropping systems. The systems are described in detail by Manevski et al. (2017).

Table 1
Monthly average temperature, global radiation and precipitation during the study years 2013 and 2014 at Foulum site in Denmark.

Month	Temperature (°C)		Global radiation (MJ m^{-2})		Precipitation (mm)	
	2013	2014	2013	2014	2013	2014
Jan	−0.6	1.4	622.6	289.4	48.8	76.4
Feb	−1.1	4.1	1082.1	1048.8	21.0	55.2
Mar	−1.3	5.5	3804.2	3010.5	3.9	38.0
Apr	5.1	8.5	5268.5	4839.1	26.9	39.6
May	12.1	11.4	6428.1	6489.1	63.5	87.4
Jun	13.5	14.5	6684.1	7641.4	73.7	26.8
Jul	16.7	18.9	7533.6	7061.1	19.9	77.8
Aug	16.2	15.2	5408.4	5010.4	74.0	116.1
Sep	12.4	13.9	3129.8	3628.7	84.2	61.8
Oct	10.2	11.5	1755.6	1584.5	113.0	106.2
Nov	4.9	6.9	810.2	541.6	76.7	74.2
Dec	5.1	2.5	378.4	416.6	84.0	102.0