Journal of Cleaner Production 162 (2017) 875–881

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Lactic acid fermentation for refining proteins from green crops and obtaining a high quality feed product for monogastric animals

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ARTICLE INFO

Article history:
Received 7 November 2016
Received in revised form
13 June 2017
Accepted 13 June 2017
Available online 14 June 2017

Keywords:
Biorefinery
Green crops
Protein feed
Organic farming
Monogastric animals

ABSTRACT

Nowadays, the organic farming sector is growing at a fast pace in Europe while needs to face the lack of organic protein sources and in particular, feeding monogastric animals is becoming more and more urgent. Green biorefinery concepts might become the suitable solution for the production of organic protein-rich feeds from green crops. In this context, red clover, clover grass, alfalfa and oilseed radish were studied as possible feedstocks for the development of an organic biorefinery system in Northern Europe. For this purpose, the green crops were processed into a nitrogen-rich protein concentrate, a fiber-rich press cake, and a residual stream of soluble nutrients (brown juice). The process, which involved a novel protein refining technique using lactic acid fermentation, yielded between 6 and 13 kg of dry organic protein product per ton of fresh crop. The protein products of the different crops presented balanced amino acid composition compared to soybeans, which are commonly used in organic farming. Moreover, methionine contents between 7.8 and 9.1 g/kg DM were obtained in the protein products, which is more than the typical concentration found in animal feeds (5.2 g/kg DM in soybeans). This makes the organic protein product produced very promising as a feed ingredient for organic farming of monogastric animals in Europe.

1. Introduction

The supply of organic protein feed for monogastric animals (i.e. poultry and pigs) with a suitable amino acid profile at a competitive price is one of the major challenges for organic agriculture nowadays. Increasing difficulties to obtain GMO-free organic soy protein, which is currently the main feed source in organic agriculture, coupled with the fact that the use of up to 5% conventional (non-organic) feed in organic agriculture is to be phased out by 2017 in EU (EU Regulation No 836/2014), are hindering this sector. Moreover, the price for organic soybeans is expected to increase in the coming years due to increasing demand for organic products and due to environmental concerns as deforestation in Brazil or impact on soil carbon stocks. Today, the European Union imports around 70% of its need for protein-rich feed being soybeans the leading imported feed ingredient (Topp et al., 2014; De Visser et al., 2014). Indeed, the net import of soybeans in Europe is 13.9 Mt on average per year (2006–2013) mostly from Brazil and the United States (FAOSTAT, 2015). In the case of Denmark, the current soybeans net imports are around 86 kt per year (average 2006–2013) (FAOSTAT, 2015). With regard to the organic sector, soybeans are mainly imported from Asia in order to ensure GMO free products. Actually, Denmark has the highest market share for organic egg production, namely 22% of the total egg production as percentage of the total market share in Denmark (Danish Poultry Council, 2014). Accordingly, there is an urgent need of finding alternative protein sources for the organic farming sector and particularly for monogastric animals, which have specific requirements for certain amino acids. In addition, Europe has the necessity of developing more sustainable farming systems based on the utilization of locally produced feeds. Legumes like faba beans, peas and lupin can grow in temperate climates and could increase the amount of locally grown

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http://dx.doi.org/10.1016/j.jclepro.2017.06.115
0959-6526/

protein sources for organic poultry, but the content of methionine and cysteine, essential for poultry, are low compared to soybeans (Petterson, 2000), and therefore not useful as an alternative to soybeans. In addition, the value of grain legumes as protein source in organic poultry diets are also dependent on the content of anti-nutritional factors (ANFs) such as protease inhibitors, lectins and tannins, as well as the content of non-starch polysaccharides (NSP), which can vary among cultivars and limit the inclusion level. For instance, a lupin-based diet resulted in a lower performance of hens when the lupin inclusion level was 25% compared with 0 and 15% inclusion levels (Hammershøj and Steenfeldt, 2005). Petterson (2000) reviews the effect of different anti-nutritional factors in legumes focusing on the amounts found in lupin, which generally is lower than in soybeans and there is no need of heat inactivation of lectins. The solubility of NSPs in lupins was also higher than in other grain legumes such as peas, faba beans, and chick peas (Perez-Maldonado et al., 1999). Alternatively, green biorefinery concepts may become an attractive solution for providing alternative protein feeds by means of the extraction of proteins from green grasses (Dale et al., 2009). Parajuli et al. (2015) point that green biorefining could be important not only by substituting import of protein sources but also for allowing processing of residues. Furthermore they discussed the sustainability of biorefining and the importance of performing life cycle assessments on the whole biorefining chain. The production of protein concentrates is not a new concept since Pirie (1969) already proposed that leaf proteins could be utilized as supplements in protein-deficient diets. However, an increased interest in such green biorefinery concepts has occurred in recent years, especially in organic farming, mainly due to the challenge with 100% organic feed and the search for new protein sources as an alternative to soybeans. Further, there is an increasing interest in the use of legumes in crop rotations for their multiple benefits with respect to less use of fertilizer, pesticides and a high biomass yield.

Plant proteins are mainly concentrated in the leaves, Rubisco (ribulose 1, 5-bisphosphate carboxylase) being the most abundant soluble protein in green biomass (Lamsal et al., 2007). Extraction of proteins contained in green biomass like grass requires an initial wet fractionation step in which the fresh biomass is pressed with a mechanical press (Kamm et al., 2009) or by extrusion (Colas et al., 2013a). This step results into a solid fraction rich in fibers and a liquid fraction or juice containing the soluble compounds including proteins (Arlabosse et al., 2011). Up to date, the extraction of proteins from plant juices has been performed by different procedures. For instance, Baraniak (1990) compared the production of alfalfa juice concentrates by heat coagulation at 85 °C, by acid precipitation at pH 3.5 and by the addition of cationic or anionic flocculants. Acidification was the method with the highest content of essential amino acids in the protein concentrate. Likewise, heat coagulation at temperatures between 80–90 °C was used by Aletor et al. (2002) in order to produce leaf protein concentrates from leafy vegetables, which seemed suitable as food additives according to their functional properties. Indeed, the extraction method affects the proteins functional properties such as the N solubility and emulsifying capacity, as reported by Lamsal et al. (2007). Prevot-D'Alvise et al. (2004) proposed the application of proteolytic enzymes in order to improve both the nutritional and functional properties of the alfalfa leaf proteins produced by acidification at pH 4.5. Besides, ultrafiltration was proposed as a method for the production of leaf protein concentrates and when compared with heat coagulation, resulted in slightly higher protein recoveries for ryegrass juice while no difference between methods was observed for alfalfa juice (Koschuh et al., 2004). Acidification with HCl (pH = 3.3), heat (21, 100 or 140 °C) and zinc treatment were combined in the production of leaf proteins from orchardgrass and switchgrass and in particular, heat and zinc treatments decreased the protein ruminal degradation (Kammes et al., 2011). To ensure the quality of the protein it is important to avoid high temperatures and improper chemical treatments which may change the protein structure and might reduce the digestibility of proteins. In particular, excessive heating might limit the availability of lysine and other amino acids as a result of Maillard reactions and formation of amino acid complexes (Boisen et al., 2000).

Proteins from organically grown green biomass extracted in the biorefinery concept can particularly be utilized to satisfy the imminent demand for protein rich organic feed for monogastric animals. The amino acid profiles of proteins extracted from green leaves are generally comparable with those from current protein sources like soybeans, meat, fish or eggs except for lower methionine content (Aletor et al., 2002). In this study, a novel extraction method was used which includes lactic acid fermentation of the plant juice triggering a pH decrease and hence, the precipitation of proteins (Kiel et al., 2015). In this manner, the extraction method does not require the use of inorganic acids or organic solvents and the product contains lactic acid, which may be beneficial for the gut health as well as reports have indicated that some of these mild organic acids can lower the total amount of feed needed with same growth (Kahn and Iqbal, 2016). Moreover, the protein rich organic feed product might be able to substitute soybeans protein meal used for conventional animal feeding. For this purpose, the costs of production have to be minimized to compete with the current market prices without compromising the product quality and amino acid composition.

This study was conducted in order to apply a mild extraction process to produce protein concentrates that comply with organic farming methods. Four different crops, red clover, clover grass, alfalfa and oilseed radish, were studied as possible feedstocks for the development of the biorefining protein process. The recovery of proteins from those four green crops into their respective protein concentrates was studied and assessed for protein extraction yields. The quality of these protein concentrates was evaluated in terms of amino acid composition.

2. Material and methods

2.1. Organic crops: agricultural practices and sampling

Four different crops or crop mixtures were studied, namely red clover (*Trifolium pratense*), clover grass (mixture of *Trifolium pratense* and *Lolium multiflorum*), alfalfa (*Medicago sativa*) and oilseed radish (*Raphanus sativus* var. *Oleiferus*). Red clover was harvested in May 2014 in Vamdrup, Denmark (55° 25′ 48.1″ North, 09° 17′ 14.5″ East) when the flowering was not started. Clover grass was harvested in May 2014 in Orten, Denmark (55° 39′ 49.9″ North, 08° 25′ 59.6″ East), and in this case the height of the crop was around 50 cm and the stems of the grass were rather woody. Alfalfa and oilseed radish were grown in Taastrup, Denmark (55° 40′ 11.4″ North, 12° 18′ 26.1″ East). Alfalfa was sown in April 2013 together with barley and grew alone since August 2013. Two cuts were performed in 2014 (June and August), thus alfalfa plants had grown for 2 months at the harvesting time in October 2014. Oilseed radish was sown in August 2014 and hence, oilseed radish plants had grown for 2 months at the harvesting time. Harvesting was performed in October 2014. No fertilizer was used in cultivation of any of the crops. Red clover, clover grass and alfalfa were processed immediately after the harvest. Oilseed radish was spread for drying outdoor at relatively cold conditions (below 10 °C) overnight to reduce its high water content. Then, it was processed the day after the harvest.