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Extractability and characteristics of proteins deriving from wheat DDGS

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ABSTRACT

Wheat Distillers' Dried Grains with Solubles (DDGS) and in-process samples were used for protein extraction. Prolamins were the predominant protein components in the samples. The absence of extractable α- and γ-gliadins in DDGS indicated protein aggregation during the drum drying processing stage. Prolamin extraction was performed using 70% (v/v) ethanol or alkaline–ethanol solution in the presence of reducing agent. DDGS extracts had relatively low protein contents (14–44.9%, w/w), regardless of the condition applied. The wet solids were the most suitable raw material for protein extraction, with recovery yields of ~55% (w/w) and protein content of ~58% (w/w) in 70% (v/v) ethanol. Protein extracts from wet solids were significantly rich in glutamic acid and proline. Mass balance calculations demonstrated the high carbohydrate content (~50%, w/w) of solid residues. Overall, the feasibility of utilising in-process samples of DDGS for protein extraction with commercial potential was demonstrated.

1. Introduction

Distillers' Dried Grains with Solubles (DDGS) is the principal by-product of the dry-grind distillation process, generated mainly from beverage alcohol plants (e.g. whisky and neutral spirits distilleries) or from grain-based fuel-ethanol plants. In the case of distilleries, single or blended grains including wheat, barley, maize and rye can be utilised as feedstock, whereas fuel-ethanol plants use either corn (maize) (US) or wheat (Europe) as starting materials.

During the dry-grind process, in the case of bioethanol production, whole grains are milled and liquefied, followed by the addition of amylolytic enzymes for starch conversion into fermentable glucose. In distillery plants, saccharification of the milled grain is carried out using malted barley instead of external enzymes and a food-grade process is followed, as the end-product (potable ethanol) is intended for human consumption. For both bioethanol and potable ethanol production, yeast is added to ferment the sugars into ethanol and carbon dioxide. At the end of the fermentation, the whole stillage undergoes distillation by direct steam injection. Ethanol is further purified via dehydration, whereas the non-volatile components (spent solids) are centrifuged to produce a liquid fraction (thin stillage) and a solid fraction (wet solids). Around 15% or more of the thin stillage is recycled to the liquefaction process of the ground grain, whereas the remaining is concentrated in a series of steam driven evaporators, mixed with wet solids and drum dried to produce the final DDGS (Kim et al., 2008; Liu, 2011). The drying process applied at the last stage is intensive, as the air temperature can be over 500 °C at the dryer inlet and over 100 °C at the dryer outlet. Partial recycling of DDGS to the drum dryer can also occur in order to increase the drying efficiency of the equipment and improve the consistency of the produced DDGS (Kingsly et al., 2010). Overall, for 100 kg of grain approximately 40 L of ethanol, 32 kg of DDGS and 32 kg of CO_2 are generated (Schingoethe, 2006).

Because it is enriched in protein, as well as in water-soluble vitamins and minerals, DDGS has been long marketed as feed for livestock (including poultry) (Klopfenstein, Erickson, & Bremer, 2008; Schingoethe, Kalscheur, Hippen, & Garcia, 2009). DDGS derived from wheat contains around 28–38% (w/w) of protein, whereas for maize DDGS the protein levels range within 28–31% (w/w) (Kim et al., 2010). The major parameters influencing the cost-effectiveness of bioethanol production from cereal grains include the cost of raw materials, as well as the revenue derived from DDGS. In Europe, bioethanol production is currently driven by the EU mandates on biofuel framework (Directive 2009/28/EC), thus the increased bioethanol demand is likely to result in increased DDGS availability. As a result, current research is focused on identifying alternative uses of DDGS, other than animal feed. To this end, existing bioethanol or distillery companies could

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implement a biorefinery approach, where DDGS is fractionated into several added value compounds including proteins, carbohydrates and phytochemicals.

In contrast to the literature on maize DDGS, a limited number of studies have investigated the extraction of protein from wheat-based DDGS (Bandara, Chen, & Wu, 2011; Hong et al., 2012; Xu, Reddy, & Yang, 2007). Wheat grains contain gluten proteins that account for 80% of the total wheat protein, with the remaining 20% corresponding to a heterogeneous group of structural and metabolic proteins, including a major group of water soluble components with molecular weight (MW) lower than 25 kDa (Veraverbeke & Delcour, 2002). By contrast, gluten proteins are largely insoluble in water due to their high non-polar amino acids content (in particular proline and glutamine) and serve as storage reserves in the wheat grain (prolamins) (Shewry, 1999). Prolamins comprise both alcohol-soluble monomers (gliadins) and alcohol-insoluble polymers (glutenins) with the individual glutenin subunits being alcohol-soluble in their reduced state. Prolamin monomers and subunits show considerable diversity in MW, ranging from 10 to 100 kDa (Shewry & Halford, 2002). The extraction of proteins from DDGS at high yield and purity remains a challenge; DDGS proteins often show low extractability possibly due to the intensive heating applied at the final stage of the production process. Looking towards potential applications, DDGS proteins can be exploited for the production of biodegradable films, coatings and biodegradable plastics, which can be used for food, agricultural and industrial applications (Day, Augustin, Batey, & Wrigley, 2006). Wheat protein (gluten) has therefore been extensively studied as a natural starting material for the development of biodegradable films, due to its remarkable cohesive and elastic properties, as well as its susceptibility to chemical modifications (Irissin-Mangata, Bauduin, Boutevin, & Gontard, 2001; Kuktaine et al., 2011). Further applications of gluten include in aquaculture feed and in pet food, as an adhesive material in tapes and medical bandages, or as a biodegradable polymer material for the slow release of pesticides or fertilising agents (Day et al., 2006; Majeed, Ramli, Mansor, & Man, 2015).

The aim of this study was to investigate the extractability of proteins from various samples originating from a distillery plant, i.e. wheat DDGS, wet and spent solids (the latter also known as whole stillage). The composition of the extracted proteins and their amino acid content were determined and are discussed in order to evaluate the effect of the multi-step DDGS production process on the properties of the proteins at each stage of production.

2. Materials and methods

2.1. Raw materials

Distillers' Dried Grains with Solubles (DDGS) and in-process samples of wet solids and spent solids were kindly provided by a distillery plant in UK. The distillery plant uses a mixture of 95% (w/w) wheat and 5% (w/w) barley as starting material for potable ethanol manufacture. After being received, samples were frozen at −80 °C. After determination of their moisture content (Section 2.2), samples were lyophilised in a VirTis Bench Top (USA) freeze-drier, initially set at −55 °C for 48 h, packed in polyethylene bags and subsequently stored at −20 °C, until further analysis.

2.2. Compositional analysis of samples

All samples were milled using a conventional coffee grinder in order to reduce their particle size to less than 0.5 mm. The moisture content was determined by drying at 105 °C until a constant weight was reached (at least 18 h of drying needed). Ash was determined after drying the samples in a muffle furnace at 550 ± 10 °C for at least 6 h until a constant weight was reached. Kjeldahl analysis was used to determine total protein using $N \times 5.7$ as the conversion factor. Starch content was measured using the Megazyme total starch assay kit (Megazyme International, Ireland). The lipid content was measured gravimetrically after extraction with a Soxhlet apparatus using petroleum ether (Merck, Germany) as solvent.

The composition of the carbohydrates in the samples was determined after a two-step acid hydrolysis procedure according to the National Renewable Energy Laboratory protocol (NREL/TP-510-42618). The material (300 mg) was first hydrolysed with 72% v/v of sulphuric acid at 30 °C for 1 h and then in diluted acid (4%, v/v) at 121 °C for 30 min. During hydrolysis the polysaccharides are hydrolysed into monosaccharides (glucose derived from cellulose and β-glucan, and xylose and arabinose derived from hemicellulose) which were quantified by HPLC (Agilent, 1100 series) with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, California, USA) and a refractive index detector. The operating conditions were: sample volume 20 μL; mobile phase 0.005 M H_2SO_4; flow rate 0.6 mL/min; column temperature 65 °C. According to the NREL protocol, during acid hydrolysis lignin is fractionated into acid soluble and acid insoluble material. Acid-soluble lignin was measured with a UV–Vis spectrometer at 320 nm and acid-insoluble lignin gravimetrically after subtracting the ash and protein contents of the samples. The lignin content of samples is presented as the sum of acid soluble lignin and acid insoluble residue.

2.3. Osborne fractionation of DDGS and in-process samples

DDGS, wet and spent solid samples were subjected to Osborne fractionation according to the method of Lookhart and Bean (1995). Briefly, 100 mg of sample were sequentially extracted with deionised water, 0.5 M NaCl (Sigma, UK), 70% (v/v) aqueous ethanol (Sigma, UK) and 50% (v/v) 1-propanol (Merck, Germany) with 1% (w/v) dithiothreitol (DTT) (Sigma, UK), in order to extract the water-soluble albumins, salt-soluble globulins, ethanol-soluble prolamins and ethanol-insoluble prolamins (as reduced subunits), respectively. A 1:10 (w/v) solids-to-liquid ratio was used for the extractions, which were performed in a thermomixer (Eppendorf, UK) with constant mixing (1400 rpm), at 60 °C for 30 min. Extractions for each sample were done in duplicate and the supernatants were collected by centrifugation (8000×g for 5 min). In the case of sodium chloride, an additional wash with deionised water was performed in order to remove the residual salt. The protein contents of the Osborne fractionated supernatants were determined using the Bradford reagent assay (Sigma, UK) (Bradford, 1976).

2.4. Protein extraction

2.4.1. Aqueous-ethanol extraction of proteins

Lyophilised and milled samples were subjected to protein extraction using different extraction conditions. Initially, all samples were treated with hexane at a 1:10 (w/v) solid-to-hexane ratio at room temperature for 8 h in order to remove the oil content. Hexane was removed by filtration though a Whatman No 1 paper and the solids were placed in an oven at 45 °C overnight to remove any residual hexane. A two-stage process was subsequently applied to the de-fatted samples to extract the water insoluble proteins. Specifically, 10 g of each sample were mixed with 70% (v/v) aqueous ethanol in a 1:10 (v/w) ratio and incubated under constant shaking for 30 min at different temperatures (50, 70 and 90 °C). Supernatants were removed by centrifugation (8000×g, 15 min) and the residues mixed with 70% (v/v) of aqueous ethanol in a 1:10 (v/w) ratio containing varying concentrations of sodium metabisulphite (Fluka, UK) (0.5, 1.0 or 1.5% w/v) as reducing agent.