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Development and characterisation of protein films derived from dried distillers' grains with solubles and in-process samples

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ABSTRACT

Polymer films were developed utilising proteins extracted from wheat distillers' dried grains with solubles (DDGS) and in-process samples (wet solids), both by-products of bioethanol production process. Structural characterisation of DDGS and wet solids films indicated a change in the secondary structure of the proteins, reflecting the impact of DDGS production process such as effect of enzyme on protein properties and consequently on the film properties; whereas the developed films exhibited a rough surface with voids. Determination of moisture sensitivity indicated that DDGS films exhibited more hydrophilicity than wet solids films, with the same trend being observed for their water solubility and water uptake. The moisture content and solubility of DDGS films ranged from 10.2–14.2% and 32.3–41.8% respectively whereas those for wet solids' film ranged from 18.9–19.8% and 23.8–24.2% respectively. The mechanical properties of DDGS and wet solids (ranging from 0.27–0.32 MPa) were comparatively lower than commercial wheat gluten film (0.6 MPa). The poor mechanical properties and high water vapour permeability of DDGS and the wet solids films limit their application as biodegradable packaging materials. However, based on their hydrophilicity, the developed films have potential applications in agriculture and horticulture as controlled release matrices and soil conditioners.

1. Introduction

Distillers' dried grains with solubles (DDGS) is a by-product of bioethanol and distillery industries, which is produced in large quantities annually worldwide (Hu et al., 2011). Distillers grains typically comprise proteins (27–33%), hemicellulose (20%), cellulose (15%), crude lipids (10%), as well as trace amounts of starch and lignin (Xiang et al., 2014). The production of DDGS has been increasing significantly during the past decade due to the growth of the biofuel industry. According to the Organization of Economic Corporation and Development, the growth rate in the production of ethanol is projected to be 19 billion litres in the next ten years (OECD, 2017). Based on these projections, such increased growth of the bioethanol industry is expected to have a direct influence on the production and availability of DDGS. It is estimated that the total production of bioethanol derived DDGS in U.S and Europe would be 44 and 9 million tonnes respectively by 2018 (OECD, 2009).

Currently, DDGS is regarded as a low-value by-product that is mainly used as a protein-rich animal feed (Muniyasamy et al., 2013). However, studies have shown that the compositional variability of DDGS can affect its nutritional quality and digestibility and may result in health problems in animals (Wu and Munkvold, 2008). This often limits the application of DDGS as an animal feed. However, this heterogeneous by-product can also be utilised as starting material for the development of added-value products that may open new income streams for bioethanol producers and distillers.

One of the first studies in this regard was the valorisation of DDGS by incorporating it with polyolefine polymers such as polypropylene (PP) and polyethylene (PE) (Julson et al., 2004). There are reports on the development of composites from DDGS by chemical modification and blending with other polymers (Li and Sun, 2011; Tatara et al., 2009). The possible applications of DDGS as a bio-filler along with other polymers and resins for the synthesis of biocomposites have also been studied (Hu et al., 2011; Tatara et al., 2009). Although Julson et al. (2004) concluded that DDGS cannot be accepted as a bio-filler since the thermoplastics incorporated with DDGS lacked of mechanical strength, further research showed that DDGS can be used as a bio-filler with phenolic resin to increase the biodegradability of the resulting composite (Tatara et al., 2009). Biocomposites of DDGS/polylactic acid (PLA) prepared by thermal compounding were also shown to exhibit

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mechanical properties similar to that of pure PLA (Li and Sun, 2011). In another study, DDGS was modified by green surface treatment and used with polyhydroxy (butyrate-*co*-valarate) for the preparation of bioplastics (Zarrinbakhsh et al., 2011).

Corn (maize) DDGS has been used for the preparation of thermoplastic films that possessed satisfactory mechanical properties (Hu et al., 2011), while Reddy et al. (2014) grafted methacrylates on to sorghum-DDGS to produce films with low water stability. Cellulose extracted from DDGS has also been used to prepare films to be used as water absorbents (Xu et al., 2009) while an alkali-soluble hemicellulose-rich biopolymer fraction from DDGS was made into standalone films (Xiang et al., 2014). However, there are few reports on the development of films from proteins extracted from wheat DDGS.

The major protein component of wheat DDGS, is gluten, which is well-characterised because its visco-elastic properties underpin the use of wheat in bread-making and other food systems. It also has excellent film-forming properties, with gluten films being excellent barriers towards gases such as oxygen and carbon dioxide at low relative humidities (Coltelli et al., 2016). A study of the use of gluten films for wrapping refrigerated strawberries indicated that gluten films are good candidates for controlling the decay of fruits (Tanada-Palmu and Grosso, 2005). However, the proteins derived from DDGS (or its intermediate products) are likely to exhibit different properties to those of commercially available wheat gluten, due to effects of enzyme action and processing conditions during DDGS production.

Earlier, we have carried out the chemical composition of DDGS and wet solids and observed that the protein and lignin contents were lower for wet solids (19.8 and 4.1% respectively) than DDGS (29.1 and 5.3% respectively) whereas carbohydrate content was slightly lower for DDGS (68%) and wet solids (73%) (Chatzifragkou et al., 2016). The dry matter content of DDGS and wet solids was obtained to be 96.6 and 33.2% respectively. In terms of crude fat, DDGS and wet solids contained 3.4% and 2.9% respectively and ash content was observed to be 3.9% for DDGS and 2.1% for wet solids.

Thus, the main objective of the present study was to develop films from DDGS-derived gluten, comparing the final dry product with an in-process sample of wet solids to determine whether the final drying step affects the properties of the extracted proteins. To this end, various functional properties of the protein fractions of DDGS and wet solids and their respective films were determined and compared.

2. Materials and methods

2.1. Materials

DDGS and wet solids were provided by Strathclyde distillery in UK (Chivas Brothers Ltd). The samples were lyophilized in a VirTis Bench Top (USA) freeze-drier for 48 h at −55 °C and stored at −20 °C. Commercial gluten and ammonium hydroxide was purchased from Sigma Aldrich and glycerol from Fisher scientific. All other chemicals used were of analytical grade.

2.2. Film preparation

Proteins were extracted from DDGS and wet solids using aqueous ethanol and alkaline-aqueous ethanol. The proteins were extracted from DDGS and wet solids by a two step process as explained by Chatzifragkou et al. (2016). The samples (10 g) were mixed with 70% (v/v) aqueous ethanol (1:10 v/w) and incubated at 70 °C for 30 min under constant stirring, followed by centrifugation (8000*g*; 15 min). The residue was treated with 70% (v/v) of aqueous ethanol (1:10 v/w) containing 1.0% (w/v) sodium metabisulphite as reducing agent. The samples were incubated at 70 °C for 30 min followed by centrifugation (10000 × *g*; 10 min) at 25 °C and second step of extraction was repeated. The concentration of ethanol in the supernatant was reduced to below 20% (v/v) by diluting with deionised water and placed at −20oC for 4 h for precipitation of proteins. The precipitated proteins were centrifuged (15000 × *g*; 20 min) at 2 °C, washed with deionised water, lyophilized in a VirTis Bench Top (USA) freeze-drier for 48 h and stored at −20 °C. Proteins were also extracted from alkaline-ethanol extraction method. Alkaline conditions were incorporated at the second stage of two-step extraction process, in which aqueous ethanol was mixed with 1.0 M sodium hydroxide (NaOH) and 1.0% sodium metabisulphite with a solid-to-liquid ratio of 1:10. After centrifugation, extracted proteins were precipitated using hydrochloric acid (HCl) at pH 5.5, collected by centrifugation, washed, lyophilized and stored at −20 °C. The carbohydrate content in the proteins extracted from DDGS and wet solids were 4.2 and 2.4% respectively. The molecular masses of extracted proteins were previously determined by SDS-PAGE (Chatzifragkou et al., 2016) and were in the range of 15–50 kDa.

For the preparation of the films, dried protein extracts (2.5 g for protein extracted from DDGS and 2.2 g for those extracted from wet solids) were dispersed in 10 mL aqueous ethanol solution followed by the addition of 30% (w/w) glycerol. The solution was stirred continuously (120 rpm) after adjusting its pH to 11.0 ± 0.5 (using ammonium hydroxide solution) and temperature at 75 °C, respectively, and then poured into a petri dish and dried at 40 °C. Films were also prepared from commercial gluten (from Sigma Aldrich) using the same procedure. The films obtained from DDGS and wet solids using the aqueous ethanol and alkali aqueous ethanol extraction solvents are referred to as DDGS_EtOH, DDGS_NaOH, wet solids_EtOH and wet solids_NaOH respectively.

2.3. Colour analysis of films

The colour of the films was determined using a sph850 spectrophotometer (ColorLite GmbH, Katlenburg-Lindau, Germany) calibrated using a white standard. The parameters measured were L* (luminescence), a* (red tone) and b* (yellow tone) which represent the lightness, red to green and yellow to blue colour dimensions, respectively. The ranges of the colour coordinates were: L* − 0–100 (black to white), a* [red (−) to green (+)] and b [(yellow (−) to blue (+)] (Ordidge et al., 2012).

2.4. Fourier transform infrared analysis (FTIR) of films

The films were structurally characterised using a Perkin-Elmer spectrum 100 FTIR spectrometer (Perkin-Elmer, UK) equipped with diamond-attenuated total reflectance (ATR) scanning accessory. The spectra were recorded from 4000 to 500 cm^{-1} at a resolution of 4 cm^{-1} and scan frequency of 32 scans.

2.5. Scanning electron microscopy (SEM)

The surface morphology of the films (at 500 x magnification) was determined using FEI Quanta FEG 600 Environmental Scanning Electron Microscope. Films were cut into small pieces and mounted on aluminium stubs with carbon cements, both sides of which are lined with a thick conductive adhesive. The images were taken at an accelerating voltage of 5 kV after coating with gold under vacuum for 180 s at 40 mA.

2.6. Thermogravimetric analysis (TGA) of films

Thermal characterization of the films (10–20 mg) was carried out using a thermogravimetric analyser, TGA Q50 (TA instruments, TA universal analysis software, UK)). Film, placed on a platinum pan was heated from 30 to 800 °C at a rate of 10 °C/min. The analysis was performed in a nitrogen atmosphere at a flow rate of 40 mL/min. The loss of weight of the films was recorded as a function of temperature.