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Abstract

Distillers' Dried Grains with Solubles (DDGS) is the major by-product of bioethanol and distillery plants. Due to its high content of proteins, water-soluble vitamins and minerals, DDGS has been long marketed as animal feed for livestock. EU legislation on liquid biofuels could raise the demand on bioethanol production in Europe, with a resulting increase in DDGS availability. DDGS contains a spectrum of complex organic macromolecules, particularly polysaccharides, in addition to proteins and vitamins, and its use as a starting raw material within a biomass-based biorefining strategy could lead to the development of multi-stream processes for the production of commodities, platform molecules or speciality chemicals, with concomitant economic benefits and waste reduction for bioethanol plants. The present review aims to outline the compositional characteristics of DDGS and evaluate its potential utilisation as a starting material for the production of added-value products. Parameters of influence on the chemical and physical characteristics of DDGS are discussed. Moreover, various pre-treatment strategies are outlined in terms of efficient DDGS fractionation into several added value streams. Additional processing steps for the production of medium and high added value compounds from DDGS are evaluated and their potential applications in the food and chemical industry sector are identified.

Keywords: DDGS, pre-treatment, biorefinery, added-value products, bioethanol

1. Introduction

Bioethanol represents one of the most important biofuels for automotive transportation. In 2013, global bioethanol production reached 88 billion litres, with economic projections estimating further increases in annual production until 2020 [1]. US contributions account for almost half of the total worldwide bioethanol production, followed by Brazil and European Union (EU). On the basis of feedstock, the USA and EU produce bioethanol through the utilisation of grains (maize and wheat, respectively), while Brazilian plants employ sugar cane as raw material. Based on the OECD-FAO Agricultural Outlook for 2011-2020, the major producers of grain-based ethanol are USA, Canada and the EU.

The production of grain-based ethanol results in the generation of distillers dried grains with solubles (DDGS) as a by-product. A schematic representation of the dry grind bioethanol production process and by-product streams is given in Figure 1. Briefly, the whole grain is milled and liquefied, while the addition of amylolytic enzymes facilitates the conversion of starch into fermentable glucose. Then, yeast is added to ferment the available carbon into ethanol and carbon dioxide. Ethanol is distilled and dehydrated, whereas the non-volatile components are centrifuged to produce a liquid fraction (thin stillage, TS) and a solid fraction (wet distillers' grains, WDG). Around 15% or more of the thin stillage is used as backset (i.e. added to the new batch) for the liquefaction of the ground grain and the rest is concentrated into condensed distiller soluble (CDS). CDS is mixed with WDG and drum dried at high temperatures to produce the final DDGS. Partial recycling of DDGS to the drum dryer is also a common practice in the ethanol industry, in order to increase the drying efficiency of the equipment [2]. It is estimated that in the dry milling process, the utilization of 100 kg of grain results in 40.2 litres of ethanol, 32.3 kg of DDGS and 32.3 kg of CO_2. As far as global bioethanol derived DDGS production is concerned, OECD-FAO projections estimate that the USA will reach 44 million tonnes by 2018, whereas EU and Canada contributions are expected to be equal to nine and one million tonnes, respectively [3].

Another industry that contributes to the global surplus of DDGS is the beverage alcohol industry (*e.g*. distilleries for whisky and other spirits). The production process is similar to that of dry grind bioethanol, although considerable emphasis is placed on Good Manufacturing Practices and hygiene aspects since the final product (potable ethanol) is intended directly for human consumption. It is also worth noting that grain whisky distilleries often utilise blended grains as raw materials that may include wheat, barley, maize and rye. As a result, the final composition of DDGS may vary more than that of strictly corn or wheat derived DDGS.

DDGS has been recognised as an important source of energy, protein, water-soluble vitamins and minerals and for this reason it has been long marketed as feed for livestock [4, 5]. This exploitation contributes significantly to the profitability of distillery and bioethanol plants. In 2014, the annual market price for wheat DDGS in the United Kingdom averaged around £230 per tonne, while the respective price for maize DDGS the same year, mainly produced in the USA, was within the range of $225-240 per tonne (source UK Home Grown Cereal Authorities-HCGA). During the first quarter of 2015, around 49.5 thousand tonnes of distillery by-products were used for the production of animal feed in the UK, increased by 46% compared to the first quarter of 2014 as reported by the UK Department for Environmental Food and Rural Affairs [6].

The production of bioethanol as “first generation” biofuel is likely to rise in future years in Europe as the Directive of EU regulatory framework for biofuels [7] requires that 10% of the energy used in transport should be of a renewable nature by 2020, the majority of which is anticipated to correspond to liquid biofuels. This fact is likely to increase the demand on bioethanol in Europe with a resulting increase in DDGS availability. Moreover, it is of importance to state that the addition of DDGS to livestock feed can account for up to 30% (dry matter basis) of the diet, as higher levels may cause palatability and excessive protein consumption issues [5]. Additionally, the compositional variation in DDGS in relation to its nutritional value and quality still constitutes an obstacle to its primary use as animal feed supplement for ruminants [8, 9]. Taking these into account, the need to find alternative routes to exploit and upgrade DDGS can be considered imperative. In 2011, the Integrated Biorefining Research and Technology Club (IBTI) of the UK Biotechnology and Biological Sciences Research Council (BBSRC) awarded in excess of £2.5M in research grants as part of an initiative to identify alternative ways to enhance the value of DDGS. Moreover, earlier in 2010, the Home Grown Cereals Authority (HGCA) in UK co-funded a collaborative 3-year project named ENBBIO LINK, aiming to identify routes to improve the nutritional value of DDGS as feed for both ruminant and non-ruminant species.

DDGS contains a spectrum of complex organic macromolecules, such as carbohydrates, proteins and oil. Its incorporation as a starting raw material within a biomass-based biorefining strategy could therefore lead to the development of multi-stream processes for the production of commodities, platform molecules or specialty chemicals, with concomitant economic benefits and waste reduction for bioethanol plants. The scope of the present review is to outline the characteristics of DDGS, with respect to its components, and investigate its potential utilisation for the production of added-value products, within a biorefinery concept.

2. Chemical composition of DDGS

2.1 Compositional variation of DDGS

The composition of DDGS is of great interest, particularly in relation to animal nutrition. To this end, parameters such as nutrient composition, digestibility, and amino acid and mineral profiles have been investigated by a number of research groups [10,11, 12]. The nutrient contents of DDGS have been reported to vary according to the nature of the raw material, e.g. wheat or maize, but also among production plants or even between batches from the same plant [13]. This variation can be directly correlated with compositional differences in the wheat and maize grains, the growing, harvesting and handling conditions of grains, but also with the addition of distillers' solubles in the dried grains, and the dehydration process as applied by each manufacturer [2, 14].

A summary of representative studies on the chemical composition of DDGS deriving from various starting materials is presented in Table 1. In the case of maize and wheat DDGS, a comparison of their chemical characteristics often reveals differences in the percentages of oil, protein, as well as in acid and neutral detergent fibre (ADF and NDF, respectively) (Table 1). Maize bioethanol DDGS is often richer in oil (11-15%, w/w) compared to wheat bioethanol DDGS (4-6%, w/w), although in both cases the lignin content is low (3-5%, w/w) and is often expressed as acid detergent fibre (ADF), including the recalcitrant cellulose [12, 15]. On the other hand, distillery DDGS can be differentiated in terms of its protein and NDF content, mainly due to the fact that distillery plants utilise blended grains, such as wheat, barley, maize and rye, instead of a single type of grain. Therefore, the choice of the starting material is a determinant factor for the final DDGS composition. Additionally, variation in the production process of DDGS between plants directly affects the chemical composition of the by-product. Spiehs et al. [16] investigated the variation in the composition of maize DDGS from ten ethanol plants in Minnesota and South Dakota. The coefficients of variation for protein, oil and crude fibre were reported to be lower than 10%, whereas even less variation was estimated for dry matter. Variation in the nutrient content of DDGS was mostly attributed to the maize grain used, the percentage of solubles added back to distillers' dried grains, as well as to possible deviations from the standard practices followed during the fermentation process. As far as wheat DDGS composition is concerned, Jarret et al. [17] characterized the chemical composition of wheat DDGS samples supplied by seven European ethanol plants. Differences in the origin and process of biofuel production between plants were directly related to the variation in the percentage of fibre (NDF and ADF) and to possible Maillard reactions taking place during the process. Furthermore, Cromwell et al. [14] compared seven sources of DDGS deriving from beverage alcohol manufacturers and two sources of DDGS from bioethanol plants, in order to evaluate their nutritional value for non-ruminants. Physical characteristics, such as odour and colour, reflected differences in the drying processes and were directly correlated with the nutritional properties of DDGS, whereas notable variation was identified in terms of the oil, fibre and ash contents between samples which could be attributed to grain variety. In another study, Pedersen et al. [18] reported the compositional variation in DDGS from various bioethanol plants, including maize, wheat and mixed DDGS (containing wheat, triticale, barley and rye, in unknown proportions). Maize DDGS presented higher amounts of oil compared to the other DDGS tested, while wheat and mixed DDGS composition in terms of protein, total sugars and ash were similar, indicating that wheat was the major grain in mixed DDGS.

2.2 Effect of processing on DDGS chemical composition

From a processing point of view, it has been demonstrated that the mixing ratio of wet distillers' grains (WDG) and condensed distillers' soluble (CDS) can considerably affect the chemical composition of the DDGS [2, 19]. The removal of starch during the fermentation step, as well as the thermal treatment of CDS and WDG, can lead to an approximately 3-fold concentration of the remaining macromolecules in DDGS, such as carbohydrates, protein and oil, whereas the inorganic content can be also substantially increased during the production process [20]. Generally, WDG contains higher amounts of insoluble fibre, whereas CDS contains soluble oligosaccharides, ash, as well as organic acids and glycerol generated as by-products during the ethanol fermentation process [2, 13]. In terms of insoluble carbohydrates, it has been reported that after completion of the fermentation, more than 60% of the initial water-insoluble glucan from cellulose is left in WGD, whereas for hemicellulosic components, approximately 55% of the initial xylan and 65% of the initial arabinan remained in the insoluble fraction, indicating the partial degradation of cellulose, xylan and arabinan during the process [21]. As far as protein is concerned, the liquefaction and subsequent fermentation of starch results in an approximate 2.5 to 3-fold increase in the DDGS protein content, taking also into account the contribution of yeast, which is estimated to be around 20% [20]. However, over half of DDGS protein may become insoluble during the dry-grind ethanol process [22, 23]. In terms of amino acids, these are concentrated in the WGD fraction and the addition of CDS prior to the drying process is reported to slightly decrease the overall amino acid content in DDGS [2]. Yeast protein demonstrates a better amino acid profile, particularly with regards to limiting amino acids such as lysine, and its presence influences the amino acid profile of downstream products [13, 20].

3. Treatment strategies for DDGS

Several studies have reported the use of various treatment steps in order to extract and further process macromolecules contained in DDGS. As mentioned above, DDGS is characterized by a complex structure, consisting of hemicellulose, cellulose and proteins; therefore, an optimum combination of different treatment steps is often necessary for the efficient fractionation of its components. Due to the absence of a rigid lignocellulosic structure, DDGS is amenable to relatively mild processing that can lead to the production of several value-added streams, which can act either as end-products or starting materials for secondary processing; the types of value-added products that can be derived from DDGS are discussed in section 4. The processing steps may include physical treatments to improve the material texture, chemical processes for the fractionation of compounds of interest and subsequent extraction and purification, enzyme-assisted processes, or a combination of these. The efficiency of such treatment steps on DDGS valorisation is summarised and discussed in the following sub-sections.

3.1 Physical treatments

DDGS samples can show significant variation in terms of their particle size distribution, ranging from 0.11 to 3.66 mm, a fact that reflects the highly heterogeneous distribution of nutrients among the different size fractions [24]. The reduction of particle size by mechanical stress is often the first pre-treatment step of the solid starting materials, in order to facilitate subsequent chemical or enzymatic hydrolysis. Generally, small particles up to 0.40 mm are preferred for the efficient enzymatic hydrolysis of the solid materials [25], due their higher specific surface area, while for compounds such as cellulose, reductions in both the degree of polymerisation (DP) and crystallinity can be achieved this way [26]. Moreover, the particle size distribution is associated with the chemical and physical characteristics of DDGS and related materials, affecting aspects of the handling systems used, the processing facilities, as well as the digestibility and nutrient availability of DDGS feed [27]. Apart from this, a minimal particle size reduction is needed in most pre-treatment strategies, in order to overcome mass and heat transport issues.

In addition, the particle size distribution could determine the initial steps required for the fractionation of DDGS, aiming to generate compositionally enriched fractions. Based on this, the combination of sieving and air classification (also known as the Elusieve process), has been shown to effectively separate fibre from DDGS [28, 29]. Pilot scale experiments on maize DDGS samples demonstrated that through this approach, DDGS is separated into fibre and an enhanced fraction with lower fibre and 4.8% more protein than the initial material, which can be potentially more suitable for non-ruminant animals [29]. The Elusieve process is a simple, non-intrusive method that can be operated at the end of the dry-mill process with a capital investment estimation of \$1.4 million, which includes an equipment purchase cost of around \$0.43 million [29]. However, the highest revenue potential can be acquired only by the protein-enriched DDGS fraction, whereas the conversion of the low fibre fraction to ethanol is not currently economically feasible and therefore its exploitation will only be profitable if the fibre market value is high [28, 29].

3.2 Chemical and physicochemical treatments

A number of chemical treatment strategies have been studied for their efficiency for the fractionation or degradation of the structural components of DDGS. These include either the use of concentrated and diluted acid and alkali, or a combination of chemical and physical processing, as in the case of ammonia fibre explosion (AFEX) and liquid hot water treatment. Depending on the treatment of the raw material, however, different types of components might be formed that can act as inhibitors and hinder subsequent processing, such as enzymatic hydrolysis or fermentation. These inhibitors are degradation products and include organic acids (mainly acetic, levulinic and formic acid), furan aldehydes, such as furfural deriving from xylose and 5-(hydroxymethyl)-furfural (5-HMF) deriving from glucose, as well as phenolic acids and aromatic compounds formed from lignin [30]. Therefore, the effectiveness of the chosen chemical pre-treatment is determined by criteria such as high conversion yields, minimum formation of toxic degradation products, efficient waste treatment and minimum energy input [31]. A summary of the chemical treatments applied for DDGS and related by-products is given in Table 2.

3.2.1 Ammonia fibre expansion (AFEX)

Ammonia fibre expansion (AFEX) technology possesses the advantage of combining physical (high pressure and temperature conditions) and chemical (ammonia) processes for the efficient pre-treatment of lignocellulosic materials. The incorporation of AFEX as a pre-treatment step leads to biomass swelling and consequently increases the accessible surface area, while supporting cellulose decrystallisation. A minor part of hemicellulose is solubilised into its respective monomers, whereas the lignin structure is rigorously altered and thus rendered more susceptible to digestion [26, 32]. In the case of DDGS, AFEX can be performed under relatively mild conditions (temperatures below 90°C and pressure range between 200-400 psi), due to the low lignin content, with the aim to increase subsequent enzymatic digestibility targeting monosaccharide production [33, 34, 35]. Bals et al. [33] evaluated the efficacy of AFEX pre-treatment on the enzymatic hydrolysis of maize DDGS and reported AFEX conditions of 70°C and 0.8:1 kg/kg ammonia loading as optimal for subsequent enzymatic hydrolysis of the pre-treated DDGS samples. AFEX is an advantageous method for DDGS treatment due to the low lignin content, whereas moderate operation conditions and short residence times minimise the formation of microbial inhibitors such as furfural and 5-hydroxylmethylfurfural (5-HMF). Moreover, the potential of ammonia recovery and recycling minimises chemical usage, and carrying out the process as a continuous operation is a viable option. On the other hand, application of AFEX on a large scale is still influenced considerably by the cost of ammonia, as well as by environmental concerns related to its unpleasant odour [26]. Additionally, AFEX treatment does not convert xylan into xylose monomers. In the case of DDGS, xylan represents around 35-40% of the total carbohydrate content; thus, the combination of AFEX treatment with hemicellulosic enzymes would be necessary in order to convert all the available DDGS carbohydrates into fermentable monosaccharides.

3.2.2 Liquid hot water (LHW)/ Autohydrolysis

Liquid hot water falls into the category of hydrothermal treatments, applied in order to solubilise hemicelluloses and disrupt the cellulose and cell wall structure. These processes are also known as autohydrolysis, hot compressed water (HCW) or hydrothermolysis. The autohydrolysis mode of action lies on the weakening of H-bonding during exposure of materials to water at high temperatures (150-240°C). Water is auto-ionised into acidic hydronium ions (H_3O^+) that act as catalysts on the glycosidic bonds. Additionally, hydronium ions are formed from the cleavage of O-acetyl groups and uronic acid substitution on arabinoxylan (glucuronoarabinoxylan), which further enable the catalysis of hemicellulose into oligosaccharides or monomeric sugars [36]. However, the latter mechanism can cause further degradation of monosaccharides into aldehydes (furfural from pentoses and 5-hydroxymethyl furfural from hexoses) that can hinder subsequent microbial fermentation. The formation of inhibitors can be reduced by controlling the pH in the range of 4-7 during the process. This type of pre-treatment produces mainly oligosaccharides [37, 38]. Moreover, since cellulose and lignin are hardly modified, they are amenable for recovery and further processing [39]. Recently, Samala et al. [40] studied the effect of autohydrolysis on maize DDGS fibre, separated using the Elusieve method. Under optimum conditions (180°C, 20 min), 54.6% of the initial xylan content was hydrolysed to xylooligoasaccharides (XOS) (reported DPs up to 6), followed by traces of degradation products. The application of LHW on maize fibre has shown to yield 80% of soluble oligosaccharides and 20% of monosaccharides, while less than 1% of the initial carbohydrate content is lost due to the formation of degradation products [41]. DDGS pre-treatment with LHW has been reported to significantly increase the rate of the enzymatic hydrolysis of the samples post-treatment, leading to the generation of monosaccharide-rich streams, with glucose hydrolysis yields higher than 90% [34, 35]. LHW treatments attract interest due to the lack of a requirement for a catalyst and the low-corrosion potential. However, the process requires large volumes of water and high energy input. In the same manner as AFEX, LHW treatment requires subsequent enzymatic hydrolysis of the hemicellulosic content in the case of DDGS or related materials with high arabinoxylan presence.

3.2.3 Dilute acid hydrolysis

Dilute acid treatment has been extensively investigated as the means for enhancing biomass digestibility through the breakage of rigid lignocellulosic structures. Hydrochloric, nitric and sulphuric acids have been evaluated for biomass treatment, with the latter being the most common acid of choice [36, 42, 43]. A disadvantage of this method is that depending on the hydrolysis conditions, high levels of sugar degradation compounds such as furfural and 5-HMF, as well as aromatic lignin degradation compounds can be formed. A number of studies have reported the feasibility of using dilute sulphuric acid treatment for DDGS. For instance, Noureddini et al. [44] performed a three-step acid pre-treatment followed by a single step enzymatic hydrolysis of maize DDGS, yielding 128 g/L of total monosaccharides (xylose and glucose monomers) that could result in about 6.4 wt. % ethanol. The effects of reaction temperature, time and acid concentration on the yields of monomeric sugars, namely xylose, arabinose and glucose, have been primarily investigated [45, 46, 47]. Low biomass concentrations (5.0% -10.0%, w/v) have been found to favour hydrolysis of the hemicelluloses in DDGS samples, whereas increased acid concentrations (3.0%-4.0%, v/v) decreased the duration of hydrolysis down to 30 min. However, the temperature of the treatment is critical since high temperatures (up to 140°C) promote the formation of pentose degradation products (furfural and furan resins) [45].

3.2.4 Alkali pre-treatment

Apart from the use of ammonia in AFEX technology as discussed above, bases such as sodium, potassium, calcium and ammonium hydroxide have been evaluated for biomass pre-treatment. In the presence of alkali, ester and glycosidic side chains are degraded whereas structural alteration of lignin and partial solubilisation of hemicellulose can occur [31] which provide the opportunity to separate intact hemicellulose components, such as arabinoxylan. Moreover, the chemical swelling of cellulose via the disruption of crosslinks between hemicelluloses and other components increases the porosity of biomass rendering it more accessible to enzymes [48].

Alkaline pre-treatments offer the advantage of low temperature operation compared to other chemical treatments [49]. However, long residence time is needed followed by neutralisation of the generated slurry in order to remove lignin and other inhibitors (phenolic acids, aldehydes, furfural and salts) of enzymes. Moreover, alkaline treatment has been used on maize fibre for hemicellulose extraction [50, 51], and more recently for DDGS, resulting in the isolation of a hemicellulose-rich biopolymer [52]. Xu et al. [53] utilised a combination of alkali and xylanase pre-treatment in order to extract cellulose from DDGS, achieving a crude cellulose yield of 7.2 % (w/w) with a cellulose content of 81% (w/w). Recently, lime has been proposed for biomass pre-treatment, offering the advantage of lower cost and less safety requirements compared to other alkaline compounds [31]. Additionally, lime can be easily recovered from aqueous solutions as insoluble calcium carbonate by reaction with CO_2 [36].

3.3. Biological treatments

The application of enzymes is considered an efficient approach for the successful valorisation of materials consisting of cellulose and hemicellulose. Enzymatic hydrolysis is often a secondary treatment step and is required for the conversion of previously generated carbohydrate-rich streams into their respective monomers. These can then be utilised as feedstock for the production of chemicals through microbial fermentation and enzymatic or chemical synthesis reactions. Aspects, such as the nature of the hemicellulose as well as the desired end-products of the bioconversion define the choice of enzymes in this step.

The main enzymes used in hydrolysis of physically and/or chemically pre-treated DDGS are hemicellulases and cellulases, often co-operating in a synergistic fashion for the degradation of the hemicelluloses and cellulose present. A summary of the most frequently used enzymes employed in hydrolysis of hemicellulosic materials is presented in Table 3.

3.3.1 Cellulases

Cellulases are derived from microorganisms or plants; they constitute a mixture of several enzymes and are responsible for hydrolyzing cellulose to soluble monosaccharides. Based on their structural properties, three major types of cellulase activities can be distinguished: endo-1,4-β-glucanases (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.176), exo-1,4-β-glucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) [54,55]. Endo-glucanases cleave cellulose chains in low crystallinity regions of the cellulose fibre and create free-chain ends that can be further attacked by exo-glucanases, acting from the non-reducing end, or by cellobiohydrolases acting progressively from the reducing end of cellulose both releasing cellobiose units. The latter are hydrolysed by β-glucosidase to produce glucose. In lignocellulosic biomass, the lignin can block the access of cellulases to cellulose; therefore, pre-treatment processes that separate lignin from cellulose and the hemicellulose component can substantially increase hydrolysis rates [31]. However, DDGS contains relatively low amounts of lignin 3-5% (w/w) and therefore a delignificationpre-treatment step is not required.

3.3.2 Hemicellulases

Hemicellulose is a heterogeneous mixture of polysaccharides and, as a consequence, a range of enzymes is needed in order to achieve effective hydrolysis. The major hemicellulose in cereal grains is arabinoxylan and enzymes involved in its degradation can be divided into depolymerising enzymes, which act on the xylan backbone, and accessory enzymes that remove substituent groups [55]. The principal hydrolytic enzymes employed for xylan degradation to monomers are endo-1,4-β-xylanase (EC 3.2.1.8), which attack the xylan backbone and yield short-chain oligosaccharides, and β-xylosidase (EC 3.2.1.37), which cleave oligosaccharides into xylose monomers. Moreover, the xylan backbone can be decorated with various substituents, such as arabinose and galactose, ferulate and acetate, so the action of ancillary enzymes is required to remove these substituent groups and facilitate backbone degradation [55]. To this end, α-arabinofuranosidase (EC 3.2.1.55), feruloyl esterase (EC 3.1.1.73), α-galactosidase (EC 3.2.1.22) acetyl xylan esterase (EC 3.1.1.72) and xylan α-1,2-glucuronidase (EC 3.2.1.131) act synergistically with xylanases and xylosidases to achieve complete xylan hydrolysis [56].

3.3.3 Enzymatic degradation of DDGS

For the enzymatic hydrolysis of DDGS and related materials, the choice of enzymes is related to the desired end-product. A summary of enzyme combinations that have been employed for DDGS hydrolysis and their respective conversion yields is presented in Table 4. If the DDGS hydrolysate is intended to be utilized as a fermentation feedstock (e.g. for production of ethanol or platform chemicals), cellulose-degrading enzymes can be used for the release of the glucose monomers [57]. Cellulose conversion rates from untreated DDGS are reported to be relatively higher in the presence of cellulase and β-glucosidase enzyme mixtures, compared to other biomass by-products such as maize stover. Glucose yields of 76% were achieved after 72 h of hydrolysis of maize DDGS with low solid loadings (5%, w/w) [35]. On the other hand, pre-treatment of DDGS is highly advantageous for nearly complete cellulose hydrolysis (98%) within the same time [35]. WDG can be less susceptible to hydrolysis, showing lower yields by approximately 30% in high substrate loadings (15%, w/w). This can be overcome through the use of auxiliary enzymes (xylanases, ferulic acid esterases) that act on the hemicellulose structure during the course of hydrolysis, and as more sites become susceptible to cellulase attack, glucose yield is increased [35]. Additionally, compounds produced during the pre-treatment step, such as lignin-derived phenolics as well as xylan oligomers, can act as inhibitors of cellulases [58]. Due to the fact that cellulases have a minor impact on hemicellulose hydrolysis, further digestion with xylanase and ferulic acid esterase mixtures is required for the production of hemicellulose-derived pentosans

[59]. However, Dien et al. [34] observed that additions of the above mentioned commercial enzymes did not favour the release of xylose and arabinose from pre-treated DDGS. On the contrary, cellulase blends with pectinase and ferulic acid esterase, increased the hemicellulose conversion yields. Although DDGS does not contain any pectin, commercial pectinases usually contain multiple side-activities and may contribute to achieving increased monosaccharide yields [34]. Banerjee et al. [60] reported that increased levels of mannanase were also needed in order to enhance the release of glucose from AFEX-treated DDGS. In addition to glycosyl hydrolases, proteolytic enzymes can be applied for the extraction of proteins from DDGS [22, 33], as the means for increasing arabinoxylan extraction [61].

The choice of the pre-treatment strategy for DDGS depends greatly on the aims of the biorefinery. On one hand, enzymatic hydrolysis is a less energy intensive process as opposed to chemical treatments, offering the advantage of selective catalysis of carbohydrates, generating monosaccharide-rich streams suitable for microbial conversion. However, enzymatic pre-treatment is often hindered by substrate concentration, enzyme activity and end-product inhibition. To this end, the production of tailored multi-enzyme cocktails (containing optimised cellulase/hemicellulase proportions) with higher specific activities compared to current commercial enzymes, obtained through screening or protein engineering approaches, is expected to reduce capital costs associated with the pre-treatment step. Physico-chemical treatments such as steam explosion are considered cost-effective and have a realistic potential for industrial scale processing. They can offer high yields of monomeric sugars and enhanced hemicellulose hydrolysis. However, their combination with subsequent enzymatic processes is often problematic due to the formation of inhibitory compounds during the pre-treatment process (e.g. in the case of dilute acid hydrolysis) or to the requirement for additional steps prior to enzyme hydrolysis (e.g. neutralisation step in the case of alkaline treatment). Thus, it seems rather unlikely that a process aiming to fully exploit DDGS will rely on a single treatment step due to the complex structure of DDGS.

4. Value-added products from DDGS

The heterogeneous nature of DDGS allows its biotransformation into several added-value products. These can either be subjected to further purification leading to primary products, or used as starting materials for secondary processing, as part of a biorefinery strategy. A schematic representation of various added-value products from DDGS based on the biorefinery concept is given in Fig. 2. These include biofuels, biopolymers, platform chemicals, prebiotic oligosaccharides as well as packaging materials. All the above mentioned products could be derived by effectively exploiting two principal components that account for 65-70% of the total DDGS composition, i.e. carbohydrates and proteins, and have a variety of potential applications in industrial sectors such as food, chemicals and packaging. Currently, the bioethanol production process generates DDGS and CO_2 as co-product streams, both of which have market values for the industry. Therefore, the choice of product(s) deriving from DDGS should be of higher added value in order to compensate for the additional energy and equipment costs. Ideally, the additional process should be easily incorporated into existing production processes. Moreover, a successful process should not be affected by feedstock variability, which could stem from the use of blended cereals as raw materials for bioethanol production. A biorefinery strategy could aim to use intermediate products of the DDGS biotransformation process as starting materials for the generation of added-value components. From an economic perspective, in the bioethanol production process, apart from feedstock price fluctuation, the thermal processing of the WDG-CDS mixture is the most costly part of production [62]; however it is required in order to confer shelf-life stability during transportation of the DDGS used as animal feed. Taking this into account, WDG could be used as substrate for chemical/enzymatic treatments as it has been shown to contain higher amounts of total carbohydrate and protein (on a dry matter basis) compared to DDGS [13]. Another in-process sample that can be utilised for the production of added-value components is thin stillage (TS). TS contains a complex mixture of carbon sources, such as soluble sugars, by-products of fermentation, such as glycerol and organic acids, and also yeast cells [13] that can serve as an ideal source of nutrients for microbial fermentations. A number of studies have demonstrated the feasibility of using TS directly as a fermentation feedstock or as source of liquid nutrients supplemented with additional carbon sources for the production of microbial metabolites such as lipids, solvents, organic acids and extracellular polysaccharides (Table 5). An additional advantage reported in these studies is the potential remediation of TS through the reduction of their total solids and chemical oxygen demand (COD) [63, 64].

4.1 Biofuels, platform chemicals and biopolymers

One of the most studied biotechnological processes for DDGS upgrade is bioethanol production, as the means for generating additional profit to bioethanol plants, through the microbial conversion of non-starch carbohydrates. Initial studies aimed to produce a cellulose-derived glucose-rich stream from DDGS which can be fermented by hexose-consuming wild-type microbial strains that exhibit high ethanol tolerance, such as *Saccharomyces cerevisiae* and *Zymomonas mobilis*. However, genetic engineering has since allowed the development of modified strains capable of fermenting pentoses (i.e. xylose and arabinose) by introducing pentose-metabolizing pathways from bacterial strains of *E. coli* or natural xylose-fermenting yeasts such as *Pichia stipitis* and *Candida shehatae* to *S. cerevisiae* strains [65]. More recently, the concept of consolidated bioprocessing (CBP) has emerged, aiming to reduce the cost of added enzymes in the pre-treatment step. In CBP, lignocellulosic materials can be directly fermented into the desired products in a single step by microorganisms performing simultaneous saccharification and fermentation of the substrate [66, 67]. CBP benefits from the elimination of the enzyme production process, since engineered yeast strains capable of secreting hydrolytic enzymes, such as cellulases, can be used. However, in some cases, high density cultures (100 g/L wet cell weight) are required for the effective hydrolysis of the raw materials [68]. A major obstacle in the process is the difference in the optimum temperatures between saccharification and fermentation [69]. To this end, research on the construction of thermotolerant recombinant yeast strains is ongoing [70]. Apart from DDGS, complementary ethanol production can be achieved through the direct fermentation of TS. A metabolically engineered *Escherichia coli* strain was capable of ethanol production, by utilizing simultaneously glycerol and the sugars present in TS media, after supplementation with mineral salts [71].

Typically, DDGS contains around 14-18% of cellulose. Based on literature data, the combination of AFEX treatment and subsequent enzymatic hydrolysis can convert up to 93% of cellulose to fermentable glucose. If the hemicellulose content (accounting for around 25-28% of total DDGS composition) is further hydrolysed, an overall yield of 92% of total hemicellulose and cellulose conversion into fermentable hexoses and pentoses can be achieved (Fig 2). In the ideal scenario of a complete fermentation of the available sugars and the absence of inhibitory parameters, the process may contribute up to 15% more ethanol than the conventional dry-grind process, whereas the generated DDGS in such a process would be enriched with protein (30-40% of total mass, compared to ~30% in standard DDGS) and could be marketed as a livestock feed at a higher price than its current price, especially if it provides the amino acid requirements for animal feeds, in terms of lysine content [72]. Kim et al. [72] investigated three case studies of process alternatives based on recycling the pre-treated and hydrolysed distillers' grains, and assessed their effect on the overall ethanol yields. They concluded that a 14% ethanol yield increase could be achieved by releasing the additional fermentable sugars present in distiller's grains by further processing and hydrolysis of fermentable glucans [72]. However, the cost of cellulosic ethanol is still high (estimated typically around £0.6 per litre) [73]. It has been proposed that the combination of reduced enzyme costs and the higher market price of DDGS enriched in protein could render the 'DDGS to bioethanol' process a viable prospect for the biofuel industry [74].

Another approach towards the production of added-value compounds is the microbial transformation of DDGS hydrolysates into platform chemicals, such as succinic acid. The latter can be used as a precursor for a variety of chemical compounds that have a number of applications in the food, pharmaceutical, and plastic industries [75]. The potential of replacing a petroleum-based chemical process with a bio-based process for succinic acid production attracts much research interest recently. The current market price of succinic acid is estimated as around £4,000-6,000 per tonne, depending on its purity [76]. Microbial production of succinic acid by strains of *Anaerobiospirillum succiniciproducens* can be achieved at conversion yields as high as 91% (on glucose-based substrates) [77]. Based on the same scenario, which includes the conversion of cellulose to glucose, around 19% of the initial DDGS amount could be converted into succinic acid, taking into account an optimum bioconversion yield of 91% (Fig 2). DDGS bioconversion to succinic acid could be further enhanced, since most of succinic acid-producing strains (*Actinobacillus succinogenes*, *Mannheimia succiniciproducens*) are capable of utilising pentose sugars as carbon substrates with satisfactory conversion yields (55-80%) [78, 79].

An additional promising bioconversion route of DDGS hydrolysates includes the microbial production of biodegradable biopolymers, such as polyhydroxyalkanoates (PHAs). The biodegradable plastics industry is currently growing fast, with world production reaching nearly 740,000 tonnes in 2013, while projections estimate that the total production volume will reach approximately 2.96 million tonnes by 2021 [80]. PHAs are polyesters that contain hydroxyl-alkanoic acids as monomers and exhibit resistance against high temperatures (up to 180°C) as well as oxygen barrier properties. Among the PHAs, polyhydroxybutyrate (PHB) is the most common biopolymer with a wide spectrum of applications. PHAs are synthesized intracellularly by a number of bacterial strains such as *Cupriavidus necator*, *Bacillus* sp., *Pseudomonas* sp. or *Aeromonas* sp. [81]. PHAs market price is still much higher than those of other bio-based polyesters (approx. £7-9/kg), [80] whereas around 50% of the total PHAs cost is due to the substrate cost [82]. The use of low-value feedstocks derived from waste streams in combination with an environmentally friendly and cost effective extraction step, could potentially lead to the establishment of a competitive PHA production process based on DDGS.

Based on the applied pre-treatments, DDGS hydrolysates can contain a mixture of glucose, xylose and arabinose as carbon sources for microbial conversions. A number of PHA-producing strains have been reported to catabolise xylose, the majority of which however demonstrate low specific PHA rates and production yields [83,84] compared to those achieved in glucose or sucrose-based media [85, 86]. Taking a best case scenario based on literature data showing a 38% of DDGS cellulose-derived glucose after AFEX treatment [34], approximately 8 kg of PHB per 100 kg of DDGS can be potentially achieved (calculations based on glucose conversion data from Ryu et al. [87]).

Poly-lactic acid (PLA), originating from lactic acid polymerisation, represents another important polymer in the field of biodegradable materials. PLA has unique biodegradability and biocompatibility properties, with potential applications in packaging and agricultural products, as well as in medical and textile industries [88]. In 2013, about 143,200 tonnes of PLA were produced worldwide; the total PLA market volume for 2021 is forecasted to rise to approximately 465,500 tonnes with a rise in demand of around 16%, and its current price is around £2-4/kg [80]. Europe is the third largest market after North America and Asia-Pacific [80]. The building monomer for PLA, lactic acid, occurs in two optical isomers, L- and D-lactic acid, which can be obtained via chemical synthesis (hydrolysis of lactonitrile) or through microbial fermentation. In the latter case, the enzymatic capacity of bacterial strains (*Lactobacillus* spp.) determines the stereo specificity of the lactic acid produced. For this reason, obtaining optically pure lactic acid is of great importance [89]. As is the case for most microbial conversions, the operation and purification costs are also of primary importance. In lactic acid bacteria (LAB), hexose catabolism is usually performed via the homofermentative pathway, producing solely lactic acid. On the other hand, most LAB catabolise pentoses via the heterofermentative pathway, generating by-products such as acetic acid and ethanol. This causes a decrease in lactic acid yield. Although a number of novel lactic acid-producing strains have been reported to efficiently ferment xylose to lactic acid with high yields and optical purity (95% and 99.6%, respectively) [90], the microorganism of choice should be capable of utilising simultaneously the mixed sugars present in the hemicellulosic hydrolysates. Recently, Tsuge et al. [91] reported the homofermentative D-lactic acid production by an engineered *L. plantarum* strain capable of simultaneously catabolising xylose and glucose in a two-step production system, based on the sequential cultivations of growing and resting cells. Lactic acid production yields were higher than 90% (w/w). In such a case, the fermentation of the mixed sugars contained in a DDGS hydrolysate could potentially lead to approximately 28 kg of lactic acid per 100 kg of DDGS.

4.2 Xylan and xylo-oligosacchrides

Xylan constitutes part of the hemicellulosic fraction and represents the major polysaccharide in bioethanol DDGS, accounting for approximately 35-40% of the total carbohydrates (Kim et al. 2008a). In the wheat grain cell, the xylan consists of a linear backbone of D-xylopyranosyl units, which may be mono-substituted with *a*-L-arabinofuranosyl residues on position O-3 (~21%) or di-substituted on positions O-2 and O-3 (~13%) [92, 93]. Glucuronic acid or its 4-methyl ether derivative can also be linked in the O-2 position of xylopyranosyl residues. Arabinofuranosyl residues linked on position O-3 of the xylose units may be ester-linked to ferulic acid, which may undergo oxidative dimerization to form covalent cross-linkages between the xylan chains [93]. These cross-links, in addition to the interactions of arabinoxylans (AX) with other cell wall components such as cellulose and lignin, are responsible for the water-insoluble nature of a high proportion of wheat grain arabinoxylan. In wheat flour, water-soluble AX account for 25% of the total AX content, but the proportion is much lower in bran and whole grain [94]. The structure and chemical properties of soluble and insoluble AX in the wheat grain have been intensively studied, however for DDGS limited information is available. Most studies on DDGS exploitation are focused on the solubilisation of the insoluble AX fraction, while hardly any information is available on the effect of the DDGS production process on the solubility of AX. In a recent study [18] comparing the composition of maize grain to that of maize DDGS, an increase in the soluble AX content in DDGS compared to grain was observed, which suggests that the non-starch polysaccharide fraction is modified during the fermentation process and the subsequent drying process. This can be attributed to factors such as the presence of exogenous or yeast -derived fibre degrading enzymes, as well as to the mechanical and heat treatments during DDGS production [18].

Apart from xylan hydrolysis to its respective monomers, an alternative way for the efficient valorisation of DDGS xylan is its conversion to xylo-oligosaccharides (XOS) or arabinoxylo-oligosaccharides (AXOS), compounds that exert potential prebiotic health effects. According to Gibson et al. [95], “prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits”. Prebiotics stimulate the population of beneficiary bacteria (e.g. *Bifidobacterium* spp. and *Lactobacillus* spp.) leading to the production of short-chained fatty acids (SCFAs), mainly acetate, propionate and butyrate. SCFAs are used as energy source by colonic epithelial cells and may function as primary protective agents against colonic disorders, inhibit the growth of pathogenic microorganisms, while they also have immunomodulatory properties. The main commercial prebiotics include fructo-oligosaccharides (FOS), inulin-type fructans and galacto-oligosaccharides (GOS). In the case of XOS (mainly mixtures of DP2 and DP3 are produced commercially) and the AXOS prebiotic effects have been shown primarily *in vitro*, whereas data from human studies are limited although a study has been recently published [96].

A small number of studies have exploited the isolation of insoluble xylan from cereal- based by-products, such as maize fibre or maize cobs. Different methods have been assessed for their efficiency towards xylan extraction, including chemical (alkaline, acid, bleach, organic solvents), enzymatic (xylanases) and mechanical assisted treatments (extrusion, hydrothermal, ultrasound and microwave) [97]. DDGS is an advantageous starting material for xylan extraction as it contains low amounts of lignin (3-5%), therefore a delignification step is not needed. Yields of up to ~25% were obtained from DDGS in a process consisting of alkaline extraction and ethanol precipitation [53].

DDGS xylan has been previously evaluated as an additive for the preparation of gluten-based biodegradable films [98]. The water vapour transfer rate of the films was not affected by xylan addition, whereas the production conditions and xylan origin influenced their mechanical and solubility properties. More recently, the feasibility of producing films from hemicellulose-rich fractions of DDGS was evaluated [53]. The extracted fraction contained around 52% hemicelluloses (mainly arabinoxylan) and 18% protein. The films produced from this fraction were stiff and had a high glass transition temperature, as a result of a greater degree of polymerisation in DDGS arabinoxylans, and due to the presence of impurities in the extracted fraction. However, when tested as paper coating, the DDGS-derived arabinoxylan/protein mixture increased considerably the paper tensile strength. Although a promising application has been identified, optimisation of the extraction procedure is needed in order to increase the purity of the extracted xylan, decrease the environmental impact of the extraction process and eliminate the presence of impurities, such as proteins and crude fat. Finally, it is worth mentioning that a xylan-based packaging material is currently marketed by Xylophane under the commercial name Skalax®. Specifically, cereal hulls and husks are used as starting materials and the extracted material is used as paper coating, acting as a migration barrier.

4.3 Protein

DDGS contains substantial amounts of protein (~30-35%, w/w), that justifies its application as a dietary supplement in livestock feed. Wheat proteins comprise gluten storage proteins, which account for about 80% of the total grain protein, and a heterogeneous range of non-gluten proteins (~20%). The non-gluten proteins comprise structural and metabolic components as well as storage components, and include abundant water-soluble (albumin) components of mass below about 25kDa [99]. By contrast, gluten proteins are not soluble in water and are classically divided into monomeric gliadins and polymeric glutenins. Both groups are defined as prolamins as they are soluble in alcohol-water mixtures, either as native monomers (gliadins) or after reduction of the inter-chain disulphide bonds (glutenin subunits) [100, 101]. Based on their genetics, structure and evolution, wheat prolamins can be categorised in three major groups: sulphur-rich (S-poor) prolamins which correspond to ω-gliadin monomers, sulphur-poor (S-rich) prolamins corresponding to of α- and γ-gliadins monomers and low molecular weight subunits of glutenin in wheat, and high molecular weight (HMW) prolamins corresponding to high molecular weight subunits [101]. The maize prolamins, known as zein, account for almost 80% of the total grain protein. *a*-Zeins are the major prolamin group occurring as monomers or oligomers, whereas minor zien groups (*β*-, *γ*- and *δ*-zeins) occur as polymers [101].

Praire Gold Inc. developed a process named COPE (Corn Oil and Protein Extraction) for the extraction of zein and oil from maize DDGS; this was achieved by fractionation at the front-end of the dry-grind ethanol process. Through this technology, several grades of high quality zein fractions are produced, containing varying amounts of xanthophylls. However, zein yields are low (2-5%, w/w) and high amounts of solvents are required in the process. On the other hand, a back-end process for protein extraction is more attractive since DDGS contains high amounts of protein as a result of starch removal and mass reduction [102]. Nevertheless, a commercial back-end extraction of proteins from DDGS protein has not been applied.

Several different approaches have been proposed for DDGS protein extraction, including aqueous ethanol, alkaline-ethanol and enzyme treatments. Bandara et al. [103] investigated the efficiency of protein extraction from triticale DDGS and concluded that treatment with alkaline ethanol gave maximum protein purity of 66% (w/w); however, extraction yields were limited to 21-30% (w/w). For corn DDGS, higher purities of extracted protein have been reported (90% w/w) accompanied by average extraction yields of 44% (w/w) using aqueous ethanol extraction in the presence of reducing agents [104]. The purity and yield of the extracted proteins from DDGS still remains a challenge, since an ideal method should provide high protein purity without compromising extraction yields. DDGS proteins often show low extractability, possibly due to the heating process that is applied and can cause denaturation of the proteins and changes in their properties [103]. During the final stage of the dry-grind ethanol production process (Fig. 1), the WDG and CDS mixture is subjected to intense thermal treatment. The extent of heating varies between plants for DDGS production but can reach up to 200°C. It is possible that the utilisation of in-process samples, such as whole stillage or WDG, could lead to the extraction of proteins with higher yields and purity, since up to that point of the process, mild heating steps are applied during the liquefaction of biomass (~50°C) and the distillation of ethanol (~80°C). Looking towards the commercialisation of a large-scale protein extraction process from DDGS, environmental aspects should also be taken into account, with respect to solvent selection and extraction method, as well as energy usage.

DDGS protein can be exploited in a variety of medium-value industrial applications, such as for the production of biodegradable films, coatings and biodegradable plastics, which can be used in food and agricultural applications [105, 106]. In particular, wheat gluten has been extensively researched 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 [107, 108]. For the production of protein-based films, plasticisers are usually added in levels of 15-40% of protein weight and contribute to the improvement in the flexibility and extensibility properties of films. Low molecular size components of low volatility, such as sorbitol, xylitol, glycerol, mannitol, diglycerol and polyvinyl alcohol, have been tested as plasticisers for wheat gluten films [109]. Among those, glycerol has many advantages as it is non-toxic and suitable for use in the food industry. Wheat gluten-based films are water-insoluble and present properties similar to those of zein films [110]. They possess higher water vapour permeability but their mechanical properties are inferior compared to most synthetic films [111]. By contrast, starch-based films are used primarily in food packaging, and possess excellent oxygen-barrier properties but poor mechanical properties. Moreover, cellulose-based films hold their share of the market, producing tough, flexible and transparent films, resistant to fats and oils and sensitive to water. Gluten-based films possess better mechanical and gas barrier properties compared to polysaccharide films, while their mechanical stability can be improved by the incorporation of plasticisers [112]. The commercial production of gluten-based films is yet to be established, whereas starch-based biodegradable products hold a major share, with a market volume of 162,500 tonnes in 2013 [80]. Attempts have been made to modify the structure and improve the functionality of gluten for films using a variety of methods, including incorporation of hydrophobic compounds [113], enzymatic cross-linking [114], controlled thermal treatment [115] and gamma-irradiation [116]. Further research is needed in order to develop processes and products that can be applied on a commercial scale and compete in terms of price and functionality with petroleum-derived polymers.

4.4 Phenolic acids

DDGS is a potential source of phytochemicals and in particular phenolic compounds, including ferulic, sinapic, *p*-coumaric, caffeic and vanillic acids. Among these, ferulic and *p*-coumaric account for 80% of the total phenolics [117]. Luthria et al. [118] reported a total phenolic acid concentration of 5.99 mg/g for DDGS, consisting of 4.59 mg/g ferulic acid and 0.72 mg/g *p*-coumaric acid. Additionally, it has been demonstrated that the phenolic content of DDGS is enhanced approximately 3-fold (in dry basis) compared to the starting material before fermentation as a result of starch depletion [119], whereas the effect of the dry mill processing on phenolic acid content is minimal [120]. Due to their unique physiological properties, phenolic acids have been proposed to have numerous health benefits due to their radical scavenging ability, inhibition of lipid peroxidation and protection against LDL oxidation in the human body [121]. For this reason, they could be marketed as nutraceuticals, and more specifically as natural sources of antioxidants in foods and dietary supplements [122]. Moreover, ferulic acid can be used for the commercial production of bio-vanillin, an aromatic flavour compound used by the food, pharmaceutical and cosmetics industries, via microbiological conversion routes [123,124].

Ferulic acid is predominantly bound on the cell wall AX components, with dimeric forms accounting for between 4.2 and 8.6% in wheat cultivars [125]. An enzymatic hydrolysis process utilising feruloyl esterases, in synergy with main-chain degrading enzymes such as endo-xylanases and pectinases, can lead to the extraction of ferulic acid and its respective dimers [126,127]. The combination of xylanase and ferulic acid esterase has been reported to release up to 86% (w/w) of the total ferulic acid in wheat aleurone [128]. In the case of DDGS, solvent-assisted methods, such as aqueous ethanol, or ultrasound pre-treatments, have been studied for the extraction of phenolic acids from DDGS. Ultrasound pre-treatment of DDGS was reported to increase the extraction yield of phenolic compounds by 14.9%, as opposed to non-treated DDGS [129] Additionally, the application of microwave irradiation in 50% aqueous ethanol solutions of DDGS led to the production of extracts with 12 mg/g of phenolic content [117]. So far, lab-scale studies have indicated the potential of producing phenolic-rich extracts from DDGS. Future work is needed in order to evaluate the scalability of the technologies and assess the economic implications of such processes.

4.5 Oil and Biodiesel

Typically, DDGS contains around 10-12% (w/w) of oil. The fatty acid composition of DDGS oil resembles that of the starting grain (usually maize or wheat), being rich in linoleic acid (~55%, w/w), while it also contains substantial amounts of oleic (~28%, w/w) and palmitic acid (~16%) [130]. Extracting oil from DDGS creates an additional profit to bioethanol plants as the crude maize oil price was estimated at around £500 per ton in 2013. The extracted oil is marketed either for biodiesel production or as refined maize oil. Oil removal leads to the production of DDGS with a higher protein content, a valuable feed component which due to its low residual oil content (5-9%, w/w compared to ~ 10-14% in DDGS) can be marketed for non-ruminant diets (*e.g.* swine). Currently in the US, more than 50% of maize-based bioethanol plants are extracting oil, the majority of which is channelled towards the biodiesel industry and the rest is used in blended feed-fats, mainly by the poultry industry.

Maize oil is either extracted from the germ of the grain prior to fermentation via a solvent/pressing-assisted process, or post-fermentation from the whole or thin stillage (back-end extraction process). In the latter case, oil is extracted by a series of centrifugation, heating and condensation steps, yielding 60-75% of the total oil content. Moreover, DDGS extracted oils were found to contain increased amounts of tocotrienols and carotenoids (1762 and 75 μg/g, respectively) compared to maize germ oil (235 and 1.3 μg/g, respectively); this offers the advantage of increased stability for crude maize oil as opposed to maize germ oil due to the antioxidant activity of the above compounds [130]. In the case of a DDGS biorefinery, the formation of glycerol as a by-product of the biodiesel process could be potentially used as a plasticiser for the production of biodegradable films from DDGS proteins (Fig. 2).

5. **Conclusions**

DDGS constitutes a by-product with potential for transformation into numerous added-value products. Due to its heterogeneous nature several pre-treatment steps have been proposed targeting specific compounds of interest as primary products or starting materials for subsequent bioconversion processes. The parent grains as well as the processing systems have been shown to significantly influence the physical and chemical characteristics of DDGS, and consequently the availability and extractability and of its components. The development of a commercially viable process scheme for the valorisation of DDGS within the biorefinery concept requires the production of medium to high added-value compounds in order to counterbalance capital investment and operating costs. Research thus far has demonstrated that this is feasible at the laboratory and in some cases pilot scale although more industrial research coupled with detailed process economics are needed before leading to commercial realisation and exploitation.

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Figure legends:

Figure 1 Simplified schematic representation of a dry-mill bioethanol production process and by-product production streams

Figure 2 DDGS valorisation based on a conceptual biorefinery strategy

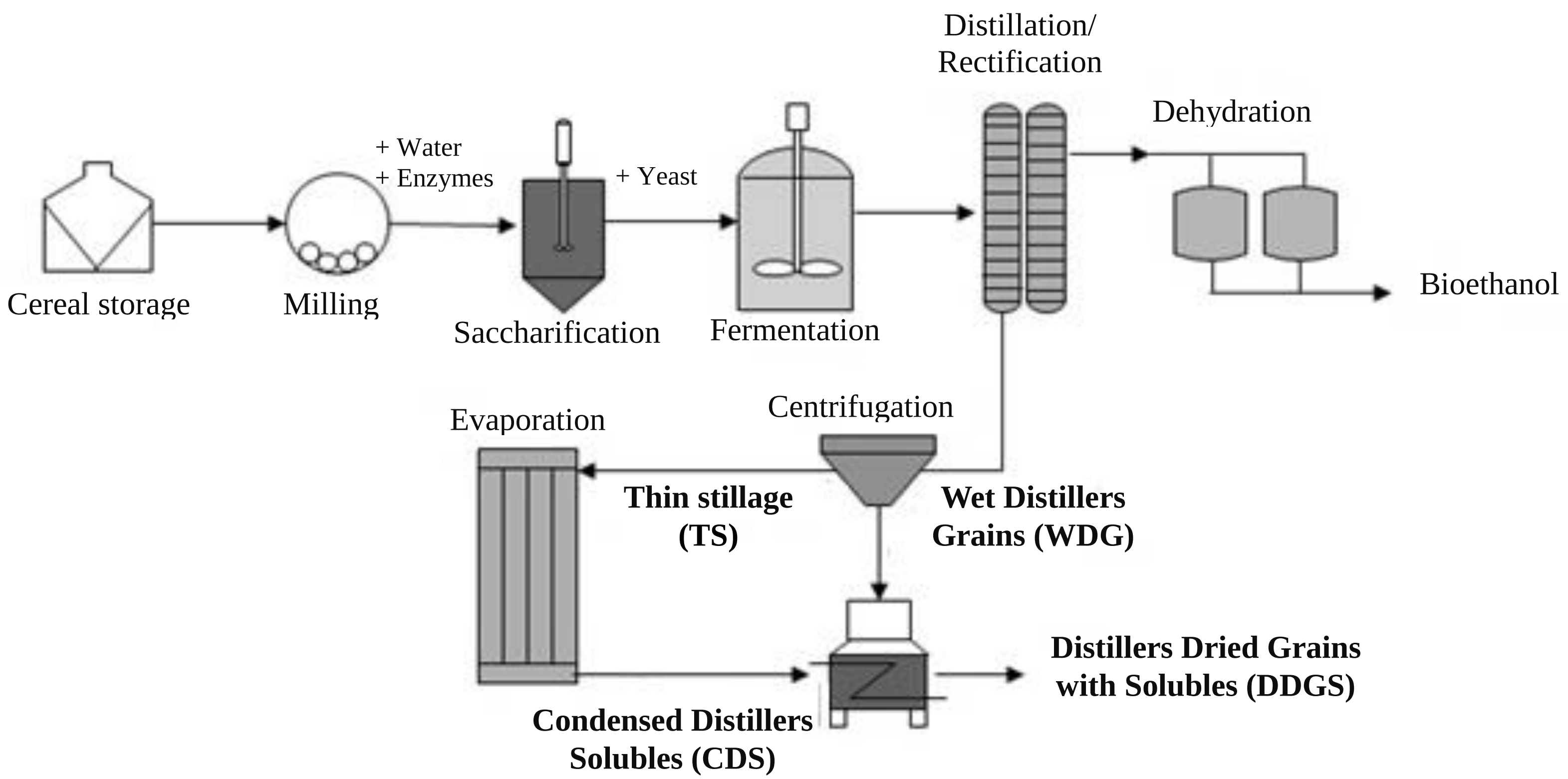
Distillation/
Rectification
Dehydration
+ Water
+ Enzymes
+ Yeast
Cereal storage
Milling
Saccharification
Fermentation
Bioethanol
Evaporation
Centrifugation
Thin stillage
(TS)
Wet Distillers
Grains (WDG)
Distillers Dried Grains
with Solubles (DDGS)
Condensed Distillers
Solubles (CDS)

Fig 1

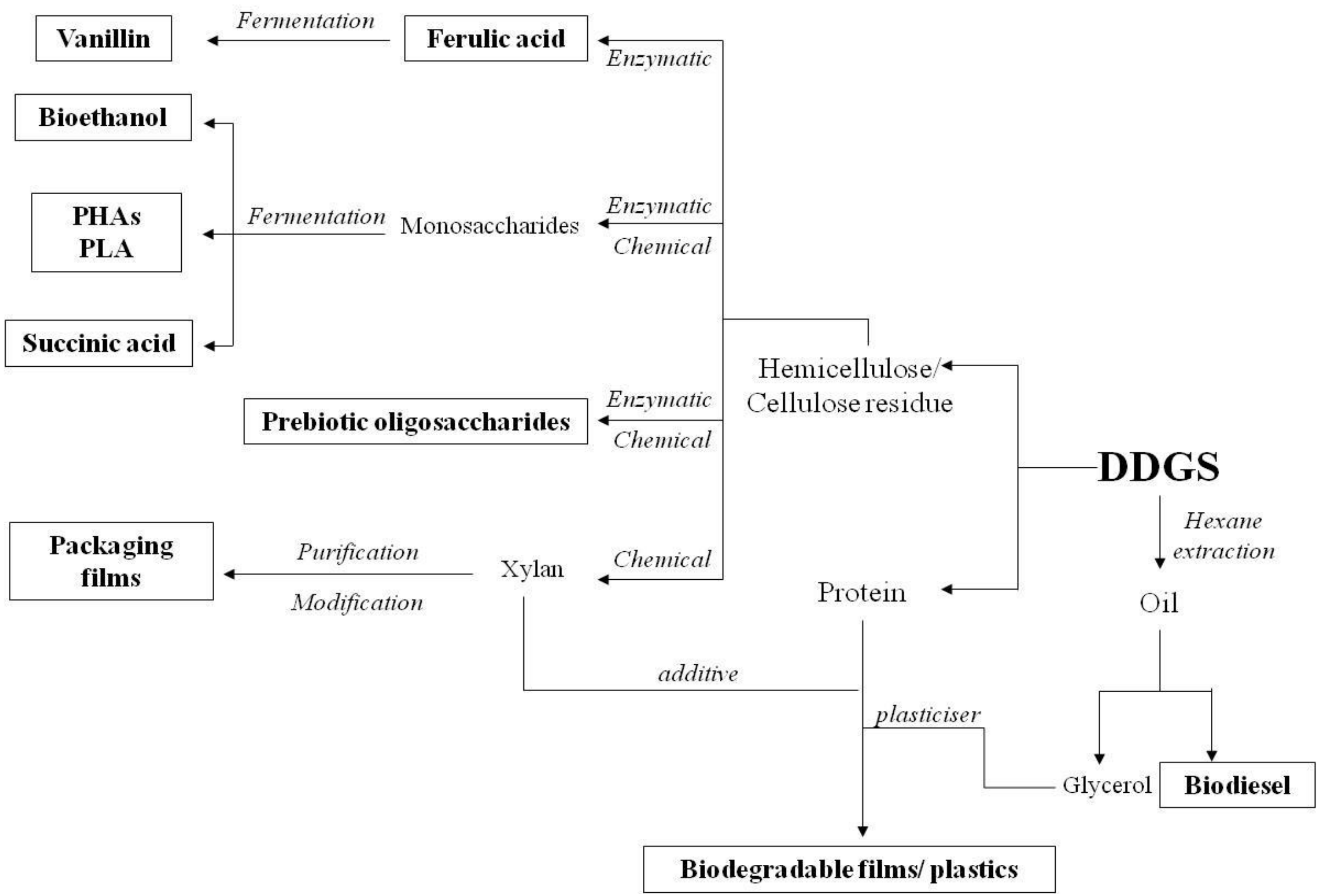
Vanillin
Fermentation
Ferulic acid
Enzymatic
Bioethanol
PHAs
PLA
Fermentation
Monosaccharides
Enzymatic
Chemical
Succinic acid
Hemicellulose/
Cellulose residue
Prebiotic oligosaccharides
Enzymatic
Chemical
DDGS
Hexane
extraction
Packaging
films
Purification
Modification
Xylan
Chemical
Protein
Oil
additive
plasticiser
Glycerol
Biodiesel
Biodegradable films/ plastics

Fig. 2

Table 1. Composition of DDGS from different plants and sources (expressed in %, dry matter basis)

	Maize DDGS[16[	Wheat DDGS[17]	Distillery DDGS[14]	Mixed DDGS[18]a
Dry matter	87.2-90.2	89.3-94.4	90.5-92.7	87.3-92.6
Oil	10.2-11.4	3.6-5.6	8.1-12.8	11.0-12.4
Protein	28.7-31.6	32.6-38.9	23.4-27.9	33.8-38.3
Crude fibre	8.3-9.7	6.2-10.9	9.6-10.6	5.6-7.6
ADF	13.8-18.5	7.7-17.9	15.4-19.3	11.5-12.3
NDF	36.7-49.1	25.1-33.8	34.8-40.3	28.9-31.2
Ash	5.2-6.7	4.3-6.7	3.4-7.3	8.0-10.2

[a]: Parent grains of mixed DDGS were wheat, triticale, barley and rye.

Table 2. Summary of main products and yields from DDGS chemical pre-treatments

Raw material	**Treatment**	**Conditions**	**Main products**	**Yield**	**Reference**
Maize Distillers Grains	Dilute acid hydrolysis	Acid conc. 1.0% Solid load. 10% Temp. 140°C	Monosaccharides (Xyl, Ara, Gluc, Gal)	61.3 g/100 g carbohydrates	[45]
Maize DDGS	Dilute acid hydrolysis	Acid conc. 3.1% Solid load. 15% Temp. 112°C	Monosaccharides (Xyl, Ara, Gluc)	43.4 g/100 g dry matter	[47]
Maize DDGS	Three stage dilute acid hydrolysis	Acid conc. 1% Solid load. 15% Temp. 120°C	Monosaccharides	35.8 g/100 g carbohydrates	[44]
Maize DDGS fibre	Autohydrolysis	Temp. 180°C, 15 min Solid load. 10%	Xylo-oligosaccharides	18.6% (w/w) of feedstock	[40]
Maize DDGS	Liquid hot water	Solid load. 15.7% Temp. 160°C, 20 min	Monosaccharides	86% Glu, 29% Xyl, 37% Ara	[34]
Maize DDGS	AFEX	Solid load. 25g Ammonia load. 80% Temp. 70°C, Pressure 350-430 psi	Monosaccharides	93% Glu, 14% Xyl, 20% Ara	[34]

Table 3. Enzymes involved in the degradation of cellulosic and hemicellulosic materials

Category	Enzymes	Linkage hydrolysed	Products
Cellulases	Endo-1,4-*β*-glucanase	Internal *β*-1,4	Cellobiose
	Cellobiohydrolase	Terminal *β*-1,4 (reducing end)	Cellobiose
	Exo-1,4-*β*-glucanase	Terminal *β*-1,4 (non-reducing end)	Cellotetrose, Cellobiose
	β-glucosidase	Terminal *β*-1,4 (non-reducing end)	Glucose
Hemicellulases	Endo-1,4-*β*-xylanase	Internal *β*-1,4	Xylo-oligosaccharides
	Exo-1,4-*β*-xylanase	Terminal *β*-1,4 (reducing end)	Xylose, xylobiose
	β-Xylosidase	Terminal *β*-1,4 (non-reducing end)	Xylose
Accessory xylanolytic enzymes	*α*-L-Arabinofuranosidases	Terminal *α*-1,2/ *α*-1,3/ *α*-1,5 (non-reducing end)	Arabinose
	α-D-Glucuronidases	*α*-1,2-glycosidic bond	Methylglucuronic acids
	Acetyl xylan esterase	Acetyl ester bond	Acetic acid
	Feruloyl esterase	Ester bond	Ferulic acid
	p-Coumaroyl esterase	Ester bond	Coumaric acid

Table 4. Enzyme combinations and main product yields from chemically pre-treated DDGS

Raw material	**Pre-treatment**	**Enzymes**	**Yields**	**Reference**
Maize DDGS	LHW AFEX	Cellulase (GC220), *β*-glucosidase (Novo188), multifect pectinase, feruloyl esterase (Depol 740L)	91% Glu, 82% Xyl, 70% Ara 100% Glu, 81% Xyl, 98%, Ara	[34]
Maize DDGS	None	Cellulase (Spezyme CP), *β*-glucosidase (Novozyme 188)	76% Glu	[35]
Maize WDG	LHW AFEX	Cellulase (GC220), *β*-glucosidase (Novozyme 188), Xylanase (Multifect Pectinase), feruloyl esterase (Depol 740L)	77% Glu, 41% Xy 72% Glu, 45% Xyl	[35]
Maize DDGS	Dilute acid hydrolysis	Cellulase & *β*-glycosidase (Sigma)	80% Glu, 82% Xyl	[44]

Table 5. Microbial conversions of DDGS-derived hydrolysates and thin stillage

Feedstock	Microorganism	Carbon source	Product	Yield [a]	Reference
Pre-treated DDGS	*Clostridium acetobutylicum*	Mixed sugars	ABE	34 %	[131]
	Saccharomyces cerevisiae	Glucose-Xylose	Ethanol	49 %	[35]
Thin stillage	*Mucor circinelloides*	Mixed sugars	Microbial oil	46 % [b]	[63]
	Aureobasidium sp.	Mixed sugars	Pullulan	21 %	[132]
	Rhizopus oligosporus	Glycerol	Single cell protein	43 %	[133]
	Gluconacetobacter xylinus	Glucose	Bacterial cellulose	57 %	[134]
	Cl. pasteurianum	Glycerol	Butanol	44 %	[135]
	Escherichia coli (recombinant)	Glycerol	Ethanol	40 %	[136]
	Aspergillus niger	Glycerol-Mixed sugars	Malic acid	80 %	[137]
	Lactobacillus panis (recombinant)	Glycerol- Glucose	1,3- Propanediol	74 %	[138]
	Lactobacillus rhamnosus	Mixed sugars	Lactic acid	96 %	[139]

[a] Expressed as %, w/w of consumed substrate

[b] Expressed as % w/w of produced biomass