Received: 24 February 2021 | Revised: 22 April 2021 | Accepted: 28 April 2021

DOI: 10.1002/cche.10445

REVIEW

Corn distillers dried grains with solubles: Production, properties, and potential uses

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Abstract

Background and objectives: The grain-based ethanol production has been increasing as the demand for biofuel additives increased, and the fossil fuel sources decreased. Distillers dried grains with solubles (DDGS) are a primary coproduct of bioethanol plants of the conventional corn dry-grind process. With increasing corn-based ethanol production, DDGS production is increased, and it has become a global commodity. Despite the high nutritional value of DDGS, however, its uses are still not fully explored. This review aims to provide a comprehensive summary of the DDGS production process, chemical and physical properties, nutritional value, and the potential of using DDGS in cattle, fish, poultry feed, and human foods. The challenges of using DDGS in animal feed and human food are discussed.

Findings: Distillers dried grains with solubles has been identified as a protein substitute that is both readily available and competitively priced (per unit protein) compared to other conventional alternative protein sources. Researchers have discovered measures to boost DDGS quality and safety. Several approaches have been proposed to improve the DDGS digestibility and nutritional value. Although the benefits and risks associated with the DDGS uses are identified, more research is required to regulate the quality and safety standards for DDGS.

Conclusions: Versatile applications of DDGS will valorize this coproduct. The development of DDGS utilization enhances health and functional benefits to animals and humans; it also assists bioethanol plants economically.

Significance and novelty: The information will not only provide knowledge of DDGS in terms of its production process, nutritional value, and applications but, more importantly, explore the opportunities of developing new uses of this underutilized commodity.

KEYWORDS
biofuels, corn, DDGS, ethanol, feed and food nutrition, mycotoxin, quality

Tweetable highlights: Uses of DDGS (a coproduct from grain-based ethanol plants) has been extended in animal feed, human food, fermentation process, etc. Bioethanol plants, feed, and food industries will benefit economically from diversifying DDGS applications.

1 | INTRODUCTION

The use of ethanol as a safe and environmentally friendly biofuel has monumentally increased in recent decades. Fuel substituted with up to 10% alcohol can be used in all vehicles in the US without any modifications; while using fuel with 15% substitution with ethanol requires some engine modifications (Mosier & Ileleji, 2020).

Starch, as the main ingredient of the corn kernels, is converted into ethanol through the fermentation process. Global corn production and fuel ethanol production have dramatically increased in the past few decades due to the rapid growth of corn ethanol production. For example, corn ethanol production in the United States has tripled in the last decade (Ray & Ramachandran, 2018). According to the Economic Research Service at the US Department of Agriculture, about 29% of US corn was used for fuel ethanol production in 2017 (USDA ERS, 2017). The establishment of the Renewable Fuel Standard (RFS) in 2005 has triggered the increase in ethanol production from 4.5 billion gallons in 2017 to more than 17 billion gallons in 2018 by using 37% of US corn for ethanol production (Olson & Capehart, 2019).

The bioethanol production process results in ethanol, byproducts such as corn oil, and coproducts such as DDGS (Iram et al., 2020; Mohammadi Shad et al., 2020). Distillers grains can be obtained from the distillation process of cereals such as corn, wheat, rice, barley, oats, and soybean, depending on geographical location, cost, and availability of the grains. The coproduct of the ethanol production from the grains varies depending on moisture content, starch quantity/quality, and ethanol yield. In the United States, the DDGS is primarily produced in the ethanol processing of corn, and a small portion is produced in sorghum-based ethanol plants. Corn is also the predominant source of fermentable starch with the highest ethanol yield. Therefore, the ingredient mainly used in ethanol production is corn in most areas, such as the United States, European Union (E.U.), and China (Vohra et al., 2014).

Among several corn conversion processes into ethanol, the dry-grind process is one of the most common approaches with 82% application in bioethanol plants (Kingsly & Ileleji, 2009). The dry-grind process involves grinding the whole corn grains and liquefaction with enzymes followed by saccharification and fermentation to convert starch into ethanol (Saggi & Dey, 2019). In bioethanol plants, corn (25.40 kg) is transformed into ethanol (11.8 liters), DDGS (7.7 kg), corn oil, and carbon dioxide (CO_2). Dry-grind fuel ethanol production is the major contributor of the distiller's grains (about 98%), although the traditional sources like breweries also contribute a small portion (1%–2%).

Non-fermentable residues from the corn bioethanol production plants are divided into two typical types, condensed distillers solubles (CDS) and wet distillers grains (WDG). CDS is the concentrated form (35%–40% solids) of thin stillage (5%–10% solids). WDG has a high moisture content (70%) that makes the shelf life of WDG limited to 4–5 days, depending on storage environments (Kingsly & Ileleji, 2009). However, the DDGS is the dried WDG mixed with thin stillage or CDS. The low moisture content of DDGS (10%–12%) makes it more storable with longer shelf life (almost indefinite), which ensures DDGS can be shipped anywhere (Mohammadi Shad et al., 2020; Vohra et al., 2014).

2 | ETHANOL PRODUCTION PROCESSES

Two primary commercial processes that convert corn into fuel ethanol are wet milling (corn fractionation into germ, fiber, and starch) and dry-grinding (without corn fractionation). Dry milling is sometimes incorrectly used as a dry-grind process. The dry milling is mainly intended for food production such as flaking grits, smaller grits, meals, or flours low in protein, fat, fiber used in breakfast cereals, snacks, bakery, and hominy feed used in feed industries (Rausch & Belyea, 2006).

About 90% of the US ethanol production occurs through the corn dry-grind process, while the wet mills contribute to only about 10% of the US ethanol production (Fang, 2017). The dry-grind process is predominant because it is more efficient in ethanol production than the wet milling process, and less investment is required to build such a plant (Mohammadi Shad et al., 2020; Moiser et al., 2020). Different kernel fractions are separated in the wet milling process, including starch, gluten, fiber, and germ (Iram et al., 2020). Though higher investment is needed for the wet milling process, very diverse products can be produced in this process, including high fructose corn syrup, biodegradable plastics, citric acid, xanthan gum, corn oil, and animal feed (Mosier & Ileleji, 2020).

2.1 | Dry-grind ethanol production

The steps involved in the dry-grind process are shown in Figure 1. The dry-grind plants are smaller and require lower capital costs than wet milling plants and are mostly producers-owned. The cost for constructing a dry-grind facility for processing 1,000 metric t/d, which produces 150 million L/yr of ethanol, is about $50 million in the United States. The DDGS is one of the primary coproducts available for marketing in the conventional dry-grind processes (Kumar & Singh, 2019). Corn kernels are subjected to grinding to reduce the particle size by hammer mills into a coarse powder to maximize ethanol yield due to increased accessibility of the nutrients by microbes and enzymes more

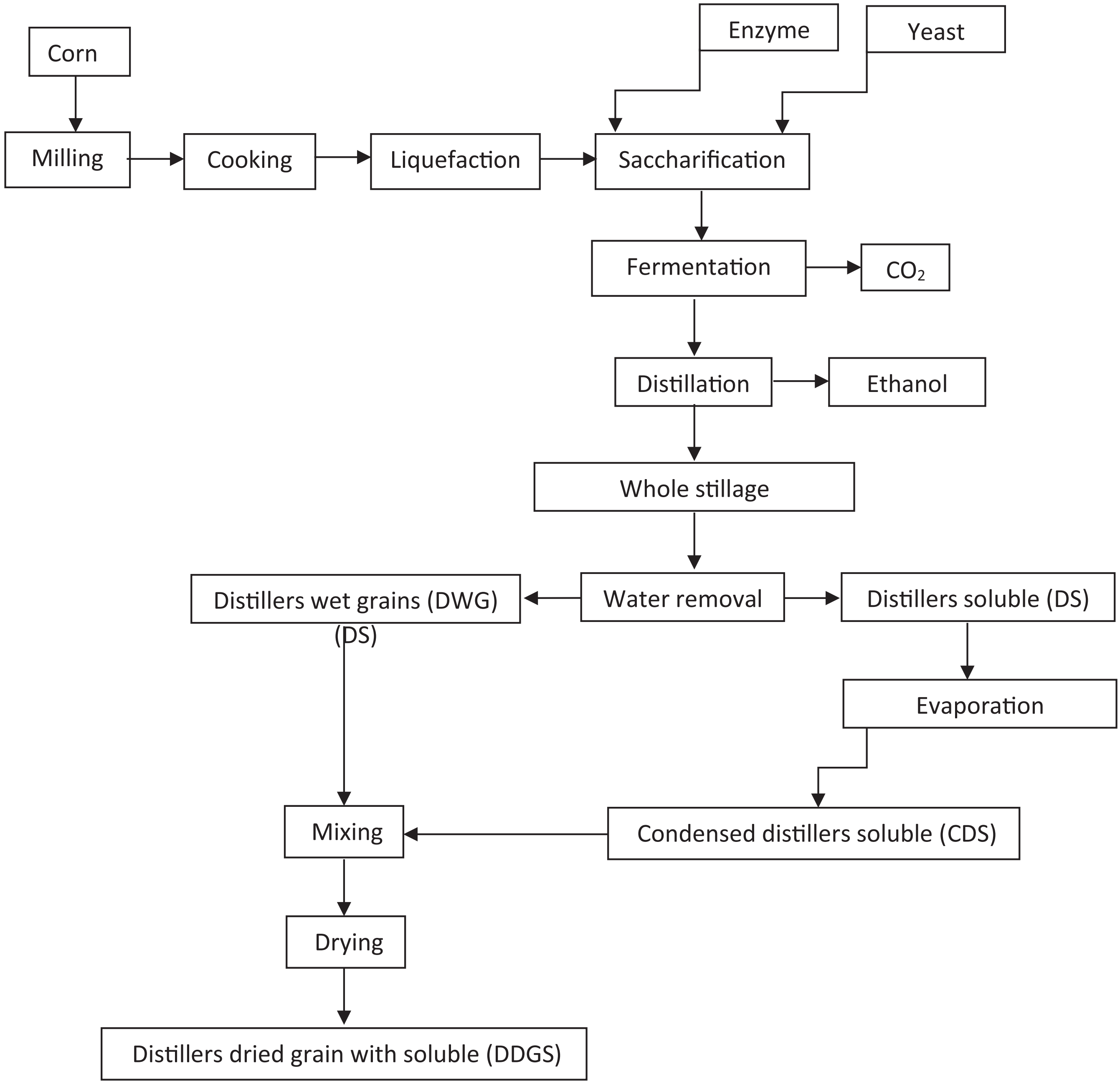


FIGURE 1 Different steps involved in dry-grind corn ethanol production

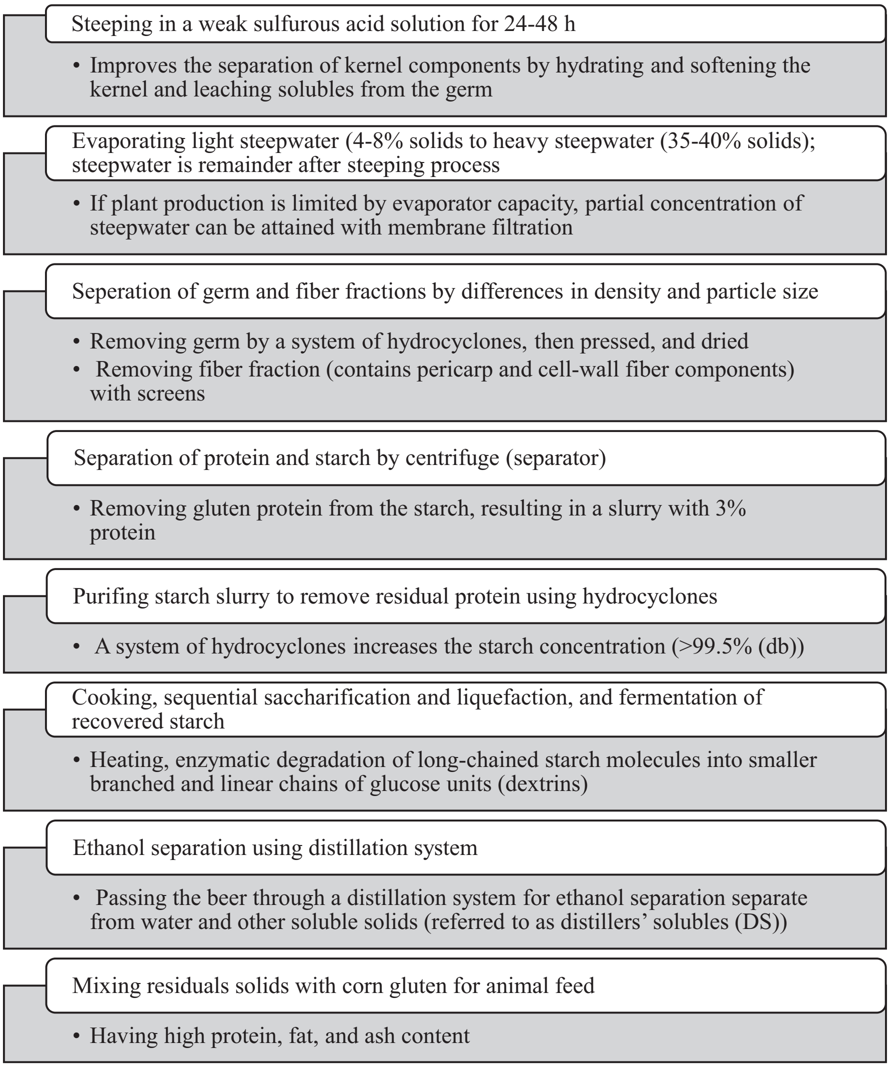
efficiently (Zeng et al., 2007) and facilitate water penetration during the cooking process (Kim, Hendrickson, et al., 2008; Rausch & Belyea, 2006). After adding water and recycled stillage to help leaching of soluble proteins, sugars, and non-starch bound lipids, the mixture is cooked (40–60°C in the pre-mixing tank, 90–165°C for cooking, and 60°C for liquefaction); along with the addition of amylolytic enzymes (amylase) to hydrolyze starch into glucose (Vohra et al., 2014); this helps to convert glucose into ethanol by yeast easily.

The mixture is then cooked at a jet cooker at 120°C (Ramirez-Cadavid et al., 2014). Next, the mash is passed to the liquefaction chamber set at 80–90°C and retained for 30 min after adding heat-stable amylase (α-amylase). During the liquefaction process, the long chains of starch are hydrolyzed and broken into smaller chains, increasing the amount of reducing sugars such as glucose, resulting in lower viscosity. Next, the sugars are converted to alcohol by yeast (*Saccharomyces cerevisiae*) in the fermentation step set at 33°C and a pH of about 4.0 for 48–72 hr. The microbial cross-contamination should be avoided in the fermentation process to preserve ethanol yield and plant productivity. One of the most common contamination is lactobacilli, which produces lactic acid, and the lactic acid inhibits *S. cerevisiae* activity. After fermentation, CO_2 is either collected in a separate column in few processing plants or released into the air. The ethanol is also distilled using a stripper and recovered and then purified with a molecular sieve system to remove water using adsorption technology. After purification, the fuel-grade ethanol is produced by mixing ethanol with a small amount of gasoline (Rosentrater, 2011).

The fiber, water, oil, protein, other unfermented components of the grain, and yeast cells that remain after distillation of ethanol are called whole stillage. This mixture is usually centrifuged to separate solids (WDG) from liquid (thin stillage); the latter is recycled and used at the front-end of the process to make a slurry of the ground grain (Kim, Hendrickson, et al., 2008). The remaining thin stillage is concentrated through evaporators to remove additional moisture and produce CDS with 30% dry matter (Kim, Hendrickson, et al., 2008). The WDG, CDS, or the combination of both (wet distillers' grains with solubles, WDGS) can be sold locally to animal feed manufacturers or combined with the coarse solids fraction and dried to produce DDGS with 88% dry matter having a longer shelf life. Another coproduct from the ethanol production plants is corn oil. Before stillage drying and DDGS production, the corn oil is obtained from the thin stillage portion through two-steps centrifugation. Thin stillage contains approximately 30% of the total corn's oil (Kwiatkowski et al., 2006).

2.2 | Wet milling

The wet milling process accounts for about 10% of US ethanol production, in which several process streams and byproducts are developed due to corn fractionation into germ, fiber, protein, and starch. The primary byproducts from these plants are wet or dried corn gluten feed, corn gluten meal, crude corn oil, and corn germ meal (Rausch & Belyea, 2006; Shurson, 2005). Wet milling plants are corporate-owned. Although wet mill systems generate large ethanol volumes, the systems are equipment and capital intensive (Rausch & Belyea, 2006). The byproducts are the source of income to make up for ethanol production costs (Rausch & Belyea, 2006). This process is called wet milling because the first step of this process is soaking the grains in water to



Steeping in a weak sulfurous acid solution for 24-48 h

- Improves the separation of kernel components by hydrating and softening the kernel and leaching solubles from the germ

Evaporating light steepwater (4-8% solids to heavy steepwater (35-40% solids); steepwater is remainder after steeping process

- If plant production is limited by evaporator capacity, partial concentration of steepwater can be attained with membrane filtration

Seperation of germ and fiber fractions by differences in density and particle size

- Removing germ by a system of hydrocyclones, then pressed, and dried
- Removing fiber fraction (contains pericarp and cell-wall fiber components) with screens

Separation of protein and starch by centrifuge (separator)

- Removing gluten protein from the starch, resulting in a slurry with 3% protein

Purifing starch slurry to remove residual protein using hydrocyclones

- A system of hydrocyclones increases the starch concentration (>99.5% (db))

Cooking, sequential saccharification and liquefaction, and fermentation of recovered starch

- Heating, enzymatic degradation of long-chained starch molecules into smaller branched and linear chains of glucose units (dextrins)

Ethanol separation using distillation system

- Passing the beer through a distillation system for ethanol separation separate from water and other soluble solids (referred to as distillers' solubles (DS))

Mixing residuals solids with corn gluten for animal feed

- Having high protein, fat, and ash content

FIGURE 2 Corn wet milling process flow diagram soften the grains for better components fractionation (Mosier & Ileleji, 2020). The steps involve in wet milling are explained in Figure 2. Wet milling aims to separate starch from other macromolecules such as germ, fiber, and protein.

3 | CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF DDGS

The composition and the nutritional value of DDGS depend on the source of raw material, production plants, and production procedure; therefore, there is no standard nutrient profile available for DDGS (Liu, 2011). DDGS is a good source of neutral detergent fiber (36.74% db), crude protein (29.93% db), and acid detergent fiber (16.2% db). DDGS also contains crude ash (12.82% db) and starch (11.07% db) (Bhadra et al., 2007). Corn DDGS has higher energy values than raw corn, indicating a superior substance in beef and dairy cattle diets (Nuez Ortín & Yu, 2009).

One of the factors affecting the storability of DDGS is phytochemical components, namely vitamin E, ferulic acids, carotenoids, and xanthophylls. The phytochemical components have antioxidant effects contributing to the betterment of human and animal health. These components are necessary to protect lipids in DDGS against oxidation. DDGS has a high concentration of neutral detergent fiber (NDF), lignin, cellulose, and hemicellulose (Anderson et al., 2006); these ingredients are concentrated in DDGS (three times) compared to initial corn grains. The extensive processing, such as high drying temperature, may negatively affect these ingredients and reduce the DDGS quality by lipid oxidation (Anderson et al., 2006; Shin et al., 2018).

Corn DDGS contains higher crude fat (165 versus 49 g/kg DM), sulfur (7.2 versus 3.9 g/kg DM) but lower crude protein (320 versus 393 g/kg DM) than wheat DDGS (Nuez Ortín & Yu, 2009). Conventional corn DDGS contains high levels of unsaturated fatty acids. The linoleic (C18:2), oleic (C18:1), and palmitic (C16:0) acids are the most abundant fatty acids, representing about 89% of the total fatty acids in DDGS (Díaz-Royón et al., 2012). Although the high content of linoleic and oleic acids as unsaturated fatty acids contributes to the high energy value of DDGS, it causes a susceptibility of DDGS oil to oxidation (Winkler-Moser & Breyer, 2011).

The fatty acid profile of DDGS plays an essential fingerprinting role to classify DDGS in terms of grain varieties, geographical origin, and year of harvest (Tres et al., 2014). A study by Tres et al. (2014) showed that fatty acids in DDGS originated from corn is different from that of wheat with having a high level of C18:1 *n*-9 (oleic), C18:0 (stearic), and C20:0. Wheat DDGS has higher monounsaturated fatty acids (*n*-6 and *n*-3 families), including linoleic (C18:2 *n*-6) and linolenic acid (C18:3 *n*-3). The fatty acid profile of US-originated corn DDGS showed a higher contribution of monounsaturated fatty acids of the *n*-9 series such as C18:1 *n*-9 and C20:1 *n*-9 than DDGS from Canada, France, Spain, Poland, and China (Tres et al., 2014).

In corn, prolamins named zein are the main proteins (50%–60% of the protein in whole grain). Zein has many applications in paper, paint, textile, packaging, and biodegradable composites. DDGS is a good source of zein (12% of the dry mass [w/w]) depending on corn variety and processing methods. DDGS zein has been recovered in many dry-grind processing plants to improve process efficiency and produce high-quality zein as a valuable byproduct in this process (Guardiola-Ponce et al., 2020; Paraman & Lamsal, 2011). The high-quality zein is usually extracted before the fermentation step to avoid heat treatment; zein extraction at this stage requires more solvents because of protein dilution. After fermentation, zein is more concentrated for extraction, but several process modifications are needed to reduce the impact of thermal processing on proteins in conventional processes. One of the recommended technologies is the cold dry-grind process, in which the cooking step before fermentation to gelatinized starch is eliminated. In this technology, enzymes are used to remove proteins surrounding starch granules (Paraman & Lamsal, 2011). A solvent treatment including propanol and ethanol is recommended to improve the efficiency of the process. Hydrolytic enzymes have been shown to increase the zein's purity extracted from DDGS to produce a smooth and clear film (Anderson et al., 2012). Xylene is a major part of hemicellulose found in DDGS, which can be used to produce functional products like xylo oligosaccharides, xylitol, and furfural. The ultrasonic technology can increase the yield of xylene extraction and protein rate in final DDGS (Gu et al., 2019).

Distillers dried grains with solubles is also a rich source of phytochemical compounds providing antioxidant and health benefits. The phytochemical content of DDGS varies based on the processing and grain variety. Antioxidants in DDGS can prevent cancer, coronary heart disease, and strikes related to oxidative activities in human cells. Phenolic compounds are major antioxidants in corn with 80% of antioxidant capacity. The phenolic compounds in corn are concentrated in DDGS more than three times (Luthria et al., 2012). DDGS had a greater concentration of xanthophyll lutein, tocopherols, and tocotrienols (lipid-soluble antioxidants) than corn. Antioxidants in DDGS are also essential to prevent oxidative reactions in animal feed during storage, which will impact the shelf life of these commodities. DDGS also contains xanthophylls components. Xanthophylls are also another antioxidant that increases the yellow color in egg yolk (Shin et al., 2018). Xanthophylls are plant carotenoids that are beneficial for human eye health. Xanthophylls such as lutein and zeaxanthin prevent cancer and aging disease (Li & Engelberth, 2018). Peptides released from DDGS protein will function as antioxidants in bulk oils, ground meat, pet, and animal feed. Two enzymes used for this purpose are Alcalase and Neutrase, metabolized by Bacillus licheniformis and Bacillus amyloliquefaciens, respectively (Hu et al., 2020).

Physical attributes of DDGS varies based on the specification of the raw material, processing set up, storage condition, etc. The physical properties are important because of their impact on DDGS shelf life, flowability, and handling of this material. The high moisture content of WDGs limits storage duration to a maximum of 3–7 days, while DDGS is stable for a maximum of 1 year. The moisture content of DDGS affects DDGS flowability. DDGS exposure to an environment with higher relative humidity will increase the moisture content of DDGS, resulting in higher compressibility and lower flowability (Ganesan et al., 2008). The low water activity (0.42–0.63) and moisture content (<12%) of DDGS are preventative factors for microbial spoilage (Bhadra et al., 2007; Rosentrater, 2006). In a study, the water activity of DDGS was significantly correlated with other DDGS physical properties such as bulk density, thermal properties, and color. Also, there was a correlation between bulk density and thermal properties of DDGS as bulk density (surface area and void spaces between particles) affects heat transfer property (Rosentrater, 2006). The waster activity of DDGS was highly and negatively correlated with bulk density. However, the water activity showed a moderate and positive correlation with color, resistivity, and diffusivity. The bulk density of DDGS was moderately correlated with thermal properties. DDGS with higher bulk density is a more compacted DDGS that can increase the ability to transmit heat energy by providing more surface contact and the interstitial air spaces between particles (Rosentrater, 2006). The bulk density of DDGS is an essential factor in determining the required storage and packaging capacity, and storage and transportation cost. DDGS with lower bulk density will have a higher transportation cost. During transportation, DDGS with lower bulk density is at the risk of particle segregation so that fine particles sink to the bottom of the load, and coarse particles remain at the top (Clementson & Ileleji, 2010).

In a recent study, the moisture content, true density, and particle size of DDGS were dependent on the ratio of WDG and CDS; with fewer CDS added, less true density and particle size of DDGS were seen (Kingsly et al., 2010). However, the fiber and protein contents of DDGS increased with the reduction of CDS added to WDG. DDGS particle size influences DDGS safety in terms of microbial infection and pest infestation. Larval weight of *Tribolium castaneum* increased significantly on a diet with ground DDGS than raw DDGS with bigger particle sizes (Fardisi et al., 2019). Therefore, storing DDGS as a raw ingredient or pelletize DDGS is highly recommended to avoid pest infestation. DDGS particle size is essential as the high variation in particle size will lead to poor sampling, inaccurate analysis, and therefore variability in nutritional parameters for feed and food prepared with DDGS (Clementson & Ileleji, 2010).

4 | NUTRITIONAL VALUE

Distillers dried grains with solubles, being rich in energy, protein, fat, minerals (phosphorus), and vitamins, is one of the best and low-cost alternatives for feed ingredients, particularly for livestock and poultry diets. Thus, DDGS has been found as a suitable substitution for a few of the more costly feed ingredients such as corn (source of traditional energy), soybean meal (source of protein), and mono- or dicalcium phosphate (source of phosphorus) in animal diets. DDGS utilization in the animal diet has contributed to animal wellbeing, better performance, and animal product quality.

The byproducts from corn-based ethanol plants have been evolving due to process modifications such as fractionation added to the traditional process (Heuzé et al., 2015). The fractionation processes such as quick germ (QG) and quick germ quick fiber (QGQF) are designed to separate germ and fiber from corn grains before fermentation. Because germ (rich in oil) and fiber are removed in the early steps of the process, protein is concentrated in the final distillers' dried grain called high protein distillers grain (HPDG) (Hoffman & Baker, 2010; Kelzer et al., 2011; Mohammadi Shad et al., 2020). In addition, researchers have tried to further increase the protein content in DDGS by using high protein sources such as food waste or mutant corn that contain high crude protein for DDGS production. The DDGS from mutant hybrid corn varieties showed higher protein contents and essential amino acids such as lysine (Corray et al., 2019; Mohammadi Shad et al., 2020; Ramchandran et al., 2017). The higher protein content in DDGS than its substrate (corn) is also contributed by yeast protein accumulated in the resultant DDGS. The fermentation and heating process also contribute to lower anti-nutritional factors in DDGS than that in corn and other feed ingredients such as soy and canola meals, which contain trypsin inhibitors and glucosinolates, respectively (Becker & Wittmann, 2012; Han & Liu, 2010; Zschetzsche, 2019).

Although DDGS is a concentrated nutritious form of its substrate (corn), there is a nutritional variability in DDGS. This variability is due to the variability of nutrient levels in the corn sources, the proportion of distiller's soluble added to DDG before drying, the efficiency of converting starch to ethanol, temperature, and drying duration (Carpenter, 1970; Martinez-Amezcua et al., 2007; Salim et al., 2010).

The chemical composition of DDGS is an indicator of market value; the variation in chemical composition affects DDGS quality (such as the nutrient content, total digestible nutrients, and amino acid digestibility), stability, its feed value, and thereby the economics of ethanol production.

TABLE 1 Methods to improve the digestibility and nutritional value of distillers dried grain with solubles (DDGS)

Target	Method	Challenge	Reference
Improve non-starch polysaccharides (NSP) degradability through polymers cleavage or side chains removal	Cell wall degrading enzymes (Xylanases)	The enzyme effectiveness depends on the extent of modification of cell-wall structure during processing	Zijlstra et al. (2010)
Improve the degradability of easily solubilizable NSP	Hammer milling and pelleting (Common feed processes)	Might not be sufficient to affect more recalcitrant NSP structures, such as arabinoxylans in maize.	de Vries et al. (2012)
Improve the digestibility of DDGS dry matter (8%–11%) by increasing protein digestion	Wet milling, extrusion, and dilute hydrothermal acid treatment	It requires severe hydrothermal acid treatments to effectively solubilized NSP	de Vries et al. (2013)
Increase digestibility of DDGS dry matter (18%–34%) by increasing protein and NSP solubilization	Hydrothermal acid treatment (maleic and sulfuric acid)	Potential high residual acid	de Vries et al. (2013)
Higher amino acid and energy digestibility with the production of a new DDGS-HP called DDGS-$\mathrm{HP}_{\mathrm{Lincolnway}}$ (38%–44% versus 27% conventional DDGS); this DDGS also has a higher lysine level	Fiber separation according to its solubility before fermentation and oil extraction after fermentation	Limited data on the nutritional value of DDGS-$\mathrm{HP}_{\mathrm{Lincolnway}}$	Espinosa and Stein (2018)

By increasing CDS into the DDGS, the crude protein, amino acid content, acid detergent fiber (ADF), and neutral detergent fiber (NDF) are decreased, while fat, ash, minerals, sugars, and glycerol content increased. Several approaches are used, as shown in Table 1, to improve the nutritional value, biodegradability, and digestibility of DDGS. Arabinoxylan is a non-starch polysaccharide (NSP) found in the agricultural coproduct, preventing feed's digestibility due to high viscosity (Bell, 2015). One of the strategies to improve the DDGS digestibility is to use the xylanases enzyme to hydrolyze arabinoxylans (Zijlstra et al., 2010). Also, different technologies are available to increase the digestibility of NSP and improve the fiber digestibility (4–16 times higher) with simultaneous enzyme addition. Grain grinding using a hammer or roller mill is a physical method that can improve the NSP. Another technology is thermal processing which either individually or mixed with shear stress can improve digestion of NSP (de Vries et al., 2012). Lincoln way Energy in Nevada, IA, has introduced a patented technology in which fiber is removed by a mechanical method before fermentation and oil is taken after fermentation. The Lincoln way technology provides DDGS with higher protein content and nutritional availability of amino acids and proteins than conventional technologies (Espinosa & Stein, 2018).

5 | USES OF DDGS

The DDGS resulting from ethanol production plants gets a lower value than corn even though the DDGS has a higher benefit than corn as a feed ingredient. By increasing ethanol production (Figure 3), bioethanol plants will end up with a considerable number of coproducts, primarily DDGS. To support the US ethanol industry and ultimately the US crop growers and energy consumers, the coproducts from corn, such as DDGS, should be priced appropriately as their high-value energy. Reviving and diversifying the DDGS market leads to higher corn prices and more energy production (Babcock et al., 2008). Also, the diversified potential application of DDGS boosts the economic benefits of bioethanol production, particularly during uncertain times. In uncertain situations such as the COVID-19 pandemic in 2020, many bioethanol plants had to shut down either temporarily or permanently because they were faced with a limited ethanol demand and increased corn prices (Hart et al., 2020; Mohammadi Shad et al., 2020). Thus, finding alternative uses for DDGS such as fermentation feedstock, feed and food ingredients and the increased utilization of DDGS by improving its digestibility and safety will help corn farmers. The developed DDGS application will help the bioethanol industries retrieve their business, benefit economically and protect the environment from DDGS disposal into the landfills.

A part of distillers grain coproducts is exported, and a high portion of the coproducts (70%) is used in beef, dairy, swine, and poultry feed in the United States. DDGS is used primarily in beef cattle diet followed by dairy cattle, swine, and poultry. Being rich in energy, protein, amino acids, and phosphorous, DDGS is used in the feed ingredients of the ruminants, swine, poultries, and fish; DDGS as a feed ingredient can partially replace some of the expensive feedstuffs such as corn (energy source), soybean (protein source), and mono- or di-calcium phosphate (phosphorous source) (Shin et al., 2018). The applications of DDGS in animal feed and human food are described below.

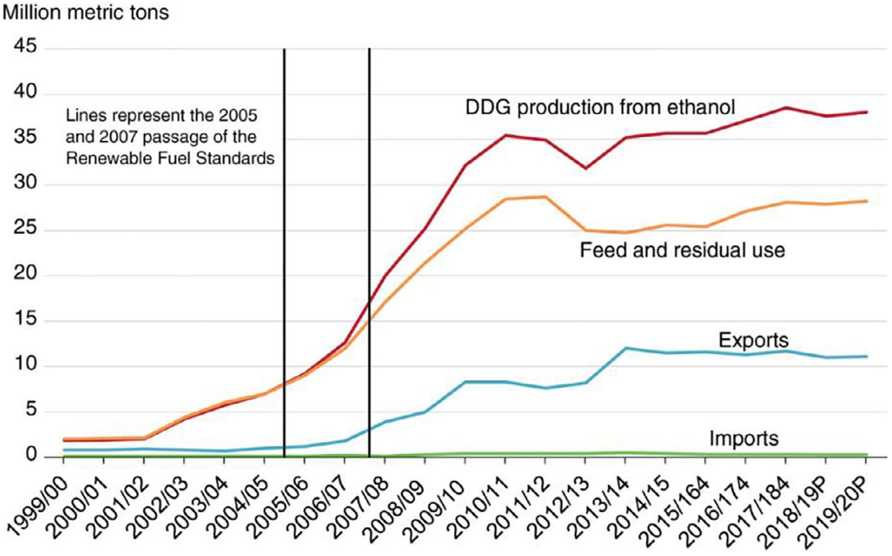


FIGURE 3 Trends and market of distillers dried grains (DDGs) in 1999–2020 (Olson & Capehart, 2019). Notes: P= projection. 2018/19 and 2019/20 data are projections. DDG, Distillers dried grains [Colour figure can be viewed at wileyonlinelibrary.com]

5.1 | DDGS in cattle diets

The US beef cattle industry has been a critical customer of distiller coproducts, and the largest purchaser of DDGS in 2017, accounting for 44% of overall domestic consumption. Using DDGS in cattle feeds provides substantial energy (118%–130% of the energy value of corn) and low starch and readily fermentable fiber content, reducing the risk of rumen acidosis with feeding dry-rolled corn (Ahern et al., 2011).

Use of DDGS in cattle diets has been a way to strengthen the economic sustainability of dry-grind ethanol plants. However, the high concentration of fiber causes limited use of these products, mainly in ruminant diets. Variability in composition driving inaccurate diet formulation and high-water removal costs needs to be addressed and solved (Rausch & Belyea, 2006). DDGS, as a rich source of protein, can replace part of the total mix ration (TMR) to feed dairy cattle, while it can also be a source of energy to replace some of the grains in dairy cattle feed (Zhang et al., 2010). In the United States, the partial substitution of ingredients used in TMR has been successfully carried out by adding up to 40% of DDGS without any significant impact on weight gain or pregnancy (Arias et al., 2008). The WDG and DDGS were used to replace part of corn silage and fed to Heifer beef cattle. The feed consumption, in terms of dry matter intake, decreased in cows fed with corn silage with dry distiller's grains. However, there was no significant difference in weight gain when Heifers were fed with corn ensilaged with DDGS (Arias et al., 2008). The average daily weight gain of steers cattle fed with DDGS and WDGS was not significantly different; however, the cattle fed with WDGS had lower body mass index (BMI) than those fed with DDGS (Ahern et al., 2016; Nuttelman et al., 2011).

The impact of replacing part of TMR fed to milking Holstein cows with different DDGS products (15% substitution) on dry mater adsorption in rumen fluid and total milk production rates were investigated. The DDGS addition did not negatively affect the feed digestibility, total milk production, and composition. The partial replacement of TMR with DDGS had some positive effects on feed efficiency (Kelzer et al., 2009). The partial replacement of barley grain or silage with DDGS (20%) increased milk production, protein, and lactose contents. Simultaneously, rumen pH was not impacted, meaning that there is no concern regarding acidosis in the rumen (Zhang et al., 2010).

Because DDGS is high in protein and fiber, using this ingredient in TMR reduces the alfalfa, soybean, and ground corn requirement in the diet formula. Considering the lower price of DDGS than these ingredients with no negative impact on milk production, DDGS reduces the cost of milk production and increases dairy farmers' revenue (Kelzer et al., 2009).

The methane (CH_4) emission in agriculture is a critical factor for environmental contamination. The dairy cattle have a 34% contribution to total agriculture practices (EPA, 2006; FAO, 2006). The partial substitution of TMR with DDGS would reduce CH_4 emission by dairy cows without reducing the nutritional intake and milk production or impacting the milk composition (Benchaar et al., 2013). The DDGS contribution in the feedstuff composition for dairy and beef cattle is 20%, while swine and poultry are 10% and 5%, respectively (Lemenager et al., 2006).

5.2 | DDGS in sheep and goat diets

Usage of DDGS in TMR of sheep is less widespread than other livestock animal farms (Şahin et al., 2013). Proteins are the most expensive nutrient for animal feeding, which are highly concentrated in DDGS, so usage of this material in sheep feeding is highly economical (Pezzanite et al., 2010). Maximum 20% DDGS inclusion in TMR for lambs feeding is recommended without impacting feed intake, weight gain, and rumen fluid composition (Şahin et al., 2013).

No negative impact on rumen fermentation was found using DDGS, up to 100% replacement, in the composition of sheep feeds (Pecka-Kielb et al., 2017). Replacing soybean meal and part of corn by DDGS in the lambs feeding was found to have no negative impact on acidosis and the carcass' quality after slaughter (Huls et al., 2006). Sunflower meal replacement with wheat DDGS in the feed mixture of dairy sheep caused no significant difference in milk production, milk technological features, and lamb weight (Dimova et al., 2009). Adding DDGS to a goat's diet is possible without a negative impact on feed consumption, weight gain, and carcass attributes (Maynard, 2015).

5.3 | DDGS in swine diets

In all stages of development, DDGS may be used in the diets of pigs. DDGS is recommended at all stages of swine husbandry and pregnant pigs, with 30% incorporation of DDGS in their diets without any negative impact (Stein & Shurson, 2009). However, due to high polyunsaturated fatty acids in DDGS, it is sensitive to get peroxidized (Song & Shurson, 2013). The peroxidized lipids could impair the growth rate in finishing male pigs (Boler et al., 2012). It may also reduce vitamin E in blood serum and cause heart disease (AASV, 2009). The impact of DDGS inclusion in pregnant pigs was checked for possible growth failure and disease in their upcoming piglets. Even though DDGS may contain oxidized lipids, sulfur-containing amino acids available in this material prevent growth failure and heart disease triggered by peroxidized fat (Hanson et al., 2015). Toxin binder ingredients were recommended to be used in TMR, which contains DDGS with low mycotoxin contamination. This helped to improve weight gain and feed intake in pigs. The inclusion of the high protein DDG in the pig feed negatively impacted growth performance while enhancing the pork fat (Yang et al., 2020).

5.4 | DDGS in poultry diets

With the increase in the cost of soybean meal and yellow corn, using DDGS in poultry diet is the best strategy to reduce the cost of feeding (El-Hack et al., 2019), which is a good source of xanthophylls, especially Lutein and Zeaxanthin (Sommerburg et al., 1998). Maximum 25% inclusion of DDGS in broiler feed mix ration was suggested by Waldroup et al. (1981). The use of DDGS in poultry feed improves the market acceptance of agricultural products like egg and broilers by improving yellow, red color in skin and egg (Leeson & Caston, 2004; Perez-Vendrell et al., 2001). The amount of DDGS in the poultry diet should be limited to 6% in starter (0–16 days) and 12%–15% in grower (17–31 days) and finisher (32–42 days) periods. The efficiency of weight gain was found to be higher in the chickens receiving more DDGS in the diet (Lumpkins et al., 2004). A maximum of 12% DDGS inclusion in hens' diet was recommended for having maximum egg weight and yellow color in egg yolk (El-Hack et al., 2019).

5.5 | DDGS in fish diets

To reduce the production cost of gilthead seabream (Sparus aurata) juveniles, soybean meal (SBM) in the TMR was substituted with DDGS up to 100% replacement, equivalent to 35% contribution in the diet. Incorporation of dietary DDGS had no effect on growth performance, voluntary feed consumption, feed efficiency, protein, or energy retention. Furthermore, adding DDGS to the fish diet reduced the cost of feeding per kilogram of fish produced by 5.7% (Diógenes et al., 2019).

Channel catfish and tilapia diet can be fed using 20%–40% DDGS in the diet, while in trout fish diet maximum of 15% DDGS incorporation was recommended (Lim & Yildirim-Aksoy, 2008). However, due to the lysine deficiency, more DDGS inclusion (up to 40%) is recommended if it is supplemented with lysine (Lim et al., 2007). Yeast available in DDGS can empower the immune system in fish, making them resistant to diseases (Lim et al., 2011). DDGS added up to 20%–40% in the feeding diet of channel catfish (Ictalurus pinctatus), significantly improved the fish's immune system by increasing immunoglobulin in the blood (Lim et al., 2009). Xanthophyll present in DDGS can also improve yellow color in fish (Lim et al., 2011). The growth performance of the channel catfish fed with diet treatments containing different distillers coproducts was investigated with the objective to examine which ingredient is responsible for the improvement of growth performance. The diet containing distillers solubles (DS), endosperm distillers solubles (EDS), and DDGS had higher performance than regular plant diet, and high protein distillers dried grain. The high-fat content in DDGS and yeast cells contributed from dried soluble might be responsible for growth improvement in channel catfish fed with DDGS, DS, and EDS (Li et al., 2011). The DDGS inclusion in the fish feed ingredients was also investigated related to body quality. It was found that DDGS inclusion increased the body lipids and decreased the moisture compared to control treatments (Lim et al., 2009).

5.6 | DDGS in human food

To increase the profitability of ethanol manufacturing plants, the use of the coproducts, including DDGS, should be extended in human food (Rosentrater & Krishnan, 2006). The use of DDGS in human diets is beneficial, considering global food demand that is expected to rise by 60% by 2050 (Alexandratos & Bruinsma, 2012). DDGS is regarded as a functional food because of its high protein and fiber content which raises interest in using DDGS in different human food recipes (Singha, 2017; Singha et al., 2018). Few uses of DDGS in food products are summarized in Table 2. The DDGS inclusion in food is beneficial for people with health problems like diabetes and celiac disease (Saunders et al., 2013). A lower glycemic index was reported in pita bread incorporated with DDGS and chickpea, individually or in a mixture compared to the recipe containing 100% wheat flour (Alrayyes, 2018). The DDGS fiber can be converted to xylo oligosaccharides (XOS) and then be used to produce functional foods because of its health benefits as a prebiotic (Srinivasan & Samala, 2012). Auto-hydrolysis is a method to produce XOS in which water is added to fiber and cooked to make XOS (Samala et al., 2012). The production efficiency of XOS is related to the time and temperature; the higher the temperature, the better performance of XOS production was found at the same time (Samala et al., 2015). The XOS production from fiber separated from DDGS was investigated in different thermal conditions. It was indicated that maximum XOS production efficiency could happen at 180℃, while hydroxymethylfurfural (HMF) was generated at a higher temperature, which is a process contaminant (Srinivasan & Samala, 2012).

Incorporating DDGS in bread, snacks, and confectionery products can improve nutritional values and provide additional health benefits like controlling glycemic impact (Liu et al., 2011). Different percentages of DDGS were incorporated in the cornbread recipe; the results showed that a maximum of 20%–25% DDGS could be added without deterioration in the texture of the bread or rheology of dough (Liu et al., 2011). Cornbread with DDGS had higher volume in the loaf and softer texture than the control sample. At the same time, the color was a little darker, and interestingly it improved the quality during storage (Liu et al., 2011).

Several steps were suggested to improve consumer acceptance of DDGS incorporated products, including deodorizing, bleaching, and milling (Saunders et al., 2013).

TABLE 2 Inclusion of corn distillers grain in different foods

Food product	Main ingredients	Substitution level (%)	Comments	Reference
Sugar cookies	Distillers dried grains flour (DDGF)	15, 25	Darker color and reduced width and thickness,15% inclusion in the recipe is recommended	Tsen et al. (1982)
Blended foods corn-soy-milk	Corn protein concentrate (CPC), Corn distillers grain (CDG), Corn distillers grains with solubles	2.5, 5, 10	CDG (2.5%) was recommended for this blend, while CDGS and CPC usage were rejected. Source of raw material and processing condition influence the flavor of the coproducts	Bookwalter et al. (1984)
Spaghetti	Corn distillers grains (CDG) extracted with hexane-ethanol azeotrope	5, 10, 15	Max 10% CDG addition recommended; More protein, fat, fiber, and amino acid compared to conventional spaghetti	Wu et al. (1987)
Pita bread	Food grade distillers dried grain (FDDG)	Max 15	Increased amino acid content, protein and fat content	Krishnan (2016)
Extruded snacks	Food grade distillers dried grain with solubles (FDDGS)	Max 20 DGS	A direct relation between viscosity and DDGS inclusion rate	Singha et al. (2018)
Pita bread	Food grade distillers dried grains (FDDGs)+Chickpea	10, 20	Positive impact on amino acids and dietary fiber without significant impact on taste; increased shelf life. The higher force required to tear the bread	Alrayyes (2018)
High energy biscuit (HEB)	Food grade distillers dried grains (FDDGs)+Chickpea	25, 50	Increased protein and dietary fiber and had acceptable sensory quality	Alrayyes (2018)
Chinese steam bread (CSB)	Food grade distillers dried grain (FDDG)	0–25	Gluten strength reduced, Bread hardness increased, weaken bread texture and more dense bread with lower volume; Maximum 15% addition recommended without any negative impact	Li et al. (2020)

TABLE 3 Chemical and physical pretreatment on DDGS used as fermentation feedstock

Method	Treatment	Result	Reference
Chemical	Soaking in aqueous ammonia and subsequent enzymatic/ Dilute acid hydrolysis	DDGS pretreatment by 15% w/w NH_4OH solution at a solid/liquid ratio of 1:10 and subsequent enzymatic hydrolysis at 60°C and 24 hr showed the highest glucose yield (91%)	Nghiem et al. (2016)
	Ammonia fiber expansion (AFEX)	AFEX pretreatment led to 190 g glucose/kg dry biomass or virtually complete conversion of cellulose after 72 hr	Bals et al. (2006)
	Dilute acid	Dilute acid (1% w/w sulfuric acid) treated DDGS resulted in 101.9% glucose yields for enzymatic hydrolysis and led to 15.1% increase in ethanol production with 86.5% increase in cellulose conversion	Li et al. (2019)
Physical	Ultrasound	Anaerobic digestion of DDGS for methane production; ultrasound reduced the particle size of DDGS by 45%	Wu-Haan et al (2010)
	Steam explosion	Very low reducing sugars were yielded from DDGS pretreated with steam explosion compared to acid treatment. Total reducing sugar from steam explosion treatments ranged from 0.016 g/g (g TRS/g DDGS, db.) treated at 120℃ for 5 min to 0.055 g/g treated at 180℃ for 15 min	Iram et al. (2019)
	Electrolyzed water	23.25 g of monosaccharides was released per 100 g of DDGS by using electrolyzed water for pretreatment of DDGS	Wang et al. (2013)

5.7 | DDGS as a fermentation feedstock

To valorize DDGS, recent studies have focused on developing new application areas for DDGS, such as fermentation in which DDGS can be used. As DDGS is a good source of carbon and nitrogen, both required for microorganism metabolism, it can be used as a fermentation raw material. However, lignin in DDGS has a negative impact on the fermentation process; therefore, pretreatment steps are required to use this ingredient as fermentation feedstock (Iram et al., 2020). Microbial fermentation can increase DDGS profitability by converting it to a more value-added product. Being rich in protein (26%–33.3% on a dry basis), fiber (16% cellulose, 13.5% hemicellulose), fat (9.1%–14.1%), starch (5.2%), and vitamins, DDGS is able to provide essential nutrients such as carbon, nitrogen, and other micronutrients for microbial fermentation to produce ethanol or butanol (Iram et al., 2020; Wang et al., 2009). The pretreatment of DDGS as fermentation feedstock is necessary because microbes such as *clostridia* cannot efficiently hydrolyze and utilize fiber content in the agricultural residue. Lignocellulosic containing lignin is a challenge in the fermentation of DDGS as lignin produces microbial inhibitors. Several mechanical, chemical, physical, and biological pretreatments are required to break down the fiber (lignin barrier) into fermentable and simple sugars such as glucose, arabinose, mannose, xylose, and other carbohydrate monomers.

Physical technologies (liquid hot water and steam pretreatment) have been used for DDGS pretreatment prior to enzymatic hydrolysis using proteases, cellulases, and xylanases. Mechanical pretreatments include extrusion, grinding, sieving, and elutriation. Grinding helps to decrease DDGS particle size (0.11–3.66 mm) and to get even distribution of nutrition in DDGS. Although extrusion pretreatment is applied to DDGS, it mostly improves DDGS quality as an animal feed. Extrusion and other mechanical pretreatments need to be more investigated for their impact on microbial fermentation of DDGS (Iram et al., 2020). Chemical methods include acids such as sulfuric acid, alkaline such as ammonia, or other chemicals. Recent physical and chemical pretreatments on DDGS for fermentation are shown in Table 3. For a better result, chemical methods are combined with physical pretreatments. For example, a technique called "ammonia fiber explosion" using liquid ammonia and high pressure could efficiently decrystallize and hydrolyze the fiber (cellulose and hemicellulose) (Iram et al., 2020). Table 3 shows that chemical pretreatment of DDGS led to more carbohydrate yields than physical pretreatments. Among chemical approaches, acid hydrolysis is a common and efficient pretreatment method. However, several parameters, including the concentration of acid, ratio of DDGS-to-liquid, temperature, pressure, and time should be optimized for better hydrolysis (Iram et al., 2020). Scanning electron microscopic (*SEM*) images showed that pretreatments with alkaline electrolyzed water and acid sulfuric led to large cracks on the fiber matrix and disruption of the DDGS crystalline structure (Wang et al., 2009). Around 23.5 g/L and 22 g/L glucose were produced when DDGS was hydrolyzed with 0.25% acid sulfuric and electrolyzed water (40% solids loading) pretreatments, respectively (Wang et al., 2009). Acid hydrolysis of DDGS using sulfuric acid (H_2SO_4; 3.3% w/w) at 140°C for 20 min led to 13 g of glucose yield/100 g of dry input feedstock. A further 16 g/L of monosaccharides was achieved when DDGS was treated with the same acid hydrolysis at 130°C combined with liquid hot water treatment (Chen & Liu, 2015; Tucker et al., 2004). Total sugar and glucose yield of pretreated DDGS with 0.5% sulfuric acid at 1 mg/g of Viscozyme L was significantly higher than other pretreatment methods such as liquid hot water, 1%–4% ammonium hydroxide. Pretreatment of DDGS with 0.5% sulfuric acid at 1 mg/g of Viscozyme L resulted in 17.4 mg/ml glucose and 33.22 mg/ml total sugar; however, these concentrations were 15.88 mg/ml and 22.38 mg/ml for liquid hot water, 11.24 mg/ml and 16.15 mg/ml for 4% ammonium hydroxide, and 0.17 mg/ml and 14 mg/ml for 1% ammonium hydroxide at the same enzyme dosage.

Although the acidic pretreatment and higher solids loading made significantly higher glucose levels, the *Clostridium acetobutylicum* cell growth was sensitive to acidic conditions (H_2SO_4 0.25% v/v) and high-solids loading. There might be more inhibitory products in pretreatments with higher solids that negatively affect cell growth. The DDGS hydrolysate obtained from alkaline electrolyzed water pretreatment at 30% (w/w) solids was the most favorable to Acetone-Butanol-Ethanol (ABE) production (Wang et al., 2009). DDGS pretreatments by liquid hot water and ammonia fiber expansion increased enzymatic digestibility of distiller's grain, resulting in more than 90% glucose yield with 15 FPU cellulase and 40 IU β-glucosidase per gram of total glucan within 24 (Kim, Mosier, et al., 2008).

Microbial fermentation of DDGS results in diversifying DDGS markets and produces organic acids (succinic acid, fumaric acid, and lactic acid), acetone, methane, hydrogen, and many other value-added products (Iram et al., 2020). Organic acids are the versatile precursor for many necessary industrial chemicals such as 1,4-butanediol (BDO), N-methyl pyrrolidinone (NMP), butyrolactone (GBL), tetrahydrofuran (THF), and 2-pyrrolidinone (2P), and noncorrosive deicers (Nghiem et al., 2016). The inhibitory compounds such as salts, phenolics, acids such as acetic, ferulic, glucuronic, and ρ-coumaric acids are mainly produced during pretreatment and hydrolysis of fiber-rich agricultural biomass such as DDGS. These inhibitors negatively impact the conversion of DDGS to ABE by damaging the hydrophobic sites of the bacterial cells, inhibiting cell growth, sugar utilization, and fermentation (Ezeji et al., 2007). Although severe pretreatment condition such as high temperature and acid concentration will result in more glucose and other monosaccharides, the severe degradation and hydrolysis pretreatments of DDGS may result in inhibiting further microbial growth by producing furfural, hydroxymethylfurfural (HMF), syringaldehyde, glucuronic acid, p-coumaric acid, ferulic acid, and other phenolic compounds (Duwe et al., 2017).

The inhibitory effect of syringaldehyde, ferulic and p-coumaric acids on solventogenic *clostridia*'s ability to ferment DDGS hydrolysates was more evident than furfural and HMF. Interestingly, furfural and HMF stimulated solventogenic *clostridia*'s growth at their concentrations in the range of 0.5–2.0 g/L (Ezeji & Blaschek, 2008).

6 | CHALLENGES

The distillers' grains contain high moisture content that needs to be reduced before transportation and storage to increase shelf life, transportation efficiency and reduce transportation costs over longer distances. The US ethanol plants produce a considerable quantity of DDGS (on average, nearly 90,000 tons per week), a large portion of which is exported, and a part is sold domestically. The DDGS quality is related to the variety of the grain, process efficiency, and the thermal processing used at the drying stage (Lim et al., 2011). There is a relationship between the color of DDGS samples and amino acid availability. When the darkness rate reaches a specific limit, the nutritional value shows a drastic fall in a way that less amino acids and energy availability were found in DDGS; this was related to the Maillard reaction in the DDGS that makes lysine unavailable by binding it to carbohydrates due to intensive heating (Fastinger et al., 2006).

Variation in the nutritional value of DDGS is another challenge. The level of digestible nutrients in DDGS varies based on the process used in ethanol production. The proportion of the CDS fraction added to the grain fraction before drying differs substantially in the dry-grind ethanol industry affecting the protein, fat, and phosphorus content of DDGS. Therefore, DDGS produced by multiple processing plants varies in nutrient content, digestibility, and physical characteristics such as particle size. Feed industries prefer to purchase and use ingredients that do not differ substantially in nutrient content, particle size, and quality; livestock and poultry diets should contain the desired level of nutrients to support optimal animal performance for their customers. The particle size should not be very small or large to avoid less flowability through storage bins and feeders and ingredient segregation in complete feeds, respectively (Shurson, 2005).

Customers' expectation has not always been met because there is a lack of a quality grading system and sourcing for DDGS; which can also happen because of non-standardized testing procedures to determine the nutrient content of DDGS. Identifying a standardized testing procedure is necessary to reduce variation in analytical results among laboratories using these procedures for distiller's coproducts (Shurson, 2005). Product quality predictability and consistency are essential for the feed nutritionists to minimize the risk of not meeting desired nutrient levels in complete manufactured feeds (Shurson, 2005). One of the nutrients having high variability in DDGS is the amino acid. This nutrient variability makes it difficult to develop an appropriate formula to feed animals. A procedure was created to help nutritionists prepare efficient

TABLE 4 The occurrence of mycotoxins in distiller's dried grains with solubles (DDGS)

Region	Mycotoxins	Analysis technique	Notes	Reference
Canada	Deoxynivalenol	LC/MS/MS and ELISA	Significantly higher concentration of DON in the DDGS than corn grain	Schaafsma et al. (2009)
United States, North and South-east Asia and Oceania	Aflatoxins, Zearalenone, Deoxynivalenol, Fumonisins B1 and B2, Ochratoxin	HPLC and ELISA	DON, ZEA and FUM were the main mycotoxins in DDGS	Rodrigues and Naehrer (2012)
Mid-west United States	Aflatoxin, Deoxynivalenol, Fumonisins, T−2 toxins, Zearalenone	Fluorescence detector, UV detector, TLC	DON was more than the acceptable limit in 12% of the samples. FUM more than FDA limit in 6% samples, T−2 toxin in all the samples were below FDA limit	Zhang & Caupert (2012)
Swine farms in central areas in Thailand	Fumonosin B1, Deoxynivalenol, Zearalenone, Beauvericin	LC-MS/MS and ELISA	FB1 and FB2 and BEA were found in 60% of the samples, followed by ZEN and DON with 49% and 30%, respectively	Tansakul et al. (2013)
China (DDGS as feed ingredient)	Aflatoxin B1, Deoxynivalenol, Zearalenone, Ochratoxin A	HPLC in combination with UV or Fluorescence Detection and ELISA	DON and ZEA were the most prevalent mycotoxins found in 100% samples with an average 1.36 ppm and 882.7 ppb, respectively	Li et al. (2014)
Imported DDGS from the United States into Saudi Arabia	Aflatoxins, Deoxynivalenol, Fumonisins, Zearalenone	HPLC	ZEA was the most predominant mycotoxin (average of 167.6 μg/kg); DON was found in 28.7% of samples (average of 3.0 mg/kg); FUM found in 25.3% of samples (an average of 1.01 mg/kg), aflatoxins found in 14.0% of samples with an average of 6.3 μg/kg	Abudabos et al. (2017)

feeding formulas for poultries that identify standardized ileal digestible content (SIDC), used as an ingredient in poultry feed. Use of this formula increases the accuracy of estimation while it reduces the cost and speed compared to the current practices. DDGS with higher amino acid content was found to have a higher concentration of SIDC (Zhu et al., 2018). Digestibility and SID of lysine are representative of DDGS nutritional quality. The level of reactive lysine is directly related to its digestibility in the intestine. The procedure called homoarginine was developed to measure reactive lysine. Another method to measure reactive lysine is a furosine procedure that measures uncreative lysine and then reduces it from total lysine (Pahm et al., 2008).

Expanding and developing markets for DDGS will strengthen the economic sustainability of dry-grind ethanol plants. However, the challenges of high concentrations of fiber causing limited use of the coproducts mainly to ruminant diets, high levels of phosphorus because of waste disposal issues, variability in composition causing inaccurate diet formulation, and high cost of water removal need to be addressed and solved (Rausch & Belyea, 2006). Also, the relatively high level of crude fat and unsaturated fatty acids in conventional corn DDGS is another concern that limits DDGS use in lactating dairy cow diets because of its negative impact on milk fat content. However, the bioethanol process advancement has led to reduced oil DDGS, which lowers the risk of reduced milk fat (Ramirez-Ramirez et al., 2016). The oil extracted from DDGS is not suitable for food and feed but usable for biodiesel (Mohammadi Shad et al., 2020).

The safety of DDGS is crucial because not only it affects animal health and productivity, but it also has a significant impact on the safety of animal-derived food products and human health. For this reason, some of the US ethanol plants are implementing safety regulations to comply with the Food Safety Modernization Act (FSMA), Good Manufacturing Practices (GMP), and Feed Certification to provide safe coproducts such as DDGS for food and feed markets. There is a negligible risk of *E. coli* O157:H7 or Salmonella shedding affected by DDGS content in animal's diet (Jacob et al., 2009, 2010). However, DDGS is reported as one of the most high-risk materials regarding mycotoxins contamination (Zhang and Caupert, 2012). More than 300 types of mycotoxins have been identified in food and feed (Oplatowska-Stachowiak et al., 2015). Major mycotoxin contaminants are listed as aflatoxins, deoxynivalenols (DON), fumonisins, zearalenone (ZEA), T-2 toxins, ochratoxins (OTA), and ergot toxins. Mycotoxin contamination may happen in the field before harvest or during storage due to poor storage management. To avoid mycotoxin contamination after harvest, grains should be intact and stored below 14% moisture content (Richard, 2007). One-third of the volume of the grains used in bioethanol production is converted to DDGS; this may lead to concentrating the quantity of mycotoxin available in the grains to the value of three times in DDGS (Zachariasova et al., 2014). Considering that mycotoxins can lead to chronic diseases and mortality in animals, these contaminants in DDGS are a critical safety issue.

Furthermore, mycotoxin contamination in feed can lead to food contamination produced by animals. Considering that mycotoxins are resistant to thermal processing, a finished product made by these products will be a risk for human health (Mohammadi Shad et al., 2019). As shown in Table 4, various types of mycotoxin have been reported in DDGS worldwide. Mycotoxin assessment on imported DDGS in Saudi Arabia using the HPLC method showed that ZEA was the most prevalent mycotoxin found in 34% of the samples. The second most detected mycotoxin was DON, found in one-fourth of the samples. On the contrary, OTA and aflatoxins were the least common mycotoxins detected in DDGS (Abudabos et al., 2017). In another study, the ultra-high performance liquid chromatography–tandem mass-spectrometry (UHPLC–MS/MS) method was used to measure mycotoxin contamination in DDGS samples present in Europe from different resources such as wheat, maize, barley, and mixed grains. Mycotoxin found in these samples varied depending on the source of the grains. The high quantity and diverse types of mycotoxins were found in the mixed samples. This issue implied the concern regarding the intensified impact of mixed mycotoxin on animal health and suggested that multigrain DDGS usage for animal feed should be avoided (Oplatowska-Stachowiak et al., 2015). Mycotoxin contamination in different DDGS samples collected from other ethanol plants in the Midwest United States and cargoes exported before and after shipping to the receiving country were investigated. Mycotoxin contamination was found in all the samples. The mycotoxin level in samples complied with FDA regulation for animal feed except for fumonisin and DON, which were marginally above the regulation in 10% of samples which could become below the limit because DDGS is usually mixed with other ingredients to feed animals (Zhang et al., 2009).

Biofuel processing steps have been shown to influence mycotoxin levels. DON and DON-3-glucoside (DON-3-Glc) level during different stages before fermentation (liquefaction and saccharification) and initial phase of fermentation increased markedly, followed by a reduction during the second step of fermentation. The mycotoxin reduction during fermentation might be related to adsorption to the yeast body and biochemical reaction (Dzuman et al., 2016). Also, Trichothecene 3-O-acetyltransferases enzyme can convert DON to a 1.5 times less toxigenic derivative, named triacetyldeoxylevalenol. This enzyme is exerted by yeast during the fermentation stage in the biofuel ethanol production process from barley (Khatibi et al., 2011). However, fumonisin B1 (FB1) showed an increasing trend during the process's initial steps and continued to increase in the second fermentation phase. This FB1 increase might be related to the enzyme produced by yeast, which is responsible for detaching FB1 from protein and starch content in maize. During the next steps in which the CDS and WDG mixture are dried at high temperatures (500°C) in rotary drum dryers, the mycotoxin concentration was drastically decreased. The FB1 was an exception in which its concentration multiplied by two times (Dzuman et al., 2016).

7 | EMERGING TECHNOLOGIES FOR DDGS UTILIZATION

The modification in the process and usage of DDGS will result in a rapid return on investment and increased ethanol plant revenue from producing and marketing a safe and high nutritious coproduct, an excellent and economical energy source.

Several process modifications such as QG, QGQF, and enzymatic milling (E-Mill) have been developed in dry-grind ethanol production to increase the amount of corn processed, increase the capacity of ethanol production, and improve the quality, digestibility, and protein content of DDGS (Wahjudi et al., 2000). These modifications help recover high-quality germ, pericarp fiber, and endosperm fiber at the beginning of the dry-grind corn process (Figure 4).

The byproducts and coproducts from the modified processes can reduce the cost of ethanol production. After soaking (6–12 hr) and grinding of corn kernels, the mixture is incubated with protease (increases the rate of fermentation by hydrolyzing protein into free amino nitrogen) and starch degrading enzyme (amylase) for 2–4 hr; this helps to recover germ and pericarp in the following steps (Singh et al., 2005). Then, a mesh sieve system is used to recover endosperm fiber. Next, the slurry goes through the conventional processing step to generate ethanol and other coproducts such as DDGS.

The recovery of germ, pericarp fiber, and endosperm at the beginning helps to increase fermentation capacity by preventing these byproducts from entering the fermentation process. Simultaneously, the pre-recovery of these fractions increases the fermentable substrate input to the process with a higher rate of fermentation due to better mixing of mash and more rapid heat transfer (Singh et al., 2005; Taylor et al., 2001). The resultant DDGS has lower fiber and higher protein content than DDGS obtained from the conventional dry-grind corn process (Table 5), a better feed source for non-ruminant animals (Mohammadi Shad et al., 2020; Singh et al., 2005). Besides, this fiber can be used as a raw material to be used for ethanol production (Srinivasan et al., 2007).

In a new development in the ethanol production process, heat treatment is replaced by enzyme treatment, and germ and bran and endosperm are separated before the fermentation phase. This practice improves these coproducts' quality because of the less thermal processing (Kelzer et al., 2009). The addition of B-vitamin and germ soak water enhanced the fermentation process and increased ethanol production, which requires saccharification and fermentation to be conducted simultaneously (Murthy et al., 2006).

Another improvement in DDGS quality, incorporation levels of DDGS, and industry's profit is using hydrolytic enzymes during the process. The application of these enzymes modifies non-starch polysaccharides to feed animals more favorably and produces DDGS with reduced oil content. The 4% oil in corn is concentrated to 14% in DDGS; this high oil in DDGS is undesirable for some animals to feed. For example, milk production in cattle and the bacon texture in DDGS-fed swine can be negatively affected (Luangthongkam et al., 2015).

The use of fiber cellulase enzymes was also suggested to reduce the fiber content of DDGS and convert this component to sugar monomers and increase alcohol production efficiency regarding volume and cost of production. The addition rate of cellulase enzyme is an essential factor to control the ethanol production yield. During saccharification and fermentation, the addition of 30 FPU/g fiber cellulase showed just below a 20% increase in ethanol production. In contrast, excessive addition at the rate of 120 FPU/g caused a reduction of ethanol production than the control experiment (Kurambhatti et al., 2018).

An additional new approach adopted by bioethanol plants is called the "cold-cook" process, which uses granular starch hydrolyzing enzymes (GSHE), resulting in more ethanol concentration. The use of GSH enzymes in the cold-cook process produces lower concentrations of glucose and glycerol (47% reduction), preventing osmotic stress to yeast compared to the conventional dry-grind process (Kumar et al., 2020).

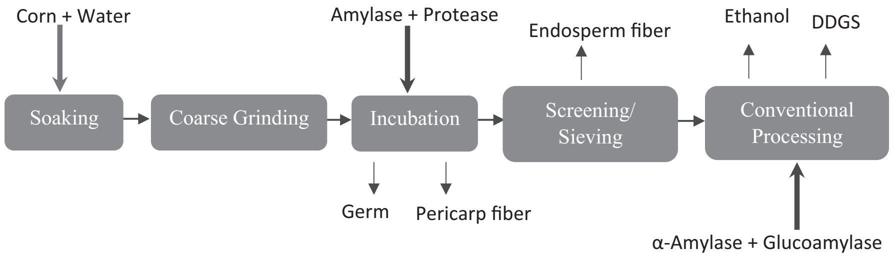


FIGURE 4 Schematic diagram of modified corn dry-grind ethanol Production (enzymatic milling [E-Mill])

TABLE 5 The differences in DDGS composition and other process features between conventional and the modified milling processes

	CP[a]	QG[a]	QGQF[a]	EM[a]
Crude protein (%)	28.50	35.91	49.31	58.50
Crude fat (%)	12.70	4.83	3.85	4.53
Ash (%)	3.61	4.05	4.13	3.24
Acid detergent fiber (%)	10.8	8.22	6.80	2.03
Rate of fermentation (g/L/h)	1.41	1.73	2.9	3.8
Ethanol yield (L/Kg)	0.34	0.32	0.32	0.33
Coproduct yields				
Germ (%)		6.1	7.7	8.1
Pericarp fiber (%)		-	9.1	10.2
Endosperm fiber (%)		-	-	4.6

[a] Conventional processing (CP), quick germ (QG), quick germ and quick fiber (QGQF), enzymatic milling (EM).

The GSHE include α-amylases, GAs, α-glucosidases, isoamylases, β-amylases, and maltogenic β-amylases that synergistically hydrolyze raw granular starch without requiring it to be liquefied or gelatinized. Lowering glycerol production, a byproduct, during fermentation can result in the utilization of sugars primarily for ethanol production and higher ethanol yield. Therefore, the cold-cook process increases process efficiency and ethanol yield in conventional dry-grind (Kumar et al., 2020; Sharma et al., 2016).

In a study by Kumar et al., 2020, GSH enzymes and advanced yeast strain could decrease glycerol production and improve ethanol yield. Therefore, the resultant DDGS had lower residual starch content (about 15% at an enzyme dosage of 4.8 kg/MT grains and higher). However, DDGS contained more residual glucose and maltose at higher fermentation temperature (36°C) than no residual glucose and maltose at 32°C (Kumar et al., 2020).

8 | CONCLUSION AND PERSPECTIVES

The increasing trend of biofuel production from corn ethanol leads to increased quantities of coproducts from corn. The DDGS is the main coproduct produced in bioethanol plants, mainly by the dry-grind process, which has been used as a low-value animal feed. The producers need to improve the marketability and practical uses of DDGS to have a viable economy of the ethanol plant operation. In this review, several topics, including procedures of ethanol production, DDGS properties, mycotoxin occurrence, nutritional value, and DDGS as feed and food, are covered. The challenges and limitations on the production and use of DDGS are discussed as well. The high nutritional value of DDGS makes it a good source for animal and human food, although the nutritional value varies due to the raw material, processes, etc. This review provides critical information on bioethanol plants' challenges and measures to avoid mycotoxin contamination, emphasizing that the source needs to be monitored and controlled for compliance. Besides, the development of prevention strategies and detoxification techniques are necessitated to lower the mycotoxin level. As grain-based ethanol production will continue to grow, the resulting DDGS is still a primary coproduct as a global commodity, emphasizing the need to improve and further incorporate DDGS in animal feed and human food.

As DDGS is a good source of nutrients with a lower price than corn, there has been researched underway to increase DDGS use in animal feed and human food. However, more future work is needed to develop a solidate plan to standardize the nutritional quality of DDGS. The production of high protein DDGS needs to be expanded to satisfy the future market in the international market. Also, the safety-associated risk with DDGS needs to be evaluated more regularly to control contaminants' levels, particularly mycotoxins. The versatile uses of DDGS play an essential role in ethanol industry profits by adding another revenue source for ethanol plants. However, the producers will need to ensure high safety and quality DDGS to the global market.

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How to cite this article: Mohammadi Shad Z, Venkitasamy C, Wen Z. Corn distillers dried grains with solubles: Production, properties, and potential uses. *Cereal Chem*. 2021;98:999–1019. https://doi.org/10.1002/cche.10445