



# Effect of co-products of enzyme-assisted aqueous extraction of soybeans, enzymes, and surfactant on oil recovery from integrated corn-soy fermentation



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## ARTICLE INFO

### Keywords:

Enzyme-assisted aqueous extraction of soybeans  
Bioethanol  
Corn fermentation  
Oil recovery  
Enzymes  
Surfactant

## ABSTRACT

Integrated corn-soy fermentation, utilizing co-products of soybean enzyme-assisted aqueous extraction process (EAEP) of soybeans in corn fermentation, has shown potential for enhancing bioethanol production compared to corn only fermentation. To maximize economic returns, oil may be recovered. In the present study, the effect of skim and insoluble fiber, and oil extraction aids on ethanol yield, oil partition, and oil recovery, and quality of Distillers Dried Grains (DDG) was investigated. Two fiber hydrolyzing enzymes (pectinase and cellulase), an acid protease (Fermgen<sup>®</sup>), and a surfactant (Tween 80) were evaluated. Addition of skim, mixture of skim and insoluble fiber, or Fermgen<sup>®</sup> to corn fermentation resulted in a ~32 h decrease in fermentation time. Addition of soy co-products also resulted in ~10–28% increase in oil partition in thin stillage with no additional enzyme or surfactant treatment. Addition of insoluble fiber alone resulted in ~19% decrease in solids partition in thin stillage. Maximum free oil recovery, 22.5 ± 4.5%, was achieved from corn-insoluble fiber thin stillage with a combined treatment of enzymes (pectinase, cellulase, and Fermgen<sup>®</sup>) and surfactant (Tween 80). Maximum extractable oil recovery, 67 ± 3.2%, was achieved with the enzyme treatment alone. Corn-soy DDG has ~11% higher protein, ~2% lower fiber, and ~2% lower fat contents compared to corn DDG. The fiber content was further reduced to ~2% after enzyme treatment. This study demonstrates an efficient use of soy EAEP co-products and enzymes to maximize oil partition in thin stillage, and produce a high quality corn-soy DDG.

## 1. Introduction

Fuel ethanol consumption in the U.S. over the past ten years has increased by 64%, with over 15.3 billion gallons of bioethanol produced in 2016 (RFA, 2017; USDA/ERS, 2017). Corn accounts for major (~95%) bioethanol production, mostly (~90%) by dry grind process (RFA, 2017). The market for the U.S. bioethanol and its co-products is estimated at \$23,333 million and \$7429 million, respectively. On average, from 24.5 kg (1 bushel) of corn processed by dry grind ethanol biorefinery, 10.7 L (2.85 gallons) of ethanol, 7.5 kg of Distillers Dried Grains (DDG), 0.29 kg of Corn Distillers Oil (CDO) and 7.7 kg of biogenic carbon dioxide are produced (RFA, 2017).

Uncertainty in crude oil production and oil prices due to fluctuation in domestic oil production and geopolitical risks demand higher production of fuel from renewable sources (RFA, 2017; Saefong and Alessi,

2017). This may be accommodated by using alternate substrates and fermentation aids. An integrated corn-soy biorefinery, which incorporates co-products of the enzyme-assisted aqueous extraction process (EAEP) of soybeans in the dry grind corn fermentation has shown potential with significant increase in ethanol yield (20%) and decrease in fermentation time (44 h) compared to corn only fermentation (Sekhon et al., 2015; Yao et al., 2011).

EAEP is an environmentally friendly alternative to conventional oil extraction methods (d Moura et al., 2011; Jung et al., 2009; Li et al., 2013). While mechanical extraction results in low oil recovery and denaturation of proteins, chemical extraction method uses hexane, an environmental hazard. EAEP can achieve high oil recovery (~97%) by using a protease, and simultaneously extract oil and protein using water as the extracting medium (d Moura et al., 2011; Campbell et al., 2011). EAEP of soybeans entails mechanical pretreatment, enzyme-assisted

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<https://doi.org/10.1016/j.indcrop.2018.05.033>

Received 21 December 2017; Received in revised form 5 May 2018; Accepted 15 May 2018

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aqueous extraction, separation of cream and co-products, and demulsification of the cream fraction to recover free oil (d Moura et al., 2011). The process offers safer operation, less initial capital investment, equivalent oil recovery (~97%), and production of a variety of products. However, considerable amounts of co-products, skim (protein-rich watery fraction) and insoluble fiber (carbohydrate-rich semi-solid fraction), are produced in large quantity in the process. Skim (57.6% db protein; 6.3% db oil) and insoluble fiber (7.7% db protein; 4.7% db oil) may be utilized in drygrind corn fermentation to maximize economic returns by enhancing ethanol production along with high value co-products. Though many studies have evaluated enzyme-assisted aqueous extraction of oil from oilseeds (Yusoff et al., 2015; Petts et al., 2017; Liu et al., 2016; Teixeira et al., 2013; Do and Sabatini, 2010; Tabatabaei and Diosady, 2013), no study till date has explored utilization of co-products from such methods.

Two high-value products of bioethanol corn fermentation, along with ethanol, are CDO and DDGS, ~42 million metric tons/year, of which CDO accounts for 1.3 million tons/year (RFA, 2017). While oil from corn fermentation is used for biodiesel production and animal feed, DDGS is primarily used as animal feed source (Wiese, 2014). Therefore, higher oil recovery and DDGS with higher nutritive value are desired to increase the value of the corn bioethanol industry. The goal of this study was to efficiently use skim and insoluble fiber to enhance ethanol production, oil partition, and quality of DDG in corn fermentation.

In the dry grind ethanol production, oil can be extracted in the front-end (before fermentation) or at the back-end (after fermentation) (Cantrell and Winsness, 2012; Fang et al., 2015; Moreau et al., 2013; Reis et al., 2017). At the back-end of the process, first ethanol is separated from the fermented corn slurry by distillation (Fig. 1a). Then whole stillage left after distillation is centrifuged to separate out solid fraction, called wet cake, from the liquid fraction, called thin stillage. Thin stillage is further concentrated to produce thick stillage or Condensed Corn Distillers Solubles (CCDS). Oil can be recovered from whole stillage, thin stillage, or CCDS. Most often the oil is recovered from CCDS in two steps: heat treatment at 65–100 °C, preferably at 82 °C, followed by centrifugation (Cantrell and Winsness, 2012). This method results in only ~25% of the total oil recovery from thin stillage

(Johnston et al., 2014; Moreau et al., 2013). Furthermore, high temperature treatment may negatively affect protein quality of the DDGS (Moreau et al., 2013). Therefore, various oil extraction methods are being explored to enhance oil recovery from corn fermentation.

Oil in ground corn could be present as oil-in-water emulsion stabilized by proteins; oil droplets bound to hydrophobic protein and/or cell wall fractions; oil trapped in unbroken endosperm and germ particles; and free intact oil bodies released from broken cells (Majoni et al., 2011a; Wang et al., 2008). Different types of oil extraction aids (chemicals, enzymes, and surfactants) have been explored to maximize oil recovery. Although polar solvents (isopropanol, butanol, and ethanol) have been used to extract oil from aqueous CCDS phase, a mixture of polar and non-polar solvents (ethanol and hexane) have also been tested to remove hydrophobic zein protein responsible for strong oil emulsion (Majoni et al., 2011a). Proprietary polyol blends from sorbitol with surfactant properties have been evaluated alone or with hydrophobic silica (Shepperd et al., 2014; Lewis and Shepperd, 2016). Silica has a steric effect at the interface and aids in breaking the emulsion. Other proprietary blends have been used such as a homogenized mixture of ethoxylated C8-22 mono- and diglycerides, a liquid oil and a metal oxide mixture by Wiese (2016, 2017), and a mixture of polyethylene glycol (PEG) ester, silica, and potassium aluminum sulfate by Murphy and Fowle (2017). Cell wall hydrolyzing enzymes, acid protease alone or with surfactants have also been explored as a sustainable method of enhancing oil recovery (Majoni et al., 2011b; Johnston et al., 2014; Wang et al., 2010; Majoni et al., 2011b; Fang et al., 2018; Luangthongkam et al., 2015). Fiber hydrolyzing enzymes, when added during fermentation, break down cell wall polysaccharides releasing oil, and in the process decrease the viscosity of the fermentation slurry. On the other hand, a protease and/or a surfactant are used to destabilize oil-in-water emulsion, protease by breaking down the proteins surrounding the oil droplet and surfactants by displacing the same proteins. The purpose of this study was to investigate the effect of soy co-products, skim and insoluble fiber, enzymes, and/or surfactant on oil partition into and oil recovery from thin stillage, and on the quality of DDG. Specific objectives were:

1. To determine the effect of soy co-products, skim and insoluble fiber,

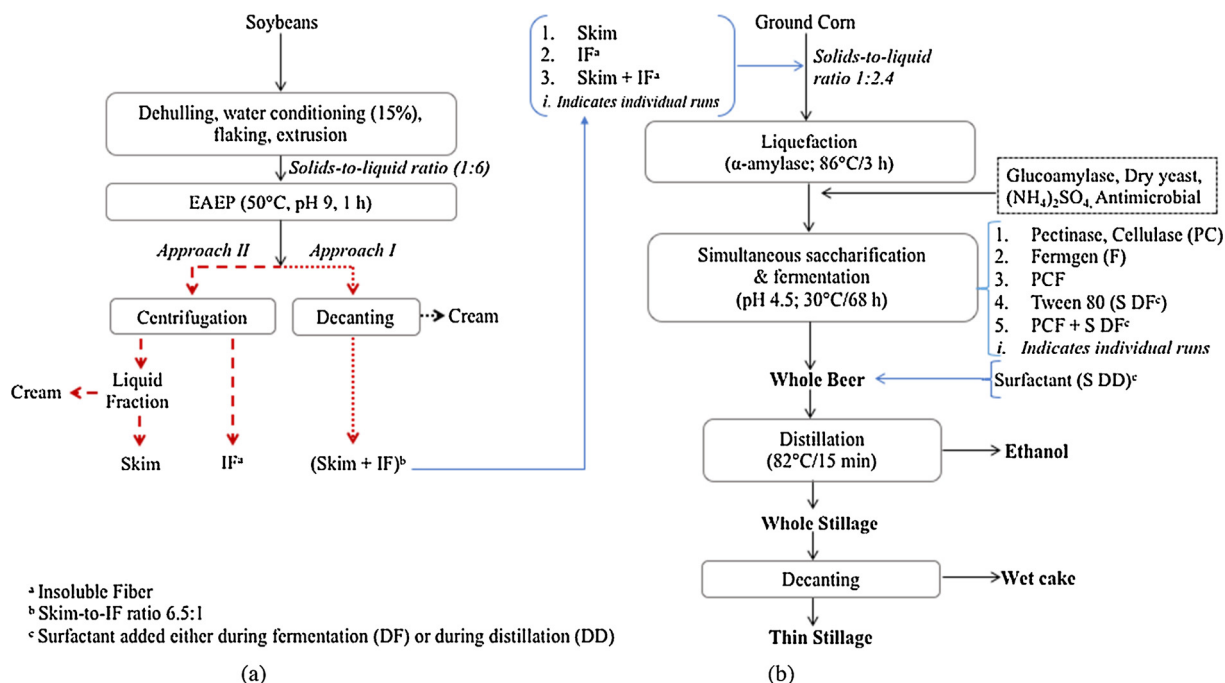


Fig. 1. Schematic flow diagram of the integrated corn-soy biorefinery (a) enzyme-assisted aqueous extraction process (EAEP) of soybeans, and (b) corn fermentation with the addition of soy co-products, enzymes, and surfactant.

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