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Physical and Chemical Processes to Enhance Oil Recovery from Condensed Corn Distillers Solubles

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Abstract Oil recovery from corn fermentation co-products can provide feedstock for biodiesel production. The effects of physical and chemical processes on oil recovery from condensed corn distillers solubles (CCDS) were investigated. Heating disrupted physical interactions in the CCDS and increased oil recovery by 2.5-fold when temperature was increased from 25 to 59 °C. Oil recovery at acidic pH conditions was significantly greater than at alkaline pH. Oil recovery at alkaline pH was increased by heating and addition of the reducing agent, sodium metabisulfite. Oil extraction using polar solvents isopropanol and butanol achieved greater than 80% oil recovery. When oil was co-extracted with zein using hexane and ethanol as co-solvents, the greatest total oil recovery was achieved, 89%. Churning CCDS at pH 3.5, 50 °C for 3 h achieved up to 80% oil recovery. This study provides data for designing further effective methods for oil separation from corn ethanol co-products.

Keywords Condensed corn distillers solubles (CCDS) · Heating · pH · Solvent extraction · Oil recovery

Introduction

The dry-grind corn fermentation process produces dried distillers grains with solubles (DDGS), which is a combination of the condensed corn distillers solubles (CCDS) and the wet distillers grains. About 23 million metric tons of DDGS were produced in 2008 as reported by the Renewable Fuels Association, and this can translate to about 2.76 million metric tons of oil. Assuming 70% of this oil can be recovered, then about 2.2 billion liters (547.6 million gallons) of biodiesel can be made. Oil in animal feed can be a positive energy source, but there are also problems with the high oil content in DDGS when used as feed ingredient [1]. Therefore, removal of the oil from DDGS or CCDS is expected to improve feed quality and present a significant source for biofuel production.

Several strategies have been explored to recover oil from the dry-grind ethanol co-products. Oil extraction by conventional solvent methods from DDGS is not feasible due to its relatively low oil content, however, oil recovery by centrifugation of the liquid is generally regarded as a viable method. Normally CCDS contains about 65% moisture, 14% protein, and 20% oil on a dry weight basis, and DDGS contains about 11% moisture, 30–31% protein, and 11–12% oil on a dry weight basis [1]. We have demonstrated that more oil can be partitioned in the liquid fraction by upstream processing [2, 3], however, the high oil content is always positively correlated with the total solids that partitioned to the liquid phase. It appears that the oil is strongly associated with the solids, and it has been challenging to completely remove the oil from the condensed liquid. We have conducted a study to examine enzymatic means to improve oil recovery, and this paper describes the physical and chemical means we have explored.

CCDS is composed of protein, lipid, fine fiber, and residual starch. It resembles syrup, and it is also referred to as thick stillage. It is a relatively stable emulsion. The oil may be present in four forms in CCDS based on our experience and observations: (1) oil-in-water emulsion that is stabilized by proteins and phospholipids (2) minute oil

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droplets that are bound to hydrophobic protein, such as zeins, and cell wall components (3) oil in intact oil bodies in the unbroken endosperm and germ particles and (4) oil in intact oil bodies released from broken cells. Oil recovery by centrifugation alone has not been satisfactory, and other treatments before centrifugal separation may be beneficial.

There have been considerable efforts in developing enzyme-based technologies for extracting oil from oilseeds, but the high cost of biocatalysts has slowed the technological acceptance in industry [4]. Enzyme-assisted aqueous extraction processes have been used to recover edible oil, eliminating the use of organic solvents and achieving oil recoveries ranging from 53 to 97% [5–8]. The use of enzymes for extracting oil from CCDS has been reported in our previous paper [9] and the enzymes showed some effectiveness when used in combination and with further particle size reduction, but such treatment with moderate benefit may not be practical.

In the present study, we intended to evaluate the use of physical and chemical processes, such as heating, pH changes, high-pressure and temperature, churning, polar solvent extraction, and co-extraction of oil and zein, for oil recovery from CCDS. Heating provides energy required to break emulsion and possibly weaken physical interactions between protein and lipid or carbohydrates and lipid so that oil recovery may be increased [10]. Increasing or decreasing pH beyond the isoelectric point increases the net negative or positive charges on the proteins, therefore affecting protein solubility as an emulsifier [10]. At alkaline pH, proteins are typically easily solubilized, which may make them better emulsifiers [10, 11], or make them release the bound oil. These physical processes may effectively free the oil from its interaction with protein and cell wall materials [5, 12].

Industrial processes for oil extraction from oilseed generally use organic solvents and the solvent of choice is hexanes [13]. Because of environmental and regulatory issues, there is a great interest in developing alternative solvent separation technologies. Isopropanol and *n*-butanol have higher boiling points than hexane, 83 and 118 versus 69 °C, thus the chance of evaporative loss is lower but energy required for evaporating the solvents is greater [13]. In the present study, isopropanol and butanol were chosen to extract oil from the CCDS residue after the removal of free oil and water by centrifugation, because these solvents can be used for moist materials such as wet CCDS. In addition, these solvents can be generated from renewable resources.

High pressure and temperature treatment may cause extensive hydrolysis of the protein and carbohydrates, thus releasing oil. Winsness et al. [14] patented high-temperature and pressure cooking for releasing bound oil from whole and thin stillage and reported high oil recovery but without detailed quantitative information. Therefore, we evaluated the effect of autoclaving on oil recovery from CCDS.

Reducing agent, sodium metabisulfite, breaks or rearranges disulfide bonds between protein subunits or within a peptide chain and makes them more soluble [7]. The major protein in corn endosperm, zein, has disulfide bonds [15]. It is possible that the hydrophobic zein stabilizes the oil-in-water emulsion and may be destabilized by reducing or rearranging the disulfide bonds and changing its properties. When the protein becomes soluble, oil may be more easily released and removed. In addition, co-extracting zein with oil may result in the removal of zein, disruption of the oil–protein interactions, therefore, improvement of oil extraction.

The objective of the present study was to determine the effects of physical and chemical processes including heating, pH changes, autoclaving, reducing agents, churning, alternative solvent extraction, and co-extraction of zein on oil recovery from CCDS.

Materials and Methods

Condensed Corn Distillers Solubles

CCDS was obtained from LincolnWay Energy, a typical ethanol plant in Nevada, IA, and it was stored in refrigerator at 4 °C until use. To prevent mold growth, sodium azide was added to the CCDS. Different batches of CCDS were obtained at different times and used in various experiments of this study. For all treatments in each experiment, the same batch of material was used. The most important factor, oil content of each batch, was determined by the acid hydrolysis method and it was used to calculate oil recovery. CCDS of 30% solids content was used unless otherwise stated.

Chemicals

Isopropanol, butanol, hexanes, petroleum ether, ethyl ether, sodium metabisulfite, sodium hydroxide, hydrochloric acid were obtained from Fisher Scientific (Fairlawn, NJ, USA). Ethanol was obtained from Underwriters Laboratories (Northbrook, IL, USA).

Enzymes

Enzymes were obtained from Genencor International Inc. (Rochester, NY, USA) and were liquid commercial preparations. They were stored at 4 °C until used. Multifect® CX GC Genencor cellulase has cellulase and hemicellulase activities. The cellulase is derived from a selected strain of *Trichoderma reesei*. Suggested optimum activity is at pH 4

and 55 °C. Experimental soy blend is a Genencor cellulase. It is a blend of Multifect ® CX B cellulase (42%), Multifect® CX GC cellulase (33%), and Multifect® Pectinase FE (25%). Multifect® Pectinase FE is a concentrated liquid pectinase complex from *Aspergillus niger* and exhibits pectinase, cellulase, and hemicellulase activities. The optimum temperature of the enzyme is 45 °C and the optimum pH is 3.8. Protex™ 15L is an acid fungal protease obtained from a genetically modified selected strain of *T. reesei* whose optimum pH and temperature are 4.5 and 55 °C, respectively.

Determination of Composition of CCDS

Moisture content was determined by using a vacuum drying oven at 50 °C until constant moisture content was obtained. Since CCDS caramelizes at higher temperatures, 50 °C was chosen as being ideal for moisture determination. The AOAC official combustion method (AOAC 990.03) [16] and a VarioMax Carbon Nitrogen analyzer (Elementar Analysensysteme, Hanau, Germany) were used for determining the protein content in CCDS and in the ethanol extract as described in the following section. Total oil content was determined by acid hydrolysis (AOAC 922.06) [16]. The moisture and oil content were measured in duplicate for every new batch of CCDS supplied during the study.

Electron Microscopy Sample Preparation and Imaging

CCDS was subjected to transmission electron microscopy (TEM) imaging to observe the forms of oil. The preparation and TEM micrograph interpretation were provided by experts from the microscopy facility. For sample preparation, contents in CCDS were fixed, washed, dehydrated, embedded with epoxy resin as previously described [9]. A JEOL 2100 scanning and transmission electron microscope (Japan Electron Optic Laboratories, Peabody, MA, USA) was used to capture the images.

Effects of Heating Treatment on Oil Recovery

For each of the two replicates, 40 g of CCDS was used. The samples were either subjected to heat treatment at a specified temperature with incubation time of 10 min at the temperature or left at room temperature of 25 °C. The heat-treated samples were placed in a shaker water bath (Model-R-76, New Brunswick Scientific Co. Inc., NJ, USA) except for the 100 °C treatment samples, which were placed in a beaker containing boiling water. Following heat treatment, oil separation was done using a Centra MP4 centrifuge (International Equipment Company, Needham Heights, MA, USA) fitted with a 854 rotor, fixed angle of 20°, 7.6 cm radius at 10,000 rpm (8,500×*g*) for 10 min followed by transferring the separated oil by using hexane (5 times of washing top layer using 10 mL hexane each time). Removal of solvent was done by distillation using a rotary evaporation system at 60 °C. Residual solvent was removed using a vacuum oven at 25 °C (National Appliance Company, Portland, OR, USA) for 24 h. The weight of the oil was determined gravimetrically.

Effects of pH on Oil Recovery

CCDS was adjusted to pH 1, 2, 3, 4, 9, 10, 11, and 12 using aqueous 20% (w/v) sodium hydroxide or 20% (v/v) hydrochloric acid. The pH-adjusted CCDS samples of about 40 g were placed in 50-mL centrifuge tubes. The treatment conditions, in addition to the different pHs, were ambient temperature (25 °C) as control, use of heating at 100 °C for 60 min, and use of reducing agent (sodium metabisulfite) with heating at 100 °C for 60 min. The concentration of the reducing agent was 1.5% w/w (based on solids content of CCDS). Following the treatments, oil extraction and quantification were performed as previously described.

Oil Extraction from the Solid Residue with Polar Solvents

CCDS was placed into 250-mL centrifuge bottles (~100 g). The treatments were carried out using 91, 81, and 71% v/v (based on total liquid volume) isopropanol and butanol for extracting oil from the residue of the CCDS after centrifugation and removal of free oil and water. The experimental protocol was as follows: the CCDS in 250-mL centrifuge bottles was placed in a water bath at 100 °C for 10 min to allow heat to destabilize the CCDS matrix. Free oil was separated by centrifugation at 4,000 rpm (2,710×*g*) for 10 min. The clear aqueous supernatant was removed, the residue was weighed, and moisture content was measured by using an infrared moisture analyzer (Sartorius MA-30, Elk Grove, IL, USA). The moisture content was used to calculate how much pure solvent was required to make 91, 81, and 71% v/v isopropanol and butanol. Oil was extracted from the residue twice using 450, 184, and 106 mL of isopropanol for the 91, 81, and 71% extraction treatments, respectively. For butanol, 494, 202, and 119 mL of butanol were used for the 91, 81 and 71% treatments, respectively. The incubation time for each extraction was 30 min. For isopropanol, the temperature used for oil extraction was 80 °C, which was 3 °C below the boiling point, and for butanol, the temperature was 90 °C, which was 28 °C below the boiling point.

After each extraction, the mixture was filtered while hot in a sintered glass funnel (about 200 micron pore size)

under a vacuum in order to separate the extract from the solid residue. The filtrates were pooled in a round-bottom flask and concentrated by using a rotary evaporator at 70 °C. Since the solvents extracted non-lipid materials, hexane was used to dissolve and extract lipids from the extracts. The hexane extraction was done 3 times to ensure that all the oil was extracted. The extracts were placed in a pre-weighed round-bottom flask and the solvent was removed using a rotary evaporator at 60 °C. Residual solvent was removed as previously described and oil was weighed.

Oil and Zein Co-Extraction

The extraction of oil and zein was done in three steps as illustrated in Fig. 1. For free oil extraction by centrifugation, CCDS was either subjected to enzyme hydrolysis prior to co-solvent extraction of the residue, or incubated using the same conditions as used for enzyme treatment but with no-enzyme, or left at room temperature for 3 h. For enzyme hydrolysis, Multifect® CX GC Genencor® cellulase and Experimental soy blend Genencor® cellulase at 4% v/w enzyme dosage (based on CCDS dry matter) were used. Incubation was at 50 °C, pH 4 for 3 h. Following incubation, the free oil (oil A) was recovered by centrifuging at 4,000 rpm (2,710×*g*) for 10 min. Oil transfer and quantification was done as previously described. Residue was separated from the supernatant and moisture content of residue was determined and it was used to determine the amount of pure ethanol needed to give 70% v/v ethanol concentration as required for zein extraction. The oil and zein were simultaneously extracted from the residue by using hexane for oil extraction (oil B) and 70% v/v ethanol for zein extraction at the same time at 40 °C for 3 h with stirring. For all treatments, 250-mL hexanes was used for oil extraction. Co-extraction was done twice. Following extraction, the mixture was centrifuged in 1-L centrifuge bottles using RC 3B Plus Sorvall® centrifuge with H-6000A swinging bucket rotor (Kendro Laboratory Products, Newton, CT, USA) at 4,500 rpm (3,493×*g*) for 10 min. The hexane layer was removed to quantify oil and the ethanol layer was collected for quantifying protein. For zein quantification, the micro-Kjeldahl method was used (AOAC 960.52) [16].

Effect of Churning Treatment on Oil Recovery

A laboratory stirrer (Eurostar power-b IKA®-Werke lab stirrer IKA®-Works, Wilmington, NC, USA) equipped with a stirring shaft with paddles at a stirring speed of

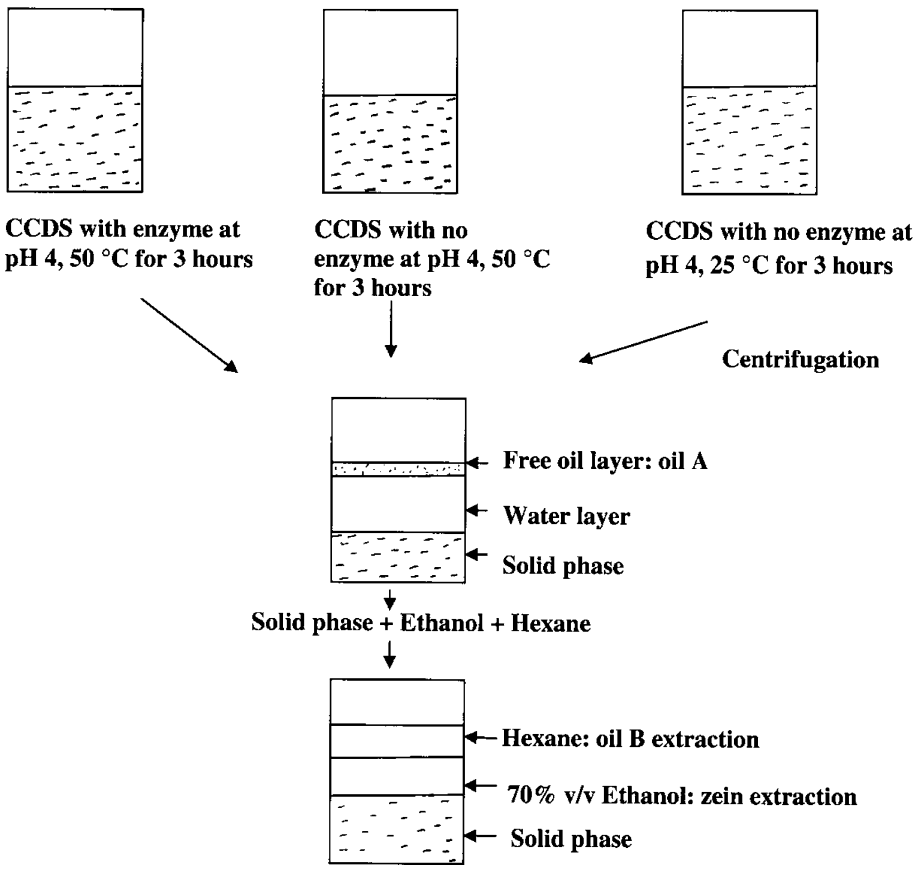

Fig. 1 Schematic representation of the oil and zein co-extraction

Table 1 Oil, protein, and moisture contents (%) of three batches of CCDS used in this study

Composition	Batch	% Average ± SD
Oil (dry basis)	1	17.9[a]
	2	19.4 ± 0.1
	3	21.4 ± 0.6
Protein (dry basis)	1	14.1 ± 0.1
	2	18.7 ± 0.1
	3	ND
Moisture	1	65.9 ± 0.1
	2	68.4 ± 0.1
	3	68.3 ± 0.2

CCDS condensed corn distillers solubles

ND not determined

[a] Analyzed by Eurofins Scientific Inc., Des Moines, IA, by acid hydrolysis method

50 rpm was used to mimic butter churning and to facilitate the coalescence of free oil droplets in the CCDS. The solids content of CCDS was adjusted to 25% to decrease viscosity and facilitate stirring. Incubation conditions were 50 °C, pH 3.5 for 3 and 6 h, separately.

Effects of High Temperature and Pressure Treatment on Oil Recovery

The CCDS (100 g) was incubated at pH 3.5, and 50 °C for 6 h in a shaker water bath. This was used as the control for the enzyme treatment. Following incubation, the CCDS was autoclaved (Tomy Tech Inc., ES-215/315, Fremont, CA, USA) at 121 °C and 103.7 kPa (15.04 psi) for 60 min. For enzyme hydrolysis treatment prior to autoclaving, Multifect® Pectinase FE and Protex™ 15L acid protease were used at 4% enzyme dosage (based on CCDS dry matter). The enzyme incubation conditions were pH 3.5, at 50 °C for 6 h. Following autoclaving, the CCDS was placed in a water bath at 80 °C to ensure that the same temperature was maintained for all the treatments prior to centrifugation. For oil separation, centrifugation was done at 2,710×*g* for 10 min as previously described.

Statistical Analysis

Each experiment was treated as an individual trial with completely randomized treatment design. Statistical analysis to determine significant difference among the different treatments within an experiment was performed using SAS 9.1 (Cary, NC, USA), analysis of variance (ANOVA). Least significant differences (LSD) were calculated at $P = 0.05$. All treatments were carried out in duplicate and results are shown as the means of two replicates ± standard deviation (SD).

Results and Discussion

Composition of CCDS

The composition of the CCDS of the different batches ranged from 18 to 21% total lipids, 14–19% protein, and 66–68% moisture content (Table 1). Oil content and moisture level are the most important parameters for this study. Oil recovery was calculated based on the oil content determined by acid hydrolysis for the batch of CCDS used.

Transmission Electron Microscopy Imaging

CCDS was subjected to Transmission Electron Microscopy (TEM) in order to determine how the oil is associated with other components in CCDS. The lipid droplets are visible as dark spheres and they are surrounded by dispersed protein (Fig. 2), as interpreted by a TEM expert (Tracy

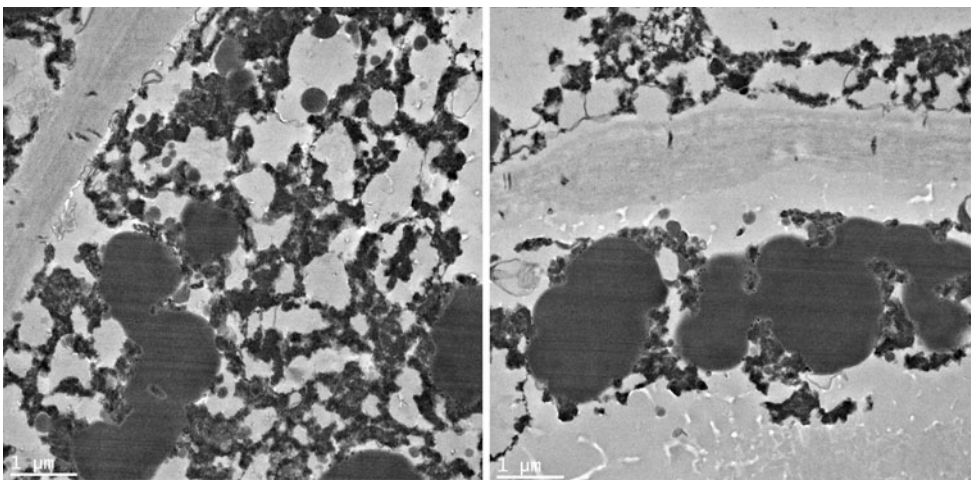

Fig. 2 Transmission electron microscopy (TEM) images of the CCDS. The lipid droplets are shown as *dark spheres* dispersed throughout the cell (*left*). The lipid droplets are also shown interacting with the protein of the *small* and *irregularly shaped* particles (*right*)

Pepper, at Iowa State University). The proteins are seen as a dense network having granular appearance in the cytoplasm. Intact cell walls were seen with two cells attached to each other. Therefore, CCDS has intact cells possibly from the large pieces of endosperm and germ. In our previous research, Majoni et al. [9] also showed free droplets of oil attachment to broken cell debris. In addition, if heating led to free oil release due to the destabilization of the CCDS matrix, it would indicate the presence of oil-in-water emulsions. Therefore, all observations confirm the four forms of oil's presence in CCDS: (1) oil-in-water emulsion stabilized by proteins and phospholipids (2) oil bound to hydrophobic protein and cell wall components (3) oil in intact oil bodies in large endosperm and germ particles and (4) oil in intact oil bodies released from broken germ and corn kernel, which may not be distinguished from the oil-in-water emulsion particles. Various physical and chemical treatments may have different effects on these forms of oil. This study was intended to be a preliminary screening and an observational study. Study of mechanisms and quantification of interactions of oil–proteins and oil–carbohydrates may be conducted in future investigations.

Effects of Temperature on oil Recovery

Increasing temperature increases oil recovery from CCDS as shown in Fig. 3. At 25 and 42 °C oil recovery was not significantly different. When the temperature was increased to 59 °C, however, oil recovery sharply increased. Oil recoveries at 59, 70, 85, and 100 °C were not significantly different. Much of the oil in the CCDS may be in form of oil-in-water emulsion with proteins and phospholipids acting as emulsifiers. A practical means of de-emulsifying is by heating [17] as protein denaturation occurs. Thus, a temperature of about 60 °C resulted in the breaking of the CCDS oil-in-water emulsion. For this batch of CCDS, about 50% oil was present in o/w emulsion. The free minute oil droplets attached to hydrophobic surface, stable oil bodies, or oil bodies in intact cells should not have been significantly affected by heating and these oils may not be recovered by this means.

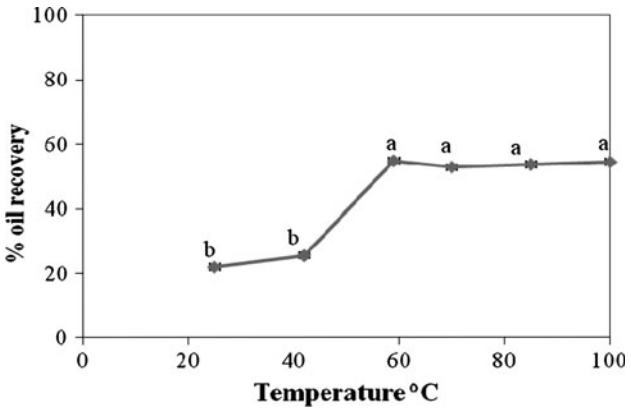

Fig. 3 Effect of heating on oil recovery from CCDS. Means followed by different letters are significantly different ($P < 0.05$)

Effect of pH on Oil Recovery

Oil recoveries at acidic pHs (pH 1, 2, 3, and 4) were significantly greater than at alkaline pHs (pH 9, 10, 11 and 12) as shown in Fig. 4a. At acidic pHs oil recoveries were not significantly different, with an average of 65%. Oil recovered at pH 9 was significantly greater than at pH 10, 11 and 12 but lower than at acidic pH. Our results are in agreement with Wu et al. [18] who recovered less free oil at neutral to alkaline pHs (pH 7 and 8) from cream de-emulsification. In general, the lower oil recoveries at

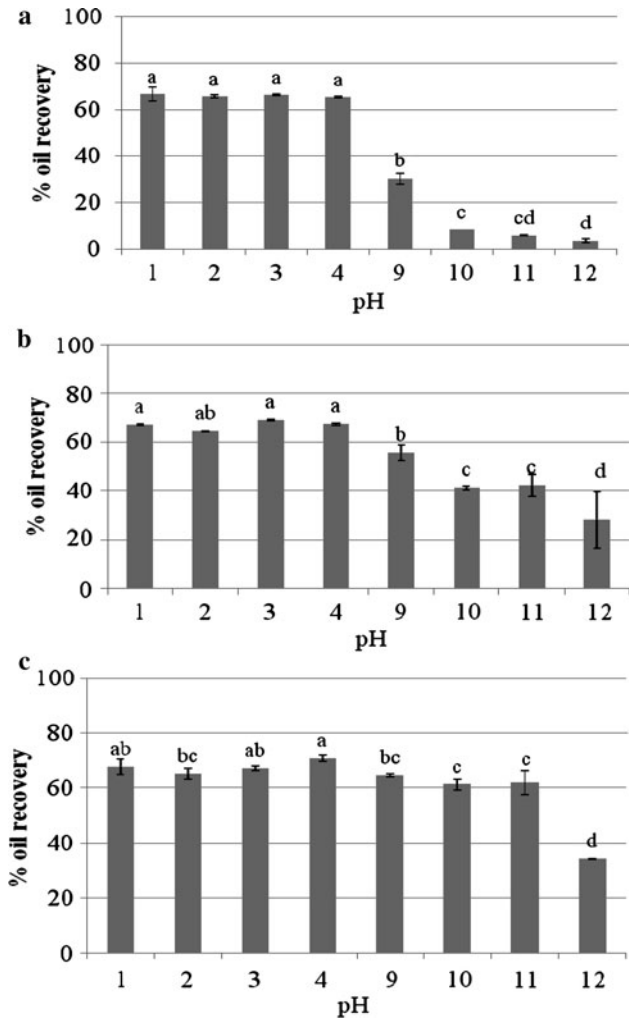

Fig. 4 **a** Effect of pH at 25 °C on oil recovery from CCDS. **b** Effect of pH and heating at 100 °C (60 min) on oil recovery from CCDS. **c** Effect of pH, heating at 100 °C (60 min) and sodium metabisulfite on oil recovery from CCDS. Means followed by different letters are significantly different ($P < 0.05$)

alkaline pHs suggest that the solubilized proteins may have served as better emulsifiers. We hypothesized that solubilized protein may release the oil more readily, but this was not the case. The major endosperm protein in corn, zein, has an isoelectric point of 6.2 [19], therefore, zein will have lower solubility at pH close to isoelectric point and we expected the uncharged zein would interact with oil even more, giving low oil recovery. These data suggest that acidic pHs are ideal for increasing oil recovery from CCDS. The natural pH of CCDS is about 4.5, which is suitable for oil separation without pH adjustment.

Oil recovery from CCDS at elevated temperature is also dependent on pH. A similar trend was observed (Fig. 4b) in which oil recovery at acidic pHs was significantly greater than at alkaline pHs. Oil recovery increased at alkaline pHs compared with the same treatments which were not heated. Oil recovery at pH 9 increased from 30 to 56%, indicating that heating facilitated the breaking of the stabilized oil-in-water emulsion.

Sodium metabisulfite was beneficial in improving oil recovery at alkaline pHs but not helpful at acidic pHs (Fig. 4c). The greatest oil recovery without sodium metabisulfite at pH 9 was 56% but with it, oil recovery increased to 65%. Sodium metabisulfite breaks disulfide linkages between protein subunits or chain segments [10], such that protein configuration may have been altered to become a less effective emulsifier. Zein protein exists in four forms, α, β, γ, δ, and β, and γ proteins are involved in intra and intermolecular disulfide crosslinking. They contain large amounts of cysteine residues and require a reducing agent to solubilize them [15]. The β and γ zeins represent 5 and 20% of the total zein.

The forms of oil which were not significantly affected by pH changes may be free oil bodies and the oil bodies in the large endosperm and germ particles. Therefore, about 30% of oil may be present in such forms.

It is worth noting that oil recovery at 25 °C and pH 4 was higher in this experiment than that in the heating experiment (Fig. 3 at 25 °C). It is possible that since the results were derived from different CCDS batches, and large batch-to-batch variations do exist and it may have contributed to this difference. Another factor is cold room (5 °C) storage of CCDS. We observed the destabilization of CCDS emulsion, as shown by the appearance of a free oil layer during prolonged storage. Additional research is needed to study batch-to-batch differences and the causes from the ethanol plants.

Effect of Alternative Solvents on Oil Recovery

Isopropanol and butanol were the solvents of choice mainly because they are polar and can be used to extract oil from wet plant materials, such as CCDS. Isopropanol and butanol have lower latent heat of vaporization compared to ethanol, 159.3 and 141.3 versus 204 cal/g, respectively [13]. Lower latent heat of vaporization suggests less energy is required to vaporize the solvent.

In this experiment, free oil is the oil that can be separated after centrifugation of the CCDS and trapped oil is the oil that remains in the residue/cake of CCDS after centrifugation and that can be extracted by solvents. The purpose for extracting oil from the residue/cake after free oil extraction was to recover all available oil in CCDS. The solubility of oil in alcohols is dependent on temperature, and solubility increases as temperature increases [13], so we used elevated temperatures. The feasibility of using isopropanol of 91% v/v for extracting oil from cottonseed was studied in the 1940s [20], therefore, this concentration was chosen as the upper limit for both solvents. The other two concentrations, 81 and 71% v/v were chosen for comparison purposes. Lower solvent concentration is more desirable if it can equally extract the oil considering the high moisture content in the CCDS residue.

Oil recovery of the free, trapped and total oil is shown in Table 2. Free oil recovery was not significantly different among all treatments. This was expected since the treatments were subjected to the same experimental conditions. For trapped oil, differences were observed when 71% isopropanol was used, with oil recovery being significantly less (5.0%) than the other treatments. For total oil recovery, there were no significant differences among 71% butanol, and 81 and 91% isopropanol and butanol treatments. Total oil recovery was approximately 84% except for that by 71% isopropanol, which was 57%. These data suggest that the two-stage oil recovery process is effective as it has higher oil recovery compared to the previous experiment on effect of pH changes on oil recovery. Butanol is a better solvent because at 71% v/v it can extract as much as 82%

Table 2 Oil recovery from CCDS by centrifugation and solvent extraction

Solvent concentration (v/v)	% Free oil	% Trapped oil	% Total (free + trapped oil)
Isopropanol (%)			
71	51.6 ± 4.9a	5.0 ± 3.8b	56.6 ± 1.1b
81	56.5 ± 2.7a	26.2 ± 4.0a	82.8 ± 1.3a
91	53.6 ± 6.4a	31.8 ± 3.1a	85.3 ± 3.3a
Butanol (%)			
71	51.1 ± 0.5a	30.7 ± 4.0a	81.8 ± 4.5a
81	52.4 ± 1.9a	30.9 ± 1.5a	83.3 ± 0.4a
91	50.8 ± 2.4a	33.6 ± 1.3a	84.5 ± 1.2a
LSD $_{0.05}$	8.9	8.0	6.0

Means within a column followed by different letters are significantly different ($P < 0.05$)

Table 3 Oil recovery from CCDS after co-extraction with zein by hexane and ethanol

Treatment	% Free oil (A)	% Co-extracted oil (B)	% Total oil (A + B)
Multifect® CX GC + Experimental soy blend	77.0 ± 0.9a	6.8 ± 0.3c	83.8 ± 0.6b
No enzyme	67.7 ± 0.7b	21.3 ± 0.5b	89.0 ± 1.2a
No incubation	15.0 ± 2.1c	69.9 ± 3.1a	84.9 ± 0.9b

Means within each column followed by with different letters are significantly different ($P < 0.05$)

oil. The economy and feasibility of such extraction should be further investigated.

Effects of Oil and Zein Co-Extraction on Oil Recovery

The rationale of using co-extraction for oil and zein was that removal of the hydrophobic protein, which is responsible for the strong binding with oil [9], would increase oil recovery. The combined treatment of enzyme and solvent was done in order to determine if more oil could be co-extracted with zein from the CCDS residue when enzyme hydrolysis of cellular materials is applied. Co-extraction of oil and zein resulted in total oil recoveries (free + trapped oil) of up to 89% as shown in Table 3. When the oil cannot be freed by centrifugation alone, it can be extracted as trapped oil by co-extraction. When such co-extraction was used, enzyme hydrolysis did not seem to be very beneficial. This indicates that the cellulases used may not have effectively hydrolyze the intact cells. About 11% oil may still be trapped in the intact cells. Majoni et al. [9] observed slight improvements in oil recovery from CCDS when cellulase was used alone, and this may be due to the hydrolysis of cell debris and release of oil from the interaction between broken cell wall and oil.

These oil recoveries were greater compared to the heat and pH treatments suggesting co-extraction of oil and zein can be an effective means of oil recovery. It is likely that when the process is further optimized for zein extraction, even higher oil recovery can be reached. More research should be done to further explore this novel co-extraction process.

The total protein content in CCDS ranged 14–19% on dry weight basis (Table 1). The amount of zein extracted (measured the same way as for protein quantification in CCDS) relative to the protein content varied depending on whether the sample had been subjected to enzyme hydrolysis. For the enzyme-hydrolyzed samples, the amount of zein recovered relative to total protein content was 14.2 ± 2.5%, whereas for the non-enzyme treatment, the amount of zein recovered was 18.0 ± 0.7% of the total protein present in CCDS. Certain hydrolysis of zein must have occurred by the enzyme treatment, resulting in the low total protein in the extracted zein fraction. It is a well known fact that commercial cellulase preparations have protease activities. As for alternative solvent extraction of oil, the commercial feasibility of such co-solvent extraction should be further evaluated.

Effect of Churning on Oil Recovery from CCDS

In traditional batch butter churning the oil-in-water emulsion (cream) is inverted to water-in-oil emulsion [21]. The cream is destabilized by slowly rotating the churn such that fat globule membranes are disrupted resulting in fat release and coalescence. During oil extraction from olives, the crushed olives in water undergo mixing with rotating stainless steel blades at 15–20 rpm in order to break the oil-in-water emulsion and to also facilitate coalescence of the small oil droplets to form larger oil droplets [22].

Churning favored the formation of large oil droplets (coalescence) in the CCDS matrix and subsequently increased oil recovery as shown in Table 4. Following 6 h of incubation, oil floated as a layer on the surface of the CCDS and large oil droplets could be seen throughout the CCDS matrix, indicating that stirring allowed the oil droplets to coalesce and float. After 6 h incubation without centrifugation, 47% of the oil could be recovered. With centrifugation, oil recovery was 75%. The 3-h incubation showed oil droplets throughout the CCDS matrix but no separate oil layer. After centrifugation, 80% oil was recovered. Therefore, if free oil recovery with no centrifugation is to be done, churning can be done for a long period of time. If a centrifuge is available, then less time is needed for churning and high oil recovery can be achieved by centrifugation. There is a great potential for an optimized churning process.

Table 4 Effect of churning at 50 °C on oil recovery from CCDS

Treatment	% Oil recovery
3 h incubation	79.7 ± 1.9a
6 h incubation	75.0 ± 1.3b

Means followed by different letters are significantly different ($P < 0.05$)

Effects of High Temperature and Pressure on Oil Recovery

We hypothesized that autoclaving would hydrolyze CCDS solids (conversion of the suspended solids to dissolved solids), as also reported by Winsness et al. [14]. The matrix had a pH of 4.5 which may be active enough to catalyze some hydrolysis of the CCDS solids at 100 °C. When comparing oil recoveries, the enzyme-hydrolyzed and then autoclaved CCDS had significantly higher ($P < 0.05$) oil recovery (74%) compared to autoclaving alone with 63% oil recovery. However, autoclaving alone gave slightly lower (not statistically significant) oil recovery (63%) than that from 80 °C incubation (66%). Therefore, autoclaving alone was not effective for CCDS oil separation under our conditions, and Winsness' result could not be duplicated. Autoclaving did not seem to result in extensive hydrolysis of proteins and fiber in the CCDS matrix, because there was no apparent viscosity change after the treatment.

We should note that a factor contributing to the apparent low oil recovery in our study is the fact that all calculations were based on total oil contained in the CCDS as measured by the acid hydrolysis procedure. This method gives higher total oil values compared to solvent extraction because it results in the total hydrolysis of cellular components. Acid hydrolysis determines both free and bound oil whereas solvents extract only the free oil.

Conclusion

Increasing temperature to about 60 °C increased oil recovery from CCDS since heat can break the oil-in-water emulsion. Oil recovery from CCDS was greater at acidic pHs and a pH of 3–4 was adequate. Use of solvents, such as butanol, may increase total oil recovery up to 85%. Churning could be an ideal process for increasing oil recovery from CCDS because after a long incubation period oil can float on top of the CCDS matrix. The oil can be scraped off without the need for centrifugation. Co-extraction of zein with oil was also effective in improving oil recovery with a major drawback being the labor and cost, however, a major advantage being production of zein as a co-product.

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