Efficient and eco-friendly extraction of corn germ oil using aqueous ethanol solution assisted by steam explosion

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Abstract An improved aqueous extraction method has been established for extraction of oil from corn germs. This method primarily included steam explosion pretreatment and aqueous ethanol extraction. Process variables such as steam pressure, resident time, particle size and ethanol concentration were investigated. The highest yield of 93.74 % was obtained when ground steam-exploded corn germ (1.3 MPa, 30 s, 30–35 µm particle size) was treated with 30 % (v/v) aqueous ethanol for 2 h, at 60 °C and pH 9.0. The residual oil content in water and sediment phase decreased dramatically to 4 % and 3 %, respectively. The enhancement mechanism of the process induced by steam explosion was analyzed by confocal laser scanning microscope (CLSM). The quality of extracted crude oil was also investigated. The results showed that the quality of extracted oil was superior to commercial oils.

Keywords Corn germ · Aqueous ethanol extraction · Steam explosion pretreatment · Free oil yield

Introduction

Corn germ is a substantial byproduct of the wet milling and alcohol industry of corn. Corn germ (dry) contains 35–56 % oil, with linoleic acid (C18:2) being the predominant fatty acid (49–61.9 %) and the highest level of tocopherol and phytosterol amongst all vegetable oils, which is a cost-effective resource for healthy nutritious oil. As estimated 2.4 million metric tons corn oil is produced annually worldwide (Moreau 2011). Because of the LDL cholesterol-lowering peculiarity (Ostlund et al. 2002), corn germ oil and its production method are receiving great attention recently. Currently, commercial corn germ oil is produced by pressing and/or hexane extraction (Moreau and Hicks 2005), in which oil is obtained from conditioned (heated) germs, followed by mechanically expelled ('pre-pressed') and then hexane extraction (Moreau 2011). However, the hyperthermal treatment of pressing, the toxicity and flammability of hexane often brings the growing nutritional and environmental concerns (Long et al. 2011).

To obtain healthy nutritious oil through eco-friendly process, several green extraction methods have been researched at laboratory scale, including aqueous enzymatic method (Dickey et al. 2010, 2008; Karlovic et al. 1994; Moreau et al. 2009) and supercritical fluid extraction (Rebolleda et al. 2012; Shi et al. 2015). However, the aqueous enzymatic method of extraction has several critical problems which restricted its large-scale industrialization. First, in traditional aqueous enzymatic extraction processing, oil is obtained in the form of mixture of free oil and emulsified cream. Demulsification is operated after separating emulsion from slurry, while this kind of emulsion is a highly stable oil-in-water state and difficult to break completely (Campbell et al. 2011). The low free oil yield created challenges in practical application. Second, the process time was relatively long (6 ~ 24 h) but which was necessary for hydrolyzing cell wall. Third, the large dosage (1 ~ 2 % (w/w)

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usually) of enzymes created high production cost which was not commercially feasible. Hence, investigation into an improved corn oil aqueous extraction processing to solve these problems seems justifiable.

Ethanol is a non-toxic and efficient emulsion splitter which has been used in vegetable oil separate emulsion to recover free oil (Chi et al. 2014). In the present study, modest ethanol was added into water to be an extraction agent to obtain free oil from corn germ slurry. The product of aqueous ethanol extraction (in appropriate concentration) was free oil directly rather than highly emulsified cream after centrifuge. The internal reaction mechanism is that diluted aqueous ethanol solution could change the local micro-environment of emulsion and collapse of the structure of orderly water molecular around emulsion drops (Jr. et al. 1990). Thus before forming a stable oil-in-water state, all emulsion in slurry has been demulsified into free oil, which enhances the free oil recovery and shortens the incubation time significantly. In addition, the unhazardous and the easy-recycle characters of ethanol are both favorable for clean and economical production of large-scale industry.

Pretreatment as an essential process, such as ultrasonic (Shah et al. 2005) and microwave (Cheng et al. 2011; Taghvaei et al. 2015), have been developed in extracting oil from plants to enhance oil yield. Among these new technologies, steam explosion is an energy-saving and non-polluting treatment (Chen and Chen 2011). Steam explosion is the most commonly used pretreatment method for lignocellulosic materials (Chen and Chen 2011), which could be performed continuously on a large scale. This method is based on high-pressure saturated steam and forcing steam into cells of material, followed by rapidly releasing the pressure to atmosphere in an explosive decompression (Zoulikha et al. 2015). The machine adopts a structure in a catapult explosion mode that is principally composed of a material vessel and piston valve, which could accomplish the explosion within 0.0875 s (Yu et al. 2012). The completion of high-pressure saturated steam releasing in an extremely short time could provide enough force to split cell wall and break the cellulose chain, and avoid a long time of violent treatment under high temperature. In recent years, the utilization of steam explosion has adequately expanded to variety biomasses, such as microalgal (Cheng et al. 2015), wool (Tonin et al. 2006) and feather (Zhang et al. 2014; Zhao et al. 2012). Nevertheless, the report of using steam explosion in oilseeds for enhancing free oil yield is exceedingly limited.

The objectives of this study were (1) to investigate the feasibility of using steam explosion treatment to enhance oil yield from corn germ; (2) to elucidate the underlying mechanisms of steam explosion on corn germ through confocal laser scanning microscope (CLSM) and (3) to optimize the conditions of steam explosion and aqueous ethanol extraction of corn germ oil.

Materials and methods

Materials

Dry corn germ was purchased from Xing Mao Corn Development Co. Ltd., Shandong Province, China. The raw material (dry corn germ) contained 6–7 % (w/w, wet basis) moisture content, 16 % (w/w, wet basis) crude protein and about 37 % (w/w, wet basis) crude oil. Different treatment conditions, such as grinding might cause modest change on oil contents of corn germ which were tested by Soxhlet extraction. Hence, oil contents of different conditions were analyzed after finely ground with a three roll milling machine (150, Changzhou Wujin Xingye Machinery Equipment Co., Ltd., China). The Soxhlet extractions were operated by Automatic Soxhlet Extractor (SZC-101, Shanghai Qianjian Instrument Co., Ltd., China), taking petroleum ether (30–60 °C) as solvent, conducting a three phase extraction (30 °C soaking 120 min, 60 °C extraction 120 min, 80 °C recycling 30 min). All chemicals were of analytical grade.

Steam explosion and aqueous ethanol extraction

In each extraction, the entire process was divided into several parts (Fig. 1), for instance, steam explosion, grinding and extraction.

Because varieties of factors would affect oil yield, based on the results of preliminary studies, several most relevant factors were chosen to design our experiments and discuss the results. The variables studied were (a) steam explosion pressure and residence time; (b) particle size; and (c) ethanol concentration.

Steam explosion process

Prior to steam explosion, the corn germ was steeped by citric acid solution (0.05 M) for 2 h. The solids-to-solvent ratio is 1:2(kg/L). After soaking, the wet corn germ was sent to steam explode immediately.

All steam explosion experiments were carried out in the QBS-200B SE device with a 5 L chamber from Gentle Science & Technology Co. Ltd., China. The machine consists of a steam generator, material vessel, receiver and rapid-opening piston valve. 1500 g of soaked corn-germ was placed inside the vessel and exposed to the saturated steam in each bath. The steam pressures were set at 0–1.8 MPa. The resident time was performed in the range of 0–120 s. The time started to count when steam pressure in the vessel reached expected value (3–5 s). After the elapsed time, the piston valve opened fleetly and the steam pressure decreased dramatically in an instant (within 0.1 s). The exploded materials were collected in the receiver, filtered out the excess liquid, and then dried in a vacuum oven at 60 °C.

Fig. 1 The diluted aqueous ethanol assisted by steam explosion treatment process diagram

Grinding steam exploded corn germ

In this step, grinding was divided into two parts: first coarsely ground, and then finely crushed. High speed Chinese medicine grinder (DFY-500, Wenling LinDa Machinery Co., Ltd., China) was used for coarsely ground, 1 time lasting 30s. The three roll milling machine mentioned above was used for fine grinding. Coarse grind material was sampling for control group, labeling it as “0” (finely ground 0 time); Experimental groups were samples of finely crushing different times (1–5 times), labeling them from “1” to “5”. After grinding, particle size of different degrees of ground material were measured using Laser particle size analyzer (S3500, Microtrac Company, America). The milled corn germs was stored in 4 °C fresh keeping cabinet until used for the extraction experiments.

Oil extraction

Corn germ oil was extracted from milled corn germ using diluted aqueous ethanol as solvent. Batch extractions were carried out in 500 mL glass reactor vessels which were series connected with a constant temperature water bath machine (MP-501 A, Shanghai Yiheng Scientific Instrument Co., Ltd., China). The containers were placed on the plate of magnetic stirrer (84–1, Shanghai Meiyongpu Instrument Manufacturing Co., Ltd., China). Each extraction was carried out with 40 g of milled corn germ, solid-liquid ratio was set as 1:7 (w/v, kg/L), and the ethanol concentration was set from 0 to 30 % (v/v), and stirring was done for 2 h at 60 °C. The mixtures were added into containers until the water bath temperature reached 60 °C, time counted after the pH value of system was adjusted to 9.0 using 10 % (w/w) NaOH solution. After incubation, each extraction system was poured into one 500 mL centrifugal cup (with the large capacity low speed centrifuge, LXJ-IIB, Shanghai Anting Scientific Instrument Factory, China). Free oil was collected after centrifugation at 5000 rpm for 15 min and weighed.

Yield of oil was expressed in this way:

$$\text{Yield}(\%) = \frac{\text{free oil weight (g)}}{\text{total oil in ground corn germ(g/40 g corn germ)}} \times 100$$

The volume of water phase was measured and transferred into a liposuction tube to determine oil content by Rose-

Gettlieh method. The remaining water phase was used to recovery protein and ethanol. The sediment phase was weighed after drying in the oven, its oil content was tested by Automatic Soxhlet Extractor (reference to raw material determination process). The sediment phase was employed to be animal feed which contained carbohydrate and protein.

Microstructural analysis

Microstructural analysis was performed with a confocal laser scanning microscope (LSM 710, Carl Zeiss Company, Germany). The specimens were immobilized by 2 % (v/v) glutaraldehyde solution for 24 h, and then rinsed with sodium phosphate buffer solution (pH 7.2). The prepared specimens were embedded in the tissue freezing medium (Leica, Germany) on small metal plates, frozen, and then sectioned to 20 μm thickness with a frozen section machine (CM1950, Leica, Germany). These slices were stained by FITC and Nile red.

Quality analysis of extracted corn germ oil

Simple refining process contained alkali and water washing followed by bleaching. Alkali washing was conducted using 10 % (w/w) alkali solution for 30 min under 60 °C. The amount of alkali used was calculated from the following formulas:

$$\text{Alkali amount} = \frac{7.13 \cdot AV \cdot G}{10000} \quad (\text{a})$$

$$\text{Extra alkali amount} = 0.2\% \cdot G \quad (\text{b})$$

$$\text{Total alkali amount} = \frac{\text{Alkali amount} + \text{Extra alkali amount}}{c} \quad (\text{c})$$

(a)(b): G - the weight of crude oil, g; AV - the acidity value of crude oil, mg KOH/g oil; (c): c - the concentration of alkali solution, w/w.

Alkali refined oil was water-washed 3 times for 30 min/time at 60 °C. Activated carbon was used to remove colour compounds for 30 min at 80 °C. Then, the oil was vacuum filtered to collect refined oil.

Acid value, peroxide value, saponification value, tocopherol content, trans fatty acid content and methylation were

determined using ISO methods (660, 3960, 3657, 9936, 15304, and 5509, respectively).

Statistical analyses

Each experiment was carried out at least in triplicate. All statistical analyses were conducted with SPSS Statistics software (Version 17.0), and $P < 0.05$ was used to determine statistical significance in all tests.

Results and discussion

Comparison between different pretreatment

The oil distribution in three phases (free oil, water phase and sediment phase) of untreated, steam exploded and citric acid soaked-steam exploded corn germ are demonstrated in Fig. 2. Compared steam exploded corn germ (1.3 MPa, 30 s) with untreated sample, free oil yield raised from 76.33 % to 88.51 %, meanwhile residual oil content declined 2.88 % and 9.35 % respectively in water phase and sediment phase. It verified the feasibility of using steam explosion treatment to enhance free oil yield from corn germs. Steam explosion pretreatment played an obvious positive effect on enhancing free oil yield. However, the free oil yield of 88.51 % was insufficient to be industrialized.

In order to enhance oil yield further, corn germs were steam-exploded after citric acid soaking. Dilute acid immersion is a conventional procedure prior to thermal treatment of

oilseeds. Dickey L. C et al. used 3 % acetate buffer (pH 4.1) as soaking solution before cooking corn germs (Dickey et al. 2008). While in this study, citric acid solution was chosen instead of acetate buffer. Citric acid is a common additive employed in food industry. It could significantly increase the oil recovery when added prior to malaxation (Chih et al. 2013). Moreover as an antioxidant synergists (Codex Alimentarius Commission 2013), it could scavenge the available oxygen radicals and subsequently inhibit the over-rapid oxidation in the period of oil storage. Thus, citric acid was a suitable immersion solution before corn germ exploded. The result of 0.05 M citric acid soaked-steam exploded (1.3 MPa, 30 s) sample was shown in Fig. 2. After citric acid soaked-steam exploded treatment, the free oil yield could reach 93.74 % from 76.33 % of the original untreated sample, residual oil content in water and sediment phase decreased to about 3 % both. Hence, the processing of citric acid soaking followed by steam explosion was chosen as the pretreatment mode.

Mechanisms of steam explosion treatment on enhancing free oil yield

To investigate the enhancement mechanism of steam explosion on free oil, the microstructure of untreated and citric acid soaked-steam exploded corn germ were observed by CLSM. The protein and oil distribution states of untreated and treated corn germ were illustrated in Fig. 3(a)–(d). The 2D photo was the overlay photo of protein (green) and oil (red) in split photo.

As shown in photos of untreated sample (Fig. 3(a) and (b)), the red spots (oil) and green spots (protein) were completely coincident and formed simplex yellow spots through superposition (Fig. 3(a)). It indicated that oil did not exist independently in corn germ cell, but formed complex body with protein in its nature state. This state was different from general oilseeds like peanut, flaxseed and sunflower seed, whose oil and protein were disconnected. In addition, oil-protein complex body was surrounded by a layer of confining cell wall which limited the mobility of complex body. While as shown in treated sample (Fig. 3(c) and (d)), red spots and green spots were unable to be coincident and almost all yellow spots disappeared. It elucidated that citric acid soaking-steam explosion treatment could destroy most of the oil-protein complexes and cell wall and release free oil from complexes.

In citric acid soaking-steam explosion treatment, saturated steam firstly penetrated the cell wall of corn germs with a powerful seepage force, the corn germs were rapidly heated by steam (Kumar and Murthy 2011), meanwhile, the cellulose and hemicellulose contained in cell wall were hydrolyzed by citric acid intercepted in corn germ cell under the high pressure (Singh et al. 2015). After maintaining this state for a short time, the pressure occurred an explosive decompression

Fig. 2 Oil distribution in three phases of untreated, steam exploded and citric acid soaked-steam exploded samples. Steam explosion conditions: 1.3 MPa, 30 s; citric acid soaking conditions: 0.05 M citric acid solution, solid-to-liquid ratio 1:2 (w/v), 2 h. Extraction conditions: extraction solvent, 30 % (v/v) aqueous ethanol solution; solid-to-solvent ratio, 1:7 (g/mL); extraction temperature, 60 °C; extraction pH (using 10 % (w/w) NaOH solution), 9.0; extraction time, 2 h

Fig. 3 Microstructure of untreated and citric acid soaked-steam exploded corn germ. **(a)** 2D CLSM photo of untreated sample; **(b)** split CLSM photo of untreated sample; **(c)** 2D CLSM photo of citric acid soaked-steam exploded sample (1.3 MPa, 30s); **(d)** split CLSM photo of citric acid soaked-steam exploded sample (1.3 MPa, 30s)

(within 0.0875 s) in which most of the steam in corn germ cells would quickly expand. Accordingly, the mechanical effect (Zhang et al. 2014) of huge impulsion force disconnected the combination between oil and protein when the steam quickly rushed out. Hence, the oil recovery enhancement mechanism of citric acid soaking-steam explosion method came from the cellulose and hemicellulose hydrolysis by citric acid and the mechanical effect of huge impulsion force on oil-protein complexes.

Effect of steam explosion conditions on oil recovery

Many pretreatment methods had been employed to enhance oil yield, such as heat and humidity cook, baking in high-temperature oven (Moreau et al. 2005) and microwave treatment (Dickey et al. 2007). Separated oil and damaged cell wall induced by steam explosion had been seen in CLSM photos, hence it was expected to be effective in enhancing yield. Investigation of the effect of steam explosion conditions on free oil yield were carried out by extracting oil from citric acid soaked corn germs (Fig. 4(a)-(b)).

As shown in Fig. 4(a), steam explosion treatment, resulted into the highest oil yield of 93.6 % at 1.3 MPa from the yield of 76.33 % for untreated sample. Simultaneously, residual oil content in water and sediment phase were gradually decreased with the increase of pressure from 0 MPa to 1.3 MPa. However

after 1.3 MPa, raising pressure could not improve free oil yield but lead to a lower oil recovery and a slight raise of water/sediment residual oil content. The loss of free oil was likely to blame to protein degradation. Apart from steam pressure, residence time was another main parameter of steam explosion (Chen and Chen 2011). As shown in Fig. 4(b), the free oil yield showed a peak at 30 s, after which it decreased. Residence time would affect the permeation of steam (Chen and Chen 2011). Therefore, appropriate resident time could strengthen the performance of steam explosion treatment, destroy cell wall and disconnected complex body. However, excessive pressure or time would probably urge protein denaturation and aggregation, the aggregated structure became a physical barrier and obstructed free oil release. Thus, in consideration of oil recovery and energy saving, 1.3 MPa, 30 s were taken as the optimal steam explosion conditions of treating corn germs.

Under optimal steam explosion conditions (1.3 MPa, 30 s), the free oil yield would reach 93–94 % at equilibrium, oil content of water and sediment phase were less than 4 % and 3 % respectively.

Effect of ethanol concentration and particle size in aqueous ethanol solution method on free oil yield

Traditional aqueous and aqueous enzymatic method used water as extraction medium, dissolving soluble cellular materials

Fig. 4 Effect of steam explosion condition on oil distribution in three phases: (a) the effect of steam pressure, residence time was kept constant for 30 s; (b) the effect of residence time, steam pressure was kept constant for 1.3 MPa; other conditions: citric acid concentration, 0.05 M; extraction solvent, 30 % (v/v) aqueous ethanol solution; solid-to-solvent ratio, 1:7 (g/mL); extraction temperature, 60 °C; extraction pH(using 10 % (w/w) NaOH solution), 9.0; extraction time, 2 h

(protein mainly) and releasing oil into water phase, from which the oil could be recovered by centrifugation but in the form of a cream emulsion (Campbell and Glatz 2009). The stable emulsion need to be broken to recover free oil, otherwise yield may not reach to the highest level. High concentration of ethanol was reported to be widely-used as demulsifier (Chi et al. 2014). In addition, it has been used as extraction solvent in earlier report (Kwiatkowski and Cheryan 2002), but the extracted oil contained much soluble saccharide and colloid substance which was easy to plug the pipeline and limited its industrial application. Hence, in the present study, diluted aqueous ethanol was used as solvent and chemical demulsifier because of brilliant demulsification performance.

Ethanol is a non-toxic surfactant, which would change the interfacial tension (IFT) (Kadioglu et al. 2011) when added into oil extraction system. The IFT of ethanol is 22.39 mN/m (20 °C), lower than that of water 72.75 mN/m (20 °C), therefore, when the ethanol concentration increases in solution, the interfacial tension and the polarity of alcohol-water system will reduce. IFT reduction would promote the mechanisms of oil liberation and enhance the oil extraction yield (Miñana-Perez et al. 1995).

Figure 5(a) illustrated the effect of ethanol concentration on free oil yield. As shown in Fig. 5(a), free oil was constantly increased to 93 % from 56 % with the increased ethanol concentration. Contrarily, emulsion oil content was lessened observably with the growth of ethanol concentration, and when alcohol concentration reached 30 % (v/v), the emulsion phase disappeared. This result corroborated the relationship between IFT and free oil yield. The internal reaction mechanism was that the added ethanol changed the local micro-environment

Fig. 5 Influence of ethanol concentration and particle size on oil distribution in each phase. (a) the influence of ethanol concentration; (b) the influence of particle size; other conditions: citric acid concentration, 0.05 M; steam pressure, 1.3 MPa; resident time, 30 s; solid-to-solvent ratio, 1:7 (g/mL); extraction temperature, 60 °C; extraction pH(using 10 % (w/w) NaOH solution), 9.0; extraction time, 2 h

of emulsion and collapsed the structure of orderly water molecular around emulsion drops, finally crushed all emulsion (Jr. et al. 1990). Figure 5(a) demonstrated diluted aqueous ethanol was an admirable oil extraction solvent and emulsion breaker. 30 % (v/v) was the optimal concentration of ethanol in extraction of corn germ oil.

Besides the ethanol concentration, particle size of corn germ powder could also influence the oil recovery. Grinding oilseeds prior to oil extraction may enlarge the interface between material particles and aqueous solvent or enzyme, and then enhance free oil yield. In previous research, colloid milling was employed to mill corn germs (Dickey et al. 2010). In this study, a dry milling method was developed to grind corn germ. Compared with colloid milling, dry milling method could produce more uniform and smaller particles, which increased oil extraction.

Figure 5(b) displayed the influence of particle size on oil yield. As shown in Fig. 5(b), the particle size greatly decreased with the increasing of grinding times, and the change trend slowed down when ground times reached 2 (the present particle size was 43.83 μm). At the same time, with the increasing of ground times to 4, free oil yield dramatically increased from 80.40 % to 93.54 % ($p < 0.05$). Represented in the CLSM photos, the cell size of corn germ was approximately 50 μm . Therefore, when particle size of ground germ was smaller than cell size, the cell wall was very likely to be severely damaged and then oil would dissociate from damaged cells to create a high free oil yield. Hence, finely ground exploded germs 2 times (particle size $< 50 \mu\text{m}$) was enough in theory for further oil extraction, but finely ground 4 times (particle size: 30–35 μm) was the optimal condition according to Fig. 5(b) and extra ground times did not impact oil yield significantly. In practical production, the choice of ground times should consider the balance between economical/power input and oil yield.

Quality analysis of corn germ oil extracted with aqueous ethanol assisted by steam explosion

Oil quality is the one of foremost scaleplates to estimate the superiority of production method. Hence, we investigated

the physicochemical characteristics of crude corn germ oil and refined corn germ oil which extracted with aqueous ethanol assisted by a short time steam explosion. Meanwhile, two commercial oil (extracted by hot-pressing and/or solvent extraction) were also selected for comparison. Table 1 showed several quality indexes of extracted oil and commercial oil. Acidity and peroxide value are two most idiocratic physicochemical index. As shown in Table 1, the acidity of crude corn germ oil was only 0.77 mg KOH/g oil, the peroxide value was only 5.08 meq/kg oil, while the CAC standard (Codex Alimentarius Commission 2013) of crude corn germ oil was acidity ≤ 4.0 (mg KOH/g oil), peroxide value ≤ 15 (meq/kg oil) and acidity ≤ 0.6 (mg KOH/g oil), peroxide value ≤ 10 (meq/kg oil) in refined corn germ oil. Therefore, our results of crude corn germ oil was much lower than the standard of crude oil and also close to or reaching the standard of finished oil, which indicated that oil extracted in this way just need a simple refining. After simple refining, the acidity value decreased to 0.25 mg KOH/g oil and the peroxide value was 3.04 meq/kg oil. It was interesting to note that the commercial oil was very likely to be overly refined because the acidity was only 0.17 mg KOH/g oil in A and 0.22 mg KOH/g oil in B, while the saponification value was over-standard(CAC standard: saponification value 187–195 mg KOH/g oil).

A simple refining not only saved cost, but also maximized the beneficial ingredients content in oil, such as tocopherol. As shown in Table 1, the tocopherol content in crude oil and refined oil were 106.24 mg/100 g oil and 105.76 mg/100 g oil respectively, which verified the progressiveness of simple refining.

Trans-fatty acid (TFA) was associated with increased risk of cardiovascular disease (Lichtenstein 2014), which considered forming in oil refining process. As shown in Table 1, the content of trans-fatty acid in crude oil and refined oil were both lower than 0.2 %, it could be recognized as ‘TFA-Free’ according to different national standards(Health-Canada 2009; Wang and Sun 2014), while in commercial oil A was up to 0.73 % and in commercial oil B was up to 0.80 %. This result proved the preponderance of aqueous ethanol method again.

Table 1 Quality index of crude oil, refined oil and two commercial oil

Item	Acidity (mg KOH/g oil)	Peroxide value (meq/kg oil)	Saponification value (mg KOH/g oil)	Tocopherol content (mg/100 g oil)
Crude oil	0.77 $\pm$ 0.01	5.08 $\pm$ 0.02	187.62 $\pm$ 0.34	106.24 $\pm$ 0.68
Refined oil	0.25 $\pm$ 0.01	3.04 $\pm$ 0.02	189.58 $\pm$ 0.12	105.76 $\pm$ 0.38
Commercial oil A	0.17 $\pm$ 0.01	4.11 $\pm$ 0.02	199.22 $\pm$ 0.52	105.29 $\pm$ 0.75
Commercial oil B	0.22 $\pm$ 0.01	2.64 $\pm$ 0.03	201.69 $\pm$ 0.48	141.44 $\pm$ 0.23
Crude oil (CAC Standard)	4.0	15	187–195	33–372
Refined oil (CAC Standard)	0.6	10	187–195	Not mentioned

Conclusion

In this study, an efficient and eco-friendly extraction process was developed by the aqueous ethanol extraction with steam explosion pretreatment for obtaining corn germ oil. The free oil recovery was influenced by the pretreatments, the operating conditions of steam explosion (pressure and resident time), the particle size of ground corn germ and the concentration of ethanol used. The yield enhancement mechanism of steam explosion was interpreted as the cellulose and hemicellulose hydrolysis by citric acid and the mechanical effect of huge impulsion force on oil-protein complexes. The highest yield of 93.74 % was obtained by using this process. In addition, the quality of extracted crude or simple refined oil was superior to commercial oils.

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