Combined effect of ohmic heating and enzyme assisted aqueous extraction process on soy oil recovery

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Abstract This research describes a new technological process for soybean oil extraction. The process deals with the combined effect of ohmic heating and enzyme assisted aqueous oil extraction process (EAEP) on enhancement of oil recovery from soybean seed. The experimental process consisted of following basic steps, namely, dehulling, wet grinding, enzymatic treatment, ohmic heating, aqueous extraction and centrifugation. The effect of ohmic heating parameters namely electric field strength (EFS), end point temperature (EPT) and holding time (HT) on aqueous oil extraction process were investigated. Three levels of electric field strength (i.e. OH600V, OH750V and OH900V), 3 levels of end point temperature (i.e. 70, 80 and 90 °C) and 3 levels of holding time (i.e. 0, 5 and 10 min.) were taken as independent variables using full factorial design. Percentage oil recovery from soybean by EAEP alone and EAEP coupled with ohmic heating were 53.12 % and 56.86 % to 73 % respectively. The maximum oil recovery (73 %) was obtained when the sample was heated and maintained at 90 °C using electric field strength of OH600V for a holding time of 10 min. The free fatty acid (FFA) of the extracted oil (i.e. in range of 0.97 to 1.29 %) was within the acceptable limit of 3 % (oleic acid) and 0.5–3 % prescribed respectively by PFA and BIS.

Keywords Ohmic heating · EAEP · Electric field strength · End point temperature · Holding time · Percentage oil recovery and FFA

Soybean (*Glycine Max*) is often called the “miracle bean” because of its chemical composition and diverse applications for food; feed and non food uses (Weingartner 2008). It is one of best sources of high quality plant protein and oil. Organic solvents (e.g. hexane, petroleum ether and ethanol) have been the preferred extraction solvents for soybean oil for a long time. The main drawback of these solvents are related to economic, environmental and safety aspects (Kalia et al. 2002; Sinerio et al. 1998). Also residual solvent retention in oil and cake has potential health hazards. Removal of solvent (desolventizing) requires high temperature, which reduces the nutritional and functional properties of the proteins in the meal. Moreover, the initial equipment costs of solvent extraction process are high and throughput required for cost efficiency is very high (Weingartner 2008). Therefore, the development of eco friendly-innovative technologies leading to higher oil yield coupled with lower the processing cost is the need of the hour.

Extraction of vegetable oils either through enzymatic treated fruits and seeds or Enzyme assisted aqueous extraction process (EAEP) might be a potential alternative method. Various forms of the enzymatic pretreatment and enzyme-assisted aqueous extraction process (EAEP) have been investigated for several oil-bearing materials such as soybeans (Rosenthal et al. 2001), corn germ (Moreau et al. 2004), rapeseed (Zhang et al. 2007; Sarkar et al. 1998), rice bran (Sharma et al. 2001), sunflower (Sinerio et al. 1998) and sesame (Sandhu et al. 2008). The low oil recovery in aqueous extraction process and enzyme assisted aqueous extraction process has been related to the inadequacies of pre-treatment

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at disrupting the cellular structure of oil-bearing materials (Rosenthal et al. 1996).

Ohmic heating, which utilizes the inherent electrical resistance of food materials to generate heat, is becoming a promising method for food processing (Wang et al. 2007). There has been some effort to use the attributes specific to ohmic heating to improve various aspects of food and industrial materials processing. Electric fields were used to increase the efficiency of sucrose extraction from sugar beet (Katrokha et al. 1984). Kim and Pyun (1995) used ohmic heating to enhance the diffusion of soy milk from soybeans. Solid and protein yields were enhanced approximately 16 % and 25 %, respectively, when the soy slurry was heated using a 12.5 V/cm voltage gradient. Praporscic et al. (2006) studied the effect of ohmic heating on juice yield from potato and apple tissues. The investigations show how tissue disintegration degree and juice yield depend on the field intensity, temperature, treatment duration and type of plant tissue. The best juice extraction was observed when the plant tissue was treated electrically at a moderate temperature of 50 °C

In a review of this attractive technology, the use of ohmic heating to oil bearing material as a pre-treatment prior to oil extraction is also reported to have resulted in higher oil yield (i.e. 93 % recovery of oil from rice bran, Rao et al. 2004). Based on literature result one can hypothesize that the combination of ohmic heating and enzyme assisted aqueous extraction process (EAEP) may increase the soy oil recovery. The objective of this study is that to determine the effect of ohmic heating conditions namely electric field strength, end point temperature and holding time on soy oil recovery from

enzymatically hydrolyzed soybean seed and compare the oil yield with control treatment (i.e. EAEP only).

Materials and methods

Soybean seed ('JS-9041') procured from Director of farms, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P), India. The samples were cleaned from impurities and thoroughly cleaned, undamaged and bold kernels were selected for the study. Moisture content of the seed was determined by oven drying to the constant weight at 105 °C (AOAC Official Method 925.40) and was found to be 7 % (w.b.). Soybean dehuller (Make: CIAE, Bhopal, capacity-100 kg/h) was used for dehulling of soybean seeds. The dehulled soy splits were then packed in 1 kg polythene bags sealed and stored in a refrigerator at 4 °C until used. Commercial cellulase enzyme procured from KPS Biotech, Mysore was used for enzymatic treatment. It was stored in a refrigerator at 4 °C, so that its enzymatic activity did not change significantly during the period of the study. Soxhlet apparatus (Make: Perfit India, Gupta Scientific Industries Model: HT 1043) was used to measure the oil content of seed. Soybean oil yield obtained by solvent extraction was 18.42 g per 100 g milled soybean.

Ohmic heating of soybean slurry

The experimental apparatus used for ohmic heating can be viewed in Fig. 1. The device employed a T-shape cylindrical geometry in which the samples (i.e. soybean slurry) were

Fig. 1 Schematic diagram of ohmic heating system.

1-Control panel; 2-Digital ammeter; 3-6A-AC variate; 4-Digital voltmeter; 5-Temperature control system with probe; 6-Temperature sensor lid; 7-Power supply to electrode (50 Hz, 220 V); 8-Electric connector to electrode; 9-Two disk electrodes; 10-Three insulator cap made of wood; 11-Supporting ring shape structure made of wood; 12-Temperature probe insert in geometric center of heating container; 13-Base; 14-T-shape-Heating container

held and heated. The heating container was made of PVC material of 75 mm Internal Diameter, 165 mm length and 6.35 mm thick. Two stainless steel electrodes of 2.5 mm thick were securely placed at left and right ends of container and connected to an AC power supply (50 Hz, 0–240 V). The gap between two electrodes was fixed at 0.16 m. Three end caps, made of wood, were provided in all three ends of cylinder for protection and to prevent heat loss. The K-type thermocouple was placed in geometric centre of the container. Voltage, current and temperature were also recorded in every 30s. This approach was necessary in order to compute the electrical conductivity of soybean slurry. Electric conductivity depends on several factors like temperature, time, ionic constituents, material microstructure and field strength (Halden et al. 1990). To evaluate the effect of time on

electrical conductivity, experiments were planned to examine the time dependence of electrical conductivity. For this, once sample was attaining the desire temperature (i.e. end point temperature), the system was automatically cut off and again started when temperature is lower than end point temperature (EPT). Sample kept in that temperature for some time (i.e. holding time). In case of measurement of electrical conductivity current and voltage were noted. Three levels of electric field strength (i.e. OH600V (96 V/cm), OH750V and OH900V), 3 levels of end point temperature (i.e. 70, 80 and 90 °C) and 3 levels of holding time (i.e. 0, 5 and 10 min.) were taken as independent variables. The temperature and current during ohmic heating were recorded for all 27 experiments. The volume of oil layer, creamy layer, aqueous phase and solid cake separated by centrifugation.

Experimental procedure

The experimental process consisted of following basic steps namely dehulling, wet grinding of soybean seed, treating it with cellulase enzyme, incubating it for 16 h, ohmic heating

Fig. 2 Process flow chart of combined application of enzyme assisted aqueous extraction process and ohmic heating for soy-oil recovery along with process parameters

Table 1 Fixed parameters and their values used in the study

Fixed parameters	Value	Decided through
Wet grinding		
Sample size (Soybean seed)	100 g	Oil extraction evaluated through 100 g basis
Solid to water ratio during grinding	1:2	Preliminary experiments
Grinding time	90 min	
Enzyme treatment		
Enzyme	Cellulase enzyme	Kisinegar and Hock (1948)
		Bhatnagar and Johri (1987)
Enzyme concentration	1 % dry basis	Preliminary experiments
Incubation temperature	50 °C	
Incubation period	16 h	
Ohmic heating		
Solid to water ratio	1:4	Preliminary experiments
Aqueous extraction		
Agitation speed	400 rpm	Preliminary experiments
Extraction pH	4	
Extraction time	60 min	
Centrifugation		
Centrifugation time and speed	20 min at 4,500 rpm	Preliminary experiments

g gram; h hour; min minute; rpm rotation per minute

Table 2 Analysis of variance (ANOVA) for soy-oil recovery (%) obtained through combined application of ohmic heating and enzyme assisted aqueous extraction process

Source of variation	DF ^a	SS ^b	MSS ^c	F _{cal}	p-value
Electric field strength	2	34.932	17.466	4.46	0.025
End point temperature	2	379.446	189.723	48.46	0.000
Holding time	2	115.127	57.563	14.70	0.000
Error	20	78.306	3.915		
Total	26	607.810	23.377		

^a DF Degree of freedom; ^b SS Sum of square; ^c MSS Mean sum of square

Fig. 3 Effect of (a) End point temperature and electric field strength (b) holding time and electric field strength (c) Holding time and end point temperature on soy-oil recovery ($n=3$)

Fig. 4 Variation of electrical conductivity of soybean slurry with temperature at different electric field strengths applied during ohmic heating ($n=3$)

dry oil separated to the amount of oil initially present into the material. The increased oil recovery in percentage

over control treatment was also calculated by following equation:

$$\% \text{ Oil recovery increased} = \frac{\% \text{ Oil recovery by (EAEP + ohmic heating)} - \% \text{ Oil recovery by EAEP only}}{\% \text{ Oil recovery by EAEP only}}$$

Determination of free fatty acids

To determine the free fatty acids in extracted oil, about 10 g of oil was weighed, dissolved in hot 100 ml of neutralized ethanol, and titrated with 0.1 N standard NaOH solution using phenolphthalein as an indicator (AOAC 1997). The % free fatty acids (as % oleic acid by weight) were calculated using the following expression:

$$\text{Free fatty acid (FFA)} = \frac{28.2 \times \text{TV} \times \text{N}}{\text{W}}$$

Where,

TV Volume in ml of NaOH solution used
N Normality of NaOH solution and
W Weight in g of sample taken for analysis

Statistical analysis

Experiments were carried out in triplicates ($n=3$) and the full factorial experimental design was adopted for experiments. Statistical analysis of data in terms of ANOVA and correlation analysis between independent variables and dependent variables was conducted using Minitab and Design Ease 7.1 (Trial pack) statistical software package.

Results and discussion

Percentage oil recovered from soybean through combined application of ohmic heating and enzymatic hydrolysis were

varies from 56.86 to 73 %. Thus incremental oil recovery through enzymatic hydrolysis coupled with ohmic heating varied from 7 % to 37 % over controlled treatment (i.e. 53.12 %). The maximum oil recovery (73 %) was obtained when the sample (at enzyme concentration of 1 % dry basis, incubation period of 16 h and incubation temperature of 50 °C) was heated and maintained at 90 °C using electric field strength of OH600V for a holding time of 10 min. The ANOVA of percentage soy-oil recovery of enzyme assisted aqueous extraction process coupled with ohmic heating (Table 2) indicated that the effect of all the studied variables i.e. electric field strength ($p<0.05$), end point temperature ($p<0.01$) and holding time ($p<0.01$), on oil recovery was highly significant. Although oil recovery was significantly affected by all studied variables yet the end point temperature ($F_{\text{cal}} = 48.46$) and

Table 3 Analysis of variance (ANOVA) for free fatty acid (% oleic acid) of extracted soy- oil

Source of variation	DF ^a	SS ^b	MSS ^c	F _{cal}	p-value
Electric field strength	2	0.05087	0.0254	13.73	0.001
End point temperature	2	0.1095	0.0547	29.56	0.001
Holding time	2	0.00046	0.00023	0.13	0.882
Error	20	0.0037	0.00185		
Total	26	0.1979	0.00076		

^a DF Degree of freedom; ^b SS Sum of square; ^c MSS Mean sum of square

holding time ($F_{\text{cal}} = 14.70$), had much stronger influence compared to the electric field strength ($F_{\text{cal}} = 4.46$). In all the cases oil recovery increased with holding time. The variation of oil recovery with interaction effect of different process parameter was shown in Fig. 3a, b and c.

It may be seen that the oil recovery was highest at interactions of end point temperature and holding time. In traditional methods of oil expression or extraction, time–temperature relationship plays a vital role on oil recovery. Total heat input loosens the bond holding lipid bodies with proteinaceous bodies in oil-bearing material. This increasing the oil fluidity and oil goes into aqueous medium. The improvement in extraction yield was not only caused by combination of time-temperature relationship but another mechanism, most likely electroporation, is also involved. Figure 4 indicated that electric conductivity was maximum (1.36 S/m), when enzymatic hydrolyzed soybean slurry were ohmically heated at electric field strength of OH600V upto an end point temperature of 90 °C. This finding could be significant for the correlating the electric conductivity with electroporation effect. Thus the combined effect of possible electroporation and thermal softening of the tissues may explain the increased oil yield (Praporscic et al. 2006). The free fatty acid of the extracted oil (i.e. in range of 0.97 to 1.29 %) was within the acceptable limit of 3 % (oleic acid) and 0.5–3 % prescribed respectively by PFA and BIS. ANOVA of free fatty acid (Table 3) shows that the electric field strength ($p < 0.01$) and end point temperature ($p < 0.01$) was significant effect to FFA of oil content while holding time is non-significant effect.

Conclusion

Oil recovery from EAEP and EAEP coupled with ohmic heating were 53.12 % and 56.86 to 73 % respectively. Thus incremental oil recovery through enzymatic hydrolysis coupled with ohmic heating varied from 7 % to 37 % over controlled treatment. The maximum oil recovery percentage of 73 % was obtained, when enzymatic hydrolyzed soybean slurry were ohmically heated at electric field strength of OH600V upto an end point temperature of 90 °C and held for 10 min. High temperature and longer time increased the extraction yield possibly due to electroporation effect with thermal softening of tissues and that may be more pronounce due to higher electric conductivity of soybean slurry. These results indicate that combined application of enzyme

assisted aqueous extraction process and ohmic heating is potential alternative method for oil extraction from oilseed.

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