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Oil extraction through hydraulic pressing from Cambodian soybean seeds and analysis of its physicochemical quality

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ABSTRACT

The objective of this study was to investigate the effects of hydraulic pressing conditions on the extraction yields of Cambodian soybeans using hydraulic press machine. The extraction of soybean oil through hydraulic pressing is a chemical-free mechanical method that involves the compression of the soybean seeds using heat and pressure. The different pressures (30, 40, and 50 MPa) and pressing times (15, 20, 25, 30, 40, 50, and 60 min) were performed on the extracted soybean oil yield. The physicochemical properties and fatty acid profiles of the extracted soybean oil were also analyzed. The physicochemical properties such as peroxide value, iodine value, acid value, and color were determined by AOAC methods. And the fatty acid profiles, including linoleic acid, oleic acid, linolenic acid, palmitic acid, and steric acid, were characterized by gas chromatography with a frame ionization detector. The results demonstrated that the highest yield of 12.49% was achieved at a pressure of 50 MPa and a pressing time of 60 min. The crude soybean oil obtained exhibited acceptable physicochemical quality and fatty acid profiles. The fatty acids of soybean oil were found to be rich in unsaturated fatty acids, with 52.69% of linoleic acid and 25.24% of oleic acid. However, it is important to note that this study has limitations, and further research should investigate additional factors, particularly the effect of temperature in hydraulic pressing, to optimize oil yield.

Contribution/Originality: Nowadays, Cambodia imports a huge amount of soybean oil for cooking, even though it is an agricultural country. This study focuses on the investigation of different pressures and pressing times of a high-pressure hydraulic pressing machine for extracting soybean oil from Cambodian soybean seeds to achieve a higher oil yield.

1. INTRODUCTION

Soybean, known as *Glycine max (L.) Merr*, from the Fabaceae family, originated in southeastern Asia (de Almeida Chuffa, Vieira, da Silva, & Franco, 2014). The most cultivated soybean oilseed crops in the world are located in the Americas and Asia (Fargione, Hill, Tilman, Polasky, & Hawthorne, 2008). In Cambodia, soybean production has been growing in the main areas of Ratanakiri and Preah Vihear provinces since 1980 (Belfield, Brown, & Martin, 2011; Nget et al., 2021). In the dry soybean seeds, there are around 18% to 20% of extractable oil by weight (de Almeida Chuffa et al., 2014). Soybean oil contains a large amount of unsaturated acids such as Alpha-linolenic acid, linoleic acid, camar-linolenic, arachidonic acid, and oleic acid that are very important in human nutrition(Nikolić, Cakić, Novaković, Cvetković, & Stanković, 2009; Olguin et al., 2003). Moreover, soybean oil can be produced either by solvent extraction or mechanical processing. Solvent extraction with n-hexane is a commonly applied for extracting oil from soybean and other plant seeds (Dutta, Sarkar, & Mukherjee, 2015; Nikolić et al., 2009). However, the organic solvents used in the extraction have been determined to cause human health, safety, and environmental contamination (De Oliveira, De Barros, & Gimenes, 2013; Li, Pordesimo, & Weiss, 2004). Consequently, mechanical processing has been applied to oil production for food and industrial purposes (Nelson, Wijeratne, Yeh, Wei, & Wei, 1987). Hydraulic pressing is a popular mechanical oil extraction method that places the oil product under high pressure for a reasonable period from the compressed kernel mass (Mwithiga & Moriasi, 2007).

Hydraulic pressing is very common for small-scale processors because it provides low cost, performs well in hot conditions, and uses incompressible fluid, which results in higher efficiency (Joshi,IITs, & IISc, 2016). There are three stages in the hydraulic system: the initial, dynamic, and final stages. The initial stage happens before the oil starts to leave the mass of grains. The commencement of the dynamic stage occurs when the oil is initially released and escapes from the reservoir. Then, the final stage starts when the maximum instantaneous flow rate occurs (Pighinelli & Gambetta, 2012).

The objective of this research was therefore to study the effects of hydraulic pressing conditions on the extraction yields of Cambodian soybeans. Moreover, the fatty acid profiles and the physicochemical properties of the crude soybean oil were also determined.

2. METHODOLOGY

2.1. Materials

Soybean seeds were locally collected from Ratanakiri Province, Cambodia. The soybean seeds were manually cleaned to remove the impurities and then ground into powder using a portable gate valve grinder machine (Model SL-30, Thailand). The samples were then kept in the plastic zipper bags and stored at room temperature for further extraction. The reagents used in the experiments were acetic acid, ethanol (Daejung, Korea), chloroform, potassium iodine, cyclohexane (Fisher, Belgium), sodium thiosulfate, potassium hydroxide (Supelco, Germany), starch (Millipore, Germany), Wijs solution (Thermo Scientific, Belgium), and phenolphthalein (Supelco, China) for analyzing the physicochemical parameters of crude soybean oil after the extraction by hydraulic pressing.

2.2. Oil Extraction

In the soybean oil extraction by using a hydraulic oil press machine (BY-180, China), 350 g of the soybean powder was put in a press cloth and placed into the pressing metallic cylinder. Then, the samples were preheated at 60℃ for 15 minutes to balance the temperature of the whole sample. After that, the samples were pressed immediately at various pressures (30, 40, and 50 MegaPascal or MPa) and pressing times (15, 20, 25, 30, 40, 50, and 60 min). Finally, the soybean oil samples after the extraction were stored in the refrigerator at −21℃ prior to analysis. The soybean oil yield was determined by using the ratio between the mass of oil extracted and the mass of the sample that was put inside the metallic cylinder, as shown in Equation 1.

$$\text{Oil yield }(\%) = \frac{\text{Weight of oil extracted (g)}}{\text{Weight of soybean used (g)}} \times 100 \qquad (1)$$

2.3. Fatty Acid Profiles Analysis

First, 100 mg of the oil sample was dissolved in 2 mL of hexane. Then, 0.1 mL of 2 M KOH was added to the methanol using a vortex mixer for 15 seconds. Next, the mixture was centrifuged at 4,000 rpm for 5 minute and filtered with 0.22-µm Polytetrafluoroethyle (PTFE). It was then analyzed by using a gas chromatography with a frame ionization detector (GC-FID), which was an Agilent 6890 series gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a spilt-splitless injector and Specialty Gas Equipment with 70% Cyanopropyl polysilphenylene-siloxane (SGEBPX70)GC-column (25 m × 0.32 mm, 0.25 µm film thickness) (Trajan Scientific, Victoria, Australia). The injector was set at 250℃ and operated using a spill mode ratio of 20:1. And the detector was set at 280℃ and the gas flow rates were set as follows: hydrogen gas at 40 mL/min, air at 450 mL/min, and nitrogen gas at 25 mL/min. The pressure was set at 10 psi. Then, the oven temperature ramp was set for 5 minute at 100℃, followed by increasing heat by 4℃/min up to 240℃, and then a holding period of 20 minutes. Finally, the fatty acid composition of the soybean oil samples was quantified by using the area normalization method. Pure standards of FAMEs at each retention time were used for comparison and identification of peaks on the chromatogram. The percentage of each major fatty acid composition was calculated by dividing the peak area of each fatty acid by the total peak area of all fatty acid majorities.

2.4. Physicochemical Analysis

The crude soybean oil was analyzed for its physicochemical properties by following the AOAC (1990) official method, such as peroxide value, iodine value, acid value, and color. The analysis procedure for each selected parameter is described in the following sections:

2.4.1. Peroxide Value

5g of soybean oil was weighed, dissolved, and mixed well with 30ml of $CH_3COOH\text{-}CHCl_3$ (3:2)in the Erlenmeyer. Then, 0.5ml of saturated KI solution was added, followed by 30ml of distilled water, and the solution was mixed well. The mixture solution was titrated with 0.01M $Na_2S_2O_3$ until the blue or dark blue color disappeared. The 0.5% starch solution was used as an indicator. The peroxide value in the soybean oil was calculated by using the following Equation 2:

$$\text{Peroxide value}\left(\frac{\text{meq}}{\text{kg}}\text{oil}\right) = \frac{S \times M \times 1000}{W} \quad (2)$$

Where:

S = ml of Na_2SO_3 (blank corrected).

M =Molarity of Na_2SO_3 (0.01M).

W = Weight of oil sample in g.

2.4.2. Iodine Value

0.1g of crude soybean oil was dissolved in 15ml of cyclohexane and mixed with15ml of Wijs solution. The solution was incubated in a light-restricted environment for duration of 30 minutes at ambient temperature. Then, 20 ml of 10% aqueous KI and 100 mL of distilled water were added to transform leftover iodine into iodine. The final content was titrated with 0.1M $Na_2S_2O_3$ by using 0.5% starch solution as an indicator. Finally, the iodine value in the soybean oil was calculated by following the formula in Equation 3:

$$\text{Iodine value}\left(g\,\frac{I_2}{g}\,\text{oil}\right) = \frac{12.69\,(B\text{-}S) \times N}{W} \quad (3)$$

Where:

B = ml of Na_2SO_3 solution required for blank.

S = ml of Na_2SO_3 solution required for sample.

N = Normality of the standard solution (0.1N).

W = Weight of oil sample in g.

2.4.3. Acid Value

In the titration method for determining acid value, 5 g of the soybean oil was weighed and dissolved with 80ml of freshly neutralized hot ethyl alcohol. Then, the solution was titrated with potassium hydroxide (KOH), and phenolphthalein solution was used as an endpoint indicator. Finally, the acid value in the soybean oil was calculated using the formula in Equation 4:

$$\text{Acid value}\left(\text{mg}\frac{\text{KOH}}{\text{g}}\text{oil}\right)=\frac{56.1\times V\times N}{W} \quad (4)$$

Where:

V = Volume in ml of standard KOH used.

N = Normality of KOH solution (0.1N).

W = Weight of oil sample in g.

56.1 = KOH molecular weight.

2.4.4. Color

The color of crude soybean oil was measured using a chroma meter (CR-400, Japan) and considered by the CIELAB color system (L*, a*, b*), where L* is used to describe relative lightness, a* represents relative redness-greenness, and b* represents relative yellowness-blueness.

2.5. Statistics Analysis

All measurements were reported as mean ± standard deviation (SD). The data was then analyzed using analysis of variance (ANOVA) using IBM® Statistical Package for the Social Sciences (SPSS) Statistics version 29 (SPSS Inc., USA). The treatment differences were performed for Duncan's multiple range test (DMRT) by a post hoc test at a confidence level of 95%.

3. RESULTS AND DISCUSSION

The experimental results of soybean oil extraction by hydraulic pressing are presented in Table 1. The lowest yield of soybean oil was 7.79% by using the following hydraulic pressing conditions: 30 MPa of pressure and 15 minutes of pressing time. The highest yield of soybean oil was 11.01% when hydraulic pressing conditions such as 50 MPa of pressing pressure and 25 minutes of pressing time at a temperature of 60℃ were applied. Moreover, by comparing with the previous study, the percentage of soybean oil extracted by mechanical screw press at the temperature of 60℃ was 9.51% (Moses, 2014).

Table 1. Experimental results for soybean oil extraction by hydraulic pressing.

Std.	Run	Pressure (MPa)	Time (Min.)	Oil yield (%)
10	1	40	20	9.40
1	2	30	15	7.79
2	3	50	15	8.76
9	4	40	20	9.60
6	5	50	20	9.86
7	6	40	15	8.60
4	7	50	25	11.01
11	8	40	20	9.31
8	9	40	25	10.13
3	10	30	25	9.90
5	11	30	20	9.31

The analysis of variance in Table 2 showed that the two factors, including pressure and pressing time of hydraulic pressing, had a significant effect ($p<0.05$) on the soybean oil yield. As a result, the higher the pressure applied, higher the soybean oil yield obtained. However, the pressure in this study could not be increased higher than 50 MPa due to the capacity of the available hydraulic press machine.

3.1. Effect of Pressure on Soybean Oil Yields

From the ANOVA result, the *p*-value for factor A (pressure) was less than 0.05. This observation suggested that the magnitude of pressure exerted during the hydraulic pressing process has had a substantial impact on the production of soybean oil. As shown in Figure 1, the average soybean oil yield increased from 7.79% to 11.01% due to the increase of pressure from 30MPa to 50MPa during hydraulic pressing operation. In a previous study, Smith, Agrawal, Sarkar, and Singh (1993) discovered the optimal pressure of mechanical expelling for soybeans and reported that an increase in pressure improved oil extraction.

Table 2. Analysis of variance (ANOVA).

Source	Sum of squares	Df	Mean square	F-value	*p*-value	Remark
Model	6.93	2	3.47	80.86	<0.0001	Significant
A-pressure	1.15	1	1.15	26.88	0.0008	Significant
B-pressing time	5.78	1	5.78	134.84	<0.0001	Significant
Residual	0.343	8	0.0429			
Lack of fit	0.299	6	0.0498	2.26	0.3380	Not significant
Pure error	0.0441	2	0.0220			
Cor total	7.28	10				

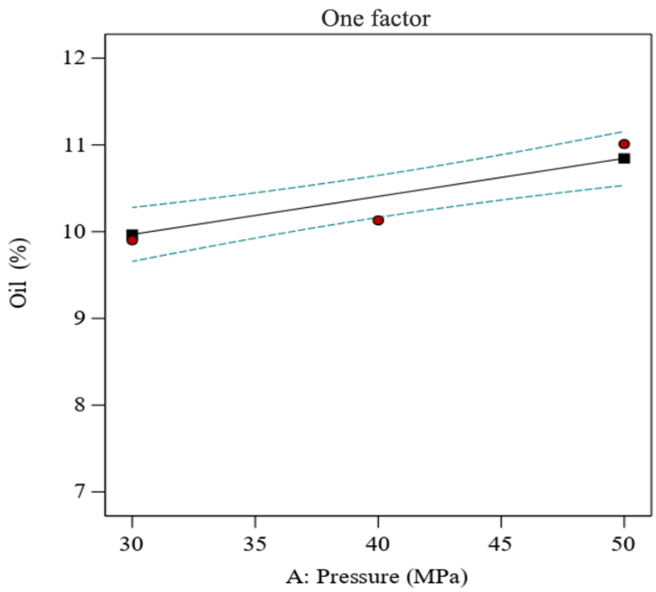

Figure 1. Effect of pressure on soybean oil yields by using hydraulic pressing.

3.2. Effect of Pressing Time on Soybean Oil Yields

For factor B (pressing time), it significantly affects the soybean oil yield during the extraction period by hydraulic pressing ($p<0.05$), as indicated in Figure 2. The result also showed that the average soybean oil yield increased from 7.79% to 11.01% as pressure increased from 30 to 50 MPa. The previous study on sunflower oil extraction by pressing also reported that the pressing time had a very significant ($p<0.05$) impact on the oil yield (Singh, Labana, & Virk, 1984).

3.3. Effect of Pressure and Pressing Time of Hydraulic Pressing on Soybean Oil Yields

The results of soybean oil yields are presented in Table 3. There are significant differences in soybean oil yields at different pressing conditions ($p<0.05$). The lowest yield of soybean oil was 7.79% ± 0.46 by using the following hydraulic pressing conditions: 30 MPa of pressure and 15 min of pressing time. The highest yield of soybean oil was 12.49% ± 0.53, and hydraulic pressing conditions such as 50 MPa of pressing pressure and 60 minutes of pressing time at a temperature of 60°C were applied. In addition, there are no significant differences ($p< 0.05$) in oil cake and total mass percentage between each treatment.

As a result, the higher the pressure applied, higher the soybean oil yield obtained. Moreover, the longer the pressing time of the hydraulic press machine, the higher of soybean oil yield was observed. In addition, heating before pressing increases oil yield because of the breakdown of oil cells, coagulation of proteins, adjustment of moisture content to the optimal value for pressing, and decreased oil viscosity, which allows the oil to flow more quickly (Augustine, 1993). However, there were no statistically significant differences between the oil cake and total mass of oil and cake when different pressures and times were applied.

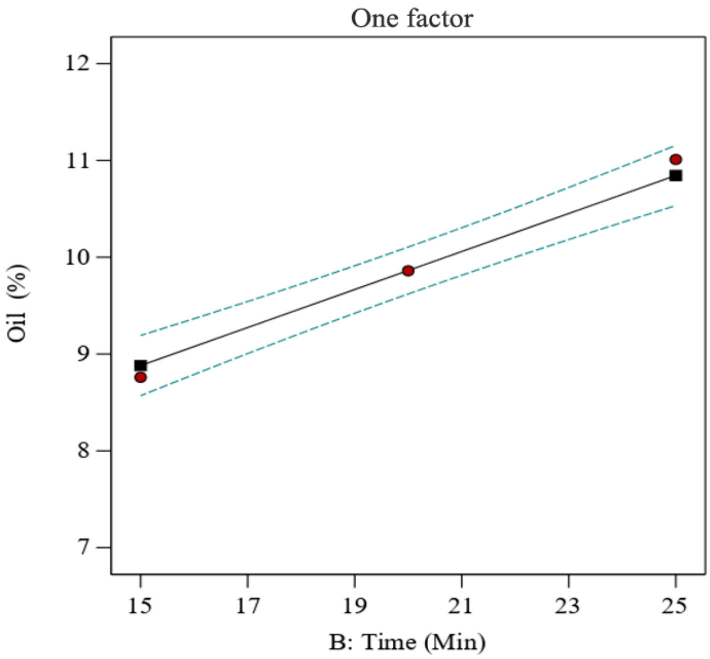

Figure 2. Effect of pressing time on soybean oil yields by using hydraulic pressing.

Table 3. The percentages of oil yield, oil cake, and total mass balance obtained from soybean oil extraction by using hydraulic pressing.

Pressure (MPa)	Time (Min.)	Oil yield (%)	Oil cake (%)	Total mass (%)
	15	7.79 ± 0.46[a]	88.68 ± 0.52	96.46 ± 0.99
	20	9.31 ± 0.36[a, b, c]	89.91 ± 0.04	99.22 ± 0.31
30	25	9.90 ± 0.14[b, c, d]	88.55 ± 0.47	98.45 ± 0.61
	15	8.60 ± 0.53[a, b]	88.91 ± 3.31	97.51 ± 2.78
	20	9.46 ± 0.20[a, b, c, d]	87.94 ± 0.72	97.40 ± 0.92
40	25	10.13 ± 0.30[b, c, d, e]	87.58 ± 1.31	97.71 ± 1.00
	15	8.76 ± 0.38[a, b]	88.97 ± 0.15	97.73 ± 0.54
	20	9.86 ± 0.41[b, c, d]	89.14 ± 2.26	99.00 ± 1.85
	25	11.01 ± 0.26[c, d, e, f]	88.00 ± 1.13	99.01 ± 1.39
	30	11.14 ± 0.04[d, e, f]	86.90 ± 0.06	98.04 ± 0.10
	40	11.73 ± 0.34[e, f]	87.67 ± 1.83	99.40 ± 1.49
	50	12.07 ± 2.28[f]	86.47 ± 1.51	98.54 ± 3.79
50	60	12.49 ± 0.53[f]	87.80 ± 0.52	100.28 ± 1.05

Note: All values are means ± standard deviation and Means across a column with a different superscript (a, b, c, d, e, f) differ significantly ($p< 0.05$).

3.4. Fatty Acid Profiles of Extracted Soybean Oil

The fatty acid profile of Cambodian crude soybean oil by hydraulic pressing is illustrated in Table 4. The highest values of fatty acid profiles were linoleic acid (52.69%), and then oleic acid (25.24%). Since the oil has a high percentage of unsaturated fatty acids, which usually are linoleic acid and oleic acid (Jokić et al., 2013), Linoleic acid is utilized to make hormone-like substances that control a variety of body processes, immune system responses, and the inflammation response to injury and infection. Moreover, it is useful in beauty products (Sodamade, Oyedepo, & Bolaji, 2013). And oleic acid helps lower total cholesterol (10%) and low-density lipoprotein (LDL) cholesterol content in the blood (Grundy, 1989; Sodamade et al., 2013). The most important unsaturated fatty acid was linolenic acid, which was about 7.04%. The amount of linolenic acid is approximately 10% of the total fatty acids that reduce the oxidative stability of the oil (Clemente & Cahoon, 2009). The saturated fatty acids obtained were 11.03% of palmitic acid and 3.97% of steric acid.

Table 4. Comparing the percentages of fatty acid profiles of the crude soybean oil.

Fatty acid profile	Current study	Chowdhury, Banu, Khan, and Latif (2007)	Park (2012)
Linoleic acid	52.69%	52.18%	55%
Oleic acid	25.24%	26.27%	18%
Linolenic acid	7.04%	5.63%	10%
Palmitic acid	11.03%	14.04%	13%
Steric acid	3.97%	4.07%	4%

The oils, which are primarily composed of triacylglycerols, are vital nutrition and energy sources. Dietary triacylglycerols are made up of fatty acids that vary in chain length, unsaturation level, isomeric orientation of double bonds, and position within the triacylglycerol molecule (Sodamade et al., 2013). According to Chowdhury et al. (2007), the values of total fatty acids in soybean oil were 52.18% of linoleic acid, 26.27% of oleic acid, 5.63% of linolenic acid, 14.04% of palmitic acids, and 4.07% of steric acid. By comparing them with previous study, both results were quite similar. However, there were a bit of difference in the values of some fatty acids, such as linolenic acid and palmitic acid because of the different soybean seeds used and the oil extraction method applied. And Park (2012) found that in the commodity of soybean oil, there are five fatty acids, such as 55% of linoleic acid (18:2), 18% of oleic acid (18:1), 13% of linolenic acid (18:3), 10% of palmitic acid (16:0), and 4% of steric acid (18:0).

3.5. Physicochemical Properties of Extracted Soybean Oil

The physicochemical properties of soybean oil after pressing by using a hydraulic press machine are shown in Table 5. The soybean oil had a peroxide value of 1.38 ± 0.26 meq/kg. Since the high peroxide value indicates an oxidized oil signed by rancid taste and smell, a lower peroxide value indicates a good quality of oil and preservation status (Mariana, Susanti, Hidayati, & Wahab, 2020). Oil quality with a peroxide value below 10 meq/kg is considered fresh oil, but when the peroxide value is between 20 and 40 meq/kg, the oil becomes rancid (Low & Ng, 1987). The iodine value was 126.01 ± 1.68 g I2/g oil since the higher the iodine value, the greater the number of double bonds (Yildiz, Alfeen, & Yildiz, 2019).

Table 5. Physico-chemical properties of soybean crude oil compared with Codex standard (2013).

Parameter	Value ±SD	Codex standard (2013)
Peroxide value (meq/kg oil)	1.38 ± 0.26	≤ 15
Iodine value (g I_2/g oil)	126.01 ± 1.68	124–139
Acid value (mg KOH/g oil)	0.67 ± 0.00	≤ 4.0
Color	L*= 34.77 ± 0.17	-
	a*= -0.33 ± 0.16	-
	b*= 4.57 ± 0.52	-

Note: L*: Lightness, a*: Redness-greenness, b*: Yellowness-blueness.

Moreover, the acid value was 0.67 ± 0.00 mg KOH/g oil, while the decomposition of the acid value is accelerated by heat and light (Kim & Siang, 1987). An increase in acid value is a sign of oil deterioration, which is caused by the degradation of chemical bonds in oil at high temperatures (Dawodu, Olutona, & Obimakinde, 2015). In contrast, the lower the free fatty acids, better the oil quality for human consumption (Amos-Tautua & Onigbinde, 2013). According to Bello, Akindele, Adeoye, and Oladimeji (2011), 0.00 to 3.00 mg KOH/g of acid value of oil is required for food preparation.

Another parameter of soybean oil quality is the color of the oil after extraction. CIELAB was used to describe the precise 3-D location of color, and L* is used to describe relative lightness, which ranges from 0 is absolute black to 100 is absolute white. a* represents relative redness-greenness, where the positive a* is red and the negative a* is green. b* represents relative yellowness-blueness, where the positive b* is yellow and the negative b* is blue. Therefore, the color of soybean oil extracted by hydraulic pressing was dark yellow (Ramos-Escudero, González-Miret, Viñas-Ospino, & Ramos Escudero, 2019). The degree of color changes due to the nature of raw materials, refining condition of oil, storage period, temperature, intensity of light, and contact surface with the air (Komoda, Ōnuki, & Harada, 1966).

4. CONCLUSION

Soybean oil extraction using a hydraulic press resulted in the highest soybean oil yield of 12.07% when applying a pressure of 50 MPa and a pressing time of 50 min. However, it is important to note that mechanical extraction through hydraulic pressing still has limitations when pressing soybean oilseeds with lower oil content. The pressing time and pressure applied in the hydraulic pressing machine significantly influenced the soybean oil yield, indicating that higher yields were achieved with increasing pressing pressure and time. Furthermore, the fatty acid profiles of the extracted soybean oil were found to be rich in unsaturated fatty acids, particularly linoleic acid. The physicochemical properties of the soybean oil, including peroxide value, iodine value, and acid value, complied with the Codex standard. These findings show that the obtained crude soybean oil compiles with standards' quality requirements. The color of the soybean oil appeared as a dark yellow shade. Nevertheless, further research should focus on exploring different temperatures and pretreatment conditions to optimize the oil yield.

Abbreviation	**Full name**
AOAC	Association of official agricultural chemists
AV	Acid value
CIELAB	International commission on illumination
IV	Iodine value
KOH	Potassium hydroxide
MPa	Megapascal
PTFE	polytetrafluoroethylene
PUFAs	Polyunsaturated fatty acids
PV	Peroxide value
SPSS	Statistical package for the social sciences

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Transparency: The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.
Data Availability Statement: The corresponding author can provide the supporting data of this study upon a reasonable request.
Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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