TEKNIKA: JURNAL SAINS DAN TEKNOLOGI

Homepage jurnal: http://jurnal.untirta.ac.id/index.php/ju-tek/

The effect of solvent type on the extraction of soybean crude oil and its laboratory-scale cost analysis

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ARTICLE INFO

Article history:
Submitted 17 September 2023
Received 18 September 2023
Received in revised form 25 October 2023
Accepted 25 November 2023
Available online on 30 November 2023

Keywords:
Fatty acid, extraction, soybean, solvent, cost analysis

Kata kunci:
Asam lemak, ekstraksi, kacang kedelai, pelarut, analisis biaya

ABSTRACT

Soybeans (*Glycine max*) are a type of legume with moderately high fat content. Appropriate and safe types of solvents are essential in extracting fatty acids for consumption. This study aims to find an alternative solvent to replace n-hexane for extracting soybean oil and analyze its lab-scale production cost. Soybean oil was extracted by soxhlet extraction using ethanol, ethyl acetate, and n-hexane with a soybean-to-solvent ratio of 1:4 and 2:4 (w/v) and an extraction time of 1 and 2 hours. The results show that solvent polarity and soybean: solvent ratio affect the yield and density of the resulting extract. The greater the soybean-to-solvent ratio, the more oil is extracted until equilibrium. The highest yield was 36.25%, obtained at a material-to-solvent ratio of 2:4 (w/v) within 2 hours with ethyl acetate, and the oil density was 0.92 g/mL. The Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis on the sample with the highest yield showed that the fatty acid composition was 34.36% linoleic acid, 22.12% palmitic acid, 19.40% stearic acid and 19.43% octadecadienoic acid. Ethyl acetate produces better yields and can be recommended as an alternative solvent to replace n-hexane for soybean oil extraction. The total extract production costs for 70% ethanol, 90% ethyl acetate, and 95% n-hexane solvents are IDR 780,847; 647,232; and 692,050, respectively.

ABSTRAK

Kedelai (*Glycine max*) merupakan salah satu jenis kacang-kacangan dengan kandungan lemak cukup tinggi. Pemilihan jenis pelarut yang tepat dan aman sangat penting dalam mengekstraksi asam lemak untuk dikonsumsi. Penelitian ini bertujuan untuk mencari pelarut alternatif pengganti n-heksana pada ekstraksi minyak kedelai dan menganalisis biaya produksi skala laboratoriumnya. Minyak kedelai diekstraksi dengan metode ekstraksi soxhlet menggunakan etanol, etil asetat, dan n-heksana dengan perbandingan kedelai terhadap pelarut 1:4 dan 2:4 (b/v) serta waktu ekstraksi 1 dan 2 jam. Hasil penelitian menunjukkan bahwa polaritas pelarut dan rasio kedelai:pelarut berpengaruh terhadap rendemen dan densitas ekstrak yang dihasilkan. Semakin besar rasio kedelai terhadap pelarut, semakin banyak minyak yang terekstraksi hingga mencapai kesetimbangan. Rendemen tertinggi sebesar 36,25% diperoleh pada perbandingan bahan terhadap pelarut 2:4 (b/v) dalam waktu 2 jam dengan etil asetat, dan densitas minyak sebesar 0,92 g/mL. Analisis *Gas Chromatography-Mass Spectrophotometry* (GC-MS) pada sampel dengan rendemen tertinggi menunjukkan komposisi asam lemak yaitu asam linoleat 34,36%, asam palmitat 22,12%, asam stearat 19,40%, dan asam oktadekadienoat 19,43%. Etil asetat menghasilkan rendemen yang lebih baik dan dapat direkomendasikan sebagai pelarut alternatif pengganti n-heksana untuk ekstraksi minyak kedelai. Total biaya produksi ekstrak untuk pelarut etanol 70%, etil asetat 90%, dan pelarut n-heksana 95% secara berurutan adalah Rp 780.847; 647.232; dan 692.050.

Available online at http://dx.doi.org/10.36055/tjst.v19i2.22059

1. Introduction

Soybeans are raw materials for food in Indonesia, such as for making tofu, tempeh, soy milk and other processed products. Generally, low-quality soybeans are used as raw materials for animal feed [1]. One can be used as raw material for processed food production to increase the value of low-quality soybeans. Soybeans contain oil as much as 18-20% of the total dry seed weight [2]. Soybean oil contains more unsaturated fatty acids than palm oil, making it healthier for the body. Unsaturated fatty acids reduce cardiovascular risk, while saturated fatty acids increase the risk [3].

An extraction method is needed to extract the oil content from soybeans efficiently. One method that can be used is soxhletation. The principle of Soxhlet extraction is repeated leaching with recycled fresh solvent to obtain good results with less solvent than the conventional method. Heat in soxhletation also increases the solvent's ability to extract insoluble compounds at room temperature. Heat-assisted soxhletation has a better yield than maceration extraction [4]. The most common solvent used in oil extraction is n-hexane. Growing concerns regarding food safety concepts and environmentally friendly processes encourage researchers to evaluate and recommend alternative solvents for vegetable oil extraction.

Many studies have been conducted as an alternative to replace the use of hexane solvents, including the use of ethanol [5], isopropyl alcohol [6], 1-butanol and ethyl acetate [7]. Meanwhile, soybean oil extraction has been carried out using ethanol [8], ethyl acetate & 1-butanol [7], and 2-methyloxolane [9].

Among these solvents, ethanol is a polar solvent that is widely produced throughout the world. 2019 world ethanol production was recorded at more than 85 million tons [10]. It is relatively cheap and environmentally safe, making it widely used in lipid extraction for food applications [11]. The high polarity of ethanol makes it penetrate cell membranes well to extract lipids [12]. Ethanol has also been commonly used in the extraction of various other components, such as anthocyanins [13], polyphenols [14], tannins [15], and flavonoids [16].

Apart from ethanol, ethyl acetate, which is a semi-polar ester compound, also has the potential to be used as an alternative solvent. Ethyl acetate is a semi-polar ester compound with a boiling point close to ethanol and is available in food-grade quality, which is preferred for extracting food. According to the study results of Lohani et al. (2015), ethyl acetate produces a yield that is competitive with n-hexane, much better than ethanol in various types of vegetable oil, so it has the potential to be used [17].

From an economic point of view, the solvent price may make the extraction process less attractive, so it is necessary to conduct a cost analysis study to obtain a feasible solvent. Techno-economic studies for the plant extraction process have been previously carried out on the lab-scale extraction of natural teak leaf dye [18]. However, to our knowledge, evaluating the cost of the lab-scale soybean oil extraction process has never been carried out. This study aims to evaluate the effect of the polarity of the alternative solvent on soybean oil extraction in terms of yield, physical characteristics, oil composition, and lab-scale costs.

2. Materials and Method

2.1 Materials

The materials used were dried soybeans from the local market. Ethyl acetate 90%, ethanol 70%, n-hexane 95% and distilled water from a local chemical store.

2.2 Extraction of Soybeans

The soybeans were cleaned of impurities and mashed with a blender. The refined soybeans were wrapped using filter paper, then put into the soxhlet extraction column. The ratio of material and solvent is 1:4 and 2:4 (w/v). For a ratio of 2:4, the amount of material used is 125 grams in 250 mL of solvent. The solvent is put into the boiling flask, then boiling stones will be added to the boiling flask. The heater is heated to a temperature of 70°C. If the temperature has reached 70°C, then the extraction stage is carried out for 1 hour at a composition of 1:2 (w/v). The same method is repeated for 2 hours, and a different composition has been determined, with a ratio of 1:4 (w/v). After extraction, the mixture was filtered and evaporated using an IKA digital rotary evaporator at 50 rpm and a temperature of 60°C. The soxhlet unit can be seen in Figure 1.

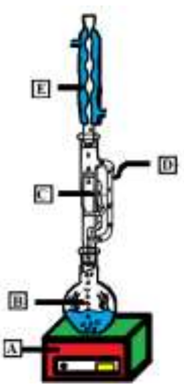


Figure 1. Soxhlet unit experiment: A: Heater; B: Boiling Flask; C: Filter Paper; D: Extractor Column; E: Condenser

2.3 Soybean Oil Analysis

The soybean oil produced was analyzed for density using a pycnometer. The composition of the soybean oil with the best yield was analyzed using gas chromatography-mass spectrometry (GCMS) at the Forensic Laboratory Center of the Indonesian National Police Headquarters (Puslabfor Sentul).

2.4 Lab-Scale Cost Analysis

The calculations include fixed and variable costs for the best yield for each solvent. The costs incurred are divided into fixed and variable costs [18]. Calculations are carried out based on one year of production with a cycle time of 4 hours, as seen in Table 1. It is assumed that there are 1920 cycles in one year.

Table 1. Extraction cycle time

Activity	Time (Hour)
Extraction equipment preparation	0.5
Soybean preparation	0.5
Extraction process	2
Cleaning up	1
Total time	**4**

3. Result and Discussion

3.1 Yield and Physical Characteristics of Soybean Extract

Based on Table 2, ethyl acetate produced the highest yield with a sample-to-solvent ratio of 2:4 (w/v) within 2 hours. Ethyl acetate is semi-polar, which can dissolve the oil in soybeans, potentially producing more oil. Even though it tends to be safer, ethanol produces less extract because ethanol is polar and struggles to extract non-polar oil. Since ethyl acetate is semi-polar, not only oil-soluble components can be extracted but also water-soluble components, which causes the resulting yield to be better than n-hexane and ethanol. According to research by Gasparetto et al. (2022), ethyl acetate produces better oil extraction rates than polar solvents [7]. Soybean composition is 15.85-19.49% oil, 6.60-7.50% fiber, 10.20-8.40% moisture, 45.64-41.67% crude protein, and 5.50-6.90% ash [19]. Solvent polarity causes differences in the compounds extracted, thus, different yields obtained. Ethyl acetate has a lower relative energy difference value than n-hexane, making it a better solvent than n-hexane for dissolving oil components [20]. The solvent purity also contributes to the resulting yield. The use of solvents containing water causes a decrease in the solubility of non-polar compounds in the solvent, thereby causing a decrease in yield [8][21] [22].

From Table 2, we also get the effect of time on the extraction results. For all samples, the longer the extraction time, the better the yield obtained. The longer the contact time, the better the mass transfer from the soybean to the solvent until it finally reaches equilibrium. Similar phenomena were also found in other studies [23][24] [25]. Table 2 shows that the more sample mass at a constant solvent volume, the greater the yield. More samples mean greater solute concentration difference between the sample and solvent, which results in better mass transfer. If extraction is continued for a longer time, the opposite may occur, where a smaller sample-to-solvent ratio will produce a better yield if equilibrium is reached, inhibiting further mass transfer. The phenomenon is supported by a study by Fahriya & Shofi (2011) where the lower sample-to-solvent ratio produces higher solute content due to the distribution of the solute in the solvent being more spread out, thereby expanding the contact surface [26]. Other studies also obtained similar results [27].

Table 3 compares this study's Soxhlet extraction conditions and yields with the reference. It was found that the yield obtained was still better than that obtained by Dari (2009), which may have occurred because of the solvent used. The results obtained were still inferior compared to the study conducted by Rosalina et al. (2018) and Gandhi et al. (2003) due to the lower purity of the solvent and extraction time used.

Table 2. The yield of soybean oil

Composition (w/v)	t (hour)	Yield (oil w/seeds w %)		Reference solvent
		Solvent		
		Ethanol	Ethyl acetate	n-hexane
2:4	1	9.06	34.78	11.83
	2	27.40	36.25	31.84
1:4	1	5.36	3.61	1.53
	2	10.96	5.15	3.13

Table 3. Comparison of soxhlet extraction results with references

Reference	Solvent	Temperature (°C)	Time (minutes)	Soybean: solvent ratio	Yield (g/100g soybean)
This study	Ethanol 70%	70	120	1:4	27,4
This study	Ethyl acetate 90%	70	120	1:4	36,25
This study	N-hexane 95%	70	120	1:4	31,84
Dari (2009) [28]	Trichloroethylene	Solvent boiling point	150	1:10	18,8
Rosalina et al. (2018) [29]	N-hexane (p.a.)	70	120	1:7,5	48,68
Gandhi et al. (2003) [30]	N-hexane (pure)	Solvent boiling point	480	1:2	99,5
Gandhi et al. (2003) [30]	Ethanol 96%	Solvent boiling point	480	1:2	95

Table 4 shows that the density of soybean extract using ethanol is higher than other samples because ethanol is polar and can dissolve other polar compounds, which might affect the resulting density. In contrast to n-hexane, which has non-polar properties, and ethyl acetate, which is semi-polar, the resulting density is close to the density range of soybean oil because it can extract more soybean oil than ethanol. Based on the results of research by Marlina and Ramdan (2019), it is known that peanut oil density is as large as 0.864 - 0.891 g/mL [31]. Soybeans contain various components, such as 13.45-20.38% oil and 38.97-44.46% protein [32]. Due to the semi-polar nature of ethyl acetate, some of the polar compounds in soybeans are also extracted and might cause the density obtained to be slightly above the density range of soybean oil.

Table 4. The density of soybean extract

Solvent	Soybean oil density (g/mL)
Ethanol	1.03
Ethyl acetate	0.92
n-hexane	0.87

From Figure 2, 3, and 4, in terms of color, ethanol produces a slightly pale color compared to ethyl acetate or n-hexane. The ethanol used is not pure and contains water. Thus, more polar components are carried into the oil [22]. It allows the dispersion of polar compounds in the oil and causes a cloudy color. In terms of color, the ethyl acetate is superior to the ethanol because the color produced is similar to the n-hexane due to better polarity and purity.

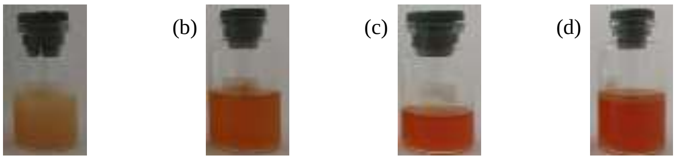


Figure 2. Soybean oil with ethanol (a) 1:4 1 hour; (b) 1:4 2 hours; (c) 2:4 1 hour; (d) 2:4 2 hours

Figure 3. Soybean oil with ethyl acetate (a) 1:4 1 hour; (b) 1:4 2 hours; (c) 2:4 1 hour; (d) 2:4 2 hours

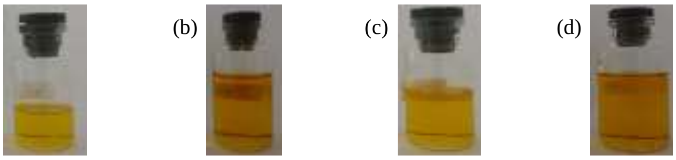


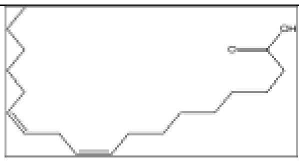
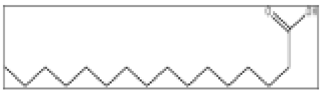
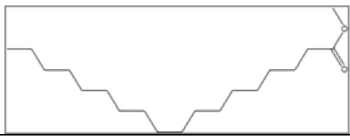
Figure 4. Soybean oil with n-hexane (a) 1:4 1 hour; (b) 1:4 2 hours; (c) 2:4 1 hour; (d) 2:4 2 hours

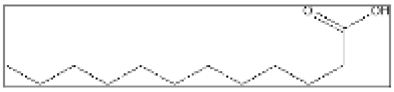
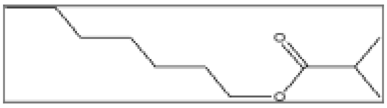
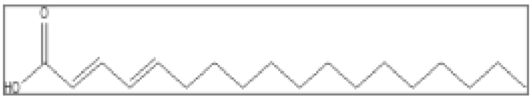
3.2 Yield and physical characteristics of soybean extract

Table 5 shows that the sample extracted using ethyl acetate contains linoleic acid, palmitic acid, stearic acid, lauric acid, and octadecadienoic acid. This result differs slightly from extraction using n-hexane, which produces stearic acid, lauric acid, and heptyl isobutyrate. Methyl ester formation is possible during material handling [33]. Ethyl acetate produces oil with higher unsaturated fatty acid concentration than n-hexane. This is very beneficial because unsaturated fatty acids are healthy for the heart, while saturated fatty acids can cause cardiovascular disease [34][35].

The fatty acid composition has several similarities and differences with the literature. Puspita (2016) found compounds in soybean oil: linoleic acid, stearic acid, and palmitic acid [36]. Other studies obtained different results, namely linolenic acid, linoleic acid, palmitic acid, stearic acid, and oleic acid, with/without elaidic acid [37]. The differences in composition are caused by the extraction method and solvent used, as occurred in other studies with different samples, namely bovine milk [38], *Tecoma stans* seed [39], and *Nannochloropsis oceanica* [11]. Genotype differences also affect the fatty acid composition [37].

Table 5. Fatty acid composition of the soybean oil

Compound		Total Area (%)	
		n-hexane	ethyl-acetate
Linoleic acid (C18:2)		-	34.36
Palmitic acid (C16:0)		-	22.12
Stearic acid (C18:0)		10.81	19.40

Lauric acid (C12:0)		54.22	4.69
Heptyl Isobutyrate		34.97	-
Octadecadienoic acid (C18:2)		-	19.43
Total unsaturated fatty acids		**54.22**	**73.19**
Total saturated fatty acids		**10.81**	**26.81**
Methyl ester		**34.97**	**-**
Ratio total unsaturated/ saturated fatty acid		**5.01**	**2.72**

3.3 Cost analysis

Based on the data in Table 6, the main obstacles in developing this extraction process are employee salaries (30.17-39.55% of total cost) and solvents (13.18-33.79% of total cost). Regarding equipment depreciation costs, the rotary evaporator is an obstacle because it produces the most considerable costs (8.60-11.28% of total cost). Zulfa and Kumalaningsih (2014) also found the same thing, stating that rotary evaporators caused obstacles to the development of laboratory-scale extraction processes [18]. In the extraction process, which is carried out on a small scale, much of it relies on human labor, so equipment depreciation costs are relatively small compared to employee salaries and solvent purchases. Investing in equipment may be greater than the employee's salary on a larger scale. Also, purchasing solvent on an industrial scale will get a relatively lower price. The extract production cost per kg was obtained with ethyl acetate solvent, 647,232 rupiah/kg, ethanol 692,050 rupiah/kg, and n-hexane 780,847 kg/rupiah. However, a more in-depth study needs to be carried out to determine the quality of the oil produced to analyze the trade-off between yield, oil quality, and costs incurred more accurately.

Table 6. Cost analysis of soxhlet extraction

Cost	Solvent								
	N-Hexane			Ethyl acetate			Ethanol		
	Annual cost (Rp)	Sub percentage*	Total percentage**	Annual cost (Rp)	Sub percentage*	Total percentage**	Annual cost (Rp)	Sub percentage*	Total percentage**
1. Fixed Cost									
a. Equipment									
Mesh filter	35,000	0.14%	0.06%	35,000	0.14%	0,06%	35,000	0.14%	0,08%
Soxhlet set 1L	300,000	1.19%	0.50%	300,000	1.19%	0,53%	300,000	1.19%	0,66%
Heater	100,000	0.40%	0.17%	100,000	0.40%	0,18%	100,000	0.40%	0,22%
Condenser pump	125,000	0.50%	0.21%	125,000	0.50%	0,22%	125,000	0.50%	0,27%
Cooling water basin 23L	80,000	0.32%	0.13%	80,000	0.32%	0,14%	80,000	0.32%	0,18%
Hose 8 meter	32,000	0.13%	0.05%	32,000	0.13%	0,06%	32,000	0.13%	0,07%
Thermometer	17,000	0.07%	0.03%	17,000	0.07%	0,03%	17,000	0.07%	0,04%
Digital scale	275,800	1.10%	0.46%	275,800	1.10%	0,49%	275,800	1.10%	0,61%
Rotary evaporator 4L	5,133,333	20.44%	8.60%	5,133,333	20.44%	9,12%	5,133,333	20.44%	11,28%
Digital timer	12,000	0.05%	0.02%	12,000	0.05%	0,02%	12,000	0.05%	0,03%
Oven 30L	855,000	3.41%	1.43%	855,000	3.41%	1,52%	855,000	3.41%	1,88%
Blender 2L	54,800	0.22%	0.09%	54,800	0.22%	0,10%	54,800	0.22%	0,12%
Measuring cup 100 mL	14,800	0.06%	0.02%	14,800	0.06%	0,03%	14,800	0.06%	0,03%
Pipette 10 mL	4,400	0.02%	0.01%	4,400	0.02%	0,01%	4,400	0.02%	0,01%
Drop pipette	2,500	0.01%	0.00%	2,500	0.01%	0,00%	2,500	0.01%	0,01%
Rag	10,000	0.04%	0.02%	10,000	0.04%	0,02%	10,000	0.04%	0,02%
Boiling stone	50,000	0.20%	0.08%	50,000	0.20%	0,09%	50,000	0.20%	0,11%
Watch glass dish	3,333	0.01%	0.01%	3,333	0.01%	0,01%	3,333	0.01%	0,01%
Glass funnel 60 mm	3,700	0.01%	0.01%	3,700	0.01%	0,01%	3,700	0.01%	0,01%
Total equipment cost	**7,108,667**	**28.31%**		**7,108,667**	**28.31%**		**7,108,667**	**28.31%**	
b. Monthly labour salary	**18,000,000**	**71.69%**	**30.17%**	**18,000,000**	**71.69%**	**31,97%**	**18,000,000**	**71.69%**	**39,55%**
Total annual fixed cost	**25,108,667**	**100%**		**25,108,667**	**100%**		**25,108,667**	**100%**	
2. Variable cost									
a. Consumables									
Soybean	4,800,000	13.89%	8.04%	4,800,000	15.38%	8,52%	4,800,000	23.53%	10,55%
Solvent	20,160,000	58.33%	33.79%	16,800,000	53.85%	29,84%	6,000,000	29.41%	13,18%
Filter paper	1,152,000	3.33%	1.93%	1,152,000	3.69%	2,05%	1,152,000	5.65%	2,53%
Ice cube	3,840,000	11.11%	6.44%	3,840,000	12.31%	6,82%	3,840,000	18.82%	8,44%

Cost	Solvent								
	N-Hexane			Ethyl acetate			Ethanol		
	Annual cost (Rp)	Sub percentage*	Total percentage**	Annual cost (Rp)	Sub percentage*	Total percentage**	Annual cost (Rp)	Sub percentage*	Total percentage**
Bottle 100mL	2,880,000	8.33%	4.83%	2,880,000	9.23%	5,11%	2,880,000	14.12%	6,33%
b. Electricity									
Heater	208,037	0.60%	0.35%	208,037	0.67%	0,37%	208,037	1.02%	0,46%
Rotary evaporator	69,346	0.20%	0.12%	69,346	0.22%	0,12%	69,346	0.34%	0,15%
Condenser pump	346,728	1.00%	0.58%	346,728	1.11%	0,62%	346,728	1.70%	0,76%
Blender	121,355	0.35%	0.20%	121,355	0.39%	0,22%	121,355	0.59%	0,27%
Oven	416,074	1.20%	0.70%	416,074	1.33%	0,74%	416,074	2.04%	0,91%
c. Water									
Washing water	540,000	1.56%	0.90%	540,000	1.73%	0,96%	540,000	2.65%	1,19%
Cooling water	27,000	0.08%	0.05%	27,000	0.09%	0,05%	27,000	0.13%	0,06%
Total variable cost	**34,560,539**	**100%**		**31,200,539**	**100%**		**20,400,539**	**100%**	
Total cost	**59,669,205**		**100%**	**56,309,205**		**100,00%**	**45,509,205**		**100%**
Production cost per kg	780.847			647.232			692.050		

***Sub percentage was calculated by dividing the cost with total cost of its group (e.g. total fixed cost or total variable cost)**

****Total percentage was calculated by dividing the cost with total cost**

4. Conclusion

The highest yield was obtained with a sample-to-solvent ratio of 2:4 (w/v) using ethyl acetate for two hours at 36.25% with a density of 0.92 g/mL. The polarity of the solvent used influences the differences in yield and density of the extract. The soybean oil color with ethyl acetate is similar to using n-hexane. Ethyl acetate extracts more unsaturated fatty acid than n-hexane. The three highest concentrations are linoleic, palmitic, and stearic acids. Ethyl acetate as a semi-polar solvent can be recommended as an alternative solvent to replace non-polar n-hexane for soybean oil extraction. The most significant cost in lab-scale development is labor costs. Regarding equipment cost, the rotary evaporator is the main obstacle to the development of lab-scale extraction. The data obtained might help optimize the soybean extraction process to be analyzed on a larger scale.

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