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Comparison of hemp (*Cannabis sativa* L.) seed oil by conventional and soxhlet extraction methods

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ABSTRACT

Hemp (*Cannabis sativa* L.) is a multi-purpose plant that recently got lots of attention in Thailand. In previous years, various studies have been done regarding the application of hemp fiber, especially in the textile sector. After the government has unlocked regulation regarding hemp cultivation and its application, hemp seed has become a remarkable part as oil content in the seeds can be used for multiple purposes. The hemp oil extraction process becomes more important to get a higher yield. So, this research interest has been focused on comparing conventional and soxhlet extraction methods using water, n-hexane, and petroleum ether as extraction solvents. The experimental results showed that solvent has an impact on the extraction efficiency of hemp seed oil as well as the extraction method. Petroleum ether as a solvent by the soxhlet extraction method showed a significantly ($P<0.05$) higher yield (24.44%) compared to those of conventional extraction (17.06%). Additionally, using n-hexane as a solvent by the soxhlet extraction method showed a higher yield (21.65%) than the conventional extraction (18.78%). However, water has better efficiency in conventional extraction (4.56%) than the soxhlet extraction method (1.27%). According to the experimental results, the soxhlet extraction method using petroleum ether as an extraction solvent is recommended to get a higher oil yield from hemp seed.

Keywords: hemp seed, oil extraction method

INTRODUCTION

Hemp (*Cannabis sativa* L.) and marijuana were classified by morphological characteristics and phytochemistry. Hemp and marijuana are plants in temperate regions of Asia. In the past, both used to benefit fiber, and the cultivation of a plant used to make a popular local narcotic. This plant is widely known to be the major source of Cannabinoids such as Cannabidiol (CBD), Tetrahydrocannabinol (THC), and cannabinol (CBN). Cannabinoids have been shown vital addictive substances and affect the brain. Cannabidiol (CBD) has active anti-tetrahydrocannabinol, which effectively reduces anxiety symptoms and pain relief. (Pinmanee, 2019).

Currently, Thailand has liberalized the permission for hemp cultivation and its application in the food, cosmetic, and medical sectors. Availability of hemp in Thailand, the Highland Research and Development Institute (Public Organization) has been developed the hemp variety and promotes the cultivation of hemp as a new economic crop with the suitable and higher potential of 4 varieties (RF1-4) (Pinmanee, 2019).

Hemp seed oil is valued primarily for its nutritional properties and the health benefits associated with it. Although its fatty acid composition is most often noted, with oil content ranging from 25-35%, whole hemp seed is additionally comprised of approximately 20-25% protein, 20-30% carbohydrates, 10-15% fiber, and 25% fat along with an array of trace minerals (Deferne and Pate, 1996). Fat consists of essential fatty acids or omega 6 vs. omega 3 at the ratio of 1:3, which is suitable for the human body.

So, this research interest has been paid to compare the hemp seed oil from 2 different extraction methods of conventional and soxhlet extraction using 3 other extraction solvents of water, n-hexane, and petroleum ether to know the most effective extraction method and appropriate extraction solvent for recommending further research and product development.

MATERIALS AND METHODS

Material

Hemp seed (*Cannabis sativa* L.) of RPF 1 variety (Figure 1) was cultivated by the Highland Research and Development Institute (Public Organization), Thailand, in 2020, and seeds were harvested from November to December 2020. The moisture content of the hemp seeds was determined according to be 10%, and seed purity was recorded at 98% (Pinmanee, 2019). After being harvested, hemp

seeds were kept at room temperature until their use for the experiments.

Figure 1. Hemp seed (*Cannabis sativa* L.) RPF 1

Composition analysis

Crude protein content of Hemp seed was determined by the Kjeldahl method using protein conversion factor (N x 6.25). Moisture and ash contents were determined using AOAC official methods (Feldsine et al., 2002). Fat content was measured by using Fat Analyzer: Soxhlet Extraction Method (FOSS, ST243 Soxtec). Crude Fiber content was determined using AOAC official methods (Feldsine et al., 2002). Total carbohydrates, including crude fiber, were calculated by then.

Conventional extraction method

Initial oil content in hemp seed was measured by a separatory funnel with water, n-hexane, and petroleum ether; 1 g of ground hemp seeds was extracted with 50 ml solvent until totally depleted (Lavenburg et al., 2021). The whole process took 5 min, and each extraction experiment was conducted three times. The resulting suspension was filtered and removed solvent in a water bath at 80°C and measure the color of the solution. The oil obtained was weighed, and the yield was calculated. Determination was done in triplicate. The extraction yield and efficiency were calculated by the following Eq's:

$$\text{Oil extraction yield (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of sample (g)}} \times 100$$

Soxhlet extraction method

Initial oil content in hemp seed was measured by automatic soxhlet extraction systems with water, n-hexane, and petroleum ether; 1 g of ground hemp seeds was extracted with 50 ml solvent until totally depleted (Porto et al., 2013). The whole process took 30 min at 105 °C. Each extraction experiment was conducted three times. The resulting suspension was filtered and removed solvent in a water bath at 80 °C, then the color of the solution was measured. The oil obtained was weighed, and the yield was calculated. Determination was done in triplicate. The extraction yield and efficiency were calculated by the following Eq's

$$\text{Oil extraction yield (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Mass of sample (g)}} \times 100$$

Color analysis

Color measurements of extracted oil were performed using a Hunter Lab colorimeter (Color Flex EZ, ASTM E380). The instrument was pre-calibrated with a standard white and blackboard before sample measurement. The color parameters were expressed in terms of L* (lightness), a* (red/green), and b* (yellow/blue) values.

Statistical analysis

The experiments were repeated three times. The experimental results obtained were subjected to analysis of variance (ANOVA), followed by Duncan's multiple range test procedures for SPSS. The significance of differences was defined at a 95% confidence level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximate composition analysis

The result of proximate analysis of hemp seed (*Cannabis sativa* L.) RPF 1 is shown in Table 1. There is a composition of 5.81% moisture content, 5.12% ash, 36.87% crude protein, 24.43% crude fat, 12.04% carbohydrate, and 0.65 water activity. According to the experimental results, especially protein content contained in analyzed hemp seeds shows higher content compared to those in egg protein (12.5%) (Rhault-Godbert *et al*., 2019), in Brazil nut seed (14.47%) (Lima, 2021), in almond seed (24.95%) (Lima, 2021), and in soy seed (14.8%) (Voleka *et al*., 2018).

Table 1. Proximate analysis of hemp seed (*Cannabis sativa* L.) RPF 1.

Parameter	Contain in Hemp seed
Moisture (%)	5.81±0.25
Ash (%)	5.12±0.08
Protein (%)	36.87±0.75
Fat (%)	24.43±0.15
Fiber (%)	12.04±2.06
Carbohydrate (%)	27.75±0.54
Water activity, Aw	0.65±0.00

Conventional and Soxhlet extraction analysis

The extraction yield and efficiency of different extraction methods and solvents were calculated (Table 2). The results showed that conventional extraction of hemp seed oil extracted by n-hexane shows the highest amount of hemp seed oil with a percent yield of 18.78%, followed by petroleum ether of 17.06%, and water of 4.56%, respectively. Additionally, the soxhlet extraction of hemp seed oil extraction by petroleum ether shows the highest amount of hemp seed oil with a percent yield of 24.44%, followed by n-hexane of 21.65% and water of 1.27%, respectively. As the results of hemp seed oil contents from different extraction solvents showed a significant difference at the 95% confidence level (P≤0.05) due to the polarity difference of the extraction solvent. According to the experimental results, it showed that oil content in RPF 1 hemp seed is higher content compared to the oil content in other seeds such as soy seed (19%) or sunflower seed (24%) (Voleka et al., 2018). Also, soxhlet extraction is one of the effective methods used for oil extraction, as shown in some previous studies. For example, Hu et al. (2021) reported that soxhlet extraction has oil yield higher than microwave-assisted extraction of *Sapindus mukorossi* seed and Eikani et al. (2012) reported that soxhlet extraction has oil yield higher than cold pressing in pomegranate seed. The colors of hemp seed oil extracted by different extraction methods and solvents are shown in Table 3. There was a significant difference (P≤0.05) in L*, a*, and b*, due to the pigment which was extracted by each solvents' ability.

Table 2. Hemp seed oil obtained from different extraction methods and solvents

Solvents	Conventional extraction method (% Yield)	Soxhlet extraction method (% Yield)
Water	4.56 ± 0.07^{cA}	1.27 ± 0.01^{cB}
n-hexane	17.06 ± 0.11^{bB}	21.65 ± 0.01^{bA}
petroleum ether	18.78 ± 0.02^{aB}	24.44 ± 0.01^{aA}

Remark: a-c shows significant difference at 95% confident level (P≤0.05) in column A-B shows significant difference at 95% confident level (P≤0.05) in row

Table 3. Colors of hemp seed oil obtained from different extraction methods and solvents

Solvents	Conventional extraction method			Soxhlet extraction method		
	L*	a*	b*	L*	a*	b*
Water	5.53^{c}	-0.41^{a}	0.07^{c}	38.22^{a}	-2.06^{c}	-5.12^{c}
n-hexane	7.94^{b}	-0.71^{b}	0.66^{a}	12.06^{b}	-1.13^{b}	2.42^{b}
petroleum ether	8.42^{a}	-0.76^{c}	0.33^{b}	3.51^{c}	-0.32^{a}	0.75^{a}

Remark: a-c shows significant difference at 95% confident level (P≤0.05) in column

CONCLUSIONS

The differences in extraction methods and solvents show differences in extraction efficiency in hemp seed oil extraction. According to the experimental results, soxhlet extraction method trends to be a more effective method than the conventional method, and petroleum ether works better as an extraction solvent in both conventional and soxhlet methods compared to those of water and n-hexane. So, it was concluded that the suitable and recommended method for getting a higher yield of hemp seed oil from RPF 1 hemp seed is the soxhlet extraction method using petroleum ether as a solvent.

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REFERENCES

Deferne, J.L., and Pate, D. W. 1996. Hemp seed oil: A source of valuable essential fatty acids. Journal of the International Hemp Association. 3: 4-7.

Eikani, M.H., Golmohammada, F., and Homami, S.S. 2012.Extraction of pomegranate (*Punica granatum* L.) seed oil using superheated hexane. Food and Bioproducts Processing. 90: 32-36.

Feldsine, P., Abeyta, C., and Andrews, W.H. 2002. Methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. Journal of AOAC international. 85: 1187-1200, https://doi.org/10.1093/jaoac/85.5.1187.

Hu, B. Xi, X. Li, H. Qin, Y. Li, C. Zhang, Z. Liu, S., and Luo, O. 2021 A comparison of extraction yield, quality and thermal properties from *Sapindus mukorossi* seed oil between microwave assisted extraction and Soxhlet extraction. Industrial Crops & Products 161: 113185.

Lavenburg, V.M., Rosentrater, K.A., and Jung, S. 2021. Extraction methods of oils and phytochemicals from seeds and their environmental and economic impacts. Processes. 9: 1839. https://doi.org/10.3390/pr9101839.

Lima, D.S. 2021. Technological quality and sensory acceptability of nutritive bars produced with Brazil nut and Baru almond coproducts. LWT- Food Science and Technology. 137: 110467.

Pinmanee, S. 2019. Hemp varieties and legally certified seeds. The Highland Research and Development Institute (Public Organization). https://www.hrdi.or.th/articles/detail/47 (in Thai)

Porto, C.A., Porretto E., and Decorti, D. 2013. Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. Ultrasonics Sonochemistry. 20: 1076–1080.

Rehault-Godbert, S., Guyot, N., and Nys, Y. 2019. The golden egg: Nutritional value, bioactivities, and emerging benefits for human health. Nutrients. 11(3): 684. https://doi.org/10.3390/ nu11030684

Voleka , Z., Ebeidb, T.A., and Uhlirova, L. 2018. The impact of substituting soybean meal and sunflower meal with a mixture of white lupine seeds and rapeseed meal on rabbit doe milk yield and composition, and the growth performance and carcass traits of their litters. Animal Feed Science and Technology. 236: 187-195.