GRASAS Y ACEITES 74 (3)
July-September 2023, e515
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.1128212

Optimization and evaluation of a hydration method for producing high quality oil and defatted meal from hemp seed kernels

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Submitted: 19 November 2021; Accepted: 29 November 2022; Published online 10 October 2023

SUMMARY: A green and efficient method for producing high quality oil and defatted meal is in high demand, which has promoted the development of a hydration method for extracting oils from hem seed kernels. The hydration method optimized in this study recovered > 96% oil, which was further proved by infrared spectra, and extracted > 91% tocopherols, vitamin A, carotenoids, coenzyme Q10, phytosterols and squalene into the oil phase; while only small portions of flavonoids, other phenolic compounds and free fatty acids were extracted. The defatted meal was rich in water-soluble vitamins (including thiamin, riboflavin, niacin, pyridoxine, folate and vitamin C), proteins, dietary carbohydrates and phospholipids. The hydration method produced oils with lower AV or PV compared to solvent extraction, supercritical CO_2 and cold-pressing methods and a defatted meal with a protein content (52.69%) which was significantly higher than that obtained by supercritical CO_2 and cold-pressing methods. The hydration method is a type of green technology for sustainablly processing hempseeds.

KEYWORDS: *Green technology; High oil recovery rate; Hydration of non-oil solids; Sustainability.*

RESUMEN: ***Optimización y evaluación de un método de hidratación para producir aceite de alta calidad y harina desgrasada a partir de semillas de cáñamo.*** Se demanda métodos verdes y eficientes para producir aceite y harina desgrasada de alta calidad, lo que promovió el desarrollo de un método de hidratación para extraer aceites de semillas de cáñamo. El método de hidratación optimizado en este estudio recuperó >96 % de los aceites que, además, se demostró mediante espectros infrarrojos, extrajo >91 % de tocoferoles, vitamina A, carotenoides, coenzima Q10, fitoesteroles y escualeno en la fase oleosa, mientras que solo se extrajeron pequeñas porciones de flavonoides, otros compuestos fenólicos y ácidos grasos libres. La harina desgrasada era rica en vitaminas hidrosolubles (incluyendo tiamina, riboflavina, niacina, piridoxina, folato y vitamina C), proteínas, carbohidratos dietéticos y fosfolípidos. El método de hidratación produjo aceites con AV o PV más bajos en comparación con los métodos de extracción por solventes, CO_2 supercrítico y prensado en frío y harina desgrasada con un contenido de proteína (52,69%) significativamente mayor que el obtenido por los métodos de CO_2 supercrítico y prensado en frío. El método de hidratación es un tipo de tecnología ecológica para el procesamiento sostenible de semillas de cáñamo.

PALABRAS CLAVE: *Alta tasa de recuperación de aceite; Hidratación de sólidos no oleosos; Sostenibilidad; Tecnología verde.*

Citation/Cómo citar este artículo: Wang S, Wu W. 2023. Optimization and evaluation of a hydration method for producing high quality oil and defatted meal from hemp seed kernels. *Grasas Aceites* **74** (3), e515. https://doi.org/10.3989/gya.1128212

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1. INTRODUCTION

The whole seeds of Hemp (*Cannabis sativa* L.) with < 0.2% δ-9-tetrahydrocannabinol, widely cultivated as an industrial crop (Petrović *et al.*, 2015) usually contain ca. 30% oil and ca. 24% protein, as well as a significant amount of dietary fiber, vitamins and minerals (House *et al.*, 2010). The Organization for Economic Cooperation and Development - Food and Agriculture Organization of the United Nations (OECD-FAO) projected that the annual per capita consumption of vegetable oils as foods may reach 27 kg in developed countries with an annual growth of 0.9% from 2020 to 2029 (OECD/FAO, 2020). Oils should also be good material for making such products as surfactants, lubricants and biodiesel (Pal *et al.*, 2019; Farooq *et al.*, 2019) and extracting fat-soluble bioactive compounds (Yara-Varón *et al.*, 2017). Furthermore, the annual protein demand for food security in the world was projected to be 31 kg per capita, while annual protein intake will reach 41 kg per capita by 2029 (OECD/FAO, 2020). Proteins are also applicable to other industries, such as protein fibers in the textile industry (Stenton *et al.*, 2021) or in medicine (DeFrates *et al.*, 2018). If animal meats are used for the protein source needed, greenhouse gas emissions (GHGE) will be huge. For example, agricultural GHGE may increase from 11 to 24% by 2029, 2/3 of it should be contributed by livestock (for obtaining animal meat proteins) (OECD/FAO, 2020). Nonmeat protein alternatives such as proteins from oilseeds are able to greatly decrease GHGE, which is valuable for environmental protection. Therefore, a method which can simultaneously produce high quality oil and defatted meal from hemp seeds is very valuable.

Solvent extraction is currently the most common method used for the commercial separation of seed oils, but the disadvantages of time-consuming, solvent consumption, high fire or explosion risk, residual toxicity, adverse effect on environment and high equipment investment costs have also been reported (Escorsim *et al.*, 2018; Crimaldi *et al.*, 2017). The application of supercritical fluids (such as supercritical CO_2) is presently restricted because this method can only be operated in batches and production on a large scale is difficult (Aladic *et al.*, 2014). Cold pressing has a low recovery rate (< 80%) of hemp seed oils (Crimaldi *et al.*, 2017). Hot pressing produces defatted meal with very poor quality.

Therefore, the purpose of this study is to develop a new method, i.e. hydration, for the production of oils which are rich in fat-soluble bioactive compounds and defatted meal which is rich in proteins and water-soluble bioactive compounds from hemp seeds. Experimental works include the optimization of operating conditions of the method and the evaluation of its efficiency compared to other methods.

2. MATERIALS AND METHODS

2.1. Materials

Hempseeds (grown in Bama, Guangxi, China) were bought from Fangyitan Economy and Trade Co. Ltd, Changchun, Jilin and stored in a cool, dry, sealed bag. Hulled hempseeds contained 6.12% water and 48.10% fat (on wet basis), and were used in all experiments. NaCl (salt) was food and analytical grade.

2.2. General procedure used in the experimentation

The hulled hemp seeds were heated in a microwave oven (TOSHIBA, ER-SS20CNW) at certain output power (variable) and time (variable), cooled, pre-crushed using a blade crusher for 30 s and finally ground by hand to pass through a sieve with certain meshes (variable). The ground hemp seed kernel (GHSK; 10.00 g) and a certain amount (variable) of pure water with a certain amount (variable) of sodium chloride were placed in a 50 mL centrifuge tube. The mixture in the centrifuge tube was agitated for a certain time (variable) and at a certain speed (variable) at room temperature (20 °C) by using a digital display electric mixer (JJ-1A 100W) with a self-made screw-type mixing head. Free oil was collected by centrifugation at 4000 rpm and weighed. The centrifugation residue was quantitatively collected and cold-pressed three times. The cold-pressed residue was quantitatively collected, dried at 50 °C and weighed. All measurements were repeated three times.

2.3. Condition optimization by single factor experimentation

According to the steps (Figure 1) and general procedure ("2.2.") described above, the single fac-

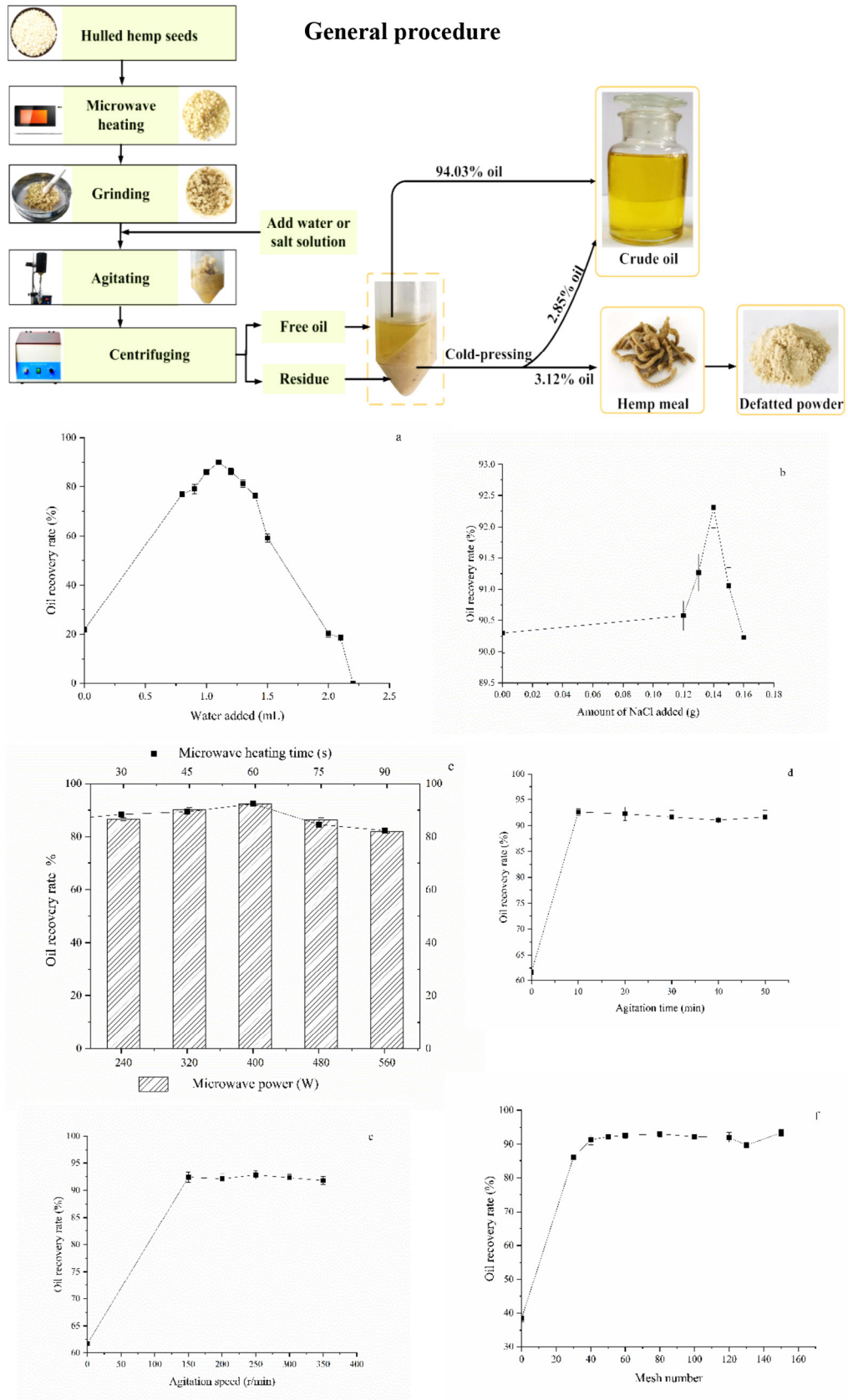

Figure 1. General procedure of hydration method and the effect of extent of hydration, salt, microwave power and microwave heating time, agitation time and speed, and mesh number of sieve on the oil recovery rate (mean±SD: n=3). Note: The average of 3 replicates was calculated.

tor experiment was performed to optimize the extraction conditions. The effect of different amounts (0, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 2.0, 2.1 and 2.2 mL/10 g) of water added on oil recovery rate (ORR) was investigated, while other parameters were fixed to be heated at 400 W for 60 s, the GHSK was passed through a 60-mesh sieve and agitated at room temperature (20 ºC) at 150 r/min for 10 min. The effect of different amounts (0, 0.12, 0.13, 0.14, 0.15 and 0.16 g/10 g) of NaCl added on ORR was investigated, while other parameters were fixed to be heated at 400 W for 60 s, the GHSK was passed through a 60-mesh sieve, 1.10 mL water were added and the mixture was agitated at room temperature (20 ºC) at 150 r/min for 10 min. The effect of different microwave heating times (0, 30, 45, 60, 75 and 90 s) on ORR was investigated, while other parameters were fixed to be heated at 400 W, the GHSK was passed through a 60-mesh sieve, 1.10 mL water and 0.14 g NaCl were added and the mixture was agitated at room temperature (20 ºC) at 150 r/min for 10 min. The effect of different microwave power settings (240, 320, 400, 480 and 560 W) on ORR was investigated, while other parameters were fixed to be heated for 60 s, the GHSK was passed through a 60-mesh sieve, 1.10 mL water and 0.14 g NaCl were added and the mixture was agitated at room temperature (20 °C) at 150 r/min for 10 min. The effect of different agitation speeds (0, 150, 200, 250, 300 and 350 r/min) on ORR was investigated, while other parameters were fixed to be heated at 400 W for 60 s, the GHSK was passed through a 60-mesh sieve, 1.10 mL water and 0.14 g NaCl were added and the mixture was agitated at room temperature (20 ºC) for 10 min. The effect of different agitation times (0, 10, 20, 30, 40 and 50 min) on ORR was investigated, while other parameters were fixed to be heated at 400 W for 60 s, the GHSK was passed through a 60-mesh sieve, 1.10 mL water, and 0.14 g NaCl were added and the mixture was agitated at room temperature (20 ºC) at 150 r/min. The effect of different sieve meshes (30, 40, 50, 60, 80, 100, 120, 130 and 150) on ORR was investigated, while other parameters were fixed to be heated at 400 W for 60 s, 1.10 mL water and 0.14 g NaCl were added and the mixture was agitated at room temperature (20 ºC) at 150 r/min for 10 min.

The residual oil content in the cold-pressed residue was determined by the Soxhlet method (using petroleum ether as a solvent). The oil recovery rate (ORR) was calculated using the following formula:

$$\text{ORRC (\%)} = \frac{\text{Oil}_c}{\text{Oil}_t} \text{x100 or}$$

$$\text{ORRCP (\%)} = \frac{\text{Oil}_t - \text{Oil}_r}{\text{Oil}_t} \text{x100}$$

In the formula, Oil_c represents the weight of free oil obtained by centrifugation (g); Oil_t represents total oil in 10 g GHSK (g; 10 g x oil fraction in GHSK); Oil_r represents oil in the centrifuged and then cold-pressed residue (g; the weight of quantitatively collected dry cold-pressed residue x its oil fraction); ORRC represents the oil recovery rate obtained by centrifugation; ORRCP represents the oil recovery rate obtained by centrifugation followed by cold-pressing.

2.4. Extraction of oils by solvent

The hulled hemp seeds were heated at 330 W for 60 s, cooled and crushed for 30 s using a blade crusher. The crushed hemp seed was extracted with Petroleum ether at 50 ºC for 7 h in a Soxhlet extractor. The residual solvent in the extract was completely removed by vacuum evaporation. The extracted oil was then put into a blast drying oven at 50 ºC and dried to constant weight with the quantity difference of less than 2 mg. The refining of crude oil was carried out according to the method reported by Ma *et al.* (2017).

2.5. Analytical methods

The Chinese National Standard Methods (GB/T 14488.1-2008, GB 5009.229-2016, GB 5009.227-2016, GB 5009.236-2016, GB 5009.262-2016, GB 5009.5-2016, GB/T25223-2010, GB5009.83-2016, GB 5009.82-2016, GB 5009.84-2016, GB 5009.85-2016, GB 5009.89-2016, GB 5009.211-2014, GB 5009.82-2016, GB 5009.86-2016, GB 5009.272-2016, GB 5009.4-2016, GB/T 5510-2011, GB 5009.88-2014, GB 5009.8-2016 and GB 5009.153-2016) were followed to determine the content of crude oil, acid value (AV), peroxide value (PV), moisture and volatile, residual solvent, crude protein, phytosterols, carotenoids, vitamin A, thiamine, riboflavin, niacin, pyridoxine, folate, vitamin C, phospholipids, ash, free fatty acids, fibers, sucrose/glucose and phytates, respectively. The content of total phenolic

compounds was determined according to the methodology reported by Wu and Sun (2011) while that of total flavonoids was measured by the method defined by Han *et al.* (2019). Cannabinoid content was determined following the method developed by Pellegrini *et al.* (2005). After completely extracting the lipids of GHSK and defatted GHSK samples three times (each time 4 h) with petroleum ether by the Soxhlet method and estimating the crude oil contents of the samples, the wax content in the extracted crude oil was then measured according to the method published by The International Olive Council (2017), while squalene and coenzyme Q10 (Co-Q10) were determined according to LS/T6120-2017 (2017) and the method developed by Karpinska and Mularczyk (2004), respectively. The color was analyzed with a colorimeter (HanterLab UltraScan Pro., Hunter Associates Laboratory, Inc., Reston, VA).

2.6. Infrared spectra

A Fourier Transform Infrared Spectrometer with ATR accessory (Spectrum 100, PerkinElmer, Inc.) was used to reveal the functional groups in oils extracted (HMEO) and defatted meal (HMDM) obtained by the hydration method or that (SEO and SEDM) obtained by solvent extraction, respectively. The proper amount of sample ground to pass a 60-mesh sieve was put on an ATR plate which was washed with ethanol before and after the test to remove any oil residue. The sample spectra were recorded in the wave number range of 4000-600 cm^{-1} by cumulating 16 scans at a resolution of 4 cm^{-1}.

2.7. Statistical analysis

Each sample was analyzed in triplicate. Data were obtained as means ± standard deviations (SD) and analyzed by a one-way analysis of variance (ANOVA) and least-significant difference. Differences in paired data and those of more than 3 were estimated by t-test and F-test, respectively.

3. RESULTS AND DISCUSSION

3.1. Optimization of hydration method

3.1.1. Division 1. Optimization by single factor experiments

Hydration. The effect of the extent of hydration on ORR is shown in Figure 1a. Only 21.9% of the total oil from GHSK was recovered by centrifugation without the addition of water. Increases in water addition from 0.00 to 1.10 mL/10 g GHSK significantly increased ORR. However, when water addition increased to be > 1.10 mL/10 g GHSK, ORR gradually decreased. The addition of 1.10 mL water/10 g GHSK resulted in the maximum ORR (90.30%); whereas that of 2.20 mL water/10 g GHSK decreased ORR to zero.

The ORR (90.30%) obtained by the absorption of 1.10 mL water for 10 g GHSK should be considered meaningful since it is much higher than that obtained by cold-pressing (Crimaldi *et al.*, 2017) and enzymatic aqueous extraction (Hou and Wu, 2014). It should be noted that the ORR at 1.10 mL water/10 g GHSK was obtained before the optimization of other conditions. This means that higher ORR is possible when other conditions are optimized.

Another important characteristic of obtaining the maximum ORR at 1.10 mL water/10 g GHSK was that the moisture content of defatted meal was only ca.25%. This moisture content means that drying the defatted meal is quite easy. Therefore, oil separation by hydration is superior to traditional aqueous (including enzyme-assisted) methods in that the discharge of waste water is huge.

Salt addition. The effect of NaCl addition on ORR is shown in Figure 1b. As the amount of NaCl increased from 0.00 g to 0.14 g, ORR continued to increase. However, the addition of more than 0.14 g continued to decrease ORR. The maximum ORR (92.31%) was obtained with NaCl addition at the level of 0.14 g/10 g GHSK, which was significantly higher than that (90.30%) obtained without NaCl addition ($p < 0.05$), but the absolute difference between the ORR obtained with and without the addition of NaCl was only 2.01%, which was not significant ($p > 0.05$).

The amount of salt needed to obtain the maximum ORR only resulted in a salt content of ca. 2% on wet weight basis and ca. 2.7% on dry weight basis in defatted meal. The defatted meal with this range of salt content should be consumable directly. Furthermore, the defatted meal with this range of salt should be applicable for making products such as meat substitute.

Microwave heating time and power. The effect of microwave heating time and power on ORR is shown in Figure 1c. It can be seen that only 86.14%

ORR was obtained without microwave heating. When microwave power was fixed at 400 W, ORR gradually increased as heating time increased from 0 to 60 s, but further extension of heating time gradually reduced ORR. The maximum ORR (92.31%) was obtained with heating for 60 s, which was higher than that obtained by using any other heating time ($p < 0.05$). When heating time was constant at 60 s, increases in microwave power from 240 to 400 w significantly increased ORR, but higher than 400 w gradually reduced ORR. The maximum ORR obtained was not significantly different from that obtained in the experiment on any other microwave power ($p > 0.05$).

The microwave oven used was able to treat 800 g samples. The electricity consumption of microwave treatment for obtaining the maximum ORR was only ca. 0.0083 kwh/kg (i.e. 0.4 (kw) x 1/60 (h) ÷ 0.8 (kg)). This means that microwave treatment not only has a high efficiency for recovering oils, but also saves energy and time, which is beneficial to environmental protection and economics.

Agitating time and speed. The effects of agitating time and speed on ORR are indicated in Figures 1d and 1e, respectively. Figure 1d shows that ORR was only 61.74% when agitation was not carried out, but ORR increased rapidly to 92.50% when the mixture of water and GHSK was agitated for 10 min. As the agitation time extended from 10 min to 50 min, ORR remained relatively stable (not significantly different; $p > 0.05$). Figure 1e shows that increases in the rotating speed of the screw head of the agitator from 150 to 350 r/min had little effect on ORR.

The agitation time of 10 min for obtaining the maximum ORR should facilitate the shortening of the total process circle. This should significantly reduce the risk of microbial growth, save time and energy, and decrease production costs. The range of 0 – 150 r/min was not investigated for the rotation speed of the screw head of the agitator, which does not mean that this range is not worth investigating in future.

Mesh number of sieve. The effect of the sieve mesh number for passing GHSK is indicated in Figure 1f. Crushing the baked HSK for 30 s by using a blade crusher resulted in only 38.46% ORR. When the crushed HSK was further ground to pass through a sieve with 30 – 40 mesh numbers, ORR increased from 86.07% to 92.90%. ORR remained almost the same (ca. 93.20%) when crushed HSK was further ground to pass through a sieve with 60 – 150 mesh numbers, but was not significantly different from that obtained by passing through a number 40 mesh ($p > 0.05$).

A sieve with 40 or 60 mesh number has a pore size of 0.38 mm or 0.25 mm, respectively. HSK ground to pass through a sieve in these ranges of pore size is quite easy to achieve. The ORR obtained by grinding HSK to pass through a sieve with mesh number 40 should be meaningful since it is higher than 92.90%. As viewed from the achievement of the lowest processing cost, the sieve with smaller mesh numbers may be preferred. However, grinding HSK to pass through a sieve with mesh number 60 should also be applicable since this operation is quite easy to achieve and not costly.

Optimal conditions. The optimal operating conditions obtained by single factor experimentation was as the following: heating HSK for 60 s by 400 w microwave, preparing GHSK by crushing the baked HSK for 30 s followed by grinding to pass through a sieve with 40 or 60 mesh number, adding 1.10 mL water and 0.14 g salt (optional), agitating the mixture of GHSK, water and salt for 10 min at 150 r/min and room temperature. Under the conditions established in the general procedure described in section 2.2., and employing the optimal conditions mentioned above, the maximum ORR obtained reached 93.20%.

3.1.2. Division 2. Further optimization by response surface method

The experimental results obtained by response surface method are shown in Table 1. The highest ORR obtained was 92.52% (run 17); whereas the lowest ORR was only 77.23% (run10). The quadratic multiple regression model based on coding factors was as follows:

$$ORR = 90.73 + 4.08A + 0.7710B - 1.13C + 0.0606D - 2.13AB + 2.08AC - 1.22AD + 1.33BC - 2.65BD + 0.7277CD - 2.06A^2 - 1.80B^2 - 4.32C^2 - 2.71D^2$$

(actual equation: $ORR = -727.649000 + 769.282740A + 5593.728340B - 0.777662C + 0.006605D - 2130.977130AB + 1.386000AC - 0.152677AD + 8.835760BC + 3.313410BD + 0.000606CD - 205.561330A^2 - 17957.380460B^2 - 0.019185C^2 - 0.000423D^2$)

The statistical significance of the response surface model is shown in Table 2. The model was extremely

Table 1. Original and coded values of process parameters, experimental design matrix and results of response surface experimentation

Run	A: Water added (mL)	B: NaCl added (g)	C: Microwave heating time (s)	D: Microwave power (W)	Oil recovery rate (%)
1	-1	0	0	-1	80.87
2	0	0	1	1	82.95
3	1	0	0	1	89.40
4	0	0	0	0	90.44
5	1	0	1	0	90.23
6	0	1	0	1	88.77
7	-1	-1	0	0	79.31
8	0	1	-1	0	85.45
9	0	0	0	0	91.06
10	-1	0	1	0	77.23
11	-1	0	0	1	85.14
12	0	1	0	-1	85.86
13	1	1	0	0	89.50
14	0	0	0	0	89.81
15	-1	1	0	0	84.82
16	0	0	0	0	90.64
17	1	-1	0	0	92.52
18	0	0	-1	1	84.72
19	0	-1	-1	0	87.01
20	0	-1	0	-1	88.88
21	0	0	0	0	91.68
22	0	-1	1	0	81.91
23	-1	0	-1	0	82.54
24	0	0	1	-1	80.56
25	1	0	-1	0	87.21
26	1	0	0	-1	90.02
27	0	0	-1	-1	85.24
28	0	-1	0	1	81.19
29	0	1	1	0	85.65

Variable	Symbol	Coded variables levels		
		-1	0	1
Water added/mL	A	1.0	1.1	1.2
NaCl added/g	B	0.13	0.14	0.15
Microwave heating time/s	C	45	60	75
Microwave power/W	D	320	400	480

significant ($p < 0.01$), whereas the lack of fit was not statistically significant ($P > 0.05$), indicating that the model was effective. Furthermore, the R^2 (= 0.9574) of the quadratic model and the low variance (1.39%) indicate that the multiple quadratic regression model (established on the basis of experimental values) simulated the relationship well among all the conditions examined and ORR with high accuracy, high reliability and repeatability. The quadratic multiple regression equation model predicted that the optimal process conditions for extracting hemp seed oil according to the hydration method were as follows: A-1.28 mL, B-0.13 g, C-61 s, D-330 W. The ORR was theoretically predicted to be 94.78%. Under the conditions of the general procedure described in section 2.2. and employing the optimal conditions mentioned above, a verification study was carried out. The study found that the actual ORR was 94.03% while the residual standard error (RSD) was 0.83% and therefore the measured value was basically in line with the predicted value (Less than ± 5% of the RSD should indicate that the model was reliable and the optimization result was credible).

In addition, the effect of the amount of water (A) and salt (B) added, microwave treating time (C) A^2, B^2, C^2, D^2 (microwave power) as well as the interaction of water and salt (A×B), water and microwave treating time (A×C), salt and microwave treating

time (B×C) or salt and microwave power (B×D) on ORR was statistically significant ($p < 0.05$). The contours and response surfaces for the effect of all parameters studied are shown in Figure 2.

The surface response method further optimized operating conditions. The optimal condition combination was different from that obtained by single factor experimentation, but the difference (94.03% – 93.20% = 0.83%) in the ORRs obtained by using the two optimal condition combinations was not significant ($p > 0.05$).

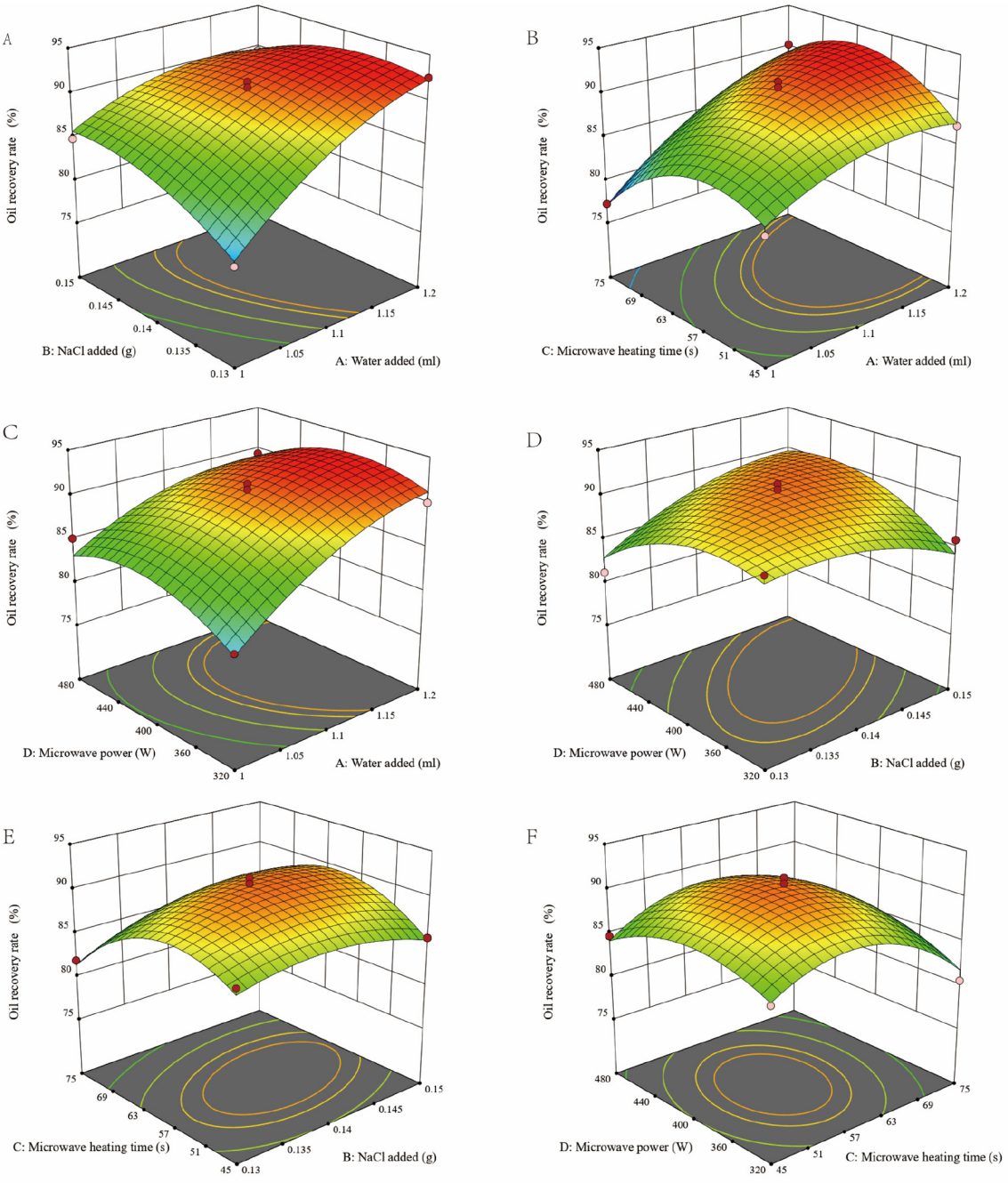

Figure 2. Response surfaces for the cross-effect of the addition of water and NaCl, water and microwave time, microwave power and the addition of water, microwave power and NaCl added, microwave heating time and NaCl added as well as microwave heating time and microwave power on the oil recovery rate.

Table 2. ANOVA for response surface quadratic model and the analysis of variance table (partial sum of squares—Type III); response: oil recovery rate

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	454.12	14	32.44	22.49	< 0.0001**
A-Water added	199.76	1	199.76	138.49	< 0.0001**
B- NaCl added	7.13	1	7.13	4.94	0.0431*
C- Microwave time	15.45	1	15.45	10.71	0.0056**
D- Microwave power	0.0441	1	0.0441	0.0306	0.8637
AB	18.16	1	18.16	12.59	0.0032**
AC	17.29	1	17.29	11.99	0.0038**
AD	5.97	1	5.97	4.14	0.0614
BC	7.03	1	7.03	4.87	0.0445*
BD	28.11	1	28.11	19.49	0.0006**
CD	2.12	1	2.12	1.47	0.2457
A^2	27.41	1	27.41	19.00	0.0007**
B^2	20.92	1	20.92	14.50	0.0019**
C^2	120.86	1	120.86	83.79	< 0.0001**
D^2	47.47	1	47.47	32.91	< 0.0001**
Residual	20.19	14	1.44		
Lack of Fit	18.24	10	1.82	3.73	0.1079
Pure Error	1.95	4	0.4884		
Cor Total	474.32	28			
R^2	0.9574				
Adj R^2	0.9149				
C.V.	1.39				

Note: "*" indicates significant, p < 0 .05; "**" indicates extremely significant, p < 0 .01; Values greater than 0.1000 indicate the model terms are not significant.

3.2. Efficiency of methods for free oil collection

The ORR described in section 3.1., *Divisions 1* and *2* was calculated on the basis of oils collected by centrifugation at 4000 rpm. The maximum ORR (94.03%) corresponded to 5.24% residual oil content in the defatted meal obtained by only centrifugation. Part of the small amount of residual oil in the centrifugation residue can be recovered by cold-pressing. The residual oil in the defatted residue obtained by centrifugation combined with cold-pressing was as low as 2.81%, which corresponded to 96.88% ORR. However, it should be noted that cold-pressing alone without centrifugation to obtain defatted meal with residual oil content as low as 2.81% was found to be difficult.

3.3. Evaluation of efficiency of hydration method by comparison with other methods

A comparison of the hydration method with other methods in terms of extraction conditions, oil extraction or recovery rate as well as oil and defatted meal qualities is shown in Table 3. Although the hydration method had a lower oil extraction rate, it had an identical ORR compared to solvent extraction, which produced crude oil which requires refining responsible for leading to significant losses in neutral oils and bioactive compounds. The hydration method had significantly higher ORR compared to supercritical CO_2 and cold-pressing methods. Figure 4 indicates that absorption peaks at 3008, 2924, 2854 and 1744 cm^{-1} corresponding to oils disappeared in the spectrum of defatted meal (containing 1.37% residual lipids) obtained by solvent extraction. This may mean that the 1.37% residual lipids were not oils. Therefore, the maximum residual oil in the defatted meal obtained by the hydration method might be only 1.44% (i.e. 2.81% – 1.37%), so that the real ORR of this method should be higher than 96.88%.

Table 3 also indicates that the AV and PV of oils extracted by the hydration method were lower than those obtained by solvent extraction or cold-pressing. Only a small amount of water and volatiles (0.06%) were found in the oil sample. Compared to solvent extraction, the hydration method had the advantage of no residual solvent in the extracted oil, which means it can be directly consumed.

Table 3. Comparison of hydration method with other methods in terms of extraction conditions, oil extraction or recovery rate as well as partial oil and defatted meal quality.

Items	Hydration method[a]	Solvent extraction[b]	Supercritical CO_2 extraction[c]	Cold-pressed
Extraction conditions	Material-to-liquid ratio:1:0.13, microwave heating at 330 W for 61 s, GHSK passed a sieve with 60 meshes (0.42 mm)	Microwave heating at 330 W for 61 s, extracting at 50 °C for 7 h	40 °C, 300 bar and, 195 min, <1 mm particle size, 10 mL/min CO_2	Temperature of output press head at 60 °C, frequency of 20 Hz, and nozzle of ID 6 mm (Aladić *et al.*, 2014)
Oil extraction (%)	96.88±1.86	98.63±1.21	93.19	70.00 (Aladić *et al.*, 2014)
ORR (%)	96.88±1.56	94.21±1.32	93.19	-
AV (mg KOH/g)	1.09±0.15	2.19±0.21	-	1.76 (Teh and Birch, 2013)
PV (mmol/kg)	0.93±0.06	1.89±0.18	2.75	1.94 (Teh and Birch, 2013)
Moisture and volatile content (%)	0.06±0.01	-	-	0.08 (Aladić *et al.*, 2014)
Residual solvent (g/kg)	-	1.59±0.17	-	-
Lipid content in defatted meal (%)	2.81±0.19	1.37±0.21	3.27	10.33 (Aladić *et al.*, 2014)
Protein content in defatted meal (%)	52.69±1.25	54.30±1.16	51.20	45.63

[a,b]The average of 3 replicates was calculated; [c]Aladić *et al.* (2014).

Figure 3 also shows that the spectrum of oils extracted by the hydration method was identical to that of the oils extracted by solvent extraction. This may mean that the major compounds in the oils extracted by both methods were identical.

Table 4 indicates that more than 91% of some fat-soluble bioactive compounds were extracted into the oil phase by the hydration method. Although solvent extracted more fat-soluble bioactive compounds into the oil phase, the majority of them should be lost during refining. Compared to solvent extraction, the hydration method had another advantage in that the extraction rate of compounds containing hydrophilic groups into the oil phase was much lower, especially those adversely affecting the stability of the oils such as waxes and free fatty acids.

Table 3 also shows that the hydration method produced the defatted meal with higher or slightly lower protein contents compared to supercritical CO_2 and cold-pressing methods or solvent extraction, respectively. The defatted meal produced by the hydration method should be a good material for making meat analogue or texturized protein in the food industry since it had a low residual oil content (< 5%) and high protein content (> 45%). It also shows potential for many applications in other products in the food industry. It can be a good material for making many products in other industries, such as solar energy conversion or absorption (strong alkali treatment to form black protein) products and graphene oxide-protein conjugates (Simsikova and Sikola, 2017).

In addition, defatted meal obtained by the hydration method was rich in some major water-soluble bioactive compounds: thiamin (2.75 ± 0.11 mg/100 g), riboflavin (0.59 ± 0.05 mg/100 g), niacin (20.07 ± 1.93 mg/100 g), pyridoxine (1.33 ± 0.09 mg/100 g), Folate (0.24 ± 0.02 mg/100 g) and vitamin C (1.07 ± 0.08 mg/100 g). It contained 10.33% total carbohydrates (including insoluble and soluble fibers), 14.39% phytates and 11.95% ash in dry matter.

Table 5 shows that the optimal conditions for the extraction of hemp seed kernel oils by the hydration

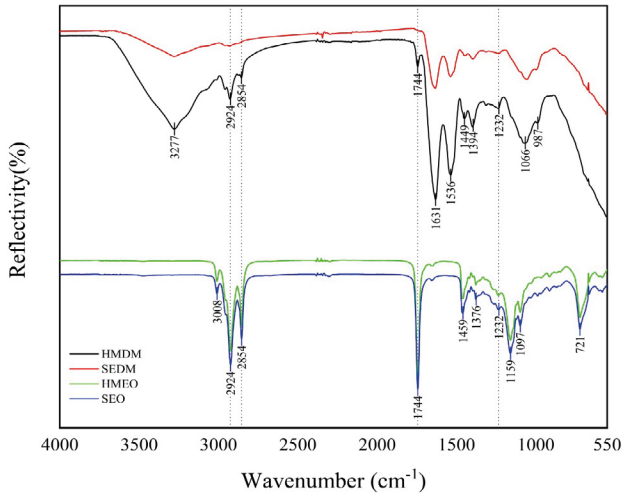

Figure 3. FTIR spectra of hemp oil obtained by different methods and corresponding defatted samples. HM-DM and SE-DM: defatted meal of the hydration method and solvent extraction, respectively; HMEO and SEO: oil extracted by hydration and solvent, respectively. Main band contribution of functional groups (ν: Telescopic or frame vibration; δ: Bending vibration; νf: skeleton; Amide II: δ(N-H)+ν(C-H)): 3277-ν(O–H) of hydrogen bonds from water and polysaccharides; 3008-ν(=CH) from lipids; 2924 and 2854-ν(C–H) of methyl (CH_3) and methylene (CH_2) groups from lipids; 1744-ν(C=O) of carbonyl and ester groups from pectin, xylan and lipids; 1631-δ(N-H) from water and protein; 1536- amide II from protein; 1459 and 1449-δ(CH_2) from polysaccharides; 1394 and 1376-δ(CH_2), n(CC), ns(COOH) from polysaccharides (pectin); 1232-ν(C-O) from polysaccharides (disaccharides sucrose); 1159-n(C–O–C), ring from polysaccharides (pectin); 1097-n(CO), n(CC), ring from polysaccharides (pectin); 1066-ν(CO), ν(CC), δ(OCH) from polysaccharides (cellulose); 987-OCH_3 from polysaccharides (cellulose); 721-νf(C-C).

method established in this study were significantly different from those developed for extracting oils from other oilseeds. Therefore, this study obtained new findings.

The results of these evaluation studies proved that the hydration method developed was efficient for the simultaneous production of high-quality oil and defatted meal as compared to other methods. This should mean that the hydration method has potential for replacing other methods which are currently used for the commercial separation of oil and defatted meal.

3.4. Analysis of the environmental impact of the hydration method

Oil extraction by the hydration method uses quite a small amount of water (a green reagent) which is completely absorbed by the solids of GHSK so that no wastewater is discharged into the environment during the extraction process. Another characteristic of the hydration method is that it avoids harmful chemical input and output. Furthermore, the hydration method utilizes whole hemp seed kernels so that no solid waste is discarded into the environment. The utilization of whole raw material saves valuable resources such as proteins and other bioactive com-

Table 4. Comparison of percentage of bioactive substances with different polarity extracted into the oil phase from hemp seed kernels by hydration method or solvent extraction

Bioactive substances	Hempseed kernel (mg/100 g)	Defatted meal (mg/100 g)		Percentage extracted into oil phase (%)	
		Hydration method	Solvent extraction	Hydration method	Solvent extraction
Total tocopherols	40.01±0.68	7.34±0.18	3.31±0.16	91.60±1.16	96.21±1.54
Vitamin A	1.60±0.17	0.31±0.06	0.15±0.02	91.06±1.11	95.65±1.44
Carotenoids	1.76±0.12	0.27±0.02	0.14±0.03	92.90±1.22	96.28±2.01
Co-Q10[a]	2.22±0.11	0.41±0.03	0.08±0.01	91.48±1.49	98.37±2.09
Phytosterols	432.44±2.68	78.02±1.19	51.20±1.02	91.74±1.35	94.58±1.26
Squalene	27.81±0.61	5.05±0.22	2.20±0.12	91.68±1.25	96.38±1.51
Cannabinoids	198.16±1.46	427.62±1.22	380.30±1.82	1.21±0.15	12.14±1.21
TF[b] (as QE equivalent)	291.00±5.01	620.00±3.01	614.00±2.02	2.43±0.37	3.40±2.24
TPC[c] (as GAE equivalent)	276.00±3.03	572.00±8.18	557.00±2.03	5.17±5.15	7.67±0.16
Phospholipid	2397.69±12.30	2890.00±18.11	1320.35±7.01	1.78±0.19	25.21±0.24
Free fatty acids (as oleic acid equivalent)	0.85±0.29	1.28±0.11	0.70±0.06	31.03±0.29	62.35±0.20
Waxes	4.73±0.39	10.32±0.98	0.86±0.07	undetectable	91.68±1.26

[a]Coenzyme Q10; [b]Total flavonoids while QE represents quercitin; [c]Total phenolic compounds while GAE represents gallic acid; [d]the average of 3 replicates was calculated.

Table 5. Comparison of process conditions optimized for processing different oilseeds by the hydration method

Oilseeds	Process conditions							
	H or P[a]	BT[b]	Bt[c]	PSSP[d]	W or S[e]	AT[f]	At[g]	ORR[h]
Peanut[1]	Hulled, peeled	110	90	150	1.5 mL H_2O+0.1 g NaCl per 10 g kernel slurry	20	Till free oil observed	96
Walnut[1]	Hulled	115	90	150	1.5 mL H_2O+0.1 g NaCl per 10 g kernel slurry	20	Till free oil observed	97
Sunflower[1] seed	Hulled	115	110	48	1.8 mL H_2O+0.1 g NaCl or 0.03 g Na_2CO_3 per 10 g kernel slurry	70	Till free oil observed	95
White sesame[2]	-	115	1	154	1.95 mL 6.00% (w/w) salt solution per 10 g ground seeds	65	25	96.06
Black[3] sesame	-	115	1	154	1.95 mL 6.79% (w/w) salt solution per 10 g ground seeds	65	30	96.54
Almond[4]	Hulled	110	3	58	1.37 or 1.40 mL H_2O per 10 g kernel slurry	RT[i]	40 or 45	96.38
Tea seed[5]	Hulled	115	90	74	1.2 mL H_2O+0.1 g NaCl or 0.08 g Na_2CO_3 per 10 g kernel slurry	65	40	94.47
Rape seed[6]	Hulled	115	2	61	1.5 mL H_2O per 10 g kernel slurry	50	30	94.64
Soybean[7]	Peeled	120	5	150	1.3 mL H_2O+0.08 g NaCl per 10 g kernel slurry(3 parts oil+5 parts powder)	75	30	81
Pumpkin seed[8]	Hulled	110	1	150	1.6 mL H_2O+0.08 g NaCl per 10 g kernel slurry	30	30	94.08
Hemp seed	Hulled	330 W microwave	1.02	380 or 250	1.28 mL H_2O+0.13 g NaCl per 10 g kernel slurry	RT[i]	10	96.88

[a]Hulling or peeling; [b]Baking temperature (°C) in oven; [c]Baking time (min) in oven; [d]Pore size (μm) of sieve passed through by ground oilseeds or kernels; [e]Water or salt added; [f]Agitation temperature (°C); [g]Agitation time (min); [h]Oil recovery rate (%); [i]Room temperature; [1]Tu *et al.* (2017); [2]Lv and Wu (2020); [3]Lv and Wu (2021); [4]Fu and Wu (2019); [5]Lv and Wu (2019a); [6]Lv and Wu (2019b); [7]Tu and Wu (2019); [8]Fu and Wu (2022).

pounds so that energy input or land for agricultural production can be significantly reduced and greenhouse gas emission is decreased. In particular, the high-quality protein in the defatted meal obtained by the hydration method may be a good material for making alternative meat products, which can significantly reduce the production of animal meats and therefore decrease greenhouse gas or methane emissions. Therefore, the hydration method is undoubtedly a kind of green technology for simultaneously producing high-quality oils and defatted meal from hempseeds or other oilseeds.

4. CONCLUSIONS

It was concluded that the hydration method optimized in this study was able to efficiently produce high-quality oils and defatted meal with an identical ORR compared to solvent extraction and a significantly higher ORR compared to supercritical CO_2 and cold-pressing methods. This method extracted more than 91% tocopherols, vitamin A, carotenoids, coenzyme Q10, phytosterols and squalene into the oil phase while only small portions of flavonoids, other phenolic compounds and free fatty acids were extracted. Defatted meal was rich in water-soluble vitamins including thiamin, riboflavin, niacin, pyridoxine, folate and vitamin C as well as proteins, dietary carbohydrates and phospholipids. The hydration method produced oils with lower AV or PV compared to solvent extraction, supercritical CO_2 and cold-pressing methods and defatted meal with protein content significantly higher than that obtained by supercritical CO_2 and cold-pressing methods. The hydration method is a kind of green

technology for environmentally-friendly and simultaneous production of high-quality oils and defatted meal from hempseeds or other oilseeds.

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