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An advanced aqueous method of recovering pumpkin seed kernel oils and de-oiled meal: Optimization and comparison with other methods

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SUMMARY: The optimal process conditions of the advanced aqueous method for recovering oil and de-oiled meal from pumpkin seed kernels were: baking the kernels at 110 °C for 1 min, grinding them to pass through a sieve of 150 μm pore size, adding 1.60 ml brine to 10.00 g ground kernels, stirring for 30 min at 30 °C, centrifuging at 4000 r/min for 30 min and cold-pressing the residue from centrifugation. This method recovered > 94% oil. Its oil recovery rate was comparable to that of solvent extraction and higher than that of enzyme-assisted aqueous method or hot-pressing. It recovered edible oil with higher quality and level of coenzyme Q10, tocopherols, carotenoids, total phytosterols and squalene as compared to solvent extraction or hot-pressing and requirements of China's national standard. It is superior to enzyme-assisted aqueous method or hot-pressing for recovering de-oiled meal which is suitable for making texturized protein.

KEYWORDS: *Optimizing parameters; Comparing methods; Water; Bioactive compounds; Green technology.*

RESUMEN: ***Método acuoso avanzado para recuperar aceites de pepitas de calabaza y harina desengrasada: optimización y comparación con otros métodos.*** Las condiciones óptimas del proceso del nuevo método acuoso para la recuperación de aceite y harina desengrasada de las pepitas de calabaza fueron: horneado a 110 °C durante 1 min, molienda para que pasen por un tamiz con un tamaño de poro de 150 μm, adición de 1,60 ml de salmuera a 10,00 g de pepita molida, agitando durante 30 min a 30 °C, centrifugación a 4000 r/min durante 30 min y presión en frío del residuo de la centrifugación. Este método recuperó > 94% de aceites. Esta tasa de recuperación de aceite fue comparable a la de la extracción con solvente y más alta que la del método acuoso asistido por enzimas o prensado en caliente. Se recuperó aceite comestible con mayor calidad y nivel de coenzima Q10, tocoferoles, carotenoides, fitoesteroles totales y escualeno en comparación con la extracción con solvente o prensado en caliente cumpliendo los requisitos de la norma nacional de China. La extracción es superior a la obtenida mediante el método acuoso asistido por enzimas o al prensado en caliente por lo que se recupera una harina desengrasada adecuada para hacer proteínas texturizadas.

PALABRAS CLAVE: *Agua; Comparación de métodos; Compuestos bioactivos; Optimización de parámetros; Tecnología verde.*

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1. INTRODUCTION

The annual consumption of edible oil per capita in 2019 have been up to 27 kg per capita (OECD/FAO, 2020). Large amounts of oils or fats may also be applied to other industries, for example, efficient solvents for extracting nonpolar bioactive substances and materials for making many valuable products (lubricants, biodiesel, linoleum, etc.) (Pal *et al.*, 2019; Kumar *et al.*, 2016).

The efficient utilization of proteins in defatted meal is essential for obtaining the maximum profit from the oilseed processing industry for the production of oils. For food security, 31 kg per capita per year by 2029 is projected to be required while actual annual consumption per capita may be up to 41 kg (OECD/FAO, 2020). Furthermore, large quantities of proteins may be consumed by food and other industries, for example textile or medicinal industries (Stenton *et al.*, 2021; DeFrates *et al.*, 2018; Asgar *et al.*, 2010).

Pumpkin seed kernels (PSKs) contain 36-50% oil, 36-50% protein and significant amounts of other bioactive compounds (Veronezi and Jorge, 2012). Therefore, pumpkin seed kernels are a very good source of oil and protein and the simultaneous production of oil and de-oiled meal which are rich in protein is important. This means that an efficient, environmentally-friendly, safe, un-costly and sustainable method for recovering oil from PSKs is in high demand.

Recently, the enzyme-assisted aqueous method (EAAM) has been studied extensively because it has advantages over solvent extraction (SE)/or cold-pressing-solvent extraction (CP-SE) (e.g. hexane emitting harmful solvent, terpenes (e.g. α-pinene, p-cymene and d-limonene) being expensive and hardly available in large quantities), critical CO_2 extraction (small scale and high pressure needed) and other methods (e.g. hot-pressing, HP; low value of de-oiled meal) which have disadvantages (Environmental Protection Agency, 1999; Yusoff *et al.*, 2014). EAAM uses large quantities of water and enzymes to disperse (or solubilize) hydrophilic compounds such as proteins, phospholipids and free fatty acids and separate oils. The application of EAAM in oilseed processing on an industrial scale is still not successful because of the use of expensive enzymes, quite low oil recovery rate (ORR; 64.2 to 89.1% (Zhang *et al.*, 2018; Konopka *et al.*, 2016; Li *et al.*, 2016; Jiao *et al.*, 2014; Hu and Zou, 2013) caused by serious emulsion, difficulty and high cost of treating large quantities of water, losses in large quantities of valuable compounds with waste water, and high production cost as compared to other methods (Yusoff *et al.*, 2014).

Recently, an advanced aqueous method (AAM) has been developed for efficiently processing tea seeds (Lv and Wu, 2019a), almonds (Fu and Wu, 2019), white sesame (Lv and Wu, 2020), soybeans (Tu and Wu, 2019a), rapeseeds (Lv and Wu, 2019b), sunflower seeds, peanuts, or walnuts (Tu *et al.*, 2017; Tu and Wu, 2019b), respectively. A comparison of optimized process conditions of the advanced aqueous method for different oilseeds is shown in Table 1. The table indicates that different oilseeds had different optimized conditions for obtaining the maximum oil recovery rate (ORR). The variation should be caused by the variation in the chemical composition and physicochemical properties of different oilseeds.

This research aimed to optimize process conditions of AAM for recovering high quality edible oil and de-oiled meal from PSKs and evaluate them as comparing with HP, EAAM and SE. All experiments carried out, their results and discussion with respect to industrial processing are reported here.

2. MATERIALS AND METHODS

2.1. Materials

Raw pumpkin seeds bought from Inner Mongolia, China via Mobile Phone Taobao (https://www.taobao.com/) were manually peeled without any heat treatment. All reagents used in the experiments were analytically pure.

2.2. Optimization of process conditions for recovering oils by AAM

Figure 1 shows the flow chart for recovering oils by the AAM studied. All measurements were carried out in triplicate.

2.2.1. Division 1 Optimization by single factor experiments.

Only one process condition varied with all others fixed and its level producing the maximum ORR was selected for the subsequent experiment. AAM procedures for investigation into the effect

TABLE 1. Comparison of optimized process conditions for different oilseeds by the new aqueous method

Oilseeds	Process conditions							
	H or P[a]	BT[b]	Bt[c]	PSSP[d]	W or B[e]	AT[f]	At[g]	ORR[h]
Peanut[1]	Hulled and 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 6.0% (w/w) salt solution per 10 g ground seeds	65	25	96.06
Almond[3]	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[4]	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[5]	Hulled	115	2	61	1.5 ml H_2O per 10 g kernel slurry	50	30	94.64
Soybean[6]	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

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

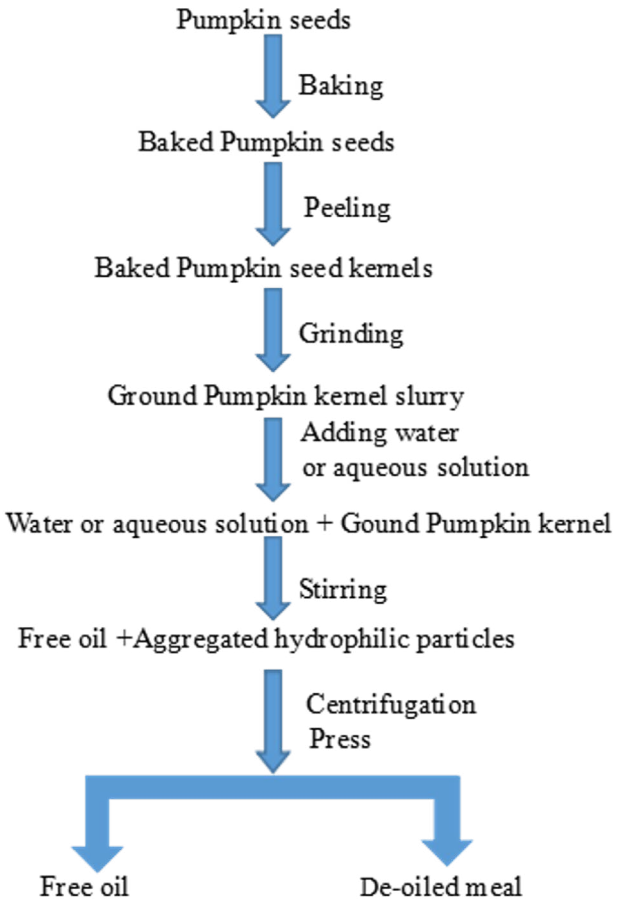

FIGURE 1. Flow chart of recovering pumpkin seed kernel oil and de-oiled meal by using a small amount of aqueous solution.

of baking time on ORR were as follows: "Raw PSKs were baked for 0, 1, 2, 3, 4, or 5 min, respectively, at 105 ºC, peeled, crushed and ground to pass through a sieve with 105 μm pore size producing ground PSK (GPSK). Water (1.50 ml) was added to a 20 ml centrifugal tube with 10.00 g GPSK. After stirring at room temperature (25 ºC) for 30 min, the oil was collected by centrifuging at 4000 r/min (1435 g) for 30 min thrice with 80-2 centrifuger (manufactured by Honghua Instrument Factory, China) and weighed. All residues from centrifugation were quantitatively collected and cold-pressed once with a presser (made by Ai Bang Agricultural and Horticultural Machinery Plant, China). All the de-oiled meal from the presser was quantitatively collected, dried, weighed and ground to pass through a sieve with 154 μm pore size for determining oil content according to the Soxhlet method. The extraction yield of oil (EYO) was calculated as follows:

$$EYO\ (\%) = (X_1\text{-}X_2) \div X_1 \times 100$$

In the formula, X_1 (g) represents the total oils of GPSK [10 g × crude oil fraction of GPSK], while X_2 (g) represents the total oils of de-oiled meal (the amount of de-oiled meal (g, dry basis) × its oil fraction). For AAM, EYO is the same as ORR.

AAM procedures for the investigation into the effect of baking temperature on ORR. Raw PSKs were baked for 1 min at 100, 105, 110, 115, 120, or 125 ºC, peeled, crushed and ground to pass through a sieve of 105 µm pore size to produce ground PSK (GPSK). Water (1.50 ml) was added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at room temperature (25 ºC) for 30 min, the rest of the procedure was the same as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for the investigation into the effect of water added on ORR. Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through a sieve of 105 µm pore size to produce ground PSK (GPSK). Water (0.00, 0.50, 1.00, 1.20, 1.40, 1.50, 1.60, 1.70, 1.80, or 1.90 ml) was added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at room temperature (25 ºC) for 30 min, the rest of the procedure was carried out in the same manner as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for the investigation into the effect of sieve pore size on ORR. Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through sieves with 550, 270, 250, 212, 160, 150, 105, 75, or 58 µm pore sizes to produce ground PSK (GPSK). Water (1.60 ml) was added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at room temperature (25 ºC) for 30 min, the rest of the procedure was the same as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for investigation into effect of stirring time on ORR. Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through a sieve with 150 µm pore size to produce ground PSK (GPSK). Water (1.60 ml) was added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at room temperature (25 ºC) for 0, 15, 20, 25, 30, 35, 40, or 45 min, the rest of the procedure was carried out in the same manner as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for the investigation into effect of stirring temperature on ORR. Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through a sieve with 150 µm pore size to produce ground PSK (GPSK). Water (1.60) was added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at 25, 30, 35, 40, 45, or 50 ºC for 30 min, the rest of the procedure was the same as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for the investigation into the effect of salt added on ORR . Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through a sieve with 150 µm pore size to produce ground PSK (GPSK). Water (1.60 ml) and salt (0.00, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, or 0.12) were added to a 20-ml centrifugal tube with 10.00 g GPSK. After stirring at 30 ºC for 30 min, the rest of the procedure was the same as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

AAM procedures for the investigation into the effect of water added with the presence of 0.08 g salt on ORR. Raw PSKs were baked for 1 min at 110 ºC, peeled, crushed and ground to pass through a sieve with 150 µm pore size to produce ground PSK (GPSK). Water (0.00, 0.50, 1.00, 1.20, 1.40, 1.50, 1.55, 1.60, 1.65, 1.70, 1.75, or 1.80 ml) was added to a 20-ml centrifugal tube with 10.00 g GPSK plus 0.08 g salt. After stirring at 30 ºC for 30 min, the rest of the procedure was the same as that described above in "AAM procedures for the investigation into the effect of baking time on ORR".

2.2.2. Division 2 Further optimization by response surface method

According to the results of the single factor experiments, sieve pore size (A), stirring time (B), water addition (C), and baking temperature (D) were selected for further optimization using the response surface method with all other process conditions fixed. For this purpose, Box-Benhnken's central combined experimental design was employed.

2.3. Recovering oils through SE

Pumpkin seeds were baked at 105 ºC for 1 min, cooled, pulverized, and extracted for 10 h at 85 ºC in a water bath using a Soxhlet extractor with n-hexane as solvent. The extract was evaporated in a rotary evaporator until the n-hexane was completely removed. Free oil was vacuum dried at 50 ºC to a constant weight. The oil content in the de-oiled meal

was determined. EYO was calculated following a method similar to that described in "*2.2.1. Division 1*". The crude oil obtained was refined by a method similar to that optimized by Ma *et al.* (2017). ORR was calculated by: [total refined oil obtained (g)/total crude oil extracted (g)] x 100.

2.4. Recovering oils by HP

Pumpkin seeds were baked at 105 ºC for 1 min and hot-pressed twice. Oil content in dark de-oiled meal was determined by the Soxhlet method. EYO was calculated by the method similar to that described in "*2.2.1. Division 1*".

2.5. Analytical methods

Transparency or smell or taste, solvent content, water content, phosphorus content, peroxide value, acid value, crude oil content, crude protein content, total phytosterols and carotenoids were determined by using Chinese National Standard Analytical Methods: GB/T5525-2008, GB5009.262-2016, GB5009.3-2016, GB5009.87-2016, GB5009.227-2016, GB5009.229-2016, GB5009.6-2016, GB5009.5-2016, GB/T25223-2010 and GB5009.83-2016, respectively. Tocopherols and squalene were estimated according to Wong *et al.* (1988) and LS/T6120-2017, respectively.

2.6. Statistical analysis

One-way ANOVA was conducted with IBM SPSS Statistics 23. The P-value determined significant difference between two groups. Significance between two groups of data was obtained by post-test and multiple-comparison. Response surface analysis was conducted with Design-Expert 8.0.6 software.

3. RESULTS AND DISCUSSION

Some important compositions of experimental materials (PSKs) were as follows: contained 44.68% crude fat (dry basis), 31.24% crude protein (dry basis) and 6.18% water. ORR calculation was based on these measurements.

3.1. Optimization of process conditions of recovering oils by AAM

3.1.1. Division 1. Optimization by single factor experiments

Figure 2 shows the effect of water addition, baking time or temperature and sieve pore size on

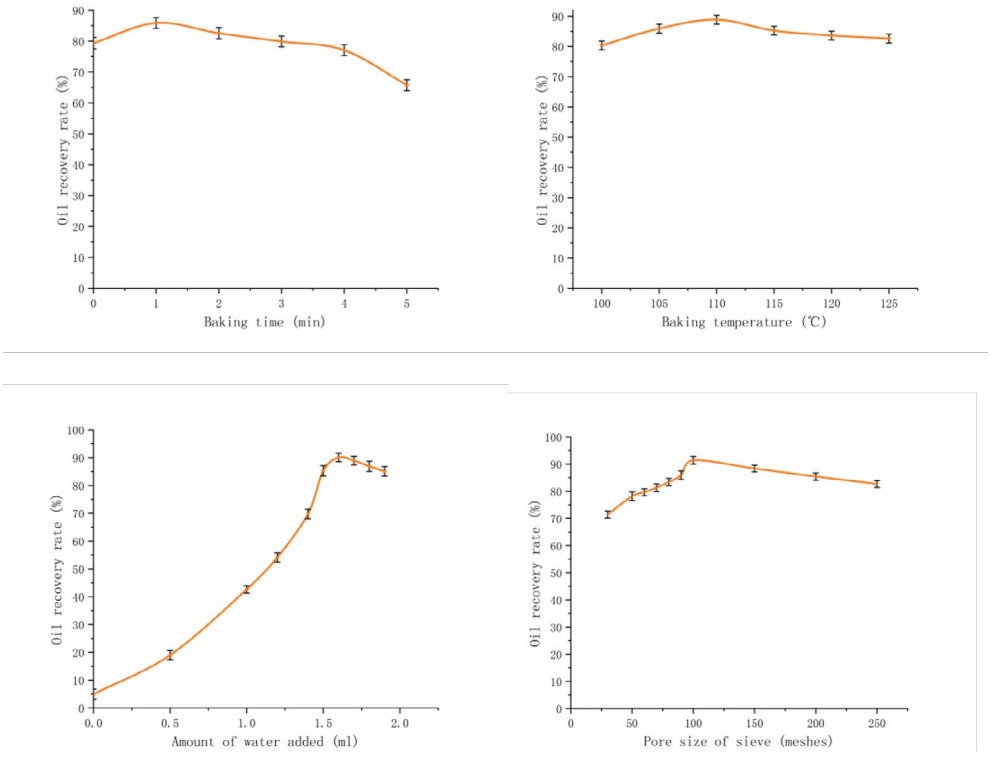

FIGURE 2. Effect of baking time or temperature, amount of water added and sieve pore size (meshes) on oil recovery rate (mean ± SD; n=3).

ORR. The effects of all these three factors were significant with different priorities.

ORR increased from 5.03 to 90.11% as water addition increased from 0 to 1.60 ml, but it continuously decreased when > 1.60 ml was added. The ORR of only 5.03% without water addition indicated that water addition was the major, essential and critical factor for efficiently separating oils. When water addition was at the level of 1.60 ml, solid particles absorbed all added water and aggregated together and the maximum release of free oils occurred. According to quantum chemistry theory, the aggregation of solid particles should be caused by the formation of hydrogen bonds because of water addition. However, the addition of too much water might cause the solubilization of some hydrophilic compounds, increase their movability, reduce the size of solid particles and therefore facilitate the formation of emulsion so that ORR decreased. The maximum ORR obtained was meaningful since it was higher than that obtained by EAAM (Hu and Zou, 2013). The amount (1.60 ml) of water added to 10.00 g raw materials (GPSK) for obtaining the maximum ORR did not cause any free water or result in waste water during the extraction of oils by AAM.

ORR increased from 79.01 to 86.02% as baking time increased from 0 to 1 min, but it continuously decreased for longer than 1 min while it increased from 80.31 to 89.02% as baking temperature increased from 100 to 110 ºC, although it continuously decreased at temperatures higher than 110 ºC. Proper heating treatment should deactivate lipase, which may reduce the losses in neutral oils during oil extraction and therefore elevate ORR. On the other hand, excessive heating (> 1 min and > 110 ºC) may cause the denaturation of proteins and increase their oil absorption capacity, which could therefore reduce ORR. The extent of variation in ORR caused by the variation in the heat treatment was much smaller than that caused by the variation in water addition, which means that heating treatment is a minor or an assistant factor.

ORR increased from 71.4 to 91.36% as sieve pore size reduced from 550 to 150 μm, but it continuously decreased when < 150 μm was used. Grinding PSKs to pass through a sieve with proper pore size should be necessary for fully breaking oil bodies and cell walls to release oils so that proper grinding increased ORR. However, excessive grinding might produce fine particles which are too small in size, which could increase the surface areas of the particles, elevate their ability to stabilize the emulsion (Dickinson, 2006) and therefore decrease ORR. This factor also had a smaller extent of variation in ORR compared to water addition, which should also be an assistant factor.

Figure 3 indicates the effect of stirring temperature or time, NaCl addition and water addition with the presence of 0.08 g NaCl on ORR. These three factors also significantly affected ORR to different extents.

ORR increased from 91.30 to 92.48% as stirring temperature increased from 25 to 30 ºC, but it continuously decreased at temperatures higher than 30 ºC. A reduction in oil viscosity and an increase in the intensity of molecular motion of oils and hydrophilic compounds might occur when stirring temperature is elevated, which might facilitate the aggregation of hydrophilic groups via hydrogen bonds as well as oil coalescence and release from the surface of aggregated solid particles so that ORR increased. On the other hand, too high a temperature (≥ 30 ºC) might lead to an increase in dispersity of surfactants, which might increase the formation of emulsion and thus reduce ORR. This factor also produced a smaller extent of variation in ORR compared to other factors, which should therefore be an assistant factor. ORR increased from 58.52 to 92.56% as stirring time increased from 0 to 30 min, but it continuously decreased after longer than 30 min. Proper stirring time should be necessary for the excess of water to hydrophilic groups of solid particles and the formation of hydrogen bonds. This might facilitate the aggregation of solid particles and the release of free oils from their surface. Excessive stirring might elevate the dispersity of surfactants which might cause emulsion and thus reduce ORR.

ORR increased from 92.40 to 94.07% as NaCl addition increased from 0.00 to 0.08 g, but it continuously decreased when > 0.08 g was added. With the presence of 0.08 g NaCl, ORR increased from 4.83 to 94.14% as water addition was increased from 0.00 to 1.60 ml, but it continuously decreased when > 1.60 ml was added. The addition of NaCl might increase the density or surface charge of aggregated solid particles and therefore facilitate the separation of oils. The addition of NaCl only increased ORR by 1.67% compared to the addition of pure water. Therefore, AAM without the addition of NaCL might also be meaningful in pumpkin seed processing practices.

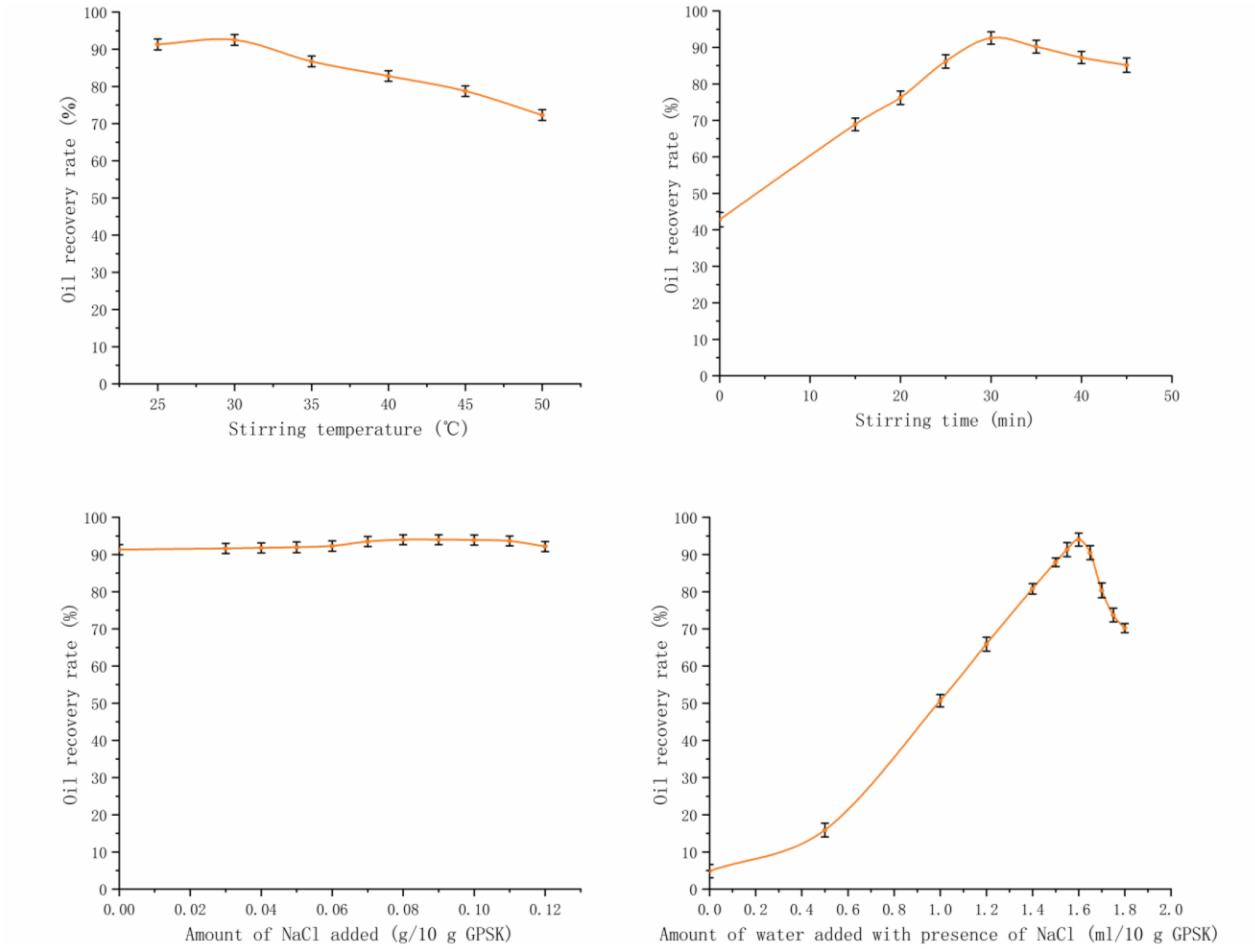

FIGURE 3. Effect of amount of salt added, amount of water added with the presence of NaCl, stirring temperature or time on oil recovery rate (mean ± SD; n=3).

Therefore, the optimal process conditions of AAM obtained by single factor experimentation were as follows: baking Pumpkin seeds at 110 °C for 1 min, crushing PSKs and grinding them to pass through a sieve with 150 μm pore size to obtain GPSK, adding 1.60 ml water and 0.08 g NaCl to 10.00 g GPSK, stirring for 30 min at 30 °C, centrifuging at 4000 r/min (1435 g) for 30 min thrice, and cold-pressing the residue from centrifugation once. The verification test proved that ORR was 94.08% under the optimal process conditions with the general procedures (see "2.2") followed. Under the optimum conditions, centrifugation and the subsequent cold-pressing recovered 90.15% and 3.93% of total oils from GPSK, respectively. The AAM process conditions optimized in this study for obtaining the maximum ORR from PSKs were significantly different from those for processing other oilseeds previously published (Table 1).

3.1.2. Division 2. Further optimization by response surface method

The response surface method associated with sieve pore size (A), stirring time (B), water addition with the presence of NaCl (C) and baking temperature (D) did not improve ORR as compared to single factor experimentation. The results obtained by the response surface method are indicated in Table 2. However, Figures 4-6 show that AB, AC and AD had significant cross-effects on ORR. The second-order response surface equation (R^2 = 0.9943) was obtained to represent the final oil yield.

$$ORR = +93.65 + 5.15*A+0.1667*B + 0.5517*C + 0.1217*D - 0.0525*AB - 0.5525*AC - 0.17*AD + 0.1725*BC - 0.18*BD + 0.365*CD - 6.2*A^2 - 0.9242*B^2 - 1.834*C^2 - 0.7643*D^2$$

TABLE 2. Design and results of response surface experimentation

Run	A: Sieve pore size (meshes)	B: Stirring time (min)	C: Water added (ml) with 0.08 g NaCl	D: Baking temperature (ºC)	*ORR (%; mean ± SD; n=3)
1	100	25	1.55	110	90.02±1.16
2	100	25	1.60	105	91.01±1.18
3	120	25	1.60	110	92.19±0.65
4	80	25	1.60	110	81.82±1.28
5	100	25	1.60	115	91.89±0.62
6	100	25	1.65	110	90.79±0.92
7	100	30	1.55	105	91.14±1.12
8	80	30	1.55	110	78.82±0.42
9	120	30	1.55	110	90.53±1.13
10	100	30	1.55	115	90.82±1.15
11	80	30	1.60	105	81.48±0.88
12	120	30	1.60	105	91.87±0.66
13	100	30	1.60	110	93.57±0.79
14	100	30	1.60	110	94.08±0.64
15	100	30	1.60	110	93.68±0.61
16	100	30	1.60	110	93.72±0.67
17	100	30	1.60	110	93.18±0.62
18	120	30	1.60	115	91.33±1.38
19	80	30	1.60	115	81.62±1.33
20	100	30	1.65	105	91.31±0.81
21	120	30	1.65	110	90.72±0.76
22	80	30	1.65	110	81.22±0.96
23	100	30	1.65	115	92.45±1.03
24	100	35	1.55	110	90.42±0.90
25	100	35	1.60	105	91.82±0.88
26	120	35	1.60	110	91.89±0.95
27	80	35	1.60	110	81.73±0.84
28	100	35	1.60	115	91.98±0.98
29	100	35	1.65	110	91.88±1.03

*Oil recovery rate.

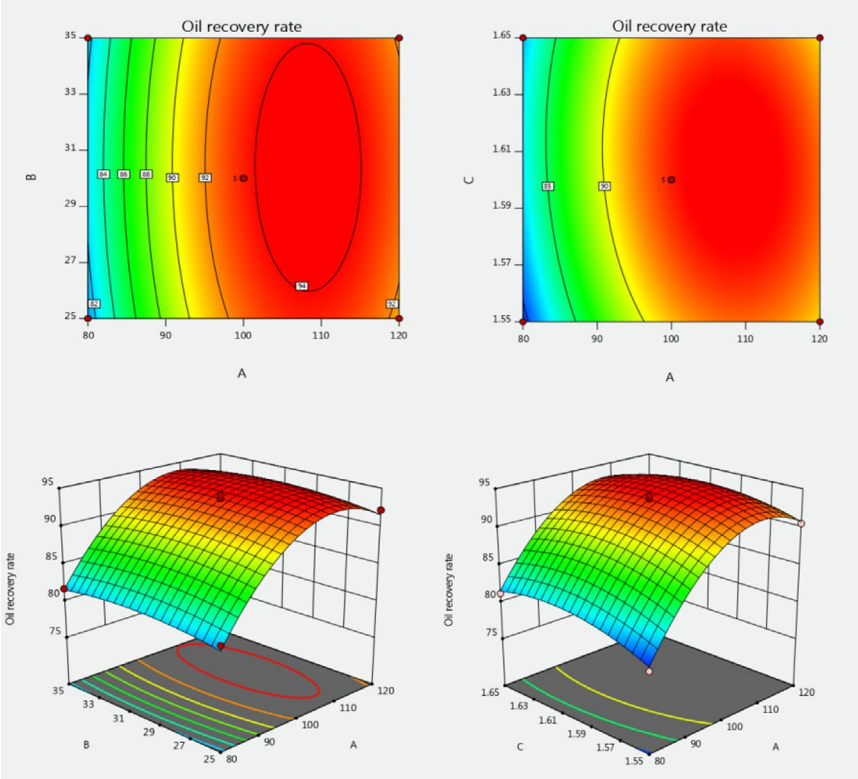

FIGURE 4. Effect of interaction between sieve pore size (meshes; A) and stirring time (B) or water addition with the presence of NaCl (C) on oil recovery rate (%; mean ± SD; n=3).

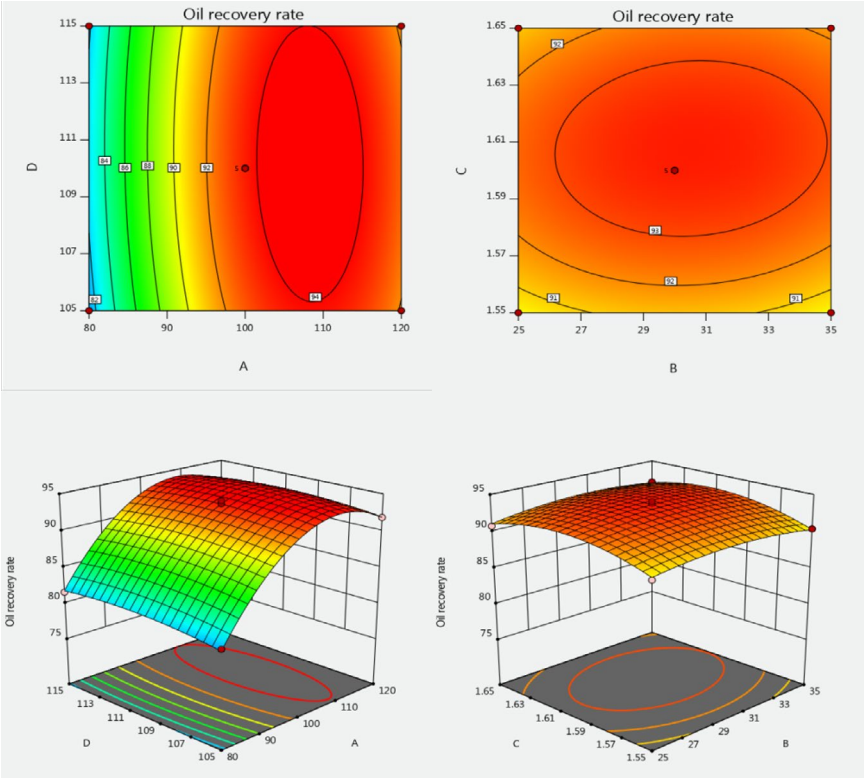

FIGURE 5. Effect of interaction between sieve pore size (meshes; A) and baking time (D) or between stirring time (B) and water addition with the presence of NaCl (C) on oil recovery rate (%; mean ± SD; n=3).

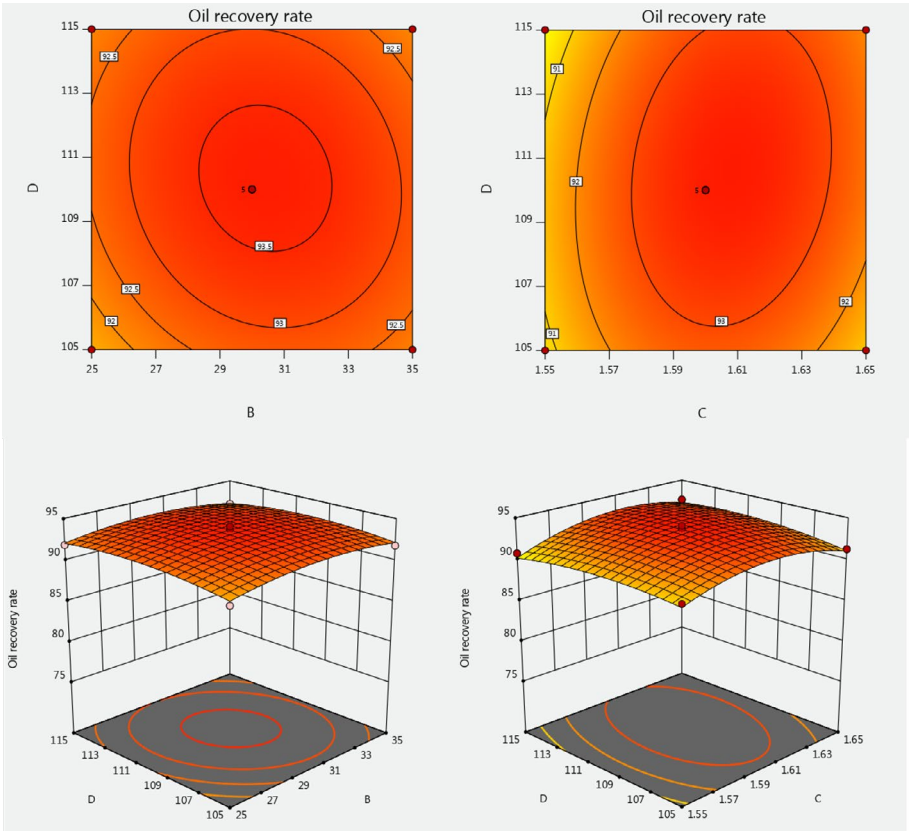

FIGURE 6. Effect of interaction between stirring time (B) and baking time (D) or between water addition with the presence of NaCl (C) and baking time (D) on oil recovery rate (%; mean ± SD; n=3).

Single factor experimentation adequately optimized process conditions since the surface response method did not find different process conditions to give higher ORR. Adding the proper amount of water was essential and critical while baking seeds, grinding PSKs, stirring GPSK and adding NaCl significantly affected ORR. The optimal conditions established can provide useful scientific basis for process engineering purposes. According to the results of some (including experiments on baking, water or salt addition, sieve pore size and stirring) of this study, bakers (e.g. tunnel furnace) with the accurate control of heating temperature (110 °C) and time (1 min), grinders (e.g. roller-type) which can grind PSKs to pass through a sieve with 150 μm pore size (or destruct all oil bodies and cell walls without production of too fine solid particles) and stirrers (e.g. worm-type) with automatically quantitative addition of water or brine solution and feeding of GPSK are necessary for producing free oils and aggregated solid particles from PSKs on an industrial scale. Centrifugers and cold-pressers are good selections for collecting free oils and de-oiled meal.

3.2. Comparison of efficiency of different methods

Table 3 compares the efficiency of AAM, SE, EAAM and HP for recovering PSK oil. It was found that ORR of AAM was comparable to that of SE and significantly higher than that of EAAM or HP. Although AAM had a lower EYO compared to SE, the crude oil obtained by SE needs to be refined for safe consumption. The oil refinery process can cause losses in neutral oil so that the ORR of SE was much lower than its EYO, which can increase the production cost of edible oil. The main purpose of extensive studies on EAAM is to improve the ORR of the aqueous method, but EAAM has not been successful yet because of a serious un-resolved emulsion problem. Therefore, the AAM for recovering PSK oil developed in this study was advanced. AAM should have the potential to replace SE for efficiently recovering PSK oil with acceptable ORR.

Table 3. Comparison of efficiency of recovering PSK oil by AAM and other methods or Chinese National Standard (CNS)

Items	CNS	AAM (mean ± SD; n=3)	EAAM	SE (mean ± SD; n=3)	HP (mean ± SD; n=3)
Extraction yield of oils (%)	_	94.08±0.52	-	98.34±0.43	87.14±0.79
Oil Smell, taste[b]	b	b	_	b	b
Water in oil (%)	-	0.058±0.011	-	0.037±0.012	0.065±0.021
Phosphorus (mg/kg oil)	-	40±1	-	400±9	405±9
Oil transparency	C,T	C,T[c]	_	C,T	C,T
AV (mg KOH/g oil)	2.5 (pressing) 2.0 (solvent)	0.25±0.01	_	0.63±0.01	0.41±0.02
PV (mmol/kg oil)	6.2 (pressing) 4.1 (solvent)	2.58±0.14	_	2.95±0.19	4.08±0.16
Residual solvent (mg/kg oil)	ND[d]	ND[d]	ND[d]	25±1	ND[d]
Oil content in de-oiled meal	_	4.60±0.15	_	1.32±0.09	9.41±0.31
ORR (%)	_	94.08±0.37	89.12[a]	93. 87±0.27	82.69±0.19
Coenzyme Q10 (mg/kg edible oil)	_	146±1	_	105±1	68±1
Tocopherols (mg/kg edible oil)	-	150±2	-	110±2	115±2
Carotenoids (mg/kg edible oil)	-	82±1	-	62±1	71±1
Total phytosterols in edible oil (%)	-	0.5±0.02	-	0.30±0.01	0.40±0.02
Squalene (mg/kg edible oil)	-	4562±111	-	2500±121	3541±112

[a]The highest extraction yield of PSK oil by EAAM reported (Hu and Zou, 2013). [b]Having the inherent smell and taste of PSK oil, no adverse smell. [c]C,T-Clarify, transparent. [d]ND-undetectable.

Table 3 also indicates that AAM-recovered oils with a significantly lower acid value or peroxide value, significantly higher concentrations of tocopherols, carotenoids, coenzyme Q10, total phytosterols or squalene, low water or phosphorus content and other better quality indexes compared to SE or HP, which are currently used as commercial production methods. In particular, the acid value or peroxide value of the oil obtained by AAM was better than the mandatory China National Standard (CNS) requirement, although this was not normally achieved by SE or HP. Although the level of fat-soluble bioactive compounds (including tocopherols, etc. mentioned above) in crude oils recovered by SE was significantly higher, it was significantly lower because oil refinery required for edible purpose caused losses compared to AAM. Furthermore, high temperatures treatment during the refining process can significantly increase the trans-fatty acid content, which is harmful to the cardiovascular system. With regard to these aspects, AAM should be superior to SE, HP and EAAM.

De-oiled meal obtained by AAM contained 4.6% residual oil and 60.7% proteins (dry-weight basis). This level of residual oil content and protein content met the requirements for making texturized protein (Crowea and Johnson, 2001). This means that the whole PSKs can be completely utilized by employing AAM to recover oil. On the other hand, HP produced dark de-oiled meal with completely denatured proteins which had little value for applying to the food industry. EAAM was not able to achieve the complete utilization of whole PSKs since significant amounts of compounds were discarded with large quantities of waste water. With regard to this aspect, AAM should be better than EAAM and HP.

AAM used only a small amount of water and caused no waste water during oil separation and NaCl to be consumable without desalting. Since only water vapor goes into the atmosphere, recovering oil by AAM may be considered a method with zero discharge of wastewater. Therefore, this method should be a green technology. EAAM discharges

large quantities of waste water with high COD or BOD or alkali or acid or other chemicals during oil extraction. The refinery process of crude oil obtained by SE also produces significant amounts of waste water. With regard to this aspect, AAM should be superior to SE and EAAM.

4. CONCLUSIONS

Process conditions for AAM were optimized by single factor experimentation followed by the surface response method. A liquid:solid (water:raw material (GPSK)) ratio of 1.6:10 was able to recover more than 94% oils. The addition of water was found to be the major, essential and critical factor for efficiently separating oils while other conditions were minor or assistant factors. The AAM recovered oils with higher quality or level of fat-soluble bioactive compounds from PSKs compared to SE, EAAM and HP, which should be green technology. With regard to producing de-oiled meal suitable for making texturized protein, AAM should be better than EAAM and HP.

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