Study on Extracting Avocado Oil from Avocado Pulp by Aqueous Extraction

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Abstract. The optimum process for extracting avocado oil from avocado pulp was studied by using the water method. The effects of material-liquid ratio, temperature, and time and pH value on oil extraction rate and oil recovery rate were studied. The optimum extraction conditions of avocado oil were determined by orthogonal test, and the physicochemical properties and fatty acid composition were analysed. The results showed: material-liquid ratio of 1:3 g/mL, extraction temperature of 75 °C, extraction time of 150 min, pH of 8 could be obtained on the optimal extraction conditions, and the oil extraction rate and the oil recovery rate of avocado oil were 78.95%, 37.21%, respectively. The acid value of avocado oil is 0.86 mg / g, the iodine value is 154.41 g / 100g, the saponification value is 198.0 g / 100g, water and volatile matter is 0.13%. The main fatty acid composition of avocado oils were oleic acid (71.93%), palmitic acid (13.33%), linoleic acid (8.51%), palm oil Acid (5.28%), total unsaturated fatty acid content 85.72%.

1. Introduction
Avocado is a tropical and subtropical fruit. The main producing country is Mexico, with an annual output of 5.6 million tons and a total production of 34% of the world.[1, 2] Because of its rich nutritional value and high economic value, it is called “net red fruit”[3]. In recent years, China has started large-scale planting in Yunnan, Guangdong, and Fujian and Hainan province. At present, because the domestic fresh-selling market is larger than the output, there is no related avocado deep processing factory in China. In the next few years, the production of avocado in China will reach a certain level, and there will be overcapacity, and the fresh fruit is perishable and the storage requirements are high.[4] It is necessary to processed further. The extracted avocado oil has been extracted by centrifugation methods[5], pressed methods[6], supercritical carbon dioxide extraction[7, 8], aqueous enzymatic extraction[9-12], and ultrasonic assisted extraction[13], microwave assisted extraction[14], and its effect on the stability during heat treatment[15, 16], physicochemical and oxidative stability of the modified emulsion[16, 17]. Without considering the addition of emulsifier, the effects of time, temperature and other factors on the extraction rate of avocado oil and the yield of
clear oil have not been reported. Based on this, this study investigated the extraction of avocado oil from avocado pulp by single factor and orthogonal test. The best extraction parameters provide basic data support for China's avocado processing industry.

2. Materials and methods

2.1. Materials

Avocado (Persea americana Mill), a variety of 'Hass', is produced in Chile and purchased from a local Wal-Mart supermarket.

2.2. Experimental design

2.2.1. Single factor test design. 1000 g of avocado pulps were beat to slurry at a ratio of 1:4, and milled in a colloid miller (ZVF300-G5R5/P7R5T4MD, Shanghai Cheoke Machinery Co., Ltd., China) for 1 min. Then, the pH of the slurry was adjusted to a certain value with a 1.00 mol/L sodium hydroxide solution or 1.0 mol/L hydrochloric acid solution. And the slurry was stirred at a low speed for a certain time and temperature in water bath. Next, the slurry was centrifuged at 10000 r/min for 10 min in a centrifuge (GR22gII, Hitachi, Japan). Then, the surface layer of clear oil could be collected and weighed, and the oil content of pulp residue measured by Soxhlet extraction method, and the avocado Oil extraction rate and oil recovery rate calculated.

The material-liquid ratio was 1:3, the extraction temperature was 50 °C, the extraction time was 90 min, and the pH value was 8 as the basic conditions. The effects of material-liquid ratio, extraction temperature, extraction time and pH on the oil extraction rate and oil recovery of avocado were investigated. The ratio of material to liquid (g/mL) was 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8. Extraction temperature (°C) is 35, 45, 55, 65, 75, and 85. Extraction time (min) is 30, 60, 90, 120, 150, and 180. The pH values are 5, 6, 7, 8, 9, and 10.

2.2.2. Orthogonal optimization test. On the basis of single factor experiment, the ratio of material to liquid (A), extraction temperature (B), extraction time (C) and extraction pH (D) were selected as orthogonal test with the oil extraction rate and the oil recovery rate of avocado oil as indicators. The L9 (3^4) orthogonal table was used for the test, and the oil extraction rate and the oil recovery rate were used as indicators. The orthogonal test design results are shown in the following table 1.

| Levels | A   | B  | C  | D  |
|--------|-----|----|----|----|
| 1      | 1:3 | 65 | 90 | 7  |
| 2      | 1:4 | 75 | 120| 8  |
| 3      | 1:5 | 80 | 150| 9  |

2.3. Determination of indicators

2.3.1. Avocado oil content. The oil content of avocado is determined according to GB/T 14488.1-2008.

2.3.2. Residual oil rate. The residual oil rate in the cake was determined according to GB 5009.6-2016.

2.3.3. Avocado oil extraction rate. Avocado oil is extracted according to the test method of 2.2.1, and the oil extraction rate is calculated, and the calculation is as shown in Eq. (1).

$$E_1 = \frac{m_1 \times \omega_1 - m_0 \times \omega_0}{m_0 \times \omega_0} \times 100\%$$

Where: $E_1$-oil extraction rate, %; $m_0$-sample quality, g; $\omega_0$-sample oil content, %; $m_1$-slag dry weight, g; $\omega_1$-slag residual oil rate, %.
2.3.4. Avocado oil recovery rate. The avocado oil was extracted according to the test method of 2.2.1, and the oil yield was calculated and calculated as Eq. (2).

\[ E_2 = \frac{m_2}{m_0 \times \omega_0 \times m_1 \times \omega_1} \times 100\% \] (2)

Where:
- \( E_2 \) - oil recovery rate, %;
- \( m_0 \) - sample quality, g;
- \( \omega_0 \) - sample oil content, %;
- \( m_1 \) - slag dry weight, g;
- \( \omega_1 \) - slag residual oil rate, %;
- \( m_2 \) - upper oil quality, g.

2.4. Data analysis
Orthogonal test design and analysis of variance were performed using statistical analysis software SPSS 22.0. Origin analysis software was used for mapping. All experimental data were measured 3 times and averaged.

3. Materials and methods

3.1. Effects of material-liquid ratio on oil extraction rate and oil recovery rate
The effects of the ratio of material to liquid on the extraction of avocado oil from avocado pulp by the aqueous extraction is shown in Figure 1. It can be seen from Figure 1 that in the selected range, with the increase of the ratio of material to liquid, the oil extraction rate and the oil recovery rate tend to increase first and then decrease. When the ratio of material to liquid is 1:3, the oil extraction rate has the maximum value, 84.90%. When the ratio of material to liquid is 1:4, the oil recovery rate has the maximum value of 43.04%. Because of the ratio of material to liquid is too small, the viscosity is too large, and it affects the diffusion speed of oil molecules, and causes the oil not to be easily separated. Therefore, the oil extraction rate and oil recovery rate are not reaching the maximum at the same time.

When the ratio of material to liquid is too large, water soluble substances such as proteins are easily spilled. Under the disturbance, the oil is difficult to separate, and the emulsification phenomenon causes the oil extraction rate and the oil recovery rate to decrease.

3.2. Effects of temperature on oil extraction rate and oil recovery rate
The effects of the temperature on the extraction of avocado oil from avocado pulp by the aqueous extraction is shown in Figure 2. It can be seen from Figure 2 that within the selected range, with the extraction temperature increased, the oil extraction rate and the oil recovery rate show an increasing trend, and the difference between the oil extraction rate and the oil recovery rate gradually decreases. The oil extraction rate and oil recovery rate under the action of 35 °C were 72.97% and 3.13%, respectively. The oil extraction rate and oil recovery yield under the action of 85 °C were 83.38% and 74.62%, respectively. The difference between the two was 69.83% and 8.6% respectively. When the temperature is low, the slurry is too viscous, the oil and water are difficult to separate, and the oil yield is low. With the increase of temperature, the emulsion system is destroyed, and the oil is gradually released, and the yield is gradually increased, and the oil extraction rate and the oil recovery rate are gradually increased. The difference is gradually reduced.

![Figure 1. Effects of materials-to-liquid ratio on extraction of avocado oil from avocado pulp by aqueous extraction.](image1)

![Figure 2. Effects of temperature on extraction of avocado oil from avocado pulp by aqueous extraction.](image2)
3.3. Effects of time on oil extraction rate and oil recovery rate
The effects of the time on the extraction of avocado oil from avocado pulp by the aqueous extraction is shown in Figure 3. It can be seen from Figure 3 that within the selected range, with the increase of extraction time, the oil extraction rate and oil recovery rate showed a trend of increasing first and then decreasing. When the extraction time was 120min, the oil extraction rate and oil recovery yield were the highest, 75.08% and 83.27%, respectively. If the extraction time is too short, the water will replace the oil in the pulp less; if the time is too long, the oil in the slurry will be replaced, and the emulsion layer will be easily formed by the stirring force for a long time, resulting in a decrease in the yield of the oil. During the oil extraction process, it takes a certain time for the oil to be displaced from the avocado slurry, but the time is too long, and it is easy to form an emulsified layer with water and protein under the action of stirring force, and the effect of centrifugal force during solid-liquid separation. The oil-protein emulsification system forms a precipitate and accumulates to the bottom, resulting in a decrease in the oil extraction rate; the oil and water form an emulsification system, resulting in a decrease in the rate of the oil.

![Figure 3. Effects of time on extraction of avocado oil from avocado pulp by aqueous extraction.](image)

![Figure 4. Effects of the pH on extraction of avocado oil from avocado pulp by aqueous extraction.](image)

3.4. Effects of pH on oil extraction rate and oil recovery rate
The effects of the pH on the extraction of avocado oil from avocado pulp by the aqueous extraction is shown in Figure 4. It can be seen from Figure 4 that within the selected range, with the pH value increases, the oil extraction rate and the oil recovery rate generally increase first and then decrease. At pH 8, the oil extraction rate and the oil recovery rate have the maximum values were 76.2% and 59.19%, respectively. The pH changes the solubility characteristics of the protein and the stability of the emulsion system to some extent. When the pH is 5-8, the emulsion system is continuously destroyed, the oil extraction rate and the oil recovery rate increase. When the pH is greater than 8, the oil may be alkaline. The hydroxide ions react to form a salt substance, and the solubility of the oil and fat in water increases, resulting in a decrease in the oil extraction rate and the oil recovery rate.

3.5. Orthogonal test results
The optimal parameters of each factor were determined by single factor experiment. Based on the extraction rate of avocado oil and the yield of clear oil, the ratio of material to liquid (A), extraction temperature (B), extraction time (C) and Four factors were extracted for pH (D). The orthogonal test was used to optimize the process. The results of orthogonal test are shown in the table. The results are shown in Table 2.

From the extreme R value of Table 2, the biggest factor affecting the extraction rate of avocado oil is the pH value, followed by the extraction temperature, while the ratio of material to liquid has the least influence on the oil extraction rate, pH> temperature> time> material to liquid rate; the biggest factor affecting the yield of avocado oil is the ratio of material to liquid, followed by the extraction time, and the extraction temperature has the least effect on the oil recovery rate, the material to liquid rate> temperature> time> material to liquid.
rate > time > pH > temperature. It can be concluded from Table 2 that the optimal combination of oil extraction rate and oil recovery rate should contain A1D2, and single factor analysis can obtain 150min higher oil extraction rate and oil recovery rate than 90min, so C3 condition is better than C1, 75 °C is higher than 65 °C oil extraction rate and oil recovery rate, so B2 condition is better than B1, so the optimal combination is A1B2C3D2; As can be seen from Table 3, when the oil extraction rate is the indicator, the pH value is significant at 0.05 level. As can be seen from Table 4, when the oil recovery yield is used as an index, the ratio of material to liquid is significant at 0.05 level.

Table 2. Orthogonal test results for optimization of extraction conditions of avocado oil.

| Number | A/(g/mL) | B/℃  | C/min | D     | Oil extraction rate/% | Oil recovery rate/% |
|--------|----------|------|-------|-------|-----------------------|---------------------|
| 1      | 1(1:3)   | 1(65)| 1(90) | 1(7)  | 65.73                 | 21.36               |
| 2      | 2(1:4)   | 1    | 2(120)| 2(8)  | 69.71                 | 11.90               |
| 3      | 3(1:5)   | 1    | 3(150)| 3(9)  | 67.15                 | 10.77               |
| 4      | 2        | 2(75)| 1     | 3     | 68.07                 | 6.92                |
| 5      | 3        | 2    | 2     | 1     | 58.23                 | 5.91                |
| 6      | 1        | 2    | 3     | 2     | 71.98                 | 34.22               |
| 7      | 3        | 3(85)| 1     | 2     | 66.13                 | 6.39                |
| 8      | 1        | 3    | 2     | 3     | 58.22                 | 27.19               |
| 9      | 2        | 3    | 3     | 1     | 56.22                 | 9.77                |

oil extraction rate

|     | k1     | k2     | k3     | R      |
|-----|--------|--------|--------|--------|
| k1  | 65.31  | 64.67  | 63.84  | 1.47   |
| k2  | 67.53  | 66.09  | 60.19  | 7.34   |
| k3  | 66.64  | 62.05  | 65.12  | 4.59   |
| R   | 60.06  | 69.27  | 64.48  | 9.21   |

optimal combination A1B1C1D2, D>B>C>A

|     | k1     | k2     | k3     | R      |
|-----|--------|--------|--------|--------|
| k1  | 27.59  | 9.53   | 7.69   | 19.90  |
| k2  | 14.68  | 15.68  | 14.45  | 1.23   |
| k3  | 11.56  | 15.00  | 18.25  | 6.70   |
| R   | 12.35  | 17.50  | 14.96  | 5.16   |

optimal combination A1B2C3D2, A>C>D>B

Table 3. Analysis of variance by oil extraction rate.

| source of variance | sum of square | degree of freedom | Average sum of squares | F value | Sig     |
|--------------------|---------------|-------------------|------------------------|--------|---------|
| A materials to liquid ratio | 3.27          | 2                 | 1.637                  | 0.039  | 0.962   |
| B temperature      | 32.78         | 2                 | 16.391                 | 0.257  | 0.795   |
| C time             | 127.40        | 2                 | 63.709                 | 38.65  | 0.025*  |
| D pH value         | 254.24        | 8                 |                        |        |         |

* indicates significant at the 0.05 level, the same below.

Table 4. Analysis of variance by oil recovery rate.

| source of variance | sum of square | degree of freedom | Average sum of squares | F value | Sig     |
|--------------------|---------------|-------------------|------------------------|--------|---------|
| A materials to liquid ratio | 725.56        | 2                 | 362.780                | 19.831 | 0.002*  |
B temperature 2.59 2 1.293 0.009 0.991  
C time 67.29 2 33.643 0.263 0.777  
D pH value 39.89 2 19.945 0.150 0.863  
Total 835.33 8  

3.6. Physical and chemical properties of avocado oil

The physicochemical properties of the avocado oil obtained by the aqueous extraction are shown in Table 5. It can be seen from Table 5, the acid value of the avocado oil obtained from the aqueous extraction is 0.86 mg/g, the iodine value is 154.41 g/100g, the peroxide value is 0.07 g/100g, the moisture and volatile matter are 0.13%, the saponification value is 198 mg/g, and the saponified product is 0.26%. Compared with the oil obtained from the n-hexane extraction and cold-pressed, the amaranth oil obtained from the aqueous extraction has the lowest acid value, iodine value, moisture, volatile matter and unsaponifiable content, the highest iodine value and saponification value, and the acid value and iodine value. The difference between moisture, and volatile matter content, and unsaponifiable matter are significant, so the physicochemical quality of avocado oil obtained from the aqueous extraction is the best.

Table 5. Physicochemical properties of avocado oil.

| items                  | aqueous extraction | n-hexane extraction | cold-pressed |
|------------------------|--------------------|---------------------|-------------|
| acid value/(mg/g)      | 0.86±0.09a         | 1.13±0.07b          | 1.16±0.06b  |
| iodine value/(g/100g)  | 154.41±4.45a       | 96.90±0.63b         | 139.61±1.86c|
| peroxide value/(g/100g)| 0.07±0.00a         | 0.15±0.00b          | 0.17±0.00a  |
| moisture and volatile matter/% | 0.13±0.01a | 15.10±0.06b | 0.44±0.11c |
| saponification value/(mg/g) | 198.0±1.2a | 162.0±9.5b | 187.0±15.0a |
| unsaponification value/% | 0.26±0.20a | 3.41±0.24b | 3.66±0.17b |

3.7. Determination of fatty acid content in avocado oil

The fatty acid content of the avocado oil obtained from the aqueous extraction is shown in Table 6. It can be seen from Table 6 that seven fatty acids were detected in the avocado oil obtained by the water method. The main fatty acids were composed of oleic acid, palmitic acid, linoleic acid and palmitoleic acid. Compared with the n-hexane extraction and cold pressing method, the water generation method The obtained avocado oil had the highest content of palmitic acid and palmitoleic acid, and the highest content of total unsaturated fatty acids was 85.72%. The difference of palmitic acid and palmitoleic acid was significant. Considering the main fatty acid composition of avocado oil by the water method. The extraction efficiency is higher.

Table 6. The fatty acids composition and relative percentage of avocado oil.

| fatty acids | retention time | aqueous extraction/% | n-hexane extraction/% | Cold-pressed/% |
|-------------|----------------|----------------------|-----------------------|---------------|
| C16:0       | 24.280         | 13.33±2.15a          | 12.99±0.17b           | 12.75±0.30b   |
| C16:1       | 23.249         | 5.28±0.50a           | 4.12±0.01b            | 4.51±0.10b    |
| C18:0       | 33.255         | 0.65±0.09a           | 0.62±0.03a            | 0.75±0.00a    |
| C18:1       | 32.350         | 71.93±3.45a          | 71.2±0.58a            | 73.63±0.43a   |
| C18:2       | 31.820         | 8.51±1.86a           | 9.32±0.06a            | 6.55±0.20b    |
| C20:0       | 38.200         | 0.12±0.01a           | 0.12±0.00a            | 0.14±0.01a    |
| Squalene    | 40.595         | 0.09±0.04a           | 0.16±0.13b            | 0.09±0.01a    |
| SFA         |                | 14.10                | 13.81                 | 13.72         |
1. C16:0 Palmitic acid, C16:1 Palmitoleic acid, C18:0 Stearic acid; C18:1 Oleic acid; C18:2 Linoleic acid; C20:0 Eicosanoic acid.

2. SFA total saturated fatty acids, MUFA total monounsaturated fatty acids, PUFA total polyunsaturated fatty acids, UFA total unsaturated fatty acids, FA total fatty acids.

3. a, b Means are compared only within a row; mean values with different letters are significantly different (P <0.05).

4. Conclusion

(1) In this experiment, the process conditions of extracting avocado oil by aqueous extraction were studied. The optimum process parameters of avocado oil extraction were obtained with oil extraction rate and oil recovery yield: the ratio of material to liquid was 1:3 (g/mL), extraction temperature is 75 °C, extraction time is 150 min, and pH value is 8. After 3 parallel tests, the extraction rate of avocado oil can reach 78.95% and the yield of clear oil can reach 37.21%. It is indicated that the water generation method is feasible to extract avocado oil from the avocado pulp. The development of avocado oil water generation technology will provide a new process route for avocado oil extraction, which has obvious theoretical and practical significance. However, it is necessary to further study the emulsification problem of avocado oil in the extraction process to improve the oil recovery of avocado.

(2) The experiment studied the physicochemical properties and fatty acid composition of the avocado oil obtained by the water method. The acid value of the avocado oil obtained by the water method was 0.86 mg/g, the iodine value was 154.41 g/100g, and the saponification value was 198.0 g/100g. The moisture and volatile matter are 0.13%, which is in line with the national edible oil quality standard; the main fatty acid compositions of avocado oil are oleic acid (71.93%), palmitic acid (13.33%), linoleic acid (8.51%), palmitoleic acid (5.28%), the total unsaturated fatty acid content is 85.72%. Compared with the avocado oil obtained by the n-hexane extraction method and the cold-pressing method, the abalone oil obtained by the water method has better quality, and the water generation method has obvious advantages.

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References
[1] Vallejo Pérez, M. R., Téliz Ortiz, D., De La Torre Almaraz, R., et al. (2017) Avocado sunblotch viroid: Pest risk and potential impact in México. Crop Protection, 99: 118-127.
[2] Ramírez-Gil, J. G., Morales, J. G. and Peterson, A. T. (2018) Potential geography and productivity of “Hass” avocado crops in Colombia estimated by ecological niche modeling. Scientia Horticulturae, 237: 287-295.
[3] Chen J B, Zhou S Y, Li Y S, et al. (1981) Preliminary study on the chemical constituents of avocado oil. Yunnan Hot Work Technology, (01): 45-48.
[4] Pedreschi, R., Hollak, S., Harkema, H., et al. (2016) Impact of postharvest ripening strategies on ‘Hass’ avocado fatty acid profiles. South African Journal of Botany, 103: 32-35.
[5] Wong M., EyresL., Ravetti L. 2 - Modern Aqueous Oil Extraction—Centrifugation Systems for Olive and Avocado Oils[M]. AOCS Press, 2014:19-51.
[6] Shen H., Wang M. (1999) Study on the Preparation of Avocado Edible Oil by Pressing. Chinese fat, (01): 12-14.
[7] Barros, H. D. F. Q., Coutinho, J. P., Grimaldi, R., et al. (2016) Simultaneous extraction of edible oil from avocado and capsanthin from red bell pepper using supercritical carbon dioxide as solvent. The Journal of Supercritical Fluids, 107: 315-320.

[8] Corzzini, S. C. S., Barros, H. D. F. Q., Grimaldi, R., et al. (2017) Extraction of edible avocado oil using supercritical CO2 and a CO2/ethanol mixture as solvents. Journal of Food Engineering, 194: 40-45.

[9] Domínguez, H., Núñez, M. J. and Lema, J. M. (1994) Enzymatic pretreatment to enhance oil extraction from fruits and oilseeds: a review. Food Chemistry, 49 (3): 271-286.

[10] Wang L Y., Yang Z W. (2018) Optimization of aqueous enzymatic extraction of avocado oil by response surface. Food technology, 43 (04): 235-241.

[11] Xu L, Ye L J., Huang X S. (2014) Study on the Process of Improving the Yield of Avocado Oil by Pineapple Juice Treatment. Food industry technology, 35 (08): 223-227.

[12] Chen K J., Chen J M. (2018) Optimization of Process for Extracting Avocado Oil from Papaya Juice. Chinese fat, 43 (08): 15-18.

[13] Martínez-Padilla, L. P., Franke, L., Xu, X.-Q., et al. (2018) Improved extraction of avocado oil by application of sono-physical processes. Ultrasonics Sonochemistry, 40: 720-726.

[14] Fuentes, E., Báez, M. E. and Díaz, J. (2009) Microwave-assisted extraction at atmospheric pressure coupled to different clean-up methods for the determination of organophosphorus pesticides in olive and avocado oil. Journal of Chromatography A, 1216 (51): 8859-8866.

[15] Jiménez, P., García, P., Bustamante, A., et al. (2017) Thermal stability of oils added with avocado (Persea americana cv. Hass) or olive (Olea europaea cv. Arbequina) leaf extracts during the French potatoes frying. Food Chemistry, 221: 123-129.

[16] Wang, J.-S., Wang, A.-B., Zang, X.-P., et al. (2018) Physical and oxidative stability of functional avocado oil high internal phase emulsions collaborative formulated using citrus nanofibers and tannic acid. Food Hydrocolloids, 82: 248-257.

[17] Wang, J.-S., Wang, A.-B., Zang, X.-P., et al. (2019) Physicochemical, functional and emulsion properties of edible protein from avocado (Persea americana Mill.) oil processing by-products. Food Chemistry, 288: 146-153.