The research on extracting oil from watermelon seeds by aqueous enzymatic extraction method

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Abstract

Watermelon kernels were used as raw materials in this study and treated with ultrasound. The effects of different factors on oil extraction from watermelon kernel were studied during the experiments. The optimum parameters obtained from single-factor experiments and response surface method optimization were as follows: enzyme additive amount 2.63\%, hydrolysis temperature 47.13 °C, hydrolysis time 4.29h, materials to water rate 1:4.35 and pH 7.89. The optimum oil extraction under the above optimum condition was 97.92\%.

1. Introduction

Watermelon Seed Oil is also known as Ootanga Oil and Kalahari Oil. In Africa, watermelon seeds have been prized for the highly nutritive oil that they contain. Traditionally, the seeds are removed from the rind and then allowed to dry outside in the sun. Once dried, the seeds are then pressed to extract the

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beneficial oil. Watermelon seed oil contains high amounts of unsaturated fatty acids with linoleic and oleic acids as the major acids [1~2].

Aqueous enzymatic extraction has been extensively studied. It has received much interest, and viewed as an alternative method to extract oil from oil-bearing seeds [3]. Not only this green technology is beneficial to people’s health but also is environmental friendly. In this study, Protex 6L was used in the extraction of oil. The single factor experiments and response surface methods were employed to optimize the parameters in oil extraction process [4].

2. Methods

2.1. Materials

Watermelon seeds were purchased from local market. Protex 6L (NOVO Company), NaOH and ethanol agent were used in this experiment. LDZ5-2-type low-speed desktop centrifuge, electronic balance, F2102 type of plant specimen grinder, electric heated water bath, precision electric mixer, Soxhlet extraction, pH5-25 meter and electro-thermostatic blast oven were used in this experiment.

2.2. Procedure

2.3. Analytical methods

Determination of water: according to GB 5009.3-2010
Determination of fat: according to GB/T 14772-2008
Determination of crude protein: according to GB 5009.5-2010
Determination of ash: according to GB/T 5505-2008

2.4. Response surface method analysis

The range of level values of each factors were determined based on the single-factor experiments parameters. The response surface method was employed to analysis the effects of each factors on total protein extraction rate. Five factors (enzyme additive amount, hydrolysis temperature, hydrolysis time,
materials to water ratio and pH) were selected as independent variable and total protein rate was chosen as variable [5].

Weigh 50g watermelon seeds grinding powder, and then mixed with distilled water according to a certain materials to water ratio. Then, transfer the mixture into water bath with continuous stirring and adjust the mixture pH using NaOH. After then, transfer the mixture to ultrasonic bath. Protex 6L was added into the mixture and kept the hydrolysis temperature during the enzymatic hydrolysis process. Then, inactivate the enzyme by increasing temperature to 100 ºC for 5 min and centrifuge the mixture. After then, absorb the oil in upper layer and dry the residual materials.

Equation 1 is used to calculate the total oil extraction rate of watermelon seeds:

\[
\text{Total oil extraction rate (\%)} = \frac{\text{Total oil in watermelon seed} - \text{oil in residual materials}}{\text{Total oil in watermelon seed}} \times 100
\]

(1)

3. Results and discussion

3.1. The analysis of single factor experiment

3.1.1 The effect of materials to water rate on oil extraction rate

Fig.1 shows that the oil extraction rate is increased with the ascending of materials to water rate and reaches the highest point at 1:5. The oil extraction rate falls when the materials to water rate beyond 1:6.

![Fig.1 The effect of materials to water rate on oil extraction rate](image)

3.1.2 The effect of enzyme additive amount on oil extraction rate

From Fig.2 we know that the oil extraction rate increases continually with the increasing of enzyme additive amount. However, the rate keeps stable when the enzyme additive amount beyond 2.5%.
3.1.3 The effect of pH on oil extraction rate

Fig. 3 shows that the oil extraction rate increases first and decreases at the pH 8 which indicates that pH 8 is the suitable condition for hydrolysing.

3.1.4 The effect of hydrolysis temperature on oil extraction rate

Fig. 4 indicates that oil extraction rate increases with the increasing hydrolysis temperature and the rate reaches its highest point at 50 °C. The rate decreases when the temperature continuous increasing which means the higher temperature has disadvantage on the activity of enzyme and then influence the oil extraction.
3.1.5 The effect of hydrolysis time on oil extraction rate

Fig.5 shows that oil extraction rate sharply increases with the prolonging hydrolysis time.

3.2. Response surface analysis

The range of each factor’s level is determined based on the data of single factor experiment. The response surface analysis method is employed to design and optimize the parameters in the experiment. The independent variables are as follows: materials to water rate (x1), pH (x2), hydrolysis time (x3), enzyme additive amount (x4) and hydrolysis temperature (x5). The response value is total protein extraction rate. Tab.1 is the level coding and Tab.2 is the design and result of this experiment.

Table.1 Encode table of factors and levels

| Code | Factor                | Materials to water rate x1 | pH x2 | Hydrolysis time x3 (h) | Enzyme additive amount x4 (%) | Hydrolysis temperature x5 (ºC) |
|------|-----------------------|----------------------------|-------|------------------------|-----------------------------|-------------------------------|
| -2   | 1:4                   | 7                          | 2     | 1.5                    | 45                          |
| -1   | 1:5                   | 8                          | 2.5   | 2                      | 47.5                        |
| 0    | 1:5.5                 | 8.5                        | 3     | 2.25                   | 50                          |
| 1    | 1:6                   | 9                          | 4     | 2.5                    | 55                          |
| 2    | 1:7                   | 10                         | 5     | 3                      | 60                          |

Table.2 Design and results of response surface analysis

| Run | Materials to water rate x1 | pH x2 | Hydrolysis time x3 (h) | Enzyme additive amount x4 (%) | Hydrolysis temperature x5 (ºC) | Total oil extraction rate y(%) |
|-----|----------------------------|-------|------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1   | 6                          | 8.5   | 5                      | 2.25                          | 47.5                          | 0.9353                        |
| 2   | 5.5                        | 8.5   | 4                      | 2.25                          | 47.5                          | 0.8575                        |
| 3   | 6                          | 8.5   | 4                      | 2                             | 47.5                          | 0.7271                        |
| 4   | 5.5                        | 9     | 4                      | 2.25                          | 50                            | 0.7622                        |
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| 5 | 5.5 | 9 | 4 | 2 | 47.5 | 0.6597 |
| 6 | 5 | 8.5 | 5 | 2.25 | 47.5 | 0.8391 |
| 7 | 6 | 8.5 | 3 | 2.25 | 47.5 | 0.8301 |
| 8 | 6 | 8.5 | 4 | 2.5 | 47.5 | 0.9082 |
| 9 | 5.5 | 9 | 3 | 2.25 | 47.5 | 0.6642 |
| 10 | 5.5 | 8.5 | 5 | 2.25 | 45 | 0.912 |
| 11 | 5.5 | 8 | 4 | 2 | 47.5 | 0.8038 |
| 12 | 5.5 | 8 | 4 | 2.5 | 47.5 | 0.9868 |
| 13 | 5.5 | 8.5 | 3 | 2 | 47.5 | 0.8163 |
| 14 | 5.5 | 8.5 | 3 | 2.25 | 45 | 0.8477 |
| 15 | 5.5 | 8.5 | 4 | 2.25 | 47.5 | 0.9309 |
| 16 | 5.5 | 8.5 | 3 | 2.25 | 50 | 0.8704 |
| 17 | 6 | 8 | 4 | 2.25 | 47.5 | 0.8511 |
| 18 | 5.5 | 8 | 3 | 2.25 | 47.5 | 0.7387 |
| 19 | 5 | 8.5 | 4 | 2.25 | 45 | 0.8256 |
| 20 | 5 | 8.5 | 4 | 2.25 | 50 | 0.8291 |
| 21 | 5.5 | 8.5 | 5 | 2.25 | 50 | 0.9102 |
| 22 | 6 | 8.5 | 4 | 2.25 | 50 | 0.8938 |
| 23 | 5.5 | 8.5 | 4 | 2.25 | 47.5 | 0.8575 |
| 24 | 5.5 | 8.5 | 4 | 2.5 | 45 | 0.9258 |
| 25 | 6 | 9 | 4 | 2.25 | 47.5 | 0.6906 |
| 26 | 5.5 | 9 | 4 | 2.5 | 47.5 | 0.8806 |
| 27 | 5.5 | 8.5 | 4 | 2 | 45 | 0.7941 |
| 28 | 5.5 | 8.5 | 5 | 2.5 | 47.5 | 0.862 |
| 29 | 5.5 | 8.5 | 4 | 2.5 | 50 | 0.9397 |
| 30 | 5 | 8.5 | 4 | 2.5 | 47.5 | 0.8967 |
| 31 | 5 | 8.5 | 3 | 2.25 | 47.5 | 0.8153 |
| 32 | 5 | 8 | 4 | 2.25 | 47.5 | 0.8543 |
| 33 | 5.5 | 8 | 5 | 2.25 | 47.5 | 0.8774 |
| 34 | 5.5 | 9 | 4 | 2.25 | 45 | 0.674 |
| 35 | 5.5 | 8 | 4 | 2.25 | 50 | 0.7586 |
| 36 | 5.5 | 8.5 | 5 | 2 | 47.5 | 0.8254 |
| 37 | 5.5 | 8.5 | 3 | 2.5 | 47.5 | 0.8261 |
| 38 | 5.5 | 8.5 | 4 | 2.25 | 47.5 | 0.9309 |
| 39 | 5.5 | 9 | 5 | 2.25 | 47.5 | 0.7351 |
| 40 | 6 | 8.5 | 4 | 2.25 | 45 | 0.8611 |
| No. | pH  | Materials to water rate | Hydrolysis time (h) | Hydrolysis temperature (°C) | Oil extraction rate (%) |
|-----|-----|-------------------------|---------------------|--------------------------|------------------------|
| 41  | 5.5 | 8.5                     | 4                   | 2.25                     | 47.5                   | 0.924                  |
| 42  | 5.5 | 8.5                     | 4                   | 2                        | 50                     | 0.7922                 |
| 43  | 5.5 | 8                     | 4                   | 2.25                     | 45                     | 0.8581                 |
| 44  | 5   | 9                      | 4                   | 2.25                     | 47.5                   | 0.7933                 |
| 45  | 5   | 8.5                    | 4                   | 2                        | 47.5                   | 0.7923                 |
| 46  | 5.5 | 8.5                    | 4                   | 2.25                     | 47.5                   | 0.8079                 |

Fig. 6 Response surface analysis of significant effective interaction items of different hydrolysis parameters on extraction rate of oil.
3.3. Pine kernel oil fatty acid analysis

Gas chromatography was used to identify components in the pine kernel oil. The fatty acid contents of pine kernel oil are as follows: palmitic acid 33.660%, oleic acid 0.723%, linoleic acid 39.485%, linolenic acid 0.432% and stearic acid 19.890% (Fig. 7).

![Graph showing gas chromatography analysis of watermelon seed oil](image)

Fig. 7 Gas chromatography analysis of watermelon seed oil

4. Conclusion

The response surface analysis method was employed to optimize the parameters in the experiment. The optimum parameters are as follows: enzyme additive amount 2.63%, hydrolysis time 4.29h, hydrolysis temperature 47.13 °C, materials to water rate 1: 4.35, pH 7.89 and total oil extraction rate 97.93%.

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