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# Ultrasonic-Supercritical $CO_2$-Assisted Extraction and Component Analysis of Navel Orange Peel Pigment

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Navel orange peel pigment was extracted using an ultrasonic-supercritical $CO_2$-assisted method. Extraction process was optimized using response surface methodology and pigment components were analyzed. Results of infrared spectroscopy and second-derivative analysis indicated that navel orange peel pigment principally contained aromatic ring, sugars and alkenes. Response surface methodology identified that the optimized conditions to obtain 72.34 % yield were as follows: ultrasonic power, 250 W; supercritical $CO_2$ flow rate, 20 L/h; extraction pressure, 7.7 MPa; temperature, 53 °C; and time, 38 min.

**Keywords: Navel orange peel pigment, Extraction of ultrasonic-supercritical $CO_2$, Process optimization, Component analysis.**

## INTRODUCTION

Navel orange peel pigment principally contains a mixture of limonene and carotenoids, vitamin E and the selenium[1], which are rich and important natural food colours and supplements. Thus, this pigment, which is also used as a food colouring agent and an herbal medicine, is worthy of development and utilization.

Several new approaches have recently been reported for the extraction of navel orange peel. These approaches include extraction with ethanol-water as solvent[2] as well as ultrasonic-[3] and microwave-assisted extractions[4] with ethyl acetate and water as solvents, respectively. These methods and the required instruments are simple, easy and available. However, these methods involve long-term leaching and perform low-efficiency extractions.

Supercritical $CO_2$ extraction has many unique advantages, such as high extraction rate, low temperature, nontoxicity, absence of residue and easy operation. This technique can retain the active substance and requires no concentration of bioactive substances for separating and purifying unstable products[5]. Ultrasonic cavitation can overcome long-term leaching and low-efficiency extraction by effectively crushing and dissolving active ingredients from cells. The combination of ultrasonic and supercritical $CO_2$ has been proven to be highly effective[6]. In this study, the navel orange peel pigment was extracted for the first time using ultrasonic-supercritical $CO_2$-assisted method. The extraction conditions were optimized using response surface methodology (RSM). This study may serve as a theoretical basis for the development of a new method to utilize navel orange peel pigment.

## EXPERIMENTAL

Fresh navel orange peel was purchased from a local supermarket. The main equipment included a supercritical $CO_2$ extraction machine (HA221-40-48-C, China), an ultrasonic generator (VGT-2013T, China), a high-performance liquid chromatograph (LC-100, Japan), a Fourier-transform infrared (FTIR) spectrometer (Varian 2000, German), an electronic analytical balance (BH2, China) and an electrically heated drying oven (DHG-9140A, China).

**Pre-processing of navel orange peel:** The navel orange peel was washed with distilled water and placed into an electric oven at constant temperature (70 °C) until its moisture content was 8 %. Then, the peel was powdered to 60 mesh using a high-speed disintegrator.

**Ultrasonic-supercritical $CO_2$-assisted extraction:** A supercritical $CO_2$ extraction instrument was charged with navel orange peel powder and dehydrated alcohol (solid-liquid ratio of 8:1). The pH of the mixture was adjusted to 3.5 and ultrasonic power preprocessed sets were installed. The pigment was extracted with different extraction times, temperatures and pressures. After being cooled to room temperature, the mixture was extracted by two filtration and centrifugation cycles to obtain the pigment solution.

**Detection:** The pigment yield of navel orange peel was detected by high-performance liquid chromatography. The sample was filtered using a filter membrane (0.45 μm). The mobile phase was water with a flow rate of 1 mL/min. The injection volume was 10 μL and the column temperature was 75 °C. A sugar pak column (16.5 × 300 mm) was used in the analysis. The yield of navel orange peel pigment was calculated as

$$y = \frac{\text{Actual extraction value of navel orange peel pigment}}{\text{Theoretical value of navel orange peel pigment}} \times 100\,\% \quad (1)$$

## RESULTS AND DISCUSSION

**Analysis by single-factor test:** Supercritical $CO_2$ was controlled at 20 L/h on the basis of exploratory experiments. Single-factor test was used to optimize the ultrasonic power, time, pressure and temperature during the pigment extraction of navel orange peel to obtaining the best yield.

Under 8 MPa extraction pressure and 20 L/h supercritical $CO_2$ flow of at 50 °C, was compared with different extraction times and ultrasonic power (Fig. 1). Pigment yields increased with extraction time. At 250 W ultrasonic power and 35 min extraction time, the condition was proven to be highly effective and resulted in 71.63 % yield, but the yield decreased subsequently. This phenomenon may have been caused by reduced stability of pigment molecules at high ultrasonic wave and pressure. Thus, 250 W ultrasonic power and 35 min extraction time were chosen for further optimization.

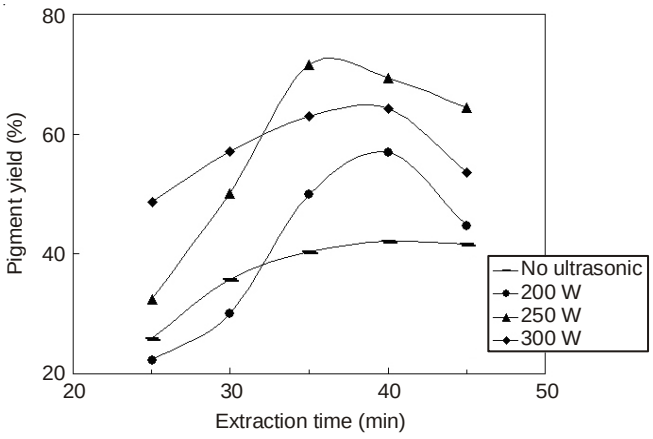


Fig. 1. Effects of extraction time on pigment yield

Pigment yield was extracted at different extraction pressures (7, 7.5, 8, 8.5 and 9 MPa) and ultrasonic power for 35 min at 50 °C. Pigment yield rapidly increased at extraction pressures below 8 MPa and then decreased (Fig. 2). A 70.41 % pigment yield was obtained at 250 W ultrasonic power and 8 MPa extraction pressure. The dissolution of navel orange peel pigment improved with increasing extraction pressure, supercritical $CO_2$ density and transfer efficiency between the solute and solvent[7]. However, the transfer efficiency between the solute and solvent decreased as the pressure continued to increase. Hence, 8 MPa extraction pressure was selected for further test.

Pigment yield was obtained at different extraction temperatures and ultrasonic power at 8 MPa extraction pressure

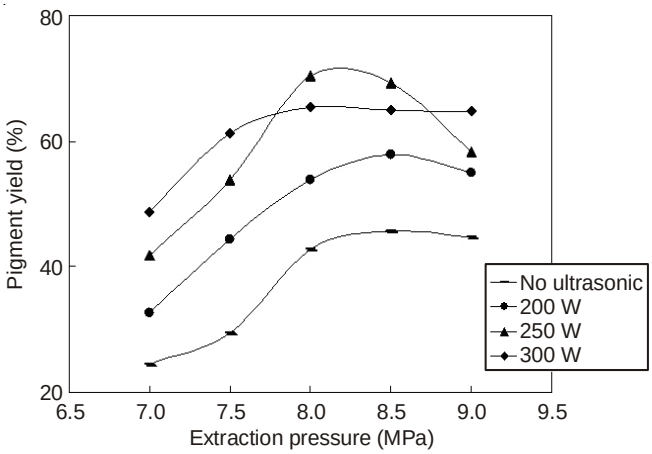


Fig. 2. Effects of extraction pressure on pigment yield

and 35 min extraction time. Pigment yield increased with increasing extraction temperature (Fig. 3). The yield was 69.47 % at 50 °C and 250 W ultrasonic power. However, the yield decreased at > 50 °C. These phenomena were due to the high solubility with increasing vapor pressure and mass transfer coefficient near 50 °C, but higher temperatures reduced the supercritical fluid density and $CO_2$ solvation effect[8]. Thus, the extraction temperature of 50 °C was selected.

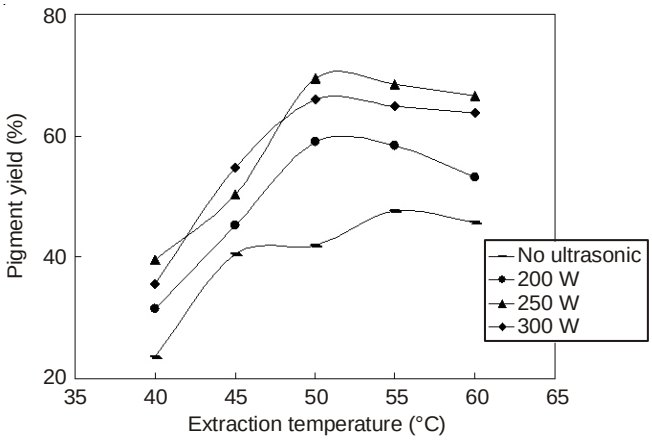


Fig. 3. Effects of extraction temperature on pigment yield

Ultrasonic power is crucial in the pigment extraction of navel orange peel. Figs. 1-3 show a relationship between ultrasonic power and pigment yield. Pigment yield increased with ultrasound assistance. The highest yield was obtained at 250 W ultrasonic power because the multi-level physical effects of ultrasonic cavitation, mechanical vibration, acoustic streaming micro jets and micro acoustic streaming could induce the deformation of micro-orange skin and other tissues to improve the pigment yield of the follow-up process[9,10]. However, the continuous increase in ultrasound power resulted in rapid cavitation bubble generation and increased energy dissipation, which led to difficulty in the collapse of cavitation bubbles and decreased the yield. Therefore, 250 W ultrasonic power was selected.

**Response surface methodology analysis:** To obtain the best yield, the extraction temperature, time and pressure were evaluated and designed for three factors and three levels to response surface with 250 W ultrasonic power to make the

extraction more scientific and reasonable on the basis of single-factor test[11] (Table-1).

According to response surface methodology, the secondary multiple regression equation was

$$Y = 74.45 + 2.07A + 4.89B - 1.13C + 2.21AB - 1.72BC - 10.41A^2 - 5.78B^2 - 2.62C^2 \quad (2)$$

TABLE-1
LEVEL AND CODE OF VARIABLES CHOSEN FOR RSM DESIGN

| Name | Code | Level | | |
|---|---|---|---|---|
| | | -1 | 0 | 1 |
| Extraction temperature (°C) | A | 45 | 50 | 55 |
| Extraction time (min) | B | 30 | 35 | 40 |
| Extraction pressure (MPa) | C | 7.5 | 8 | 8.5 |

Results from ANOVA are shown in Table-2. The model is highly significant as shown by its P value < 0.0001, whereas that for the missing item is 0.3079, which is higher than the significance value of 0.05. The complex correlation is 0.9893, which is very close to 1, which indicates a high degree of agreement with the actual model.

The contour plot of the response surface model is shown in Fig. 4. The closed oval curve indicates the existence of the optimal value of the quadratic regression equation. The combination of extraction temperature, time and pressure strongly influenced pigment yield. Pigment yield rapidly increased when two variables were increased and one was fixed. However, the yield decreased when the peak values were surpassed. Thus, rational optimization of extraction temperature, time and pressure is necessary to improve the yield.

Regression eqn. 2 shows that A, B and C are the significant factors affecting pigment yield and the order is A > B > C. Extraction temperature had the greatest effect, but extraction pressure had minimal effect on pigment yield. Thus, the optimized conditions for obtaining the maximum yield of 73.01 % were identified: extraction pressure, 7.7 MPa; temperature, 52.72 °C; and time, 38.27 min.

Three extraction cycles of verification experiment were performed at 73 °C under 7.7 MPa for 38 min. The average yield of pigment was 72.34 %, which was close to theoretical value.

**FTIR spectra of pigment:** Fig. 5 shows the FTIR spectra of navel orange peel pigment. A low-resolution image is shown because of the complex chemical composition of peel pigment and functional groups displayed on broad or overlapping peaks.

Peak position of the second derivative FTIR spectra corresponded to the peak in the original spectrum, which highlighted the characteristics of the spectrum and clearly distinguished overlapping and small peaks. The FTIR spectral analysis of the second derivative showed that the navel orange peel pigment

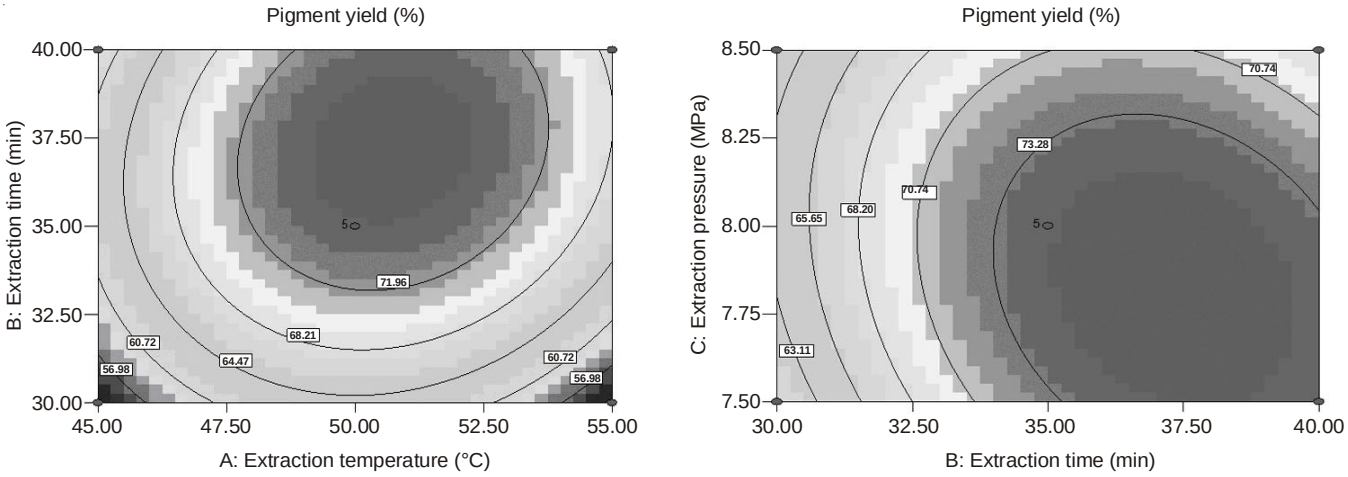


Fig. 4. Contour plot of the response surface model

TABLE-2
MODEL AND VARIANCE

| Source | Sum of squares | df | Mean square | F value | P-value | Significance |
|---|---|---|---|---|---|---|
| Model | 951.78 | 9 | 105.75 | 71.77 | < 0.0001 | ** |
| A | 34.3 | 1 | 34.28 | 23.26 | 0.0019 | ** |
| B | 190.91 | 1 | 190.91 | 129.55 | < 0.0001 | ** |
| C | 10.17 | 1 | 10.17 | 6.9 | 0.0341 | * |
| AB | 19.49 | 1 | 19.49 | 13.23 | 0.0083 | ** |
| AC | 6.48 | 1 | 6.48 | 4.4 | 0.0742 | - |
| BC | 11.8 | 1 | 11.8 | 8.01 | 0.0254 | * |
| $A^2$ | 456.7 | 1 | 456.7 | 309.93 | < 0.0001 | ** |
| $B^2$ | 140.9 | 1 | 140.9 | 95.62 | < 0.0001 | ** |
| $C^2$ | 29.01 | 1 | 29.01 | 19.69 | 0.0030 | * |
| Residual | 10.31 | 7 | 1.47 | - | - | - |
| Lack of fit | 5.75 | 3 | 1.92 | 1.68 | 0.3079 | Not significant |
| Pure error | 4.57 | 4 | 1.14 | - | - | - |

$R^2$ = 0.9893; ** highly significant; * significant

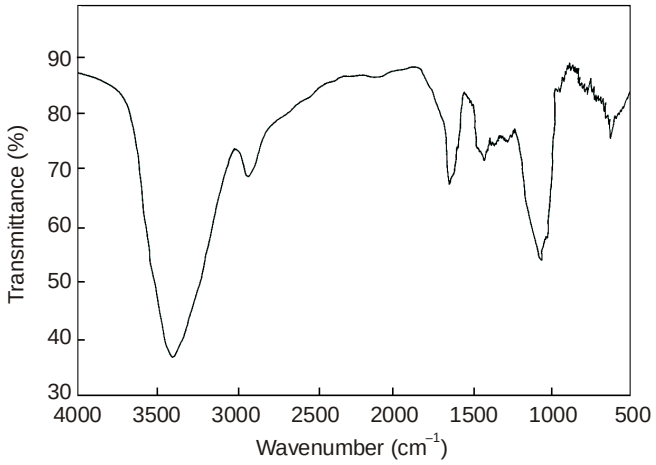


Fig. 5. FTIR pattern of navel orange peel pigment

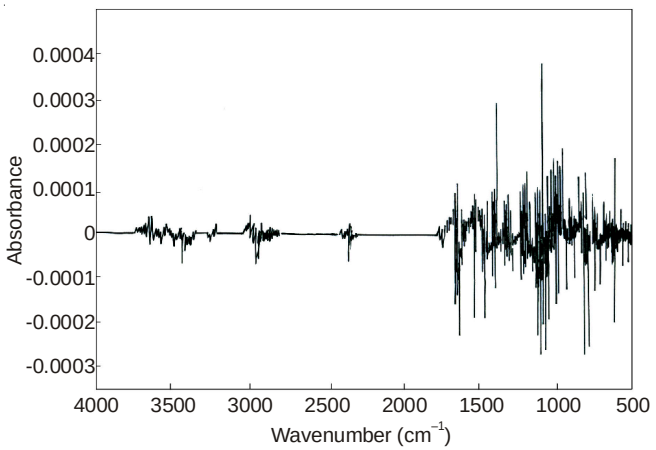


Fig. 6. Second-derivative FTIR spectral pattern of navel orange peel pigment

contained mainly aromatic ring, carbohydrates and olefin compounds (Fig. 6). The FTIR-spectral analysis is shown in Table-3.

TABLE-3
SECOND-DERIVATIVE FTIR SPECTRA OF NAVEL ORANGE PEEL PIGMENT

| Frequency of infrared absorption peak (cm$^{-1}$) | Explanation |
|---|---|
| 3088 | Olefins $CH_2$ antisymmetric stretching vibration |
| 2989 | Olefins $CH_2$ symmetrical stretching vibration |
| 1655, 1638, 1618 | Aliphatic chain C=C stretching vibration |
| 1606, 1472, 1378 | Aromatic ring C=C stretching vibration |
| 1458 | Alkyl $CH_3$ dissymmetry variable angle vibration |
| 1416, 1404 | Olefins $CH_2$ angular vibrations |
| 1374 | Alkyl $CH_3$ symmetry variable angle vibration |
| 1104, 1073, 1050, 1024, 1010 | Sugar and polysaccharide C-OH stretching vibration |
| 982 | Olefins $CH_2$ twisting vibration |
| 890 | Olefins $CH_2$ wagging vibration |
| 731, 722 | Olefins $CH_2$ rocking vibration |

## Conclusion

The proposed ultrasonic-supercritical $CO_2$-assisted method can highly improve the efficiency and shorten the duration of pigment extraction. The main components of navel orange peel pigment were aromatic ring, sugars and alkenes. The optimized conditions to obtain 72.34 % yield were as follows: ultrasonic power, 250 W; supercritical $CO_2$ flow rate, 20 L/h; extraction pressure, 7.7 MPa; temperature, 53 °C; and time, 38 min.

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