



Study on Extraction and Purification Process of Capsicum Red Pigment

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Abstract

Capsicum red pigment extracted from the dry pepper is a kind of high-quality natural dye which has anticancer and cosmetic properties. First, Qiu North spicy was selected as the experimental objects by comparing the peel meal rate and relative amount of pigment of *Fructus capsici*, Qian chilli 2, and Qiu North spicy. Then, before extracting paprika dye, sodium hydroxide was used to eliminate the piquancy. Capsicum red pigment was extracted in Soxhlet extractor with 95% ethanol. The elution condition of column chromatographic separation was obtained by thin-layer chromatography, and the purity of capsicum red pigment was identified by methods of infrared spectrum(IR). Finally, the color value of capsicum red pigment was determined by spectrophotometer with 460nm. Results showed that the optimal process: Concentration of peppery removal agent (sodium hydroxide) was 10%, the holding temperature was 80°C, solid-liquid ratio was 1:20 (g/ml), the extraction time was 120min and the times of extraction were twice. Eluent of silica gel column chromatography separation consisted of petroleum ether and 90% ethanol with the volume ratio of 2:1. The color value of capsicum red pigment ($E_{1cm}^{1\%460nm}$) could reach to 125 and 2.88% of yield could be gained. The process was good to extract and purify capsicum red dye whose stability was high at neutral and weak acid solution.

Keywords: Capsicum red pigment, Extraction, Purification, Process, Stability

1. Introduction

Recently, the harm of synthetic pigments to human is becoming noticeable, and some of them which are even teratogenic and carcinogenic and induce chromosome variation have been forbidden using. Natural dyes have many excellent properties such as little side effect, high safety factor, biodegradable, green environmental protective. Some natural dyes have certain therapy of effect and health function. At present, researchers paid increasing attention to natural dyes and obtained some infusive findings.

Ripe fruits of red pepper (*Capsicum annuum* L.) are widely consumed as vegetables and are used as food colorants because they are a good source of the red carotenoids capsicum red pigment and capsorubin. Capsicum red pigment accounts for 30–60% of total carotenoids in fully ripe fruits. It contains 11 conjugated double bonds, a conjugated keto group, and a cyclopentane ring, and has stronger antioxidative effects than β -carotene. These structural characteristics give rise to singlet oxygen-quenching ability and prevent colon carcinogenesis.

To explore the optimal extraction and purification process of capsicum red pigment from dry pepper, this experiment selected as the experimental objects, and studied and analyzed its preparation process. We also analyzed the pH stability (acidic and basic stability) of the pigment purified.

2. Experimental

2.1 Materials

Pepper: *Fructus capsici*, Qian chilli 2, Qiu North spicy

Reagents: Ethanol (analytically pure, Tianjin Kermel Chemical Reagent Co, Ltd.), Sodium hydroxide(analytically pure, Tianjin Kermel Chemical Reagent Co, Ltd.), Hydrochloride (analytically pure, Tianjin Reagent Factory), Leveling

agent FFA, Penetrating agent JFC.

Instruments: Soxhlet extractor, High-speed universal pulverizer (FW-80, Tianjin Taisite Instruments Co, Ltd.), Electric blastdrying oven (DL-101-1B, Tianjin Zhonghuan experiment electric stove Co, Ltd.), temperature adjusting electric heating-jacket (ZDHW, Huanghua ZTE Instrument Co, Ltd.), thermostat water bath cauldron (Tianjin Zhonghuan experiment electric stove Co, Ltd.), Electronic balance (LD, Shenyang Dragon Electronics Co. Ltd.), Visible spectrophotometer (VIS-723, Shanghai Jingke Industrial Co, Ltd.), Electric thermal-vacuum drying oven (DZG-04, Tianjin Tianyu Experimental Instrument Co, Ltd.).

2.2 Preparation of dry capsicum powder

Before experiment, three kinds of dry capsicum must be removed seeds and stalks and washed using tap water twice to three times. They were put into electric blastdrying oven maintaining 60°C for 2hs and milled into powder. Subsequently, the dry capsicum powder was sift through 10-20 meshes.

A gram of dry capsicum powder was mixed with sodium hydroxide to eliminate the piquancy in the beaker, the concentration of which was 5%, 10%, 15%, 20%, 30%, respectively. Finally, dry capsicum powder eliminated the piquancy was obtained after vacuum filtrating and drying in the Electric thermal-vacuum drying oven.

2.3 Extraction of capsicum red pigment

Dry capsicum powder was transferred to Soxhlet extractor which was added into 95% ethanol. To ensure the optimal extraction process, the temperature was maintained at 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C, respectively; solid-liquid ratio was 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, respectively; time was 30min, 60min, 90min, 100min, 110min, 120min, 150min respectively and reflux times were once, twice, third, four, five, six times. Finally, head product was obtained after vacuum distillation and recovering ethanol.

2.4 Purity of capsicum red pigment

Silica gel column chromatography was selected to purify capsicum red pigment: elution condition was ensured by thin layer chromatography. First, concentration extracted was injected into the column with 100cm in high, 10cm in diameter and was eluted by eluent selected. Afterward, several parts of solution were gained and red part was collected and vacuum-concentrated to relatively pure capsicum red pigment. At last, the capsicum red pigment was analyzed by methods of infrared spectrum (IR).

2.5 Elemental analysis

2.5.1 Measurement of color value

A certain quality sample (accurate to 0.0002g) weighted was diluted with acetone, and using colorimetric utensil of 1cm thickness to measure it with spectrophotometer where the wavelength (λ) is 460nm. When the dilution multiple falls in a certain range, the wavelength is proportional to the content of capsicum red pigment. The color value was expressed as followed:

$$E_{1cm}^{1\%} 460nm = \frac{Af \times 1\%}{m}$$

Where, $E_{1cm}^{1\%} 460nm$ is the color value measured under the conditions of mass fraction of 1%, colorimetric utensil of 1cm, maximum absorption peak of 460nm, A is absorbance of measured sample, f is dilution multiple, and m is mass of sample.

2.5.2 Determination of yield

yield (%) = volume of capsicum red pigment (ml) / total mass of capsicum powder (g) $\times$ 100%

2.5.3 pH stability

First, capsicum red pigment was divided into ten portions adjusted to represent the pH from 1 to 10, respectively. Finally, absorbances (λ) of those ten portions were measured.

3. Results and discussion

3.1 Selection of species of pepper

Capsicum red pigment mainly exists in peel of pepper, so the larger peel meal rate is, the better effect of extraction becomes. Moreover, the larger relative amount of pigment is, the better effect of extraction becomes. In the same condition, the peel meal rate and the relative amount of pigment for three specie of pepper were showed on tab.1

Tab.1 shows that, the maximum peel meal rate is Qiu North spicy (61.2%), and the minimum is Qian chilli 2 (50.4%); the maximum relative amount of pigment is Qiu North spicy (14.62), and the minimum Fructus capsici is (7.32). Comprehensive consideration on the factor of peel meal rate and relative amount of pigment, the optimal raw material is Qiu North spicy.

3.2 Selection of the concentration of peppery removal agent (sodium hydroxide)

From observing the color change of sodium hydroxide mixed with dry capsicum powder, the peppery removal effect is good when the concentration of sodium hydroxide was 10%, 15%, 20%, 30%. However, for the concentration of 15%, 20%, 30%, the solution appeared turbid and caking during the holding process. So we selected the optimal concentration of sodium hydroxide is 10%.

3.3 Selection of extraction temperature

Before extracted, capsicum red pigment exists in cell tissue. Capsicum red pigment is protected by membrane and some components in cell tissue to form lipid, so it has high stability. Since capsicum red pigment extracted loses the protection from membrane, self-oxidation occurs easily, particularly in High-Temperature condition, color of solution fades obviously. The effect of temperature is the primary considered factor. The larger relative quantity of solvent and the longer extraction time is, the better extraction effect is. So less solid-liquid ratio and longer extraction time is the selection in this part.

Experimental Conditions is listed as follow: solid-liquid ratio is 1:35, the extraction time is 160min and the time is once. Absorbance (λ) measured in different temperature is showed in Fig.1.

Fig.1 shows that, with extraction temperature increasing, the absorbance (λ) increases gradually and reaches the peak at extraction temperature of 80°C, then it goes down. When extraction temperature at the range from 0°C to 80°C, the increase of temperature promotes the dissolution of capsicum red pigment, when extraction temperature exceeds 80°C, the increase of temperature turns to damage capsicum red pigment for self-oxidation as introduced above. The optimal extraction temperature is 80°C.

3.4 Selection of solid-liquid ratio

Experimental Conditions is listed as follow: the extraction temperature is 80°C, the time is 160min and the time is once. Absorbance (λ) measured in different solid-liquid ratio is showed in Fig.2.

Fig.2 shows that, with solid-liquid ratio increasing, the absorbance (λ) increases significantly and reaches the peak at solid-liquid ratio of 1:20, then it changes little and basically maintains at about 8.0-8.5. When extraction temperature at the range from 1:5 to 1:20, the solubility is the main factor of extraction effect, when extraction temperature exceeds 1:20, the solubility is not the main factor of extraction effect in that capsicum red pigment has been dissolved completely. The optimal extraction temperature is 1:20.

3.5 Selection of extraction time

Experimental Conditions is as the following: the extraction temperature is 80°C, solid-liquid ratio is 1:20 and the times are once. Absorbance (λ) is measured in different extraction time is showed in Fig.3.

Fig.3 shows that, with extraction time increasing, the absorbance (λ) increases gradually and reaches the peak (0.587) at extraction time of 120min, then it goes down. When extraction time at the range from 30min to 120min, the increase of time promotes the dissolution of capsicum red pigment, when extraction time exceeds 120min, the increase of time turns to damage capsicum red pigment for self-oxidation in High-Temperature. The optimal extraction time is 120min.

3.6 Selection of extraction times

Experimental Conditions is as the following: the extraction temperature is 80°C, solid-liquid ratio is 1:20 and the extraction time is 120min. Absorbance(λ) is measured in different extraction times is showed in Fig.4.

Fig.4 shows that, capsicum red pigment has been extracted completely after twice extraction, and absorbance (λ) is not significant change and basically maintains at about 1.01-1.02. Considering the energy efficiency, the optimal extraction time is twice.

3.7 The purification of capsicum red pigment

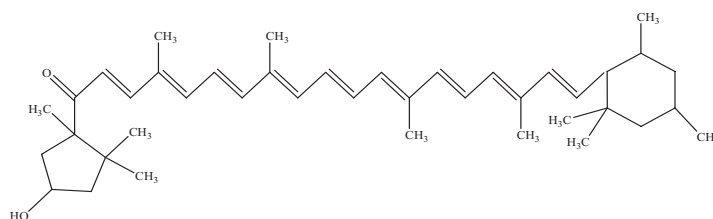
3.7.1 The selection of elution condition

Thin layer chromatography (TLC) was used to ensure the elution condition of column chromatographic separation. Comprehensive consideration on the factor of solubility, affinity, resolution and the elution curve of petroleum ether, ethyl acetate, ethanol and n-hexane, the results showed that the effect of exhibition layer is the best with petroleum ether-90% ethanol (2:1) as developer. The optimal eluent of silica gel column chromatography separation is petroleum

ether-90% ethanol (2:1).

3.7.2 Analysis on purity

The structure molecular formula of capsicum red pigment is showed as follows:



The characteristic absorption peak of Pure capsicum red pigment is as follows: the characteristic absorption peak of cyclopentane and cyclohexane is at $2950\text{--}2800\text{cm}^{-1}$, the stretching vibration absorption peak of carbonyl($\text{C}=\text{O}$) is at $1720\text{--}1710\text{cm}^{-1}$, the scissors vibration absorption peak of methylene(CH_2) and anti symmetric deformation absorption peak of methyl (CH_3) is at $1465\pm 20\text{cm}^{-1}$, the stretching vibration absorption peak of methoxy($\text{C}-\text{O}$) is at $1170\text{--}1150\text{cm}^{-1}$ [10]. Fig.5 represents the infrared spectrum of capsicum red dye prepared, and shows the infrared absorption band of capsicum red pigment extracted and purified is consistent with the characteristic absorption peak of Pure capsicum red pigment. Capsicum red pigment extracted and purified in this experiment has high purity and meets the need of staining.

3.8 pH stability

First, the pigment sample is diluted with the concentration 1:200, and then is divided into ten portions adjusted to represent the pH from 1 to 10, respectively. Finally, absorbances (λ) of those ten portions are measured and showed in Tab. 2.

Tab.2 shows that, when pH is at the range of 1-2, a slight increase of pH will bring a significant increase in absorbance (λ) and when pH is at the range of 6-7, a slight decrease of pH will bring a significant decrease in absorbance (λ), and when pH is at the range of 2-6 and 7-10, the alter of absorbance (λ) is not noticeable with the increase of pH. It is clear that capsicum red pigment can be damaged in strong acid or alkaline condition, and keep relatively stable at neutral and weak acid solution.

4. Conclusion

Results showed that the optimal process was listed as follow: the raw material was Qiu North spicy, concentration of peppery removal agent (sodium hydroxide) was 10%, the extraction temperature was 80°C , solid-liquid ratio was 1:20 (g/ml), the extraction time was 120min and the time of extraction was twice, eluent of silica gel column chromatography separation was petroleum ether-90% ethanol (2:1). The color value of capsicum red pigment ($\text{E}1\text{cm}1\%$ 460nm) could reach to 125 and 2.88% of yield could be gained. The infrared absorption band of capsicum red pigment extracted and purified is consistent with the characteristic absorption peak of Pure capsicum red pigment. The process was good to extract and purify capsicum red pigment and the stability of which was high at neutral and weak acid solution.

References

- Chen, C., W., Lee, T. C., & Ho, C. T. (1996). Antioxidative Effect and Kinetics Study of Capsanthin on the Chlorophyll-Sensitized Photooxidation of Soybean Oil and Selected Flavor Compounds. *Spices, Flavor Chemistry and Antioxidant Properties*, 188–198.
- Dong, Qingnian. (1979). Infrared Spectroscopy. *Chemical Industry Press*, 99-229.
- Liu, Zhiguo, Zhang, Yu, Zhang, Zhenxin, & Yu, Feng. (2005). Optim Ization of Extraction Cond Ition of Rose Rugosa Pigment. *Journal of Wuhan Polytechnic University*, 24, 1-3.
- Ma, Jianmin, & Gong, Wenjie. (2008). Study on Experimental Method and Skills of Chromatography Fractionation. *Chinese Journal of Health Laboratory Technology*, 18, 745-762.
- Minquez, Mosquera, I. M., & Hornero-Mendez, D. (1994). Formation and Tranformation of Pigments During the Fruit Ripening of Capsicum annum cv. Bola and Agridulce. *J. Agric. Food Chem*, 42, 38-44.
- Nie, Jing, & Qi, Xingjuan. (2002). Research Trends of Synthetic Food Dyes. *Chinese Journal of Food Hygiene*, 14, 58-60.
- Schweiggert, Ute, Kurz, Christina, Schieber, Andreas, & Carle, Reinhold. Effects of processing and storage on the stability of free and esterified carotenoids of red peppers (*Capsicum annum* L.) and hot chilli peppers (*Capsicum*

frutescens L.). *European Food Research and Technology*, 225, 261-270.

Wang, Taoyun, Dan, Zhiyong, Wu, Weijun, Ding, Jiangyan, & Tian, Miao. (2004). Extraction Technics and Stability of Yellow Pigment from Pumpkin. *Journal of University of Science and Technology of Suzhou(Natural Science)*, 21, 44-52.

Yao, Zhufeng, Jiang, Hongjie, & Zhang, Shiwen. (2006). Research on the Chili Red. *Journal of Jishou University(Natural Science Edition)*. 21, 48-52.

Zhou, Wenwen, Li, Xiangzhou, & Zhang, Yanqiang. (2005). Study Progress on Capsanthin. *Yunnan Chemical Technology*, 10, 52-54.

Table 1. Property of different species of pepper

Species	Dry capsicum weight (g)	Peel meal weight (g)	Peel meal rate (%)	Absorbance	Relative amount of pigment
Fructus capsici	250	141.2	59.3	0.387	7.32
Qian chilli 2	250	125.1	50.4	0.452	8.64
Qiu North spicy	250	150.5	61.2	0.683	14.62

Table 2. Stability influence of different pH Values

pH	1	2	3	4	5	6	7	8	9	10
λ	0.057	0.535	0.448	0.458	0.564	0.629	0.091	0.077	0.221	0.046

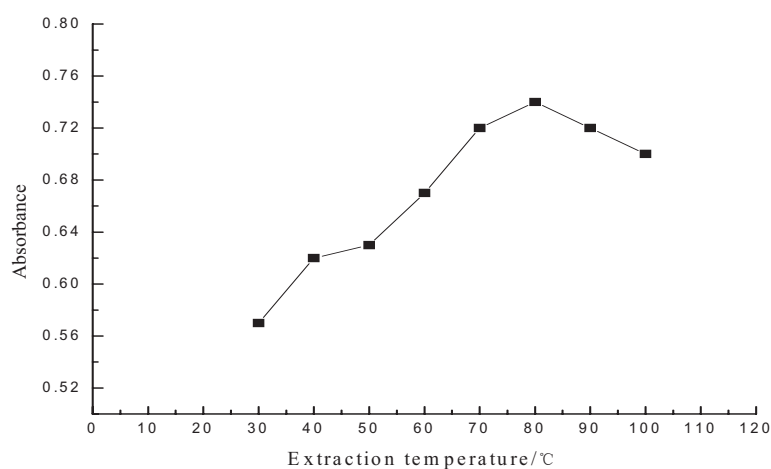


Figure 1. Extraction effect of extraction temperature

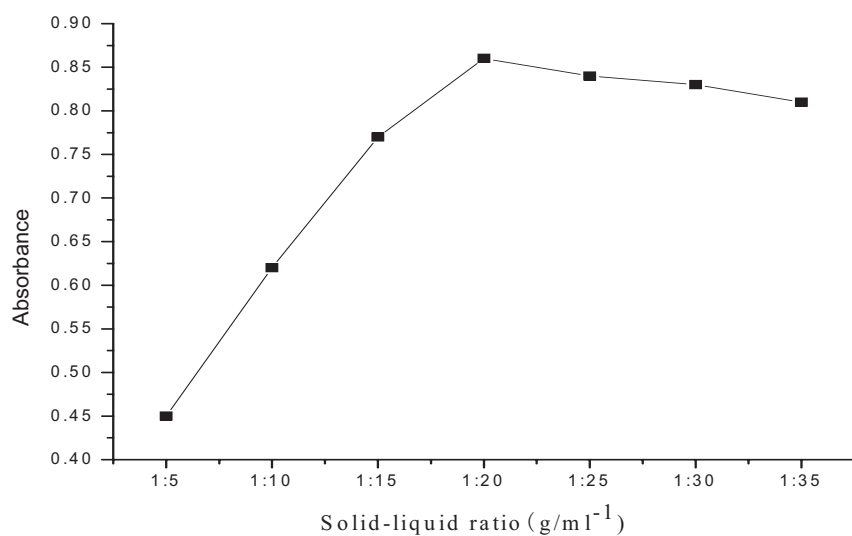


Figure 2. Extraction effect of solid-liquid ratio

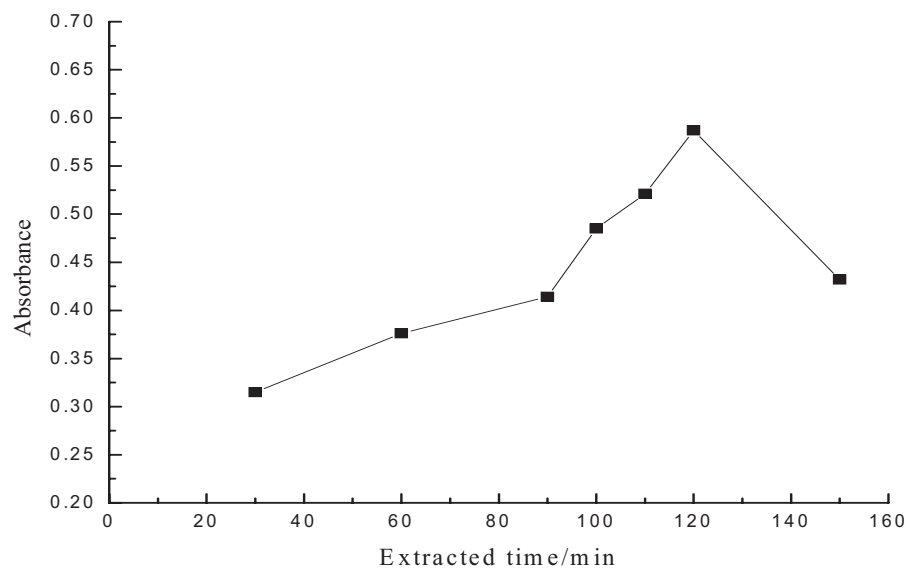


Figure 3. Extraction effect of extraction time

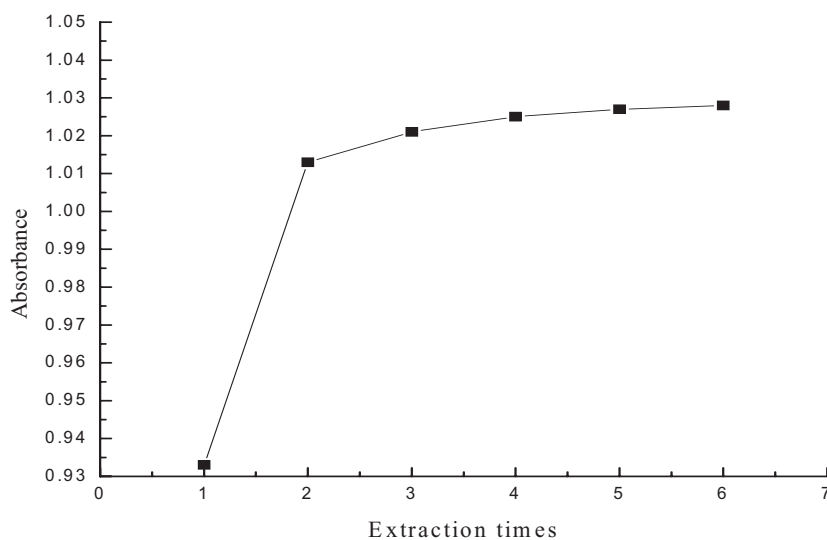


Figure 4. Extraction effect of the extraction times

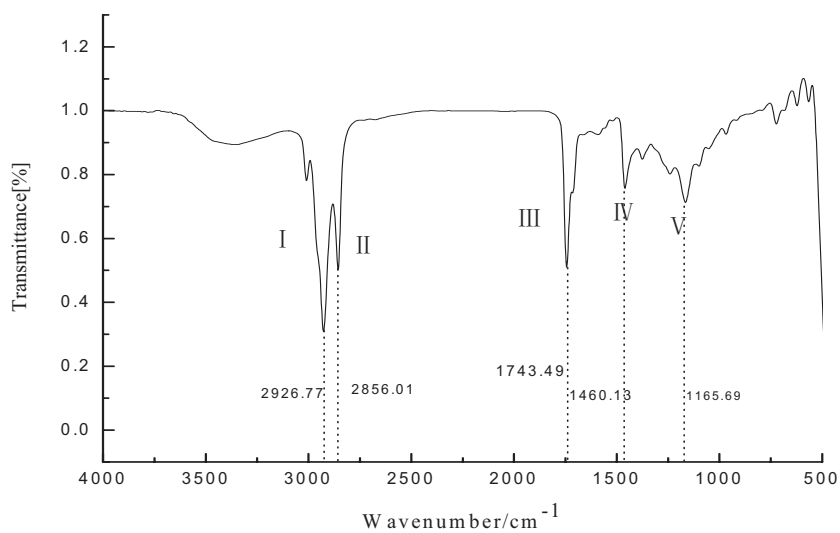


Figure 5. Infrared spectrum of capsicum red pigment