Research Progress on Purification Process Optimization and Content Determination of Zeaxanthin

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Abstract At present, the purification process of zeaxanthin mainly includes organic solvent extraction, ultrasonic-assisted extraction and enzyme extraction, and the content determination technology mainly includes ultraviolet-spectrophotometry and high performance liquid chromatography. In this paper, the purification process and content determination technology of zeaxanthin in recent years are reviewed in order to provide ideas and theoretical basis for further research and application of zeaxanthin.

Key words Zeaxanthin, Carotenoid, Purification process, Content determination

1 Introduction

Carotenoids are a kind of important natural pigments. At present, more than 800 kinds of natural carotenoids have been found, which are ubiquitous in animals, higher plants, fungi and algae[1]. Carotenoids are terpenoids with multiple conjugated double bonds. According to chemical structures, carotenoids can be divided into two categories, one is carotene (containing only two elements of hydrocarbon and hydrogen, but not oxygen), and the other is lutein (including oxygen-containing functional groups such as hydroxyl, ketone, carboxyl and methoxy)[2]. Zeaxanthin is a dihydroxy derivative of β-carotene, which is isomeric with lutein. It is a fat-soluble pigment in nature, which exists in a large number of plant tissues such as yellow maize, medlar, sour berry and some non-photosynthetic bacteria, and can effectively prevent cancer, cataract, cardiovascular diseases and age-related macular degeneration. As a natural pigment, zeaxanthin has been recognized as a safe natural food pigment by *Hygienic Standard for the Use of Food Additives*, and is gradually replacing synthetic pigments such as lemon yellow and being widely used in food industry[3]. Besides coloring function, zeaxanthin also has many pharmacological effects, and it can effectively prevent the occurrence of cancer and age-related macular degeneration[4]. In this paper, the purification process and content determination technology of zeaxanthin are reviewed in order to provide a theoretical basis for the research and development and application of zeaxanthin.

2 Purification process of zeaxanthin

At present, the main raw materials for industrial production of zeaxanthin are maize, marigold and citrus peel, and its purification processes mainly include organic solvent extraction, ultrasonic-assisted extraction, microwave extraction, enzyme extraction, supercritical fluid extraction, membrane-assisted separation and extraction, *etc.*

2.1 Organic solvent extraction method This method is a traditional method for extracting bioactive substances by using organic solvents such as ethanol, petroleum ether and chloroform, which has the advantages of simple operation and high extraction efficiency. The filter residue in the extraction process can be extracted twice, and the distilled solvent can also be used twice, which effectively improves the utilization rate of raw materials and reduces the extraction cost. Zhang Zhihua *et al.* used organic solvent extraction method to dissolve maize gluten meal in ethanol, obtain the extract by extraction, filter it for secondary extraction, and remove impurities to obtain zeaxanthin. The optimum extraction conditions were determined by orthogonal test of three factors and four levels: ethanol concentration, the ratio of maize gluten meal weight (g) to extraction amount (mL), extraction temperature and extraction time were 95%, 1∶16, 65 ℃ and 4 h[5], respectively. Xiong Wanyang *et al.* optimized the extraction process of zeaxanthin from maize yellow flour by response surface methodology. maize yellow flour was dissolved in ethanol, extracted by oil bath reflux and centrifuged, and its supernatant was the extract. According to the central combination design principle of Box-Benhnken in Design Expert software, the orthogonal experiment of three factors and three levels was carried out. The optimum purification conditions of ethanol volume fraction, extraction temperature and extraction time were 83.36%, 62.13 ℃ and 5.79 h, respectively, and the yield of zeaxanthin reached 0.21%[6]. Di Huoli *et al.* used organic solvent extraction method to dissolve lutein in dimethyl sulfoxide (DMSO), heated it in oil bath, added potassium hydroxide (KOH) and dried it to obtain zeaxanthin crystals. The optimum purification process of zeaxanthin was determined as follows: molar ratio of KOH to xanthophyll, molar concentration of KOH, reaction temperature, solid-liquid ratio and reaction time were 56∶1, 12 mol/L, 90 ℃, 1∶25

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and 1 h, respectively. The purity of zeaxanthin obtained under these conditions was over 90% by UV spectrophotometer and high performance liquid chromatograph[7]. Extrusion is an effective pretreatment method, which is widely used in the modification of starch and protein. Jiao Yan *et al.* extruded maize gluten meal, then crushed it, added organic solvent to extract it, and separated the supernatant to obtain zeaxanthin. Finally, through three-factor and three-level orthogonal test, the optimum extrusion conditions were determined as follows: the temperature of extrusion tube was 150 ℃, the moisture content of maize gluten meal was 18% and the screw speed was 160 rpm[8].

2.2 Ultrasonic-assisted extraction method This method is a commonly used purification process in recent years, which has the advantages of wide applicability, short extraction time, high extraction efficiency, good product stability, simple operation and so on, and is suitable for the extraction of most effective components. Li Xiuxin *et al.* extracted zeaxanthin from maize yellow flour by ultrasonic-assisted method, mixed maize yellow flour and sunflower oil microemulsion evenly for extraction, and its absorbance was detected at 446 nm. The results showed that the extraction rate of ultrasonic-assisted microemulsion extraction method was almost twice as high as that of water bath method. The optimum ratio of solid to liquid, temperature and time were determined to be 1∶40 (g/mL), 40 ℃ and 110 min by orthogonal test[9]. Li Wei *et al.* extracted zeaxanthin from maize gluten meal by ultrasonic and enzyme extraction, mixed maize gluten meal with ethanol, added neutral protease to obtain the extract by ultrasonic treatment. The results showed that compared with the conventional ethanol extraction method, the ultrasonic extraction with enzyme had shorter time and higher extraction rate. The optimum extraction ratio, ultrasonic power, enzyme amount and extraction time were 1∶25, 100 W, 1.5×10^4 U/g and 5 min, respectively[10].

2.3 Enzyme extraction method This method mainly uses enzymes to destroy the cell wall structure, reduce the resistance of effective components diffusing from the intracellular extraction medium, and then improve the extraction efficiency. At the same time, the specificity of enzymes can avoid the destruction of substances other than substrates. When extracting chemical components with poor thermal stability or less content, the advantages of enzymatic extraction are more obvious. Zhu Lei *et al.* extracted zeaxanthin from maize gluten meal by enzyme extraction, dissolved maize gluten meal in neutral enzyme buffer, then added organic solvent for extraction, and measured the absorbance value at 445 nm. The results showed that the extraction rate of zeaxanthin was obviously improved. The optimum enzyme concentration, pH and temperature were 1.4%, 7.0, 40 ℃ and 6 h, respectively through single factor test and orthogonal test[11].

2.4 Microwave extraction method This method, also known as microwave-assisted extraction, is a method to extract various chemical components from plants, animals, minerals and other tissues by using solvents in microwave reactor, which has the advantages of high efficiency, energy saving, safety and environmental protection. Liu Zhenchun *et al.* used microwave extraction method, combined with enzyme extraction method, and added extractant to maize gluten meal. After enzymatic hydrolysis, microwave treatment significantly improved the extraction effect and shortened the extraction time and production cycle. Verified by orthogonal test, the optimum microwave power, heating time, enzyme amount, substrate concentration and enzymatic hydrolysis time were determined to be 480 W, 35 s, 1.4%, 5% and 6 h, respectively[12].

2.5 Supercritical extraction and separation This method is a new extraction technology, which uses supercritical fluid as extractant to extract specific components from liquid or solid. It has the advantages of high extraction efficiency, short extraction time and stable products. Zhang Min *et al.* successfully obtained high purity zeaxanthin by supercritical propane extraction under the conditions of pressure of 8.5 – 10.0 MPa and temperature of 96.8 ℃ for 4 h[13]. Sun Lili *et al.* used supercritical CO_2 extraction to extract hydrolyzed maize gluten meal and dissolved it with absolute ethanol to obtain zeaxanthin. The technological conditions of supercritical CO_2 extraction of zeaxanthin were optimized by single factor experiment and orthogonal experiment, and the content of zeaxanthin in the extract was detected by HPLC. The optimum extraction time, CO_2 flow rate, extraction pressure and extraction temperature were determined to be 2 h, 35 kg/h, 30 MPa and 45 ℃, respectively. Under these conditions, the yield of zeaxanthin could reach 210.97 μg/g[14].

2.6 Membrane-assisted separation and extraction method This method is a new technology for separation, concentration and purification with separation membrane as the core. Tsui *et al.* used membrane technology to extract lutein from maize, ground the maize material, added ethanol for extraction, and obtained zein by ultrafiltration (U20T and RTM-PX membrane) and separation. Zeaxanthin was separated and purified by nanofiltration (TFC-SR1 membrane) and chromatography. This method has the advantages of strong selectivity, simple operation process, wide application range, low energy consumption, *etc.* It is widely used in food, medicine, biology, environmental protection, chemical industry and other fields, and has become one of the most commonly used means in today's purification process[15].

3 Content determination technology of zeaxanthin

Content determination is one of the core steps of extracting active components from traditional Chinese medicine, and it is also an important means to evaluate the quality of drugs and ensure the safety of medication. At present, the content determination methods of traditional Chinese medicine mainly include chemical analysis and instrumental analysis, and the content determination methods of zeaxanthin mainly include spectrophotometry, high performance liquid chromatography and thin layer chromatography.

3.1 Spectrophotometry Xu Xiuhong *et al.* selected six fresh maize varieties as raw materials, analyzed the zeaxanthin content of different varieties of maize grains by spectrophotometry, made fresh maize grains into dry powder, dissolved them, extracted su-

pernatant, measured the absorbance value at wavelength 445 nm, and calculated the zeaxanthin content. The results showed that there were significant differences in the highest zeaxanthin content among different varieties, and the zeaxanthin content of Yuetian 3 was the highest, reaching 14.809 mg/kg[16]. Deng Xiaojing *et al.* used spectrophotometry to analyze the effects of maize type, grain color and grain type on zeaxanthin content in grain. 123 maize inbred lines were selected from 3 200 maize inbred lines as research materials for grinding, and then the supernatant was extracted by organic solvent to measure the absorbance value at wavelength 445 nm. The results showed that maize type and grain color had great influence on zeaxanthin content, and the zeaxanthin content in yellow maize was the highest, reaching 2.2 mg/kg[17].

3.2 Chromatography Compared with ultraviolet-spectrophotometry, chromatography has the advantages of high separation efficiency and high sensitivity. According to the state of mobile phase, it can be divided into four categories: gas chromatography, liquid chromatography, electrochromatography and supercritical fluid chromatography. Li Huanpeng *et al.* determined the content of zeaxanthin in milk powder by high performance liquid chromatography (HPLC), dissolved milk powder in water before adding protease, and it was repeatedly extracted with n-hexane: acetone (1∶9) as extraction solvent and filtered to obtain sample solution. It was determined by Si60 chromatograph with n-hexane: ethyl acetate (65∶35) as mobile phase. The results showed that when zeaxanthin content was 63.3, 126.7, 316.7 μg/100 g, the recovery rate was 95.8% -96.7%, the relative standard deviation was 1.1% -3.9%, the detection limit was 20 μg/100 g, and the quantitative limit was 50 μg/100 g[18]. The method has the advantages of simple process, easy operation, high sensitivity and good recovery rate. Wang Hongmei *et al.* determined the content of zeaxanthin in marigold by normal phase high performance liquid chromatography (NP-HPLC). After dissolving dried marigold flowers in water, they were added into the mixed solution of n-hexane and ethyl acetate (72∶28), extracted by ultrasound and filtered to obtain the sample solution. The sample solution and the mixed reference solution were determined by chromatograph with the detection wavelength set at 450 nm. The results showed that the average dry weight content of zeaxanthin in marigold was 1.58 g/kg[19].

Ultra-high performance liquid chromatography (UPLC), as a new liquid chromatography technology, has been widely used in chemistry, biology, pharmacy, environmental protection, detection and other scientific fields because of its good separation efficiency, high sensitivity and superiority for the separation of complex systems. Meng Fanlei *et al.* used ultra-high performance liquid chromatography to determine the content of zeaxanthin in different varieties of yellow maize[20]. After crushing yellow maize grains, ultrasonic extraction and filtration, the sample solution was obtained, and the sample solution and mixed reference solution were determined by chromatograph with the detection wavelength set at 445 nm. The results showed that the content of zeaxanthin in different varieties of yellow maize ranged from 0.69 mg/kg to 7.43 mg/kg, and there was a significant difference. The detection limit of this method was 0.03 mg/kg, the quantitative limit was 0.10 mg/kg, the average recovery rate was between 92.67% and 94.67%, and the *RSD* was between 1.26% and 4.12%, with the advantages of simple operation, good repeatability and high accuracy.

4 Conclusion

In recent years, with the deepening of research, the purification process of zeaxanthin has also been continuously improved. Compared with the traditional organic solvent extraction method, the new purification process taking supercritical extraction as an example has the advantages of low energy consumption, simple operation, wide applicability, mild conditions and high extraction efficiency, which provides better technical support for the subsequent determination of zeaxanthin content. However, it is still necessary to optimize the purification process conditions in order to further reduce production costs and environmental pollution. In addition, the determination technology of zeaxanthin content is also developing in a more accurate direction. The existing HPLC and other methods generally have the advantages of high efficiency, high sensitivity and accurate results, but the instruments are usually expensive, which leads to high economic cost, so it is difficult to be applied on a large scale. To sum up, there is still much room for development in the purification process and content determination technology of zeaxanthin. Establishing a more perfect theoretical method and mature technical system, overcoming the shortcomings of the new purification process and content determination technology in industrial application, reducing the production cost and improving the stability of the product while ensuring the quality and safety of the product, will help to further improve the application value of zeaxanthin.

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