

## **Research Article**

### **Effect of nano materials on lutein extraction from collard using ethanol assisted with ultrasonic**

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**Abstract:** In the process of extracting lutein from collard using 95% ethanol assisted with ultrasonic, the effects of nano silicon dioxide, hydrophilic nano titanium dioxide and lipophilic nano titanium dioxide on the lutein yield of collard were investigated. The result showed that lipophilic nano titanium dioxide might significantly increase lutein yield. When the collard was extracted with 95% ethanol for 30 minutes, the lutein yield was 9.24 mg/100g under 700 W ultrasonic power through sample to solvent ratio 1:15 at 65 °C with the addition of lipophilic nano titanium dioxide of 5mg/15mL 95% ethanol.

**Keywords:** lutein, collard, nano materials, ultrasonic, ethanol.

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#### **INTRODUCTION**

Lutein (C<sub>40</sub>H<sub>56</sub>O<sub>2</sub>), one of carotene derivatives, has a variety of physiological functions, suggested as cancer preventive agent, ulcer inhibitor and coronary artery disease inhibitors. Especially in the prevention of retina macular degeneration, it showed positive curative effect [1]. Currently, lutein was mainly extracted from marigold as medicine and food additives. Research suggested that collard also contained abundant lutein [2]. Collard belongs to a cabbage variety of brassica of cruciferae and is biennial herb. Food originating of collard was recommended by the United Nations for the preventing nyctalopia of child [3].

Ultrasonic, an electromagnetic wave with the length of 20 ~ 50 MHz, can generate strong energy. Its strong cavitation effect, mechanical vibration and high acceleration may cause cell wall rupture. Moreover, the cell wall rupture process was instantly finished, thereby increasing molecular motion frequency and speed, enhancing solvent penetrating force, and promoting effective ingredient into solvent. Meanwhile, the vibration effect caused by ultrasonic wave strengthens the release, diffusion and dissolution of product in cell, improving extraction efficiency [4]. Ultrasonic technology has been used for the extraction of many food ingredients such as polysaccharides, flavonoids, polyphenols and so on [5, 6, 7].

Nano technology is a vigorous new technology, emerging in the late 1980s. It is now widely used in the fields of materials, chemical engineering and medicine. Nano is a unit of length and nano structure usually

refers to the tiny size below 100nm. Nano materials have special effects such as surface effect, small size effect, quantum effect and macro tunnel effect, and conventional materials have no these effects[8]. Currently, in research field, nanotechnology had been applied in food packaging, preservation, processing, detection, and so on [9, 10, 11, 12].

At present, ultrasonic or microwave was used to assist lutein extraction, but there was no report of assisting extraction of lutein using nano material. In this experiment, the effect of nano materials on lutein extraction from collard using ethanol assisted with ultrasonic was investigated. This experiment is to explore the effect of nano materials on lutein extraction.

#### **MATERIALS AND METHODS**

##### **Materials and Reagents**

Collard (*Brassica oleracea* var. *acephala* f. *tricolor*) was originated of Linfen, Shanxi province, China. nano silicon dioxide (50nm), hydrophilic nano titanium dioxide (60nm, Anatase) and lipophilic nano titanium dioxide (100nm, Rutile) were purchased from Shanghai crystal pure Industrial Co., Ltd. Ethanol(95%) was purchased from Beichen Tianjin Founder Reagent Factory. (Tianjin, China).

##### **Equipments and instruments**

GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China; JY92- II N Ultrasonic Cell

Disruption System, Ningbo Scientz Biotechnology Co, Ltd., Ningbo, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China; Eppendorf 5417R centrifuge, Gene Company, Germany.

**Extraction of lutein from collard**

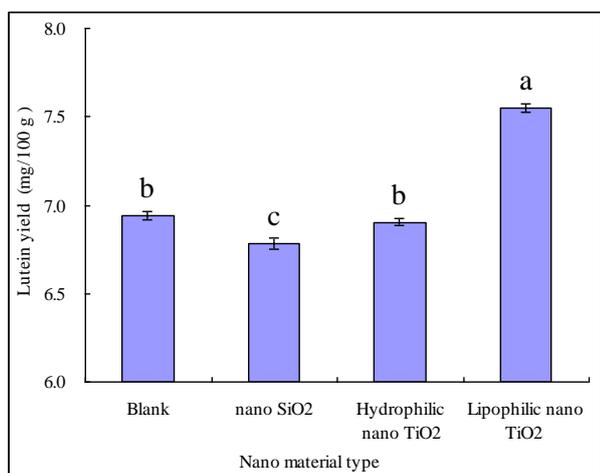
The fresh leaves of collard was dried under 50°C using blast drying box, and then they were smashed into 100 mesh powder. 1g of collard powder was added into a triangular flask of 100 mL, and appropriate amount of 95% ethanol and nano materials was added into triangular flask. The suspension was treated with ultrasonic for 30 minutes. Afterward, the lutein content of extracts was assayed. In this experiment, each treatment was repeated three times.

**Determination of lutein**

The lutein content of extracts was determined using spectrophotometry [13]. The extracts was centrifuged using refrigerated centrifuge for 15 min at 4°C, and the supernatant was measured at 445 nm wavelength using absolute ethanol as blank. Lutein content was calculated according to following formula.  $X = (A \times Y) / (A^{1\%} \times 100)$ , where X was lutein content (mg/100g), A was absorbance at 445 nm, Y was extract volume, and  $A^{1\%}$  was specific absorptivity and the specific absorptivity of lutein in ethanol was 2550.

**RESULT ANALYSIS AND DISCUSSION**

**Selections of nano materials**

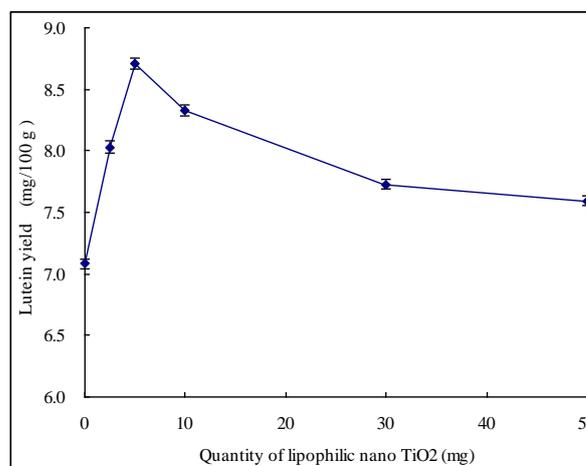


**Fig-1: Effect of nano material type on lutein yield of collard**

In 15 ml 95% ethanol containing 1 g of collard powder, 50 mg of different nano material was added the suspensions. Subsequently, the mixture suspensions were treated with 500W power ultrasonic for 30 minutes under ambient temperature. As shown in Fig.1, the effect of each nano materials on lutein yield of collard was different. Nano silicon dioxide decreased lutein yield, hydrophilic nano titanium dioxide had little

effect on lutein yield, but lipophilic nano titanium dioxide significantly increased lutein yield compared to control sample (P<0.05). The lutein yield of adding lipophilic nano titanium dioxide increased 8.7% higher than that of control sample. Lutein, a liposoluble substance, might easily interact with the surface of lipophilic nano titanium dioxide. Moreover, lipophilic nano titanium dioxide had very large specific area that accelerated lutein migration from collard powder. Thus, lipophilic nano titanium dioxide increased lutein yield. In the following experiments, lipophilic nano titanium dioxide was selected for extracting lutein from collard.

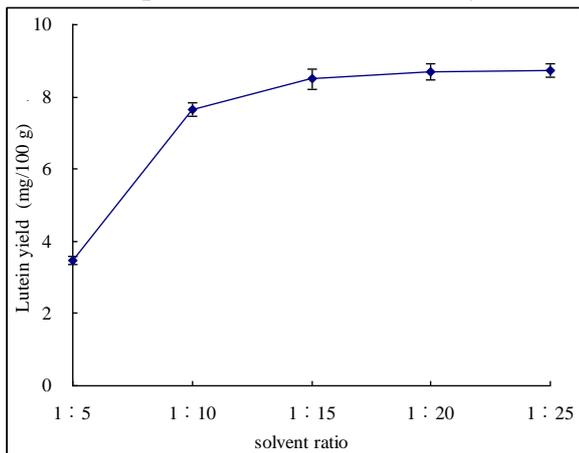
**Effect of lipophilic nano titanium dioxide quantity on lutein yield**



**Fig-2: Effect of lipophilic nano titanium dioxide quantity on lutein yield of collard**

In 15 ml 95% ethanol containing 1 g of collard powder, the different quantities of lipophilic nano titanium dioxide were added into suspensions. Subsequently, the mixture suspensions were treated with 500W power ultrasonic for 30 minutes under ambient temperature. As shown in Fig.2, with lipophilic nano titanium dioxide quantity enhancement, lutein yield first increased and then decreased. When lipophilic nano titanium dioxide quantity was added from 0 to 5mg, lutein yield increased. At 5 mg, lutein yield was the maximum of 8.71 mg/100g, which was 23% higher than that of control sample. With lipophilic nano titanium dioxide quantity from 5 to 50mg, lutein yield decreased. Especially at 50mg, lutein yield was 7.59 mg/100g, which was 12.8% lower than that of 5 mg. The concentration effect mechanism of lipophilic nano titanium dioxide on the lutein extraction of collard should be further investigated in future.

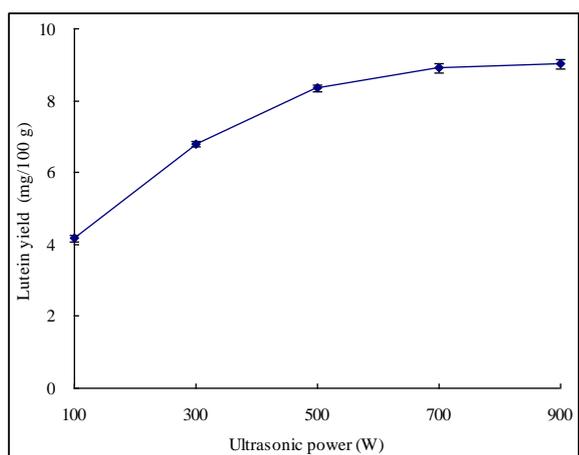
**Effect of sample to solvent ratio on lutein yield**



**Fig-3: Effect of sample to solvent ratio on lutein yield of collard**

1 g of collard powder and 5 mg of lipophilic nano titanium dioxide were added into different volume of 95% ethanol, and the mixture suspensions were treated with 500 W ultrasonic for 30 minutes under ambient temperature. With the increase of sample to solvent ratio, the lutein yield of collards showed an increasing trend (Fig. 3). When sample to solvent ratio was 1:15, lutein yield was close to equilibrium of 8.49 mg/100g. When sample to solvent ratio continuously expanded, lutein yield was almost no increase. Extraction was a process of molecule migration from solid powder to solution. When sample to solvent ratio was lower, the lutein concentration of solution was higher and was not conducive to migration from collards power to ethanol [14]. Therefore, the appropriate increase of sample to solvent ratio was helpful to lutein migration.

**Effect of ultrasonic power on lutein yield**

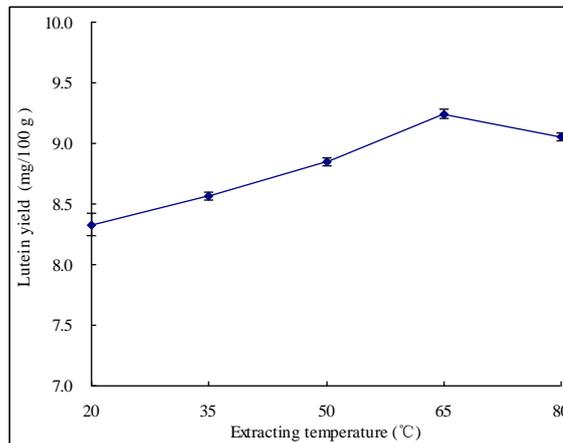


**Fig-4: Effect of ultrasonic power on lutein yield of collard**

In 15 ml 95% ethanol containing 1 g of collard powder and 5 mg of lipophilic nano titanium dioxide, the mixture suspensions were treated with different

power ultrasonic for 30 minutes under ambient temperature. As described in Fig.4, the lutein yield of collard increased with ultrasonic power enhancement. At 700W, lutein yield reached to 8.90 mg/100g, being almost close to equilibrium. With the further increase of ultrasonic power, lutein yield did not obviously increase. Therefore, at 700W, the effect that ultrasonic crushed the collard cell probably approached limitation.

**Effect of ultrasonic temperature on lutein yield**



**Fig-5: Effect of ultrasonic temperature on lutein yield of collard**

In 15 ml 95% ethanol containing 1 g of collard powder and 5 mg of lipophilic nano titanium dioxide, the mixture suspensions were treated with 700 W ultrasonic for 30 minutes at different temperature. As shown in Fig.5, the lutein yield of collard first increased and then decreased with temperature increasing. When temperature reached to 65 °C, lutein yield achieved maximum for 9.24 mg/100g, which was 11.0% higher than that of 20 °C. As temperature continuously rose to 80 °C, lutein yield began to drop. Appropriate high temperature might accelerate the molecular motion of lutein, promoting lutein migration into solution from collard powder. Therefore, lutein yield increased with temperature from 20 °C to 65 °C. However, excessively high temperature might destroy the lutein of collard, resulting in lutein yield decrease.

**CONCLUSION**

In the process of extracting lutein from collard using 95% ethanol assisted with ultrasonic, adding lipophilic nano titanium dioxide might significantly increase lutein yield. When the collard was extracted with 95% ethanol for 30 minutes, the lutein yield was 9.24 mg/100g under 700 W ultrasonic power through sample to solvent ratio 1:15 at 65 °C with the addition of lipophilic nano titanium dioxide of 5mg/15mL 95% ethanol.

### Acknowledgments

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