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Microwave and enzyme co-assisted aqueous two-phase extraction of polyphenol and lutein from marigold (*Tagetes erecta* L.) flower

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ABSTRACT

In this study, a novel method of simultaneous extraction and enrichment of total polyphenol (TP) and lutein from marigold flower was developed by microwave and enzyme co-assisted aqueous two-phase extraction (ATPE) (MEAATPE). In this study, ethanol/ammonium sulfate was chosen to construct aqueous two-phase system (ATPS) due to its fine partition and recycling behaviors. The optimal MEAATPE condition was obtained, that is, 28% (w/w), ethanol/20% (w/w) ammonium sulfate, 0.45 U/g enzyme concentration, enzymolysis time of 150 min, enzymolysis temperature of 45 °C, microwave time of 120 s, and microwave power of 270 W, by investigating the effects of ATPS composition and other factors on the yield of target products. Under this optimal condition, the yields of TP and lutein reached 84.61 and 7.32 mg/g with the corresponding recovery rates of 95.35% and 99.85%, respectively. Compared with enzyme-assisted ATPE, microwave-assisted ATPE, ATPE, and Soxhlet extraction (SE), the extraction yield of TP by MEAATPE was the highest, which is 71.14% higher than that of SE. Scanning electron microscopy analysis further showed an optative pretreatment effect of marigold flower powders. Thus, MEAATPE was an efficient, rapid, and environmental-friendly method used for the extraction of natural products.

1. Introduction

Marigold (*Tagetes erecta* L.) is also called smelly hibiscus and gold chrysanthemum and belongs to family Asteraceae (Schiavinato et al., 2017). Marigold is a common ornamental plant with yellow or dark orange tongue flowers, and available in many parts of the world (Li et al., 2007) and originated in Mexico. Marigold meals and extracts are most widely accepted products among natural pigments (Wang et al., 2017). Li Wei's study (Li et al., 2007) indicated that phenolic, flavonoid, and lutein ester are detected in Chinese marigold. Augusti Paula Rossini (Augusti et al., 2017) evaluated the possible protective effect of lutein extracted from marigold flowers against microcystin-LR toxicity. Marigold flower is a good source of total polyphenol (TP) and lutein, and lutein accounts for 70% to 79% of the carotenoid content. Interest in the study of TP and lutein is increasing because of their various biological properties such as antioxidant, antibacterial, antidiabetic, anti-obesity, anti-inflammatory, anti-cancerous effects of TP (Borutinskaitė et al., 2017). Lutein has been applied in nutritional, cosmetic, and pharmaceutical industries as an excellent antioxidant (Tekwani and D'Mello, 2010). Lutein is predominately present in the macular region and acts as an efficient pigment in absorbing high-energy blue light (Zhang et al., 2016). Lutein is also effective in reducing the risk of developing advanced age-related macular degeneration and notably exhibits anti-proliferative activity (Woo et al., 2013; Nalawade and Gajjar, 2016). Previous studies showed that TP and lutein have beneficial effects on human health (Castro-López et al., 2017; Xu et al., 2015; Lin et al., 2015).

Aqueous two-phase extraction (ATPE) is a new type of technology of extraction and separation, which has aroused increasing attention among the majority of scholars due to its biphasic extraction capacity and selectivity (Yau et al., 2015; Soares et al., 2015). ATPE targets constituents and impurities to be extracted into the top and bottom phases, respectively (Iqbal et al., 2016; He et al., 2016; Xavier et al., 2017) and integrates extraction and enrichment into a single step. To improve the separating–extraction effect of ATPE, researchers have proposed microwave-assisted ATPE (MAATPE) combined with the advantages of microwave-assisted extraction (MAE) and ATPE (Ma et al., 2013; Xie et al., 2017; Dang et al., 2014). Enzyme-assisted extraction

Abbreviations: TP, total polyphenol; MEAATPE, microwave and enzyme co-assisted aqueous two-phase extraction; ATPS, aqueous two-phase system; SD, standard deviation; EAATPE, enzyme-assisted aqueous two-phase extraction; MAATPE, microwave-assisted aqueous two-phase extraction; ATPE, aqueous two-phase extraction; SE, Soxhlet extraction; AMD, age-related macular degeneration; MAE, microwave-assisted extraction; EAE, enzyme-assisted extraction; DHP, disodium hydrogen phosphate; *Y*, yield; *R*, recovery; *K*, partition coefficient; SEM, scanning electron microscope; IC_{50}, 50% DPPH inhibition; DPPH, 1,1′-diphenyl-2-picrylhydrazyl free radical

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(EAE) is commonly used in the food industry due to its several advantages such as high efficiency, environmental friendly nature, and ease of operation (Zhao et al., 2016). Sadineni Varakumar (Varakumar et al., 2017) extracted oleoresin from ginger rhizome powder using enzyme-assisted three-phase partitioning, and Tan Zhijian (Tan et al., 2016) already explored this topic by using enzyme-assisted three-phase partitioning to extract oil from flaxseed. However, exploring the combination of MAE, EAE, and ATPE and comparing the pretreatment effect of MAE and EAE have not been conducted. In this study, on the basis of thermal effect of MAE, high efficiency of EAE and ATPE in extraction, and enrichment of TP and lutein, researchers developed a more efficient and environmentally friendly extraction method, which is defined as microwave and enzyme co-assisted ATPE (MEAATPE). This method may be an alternative in extracting TP and lutein from marigold flowers and may be a promising technique in extracting bioactive components from plants and herb analysis.

2. Materials and methods

2.1. Materials and reagents

Fresh marigold flowers were obtained in September 2016 from Xinjiang Province and dried at room temperature in the dark. The moisture content of marigold flower was 6.67%. Subsequently, universal high speed pulverizer was used to ground these flowers into powders, and 160 mesh was used to make to particle size of similar size. Then, powders were stored in a sealed bag at −4 °C. Pectinase (30 U/mg) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). Folin–Ciocalteu's phenol reagent was procured from Xiya Chemical Industry Co., Ltd. (Shandong, China). Lutein standard was obtained from ChromaDex Co., Ltd. (America), and gallic acid standard was purchased from Aladdin Co., Ltd. (Shanghai, China). Other reagents were all of analytical grade.

2.2. MEAATPE procedures

A total of 0.22 g marigold flower powders, 4.0 g disodium hydrogen phosphate–citric acid buffer solution, and 0.45 U/g pectinase were first added to a 10 mL graduated test tube and then mixed evenly by a vortex mixer and placed in water bath at a certain constant temperature. Then, ammonium sulfate and ethanol were added into the enzymatic slurry and vibrated for 10 min by a vortex mixer to completely dissolve salt. The suspension was irradiated in a microwave reactor (MCR-3, Shanghai Xingchuang Science Instrument Equipment Co., Ltd.). After microwave treatment, the mixture was mixed well and then set at room temperature for 30 min to form aqueous two-phase system. Finally, the mixture was centrifuged at 4000 rpm for 10 min to facilitate phase separation, and the volume of the upper and bottom phases of system were recorded after phase separation was completed. Then, the yields of TP and lutein were determined. The yield (*Y*), recovery (*R*), and partition coefficient (*K*) of TP and lutein serve as the indices of extraction abilities, and the parameters were defined according to Eqs. (1–3) (Dang et al., 2014; Qin et al., 2017), as follows:

$$Y\,(mg/g) = \frac{C_t V_t}{M} \tag{1}$$

$$R(\%) = \frac{C_t V_t}{C_t V_t + C_b V_b} \times 100 \tag{2}$$

$$K = \frac{C_t}{C_b} \tag{3}$$

where C_t and C_b represent TP and lutein concentrations in the top and bottom phases, respectively, and V_t and V_b are the volumes of the top and bottom phases, respectively. *M* is the total mass of marigold flower powders.

2.3. Determination of TP and lutein contents

2.3.1. Determination of TP content

TP content was determined by a slightly modified version of Folin–Ciocalteu method (Luquerodríguez et al., 2006). A total of 0.2 mL of diluted extracts, 10 mL of distilled water, 1 mL of Folin–Ciocalteu's phenol, and 3 mL of 20% sodium carbonate solution were added to the colorimetric tube successively and reacted at 50 °C for 5 min. The absorbance of sample was finally measured at 765 nm by an UV–vis spectrophotometer (model: LAB-014/000139) after standing for 30 min. The calibration curve was obtained using gallic acid as the standard. ($y = 2.0861x + 0.0003$, $R^2 = 0.9985$).

2.3.2. Determination of lutein content

Lutein content was determined by HPLC (Agilent, USA) analysis (Bhattacharyya et al., 2010). High performance liquid chromatographic (HPLC) conditions are supplemented as follows: column, C_{18} (5 μm, 4.6 mm × 250 mm); mobile phase, ethyl acetate: methanol: acetonitrile = 5:5:90; detection wavelength, 445 nm; flow rate, 1.0 mL/min; injection volume, 1 μL; and column temperature, 30 °C. Calibration curve was generated using lutein as the standard. ($y = 0.1805x + 0.0238$, $R^2 = 0.9990$).

2.4. Comparison of different methods

The extraction effect of MEAATPE for TP and lutein from marigold flowers was compared with the effects of enzyme-assisted ATPE (EAATPE), MAATPE, ATPE, and soxhlet extraction (SE). All conditions used for TP and lutein extraction by MEAATPE, EAATPE, MAATPE, and ATPE were the corresponding optimal conditions. The conditions for SE processes are as follows: 1 g marigold flower powders and 80 mL of ethanol were added to the Soxhlet extractor and placed in water bath for 8 h and kept at a constant temperature of 95 °C.

2.5. Surface morphology of marigold flower powder

The surface morphologies of marigold flower powders (before and after extraction) were observed using a scanning electron microscope (SEM, Sigma 300, Carl Zeiss Jena, Germany) at different magnifications.

2.6. Statistical analysis

All the experiments were carried out in triplicates, and the results were expressed as the mean ± SD of three determinations.

3. Results and discussion

3.1. Determination of the optimal composition of aqueous two-phase system

3.1.1. Effects of ethanol concentration on system

As shown in Fig. 1, the yields of TP and lutein were gradually increased with the increase in ethanol concentration. Maximum yields appeared when the ethanol concentration was 28% (w/w), and then the yields all have shown a downward trend with the continuous increase of ethanol concentration. This phenomenon can be interpreted that TP and lutein prefer a top phase, the volume of the top phase was increased with the increase in ethanol concentration, and the capability that compete for water molecules was increased. These results contributed to the enrichment of TP and lutein in the upper phase. However, excessively high ethanol concentration can lead to salt precipitation (Xie et al., 2017). Therefore, 28% (w/w) of ethanol was selected for the next experiment.

3.1.2. Effects of ammonium sulfate concentration on system

The effects of ammonium sulfate concentration on the partition