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Optimization by response surface methodology of lutein recovery from paprika leaves using accelerated solvent extraction

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ABSTRACT

In this study, we used response surface methodology (RSM) to optimize the extraction conditions for recovering lutein from paprika leaves using accelerated solvent extraction (ASE). The lutein content was quantitatively analyzed using a UPLC equipped with a BEH C18 column. A central composite design (CCD) was employed for experimental design to obtain the optimized combination of extraction temperature (°C), static time (min), and solvent (EtOH, %). The experimental data obtained from a twenty sample set were fitted to a second-order polynomial equation using multiple regression analysis. The adjusted coefficient of determination (R^2) for the lutein extraction model was 0.9518, and the probability value ($p = 0.0000$) demonstrated a high significance for the regression model. The optimum extraction conditions for lutein were temperature: 93.26 °C, static time: 5 min, and solvent: 79.63% EtOH. Under these conditions, the predicted extraction yield of lutein was 232.60 μg/g.

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1. Introduction

Paprika (*Capsicum annuum* L.) has been cultivated in many countries. The uses of it range from being a fresh vegetable to a condiment because of its colors, while other functions include being ingredients for some foods such as soups, stews, sausages, salad dressings, and sauces (Nieto-Sandoval, Fernandez-Lopez, Almela, & Munoz, 1999; Silva, Azevedo, Pereira, Valentao, & Andrade, 2013). Furthermore, blanched young leaves of *Capsicum* spp. have been dietary eaten as side dishes in Korea (Kim et al., 2011). Increasing paprika consumption has led to the discarding problem of paprika leaves and stems. However, paprika leaves have amounts of lutein and tocopherol than paprika (Kim et al., 2011). Some of the possible usages of these paprika leaves are biological functions including free radical scavenging, antimicrobial, and tyrosinase inhibitory activities (Kim & Kim, 2012; Ma et al., 2012). Although these potential usages for paprika leaves exist, detailed research on these paprika leaves and their biological activity is not yet fully available.

Lutein, an oxygenated carotenoid, is in some types of food including egg yolks, dark green leafy vegetables, and colored fruits. Research shows that the lutein in these types of foods can help eye health (Ma et al., 2012; Vishwanathan, Neuringer, Max Snodderly, Schalch, & Johnson, 2013). It is distributed ubiquitously in body tissues and tends to be the dominant carotenoid in central nervous tissues. Lutein, along with zeaxanthin, is a main carotenoid in the macula of primate retina that acts as a blue-light filter and antioxidant, and might combat age-related macular degeneration (AMD), a leading cause of visual impairment and blindness in the United States (Johnson & Record, 2014).

Accelerated solvent extraction (ASE) is an automated extraction technique that uses elevated temperatures and pressures to achieve efficient extraction in very short time. In addition, this technique eliminates many manual steps involved in preparing food samples for analysis, which helps ensure increased reproducibility and accelerates the process significantly (Richter, Jones, Ezzell, & Porter, 1996). As the temperature increases, several changes occur to the extraction in relation to the heat. As the temperature increases, the solubility and diffusion rates of the extracting solvents increase. On the other hand, solvent viscosities and solute–matrix interactions decrease (Jentzer, Alignan, Vaca-garcia, Rigal, & Vilarem, 2015). Compared to the conventional extraction (CE) techniques, ASE has more advantages, including a higher automation, extraction yields, and recovery of target compounds (Heo, Kim, Kang, & Moon, 2014). Other advantages of using ASE include a lower solvent volume (15–40 mL), shorter extraction time (15–20 min), and lower toxicity of solvents compared with other CE methods. ASE also utilizes nontoxic solvents like ethanol, water or carbon dioxide (Kukla-Koch et al., 2013). Furthermore, if

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sufficient pressure is exerted on the solvent during the extraction, a lower boiling point can be used. Hence, the combination of elevated temperature and pressure allows complete extraction to occur rapidly.

Surface methodology (RSM) examines the relationships between several explanatory variables and one or more response variables (Box & Wilson, 1951). RSM is used and analyzed through different methods including experimental strategies, mathematical methods, and statistical inference. Using these methods, the RSM focuses on the optimal condition in foods and pharmaceutical research by examining the different variables and their effect (Xu et al., 2008; Zhu, Heo, & Row, 2010). Recently, extraction has been efficiently applied using both equipment and statistical experimental design by depicting the combined effect of all the factors, as well as the time and labor consumed in conventional and classical methods (Ravikumar, Ramalingam, Krishnan, & Balu, 2006).

In this study, we obtained the optimum conditions through RSM for lutein recovery from paprika leaves by ASE, and, after validating the method, performed a qualitative and quantitative analysis using ultra performance liquid chromatography (UPLC). In addition, we analyzed γ-tocopherol in all extracts and determined the antioxidant activities.

2. Materials and methods

2.1. Samples and chemicals

Paprika leaves provided by Nongsan Trading (Iksan, Jeollabuk-do, Korea) were washed, drained and freeze-dried (PVTFD10R, Gyeonggi-do, Korea). Dried leaves were ground (HMF-3000S, Gwangju, Korea), and stored at −70 °C for analysis. Lutein (⩾97.0% (HPLC) purity) and β-carotene (⩾95% (HPLC) purity) were purchased from CaroteNature GmbH (Lupsingen, Switzerland), and acetonitrile (99.9% purity), acetone, methanol (MeOH), and ethanol (EtOH) were obtained from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals used in this study were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Experimental design by RSM

The experimental ranges of the selected variables, including temperature, static time and EtOH concentration, are shown in Table 1. Twenty sample sets of extraction were repeated three times according to the CCD matrix (Table 3), and the lutein contents were quantitatively analyzed using UPLC. The experimental data were statistically analyzed using the software Minitap 14 (Minitap Inc., State College, PA, USA). Analysis of variable (ANOVA) was used to examine the developed model's adequacy and the statistical significance of the regression coefficients. Response surface contour plots analyzed how the independent variables interacted and how those interactions impacted the overall response (Sinha, Chowdhury, Saha, & Datta, 2013). The quadratic regression model equation for the independent and dependent variables is as follows:

$$Y_{yl} = a + bX_1 + cX_2 + dX_3 + eX_1^2 + fX_2^2 + gX_3^2 + hX_1X_2 + iX_1X_3 + jX_2X_3$$

Table 1
Independent variables and their levels used for central composite design (CCD).

Variable	Coded X_i	Experimental design (central composite design) Coded level				
		−1.682	−1	0	1	1.682
Temperature (°C)	X_1	60	90	120	150	180
Static time (min)	X_2	1	2	3	4	5
EtOH in water (%)	X_3	60	70	80	90	100

where Y_{yl} is the extracted lutein content, and X_1, X_2, and X_3 are selected variables that affect the extraction.

2.3. ASE extraction

All extractions were carried out with 1 g of sample in a 22 mL stainless steel vessel using an ASE 150 system (Dionex, Sunnyvale, CA, USA). The stagnant volume of each vessel was filled with diatomite and the extraction was performed with various solvents (60%, 70%, 80%, 90%, and 100% EtOH/water), temperatures (60, 90, 120, 150, and 180 °C), and static times (1, 2, 3, 4, and 5 min) at a high pressure of 10 MPa. All extracts were evaporated to a volume of 45 mL with ASE 150 system.

For saponification, 10 mL of extracts were incubated with 3 mL of diethyl ether, 3 mL of MeOH, and 1 mL of 30% KOH/MeOH for 150 min at room temperature in darkness, and then fractionated with diethyl ether three times for collecting lipophilic phase. The combined lipophilic extracts were washed repeatedly with distilled water to remove hydrophilic components, and added with 10 mL of 10% NaCl (10 mL) for removing remained distilled water, and then finally added 10 mL of 2% Na_2SO_4 for complete removing of distilled water. The final extracts were evaporated using a TurboVap LV evaporator (Biotage, Uppsala, Sweden), redissolved in acetone (3 mL), and then stored at −20 °C under nitrogen until analysis.

2.4. Lutein analysis using UPLC and method validation

Lutein was analyzed using a UPLC system (Waters Co., Milford, MA, USA) equipped with an Acquity UPLC BEH C18 column (100 × 2.1 nm, 1.7 μm), auto-sampler, quaternary pump system, tunable ultraviolet (TUV) detector, thermostated column compartment, and degasser. Water and MeOH (15:85 v/v) was used as the mobile phase with a flow rate of 0.5 mL/min, injection volume of 1.0 μL, column temperature of 40 °C, and detection wavelength of 470 nm. The UPLC method for lutein analysis in paprika leaves was validated in terms of limits of detection (LOD) and quantification (LOQ), linearity, inter- and intra-day precision, and accuracy. Three calibration curves were plotted using 11 different concentrations (2.5, 5, 7.5, 10, 12.5, 25, 50, 75, 100, 125, and 150 μg/mL), which were prepared from a stock solution (1 mg/mL) in dimethyl sulfoxide (DMSO), to evaluate the linearity of the analytical method. To evaluate the sensitivity of the method, the LOD and LOQ values were calculated using the following equations: LOD = 3.3 × (standard deviation/slope of calibration curve) and LOQ = 10 × (standard deviation/slope of calibration curve). Lutein at four different concentrations (10, 25, 50, and 100 μg/mL) was analyzed with five replicates on the same day to determine the intra-day precision and on three different days to determine the inter-day precision. Both precision values are expressed as relative standard deviations (RSD, %).

2.5. γ-tocopherol analysis

γ-Tocopherol was analyzed using an HPLC system (Waters Co., Milford, MA, USA) equipped with a Shiseido Capcell Pak C18 column (4.6 × 250 mm, 5 μm), auto-sampler, binary HPLC pumps featuring on-board pulse dampening and an efficient mixing system, Waters 2489 UV/Visible (UV/Vis) detector, thermostated column compartment, and degasser. Water and MeOH (20:80 v/v) was used as the mobile phase with a flow rate of 1.2 mL/min, injection volume of 20 μL, oven temperature of 25 °C, and detection wavelength of 292 nm.

2.6. Determination of anti-oxidant activity

To measure the antioxidant activity, an ABTS assay was performed using the method of Zanfini, Corbini, La Rosa, and Dreassi

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