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Tulip petal as a novel natural food colorant source: Extraction optimization and stability studies

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ABSTRACT

Production of food ingredients from natural sources has attracted a great attention since people tend to consume foods prepared with natural additives. In the present study, potential usage of tulip petal waste was investigated to produce alternative food colorants. This study involves three parts: (i) solvent optimization (ethanol and water), (ii) process optimization (temperature and time) for anthocyanin extraction and (iii) determination of stability of anthocyanins extracted under optimum conditions determined with respect to pH and temperature. Optimum solvent was found as 35% ethanol and 65% water using mixture design. Response surface methodology was applied to determine optimum extraction process for obtaining maximum anthocyanin amount which was extracted at 54 °C for 116 min. Stability tests were perfomed to determine stability of anthocyanins at different temperatures (60–90 °C) and pH levels (2, 4 and 6). An increase in temperature and pH levels caused an increment in the degradation kinetics of the anthocynains. The results of the present study highlighted that tulip petal wastes could be utilized as a novel natural source for the recovery of the natural food colorant.

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1. Introduction

Appearance of the food products is one of the most important preference criteria determining consumer acceptability of the product. The first impression associated with the food products is appearance; therefore the rejection or acceptance of the products is mainly dependent on the appearance. Among the appearance parameters, color is one of the most important one. Due to the significance of the color during the consumer acceptability of the product, producers exert an effort to improve the color of the corresponding material by adding natural or synthetic colorants according to the regulations. Synthetic colorants are widely used in the food industry due to their physicochemical properties. Although they surpass the natural ones considering their characteristics such as encoloring performance, color ranges, stability properties, producers tend to substitute them with the natural ones due to the toxic effects of some of the synthetic colorants (Chou et al., 2007). Some synthetic colorants promote hypersensitivity reactions involving urticaria, angioneurotic edema, and asthma (Vidotti et al., 2006). Carotenoids and anthocyanins are accepted as the mostly used vegetable based natural colorants in the food industry. Anthocyanins are included in the class of flavonoids which are responsible for coloring of many plants (Davies et al., 2012). Anthocyanins give shiny orange, pink, red, violet and blue colors to many flowers and fruits (Castañeda-Ovando et al., 2009). Moreover, anthocyanins have drawn significant attention due to their antioxidant activities, which can prevent or reduce the risk of neuronal and cardivascular illnesses, cancer and diabetes (Konczak and Zhang, 2004). When considering toxic effects of the synthetic colorants and beneficial effects of the natural anthocyanins, in recent years, researchers have interested in determining novel natural anthocyanin sources to produce natural food colorants. One of the potential anthocyanin source is tulip varieties.

Tulipa L. is a one of the colored flowers and they are a member of *Liliaceae* and family represented by 16 taxa (15 species), and two of these species are endemic in Turkey (Mlcek and Rop, 2011). They are generally used as ornamentation purposes. Anthocyanin varieties present in the tulip structure determine the color of them. As delphinidin type tulips, containing delphinidin more than 50% of the total anthocyanidin content, have black, black-purple, fade-sky, violet and purple, cyanidin types have red-purple, pink, black-red, deep crimson, crimson, deep-red, dark red-orange and red-orange

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flowers, those are orange and flesh pink color for pelargonidin types (Shibata and Ishikura, 1960). Torskangerpoll et al. (2005) studied the relation between colour and antocyanin profile of the tulips. Annually 50 millions tulip petal were waste in Turkey, which is equally 250 millions gram. When considering 80% of the tulip petal composes of dry matter, there are 50,000,000 g (50,000 kg) dried tulip waste in Turkey. Sagdic et al. (2013) reported that anthocyanin content of different tulip flowers changed between 236 and 839 mg pelargonidin 3-glucoside/kg dry extract. This anthocyanin amount could be increased by application of different treatments such as using optimum solvent or ultrasonic application. If the anthocyanin content of the tulip flower is accepted as 800 mg anthocyanin/kg dry extract, 40,000 g anthocyanin could be produced from tulip petal wastes in Turkey. As the maximum colorant limit is taken into consideration, 2,000,000 kg edible ice or food ornamentation materials or 135,000 kg confectionery products or 400,000 kg jam, marmalade or jelly could be produced by addition of the food colorants extracted from tulip petal waste in Turkey. Therefore, assessment of those tulip wastes is very important for the food industry, which provides substantial economic gain to the countries. There is no problem about the usage of tulip extracts in the food industry since it was reported that the orange red, pink and violet extracts had no cytotoxic activity against MCF-7 cell lines (Sagdic et al., 2013). Therefore, increasing extraction yield is very necessary for increasing economic gain.

In order to increase extraction yield, optimization of extraction solvent is required. The influence of different solvent or solvent combinations on the phenolic content of the extracts obtained from different materials was found as statistically important in different studies (Karaman et al., 2014; Ozturk et al., 2014). From this reason in such studies, mixture design could be performed to determine optimum solvent combination to extract corresponding compounds in maximum amount. After determination of optimum solvent, the extraction process parameters such as temperature, time, pH and ultrasonic power etc. should be also optimized for increasing the yield. This optimization is especially required for the extraction of anthocyanins since they are very instable compounds; therefore, the extraction conditions should be optimised. The color characteristics and stability of anthocyanins are influenced by several factors such as structure and concentration of the pigment, pH, temperature, light intensity and quality and the presence of copigments (Cevallos-Casals and Cisneros-Zevallos, 2004). Response surface optimization could provide information about extraction temperature and time ranges. However, in the literature there are no studies related with extraction optimization of anthocyanins from the tulip petals as a novel food colorant source.

In this study, it was aimed to produce natural food colorants from the tulip petal waste which could be used as a novel source. This study is composed of three parts: i.) Extraction solvent will be optimised performing mixture design, ii.) Extraction temperature and time will be optimised using response surface methodology and iii.) Determination of temperature and pH stability characteristics of the anthocyanins extracted with optiumum solvent under optimum conditions.

2. Materials and methods

2.1. Materials

Tulip (*T. gesneriana* L.) flowers were acquired from Istanbul Wood and Landscape Corporation (Istanbul Agac and Peyzaj A.S., Istanbul, Turkey). The tulip petals were dried at 50 °C by vacuum drier and kept at room temperature in polyethylene bags until analysis. The chemicals used in this study were obtained from Merck (Darmstadt, Germany).

Table 1
Anthocyanin contents of the samples extracted from dried tulips using different solvents determined by mixture design.

Points	Solvent Concentration		Total anthocyanin content (ppm)
	Ethanol (%)	Water (%)	
1	0	100	378
2	0	100	372
3	25	75	468
4	50	50	447
5	50	50	457
6	75	25	246
7	100	0	25
8	100	0	27

2.2. Methods

2.2.1. Solvent optimization

Optimum solvent concentration for the extraction of maximum amount of anthocyanin was determined using simplex lattice mixture design. Water and ethanol were selected as independent variables in the mixture design, and total anthocyanin content was selected as the dependent response. Each solvent concentration was ranged from 0 to 100% concentration. Total solvent concentration was adjusted to 100%. Eight experimental points determined using model are shown in Table 1. Design Expert software (Version 7 Stat-Easy Co., Minneapolis, MN, USA) was used to carry out simple lattice mixture design analysis. All experiments were performed in triplicate.

2.2.2. Process optimization

After determination of optimium solvent mixture, process optimization was carried out using response surface methodology (RSM) for aquiring maximum amount of anthocyanin. The design is composed of two factors, namely temperature and time of extraction process between 25 and 55 °C and 30–120 min, respectively. Two-factor-3-level Central Composite Design including 3 center points were performed in the present study to optimize extraction process. Total anthocyanin content (mg/L) was selected to be a response of the corresponding established model. Eleven experimental points set by the use of the Design Expert software (Version 7 Stat-Easy Co., Minneapolis, MN, USA) are presented in Table 2 where actual variables of the design were presented. The following second-order polynomial equaiton was used to mathematically express the magnitude of anthocyanin content as a function of extraction temperature and time.

$$y = \beta_0 + \sum_{i=1}^{N} \beta_i x_i + \sum_{i=1}^{N} \beta_{ii} x^2 + \sum_{\substack{i=1 \\ i<j}}^{N} \beta_{ij} x_i x_j \quad (1)$$

Table 2
Anthocyanin contents of the extracts obtained from dried tulips under different conditions determined by response surface methodology.

Points	Extraction Conditions		Total anthocyanin content (ppm)
	Temperature (°C)	Time (min)	
1	25	30	351
2	25	75	519
3	25	120	616
4	40	30	360
5	40	75	524
6	40	75	540
7	40	75	548
8	40	120	642
9	55	30	393
10	55	75	587
11	55	120	680

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