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Determination of Anthocyanin Content in Saffron Petals of Herat Province

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ABSTRACT

Saffron is a valuable product that is semi-resistant to drought and is used in various cases, including in the food industry as a flavoring agent and in the medical industry as a sedative, anti-depressant, and anti-heart disease. In Afghanistan, only saffron stigma is used, and thousands of tons of saffron petals are thrown away as waste. In contrast, saffron petals have a high amount of anthocyanin that can be used in the food industry instead of artificial colors, which, in addition to having a bright color, is not harmful and has anti-cancer and anti-virus properties. An experiment was conducted to determine the amount of anthocyanin content in saffron petals on 80 samples from Ghorian, Pashtoonzarghon, Gozarah, and Karokh districts in the autumn of 1397 using a spectrometric method in the Food Technology Laboratory Faculty of Agriculture, Herat University. The results of this study showed that the Gozarah District with (1691 mg / L) had the highest amount of anthocyanin in petals, and the lowest amount of anthocyanin (1469 mg / L) was recorded in saffron petals of the Ghorian District. The difference in the amount of anthocyanin among different regions depends on different environmental factors, such as agricultural operations and the type of saffron corn. The findings of the research showed that by extracting anthocyanin from saffron petals and using it in the food industry and medicine, not only the loss of this major and valuable part of the saffron flower is prevented, but it will help in the economic development of farmers and the country.

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Introduction

Saffron (*Crocus sativus* L.) is a multiyear plant that reaches 10 to 30 cm tall. The flowers have six purple petals and one stigma, which consist of three styles. The portion known as saffron is a stigma with three branches, and 150,000 flowers are needed to produce one kilogram of saffron. Therefore, about 75 kg of saffron flowers are wasted to obtain this dry stigma (Kakouri et al., 2017). Hemmati et al. (2009) reported that saffron petals are discarded as

waste after separating the stigma, while saffron petals comprise an average of 86.4% fresh weight and 96.36% dry weight of flowers. The color of saffron petals is due to the presence of anthocyanin and flavonoids, which are natural compounds in the group of secondary metabolites belonging to the flavonoid family (Erlie et al., 2014). Anthocyanin and flavonoids produce red, purple, and blue colors in many flowers, fruits, and vegetables, including saffron. These compounds are of great medicinal importance due to their unique biological properties (Nickhah et al., 2010). Today, the use of artificial colors in the food industry has increased due to their attractiveness. At the same time, there is no awareness of their harmful effects, so anthocyanin is a good alternative to synthetic colors because, in addition to having a bright color, anti-cancer and anti-viral properties have been reported(Andersen et al., 2010). Saffron petals have been introduced to prevent asthma, whooping cough, inflammation, and anti-depressants and reduce premenstrual symptoms in women (Hoseini et al., 2008). Saffron petals have an important role in the prevention of various diseases by having antioxidant activity; antioxidants are compounds that effectively prevent the reaction of free radicals in various ways and lead to reduction of damage or reduction of cell death, reduction of heart disease, vascular and cancers (Sharififar et al., 2007). Research shows several synthetic antioxidants used in the food industry as preservatives have side effects. Still, no side effects from the use of natural antioxidants in saffron petals have been reported in various studies (Velioglu et al., 1998). Saffron petals, which are disposed of as waste, have several properties, including anti-depressant, anti-inflammatory, analgesic, anticoagulant, and antihypertensive, and are used to treat neurological disorders (Bagherzade and Manzaritavakoli, 2015). Saffron is a plant commonly used for its stigma part of the flower, and the rest of the saffron petals, which make up about 90% of its weight, are wasted. Nowadays, saffron petals, due to the richness of anthocyanin, an antioxidant, their role in human health, and their application in the food industry, have been attractive in recent years (Tuberoso et al., 2016).

Due to the high annual production of saffron in Herat province, after the stigma is removed, a large amount of this valuable material remains unused because no such research has been done on the importance of this issue in the country. This research was conducted to investigate the amount of anthocyanin in saffron petals of Herat province and identify the difference in the amount of anthocyanin in different regions to prevent its wastage and help farmers' economies.

Materials and Methods

Study Area

The following research was conducted in the Food Technology Laboratory, Faculty of Agriculture of Herat University in the fall of 1397 to investigate the amount of anthocyanin in the saffron petals of Herat province.

Samples Collection

From the districts with the highest production of saffron, 80 samples of saffron flowers and 20 samples from each district were collected, and the areas were numbered randomly. 200g of petals from each sample were dried for two hours in an electric oven at 50°C, and then saffron petals were powdered and passed through a 40 Mesh sieve. To extract the anthocyanin of saffron petals from each sample, 1g of dried saffron petals were dissolved in 250 ml of a hydrochloric acid solution of 0/1 normal; then the mixture was stirred for 15 minutes with a magnetic stirrer at room temperature; subsequently, it was left at room temperature for 5 minutes to settle the sediment, after that the solution was filtered through filter paper Whatman No. 1. To determine the amount of anthocyanin in saffron petals using the differential pH method adopted from (Lee, J., 2005). For this purpose, the extracted were added to potassium chloride buffer pH 1.0 and sodium acetate buffer pH 4.5 and left for incubation for 20 minutes at room temperature; after that, they were studied by spectrophotometer model(UV752D) with wavelengths of 700 and 516 nm and the numbers obtained from the device were calculated in terms of milligrams per liter using the following formula.

Equation (1)

$A = (A516 — A700)\ pH\ 1.0 — (A516 — A700)\ pH4.5$

Equation (2)

$\text{Anthocyanin level of monomer} = (A \times MW \times DF \times 1000) / (\varepsilon \times L)$

A: Amount of sample adsorption

MW: Molecular weight of cyanidin triglyceride (based on gram per mole)

DF: dilution factor

ε: Molar absorption of cyanidin triglyceride (26900)

L: cell diameter of the spectrophotometer (in terms of 1 cm)

The data obtained were subjected to statistical analysis as per the guidelines suggested by Panse and Sukhatme (1978).

Results and Discussion

Gozarah District

There was a high amount of anthocyanin in the saffron petal of Gozarah district based on the results obtained in Figure 1. According to this result, sample 16 with (1713mg / L) had the highest amount of anthocyanin, and the 12th sample had the lowest amount (1663mg / L) of anthocyanin. The average amount of anthocyanin in the saffron petal of Gozarah district was 1691mg/L. Our result shows that the amount of anthocyanin in the saffron petals of Herat is much higher than the saffron petals of Iran, which is the largest producer of saffron so that Ainafshar 2017 reported the amount of anthocyanins in saffron petals as 999 mg/liter. The

findings of this study make it clear that there is a sufficient amount of anthocyanin in the saffron petals of Herat. Using it in the food and medicine industries will prevent the waste of this useful and economical product. The difference in the amount of anthocyanin in saffron petals in different regions may depend on different agricultural factors such as plowing, fertilizing, irrigation, etc.; according to our findings, Rasouli et al. (2015) stated that the use of organic fertilizer in saffron fields increased the amount of crocin, picrocrocin, safranal and anthocyanin content of saffron.

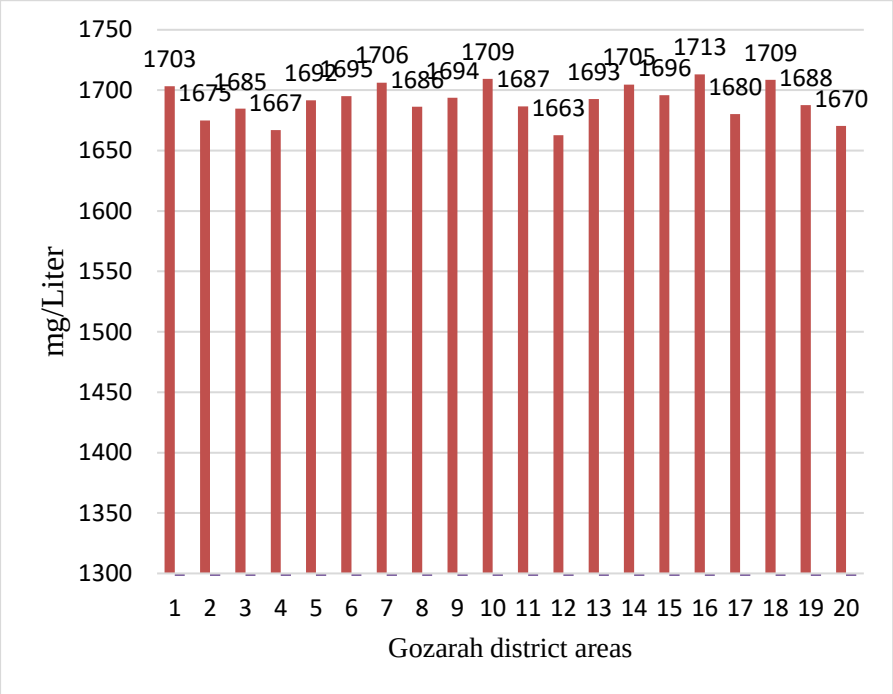

Figure 1: *Anthocyanin content of saffron petals in Gozarah district*

Ghorian District

The data in Fig 2 indicated a high amount of anthocyanin in the saffron petals of the Ghorian district. It showed that the highest amount of anthocyanin (1495 mg / L) was present in the sample (8). The lowest amount of anthocyanin (1440 mg / L) was presented in the sample (2), and other samples with different amounts of anthocyanin were located between these two samples, and the average amount of anthocyanin in saffron petals of Ghorian district was (1469mg / L). The results showed that high anthocyanin levels were present in this district's saffron petals, which can be used due to their medical and industrial importance. By comparing the anthocyanins of saffron petals with the anthocyanins found in other products, it can be said that saffron petals are a very rich source of all kinds of anthocyanins. According to this result, Kirka et al. (2007) reported that the amount of anthocyanin in black carrots was equal to 1750 mg/kg of fresh carrots. The difference in the amount of anthocyanin in different areas may be due to agricultural operations, genetic diversity, moisture content, light, oxygen, etc., which are dependent factors. The results confirm the studies by Stintzing and Carle (2004), which reported that various factors such as chemical structure, temperature,

light, moisture, oxygen, solvents, enzymes, and flavonoids are effective in the stability of anthocyanin.

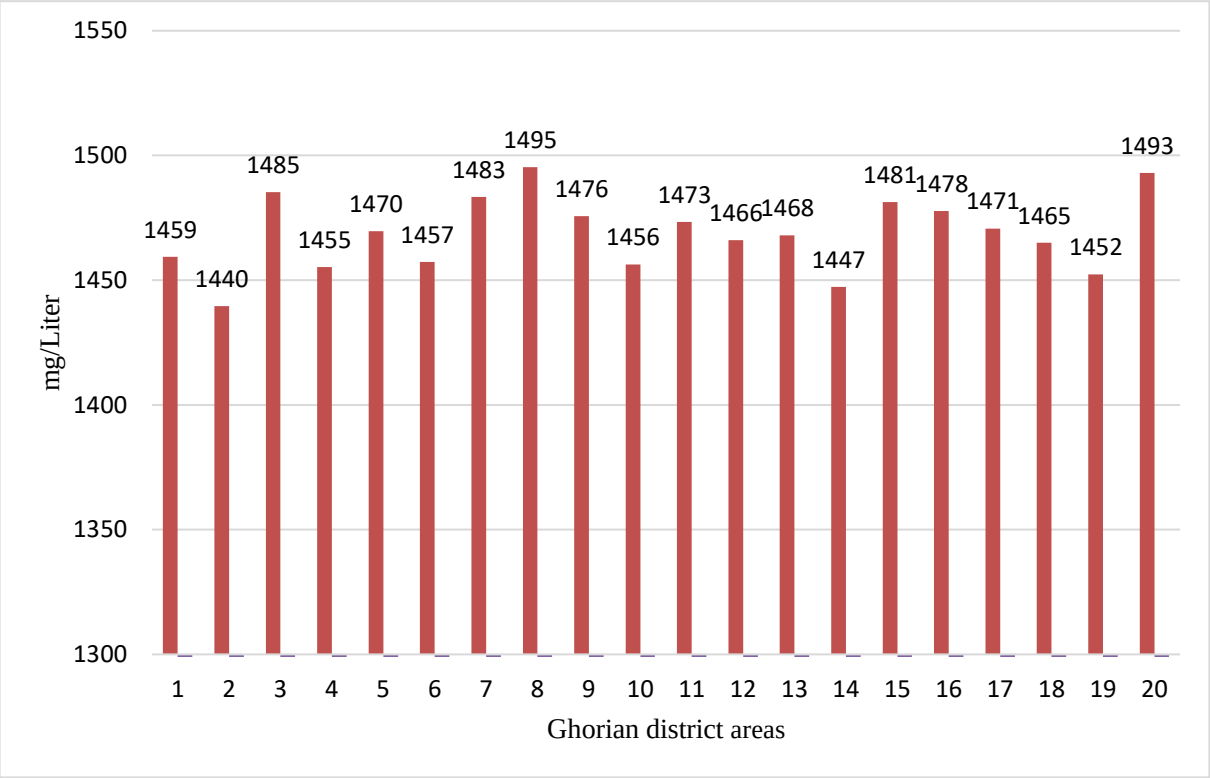

Figure 2: *Anthocyanin content of saffron petals in Ghorian district*

Pashtunzarghon District

The amount of anthocyanin in the saffron petals of Pashtunzarghon district is shown in Figure 3. This finding indicated that sample 7 with (1595mg /L) had the highest amount of anthocyanin, and sample 9 with (1542mg /L) had the lowest amount of anthocyanin. The average amount of anthocyanin in the saffron petals of Pashtunzarghon district was 1571 mg / L. It showed that the amount of anthocyanin in saffron petals of Pashtunzarghon district was high and can be used in the food industries as a natural color. This research (Hemmati, 2009) also compared the amount of anthocyanin in saffron petals with other anthocyanin sources. It stated that anthocyanin in saffron petals is higher than in many natural coloring products. The difference in anthocyanin content of Pashtunzarghon district depends on different environmental conditions and agricultural operations. The results agree with the findings of Vakili et al. (2016) that altitude and the length of cold nights affect the quality of saffron, and saffron produced in mountainous areas is higher than saffron grown in desert areas.

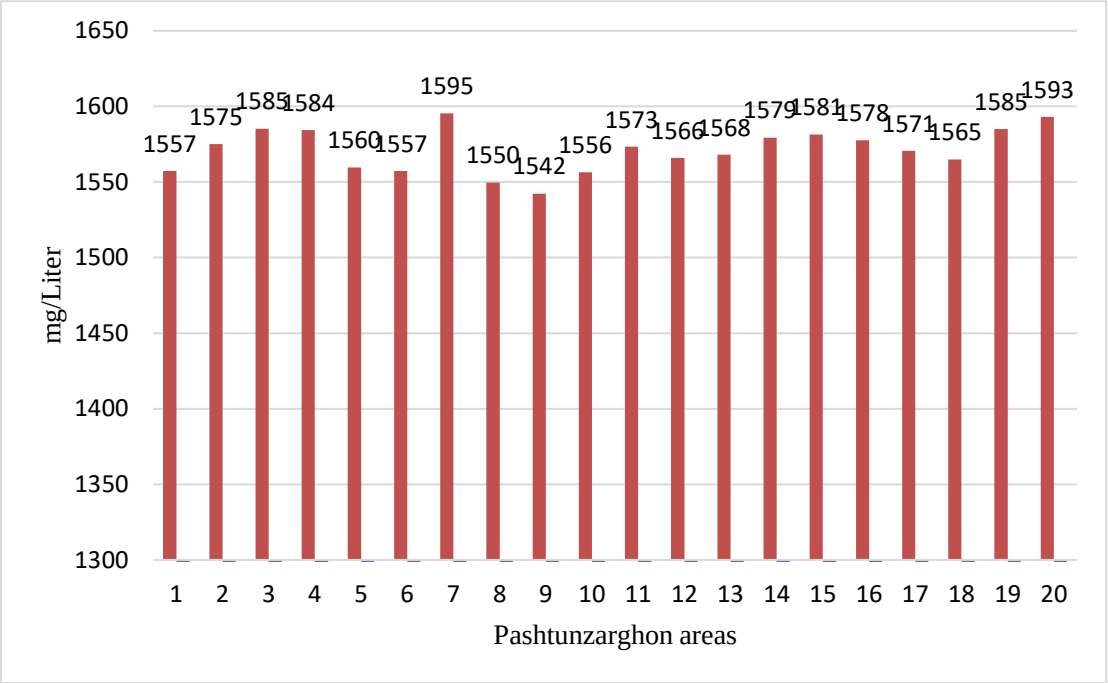

Figure 3: Anthocyanin content of saffron petals in Pashtunzarghon district

Karokh District

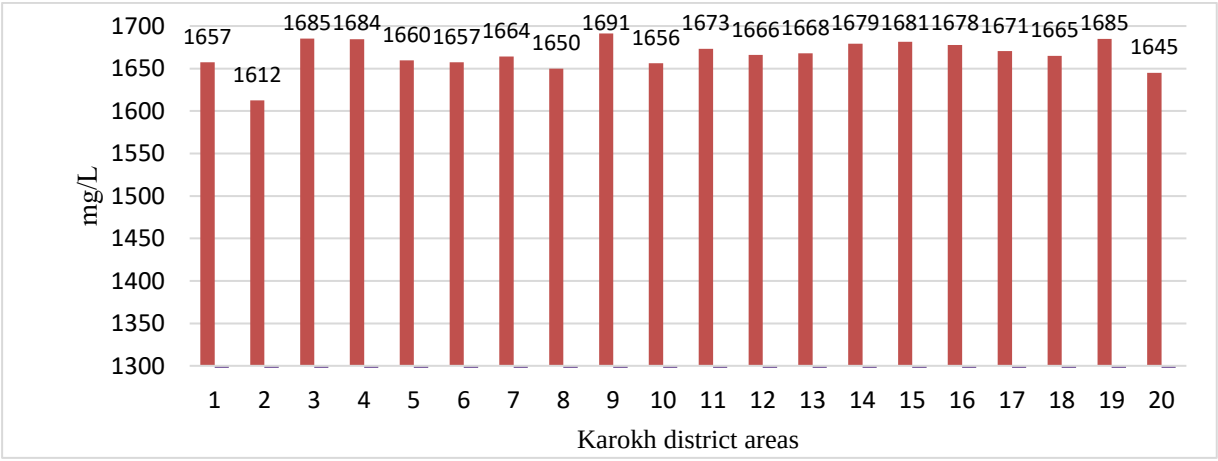

Figure 4: *Anthocyanin content of saffron petals in Karokh district*

To determine the amount of anthocyanin in saffron petals of the Karokh district, 20 samples were analyzed, and the results of this study showed that sample 9 with (1691mg / L) had the highest amount of anthocyanin and the lowest amount (1612mg / L) was recorded in the sample (2). The average amount of saffron anthocyanin in Karokh district was 1666 mg / L (Figure 4). It showed that the amount of anthocyanin in Herat saffron petals is high and can be a good alternative to artificial colors used in the food industry. The results conform to the work of Khazaei et al. (2013), who also reported the amount of anthocyanin in saffron petals of Iran as 1612 mg/liter. The difference in the amount of anthocyanin in saffron petals in different regions may depend on agricultural operations, varieties used, transportation, and

environment. Caballero et al. (2007) stated that onion type, agricultural operations and environmental factors significantly affect saffron's secondary metabolites.

Conclusion

The result showed that the amount of anthocyanin extracted from saffron petals of Herat province is very high, and saffron petals can be introduced as a potential replacement for producing natural anthocyanin color in food and pharmaceutical industries. The results of laboratory tests on 80 saffron samples taken from the four districts of Herat province show significant differences among different regions. Based on this, the highest amount of (1691mg/L) and (1666mg/L) anthocyanins was in the saffron petals of Gozara and Karokh districts, respectively, while Pashtunzarghon and Ghorian by having (1571mg /L), (1469mg/L) anthocyanin in the saffron petals located in the third and fourth place. The results of this study showed that high amounts of anthocyanin are present in saffron petals of Herat province, and it is used in food and medicine industries, not only prevent the side effects of artificial colors on human health but also help to raise the economy of saffron farmers. Because 86 kg of saffron petals from 100 kg of fresh flowers, discarded as waste, can be used as an economical product.

Table 1: Average of anthocyanin content in saffron petals of Herat

Areas	Anthocyanin content (mg/L)
Gozara District	1691[a]
Krokh District	1666[b]
Pashtonzarghun District	1571[c]
Ghorian District	1469[d]
CD @ 1%	13.05
Significance	**

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