Effect of Ascorbic Acid on the Stability of Pigmented the Waste Saffron Flower in Food Product

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Abstract

Wasted saffron flowers (sepals and petals) is one source of antioxidants in it is not no use and be discarded. This study examined the effect of saffron petals synthetic color stability during storage was studied. For this purpose, firstly extracted by ultrasonic extract of saffron petals by solvent (ethanol acidic) to ratio of about 50 with three factors of time (5, 10, 15) and three intensities (20, 60 and 100%) were the best conditions to extract the sound intensity of 100% and took 15 minutes to determined and Then stability of ascorbic acid, 10%, 20% and 30% at 25 and 35°C and 45°C were measured within 5 days. The results are treated as destruction effect of ascorbic acid on the pigment of saffron petals and the destruction increases at higher temperatures and it is worth noting that this course destruction in the presence of ascorbic acid at the temperature of 45°C has reached its maximum.

Keywords: Saffron waste; Sound waves; Pigments; Ascorbic acid

Introduction

Saffron is a plant of Zanbagh family, multi-year scientific name Sativus Crocus, native regions, particularly Asia, southwest Asia, southern Europe and Spain, but it is cultivated in other parts of the world [1-7].

Many of carotenoid pigments [8,9], both water- soluble and fat-soluble lycopene, alpha carotene, beta-carotene and carotenoids Zagazyn and the water-soluble glycosides, krocin, which is a mixture of glycosides is more important. Additionally it contains saffron crocetin free aglycones and it's a small amount of anthocyanin pigments [1,3].

It should be noted that the pigments in nature due only to provide certain appearance, not other tasks they are doing. for example the Sun’s energy chlorophyll absorb, or oxygen, by carbon dioxide, of hemoglobin in the body and The transfer of finds [8-10].

The food industry is trying as much as possible original colors used in foods, such as orange soda to make the attempt to emulate the color orange.

Food tinters are both naturally occurring and synthetic, which must be approved by the Administration flavors, food, and pharmaceutical and cosmetic, are divided [10-12].

It is important to note that natural dyes do not require approval of the department. In general, the synthesis of dyes from simple materials, often aromatic hydrocarbons such as benzene, toluene and naphthalene, it starts with a combination of amine groups, nitro or halogen done [13,14].

The effect pigments contained in saffron with cancer fighting properties and are used as a sedative in patients of neurology [15].

Materials and Methods

These experiments and carried out research during the color materials from waste saffron and near period storage of ascorbic acid and were measured compound and carotenoids anthocyanin dye includes are:

Preparation of saffron petals

Fifteen kg of saffron flower, Crocus satirus of Agriculture Natural Resources Research Center of Khorasan Razavi province were created, then immediately excised stigmas and stamens of flowers and flags separated by using the natural airflow and dry in the shade. Flag dried using the powder mill. Powdered samples in nylon bags in a dark place with a temperature of 18°C until the time of extraction were stored.

Extraction

To identify the color of the saffron plant the flag of the extraction solvent, acid, ethanol and n- hexane was used to extract anthocyanin and carotenoids. The new process to achieve optimum extraction of ultrasound intensity of 20, 60 and 100 at 5, 10 and 15 were performed.

Extraction of anthocyanin’s compound

Compound anthocyanin’s extracted with cold solvent (using a dipping method): Powdered samples were pooled and the solvent ratio of 1 to 50. Solvent consisting of a mixture of ethanol 96 and Hydrochloric Acid 3/1 was normalized to the ratio of 85°C to 15°C. Mixed with a magnetic stirrer for 15 minutes at ambient temperature was too. The first stage of filtration using Whatman filter paper No.1 was performed with a vacuum pump and the resulting precipitate was extracted again under the same conditions. After mixing, the extract filtered, most of the solvent under vacuum using a rotary evaporator at 40°C was removed and finally extract spread on the plate surface and the vacuum oven was cooled below 40 degrees. After placing it on the DL until constant weight decicatore extraction efficiency calculation resulting extracts shaved and dark-colored containers were stored in a cool, dry place [16,17].

Compound anthocyanin extraction with ultrasound: Powdered samples were pooled and the solvent ratio of 1 to 50. Ethanol mixed...
solvent consisting of 70°C and Hydrochloric Acid 5/1 was normalized to the ratio of 85°C to 15°C. Mixed with a magnetic stirrer for 15 minutes at ambient temperature was too. After the time has elapsed, the mixture of sample and solvent at a fixed frequency of 24 kHz ultrasonic waves at room temperature in 5, 10, 15 minutes and three intensities of 20, 60 and 100 Hz were. Ultrasound uses ultrasonic Hilshir German Company UPS Model 400 SS with 400 watts of power and H7 type titanium probe with a diameter of 7 mm and a length of 100 mm was cycle1 [18-20]. The first stage of filtration using Whatman filter paper No. 1 was performed with a vacuum pump and the resulting precipitate was extracted again under the same conditions. After mixing, the extract filtered major part of the solvent using a rotary evaporator under 40°C and this extract was removed and the extract spread on the plate surface and the vacuum Avon was cooled below 40 degrees.

Calculating the extraction rate: The measurement of initial and final weight plates containing dry it persists (in the vacuum oven); dry matter extracted was calculated and expressed as percentage.

Measurement of total anthocyanin compounds: To measure anthocyanin’s used the pH differential method; Rulastad procedure (1976) is performed. For this purpose, we used the two buffers with a pH of 1 (KCl-HCL) and 4.5 (sodium acetate), 200 micro liters of sample buffer to a volume of 3 cc to accomplish and then at two wavelengths 520 and 700 nm absorption once with buffer 1 and once with buffer 2 to the reading of was calculated based on 3-1 equation (21-22).

\[
A = (\text{Abs} \cdot \text{max} - \text{Abs} \cdot \text{700}) \cdot \text{pH} = 1 - (\text{Abs} \cdot \text{Vis} \cdot \text{max} - \text{Abs} \cdot \text{700}) \cdot (\text{pH} = 4.5)
\]

\[
\text{A} = \text{Absorption Difference between pH = 4.5 and pH = 1}
\]

\[
\text{Mw: molecular weight Cyanidin-3 glucoside (449.2 g mol)}
\]

\[
\text{E: molar absorption cyanidin-3 glycosides (26900)}
\]

\[
\text{DF: dilution factor}
\]

\[
\text{L: length of the cell}
\]

Prepare buffers the required:

Sodium acetate buffer amount of 54.43 g sodium acetate with concentrated hydrochloric acid and distilled water to make it to the pH = 4.5’ve raised the volume to 1000 ml. Potassium Chloride Buffer: The 1.86 g of potassium chloride in concentrated hydrochloric acid solution with distilled water and brought it pH=1’ve raised the volume to 1000 ml [24-26].

Statistical analysis:

All tests in completely randomized design (ANOVA1) and tests based on factorial experiment were performed in triplicate. Means MStatC software based on Duncan’s test at the five percent (05/0>p) were compared.

Results and Discussion:

Effects of ascorbic acid on anthocyanin component at three temperatures 25, 35 and 45°C were evaluated. According to data obtained from analysis of variance was observed that by increasing the percentage of AA and temperature in comparison with the control sample and the amount of Antoine compounds cyanine declined. As shown in Table 1 is observed between the data is quite significant. It is worth noting that the rate of decline Antoine cyanine compounds in the control sample at a temperature of 35°C is higher than other treatments, but the rate of decline Antoine cyanine compounds follows the same pattern in other treatments.

Independent effect of ascorbic acid on anthocyanin compounds at 25°C

According to the Figure 1 is observed with an increase in the percentage of ascorbic acid, reduced levels of anthocyanin compounds. However, this reduction between the treatment and control samples containing 10% ascorbic acid and most of the samples containing 10%, 20% and 30% less. Between data is also quite significant.

Independent effect of ascorbic acid on the rate of anthocyanin compounds at temperatures of 35°C

According to data obtained from analysis of variance was observed at the same temperature of 35°C to 25°C, with the temperature held constant with increasing ascorbic acid levels anthocyanin compounds was reduced to.

Temperature of the decline in 35 temperatures greater than 25°C and the difference between the sample and control sample containing ascorbic acid is more significant (Figure 2).

Independent effect of ascorbic acid on the rate of Antoine cyanine compounds at a temperature of 45°C

As indicated in the Figure 3 is observed, with an increase of ascorbic acid compared to the control sample, the amount of anthocyanin compounds are reduced. Course, it is worth noting that the independent effect of comparison charts, indicating the fact that the rate of temperature increase decline in the percentage of anthocyanin compounds, and ascorbic acid increases.

Well compared to control treatments at three temperatures 25, 35 and 45°C, confirming the above requirements” three control treatments can be seen that with increasing temperature, has resulted in reduced anthocyanin compounds.

Independent effect of time on the amount of anthocyanin compounds at 25°C

Figure 4 indicates that with time at 25°C are reduced anthocyanin compounds. The difference between the data is quite significant. This indicates that at constant temperature, over time, will lead to increased loss of anthocyanin compounds.

Independent effect of time on the amount of anthocyanin compounds at temperature of 35°C

Figure 5 indicates that over time the amount of anthocyanin compounds are reduced. However, the decrease in temperature from

| Mean-square | Source                  |
|-------------|-------------------------|
| 45°C        | 3752.63**               |
| 35°C        | 244124.139**            |
| 25°C        | 244124.139**            |
|             | treatment (A)           |
| 233762.260**| Time Storage (B)        |
| 2232.843**  | 2299.812**              |
| 963.935**   | A+B                     |
| 17.282      | 21.32                   |
| 18.404      | 40                      |
| Error       |                         |

Table 1: ANOVA treatment effect (control and different levels of ascorbic acid) and storage on anthocyanin extract of saffron 3 temperatures 25, 35 and 45°C.
35°C to 25°C is much higher than the temperature. The results also indicate that the difference between the data is quite significant.

**Independent effect of time on the amount of anthocyanin compounds at temperature of 45°C**

The results of the analysis of the data indicates that at the same temperature 45°C, 35.25°C, over time, reduced anthocyanin compounds, However, an independent effect of the chart revisions and accuracy at three times the rate of anthocyanin compounds can be observed that over time decreases the amount of anthocyanin compounds it is the rise in temperatures intensifies this Destruction. It can be seen that the difference between the data is quite significant (Figure 6).

**Interactive effects of temperature and of the amount of ascorbic acid, anthocyanin compounds extracted from the petals of saffron**

Comparing the data in Table 2, (we added it in appendage), shows that in control samples over time from the first day to the fifth drop anthocyanin compounds in samples stored at 25°C compared to samples stored at temperatures 35 and 45°C is lower. This indicates that the minimum loss of anthocyanin compounds in control samples stored at 25°C for one day and the maximum decline, the control sample maintained at a temperature of 45°C is 5 days. Results of the control sample Comparison with a sample containing 10, 20% and 30% compared to ascorbic acid. Is viewed as control samples with increasing storage time and temperature drop is increased anthocyanin compounds, and point to note that relative to be control sample, increases ascorbic acid, time and temperature acts as an aggravating factor that drop anthocyanin compounds.
Table 2: Interactive effects of temperature and of the amount of ascorbic acid, on the extraction of anthocyanin compounds from the petals of saffron.

| Temperature (°C) | 20% Vitamin C | 40% Vitamin C | 60% Vitamin C |
|-----------------|----------------|----------------|----------------|
| 45°C            | 586.60 ± 2.81* | 608.30 ± 1.05* | 619.20 ± 1.07* | 1 |
| 35°C            | 522.80 ± 4.75* | 553.90 ± 2.41* | 586.60 ± 1.05* | 2 |
| 25°C            | 425.10 ± 5.76* | 499.50 ± 2.83* | 517.00 ± 2.89* | 3 |
| 15°C            | 373.20 ± 4.70* | 489.55 ± 10.09*| 517.00 ± 2.89* | 4 |
| 5°C             | 262.80 ± 5.63* | 432.00 ± 3.96* | 423.00 ± 3.96* | 5 |
| 25°C            | 464.90 ± 4.56* | 514.30 ± 3.64* | 423.00 ± 3.96* | 1 |
| 45°C            | 608.30 ± 1.05* | 517.00 ± 2.89* | 423.00 ± 3.96* | 2 |
| 35°C            | 373.20 ± 4.70* | 489.55 ± 10.09*| 423.00 ± 3.96* | 3 |
| 25°C            | 262.80 ± 5.63* | 432.00 ± 3.96* | 423.00 ± 3.96* | 4 |
| 5°C             | 464.90 ± 4.56* | 514.30 ± 3.64* | 423.00 ± 3.96* | 5 |

Figure 6: Independent effect of time on the amount of anthocyanin compounds at a temperature of 45°C.

Figure 7: Interactive effects of time and temperature on the rate of anthocyanin extraction with 45°C.

Acknowledgments

It is recommended to use pigmented wastes obtained from the saffron plant, besides ascorbic acid in food products should be use such that the quickly of production.

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