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Original paper

Stability of anthocyanin extracts from tall currant (*Ribes altissimum*) fruits

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Abstract
Plant anthocyanins are colored pigments that are usually used as a food colorant. In addition, anthocyanins have been widely studied for its medicinal values as an attracting potential pharmaceutical ingredient. This study investigated the anthocyanins stability and extraction rate from tall currant extracts. The results showed that tall currant fruits are rich in anthocyanin content, providing 1.93%–2.70% anthocyanin in different extraction solvents. The stability of anthocyanin extracts was significantly affected by light exposure as indicated by 34.96%–56.64% degradation. The optimal condition for anthocyanin extraction from tall currant fruits was achieved with 70% ethanol/citric acid solution (pH = 2.50, and solid/liquid ratio of 1:20) when the extraction was carried out at 70°C for 90 minutes. This condition corresponded to anthocyanin content of 2.39% or 2390 mg anthocyanin in 100 g of dry plant materials. The findings of this study will be useful to predict the quality changes that might occur in preparing food colorants from tall currant (*Ribes altissimum*) in terms of thermal processing and prevent the degradation of the anthocyanins for the beverage and food industry in Mongolia.

Keywords: Food coloring, extraction, *Ribes altissimum*, stability

1 Introduction

Anthocyanins are water-soluble colored pigments in the forms of anthocyanidin glycosides and acylated anthocyanins that belong to one of the subclasses of phenolic phytochemicals. The general molecular structure of anthocyanin is shown in Figure 1.
These are responsible for the red, purple, and blue colors in plants, like flowers, fruits and vegetables. Especially, wild berries, currants, and grapes as well as some tropical fruits have high content of anthocyanins [1].

Fig. 1 Molecular structure of anthocyanin

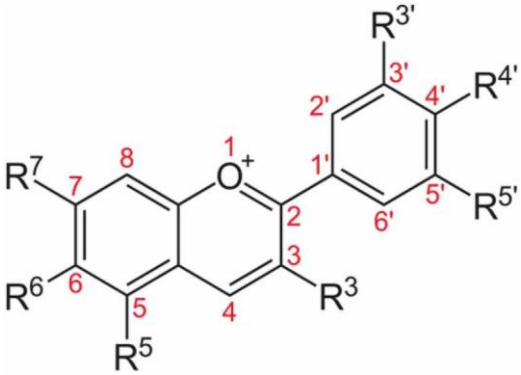


Table 1 Solvent system for the anthocyanin extraction

Extracts	Solvent system
Extract I	70% ethanol/acetic acid, (95:5), pH = 3.3
Extract II	30% ethanol/acetic acid (95:5), pH = 2.6
Extract III	70% ethanol/citric acid (100:5), pH = 2.5
Extract IV	30% ethanol/citric acid (100:5), pH = 2.0

Numerous studies have shown that plant anthocyanins possess antidiabetic, anticancer, anti-inflammatory, antimicrobial, anti-mutagenic, anti-neurodegenerative and anti-ageing activities and have anti-obesity effects and prevent from cardiovascular diseases (CVDs) [2,3,4]. Therefore, anthocyanin extracted from plants could be attracting potential pharmaceutical ingredients [1].

Since natural anthocyanin pigments are traditionally used as colorant in food industry, it has been receiving increasing attention. Anthocyanin pigments are permitted as natural food colorants in the USA under the category of fruits (21 CFR 73.250) and vegetables (21 CFR 73.260), and the EU classification number is E163 [5].

In this paper, we focused to anthocyanin as a food coloring. Though they are widely used as natural colorants in food industry, the poor stability of anthocyanin is the limiting factor in their application. The extracted compounds are susceptible to degradation and its color and stability can be affected by several factors, including pH, temperature, light, its structure and concentration, metal ions, enzymes, oxygen, and the presence of other contamination [1,6,7].

Regardless, anthocyanin extracts, like from elderberry and elm leaf blackberry [8] were used to color pastry product, and have shown a good coloring capacity without disturbing the nutritional value of the products. Also, purple sweet potato anthocyanin are widely used as a natural colorant in various pastries, sweets, jams, and beverages in Japan [9].

Mongolia is a rich country of plant diversity, especially in wild berries, such as red, black, and tall currant, strawberry, blueberry. Tall currant is widespread in most provinces of Mongolia in the forest and forest-steppe and steppe zones, and grows in groups of 2-3 m tall shrubs in the upper part of the forest, on the rocky slopes. Tall berries are rich in vitamins P and C, sugars, anthocyanin, pectin and glycosides, but less in organic acids and essential oils than other berries. In Mongolian folk medicine, tall currant is used for diarrhea and pneumonia in cases of gastroenteritis. It also has anti-inflammatory and anti-toxic properties, increases urine and bile secretion, regulates digestion and closes pus. Earlier study by Badgaa et al. showed that anthocyanin is abundant in blackcurrant (*Ribes nigrum*), blueberry (*Vaccinium uliginosum*) and especially in tall currant (*Ribes altissimum*) berries that grow in Mongolia [10].

Therefore, the aim of this study was to determine an optimized condition for the high yield extraction of anthocyanin from tall currant berries (*Ribes altissimum*) that grow in Mongolia and investigate its stability focusing on the effect of temperature, time, and natural light.

2 Materials and Methods

Materials

Tall currant (*Ribes altissimum*) fruits were collected from Western province, Zavkhan in the beginning of late summer. Fruits were frozen immediately and kept at -20°C. All reagents and solvents used were of analytical grade. The experiment was carried out in triplicate.

Extraction of anthocyanin

A modified method from the methodology by Jia et al. [11] and Fera et al. [12] was used for the extraction. An ethanol solution of 70% and 30% in water, acidified with acetic and citric acids, were used as solvents, as shown in Table 1. These solvent systems were tested to find the best solvent to yield the high content of anthocyanin from tall currant.

Fruits were dried and one gram (1g) grounded dry berries was mixed with 20 ml of each solvent in Beaker glass. Samples were left for overnight at room temperature for the extraction. Then, extracts were filtered under vacuum filter. The filtrates were diluted to 100 ml with solvent, separately, and subjected to the total anthocyanine content determination.

Table 1. Solvent system for the anthocyanin extraction

Extracts	Solvent system
Extract I	70% ethanol/acetic acid, (95:5), pH = 3.3
Extract II	30% ethanol/acetic acid (95:5), pH = 2.6
Extract III	70% ethanol/citric acid (100:5), pH = 2.5
Extract IV	30% ethanol/citric acid (100:5), pH = 2.0

Determination of total anthocyanin content

The total anthocyanin content was determined by pH differential spectrophotometric method as decribed by Fela [12] with a slight modification. Briefly, an aliquot of 0.4 ml of each samples was added to the 3.6 ml of each 0.025 M potassium chloride (pH = 1.0) and 0.4 M sodium acetate (pH = 4.5) Then, mixtures were left in darkness. Absorbance was measured at 520 nm and 700 nm within 30 min to 1 h after mixing solutions in UV-Visible spectrophotometer. The absorbance (A) of the extracts was calculated with the following formula:

$$Ab = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

Sample was dissolved in 0.025 M potassium chloride (pH = 1.0) and 0.4 M sodium acetate (pH = 4.5) based on the dilution factor. While dilution factor was determined by dissolving the sample in 0.025 M potassium chloride (pH = 1.0) and 0.4 M sodium acetate (pH = 4.5) until its absorbance obtained less than 1.2 at 520 nm.

The absorbance of each sample was then read versus the buffer solutions pH 1.0 and pH 4.5 as the blank at λ = 520 nm (for the cyanidin 3-glucoside) and λ= 700 nm (for correction factor).

Then, the final absorbance was employed to calculate the total anthocyanin in percentage using the following formula with a slight modification:

$$\text{Total anthocyanins (\%)} = Ab \times MW \times DF \times 100/(\varepsilon \times L)/1000$$

Where, MW - molecular weight of cyanidin 3 - glucoside (449.2 g/mol^{-1}), DF - dilution factor, ε - extinction coefficient of cyanidin 3 - glucoside (226,900 L/mol.cm^{-1}), cyanidin-3-glucoside is the major anthocyanin in plants, L - cuvette width (1 cm), and 100 - filtrate volume (volume of the filtrate after overnight brought to 100 ml).

Determination of anthocyanin stability to light and temperature

The stability of anthocyanin extracts was conducted by exposure to the natural light for 28 days at room temperature. Anthocyanin content was determined as an indicator to evaluate the effect of light on tall currant stability on the 1, 7, 14, 21, and 28th days.

While stability of anthocyanin extracts to temperature was studied by transferring the aliquots of 10 ml of each extract to 15 ml brown glass vials and covered with a plastic cap to avoid the evaporation. The vials were placed in a thermoelectric water bath, preheated to the temperatures at 25°C, 40°C, 55°C, 70°C, and 85°C for 60 minutes.

Furthermore, in order to determine time-depending stability of anthocyanin, separate extractions were kept at 70°C for 30, 60, 90, and 120 minutes and cooled in an ice bath to stop thermal degradation.

3 Results and Discussion

There are approximately 2000 edible and medical plants in Mongolia. However, scientific research into biological activities and phytochemicals of Mongolian plants is rarely carried out [12]. In this study, the anthocyanin rich extract prepared with tall currant berries in four different extraction systems. The tall currant berries are widely used in the production of jams and juices in Mongolia.

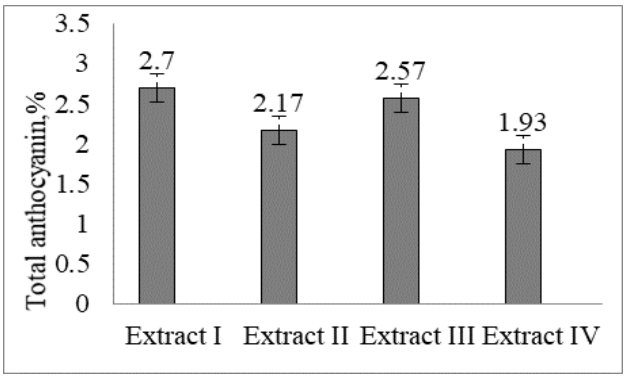
In the present study, the berries were freeze-dried and used for further study. The grounded dry tall currant (*Ribes altissimum*) berries were extracted in four different solvent systems of an acidified ethanol as described in the Table 1.

In general, ethanol is a suitable solvent for extraction of polyphenols from various plants. As stated by Khoo [1] the polyphenolic structure adds a hydrophobic characteristic to anthocyanins that makes it soluble in organic solvents, such as ethanol and methanol. While the solubility of anthocyanins in water increases at lower pH values

where strong protonation occurs [14]. Thus, the addition of HCl to alcohol increases the solubility of anthocyanins [15].

The results, as in the Figure 1, showed that the I and III extracts, containing 70% of ethanol, acidified with acetic (pH 3.3) and citric (pH 2.5) acids yielded the highest concentration of anthocyanin, corresponding to 2.70% and 2.57%, respectively. While the extraction efficiency of solvent systems II (2.17%) and IV (1.93%), containing 30% of ethanol were lower than solvent systems I and III.

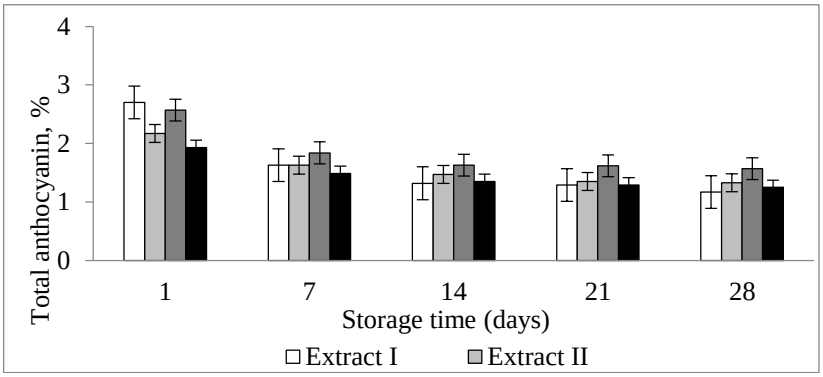
Fig. 1 Anthocyanin content in solvents



In this study, we used an acidified ethanol for the extraction since anthocyanins are not stable in neutral or weak alkaline solutions. Consistent with literatures reported previously by Shil the extraction efficiency was much greater with 70% ethanol than with distilled water [16]. Also, as described by Metivier [17], the anthocyanin extraction from grape pomace was more effective with ethanol in comparison with methanol and water.

Anthocyanins chemical stability is the main focus due to their abundant potential applications, beneficial effects and use as alternative to artificial colorants in foods. However, anthocyanins are highly susceptible to degradation caused by various factors. Stability is dependent on the type of its pigment, copigments, light, temperature, pH, metal ions, enzymes, oxygen, and antioxidants [18]. In this part, we report the stability of anthocyanin to light and temperature. Influence of light to the stability of anthocyanins in different extracts was studied for 28 days. Here, anthocyanin content was determined as an indicator to evaluate the effect of light at 1, 7, 14, 21 and 28th days. The effect of light on accelerating the destruction of anthocyanin in the tall currant extracts has been presented in Figure 2.

Fig. 2 Degradation of anthocyanins in tall currant extract in the exposure to the indoor light



As expected, significant decrements in content have been shown in each extracts. According to results, anthocyanin degradation has appeared relatively higher in the extract I; 56.46% of anthocyanin content was degraded from an initial amount after 28 days. While the extract III has shown to be the most stable to light, showing the least degradation of anthocyanin counted at 38.85% after 28 days of storage. Thus, the anthocyanin degradation has appeared differently in solvents with respect to storage time at light exposure.

Anthocyanin extract of redcurrant, gooseberry and golden currant stored in refrigerator without light exposure for 84 days showed 10%–20% degradation, whereas black currant anthocyanin extract suffered 50% degradation. By studies of Tensiska [19], it showed that purple sweet potato anthocyanin added to a jelly drink was more stable in refrigerator without light exposure.

Similar findings by Jia [11] were reported that the anthocyanins content of black currant exposed to natural outdoor/indoor light and kept in the dark had losses of 89.76%, 55.41% and 45.29% on the 25th day of storage, respectively. Also, light exposure of the mulberry fruit extract significantly worsened total anthocyanin [7].

In accordance, the stability of tall currant berry anthocyanin could be adjusted by optimizing the storage condition during food and/or beverage preparation.

Temperature also is another factor, which has a role in destabilizing the anthocyanin molecular structure. Also, several studies reported that the duration of heating has a much influence on anthocyanin stability [5,9,19].

Thus, the extract III that was the most stable during storage and gave higher extraction rate yield of anthocyanin, has been chosen for further experiment to establish the thermal influence. Temperature-dependent stability of anthocyanins in extract III was investigated at 25°C, 40°C, 55°C, 70°C, and 85°C for 60 minutes. As indicated in Figure 3, anthocyanin extraction efficiency rate in the solvent system III was increasing as temperature arises and reached its highest at 70^0C. Each vials kept in various temperatures were subjected to determine the content of anthocyanin, which showed that the anthocyanin content at 70° C was 2.21%, while at 85°C it was decreased till 1.51%.

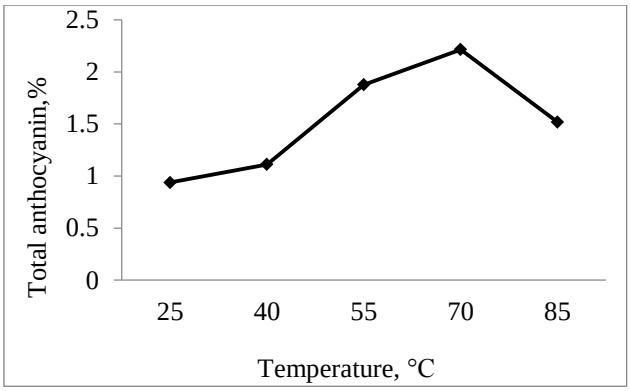


Fig. 3 Temperature-dependent stability of anthocyanin (extract III)

Then, the extraction yield of anthocyanins were studied in different extraction periods for 30, 60, 90, and 120 min, respectively to determine the relation between extraction rate and time. As shown in Figure 4, the extract was relatively stable till 90 minutes and degradation occurred as the extraction time extended to 120 minutes. Consequently, the anthocyanin content extracted at 70° C for 90 minutes was the highest at 2.39% and reduced to 1.65% at 120 minutes of continuous extraction.

Some studies have shown that extraction yield of anthocyanins from purple sweet potato was highest at 80°C for 60 minutes [9], and ultrasound-assisted extraction of blueberry anthocyanins yeild was optimal in 70% ethanol at 40°C for 40 minutes [20].

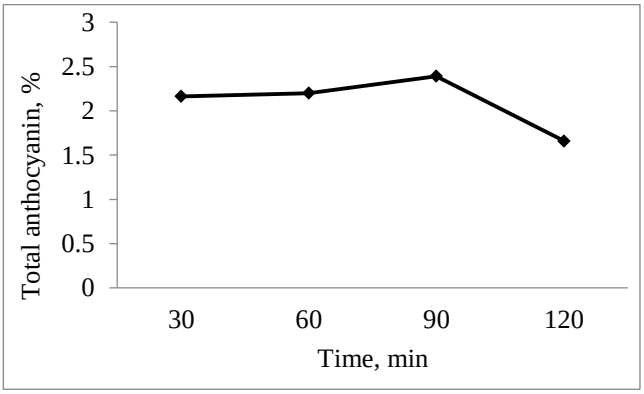


Fig. 4 Time-dependent stability of anthocyanin (extract III)

Usually, food thermal processing takes at heating to temperatures from 50 to 150^0C, depending upon pH of the product and desired shelf life [5]. A previous study reports that heat treatment at a maximum of 35°C reduced the total anthocyanin content in the grape to less than half the amount in control berries at 25°C [21].

In contrast, heat treatment of an anthocyanin-rich extract solution may not cause a degradation of anthocyanin pigments. This is because the extract commonly contains phenolic compounds that are enzymatically degraded by polyphenol oxidase as stated by Bridle at al. [22]. Most of the anthocyanin pigments have a high stability in acidic conditions compared with bases, and degradation occurs at higher pH [1].

Lately, synthetic food dyes have been attracted public concern regarding the adverse effect on human health, particularly neurological functions and behavioral effects [23]. Therefore, this finding has drawn great interest in exploring natural food colorants such as anthocyanin as a promising alternative food dyes [1]. Anthocyanins, as natural colorants extracted from plants, have value-added properties [22]. The use of natural colorant and additives in processed foods and beverages is important for increasing consumer acceptability of these products due to their low to no toxicity. Food additive, like E163, is one of the commercial additives derived from grape skin anthocyanin. It is a purple colored food additive for the use in the production of purple-colored jam, confectionaries, and beverages. Also, the use of anthocyanin-based colorants in yogurt drink and some mixed fruit juice is becoming more popular [1].

4 Conclusion

Our study was conducted to establish the solvents for the extraction of anthocyanin from tall currant berries that widely wild grown in Mongolia, the storage in the indoor light, and its stability to various factors. The results suggested that the high yield was achieved with extraction of 70% ethanol/citric acid solution (pH = 2.55) at 70°C for 90 minutes and this condition has provided the best stability for anthocyanin under the light exposure. This condition corresponded to anthocyanin content of 2.39% or 2390 mg anthocyanin in 100 g of dry plant materials. Our findings will be useful in preparing food colorants from tall currant (*Ribes altissimum*) berry in terms of thermal processing for the beverage and food industry in Mongolia.

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