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**Research Paper / Araştırma Makalesi**

# Anthocyanin-Based Natural Food Colorant from Fresh Waste Carnation Flower Petals: Effect of pH, Temperature, and Drying Method on its Degradation Kinetics and its Use in Ice Cream

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## ABSTRACT

The carnation flowers (*Dianthus caryophyllus* L.) with broken stems or overgrown buds remain in the greenhouse and are discarded after all cutting operations are completed. Waste flowers are also separated while bouquets are being prepared. Therefore, this study aimed to utilize these fresh waste flowers in the production of natural food colorants. Anthocyanin extract was obtained from waste carnation flowers and turned into powder products via freeze- and spray-drying. Various properties of the powders were analyzed and statistically compared. Since degradation parameters of anthocyanins should be taken into consideration during the planning of the materials to which anthocyanin-based colorant will be added, the kinetic parameters of carnation anthocyanins were calculated at different pH (2.6, 4.0, and 6.0) and temperature (70, 80, and 90°C) values. Except for the liquid extract, the activation energies of all samples sharply decreased when the pH changed from 2.6 to 6.0. The most and least susceptible samples to the temperature elevation were freeze-dried samples at pH 2.6 and spray-dried samples at pH 6.0, respectively. Across all the data, the activation energies of the liquid extract sample at pH 2.6 and pH 4.0 were not significantly different from each other ($p<0.05$). Moreover, the produced colorant was tested in ice cream as a model food. There was no statistically significant difference between the total color differences of ice creams prepared with the commercial colorants and carnation flowers-based colorants.

**Keywords:** Anthocyanin, Carnation flower, Colorant, Degradation kinetics, Stability

## Taze Atık Karanfil Çiçeği Taçyapraklarından Üretilen Antosiyanin Bazlı Doğal Gıda Renklendiricisi: Bozunma Kinetiği Üzerine pH, Sıcaklık ve Kurutma Yönteminin Etkisi ile Dondurmada Kullanılması

## ÖZ

Karanfil çiçeği (*Dianthus caryophyllus* L.) üretimi sırasından kısa boylu, kırık saplı veya tomurcuğu fazla açılmış çiçekler serada kalmakta ve tüm kesim işlemleri tamamlandıktan sonra sökülüp atılmaktadır. Kesimi yapılan çiçeklerin buket haline getirilmesi aşamasında da yine atık çiçekler ortaya çıkmaktadır. Bu nedenle mevcut çalışmada bu taze atık çiçeklerin doğal gıda renklendiricisi üretiminde değerlendirilmesi amaçlanmıştır. Atık karanfil çiçeklerinden antosiyanin ekstraktı elde edildikten sonra donuk ve püskürterek kurutma yöntemleriyle toz ürün formuna getirilmiştir. Tozların çeşitli fiziksel özellikleri analiz edilmiş ve istatistiksel olarak karşılaştırılmıştır. Antosiyanin bazlı renklendirici ilave edilecek malzemelerin planlanmasında antosiyaninlerin bozunma parametrelerinin dikkate alınması gerektiğinden karanfil antosiyaninlerinin farklı pH (2.6, 4.0 ve 6.0) ve sıcaklık (70, 80 ve 90°C) değerlerinde kinetik parametreleri hesaplanmıştır. pH 2.6'dan 6.0'a yükseldiğinde, sıvı ekstrakt hariç tüm örneklerin aktivasyon enerjileri

keskin bir şekilde azalmıştır. Sıcaklık artışına en çok ve en az duyarlı numuneler sırasıyla pH 2.6'daki donuk kurutulmuş numuneler ve pH 6.0'daki püskürtülerek kurutulmuş numuneler olmuştur. Tüm veriler incelendiğinde, sadece sıvı ekstrakt örneğinin pH 2.6 ve pH 4.0'daki aktivasyon enerjileri birbirinden önemli ölçüde farklı olmadığı görülmüştür ($p<0.05$). Ayrıca elde edilen renklendiriciler, model gıda olarak dondurmada test edilmiştir. Ticari renklendiricilerle hazırlanan dondurmalar ile karanfil çiçeği bazlı renklendiricilerle hazırlanan dondurmaların toplam renk farklılıkları arasında istatistiksel olarak anlamlı bir fark bulunmamıştır.

**Anahtar Kelimeler:** Antosiyanin, Karanfil çiçeği, Renklendirici, Bozunma kinetiği, Stabilite

## INTRODUCTION

The consumption of processed food is on the rise in many countries. The color of a food item may alter during processing or due to the formulation it underwent. A food item that does not have the expected color can affect the taste perception and purchasing preference. In that case, manufacturers add colorants to attain the market demand, retain quality and gain enough income [1].

Synthetic colorants are cheaper and easier to produce in addition to higher stability during food processing. Although fewer amounts are enough for the desired coloring, safety concerns about synthetic colorants are increasing. The number of legally permitted colorants is decreasing due to their negative biological effects or side effects. Thus, natural colorants are once again becoming the center of attention [2]. These natural colorants can be used in foods, cosmetics, and textiles, especially child clothes. They are safe for such applications where non-toxicity is a must. Recent trends in health and wellness provide new opportunities to use agricultural crops as renewable resources to partially replace synthetic components in food [3].

Pigments, which have different chemical and physical properties, bless natural colors to fruits, vegetables, and flowers. Many are affected by pH, temperature, presence of oxygen and light. While, some are soluble in water, others are soluble in oil or organic solvents [4]. To coup this situation, producers of natural food colorants have focused on three main categories: processing technology, formulation, and alternative pigment sources. Although natural food colorants have a limited color range and stability, they can be produced from herbal, animal, microbial and mineral sources using appropriate technologies [2, 4].

Anthocyanins are the most important group of commercial natural food colorants. They are soluble in water and turn into pink, red, violet, blue, or purple depending on pH of the media. Anthocyanidins are phenolics and constitute the aglycone part of anthocyanins. One or more sugar moieties can be linked to an anthocyanidin with glycosidic bonds. Anthocyanins often exist in the acylated form due to acylation with organic acids. Furthermore, they are highly reactive as it lacks an electron in their flavylium nucleus [5, 6].

Due to high reactivity, anthocyanins easily degrade and turn into unwanted brown-colored or colorless compounds. Stability of anthocyanins depends on their structures and copigmentation. While the number of methoxyl groups in the B ring increases stability, the number of hydroxyl groups decreases it. In acidic pH, four different anthocyanin structures (flavylium cation, quinoidal- base, carbinol pseudo-base and chalcone) are in equilibrium, although their relative amounts may vary. Anthocyanins are generally more stable in acidic media. Intermolecular copigmentation can also increase their stabilities. Aqueous extracts of fruits, vegetables and flowers have a composition that can include various compounds acting as copigments. However, if there are free sugar molecules, they turn into furfurals after the Maillard reaction and tend to speed up the degradation of anthocyanins. The degradation rate is correlated with the type of sugar moiety [6-8].

Worldwide, anthocyanin-based commercial colorants are commonly produced from wine production waste and black carrot. Anthocyanins have been extracted from various plants such as red cabbage, grape, black carrot, radish, jamun, sweet potato and purple corn [6, 9-12]. It is also possible to extract anthocyanin pigment from flowers that are edible and non-toxic.

Edible flowers are a good source of natural food colorants, and they contain high amount of acylated anthocyanins. They may be also consumed owing to their special taste and impressive colors [3, 13]. Cut flower industry is a large industry worldwide that has attractive outcomes in sense of trade and product variations. Carnation (*Dianthus caryophyllus* L.) is a member of the Caryophyllaceae family, whose homeland is the Mediterranean region, and is classified as an edible flower. Compared to the anthocyanins obtained from fruits or vegetables, anthocyanins extracted from carnation are stable at different pH and high temperatures owing to their highly acylated structure. Specifically, anthocyanins acylated with malic acid have been reported only in species within the Dianthus genus, such as *D. barbatus, D. caryophyllus, D. chinensis and D. deltoides* [14]. According to TÜİK (Turkish Statistical Institute) 2021 data, the carnation flower had a share of 35.5% out of total cultivation of ornamental plants in Türkiye. During the production of carnation flowers in the greenhouse, flowers with suitable heights and bud openings are collected. Flowers with short, broken stems or too open buds are discarded. While the cut flowers are used to prepare bouquets, a second batch of waste flowers come out. These fresh wastes are only used as animal feed or fertilizer.

Among the literature regarding the extraction of anthocyanin, limited information is available to obtain the food colorants from petals [13, 15-17]. Furthermore,

no study using carnation flower was found as a source of natural food colorant.

During the production of carnation flowers, the flowers with the appropriate size and bud opening are mostly selected. The flowers with broken stems or overgrown buds remain in the greenhouse and are discarded after all cutting operations are completed. Waste flowers are also separated while bouquets are being prepared. Therefore, this study was aimed to utilize these fresh waste cut flowers in the production of natural food colorants. Since degradation parameters of anthocyanins should be taken into consideration during the planning of the materials to which anthocyanin-based colorant will be added, it was also aimed to calculate the kinetic parameters of carnation anthocyanins at different pH and temperature values Moreover, the produced colorant was tested in ice cream as model food.

## MATERIALS and METHODS

### Materials

Fresh waste carnation flowers, cultivars of "Osiris", were obtained from a local carnation producer. The chemicals were purchased from Sigma-Aldrich and Merck (Darmstadt, Germany).

### Extraction of Anthocyanins

The petals were cut by a sharp knife from the flowers and mixed in the acidified ethanol solution (80%, diluted with 0.1N HCl solution) in 1/20 solid-liquid ratio. The mixture was blended (Ultra-Turrax, IKA T18 Digital, Staufen, Germany) at 13,000 rpm for 5 seconds thrice. Anthocyanins were extracted in a shaking water bath (Digital precise shaking water bath, WSB-30, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea), maintained at 30°C and 178 rpm for 20 minutes [18]. The obtained mixture was filtered through 4-7 µm filter paper using Buchner funnel equipped with a vacuum pump (BFC, BF-S2500 Diaphragm vacuum/pressure pump, Shanghai, China). The filtered extract was concentrated to 10°Bx by using a rotary evaporator (IKA RV-10, Staufen, Germany) at 53 rpm rotation speed, 50°C water bath temperature, and 153 mbar absolute pressure to remove the residual ethanol [19].

### Encapsulation of Anthocyanins

Concentrated anthocyanin extract was diluted with distilled water until 5°Bx to obtain the standard extract (E). Subsequently, maltodextrin (DE-12) was added in such an amount that the final concentration was 20°Bx. The mixture was stirred with a magnetic stirrer (Wisestir MSH-20A, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) for 10 min to completely dissolve maltodextrin and homogenized with a disperser (Ultra-Turrax, IKA T18 Digital, Staufen, Germany) at 10000 rpm for 15 min at ambient temperature [19, 20]. The final mixture was then divided into two parts for encapsulation processes (spray-drying (S) and freeze-drying (F))

### Encapsulation by Spray-Drying

Spray-drying was carried out using a lab-scale dryer (Büchi B-290, Essen, Germany) under the following operating conditions: drying air inlet temperature, 180°C; nozzle air flow rate, approximately 500 L/h; and aspiration, 70%. The outlet temperature was held constant at 90°C by adjusting the liquid feed volumetric flow rate (from 100 to 500 mL/h) [20]. The powder was immediately transferred into an amber glass container, closed, and stored at -18°C until analyses.

### Encapsulation by Freeze-Drying

Lyophilization was carried out on a lab-scale freeze dryer (Operon, FDU-7003, Gyeonggi-do, South Korea). The final mixture was poured into trays that belong to the freeze-dryer and frozen at -76°C for 2 h in an ultra-freezer (Ultra-low temperature freezer, U410-86, New Brunswick Scientific, Hertfordshire, England). Then, the trays were placed into a freeze-dryer under the following drying conditions: -76°C and 0.38-0.40 mmHg absolute pressure. The sample was left to dry for 48 hours [19]. Dried sample was ground and immediately transferred into an amber glass container, closed, and stored at -18°C until analysis.

### Powder Analyses

### Powder Yield

Powder yield was gravimetrically calculated as the ratio of the dry matter of powder to the total dry matter of the feed solution.

### Water Activity and Moisture Content

The water activity and the moisture content of the powders were measured with a water activity meter (Aqualab 4TE: Decagon Devices, Pullman, WA, USA) and a moisture analyzer (Kern DBS 60-3, Balingen, Germany), respectively.

### Color Measurement

The color values of the powdered samples were determined using a colorimeter (UltraScan VIS HunterLab, Reston, VA, USA). Colorimeter was calibrated with black and white calibration plates prior to measurements, respectively. Then, the sample was filled to the vessel of the device. The measurement was carried out at three different points. The color of the samples was recorded as L* (darkness-lightness), a* (greenness-redness), b* (blueness-yellowness). Hue angle and chroma value were calculated from the a* and b* values using Equations 1 and 2 [21]:

$$Hue\ angle = \frac{180}{\pi} * arctan\frac{b^*}{a^*} \quad (1)$$

$$Chroma = \left(\sqrt{a^{*2} + b^{*2}}\right) \quad (2)$$

Total color difference (TCD) was also computed from the L*, a* and b* values using Equation 3:

$$TCD = \sqrt{(L_F^* - L_S^*)^2 + (a_F^* - a_S^*)^2 + (b_F^* - b_S^*)^2} \quad (3)$$

while $L_F^*, a_F^*$ and $b_F^*$ are color values of freeze-dried powder, whereas $L_S^*, a_S^*$ and $b_S^*$ are color values of spray dried powder [22].

### *Solubility*

Solubility was determined with the method used by [23] with slight modifications. For this purpose, 0.5 g of the powder (dry basis) was solved in 50 mL of distilled water by stirring in a beaker for 5 min by using a magnetic stirrer (Wisestir MSH-20A, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) at 600 rpm. Solution was poured into a tube and centrifuged at 3000×g for 5 min. Then, 10 mL of the supernatant was poured into a pre-weighed Petri dish and dried in an oven at 70°C till the weight became constant. Then the solubility (%) was calculated by difference of weight.

### Bulk Density

Bulk density of the powders was measured according to the method performed by [24] with slight modifications. The amount of 0.5 g powder was filled in a 10 mL graduated cylinder. Then the final volume was read from the scale of the cylinder. Bulk density was determined as the ratio of powder mass to the volume.

### Turbidity

Turbidity of aqueous solutions prepared with the powders was measured by a turbidimeter (Hach 2100 N, Loveland, CO, USA). Results were described as nephelometric turbidity units (NTU).

### Determination of Total Monomeric Anthocyanin Content

Total monomeric anthocyanin (TMA) contents were determined using the pH-differential method [25]. The samples were individually diluted with buffer solutions. The dilution factors were predetermined to be into the linear region of Lambert–Beer law. The absorbances of the solutions were recorded against distilled water at 513 ($\lambda_{max}$) and 700 nm. The $\lambda_{max}$ is the wavelength of the highest absorbance in the spectrum. The net absorbances and TMA contents (as cyanidin-3-glucoside equivalents/L) of the solutions were calculated using Equation 4 and 5:

$$A = (A_{\lambda vis-max} - A_{700\,nm})_{pH1.0} - (A_{\lambda vis-max} - A_{700\,nm})_{pH4.5} \quad (4)$$

$$TMA\ (mg/L) = \frac{A*MW*DF*1000}{\varepsilon*L} \quad (5)$$

where, A=absorbance, MW=449.2 g/mol (molecular weight of cyanidin-3-glucoside), DF=dilution factor, 1000=conversation from gram to milligram, ε=26900 L/mol·cm (molar extinction coefficient for cyanidin-3-glucoside) and L=1 cm (path length).

### Thermal Degradation Kinetics of Carnation Anthocyanins

Thermal stability of the extract (E) and the powders (S and F) was determined at different pH (2.6, 4.0, and 6.0) and temperatures (70, 80 and 90°C). Citrate-phosphate buffer was used to maintain the determined pH. Certain amounts of samples (E, S and F), containing approx. 4 mg/L TMA, were dissolved in citrate-phosphate buffer solutions at different pH. Aliquots of 10 mL solution were transferred into test tubes, and they were tightly closed with screw caps. The tubes were incubated in a water bath (Digital precise shaking water bath, WSB-30, Daihan Scientific Co. Ltd., Gangwon-Do, South Korea) operated at selected temperature. Two tubes were sampled per hour throughout the incubation period and instantly cooled in an ice bath. TMA content of the tubes was analyzed. Earlier studies showed that thermal degradation of anthocyanins followed a first-order reaction [2, 26, 27]. Following equations were used to determine the thermal degradation behavior of the anthocyanins at different conditions:

$$\ln(\frac{C_t}{C_0}) = -kt \quad (6)$$

$$t_{\frac{1}{2}} = -\frac{\ln(0.5)}{k} \quad (7)$$

The temperature-dependence of the degradation rate constant was determined by using the Arrhenius equation:

$$k = k_0 * e^{-Ea/RT} \quad (8)$$

The decimal reduction time (D-value), which is the required time for a decuple decrease of the initial concentration at a particular temperature, was calculated by using Equation 9:

$$D = \frac{\ln(10)}{k} \quad (9)$$

The temperature coefficient ($Q_{10}$) was calculated using Equation 10:

$$Q_{10} = e^{(\frac{Ea}{R})(\frac{10}{T_2*T_1})} \quad (10)$$

where (in Eqs: 6-10) $C_0$ is the initial value of TMA content, $C_t$ is the TMA content after t minute heating at the determined temperature, t is time (min) $t_{1/2}$ is the half-life (min), k is the first order kinetic rate constant ($min^{-1}$), $k_0$ is the frequency factor ($min^{-1}$), $E_a$ is the activation energy (kJ/mol), D is the decimal reduction time, $Q_{10}$ is the temperature coefficient, R is the universal gas constant (8.314 J/mol·K) and T is the absolute temperature (Kelvin) [9, 28].

### Pesticide Residue Analysis

Pesticide residue in the extract was determined according to AOAC 2007.01 method via LC-MS/MS by Akdeniz University Food Safety and Agricultural Research Center [29].

### Application in Food Model System

The carnation-based colorant was tested in a model food, ice cream. The ice cream mix was prepared in accordance with the formulation specified in the study by [30], and divided into four equal portions. Citric acid (5%) and certain amounts of colorants (black carrot anthocyanin-based commercial liquid colorant, E, S, and F), containing the same amount of TMA, were added to each one. The colored ice cream mixtures were frozen with a kitchen-type ice cream machine and stored at -20 °C. Color values (L*, a*, b*) of ice creams were measured during storage.

### Statistical Analyses

The data were subjected to the analysis of variance using the SAS 9.0 (SAS Institute, Cary, NC, USA) software package, significant differences were revealed by the Duncan's Multiple Range Test and Independent Sample T-Test at a confidence level of 5%.

## RESULTS and DISCUSSION

### Powder Properties

Results of powder analyses were given in Table 1. All properties of the samples were found significantly ($p$<0.05) different from each other, except solubility and turbidity. In general, physical properties of the powder sample obtained via freeze-drying technique were superior in quality than those obtained *via* spray-drying technique. There is a similar report where freeze-drying provided efficient results than spray-drying for different type products [20].

Table 1. Physical properties of powdered samples*

| Source | Freeze dried powder (F) | Spray dried powder (S) |
|---|---|---|
| Powder yield (%) | $91.56^{a}$±0.22 | $61.30^{b}$±0.18 |
| Moisture (%) | $4.34^{b}$±0.01 | $6.33^{a}$±0.03 |
| Water activity | $0.24^{b}$±0.02 | $0.41^{a}$±0.00 |
| Color | | |
| *L** | $47.07^{b}$±0.04 | $55.38^{a}$±0.00 |
| *a** | $37.03^{b}$±0.20 | $46.71^{a}$±0.02 |
| *b** | $5.66^{b}$±0.03 | $6.28^{a}$±0.01 |
| *h°* | $8.69^{a}$±0.00 | $7.65^{b}$±0.00 |
| *C** | $37.46^{b}$±0.20 | $47.12^{a}$±0.02 |
| Solubility (%) | $81.01^{a}$±0.17 | $80.39^{a}$±0.06 |
| Bulk density (kg/m$^3$) | $500.10^{a}$±0.10 | $244.19^{b}$±5.91 |
| Turbidity (NTU) | $14.95^{a}$±0.25 | $14.85^{a}$±0.15 |

*: Results are the mean ± standard error; values within a row with different superscript letters are significantly ($p < 0.05$) different *according to* T-test.

Yield of the freeze-dried powder (91.56%) was much higher than the spray dried powder (61.30%). Similar results were also reported by Laokuldilok and Kanha (2015). Major reason of low powder yield for spray-drying is probably the stickiness problem on the drying chamber surface. According to [31] spray drying process can be considered successful if the product yield is >50%.

The moisture content of a powdered food is often affected by its composition, quality, and stability, that could further affect storing, packing, and processing (Tonon et al., 2010). Moisture content is different from $a_w$, as the moisture content shows the amount of water in a food system whereas $a_w$ expresses the presence of free water that is usable for any biochemical reactions. As water activity increases, the rate of microbial spoilage and degradation of chemical components accelerates, thereby shortening the shelf-life of the product [32]. Moisture contents of the freeze-dried and the spray dried powders in this study were found 4.34% and 6.33%, respectively. Similar range of moisture contents were reported by [23] for freeze- and spray-dried blackberry powders (6.11% and 3.7%, respectively) and by [20] for freeze- and spray-dried black glutinous rice (*Oryza sativa* L.) bran powders (7.63% and 2.47%, respectively). The average water activities of the freeze- and the spray-dried powders in this study were determined as 0.24 and 0.41, respectively. These values can be deemed microbiologically safe and stable when the water activity ($a_w$) is below 0.6, as it prevents hydrolytic and enzymatic degradation [20].

Color is an important quality parameter, particularly for the kind of products which are produced as colorants. Since color values substantially vary by the raw materials, comparing powders produced from different raw material could be misleading. For this reason, the obtained color results were compared with each other. The color values of the powders revealed that the freeze-dried powder was darker (lower L* value) and had a lighter red color (lower a* value) than those of spray dried powder with the higher L* and a* values. The color of the spray dried powder was close to intense red (0°) with lower hue angle and higher chroma values according to the CIELAB color space diagram. TCD was calculated as 12.77 between the freeze-dried powder and the spray dried powder. According to [22], color differences can be analytically classified as very distinct

(TCD>3), distinct (1.5<TCD<3), and slightly distinct (TCD<1.5). In this case, the color difference between the freeze- and the spray-dried powders was categorized as "very distinct" in this study.

Bulk density is related to the size, shape, and surface properties of particles. Powders with a smooth and uniform surface have higher bulk density than those with a rough and non-uniform surface. A decrease in particle size allows less void space between neighboring particles, hereby, the bulk density increases as there are more particles in a certain volume. The aim is to obtain high bulk density powder to reduce shipping and packaging costs. Furthermore, bulk density also affects flowability and solubility of powders [33]. Bulk density of the freeze-dried powder (500.10 kg/m$^3$) was two times higher than that of the spray-dried powder (244.19 kg/m$^3$). These results concurred with several reports like [32] for spray dried black mulberry juice powder (350-550 kg/m$^3$); by [24] for spray-dried açai juice (390 kg/m$^3$); by [20] for freeze- and spray- dried of black glutinous rice (*Oryza sativa* L.) bran (350 and 240 kg/m$^3$, respectively); and by [23] for freeze- and spray-dried blackberry powders (450 and 430 kg/m$^3$, respectively).

Solubility is an essential property especially for powdered additives. In addition to the raw materials and carrier materials used, the moisture content of the obtained powder, the particle size and the physical state of the particles have a significant effect on the solubility [33]. No significant difference was found between solubilities of the freeze- and spray-dried powders (81.01 and 80.39%, respectively), probably due to the fact that same carrier material (maltodextrin) was used in both cases, and they were amorphous. These results show coherence with the findings of several studies [20, 23, 32].

## Stability of Carnation Anthocyanins

Stability of carnation anthocyanins was investigated depending on different pH (2.6, 4.0, and 6.0) and temperature (70, 80 and 90 °C) values to evaluate the potential of liquid and powder samples as natural food colorant. This step was carried out to determine the degradation kinetics of the anthocyanins for each sample. $R^2$ values (0.886-0.997) indicated that the first order model was appropriate to predict anthocyanin content as a function of time. Thus, data were fitted to the first order model equations. Calculated parameters were shown in Table 2. Previous studies have also shown that thermal degradation of monomeric anthocyanins followed a first-order reaction [28, 34, 35].

According to Table 2, degradation rate constants (k values), especially the general mean of temperature, showed that the thermal stability of the anthocyanins decreased with increasing temperature for each sample. The lowest k value was observed at the extract pH 4.0 and 70°C. Furthermore, highest k values were determined at pH 2.6 and 90°C for each sample. There was no significant difference between extract and spray-dried powder, contrast to freeze-dried powder that has the highest general mean for the k value. Moreover, there was a significant difference between general means of temperature contrary to general mean of pH. The results indicated that temperature had a stronger influence on the stability of the anthocyanins.

As expected, degradation rate increased at higher temperatures while shortening the half-life ($t_{½}$) (Table 2). The longest half-life was estimated for the extract at pH 4.0 and 70°C. Furthermore, the shortest half-lives were determined at pH 2.6 and 90°C for each sample. Anthocyanins in the freeze-dried powder were more vulnerable to high temperatures in comparison to the others. A significant difference between only the mean of temperature among other general mean values was determined. According to the general mean value of temperature, it was found that an increase in temperature shortened the half-life extremely. There are several reports stating that anthocyanin loss accelerates at higher temperatures [6, 28].

Activation energy ($E_a$) is the energy of reaction required to reach the transition state. High $E_a$ value indicates strong temperature dependence, which means that the reaction carries out slowly at low temperatures, but relatively fast at high temperatures [28]. The $E_a$ values computed from experimental data were presented in Table 2. Except of liquid extract sample, the $E_a$ values of all samples sharply decreased when pH value changed from 2.6 to 6.0. The most and least susceptible samples to the temperature elevation were freeze-dried at pH 2.6 and spray-dried at pH 6.0, respectively. Moreover, across all the data, the activation energies of the liquid extract sample at pH 2.6 and pH 4.0 were not significantly different from each other. According to general mean of pH values, pH 2.6 and pH 6.0 values were statistically different from each other but not from pH 4.0 values. The general mean of spray-dried powder samples was observed lower than the others. Thus, spray-dried powder is comparatively less susceptible to temperature elevation.

The behavior of temperature coefficient ($Q_{10}$) was like $E_a$ when the effect of temperature on anthocyanin degradation was analyzed. This coefficient is a ratio between two rate constants that belong to temperatures having 10°C difference in between. It can also be calculated via $E_a$ without experimental data [28]. The $Q_{10}$ values calculated from $E_a$ are presented Table 2. The highest $Q_{10}$ values were determined when temperature was changed from 70 to 80°C at pH 2.6 for all samples. In addition, there was a significant ($p<0.05$) difference between general mean of pH while samples at pH 2.6 had the highest mean. According to the general mean of sample, mean of freeze-dried sample was statistically lower comparatively. Thereby, the rate constant of the freeze-dried sample was less susceptible to temperature change of 10°C.

Table 2. Effect of temperature, pH and, drying methods on the degradation kinetics parameters*

**Degradation rate constants (k, k x10$^3$ min$^{-1}$)**

| Temperature (°C) | pH | Sample: Extract (E) | Sample: Freeze Dried Powder (F) | Sample: Spray Dried Powder (S) | General mean of temperature |
|---|---|---|---|---|---|
| 70 | 2.6 | 0.402$^{F}$ | 0.405$^{G}$ | 0.453$^{F}$ | 0.513$^{C}$ |
| | 4.0 | 0.354$^{cF}$ | 0.603$^{aF}$ | 0.490$^{bF}$ | |
| | 6.0 | 0.506$^{cE}$ | 0.776$^{aE}$ | 0.625$^{bE}$ | |
| 80 | 2.6 | 0.905$^{bC}$ | 1.184$^{aD}$ | 0.938$^{bD}$ | 1.050$^{B}$ |
| | 4.0 | 0.786$^{bD}$ | 1.503$^{aC}$ | 0.667$^{cE}$ | |
| | 6.0 | 0.927$^{bC}$ | 1.607$^{aC}$ | 0.936$^{bD}$ | |
| 90 | 2.6 | 2.339$^{bA}$ | 3.178$^{aA}$ | 2.150$^{cA}$ | 2.291$^{A}$ |
| | 4.0 | 1.967$^{bB}$ | 3.028$^{aA}$ | 1.563$^{cB}$ | |
| | 6.0 | 2.274$^{bA}$ | 2.628$^{aB}$ | 1.489$^{cC}$ | |
| General mean of sample | | 1.162$^{b}$ | 1.657$^{a}$ | 1.035$^{b}$ | |
| General mean of pH | 2.6 | 1.328 | | | |
| | 4.0 | 1.218 | | | |
| | 6.0 | 1.308 | | | |

**Half-lives ($t_{1/2}$, h)**

| Temperature (°C) | pH | Extract (E) | Freeze Dried Powder (F) | Spray Dried Powder (S) | General mean of temperature |
|---|---|---|---|---|---|
| 70 | 2.6 | 28.77$^{B}$ | 28.53$^{A}$ | 25.50$^{A}$ | 23.82$^{A}$ |
| | 4.0 | 32.61$^{aA}$ | 19.18$^{cB}$ | 23.59$^{bB}$ | |
| | 6.0 | 22.85$^{aC}$ | 14.92$^{cC}$ | 18.48$^{bC}$ | |
| 80 | 2.6 | 12.77$^{aE}$ | 9.76$^{bD}$ | 12.35$^{aE}$ | 11.85$^{B}$ |
| | 4.0 | 14.71$^{bD}$ | 7.69$^{cE}$ | 17.32$^{aD}$ | |
| | 6.0 | 12.51$^{aE}$ | 7.2$^{bE}$ | 12.34$^{aE}$ | |
| 90 | 2.6 | 4.94$^{bF}$ | 3.64$^{cF}$ | 5.37$^{aG}$ | 5.36$^{C}$ |
| | 4.0 | 5.87$^{bF}$ | 3.82$^{cF}$ | 7.39$^{aF}$ | |
| | 6.0 | 5.08$^{bF}$ | 4.40$^{cF}$ | 7.76$^{aF}$ | |
| General mean of sample | | 15.57 | 11.01 | 14.46 | |
| General mean of pH | 2.6 | 14.62 | | | |
| | 4.0 | 14.69 | | | |
| | 6.0 | 11.73 | | | |

**Activation energies ($E_a$, kJ/mol)**

| pH | Extract (E) | Freeze Dried Powder (F) | Spray Dried Powder (S) |
|---|---|---|---|
| 2.6 | 91.12$^{bA}$ | 106.73$^{aA}$ | 80.60$^{cA}$ |
| 4.0 | 88.69$^{aA}$ | 83.70$^{bB}$ | 59.84$^{cB}$ |
| 6.0 | 77.71$^{aB}$ | 63.31$^{bC}$ | 44.91$^{cC}$ |
| General mean of sample | 85.84$^{a}$ | 84.58$^{a}$ | 61.78$^{b}$ |
| General mean of pH 2.6 | 92.81$^{A}$ | | |
| 4.0 | 77.41$^{AB}$ | | |
| 6.0 | 61.98$^{B}$ | | |

**Temperature coefficients ($Q_{10}$)**

| Temperature (°C) | pH | Extract (E) | Freeze Dried Powder (F) | Spray Dried Powder (S) | General mean of temperature |
|---|---|---|---|---|---|
| 70-80 | 2.6 | 2.47$^{bA}$ | 2.88$^{aA}$ | 2.22$^{cA}$ | 2.18 |
| | 4.0 | 2.41$^{aB}$ | 2.29$^{bD}$ | 1.81$^{cD}$ | |
| | 6.0 | 2.16$^{aF}$ | 1.87$^{bG}$ | 1.56$^{cG}$ | |
| 80-90 | 2.6 | 2.35$^{bC}$ | 2.72$^{aB}$ | 2.13$^{cB}$ | 2.09 |
| | 4.0 | 2.30$^{aD}$ | 2.19$^{bE}$ | 1.75$^{cE}$ | |
| | 6.0 | 2.07$^{aG}$ | 1.81$^{bH}$ | 1.52$^{cH}$ | |
| 90-100 | 2.6 | 2.24$^{bE}$ | 2.58$^{aC}$ | 2.04$^{cC}$ | 2.01 |
| | 4.0 | 2.20$^{aEF}$ | 2.10$^{bF}$ | 1.70$^{cF}$ | |
| | 6.0 | 1.99$^{aH}$ | 1.75$^{bI}$ | 1.49$^{cI}$ | |
| General mean of sample | | 2.24$^{a}$ | 1.80$^{b}$ | 2.24$^{a}$ | |
| General mean of pH | 2.6 | 2.40$^{A}$ | | | |
| | 4.0 | 2.08$^{B}$ | | | |
| | 6.0 | 1.80$^{C}$ | | | |

**Decimal reduction times (D-value, h)**

| Temperature (°C) | pH | Extract (E) | Freeze Dried Powder (F) | Spray Dried Powder (S) | General mean of temperature |
|---|---|---|---|---|---|
| 70 | 2.6 | 95.54$^{B}$ | 94.75$^{A}$ | 84.72$^{A}$ | 79.14$^{A}$ |
| | 4.0 | 108.30$^{aA}$ | 63.71$^{cB}$ | 78.37$^{bB}$ | |
| | 6.0 | 75.90$^{aC}$ | 49.54$^{cC}$ | 61.39$^{bC}$ | |
| 80 | 2.6 | 42.41$^{aE}$ | 32.41$^{bD}$ | 41.03$^{aE}$ | 39.36$^{B}$ |
| | 4.0 | 48.87$^{bD}$ | 25.55$^{cE}$ | 57.52$^{aD}$ | |
| | 6.0 | 41.55$^{aE}$ | 23.92$^{bE}$ | 40.98$^{aE}$ | |
| 90 | 2.6 | 16.42$^{bF}$ | 12.08$^{cF}$ | 17.85$^{aG}$ | 17.82$^{C}$ |
| | 4.0 | 19.51$^{bF}$ | 12.69$^{cF}$ | 24.55$^{aF}$ | |
| | 6.0 | 16.88$^{bF}$ | 14.62$^{cF}$ | 25.78$^{aF}$ | |
| General mean of sample | | 51.71 | 36.59 | 48.02 | |
| General mean of pH | 2.6 | 48.58 | | | |
| | 4.0 | 48.79 | | | |
| | 6.0 | 38.95 | | | |

* Results are the mean ± standard error; values within a row with different superscript lower cases and values within a column with different superscript upper cases are significantly ($p < 0.05$) different *according to Duncan's Multiple Range Test*.

Table 2 shows decimal reduction time (D-value), which indicates the time required for decay 90% of total anthocyanin. The extract at pH 4.0 showed the highest D-value at 70°Cwhile the lowest D-values were determined at 90°C and for the samples at all pH values for freeze-dried sample. There was a significant ($p$<0.05) difference between general means of temperature on the contrary to general means of sample and pH. Temperature elevation had a strong influence on D-value.

Stability of anthocyanins obtained from various plants such as poppy, tulip, rose, rosella, black carrot, grape and some berries has been investigated by many researchers [9, 13, 16, 34, 36, 37]. However, to the best of our knowledge, there has been no report about the degradation kinetics of carnation flower anthocyanins. Our results were found in agreement with those of the previous studies with slight differences arose due to the source and/or processing conditions.

In the present study, degradation rates (k) of carnation extract were found between $0.354x10^{-3}$ and $2.339x10^{-3}$ $min^{-1}$ under the experimental conditions (Table 2). The degradation rate of carnation anthocyanins, observed in this study, was relatively higher than poppy anthocyanins [16] and quite close to black carrot anthocyanins [37] under the same thermal conditions. Comparison of the current results with the reported ones show that the degradation rate of carnation anthocyanins was lower than the other potential colorant sources such as red tulips, roses, roselles [16], tulips [13, 34] and grapes [9]. At 90°C, degradation rate of the anthocyanins was pH 2.6>6.0>4.0, respectively. Similar results about correlation between the anthocyanin degradation and pH was reported by [38] who studied degradation of the purple-fleshed sweet potato anthocyanins and by [34] who studied tulip petal as a novel natural food colorant source.

It is well known that as the temperature increases, degradation rate also increases while a decrease occurred in half-life. In this study, half-lives ($t_{1/2}$) of carnation extract were found between 4.94 and 32.61 h as shown in Table 2. These data indicate that the carnation anthocyanins were more stable anthocyanins compared to other samples obtained from different sources [9, 13, 16, 34], except poppy [16] and black carrot [37]. The higher stability can be related with acylation and/or co-pigmentation with phenolic acids, minerals etc. [1, 2].

$E_a$ values of the carnation anthocyanins, ranged from 77.71 to 91.12 kJ/mol (Table 2), were also consistent with the literature results [9, 13, 16, 34]. Temperature dependency of the anthocyanin obtained from different sources was at proximate level under similar experiment conditions. As contrary, it was observed that poppy [16] and black carrot [37] anthocyanins were slightly outside this range i.e. 114.13 and 42.0 kJ/mol $E_a$, respectively.

$Q_{10}$ values of the carnation anthocyanins were found in range of 1.99 to 2.47 (Table 2). These values were also consistent with literature results [9, 26, 37]. Similar with [9]'s results, $Q_{10}$ values decreased with temperature elevation. This result may depend on decomposition of phenolics in extract with increasing temperature. The polymerization reaction of anthocyanins mostly occurs by reacting the monomeric anthocyanins with other phenolic compounds [27]. Thus, the observed result for the reaction could be explained by substrate limitation.

D-values of carnation extract were determined in the range of 16.42 and 108.30 h (Table 2). D- values of the carnation anthocyanin were higher, thus, time required for decay of 90% of total anthocyanin was longer than values reflected by Juçara, 'Italia' grape [9], purple-flesh potato and grape at the same temperatures [26].

## Pesticide Residue Results

The widespread usage of pesticides in agriculture poses the residue problem in the final product. The extract was analyzed for 300 different pesticides. All of them were found to be below the standard maximum residue limits (MRL) according to Regulation (EC) NO 396/2005 (Data were not shown.). It can be said that pesticides either degraded prior to harvesting or decomposed during the extraction treatment at high temperature and acidic medium [39].

## Storage Test of Ice Cream

The carnation-based colorant was successfully applied in the food model system. During storage, it was observed that the total color difference values of all ice cream samples were close to each other (Figure 1) and the hue angle (h°) values of all samples remained almost constant. Considering the initial hue angle values, it was determined that the hue angle value of the sample prepared with commercial colorant was lowest (Figure 2).

According to the Table 3, there was no difference in total color difference values between the samples prepared with liquid extract and the samples prepared with commercial colorant, and also, between the samples prepared with commercial colorant, spray-dried powder, and freeze- dried powder.

It is thought that the obtained colorants can be successfully used in ice cream by adding appropriate amounts. In the literature studies on similar model foods, color values are measured, and the results are interpreted, and it is stated that the addition of anthocyanin-based extract obtained from grapes at a rate of 0.3-0.5% by weight in dairy products creates an impressive color [1]. In a similar study, black carrot, red cabbage and grape skin extracts added to the ice cream mixture at the amount of 3 mg/100 g increased the phenolic content of the ice creams and was also liked sensory [30]. In another study, yogurt, and sour cream; prepared with extracts (5 mg/100 g) from different sources such as red radish, red cabbage and black carrot; it has been reported that red radish and black carrot give the product a pleasant red color, while red cabbage creates an attractive purple color. It has been stated that desired colors can be obtained by adding at

least 5% microcapsules to yoghurt products [5]. Considering that the a few weeks shelf life of dairy products, it is stated that natural anthocyanin sources can be used successfully in such products [2]. In another study, cornelian cherry was used for the production of a novel acidic non-carbonated red beverage with attractive red and stable color [40].

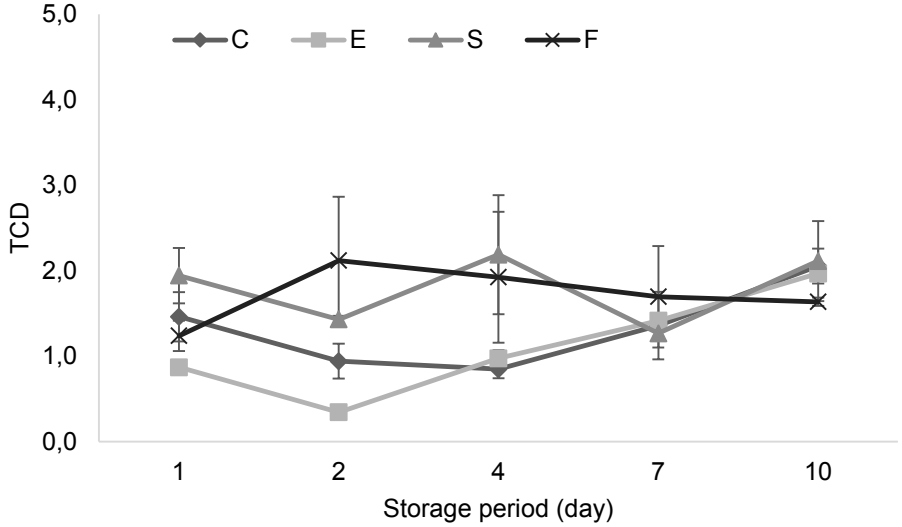


Figure 1. Total color difference values (TCD) of ice cream samples (C: Ice cream prepared with commercial liquid colorant, E: Ice cream prepared with extract, S: Ice cream prepared with spray-dried powder, F: Ice cream prepared with freeze-dried powder)

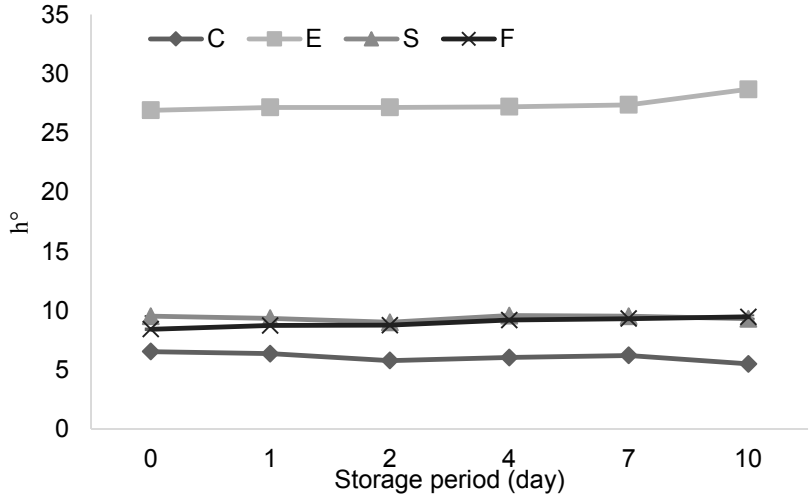


Figure 2. Hue angle (h°) values of ice cream samples (C: Ice cream prepared with commercial liquid colorant, E: Ice cream prepared with extract, S: Ice cream prepared with spray-dried powder, F: Ice cream prepared with freeze-dried powder)

Table 3. Storage analysis results of ice creams prepared with different colorants*

| Source | TCD | |
|---|---|---|
| Colorant | Commercial liquid colorant (C) | $1.33^{ab}$ ± 0.53 |
| | Extract (E) | $1.11^{b}$ ± 0.58 |
| | Spray-dried powder (S) | $1.79^{a}$ ± 0.58 |
| | Freeze-dried powder (F) | $1.72^{a}$ ± 0.66 |
| Storage period (day) | 1 | 1.38 ± 0.49 |
| | 2 | 1.21 ± 0.81 |
| | 4 | 1.48 ± 0.84 |
| | 7 | 1.43 ± 0.42 |
| | 10 | 1.94 ± 0.34 |

**: Results are the* mean ± standard error*; values within a column with different superscript upper letters are significantly ($p < 0.05$) different* according to Duncan's Multiple Range Test.

## CONCLUSION

This study evaluated the potential of carnation flower as a novel source of natural food colorant. Colorants obtained in liquid and powder forms were investigated for their stabilities under different temperature and pH conditions. Generally, the physical properties of the powder sample obtained by freeze-drying were found more efficient than the ones obtained by spray-drying. According to kinetic data, the degradation behaviors of the liquid and the powder colorants were significantly different. This difference might be caused by the percentage of acylated-anthocyanins and/or by the chemistry of anthocyanins at different pH. Moreover, pesticide analysis indicated that carnation flowers were safe from the perspective of pesticide residues and may be a novel and safe source of natural food colorants. By way of conclusion, anthocyanin-based colorant, derived from carnation, can be either used or improved for various purposes such as coloring foods and textile materials as they are stable under different pH and temperature conditions.

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### Conflict of Interests

The authors declare no conflict of interests.

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