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Natural food colorants and bioactive extracts from some edible flowers

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Summary

Consumers' interest in natural coloring has been growing in parallel with their consciousness of food-health relationship. Anthocyanin based colorings bear antioxidative features which makes this group of colorings attractive. In this research, heat stabilizations and some bioactive properties of anthocyanin-based extracts (ABE) obtained from corn poppy, tulip, rose and roselle, were determined. While the highest amount of phenolic substance was determined in the tulip (113.8 mg gallic acid equivalents (GAE) g^{-1}dry extract), the highest amount of anthocyanin is in the corn poppy (405.2 mg cyanidin-3-glucoside g^{-1} dry extract). Among the extracts, the corn poppy has been determined to have the highest antiradical capacity with 55.9 μg mL^{-1}, and the tulip, with a level of 63.4 mg of ascorbic acid equivalent (AAE) g^{-1} dry extract, has been determined to have the highest antioxidant activity. While there has been no antimicrobial effect of corn poppy extract observed on any microorganism, roselle extracts have been found to display high antimicrobial activity. Heat stability of ABEs was investigated in buffer solution pH 3.5. The flowers have high bioactive activities.

Introduction

Edible flowers originate from a wide variety of plants different in form, color and flavor. Such flowers are used in many countries to enhance the color, appearance and nutritive value of foods (KILIC and SAHIN, 2009).The petals of some flowers are used in the production of salads, desserts and ice cream as well as in food decoration. For instance, marigold (*Calendula officinalis*), crocus (*Crocus sativus*), violet (*Viola odorata*) and dandelion (*Taraxacum officinale*) are used in certain drinks and salads (MLCEK and ROP, 2011). The most common edible flowers in Turkey are saffron (*Crocus sativus*), rose (*Rosa damascena*), lavender (*Lavandula angustifolia*), violet (*Viola odorato*), roselle (*Hibiscus sabdariffa*),verbena (*Verbena officinalis*), corn poppy (*Papaver rhoeas*), pumpkin (*Curcubita pepo*) and borage (*Borago officinalis*) (KILIC and SAHIN, 2009). In addition to their beauty, recent studies on their nutritive quality are an important factor in increasing flower consumption in food sector (MLCEK and ROP, 2011).

Color, one of the most important organoleptic features of edible plants, varies depending on the carotenoid and anthocyanin content (FRIEDMAN et al., 2007). Anthocyanins, a subgroup of flavonoids, gives flowers and fruits colors varying from red to blue (LONGO and VASAPOLLO, 2006; EKICI, 2011). Natural or synthetic colors have been in use for centuries to render the color, one of the most important visual features, more attractive. However, synthetic coloring is known to affect health adversely (KAMMERER et al., 2007). Therefore, natural colorants and anthocyanin in particular have been used increasingly in food in recent years. This interest in the use of anthocyanin as a coloring agent results from their capability to make the color more appealing and their high solubility in water as well as their beneficial effects on health (EKICI, 2011).

Taken up in this study are the bioactive characteristics of the petals of corn poppy (*Papaver rhoeas*), tulip (*Tulipa gesneriana*), rose (*Rose damascene*) and roselle (*Hibiscus sabdariffa*). Corn poppy, the petals of which are used in making the traditional sorbet, is included in the family *Papaveraceae* (EKICI, 2014). It is effective on the intestinal and urinary system problems as well as with the common cold (KULTUR, 2007; ZEYBEK and ZEYBEK, 2002), bronchitis, diarrhea (SOULIMANI et al., 2001). Tulip, a member of the family *Liliaceae*, has different varieties (tulip), two of which are endemic in Turkey (MLCEK and ROP, 2011). The dark color tulips in particular are reported to have high antioxidant activity (SAGDIC et al., 2013). The rose is a sweet smelling plant of the *Rosa* variety of the *Rosaceae* family (SHIKOV et al., 2012) and has more than 200 varieties with over 18.000 cultivar (GUDIN, 2000). It is known to possess antioxidative (KIM et al., 2003; SCHIEBER et al., 2005; WANG et al., 2006) and antibacterial properties (OZKAN et al., 2004). Roselle, rich in antioxidants and used for strengthening the immune system, is consumed by different cultures (CHANG et al., 2006).

This study aims to determine (1) total phenolic, total anthocyanin, antiradical, antioxidant and antimicrobial properties of the flowers, and (2) thermal degradation kinetics of anthocyanin based extracts (ABE) at 70, 80 and 90 °C.

Material and methods

In this research the petals of corn poppy, red tulip, rose and roselle are studied. Wildly growing corn poppies were picked in the Botanical garden locality of Erciyes University and their petals separated from their roots and stems. While using poppy's petals, the black part of petal which is close to the capsule of flower, an alkaloid called 'thebaine' should be seperated. After the removal the black sections on the petals, the washed samples were dried at ambient temperature. Red tulip and rose were obtained from Istanbul Nursery and Landscaping Corporation. Red tulip and roses were also harvested, washed with tap water and dried at ambient temperature. Roselle, however, was dry when obtained from the Kayseri market. Having been dried at ambient temperature, the petals were put into colored nylon bags and stored at room temperature until extraction.

Extraction of ABE

Extraction has been carried out as described by ERSUS and YURDAGEL (2007). A mixture of ethanol (Merck, Darmstadt, Germany) and pure water (1:1) acidified with 0.01% hydrochloric acid (HCl, Merck, Darmstadt, Germany) was used as solvent for the extraction of anthocyanin pigments. The sample-solvent ratio in the extraction process was set to be 1:19 (weight/volume). The samples were extracted for 2 hours at 35 °C in a shaking-water bath, filtered and the extract was dried at 50 °C in a rotary evaporator (BUCHI, Rotavapor R-200, Switzerland). Nitrogen gas was pressed on the dried extracts placed in tubes to prevent adverse effects by oxygen, and the extracts were kept at -18 °C until use (Vestel FT 280, Istanbul, Turkey).

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Determination of total phenolic content

The total phenolic content (TPC) of ABE's has been determined using a modified version of the method developed by SINGLETON and ROSSI (1965). Briefly, 40 µl of sample were taken in test tubes. 2.4 ml of distilled water, 200 µl of Folin-Ciocalteu reagent, 600 µl sodium carbonate (20%) and 760 µl distilled water were added respectively. The tubes were mixed and allowed to stand at the dark for 2 hours. The absorbance of the samples has been determined with spectrophotometer (Varian Cary 100 Conc UV-Visible, America) at 765 nm wavelength. The TPC of the samples were calculated as gallic acid equivalent (GAE) g^{-1} dry extract.

Determination of total anthocyanin

Total anthocyanin content (TPC) of samples were determined by means of pH differential method (FULEKI and FRANCIS, 1968). Quantities of anthocyanin were determined in cyanidin-3-glucoside (MW=449.2, molar absorbance, ε=26.900) for corn poppy, rose and roselle, and in pelargonidin-3- glucoside (MW=433.2, molar absorbance, ε=22.400) for tulip materials.

Determination of antioxidant activity

The antioxidant activity (AA) of ABE's has been determined at 695 nm according to the method by PRIETO et al. (1999). A sample amount of 0.4 ml was mixed with 4 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The samples were incubated in a water bath at 95 °C for 90 min. For calculations, a series of standard solution has been prepared out of ascorbic acid within 0-1 mg mL^{-1} and, with the help of the curve obtained, the antioxidant activities of the samples was presented in mg ascorbic acid equivalent (AAE) g^{-1} dry extract.

Determination of antiradical capacity

The sentence "The antiradical capacities (AC) of ABE's have been determined with method of BRAND-WILLIAMS et al. (1995)." was changed as "The antiradical capacities (AC) of ABE's have been determined with method of BRAND-WILLIAMS et al. (1995)."

Briefly, ABEs were diluted with an ethanol-water (1:1) mixture. Then 4000 µL 0.1 mM L^{-1} DPPH (Sigma St. Louis, MO, America) were added to 200 µL of the sample. The absorbance levels of the samples, kept in the dark for 30 minutes, were measured at 517 nm.

The AC of the samples have been calculated using the following equation,

$$\%I=100 \times (1-A_s/A_c)$$

Where %I stands for DPPH inhibited by the sample, A_s: for the absorbance of the sample, A_c : for the absorbance of the control. The amount of extract required for the inhibition of 50% of DPPH (µg mL^{-1}) was calculated.

Determination of the antimicrobial activities of ABEs by agar diffusion method

The antimicrobial activity of ABEs was determined using the method of agar diffusion (SAGDIC et al., 2003). In this study, 13 microorganisms containing 11 bacteria: *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *Enterobacter cloasea* ATCC 13047, *Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 23897, *Escherichia coli* 0157:H7 ATCC 33150, *Yersinia enterocolitica* ATCC 27729, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7644, *Bacillus cereus* ATCC 33019, *Bacillus subtilis* ATCC 6630, and two yeasts: *Saccharomyces cerevisiae* BC 5461 and *Candida albicans* ATCC 1223 were used. Extracts with concentrations of 1, 2.5, 5 and 10% were prepared with ethanol: water (without acid). For agar diffusion method, 4 small wells with 4 cm in diameter were drilled with a cork bore into fresh feeding locations on petri boxes kept in the fridge for an hour. Then 40 mL of the extracts prepared in above-mentioned percentages were placed into the wells. *Y. enterocolitica* and yeasts were incubated at 27 ºC for 18-24 hours and other microorganism at 35 ºC for the same time period. The diameter of the forming inhibition zones were measured in mm.

Determination of thermal stability of ABEs

The effects of the heating process on the ABEs were determined in buffer solutions with a pH of 3.5 (EKICI, 2011). ABEs containing 4 mg anthocyanin (poppy: 0.10 g, red tulip: 2.92 g; rose: 0.34 g and roselle: 0.38 g) were added to 100 mL of sodium citrate buffer solution prepared immediately before the analysis. The samples taken every 30 min beginning from the minute zero were subjected to either a 420 min (70 and 80 ºC) or 180 min (90 ºC) heating. For this purpose, 15 mL of samples in colored buffer solutions were placed in experimental tubes and immersed in a water bath (Memmert WB-22, Germany) adjusted to the respective temperature. The temperatures of the sample in the tube were measured, and the moment when it reached the desired temperature was taken as starting point. When reaching the respective time point, the samples were immediately cooled to ambient temperature, and TAC analysis was completed.

Calculation of kinetic parameters

It has been determined in previous studies that the degradation reactions are suitable for the kinetic model of the first degree (SAGDIC et al., 2013; CEMEROGLU et al., 1994). For this reason, kinetic coefficients have been determined using the equation No.2 derived by taking the integral to the differential equation No.1, which defines the reaction appropriate for the kinetics of the first degree (SAGDIC et al., 2013; KIRCA et al., 2004).

$$-(dC/dt) = k\,C \quad (1)$$

$$\ln (C/C_0) = -k\,t \quad (2)$$

Where C_0 stands for the initial concentration of anthocyanin, C is the concentration of anthocyanin during the 't' time, k is the constant for reaction rate, t time (in hour).

The calculation of the constant rate

The losses of anthocyanin for each temperature exerted was plotted on the axis "y" and the times on the axis "x" and a linear curve has been obtained in a graphic with a semi-logarithmic scale. Linear regression analysis was applied to the curve to derive its equation. Again, use was made of the slope of this curve and the equation No.3 use was taken into consideration for the calculation of reaction speed constant (SAGDIC et al., 2013; KIRCA et al., 2004).

$$k = \text{slope} \times 2.303 \quad (3)$$

Calculation of activation energy

Dependency of the reaction on temperature was determined by calculating activation energy (E_a) using the Arrhenius equation (EKICI, 2011; SAGDIC et al., 2013; KIRCA et al., 2004).

$$k = k_0 \times \exp^{-Ea/RT} \quad (4)$$

For the calculations the formula shown in equation No.5 derived by taking the logarithm of equation No.4 was used

$$\ln k = [(-E_a/R) \times (1/T)] + \ln k_0 \quad (5)$$

For this purpose, the logarithm of the speed constants of reaction (k) was placed on the axis "y" of a graphic with arithmetic scale and the (Kelvin) equivalent (1/T) of the temperatures were placed on the axis "x" and thus, a linear curve was derived. This curve called Arrhenius graphic underwent regression analysis. Activation energy was calculated by multiplying the slope of the curve obtained by the gas coefficients (SAGDIC et al., 2013; KIRCA et al., 2004).

Calculation of half-life ($t_{1/2}$)

Half-life is the period of time needed for 50% of the studied compound to get last, and it was calculated on the basis of the equation No.6 for reactions developing according to the kinetic model of the first degree (KIRCA et al., 2004).

$$t_{1/2} = -\ln (0.5) \times k^{-1} \quad (6)$$

Statistical analysis

All the stages of the study were repeated. For the assessment of the data, one way and two way variance analyses, and the method of DUNCAN multiple comparison was used. For analyses, use has been made of SAS (version 8.2) statistical program (SAS, 2000).

Results and discussion

The yields of extracts obtained from edible flowers are given in Tab. 1. The extracts yields in diminishing order are red tulip, roselle, rose and poppy. In Tab. 1 the bioactive properties TPC, TAC, AA and AC are also presented. The highest TPC was determined in red tulip (113.8 mg GAE g^{-1} dry extract), followed by rose (43.1 mg GAE g^{-1} dry extract) while the TPC of the poppy has been found to be (29.0 mg GAE g^{-1} dry extract) and roselle (9.8 mg GAE g^{-1} dry extract). CONFORTI et al. (2009) reported that the poppy contains phenolic substance of 72 mg chlorogenic acid equivalent g^{-1} extract. In another study on the phenolic profile of the poppy its total phenolic content was found to be 31 mg 100 g^{-1} (TRICHOPOULOU et al., 2000). We determined the roselle TCP as 9.8 GAE g^{-1} dry extract. MOHD-ESA et al. (2010) have demonstrated that the total phenolic content of extract obtained from the petals of roselle (*Hibiscus sabdariffa* L.) with 80% methanol and water was 2.9 mg GAEg^{-1} dry extract and 1.9 mg GAE g^{-1} dry extract (MOHD-ESA et al., 2010). The TPC of the red tulip has been found to be 113.8 mg GAE g^{-1} dry extract. This value is consistent with the data in the literature as SAGDIC et al. (2013) have reported the TPC of the claret red, orange-red, pink, violet and yellow tulips to be 126.6, 113.8, 73.7, 80.5 and 2.6 mg GAE g^{-1} dry extract, respectively. YOUWEI et al. (2008) on the other hand, has found the TPC yellow and red tulips to be 0.5 mg g^{-1} catechin equivalent (CE) and 0.3 mg CE g^{-1}, respectively (YOUWEI et al., 2008).

With a TAC of 405.2 mg cyanidin-3-glucoside equivalents kg^{-1} dry extract, poppy extracts was found to have the richest content of anthocyanin among the species investigated. Poppy extracts was followed by the rose, tulip and roselle with dry extracts of 322.6 mg cyanidin-3-glucoside equivalents kg^{-1} dry extract, 236.5 mg pelargonidin-3-glucoside equivalents kg^{-1} dry extract and 175.2 mg cyanidin-3-glucoside equivalents kg^{-1} dry extract, respectively. In a study on 5 different colored tulips, it has been detected that while yellow tulip does not contain anthocyanin, claret red, orange-red, pink and violet tulip have a combined anthocyanin ranging from 839.1 to 236.5 mg pelargonidin-3-glucoside equivalents kg^{-1} dry extract (SAGDIC et al., 2013). Although TAC of extracts obtained from edible flowers is within a wide range, it should be emphasized that this is an expected case. Indeed, the anthocyanin compositions of raw materials and their bioactive contents are affected by variety, strain, maturity, the conditions in which they are raised and climate. In studies on different varieties, it is deemed natural that the secondary metabolites synthesized under genetic controls, namely phenolics, and accordingly their anthocyanin contents should be found different (EKICI, 2011).

Tab. 1: Yields and bioactive properties of the ABEs obtained from edible flowers

	Poppy	Red tulip	Rose	Roselle
TPC	$29.0^{C}\pm0.5$	$113.8^{A}\pm0.3$	$43.1^{B}\pm0.3$	$9.8^{D}\pm0.6$
TAC	$405.2^{A}\pm1.3$	$236.5^{C}\pm3.7$	$325.6^{B}\pm2.3$	$175.2^{D}\pm1.0$
AA	$39.1^{C}\pm0.1$	$63.4^{B}\pm1.0$	$53.6^{B}\pm1.5$	$20.0^{D}\pm0.0$
IC_{50}	$55.9^{D}\pm1.1$	$159.8^{B}\pm2.8$	$103.2^{C}\pm0.3$	$167.5^{A}\pm2.1$
Yield (%)	40.9	58.7	44.2	45.7

TPC: Total phenolic content (mg GAEg^{-1} dry extract), TAC: Total anthocyanin content (mg pelargonidin-3-glucoside equivalents kg^{-1} dry extract for red tulip, mg cyanidin-3-glucoside equivalents kg^{-1} for poppy, rose and roselle), AA: Antioxidant activities (mg AAE g^{-1} dry extract), IC_{50}: Antiradical capacity (μg mL^{-1}), For each property; ^{AB}: Means in different capital letters in the same row compare flower type and show significant differences at $P<0.05$.

AA of the ABE have been determined through the method of phosphomolybdenum and the results were presented in Tab. 1. With 63.4 AAE g^{-1} dry extract, the red tulip has been determined to had the highest antioxidant activity in this study. As for the least antioxidant activity, it was roselle extract (20.0 AAE g^{-1} dry extract). In general, an increase in antioxidant activities seems to depend on the increase in TPC (Tab. 1). It is known that the antioxidant activities of phenolic compounds are proportional to the number of the hydroxyl group to be able to enter a reaction. The studies on the flavonoids containing hydroxyl in 3', 4' and 5' positions in the ring B in different model systems revealed that they display a lot higher antioxidant activity then do their counterparts containing are hydroxyl only in the same ring (RUBERTO et al., 2007). As can be seen in Tab. 1, with a dry extract level of 405.2 cyanidin-3-glucoside equivalents g^{-1} dry extract the poppy possesses the highest amount of anthocyanin followed by tulip and roselle.

The amounts of extracts required for the ABEs to inactivate 50% of DPPH radicals appear in Tab. 1 as IC_{50} (μg mL^{-1}). Since IC_{50} is the quantity of extract that provided inhibition by 50%, the flowers with high inhibition possessed a lower IC_{50}. The IC_{50} of poppy, which has the highest AC, was found to be 55.9 μg mL^{-1}. CONFORTI et al. (2009) found in their study that the IC_{50} of the poppy was 49.0 μg mL^{-1}, which agrees with our findings. The results of antiradical analysis suggested that AC was not correlated with TPC. It was demonstrated that poppy extract, which had a relatively low TPC (29.0 mg GAEg^{-1} dry extract) among the samples, displayed the highest level of antiradical activity (55.9 μg mL^{-1}).

It was found that the AC of tulip extract, despite having a TPC of 113.8 mg GAEg^{-1} dry extract, was much lower (159.8 μg mL^{-1}) than that of the poppy. The phenolic and DPPH results may not be correlates (PILJAC-ZEGARAC et al., 2009). Additionally, it was argued it was not right to correlate AC to TPC only (MAKRIS et al., 2008). It was reported that the IC_{50} values of tulips in different colors were in the 62.46-159.8 μg mL^{-1} range. In the same study, antioxidant activities were found within the range of 23.9-48.7 mg AAE g^{-1} dry extract (SAGDIC et al., 2013).

The microbial activities of the ABEs of edible flowers

The inhibitory effect of ABEs from poppy, tulip, rose and roselle, had inhibitory effects on a total of 13 different microorganisms, two

of which were yeasts. Results are presented in Tab. 2. We found that the ethanol water (1:1) did not have any inhibitory effect on microorganisms. The antimicrobial activities of ABEs rose generally with increasing concentrations. None of ABEs showed antimicrobial effects on *S. cerevisiae* and *C. albicans*. Similarly, SAGDIC et al. (2013) had reported that tulip extracts do not affect on yeasts. Roselle was found to have the highest antimicrobial activity. In the current study, it was observed that the ABEs of the poppy flowers had no inhibitive effect on any microorganism, whereas the ABEs of red tulip flowers had inhibitive effects on bacteria only in concentrations of 10% (see Tab. 2). Rose extracts, however, have displayed inhibitive effect on all microorganisms except *Escherichia coli* O157:H7 and *Escherichia coli*. In general, the flowers with the exception of poppy, can be said to have high antimicrobial activities. In another study, the examination of *Tamarix gallica* leaves and flowers had revealed that, in general, flower extracts exhibited high antimicrobial activity compared to leaf extracts. In the same study, while *M. luteus* was determined to be the most susceptible bacterium, *E. coli* was reported to be the most resistant bacterium (KSOURI et al., 2009). KONCIC et al. (2010) had found that the diluted extracts of *Moltkia petraea* had no antimicrobial effects on fungi (*C. albicans* and *Aspergillus niger*), Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria.

The ABEs included in the study, were in general somewhat more effective in Gram positive bacteria, which was in agreement with the data in the literature. It was reported, that phenolic compounds generally had higher antimicrobial effect on Gram-positive bacteria (KATALINIC et al., 2010). The cell membranes' polysaccharide structure of Gram-negative bacteria were thought to be an important factor in these bacteria's resistance to chemical stress.

The reaction rate constants of ABEs in the buffer solution

The degradation kinetics of the buffer solutions with a pH of 3.5 were determined following its coloring with different ABEs of 4 mg anthocyanin100 mL^{-1}. Results are shown in Tab. 3 and Fig. 1. It was reported that the degradation of anthocyanin depending on the temperature during the storage process was primarily fit for the reaction kinetics (SAGDIC et al., 2013; CEMEROGLU et al., 1994). The reaction rate constant of the poppy anthocyanins were 0.20×10^{-3}, 0.60×10^{-3} and 1.10×10^{-3} min^{-1} for 70, 80 and 90 ºC, respectively. The group of anthocyanins in poppy was the most heat resistant among the investigated (Tab. 3). The most degraded group when subjected to heating was roselle anthocyanin and its degradation rate constants for 70, 80 and 90 ºC were 1.84×10^{-3}, 2.99×10^{-3} and 10.80×10^{-3} min^{-1}, respectively. WANG and XU (2007) reported that the degradation rate constants of blackberry juice for 70, 80 and 90 ºC were 1.32×10^{-3}, 2.47×10^{-3} and 3.94×10^{-3} min^{-1}, respectively. In this study, it was determined that poppy anthocyanin, in particular, possessed a much greater stability than those of blackberry, in contrast to roselle and rose, which posed weak stability. The increase in temperature shortened the half-life ($t_{½}$) while increasing the degradation rate constant, which was consistent with the data in literature. In a multitude of studies it was observed that as k rises, depending on the increases in temperature, $t_{½}$ diminished (SAGDIC et al., 2013; WANG and XU, 2007; KIRCA et al., 2003). While $t_{½}$ of blackberry for temperatures of 70, 80 and 90 ºC was 8.8, 4.7 and 2.9 hours, respectively, its activation energy was found to be 58.95 kJ mol^{-1} (WANG and XU, 2007). The $t_{½}$ of sour cherry juice for 60, 70 and 80 ºC was reported to be 54.3, 22.5 and 8.1 hours (CEMEROGLU et al., 1994). In current study for the effect of temperature on the stability of anthocyanin to be determined, the activation energy

Tab. 2: Antimicrobial activities of ABEs obtained from different flowers (inhibition zone diameter, mm)

	% Concentration	*E. coli* O157:H7 ATCC 33150	*L. monocytogenes* ATCC 7644	*S. typhimurium* ATCC 14028	*S. aureus* ATCC 25923	*E. cloacae* ATCC 13047	*Y. enterocolitica* ATCC 27729	*B. cereus* ATCC 33019	*B. subtilis* ATCC 6630	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 29213	*E. coli* ATCC 23897
Red tulip	10	9.8±1.4	16.2±3.0	9.0±1.8	14.8±3.1	7.2±0.5	20.8±2.6	12.3±2.8	9.1±2.4	7.3±0.2	-	-
	5	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-	-	-	-
Rose	10	-	14.0±0.7	14.0±0.0	18.0±1.4	18.3±15.5	12.8±0.5	14.0±0.0	13.8±1.5	37.3±4.2	14.0±0.8	-
	5	-	12.0±0.7	11.0±0.0	12.2±0.5	15.5±0.6	9.3±0.5	11.5±1.4	9.8±1.3	28.3±1.9	10.3±1.5	-
	2	-	10.0±0.7	6.8±1.0	9.8±1.5	12.3±0.5	7.5±1.0	9.5±0.7	7.0±0.0	25.3±2.1	9.0±1.2	-
	1	-	8.0±0.7	5.0±0.0	7.0±0.0	10.8±0.5	6.5±2.4	7.0±0.0	3.5±0.6	22.0±0.0	7.0±1.4	-
Roselle	10	15.0±1.2	17.0±0.8	14.0±1.2	19.8±1.0	15.3±1.0	16.5±1.0	18.5±1.0	23.5±1.0	16.0±1.2	20.3±0.0	14.3±1.3
	5	11.5±0.6	14.3±0.5	10.0±0.0	17.8±1.5	12.0±1.4	9.0±2.0	15.8±1.3	17.0±1.2	12.5±0.6	15.8±0.7	10.3±1.0
	2	8.5±0.6	10.0±1.4	7.5±1.0	13.3±1.9	7.8±0.5	5.5±0.6	12.5±1.0	13.5±0.6	10.0±0.0	13.5±0.7	6.8±0.5
	1	5.5±0.6	6.8±2.1	5.0±0.0	9.8±0.5	5.3±0.5	7.5±0.6	9.3±0.5	22.0±1.2	6.0±0.0	20.0±0.0	5.0±0.0

Poppy extracts did not exhibit inhibitive effect on all microorganisms. Red tulip, rose and roselle extracts did not exhibit inhibitive effect on *S. cerevisiae* BC 5461 and *C. albicans* ATCC 1223. -: not effective

Tab. 3: Kinetic parameters of ABEs obtained from different edible flowers at pH 3.5 buffer solution during heating at 70, 80 and 90 ºC

Flowers	Temperature (ºC)	- k x 10^{-3} (min^{-1})	$t_{1/2}$ (h)	Ea (kJ mol^{-1})
Poppy	70	0.20	25.1	88.53
	80	0.60	8.4	
	90	1.10	4.6	
Red tulip	70	1.50	7.79	76.00
	80	3.68	3.12	
	90	6.45	1.81	
Rose	70	0.92	12.5	114.13
	80	3.68	3.1	
	90	8.29	1.4	
Roselle	70	1.84	6.3	69.79
	80	2.99	3.9	
	90	10.8	1.1	

k: Reaction rate constant, $t_{1/2}$: Half-life value, Ea: Activation energy

calculated for poppy, red tulip, rose and roselle was reported, to be 88.53, 76.00, 114.13 and 69.79 kJ mol^{-1}, respectively. Similarly, SAGDIC et al. (2013) reported that the activation energy levels of tulips in different colors range from 68.69 to 76.00 kJ mol^{-1}.

We found that the sample with the highest degradation rate constant was the poppy anthocyanin, which might result from the difference in anthocyanin composition. In fact, the difference in anthocyanin degradations may result from the anthocyanin composition of the samples (WANG and XU, 2007). Furthermore, FISCHER et al. (2013) reported that intermolecular and intramolecular complex formations are very important mechanisms of copigmentation contributing to enhanced anthocyanin stability. Degradation of anthocyanin changes depending on the storage temperature and extraction conditions (PRIETO et al., 1999), and it was reported repeatedly that anthocyanin loss accelerates depending on the increase on the temperature of heating process (EKICI, 2011; SAGDIC et al., 2013; KIRCA et al., 2003; HARBOURNE et al., 2008).

Conclusion

In this research, the antimicrobial properties of some edible flowers along with their bioactive features were studied. It was ascertained that roselle extracts contain less TPC and TAC than poppy, rose and tulip extracts. The lowest AA and AC were found in roselle extracts. Findings show that red tulips possessed extremely high TPC. As for

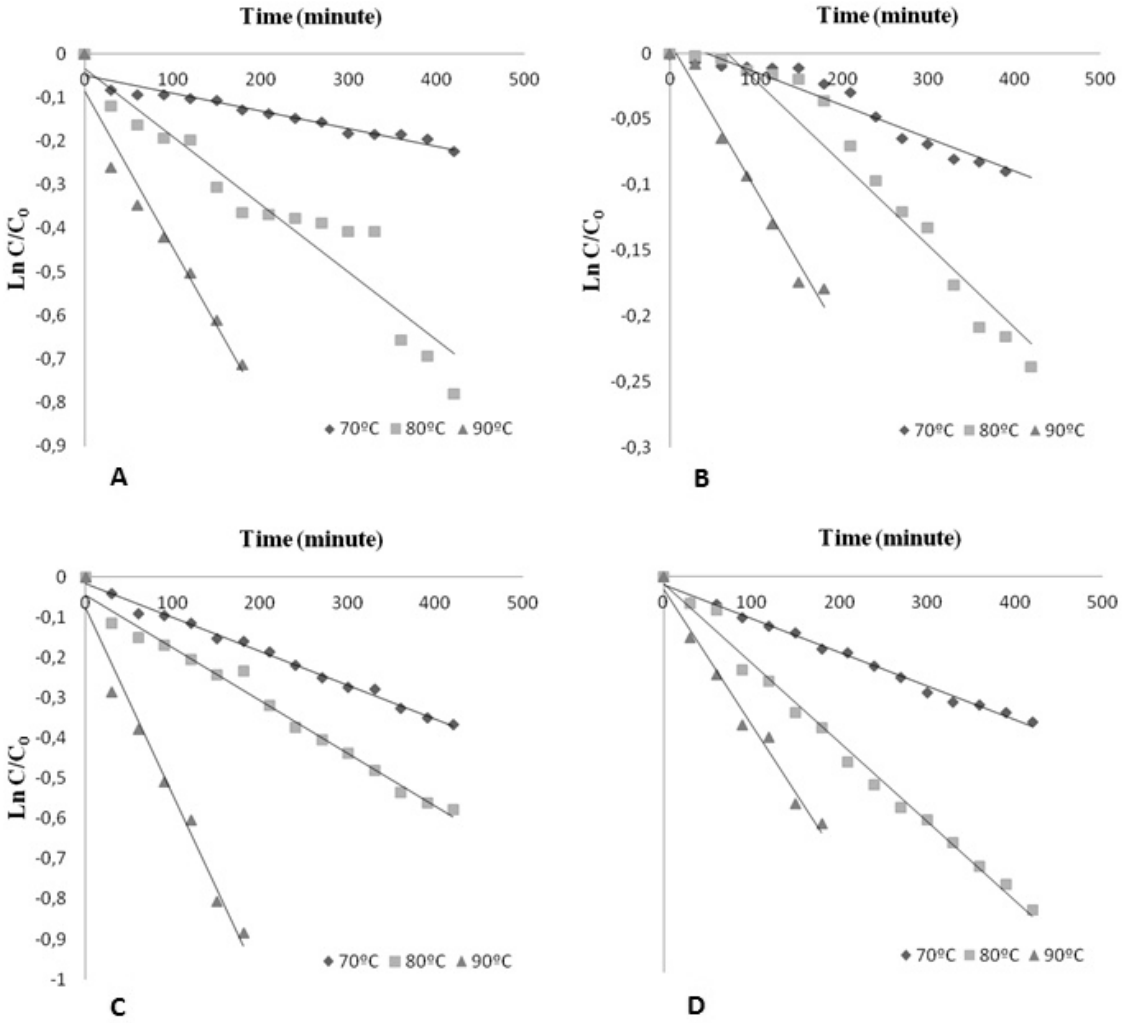

Fig. 1: Degradation of anthocyanins of edible flower extracts at pH 3.5 buffer solution. A: Rosa, B: Poppy, C: Roselle, D: Red tulip

poppy, it had both the highest TAC and AC. While it was found that the ABEs derived from roselle possess antimicrobial activity, the poppy extracts was determined not to be effective on any micro-organisms used in the study. The findings of anthocyanin degradation kinetic of extracts revealed that the most resistant to heating process was the poppy anthocyanin. Among the materials studied, the tulip petals only cannot be used for food yet. SAGDIC et al. (2013) demonstrated in their study that red, pink and violet tulip extracts did not display cytotoxic activity on MCF-7 cell lines, only yellow and claret red tulip extracts possessed somewhat cytotoxic effect. Accordingly, red tulips could be considered for natural food coloring following the study of their cytotoxic properties. Moreover, it was thought that using the red tulip as natural food coloring will be in question after the examination of its cytotoxic features. It was assumed that the use of those flowers in this study, directly in garnishing foods or in different ways in food industry, will be in question.

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