Extraction of anthocyanin pigments from different plants and study the effect of solvent , temperature and pH variation on it

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Abstract:
The anthocyanin pigment was extracted from the four different plants Roselle (*Hibiscus sabdariffia*L.), Red Beet (*Beta vulgaris* L.), pomegranate peel ,(*Punicagranatum* L.), and Grape peel (*Vitisvinifera* L.)using theacidified methanol. The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules These environmental conditions were included Appropriate solvent , various temperatures (20,30,40,50,60 and 70), three different pHs (1,7 and 14).The results of the study showed that the Appropriate solvents Ethanol and Methanol, anthocyanin extract was more stable at pH 1, and it's showed high stabilityat temperatures range (20-60)℃.

Keywords: Roselle (*Hibiscus sabdariffia*L.), red beet (*Beta vulgaris* L.), pomegranate peel ,(*Punicagranatum* L.), and Grape peel (*Vitisvinifera* L.), anthocyanin, Appropriate solvent , pH,and temperature.

Introduction

Anthocyanins are the most important group of pigments, after chlorophyll, that are visible to the human eye (Harborne,1988). Anthocyanins are an important group of water-soluble plant pigments commonly found in various fruits and vegetables. The interest arises due to the pigments wide range of attractive color spectrum from shiny orange, pink, red, purple and blue that has great potential for use as natural food colorant to replace synthetic dyes (Rein, 2005; Patras*et al*., 2010; Cavalcanti*et al*., 2011). The intensityand stability of the anthocyanin pigments is dependent on various factors including structure and concentration of the pigments, pH, temperature, light intensity, quality and presence of other pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide etc (Mazza and Minitiati, 1993; Francis, 1989). Among these factors, pH is one of the major factors significantly influenced the pigment color variations and stability. In general, anthocyanins are more stable in acidic media at low pH values than in alkaline solutions (Rein, 2005). Anthocyanins have four different structures, which are in equilibrium and include flavylium cation, quinoidal base, carbinolpseudobase and chalcon. The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Mazz and Minitiati, 1993). Some anthocyanins are more stable than other depends on their molecular structure. The example of this is the Malvidin glycosides, the major anthocyanin in grape, which due to dimethyloxylation of the molecules are more stable than other anthocyanins (Bridle and Timberlake, 1997). Moreover, hydroxylation of organic acids results in more stable molecules in most cases (Bassa and Francis, 1987; Francis, 1989).Anthocyanins play a critical role in the color quality of many fresh and processed fruits. The reasons for increasing the use of these colorants could be justified by their beneficial health effects (Torskangerpoll and Andersen, 2005). Anthocyanins have a useful potential as natural colorants due to their attractive colors (Markakis, 1982); Anthocyanins differ from other natural flavonoids due to their large range of colors and their ability to form resonance structures through pH variation (Lapidot et al., 1999).The interest of the food industry in natural colorants replacing synthetic dyes has increased significantly over the decades, mainly due to safety issues (Garcia-Falcon *et.al.*, 2007). Anthocyanins are responsible for many of the attractive colors, from scarlet to blue, of flowers, fruits, leaves, and storage organs(Harborne,1988). They are almost universal in higher plants, but in general anthocyanins seem absent in the liverworts, algae, and other lower plants, although some of them have been identified in mosses and ferns (Swain, Bate-Smith,1962).

Materials and Methods:-

Sample collection :- Roselle (*Hibiscus sabdariffia*L.), red beet (*Beta vulgaris* L.), pomegranate peel ,(*Punicagranatum* L.), and Grape peel (*Vitisvinifera* L.) Were supplied locally from the Basra governorate market then dried far of sun light, The samples were grounded by blender and kept in polyethylene bags at room temperature until used.

Anthocyanin Extraction:-

Soak a (8 g) of each plants powder in (50 ml)methanolcontaining1%ofhydrochloricacidfor (5-10) minutes, thenthe solution filtered using afilter paper (Whatman NO.1), thenthe filtrateplacedina rotary evaporatorto get thefiveoriginalsize,and leave thesolutioninplateglass(Petri dish) uncoveredinthe shade atroom temperatureto drywhere they aregettingsticky substanceamorphous(Harborne,1984).

Solvent Effect

Several solvents were used to demonstrate solvent effect on extracted anthocyanin pigments by dissolving(0.03 gm) of pigment in (5 ml)of different solvents differ in nature ,polarity and dielectric constant,(Distilled water, MeOH ,EtOH ,Acetone).

Effect of Temperature on anthocyanin pigment stability

The effect of temperature on colorant stability was studied by exposed samples (dissolving 0.1 g of anthocyaninextractedin the appropriatesolvent) to different temperatures ranging from (20-70) ℃ by raisethe temperature(10)℃aftereach(10) min andrecordedthe absorbancevalues at λ_{max}.

Effect of pH on anthocyanin pigment stability

Threesolutionsfor extracted pigments prepared and adjusted acidic functionpH,for the first solutionat pH= 1,the second at pH=7andthe third solutionat pH =14,using(1N)hydrochloricacid, (1 N) sodiumhydroxide, thecolorchanges observedfor the each solutionsafterpreparationimmediatelyandafter 24 ,48 and72 hours,measuredthe absorbanceatλ_{max}.

RESULTS AND DISCUSSION

Appropriate solvent:-

Our results showed that all extracts(anthocyanin's pigments) have a partial solubility in Distilled water, non-soluble in Acetone, while it showed height solubility in both Methanol and Ethanol,and it showed closevalues in λ_{max}.

Electronicspectruminthe visible regionshows appearance of one band in all extracts which due to n - π* transition which is specific for compounds containing non-bonding electrons (Sliversteinet *al*.,1981). The **Roselle (*Hibiscus sabdariffia*L.)**anthocyanin's extract show λ_{max} at EtOH= 544nm, in MeOH = 530 nm, for **Red Beet (*Beta vulgaris* L.)** λ_{max} at EtOH= 410nm, in MeOH = 420 nm,**pomegranate peel ,(*Punicagranatum* L.)**show λ_{max} at EtOH= 425 nm, in MeOH = 420 nm,and for **Grape peel (*Vitisvinifera* L.)** showed λ_{max} at EtOH= 540nm, in MeOH = 536 nm.

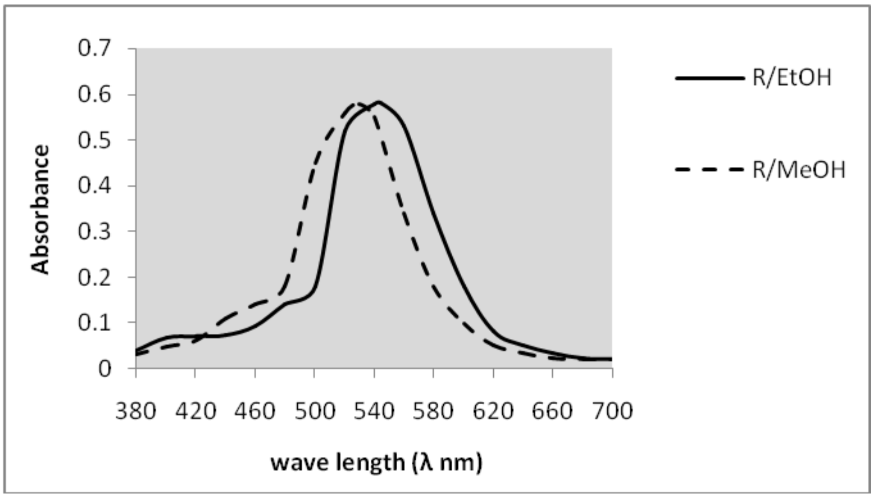

Fig 1. Absorption Spectra for anthocyanin pigment extracted fromRoselle (*Hibiscus sabdariffia*L.)

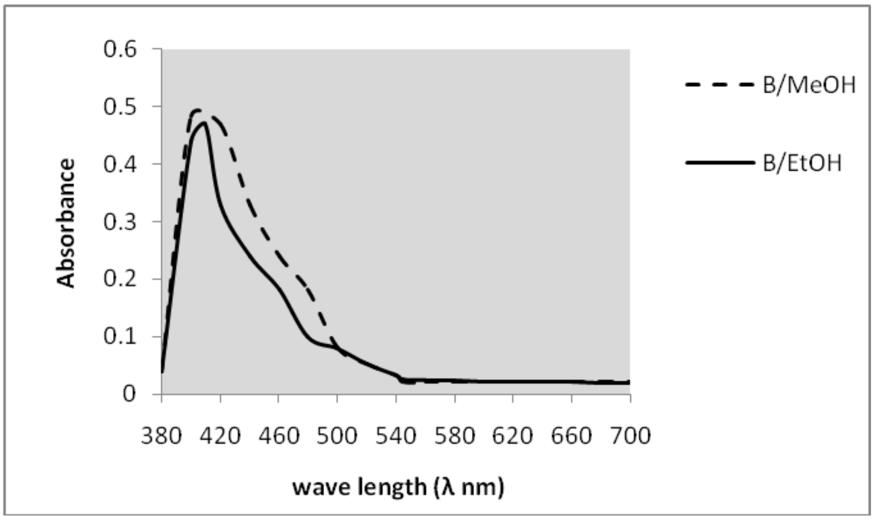

Fig 2. Absorption Spectra for anthocyanin pigment extracted fromRed Beet (*Beta vulgaris* L.)

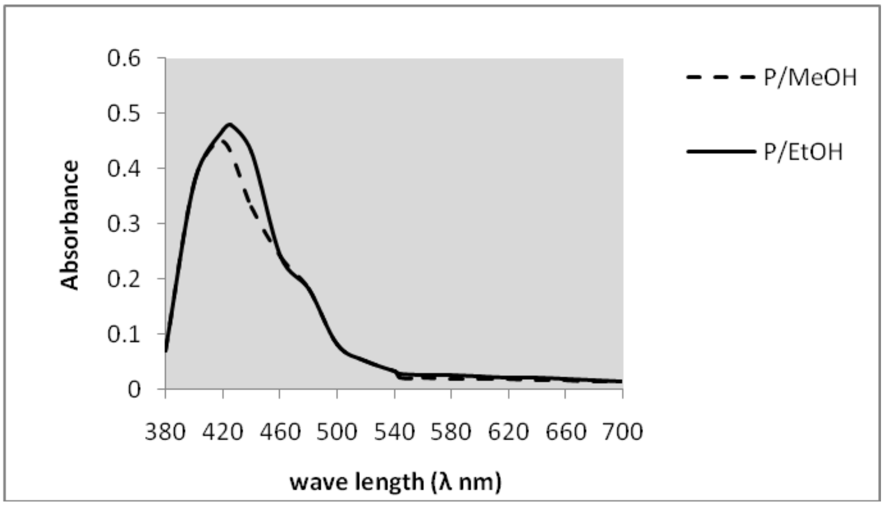

Fig 3. Absorption Spectra for anthocyanin pigment extracted frompomegranate peel ,(*Punicagranatum* L.)

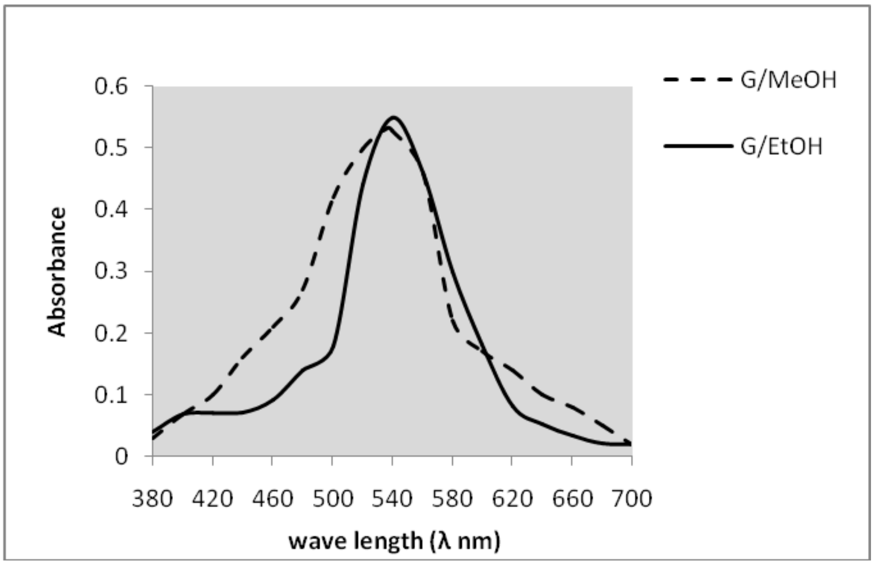

Fig 4. Absorption Spectra for anthocyanin pigment extracted fromGrape peel (*Vitisvinifera* L.)

Effect of Temperature on anthocyanin pigment stability

The effects of temperature on the stability of anthocyanins were studied by several authors and relationships between these effects and the decomposition of the anthocyanin pigments has always been observed (Kuskoski et al., 2000). Mohammed et al. (2006) reported that increasing in time and temperature of heating resulted in changes in anthocyanin and the co-pigmentation complex which resulted an increase in absorbance (Hyperchromic effects) and bathochromic effect (red shift) in solutions.Based on our results showed high stabilityat temperatures range (20-60)℃.

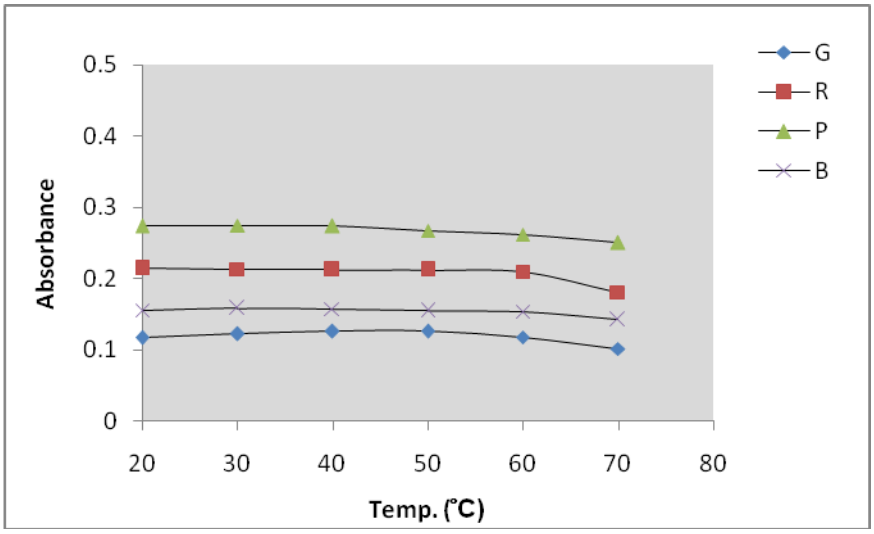

Fig 5. Temperature Effect on anthocyanin

Effect of pH on anthocyanin pigment stability

Anthocyanins may exhibit different colours, depending on their structure.pH is one of the factors which is affectson the stability of anthocyanin, Based on our results, pH had a great influence on the stability of anthocyanin pigment. The increasing pH cause greater destruction of anthocyanin in pigment structure. Flavylium salts are stable only in highly acidic conditions. These salts loose the proton in higher pH and transform intoquinoidal base, which is an unstable pigment, and immediately bond to water and form colourless compound called chromenol. Our results showed that all anthocyanin extracts have high stability at pH=1.

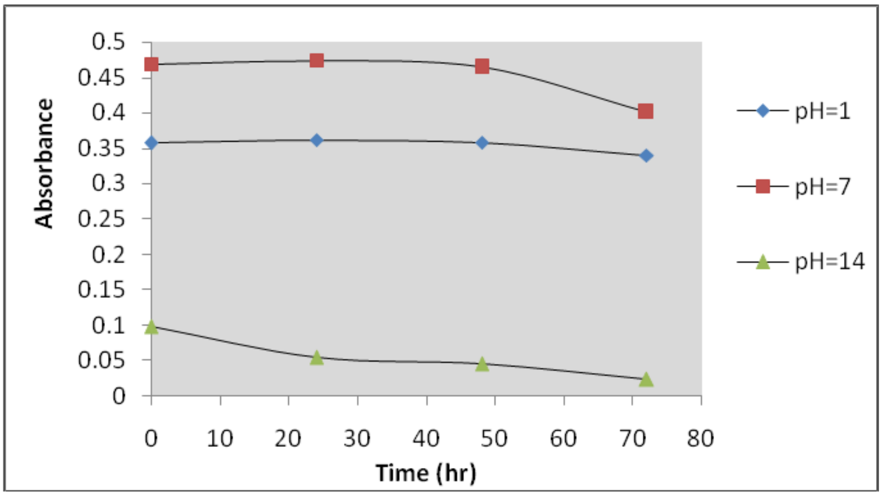

Fig 6. pH effect on anthocyanins extracted from Roselle(*Hibiscus sabdariffia*L.)

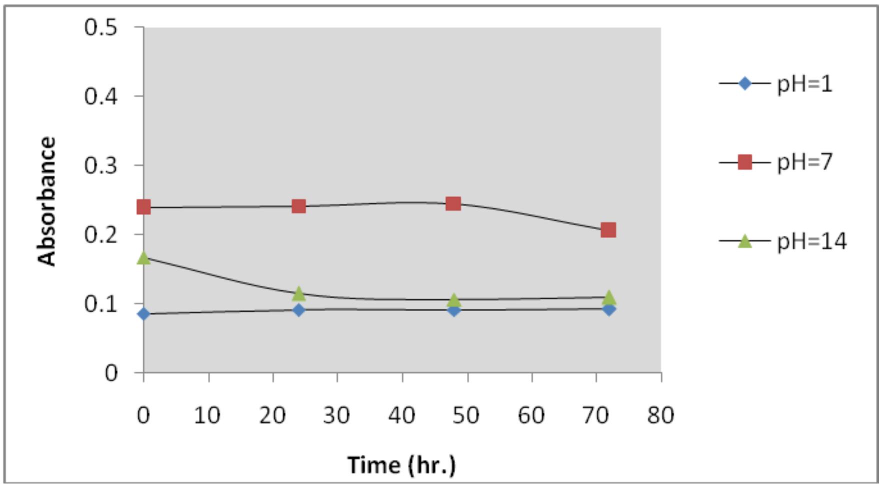

Fig 7. pH effect on anthocyanins extracted fromRed Beet (*Beta vulgaris* L.)

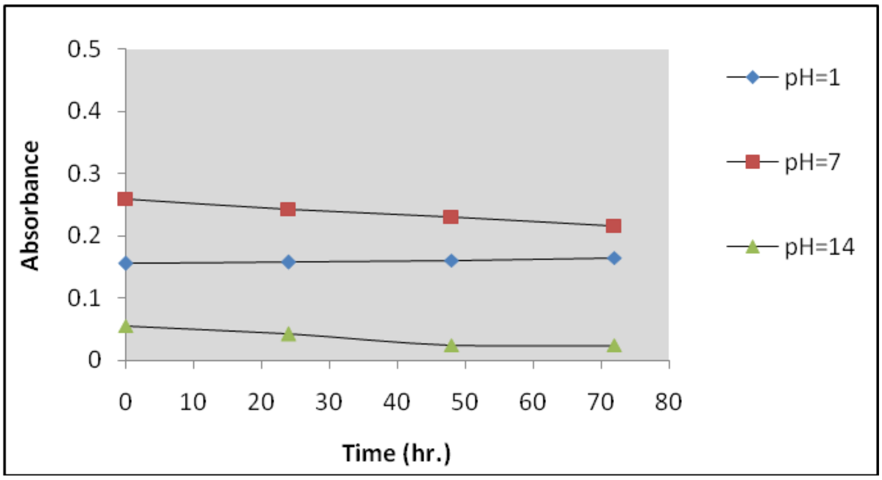

Fig 8. pH effect on anthocyanins extracted frompomegranate peel ,(*Punicagranatum* L.)

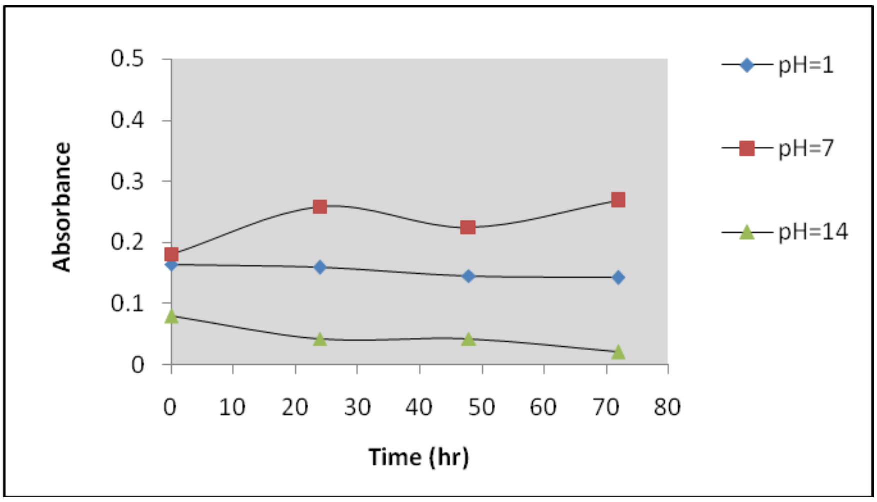

Fig 9. pH effect on anthocyanins extracted fromGrape peel(*Vitisvinifera* L.)

Conclusion:

From the results it can be concluded that anthocyanin extracted from Roselle (*Hibiscus sabdariffia*L.), Red Beet (*Beta vulgaris* L.), pomegranate peel ,(*Punicagranatum* L.), and Grape peel (*Vitisvinifera* L.) were highly or moderately resistant to the pH and temperature factors tested. And it's were more stable at pH =1, temperature at 20° C and 60° C.Increase in environmental factors like pH and temperature accelerates destruction of anthocyanins. This studies verify our results. The extracted anthocyanins were more stable to temperature and pH-induced colour loss than the anthocyanins commonly found in fruits and vegetables.these anthocyanins potentially valuable source of natural food colour.

References

- Bassa, I.A. and F.G. Francis, (1987). Stability of anthocyanins from sweet potatoes in a model beverage. Aresearch not. J. Food Sci., 52: 1753- 1754.
- Bridle, P. and C.F. Timberlake, (1997). Anthocyanins as natural food colours-selected aspects. Food Chem., 58: 103-109.
- Brouillard R, Bangles O (1994). Anthocyanin molecular interactions: The first step in the formation of new pigments during wine aging? Food Chem. 51:365-371.
- Garcia-Falcon, M.S., C. Perez-Lamela, E.Martinez-Carballo and J. Simal-Gandara, (2007). Food Chemistry , **105**, 248–259.
- Harborne, J. B. and Grayer, R. J., (1988).The anthocyanins. In: Harborne, J.B., Ed. *The Flavonoids*. London: Chapman and Hall Ltd, 1-20.
- Harborne, J. B., (1988). The flavonoids: recent advances. In: Goodwin, T. W., Ed., *Plant Pigments.* Academic Press, London, 298–343.
- Jackman RL, Yada RY, Jung MA, Speers RA (1987). Anthocyanins as food colorants – a review. J. Food Biochem. 11:201-247.
- Kuskoski EM, Marques PT, Fett R (2000). Estudo comparative da estabilidade das anthocianinas do baguacu, jambolaoeda viva ReristaBrasileiva de coranto Natural (Brasil) 4:73-76.
- Lapidot, T.; Harel, S.; Akiri, B.; Granit, R.; Kanner, J. (1999). PHDependent forms of red wine anthocyanins as antioxidants. **J**ournal of Agricultural and Food Chemistry 47: 67-70.
- Markakis, P., (1982). Introduction In Anthocyanin in fruits, vegetables, and grain (pp. 1.28) Boca Roton,FL: CRC Press (Chapter 1).
- Mazza, G. and E. Minitiati, (1993). Introduction. In Anthocyanin in Fruits, vegetables, and grains (pp: 1-28). Boca raton, FL: CRC Press(Chapter 1).
- Mohammed A, Reza H, Rashid J (2006). The effects of Heating, UV irradiation and pH on stability of *Sahesardasht*Grape anthocyanin – copigment complex .
- Patras, A., Brunton, N. P., O'Donnell, C. and Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanism and kinetics of degradation. Trends in Food Science and Technology 21: 3-11.
- Rein, M. (2005). Copigmentation reactions and color stability of berry anthocyanins. Helsinki, Russia: University of Helsinki, Phd thesis.
- Sliverstien, Bassler and Morril (1981). Spectrometric Identification of Organic Compounds, 4^{th} ed., John Wiley and Sons. Inc, New York.
- Swain, T. and Bate-Smith, E. C., (1962).Flavonoid Compounds. In: Florkin, M. and Mason, H. S., Eds., *Comparative Biochemistry Vol. III: Constituents of Life. Part A*, Academic Press, New York, 755–809.
- Torskangerpoll, K.; Andersen, O.M. (2005). Color stability of anthocyanins in aqueous solutions at various pH values. Food Chemistry 89: 427-440.