

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 sabdariffa* L.), Red Beet (*Beta vulgaris* L.), pomegranate peel, (*Punicagranatum* L.), and Grape peel (*Vitisvinifera* L.) using the acidified 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 stability at temperatures range (20-60)°C.

Keywords: Roselle (*Hibiscus sabdariffa* 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; Patraset *al.*, 2010; Cavalcantiet *al.*, 2011). The intensity and 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 flavylum 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 sabdariffa* 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) methanol containing 1% of hydrochloric acid for (5-10) minutes, then the solution filtered using a filter paper (Whatman NO.1), then the filtrate placed in a rotary evaporator to get the five original size, and leave the solution in plate glass (Petri dish) uncovered in the shade at room temperature to dry where they are getting sticky substance amorphous (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 anthocyanin extracted in the appropriate solvent) to different temperatures ranging from (20-70) °C by raise the temperature (10)°C after each (10) min and recorded the absorbance values at $\lambda_{\max}$.

Effect of pH on anthocyanin pigment stability

Three solutions for extracted pigments prepared and adjusted acidic function pH, for the first solution at pH= 1, the second at pH=7 and the third solution at pH =14, using (1N) hydrochloric acid, (1 N) sodium hydroxide, the color changes observed for the each solutions after preparation immediately and after 24, 48 and 72 hours, measured the absorbance at $\lambda_{\max}$.

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 close values in $\lambda_{\max}$.

Electronic spectrum in the visible regions shows appearance of one band in all extracts which due to $n - \pi^*$ transition which is specific for compounds containing non-bonding electrons (Sliverstein *et al.*, 1981). The **Roselle (*Hibiscus sabdariffa* L.)** anthocyanin's extract show $\lambda_{\max}$ at EtOH= 544nm, in MeOH = 530 nm, for **Red Beet (*Beta vulgaris* L.)** $\lambda_{\max}$ at EtOH= 410nm, in MeOH = 420 nm, **pomegranate peel, (*Punicagranatum* L.)** show $\lambda_{\max}$ at EtOH= 425 nm, in MeOH = 420 nm, and for **Grape peel (*Vitis vinifera* L.)** showed $\lambda_{\max}$ at EtOH= 540nm, in MeOH = 536 nm.

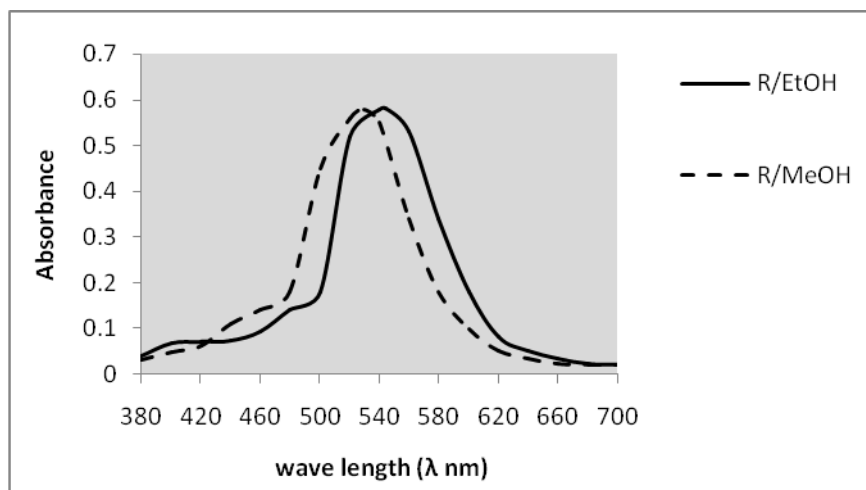


Fig 1. Absorption Spectra for anthocyanin pigment extracted from Roselle (*Hibiscus sabdariffa* L.)

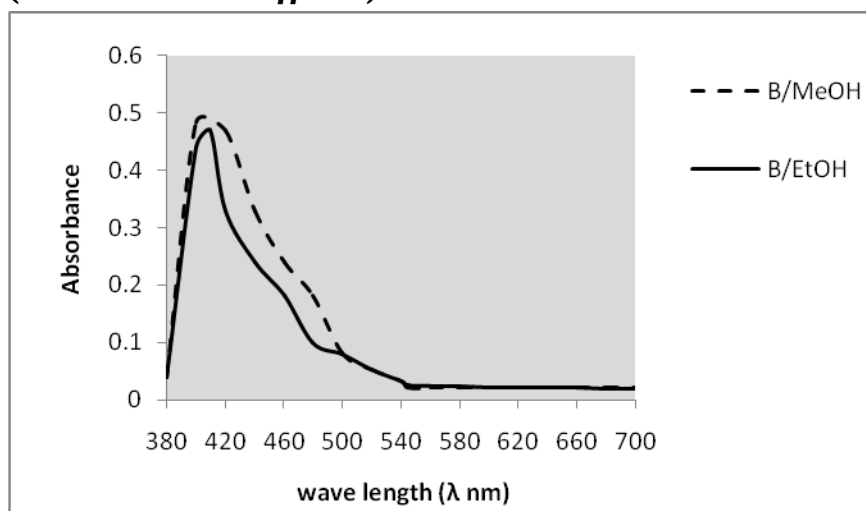


Fig 2. Absorption Spectra for anthocyanin pigment extracted from Red Beet (*Beta vulgaris* L.)

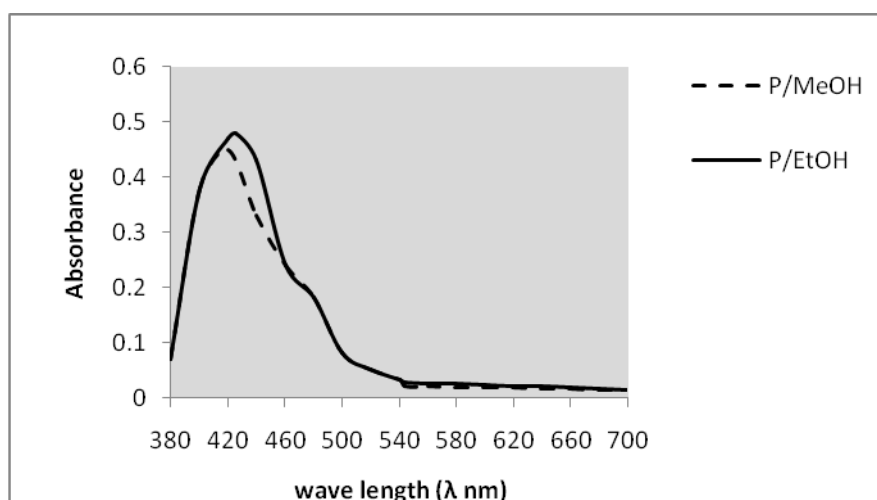


Fig 3. Absorption Spectra for anthocyanin pigment extracted from pomegranate peel, (*Punicagranatum* L.)

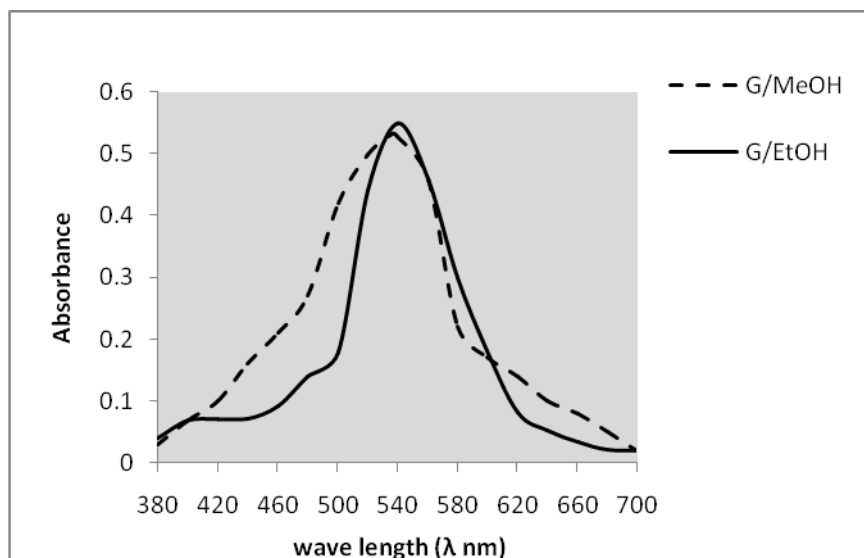


Fig 4. Absorption Spectra for anthocyanin pigment extracted from Grape peel (*Vitis vinifera* L.)

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 stability at temperatures range (20-60)°C.

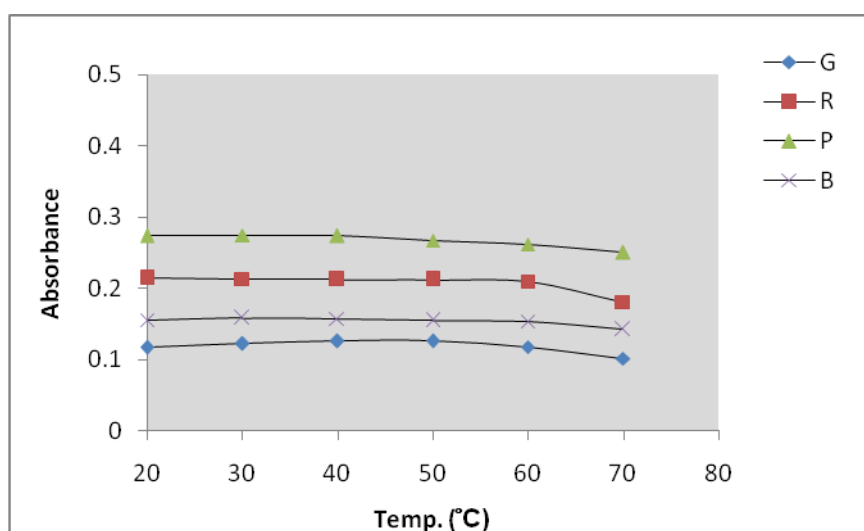


Fig 5. Temperature Effect on anthocyanin

Effect of pH on anthocyanin pigment stability

Anthocyanins may exhibit different colours, depending on their structure. pH is one of the factors which affects 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.

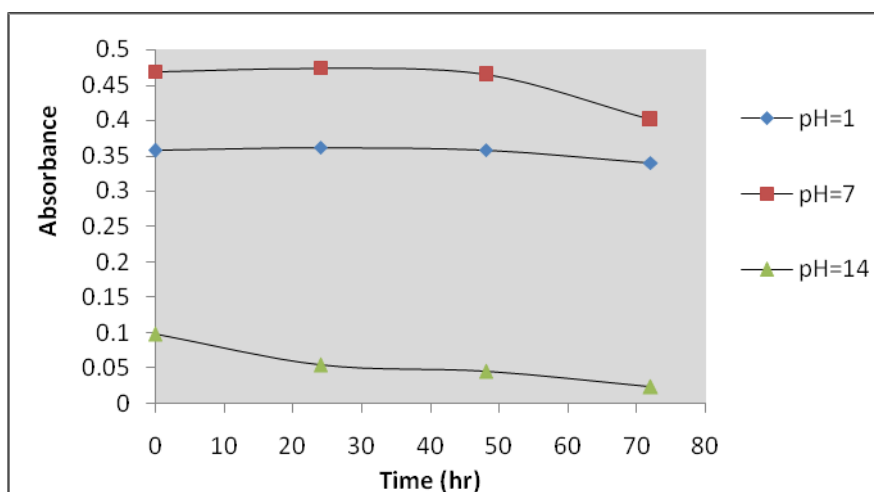


Fig 6. pH effect on anthocyanins extracted from Roselle(*Hibiscus sabdariffa*L.)

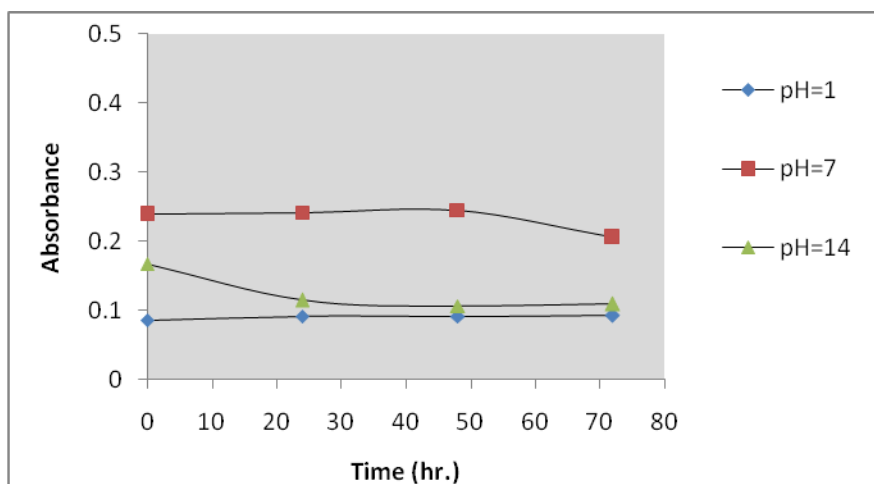


Fig 7. pH effect on anthocyanins extracted from Red Beet (*Beta vulgaris* L.)

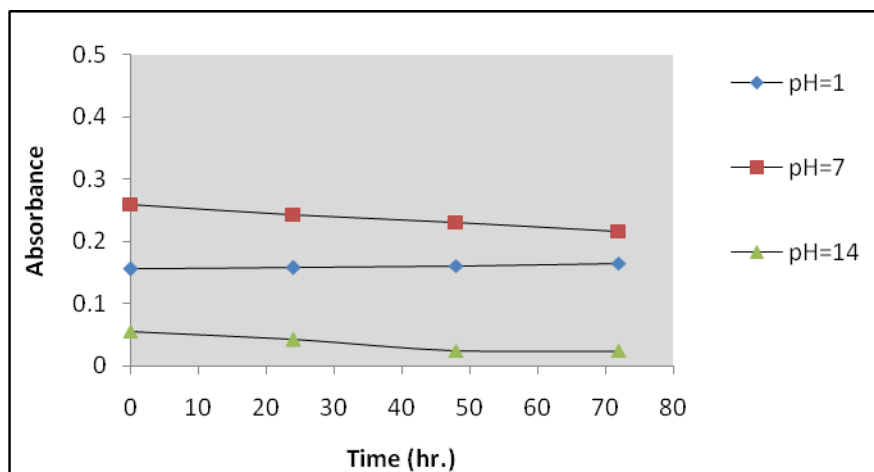


Fig 8. pH effect on anthocyanins extracted from pomegranate peel ,(*Punicagranatum* L.)

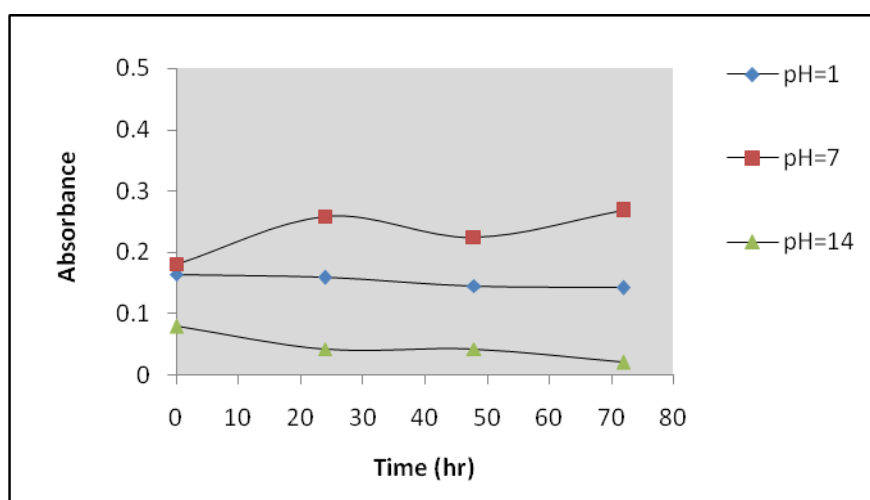


Fig 9. pH effect on anthocyanins extracted from Grape peel(*Vitisvinifera* L.)

Conclusion:

From the results it can be concluded that anthocyanin extracted from Roselle (*Hibiscus sabdariffa* 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 comparativo da estabilidade das anthocianinas do baguacu, jambolaoeda viva *Revista Brasileira 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. *Journal 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*, 4th 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.