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Spectrophotometric characterizations of anthocyans extracted from black grapes skin

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Abstract. The aims of this study consist on anthocyans isolation from black grapes skin by different extraction methods in order to select the best choice, as well as the spectrophotometric characterization of anthocyans extracts (quantitative analysis, indices for pigment degradation, color density, polymeric color and stability study during time at ambient temperature). The study is justified by two major reasons: first the proved antioxidant effect of anthocyans and their importance for health and the second the worldwide trend for replacing synthetic foods colorants with natural pigments among them anthocyans being situated in first place because of their intense and diverse colors as well as for their protective effect.
The results show that black grapes skin has rich anthocyans content (325.216- 323.456 mg/100g fresh products), the index for pigment degradation are between 40.4% - 41.77% in fresh products, the color density values are from 1.59 to 2.68 and the anthocyans extracts have a good stability during time (289.335 – 316.962 mg/100g fresh products).

Keywords: *anthocyans, index for pigment degradation, color density, polymeric color, black grapes*

1. Introduction

The color is an important factor influencing consumers' acceptability of the food. The replacement of synthetic by natural colorants as food additives substantially increased. Anthocyanins are considered as potential substitutes of synthetic colorants due to the bright and attractive colors they confer to food and they are approved as food colorants. Another favorable aspect of anthocyanins is their contribution to the antioxidant properties of certain foods; thus there is considerable interest in their health effects [1-4] Therefore, antioxidant properties, together with low toxicity, make anthocyanins an interesting class of natural pigments to use in the pharmaceutical and alimentary.

It was demonstrated that the potentially antioxidant activity is correlated with anthocyanin content. Anthocyans have been shown to be powerful antioxidants and may interfere with carcinogenicity by engaging this property [5-8].

The anthocyanidins found in taller plants are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, with a distribution in nature of 50%, 12%, 12%, 12%, 7% and 7%, respectively, and they occur almost exclusively as anthocyanins [9].

Black grape skin contains a great number of polyphenolic compounds, the concentration of which varies greatly according to the variety of grapevine and is influenced by cultivator, season and environmental factors [10]. The most abundant of these compounds in red grapes are anthocyans, mainly 3-glycosides, 3-acetylglycosides and 3-*p*-coumaroylglycosides of malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy); tartaric esters of hydroxycinnamic acids, monomeric and dimeric flavanols, flavonols and stilbenes are also found [11]

The aim of this research consists on the anthocyans isolation from black grapes skins using three different solvents in order to determine the best extraction method. Then, the spectrophotometric

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characterization of anthocyans extracts was done (quantitative analysis, degradation index, color density, polymeric color) and anthocyans extracts stability was studied in time at ambient temperature.

2. Experimental

The study was carried out with fresh skin of black grapes (*Vitis Vinifera*), harvested on optimum maturity period from Murfatlar area, Constanta county.

For obtaining the anthocyans extracts 5 g of fresh skin of black grapes (*Vitis Vinifera*) was put in contact 24 h with three different solvents as follow:

- Solvent 1: formic acid 3% aq./methanol 1:1 (pH = 5)
- Solvent 2: ethanol 20% and citric acid solution 0.1M (pH = 3)
- Solvent 3: 100 mL ethanol/1mL HCl conc. (pH = 1)

The anthocyans extracts were filtrated and after that were characterized by two different spectrophotometric methods.

The pH-differential spectrophotometric method.

It is well known that anthocyans undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra [12].

The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and allows accurate and rapid measurement of the total anthocyans, even in the presence of other interfering compounds. The absorption values of the three different anthocyans extracts were measured at 420, 520 and 700 nm wavelengths. The anthocyans content was determinate taking into consideration the absorption computed as follows:

$$A = \left(A_{\lambda vis-maz} - A_{700}\right)_{pH\,1.0} - \left(A_{vis-max} - A_{700}\right)_{pH\,4.5}$$

$$A = C \times a = C \times \frac{\varepsilon}{M}; \quad C = A \times \frac{M}{\varepsilon}$$

$$C_{sample}\% = \frac{A \times \frac{M}{\varepsilon} \times F \times (V_S \times 10^{-3})}{m_{sample}} \times 100$$

$$C_{sample}\% = A \times \frac{M}{\varepsilon} \times F \times \frac{V_S \times 10^{-3}}{m_{sample}} \times 100$$

$$\frac{g.cyanidin-3-glucoside}{100g.sample}$$

$$C_{sample}\% = A \times \frac{485\frac{g}{mol}}{25.740\frac{L}{mol \times cm}} \times F \times \frac{100mL \times 10^{-3}}{m_{sample}} \times 100;$$

$$\frac{g.cyanidin-3-glucoside}{100g.sample}$$

$$C_{sample}\% = 0.1884 \times \frac{A \times F}{m_{sample}} \quad \frac{g.cyanidin-3-glucoside}{100g.sample}$$

where:

- the absorbance of the analyzied dilluted solution by *pH-differential spectrophotometric method*;
- M-molar weight (M=485 g/mol for cyanidin-3-glucoside)
- ε- molar absortivity of cyanidin-3-glucoside (ε = 25.740 $\frac{L}{mol \times cm}$ at 520nm);
- F-dillution factor;
- m_{sample}-the mass of the analyzed sample (fresh black skins grapes, in g);
- V_S- the volumetric flask of the obtained extract (100mL)
- 10^{-3}-factor for conversion from mL to L.

Taking into account the variable pH, the potassium chloride buffer (KCl/HCl) was used for pH = 1 and the sodium acetate buffer ($H_3C\text{-}COO^-Na^+$) was used for pH = 4.5.

The absorbance readings have been achieved against water blanks and the dilution factors 40; 20; 13.3; 10, respectively 7.69.

Subtractive - Sodium metadisulfite discoloration method is based on absorption measurements at 420, 520 and 700 nm of the anthocyans extracts diluted with potassium chloride buffer at pH = 3 (KCl/HCl). The dilution factors were: F= 20; 10; 6.66; 5, respectively 4.

The degradation index (DI %) for anthocyanin of fresh black grapes extracts was derived from

absorbance readings of a samples that has been treated with sodium metabisulfite (200 mg/mL aq.). Anthocyanin pigments combine with bisulfite and form a colorless sulfonic acid adduct. The ratio between monomeric and total anthocyanin can be used to calculate the degradation index.

$$D.I. = \frac{P.C.}{C.D.} \times 100$$

The absorbance of the sodium metabisulfite - treated samples was measured at 420 nm, 520nm and 700 nm and serves as an index for browning.

Colour density (C.D.) is the sum of absorbances at the $\lambda_{vis\text{-}max}$ and at 420 nm of anthocyans extracts diluted with distilled water.

$$C.D. = [(A_{420}\text{-}A_{700}) + (A_{520}\text{-}A_{700})] \times F$$

The dilution factors were 20; 10; 6.66; 5 respectively 4.

The polymeric colour value (P.C.) is the sum of absorbance at the $\lambda_{vis\text{-}max}$ and at 420 nm of anthocyans extracts diluted with sodium metabisulfite.

$$P.C. = [(A_{420}\text{-}A_{700}) + (A_{520}\text{-}A_{700})] \times F$$

The dilution factors were the same as in case of color density determination.

The stability study of anthocyans extracts during time at ambient temperature was done by pH-differential spectrophotometric method and absorbance ratio method during one month period.

The absorption spectra were recorded on JENWAY 6300, Cambridge England Spectrometer (λ = 320-1000nm, 10 nm and 1 nm).

3. Results and Discussions

The pH-differential spectrophotometric method results show that black grapes skin has a rich content in anthocyans and also that Solvent 1 (323.456 mg/100 g fresh product) and Solvent 3 (325.216 mg/100 g fresh product) present the higher extraction capacity of anthocyans from fresh black skins grapes. (Fig. 1)

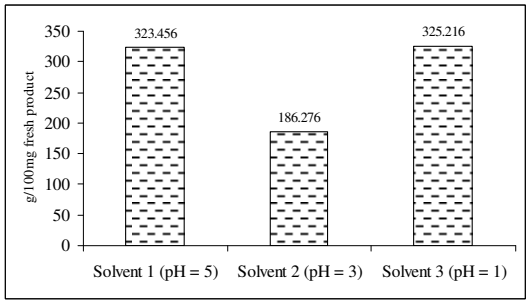

Fig. 1. Anthocyans extract contents for the analyzed samples by differential spectrophotometric method (mg anthocyans/100g fresh product)

Considering the color density, polymeric colour and calculated degradation index for anthocyans extracts of fresh black grapes (*Vitis Vinifera*) worked up by subtractive - sodium metabisulfite discoloration method, one can observe in Fig. 2 that the degradation index in case of Solvent 1 and Solvent 3 have the highest values (40.4-41.77%) due to their richest anthocyans contents.

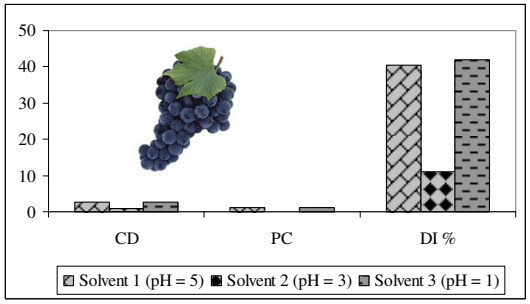

Fig. 2. Color density, polymeric colour and degradation index for the analyzed anthocyans extracts from fresh black grapes (*Vitis Vinifera*)

The stability study of anthocyans extracts from fresh black grapes (*Vitis Vinifera*) performed by pH-differential spectrophotometric method and absorbance ratio method during time at ambient temperature indicates that all extracts presents a good stability. (Fig. 3)

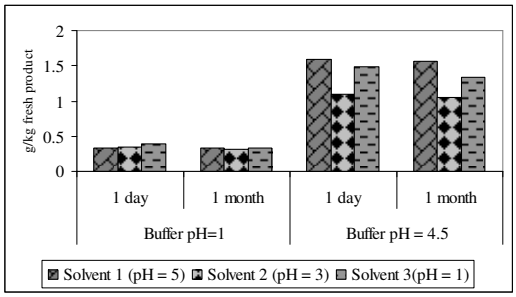

Fig. 3. Stability of anthocyans extracts from fresh black grapes (*Vitis Vinifera*) during time at ambient temperature.

4. Conclusions

The results of the two different spectrometrical methods show that black grapes skin (*Vitis Vinifera*) has a rich anthocyans content (325.216- 323.456 mg/100g in fresh products).

It was found out that Solvent 3 (ethanol 1%/HCl conc.) and Solvent 1 (formic acid 3% aq./methanol 1:1) have higher extraction capacity for the anthocyans from black grape skin than Solvent 2.

The obtained anthocyans extract from fresh black grapes (*Vitis Vinifera*) presents a good stability in time at room temperature (289.335 - 316.962 mg/100g in fresh products) therefore anthocyans content in black grapes skins can be successfully isolated and used as food colorants.

5. References

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