Spectrophotometric study on stability of anthocyanins extracts from black grapes skins

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Abstract. The aim of the study is to assess the stability of the anthocyanins extracts obtained from black grapes skins of *Vitis Vinifera.*
In order to obtain the extract with the highest concentration of anthocyanins and the one most stable under different varying factors such as: concentration of anthocyans, light exposure, temperature and oxygen, different extraction solvents were used.
The quantity of anthocyanins in the extracts and stability of anthocyanins extracts were established applying the pH differential method. The results show that black grapes skins have rich anthocyanins content (497.747 – 842.180 mg/100g in fresh products). The stability of anthocyanins extracts have been significantly affected by temperature (8.45% - 17.41% degradation) and exposure to light (37.91% - 89.48% degradation).

Keywords: anthocyanins, natural pigments, *Vitis Vinifera*, anthocyanins stability

1. Introduction

The interest of the food industry in natural colorants replacing synthetic dyes has increased significantly over the decades, mainly due to safety issues [1].

According to the numbering system used by the *Codex Alimentarius Commission,* anthocyanins (any anthocyanin-derived colorant) are listed as a natural colorant by the European Union (EU) legislation as product E163.

Anthocyanins are a group of reddish-blue, water-soluble pigments common in many flowers, fruits and vegetables and they can be included in the category of natural additives [2].

Besides their color features, anthocyanins have recently attracted even more interest due to their possible health attributes, such as reduction of coronary heart diseases, antioxidant properties, improved of visual acuity and anticancer activities [3-4].

In grapes, 5 to 20 different anthocyanins have been identified, depending on the *Vitis* genus. One of these anthocyanins, malvidin 3-glucoside (oenin) is the most important one, but also cyanidin and peonidin derivatives are common [5].

Low stability of anthocyans is the limiting factor in their application as food additives.

The influence of pH, storage temperature, presence of enzymes, light, oxygen, structure and concentration of the anthocyanins and the presence of other compounds such as flavonoids, proteins and minerals participate at anthocyanins degradation. [5-8].

The aim of the study is to assess the stability of the anthocyans extracts obtained from black grapes skins of *Vitis Vinifera.* [9-11].

2. Experimental

In order to obtain the extract with the highest concentration of anthocyanins and the one most stable under different varying factors such as: concentration of anthocyanins, temperature, light exposure and oxygen, different extraction solvents (*solvent 1*: formic acid 3% aq./methanol, 1:1 - pH = 5; *solvent 2*: ethanol 20%/ citric acid solution 0.1M, 100: 6 (pH = 3); *solvent 3*: ethanol/1mL HCl conc.,

ISSN-1223-7221

100 :1 (pH = 1); *solvent 4*: citric acid solution 1M - pH=1.5) were used.

Sampling

Fresh skins of black grapes (*Vitis Vinifera*), harvested on optimum maturity period from Murfatlar area, Constanta County, August 2009.

Anthocyanins extraction

Anthocyans extractions were obtain as follow:

- *extract 1:* $m_{sample1}$ = 5.0089 g (fresh product) and 200 mL *solvent 1*: formic acid 3% aq./methanol, 1:1 (pH 5.0)
- *extract 2:* $m_{sample2}$ = 5.0616 g (fresh product) and 200 mL *solvent 2*: ethanol 20%/ citric acid solution 0.1M, 100: 6 (pH 3.0)
- *extract 3:* $m_{sample3}$ = 5.0180 g (fresh product) and 200mL *solvent 3*: ethanol/1mL HCl conc., 100 :1 (pH 1.0)
- *extract 4:* $m_{sample\ 4}$ = 5.004 g (fresh product) and 200mL *solvent 4* : citric acid solution 1M (pH 1.5)

Overnight, grape skins samples were left to macerate in the dark in a beaker and after that anthocyanins extracts were filtrated.

For an accurate comparative analyse all extracts were brought to the same volume (200 mL) using the correspondent solvent.

Equipment

The absorption spectra were recorded on Jasco V 550 UV-Vis spectrofotometer (λ = 320 - 1000 nm, 10 nm and 1 nm).

Determination of anthocyanin concentration

All samples were analyzed by pH-differential spectrometric method [12].

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

At pH 1.0 predominates the colored oxonium form while at pH 4.5 predominates the colorless hemiketal form.

The pH-differential method is based on this reaction and permits accurate and rapid measurement of the total content anthocyanins, even in the presence of other interfering compounds.

Samples were diluted in two buffer solutions: potassium chloride buffer 0.025 M (pH 1.0) and sodium acetate buffer 0.4 M (pH 4.5).

In order to determine the appropriate dilution factor, analyzed samples were diluted with potassium chloride buffer (pH 1.0) until the absorbance of the sample at $\lambda_{vis\text{-}max}$ (520 nm) was within the linear range of spectrophotometer (absorbance values were less than 1.2).

The absorbance readings were done against water blank. Dilution factors (DF = final volume/initial volume) established for anthocyans extracts were: 20, 10, 6.66, 5 and 4.

All measurements were performed at 520 nm ($\lambda_{vis\text{-}max}$) and 700 nm (to correct the haze) from 15 min to 1 hour after sample preparation.

The absorbance (A) of the diluted samples was calculated as follow:

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5}$$

The monomeric anthocyanin pigment concentration (mg/100g fresh product, expressed as cyaniding-3-glucoside equivalents) was calculated with the following formula:

$$C(mg/100g.fresh.product) = \frac{A \times MW \times DF \times V_{flask} \times 100}{\varepsilon * l * m_{sample}}$$

where: MW is molecular weight (449.2 g/mol cyanidin 3-glucozide); DF is dilution factor (DF = 20, 10, 6.66, 5 and 4), V_{flask} is volume (mL) of volumetric flask which was brought extract/sample stock solution, ε is molar absorptivity (25 740 at λ = 520nm, in 0.1N HCl solution) and *l* is path length (1 cm).

In time stability of anthocyanins extracts at room temperature and at natural light was studied during a four weeks period.

Stability of anthocyanins extracts under the influence of temperature was studied at 35^0C, 50^0C, 65^0C and 80^0C.

Regarding the influence of oxygen, stability of anthocyanins extract was tested by air bubbling in obtained extracts for 1 to 8 h.

3. Results and Discussions

The extracts obtained with four different solvents and analyzed by pH-differential spectophotometrical method (520 and 700 nm)

relived that *extracts 1* (861.206 mg/100 g fresh product) and *4* (778.499 mg/100g fresh product) have the highest contents of anthocyanins. (**Fig. 1.**).

Extracts 2 and *3* showed close values in anthocyanin content which leads to the conclusion that *solvents 2* and *3* have similar extraction capacities (500.267 -501.358 mg/100 fresh product).

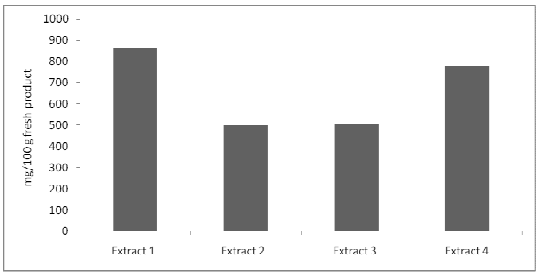
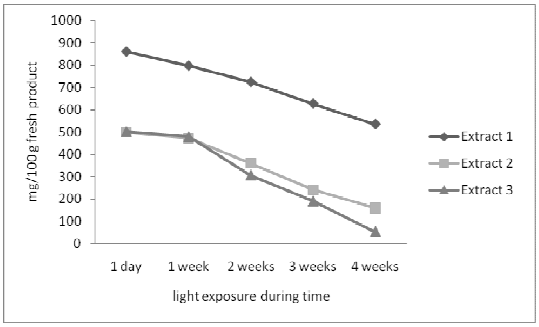


Fig.1. Anthocyanins crud extracts contents determined by pH-differential spectrometric method (mg/100g fresh product)

The influences of light exposure on the first three anthocyanins extracts in one month period demonstrate that the anthocyanins content reduces significantly. As **Fig. 2** shows *extract 1* suffered 37.91% degradation of anthocyanins content (861.206 to 534.947 mg /100g fresh product) while the *extract 2* and *extract 3* suffered 68.14% degradation (500.267 to 159.376 mg/100g fresh product) and 89.48% degradation (501.358 to 52.71 mg/100g fresh product) respectively.



Fig, 2. The influence of light exposure during one month on anthocyanins extracts (mg/100g fresh product)

The first three anthocyanins extracts subjected to different temperature values (20^0C, 35^0C, 50^0C, 65^0C, 80^0C) showed that the anthocyanins content suffered 8.45% degradation (861.206 to 788.396 mg /100g fresh product) for *extract 1*, 12.6% degradation (500.267 to 437.193 mg/100g fresh product) for *extract 2* and 17.41% degradation (501.358 to 414.049 mg/100g fresh product) for *extract 3* (see **Fig.3**).

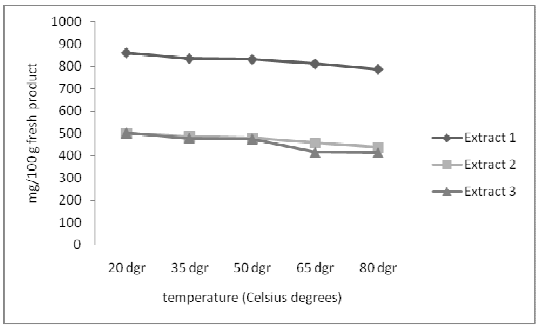


Fig. 3. The influence of temperature on anthocyanins extracts (mg/100g fresh product)

According to literature data regarding this subject the content of anthocyanins should decrease.

The degradation effect of oxygen on anthocyanins through direct oxidative mechanism increases brown products concentration.

As a result the oxidized components of the media further react with anthocyanins giving rise to colourless or brown products. [8]

Anthocyanins extracts subjected to air bubbling for a 8 h period demonstrate an increase in colour pigments (**Fig. 4.**). This fact could be a result of brown product concentration.

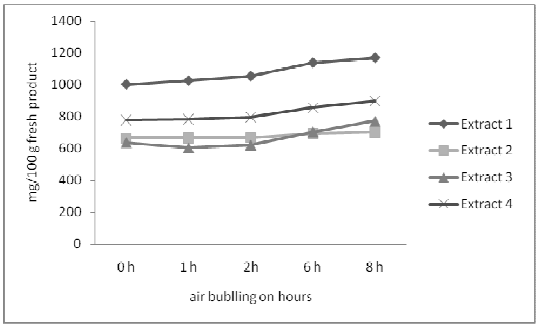


Fig. 4. The influence of oxygen on anthocyanins extracts (mg/100g fresh product)

Further investigation will be performed in order to obtain more detail results about degradation effect of oxygen on anthocyanins extracts.

Analysing data showed that *extract 1* is the most stable under light exposure (861.206 to 534.947 mg /100g fresh product) and temperature variation (861.206 to 788.396 mg/100g fresh product) from all studied extracts (**Fig.5**).

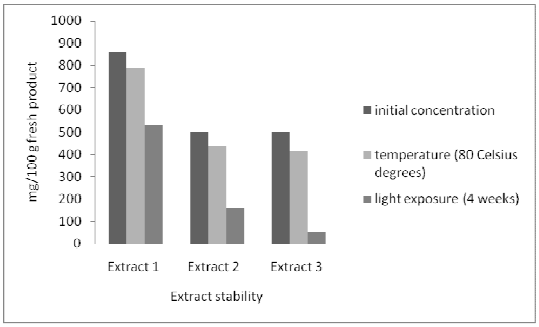


Fig 5. The stability of anthocyanins extracts subject to temperature and light exposure

4. Conclusions

Black grapes skins have rich anthocyans content (861.206 - 500.267 mg /100g in fresh products).

Solvent 1 (formic acid 3% aq /methanol 1:1) and *solvent 4* (water/citric acid 1M) proved higher extraction capacities of the anthocyanins from black grape skins then the other used solvents.

Extract 1 showed to be the most stable under light exposure (861.206 to 534.947 mg /100g fresh product) and temperature variation (861.206 to 788.396 mg/100g fresh product).

Although *extract 1* proved to be the most efficient in terms of extraction and stability properties. The food industry should take in consideration the use of *extract 2* because the solvent used to obtain it (*solvent 2)* isn't toxic.

Extract 4 proved to have beside large content of anthocyans also a large content of polysaccharides which is a perfect nutrient for microorganism's development. This fact is one of the reasons that discourage its use in food industry.

The degradation effect of oxygen on anthocyanins results in brown product concentration.

5. References

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