CHANGES IN THE ANTIOXIDANT ACTIVITY OF POLYPHENOLS FROM GRAPE POMACE AFTER INTERACTION WITH UV RADIATION

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Abstract

The aim of the study was to determine changes in the content of polyphenols and their antioxidant activity after interaction with UV radiation. An aqueous extract of grape pomace was used as a source of the tested polyphenols. Due to the differences between coloured monomeric anthocyanins and polymeric condensed tannins extracts from the skins and seeds were tested separately. It was shown that despite the light instability of coloured anthocyanins, a high content of total polyphenols remained in these solutions, and also their ability to destroy free radicals remained high even after 24 hours of their exposure to UV radiation.

Introduction

Sunscreens contain various UV absorbers which cause that the UV radiation does not get into the deeper layers of the skin. But there is a paradox – in the absorption of UV radiation they also produce large amounts of undesirable free radicals which accumulate on the surface of the skin, which is also a highly undesirable effect. Therefore, sunscreens also contain additives in the form of antioxidants (such as vitamins A, C or E) that eliminate these generated free radicals. Plant polyphenols exhibit similar properties and they have many favourable biogenic effects. For this reason we tested the resistance of plant polyphenols against UV-A radiation, changes in their content and in their antiradical power after this interaction. An aqueous extract of blue grape pomace was used as a source of polyphenols. Given the differences between monomeric anthocyanins glucosides (which are contained especially in skins of grape berries) and polymeric tannins (located mainly in grape seeds), these extracts were tested separately.

1 Theoretical part

1.1 Polyphenols

Polyphenols are a broad group of bioactive substances in plants that perform numerous tasks: they protect plant tissues against oxidative stress, UV radiation, pests and finally give plants their colour, which helps them in a competitive allogenic fight with other plants. These substances are necessary and useful even for humans, because as a part of our food they act as powerful antioxidants in the human body and help protect our cells against oxidative stress, premature aging of tissue and a number of diseases (cardiovascular disease, cancer, inflammations) [1], [2], [3].

Polyphenols exhibit a number of interesting effects, some have antimicrobial, antifungal and antiviral properties [4] (resveratrol, quercetin), polymeric tannins have astringent and antidiarrheal effects, the ability to bind metal ions and coagulate proteins, and some

polyphenols are likely to affect contractility of smooth muscle cells and anti-aggregatory effect on platelets [5]. The common characteristic of all of these substances is their strong antioxidant effect and the ability to eliminate oxygen radicals, both internally in the form of food, which acts locally in the gut as resorbed substances and their metabolites, as well as externally in contact with skin and mucous membranes. The complex of the above characteristics makes polyphenols interesting as potential candidates for substances that may assist in the treatment of wounds. Another application of polyphenols is possible where free radicals are generated by UV radiation, such as sunscreens.

1.2 Anthocyanins

Anthocyanins are plant pigments that give colour to plant flowers, leaves and sometimes the underground parts of the plant, in colour shades of orange, pink, red to purple and blue. The plants are most often found in the form of glycosides which are aglycones (anthocyanidins) coupled to a simple type of sugar, usually glucose. Malvidin (Figure 1) and cyanidin are typical for blue grapes. Their content in plants varies depending on nutrition and sunlight.

Anthocyanins are sensitive to the high temperature, pH and radiation. They absorb UV radiation and they act as the plant cell protective UV filter [6].

Anthocyanins have always been used as natural dyes; however, they have a very low light fastness, and their colour varies with variations of pH: acidic pH causes the formation of bright red flavyliumcation, which has a strong absorbance of the solution at 510-520 nm (it is used for the spectroscopic analysis).

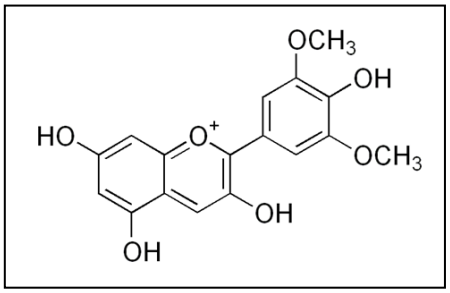

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Fig. 1: *Malvidin*

In an alkaline environment anthocyanins change gradually in the form of so-called colourless carbinol base, then they point to the blue and blue-violet or, in strong alkaline conditions, there appears their discoloration due to the opening of the pyran ring to form yellow chalcones and their irreversible degradation [7].

1.3 Tannins

Tannins are polymeric plant polyphenols with astringent tastes. They are able to precipitate proteins and have been used for tanning leather since long ago. They are divided into hydrolysable and condensed tannins. The monomer unit of hydrolysable tannins is gallic or ellagic acid, therefore called gallotannins or ellagitanins. These substances can be easily hydrolysed by action of weak acids. Tannic acid (Figure 4) is a typical gallotannin which is contained, e.g. in the oak bark or sumac.

The monomer unit of condensed tannins (called procyanidins, Figure 3) is the flavan-3-ol (Figure 2), and these units are connected covalently directly via carbons; therefore, they do not succumb to hydrolysis so easily. These compounds are structurally similar to anthocyanins, because they are created by the same metabolic pathways. Condensed tannins are typically contained just in grape seeds, which were tested in this work [9], [10].

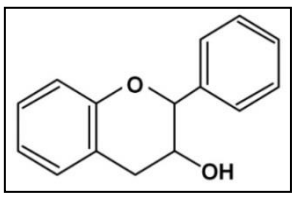

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Fig. 2: *Flavan-3-ol*

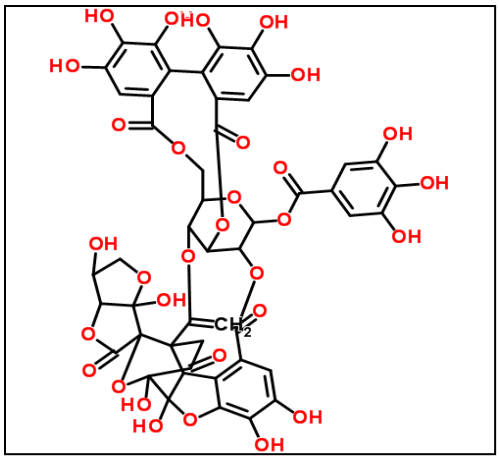

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Fig. 3: *Condensed tannin*

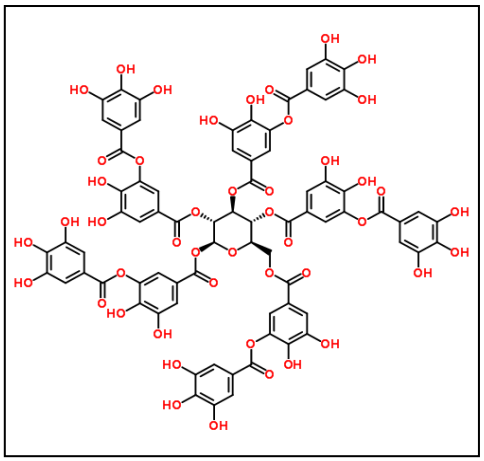

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Fig. 4: *Tannic acid*

2 Experimental part

2.1 Materials

Pomace of blue grapes varieties Fratava (Lobkowicz castle winery Roudnice nad Labem, Ltd.), stable radical 2.2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma-Aldrich), ethanol 96% for spectrometry (Lachema), Folin-Ciocalteau reagent (Penta Chrudim), HCl/KCl buffer (pH 1) (Lach-Ner), acetate buffer (pH 4.5) (Lach-Ner), anhydrous Na_2CO_3 (Lachema), gallic acid (Sigma-Aldrich), polyethylen food foil (permeable to UV) (no name).

2.2 Methods

2.2.1 Preparation of extracts from pomace

Pomace of blue grapes is separated into seeds and skins, dried at 70 °C and crushed. The exctracts were prepared as follows: 2 g of dried skins + 200 ml of distilled water, and 2 g of dried seed + 200 ml of distilled water. The extraction was carried out in closed cartridges at 95 °C for 30 minutes (skins) and 60 minutes (seeds). Thirty ml of each extract were dosed in 2x4 glass beakers, they were sealed by food foil (permeable for UV radiation) and while cooling in a water bath, the solutions were exposed to UV-A radiation for 6, 10 and 24 hours. The fourth sample was left as a non-irradiated control.

2.2.2 Content of polyphenols

All samples were filtered before their analysis through a glass fiber filter. The content of total polyphenols was determined using the Folin-Ciocalteau (FC) reagent, which arises in alkaline environment after interaction with phenolic groups of blue oxide of molybdenum. It does a maximum absorbance at 765 nm. FC reagent was diluted 10times by distilled water and for the analysis we chose the ratio 1:1:1 from diluted FC reagent, distilled water and 0.75 M solution of sodium carbonate. Then 50 µl of extract from seeds or skins were added and the resulting absorbance was measured after 60 minutes at 765 nm against distilled water in the VIS spectrophotometer Helios Epsilon (Thermo). The result was given in mg/ml of gallic acid, which was used as a calibration standard (Figure 5). All measurements were performed 3 times and averaged [11].

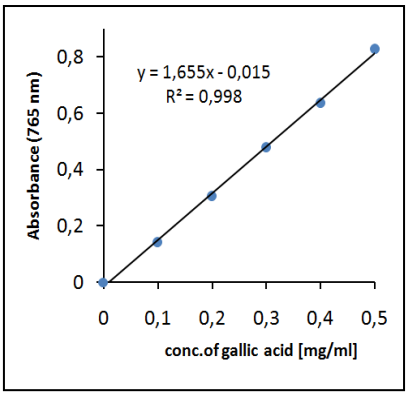

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Fig. 5: *Calibration curve of gallic acid*

2.2.3 Content of anthocyanins

The conversion of anthocyanins in flavylium salt in acidic pH was caused by reaction with HCl/KCl buffer (pH 1) which were diluted by extracts of skins in the ratio of 1:9, and the absorbance was measured at 520 nm after 30 minutes [12].

2.2.4 Antioxidant (antiradical) activity

Dissolving 8 mg of the stable radical DPPH in 50 ml of acetate buffer and 100 ml of 96% ethanol created a dark purple solution, the absorbance of which was 1.3 at the absorption maximum 520 nm. Degression of DPPH is accomplished by adding of antioxidant. This is reflected by a differently rapid discoloration of the violet solution until the yellow colour which is also associated with a decrease in absorbance at 520 nm. After addition of 100 (seeds) or 200 µl (skins) of the sample, the measured time taken for the decrease was about 1 unit of the absorption (i.e. the time from the decrease in absorbance from 1.3 to 0.3). A greater power and the ability to eliminate free radicals also affect a faster DPPH discoloration. The time was recorded in seconds at 3 measurements from which the average was calculated.

2.3 Results of measurements

2.3.1 Changes in polyphenols and anthocyanins content

As can be seen from Figures 6 and 7, after 24 hours of UV radiation, polyphenols in the extract from the skins decreased by 8% although the coloured anthocyanins content decreased by 70%. This is because a small amount of tannins is present in the skins, although having a somewhat different composition than in the seeds. In their composition there is a dominance of prodelfinidin and epigallocatechin – both are not present in seeds [10]. Polyphenols content in the extract from the seeds decreased by 16%. In addition to tannins and anthocyanins there are also a lot of other mono- and oligomeric polyphenols which influence the result of measurement.

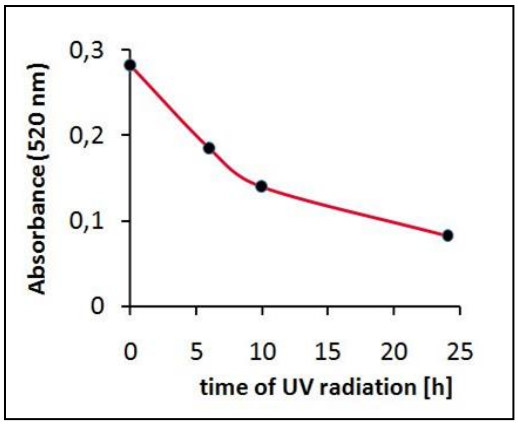

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Fig. 6: *Decrease of anthocyanins content during UV exposure*

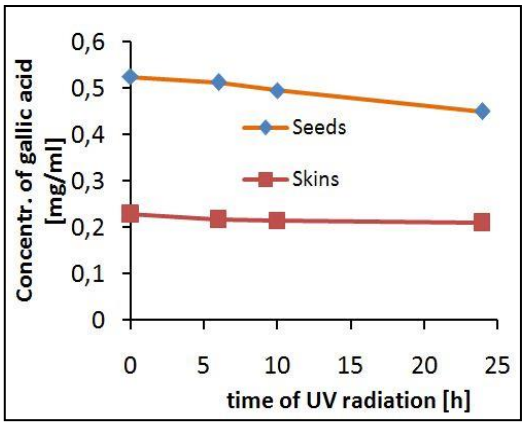

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Fig. 7: *Decrease of polyphenols content during UV exposure*

2.3.2 Changes in antioxidant (antiradical) activity

Figure 8 shows the slow destruction of DPPH radical by polyphenols after their exposure to UV radiation. The extract from the skins contains fewer polyphenols than the extract of seeds; thus for decomposition of 2 ml DPPH twice the amount of sample (200 µl) than the extract from the seeds was used. The original extract from the skins (200 µl) discolours 2 ml DPPH solution by 1 AU (520 nm) in 24 seconds, half the amount of seed extract (100 µl) in 19 sec.

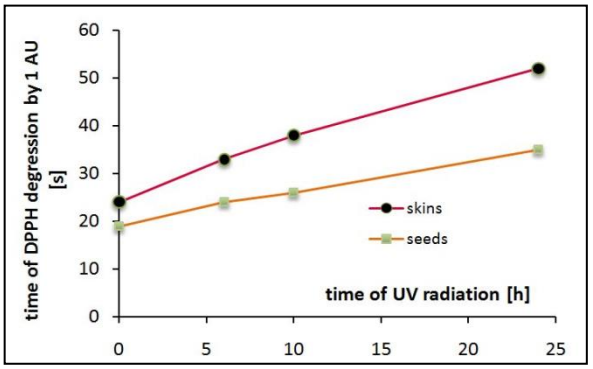

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Fig. 8: *Increase of the time needed for degression of DPPH radical after UV exposure*

24 hours of UV radiation prolong this time by 28 seconds for skins and by only 14 seconds for seeds. It is clear that the antioxidant power of polyphenols is considerable in grape seeds, which contain mainly condensed tannins.

Conclusion

The measurements showed that UV-A radiation slightly reduces the amount of polyphenols in aqueous solutions and the degradation is especially obvious in the coloured anthocyanins. Both extracts retained a significant ability to eliminate free radicals even after 24 hours of UV exposure.

The experiment showed that plant polyphenols – especially tannins from grape seeds – are very good candidates for additives in sunscreen cosmetics due to their antioxidant ability in combination with anti-inflammatory and other biological effects.

Acknowledgements

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ZMĚNY V ANTIOXIDAČNÍ AKTIVITĚ POLYFENOLŮ Z RÉVOVÝCH VÝLISKŮ PO INTERAKCI S UV ZÁŘENÍM

Cílem práce bylo zjistit změny v obsahu polyfenolů a v jejich antioxidační aktivitě po interakci s UV zářením. Jako zdroj polyfenolů byl použit vodní extrakt z výlisků z modré révy. Vzhledem k odlišnostem barevných monomerních antokyanů a polymerních kondenzovaných taninů byly testovány extrakty ze slupek a semínek odděleně. Bylo prokázáno, že i přes světelnou nestabilitu barevných antokyanů zůstal i po 24 hodinách působení UV záření v těchto roztocích zachován vysoký obsah celkových polyfenolů i vysoká schopnost zhášet volné radikály.

ÄNDERUNGEN IN DER AKTIVITÄT DER POLYPHENOLE AUS DEN REBEPRESSLINGEN NACH DER INTERAKTION MIT UV STRAHLUNG

Ziel der Arbeit ist die Ermittlung der Veränderungen von Polyphenolen und deren Aktivität als Antioxydantien nach der Interaktion mit UV Strahlung. Als Quelle der Polyphenole wurde der Wasserextrakt aus den Presslingen der Blaurebe benutzt. Im Hinblick auf die Unterschiede der farbigen Monomerantokyane und der polymeren kondensierten Tannine wurden die Extrakte aus den Schalen und den Kernen getrennt getestet. Es wurde nachgewiesen, dass trotz der Lichtinstabilität der farbigen Antokyane in den Wasserextrakten ein hoher Gehalt an Gesamtpolyphenolen auch nach 24 Stunden Einfluss der UV Strahlung und auch ihre Fähigkeit, freie Radikale zu neutralisieren, erhalten blieben.

ZMIANY W AKTYWNOŚCI ANTYOKSYDACYJNEJ POLIFENOLI Z WYTŁOKÓW WINOGRON PO INTERAKCJI Z PROMIENIOWANIEM UV

Celem pracy było określenie zmian w zawartości polifenoli i ich aktywności antyoksydacyjnej po interakcji z promieniowaniem UV. Jako źródło polifenoli użyto wodnego ekstraktu z wytłoków niebieskich winogron. Ze względu na różnice kolorowych antocyjanów monomerycznych i skondensowanych tanin polimerowych ekstrakty ze skórek i nasion badano oddzielnie. Stwierdzono, że pomimo niestabilności kolorowych antocyjanów w tych roztworach, również po 24 godzinach ekspozycji na promieniowanie UV zachowała się duża całkowita zawartość polifenoli i wysoka zdolność do eliminowania wolnych rodników.