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Characterization of grape seeds for total polyphenol content as potential source of antioxidants

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Declaration

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere for any award. Where other sources of information have been used they have been acknowledged.

Signature é

Date é .

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Summary

Biologically the term antioxidants can be defined as follows ""any substance that when present at low concentrations compared to that of an oxidizable substrate would significantly delay or prevent oxidation of that substrate"" (Wanasundara et al., 2005).

The interest to these chemical compounds is based on their ability to reduce free radicals that can rise from the oxidation of compounds contained in food that we consume and these radicals are responsible of diseases.

Oxidative stress can destroy biomolecules (lipids, proteins, carbohydrates, DNA, enzymes...) which has a huge impact on the whole organism and can lead to diseases such as cardiovascular diseases, cancer, neurodegenerative disorders, diabetes, autoimmune disorders (Sanda et al., 2002).

Based on the role of antioxidants for livings, it is of great importance to understand the functioning, to know food that contains them, their quantity, and the available quantity for reaction.

In this research, the aim was to find the total polyphenol content in grape seeds, their antioxidant activity and factors influencing their amount. For the determination of TP content we used the method of Folin-Ciocalteau reagent and DPPH for determination of antioxidant activity. Fourteen grape seeds varieties have been used for the purpose of this study and they were collected from different regions of the Czech Republic.

Results showed that the quantity of polyphenols and their antioxidants capacity varied according to growing region, colour and variety.

White varieties contain higher TP content than red varieties. We found that there is correlation between TP content and antioxidants activity which is in the same line as results found by Lachman et al., 2009 who found a significant relationship between the total concentrations of phenol compounds in conventional and ecological red and white wines and the antioxidant activity determined by DPPH assay.

Key words: grape seeds; polyphenols; antioxidant activity; DPPH.

Souhrn

Z biologického hlediska pojeru antioxidanty mŢge ýt definován následué jakákoliv láska pŚítomna v rúskgéh koncentracích ve srování s oxidovanjeu substrádeu kserá mŢzŊ výZnamnŊzpomalit nebo zabránit oxidaci substrátu (wanasundra et al., 2005). Zájem o tyto chemické látkz je yalogen na jejich schopnasti redukavat volné radikály, které mohou vziknout oxidací látek obsaģerujeh v potrovinách, které konzumujeme a kyko radikály jsouodpovedué ya vynik nemocí.

Oxidativní stres mŢzŊ znļit biomolekuly lipidy, bílkoviny, suckaridy, DNA, enzymy steré mají velky vliv na celý organismus a mŢģe vést ke vzniku nemocí jako jsou kardiovaskulárni porucy, rakovina, neurodegereationí pouckz, diabetes, autoimunné porucky (Sanda et al., 2002).

Na základŊ role antioxidantŢ proģivé organismy má velký význam porozumŊní jejich funkci, poznatky o potravinách, které je yískatelný prodalsz reakce.
V této práci cílem bzlostanovit obsah celkových polzfenolŢ v semenech révz vinné a slanovit antioxidanļni aktivitu.

Bzla pouģita semena 14 odnid vivné révy z rŢtných vinaŚkých oblastí ļské republiky. Výsledkz ukázaly, y runoģslví polzfenoli a jejich antioxidanní kapacita byly rŢtné ve vztahu z vinaíské oblasti, barvé hroynŢ a odrŢdé.

Bílé odnnidz obsahují vyģví obsah celkových polyfenolŢ (CP) NEĢĻERVENÉ ODRŢdy. Zjistili jsme, Śe existrji koulace mezi obsahem CP a antioxidanļní aktivitou, coģ souhlasí s výsleclkz Lachmana et al (2009), ZtŊí urelezli statistckz vyznamnz Vzatah mezi obsahem celkorujch polzfenolŢ v ļervených a bílých vínech pŊstovaných kouvenŊhŊ a ekologicky a antioxidaļní aktivitou stanovenou metodou DPPH.

KléŊová slova: semena vinné révy, polifenolz, antioxidaļni aktivita, DPPH.

Abbreviation

% W/W: percentage weight/weight

ABTS: 2,2ô-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)

AH: Antioxidant

ĮZU: Įeská Zemědělská Univerzita

DM: Dry Matter

DNA: Deoxiribo Nucleic Acid.

DPPH: 2,2-diphenyl-1-picryl-hydrazyl

GC: Gas Chromatography

H_2O_2: hydrogen peroxide

HPLC: High Pressure Liquid Chromatography

O_2: Superoxide Anion

OH^-: hydroxyl radical

R.O.S: Reactive Oxygen Species

RNS: Reactive Nitrogen Species

RO: Free radical

ROS: Reactive Oxygen Species

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1 Introduction

Grapes are one of the most widely grown fruit crops throughout the world, and their composition and properties have been extensively investigated, with several reports of the presence of large amounts of phenolic compounds. Most phenolic compounds found in wine can act as antioxidants. Likewise, the residues of wine production are characterized by high contents of phenolic compounds due to their incomplete extraction during vinification (Rockenbach et al., 2011).

Grape seed (*Vitis vinifera L.)* is a well known oil seed crop containing typically 8ï 20% (w/w) of oil (Passos et al., 2009; Crews et al., 2006), and 10ï 20% (w/w) of polyphenolic compounds in dry basis (Passos et al., 2010, Bail et al., 2008).

Plant origin polyphenolic compounds are intensely studied in the recent years thank to their potent antioxidant, anti inflammatory and immune modulatory properties (Nicols et al., 2010). Grapes (*Vitis vinifera*), which are one of the most widely consumed fruits in the world have enormous health benefits. They contain a great variety of polyphenolic antioxidants with preventive and also therapeutic effects in several cancers, 60ï 70% of their content being represented by proanthocyanidins, composed mainly of dimers, trimers, tetramers and oligomers of monomeric catechins (Ricardo da Silva et al., 1991). Grape seed extract can be considered among the most powerful natural nutrients efficient in the protection of skin health, proanthocyanidins from grape seeds are stronger antioxidants and free radical scavengers than ascorbic acid or vitamin E (Katiyar et al., 2008).

Grape seeds are a particularly rich source of complex polymers of flavonoids such as gallic acid, the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin 3-O-gallate, dimeric-, trimeric- and even more polymeric proanthocyanidins (Segura et al., 2003; Shi et al., 2003).

Physiochemical properties of polyphenols allow them to participate in different metabolic cellular oxidation-reduction reactions. And these abilities explain the function of polyphenols as antioxidants (Quiñones et al., 2013).

The aim of this research was the evaluation of different grape seeds varieties for contents of phenolic compounds and their antioxidant activities.

Food rich in antioxidant is beneficial to human being health in many ways, including regulation of antioxidant detoxifying enzymes and gene expression.

Antioxidants are also known for their ability to protect a target molecule exposed to a free radical source, to inhibit the oxidation of low density lipoprotein and the capacity of antioxidants to reduce cupric or ferric ions (López-Alarcón and Denicola, 2012).

2 Objectives of Thesis

Grapes seed may be rich sources of antioxidants, especially phenolic compounds. The aim of this study was the evaluation of fourteen grape seed varieties for total phenolic content in grape seeds, their antioxidant activities and factors influencing them. These grape seeds were collected from different regions of the Czech Republic (Bohemia, Mělník, Karlštejn and Prague – Grébovka).

Polyphenols are a large and integral part of the human diet; it is of great importance to understand its activities and their concentration in food. In this research, interest was paid to the concentration of polyphenols and their antioxidants activities, as well as all polyphenols are not antioxidants and have different antioxidant activity.

3 Literature review

3.1 Polyphenols

The term polyphenol include several classes of compound such as flavonoids which makes the most important group. Flavonoids can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds (anthocyanins, flavan-3-ols and flavonols).

Anthocyanins are a family of polyphenols that are directly responsible for colour in grapes and young wines. Flavan-3-ols (monomeric cathechins and proanthocyanidins) are another large family of polyphenolic compounds, which are mainly responsible for the astringency, bitterness and structure of wines (Rodriguez et al., 2005).

Chemically, polyphenols are a group of natural compounds with phenolic structural features. It is a collective term for several sub-groups of phenolic compounds.
Polyphenols are chemically characterized as compounds with phenolic structural features; this group of natural products is highly diverse and contains several sub-groups of phenolic compounds.

Due to great importance of polyphenols for wine quality there is a growing interest in the development of selective and sensitive methods for their detection and quantification. Polyphenols are well known to be electroactive due to the presence of hydroxyl groups attached to the aromatic rings, which undergo electrochemical oxidation reactions (Janeiro, et al., 2004).

Polyphenolic compounds have great importance in food technology and cosmetics due to their ability to maintain flavour and colour of the products and of food because of their ability to prevent oxidative damages due to oxygen and polyphenol makes up to 65% of antioxidants present in the plant (Milan et al., 2013).

The polyphenolic contents of wine consist of flavonoids and non flavonoids and their concentration depend on the grape variety, vineyard location, cultivation system, climate, soil type, harvesting time, production process and aging (Lachman et al., 2009).

The quantity of phenolic compounds in plant depends also on the extraction method employed, sample particle size, storage time and conditions, as well as assay method,

selection of standards and presence of interfering substances such as waxes, fats, terpenes and chlorophylls (Sanda et al., 2012).

Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols.

Phenolic compound are very important to plants themselves because phenolic compounds contribute to the physiology of plants for example organoleptic characteristics such as pigmentation of plants, odours and flavour.

Phenolic compounds help plants to resist to invaders microorganisms and insects (Visioli et al., 2000). Phenols also have an important function in matter of influencing the pools and fluxes of organic and inorganic soil nutrients; they enter soil as leachates from above the plants parts and influence composition of soil decomposers and decomposition rate (Lattanzio et al., 2006).

Polyphenols have a UV (screening protective property, consequently plants can resist to the negative impact of UV light 280-320nm) that can affect plants' DNA, proteins and membrane, thus leading to generation of Reactive Oxygen Species (ROS).

Studies showed that changes in flavonoids (main abundant polyphenol) in leaves depend to the exposure to sun, consequently plant in high mountains and in tropical areas contain more polyphenols than plants in temperate regions (Lattanzio et al., 2006).

In beverage such as tea, cider, wine... the taste of bitterness are elicited by flavonoids. The astringency increased and bitterness decreased with the mean degree of polymerization. Increasing the ethanol concentration of wine from 8% to 14% (by volume) approximately double the bitterness intensity but had no effect on astringency (Lesschaeve and Noble, 2005).

Polarity, acidity, volatility and molecular size are physiochemical properties of phenols.

Phenols are polar, thus water immiscible solvents do not extract polyphenols very efficiently. Polyphenols shows relatively high polarity. Is easy to solubilise phenols in water and organic solvent according to acidification at higher temperature because it allows release of any phenols' bounds.

Acidification increases the extractability by making the hydrogen bonds with fibrous polar matrix weaker.

Gas chromatography (GC) is usually preferred for separation of volatile compounds, but this method is not suitable phenols because are less volatile.

High pressure liquid chromatography (HPLC) is the preferable method for polyphenols extraction (Callemien and Collin, 2010).

3.1.1 Polyphenols classification

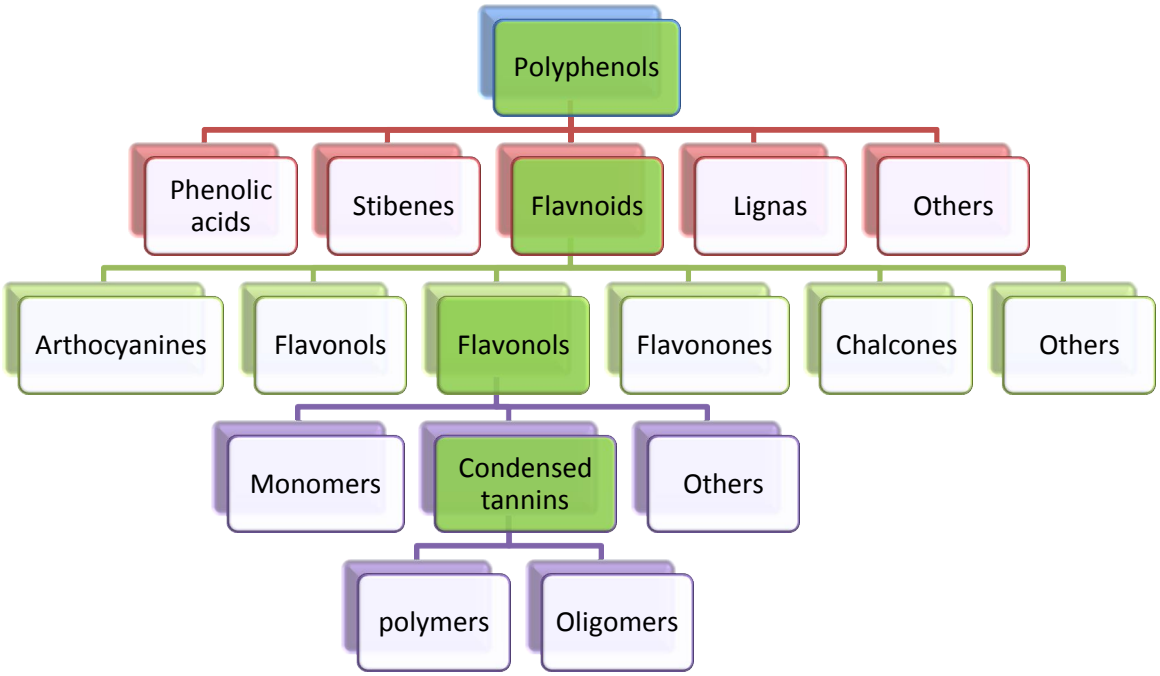
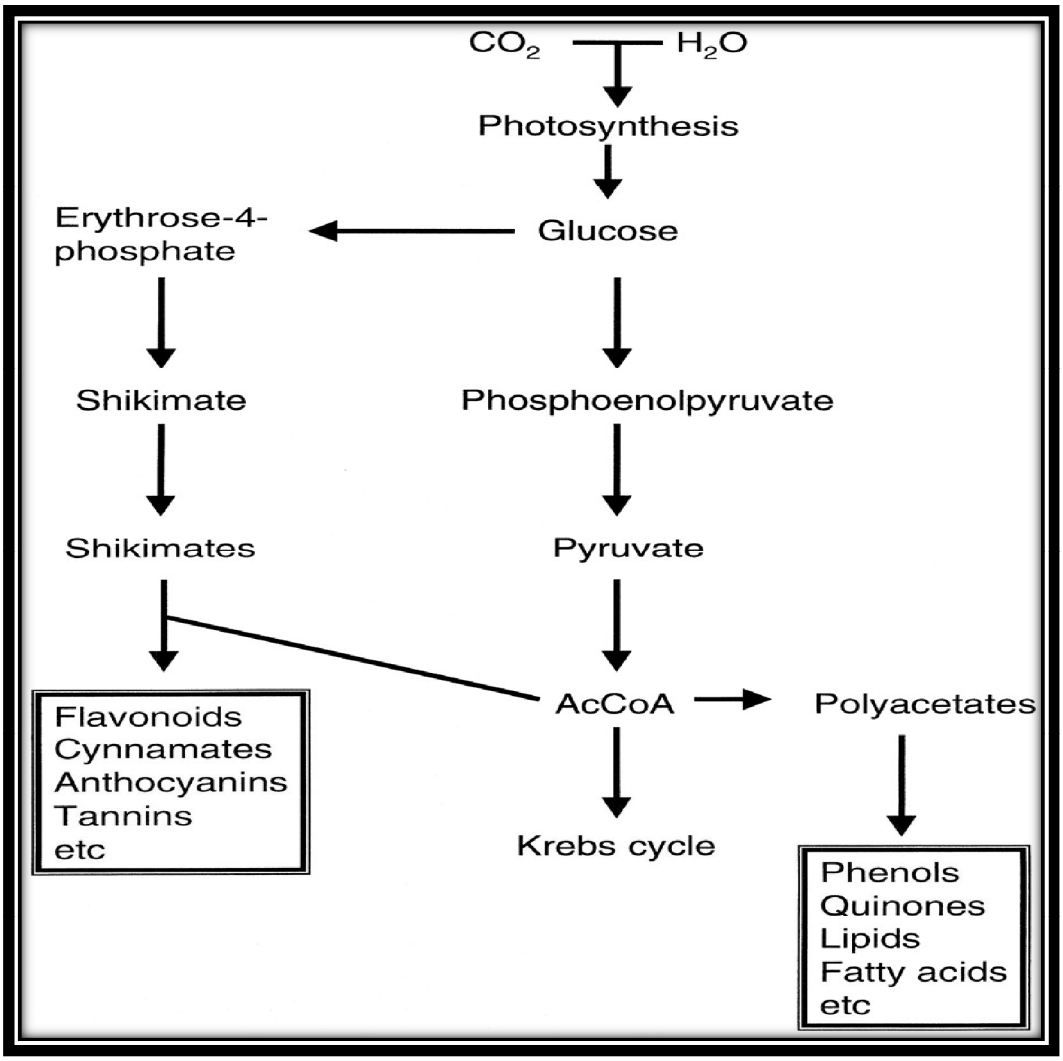


Figure 1. Polyphenols classification
(http://www.opc-1-2-3.com/polyphenols_classification.pdf)

Polyphenols can be found in black and green teas, fruits, vegetables, olive oil, Coffee, red and white wines, fruit juices, and chocolate.

For people consuming food rich in vegetables and fruits they consume a significant amount of polyphenols. Due to its organoleptic characteristics, polyphenolic compounds play an important role in the quality of grapes and wines.

3.1.2 Phenolic compounds biosynthesis



Equation 1. Phenolic compounds biosynthesis (Visioli et al., 2000).

Phenolics are derivatives of benzene (cyclic derivatives in the case of polyphenols) with one or more hydroxyl groups associated with their ring. They can be conveniently classified into at least ten different classes depending on their chemical structure (Visioli et al., 2000).

3.1.3 Polyphenol medical properties

The polyphenolic molecules have a functional role in that they behave as antioxidants against the free radicals and show a physiological role as well; in fact, they increase the antioxidant capacity in the human body after red wine consumption (Serafin et al.,1998).
ROS are results of a normal cellular metabolism and participate in vital roles in the stimulation of signalling pathways in cells in reaction to changes in intra and extracellular environmental conditions. Most ROS are generated in cells by the mitochondrial respiratory

series. During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion ($O_2^{\bar{\cdot}}$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and organic peroxides as normal products of the biological reduction of molecular oxygen. The electron transfer to molecular oxygen occurs at the level of the respiratory chain, and the electron transport chains are located in the membranes of the mitochondria. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate reactive nitrogen species (RNS). ROS/RNS can further generate other reactive species by inducing excessive lipid peroxidation. In order to combat and neutralize the deleterious effects of ROS/RNS, various antioxidant strategies have involved either the increase of endogenous antioxidant enzyme defences (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) or the enhancement of non-enzymatic defences (e.g., glutathione, vitamins) through dietary or pharmacological means. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable substrate by scavenging free radicals and diminishing oxidative stress (Sanda et al., 2012).

Polyphenols are primarily recognized for their antioxidant functions, they have many other biological activities, such as anti-histamine (Nitta et al., 2007) antibacterial, and antiviral activities (Song et al., 2005) anti-inflammatory (Macheix et al., 1990).
The antimicrobial activity has been found in grape seeds extracts, especially against Gram-positive bacteria like *S. aureus subsp. aureus* and *Listeria* as well as Gram-negative bacteria as *Pseudomonas aeruginosa. Salmonella enteric subsp. Enteric* and *Echerichia coli*. These bacteria are the most causal agents of food-borne infections in developing and developed countries. Further tests are needed to confirm these screening results in other in vitro and in vivo assays (Adámez et al., 2012).

Plants and food polyphenols have important effect in order to reduce and to fight against effect of diabetes in human being.

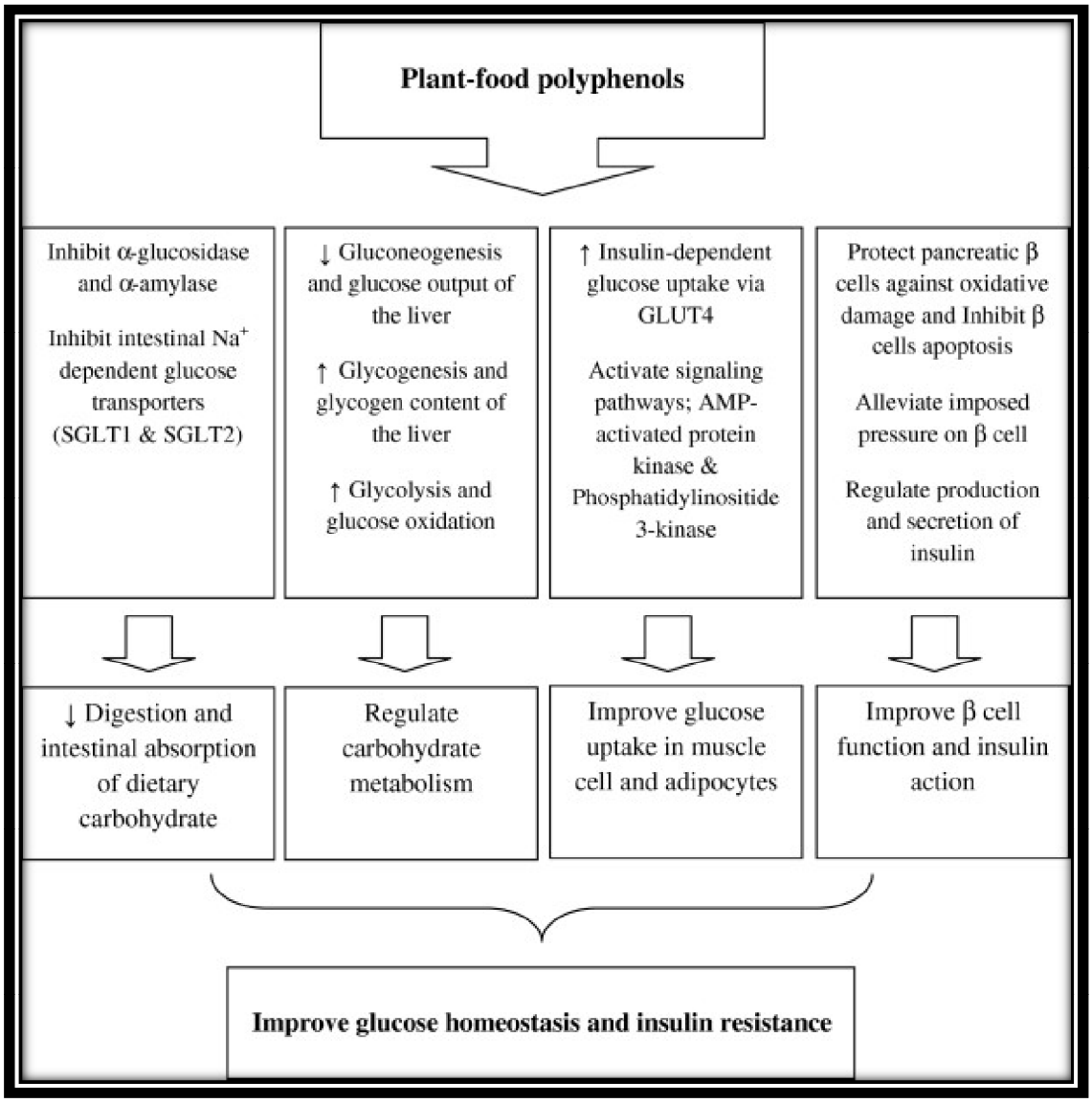


Figure 2. Polyphenols role in homeostasis and insulin resistance in plants (Zahra et al., 2013)

Polyphenols have cardiovascular defensive effects with regard to lipid metabolism, blood pressure, blood coagulation and vascular function (Zahra et al., 2013). Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations (Devasagayam et al., 2004). There is rising proof suggesting that food intake of polyphenol-rich foods and supplementation with these bioactive components could have protective effects against diabetes, induced cardiovascular pathogenesis; the mechanisms implicated in these properties mostly include regulation of lipid metabolism, reduction of oxidative break and scavenging of free radicals (Zahra et al., 2013).

Oxidative stress which is defined as the imbalance production of free radical and reactive metabolites (Oxidant or R.O.S Reactive Oxygen Species) and their elimination by antioxidants, can destroy biomolecules (lipids, proteins, carbohydrates, DNA, enzymes...) this

has a huge impact on the whole organism and can lead to diseases like Cardiovascular diseases, cancer, neurodegenerative disorders, diabetes, autoimmune disorders (Sanda et al., 2002).It was proved that cardioprotective properties of polyphenols compounds depends on the polyphenols capacity to improve endothelial functions (Demrow et al., 1995).

3.1.4 Bioavailability of polyphenols

Bioavailability of food is the quantity of that specific food that is digested, absorbed and used by the organism in ordinary situation. The bioavailability is very high linked to the bioaccessibility digestion (Hedren et al., 2002).

The food macromolecules affects the bioaccessibility and bioavailability of several nutrients, thus the bioavailability of polyphenols differ deeply depending on many factors such as food source, chemical interaction with other phytochemicals and biomolecules present in food (Manach et al., 2005; Parada and Aguilera, 2007).

3.2. Antioxidants

Unstable molecules (free radicals), cause cell damage and this is prevented by antioxidants.
Antioxidants are rich in fruits and vegetables. Beta-carotene is found in a lot of food of orange colour, as well as sweet potatoes, squash, cantaloupe, apricots, pumpkin, carrots, and mangoes, some green, leafy vegetables, together with collard greens, spinach and kale, are also rich in beta-carotene (Borek, 1997).

3.2.1 Classification of antioxidant

Antioxidants may be broadly grouped according to their mechanism of action: primary or chain breaking antioxidants and secondary or preventive antioxidants.

3.2.1.1 Primary Antioxidants

Primary antioxidants are also referred to as type 1 or chain-breaking antioxidants.
Because of the chemical nature of these molecules, they can act as free radical acceptors/scavengers and delay or inhibit the initiation step or interrupt the propagation step of autoxidation.

3.2.1.1.1 Mode of reaction of primary antioxidants

ROOÅ+ AH ⟶ ROOH +AÅ

RÅ+AH ⟶ RH +AÅ

ROOÅ+AÅ ⟶ ROOA

ROÅ+AÅ⟶ ROA

AÅ+AÅ⟶ AA

Equation 2. Mode of reaction of primary antioxidants

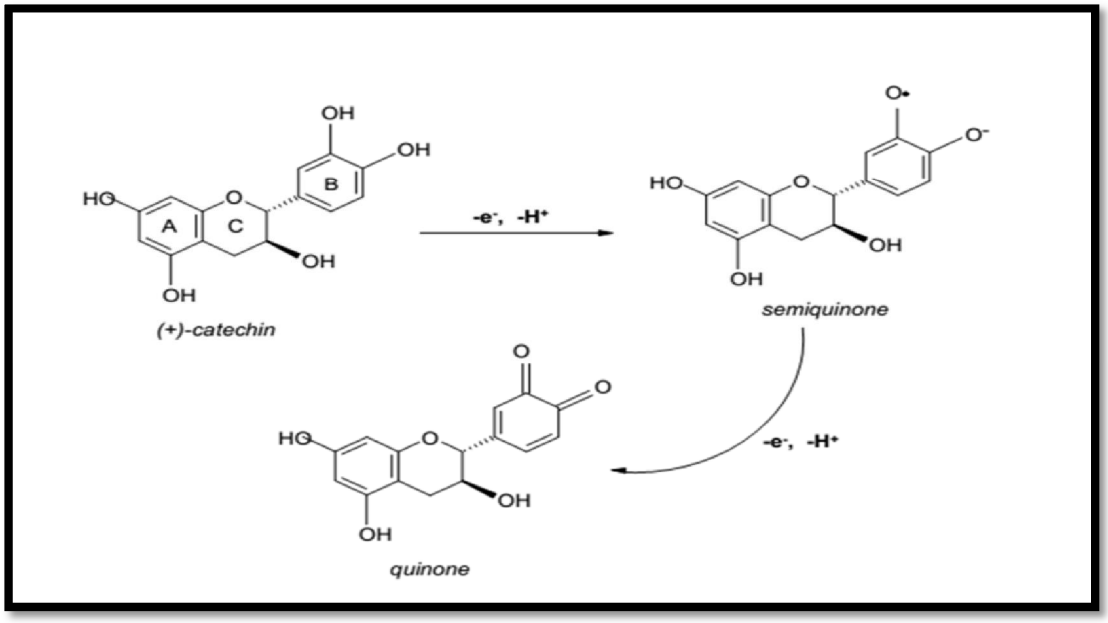
Note AH: Antioxidant

RO: Free radical

The primary antioxidants (AH) react with lipid and peroxyl radical and convert them to more stable, non radical products. Primary antioxidants have higher affinities for peroxyl radicals than lipids and react predominantly with peroxyl radicals.The antioxidant effectiveness is influenced by the chemical properties of the compound including hydrogen bond energies, resonance delocalization, and susceptibility to autoxidation (Yildiz, 2010).

3.2.2 Secondary antioxidants

Secondary antioxidants are also classified as preventive or class II antioxidants. They offer their antioxidant activity through various mechanisms to slow the rate of oxidation reactions. The main difference with primary antioxidants is that the secondary antioxidants do not convert free radicals into stable molecules. They act as chelators for prooxidant or catalyst metal ions, provide H to primary antioxidants, decompose hydroperoxide to non radical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as oxygen scavengers (Wanasundara et al., 2005).



Equation 3. Example of secondary antioxidant activity (Janeiro and Oliveira, 2004).

The oxidation of flavonoids is of great interest due to its antioxidants activities which scavenge radicals by electrotransfer processes.

3.3 Introduction to grapes

A grape is a berry fruit of the botanical genus *Vitis* . Grapes can be eaten raw or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar

Figure 3. Grape picture (http://vitalityinternational.info/wp-content/uploads/2013/03/grape-seed.jpg)

Figure 4. Grape seeds (http://www.simplyorganico.com/store/100-pure-natural-oils/grape-seed-oil/).

Grape seeds are highly on demand as sources of polyphenols. Polyphenols are receiving increasing interest from consumers and food manufacturers for several reasons. Epidemiological studies have suggested associations between the consumption of polyphenol-rich foods or beverages and the prevention of diseases. Fruit and vegetable consumption prevents cancers (Steinmetz et al, 1996).

Purple grape juice and Red wine have polymeric flavonoids with antioxidant properties supposed to be defensive against cardiovascular events but the sugar and alcohol content of these beverages has imperfect their medicinal use (Vitseva et al., 2005).

4.1 Main grape phenolic antioxidant and their classification

- Flavanols
- Hydroxybenzoic acids
- Hydroxycinnamic acids
- Tartaric acid derivatives
- Proanthocyanidins
- Phenols
- Flavonols
- Anthocyanins (coumaroylated, acylated, pyranoanthocyanins)
- Resveratrols

(Lachman et al.,2009).

Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause (Sies, 1997).
Resveratrol, which is a free radical scavenger, inhibits the risk of cardiovascular diseases. (Filip et al,. 2003; Gutiérrez et al,. 2005) resveratrol is mainly contained in the skins of grapes (Schmandke et al., 2002).
Recently, some studies indicate that aqueous extracts prepared from grape seeds can have antibacterial and antioxidant activities (Adámez et al., 2012).

4.2. Antioxidant Activities

In this research we used DPPH [2,2-Diphenyl-1-picryl-hydrazyl] and ABTS [2,2ô-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] methods to determine the antioxidants activities of 11 types of grape seeds.
Different methods for antioxidant activity determination are used in order to understand and compare food capacity to scavenge free radicals. These methods require special equipment and technical skills for the analysis. Extraction accuracy is a key factor in quantification of antioxidant activity of foods.

The antioxidant activity of phenolic acids is related to the quantity, number and position of hydroxyl groups in the molecule (Biskup et al., 2013).

Interaction between antioxidants and the environment of reaction it affect their antioxidant capacity, for example interaction between antioxidants and plasma proteins. (Aarts et al., 2001).

Phenolic compounds can act in many different ways:

- Chelating metals such as iron and copper, which can prevent their involvement in Fenton reactions that can generate high concentrations of hydroxyl radicals,
- Breaking the chain of reactions triggered by free radicals,
- Slowing down or accelerating enzyme activity (Biskup et al., 2013).

Stability of antioxidants has a huge effect on the cellular protective activities.

In consideration to hydrophilic and lipophilic characteristics of species in reaction I used DPPH and ABTS assay to determine antioxidants activities.

ABTS can be dissolved in aqueous and organic media, DPPH can only be dissolved in organic media especially ethanol, this can be a limitation in interpretation of hydrophilic antioxidants activities (Wojdylo et al., 2007).

5. Materials and methods

5.1 List of material

1. Balance
2. Volumetric flasks
3. UV-VIS Spectrophotometer
4. Centrifuge
5. Blinder electric mill HR 2185 Philips (Amsterdam, the Netherlands)
6. Ultrasound bath

Chemicals

Folin-Ciocalteau reagent (Penta Chrudim, Czech Republic)

Ethanol 80%

Na_2CO_3 (Lach-Ner, Ltd., NerAatovice, Czech Republic)

Gallic acid

5.2 Plant material collection

Plants materials used in this research have been collected in 2012 in different regions of the Czech Republic (Bohemia, MÍlník, Karlštejn, Prague, Grébovka) after grape fruit collection, seeds have been air drayed at room temperature into the chemistry laboratory of CZU. Drayed grape seeds have been grinded and the powder was used for polyphenol extraction.

5.3 Chemical polyphenol extraction and Antioxidant activity measurement

0.5 g of Grape seed powdered in HR 2185 Philips electricmill (Amsterdeam, the Netherlands) have been mixed with 10 ml methanol 80%

The solution (grape seed and methanol) was bathed in ultrasound bath for 10 min at 22ºC.

The solution was centrifuged and transferred into 25 ml volumetric flask and the extraction was repeated twice.

The extract was adjusted to 25 ml with 80% methanol and carefully stirred. For the TP determination 1 ml aliquots of sample solutions were pipette. The sample extract (1 ml) was transferred into a 50 ml volumetric flask and diluted with approximately 5 ml distilled water. Then, 2.5 ml Folin-Ciocalteau reagent (Penta Chrudim, Czech Republic) and 7.5 ml of 20% (w/w) Na_2CO_3 (Lach-Ner, Ltd., Neratovice, Czech Republic) were added, adjusted with distilled water to 50 mL, agitated and left to stand for 2 h. Absorbance of the sample was measured on the UV spectrophotometer Spectronic Heλios (Thermo Spectronic, Cambridge, Great Britain) at λ= 765 nm against a blank prepared with distilled water. Gallic acid (G.R. purity, Merck KGaA, Darmstadt, Germany) was used for calibration. The results were expressed as Gallic acid equivalents (GAE) in mg/kg DM from three replicates. This method has been also used by (Lachman et al., 2013)

Ultrasound-assisted extraction applies high-intensity ultrasound waves, which cause physical disruption of biological cell walls and cell membranes, as well as particle size reduction. These effects enable better penetration of the solvent into cellular materials which improve mass transport rates within the tissue and facilitate the transfer of components from the cell into the solvent leading to the enhancement of the extraction operation (Novak et al,. 2008).

According to some authors, the extraction of polyphenols increases with the increase of ultrasound exposure time, and the extraction yield is the highest when the ultrasound exposure time is in the range of 45ï 90 min. On the other hand, prolonged application of ultrasound may result in denaturation of phenolics, so that the sonication time should be very carefully considered (Usaquén-Cas et al., 2006).

Ultrasound effects in cell tissue are much stronger at low frequencies 18ï 40 kHz and negligible at 400ï 800 kHz, because these high frequencies may cause microfractures in the tissue (Novak et al., 2008).The extracts were centrifuged at 5000 rpm for 5 minutes at room temperature (22 °C).the aliquot was analyzed by Heλios ɔ spectrophotometer (Spectronic Unicam, GB) in both TP and antioxidant activity. The antioxidant activity was measured at wavelength of 515 nm.

DPPH test

DPPH is a free stable radical it has been used in this research, where the working solutions were prepared dissolving the solution of DPPH with Methanol. An aliquot of 100mL of the sample was placed in a tube and reacted with 2 ml of DPPH working solution. The mixture was well mixed

The absorbance was measured at 515 nm, determinations were performed in triplicate.

The DPPH test provides information on the reactivity of the test compounds with a stable free radical. The DPPH is used to determine the free radical scavenging activity. DPPH gives a strong absorption band at 515 nm in visible region and is purple in colour (Ayoola et al., 2008).

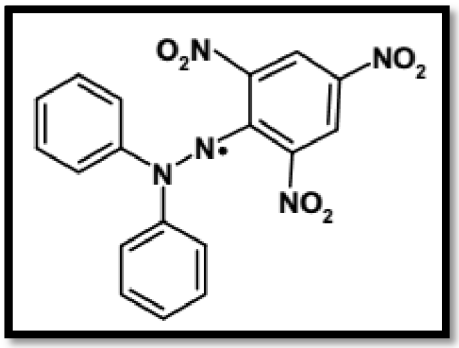


Figure 5. Chemical structure of [2,2-Diphenyl-1-picryl-hydrazyl] DPPH (Tirzitis and Bartosz, 2010).

The frequently used antioxidant methods are ABTS and DPPH. They are characterized by excellent reproducibility. They are also different in the way of reaction with antioxidants ABTS must be generated by enzymes. ABTS can be dissolved in aqueous and organic media, because of hydrophilic and lipophilic nature of the compounds in samples, but DPPH can only be dissolved in organic media, especially in ethanol, or Methanol this is an important limitation when interpreting the role of hydrophilic antioxidants by DPPH (Wojdyğo et al., 2007).

6. Results and Discussion

The discussion and analysis of results was performed using ANOVA tools. Experimental results were means ± S.D. of three parallel measurements. Data were subjected to ANOVA and differences among cultivars were tested by post hoc comparison test (Student Newman Keuls) at p= 0.05. The analysis of relations between samples and phenolic compound or antioxidant properties was performed by regression and correlation analysis.

Table 1. Evaluation of varieties in total phenol content

Variety	**Average of Total Phenols (g/kg DM)**
Cabernet	1.531^{a}
Saint Laurent	$1.661^{a,b,c}$
Hibernal	2.065^{a}
Neronet	2.190^{a}
Laurot	$2.492^{a,b}$
Zweigeltrebe	2.805^{a}
Mixture of red grapes	$3.499^{a,b,c,e}$
Saint Laurent	$3.676^{a,b,c,d}$
Pinot Noir	$4.631^{b,c,d,e}$
Chardonnay	$4.822^{a,b,c,d,e,f}$
Mixture of white grapes	$5.048^{c,d,e,f}$
Pinot Gris	$5.479^{d,e,f}$
Chardonnay	$5.691^{d,e,f}$
Welschriesling	$5.865^{e,f}$
Traminer Rot	$6.232^{e,f}$
Müller Thurgau	6.636^{f}

This table shows that there is a statistical significant difference between [Müller Thurgau (6.636^{f} mg/kg DM); Traminer Rot ($6.232^{e,f}$); Pinot Gris ($5.479^{d,e,f}$ g/kg)] and [Hibernal (2.065^{a} mg/kg DM); Neronet (2.190^{a} mg/kg DM); Cabernet (1.531^{a}mg/kg DM)]

No statistical significant difference found among Hibernal (2.065^{a} g/kg DM), Neronet (2.190 g/kg DM) and Laurot ($2.492^{a,b}$g/kg DM) as well as between Mixture of red grapes ($3.499^{a,b,c,e}$ mg/kg DM) and Pinot Noir ($4.631^{b,c,d,e}$ mg/kg DM)

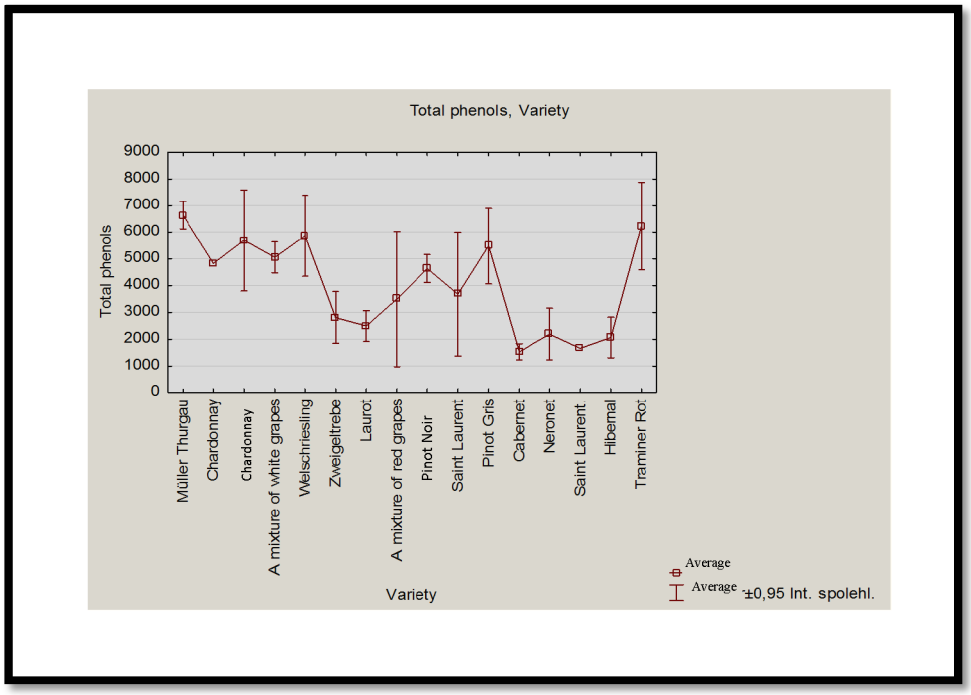


Figure 6. Evaluation of varieties in total phenol content

Table 2: Evaluation of phenol content regarding colour of grape seed

Colour	Total Phenol Average (g/kg DM)
Red grape varieties	3.193^{a}
White grape varieties	5.618^{b}

There is a statistical significant difference between red grape varieties (3.193^{a} g/kg DM) and white grape varieties (5.618^{b} g/kg DM).

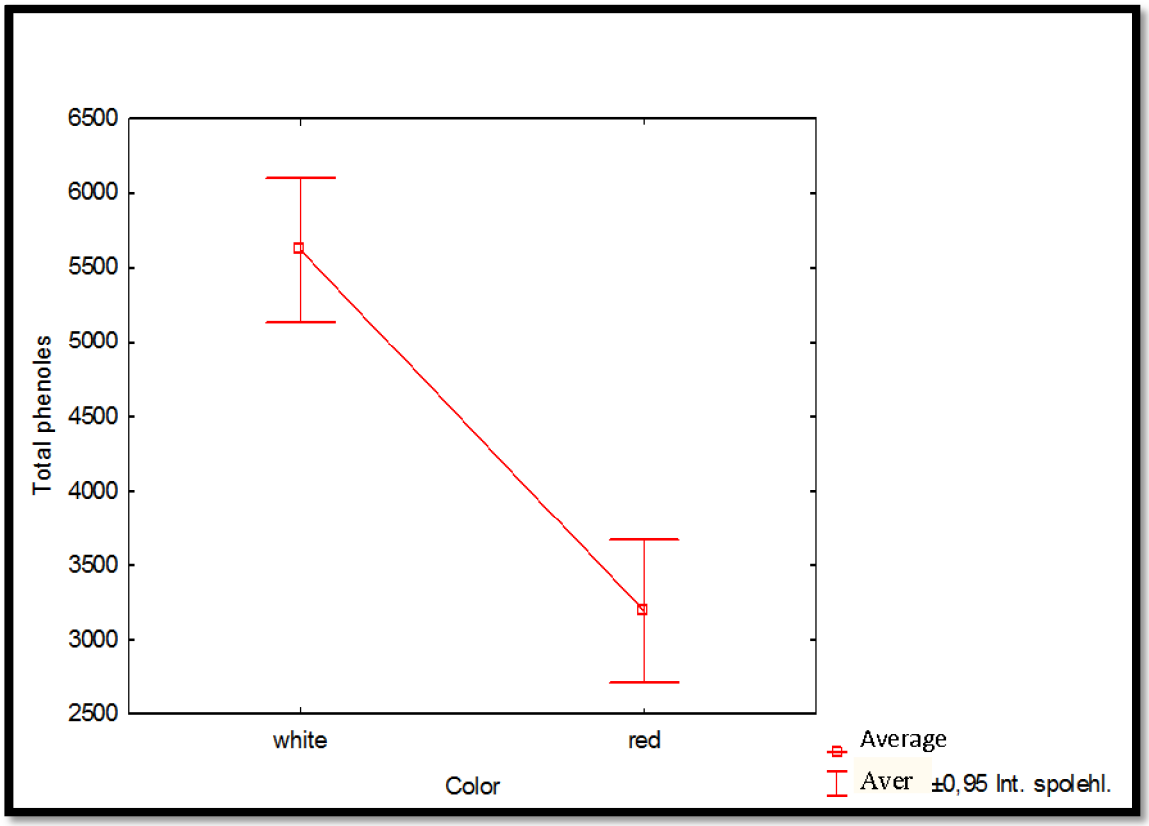


Figure 7. Evaluation of phenol content regarding colour of grape seed

6.1 Evaluation of varieties in individual localities

The method used in this research is one-way ANOVA

Table 3. Evaluation of varieties in Velké Bílovice

Varieties	**Total Phenols Average (mg/Kg DM)**
Cabernet	1.531[a]
Saint Laurent	1.661[a]
Zweigeltrebe	1.710[a]
Saint Laurent	1.891[a]
Pinot Noir	5.464[b]
Muller Thurgau	5.954[b]

We found that Cabernet (1.531[a] g/kg DM); Saint Laurent (1.661[a] g/kg DM); Zweigeltrebe (1.710[a] g/kg DM);, Saint Laurent (1.891[a] g/kg DM); are no statistically significant different in total phenol content but are statistically significant different from Muller Thurgau (5.464[b] g/kg DM); and Pinot Noir(5.464[b] g/kg DM).

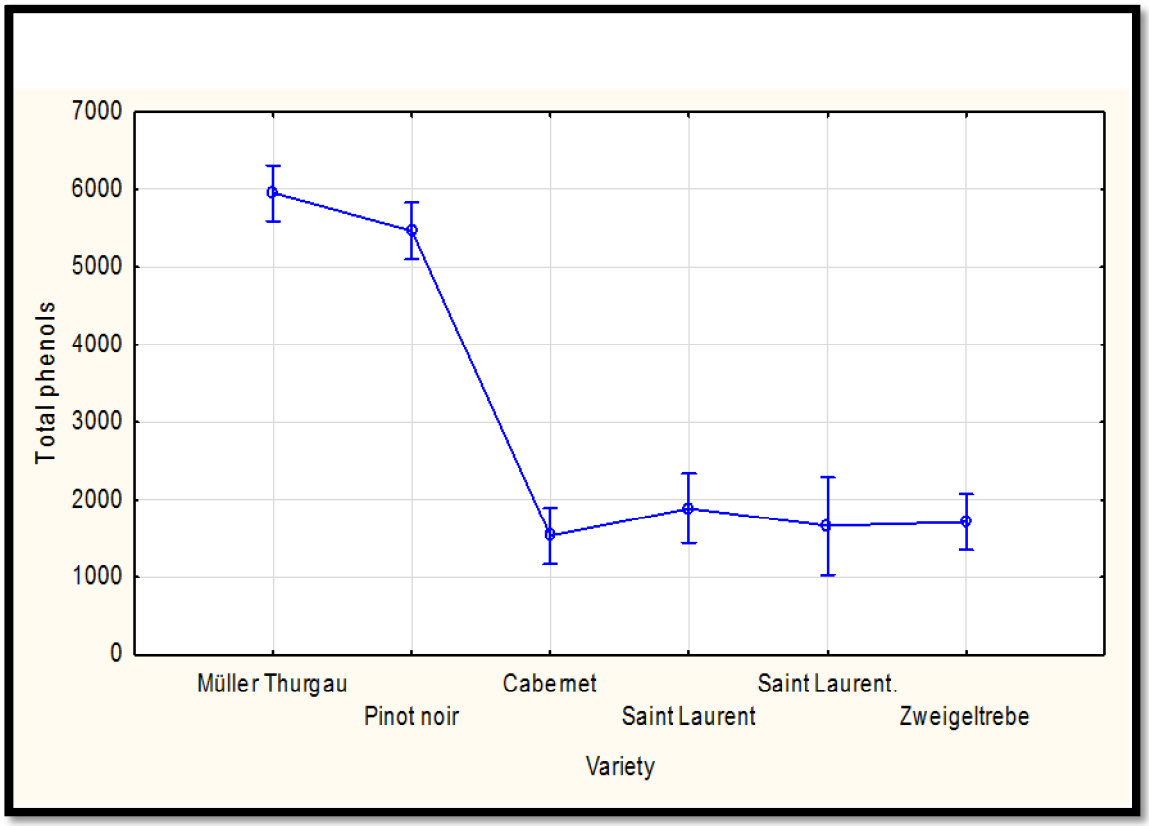


Figure 8. Evaluation of varieties in Velké Bílovice

Table 4. Evaluation of varieties in Hustopeļe

Varieties	**Total phenols average (mg/Kg DM)**
Zweigeltrebe	2.917[c]
Mixture of red grape	3.499[b,c]
Mixture of white grape	4.592[a,b]
Welschriesling	4.725[a,b]
Chardonnay	5.402[a]
A mixture of white grapes	5.504[a]

There is no statistical significant difference between mixtures of white grapes (4.592[a,b] g/kg DM) and Chardonnay (5.402[a] g/kg DM) but are statistically significant different from Zweigeltrebe (2.917[c] g/kg DM) and Mixture of red grape (3.499[b,c] g/kg DM).

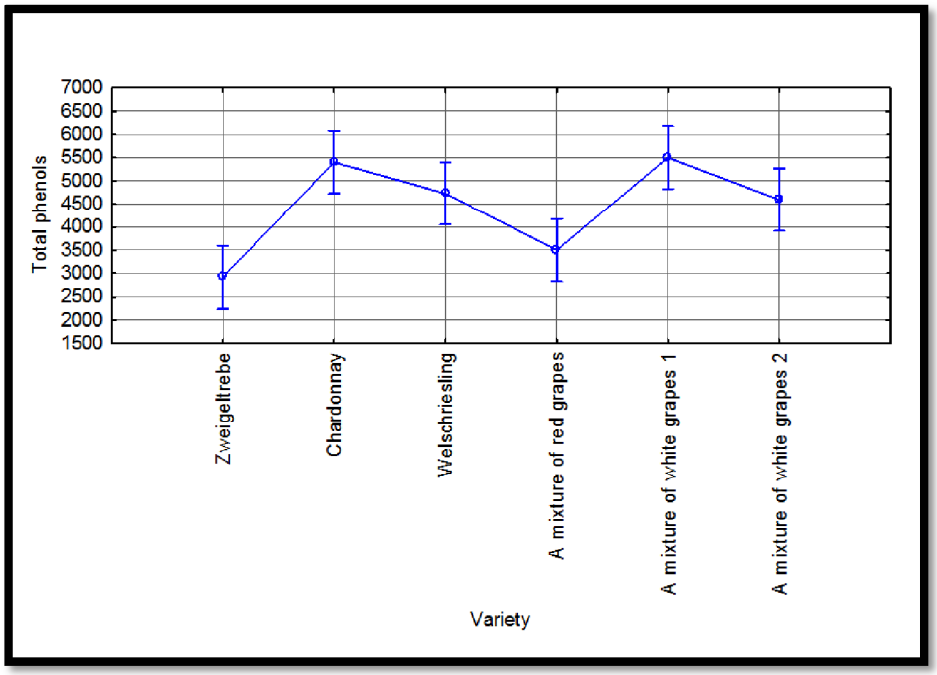


Figure 9 Evaluation of varieties in Hustopeļ e

Table 5. Evaluation of varieties in Praha-Grébovka

Varieties	Total phenols Average (mg/Kg DM)
Zweigeltrebe	1.765[a]
Hybernal	2.2065[a]
Neronet	2.190[a]
Pinot Noir	4.133[b]
Pinot Gris	4.817[b]
Muller Thurgau	6.800[c]

No statistical significant difference fond between Hybernal (2.2065[a] g/kg DM), Zweigeltrebe (1.765[a] g/kg DM), and Neronet (2.190[a] g/kg DM), but are statistically significant different from Pinot noir (4.133[b] g/kg DM) and Pinot Gris (4.817[b] g/kg DM). Muller Thurgau (6.800[c] g/kg DM) is statistically significant different from all other varieties we found in Praha-Grébovka. No statistical significant difference found between Pinot Gris (4.817[b] g/kg DM) and Pinot Noir (4.133[b] g/kg DM).

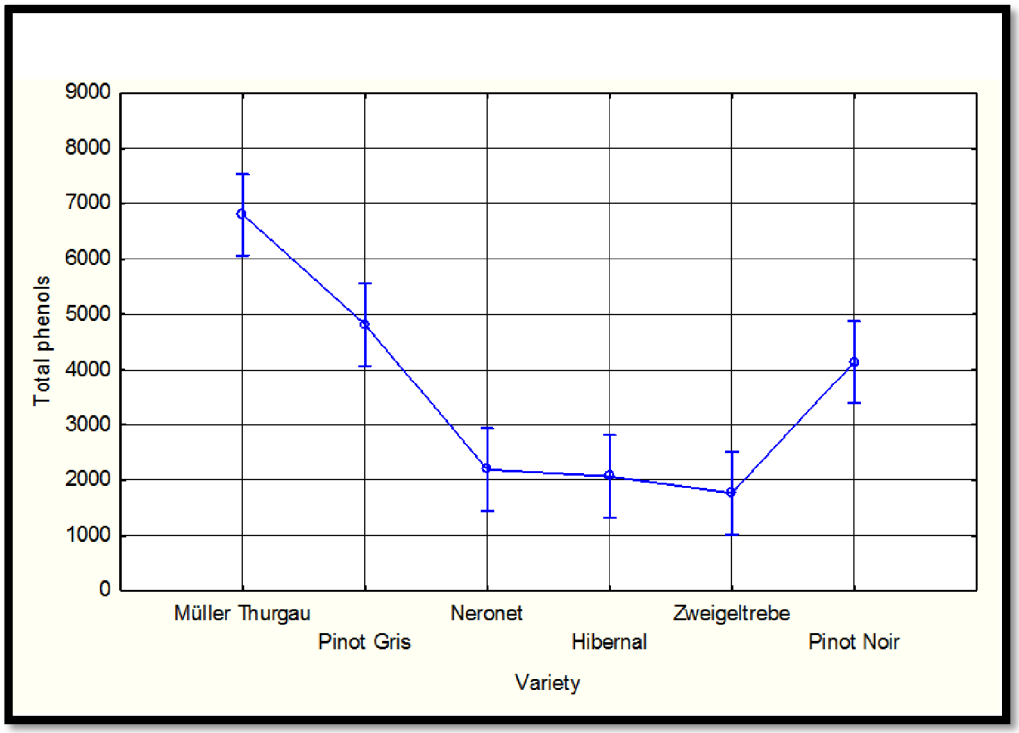


Figure 10. Evaluation of varieties in Praha-Grébovka

Table 6. Evaluation of varieties in Karlštejn

Varieties	Total phenols averages (mg/Kg DM)
Pinot Noir	4.295[a]
Zweigeltrebe	4.828[a]
Saint Laurent	4.866[a]
Pinot Gris	6.142[a]
Traminer Rot	6.232[a]
Muller Thurgau	6.254[a]

No statistical significant difference found in this region of Karlštejn in all samples we collected from this place.

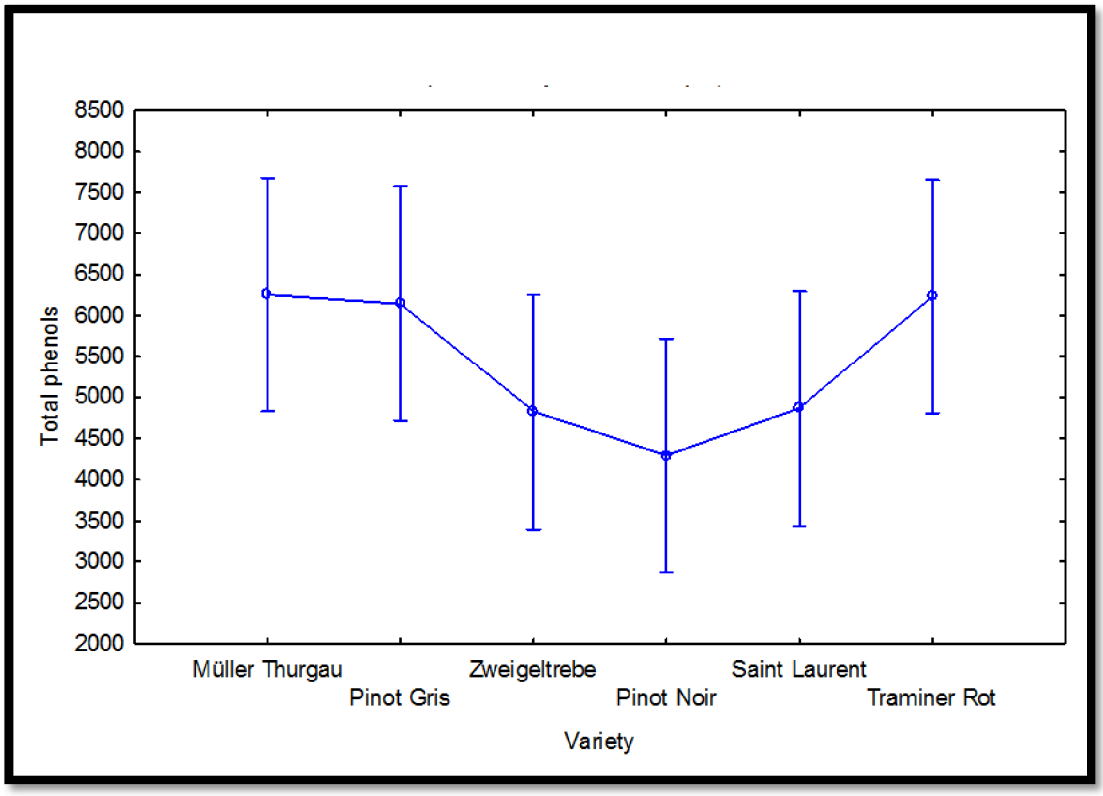


Figure 11. Evaluation of varieties in Karlštejn

6.2 Evaluation of localities

One-way ANOVA

Table 7. Evaluation phenol content of Muller Thurgau in different regions

Varieties	**Total Phenols average (mg/kg DM)**
Velké Bílovice	5.954[a]
Karlštejn	6.254[a]
Praha-Grébovka	6.800[a]
MĚlník	7.536[a]

Variety Muller Thurgau does not show any statistical significant difference in these 5 assessed regions.

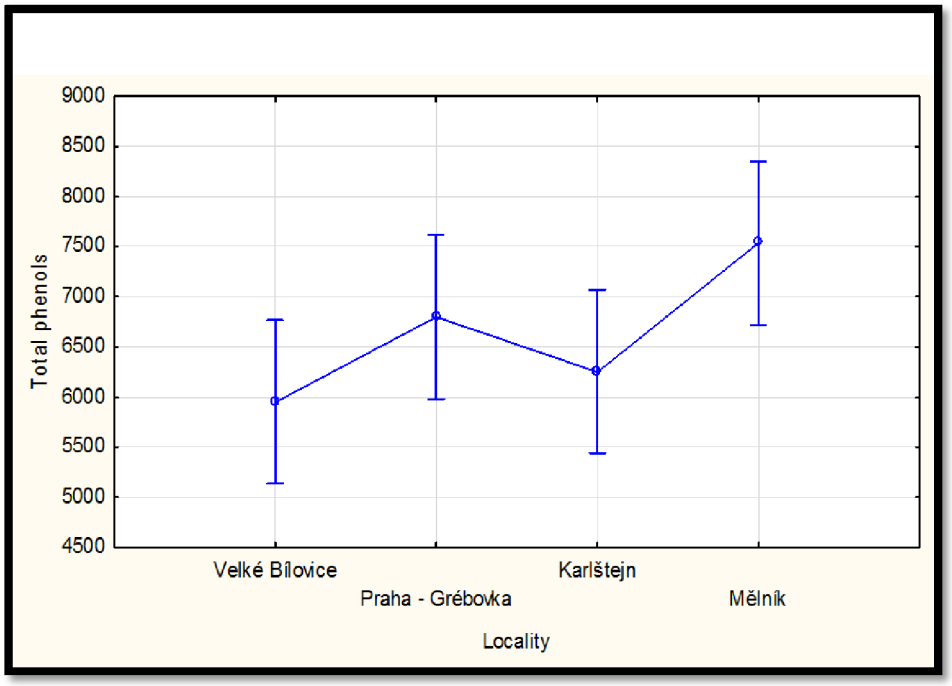


Figure 12. Evaluation phenol content of Muller Thurgau in different regions

Table 8. Evaluation phenol content of Pinot Noir in different regions

Varieties	**Total phenol average (mg/Kg DM)**
Praha-Grébovka	4.133[a]
Karlštejn	4.295[a]
Velké Bílovice	5.464[b]

The variety Pinot Noir proved a statistical significant difference between Karlštejn, Praha-Grébovka and Velké Bílovice.

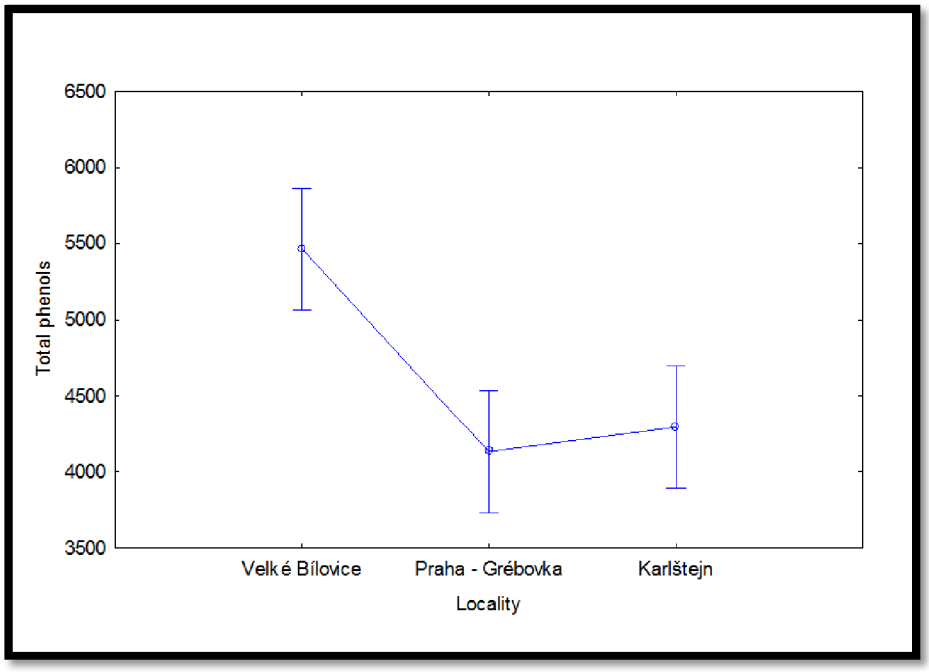


Figure 13. Evaluation phenol content of Pinot Noir in different regions

Table 9. Evaluation phenol content of Zweigeltrebe in different regions

Varieties	**Total phenols content average (mg/Kg DM)**
Velké Bílovice	1.710[a]
Praha-Grébovka	1.765[a]
Hustopeļe	2.917[a,b]
Karlģtejn	4.828[a]

No absolute statistical significant difference found among these areas.

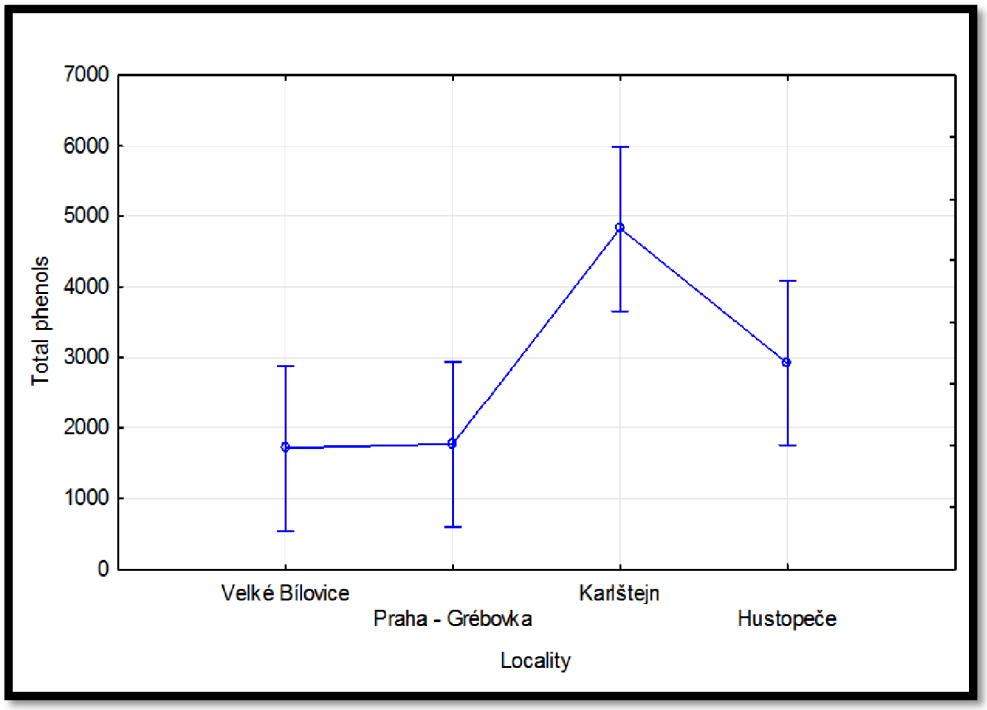


Figure 14. Evaluation phenol content of Zweigeltrebe in different regions

6.3 Antioxidant activity and total phenols relationship

For this analysis we used the method of Regression and Correlation analysis

Independent variable x: total phenols; dependent variable y: antioxidant activity

Statistical summary

R	0.510
R^2	O.260
R^2	0.230
F(1-24)	8.412
p	0.007

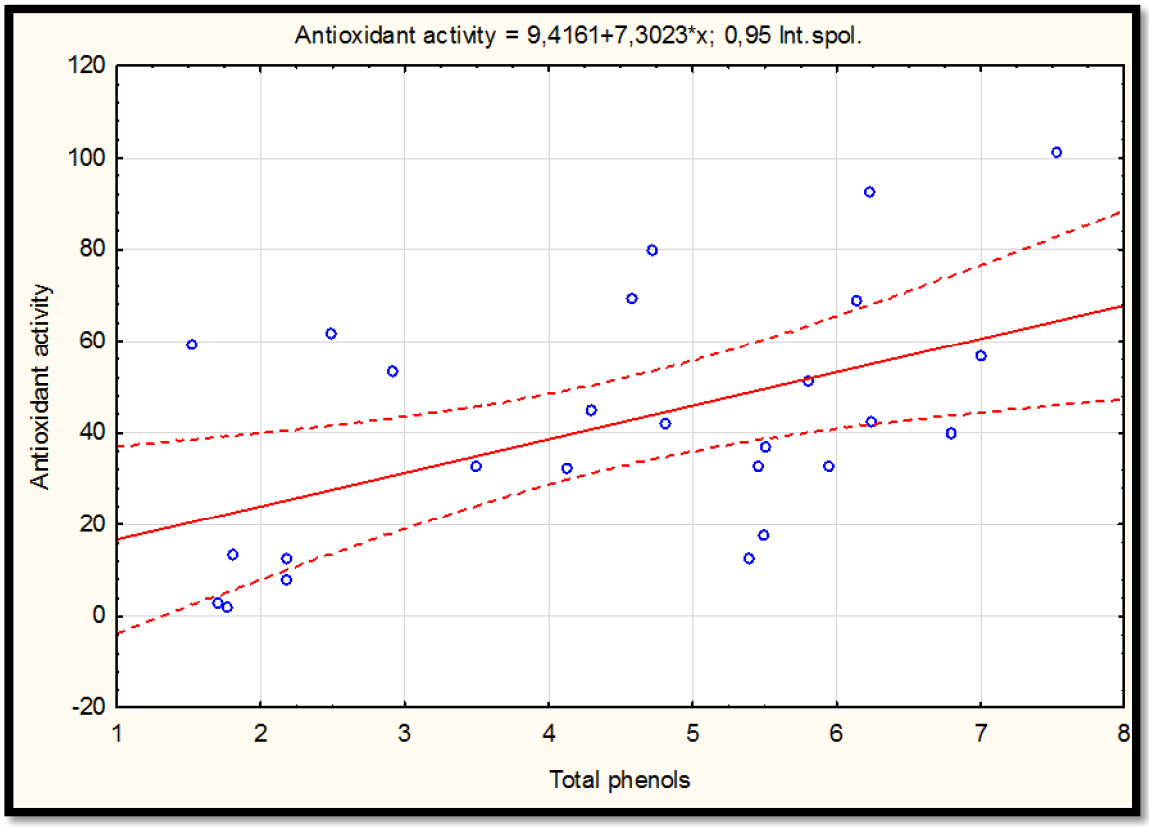


Figure 15. Scatter plot of correlation of antioxidant activity Vs total phenols.

For N=26 the Pearson correlation coefficient the critical value is R=0.496; we have correlation coefficient value R= 0.510, Because it is greater than critical value we can say that between antioxidant activity and total polyphenol content exists statistical significant correlation at pÒ0.005 (our p value is 0.008).

Table 10. Variety total polyphenol mean and standard deviation (g/kg DM)

Grape Variety	Mean	Standard Deviation
Chardonnay	5.4	0.4
Cabernet	1.5	0.1
Hibernal	2.2	0.2
Laurot	2.5	0.2
Müller Thurgau	6.6	0.7
Neronet	2.2	0.3
Pinot Noir	4.6	0.7
Pinot Gris	5.5	0.9
Welschriesling	5.9	1.6
Mixture of white varieties	5.0	0.6
Mixture of red varieties	3.5	0.8
Saint. Laurent	3.7	1.3
Zweigeltrebe	3.1	1.9
Traminer Rot	6.2	0.5

The determination of total polyphenol content by variety grouped according to colour of their grape seeds (g/kg DM)

Table 11: Total phenol content in different varieties (g/kg DM)

Variety	**Total polyphenols**	**Standard deviation**	**Variety**	**Total polyphenols**	**Standard deviation**
White varieties	**5.3**	**1.5**	**Red Varieties**	**3.0**	**1.0**
Hibernal	2.2	0.2	Cabernet	1.5	0.1
Müller Thurgau	6.6	0.7	Laurot	2.5	0.2
Pinot Gris	5.5	0.9	Neronet	2.2	0.3
Welschriesling	5.9	1.6	Pinot Noir	4.6	0.7
mixture of white	5.0	0.6	Mixture of red var.	3.5	0.8
Chardonnay	5.4	0.4	St. Laurent	3.7	1.3
Traminer Rot	6.2	0.5	Zweigeltrebe	3.1	1.9

Table 12: Average of total Phenol content in vine growing region and subregion

Region	**Total**	**Standard**	**Region**	**Total**	**Standard**

	polyphenols (g/kg DM)	deviation		polyphenols ($g.kg^{-1}$)	deviation
ohemia	**5.1**	**1.4**	**loravia**	**3.8**	**0.3**
lník	7.3	0.4	ustopeļe	4.4	1.0
arlģtejn	5.7	0.7	ednice	2.5	0.2
ague - Grébovka	3.6	2.0	elké Bílovice	3.3	2.2

Table 13: Classification according to colour of grape

White	Total phenols (g/kg	Standard deviation	Antioxidant activity (mg/g)	Red	Total phenols (g/kg	Standard deviation	Antioxidant activity (mg/g)
Müller Thurgau	6.0	0.5	32.7	Zweigeltrebe	2.9	0.2	53.2
Chardonay	5.4	0.4	12.3	Laurot	2.5	0.2	61.5
Welschriesling	4.7	0.4	79.6	Pinot noir	5.5	0.1	32.4
Müller Thurgau	7.5	0.3	101.1	Zweigeltrebe	5.8	1.4	50.9
Mixture of white varieties	4.6	0.2	69.0	Saint Laurent	5.5	1.0	36.8
Welschriesling	7.0	0.9	56.4	Cabernet	1.5	0.1	58.9
Müller Thurgau	6.8	0.2	39.8	Pinot noir	4.3	0.3	45.0
Pinot Gris	4.8	1.1	41.7	Neronet	2.2	0.3	12.4
Hibernal	2.2	0.2	7.8	Saint Laurent	1.8	0.1	13.4
Pinot Gris	6.1	0.1	68.6	Zweigeltrebe	1.7	0.1	2.5
Traminer Rot	6.2	0.5	92.1	Zweigeltrebe	1.8	0.1	1.7
Müller Thurgau	6.3	0.8	42.4	Pinot noir	4.1	0.3	32.0
Mixture of white varieties	5.5	0.2	17.3	Mixture of red varieties	3.5	0.8	32.7
Average			50.8				33.3

The analysis showed that white grape seeds have both higher antioxidants activity and TP content than red grape seeds. This proves the relationship between antioxidant activity and total phenol content. This is in the same line as Lachman et al. (2009), who found a significant

relationship between the total concentrations of phenol compounds in conventional and ecological red and white wines and the antioxidant activity determined by DPPH assay (Lachman, et al., 2009)

Table 14: Classification according to vine growing region

	Region	Name	Color	Total phenols (g/kg	Standard deviation	Antioxidant activity (ε g/g DM)
	Hustopeče	Chardonay	White	5.4	0.4	12.3
	Hustopeče	Mixture of white varieties	White	5.5	0.2	17.2
	Hustopeče	Welschriesling	White	4.7	0.4	79.6
	Hustopeče	Zweigeltrebe	Red	2.9	0.2	53.2
	Hustopeče	Mixture of white varieties	White	4.6	0.2	69.0
	Hustopeče	Mixture of red varieties	Red	3.5	0.8	32.6
Average				4.4	0.4	44.0
STD				1.038589	0.23	27.6
	Karlštejn	Zweigeltrebe	Red	5.8	1.4	51.0
	Karlštejn	Saint Laurent	Red	5.5	1	36.8
	Karlštejn	Pinot noir	Red	4.3	0.3	45.0
	Karlštejn	Pinot Gris	White	6.1	0.1	68.6

	Karlštejn	Traminer Rot	White	6.2	0.5	92.1
	Karlštejn	Müller Thurgau	White	6.3	0.8	42.4
Average				5.7	0.7	56.0
STD				0.7	0.5	20.8
	Lednice	Laurot	Red	2.5	0.2	61.5
AVERAGE				2.5	0.2	61.5
	Mělník	Müller Thurgau	White	7.5	0.3	10.1
	Mělník	Welschriesling	White	7	0.9	56.4
Average				7.3	0.6	78.7
STD				0.3	0.4	31.6
	Prague Grébovka	Müller Thurgau	White	6.8	0.2	39.8
	Prague Grébovka	Pinot Gris	White	4.8	1.1	41.7
	Prague Grébovka	Neronet	Red	2.2	0.3	12.4
	Prague Grébovka	Hibernal	White	2.2	0.2	7.8
	Prague Grébovka	Zweigeltrebe	Red	1.8	0.1	1.7
	Prague Grébovka	Pinot noir	Red	4.1	0.3	32.0
Average				3.6	0.37	22.5
STD				1.9	0.37	17.4
	Velké Bílovice	Müller Thurgau	White	6	0.5	32.7
	Velké Bílovice	Pinot noir	Red	5.5	0.1	32.4

	Velké Bílovice	Cabernet	Red	1.5	0.1	58.9
	Velké Bílovice	Saint Laurent	Red	1.8	0.1	13.4
	Velké Bílovice	Zweigeltrebe	Red	1.7	0.1	2.5
Average				3.3	0.18	28.0
STD				2.3	0.2	21.5

Grapes from Mikulník have greater antioxidants activities the average is 31.5 mg/g. In this study grapes from Velké Bílovice have the lowest level of antioxidant activities. The analysis showed that agricultural region has an influence on the antioxidant activities; this is proved by that same grape variety grown in different regions has also different antioxidant activity. Müller Thurgau grown in Velké Bílovice has 32.6 mg/g but Müller Thurgau grown in Mikulník has 101.0 mg/g and Müller Thurgau grown in Karlštejn has 42.4 mg/g.

Table 15. Classification according to grape varieties

	Region	**Name**	**Colour**	**Total phenols (g/kg DM)**	**Standard Deviation**	**Antioxidant activity (mg/g DM)**
	Velké Bílovice	Cabernet	Red		0.1	58.9
	Hustopeče	Chardonnay	White	5.4	0.4	12.3
Average				3.5	0.3	35.6
STD				2.8	0.2	32.9
	Prague Grébovka	Hibernal	White	2.2	0.2	7.8

Average				2.2	0.2	7.8
	Lednice	Laurot	Red	2.5	0.2	61.5
Average				2.5	0.2	61.5
	Hustopeče	Mixture of red varieties	Red	3.5	0.8	32.6
Average				3.5	0.8	32.6
	Hustopeče	Mixture of white varieties	White	5.5	0.2	17.3
	Hustopeče	Mixture of white varieties	White	4.6	0.2	69.0
Average				5.1	0.2	43.1
STD				0.6	0.0	36.6
	Karlštejn	Müller Thurgau	White	6.3	0.8	42.4
	Mělník	Müller Thurgau	White	7.5	0.3	101.1
	Prague Grébovka	Müller Thurgau	White	6.8	0.2	39.8
	Velké Bílovice	Müller Thurgau	White	6.0	0.5	32.7
Average				6.7	0.5	54.0
STD				0.7	0.3	31.7
	Prague Grébovka	Neronet	Red	2.2	0.3	12.4
Average				2.2	0.3	12.4
STD						0.0
	Karlštejn	Pinot Gris	White	6.1	0.1	68.6
	Prague Grébovka	Pinot Gris	White	4.8	1.1	41.7

Average				5.5	0.6	55.1
STD				0.9	0.7	19.0
	Karlštejn	Pinot Noir	Red	4.3	0.3	45.0
	Prague Grébovka	Pinot Noir	Red	4.1	0.3	32.0
	Velké Bílovice	Pinot Noir	Red	5.5	0.1	32.4
Average				4.6	0.2	36.5
STD				0.8	0.1	7.4
	Karlštejn	Saint Laurent	Red	5.5	1.0	36.8
	Velké Bílovice	Saint Laurent	Red	1.8	0.1	13.4
Average				3.7	0.6	25.1
STD				2.6	0.6	16.6
	Karlštejn	Traminer Rot	White	6.2	0.5	92.1
Average				6.2	0.5	92.1
	Hustopeče	Welschriesling	White	4.7	0.4	79.6
	Mělník	Welschriesling	White	7.0	0.9	56.4
Average				5.9	0.7	68.0
STD				1.6	0.4	16.4
	Hustopeče	Zweigeltrebe	Red	2.9	0.2	53.2
	Karlštejn	Zweigeltrebe	Red	5.8	1.4	50.9
	Prague Grébovka	Zweigeltrebe	Red	1.8	0.1	1.7

	Velké Bílovice	Zweigeltrebe	Red	1.7	0.1	2.5
Average				3.1	0.5	27.1
STD				1.9	0.6	28.9

The antioxidants activity depends on variety of grape seeds. And it is proved by that grape seed grown in the same environmental conditions have different antioxidant activity variety.

7. Conclusion

This research showed that the polyphenol content in white grape seeds varieties is higher than TP content in red grape seed varieties. The antioxidant activity is also higher in white grape seeds than in red grape seeds, the average in white is 51.0 mg/g in white varieties and 33.3 mg/g in red grape seeds varieties. We found that there is a relationship between antioxidant activity and TP concentration, this is in the same line as Lachman et al. 2009, who found a significant relationship between the total concentrations of phenol compounds in conventional and ecological red and white wines and the antioxidant activity determined by DPPH assay, this also verified our hypothesis which was supposing that there is a relationship between antioxidant activity and TP content in grape seeds.

The content of TP and antioxidant activity depends on the region of cultivation and on the variety. In our research we found that grapes seeds from MÌlník have greater antioxidants activities the average is 31.6 mg/g. In this study grapes from Velké Bílovice have the lowest level of antioxidant activities. The analysis showed that agricultural region has an influence on the antioxidant activities; this is proved by that same grape variety grown in different regions has different antioxidant activity. Müller Thurgau grown in Velké Bílovice has 32.6 mg/g but Müller Thurgau grown in MÌlník has 101 mg/g and Müller Thurgau grown in Karlštejn has 42.3 mg/g. Pearson correlation coefficient the critical value is R=0.496; we have correlation coefficient value R= 0.510, because it is greater than critical value we can say that antioxidant activity and total polyphenol content correlated. Statistical significant correlation at pÒ0.005 (our p value is 0.008).

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APPENDIXES

Annexe 1: Triplicate of TP content measurement.

	Sample	**Variety**	**(absorbance) 765,0 nm**	**Concentration**	**Errors**	**Weight**	Region	Weight
	1A	Müller Thurgau	0.301	130.105		0.508	Velké Bílovice	0.505
	1B	Müller Thurgau	0.243	107.585		0.505	Velké Bílovice	0.501
	1C	Müller Thurgau	0.282	122.917		0.501	Velké Bílovice	0.508
Average			0.300	120.200		0.500		0.505
Standard deviation			0.000	11.500		0.000		0.501
	2a	Chardonnay	0.218	97.984	None	0.508	Hustopeļe	0.508
	2B	Chardonnay	0.263	115.278	None	0.52	Hustopeļe	0.505
	2C	Chardonnay	0.273	119.151	None	0.51	Hustopeļe	0.501
Average								0.508
Standard deviation								0.505
	3A	A mixture of White gra	0.264	115.748	None	0.499	Hustopeļe	0.501

	3B	A mixture of white grapes	0.253	111.591	None	0.508	Hustopeļe	0.508
	3C	A mixture of white grapes	0.238	105.661	None	0.506	Hustopeļe	0.505
Average								0.501
Standard deviation								0.508
	4A	Welschriesling	0.180	82.925	None	0.497	Hustopeļe	0.505
	4B	Welschriesling	0.218	97.751	None	0.500	Hustopeļe	0.501
	4C	Welschriesling	0.231	102.739	None	0.502	Hustopeļe	0.508
Average								0.505
Standard deviation								0.501
	5A	Zweigeltrebe	0.131	64.096	None	0.497	Hustopeļe	0.508
	5B	Zweigeltrebe	0.111	56.080	None	0.503	Hustopeļe	0.505
	5C	Zweigeltrebe	0.108	54.891	None	0.501	Hustopeļe	0.501
Average								0.508
Standard deviation								0.505
	6A	Müller Thurgau	0.353	150.535	None	0.507	Mlýník	0.501
	6B	Müller Thurgau	0.350	149.209	None	0.512	Mlýník	0.508

	6C	Müller Thurgau	0.372	158.014	None	0.500	Mělník	0.505
Average								0.501
Standard deviation								0.508
	7A	A mixture of white grapes	0.197	89.622	None	0.505	Hustopeče	0.505
	7B	A mixture of white grapes	0.215	96.783	None	0.501	Hustopeče	0.501
	7C	A mixture of white grapes	0.198	89.993	None	0.499	Hustopeče	0.508
Average								0.505
Standard deviation								0.501
	8A	Laurot	0.085	45.931	None	0.51	Lednice	0.508
	8B	Laurot	0.097	50.766	None	0.506	Lednice	0.505
	8C	Laurot	0.106	54.347	None	0.500	Lednice	0.501
Average								0.508
Standard deviation								0.505
	9A	Welschriesling	0.269	117.812	None	0.502	Mělník	0.501
	9B	Welschriesling	0.346	147.861	None	0.513	Mělník	0.508

	9C	Welschriesling	0.374	158.86	None	0.500	Mělník	0.505
Average								0.501
Standard deviation								0.508
	10A	A mixture of red grapes	0.187	85.843	None	0.506	Hustopeče	0.505
	10B	A mixture of red grapes	0.093	49.287	None	0.526	Hustopeče	0.501
	10C	A mixture of red grapes	0.170	79.334	None	0.507	Hustopeče	0.508
Average								0.505
Standard deviation								0.501
	11A	Müller Thurgau	0.321	137.945	None	0.502	Praha - Grébovka	0.508
	11B	Müller Thurgau	0.327	140.369	None	0.502	Praha - Grébovka	0.505
	11C	Müller Thurgau	0.303	131.045	None	0.501	Praha - Grébovka	0.501
Average								0.508
Standard deviation								0.505
	12A	Pinot Noir	0.244	108.118	None	0.504	Velké Bílovice	0.501
	12B	Pinot Noir	0.257	113.030	None	0.510	Velké Bílovice	0.508

	12C	Pinot Noir	0.250	110.404	None	0.503	Velké Bílovice	0.505
Average								0.501
Standard deviation								0.508
	13A	Zweigeltrebe	0.115	57.815	None	0.506	Karlštejn	0.505
	13B	Zweigeltrebe	0.255	112.208	None	0.506	Karlštejn	0.501
	13C	Zweigeltrebe	0.282	122.622	None	0.504	Karlštejn	0.508
Average								0.505
Standard deviation								0.501
	14A	Saint Laurent	0.157	74.185	none	0.519	Karlštejn	0.508
	14B	Saint Laurent	0.227	101.193	None	0.512	Karlštejn	0.505
	14C	Saint Laurent	0.283	123.151	None	0.506	Karlštejn	0.501
Average								0.508
Standard deviation								0.505
	15A	Pinot Gris	0.177	81.736	None	0.517	Praha - Grébovka	0.501
	15B	Pinot Gris	0.289	125.348	None	0.497	Praha - Grébovka	0.508
	15C	Pinot Gris	0.181	83.352	None	0.497	Praha - Grébovka	0.505
Average								0.501
Standard								0.508

deviation								
	16A	Cabernet	0.041	28.774	None	0.503	Velké Bílovice	0.505
	16B	Cabernet	0.054	33.994	None	0.510	Velké Bílovice	0.501
	16C	Cabernet	0.046	30.607	None	0.511	Velké Bílovice	0.508
Average								0.505
Standard deviation								0.501
	17A	Pinot Noir	0.169	78.809	None	0.503	Karlštejn	0.508
	17B	Pinot Noir	0.192	87.531	None	0.501	Karlštejn	0.505
	17C	Pinot Noir	0.208	93.874	None	0.51	Karlštejn	0.501
Average								0.508
Standard deviation								0.505
	18A	Neronet	0.062	36.959	None	0.498	Praha - Grébovka	0.501
	18B	Neronet	0.102	52.606	None	0.503	Praha - Grébovka	0.508
	18C	Neronet	0.074	41.902	None	0.499	Praha - Grébovka	0.505
Average								0.501
Standard deviation								0.508
	19A	Saint Laurent	0.065	38.025		0.502	Velké Bílovice	0.505
	19B	Saint Laurent	0.065	38.075		0.504	Velké Bílovice	0.501

	19C	Saint Laurent.	0.053	33.354		0.502	Velké Bílovice	0.508
Average								0.505
Standard deviation								0.501
	20A	Zweigeltrebe	0.05	32.335		0.508	Velké Bílovice	0.508
	20B	Zweigeltrebe	0.057	34.935		0.511	Velké Bílovice	0.505
	20C	Zweigeltrebe	0.063	37.245		0.509	Velké Bílovice	0.501
Average								0.508
Standard deviation								0.505
	21A	Hibernal	0.063	37.268		0.508	Praha - Grébovka	0.501
	21B	Hibernal	0.091	48.172		0.500	Praha - Grébovka	0.508
	21C	Hibernal	0.085	45.980		0.500		
Average								0.501
Standard deviation								0.508
	22A	Zweigeltrebe	0.053	33.717		0.499	Praha - Grébovka	0.505
	22B	Zweigeltrebe	0.057	34.976		0.501	Praha - Grébovka	0.501
	22C	Zweigeltrebe	0.063	37.454		0.503	Praha - Grébovka	0.508
Average								0.505
Standard								0.501

deviation								
	23A	Pinot Gris	0.209	94.354		0.502	Karlštejn	0.508
	23B	Pinot Gris	0.323	138.649		0.51	Karlštejn	0.505
	23C	Pinot Gris	0.325	139.432		0.503	Karlštejn	0.501
Average								0.508
Standard deviation								0.505
	24A	Traminer Rot	0.267	116.804		0.509	Karlštejn	0.501
	24B	Traminer Rot	0.275	120.226		0.503	Karlštejn	0.508
	24C	Traminer Rot	0.323	138.856		0.497	Karlštejn	0.505
Average								0.501
Standard deviation								0.508
	25A	Müller Thurgau	0.289	125.635		0.496	Karlštejn	0.505
	25B	Müller Thurgau	0.237	105.089		0.505	Karlštejn	0.501
	25C	Müller Thurgau	0.338	144.532		0.5	Karlštejn	0.508
Average								0.505
Standard deviation								0.501
	26A	Pinot Noir	0.168	78.394		0.51	Praha - Grébovka	0.508
	26B	Pinot Noir	0.199	90.541		0.503	Praha - Grébovka	0.505

	26C	Pinot Noir	0.175	81.15		0.5	Praha - Grébovka	0.501
Standard deviation								0.508