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# ECONOMIC POTENTIAL OF APPLING GRAPE SEED EXTRACT AS A NATURAL ANTIOXIDANT

# EKONOMSKI POTENCIJAL PRIMENE EKSTRAKTA SEMENKE GROŽĐA KAO PRIRODNI ANTIOKSIDANT

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## ABSTRACT

*Grape growing contributes to the Serbian economy in diverse ways (exports, tourism and, of course, wines, juices, raisins and table grapes). The grape products are rich sources of health-promoting polyphenols. During winemaking, large quantities of wastes such as grape marc (the residue after pressing for white wines or vinification for red wines) stalks and seed are produced. In the Serbia, the grape industry was produced 431 000 tons of grapes in 2009. From this, an estimated near 240 million L wine and 190 000 tons pomace (which comprised the pulps and seeds left over after pressing grapes for juice and wine). Grape seed is an important by-product of grape processing. This by-product is characterized by high-phenol contents because of poor extraction during winemaking, so that its use supports sustainable agricultural production. The results of spectrophotometric analysis of Vranac grape seed extract (GSE) show high contents of total phenols (385.21±5.29 mg gallic acid equivalent g-1); hydroxyl-cinnamoyl tartaric acids (10.44±0.69 mg caffeic acid equivalent g-1) and flavonols (7.44 ± 0.52 mg quercetin equivalent g-1) reflecting their high antioxidant activity (99.02 ± 0.11%). The GSE is a complex mixture of polyphenols and antioxidant activity was expressed as the amount of extract needed to quench certain amount of 2,2`- diphenyl-1-picrylhydrazyl free radical (DPPH). The GSE, constitute a very cheap source for the extraction of antioxidants, which can be an alternative source for obtaining natural antioxidants (dietary supplements), thus providing an important economic advantages.*

***Key words:** grape seed, economic potential, polyphenol, natural antioxidant.*

## REZIME

*Vinogradarstvo doprinosi srpskoj ekonomiji na različite načine (izvozu, turizmu i naravno, proizvodnji vina, sokova, suvog i stonog grožđa). Proizvodi grožđa su bogati izvori korisnih po zdravlju polifenola. Ova jedinjenja su veoma važna za industriju hrane zbog njihovih bioloških svojstava (deluju protiv starenja, kancera, mikroba i sl.) i antioksidativne aktivnosti. U Srbiji, u 2009. godini dobijeno je 431 000 tona grožđa, a od toga oko 240 miliona L vina i 190 000 tona komine (koja se sastoji od pulpe i semenke). Ovaj nusproizvod se karakterizuje visokim sadržajem polifenola zbog nedovoljne ekstrakcije tokom proizvodnje vina, tako da je njegova primena značajna za održivu poljoprivrednu proizvodnju. Rezultati spektrofotometrijske analize ekstrakta semenke grožđa Vranca pokazuju visoki sadržaj ukupnih fenola (385,21± 5,29 mg galne kiseline kao ekvivalent g-1); estara hidroksicimetne i vinske kiseline (10.44 ± 0.69 mg kafeinska kiselina kao ekvivalent g-1) i flavonola (7,44±0,52 mg kvercentin kao ekvivalent g-1), što se reflektuje na njihovu visoku antioksidativnu aktivnost (99,02 ± 0,11%.). Ekstrakt semenke grožđa je kompleksna smeša polifenola, tako da antioksidativna aktivnost je izražena kao količina ekstrakta potrebna da "uhvati" određenu količinu 2,2`- difenil-1-pikrilhidrazil slobodnog radikala (DPPH). Ekstrakt semenke grožđa predstavlja jeftini izvor antioksidanata i kao alternativni izvor dobijanja prirodnih antioksidanata (dijetalnih dodataka) ima značajnu ekonomsku prednost.*

***Ključne reči:** semenke grožđa, ekonomski potencijal, polifenoli, prirodni antioksidant.*

## INTRODUCTION

The agricultural industries are producing increasing amounts of solid wastes. In the case of fruit process a large amount of marc is produced as residual of squeezing (*Hodur et al., 2009*). Grape growing contributes to the Serbian economy in diverse ways (exports, tourism and, of course, wines, juices, raisins and table grapes). The grape contain antioxidants and a large amount of biological active components was retained in the marc. The extraction of these utilizable component (pectin, antioxidants, polyphenols) can be beneficial from economical and environmental aspects.

The grape products are rich sources of health-promoting polyphenols. These compounds have been of great interest to the food industry due to their benefits as anti-aging, anticarcinogenic, antimutagenic, antimicrobial and as the inhibition of human low density lipoprotein oxidation (*Cook et al., 1996; Gey, 1990; Heim et al., 2002; Park et al*., 2011). Grape seeds, waste products from the wineries, are containing lipids, proteins, carbohydrates and 5-8% polyphenols depending on the variety.

During winemaking, large quantities of wastes such as grape marc (the residue after pressing for white wines or vinification for red wines) stalks and seed are produced. The placing of the enormous amount of waste grape marc generated in all over the world is an increasing problem. By processing 100 kilograms of grape-vine approximately 20-25 kilograms of grape marc is produced. For example, in Europe alone 112 million tons of grapes were processed by the wine industry in 1998, and an estimated 13% (14.5 million tons) of this amount corresponded to the by-product after pressing, consisting mainly of skins and seeds.

In the Serbia, the grape industry was produced 431 000 tons of grapes in 2009 (*Ministry of Agriculture, Forestry and Water Management of Serbia, www.minpolj.gov.rs*). From this, an estimated near 240 million L wine and 190 000 tons pomace (which comprised the pulps and seeds left over after pressing grapes for juice and wine). Grape seed is an important by-product of grape processing. Grape seed represents a rich source of various high-value products as ethanol, tartarates, malates, citric acid, grape seed oil, hydrocolloids and dietary fibre. This by-product is characterized by high-phenol contents because of poor extraction during winemaking, so that its use supports sustainable agricultural production. (*Arvanitoyannis et al., 2006; Yinronget al., 1999).* Grape from crushed seeds is used in cosmeceuticals and skincare products for many perceived health benefits. Grape seed oil is notable for its high contents of tocopherols (vitamin E), phytosterols, and polyunsaturated fatty acids such as linoleic acid, oleic acid and alpha-linoleic acid. Grape seed have been investigated previously as a potential feed for livestock and as an organic fertilizer (*Bertran et al., 2004; Celma et al., 2007; Ferrer et al., 2001*).

Grape seed extract (GSE) is a nutrient derived from the seeds of grapes which belongs to the bioflavonoid family. The active ingredients contained in grape seed extract are called "proanthocyanidins". Since the 1980s, biochemical and medical studies have demonstrated significant antioxidant properties of grape seed oligomeric proanthocyanidins. Grape seed is characterized by high-phenol contents because of poor extraction during winemaking, so that its use supports sustainable agricultural production. Polyphenols in grape seeds are mainly flavonoids, including the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin 3-O-gallate, and procyanidin dimers, trimers and more highly polymerized procyanidins (*Alonso et al., 2002; Gonzales-Paramas et al., 2004*). Together with tannins, polyphenols and polyunsaturated fatty acids, these seed constituents display inhibitory activities against several experimental disease models, including cancer, heart failure and other disorders of oxidative stress. Recent studies in animals, as well as some human studies, have shown that grape seed proanthocyanidin extracts possess a broad spectrum of biological, pharmacological and chemoprotective properties against free radicals and oxidative stress (*Heim et al., 2002*). Grape seed extract (GSE) is known as a powerful antioxidant that protects the body from premature aging, disease and decay. GSE is consumed worldwide as a dietary supplement because it exhibit both cancer-preventative and anticancer effects *in vitro* and *in vivo* (*Park et al*., 2011). In addition to their antioxidant and free radical scavenging activity, proanthocyanidins found in GSE have been reported to have antibacterial, antiviral, anti-allergic, anti-inflammatory and vasodilatory actions (*Cook et al., 1996; Gey, 1990; Radovanovic et al., 2008*). GSE has also proven to be valuable in the treatment of inadequate blood flow in the capillaries and veins. Small studies have shown increased capillary strength using as little as 50 milligrams/day, and increased venous blood flow using 150 milligrams/day. For this reason, grape seed extract has become popular in recent years as a nutritional supplement.

Recently, extraction of polyphenols from grape seeds has emerged as an opportune and vital business for the wine and food industry. The addition of antioxidants is a method of increasing shelf life, especially of lipids and lipid-containing foods. Nowadays, there has been a growing interest to use natural antioxidants especially of plant origin rather than synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The usages of these synthetic antioxidants have been restricted because they are suspected to have toxicological effects. The aim of this work is to evaluate grape seeds as a source of natural antioxidant for their possible use as dietary supplement or food antioxidants. To this purpose antioxidant capacity and total phenol, hydroxycinnamoyl tartaric acid and flavonol contents of Vranac GSE were investigated.

## MATERIAL AND METHOD

All chemicals used for analysis were of analytical grade and were obtained from Merck (Darmstadt, Germany) and Zorka (Šabac, Serbia). There was used the following chemicals: 2.2`-diphenyl-1-picrylhydrazyl free radical (DPPH), methanol, acetone, trifluoroacetic acid (TFA), quercetin, caffeic acid, gallic acid.

Analysis of GSEs were carried out by recording absorption spectra on the 8453 UV-visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Extracts were purified through a 0.45 µm syringe filter (Chromafil PVDF P-45/25; Macherey-Nagel) before analyses.

### Preparation of grape seed extracts (GSEs)

Samples of Vranac grape were collected in October 2009 grown in Niš-Moravski vineyard region (southern Serbia). Berries were carefully removed from the stalk, washed and frozen at -20°C and as that kept for analysis. The grape seeds were dried and ground to powder and were extracted with methanol/acetone/water/TFA (30:42:27.95:0.05) at room temperature and centrifugated at 4000 rpm. The procedure was repeated once more.

### Determination of total phenols, hydroxycinnamoyl tartaric acids and flavonols

Total phenol, hydroxycinnamoyl tartaric acid and flavonol contents in GSEs were determined spectrophotometrically by modified Glories method (*Mazza et al., 1999; Radovanović et al., 2010*). The grape extract sample was mixed with 20 mlL-1 HCl in 95 mlL-1 ethanol. After about 15 min the absorbances (A) at 280, 320 and 360 nm were measured using an Agilent 8453 UV-visible spectrophotometer. A280 was used to estimate total phenols (with gallic acid as standard), A320 nm was used to estimate hydroxycinnamoyl tartaric acids (with caffeic acid as standard) and A360 nm was used to estimate flavonols (with quercetin as standard).

### Determination of antioxidant activity using DPPH assay

The antioxidant activity of GSEs was analyzed by using DPPH assay (*Lachman et al., 2007; Radovanović et al., 2008, 2010*). This antioxidant assay is based on the measurement of DPPH colour loss due to the change in absorbance at 517 nm caused by the reaction of DPPH with the test sample. The reaction was monitored using an Agilent 8453 UV-visible spectrophotometer. The GSE was mixed with fresh 1 x $10^{-4}$ mol/L $DPPH^{\bullet}$ methanolic solution in a cuvette and left to incubate at room temperature. After 20 min the absorbance at 517 nm was read against a blank. The DPPH-scavenging activity of each sample was calculated from the decrease in absorbance according to the following relationship:

$$\text{Antioxidant activity (\%)} = [1 - (A_{sample} - A_{blank})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of control (1 x $10^{-4}$ $molL^{-1}$ DPPH methanolic solution), $A_{blank}$ is the absorbance of GSE sample and $A_{sample}$ is the absorbance of the GSE with the same concentration of DPPH radical. Three analytical replicates were carried out on each sample extract.

## Statistical analyses

Three analytical replicates were carried out on each GSE sample. Concentrations of phenolic compounds were measured and subjected to one-way analysis of variance (ANOVA). Measurements were averaged and results are given as mean ± standard deviation. The standard deviation was calculated by ANOVA using the Minitab statistical package (Minitab Inc., State College, PA, USA.

## RESULTS AND DISCUSSION

According to the "Food and Agriculture Organization" (FAO), 75 866 square kilometres of the world are dedicated to grapes. Approximately 71% of world grape production is used for wine, 27% as fresh fruit and 2% as dried fruit. In general, the nutritional mean value of grape was reported in Table 1.

The phenolic composition in grape varies widely and is usually determined by several factors, such as: the variety of grape and conditions under which they was grown (soil, geographical location, light exposure and weather).

Grape seeds are known to be a rich source of a number of phenolic compounds. Polyphenols in grape seeds are mainly flavonoids, including the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin 3-O-gallate, and procyanidin dimers, trimers and more highly polymerized procyanidins (*Downey, 2006*).

*Table 1. Nutritional mean value per 100 g grape**

| | |
|---|---|
| Energy | 288 kJ |
| Carbohydrates | 18.10 g |
| Sugars | 15.48 g |
| Dietary fibers | 900 mg |
| Fats | 160 mg |
| Proteins | 720 mg |
| Vitamin C | 10.80 mg |
| Niacin (Vit. $B_3$) | 0.19 mg |
| Vitamin $B_6$ | 0.09 mg |
| Vitamin $B_6$ | 0.09 mg |
| Thiamine (Vit. $B_1$) | 0.07 mg |
| Riboflavin (Vit. $B_2$) | 0.07 mg |
| Pantothenic acid ($B_5$) | 0.05 mg |
| Vitamin K | 22 μg |
| Folate (Vit. $B_9$) | 2 μg |
| Phosphorus | 20.00 mg |
| Calcium | 10.00 mg |
| Magnesium | 7.00 mg |
| Iron | 0.36 mg |
| Manganese | 0.07 mg |

* Source: USDA National Nutrient Database for Standard Reference *(Agriculture Research Service, 2010, www.nal.usda.gov*)

Vranac grape is an autochthonic variety with a very expressive vegetative power of vines. Grape berry is large or medium-large (from 150 to 300 g), round or slightly oval with a thin dark blue skin and abundant pedicellate. Grape juice is colorless and neutral smell. In juice of ripe grapes is collected from 20 to 24% sugar and total acids 6 to 7 g dm-3. The grapes are used for production top and quality red wines. In the appropriate environmental conditions, this variety has great economic importance because that the grape and wine of Vranac are very appreciated in the domestic and foreign markets.

The results of spectrophotometric analysis of GSEs show high contents of total phenols (385.21±5.29 mg gallic acid equivalent g-1); hydroxylcinnamoyl tartaric acids (10.44±0.69 mg caffeic acid equivalent g-1) and flavonols (7.44±0.52 mg quercetin equivalent g-1) reflecting their high antioxidant activity (99.02± 0.11%). The amount and types of phenol compounds present in a particular GSE can vary and is greatly influenced by the extraction process, as well as the source, variety and storage of the used seeds. Antioxidant activity is the common assay used and widely accepted by researchers as an anticancer indicator (*Tsai et al., 2005*).

Recently, the antioxidant power and health benefits of GSE have been demonstrated by scientific studies and this leads to their use as a dietary supplement and food additive (*Nakamura et al., 2003*).

## CONCLUSION

Our analysis shows that in 1 kg of Vranac grape is about 10 g of seeds, which contain almost 4 g total polyphenols. The spectrophotometric analysis of GSEs show high contents of total phenols, hydroxylcinnamoyl tartaric acids and flavonols, reflecting their high antioxidant activity. If is known that GSE has 20 times the antioxidant value of vitamin C and 50 times the antioxidant value of vitamin E, it is clear the nutritional, biological and economic power of SGE as dietary supplement and natural antioxidant. SGE is shaping up to be a powerful alternative to older traditional supplements.

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## REFERENCES

Alonso A., Guillean D., Barroso C., Puertas B, Garcia A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. Journal of Agricultural Food and Chemistry, 50, 5832-5836.

Arvanitoyannis I. S., Ladas D., Mavromatis A. (2006). Potential uses and applications of treated wine waste: a review. International Journal of Food and Science Technology, 41, 475-487.

Bertran E., Sort X., Soliva M., Trillas I. (2004). Composting winery waste: sludges and grape stalks. Bioresource Technology, 95, 203-208.

Celma A.R., Rojas S., Lopez-Rodrigez F (2007). Waste to energy possibilities for industrial olive and grape by-products in Extremadura. Biomass and Bioenergy, 31, 522-534.

Cook N.C., Samman S. (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry, 7, 66–76.

Downey M. O., Dokozlian N. K., Krstic M. P. (2006). Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review of Recent Research, American Journal of Enology and Viticulture, 3, 257–268.

Ferrer J., Paes G., Marmol Z., Ramones E., Chandler C., Marin M., Ferrer A. (2001). Agronomic use of biotechnologically processed grape wastes. Bioresource Technology, 76, 39-44.

Gey K.F. (1990). The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. Biochemcal Society Transitions, 18, 1041-1045.

Gonzales-Paramas, A., Esteban-Ruano S., Santos-Buelga C. (2004). Flavanol content and antioxidant activity in winery byproducts. Journal of Agricultural food and Chemistry, 52, 234-238.

Heim K.E., Tagliaferro A.R., Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationship. The Journal of Nutritional Biochemistry, 13, 572–584.

Hodur S., Beszedes S., Kertesz S., Szep A., Laszlo Z., Szabo G.(2009). Maximum recovery of different types of berry by products. Journal on Processing and Energy in Agriculture 13(4), 312-314.

Lachman J., Sulc M., Schilla M. (2007). Comparison of the total antioxidant status of Bohemian wines during the wine-making process. Food Chemistry, 103, 802-807.

Mazza G., Fukumoto L., Delaquis P., Girard B., Ewert B. (1999). Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir Wines from British Columbia. Journal of Agricultural of Food Chemistry, 47, 4009–4017.

Nakamura N., Nokura K., Zettsu T., Koga H., Tachi M., Terada M., Katoh H., Itoh Y., Osawa H., Ozeki T., Yamamoto H/ (2003). Neurologic complications associated with influenza vaccination: two adult cases. Internal Medicine, 42, 191-194.

Park S.Y., Lee Y-H., Choi K-C., Seong A-R., Choi H-K., lee O-H., Hwang H-J., Yoon H-G. (2011. Grape seed extract regulates androgenreceptor-mediated transcription in prostate cancer cells, through potent anti-histone acetyltransferase activity. Journal of Medicinal Food, 14, 9-16.

Radovanović A., Radovanović B., Jovančičević B. (2008). Free radical scavenging and antibacterial activities of southern Serbian red wines. Food Chemstry, 117, 326–333.

Radovanović B., Radovanović A., Souquet J-M. (2010). Phenolic profile and free radical -scavenging activity of Cabernet Sauvignon wines of different geographical origins from the Balkan region, Journal of the Science of Food and Agriculture, 90, 2455-2461.

Radovanović B., Bojić J.(2010). New spectrophotometric method for wine quality control, Journal on Processing and Energy in Agriculture 14 (3), 160-162.

Rodrígez Montealegre R., Romero Peces R.,. Chacón Vozmediano J. L, Martínez Gascueña J., Romero García E. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. Journal of Food Composition and Analysis, 19, 687–693.

Tsai T., Gombos D., Fulton L., Conway R.M., O'Brien J.M., Cronin J.E., Muthialu A. (2005). Retinoblastoma and hypochondroplasia: a case report of two germline mutations arising simultaneously, Ophthalmic Genetics, 26, 107-100

Yinrong L., Yeap Foo L (1999). The polyphenol constituents of grape pomace. Food Chemistry, 65, 1–8.

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