

# Effect of drying method on supercritical CO<sub>2</sub> extraction of grape seed oil

Utjecaj načina sušenja na ekstrakciju ulja iz sjemenki grožđa primjenom superkritičnoga CO<sub>2</sub>

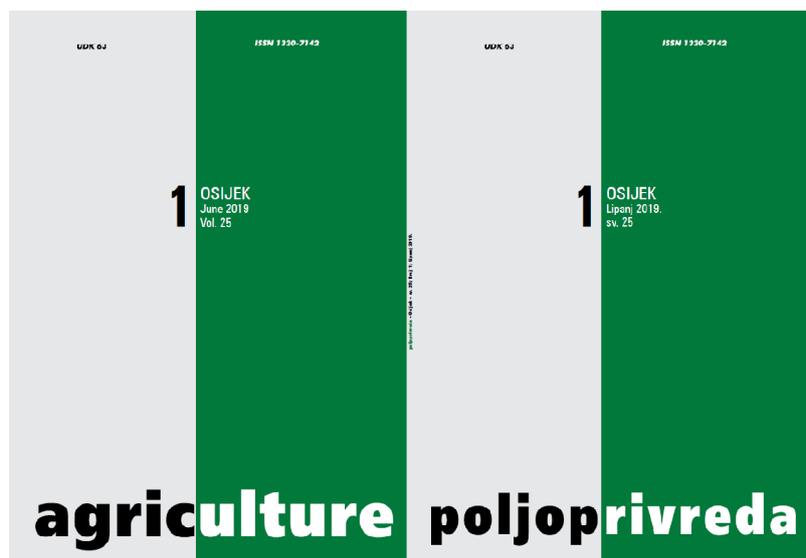
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## EFFECT OF DRYING METHOD ON SUPERCRITICAL CO<sub>2</sub> EXTRACTION OF GRAPE SEED OIL

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### SUMMARY

**Aim of this study was to monitor the influence of drying method (naturally and chamber drying) and different sample preparation on supercritical CO<sub>2</sub> extraction of oil from three grape seed varieties (Graševina, Zweigelt, Cabernet Sauvignon). The highest oil content was obtained from naturally dried screened and washed seeds of red variety Cabernet Sauvignon (14.85%) and lowest from chamber dried screened seeds of white variety Graševina (7.67%). Peroxide value ranged from 0.36 to 1.77 mmol O<sub>2</sub>/kg oil, free fatty acids 0.28-8.0%, and insoluble impurities 0.05-0.28%. Determined fatty acids were palmitic (6.98-11.58%), stearic (3.82-6.59%), oleic (14.90-19.97%) and linoleic acid (61.82-71.96%) in oil obtained from naturally dried seeds and 6.84-8.68%, 4.12-5.73%, 15.10-20.18% and 67.88-70.76% in oil from chamber dried seeds, respectively. In defatted cakes after supercritical CO<sub>2</sub> extraction, protein and fibre content ranged from 8.17 to 9.85% and 34.58 to 43.96%, respectively. According to ANOVA results, sample preparation and drying method had statistically significant influence on grape seed oil extraction.**

**Key words:** supercritical CO<sub>2</sub> extraction, oil, grape seeds, oil quality, defatted cake

### INTRODUCTION

Grape cultivation is widespread throughout the world and *Vitis vinifera* is most commonly cultivated species for wine production (Devesa-Rey et al., 2011). Data from 2013 show that world's grape production accounted 281 million tons (Barba et al., 2016). The Republic of Croatia has large area under vineyards with various grape varieties production.

By-products such as grape stalk, grape pomace, exhausted yeast, wine lee and wastewater (Barba et al., 2016) are generated during wine making wastes. Grape pomace is the main by-product which accounts for around two thirds of the solids (Duba and Fiori, 2015). In the last ten years, there has been problem due to stricter environmental regulations in the EU which prohibit the disposal of organic waste containing more than 5% organic carbon (Voća et al., 2010). Grape pomace roughly consists of grape stalks, seeds and skins (Duba and Fiori, 2015). Nowadays, grape pomace is mainly used as a soil conditioner, but it has a great potential for getting value-added products.

The share of grape seed (GS) in grape pomace can range from 20 to 38% (Duba and Fiori, 2015). GS is a

good source of oil and wide of oil yield (3.95-20%) can be obtained with differences among cultivars (Fernández et al., 2010).

Grape seed oil (GSO) is very interesting for food industry due to its composition and possibility to be used as nutritive edible oil. Since bioactive compounds (tocopherols, phenolic compounds) of unrefined oils (Bail et al., 2008) are very sensitive on processing parameters, mild extraction conditions, as those used during supercritical fluid extraction (SFE), should favour them.

SFE has attracted considerable attention in recent years as a promising alternative to the conventional solvent extraction and mechanical pressing in food pro-

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cessing. It is due to environmental advantages compared to the current classical extraction methods by organic solvents and better extraction yield compared to pressing methods (Jokić et al., 2014).

The aim of this study was to determine the influence of drying method (naturally - ND and chamber - CD drying) as well as sample preparation (seeds screened, seeds screened and washed, seed screened after drying from grape pomace) on SFE of GSO. Supercritical CO<sub>2</sub> which proved to be a highly desirable solvent in the separation processes has been considered as a green solvent in extraction of GSO, since it is non-toxic, non-flammable, tasteless or odourless, inexpensive and readily available in large quantities. It is also environmentally friendly and generally recognized as safe solvent.

## MATERIAL AND METHODS

### Material

Clusters of grape varieties *Graševina* (GR), *Zweigelt* (ZW) and *Cabernet Sauvignon* (CS) were collected during the harvest 2015 in the vineyards Kutjevo (continental Croatia). All the investigated varieties are from vineyards cultivated by standard cultivation around the vines, mulched between rows and fertilized using cow manure around grape vines every 3-4 year period, as well as foil with microelements during vegetation. The rootstocks inspected in the combinations with scions were Kober 5BB. The vines were trained on double Guyot with an average number of 16 buds per each Grape vine. The 2015 growing season in continental Croatia was very good for grapes production. The must composition was as follows: cv. *Graševina* must sugar content was 22,5 °Bx, total acidity was 6.2 g/l, for cv. *Zweigelt* the sugar content was 19.8 °Bx and the total acidity was 5.2 g/l, while in the cv. *Cabernet Sauvignon* the sugar content

was 24.5 °Bx and the total acidity was 5.1 g/l. The pH level ranged from 3.8 (cv. *Graševina* and *Cabernet Sauvignon*) to pH 3.9 (cv. *Zweigelt*). The vineyards have no irrigation system, and the protection against diseases and pests is carried out by a conventional approach to agricultural production using the permissible substances prescribed by the competent authorities.

Industry FAME mix 37 standard for FA was purchased from Restek (USA). The purity of CO<sub>2</sub> used for extraction was 99.97% (w/w) (Messer, Osijek, Croatia). Potassium hydroxide was supplied by Kemika (Zagreb, Croatia).

### Preparation of material for extraction

Separation stems were performed using a standard procedure in the production of high quality white and red wine. In the case of grape variety GR, pressing was started immediately after separation of the stems, while ZW and CS were transferred to maceration and fermentation. All three varieties were pressed by a pneumatic press (type Della Toffola PF 16, Italy). In the wine making process, a very high humidity is present due to pomace. Therefore, it is important to start the seeds separation as soon as possible, with white varieties, due to high sugar content in grape pomace. Consequently, fermentation of grape pomace and formation of harmful compounds could affect the quality of oils obtained from seeds. When producing oil from red variety seeds, this problem is much smaller, since grape pomace has already undergone a partial fermentation and is protected from decay by alcohol produced during fermentation. In further preparation process, GS listed varieties were separated by sieving from grape pomace using sieves of aperture size of 5 mm. The whole mash with seeds were dried for red grape varieties. After that drying seeds were separated from the skin. Prior extraction GS were prepared according to condition given in Table 1.

**Table 1. Characteristics of grape seeds used in this study.**

*Tablica 1. Karakteristike sjemenki grožđa korištenih u istraživanju.*

Material number <i>Broj uzorka</i>	Grape varieties <i>Sorta grožđa</i>	Preparation <i>Priprema</i>	Drying <i>Sušenje</i>
1A	Graševina	seeds screened from pomace	naturally
1B	Graševina	seeds screened from pomace and washed	naturally
1C	Graševina	seeds screened from pomace	chamber dryer
1D	Graševina	seeds screened from pomace and washed	chamber dryer
2A	Zweigelt	seeds screened from pomace	naturally
2B	Zweigelt	seeds screened from pomace and washed	naturally
2C	Zweigelt	seeds screened from pomace	chamber dryer
2D	Zweigelt	seeds screened from pomace and washed	chamber dryer
2E	Zweigelt	seeds screened from pomace after drying	chamber dryer
2F	Zweigelt	seeds screened from pomace after drying	naturally
3A	Cabernet sauvignon	seeds screened from pomace	naturally
3B	Cabernet sauvignon	seeds screened from pomace and washed	naturally
3C	Cabernet sauvignon	seeds screened from pomace	chamber dryer
3D	Cabernet sauvignon	seeds screened from pomace and washed	chamber dryer
3E	Cabernet sauvignon	seeds screened from pomace after drying	chamber dryer
3F	Cabernet sauvignon	seeds screened from pomace after drying	naturally

### Determination of initial oil and water content

The initial oil content in GS was measured by automatic extraction systems Soxterm by Gerhart with *n*-hexane (Aladić et al., 2014). Moisture content of the seeds was determined by AOAC Official Method 925.40 (2000).

### Determination of particle size distribution of GS with sieving

Rosin-Rammler-Bennet (RRB) distribution (Allen, 1981) was chosen for determination of particle size distribution. All the experiments were performed in duplicate.

### Supercritical CO<sub>2</sub> extraction of GSO

The experiment was performed in SFE system explained in detail elsewhere (Jokić et al., 2015; Jokić et al., 2014). The grounded GS of 100 g were placed into extractor vessel. Each extraction process took 90 minutes. The SFE was performed at extraction pressure of 300 bar and temperature of 40°C at mass flow rate of 2 kg/h. All the experiments were performed in duplicate.

### Oil quality parameters

Peroxide value of GSO was determined by ISO 3960(1998) and expressed as mmol O<sub>2</sub>/kg of oil. Free fatty acids (FFA) were determined by AOAC Official Methods 940.28 (1999), insoluble impurities by ISO 663(1992), moisture content in the oil by AOAC Official Method 925.40 (2000). All these determinations were carried out in triplicate.

### Determination of fatty acids (FA) composition

FA methyl esters preparation was carried out by EN ISO 5509:2000 standard. The prepared FA methyl esters were analyzed by gas chromatography according to EN ISO 5508:1995. Gas chromatograph 7890B (Agilent Technologies, Lake Forest, USA) with a capillary column HP88 100 m long with a diameter of 0.25 mm and the thickness of the stationary phase 0.20 microns (Agilent Technologies, Lake Forest, USA), a split-splitless injector (temperature 250°C) and a flame-ionization detector (temperature 280°C) was used. A sample (1 µL) was injected with a split ratio of 1:50. Start column temperature was 120°C with holding time for 1 minute. The oven temperature was increased with a rate of 10°C/min to 175°C/min, holding for 10 minutes, then at a rate of 5°C/min was heated to 210°C, holding for 5 minutes, then again at a rate of 5°C/min was heated up to 230°C hold-

ing for 5 minutes. A carrier gas was helium (99.99%) at a constant flow rate of 2 ml/min. The hydrogen flow was 40 ml/min, airflow 450 ml/min, and the makeup gas flow (nitrogen) 30 ml/min.

### Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's post-hoc test) were used to evaluate the significant difference of the data at  $p < 0.05$ . The same letter for the same determined response indicates no significant differences (Duncan's test,  $p < 0.05$ ).

## RESULTS AND DISCUSSION

Three grape varieties, two red grape varieties (ZW and CS) and one white grape variety (GR) were used for the present research. Preparations of GS samples, before extraction procedure, were performed differently given in detail in Table 1.

Extraction of GSO was performed employing the supercritical CO<sub>2</sub> extraction technique. Prior extraction, oil and moisture content (Table 2) of raw material were determined followed by milling the GS. The pressed grape residue contains a lot of moisture very susceptible to microbial growth. Activities of microorganisms and enzymes can cause development of unpleasant aroma component and it can be reflected on oil quality (Matthäus, 2008). In this study, ND and CD of GS were applied. The average moisture content in ND samples was 14.55% for GS white variety GR, 15.93% for GS red variety ZW and 14.87% for GS red grape variety CS, further in CD samples average of the moisture content was 9.13%, 13.84% and 9.19%, respectively. Results of moisture content in seeds indicate that the cause of the high moisture content in GS can be found in the natural way of drying. The average of initial oil content in ND raw samples was 9.15%, 9.56% and 12.56% for GR seeds, ZW seeds and CS seeds, respectively. Average of initial oil content was 9.61%, 12.22% and 10.16% for GR seeds, ZW seeds and CS seeds, respectively, in CD samples. The highest oil content was obtained from ND screened and washed seeds of red variety CS (14.85%) and the lowest from CD screened seeds white variety GR (7.67%). Results for oil content in our GS samples are in accordance with literature where oil content in GS varied from 8 to 16% (Baydar et al., 2007).

**Table 2. Oil and moisture content of naturally and chamber dried grape seeds.**

Tablica 2. Udio ulja i vlage u sjemenkama grožđa osušenim prirodno i osušenim u komornom sušioniku.

Properties Svojstva	Seeds screened from pomace Sjemenke prosijane iz komine			Seeds screened from pomace and washed Sjemenke prosijane i isprane			Seeds screened from pomace after drying Sjemenke prosijane nakon sušenja	
	Natural drying / Prirodno sušenje							
Sample Uzorak	1A	2A	3A	1B	2B	3B	2F	3F
Oil content (%) Udio ulja (%)	7.98 <sup>a</sup>	9.67 <sup>b</sup>	12.07 <sup>c</sup>	10.32 <sup>d</sup>	11.19 <sup>e</sup>	14.85 <sup>f</sup>	7.82 <sup>a</sup>	10.75 <sup>d,e</sup>
Moisture content (%) Udio vlage (%)	14.83 <sup>a</sup>	13.21 <sup>b</sup>	15.62 <sup>c</sup>	14.27 <sup>d</sup>	14.42 <sup>a,d</sup>	9.14 <sup>e</sup>	20.17 <sup>f</sup>	19.85 <sup>g</sup>
Chamber drying / Komorno sušenje								
Sample Uzorak	1C	2C	3C	1D	2D	3D	2E	3E
Oil content (%) Udio ulja (%)	7.67 <sup>a</sup>	12.91 <sup>g</sup>	9.88 <sup>b</sup>	11.54 <sup>e</sup>	12.36 <sup>c</sup>	10.18 <sup>d</sup>	11.39 <sup>e</sup>	10.42 <sup>d</sup>
Moisture content (%) Udio vlage (%)	9.71 <sup>h</sup>	14.63 <sup>a,d</sup>	9.02 <sup>e,i</sup>	8.55 <sup>i</sup>	12.76 <sup>j</sup>	8.10 <sup>h</sup>	14.12 <sup>d</sup>	10.45 <sup>i</sup>

The same letter in the same row indicates no significant differences (Duncan's test,  $p < 0.05$ ) for oil and moisture content, respectively

Particle size of the milled GS prepared for extraction was determined to be  $0.345 \pm 0.021$  mm. During the extraction experiments particle size kept constant because different particle size of ground GS could also effect oil yield. Reverchon and de Marco (2006) reported that the average particle size should range from 0.25 to 2.0 mm, approximately.

The following quality parameters were analysed in obtained GSO: peroxide value, FFA and insoluble impurities (Table 3).

Primary oxidation process in the oil mainly forms hydroperoxides measured by the peroxide value. Lower peroxide values are measured in CD GS samples, 0.51

mmol O<sub>2</sub>/kg, 0.55 mmol O<sub>2</sub>/kg and 0.51 mmol O<sub>2</sub>/kg, for ND GS samples peroxide value was 0.72 mmol O<sub>2</sub>/kg, 1.16 mmol O<sub>2</sub>/kg and 0.89 mmol O<sub>2</sub>/kg for GR, ZW and CS, respectively. The FFA contents were in the range of 0.54-2.64%, 1.27-4.89% and 1.10-8.00% in ND GS samples for GR, ZW and CS, respectively. For CD GS samples FFA were 0.28-0.78%, 1.31-3.12% and 1.13-3.27% for GR, ZW and CS, respectively. GSO Content obtained by SFE is in accordance with literature in range from 7% to 15% (Beveridge et al., 2005). It is very important that GSO is low in peroxide value, FFA and moisture content to maintain the quality and shelf life of the oil (Teh and Birch, 2013). The obtained GSO had very low values of insoluble impurities and results are given in Table 3.

**Table 3. Quality parameters of grape seed oil (natural and chamber drying) obtained by supercritical CO<sub>2</sub>.**Tablica 3. Parametri kvalitete ulja sjemenki grožđa (osušene prirodno i u komornom sušioniku) dobivenog ekstrakcijom superkričnim CO<sub>2</sub>.

Properties Svojstva	Seeds screened from pomace Sjemenke prosijane iz komine			Seeds screened from pomace and washed Sjemenke prosijane i isprane			Seeds screened from pomace after drying Sjemenke prosijane nakon sušenja	
	Natural drying / Prirodno sušenje							
Sample Uzorak	1A	2A	3A	1B	2B	3B	2F	3F
Peroxide value (mmol O <sub>2</sub> /kg of oil) Vrijednost peroksidnog broja (mmol O <sub>2</sub> /kg ulja)	0.88 <sup>a</sup>	0.72 <sup>b</sup>	1.06 <sup>c</sup>	0.55 <sup>d</sup>	0.99 <sup>c</sup>	0.36 <sup>e</sup>	1.77 <sup>f</sup>	1.26 <sup>g</sup>
Free fatty acids (%) Udio slobodnih masnih kiselina	0.54 <sup>a</sup>	2.70 <sup>b</sup>	4.63 <sup>c</sup>	2.64 <sup>b</sup>	1.27 <sup>d</sup>	1.10 <sup>e</sup>	4.89 <sup>c</sup>	8.0 <sup>f</sup>
Insoluble impurities (%) Udio netopivih tvari (%)	0.28 <sup>a</sup>	0.05 <sup>b</sup>	0.09 <sup>b</sup>	0.08 <sup>b</sup>	0.13 <sup>c</sup>	0.11 <sup>b,c</sup>	0.05 <sup>b</sup>	0.15 <sup>c</sup>
Chamber drying / Komorno sušenje								
Sample Uzorak	1C	2C	3C	1D	2D	3D	2E	3E
Peroxide value (mmol O <sub>2</sub> /kg of oil) Vrijednost peroksidnog broja (mmol O <sub>2</sub> /kg ulja)	0.63 <sup>h</sup>	0.59 <sup>d,h</sup>	0.47 <sup>d,i</sup>	0.38 <sup>e</sup>	0.41 <sup>i</sup>	0.50 <sup>d</sup>	0.64 <sup>h</sup>	0.55 <sup>d</sup>
Free fatty acids (%) Udio slobodnih masnih kiselina	0.28 <sup>g</sup>	1.31 <sup>d,i</sup>	1.62 <sup>h</sup>	0.78 <sup>i</sup>	1.42 <sup>j</sup>	1.13 <sup>e</sup>	3.12 <sup>k</sup>	3.27 <sup>k</sup>
Insoluble impurities (%) Udio netopivih tvari (%)	0.09 <sup>b</sup>	0.12 <sup>c</sup>	0.08 <sup>b</sup>	0.09 <sup>b</sup>	0.13 <sup>c</sup>	0.12 <sup>c</sup>	0.06 <sup>b</sup>	0.09 <sup>b</sup>

The same letter in the same row indicates no significant differences (Duncan's test,  $p < 0.05$ ) for selected properties, respectively

Gas chromatography and mass spectrometry (GC/MS) were used for determination of FA composition in GSO (Table 4). GSO has high amount of unsaturated FA and its content is 90% (Matthäus, 2008; Sabir et al., 2011). Content of individual fatty acids is in accordance with other papers ranged from 6.9 to 10.4% for palmitic acid, 2.5-5.8% for stearic acid, 13.2-24.8% for oleic acid and 60.9-75.3% for linoleic acid (Pardo et al., 2009; Ramos et al., 2009; Tangolar et al., 2009; Lutterodt et al., 2011; Shinagawa et al., 2018). It was found out that the dominant FA linoleic acid is present in the range from 61.82 to 71.96%, while the second most common FA in GSO is oleic acid in the range of 14.90-20.18%. Palmitic and stearic acid were also detected in GSO in range from

6.84 to 11.51% and from 3.82 to 6.59%, respectively. FA show slight variation in results considering different drying and preparation methods of samples. High level of unsaturated FA (90% poly- and monounsaturated FA) and low content of saturated FA are responsible for nutritional value of GSO. FA composition of the obtained GSO is very similar to those published by other authors (Crews et al., 2006; Demirtas et al., 2013). The main FA was linoleic, followed by oleic, palmitic and stearic acids. Content of linoleic acid was higher compared to many other oils such as sunflower oil, olive oil, pumpkin seed oil, coconut oil, corn oil (Boskou et al., 2006; Stevenson et al., 2007; Aladedunye and Przybylski, 2013; Garavaglia et al., 2016; Wall-Medrano et al., 2017).

**Table 4. Fatty acid composition of grape seed oil.**

Tablica 4. Sastav masnih kiselina u ulju sjemenki grožđa.

Properties Svojstva	Seeds screened from pomace Sjemenke prosijane iz komine			Seeds screened from pomace and washed Sjemenke prosijane i isprane			Seeds screened from pomace after drying Sjemenke prosijane nakon sušenja	
Natural drying / Prirodno sušenje								
Sample Uzorak	1A	2A	3A	1B	2B	3B	2F	3F
Palmitic acid (%) Palminska (%)	6.98 <sup>a</sup>	7.52 <sup>b</sup>	8.89 <sup>c</sup>	7.79 <sup>b,e</sup>	7.67 <sup>b,e</sup>	11.51 <sup>d</sup>	7.84 <sup>e</sup>	7.41 <sup>b</sup>
Stearic acid (%) Stearinska (%)	4.58 <sup>a</sup>	3.82 <sup>b</sup>	5.99 <sup>c</sup>	4.58 <sup>a</sup>	4.26 <sup>a</sup>	6.59 <sup>d</sup>	4.63 <sup>a,f</sup>	5.66 <sup>e</sup>
Oleic acid (%) Oleinska (%)	19.50 <sup>a</sup>	17.94 <sup>b</sup>	16.22 <sup>c</sup>	19.97 <sup>d</sup>	18.83 <sup>e</sup>	19.76 <sup>a,d</sup>	19.62 <sup>a</sup>	14.90 <sup>f</sup>
Linoleic acid (%) Linolna (%)	68.92 <sup>a</sup>	70.72 <sup>b</sup>	68.77 <sup>a</sup>	67.66 <sup>d</sup>	69.25 <sup>e</sup>	61.82 <sup>f</sup>	67.79 <sup>d</sup>	71.96 <sup>g</sup>
Chamber drying / Komorno sušenje								
Sample Uzorak	1C	2C	3C	1D	2D	3D	2E	3E
Palmitic acid (%) Palminska (%)	7.86 <sup>e</sup>	7.73 <sup>b,e</sup>	8.13 <sup>f</sup>	6.84 <sup>a</sup>	7.50 <sup>b</sup>	8.22 <sup>g</sup>	8.03 <sup>f</sup>	8.68 <sup>h</sup>
Stearic acid (%) Stearinska (%)	4.73 <sup>f</sup>	4.27 <sup>a</sup>	5.58 <sup>e</sup>	4.57 <sup>a</sup>	4.12 <sup>g</sup>	5.54 <sup>e</sup>	4.50 <sup>a</sup>	5.73 <sup>e</sup>
Oleic acid (%) Oleinska (%)	19.52 <sup>a</sup>	18.31 <sup>g</sup>	15.43 <sup>h</sup>	20.18 <sup>d</sup>	18.46 <sup>g</sup>	15.76 <sup>h</sup>	18.62 <sup>g</sup>	15.10 <sup>f,h</sup>
Linoleic acid (%) Linolna (%)	67.88 <sup>d</sup>	69.70 <sup>e</sup>	70.76 <sup>b</sup>	67.78 <sup>d</sup>	69.78 <sup>b,e</sup>	70.39 <sup>b</sup>	68.77 <sup>a</sup>	70.38 <sup>b</sup>

The same letter in the same raw indicates no significant differences (Duncan's test,  $p < 0.05$ ) for each fatty acid, respectively

Table 5 shows total protein and fibre content in GS meal. The total protein content was in the range of 8.17-9.85% while total fibre content in the range of 34.58-43.96%. Variation in results between ND and CD samples was negligible. Furthermore, comparing extraction yields gained employing Soxhlet method (7.98% and 9.67%, respectively) and supercritical CO<sub>2</sub> method (7.54% as well as 9.51%, respectively). It is obvious that oil from GS can be totally extracted by supercritical CO<sub>2</sub> if the appropriate extraction conditions are applied. Compared to conventional extraction, using supercritical CO<sub>2</sub> extraction the solvent distillation and oil refining stages can be omitted (Molero Gomez et al., 1996). GSO extracted by supercritical CO<sub>2</sub> had a yellow colour with

characteristic aroma and can be further used not only like dietary product but also in the pharmaceutical and cosmetic industry (Fernandes et al., 2013). Other very important advantage of this green technology is that the defatted cake remaining after SFE is free of toxic solvents, opposed to extraction with organic solvents where the presence of traces of residual solvent in the final product makes the process less attractive from health and environmental point of views. Such defatted cake which remains after supercritical CO<sub>2</sub> extraction can be used further in other processes, for example, in development of new functional and enriched products since large amounts of phenolic compounds are left in a cake (Jokić et al., 2014).

**Table 5. Total protein and fibre content in dried grape seed meals after oil extraction.**

Tablica 5. Udio proteina i vlakana u pogačama sjemenki grožđa nakon ekstrakcije ulja.

Properties Svojstva	Seeds screened from pomace Sjemenke prosijane iz komine			Seeds screened from pomace and washed Sjemenke prosijane i isprane			Seeds screened from pomace after drying Sjemenke prosijane nakon sušenja	
Natural drying / Prirodno sušenje								
Sample Uzorak	1A	2A	3A	1B	2B	3B	2F	3F
Total protein (%) Udio proteina (%)	8.17 <sup>a</sup>	8.65 <sup>b</sup>	9.12 <sup>c</sup>	9.35 <sup>d</sup>	8.74 <sup>b</sup>	9.50 <sup>d,e</sup>	9.13 <sup>c</sup>	9.63 <sup>e</sup>
Total fibre content (%) Udio vlakna (%)	40.15 <sup>a</sup>	37.83 <sup>b</sup>	34.58 <sup>c</sup>	43.08 <sup>d</sup>	41.62 <sup>e</sup>	42.55 <sup>f</sup>	41.23 <sup>e</sup>	43.70 <sup>d,g</sup>
Chamber drying / Komorno sušenje								
Sample Uzorak	1C	2C	3C	1D	2D	3D	2E	3E
Total protein (%) Udio proteina (%)	9.85 <sup>e</sup>	9.51 <sup>d,e</sup>	8.71 <sup>b</sup>	8.89 <sup>f</sup>	8.71 <sup>b</sup>	8.76 <sup>b</sup>	9.12 <sup>c</sup>	9.54 <sup>d,e</sup>
Total fibre content (%) Udio vlakna (%)	43.3 <sup>d</sup>	40.61 <sup>a</sup>	38.61 <sup>f</sup>	39.53 <sup>a,f</sup>	43.5 <sup>d</sup>	43.96 <sup>g</sup>	35.34 <sup>h</sup>	37.20 <sup>b</sup>

The same letter in the same row indicates no significant differences (Duncan's test,  $p < 0.05$ ) for total protein and fibre content, respectively

The differences in all the obtained results regarding quality and selected properties of used grape seeds and oil were statistically evaluated. Factorial analysis of variance (ANOVA) was performed and there were significant differences ( $p < 0.05$ ) between sample preparation and drying method.

## CONCLUSION

Grape pomace is a by-product in the wine production often characterized as a waste. From the results of this paper it can be seen that grape pomace may be a raw material for the production of new products with high added value. It is very important to find the best preparation and drying method for grape seeds because these parameters have statistically significant influence on grape seed oil extraction. Furthermore, the results obtained in this study it can show that the seeds should be dried in a dryer in order to reduce the moisture in the seeds and consequently in the oil. In this way we would increase the quality of oil, since the high moisture content adversely affects oil quality. The use of green technologies, such as SFE, achieves a maximum utilization of oil. However, future research should examine the use of other favourable methods of extracting GSO (cold pressing with a screw press) in order to compare the economic viability of major pomace waste management projects.

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## UTJECAJ NAČINA SUŠENJA NA EKSTRAKCIJU ULJA IZ SJEMENKI GROŽĐA PRIMJENOM SUPERKRITIČNOGA CO<sub>2</sub>

### SAŽETAK

**Cilj istraživanja bio je odrediti utjecaj načina sušenja (prirodno i u komornome sušioniku), kao i same pripreme sjemenki grožđa, na ekstrakciju ulja superkričnim CO<sub>2</sub> iz triju sorata grožđa (graševina, Zweigelt, Cabernet Sauvignon). Najveći udio ulja ekstrahirano je iz prirodno osušenih i prethodno opranih sjemenki grožđa crne sorte Cabernet Sauvignon (14,85%), dok je najmanje ulja dobiveno iz sjemenki osušenih u komornome sušioniku bijele sorte graševina (7,67%). Vrijednost peroksidnoga broja dobivenih ulja bila je u rasponu 0,36-1,77 mmol O<sub>2</sub>/kg, slobodne masne kiseline 0,28-8,0%, a netopljive nečistoće 0,05-0,28%. U ulju dobivenome iz prirodno osušenih sjemenki grožđa određene su sljedeće masne kiseline: palmitinska (6,98-11,58%), stearinska (3,82-6,59%), oleinska (14,90-19,97%) i linolna kiselina (61,82-71,96%). U ulju sjemenki grožđa prethodno osušenih u komori određene su sljedeće masne kiseline: palmitinska (6,84-8,68%), stearinska (4,12-5,73%), oleinska (15,10-20,18%) i linolna kiselina (67,88-70,76%). Nakon ekstrakcije superkričnim CO<sub>2</sub> u odmašćenim pogačama određen je udio proteina (8,17-9,85%) i vlakana (34,58-43,96%). Priprema uzorka i način sušenja sjemenki grožđa te sorta imaju značajan utjecaj na ekstrakciju i kvalitetu dobivenoga ulja.**

**Ključne riječi:** ekstrakcija superkričnim CO<sub>2</sub>, ulje, sjemenke grožđa, kvaliteta ulja, odmašćena pogača

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