

## Grape Processing By-Product as a Source of Nutraceutical Components

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The aim of this study was to evaluate the oil and bioactive contents of grape waste by both conventional and microwave-assisted extraction (MAE) methods. Other controlled factors included the segment solvent used (n-hexane-methanol, n-hexane-ethanol), the solid/solvent ratios (1 : 5, 1 : 10, 1 : 20 g/mL) and in MAE, the microwave powers (40 W, 240 W and 440 W). The extracts were analysed for the contents of fatty oil, lipid hydroperoxide content (LP), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. The optimal conditions for the grape oil recovery were at 240 W, using n-hexane-ethanol solvent, and the ratio of 1 : 10 by using the MAE method. At the same ratio conditions, the highest contents of LP and TPC were 0.032 mM (at 440 W) and 43.657 mg gallic acid (AG)/dry weight (at 240 W). At 440 W and ratio of 1 : 10, TFC peaked the highest point at 37.862 mg quercetin/g dry weight. The results indicated that grape waste is a valuable input for a wide range of applications in nutraceutical and food industries, offering an additional economic by-product for the grape processing.

### 1. Introduction

By-product produced during wine processing, which contains high level of unsaturated fatty acids and phenolic compounds with high nutritional value, affects effectively on human health (Fiori et al., 2014). The production of grape oil from the waste of the wine processing industry takes thorough advantage of the economic value of grape. According to many researches, the phenolic compounds are more biologically active, but the most common are antioxidant properties (Maier et al., 2009). The extraction of polyphenol assisted by microwave can examine the material, prepare the sample, and appreciate total polyphenol content by UV-VIS spectroscopy (Li et al., 2011). In some other studies, extraction of grape seed oil assisted by ultrasound, using n-hexane as extracting solvent, at a frequency of 20 Hz, and 150 W in 30 min brings the highest efficiency that is equivalent to the Soxhlet extraction method (as Soxhlet 100 %) (Porto et al., 2013). Fiori et al. (2015) used supercritical fluid extraction (SFE) with empirical and model methods to examine the factors that impact the recovery of the grape seed oil (Fiori and Duba, 2015). Grape seed oil is the source of unsaturated fatty acids and tocol (Fiori et al., 2014). Grape seed oil is an important source of tocol. Grape seed oil extracted by supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) method resulted in higher total tocol and carotenoid levels, associated with higher lipophilic antioxidant activity, compared to n-hexane extraction (Mohamed et al., 2016). With SC-CO<sub>2</sub>, the content of total phenolic compounds and total antioxidant capacity of the grape seed oil were higher than of pomegranate and tomato (Durante et al., 2017). The extraction solvent and grape variety had significant effect on bioactive properties (Yalcin et al., 2017). They showed that grape seed oil contained components useful for human nutrition (Juhaimi et al., 2017). Compared with the synthesis of artificial antioxidants, grape residue is considered as a source of simple, cheap, and much safer antioxidants.

The main purposes of this work were to compare several bioactive compounds in grape oil residue extracts by using microwave-assisted extraction (MAE) method under different conditions and to evaluate the antioxidant capacity as well as the potential functional properties of grape oil residue extracts for application in industry. The efficiency of the extraction was evaluated based on the recovery yields of phenolic from solvent segment, total lipid hydroperoxide content analysis and antioxidant capacity, and total phenolic content (TPC) and total flavonoid content (TFC) analysis.

## 2. Materials and Methods

### 2.1 Materials

Wine grape wastes after fermentation were recovered at a wine processing facility. Grape wastes were dried at 60 °C for 32 h until reaching constant weight. The dried materials were grounded into powder and the powder was used in the extraction process. Foline-Ciocalteu phenol reagent and Gallic acid (3,4,5-trihydroxybenzoic acid) were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and quercetin were obtained from Merck Co. The equipment used in this study were Microwave (Sanyo EM-S2086W, Japan), supersonic path (Power Sonic 410), Soxhlet extraction, filtering evaporator, rotating evaporator (model R-215, BUCHI Labortechnik AG, Switzerland) and Ultraviolet-visible spectrophotometer (DR 5000 HACH).

### 2.2 Methods

This section presented the methods used for the extraction and determination of the total lipid hydroperoxide content, the total lipid hydroperoxide content, the total phenolic compounds content and the antioxidant activity, to compare and evaluate the extraction efficiency between the two methods.

The extraction experiments were carried out by two methods, maceration and Microwave-assisted extraction (MAE). For maceration method, 5 g of grape powder with 125 mL n-hexane were loaded into 250 mL flask. The mixture was stirred continuously for 8 h/d for consecutive 12 d. Then, the extraction was immersed with methanol to determine the composition. For MAE method, 15 g of sample was used with the appropriate amount of n-hexane depending on the solid:liquid ratio of 1 : 5, 1 : 10, and 1 : 20. This corresponds to 75 mL, 150 mL, and 300 mL of n-hexane. The sequences of capacity were used in a microwave oven at: 40 W, 240 W, 440 W. The next stage is the extraction with methanol (ethanol) to determine the composition of the oil and verify the optimal extraction conditions for MAE method.

Due to the presence of unsaturated fatty acid compounds, grape seed oil contains self-oxidation of unsaturated fatty acid radicals. Lipid hydroperoxide (LP) were determined based on the reduction of those compounds from  $Fe^{2+}$  into  $Fe^{3+}$ . The content of  $Fe^{3+}$  was determined spectrophotometrically at 500nm combined with  $\epsilon = 58,440 M^{-1} cm^{-1}$ , due to the formation of the complex between  $Fe^{3+}$  and  $SCN^-$ .

The total phenolic content (TPC) of the extract was measured by Folin–Ciocalteu method. This method was based on the reaction of Folin–Ciocalteu reagent with hydroxyl radical in the phenolic compound whereby the reagent solution turns from yellow to dark blue. TPC was determined based on calibration curve of acid gallic along with the measured absorbance of the sample after reaction at 765 nm.

The total flavonoid content (TFC) of grape waste was determined by aluminium chloride colorimetric method based on the formation of a complex.

PPH (2,2-diphenyl-1-picrylhydrazyl) was commonly used to study the inhibition of free radicals. The antioxidants are neutralised by the DPPH base. This process results in the reducing of the absorption at maximum wavelength of solution (DPPH and sample) and the colour of the reaction solution would fade, turning from purple to yellow. The values used to assess strong or weak inhibition of the sample is IC50. IC50 was defined as the concentration of sample at which it could inhibit 50 % of free radicals. In general, the higher the activity pattern, the lower the IC50 value. Le et al. (2017) carried out extraction and evaluation of biological activity based on these methods with spent coffee grounds material.

## 3. Results and Discussion

### 3.1 Comparison with maceration methods

Table 1 showed that the grape seed oil and phenolic recovery efficiency by the maceration method was higher than the MAE method, but the maceration method took 12 d to achieve high retrieval while the microwave method only needs a few minutes. The indicators such as LP, TPC, TFC, and antioxidant activities were also higher by using MAE method than maceration method. This shows that MAE method was very effective, and this method was chosen to evaluate the antioxidant capacity as well as the potential functional properties of grape oil residue extracts for application in industry.

Table 1: Comparison between maceration and MAE method

Methods	Oil recovery (%)	Phenolic recovery (%)	LP (mM)	TPC (mg AG/ g dry material)	TFC (mg quercetin/ g dry material)	IC 50
Maceration	14.16	22.28	0.0265	37.653	30.339	19.285
MAE	13.39	20.45	0.0320	43.657	37.862	20.925

### 3.2 Fatty acid compositions

The analysis showed that the main constituents of grape oil were unsaturated fatty acids, of which the glyceride portion was predominantly made up of palmitic, oleic, and linoleic acids. Most of the fatty acids in the diet are long-chain as shown in Table 2. Table 2 showed that total fatty acid that was unsaturated accounted for over 80 % of the composition. Linoleic acid (C18-2) has the highest percentage, approximately 68.27 % by MAE. High amount of linoleic acid (C18-2) in grape oil is important for the human body. Palmitic acid (C16:0) makes up 12.67 % and oleic acid (C18-1) 11.46 %. The high concentration of oleic acid (C18:1), one of the drug neurotransmitters, can enhance the nutrient absorption of human body. The minority content of the oil was other acids that constitutes less than 10 % of the composition. These results were also consistent with previous reports such as the one by Blu et al. (2012) whereby grape seed oil was identified with linoleic (65.0 %), oleic (17.0 %) and palmitic (8.0 %). The fatty acid compositions from a variety of grapes in Turkey were different. The linoleic acid contents ranged from 47.34 % (Sangiovese) to 72.91 % (Cinsaut), and the oleic acid contents varied between 13.35 % (Cabernet Sauvignon) and 26.30 % (Sangiovese). The palmitic acid and stearic acid contents of grape seed oils ranged from 7.15 % (Cinsaut) to 16.06 % (Sangiovese) and from 2.43 % (Narince) to 6.55 % (Sangiovese) (Juhaimi et al., 2017).

Table 2: The fatty acids composition of grape oil residue were analysed by GC-MS

Content of fatty acid	MAE	Content of fatty acid	MAE
Dodecanoic acid (C12:0)	0.44	Cis-9-Octadecenoic acid (oleic acid) (C18:1)	11.46
Tetradecanoic acid (C14:0)	0.27	Cis-9,12-Octadecenoic acid (linoleic acid) (C18:2)	68.27
Pentadecanoic acid (C15:0)	0.06	Cis-9,12,15-Octadecenoic acid (C18:3)	1.38
Hexadecanoic acid (palmitic acid) (C16:0)	12.67	Eicosanoic acid (C20:0)	0.47
9-Hexadecenoic acid (C16:1)	0.21	11-Eicosanoic acid (C20:1)	0.16
Heptadecanoic acid (C17:0)	0.13	Docosanoic acid (C22:0)	0.6
Octadecanoic acid (C18:0)	3.63	Tetracosanoic acid (C24:0)	0.25

### 3.3 Recovery yields of oil and phenolic from extracted segment by microwave-assisted extraction (MAE).

The recovery yields of oil and phenolic from extracts were calculated by the following Eq(1) and Eq(2) formulas:

$$\text{The recovery yields of oil} = \frac{\text{weight of oil from extract}}{\text{dry weight material}} \times 100 \% \quad (1)$$

$$\text{The recovery yields of phenolic} = \frac{\text{weight of phenolic from extract}}{\text{dry weight material}} \times 100 \% \quad (2)$$

#### 3.3.1 Recovery yields of oil from solvent segment

The recovery yields of oil from solvent segment with different solid/liquid ratio and three capacity level as shown in Figure 1a and Figure 1b.

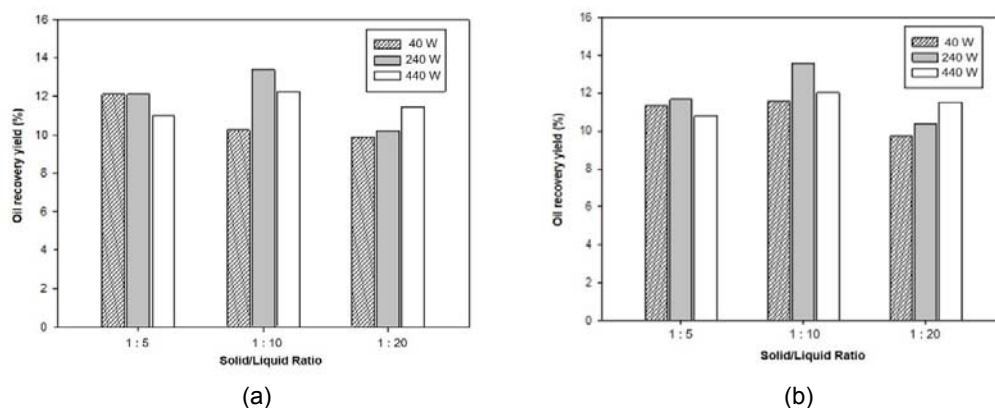


Figure 1: Recovery yields of oil vs. different solid/liquid ratio and three capacity level with solvent (a) n-hexane-methanol; (b) n-hexane-ethanol

From Figure 1a, it can be clearly seen that when using solvent n-hexane-methanol, the recovery yields of oil was the highest at 240 W and solid/liquid ratio of 1 : 10 (13.39 %), while the lowest oil recovery was at 40 W with solid/liquid ratio of 1 : 20 (9.89 %) of absolute dry weight. From Figure 1b, it can be seen that when using n-hexane-ethanol, the highest and the lowest oil recovery yield were obtained at the same ratio and capacity as when using n-hexane-methanol with the highest yield of 13.58 % (at ratio 1 : 10, and capacity 240 W) and the lowest yield of 9.74 % (at ratio 1 : 20, and capacity 40 W). This is due to the fact that the 240 W produces the right amount of heat, the vapour and the high pressure to destroy the cell. At the same time, 1 : 10 is a suitable ratio so that solvent can infiltrate easily into the material. With the use of n-hexane-ethanol solvent and at a ratio of 1 : 10, the 240 W capacity gave the highest oil recovery compared to the other ratios and capacities.

### 3.2.2 Recovery yields of phenolic from solvent segment

The efficiency of extraction of phenolic compounds from grape oil residue is presented in Figure 2a and 2b. From Figure 2a, the highest phenolic recovery efficiency was 20.45 % at 240 W and ratio of 1 : 10, while the lowest phenolic recovery was 13.5 %, compared to the absolute amount of dry material at 40 W with ratio of 1 : 5. From Figure 2b, the highest phenolic recovery was 14.51 %, at 440 W with solid/liquid ratio of 1 : 5, while the minimum yield was 10.64 % achieved by MAE at 40 W and ratio of 1 : 10. The use of n-hexane-methanol results in a higher recovery rate than n-hexane-ethanol at all capacities and ratios. This can be explained by the fact that methanol has the appropriate polarisation to extract all compounds in the grape residue.

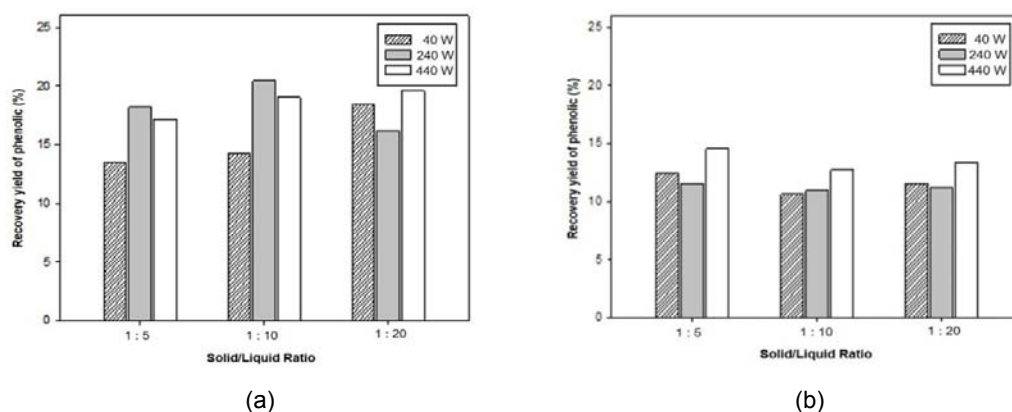


Figure 2: Recovery yields of phenolic with different ratios and solvent (a) n-hexane-methanol; (b) n-hexane-ethanol Result of total lipid hydroperoxide content analysis and antioxidant capacity

The total LP contents of grape oil residue extracted by different ratios and capacity were shown in Figure 3. When the capacity considerably rose from 40 W to 240 W and to 440 W, the LP content increased. The highest amount of LP was 0.0320 mM, found in MAE at the ratio of 1 : 10 with the capacity of 440 W. The lowest amount of LP was 0.0302 mM, found at the ratio of 1 : 5 and 40 W. The 440W produces the right amount of heat for heating and the 1 : 10 ratio was a suitable ratio for separation. Fatty acids were non-polar substances, so the use n-hexane for extraction was perfectly suited.

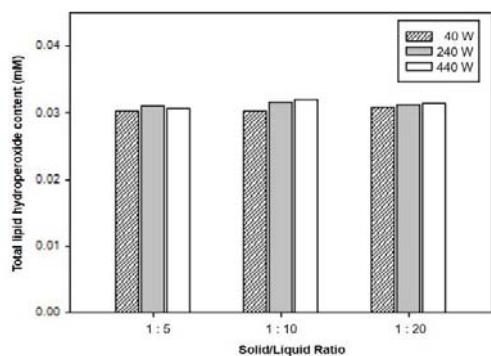


Figure 3: Result of total LP content analysis

Figure 4a and 4b showed the effects of solvent, ratios, and capacities on antioxidant activity. The activity can be evaluated through IC<sub>50</sub> value. The value ranged from the 19.7 mg/L to 29.0 mg/L. It confirmed the antioxidant potential of grape oil residue which was reported as 19.3 mg/L by Selcuk et al. (2011).

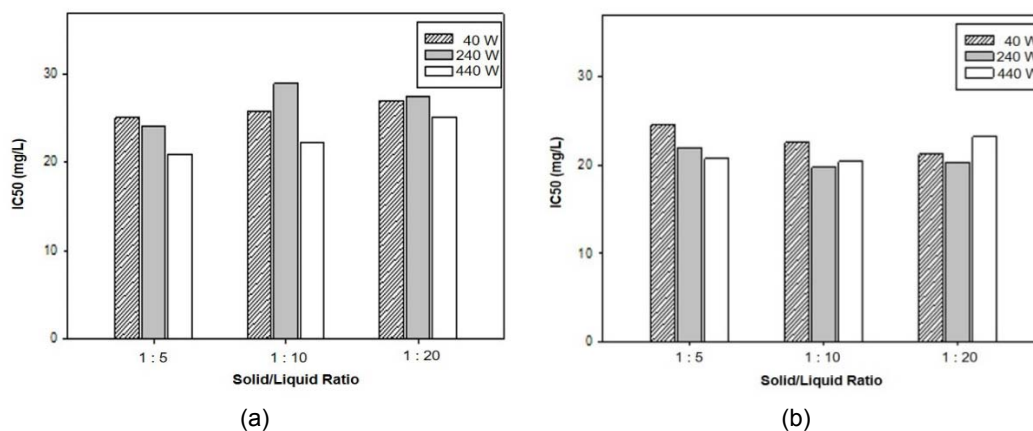


Figure 4: Antioxidant capacity of extract with solvent (a) *n*-hexane-methanol; (b) *n*-hexane-ethanol

### 3.5 Results of total phenolic content (TPC) and total flavonoid content (TFC) analysis

Figure 5a and 5b showed that the highest value of TPC was 43.7 mg AG/g dry mass which was achieved using methanol solvent at ratio of 1 : 10 and 240 W. This value is higher than the result of Bittar et al. (2013) (21.4 mg AG/g dry mass) and Marques et al. (2013) (22.6 mg AG/g dry mass). The lowest amount was 16.0 mg AG/g dry mass, obtained by using ethanol solvent. TPC content of the studies was different because grape varieties, climatic and soil conditions showed dissimilarity.

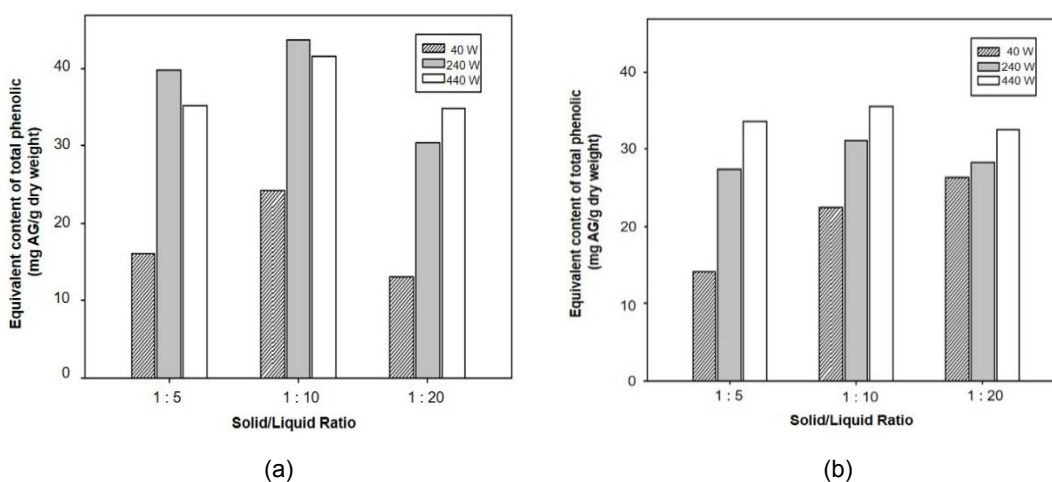


Figure 5: The result of TPC of extract vs different solid/liquid ratio with three capacity level with solvent (a) *n*-hexane-methanol; (b) *n*-hexane-ethanol

It is apparent from Figure 6a and 6b that TFC extracted by methanol solvent was much higher than when extracted by ethanol solvent. The highest value was 37.9 mg quercetin/g dry weight at ratio 1 : 10 and 440 W, which is approximately 2 times the lowest value at 1 : 10 ratio. This value is much higher than the result obtained by Selcuk et al. (2011) which is 24.2 mg quercetin/g dry mass. This trend resulted from the effects of heat and solvent. Heating caused the molecular flavonoid to be easily separated from the raw material, while the solvent has a very good solubility of flavonoid, good interaction with the sample, and proper microwave uptake. This makes the extraction of flavonoid moderately challenging.

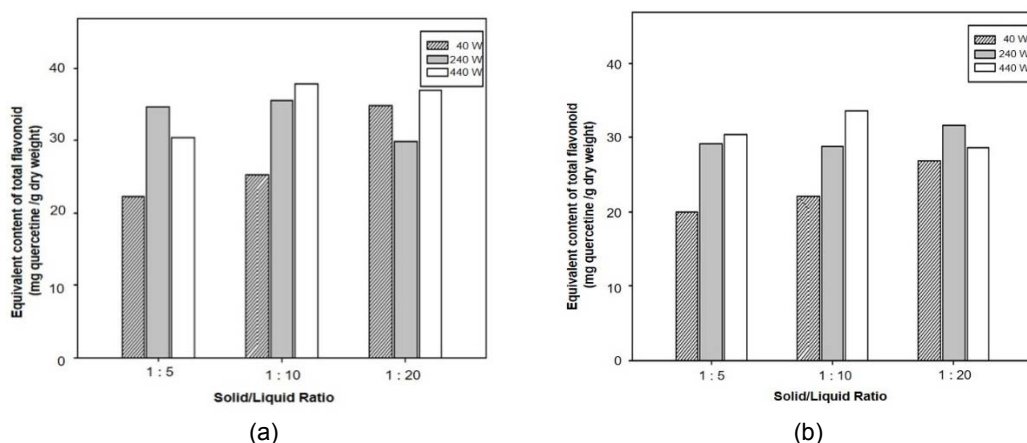


Figure 6: The result of TFC of extract vs different solid/liquid ratio with three capacity level with solvent (a)*n*-hexane-methanol; (b)*n*-hexane-ethanol

#### 4. Conclusions

This paper focused on the determination of the content of bioactive compound in grape oil residue and the efficiency of Microwave-assisted extraction. This study found that 80 % of grape oil residue is composed of palmitic, oleic and linoleic acids. MAE was the optimal method to obtain the highest recovery of both oil and phenolic compared to maceration method, based on its capacities and the solid/liquid ratios. The highest amount of total LP was achieved at ratio 1 : 10, 440 W. At the same ratio but with a different capacity of 240 W, the phenolic content reached the highest. Grape oil residue was confirmed to be a highly potential antioxidant source with 19 - 29 mg/L, enabling their application as an ingredient of functional or enriched foods.

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