Correlation between the Antioxidant, Total Flavonoid and Total Phenolic Content of Phaleria macrocarpa Fruit Extract

Yee Peng Lim, Sook Fun Pang, Mashitah M Yusoff, Jolius Gimbun

Abstract: ***The effect of temperature and extraction time on the yield of polyphenol and antioxidant activity (DPPH-RSA) of Phaleria macrocarpa fruits was studied. The extraction of polyphenols from Phaleria macrocarpa fruit was performed using a maceration technique. The (TFC) Total Flavonoid Content and Total Phenolic Content (TPC) in the sample was analysed using aluminium chloride colorimetric assay and Singleton's method, respectively. Meanwhile, the antioxidant activity was determined via DPPH assay. The optimum extraction condition was achieved at 80 ºC and 60 min which yielded 69.5 mg QE/g DW, 183.2 mg GA/g DW and 171.8 mg BHA/g DW, respectively. Pearson correlation coefficient analysis shows excellent correlation coefficient with*** $R^2 > 0.91$ ***between the TPC, TFC and antioxidant activities. The method outlined here may serve as a guide to optimize the polyphenol extraction from Phaleria macrocarpa fruit.***

Index Terms**: *Keywords: Total Phenolic Content, Total Flavonoid Content, Antioxidant Activity, Maceration.***

I. INTRODUCTION

Phaleria macrocarpa (vernacular name: '*Mahkota dewa'*) is a tropical plant native to New Guinea, Indonesia that poses many pharmacological activities including anticancer [1], antidiabetes [2], antimicrobial [3] anti-inflammatory [4] and antioxidant [5]. The aforesaid medicinal benefit of *P. macrocarpa* is due to the presence of polyphenols, such as phenolic acids and flavonoids [6], [7]. However, polyphenols from *P. macrocarpa* must be extracted before it can be routinely used.

Polyphenols are dietary antioxidants, which have a higher dietary intake (1.0 g/day) than other classes of phytochemicals [8]. These antioxidants may also relieve oxidative stress in the human body, which causes various health issues, such as cancer [9], diabetes mellitus [10]-[12], osteoarthritis [13] and male infertility [14], [15]. Furthermore, antioxidants are also capable of extending the shelf-life of food by reducing rancidity or postponing lipid peroxidation [16], [17]. Therefore, they have gained attention in pharmaceuticals and cosmetic industry for their health beneficial effects.

The most common extraction technique such as maceration has been frequently applied for harvesting the polyphenolic compounds from *P. macrocarpa* [18]-[20]. However, the optimal extraction condition of polyphenols from *P. macrocarpa* is yet to be investigated. There were few parameters influencing the extraction efficiencies of maceration, including temperature, residence time, particle size, type of solvent and ratio of solvent to plant material [21]-[23]. Among these parameters, temperature and time are the most important keys to minimise the energy usage and cost of extraction process [24]. Hence, it is necessary to elucidate the influence of temperature and extraction time to the yield of polyphenol extraction from *P. macrocarpa* fruit. Thus, the present work aims to determine the influence of temperature and extraction time to the TFC, TPC and the antioxidant activity (DPPH RSA) of *P. macrocarpa* fruit extracts using a single factor approach.

II. METHODOLOGY

A. Chemical and Plant Material

Aluminum chloride hexahydrate and DPPH (1,1-diphenyl-2-picrylhydrazyl) were acquired from Sigma-Aldrich (St. Louis, MO), whereas analysis grade methanol (99%) and Folin–Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). Ethanol absolute (99.6%) was purchased from Fisher, meanwhile butylated hydroxyanisole (BHA) (98%) was sourced from R&M Chemical.

Ethno Herbs Sdn Bhd, Malaysia supplied the dried *P. macrocarpa* fruit without seed. The plant material was oven-dried for two days at 60 ºC before the plant was grinded into powder. The powder was sieved by sieve shaker to obtain the powder with about 125-250 µm. The grinded powder has a moisture content of 11.6%.

B. Extraction of Polyphenols

The extraction was performed in a stainless-steel tube using a 40% ethanol as solvent and the ratio of solvent to plant solid was set at 40 ml/g. Temperature of the vessel was maintained by a water bath (Memmert, Germany) and duration of extraction was set according to the experimental design. After extraction, the supernatant was separated from the plant material using Eppendorf 5810R centrifuge at 12857 RCF. The total flavonoid content (TFC), total

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phenolic content (TPC), DPPH radical-scavenging activity (DPPH-RSA) of the supernatant was performed thereafter. The extraction was performed in duplicate for statistical analysis.

C. Experimental Design

One factor at time experiment was carried out to determine the optimal extraction condition of polyphenols from *P. macrocarpa* fruit. At first, temperature was fixed at 80 ºC and different extraction times ranging from 30 to 240 min were applied to extract P. macrocarpa fruit. The extracts was analysed for its TFC, TPC and DPPH-RSA. The extraction time (60 min) that produced the highest TFC, TPC and DPPH-RSA was selected to study effect of temperature. The effect of temperature ranging from 60 to 100 ºC on the TFC, TPC and DPPH-RSA were studied.

D. Total Phenolic Content

Singleton's method was used to determine the TPC. Briefly, a 0.125 ml of extract was added into 0.5 ml of ultrapure water. Subsequently, a 0.125 ml of the Folin–Ciocalteu reagent was added. After 3 minutes, 1.25 ml of 7% Na_2CO_3 solution and 1 ml ultrapure water were added. The solution was mixed homogeneously and incubated in darkness for 60 min prior to analysis. The absorbance was measured at λ = 760 nm using an ultraviolet–visible spectroscopy (Varian Cary 50). TPC of the sample was determined using the calibration curve for gallic acid ranging from 10 to 400 ppm as follows:

$y = 0.0046x + 0.0064$; $R^2 = 0.998$

E. Total Flavonoid Content

The aluminum chloride colorimetric assay was used to determine the TFC. A 4.8 mL of ultrapure water is mixed with 0.2 mL of extracts, followed by addition of 5% $NaNO_2$ (0.3 mL) before subjected to intense mixing. A 10% $AlCl_3 \cdot 6H_2O$ (0.3 mL) was added 5 min later, followed by 1M NaOH (2 mL) and ultrapure water (2.4 mL). The mixed solution was measured at λ = 414 nm by ultraviolet–visible spectroscopy. TFC of the sample was determined using the calibration curve for quercetin ranging from 50 to 1000 ppm as follows:

$y = 0.0003x + 0.0008$; $R^2 = 0.9996$

F. DPPH Radical-scavenging activity

The DPPH-RSA analysis of Mokrani and Madani [25] with some adaptations was used. A 0.04 mg/mL methanolic solution of DPPH (1.8 mL) was mixed with the sample (0.2 mL of). The solution was mixed and incubated in darkness for 20 min before measuring its absorbance at λ = 517 nm by UV-VIS. All measurements were made in duplicate. The DPPH-RSA determined as follows:

$$\% \text{ DPPH radical-scavenging} = \frac{Ao - As}{Ao} \times 100$$

where A_o and A_s were absorbance of the control (blank) and sample, respectively. The DPPH-RSA of the sample was calculated using a calibration curve of butylated hydroxyanisole ranging from 0.5 to 200 ppm as follows:

$y = 0.3770x + 2.0697$; $R^2 = 0.9951$

G. Statistical Analysis

All experiments were conducted in duplicate, and the value was reported as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed at $p < 0.05$ by using the data analysis tools in Microsoft Excel 2010. Pearson correlation analysis was performed by using Microsoft Excel 2010 to compare TPC, TFC and DPPH RSA assays obtained.

III. RESULTS AND DISCUSSION

A. Effect of Extraction Time

Extraction time is a main factor that affects the energy consumption and process cost. Thus, the influence of duration (30 to 240 min) on the extraction of polyphenols from *P. macrocarpa* fruit was investigated. Fig. 1 shows that the extraction time of 60 min yielded the maximum TPC, TFC and DPPH-RSA with values of 67.1 mg GA/g DW, 183.2 mg QE/g DW and 161.9 mg BHA/g DW, respectively. The TFC, TPC and AA yield increased by 13.5%, 12.3 % and 3.5% respectively, when duration increased from 30 to 60 min. However, further increase of extraction time from 60 min up to 240 min cause the TPC, TFC and AA yield to decrease by 27.4%, 38.2% and 30.7%, respectively. The initial increase of extraction yield can be explained by Fick's law, which stated that the solute concentration in solvent and plant matrix would eventually reach an equilibrium after a certain duration [25]. At 30 min, the solute concentration gradient may still exist between plant and solvent, thus a maximum polyphenols yield was not yet recovered. However, this gradient has reached a state of equilibrium at 60 min, in which no net solute diffusion between the plant matric and solvent, and at this point, maceration has produced the highest amount of flavonoid and phenolic contents from *P. macrocarpa* fruit. Therefore, a prolong extraction time beyond 60 min was unable to extract more polyphenols. Instead, after this optimum time is reached, the decreases of phenolic and flavonoids accompanied by reduction of antioxidant capacity were observed. Obviously, a longer extraction time would have caused denaturation and oxidation of compound in presence of light and air [26], [27]. That is was why the free radical scavenging ability of *P. macrocarpa* extract decreased after 60 min. For, instance Muhamad, Yusoff and Gimbun [28] also observed a significant degradation of *Averrhoa bilimbi*'s polyphenols as they exposed to heat. In this work, polyphenols in the extracts is exposed to hot solvent throughout the extraction process. Moreover, the long extraction duration would also cause evaporation of solvent and thus lead to solvent saturation which reduced the solvent to solid ratio [29]. Therefore, the polyphenol yields and antioxidant capacities kept decreasing from 60 min until 240 min.

The results in this work are in agreement with Bindes, Cardoso [30] who reported that the highest polyphenol yield from green tea leaves was obtained in 60 min via solvent extraction. In Bindes, Cardoso [30] study, polyphenol yield increased when extraction time increased until 60 min and started to reduce after 60 min when moderate temperatures applied (40, 60 and 80 º C). Chew, Khoo [31] also employed 60 min maceration for polyphenols extraction of Centella asiatica. Chew, Khoo [31] explained that the highest TPC was obtained at 120 min was not significantly different from

that of 60 min, but shorter time was selected to minimize the process cost. In this study, 60 min gave the highest TPC, TFC and optimal antioxidant activity; hence the extraction time is fixed to 60 min for the remainder of this work.

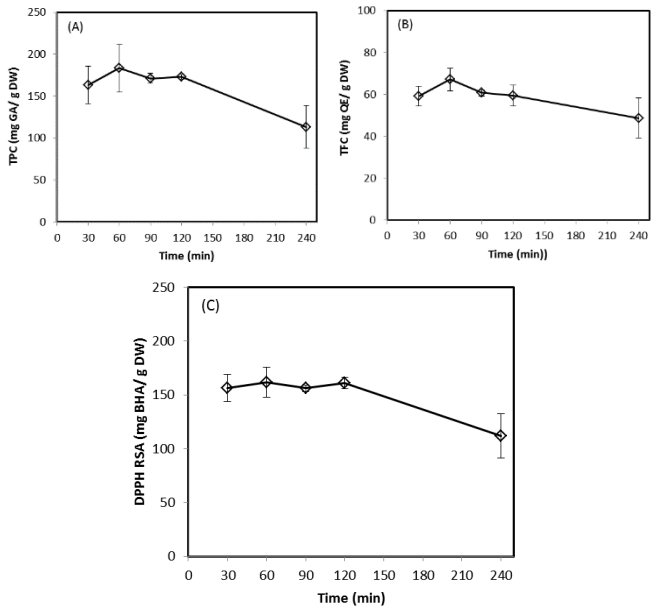

Fig. 1. Influence of duration on the extraction yield of TPC (A), TFC (B) and DPPH-RSA (C) from *P. macrocarpa* fruit.

B. Extraction Temperature

The effects of extraction temperature on TPC, TFC and antioxidant capacity (DPPH RSA) of *P. macrocarpa* extract are shown in Fig. 2. It was found that application of different temperatures significantly influenced ($p < 0.05$) the extraction of TPC, TFC and DPPH-RSA from *P. macrocarpa* fruits. The TPC and TFC yield increased from 29.4 mg GA/g DW and 53.2 mg QE/g DW to 69.5 mg GA/g DW and 183.2 mg QE/g DW, respectively when the temperature increased from 60 to 80 ºC. However, the further increase of temperature to 100 ºC caused a significant reduction of TFC yield, although the change in TPC yield is insignificantly. Generally, the TPC and TFC yield increased proportionally with increases in temperature, then reaching the highest values at 80 ºC but further increase of temperature up to 100 ºC cause a reduction in TPC and TFC. The higher temperature cause plant tissue softening and thus improved the permeability of cell wall for diffusion of compounds though cell wall. Besides, heat also weakened the phenol-protein and phenol-polysaccharide bonds in plant matric by hydrolysis to promote transfer of polyphenols into solvent [22]. The high temperature also enhanced the solubility of compound and increased the diffusion coefficient [32], [33]. Therefore, more compounds migrated into the solvent at higher temperature. However, a higher temperature beyond 80 °C may cause polyphenols degradation [34]. Similarly, Mueller-Harvey [35] also reported that the phenolic compounds, such as hydrolysable tannins, decompose very rapidly when exposure to sunlight or higher temperatures.

The antioxidant activity also increased from 73.4 mg BHA/g DW to 171.8 mg BHA /g DW when temperature increased from 60 ºC to 80 ºC, but reduced to 101.2 mg BHA/g DW when temperature continued to increase up to 100 ºC. As explained earlier, this phenomenon can be attributed to polyphenol degradation at higher temperature, and hence resulted in the loss of antioxidant ability of *P. macrocapa* fruit extract. Previous studies have demonstrated that heat promotes the extraction of antioxidants by increasing the permeability of cell wall, improving the solubility of compound, higher diffusion coefficient and thus enhancing mass transfer of compound into solvent [36]-[38]. However, the stability of compound can be significantly affected and may denature at high temperature. The results obtained in this work are in accordance with Bucić-Kojić, Planinić [39] who found an increasing trend of phenolics compounds corresponding to increase of temperature from 25 to 80 ºC and reached the optimum at 80 ºC to give maximum phenolic content of 129.6 mg/ g from grape seed. Similarly, Bindes, Cardoso [30] also reported the maximum phenolic content recovered from green tea leaves at 80 ºC. Thus, 80 ºC was employed as optimum temperature for *P. macrocarpa* fruit extract because it maximized the extraction yield of polyphenols and at the same time, minimize the polyphenols degradation.

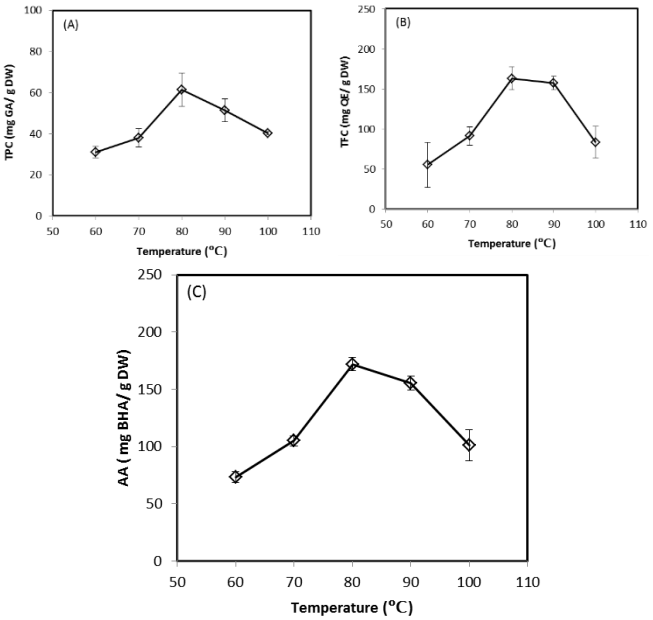

Fig. 2. Influence of temperature on the extraction yield of TPC (A), TFC (B) and DPPH-RSA (C) from *P. macrocarpa* fruit.

C. Pearson Correlation Analysis

Analysis of the relation between the TFC, TPC and DPPH-RSA for sample obtained at different extraction condition was performed to elucidate the effect of extraction condition to the in-vitro antioxidant activity of the extracts. The Pearson correlation between TFC, TPC and DPPH-RSA for sample obtained at different extraction conditions is shown in Table 1. At different extraction time, TPC and TFC were found to have a significant ($p < 0.001$) and positive correlation to antioxidant activity with the Pearson correlation coefficients (R) of 0.93 and 0.96, respectively. This indicates that the polyphenol content of *P. macrocarpa* fruit extract contributes to its hydrogen electron donating abilities. In the other words, the antioxidant activity of *P.*

macrocarpa extracts can be attributed to the presence of polyphenol. The linear positive correlation between polyphenol and antioxidant capacity was also reported by previous works [40]-[42].

Under the influence of extraction temperature, the correlation between antioxidant assays and TPC (R = 0.98) and TFC (R = 0.91) were also positively significant at $p <$ 0.001. The positive correlation over elevated temperature suggests that polyphenol compound of *P. macrocarpa* undergone thermal degradation into the products which poses higher antioxidant capacity. This phenomenon was also reported by Hossain, Barry-Ryan, Martin-Diana & Brunton [43] who found that the positive relation between antioxidant capacity and total phenolic content for increased temperature was attributed by the decomposition products of rosmarinic acid which poses higher antioxidant activity. Earlier, Moon and Terao [44] reported the degradation product from polyphenol, such as dihydrocaffeic acid has a stronger antioxidant power than caffeic acid which they come from. Earlier, other researcher [45] also found that the thermal decomposition of rosmarinic acid into 3,4-Dihydroxyphenyllactic acid contributed to the linear positive correlation with antioxidant activity. Therefore, even at high temperatures, the thermal degradation of *P. macrocarpa* may produce useful antioxidants.

Table I. Pearson Correlations coefficient (R) between different assays under influence of extraction parameters

	Time		Temperature	
	TFC	**RSA**	**TFC**	**RSA**
TPC	0.9283	0.9270	0.8841	0.9819
TFC		0.9625		0.9142

IV. CONCLUSION

One factor at time approach employed in this work successfully yielded an extract with higher TFC, TPC and DPPH-RSA from *P. macrocarpa* fruit via maceration. The best extraction condition was achieved at 60 min and 80 ºC with TPC, TFC and DPPH-RSA of 69.5 mg QE/g DW, 183.2 mg GA/g DW and 171. 8 mg BHA/g DW, respectively. It was found that TPC and TFC in the extracts is significantly and positively correlated to its in-vitro antioxidant activity. In a way, either TPC or TFC values may be used to predict the free radical scavenging activity of *P. macrocarpa* fruit extract. The method outlined in this paper may offer a useful guidance to optimize the polyphenol extraction from *Phaleria macrocarpa* fruit.

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REFERENCES

[1] Lay MM, Karsani SA, Mohajer S, Abd Malek SN. Phytochemical constituents, nutritional values, phenolics, flavonols, flavonoids, antioxidant and cytotoxicity studies on Phaleria macrocarpa (Scheff.) Boerl fruits. BMC Complementary and Alternative Medicine. 2014;14:1-12.

[2] Ali RB, Atangwho IJ, Kuar N, Ahmad M, Mahmud R, Asmawi MZ. In vitro and in vivo effects of standardized extract and fractions of Phaleria macrocarpa fruits pericarp on lead carbohydrate digesting enzymes. BMC Complementary and Alternative Medicine. 2013;13.

[3] Shodikin MA. Antimicrobial activity of mahkota dewa (Phaleria macrocarpa (Scheff). Boerl) leaf extract againts Pseudomonas aeruginosa by agar dilution and scanning electron microscopy. Folia Medica Indonesiana. 2010;46:172-8.

[4] Tandrasasmita OM, Sutanto AM, Arifin PF, Tjandrawinata RR. Anti-inflammatory, antiangiogenic, and apoptosis-inducing activity of DLBS1442, a bioactive fraction of Phaleria macrocarpa, in a RL95-2 cell line as a molecular model of endometriosis. International journal of women's health. 2015;7:161.

[5] Anggraini T, Lewandowsky P. The Exotic Plants of Indonesia : Mahkota Dewa (Phaleria macrocarpa), Sikaduduak (Melastoma malabathricum Linn) and Mengkudu (Morinda citrifolia) as Potent Antioxidant Sources. Intenational Journal On Advanced science Engineering nformation Technologi. 2015;5:115-8.

[6] Fariza IN, Fadzureena J, Zunoliza A, Chuah AL, Pin K, Adawiah I. Anti-inflammatory activity of the major compound from methanol extract of Phaleria macrocarpa leaves. J Appl Sci. 2012;12:1195-8.

[7] Shwter AN, Abdullah NA, Alshawsh MA, El-Seedi HR, Al-Henhena NA, Khalifa SA, et al. Chemopreventive effect of Phaleria macrocarpa on colorectal cancer aberrant crypt foci in vivo. Journal of ethnopharmacology. 2016;193:195-206.

[8] Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. The American journal of clinical nutrition. 2005;81:215-7.

[9] Trilaksana N, Riwanto I, Tjandrawinata RR, Winarto R. Inhibition of Mahkota Dewa (Phaleria macrocarpa) bioactive fraction on proliferation of human retinoblastoma tumor cells Y-79 through suppression of mRNA level of cyclin E. Asian Pacific Journal of Tropical Biomedicine. 2017;7(4):280-7.

[10] Sulistyoningrum E. Phaleria macrocarpa reduces glomerular growth factor expression in alloxan-induced diabetic rats. Universa Medicina. 2013;32(2):71-9.

[11] Triastuti A, Park HJ, Choi JW. Phaleria macrocarpa suppresses oxidative stress in alloxan-induced diabetic rats by enhancing hepatic antioxidant enzyme activity. Natural Product Sciences. 2009;15(1):37-43.

[12] Yanti AR, Radji M, Mun'im A, Suyatna F. Antioxidant effects of Methanolic extract of Phaleria macrocarpa (Scheff.) Boerl in fructose 10%-induced rats. International Journal of PharmTech Research. 2015;8(9):41-7.

[13] Rahmadi A, Dewi S, Nawawi A, Adnyana I, Gunadi R. Effectivity and safety of mahkota dewa fruit extract compared to meloxicam (phaleria macrocarpa fructus) on osteoarthritis. Indonesian Journal of Rheumatology. 2016;8:20-5.

[14] Bagyo DE, Utomo B, Setiabudi RS. The Effect of Mahkota Dewa (Phaleria macrocarpa) Pulp Extract by Peroral Administration Toward The Percentage of Capacitation and Acrosome Reaction in Rat (Rattus norvegicus). KnE Life Sciences. 2017;3(6):694-701.

[15] Parhizkar S, Yusoff MJ, Dollah MA. Effect of Phaleria macrocarpa on sperm characteristics in adult rats. Advanced pharmaceutical bulletin. 2013;3(2):345.

[16] Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food chemistry. 2007;100(4):1409-18.

[17] Sulistyoningrum E, Pradipta DM, Fanana S, Haikhah JA, Putro MDH. Protective effect of Phaleria macrocarpa (Scheff.) Boerl extract on the testicular damage of streptozotocin and nicotinamide-induced type 2 diabetic rats. Journal of Applied Pharmaceutical Science Vol. 2018;8(06):139-46.

[18] Oshimi S, Zaima K, Matsuno Y, Hirasawa Y, Iizuka T, Studiawan H, et al. Studies on the constituents from the fruits of Phaleria macrocarpa. Journal of natural medicines. 2008;62(2):207-10.

[19] Suparto IH, Arfianti N, Septiawati T, Triwahyuni W, Iskandriati D. Ethanol extract of Mahkota Dewa (Phaleria macrocarpa (Scheff,) Boerl.) fruit with in-vitro antidiabetic activities. Proceding Int Semin Chem. 2008:285-8.

[20] Susilawati S, Matsjeh S, Pranowo HD, Anwar C. Antioxidant activity of 2, 6, 4'-trihydroxy-4-methoxy benzophenone from ethyl acetate extract of leaves of Mahkota Dewa (Phaleria macrocarpa (Scheff.) Boerl.). Indonesian Journal of Chemistry. 2011;11(2):180-5.

[21] Pinelo M, Rubilar M, Sineiro J, Nunez M. Extraction of antioxidant phenolics from almond hulls (Prunus amygdalus) and pine sawdust (Pinus pinaster). Food Chemistry. 2004;85(2):267-73.

[22] Shi J, Yu J, Pohorly J, Young JC, Bryan M, Wu Y. Optimization of the extraction of polyphenols

from grape seed meal by aqueous ethanol solution. J Food Agric Environ. 2003;1(2):42-7.
[23] Rajha HN, El Darra N, Hobaika Z, Boussetta N, Vorobiev E, Maroun RG, et al. Extraction of total phenolic compounds, flavonoids, anthocyanins and tannins from grape byproducts by response surface methodology. Influence of solid-liquid ratio, particle size, time, temperature and solvent mixtures on the optimization process. Food and Nutrition sciences. 2014;5(04):397.
[24] Yim HS, Chye FY, Rao V, Low JY, Matanjun P, How SE, et al. Optimization of extraction time and temperature on antioxidant activity of Schizophyllum commune aqueous extract using response surface methodology. Journal of food science and technology. 2013;50(2):275-83.
[25] Mokrani A, Madani K. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (Prunus persica L.) fruit. Separation and Purification Technology. 2016;162:68-76.
[26] Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of pharmaceutical and biomedical analysis. 2006;41(5):1523-42.
[27] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-52.
[28] Muhamad N, Yusoff M, Gimbun J. Thermal degradation kinetics of nicotinic acid, pantothenic acid and catechin derived from Averrhoa bilimbi fruits. RSC Advances. 2015;5(90):74132-7.
[29] Tan M, Tan C, Ho C. Effects of extraction solvent system, time and temperature on total phenolic content of henna (Lawsonia inermis) stems. International Food Research Journal. 2013;20(6):3117.
[30] Bindes MMM, Cardoso VL, Reis MHM, Boffito DC. Maximisation of the polyphenols extraction yield from green tea leaves and sequential clarification. Journal of Food Engineering. 2019;241:97-104.
[31] Chew K, Khoo M, Ng S, Thoo Y, Aida WW, Ho C. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. International Food Research Journal. 2011;18(4):1427.
[32] Saci F, Louaileche H, Bey MB, Meziant L. Optimization of phenolic compound recovery and antioxidant activity from carob pulp using response surface methodology. International Food Research Journal. 2017;24(3):1094.
[33] Tabaraki R, Nateghi A. Optimization of ultrasonic-assisted extraction of natural antioxidants from rice bran using response surface methodology. Ultrasonics sonochemistry. 2011;18(6):1279-86.
[34] Perva-Uzunalić A, Škerget M, Knez Ž, Weinreich B, Otto F, Grüner S. Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. Food Chemistry. 2006;96(4):597-605.
[35] Mueller-Harvey I. Analysis of hydrolysable tannins. Animal feed science and technology. 2001;91(1-2):3-20.
[36] Pinelo M, Del Fabbro P, Manzocco L, Nuñez MJ, Nicoli MC. Optimization of continuous phenol extraction from Vitis vinifera byproducts. Food Chemistry. 2005;92(1):109-17.
[37] Spigno G, De Faveri DM. Antioxidants from grape stalks and marc: Influence of extraction procedure on yield, purity and antioxidant power of the extracts. Journal of Food Engineering. 2007;78(3):793-801.
[38] Dorta E, Lobo MG, Gonzalez M. Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. Journal of Food Science. 2012;77(1):C80-C8.
[39] Bucić-Kojić A, Planinić M, Tomas S, Jakobek L, Šeruga M. Influence of solvent and temperature on extraction of phenolic compounds from grape seed, antioxidant activity and colour of extract. International journal of food science & technology. 2009;44(12):2394-401.
[40] Shiraishi M, Shinomiya R, Chijiwa H. Varietal differences in polyphenol contents, antioxidant activities and their correlations in table grape cultivars bred in Japan. Scientia Horticulturae. 2018;227:272-7.
[41] Frankel EN, Waterhouse AL, Teissedre PL. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. Journal of Agricultural and Food chemistry. 1995;43(4):890-4.
[42] Burns J, Gardner PT, O'Neil J, Crawford S, Morecroft I, McPhail DB, et al. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. Journal of agricultural and food chemistry. 2000;48(2):220-30.
[43] Hossain M, Barry-Ryan C, Martin-Diana AB, Brunton N. Optimisation of accelerated solvent extraction of antioxidant compounds from rosemary (Rosmarinus officinalis L.), marjoram (Origanum majorana L.) and oregano (Origanum vulgare L.) using response surface methodology. Food Chemistry. 2011;126(1):339-46.
[44] Moon J-H, Terao J. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. Journal of Agricultural and Food Chemistry. 1998;46(12):5062-5.
[45] Vergara-Salinas JR, Pérez-Jiménez J, Torres JL, Agosin E, Pérez-Correa JR. Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (Thymus vulgaris). Journal of agricultural and food chemistry. 2012;60(44):10920-9.