Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(2): 1129-1137. **OPEN ACCESS**

Quantification of Epigallocatechin Gallate in melon manis Terengganu (*Cucumis melo* L.) by High Performance Liquid Chromatography

Ying Qian Ong[1], Sakinah Harith[1], Mohd Razif Shahril[1], Norshazila Shahidan[2] and Su Yi Sia[2]

[1]Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Nerus, Terengganu, **Malaysia**

[2]Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Tembila Campus, 22200 Besut, Terengganu, **Malaysia**

***Correspondence:** sakinahharith@unisza.edu.my **Received 24-03-2020, Revised: 23-05-2020, Accepted: 28-05-2020 e-Published: 10-06-2020**

Growing demands for *Melon Manis Terengganu* (MMT) have resulted in an increase in waste production contributed by both discarded immature MMT and the inedible portion of mature MMT. These wastes are the source of biologically active compounds such as polyphenols and epigallocatechin gallate (EGCG) which is an interesting compound due to its well-known antimicrobial, antioxidant and anti-inflammatory properties. Hence, this research aimed to identify and compare the EGCG content in different maturity levels and fruit parts (peel, flesh and seed) of MMT. Different fruit parts were dried and ground into powder form, followed by water extraction. The crude extracts were then subjected to high performance liquid chromatography. The results showed that mature MMT peel demonstrated the highest EGCG concentration of 0.042 mg/mL among all fruit parts. In terms of maturity comparison, both mature seed and peel had significantly higher EGCG concentrations (0.022 mg/mL and 0.042 mg/mL respectively) compared to their counterparts (0.008 mg/mL and 0.009 mg/mL respectively). In summary, mature MMT had higher extraction yield and EGCG concentration. The result of this study can serve as the baseline data for further applications in the food industry to develop functional food ingredients with the utilization of MMT.

Keywords: epigallocatechin gallate, *Melon Manis Terengganu*, high performance liquid chromatography

INTRODUCTION

Generally, fruits and vegetables are well known for their high concentration of polyphenols (Sommella et al., 2015). The Cucurbitacaea family has garnered a lot of attention by researchers in which *Cucumis melo* L. is the main plant of this family (Gill et al., 2011). *Melon Manis Terengganu* (MMT) is one of the *Cucumis melo* L. species and categorized under the Cantaloupe variation which originated from Terengganu, Malaysia (N. Muhamad et al., 2018). Increasing demands for this fruit have resulted in an average total waste of up to 83% contributed by discarded immature MMT (peel, flesh and seed). The inedible parts of mature MMT such as the peel and seed also contributed to waste production. However, these wastes contain sources of biologically active compounds such as polyphenols, vitamins, enzymes and dietary fibers (Sagar et al., 2018).

Fruit polyphenols consist of a wide variety of bioactive compounds such as hydroxycinnamic acids, flavan-3-ols, gallic acid derivatives, flavonols and anthocyanins (Miletić et al., 2012). Among all of the bioactive compounds,

epigallocatechin gallate (EGCG) is one of the interesting compounds being investigated by researchers due to its presumed roles in various physiological activities with its antimicrobial, antioxidant, anti-inflammatory and anti-cancer properties (Tu et al., 2019). Research has demonstrated that EGCG acts via diversified molecular mechanisms (Peter et al., 2017). EGCG is derived from the flavonoid class and categorized under flavan-3-ols class (Vasantrao and Balaraman, 2015). Fruit maturity and fruit parts can influence the composition, concentration and distribution of these phenolic compounds (Miletić et al., 2012).

It is necessary to identify the concentration of these phenolic compounds by using chromatography due to its high sensitivity (Sarnoski et al., 2012). This study indicates an initiative toward marketing it in the nutraceutical, functional food and antioxidant industry via value addition (Mahmood et al., 2012). Therefore, this study aims to determine the extraction yield and analyze the EGCG concentration in different fruit parts and different maturity levels of MMT using high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Plant Materials and Chemicals

Fresh immature MMT with 5° Brix of average weight (150 - 200 g), diameter (6 – 9 cm) and length (8 – 12 cm), as well as mature MMT with 10° Brix and average weight (650 - 700 g), diameter (10 – 12 cm) and length (11 – 13 cm), were collected from Mega Fertigation Farm, Kampung Telaga Papan, Setiu, Terengganu. HPLC grade acetic acid and HPLC grade acetonitrile were obtained from Thermo Fisher Scientific (USA) while HPLC grade methanol was purchased from O & E Technology (Malaysia). Sodium metabisulphite was bought from Nacalai Tesque (Japan) while a standard EGCG was purchased from Chemfaces (China). All chemicals and reagents used were of analytical grade. Distilled water was purified using a Sartorius water purification system (Germany).

Equipment

HPLC was conducted using a Shimadzu model LC20A system (Japan) comprising a binary pump LC-20AT with micro vacuum degassers, a thermostated column compartment, an autosampler SIL-20A, and a UV-Vis detector SPD-20A. The analytical column used in the reversed phase of HPLC was Shim-pack GIS HILIC (4.6×250 mm, 5 µm, Japan) while the guard column used was HILIC (4.0×10 mm, 5 µm, Japan).

Sample Preparation

The immature and mature MMT were washed under running tap water to remove any impurities, dust or foreign substances and separated into different parts, namely peel, flesh and seed. The peel, seed and sliced flesh were soaked in 0.2% sodium metabisulphite for 15 minutes before drying in a cabinet dryer at 50 °C for 72 hours. Then, the dried pieces of fruits parts were ground into powder form using a stainless steel grinder. The sample powder was then sieved through 500 µm mesh and stored in an airtight container in a freezer (-18°C) prior to extraction (N. Muhamad et al., 2018).

Sample Extraction

Samples were extracted with deionized water following the procedure described by Wissam et al., (2012). 200 mg of sample powder was mixed with 10 ml of deionized water and placed in a thermostatic water bath shaker at 50°C for 20 minutes. The supernatant was collected by centrifugation at 2000 rpm for 10 minutes at 25 °C (Mitchell, Robertson, and Koh 2017) and filtered through Whatman filter paper No. 1 to obtain a clear solution. Then, 10 ml of deionized water was added to the solid residue and extracted twice, following the same procedure. The supernatants were pooled and freeze dried using the VirTis™ SP Scientific (United Kingdom) pilot freeze dryer at -40 °C for 72 hours.

HPLC Analysis

Chromatographic Condition

The crude extract was dissolved in HPLC grade methanol (50 mg/ml) and filtered through a polypropylene filter unit (0.45 µm) before being injected for the reversed phase of HPLC analysis described (Pasini et al., 2019) with acetonitrile: acetic acid (98:2v/v) as mobile phase A and methanol: water: acetic acid (95:3:2 v/v/v) as mobile phase B. Separation of phenolic compounds was achieved with the following gradient as shown in Table 1 with a flow rate of 0.8 ml/min. The temperature of the column was kept at 35°C and the injection volume was 10 µL. The separation was monitored by UV-Vis at 280 nm. HPLC analysis was performed in triplicate for each sample.

Table 1 : Gradient elution of reversed-phase HPLC

Time (min)	Mobile phase A	Mobile phase B
0 – 3	93%	7%
3 – 20	93% - 84.5%	7% - 15.5%
20 – 23	84.5% - 0%	15.5% - 100%
23 – 30	0%	100%
30 – 36	0% - 93%	100% - 7%
36 - 42	93%	7%

Standard Solution Preparation

A stock solution containing the EGCG was prepared and diluted to appropriate concentrations ranging from 0.02 – 0.1 mg/ml with HPLC grade methanol. Briefly, the calibration was achieved using the standard substance EGCG. Standard calibration curve of peak area (y) against concentration (x) was plotted. A linear regression method was used to identify the slope and correlation coefficient of the linear regression equation.

Statistical Analysis

The research data were analyzed using IBM SPSS for Windows version 21.0. Data were entered, cleaned and checked before analysis. The data were assessed by descriptive analysis and presented as mean and standard deviation. Statistical analysis was conducted using one-way ANOVA test followed by Tukey's Post-hoc test to compare the extraction yield and EGCG content in different fruit parts while independent t-test was used for different maturity comparison. A p-value of ≤ 0.05 was considered as statistically significant.

RESULTS

Extraction Yield

As shown in Table 2, the yield of extract ranged from 20.00% to 31.70% in mature MMT and 15.83% to 21.50% in immature MMT. In terms of maturity breakdown, a significantly higher extraction yield was found in mature peel and flesh compared to their counterparts (***P*** < 0.05) but not for the seed.

By looking at the fruit parts, the highest yield was obtained from the flesh while seed presented the lowest yield in mature MMT. However, there was no significant difference in the extraction yield between peel and seed. On the other hand, the immature seed had the highest extraction yield, followed by flesh and peel (***P*** = 0.016).

HPLC Analysis

Chromatographic peaks in the MMT extracts were determined by tallying the retention time and UV absorption spectra with the peak in the chromatogram of EGCG standard. The retention time for EGCG standard was 18.099 minutes as shown in Figure 1. Meanwhile, the chromatogram for mature and immature MMT flesh is displayed in Figure 2 and 3 respectively. An external standard calibration method was used for EGCG quantification in the MMT extracts. Figure 4 depicts the EGCG calibration curve with R^2 = 0.995. The equation for calibration curve was y = 4.58059e+006x - 24720.3. The EGCG concentration in MMT extracts was calculated based on the calibration equation prepared by the EGCG standard as displayed in Table 3.

In terms of the fruit parts, there was no significant difference in EGCG concentration in immature MMT (***P*** = 0.106). In contrast, mature MMT showed a significant difference in which peel had the highest EGCG concentration of 0.0420 (0.0026) mg/mL, followed by seed and flesh (P < 0.0001). Next, in terms of maturity comparison, both mature seed and peel had a significantly higher EGCG concentration compared to their counterparts while the EGCG concentrations in both mature and immature flesh were similar.

Table 1 : Comparison of % extraction yield in different maturity and parts (n = 3)

Maturity	Extraction yield (%)			***P*-value[b]**
	Seed	**Peel**	**Flesh**	
Mature	20.00 (1.56)[a]	21.87 (0.15)[a]	31.70 (1.60)[b]	< 0.0001
Immature	21.50 (2.82)[a]	15.83 (0.35)[b]	18.10 (0.30)[ab]	0.016
***P*-value[a]**	0.465	< 0.0001	< 0.0001	

Data are reported as mean (SD).
Values with different superscript letters within the same row are statistically different
[a] Independent t-test was applied.
[b] ANOVA test was applied followed by Post-hoc multiple comparison test.

Table 2 : Comparison of EGCG concentration in different maturity and parts (n = 3)

Maturity	EGCG concentration (mg/mL)			*P*-value[b]
	Seed	Peel	Flesh	
Mature	0.0220 (0.0017)[a]	0.0420 (0.0026)[b]	0.0150 (0.0035)[c]	< 0.0001
Immature	0.0077 (0.0029)[a]	0.0087 (0.0006)[a]	0.0130 (0.0036)[a]	0.106
***P*-value[a]**	0.002	< 0.0001	0.527	

Data are reported as mean (SD).
Values with different superscript letters within the same row are statistically different
[a] Independent t-test was applied.
[b] ANOVA test was applied followed by Post-hoc multiple comparison test.

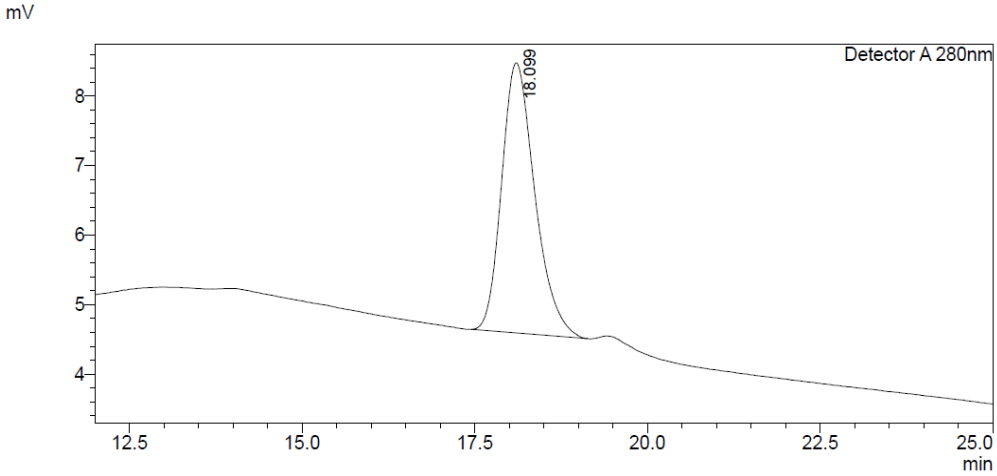

Figure 1: HPLC chromatogram of EGCG standard at 280 nm

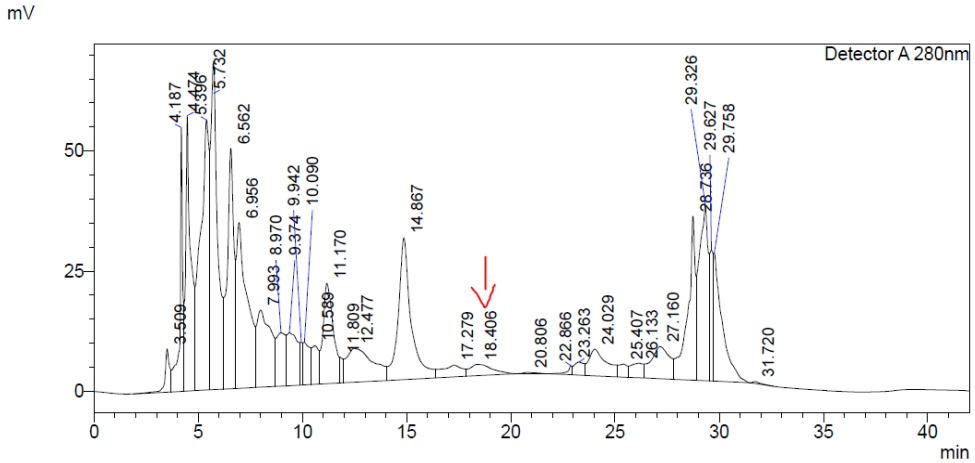

Figure 2: HPLC chromatogram of mature MMT flesh at 280 nm (red arrow: EGCG)

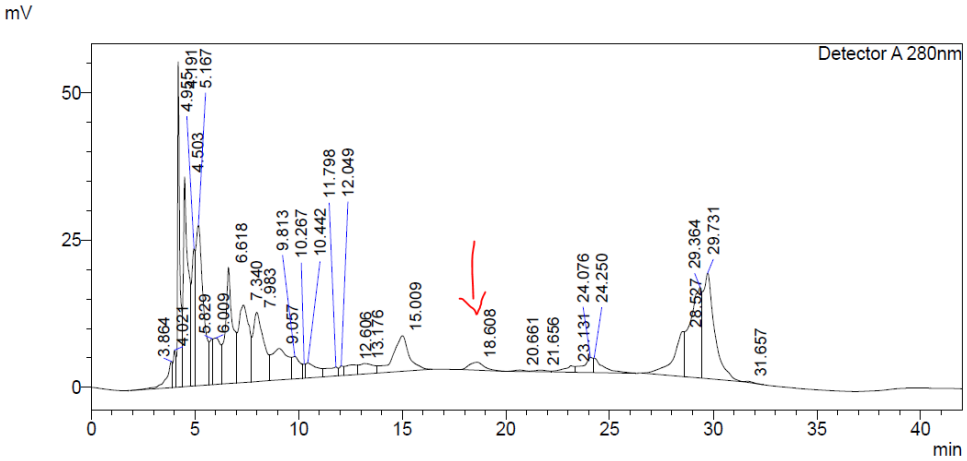

Figure 3: HPLC chromatogram of immature MMT flesh at 280 nm (red arrow: EGCG)

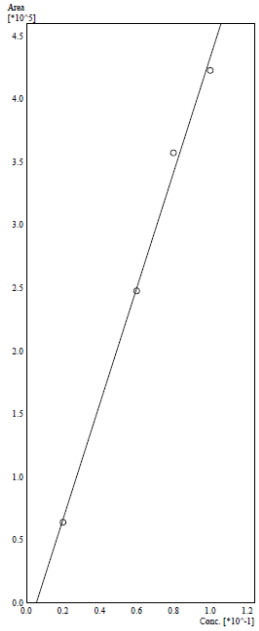

Figure 4: Standard curve for EGCG over the concentration range of 0.02–0.1 mg/ml

DISCUSSION

Extraction Yield

Extraction is the first vital step to recover and isolate bioactive compounds from plant tissues (Singh et al., 2016). Solid liquid extraction (SLE) is the most common extraction method that involves the mass transfer phenomenon in which the compounds in the solid matrix are dispersed into the extraction solvent via maceration, mixing or shaking (Miletić et al., 2012). The results of this study are in agreement with the extraction yield of pawpaw pulp (*Asimina triloba* [L.] Dunal) with ripe fruit having a higher yield than unripe fruit (J.-S. Nam et al., 2019). Next, another study also reported increased extraction yield in *Berberis buxifolia* fruit throughout the ripening process (Arena et al 2012). This is because mature fruits have a higher activity of pectinolytic enzymes (Goulao et al 2007) which assists the extraction process and results in higher extraction yield (Antonio et al., 2014). The high extraction yield is predicted to be contributed by the monosaccharide or disaccharides such as glucose, sucrose and fructose due to increased free sugar content with the ripening process (J. Nam et al., 2018).

The findings on the comparison of mature MMT parts are in accordance with the study

conducted by Ismail et al., (2010) and Marwa and Hany (2016) who reported that the highest extraction yield was in the flesh of cantaloupe and the lowest was in the seed. Another study which was carried out by Shafii et al., (2017) also reported a higher extraction yield in the flesh of *Manilkara zapota* fruit compared to the peel and seed. Low extraction yield of seed was attributed to the presence of major components such as starch and protein which are less soluble in deionized water (Ismail et al., 2010; Marwa and Hany 2016). However, a study reported a higher yield in the peel of *Carica* papaya compared to its seed (S. A. S. Muhamad et al., 2017). Research claimed that the extraction efficiencies are mostly influenced by understanding the nature of plant matrix and chemistry of the bioactive compounds (Azmir et al., 2013).

Hydrophilic Interaction Chromatography (HILIC)

HPLC is considered as the most common and crucial method to separate and quantify distinct phenolic compounds. HILIC column was used as it was expected to provide a better resolution compared to Diol column with a similar eluent mixture. This is because the HILIC column contains cross-linked diol groups which increase both its functionality and robustness compared to the Diol column. By looking at organic solvents, acetonitrile is commonly used as an eluent due to its weak polarity and samples dissolved in such solvent can be directly introduced into the HPLC system without any effect on separation efficiency (Miletić et al., 2012). Methanol is also recommended in HILIC (Sentkowska et al., 2013) as it can disturb the water layer formation by replacing the water molecules via hydrogen bonding. This results in a more hydrophobic water layer (Sentkowska et al., 2016). In addition, another study proposed that methanol is adsorbed near the stationary phase by hydrogen bonding with residual silanols while acetonitrile can react with silanols using dipol-dipol interactions (Buszewski et al., 2010).

EGCG is unstable in an alkaline medium, thus the presence of acid in the mobile phase is crucial to ensure complete resolution and peak tailing elimination (Dhanani et al., 2017). Buffers are usually used to adjust the mobile phase pH to avoid pH fluctuation of eluent in order to produce more reproducible and robust methods (Tu et al., 2019). Next, the presence of organic acid such as acetic acid in the mobile phase acts as salt to protect against attractive and repulsive electrostatic effects. Some modifications had been made to ensure complete resolution of EGCG. For example, an isocratic period was set at the initial run (held at 7% B for 3 minutes) followed by a slowed increase in the addition of the methanolic mobile phase (B) into the acetonitrile phase (A) (Kelm et al., 2006). Column temperature of 35 °C was found to aid in achieving good resolution and deviations of ±5 °C and did not exert significant effects on the separations (Robbins et al., 2009).

HPLC Analysis of EGCG

The results demonstrated that EGCG concentration was higher in mature MMT peel, which is in accordance with the study by Rolim et al., (2018) who found that the melon (*Cucumis melo* L.) peel extracts showed a higher phenolic content than the melon seed extracts. They also revealed that catechin concentrations in the melon peel aqueous extracts were higher (4.10 µg/mL) than that of the melon seed aqueous extracts (1.77 µg/mL). Besides, the peel extracts of grape varieties had high proanthocyanidins contents (PACs) which are known to have a high antioxidant activity according to Benmeziane and Cadot (2018). Wissam et al., (2012) also reported that higher PACs yield was observed in pomegranate peel extracts by using water as a solvent. Similarly, a significant portion of polyphenols is often present in high concentrations in the outer parts of fruits (Wissam et al., 2012).

Morais et al., (2015) evaluated seven tropical fruits' parts (pulp, seed and peel) and the results showed that the highest phenolic contents were found in peels as compared to pulps and seeds. Pulps were found to have a poor amount of antioxidant compounds. Ayala-Zavala et al., (2011) also reported that the content of functional compounds in different tissues of tropical exotic fruits was located mostly in peels and seeds and to a lesser extent in the pulps. The differences in phenolic compounds concentration in different fruit parts could be attributed to the variability of each part against environmental stresses that can stimulate an increase in phenolic compound contents as a natural survival instinct of the plants. Thus, peel extract had the highest EGCG concentration due to greater exposure to environmental stress conditions (Rolim et al., 2018).

Generally, the EGCG concentrations in mature MMT were higher than those of immature MMT. According to Bargui Koubala (2016), the total phenolic contents of peel, flesh and seed

varied significantly ($p < 0.05$) during the Tibish melon (*Cucumis melo* L.) fruit development. The peel was five to six times richer in phenolic compounds than the flesh and seed at the early stage of fruit maturation. Lee and Hwang (2017) reported that the total phenolics, anthocyanins, and antioxidant activities of mulberry fruits increased during ripening. An increase in the concentrations of total phenols in the grape seed during grape ripening has been reported by Abdel-Salam and Hassan (2015). A study on bitter melon proposed that catechin contents increased with increasing maturation (S. H. Lee et al., 2018). Next, catechin also increased slightly during ripening from the immature stage to the commercially mature stage as observed in cranberries (Oszmiański et al., 2018). Higher phenolic compound concentration in mature fruit could be attributed to the biosynthesis of phenolic compounds caused by enzyme hydrolysis during maturation (Kubola and Siriamornpun, 2008).

Strengths and Limitations

The presence of high organic solvent concentration in the HILIC mode enables fast separation of analytes under higher flow rates or when using a column with small particles due to lower backpressure. However, HILIC is more affected by the sample diluent composition and the retention characteristics are less predictable due to the complex retention mechanism (Sentkowska et al., 2016).

The traditional extraction method has a few drawbacks such as solvent consumption, high energy usage, thermal destruction of heat-sensitive compounds and long extraction period. Besides, this method is not selective in which other polar compounds will also be extracted together with EGCG when a polar solvent is used in the extraction (Watson, 2019).

Besides, all phenolic compounds absorbed UV light in the 280 nm region. Therefore, it is difficult to quantify EGCG in complex samples containing various interfering compounds that can be co-eluted with EGCG (Nollet and Toldrá, 2013). Thus, HPLC coupled with mass spectrometry can be performed in future studies for a more accurate EGCG quantification.

CONCLUSION

This study generated informative and novel data on the extraction yield and EGCG content with regard to different fruit parts and different maturity level of MMT. Mature MMT had both higher extraction yield and EGCG concentration. This result can serve as the baseline data for further application in the food industry to develop a functional food ingredient.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This study was funded by the Ministry of Education, Malaysia under Fundamental Research Grant Scheme (FRGS/1/2018/TK02/UNISZA/03/1). Special thanks to Centre of Research and Field Service, Universiti Malaysia Terengganu for providing HPLC services. The authors would like to extend their gratitude to everyone who helped in this research process.

AUTHOR CONTRIBUTIONS

SH, MRS and NS supervised the research process and provided critical feedback. YQO performed experiments, data analysis and wrote the manuscript with support from SYS. YQO designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Abdel-Salam M, and Manal H. 2015. Effect of CaCl2 Sprays on Anthocyanin, Pectin and Phenolic Acids Fractionation in Berry Skin of Two Red Grape (Vitis Vinifera L.) Cultivars during Ripening. Int. J. Acad. Res 7(1): 159–68.

Antonio A et al., 2014. Effect of Mash Maceration and Ripening Stage of Apples on Phenolic Compounds and Antioxidant Power of Cloudy Juices: A Study Using Chemometrics. LWT - Food Sci. Technol 57(1): 223–29.

Arena ME, Postemsky P, and Curvetto NR. 2012.

Accumulation Patterns of Phenolic Compounds during Fruit Growth and Ripening of Berberis Buxifolia, a Native Patagonian Species. New Zeal. J. Bot 50(1): 15–28.

Ayala-Zavala JF. et al., 2011. Agro-Industrial Potential of Exotic Fruit Byproducts as a Source of Food Additives. Food Res. Int 44(7): 1866–74.

Azmir J et al., 2013. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. J. Food Eng 117(4): 426–36.

Bargui Koubala B. 2016. Morphological and Biochemical Changes during Muskmelon (Cucumis Melo Var. Tibish) Fruit Maturation. J. Food Nutr. Sci 4(1): 18–28.

Benmeziane F, and Yves C. 2018. Identification of Proanthocyanidins Compounds in Skins of Some Table Grape Vitis Vinifera Varieties from Algeria Grown in Mediterranean Climate by High Performance Liquid Chromatography. Adv Food Technol Nutr Sci Open J. SE(2): S45–50.

Buszewski B et al., 2010. Study of Solvent Adsorption on Chemically Bonded Stationary Phases by Microcalorimetry and Liquid Chromatography. J. Colloid Interface Sci 349(2): 620–25.

Dhanani T, Raghuraj S, and Satyanshu K. 2017. Extraction Optimization of Gallic Acid, (+)-Catechin, Procyanidin-B2, (–)-Epicatechin, (–)-Epigallocatechin Gallate, and (–)-Epicatechin Gallate: Their Simultaneous Identification and Quantification in Saraca Asoca. J. Food Drug Anal 25(3): 691–98.

Gill NS. et al., 2011. Evaluation of Therapeutic Potential of Traditionally Consumed Cucumis Melo Seeds. Asian J. Plant Sci 10(1): 86–91.

Goulao LF, Isabel DS, and Cristina MO. 2007. Patterns of Enzymatic Activity of Cell Wall-Modifying Enzymes during Growth and Ripening of Apples. Postharvest Biol. Technol 43: 307–18.

Ismail HI, Kim WC, Abdalbasit AM, and Maznah I. 2010. Phenolic Content and Antioxidant Activity of Cantaloupe (Cucumis Melo) Methanolic Extracts. Food Chem 119(2): 643–47.

Kelm MA. et al., 2006. High-Performance Liquid Chromatography Separation and Purification of Cacao (Theobroma Cacao L.) Procyanidins According to Degree of Polymerization Using a Diol Stationary Phase. J. Agric. Food Chem 54(5): 1571–76.

Kubola J, and Sirithon S. 2008. Phenolic Contents and Antioxidant Activities of Bitter Gourd (Momordica Charantia L.) Leaf , Stem and Fruit Fraction Extracts in Vitro. Food Chem 110: 881–90.

Lee SH et al., 2018. Phenolic Acid, Carotenoid Composition, and Antioxidant Activity of Bitter Melon (Momordica Charantia L.) at Different Maturation Stages. Int. J. Food Prop 20(3): S3078–87.

Lee YC, and Keum TH. 2017. Changes in Physicochemical Properties of Mulberry Fruits (Morus Alba L.) during Ripening. Sci. Hortic. (Amsterdam) 217: 189–96.

Mahmood T, Farooq A, Mateen A, and Nazamid S. 2012. Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species from Pakistan. Int. J. Mol. Sci 13: 4591–4607.

Marwa EEI, and Gaber EH. 2016. Phenolic Content and Antioxidant Activity of Cantaloupe (Cucumis Melo Var. Cantalupensis) and Food Application. Int. J. Nutr. Food Sci 5(1): 16–24.

Miletić N, Branko P, Olga M, and Miodrag K. 2012. Phenolic Content and Antioxidant Capacity of Fruits of Plum Cv . 'Stanley' (Prunus Domestica L.) as Influenced by Maturity Stage and on-Tree Ripening. Aust. J. Crop Sci 6(4): 681–87.

Mitchell AE, Danielle R, and Eunmi K. 2017. Optimizing the Extraction of Procyanidins Oligomers through Decamer. Nutr. Food Sci. Int. J 4(3): 555–636.

Morais DR et al., 2015. Antioxidant Activity, Phenolics and UPLC–ESI (–)–MS of Extracts from Different Tropical Fruits Parts and Processed Peels. Food Res. Int 77(3): 392–99.

Muhamad N, Sahadan W, and Ho LH. 2018. Effect of Drying Temperatures and Extraction Solvents on Total Phenolic, Flavonoid Contents and Antioxidant Properties of Immature Manis Terengganu Melon (Cucumis Melo). J. Agrobiotechnology 9(1S): 114–21.

Muhamad SAS, Jamilah B, Russly AR and Faridah A. 2017. The Antibacterial Activities and Chemical Composition of Extracts from Carica Papaya Cv. Sekaki/Hong Kong Seed. Int. Food Res. J 24(2): 810–18.

Nam JS et al., 2019. Phenolic Profiles, Antioxidant and Antimicrobial Activities of Pawpaw Pulp (Asimina Triloba [L.] Dunal) at Different Ripening Stages. J. Food Sci 84(1): 174–82.

Nam JS, Hye LJ, and Young HR. 2018. Nutritional Compositions in Roots, Twigs, Leaves, Fruit Pulp, and Seeds from Pawpaw (Asimina Triloba [L.] Dunal) Grown in Korea. J. Appl. Bot. Food Qual 91: 47–55.

Nollet LML, and Fidel T. 2013. Handbook of Analysis of Active Compounds in Functional Foods. CRC Press., New York, USA

Oszmiański J, Sabina L, Józef G, and Natalia M. 2018. The Effect of Different Maturity Stages on Phytochemical Composition and Antioxidant Capacity of Cranberry Cultivars. Eur. Food Res. Technol 244: 705–19.

Pasini F, Fabio C, Maria FC, and Vito V. 2019. Recovery of Oligomeric Proanthocyanidins and Other Phenolic Compounds with Established Bioactivity from Grape Seed By-Products. Molecules 24: 677.

Peter B, Szilvia B, Robert H, and Beatrix P. 2017. Biophysical Characteristics of Proteins and Living Cells Exposed to the Green Tea Polyphenol Epigallocatechin - 3 - Gallate (EGCG): Review of Recent Advances from Molecular Mechanisms to Nanomedicine and Clinical Trials. Eur. Biophys. J 46(1): 1–24.

Robbins RJ et al., 2009. Method Performance and Multi-Laboratory Assessment of a Normal Phase High Pressure Liquid Chromatography-Fluorescence Detection Method for the Quantitation of Flavanols and Procyanidins in Cocoa and Chocolate Containing Samples. J. Chromatogr A 1216(24): 4831–40.

Rolim PM et al., 2018. Phenolic Profile and Antioxidant Activity from Peels and Seeds of Melon (Cucumis Melo L. Var. Reticulatus) and Their Antiproliferative Effect in Cancer Cells. Brazilian J. Med. Biol. Res 51(4): e6069.

Sagar NA et al., 2018. Fruit and Vegetable Waste: Bioactive Compounds, Their Extraction, and Possible Utilization. Compr. Rev. Food Sci. Food Saf 17(3): 512–31.

Sarnoski PJ et al., 2012. Separation and Characterisation of Proanthocyanidins in Virginia Type Peanut Skins by LC – MS N. Food Chem 131(3): 927–39.

Sentkowska A, Biesaga M, Pyrzynska K. 2016. Application of Hydrophilic Interaction Liquid Chromatography for the Quantification of Flavonoids in Genista Tinctoria Extract. J. Anal. Methods Chem 1: 1–9.

Sentkowska A, Magdalena B, and Krystyna P. 2013. Effects of the Operation Parameters on HILIC Separation of Flavonoids on Zwitterionic Column. Talanta 115: 284–90.

Shafii ZA, Mahiran B, Emilia AM, and Maznah I. 2017. Phytochemical and Antioxidant Properties of Manilkara Zapota (L.) P Royen Fruit Extracts and Its Formulation for Cosmeceutical Application. Asian J. Plant Sci. Res 7(3): 29–41.

Singh J, Vinti S, Surabhi S, and Awadhesh Rai. K. 2016. Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents. J. Nutr. Food Sci 6: 565.

Sommella E et al., 2015. Detailed Polyphenolic Profiling of Annurca Apple (M. Pumila Miller Cv Annurca) by a Combination of RP-UHPLC and HILIC, Both Hyphenated to IT-TOF Mass Spectrometry. Food Res. Int 76(Pt3): 466–77.

Tu LJ et al., 2019. Isolation of Epigallocatechin Gallate from Green Tea and Its Effects on Probiotics and Pathogenic Bacteria. Curr. Top. Nutraceutical Res 17(1): 69–77.

Vasantrao P, and Manohar B. 2015. Food and Bioproducts Processing Catechins: Sources, Extraction and Encapsulation. Food Bioprod. Process. 93: 122–38.

Watson RR. 2019. Polyphenols in Plants: Isolation, Purification and Extract Preparation. Ed 2. Elsevier Inc, New York, USA

Wissam Z, Bashour G, Abdelwahed W, and Khayata W. 2012. Effective Extraction of Polyphenols and Proanthocyanidins from Pomegranate's Peel. Int. J. Pharm. Pharm. Sci 4(Suppl 3): 675–82.