POLYPHENOL EXTRACTION FROM SAGO WASTEWATER
BY ION-EXCHANGE RESIN

Rizqiah Banu Binti Varusai Mohamed

Bachelor of Science with Honours
(Resource Biotechnology)
2013
Polyphenol Extraction from Sago Wastewater by Ion-Exchange Resin

Rizqiah Banu Binti Varusai Mohamed
(28021)

This project is submitted in partial fulfillment of the requirements for the degree of
Bachelor Science with Honours in Resource Biotechnology

Supervisor: Assoc. Prof. Dr. Cirilo Nolasco Hipolito

Resource Biotechnology Programme
Department of Molecular Biology
Faculty of Resource Science and Technology
University Malaysia Sarawak
ACKNOWLEDGEMENT

First of all, I would like to thank God for the strength and blessings from Him for me to complete this thesis.

I would like to express my deepest gratitude and sincere appreciation to my supervisor, Assoc. Prof. Dr. Cirilo Nolasco Hipolito for providing me opportunity to work on title proposed by me, apart from other title being provided and for his guidance and support from the beginning until the completion of this project. Special dedication to other lecturers especially Prof. Dr. Kopli Bujang in helping to obtain sample, Dr. Sim Siong Fong and Prof. Dr. Fasihuddin Badruddin Ahmad, for their advices and building comments at the very beginning of this project.

Sincere thanks go to Dr. Vimala Subramaniam, senior researcher of Medical Department of Forest Research Institute Malaysia (FRIM) for guiding me on protocols in determining polyphenol content, and also to the support staffs for their help and assistance throughout this project. My heartfelt appreciation goes to my supportive friends, classmates and peers from biochemical laboratory.

Last but not least, greatest thanks to my beloved family, especially my mom, my late dad, brothers, sisters and also to my fiance, for their unconditional love, supports and prayers.
DECLARATION

No portion of the work referred to in this report has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

(RIZQIAH BANU BINTI VARUSAI MOHAMED)

Resource Biotechnology

Department of Molecular Biology

Faculty of Resource Science and Technology

University Malaysia Sarawak.
Table of Contents

ACKNOWLEDGEMENT ....................................................................................................... ii

DECLARATION .................................................................................................................. iii

List of Abbreviations ......................................................................................................... vii

List of Tables ...................................................................................................................... ix

List of Figures .................................................................................................................... xi

Abstract .............................................................................................................................. 1

1.0 Introduction .................................................................................................................. 2

2.0 Literature Review ........................................................................................................ 4
  2.1 Sago Palm (*Metroxylon*) ......................................................................................... 4
     2.1.1 Commercial Products ....................................................................................... 5
  2.2 Sago Wastewater ........................................................................................................ 5
  2.3 Wastewater Treatment .............................................................................................. 6
  2.4 Polyphenol .................................................................................................................. 7
  2.5 Extraction of Polyphenol ........................................................................................... 8
     2.5.1 Polyphenol Extraction from Olive Mill Wastewater (OMW) ............................. 8
     2.5.2 Solid-Phase Extraction (SPE Extraction) ......................................................... 9
     2.5.3 Ion-Exchange Resins ....................................................................................... 11

3.0 Materials and Methods .............................................................................................. 15

  3.1 Materials .................................................................................................................... 15
     3.1.1 Materials Used for Primary Treatment and Membrane Filtration .................. 15
     3.1.2 Materials Used for Polyphenol Extraction ..................................................... 15
     3.1.3 Materials Used for Determination of Total Polyphenol Content ..................... 15

  3.2 Methods ..................................................................................................................... 16
     3.2.1 Sample Preparation ........................................................................................... 17
     3.2.2 Primary Treatment ............................................................................................ 18
     3.2.3 Extraction of Polyphenol (first experiment) ..................................................... 18
     3.2.4 Determination of the total phenolic content in the extracts ............................. 19
     3.2.4 The Efficiency of Amberlite IRA-400 in Cleaning the Wastewater (second experiment) 20
4.0 Results .................................................................................................................. 21
5.0 Discussion ........................................................................................................... 31
6.0 Conclusion and Recommendation ..................................................................... 36

References ............................................................................................................... 38

Appendix .................................................................................................................. 43
Appendix I ............................................................................................................... 43
Appendix II .............................................................................................................. 44
APPENDIX III ......................................................................................................... 45
APPENDIX IV ......................................................................................................... 46
APPENDIX V ......................................................................................................... 47
APPENDIX VI ......................................................................................................... 48
APPENDIX VII ....................................................................................................... 49
APPENDIX VIII ..................................................................................................... 50
APPENDIX IX ......................................................................................................... 51
APPENDIX X .......................................................................................................... 52
APPENDIX XI ......................................................................................................... 53
APPENDIX XII ....................................................................................................... 54
APPENDIX XIII ..................................................................................................... 55
APPENDIX XIV ..................................................................................................... 56
APPENDIX XV ....................................................................................................... 57
APPENDIX XVI ..................................................................................................... 58
APPENDIX XVII .................................................................................................... 59
APPENDIX XVIII ................................................................................................. 60
APPENDIX XIX ..................................................................................................... 61
APPENDIX XX ....................................................................................................... 62
APPENDIX XXI ..................................................................................................... 63
APPENDIX XXII ................................................................................................... 64
APPENDIX XXIII ................................................................................................. 65
APPENDIX XXIV ................................................................................................. 66
APPENDIX XXV ................................................................................................... 67
APPENDIX XXVI ................................................................................................. 68
APPENDIX XXVII ............................................................................................... 69
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD</td>
<td>Biochemical Oxygen Demand</td>
</tr>
<tr>
<td>C</td>
<td>intercept</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>C18</td>
<td>Carbon 18</td>
</tr>
<tr>
<td>d</td>
<td>density</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>ft</td>
<td>feet</td>
</tr>
<tr>
<td>FC</td>
<td>Folin-Ciocalteu</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic Acid Equivalent</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
</tr>
<tr>
<td>hrs</td>
<td>hours</td>
</tr>
<tr>
<td>in</td>
<td>inch</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LPPO</td>
<td>Latent Polyphenol Oxidase</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>mg/g</td>
<td>milligram per gram</td>
</tr>
<tr>
<td>mg/L</td>
<td>milligram per litre</td>
</tr>
<tr>
<td>mg/ml</td>
<td>milligram per millilitre</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>M</td>
<td>gradient</td>
</tr>
<tr>
<td>MF</td>
<td>Micro-Filtration / Membrane Filtration</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NF</td>
<td>Nano-Filtration</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OMW</td>
<td>Olive mill wastewater</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid-Phase Extraction</td>
</tr>
<tr>
<td>SPME</td>
<td>Solid Phase Micro-Extraction</td>
</tr>
<tr>
<td>SPPO</td>
<td>Soluble Polyphenol Oxidase</td>
</tr>
<tr>
<td>T°</td>
<td>Temperature</td>
</tr>
<tr>
<td>TPC</td>
<td>Total Phenolic Content</td>
</tr>
<tr>
<td>µl</td>
<td>microlitre</td>
</tr>
<tr>
<td>µm</td>
<td>micrometre</td>
</tr>
<tr>
<td>UASB</td>
<td>Up-Flow Anaerobic Sludge Blanket</td>
</tr>
<tr>
<td>UF</td>
<td>Ultra-filtration</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume (volume concentration)</td>
</tr>
</tbody>
</table>
List of Tables

Table 1: Effect of the temperature on the polyphenol adsorption ........................................ 22
Table 2: The pH of wastewater samples at different temperature before and after the treatment. ........................................................................................................................................... 24
Table 3: Light adsorption polyphenol in the wastewater samples before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 26
Table 4: Variation of pH in wastewater samples, before and after treatment with amberlite IRA-400 ........................................................................................................................................... 29
Table 5: Calibration curve concentration for gallic acid standard ........................................................................................................................................... 43
Table 6: Protocol for total phenolic content (TPC) ........................................................................................................................................... 44
Table 7: Light Adsorption of polyphenol in the wastewater samples at 30 °C, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 45
Table 8: Adsorption of light of polyphenol in the wastewater samples at 40 °C, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 46
Table 9: Adsorption of light of polyphenol in the wastewater samples at 50 °C, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 47
Table 10: Effect of the temperature on the light adsorption of polyphenol in the wastewater samples before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 48
Table 11: The pH of wastewater samples at 30 °C, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 49
Table 12: Effect of the temperature on the pH in the wastewater, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 50
Table 13: The pH of wastewater samples at 50 °C, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 51
Table 14: Changes in pH of wastewater samples at different temperature, and changes in pH based on before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 52
Table 15: Light Adsorption of polyphenol in the wastewater sample 1, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 53
Table 16: Adsorption of light of polyphenol in the wastewater samples 2, before and after the treatment with Amberlite IRA-400 ........................................................................................................................................... 54
Table 17: Adsorption of light of polyphenol in the wastewater samples 3, before and after the treatment with Amberlite IRA-400. .......................................................... 55
Table 18: The mean reading of adsorption of light of polyphenol in the wastewater samples 1, 2 and 3, before and after the treatment with Amberlite IRA-400a. ........................................ 56
Table 19: The percentage of adsorption of light of polyphenol in the wastewater samples 1, based on before and after the treatment with Amberlite IRA-400. .......................................................... 57
Table 20: The pH of wastewater sample 1, before and after the treatment with Amberlite IRA-400. .................................................................................................................. 58
Table 21: The pH of wastewater sample 2, before and after the treatment with Amberlite IRA-400. .................................................................................................................. 59
Table 22: The pH of wastewater sample 3, before and after the treatment with Amberlite IRA-400. .................................................................................................................. 60
Table 23: The mean pH of wastewater samples 1, 2 and 3, based on before and after the treatment with Amberlite IRA-400a. .......................................................... 61
Table 24: The changes in pH of wastewater samples 1, 2 and 3, based on before and after the treatment with Amberlite IRA-400a. .......................................................... 62
Figure 26: The colour changes after first round of the third cycle. ..............................................72
Figure 27: The colour changes after second round of the third cycle. ........................................73
Figure 28: The colour changes after third round of the third cycle. ..........................................73
Figure 29: The colour changes after fourth round of the third cycle. ...........................................74
Figure 30: The initial colour of the primarily treated wastewater (light-yellow colour). ..............74
Figure 31: The colour changes after first round of the first cycle. ..............................................75
Figure 32: The colour changes after second round of the first cycle. ..........................................75
Figure 33: The colour changes after third round of the first cycle. ..............................................76
Figure 34: The colour changes after fourth round of the first cycle. ...........................................76
Figure 35: The colour changes after first round of the second cycle. ..........................................77
Figure 36: The colour changes after second round of the second cycle. .....................................77
Figure 37: The colour changes after third round of the second cycle. ...........................................78
Figure 38: The colour changes after fourth round of the second cycle. .....................................78
Figure 39: The colour changes after first round of the third cycle. .............................................79
Figure 40: The colour changes after second round of the third cycle. .......................................79
Figure 41: The colour changes after third round of the third cycle. ............................................80
Figure 42: The colour changes after fourth round of the third cycle. ..........................................80
Figure 43: The steps on determining the phenolic content............................................................81
Polyphenol Extraction from Sago Wastewater by Ion-Exchange Resins

Rizqiah Bani Binti Varusai Mohamed
Resource Biotechnology
Faculty of Resource Science and Management
Universiti Malaysia Sarawak

Abstract

In Malaysia, especially in Sarawak, Metroxylon sagu, is significantly prolific for commercial starch production which is used for its conversion to syrup, derivatisation, maltodextrins, animal food and fuel ethanol. There are plenty of wastewater released from sago processing mill, as to extract starch require water supply abundantly. The sago pith residues from sago production are commonly used as diluents and supplement to green manures. Alternatively, as the presence of soluble polyphenol, flavonoids, in the sago that has been reported in the literature, therefore, this research was focused on the recovery of the polyphenols contained in the sago wastewater. The polyphenol compound was extracted due to its possible health added value. Documentation about sago wastewater is necessary for future research and effective water management. Firstly, the treatment of wastewater was conducted through a primary treatment by using a membrane filters to improve the water quality, followed by extraction of polyphenol and finally removal of polyphenol together with other mineral element. The result was obtained as clean water by primary treatment and able to collect polyphenols from sago wastewater via ion-exchange resins Amberlite IRA-400 for future studies.

Keywords: Metroxylon sagu, sago pith residues, wastewater treatment, polyphenol

Abstrak


Kata kunci: Metroxylon sagu, sisa-sisa empulur sago, rawatan air kumbahan, polifenol
1.0 Introduction

Metroxylon sagu is found in Malaysia in the states of Pahang, Johor and particularly in Sarawak. The habitats are basically found at freshwater swamps and lowland forest with near sea level but can be found at 1–700 m (3–2300 ft) with rainfall of 2000–5000 mm (80–200 in) (McClatchey et al., 2006). In Sarawak, when compared to other states in Malaysia, M. sagu is planted for commercial purpose. In the sago production industries, sago bark is one of the solid residues. The estimated productions of 5-15 tons bark per day, almost 17% of the logs are processed (Chew and Shim, 1993). While processing the pith of sago palm, there are three final products could be obtained, that are the starch, fibres and water (Yean and Lan, 1993). The main commercial manufactures are the starch, which is processed from the pith of M. sagu (Phang et al., 2000). One of the derivations of starch is the glucose and it is further processed for ethanol production (Adeni et al., 2010). Besides that, starch could be developed into syrup, derivatisation and maltodextrins (Phang et al., 2000) and the fibre is used for animal feeding purpose (Phang et al., 2000). Other than that, wastewater also is treated as fertilizer for the crops via the addition of urea.

Nevertheless, the research on the wastewater is insufficient in terms of the analysis of wastewater and the treatment of wastewater commercially. In Malaysia, there are studies on the formation of fertilizer via mixing the urea with the sago wastewater as in the case of the maize (Zea mays L.) (Omar et al., 2011) and also studies on heavy metals removal from wastewater (Rafeah Wahi et al., 2010).

The pith of the sago palm contains two soluble phenolic compounds i.e. (+)-catechin and (-)-epicatechin (flavonoids). The concentration of (+)-catechin and (-)-epicatechin was
found higher in mature sago palms while distribution of these compounds with trunk growth did not show a clear correlation (Shirlene, 2002). Furthermore, there is no research documented on the existence of polyphenol in wastewater of sago. In general, polyphenols, the aromatic rings, involves in providing health benefits via several mechanisms, including: (1) free radical quenching expressed, (2) fortification and redevelop of other dietary antioxidants, (3) chelation of metal ions (Ahmad et al., 2012). These polyphenols works as antioxidant, antibiotic or antiviral, anti-inflammatory, and defensive against diseases (Ahmad et al., 2012).

The hypotheses that was tested:

H₀: A primary treatment cannot remove the coarse solids in sago effluents and ion-exchange resins, Amberlite IRA-400, cannot recover the polyphenol compounds.

Hₐ: A primary treatment can remove the coarse solids in sago effluents and ion-exchange resins, Amberlite IRA-400, can recover the polyphenol compounds.

Then: Polyphenol concentration before and after the treatment does not change.

\[ H₀: \mu_1 = \mu_2 \]

\[ Hₐ: \mu_1 \neq \mu_2 \]

The objectives of this research are:

1) To compare the capacity of recovering the polyphenol compounds by using ion-exchange resins Amberlite IRA-400 at different temperature.

2) To remove the coarse suspended solids by a primary treatment and clean the wastewater with the usage of ion-exchange resins Amberlite IRA-400 at room temperature.
2.0 Literature Review

2.1 Sago Palm (*Metroxylon*)

Around 2.47 million ha are covered by sago palm in which, 1.4 million ha in Indonesia, 1.02 million ha in Papua New Guinea, and the remaining is in Malaysia, Thailand, Philippines and other countries (Flach, 1997). Sago palm propagates vegetative by suckers and generatively by seeds (Jong, 1995). Commonly, the palms are harvested by cutting the trees just before flowering and therefore seed production is rare (Jong, 1995). The limitations of uniform suckers are the main problem to establish large-scale plantations (Jong, 1995). Furthermore, the good-sized suckers weighs from 2 to 5 kg (Rostiwati *et al.*, 1998).

In Indonesia (East), Papua, Moluccas, sago are modified and produced as staple food for people (Flach, 1997). Sago starch not only benefits in raw materials but also in production of noodles, white bread, high-fructose syrup, biodegradable filler in plastics, animal feed, adhesive, bioethanol and many other derivative products (Flach, 1997).

Sago palm contributes in many special qualities compare to other carbohydrate-producing crops, in particularly, for its higher yield (15-25 ton dry starch/ha/year) (Flach, 1997). Riverbanks and swampy areas is the location where the palm grows and it is not suitable for other crop. Thus, the development of sago palm does not compete with the use of land for food crops and this importance is particular economic interest (Rostiwati *et al.*, 1998). In addition, sago known as perennial crop that means one-time plantation enables many years production and by managing the suckers is enough to aid in harvesting (Rostiwati *et al.*, 1998).
The preferred scientific names *Metroxylon amicarum* (H. Wendland) Beccari, *M. paulcoxii* McClatchey, *M. sangu Rottboell*, *M. salomonense* (Warburg) Beccari, *M. vitiense* (H. Wendland) H. Wendland ex Bentham & Hooker f. and *M. warburgii* (Heim) Beccari (McClatchey *et al.*, 2006). It is originates from the family of Arecaceae (palm family) and the subfamily is known as Calamoideae (McClatchey *et al.*, 2006).

2.1.1 Commercial Products

On the contrary to other sources of starch, sago produce outstandingly high productions and profits (McClatchey *et al.*, 2006). Sago (*Metroxylon sangu*) starch is obtained from the stem of palm (sago palm) feasibly the only example of commercial starch (A.A. Karim *et al.*, 2008). Based on current estimation, around 2 million ha consists of natural sago palm forests and about 0.14 million ha of planted sago palm, out of a total swamp area of about 20 million ha in Asia and the Pacific Region, majorly utilized or no utilized (A.A. Karim *et al.*, 2008). There are possibilities for sago palm to have a yield potential of up to 25 tons of starch per hectare per year by ensuring suitable environment with organized farming practices (A.A. Karim *et al.*, 2008). The sago production’s by-product and also refined fibres and waste pith used as fertilizer (McClatchey *et al.*, 2006). Appropriate fertilizers that are made via pith residue, enables competent cost-effective agricultural development for those running farm located near to commercial sago cultivating areas (McClatchey *et al.*, 2006).

2.2 Sago Wastewater

Adeni *et al.* (2010) has reviewed the progress made in these resources with emphasis on the bio-conversion into value added products as well as the environmental problems resulted from
a lacking of waste management. A large amount of water is needed for the starch extraction process from sago pith, therefore equally great amount of wastewater is discharged from sago mills (Adeni et al., 2010). In that equally total large amount of sago wastewater discharged from sago mills contains 94-97% liquid, nevertheless the sago ‘hampas’ is the solid waste would cause lots of problems in the treatment (Adeni et al., 2010). Further more, this problem also causes severe river contamination since there is no firm enforcement from the authorities that are supposed to be responsible on effluent clearance (Adeni et al., 2010). Other reports include the sago ‘hampas’ contains mainly starch and lignocellulose which is suitable to be used as substrate for solid substrate fermentation for both fungal bioconversion or by enzyme or acid hydrolysis (Adeni et al., 2013). However, sago barks have predominantly lignin that are rigid structure, conventionally it functioned as a base around the sago processing mill (Adeni et al., 2013).

About 10-22 tons wastewater is produced daily from all factories that consists of very high carbon to nitrogen ratio (105:0.12), somehow it is more compatible for fermentation via anaerobic fermentation in an up-flow packed bed digester (Phang et al., 2000).

2.3 Wastewater Treatment

A study by Banu, et al (2006) on different genus showed the research on the wastewater from tapioca starch obtained via Cassava or tapioca root (Manihot esculenta Crantz Syn. Utillisema) that is also called “sago” in India. This process takes place in over 800 small-scale units located in the Salem district of the State of Tamil Nadu, South India.
Current study is handled to treat the sago wastewater with an aid of a hybrid reactor, which mixes both the excellence of fixed-film and up-flow anaerobic sludge blanket systems (UASB) (Banu et al., 2006). It is found a great decrease in COD and efficient removal of solids are apparent after 120 d of start-up (Banu et al., 2006). There is a difference in the COD removal ranging from 91% to 83% (Banu et al., 2006).

The range of methane production throughout the research is 0.11 to 0.14 L CH$_4$/g COD-d and the percentage is from 55% to 67% (Banu et al., 2006). The findings of the study provide an opportunity for the low-cost design and compact on-site treatment systems with very short retention periods (Banu et al., 2006). This design of strategy could be applied on handling the Sago Palm (M. Sagu) wastewater generation in Sarawak in order to manage the pollution.

### 2.4 Polyphenol

The pith of the sago palm contains two soluble phenolic compounds i.e. (+)-catechin and (-)-epicatechin (Shirlene, 2002). Concentration of (+)-catechin and (-)-epicatechin is higher in mature sago palms while distribution of these compounds with trunk growth did not show a clear correlation (Shirlene, 2002). Soluble polyphenol oxidase (SPPO) activity increased while latent polyphenol oxidase (LPPO) activity decreased with increase in maturity of the sago palms (Shirlene, 2002). Thus, there are some possibilities for polyphenol to be soluble in wastewater where this wastewater is produced during the processing of sago pith.
2.5 Extraction of Polyphenol

2.5.1 Polyphenol Extraction from Olive Mill Wastewater (OMW)

The industrialization and the progressive development in olive production, for the past decades, this matter destroys the environment of olive mill wastewater due to excessive effluent discharge (Ompe et al., 2012). However, there is a prospective method on recovery of useful components such as antioxidant phenol compounds from olive mill wastewater (OMW) in order to refinance the high costs of the wastewater treatment (Ompe et al., 2012). The problem is solved via the effective technological input, membrane separation for the ecological problematic olive mill wastewater and membrane separation enables the recovery of polyphenols (Ompe et al., 2012). This eventually reduces environmental pollution, caused by OMW (Ompe et al., 2012).

Basically, either microfiltration (MF) and/or ultra-filtration (UF) are employed for the reduction of COD by retaining the suspended solids, which commonly contains organic substances (Pizzichini and Russo, 2005; Servili et al., 2011). Furthermore, pre-treatment by utilizing enzymes also supports the COD reductions (Pizzichini and Russo, 2005; Servili et al., 2011). Then, the pH value of OMW is adjusted repeatedly prevent phenolic compounds being oxidised and ensures the product quality (Ompe et al., 2012). Nanofiltration (NF) and reverse osmosis (RO) also involves in producing potential concentration of the phenolic compounds in the pre-treated wastewater (Ompe et al., 2012). The final concentration is ranged between 0.5 and 30g L-1 polyphenols that is mainly depends on initial concentration (Ompe et al., 2012).
Specifically, if the polyphenol content is at concentration of 0.5 g L⁻¹, it can be further used for industrial sector (Russo, 2007).

Therefore, the factor of the concentration is much significant than the final concentration value itself (Russo, 2007). Overall, the membrane processes proved good results on solving pollution problem of OMW and produced polyphenol concentrates, which are valuable for several industry branches (Ompe et al., 2012).

2.5.2 Solid-Phase Extraction (SPE Extraction)

Polyphenol extraction in plant materials is affected by their chemical nature, the extraction process engaged, sample particle size, storage time and conditions and also presence of interfering substances (R. Robbins, 2003). There are various types of chemical nature in plant phenolics found (R. Robbins, 2003). It differs from simple to highly polymerized substances, which involves of phenolic acids, phenylpropanoids, anthocyanins and tannins (R. Robbins, 2003).

Solid phase extraction (SPE) methods and fractionation based on acidity are usually utilized for removal unnecessary phenolics and non-phenolic substances (Antolovich et al., 2000). The type of solvent (polarity) operated, degree of polymerization of phenolics, in addition, interaction of phenolics with other food constituents and insoluble complexes formation, indicates the phenolic compounds solubility (Antolovich et al., 2000). Hence, there are no standardized techniques for extraction of all phenolics or a specific class of phenolic substances in plant materials (Antolovich et al., 2000). Methanol, ethanol, acetone, water, ethyl acetate and, to a lesser scope, propanol, dimethylformamide, and their mixtures are
commonly used for polyphenol extractions (Antolovich et al., 2000). There is a research have been documented on polyphenol extraction from de-oiled grape seed by using supercritical mixture of carbon dioxide and alcohol (Palma et al., 1999). Based on this research, it is mentioned that, methanol was a better carbon dioxide modifier than ethanol (Palma et al., 1999).

Related research also indicates the operation condition also manipulates the solvent capacity of the supercritical mixture of carbon dioxide and methanol (Murga et al., 2000). Firstly, methanol-modified carbon dioxide in order to extract catechins and epicatechins and for extraction of procyanidins from the seeds a pure methanol are utilized (Ashraf-Khorassani et al., 2004). By using 40% methanol-modified carbon-dioxide solvent system approximately 80% of catechins and epicatechins, present in the seeds, are able to extract (Ashraf-Khorassani et al., 2004). The extraction phase regularly takes about 1 min (M.L. Price et al., 1977) to 24 h (R.E. Burns et al., 1971; E.D. Maxson et al., 1972; S.J. Cork et al., 1991) varies based on condition have been reported. The phenolics will be easily oxidised if the extraction times become longer, and this could be prevented by adding reducing agents to the solvent system (S.K. Khanna et al., 1968). Methanol, ethanol, water or their combination is usually employed for flavonoids (catechin and epicatechin) extraction from plant materials but in some cases these solvents are acidified (Naczk et al., 2004).

a) Purification and Fractionation Procedures

Oleszek et al. (2001) make use of crude methanolic extract from yucca bark onto a C18 column (30 mm × 70 mm, 60m, Baker) equilibrated with water. Firstly, 40% (v/v) of methanol is used to wash the column and next with pure methanol (Oleszek et al., 2001). In the 40% methanol
elute only, the polyphenolic are identified (Oleszek et al., 2001). Subsequently, as aid to remove sugars and other contaminants from acidified extracts of raspberry, Mullen et al. (2002) used both ion-exchange column (Diaion HP-20) and column that consists of 40m C18 silica gel. Purified and fractionated phenolics extracts can be obtained via solid phase extraction or solid phase microextraction (SPME) on C18 cartridges (Mullen et al., 2002).

Auguste et al. (1984) as well as Jaworski et al. (1987) established the method to separate grape phenolics into acidic and neutral fractions by using a C18 Sep-Pak cartridge. Then, Sun et al. (1998) manage implement the C18 Sep-Pak cartridge in purpose to conduct fractionation of grape proanthocyanidins based on their degree of polymerization. The methodology involved is firstly by placing the extract of grape phenolics through the passage of two preconditioned neutral C18 Sep-Pak cartridges connected in series (Sun et al., 1998). Water is used in order to wash out the phenolic acids; catechins and oligomeric proanthocyanidins are then eluted with ethyl acetate and anthocyanidins and also polymeric proanthocyanidins with methanol (Sun et al., 1998). On the same C18 Sep-Pak cartridge, ethyl acetate fraction was replaced and diethyl ether elutes the catechins as well as methanol washes out the oligomeric proanthocyanidins (Sun et al., 1998).

2.5.3 Ion-Exchange Resins

a) Polyphenols Interchange

Originally, the proposal to Bakelite is a low-cost alternative for tannins that will be cross-linked with sulphuric acid to form an insoluble polyphenol (Burrell et al., 1938). They seem likely to "...dominate certain qualities such as cheapness, high exchange capacities, and shows..."
resistance towards aggressive waters...” and to “...either sodium or hydrogen ions will be exchanged for calcium and magnesium...” (Spiro, 2009). A proposal is made for the exchange mechanism: “Why does the base exchange shown by the material is definitely unknown. In justification with polyhydric phenol-formaldehyde resins, there is possibility some large molecules are built up normally in favour for condensations of phenol-formaldehyde to generate substances that are competent of base exchanging that is similar way to humus or lignin derivatives which is via functional phenolic groups” (Burrell et al., 1938).

b) Sorption of Phenol

The phenol and chlorinated phenols that included in wastewaters that is identified severe problem in removal of those substances (Spiro, 2009). Subsequently, a strong base anion-exchange resins are proved to have a high capacity for phenols that the mechanism are more like undergo sorption method, much considerable than ion exchange, because ions in resins are not discharged and the amounts sorbed beats its exchange capacity (Anderson et al., 1955)

Sorption isotherms are likely to be same as those recovered with charcoal (Spiro, 2009). The removal of phenols from the resin can be made by elution with methanol (Spiro, 2009). The amines sites of resins are the primary, secondary and quaternary sorb phenols except for tertiary amine resins (Chasanov et al., 1956). The binding connecting the amines and the phenols can be seen through infrared spectra (Spiro, 2009).

c) Strong Base Anion-Exchange Resins

The cultivation of amine resins with certain compounds for example m-phenylenediamine are fit for anion exchange only if the amine was protonated where subjected to use in acidic