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Moh Moh Zin

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SCAVENGING OF BIOACTIVE COMPOUNDS FROM BEETROOT (*BETA VULGARIS* L.) WASTES VIA EMERGING TECHNOLOGIES APPROACH

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MOH MOH ZIN

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The PhD School

Name: Doctoral School of Food Science

Discipline: Food Science

Head: **Dr. Livia Simonné Sarkadi**
Professor, DSc
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Nutrition

Supervisor: **Dr. Szilvia Bánvölgyi**
Associate Professor, PhD
Hungarian University of Agriculture and Life Sciences
Institute of Food Science and Technology
Department of Food Process Engineering

.....	.....
Approval of the Head of Doctoral School	Approval of the Supervisor (s)
	.....
	Approval of the Supervisor

1 INTRODUCTION

Food colourants processed via agro-industrial wastes are demanded under the heading of food waste management, at present, not only for health benefits but also it ensures the minimization of common costs. Utilizing food waste products is one of the effective ways to save the planet by diminishing overdose. Along the line, many efforts have been made to recover the valuable components of food wastes and applied in various fields with individual purposes instead of ending up as ruminant feed only. The currently developed trend of innovation has fetched the attention of food additives to be judged as worthy to incorporate in foods or not based on their nutritional values except their organoleptic properties. The bottom line is that food colour is an unavoidable additive in food processing either in one way or another. Although; it is undeniable that currently applied synthetic food dyes are neither toxic nor nutritious. In most developing countries, it is a bit challenging to substitute artificial dyes with nutritious bio-colourants since they are pricy and efficient technology supply is limited as well. However, it is insightful to apply natural food ingredients and species such as turmeric (yellow) and red pepper powder, particularly in the preparation of foods for those, especially from Asian countries due to their additional therapeutic properties.

Most food ingredients such as flavour or colour which are added in the food processing to improve sensory characteristics are known as food additives unless they are bioactive compounds with the ability to fortify the foods by their nutritional values then they are nailed as functional ingredients. Fibre, protein, energy, vitamins, minerals, and antioxidants are examples of functional ingredients. A primary metabolite (eg. carbohydrates, amino acids, proteins and lipids) is a kind of metabolite that is directly involved in the normal growth, development, and reproduction of plants whereas a secondary metabolite is not directly involved in those processes but attributes the organoleptic properties to

the host in advance and take part in some important factors like pollination and against the environmental attacks as well. Most plant-based bioactive compounds are secondary metabolites that have pharmacological and toxicological effects on living organisms attributing colour, aroma and flavour to the hosts.

Bioactive compounds can be categorized into three main groups: terpenes and terpenoids (25,000 types), alkaloids (12,000 types), and phenolic compounds (10,000 types). Most of them are applied as additives in the food industries due to their sensory attributions together with enhanced nutritional values to the food products. Bio-colourants broadly exist in the plant (flower, root, stalk, seed, fruit, peel, leaf, pomace, rhizome, and stigma), insect (cochineal), algae, bacteria, and fungi. The common natural colourants are carotenoids, chlorophylls, flavonoids, and betalains. Aside from their colour supply, bio-colourants are beneficial as supplements, for example, betalain (beetroot-based) has some benefits for skin whereas carotenoid is well known for hair proliferation. Additionally, bioactive compounds which enrich antioxidant and anti-inflammatory properties are preventive for neuroinflammation, and fatal cardiovascular and carcinogenic diseases. They can be lipophilic or hydrophilic and their constituent in foods can be classified by chromatographic and spectrometric analysis.

1.1 Objectives

Beetroot (*Beta vulgaris* L.) is a well-known vegetable consumed throughout Europe in different forms such as fresh or processed, etc. Thence, beetroot processing wastes have come to conquer the interest of environmentalists with the privilege of recoverable bio-colourant. The major focal aim of this studying is to valorize the antioxidant-rich betalain colour compounds as well as phenolic compounds from the different parts of two types

of beetroot (Cylindra and Rhonda), i.e, peel, flesh, and stalk. Accordingly, the following factors are to be inquired about:

- Can the most influential optimization parameter for the effective extractions of bioactive compounds from beetroot peel (*Beta vulgaris* L.) be approached by the response surface technology (RSM) in terms of practical industrial applications?
- Is the recovery of bioactive compounds from the waste parts of beetroot worthy enough to leverage?
- Among the updated extraction technologies, can microwave-assisted extraction (MAE) and ultrasonic-assisted extraction (UAE) boost the extractability of betalain and phenolic compounds, and antioxidant activities compared to the cultural solid-liquid extraction?
- Is the stability of the betalain colour compounds affected by changing the solvent characteristic during their processing?
- How can membrane technology assist the recovery of bioactive compounds? Will the nanofiltration (NF) and reverse osmosis (RO) membranes successfully detain colour compounds and phenolic compounds, meanwhile, concentrating the extracts under the specific operating parameters?

2 MATERIALS AND METHODS

2.1 Microwave-assisted extraction (MAE)

Realization of MAE of bioactive compounds from the peel of Cylindra beetroots (*Beta vulgaris* L.) with different types of solvent was done according to the response surface methodology (RSM) in terms of central composite design (CCD) built by Design of Expert (DOE) statistical software. Three modes of microwave power (100-800 W) together with varied treatment times (30-150 s) and solvent ratio (0.1-0.2 w/v); i.e, 0.1 w/v (1 g matrix per 10 mL solvent), 0.15 w/v (1 g matrix per 6.67 mL solvent), and 0.2 w/v (1 g matrix per 5 mL solvent); were set up and performed by a home-use microwave oven (Specs Electrolux EMM 2005). The microwave treatments were performed with intermittent mode (30 s on 15 s off, 15 s on 15 s off) and cooling in between with icy water. Four different types of solvents applied for the betalains extractions were pure water (PW), acidified water (AW), 15 % (v/v) ethanol-water (EW), and acidified ethanol-water (AEW). 0.1-0.5 % (w/v) ascorbic acid was applied for the acidification of the solvents.

For the comparison of the bioactive compound contents in the different parts of the beetroot (i.e peel, flesh, and stalk), pure water solvent extractions were carried out under three different process conditions following the pattern of the central composite design model which were: low level (microwave wattage-100 W, irradiation time-30 s, solvent ratio-0.1 w/v); medium level (microwave wattage-450 W, irradiation time-90 s, solvent ratio-0.15 w/v); high level (microwave wattage-800 W, irradiation time-150 s, solvent ratio-0.2 w/v). Meanwhile, the respective conventional solid-liquid extractions were achieved with pure water (0.1 w/v) at 70 ºC for one hour of extraction time and used as the controls. The efficiency of applied solvent types on bioactive compounds extraction from beetroot peel and flesh was investigated under the processing

conditions of microwave wattage (100 W, 450 W, and 800 W), irradiation time (30 s, 90 s, and 150 s), and solvent ratio (0.1 w/v, 0.15 w/v, and 0.2 w/v).

2.2 Ultrasound-assisted extraction (UAE)

The UAE was performed by power ultrasound (400 W, 20 kHz) produced by a generator (Weber ULC 400 Premium Ultrasonic Generator) according to the following process variables: ultrasound intensity (3.5 W/cm^2, 8 W/cm^2, and 56.5 W/cm^2); treatment time (5 min, 10 min, and 15 min); solvent ratio [0.02 w/v (1 g matrix per 50 mL solvent), 0.04 w/v (1 g matrix per 25 mL solvent), and 0.06 w/v (1 g matrix per 16.67 mL solvent)], respectively. To stabilize the heat distribution throughout the treatments, an icy water bath was used maintaining the temperature around 30 ºC. Under the scope of comparative study between ultrasonic and microwave-assisted extractions, the microwave extracts were also prepared with solvent ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v) for (45 s, 105 s, and 165 s) microwave irradiation at 800 W (50 % duty cycle) of microwave power. The control samples were achieved conventionally by a double wall jacket single-batch-extractor equipped with YELLOWLINE OST 20 digital stirrer. The temperature was fixed at 30 ºC but treatment time (5 min, 10 min, and 15 min) and solvent ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v) were varied.

2.3 Membrane separation

Both nanofiltration (NF) and reverse osmosis (RO) concentrations were performed by cross-flow filtration process with DDS Filtration Equipment (LAB 20-0.72, Denmark). Polyamide Thin Film Composite (NF 200, FILMTEC^{TM} membrane) with active surface areas of 0.144 m^2 and low fouling type Trisep X20 advanced composite RO membrane (Microdyn) with active surface areas of 0.18 m^2 were applied. Extractions were accomplished by a single batch type extractor which was designed with a thermostat water bath (LAUDA ECOLINE

E100) and stirrer (YELLOW LINE BY IKA OST 20 DIGITAL). The extraction for NF was achieved at 22 ºC for 60 minutes of extraction time with pure water and 15 % (v/v) aqueous ethanol solvent (1:10 solid-to-solvent ratio). In RO processes, aqueous extraction was attained at 40 ºC for 40 minutes with 1:20 solid-to-solvent ratio. The parameters for the filtrations were as follows: operation temperature (~30 ºC), transmembrane pressure (TMP, average 40 bars), and recirculation flow rate (400 L/h) which is calculated by the frequency of the recirculation pump (32.3 Hz). During the concentration, the time required to collect each 100 mL of filtrate was recorded for the flux calculation and the sample collections were performed at every 500 mL of permeates. Pure water flux measurements were carried out before and after the membrane filtrations to estimate membrane resistance and fouling resistance. After the concentration process, distilled water was used for rinsing and removing the polarization layer completely. The chemical cleaning of the membranes was followed upon necessary.

The different extracts were analyzed by Spectronic GENESYS 5 (MILTON ROY, U.S.A) spectrophotometer in which BX, BC, and TBC were measured by the second (Nilson's) method; TPC was analyzed by Folin's method; AA was determined by ferric reduction antioxidant power (FRAP), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods, respectively. All measurements were triplicated and averaged. Dry matter-based yield percentages were calculated by moisture analyser (KERN MLS; KERN & SOHN GmbH, Germany). Total soluble solids (TSS) were determined by ATAGO pocket refractometer. Densities of the crude extracts and retentates were measured by DMA 4500 (Anton Paar) density meter.

3 RESULTS

The navigation of the efficiency of MAE was accomplished with hundred experimental runs in total by four different types of solvents as mentioned in the materials and methods section (2.1). With PW solvent, twenty treatments were conducted according to CCD including replication of the centre points. The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds was; microwave power (800 W), operation time (150 s), and solvent ratio (0.2 w/v). Under this processing condition, the expected outcomes are TBC (10.49 mg/g DM), TPC (18.98 mg GAE/g DM), AA by FRAP method (28.56 mg ASE/g DM), and AA by DPPH (78 %), respectively. Among the thirty runs with AW solvent, microwave power (800 W), operation time (81.82 s), solvent ratio (0.1 w/v), and acid (0.5 % w/v) ensured the amounts of targeted bioactive compounds as TBC (5.79 mg/g DM), TPC (356.55 mg GAE/g DM), and AA by FRAP method (205.06 mg ASE/g DM) and DPPH method (67 %). The operating condition optimized predominantly by the model depending on the maximum yields of extracted compounds by EW solvent was microwave power (800 W), operation time (150 s), and solvent ratio (0.123 w/v). Under this processing condition, the experimental outcomes are 10.78 mg/g DM of TBC, 11.26 mg GAE/g DM of TPC, and 16.27 mg ASE/g DM (50 %) of AA, respectively. In the meantime, the RSM model estimated the scavenged amounts of TBC, TPC, and AA by AEW solvent as 8.94 mg/g DM, 328.43 mg GAE/g DM, 270.33 mg ASE/g DM or 59 %, respectively under the processing condition of microwave power (799.85 W), operation time (126.92 s), solvent ratio (0.1 w/v), and acid (0.5 % w/v).

After MAE at 800 W for 150 s with PW solvent (0.2 w/v), betalain content is the topmost in the peel i.e BC (7.06±0.07 mg/g DM), BX (5.25±0.07 mg/g DM), and TBC (12.31±0.14 mg/g DM) compared to the other parts; followed by the flesh (BC=6.61±0.26 mg/g DM, BX=2.55±0.07 mg/g DM,

TBC=9.15±0.25 mg/g DM); and the stalk (BC=0.99±0.13 mg/g DM, BX=0.32±0.06 mg/g DM, TBC=1.3±0.08 mg/g DM). Meanwhile, the recovered TBC amounts in the controls are as follows; 10.99±0.05 mg/g DM (peel), 6.32±0.24 mg/g DM (flesh), and 2.85±0.15 mg/g DM (stalk), respectively. TPC and TFC of the stalk, flesh, and peel extracts under different process conditions were examined accordingly in which TPC was outweighed in the control sample in stalk extract (39.37±0.11 mg GAE/g DM) and the flesh extract (15.61±0.32 mg GAE/g DM) whereas peel behaved a bit different due to its amount was utmost in the maximum process condition of MAE (21.94±0.54 mg GAE/g DM). In the same vein, fewer TFC amounts were displayed in the MAE extracts of the stalk (1.77± 0.0 mg QUE/g DM) and flesh (1.46±0.12 mg QUE/g DM); along with the maximum TFC observed in the MAE peel-water extract as 5±0.12 mg QUE/g DM. The maximum radical scavenging activity of 35.68±0.77 mg ASE/g DM (FRAP) is discovered in the peel-water extracts which amount was 1.3 times exceeded the control, as well as 2.5 and 2.9 times greater than the flesh and stalk extracts under the same processing conditions. In the case of DPPH, the radical scavenging activities are found in the individual extracts in the following ascending order; 22 % in the stalk (control), 39 % in flesh (control), 50 % in peel (control) while 53 %, 58 %, and 94 % of radical scavenging activities were detected in flesh, stalk, and peel extracts of MAE under the high level of processing conditions. Similarly, ABTS measurement proved that the exceeded amounts of antioxidants were extracted with MAE samples which are 23 % (stalk), 32 % (flesh), and 99 % (peel) compared to the control samples of the stalk (10 %), flesh (15 %), and peel (44 %).

From the investigation of the influence of different types of solvent on targeted compounds' extractibility from beetroot peel and flesh, the amounts of BX (5.25±0.07 mg/g DM), BC (7.06±0.07 mg/g DM), TBC (12.31±0.14 mg/g DM), and DPPH (94 %) were maximized in the peel-water extract (sample A). Whilst, TPC (156.11±11.9 mg GAE/g DM) and AA (140.58±1.03 mg ASE/g

DM) were discovered utmost in the acidified water extract (sample D). In the case of flesh extracts, the acidified solvents exhibited the greatest amounts of targeted bioactive compounds; BC (7.21±0.12 mg/g DM), and TBC (9.77±0.15 mg/g DM) with the highest antioxidant activity (221.58±3.1 mg ASE/g DM) in were observed in sample B whereas BX (3.09±0.29 mg/g DM), TPC (232.14±3.32 mg GAE/g DM), and DPPH (86 %) were maximum in sample D.

The subsequence stability test of colour compounds was done with the kinetic study. Based on the changes in the temperature from 30 ºC to 70 ºC after 5 hrs of heat treatment, the retention percentage (R %) of BC in different sample extracts varied drastically with the deduction of 82 % in sample A, 62 % in sample B, 82 % in sample C, and 79 % in sample D, respectively. In addition, R % of TBC was reduced to 66 % in sample A, 43 % in sample B, 57 % in sample C, and 68 % in sample D. Adversely, R % of BX varied slightly with temperature changes; 33 % (sample A), 37 % (sample B), 16 % (sample C), and 48 % (sample D). Regardless of the minor fluctuation in some cases, R % of BX was the topmost in the extract of PW (sample A) proving its better stability in the non-acidic medium, unlike BC.

Among the different ultrasonic probes, the probe type with 8 (W/cm^2) power intensity and solvent ratio (0.02 w/v) has ensured the topmost extractability of plant compounds from beetroot peel (Rhonda type) after 15 min of sonication. Under this circumstance, BC (13.93±0.09 mg/g DM), BX (5.79±0.11 mg/g DM), TBC (19.67±0.02 mg/g DM), and TPC (19.16±0.65 mg GAE/g DM) were scavenged. For total antioxidants by FRAP method, 56.5 (W/cm^2) power intensity and solvent ratio (0.02 w/v) seemed to be more operative with the recovered amounts of 24.39±0.18 mg ASE/g DM. Howbeit, the combined treatment of 5 min of sonication, 8 W/cm^2 power intensity, and 0.06 w/v solvent ratio has encouraged the maximum radical scavenging % of 61.17±0.91. Subsequently, the parallel extractions were achieved with solvent

ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v) via UAE (56.5 W/cm^2 power intensity for 15 min); MAE (800 W for 165 s); and CON (30 ºC for 15 min). According to the spectrophotometric analysis, TBC amount was topmost in UAE extract (19.67±0.02 mg/g DM; 8 W/cm^2, 0.02 w/v, and 15 min), afterwards, in MAE extract (16.26±0.04 mg/g DM), and it is the least in CON extract with the amount of 15.11±0.15 mg/g DM. In terms of TPC, MAE was observed to be the most effective with the utmost recovery amount of 19.49±2.54 mg GAE/g DM (165 s and 0.06 w/v) followed by UAE (13.52±0.99 mg GAE/g DM) and CON (6.95±0.77 mg GAE/g DM), respectively. In addition, the radical scavenging activity of bioactive compounds determined by the FRAP method was superior in UAE extract (56.5 W/cm^2, 0.02 w/v, and 15 min) compared to MAE and leaching methods, those are 24.39±0.18 mg ASE/g DM, 20.24±0.22 mg ASE/g DM, and 12±0.14 mg ASE/g DM. By the DPPH scavenging test, MAE (165 s and 0.06 w/v) exhibited 53.31±2.54 % of AA whereas UAE and CON expressed 44.88±1.68 % and 24.96±1.36 %, respectively. The highest yield percentages of all extraction techniques came out individually as 4.73 %, 3.06 %, and 1.65 % in the extracts of MAE, UAE, and CON with the 0.06 w/v solvent ratio. Within the study range, UAE is superior in the extractability of bio colourants to MAE and CON whilst MAE is a more effective way to extract polyphenols than UAE and CON. Moreover, MAE is a favour in terms of extraction yield as well as cost reduction as it is achieved in a short period.

After the filtration by NF membrane, the scavenged amounts of TBC, TPC, and AA were scaled up to 4 times in the final retentate of WE whereas up to 5 times of those compounds were determined in the final retentate of EWE. With RO membrane, the bioactive compounds from the beetroot peel juice could be detained in the final concentrates from 4 to 9 times compared to the crude extract. Meanwhile, 5 to 12 times of TBC, TPC, and respective AA were recovered in the beetroot flesh concentrate after the filtration process.

4 CONCLUSION AND RECOMMENDATIONS

The major purpose of this dissertation is to investigate how to boost the extractibility of bio colourants known as betalains, polyphenols, and antioxidant activities by emerging technologies including microwave, ultrasonic wave, and membrane separation for the concentration of extracts. Under the investigation of MAE efficiency on bioactive compounds extraction from the beetroot peels with the RSM approach, the following conclusions can be made:

- With PW solvent, the supreme amounts of targeted bioactive compounds were recovered under the operating variables of 800 W of microwave power, 150 s of irradiation time, and 0.2 w/v solvent ratio.
- Among the thirty experimental runs by AW solvent, the utmost amounts of TBC and TPC were scavenged at 800 W with 0.1 w/v solvent ratio and 0.5 % acid after 150 s of microwave irradiation. Meanwhile, the highest radical scavenging activity was examined by the FRAP method at 100 W, 0.1 w/v solvent ratio, and 0.5 % acid after 30 s of irradiation; and radical scavenging activity (DPPH) was measured at 450 W, 0.15 w/v solvent ratio, and 0.3 % acid after 30 s of irradiation.
- In the case of EW solvent extracts, the highest values of detained TBC, TPC, and AA by FRAP were observed in the extract of MAE at 800 W for 90 s of irradiation with 0.15 w/v solvent. 60 % of AA was found in the extract of MAE at 100 W for 150 s withal 0.1 w/v solvent ratio.
- From thirty experimental runs with AEW solvents, the maximum amounts of TBC were recovered at the processing condition of 800 W, 150 s, 0.1 w/v solvent ratio, and 0.1 % acid. TPC and AA were determined in the extracts of MAE for 30 s of irradiation at 100 W with 0.1 w/v solvent ratio, and 0.5 % acid.

Under the high level of MAE processing conditions, betalain content is the topmost in the peel compared to the other parts followed by the flesh and the

stalk. Meanwhile, the recovered total betalain amounts in the controls are in the following order as well; peel>flesh>stalk. TPC and TFC of the stalk, flesh and peel extracts under different process conditions were examined accordingly in which TPC was outweighed in the control sample in stalk and the flesh extracts whereas peel behaved a bit different due to its amount was utmost in the maximum process condition of MAE. Likewise, fewer TFC amounts were displayed in the MAE extracts of the stalk and flesh compared to peel-water extract. Subsequently, the maximum radical scavenging activity was discovered in the peel-water extracts as to the flesh and stalk extracts under the same processing conditions. From the investigation of the influence of different types of solvent extractibility on targeted compounds from beetroot peel and flesh, the amounts of total betalains were maximized in the peel-water extract. Whilst, TPC and AA were discovered utmost in the acidified water extract. In the case of flesh extracts, the acidified solvents exhibited the greatest amounts of betalains, phenolics, and antioxidants. The subsequence task of colour compounds' stability was done with the kinetic study. Based on the changes in the temperature from 30 ºC to 70 ºC, R % of TBC was reduced differently with a specific type of solvents after 5 hrs of heat treatments. R % of BX was the topmost in the extract with pure water while R % of BC was higher in the acid-induced solvent extracts.

The parallel extractions were achieved with solvent ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v) via UAE (56.5 W/cm^2 power intensity for 15 min); MAE (800 W for 165 s); and CON (30 ºC for 15 min). Under the study range, UAE is superior in the extractability of TBC to MAE and CON whilst MAE is a more effective way to extract TPC compared to UAE and CON. Moreover, MAE is a favour in terms of extraction yield as well as cost reduction as it is achieved in a short period. With NF and RO membranes, the bioactive compounds from the beetroot peel and flesh juices were successfully detained in the final concentrates compared to the crude extracts. Following our experimental results,

the conclusion comes up that membrane technology can be applied effectively in the concentration or separation of valuable compounds from vegetable wastes.

When it comes to the recommendations, further study on the effectiveness of microwave irradiation on the recovery of plant compounds has to be broadened in terms of microwave power, irradiation time, solvent characteristics, and solvent ratio. The optimizations of the scavenging of the desired compounds by the RSM approach with wider setup variables are encouraged. Different types of beetroot genres can be chosen for the investigation of bioactive compound contents in their wastes to make a comparison. Larger scale membrane concentration processes with different operating temperatures and pressure should be investigated. The spray drying can be applied to produce the powder form of beetroot waste extract for their more convenient practical usage.

5 NEW SCIENTIFIC RESULTS

From my dissertation, I have found out:

[1] The synergetic effects of the solvents' characteristics and the nature of the plant matrix have been found in microwave-assisted extraction. For example, the maximum amount of betaxanthin, betacyanin, total betalain compounds, and antioxidants (by DPPH method) were observed in the peel-water extract of Cylindra beetroot at microwave power (800 W), operation time (150 s), and solvent ratio (0.2 w/v) within the study ranges of microwave power (100 W-800 W), operation time (30 s-150 s), and solvent ratio (0.1 w/v-0.2 w/v). Whilst, total phenolic compounds and antioxidant activity (by FRAP method) were discovered utmost in the acidified ethanol-water extract at microwave power (799.85 W), operation time (126.92 s), solvent ratio (0.1 w/v), and acid (0.5 % w/v). In the case of flesh extracts, the acidified solvents were preferable by exhibiting the greatest amounts of betacyanin and total betalain compounds with the highest antioxidant activity (by FRAP method) in the acidified-water extract as well as betaxanthin, total betalain compounds, and antioxidants (by DPPH method) in the acidified aqueous-ethanol extract under the processing condition of microwave power (800 W), operation time (150 s), and solvent ratio (0.2 w/v).

[2] 0.1 %-0.5 % ascorbic acid-induced solvent (either acidified water or acidified ethanol-water) did not contribute any impact on the extractability of betalain colour compounds from the peel of Cylindra beetroot in microwave-assisted extraction techniques under the study ranges of microwave power (100 W-800 W), operation time (30 s-150 s), and solvent ratio (0.1 w/v-0.2 w/v). Whilst, improvements in polyphenol and antioxidant activities were observed in the acidified solvent extracts.

[3] The instability of betacyanin was terminated to some point in the acidic medium (0.1-0.5 % (w/v) ascorbic acid) compared to the aqueous one. On the other side, betaxanthin was found to be less stable in the acidified solvent extract than in the aqueous extract.

[4] Within the study range of ultrasonic wave-assisted extraction (400 W, 20 kHz) with ultrasound intensity (3.5 W/cm^2-56.5 W/cm^2), treatment time (5 min-15 min), solvent ratio (0.02 w/v-0.06 w/v); ultrasonic wave-assisted extraction (8 W/cm^2, 0.02 w/v, and 15 min) is superior in the extractability of bio colourants to microwave-assisted extraction (for 45 s-165 s at 800 W of 50 % duty cycle) and conventional extraction (for 5 min-15 min at 30 ºC). Whilst, microwave-assisted extraction (165 s and 0.06 w/v) is a more effective way to extract polyphenols than ultrasonic wave-assisted extraction and conventional extraction. In addition, the radical scavenging activity of bioactive compounds determined by the FRAP method was superior in ultrasonic wave-assisted extraction extract (56.5 W/cm^2, 0.02 w/v, and 15 min) compared to microwave-assisted extraction and leaching methods whereas microwave-assisted extraction (165 s and 0.06 w/v) was preferable by the DPPH scavenging test.

[5] After the filtration by nanofiltration membrane (NF 200, FILMTEC^{TM} membrane) operated at 30 ºC with 40 bars and 400 L/h recirculation flow rate, the scavenged amounts of betalains, phenolics, and antioxidants were scaled up to 4 times in the final retentate of water extract whereas up to 5 times of those compounds were determined in the final retentate of ethanol-water extract. Membrane retention for betalains, phenolic, and antioxidant activity was assumed to be 99 % hence those compounds were not detectable in the permeate of either extract.

[6] With the low fouling type Trisep X20 reverse osmosis membrane under the processing condition of temperature (27 ºC), pressure (40 bars), and recirculation flow rate (400 L/h); the bioactive compounds from the

beetroot peel juice could be detained in the final concentrates from 4 to 9 times compared to the crude extract. Meanwhile, 5 to 12 times of betalains, phenolics, and respective antioxidants were recovered in the beetroot flesh concentrate after the filtration process. The filtration processes of beetroot peel and flesh extracts by reverse osmosis membrane were succeeded in detaining the mentioned compounds at 99 % in the concentrates.

7 LIST OF PUBLICATIONS AND CONFERENCES

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