HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Scavenging of bioactive compounds from beetroot (*Beta vulgaris.* L) wastes via emerging technologies approach

Doctoral (PhD) dissertation by

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ABBREVIATIONS

Microwave-assisted extraction

c	speed of the wave ($m \cdot s^{-1}$)
D	electric displacement field of material ($C \cdot m^{-2}$)
D_f	dissipation factor
D_{vacuum}	electric displacement field of vacuum ($C \cdot m^{-2}$)
ε'	dielectric constant
E	electric field strength ($V \cdot m^{-1}$)
ε	relative permittivity
ε''	dielectric loss
ε*	dielectric permittivity
ε_d''	relative dipole loss
ε_r''	total loss factor
ε_σ''	relative ionic loss
f	frequency (Hz)
k	constant value of dissipated microwave power ($55.61 \cdot 10^{-14}\ C \cdot m^2 \cdot V^{-1}$)
P_D	dissipated microwave power
tan δ	electric loss tangent
z	depth of penetration (m)
λ	wavelength (m)

Membrane concentration

Δt	time required to collect the filtrate (h)
ΔV	volume of filtrate (L)
α_1	slope of pure water flux curve before the concentration
α_2	slope of pure water flux curve after the concentration
A_m	active surface area of membrane (m^2)
C_0	feed concentration (mg/g DM)
C_p	concentration of permeate (mg/g DM)
C_R	concentration of retentate (mg/g DM)
EWE	ethanol-water extract

J	permeate flux of pure water ($L/(m^2 \cdot h)$)
J_x	permeate flux of the sample ($L/(m^2 \cdot h)$)
NF	nanofiltration
R_f	fouling resistance (1/m)
R_m	membrane resistance (1/m)
RO	reverse osmosis
TMP	applied transmembrane pressure difference (Pa)
TSS	total soluble solids (Brix %)
V_0	feed volume (m^3)
V_P	volume of permeate (m^3)
V_R	volume of retentate (m^3)
VRR	volume reduction ratio (m^3/m^3)
WE	water extract
μ	dynamic viscosity of permeate ($Pa \cdot s$)

Analytical measurements

Δa^*	differences in redness or greenness of the sample and the standard
Δb^*	differences in yellowness or blueness of the sample and the standard
ΔL^*	differences in the lightness of the sample and the standard
A	pre-exponential factor
a^*	redness or greenness
α_3	slope of TPC calibration curve
α_4	slope of TFC calibration curve
α_5	slope of AA calibration curve
AA	antioxidant activity (mg ASE/g DM)
Abs	sample absorbance
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ASE	ascorbic acid equivalent
b^*	blueness or yellowness
BC	betacyanin compounds concentration (mg/g DM)
BI	browning index
BX	betaxanthin compounds concentration (mg/g DM)

C^*_{ab}	chroma value
C_0/ C_i	initial concentration (mg/g DM)
C_f	final concentration (mg/g DM)
CR	concentration ratio
C_t	concentration at a specified time (mg/g DM)
DF	dilution factor
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
E_a	activation energy (kJ/mol)
FRAP	ferric reduction antioxidant power
GAE	gallic acid equivalent
Hue^o	angel of hue
k	first-order degradation constant (h^{-1})
L	path length (cm)
L^*	lightness
MW	molecular weight (g/mol)
OD	optical density
Q_{10}	temperature quotient or coefficient
QUE	quercetin acid equivalent
R %	percentage of colour retention (%)
R	universal gas constant ($8.3145 \cdot 10^{-3}$ kJ/K mol)
S	amount of sample (µL)
T	absolute temperature (K)
t	heating time (h)
$t_{1/2}$	half-life (h)
TBC	concentration of total betalain compounds (mg/g DM)
TFC	concentration of total flavonoid content (mg QUE/g DM)
TPC	concentration of total phenolic compounds (mg GAE/g DM)
ΔE^*_{ab}	total colour difference
Ɛ	molar extinction coefficient (L/(mol · cm))

General terms

AEW	acidified ethanol-water

ANOVA	analysis of variance
AW	acidified water
BBD	box-behnken design
C.V. %	coefficients of variation
CCD	central composite design
CON	conventional extraction
df	degree of freedom
DOE	design of expert
EW	ethanol-water
MAE	microwave-assisted extraction
PEF	pulsed electric fields
PW	pure water
R^2	coefficient of determination
RSM	response surface technology
SS	sum of squares
Std. Dev.	standard deviation
UAE	ultrasonic wave-assisted extraction

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 (Dos Santos et al., 2016). 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 (Fernandes et al., 2019). As their enrichment in those functional ingredients and micronutrients, the daily intake of fruits and vegetables (450 – 500 g) is recommended by World Health Organization (WHO/FAO 2014). 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 (Antolak & Kregiel, 2017).

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) (Antolak & Kregiel., 2017). 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 (Aberoumand, 2011; Delagdo-Vargas et al., 2010, Shamina et al., 2007). The common natural colourants are carotenoids, chlorophylls, flavonoids, and betalains (Aberoumand, 2011, Shamina et al., 2007). 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 (Hussain, 2018). 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. Valorisation of them from food wastes or by-products via thermal and non-thermal emerging technologies has been discussed broadly in the reviews of Zin et al. (2020a) and Wani et al. (2021).

1.1 Hypothesis:

- The versatility of antioxidant-rich natural colour compounds had replaced artificial dyes mainly in food processing.
- Bioactive ingredients with high sensitivity toward pH, temperature, etc., caused a major challenge for food processors and technocrats.
- The study on the stability of native organoleptic properties of bioactive compounds that can be determined by adjusting their processing environment is thriving.
- It is still needed to leverage the practical usage of bioactive compounds in different areas with certain sources of their jeopardized behaviour.
- Tremendous amounts of plant-based compounds can be scavenged from the fruits and vegetable wastes with improved extraction technology that is considered novel in terms of low impact on the environment.

1.2 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, phenolic compounds, and antioxidants compared to the cultural solid-liquid extraction?
- Is the stability of the betalain colour compounds affected by changing the solvent characteristic during their processing and thereafter?
- 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 LITERATURE REVIEW

2.1 Food colourants

Functional additives are compulsory in food processing to enhance the native organoleptic properties of foods. Food colourants are promising in food processing as they determine the appeal of foods by improving or maintaining the existing colour or restoring the colour which is destroyed during processing. Bio-colourants can be found in plants (grain, flower, root, stalk, seed, fruit, peel, leaf, pomace, rhizome, and stigma), insects (cochineal), algae, bacteria, fungi, and microbes. Their existence is essential for pollination and seed disposal as insects and birds are attracted by their hues. Bio-colourants can be grouped as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Their applications as natural colourants in food and cosmetic industries have been named E (Europe) numbers such as carotenoids (E 160, E 161, E 164), chlorophylls (E 140), anthocyanin (E 163), and betalains (E 162). Their characteristics and extractable common sources are represented in Table 1. Since these compounds have characteristics of absorption in the visible spectrum, their quantification can be done with a UV-visible spectrophotometer within the 380 nm - 700 nm wavelength range according to Beer-Lambert's law (Zin et al., 2020a).

- Carotenoids or terpenoids possessing 40 carbon atoms are derived from the condensation of geranylgeranyl-PP molecules. They are lipid-soluble basically found in cyanobacteria, algae, plants, some fungi, and some bacteria and are produced intracellularly by bioproduction of microorganisms. Based on chemical structure, they can be classified as hydrocarbon carotenoids and xanthophylls, and their extraction can easily be performed with nonpolar solvents (Merhan, 2017; Urnau et al., 2018).
- Flavonoid (C6-C3-C6) is a class of phenylpropanoids and furnishes intense colour, texture, and taste in fruits and flowers exhibiting radical scavenging activity. The colour variety and classification differ according to the structural groups such as hydroxyl, methyl, glucosyl, and acyl (Shamina et al., 2007; Tanaka et al., 2008).
- Anthocyanin; glycosylated and acylated, is a group of flavonoids derived from phenylalanine, strongly conferring colouration from pale yellow to blue along with pH changes. Anthocyanin possesses several nutritional values and strongly exhibits

antioxidant properties. It is abundantly found in berries, blackcurrant and other purple colour giving fruits and vegetables with host taste attributes. Both anthocyanin and betalain (betacyanin) have UV protectable ability for host plant tissues. Betalains have a wider range of pH (3-7) with yellow to red colouration though less stable to temperature and light exposure as compared to anthocyanin (Shamina et al., 2007; Martín et al., 2017; Stintzing and Carle, 2004; Tanaka et al., 2008).

Table 1. Natural colours applied in the food and cosmetic industries, their E numbers, and common sources (Shamina et al., 2007; Martín et al., 2017; Stintzing and Carle, 2004; Tanaka et al., 2008)

Group	Compound/ product	Colour	Code	Common sources
-	curcumin	yellow-orange	E 100	turmeric
-	carminic acid	magenta-red/ crimson	E 120	cochineal insect
-	chlorophyll	green	E 140	grass, lucerne (alfalfa leaf), tagetes erecta (marigold flowers) and, spinach
-	caramel	brown	E 150	sugar
-	vegetable carbon	black	E 153	vegetables
carotenoids	α-carotene, β-carotene, γ-carotene	orange-red, red, yellow, amber, brown	E 160 a	carrot, pumpkin, apricot, sweet potato, beans, spinach, kale, collard greens, papaya, bell peppers, tomatoes, and green leafy vegetables
carotenoid	annatto	orange-red	E 160 b	fruit of the achiote tree
carotenoid	paprika oleoresin	red	E 160 c	fruits of capsicum annuum or capsicum frutescens (chilli)
carotenoid	lycopene	bright to deep red	E 160 d	tomato
Nitrogen-containing compound	betalains/ betanin	red violet to yellow (pH <3 (more reddish), pH >7 (more yellowish))	E 162	mangosteen, beetroot, dragonfruit, red cabbage, swiss chard, *Opuntia*
phenolic	anthocyanin	dark purple (pH 1 (red), pH 4-5 (colourless), pH <7 (purple), pH <8 (deep blue), pH <12 (yellow/brown))	E 163	black currant, berries, grape, red cabbage, *Opuntia*, rose, onions
carotenoid	saffron	yellow-orange-red	E 164	crocus sativus

Group	Compound/ product	Colour	Code	Common sources
-	riboflavin (vitamin B2)/ riboflavin-5'-phosphate	yellow-orange	E101 i/ E101 ii	eggs, green vegetables, milk and other dairy product, meat, mushrooms, and almonds
-	riboflavin-5-sodium phosphate	yellow	E106	eggs, organ meats (kidneys and liver), lean meats, milk and green vegetables
carotenoid	lutein	orange-red to yellow	E161b	green leafy vegetables and fruits, and yellowish flowers
carotenoid	canthaxanthin	violet	E 161g	mushrooms, crustaceans, fish and eggs

2.1.1 Betalain colour compounds

Betalains ($C_{24}H_{26}N_2O_{13}$) are the immonium derivatives of betalamic acid and are acidic in nature owing to carboxyl groups and are well known for their antioxidant and anti-inflammatory properties (Bastos & Gonçalves, 2017; Hussain, 2018). They are derivatives of tyrosine and are normally found in plant groups in the order of Caryophyllales including Amaranthaceae (*Beta vulgaris*), Cactaceae (*Opuntia, Pitaya or Pitahaya*), Nyctaginaceae (*Bougainvillea*), Phytolaccaceae (*Phytolacca Americana*), and Portulacaceae (*Portulaca grandiflora*) (Tanaka et al., 2008; Zin et al., 2020a). Betalains are the main compounds associated with the displayed red colour of flowers, fruits and other plant tissues which protects the host from degradation by light (Tanaka et al., 2008), however, found themselves light-sensitive and their colour stability is greater in the absence of oxygen. Betalain compounds are normally coagulated in vacuoles of plant cells enclosed by tonoplast, vacuolar membrane, accompanying other phytochemical compounds. Depending on the type of groups attached to the main stem of betalains, which is betalamic acid, [4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridene-2,6-dicarboxylic acid], two basic colour compounds (betacyanin and betaxanthin) are derived (Herbach et al., 2006; Ravichandran et al., 2013) (Figure 1).

Betaxanthin (95 % of major yellow colour giving vulgaxanthin-I) is acquired by conjugating with amines whereas betacyanin (75-95 % of betanin which is responsible for red colour) is derived from the condensation with 3, 4-dihydroxyl phenylalanine or its glucosyl derivatives (Slimen et al., 2017). Betaxanthin earlier known as flavonoid and betacyanin regarded as nitrogenous anthocyanin prior to the discovery of their betanin, betanidin and indicaxanthin derivatives following their

degradation led to the assumption of their unique nature with them later grouped as betalains in 1968 (Choo, 2019; Khan & Giridhar, 2015; Slimen et al., 2017). The colour variety of betalains is influenced by yellow colour content to some extent, and their stabilities are determined by pH, light, heat, water activity, chelating agents, antioxidants, and enzymatic reactions. Peroxidases (POX), polyphenol oxidases (PPO), *ß*-glucosidase, and betalain oxidase are the most responsible enzymes for betalain degradation during processing; those can be gotten rid by the blanching process (Bastos & Gonçalves, 2017; Zin et al., 2020a). In practice, betalain colour varies along with its structure depending on the pH of the applied aqueous medium, i.e, the colourless carbinol pseudo base is favoured at pH 7 – 8 whilst the quinonoidal form is dominated at pH ≥ 7 (Ibraheem et al., 2016).

Betacyanin is a red-violet indoline- and dihydropyridine-derived nitrogen-containing water-soluble colour compound located in most Caryophyllales families order (Kumorkiewicz-Jamro et al., 2021). It has been known as betanin and numbered (E 162) in the food processing industries. Colour variety is influenced by yellow colour content to some extent (Aberoumand, 2011) and is more stable to process conditions than betaxanthin (Lee et al., 2014). Their stabilities are decisively governed by pH, light, heat, water activity, chelating agent, antioxidants, and enzymatic reactions (Nemzer et al., 2011; Slimen et al., 2017). Most degradation processes of betacyanin are isomerization, deglycosylation, dehydrogenation, hydrolysis, and decarboxylation (Slimen et al., 2017; Hussain et al., 2018; Strack et al., 2003). The presence of glucosyl substituent in the C-6 hydroxyl group position and amine group in the ring system encourages the radical scavenging activity of betacyanin, attributes of its anti-oxidative stress-related disorders, anti-cancer, and anti-inflammatory properties in specific plants and vegetables. There has been proved the existence of betacyanin in red or yellow beetroot (*Beta vulgaris* L.), Malabar spinach (Basellaceae), Bougainvillea glabra, Chenopodium quinoa Willd., cactus (*Opuntia*, *Hylocereus*, *Mammillaria*, *Melocactus*, and *Myrtillocactus* species), etc. (Kumorkiewicz-Jamro et al., 2021).

Betanin, gomphrenin, amaranthin, and bougainvillein are four major types of betacyanin that differed by the substituent groups attached to *cyclo*-DOPA moiety in the ortho position. Phyllocathin (betanidin *5-O-ß*-malonyl-glucoside) and hylocerenin (betanidin *5-O-ß*-[3"-hydroxyl-3"-methyl-glutaryl] glucoside) are acylated forms of betacyanin. Betanidin is the basic structural unit of most betacyanin derivatives and betanin (betanidin *5-O-ß*-glucoside) comes out from glucosylation and acylation of aglycon betanidine (I). Betanin, mostly beetroot (*Beta vulgaris* L.) based betanin, is the most stable red colour compound and its antioxidant ability is based on its

donation of hydrogen and electron. Besides, it has been claimed to possess a neuroprotective effect, reduce oxidative stress, improve cognitive abilities, and protect DNA from damage (Kumorkiewicz-Jamro et al., 2021). Apart from betanin; isobetanin, betanidin, isobetanidin, prebetanin, neobetanin coexist in a small amount composing aglycone (betanidin) linked by a *ß*-glucosidic bond with the glucose unit at C-5 carbon atom. Neo-derivatives are of interest due to their yellow colour appearance derived from the thermal degradation of *Beta vulgaris* and *Opuntia* species betanin.

Betalains colourants are available in the forms of concentrates, dried powder through the air, freeze or spray drying and encapsulation as well. They have been successfully applied in food, drugs, cosmetic products, and even in the technological field for example solar cell nanoparticle fabrication, hydrogen-producing devices, fluorescent probes, and colourimetric sensors (Bastos & Gonçalves, 2017). They are normally used in dairy, confectioneries, and meat products however their sensitiveness makes them more suitable for incorporation in cold products. Furthermore, Sivakumar and coworkers (2009) investigated the extraction of betalains through the sonic extraction method for leather dying. Coating of betalain pigments extracted from the skin and pulp of *Cactus* fruit with acidified mucilage from the pulp of the fruit, and ionic gelation can extend the shelf life as well as the stability of the pigments (Delia et al., 2018; Otálora et al., 2016). Microencapsulation of beetroot pomace extracts and their applications in bakery products have also been explored (Hidalgo et al., 2018). High resistance to degradation of betalain pigments encapsulated with glycerol and freeze-dried, which is incorporated in food products (such as strawberry jam, tomato paste and burgers) was proved with colour remaining 87.31 % after incubation at 150 ºC for 6 hrs (Ibraheem et al., 2016).

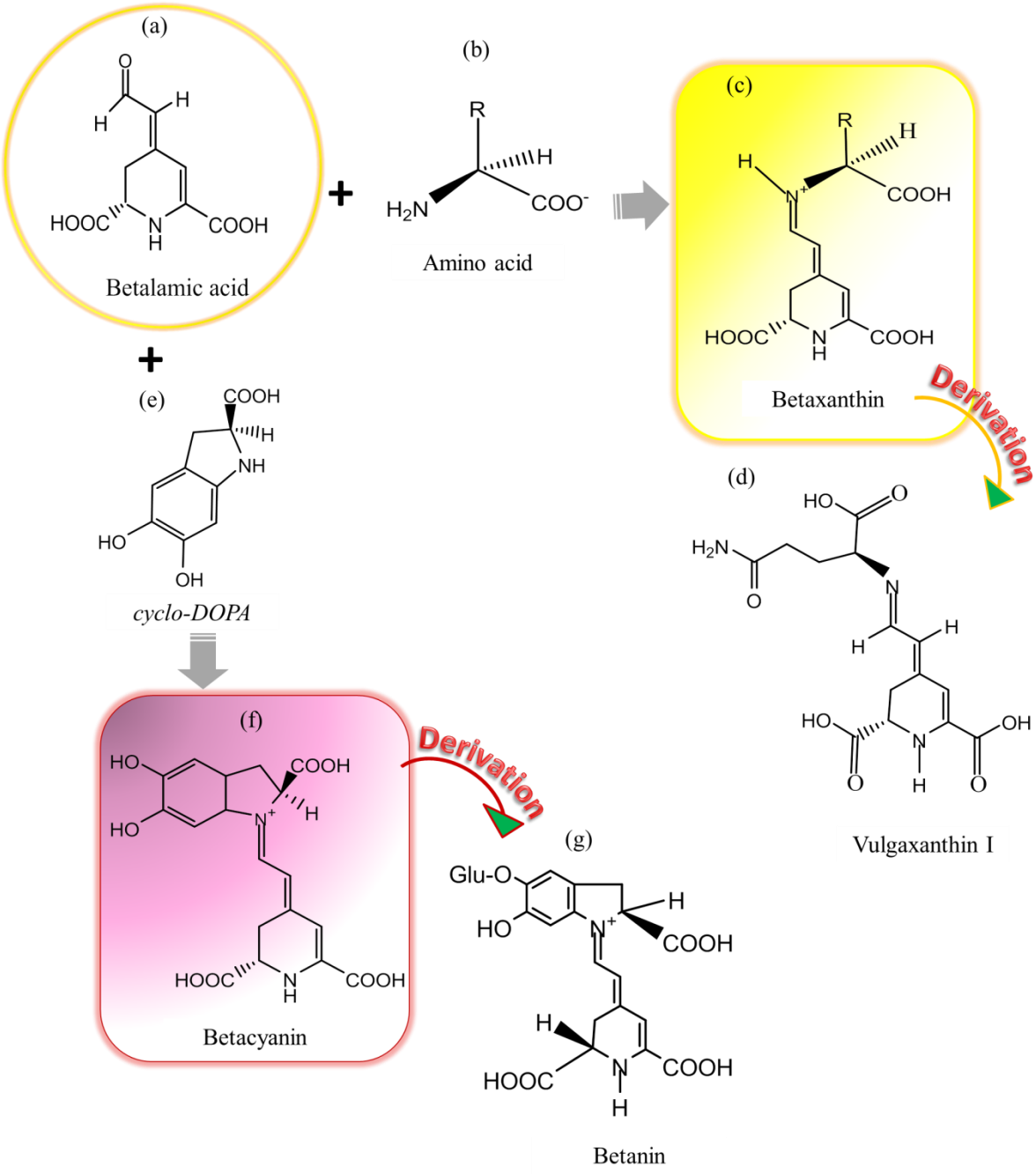
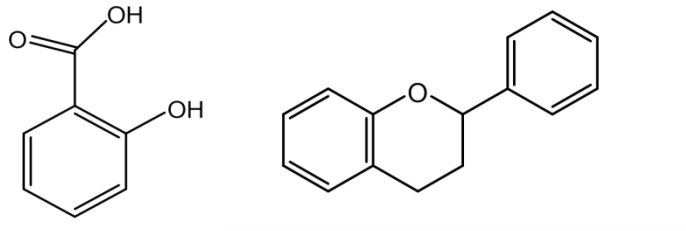


Figure 1. Derivation of betacyanin and betaxanthin colour compounds from betalamic acid: (a) Betalamic acid which is the core structure of betalain, (b) Amino acid which conjugates with betalamic acid to derive betaxanthin, (c) Yellow colour giving betaxanthin, (d) Vulgaxanthin I which is a derivative of betaxanthin, (e) cyclo-DOPA which condenses with betalamic acid to give betacyanin, (f) Red-violet colour giving betacyanin, and (g) Betanin which is a derivative of betacyanin

2.1.2 Phenolic compounds

Phenolic compounds (C_6H_5OH) with highly radical scavenging activity are plant secondary metabolites that consist of sugar moieties substituents such as glucose, arabinose, xylose, rhamnose, and galactose (Kim et al., 2003). Known as polyphenols, phenolic compounds are aromatic metabolites possessing a polar functional (−OH) group bonded directly to aromatic hydrocarbon rings which makes it easy to bond with hydrogen atoms in an aqueous medium and so raises their water solubility. Phenolic compounds are derivatives of aromatic amino acid phenylalanine ubiquitous in most plant kingdoms with different glycosylated forms ranging from simple phenolic molecules to high complex polymer molecules. They are mostly localized in plant tissues imparting pigments to their host and are protective of UV-radiation and plant resistance to infections (Manach et al., 2004). As aromatic secondary metabolites, phenolic compounds are known for the following properties; anti-oxidative, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic, antithrombotic, and anti-inflammatory activities (Kim et al., 2003). Their biological properties vary accordingly with their structure, solubility, and molecular size. Again, the specific structure of the phenolic compounds can manipulate their stability during processing and storage. Extracted amounts were even higher in the waste parts of fruits and vegetables as to the edible parts process (Balasundram et al., 2006). The presence of phenolic compounds in foods, both free and bound forms, can extend the shelf-life of food products by reducing the oxidation level of lipids and proteins. They scavenge free radicals by transferring their electrons from the outermost shell to fulfil the leakage of free radicals. Consequently, phenoxyl radicals ($PhO^{\cdot}$) are developed after transferring the hydrogen atom to the free radicals, which in turn can conjugate with adjacent hydroxyl or amine groups to enhance their stabilities, making them resistant to further oxidation process (Balasundram et al., 2006; Slimen et al., 2017).



Phenolic acid General structure of flavonoid molecule

Figure 2. Basic structures of phenolic compounds

Over 10,000 phenolic compounds (flavonoid and non-flavonoid, Figure 2) exist in several kinds of fruits and vegetables, cereals, tea leaves, and coffee beans. Their accumulation in the whole parts of the plant including root, stem, bark, flower, and leaf has been noticed (Martín et al., 2017). The nonflavonoid phenolics are classified, based on their carbon skeletons, into the following subgroups: simple phenols, phenolic acids and derivatives, phenones, phenylacetic acids and derivatives, hydrolyzable tannins, and stilbenes. Benzoic acid derivatives (C_6-C_1) and cinnamic acid derivatives (C_6-C_3) are the two main classes of phenolic acids and are richly found in red colour fruits such as berries, blackcurrant, plum, grape, and black carrot as in hydroxyl forms (Manach et al., 2004). Whereas, flavonols, flavones, flavanones, flavanols, isoflavones, flavanonols, and anthocyanidins are unique flavonoid compounds. Phenolic acids and flavonoids are common and the most active plant-based phenolic compounds (30 % and 60 % of total dietary polyphenols content) (Martín et al., 2017). Flavonoids with 15 carbon atoms are composed of two phenyl groups linked together with a heterocyclic ring and exhibit antioxidant properties. According to Moussa et al. (2019), more than 8000 flavonoids have been identified. Vitro analysis of the effect of citrus flavonoids on the inhibition of human breast cancer cell proliferation has been done by Felicia and co-workers (1996).

Since phenolic compounds are polar, extraction of them with water, supercritical or subcritical water, and alcoholic solvents is more compatible compared to non-polar solvents such as hexane or chloroform. Aqueous-alcoholic solvents are preferable rather than pure form as improvements in quality and quantity were observed with the latter. Other organic acids, pigments, proteins, and carbohydrates can be considered with them but then be cleaved off via purification or separation steps (Li et al., 2012; Flórez et al., 2015; Longhi et al., 2011; Rafiee et al., 2011). Oxidation is one of the most responsible reactions for food degradation which is why the sector of antioxidants plays an important role in food processing. Peroxidases (POX) and polyphenol oxidases (PPO) are the most responsible enzymes for phenolic degradation during processing (Bastos & Gonçalves, 2017). Phenolic compounds are found to be more stable in an acidic medium since the acid denatures the enzymes which are coming out of the cell membrane because of the grinding action, consequently, they are free from the disturbance of enzymes without going through the oxidation process. As reducing agents, phenolic compounds can retard oxidation reactions which catalyst through enzymes and can stabilize lipid peroxidases (Gallo et al., 2010).

2.2 Antioxidants

Reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive chlorine species (RCS), reactive bromine species (RBS), etc. produced by biochemical reactions are full of unstable free radicals, and their concentration can be increased depending on the level of environmental stress (Aguilar et al., 2017). Since those free radicals are with electron deficiency in their outermost shell, their attempt to grab the electrons from the other biomolecules to fulfil their leakage can damage the cells. Consequently, which behaviour leads to oxidative stress and related chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. Antioxidants are substances that have a lower concentration than the oxidizable substrates and retard their oxidation function by destroying or deactivating those free radicals. Antioxidants transfer the electrons to fulfil the lack of those free radicals by oxidizing themselves to retard the formation of more free radicals. This free radicals modulation can be enzymatic (endogenous antioxidants) or non-enzymatic (exogenous antioxidants). Metabolic processes inside our bodies synthesize endogenous antioxidants, in contrast, exogenous antioxidants are the ones that can get from the diet (Aguilar et al., 2017; Nimse & Pal, 2015; Mironczuk-Chodakowska et al., 2018; Moussa et al., 2019). Enzymatic antioxidants can break down free radicals in which superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are some primary antioxidants whilst glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) are secondary antioxidants. They are known as endogenous protein antioxidants and are major defences against oxidation metabolism (Aguilar et al., 2017; Nimse & Pal, 2015). Vitamin A, C, phenolic compounds, and betalains are water-soluble non-enzymatic antioxidants naturally formed in the cytosol whereas vitamin E and carotenoids are lipid-soluble accumulated in cell membranes (Nimse & Pal, 2015; Mironczuk-Chodakowska et al., 2018; Moussa et al., 2019). Food proteins that can combine with the excess iron species are known as antioxidants, some of them are transferrin (TF), ferritin (FER), and lactoferrin (LTF) (Mironczuk-Chodakowska et al., 2018). The composition of amino acids (parts of food protein) in betalain structure encourages the antioxidant activity of the host (Aguilar et al., 2017; Nimse & Pal, 2015; Mironczuk-Chodakowska et al., 2018; Moussa et al., 2019).

2.3 Beetroot

For the sake of highness in sugar content, beet variety is an alternative source of sugar cane supplied for sugar production industries. Beetroot (*Beta vulgaris ssp. esculenta var. rubra* L.),

belongs to Chenopodiaceae (Amaranthaceae) family, is the prominent vegetable ranked in the topmost list of highly nutrient vegetables fortified with antioxidants (Lee et al., 2014; Rubóczki & Hájos, 2018). Beetroots are worldwide known vegetables since ancient times and spread to Hungary in the 17th century (Székely et al., 2014). In Hungary, beetroot is regarded as a second crop with a growing period of 100-110 days and is mostly served in the fresh form as salad or dessert; frozen or desiccated form; processed form as pickles, chips or cold drinks and cooked form as soups. Beetroot is a chief source of sugar such as glucose, fructose, and mostly sucrose (Mirmiran et al., 2020). It is high in nitrate (NO_3^-), the precursor of nitric oxide (NO^-), so contributes to the bioactivity in the Cardiovascular system (Milton-Laskibar et al., 2021). The composition of beetroot juice was expressed as moisture content (30.88 %), ash content (10.58 %), crude fat (3.29 %), crude fibre (6.98 %), protein (4.1 %), and carbohydrate (44.17 %), respectively in the study of Shuaibu et al. (2021). Regardless of the earthy smell, beetroot is demanded as a good source of vitamins mostly immune booster vitamin C; and vitamin A for bone, skin, and eye health. It is bestowed with micro and macro elements to varying extent in the whole tuber and has been claimed as a Chempreventative (Lechner & Stoner, 2019). 100 g of beetroots contain sodium (4.17 mg), potassium (13.82 mg), magnesium (5.91 mg), phosphorous (11.57 mg), copper (0.21 mg), and iron (26.46 mg) (Shuaibu et al., 2021). Daily intake of folic acid can be accomplished by consuming 200 g of beetroot (Lorizola et al., 2018) and therefore can raise the haemoglobin level. Additionally, polyphenols, flavonoids, betalains, ascorbic acid, carotenoids, and triterpenes are the most thriving bioactive compounds and their contribution boosted the free radicals scavenging activity of beetroot. Based on the cultivar, the ratio of bio ingredients can differ. The significant influence of ripening stages on the composition of betalains and colour attribution has been reported by Montes-Lora and co-workers (2018). For example, beetroot (Dakota type) consists of 90 % moisture content and superior amounts of protein and fat were observed in the leaf part (1.7 % and 0.2 %) than in the root (1.3 % and 0.03 %) (Richardson, 2014); the Cylindre cultivar (both are autumn sowing cultivars) is higher in water-soluble content, antioxidant activity, and betalain contents compared to Alto F1 (Székely et al., 2014).

Beetroot is the most potent vegetable attributing a wide range of micronutrients and whole parts are occupied with valuable compounds for example some elements such as calcium, magnesium, potassium, sulfur, boron, and mineral contents are even higher in foliage than in root. The amount of components is quite different according to the variety of cultivars (Rubóczki &

Hájos, 2018; Székely et al., 2014). The chemical composition of the beetroot (*Beta vulgaris* L.) peel was presented as follows: moisture content (30.88 %), ash content (10.58 %), crude fat (3.29 %), crude fibre (6.98 %), protein content (4.1 %), carbohydrate (44.17 %); along with the mineral composition such as sodium (4.17 %), iron (26.46 %), copper (0.21 %), magnesium (5.91 %), potassium (13.82 %), and phosphorous (11.57 %), respectively (Shuaibu et al., 2021). Supplementation of beetroot stalks could be helpful to deduce endogenous antioxidant compounds (Lorizola et al., 2018). Beetroot stalk and leaf can protect against the oxidative damage in the liver of mice caused by the high-fat diet, according to the report of Lorizola and co-authors (2018). Of acknowledged sources of betalains, i.e. beetroot, swiss chard, dragon fruit, and prickly pear, beetroot has gained attention since besides the whole tuber even its waste parts such as stalk, peel, and pomace are rich in betalains. Additionally, betalain concentration is 40 to 70 (%) higher in peel compared to the flesh. The colour content ratio of betaxanthin to betacyanin is influenced by genetics or cultivar, the environment of cultivation throughout growing, and the stages of harvesting (Bucur et al., 2016; Lorizola et al., 2018; Nemzer et al., 2011; Pandita et al., 2020; Richardson, 2014; Sawicki et al., 2016).

Ideally, one kilogram of fresh beetroot contains 300-600 mg of betacyanin and 320-420 mg of betaxanthin (Bastos & Gonçalves, 2017). Based on the investigation of Lee et al. (2014), betalain contents in fresh beetroot cultivated from the field range from 0.65-800 mg/g FW whereas 3-4.8 mg/g FW of betaine (trimethylglycine or glycine betaine) was weighed. Additionally, freshly peeled beetroot contains 84.26 mg per 100 g of betacyanin and 27.54 mg/100 g of betaxanthin; according to De Azeredo and co-workers (2009). Wruss and co-workers (2015) expressed that 0.8 to 1.3 g/L of betalains could be preserved in fresh beetroot juice. In terms of bioactive compounds, the recovery of them from different parts of beetroot was ordered as follows: peel>flesh>stalk according to Slatnar et al. (2015). Likewise, the total phenolic contents in various root parts were found to decrease in the order of peel, crown, and flesh (Kujala et al., 2000). The characterisation of beetroot pomace has revealed the presence of moisture contents; carbohydrates such as cellulose, hemicellulose, lignin, and pectin, polyphenols; nitrogen-containing functional groups; aliphatic aldehydes, carboxylic acids, and citric acid (Kushwaha et al., 2018).

2.4 Extraction of plant compounds

Solid-liquid extraction is the process of washing or leaching out of desired components from the main matrix by proper mixing with solvent. Mass and heat transfer between two different phases are driven by molecular force in thermal separation processes. Solid size, the polarity of the solvent, and the inter miscibility matter most in solid-liquid extraction. Enough intimation time is necessary to accomplish transferring processes of key components from one phase to another. When the exchange process completes, separation of phases occurs accompanied by partial or complete separation of the mixture. In solid-liquid-based extraction, apparent equilibrium is reached when the concentration of solution inside of the capillaries is the same as that of free solution. Outlets can be separated into raffinate (rich in solvent) and extract (rich in solute) once the mass transfer process between feed and solvent is completed. In the extract phase, the mixture of solvent and key components can be expected which later on can be separated. Commonly used traditional solid-liquid extractions are; partitioning, maceration, leaching, percolation, digestion, Soxhlet, heat-flux, reflux, rotary, cold compression, enfleurage, and distillation (Renard, 2018).

According to the investigation of De Azeredo and co-workers (2009), a betalain extraction yield of 67.12 % was achieved by the following process conditions: pH, 3; solvent-to-beetroot ratio, 5:1; temperature, 70 ºC; and grinding time, 2 min. Likewise, extraction of betalains and phytochemicals from beetroot pomace had been conducted under varying process conditions. Pomace-to-solvent ratio (1:15), temperature (50 ºC), processing time (10 min), and pH (2.5) were observed to be the optimum conditions (Kushwaha et al., 2018). In our previous work, extraction of betalain pigments was accomplished by 15 % ethanol with solvent to beetroot peel ratio 0.8 w/v at 1 h treatment time and 20 ºC operation temperature. Under this condition, the amount of betaxanthin was 839.115 mg/L whereas double the amount of betacyanin, 1512.14 mg/L was recovered (Zin et al. 2020c). In the finding of Sanchez-Gonzalez and co-workers (2013), the maximum amount of betalains (92 mg/100 g of fruit) was separated by (1:4) methanol to water ratio at 10 min and 15 ºC processing time and temperature, respectively.

Some traditional extraction techniques such as distillation, solvent extraction, and cold compression have been upgraded by thermal or non-thermal emerging techniques such as microwave, ultrasonic wave, and gamma irradiation to avoid the decomposition of desired compounds as much as possible (Patel et al., 2019; Zhang et al., 2018). Meanwhile, extractions with

modified solvents such as deep eutectic, pressured liquid, and supercritical fluid have been encouraged in recent innovative extraction technology. According to Hernández-Aguirre and co-workers (2021), betalains' stability was highly improved in the beetroot waste extract of deep eutectic solvents magnesium chloride hexahydrate. Additionally, extraction yield % of 4.09 ± 0.69 withal total betacyanins content of 25.49± 1.54 mg/100 mL were extracted from red pitaya fruit (*Hylocereus polyrhizus*) peel via supercritical fluid extraction with carbon dioxide as solvent (Fathordoobady et al., 2019). Pretreatments such as solvent pre-heating and pre-maceration can be performed to improve the extraction efficiency as well (Pedroza et al., 2015; Patel et al., 2019; Zhang et al., 2018; Renard, 2018).

2.4.1 Modification of traditional extraction by acidification and enzymation

Since cell membranes or cell walls of the plant matrix are structured by polysaccharides, protein, and pectin, the denaturation of these macromolecules by heating or enzymation can enhance the extraction of bioactive compounds that are trapped inside the cell wall. Biosynthesis reaction of compounds present in the plant matrix can be prohibited by acidification of the extraction medium for example enzymatic decolourization of betanin can be retarded by ascorbic acid, which disturbs oxidative activity of polyphenol oxidases or ß-gluconolactone and inhibits ß-glucosidase (Strack et al., 2003). Apart from ascorbic acid, chelating agents such as citric acid and EDTA are also prominent for acidification extraction of bioactive compounds to boost their yield (Slimen et al., 2017). However, ascorbic acid being exogenous antioxidants, their application should be taken into consideration for antioxidant activity determination (Bastos & Gonçalves, 2017). In addition, the stability of red colour betalain was significantly improved with 0.05 % of ascorbic acid (Elbandy et al., 2008). In some cases, inorganic acid like hydrochloric acid is favourable to preventing the activity of endogenous enzymes (Delgado-Vargas et al., 2010). Kujala and co-workers (2001) adjusted the pH of the extraction medium to 2 with hydrochloric acid for the extraction of betalain and phenolic compounds from beetroot peel. Since the protein content of betacyanin is considerable and in the range of about 23.2-31.7 % in dry extract (Cai et al., 1998), enzymation is helpful to eliminate the protein contents. That can also be effective for membrane purification; however, a reduction in betalain has to be taken into account due to its presence of glucosyl derivatives (Sawicki et al., 2018). Lactic acid bacteria (Czyżowska et al., 2006), *Cysteine* (Preczenhak et al., 2019), and *Saccharomyces cerevisiae* (Castellar et al., 2008) have been applied for enzyme-assisted extraction of betalain pigments.

2.4.2 Application of Emerging Technologies in Food Processing

Handling fragile vegetables is a big challenge for food processing industries which has led to the innovation of processing techniques to minimize food loss. Minimal processing is the preparation of food with minimal treatment and the smallest changes in food quality through modern processing technology. Attempts have been made to bring practical usage of emerging technology to minimal food processing in the areas of tempering, vacuum drying, freeze-drying, dehydration, cooking, baking, roasting, pasteurization, sterilization, extraction, blanching, and direct microwave blanching (Latorre et al., 2012). Based on the characteristics of targeted products, thermal treatment methods (Ultra high-temperature processing, aseptic or semi-aseptic heat treatment, sous-vid, infrared heating, high frequency or radiofrequency heating, ohmic heating, microwave heating, inductive electrical heating, etc.) and non-thermal treatment methods (low direct current electric fields, ionizing radiation, gamma irradiation, pulsed electric fields (PEF), UV light, pulsed light, laser light heating, ultrasonic wave heating, high-pressure processing, etc.) have been developed. Two types of electro heating are evoked direct heating (ohmic) and indirect heating (microwave or radio-frequency) (Ohlsson et al., 2002). Gamma irradiation has some improvements in total phenolic compound content and antioxidant activity in water extract and it can even procure enzymatic browning (Abolhasani et al., 2017).

Radio-frequency heating applied frequency roughly from 10 to 50 MHz which is laid on two parallel electrodes (Marra et al., 2009). Fundamentally, frequencies of 13.56, 27.12, and 40.68 MHz are allowed for their practical usage in different fields. Like the microwave heating process, when the alternating electric field is applied to a substance that contains dipolar molecules or ions, two different modes of motion; dipole rotation and ionic migration to the field occur. Electrodes can be designed according to the purpose of applications for suitable distribution of electric field and heating patterns. Radio-frequency heating is widely used in food processing for drying, thawing, baking, defrosting, and sterilisation or pasteurisation (Marra et al., 2009; Ohlsson et al., 2002).

PEF is based on the principle of electropermeabilization and is suitable for the extraction of cellular compounds (Haberkorn et al., 2019). The treatment is performed by the pulse electric field induced by two electrodes, through the conductive material placed between them, with short duration pulses (μs) of high electric field (0.1 – 50 kV/cm) (López et al., 2009). Based on desired process parameters, it can be adjusted as conventional PEF (micro to millisecond) or nanosecond

PEF, and reversible or irreversible electropermeabilization (Haberkorn et al., 2019). PEF was introduced in the 1980s for the treatment of biological materials (Ohlsson et al., 2002). Later on, its application has been expanded in bio-based industries with different purposes including the extraction of bioactive compounds. The degree of cell membrane perforation depends on the number and duration of the pulses (Haberkorn et al., 2019). The efficiency of the PEF, with different pulses and electric intensities, was tested considering the transmission of betanin into the medium under the control of pH and temperature. The highest yield of betanin (90 %) subjected to pulsed electric field treatment, 5 pulses at 7 kV/cm (2.5 kJ/kg) and 10 kg/cm^2, through acidified extraction medium had been achieved by López and co-workers (2009). A recent study has pointed out that the PEF treatment with sixty 100 μs long pulses of 1.2 kV/cm (11.44 kJ/kg) at a frequency of 10 Hz on *Opuntia ficus-indica* fruit has some improvements in juice yield (3.3 times), extracted amounts of total betalains (1.48 times) and polyphenol (1.4 times) with antioxidants capacity (1.4-1.5 times) (Surano et al., 2022).

2.4.3 Microwave-assisted extraction (MAE)

The prominence of MAE as a novel technology in green chemistry originated from its advantages of diminution of reaction time via faster conversion, and lower or no solvent supplement. Dielectric radiation is non-ionizing radiation so that the electrons inside the material just move with the field instead of following them. Dielectric materials are electric insulators that polarize with the electric field in which only positive charges are aligned according to the direction of the field, in contrast, negative charges are moved in the opposite direction of the field. In dielectric heating, materials that possess dielectric properties absorb radiation. Microwave heating has been successfully applied for baking (bread, cake, pastry, etc.), cooking, tempering, drying, pasteurization, and sterilization. Compared to the conventional system, the tempering process can be done in a short time by microwave due to its high penetration depth. Notwithstanding, microwave heating is being extensively used in thawing frozen meat and food products for quick penetration. However, for appropriate and efficient treatment, differences in dissipation or loss factor followed by loss tangent should be taken into account (Figura & Teixeira, 2007; Ohlsson et al., 2002).

Electromagnetic waves with frequencies between 300 MHz and 300 GHz are noted as microwave and previously applied for navigation and telecommunication operations. Based on their application purposes, two frequency ranges are classified as 2450 MHz for home usage with

adjustable power output <1000 W and 915 MHz for industrial usage. As a segment of the electromagnetic spectrum, like visible light, the phenomenon of bending, reflection, refraction, and absorption of microwave radiation by the medium through which it passes is unavoidable. Likewise, the degree of absorptivity or transmissivity of transmitted waves relies on the electromagnetic properties of the treated object, for instance, dielectric property, polarity, permittivity, and the shape of the object. Another important factor is in situ water content of the matrix as the level of swelling and rupture of the matrix can be varied with it. The contradiction of electromagnetic waves is based on their electric and magnetic wave propagation oscillating in a perpendicular direction to each other with wavelength (1m ~ 1mm). In this case, the electric part if only absorbed by natural biological materials consequently leads to a heating process occurring in the medium with the absorption property of a microwave. Microwave radiation is non-ionized with photon energy ranging from $3.78 \cdot 10^{-6}$ eV to $1.01 \cdot 10^{-5}$ eV, and the interaction process occurs by heat convection mode. Moreover, the transformation phenomenon of kinetic to thermal energy is mainly concerned with the polarization potential of the polar molecules and their surroundings. The protocol is that once polar molecules present in a substance are hit by the electromagnetic beams, they become energetic and swing with the alternative movement of the electric field and consequently the alternative action of alignment and realignment of polar molecules creates friction between them which in turn lead to the heating up of the surroundings. Meanwhile, ionic components which are present in the substance orientate themselves according to the electric field (Figure 3). This intermolecular friction and ionic movements occur several million times ($4.9 \cdot 10^9$ times at a frequency of 2450 MHz) per second and raise the internal pressure of the cell. Consequently, higher internal pressure encountered due to rapid vaporization of in situ water could rupture the cell wall and enclosed substances inside the cell wall could be forced out of the cell at a high rate (Zin et al., 2020a).

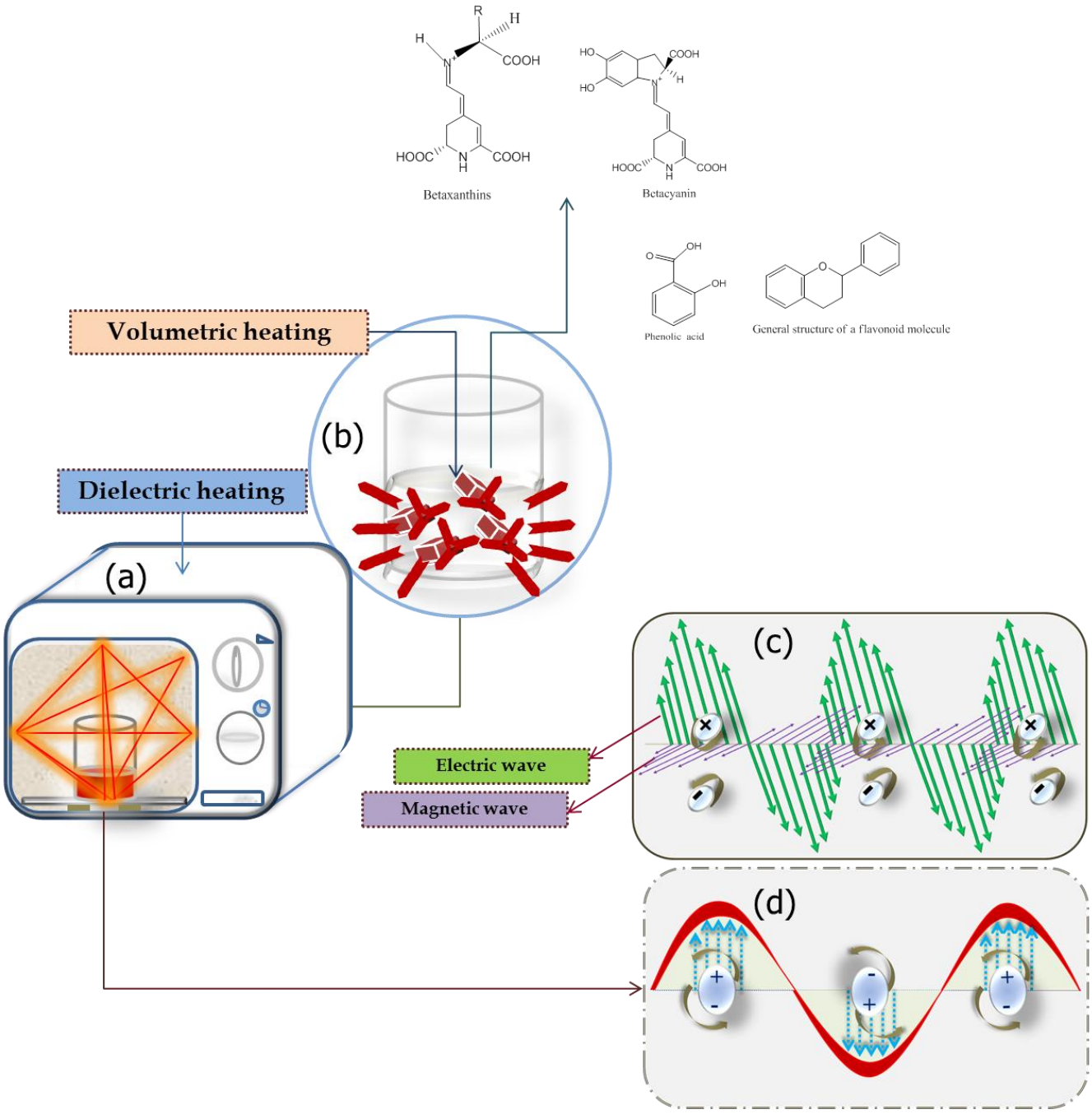


Figure 3. (a) Microwave irradiation for secondary plant metabolites extraction, (b) Heat diffusion from inside of the matrix to solvent medium and the surroundings, (c) Movements of ions with the electromagnetic wave, and (d) Alignment of dipolar molecules with electromagnetic wave

Non-polar solvent allows the microwave to pass through them with relatively poor absorption unlike polar solvent therefore specific effects of microwave power and its temperature on

reaction rate are controversial. However, the effective way of non-polar solvent application can be achieved under the specific mode of controlling process conditions (Perreux & Loupy, 2001). The solvent selection is critically based on their properties for instance non-toxic, non-corrosive, non-flammable, and most preferably renewable (Flórez et al., 2015). The temperature should be adjusted with the boiling point of the applied solvent to avoid subcritical process conditions (Seoane et al., 2017). As a strong polar universal solvent, water is an unavoidable portion in biology and a basic supplementary for the food industry (Ryynänen, 1994). It is utilized for extraction purposes by heating with radiation because of its highest dielectric losses among the polar solvent. The dielectric constant of water decreases at high temperatures and pressures and under these conditions, water can solubilize more nonpolar molecules (Filly et al., 2014). In open system microwave heating, the solvent-free reaction was preferable to ethanol leading to significant yield enhancement compared to conventional heating at the identical temperature used for conventional heating (Perreux & Loupy, 2001). Although, acceleration in the chemical reaction of targeted compounds such as epimerization, oxidation, and polarization by microwave should be considered (Bastos & Gonçalves, 2017).

Electric loss tangent (tan δ) of materials which is also known as dissipation factor (D_f) quantifies the transformation of electric and magnetic energy to thermal energy in the materials; it can be explained in terms of dielectric loss (ε") and dielectric constant (ε') as given below in (Eq. (1));

$$D_f = \tan\delta = \frac{\varepsilon''}{\varepsilon'} \qquad (1)$$

The dielectric constant expresses the capacity of a molecule to be polarized by an electric field whereas the dielectric loss factor expresses the efficiency of transformation of electromagnetic energy into heat. The greater in dielectric loss, the better the absorption of microwave: whilst the higher the dissipation energy, the better transparency to the solvent (Proesto & Komaitis, 2008). Besides, the higher the dissipation factor, the higher will be the thermal energy (Destandau et al., 2013). The absorption, transmission, and reflection abilities of different materials, known as electric permittivity, can be tracked in several ways. The absolute permittivity elucidates only how the material interacts with applied electromagnetic waves for nonionizing radiation and nonmagnetic materials. Alternatively, it can be said that nonmagnetic materials rely on dielectric permittivity (ε*) to interact with electromagnetic waves and can be expressed by dielectric constant (ε') and dielectric loss (ε") shown in (Eq. (2));

$$\varepsilon^* = \varepsilon'_r - j\varepsilon''_r \quad (2)$$

Real and imaginary parts of permittivity represent dipolar oscillation and damping and are valued depending on the motion of dipoles, nature of materials, high or low frequency, elevated or reduced temperature and also the concentration of the aqueous ionic solution. Relative permittivity (ε) can be estimated from the electric displacement field of material (*D*, C· m^{-2}) which is divided by of vacuum (D_{vacuum}, C· m^{-2}).

$$\varepsilon = \frac{D}{D_{vacuum}} \quad (3)$$

In dielectric material, the conversion of electrical energy into thermal energy is defined by its loss factor. Consequently, dipole rotation and electrical conduction are assumed as loss factors in microwave heating, (Eq. (4));

$$\varepsilon''_r = \varepsilon''_d + \varepsilon''_\sigma \quad (4)$$

where ε_r" is total loss factor, ε_d" is relative dipole loss, and ε_σ" is relative ionic loss. The dielectric properties of the common solvents used in MAE were listed in Table 2 (Flórez et al., 2015; Latorre et al., 2012; Seoane et al., 2017).

Table 2. Properties of commonly used solvents for phytochemical recovery through MAE at the frequency of 2.45 GHz

Solvent	Loss tangent (tan δ)	Dielectric constant (ε')	Dielectric loss (ε")
water	0.123	80.4	9.8892
ethanol	0.941	25.7	24.1837
methanol	0.659	32.7	21.5493
acetone	0.054	20.6	1.1124

If the components are homogeneous, their shape is more responsible for the effective permittivity of the mixtures, especially when the particle size is smaller than the wavelength. In principle, ordinary foods possess the penetration depth of electromagnetic waves (10-15 mm). The penetration level of the microwave through the substance can be traced by the degree of reflection, transmission, and absorption of the wave by the host substance; for example, pure water has a low degree of reflection and transmission but a high degree of absorption of the wave. Dissipated microwave power (P_D, W)

in a material can be estimated from electric field strength (E, V· m^{-1}), and frequency (f, Hz) as reported by (Eq. (5));

$$P_D = k \cdot E^2 \cdot f \cdot \varepsilon'' \quad (5)$$

where k is a constant value of 55.61· 10^{-14} C· $m^2 \cdot V^{-1}$. Due to the direct proportionality of dissipated microwave power to the absorptivity of the material, the attenuation of microwave differs according to the depth of penetration (z) and can be estimated as expressed in Eq. (6):

$$z = \frac{\lambda}{2\pi} \cdot \sqrt{\frac{2}{\varepsilon'\left(\sqrt{1 + tan^2\delta} - 1\right)}} \quad (6)$$

The frequency of applied radiation (f, Hz) and speed of the wave (c, m· s^{-1}) can be used to evaluate wavelength (λ, m).

$$c = f \cdot \lambda \quad (7)$$

2.4.4 Ultrasonic wave-assisted-extraction (UAE)

Sound frequencies less than 20 vibrations over a set amount of time (infrasonic sound) and above 2000 vibrations per second (ultrasonic) are regarded as inaudible by human beings. Ultrasonic wave is a series of compression and rarefaction movement that leads to kinetic energy and it is an adiabatic process since it travels at high speed. Depending on the frequency range, ultrasound can be differentiated as high power ultrasound or low-frequency ultrasound (20-100 kHz); high-frequency ultrasound (100 kHz-1 MHz); low power ultrasound or very high-frequency ultrasound (>1 MHz) (Legay et al., 2011). Ultrasound treatment is based on acoustic cavitation which propagates the tiny bubbles of gas in the liquid medium where ultrasound is applied and the continuous expansion and contraction action of gas collapse the bubbles leading them to implosion via high temperature (up to 5500 ºC) and pressure (up to 50 Mpa) (Zhang et al., 2018). Shear forces and turbulences are generated from the evolution of cavitation bubbles because of the international pressure build-up within the gland. The collapsing of those bubbles around the matrix, known as micro-jetting, is followed by fragmentation, erosion, capillarity, detexturation, and sonoporation which improve the breaking down of the layer of the matrix and so does the mass transfer. Ultrasound treatment influences the viscosity, opacity, particle size, and gel strength of the material (Chemat et al., 2017).

Uses of ultrasonic waves are degassing, defoaming, dehydration, drying, freezing and thawing, tenderization of meat, crystallization of lactose and fat, cutting, extraction, filtration and emulsification, and ageing of wines and esterification (Chemat et al., 2017; Urnau et al., 2018). Also, food preservation by ultrasonic waves by deactivating the microbes and enzymes is the trend of non-thermal food processing (Zhang et al., 2018). Upon the specific frequency, the application of ultrasound differed, for instance, an ultrasonic wave between the frequency of 20 and 100 kHz is applicable for emulsification and extraction purposes whereas the ultrasound with the frequency of 300 to 500 kHz is used for chemical reactions, pharmaceutical productions as well as wastewater treatments (Zhang et al., 2018). In advance, the application of ultrasound in plant biomolecules extraction has been lightened up due to its green effects; reducing the consumption of solvents, shortening the extraction time, and saving operation and maintenance costs. UAE has been used to extract various plant compounds and biomaterials such as polysaccharides, essential oils, proteins, peptides, fine chemicals (dyes and pigments), and bioactive molecules of commercial importance. Apart from conventional sonication, ultrasonic extraction has been improved with other techniques such as soxhlet extraction, Clevenger distillation, microwave, extrusion, and supercritical fluid extraction (Chemat et al., 2017; Urnau et al., 2018). Moreover, ultrasound-assisted and agitated enzymatic extraction enables the process duration to be reduced (Velyamov et al., 2019).

2.5 Membrane concentration

The focal aim of membrane separations of plant extracts is to separate or concentrate the thermo-sensitive bioactive compounds (Tamba et al., 2019). Another reason is to stabilize the natural pigment by reducing the factors that favour their degradation (Dos Santos et al., 2016). The expectation is to scale up the extraction of desired compounds from agro-industrial wastes through modernized concentration methods. Another purpose of filtration is as a part of preservation performed by allowing the juice to pass through the membrane with high pressure in which the microbes with bigger size than membrane pores were left behind the membrane. By doing so, the microbes-free liquid will stand longer than unfiltered ones. Membrane filtration is a promising technology with high throughput and low cost if properly applied. Its benefit in biological separation has been known by scientists for decades but it is not quite commonly used. The application of membrane technology (mostly ultrafiltration and microfiltration) to separate the various components from the feed stream has been receiving increased attention since the 1980s in different fields.

Reusing agro-industrial waste products from food industries is one of the effective ways to save the planet by diminishing the waste of fruits and vegetables (Dos Santos et al., 2016). Recovery of bioactive compounds from the waste parts of the beetroots; stalk (Maran & Priya, 2016), peel (Sawicki et al., 2016), and pomace (Vulić et al., 2013), has been accomplished through solid-liquid-based extraction methods. Notwithstanding, clarification of beetroot (stalk) extracts has been successfully realized via different membrane filtration processes such as microfiltration and ultrafiltration (Dos Santos et al., 2016). In food processing industries, membrane technology has been applied for the concentration and clarification of beverages including red wine (Bánvölgyi et al., 2006), blackcurrant juice (Bánvölgyi et al., 2009), apple juice (Vladisavljević et al., 2003), strawberry juice (Arend et al., 2017), and Indian blackberry juice (Ghosh et al., 2018).

In membrane filtration, substances that are larger in molecular size than the pore size of the membrane are rejected by the membrane. They are defined as retentate and the medium in which the rejected particles are dispersed is called concentrate. Suitable membranes for different purposes of separation are chosen according to their pore sizes. Polymeric membranes have low thermal conductivity and high resistance to almost all organic solvents at processing temperatures (Perfilov, 2018). Commonly used membranes are micro-porous membrane, homogeneous membrane, electrically charged membrane, asymmetric membranes, and liquid membrane. According to the purpose of processing, membrane operation can be distinguished as pressure-driven, concentration-driven, electric potential gradient, and temperature gradient. Transmembrane pressure (TMP) is defined as the difference in pressure between two sides of membranes (feed and permeate). When TMP is fixed for the whole process, the membrane capacity is defined as the amount of fluid passing through the membrane area. In both cross-flow and dead-end filtration systems, external surface fouling and pore-blocking are normally encountered by nanofiltration. Major substances which bring fouling are dissolved inorganic or organic components, colloidal, bacteria, or suspended solids. Additionally, hydrophobic components are much more connected to fouling the membrane than hydrophilic ones. Sometimes, the membrane manifests its fouling per its surface charge, roughness, and hydrophobicity. Another reason can be due to the trapped compounds or substances in the pores of the membrane or captured by the cake layer on the membrane when the process was carried out under high driven pressure. Biological and chemical fouling are common issues encountered in RO membranes. In all full-scale membrane filtration processes, overall removal efficiency is influenced by cross-flow rate, TMP, recovery, system arrangement, cleaning frequency, and module design.

General disadvantages of membrane fouling include flux decline, a significant requirement for an increase in TMP, biodegradation of membrane materials, and system failure (Choi, 2005).

Pressure-driven nanofiltration membrane has a higher retention rate of particles with molecular weight between 100 and 1000 Da (Dach, 2009). It has been used not only for lone filtration but also as a pretreatment for reverse osmosis feed. Along the line, the rejection rate of fresh RO membrane is 99 % and the separation mechanism concerns not only size and shape but also ionic charge as well as how the species and the membrane interact. Reverse osmosis membranes are used in water purification with the purpose of desalination. Even so, their applications in the food and beverage industries are continuously expanding mostly in the concentration of juices to retain flavour and sugars. Plant compounds such as antioxidants, carbohydrates, sugars, pectins, proteins, phenolic compounds, and colour compounds have been scavenged by RO from the fruits and vegetable wastes. Enzyme treatment prior to membrane filtrations is popular in juice clarifications however the loss in pigment needs to be considered. Along the line, physical properties of the solvents, such as polar or non-polar, molecular weight, dielectric constant, surface tension, and viscosity, are related to flux variations when it comes to the filtration of plant extracts. Especially for alcoholic solvents, the difference in the viscosities and the dielectric constant strongly affect the solvent flux. Otherwise, the pure water flux seems to be the highest compared to the other types of solvent due to its smallest molecular weight and the highest dielectric constant and polarity (Kim et al., 2002).

2.6 Modeling by response surface methodology (RSM)

RSM is an effective tool for the optimization of the processing condition through the determination of the impacts of the independent values on the dependent values under the range of investigation. Central composite design (CCD) and Box-Behnken design (BBD) are the major factorial designs for developing the RSM model. Four types of models, i.e, linear, 2FI, quadratic, and cubic can be evaluated in RSM. Properly randomizing the experiment runs withal replication of the centre points can average out the effect of extraneous factors which might exist. Data distribution can be transformed by the functions of square root, natural log, base 10 log, inverse square root, inverse, power, logit, and arcsine square root. In RSM, the significance of the model is determined by the sum of squares, mean square, degree of freedom, F-value, p-value, coefficient of determination (R^2), the adjusted and predicted (R^2) values, residuals, lack of fit, standard deviation,

mean, and coefficients of variation (C.V. %), which were placed in the analysis of variance (ANOVA) table.

- The greater F values, as well as smaller Prob>F values, are favourable.
- Theoretically, non-significant in lack of fit means the model is significant for the independent variables. The larger values of R-squared are more desirable.
- The values of adjusted R^2 and predicted R^2 differ by less than 0.2 meaning that these two values are in reasonable agreement with one another.
- Standard deviation is the square root of the error mean square.
- The C.V. % which measures the residual variability in the data is expressed as a percentage.

Once the response is modelled by a linear function of the independent variables, the following polynomial equation is applied to fit the first-order model:

$$y = \beta_0 + \beta_i x_i + \beta_{ii} x_{ii} + \cdots + \beta_k x_k + \epsilon \quad (8)$$

When there is a curvature in the system, the approximate function is the second-order model and the following equation is used for model fitting (Mahdevari & Hayati, 2021):

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2 + \sum \sum_{i<j} \beta_{ij} x_i x_j + \epsilon \quad (9)$$

Additionally, the predicted values and the actual values estimated by the model can be derived from the equations built by the model in terms of coded and actual values.

3 MATERIALS AND METHODS

3.1 Microwave-assisted extraction (MAE)

RSM in terms of CCD built by Design of Expert (DOE) statistical software, version 11.0.3, was applied to perform the process optimization based on the resulting responses for each and their mutual contents with replication that reflects the sources of variability between and within runs. The test sequence was randomized to prevent the effects of unknown nuisance variables or to minimize the potential bias. Square root transformation was chosen in order to observe which follows the Poisson distribution. Realization of MAE of bioactive compounds from the peel of beetroot with different types of solvent was done for hundred experimental runs in total accordingly to four process variables (Table 3). Aside from the peel; flesh and stalk parts of beetroot were also utilized for MAE at three different coded levels with pure water for a better comparison.

$$A = \frac{Power - 450}{350}, B = \frac{Time - 90}{60}, C = \frac{Solvent\ ratio - 0.15}{0.05}, D = \frac{Acid\ (\%) - 0.3}{0.2}$$

Table 3. Process variables for MAE of bioactive compounds from the beetroot wastes

Level	Natural Variables				Coded Variables			
	Power (W)	Time (s)	Solvent ratio (w/v)	Acid (%)	A	B	C	D
Low	100	30	0.1	0.1	-1	-1	-1	-1
Medium	450	90	0.15	0.3	0	0	0	0
High	800	150	0.2	0.5	+1	+1	+1	+1

The flowsheet of MAE processes is shown in Figure 4. Cylindra beetroots (*Beta vulgaris* L.) were supplied by a local bio farm, Cegléd, Hungary. Cleaning, peeling, and chopping processes of the beetroot parts (stalk, flesh, and peel) were completed manually. Grinding of the different portions was done using GM200 (Retsch GmbH, Germany) pulverizer to improve the efficiency of MAE by enlarging the active surface area for improved contact with the solvent. To investigate the extractability of betalains with the support of a microwave, three modes of microwave power (100-800 W) (based on the available modes in the home usage microwave oven) together with varied treatment times (30-150 s) (based on the pre-test accordingly with the handleable boiling point at

maximum microwave power) 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) as described in Table (1). 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; which was found to be efficient for direct microwave exposure of the sample with an open vessel due to its prevention of superheating effect and evaporation loss. 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. The obtained extracts were centrifuged by (Z206A; Hermle, Germany) centrifuge machine before spectrophotometric analysis.

The different MAE 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 as mentioned in section (3.4.3); 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 a moisture analyzer (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.

3.1.1 Extraction of bioactive compounds from stalk, flesh, and peel of Cylindra beetroot

For the comparison of the bioactive compound contents in the different parts of the beetroot (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) as explained in the materials and method section. Meanwhile, conventional solid-liquid extractions of bioactive compounds from stalk, flesh, and peel of Cylindra beetroot were achieved with pure water (0.1 w/v) at 70 ºC for one hour of extraction time and used as the controls.

3.1.2 The extraction efficiency of bioactive compounds from Cylindra beetroot via different types of solvents

The efficiency of different types of solvents on bioactive compounds extraction from beetroot peel and flesh was investigated with four different types of solvent (PW, AW, EW, and AEW) 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).

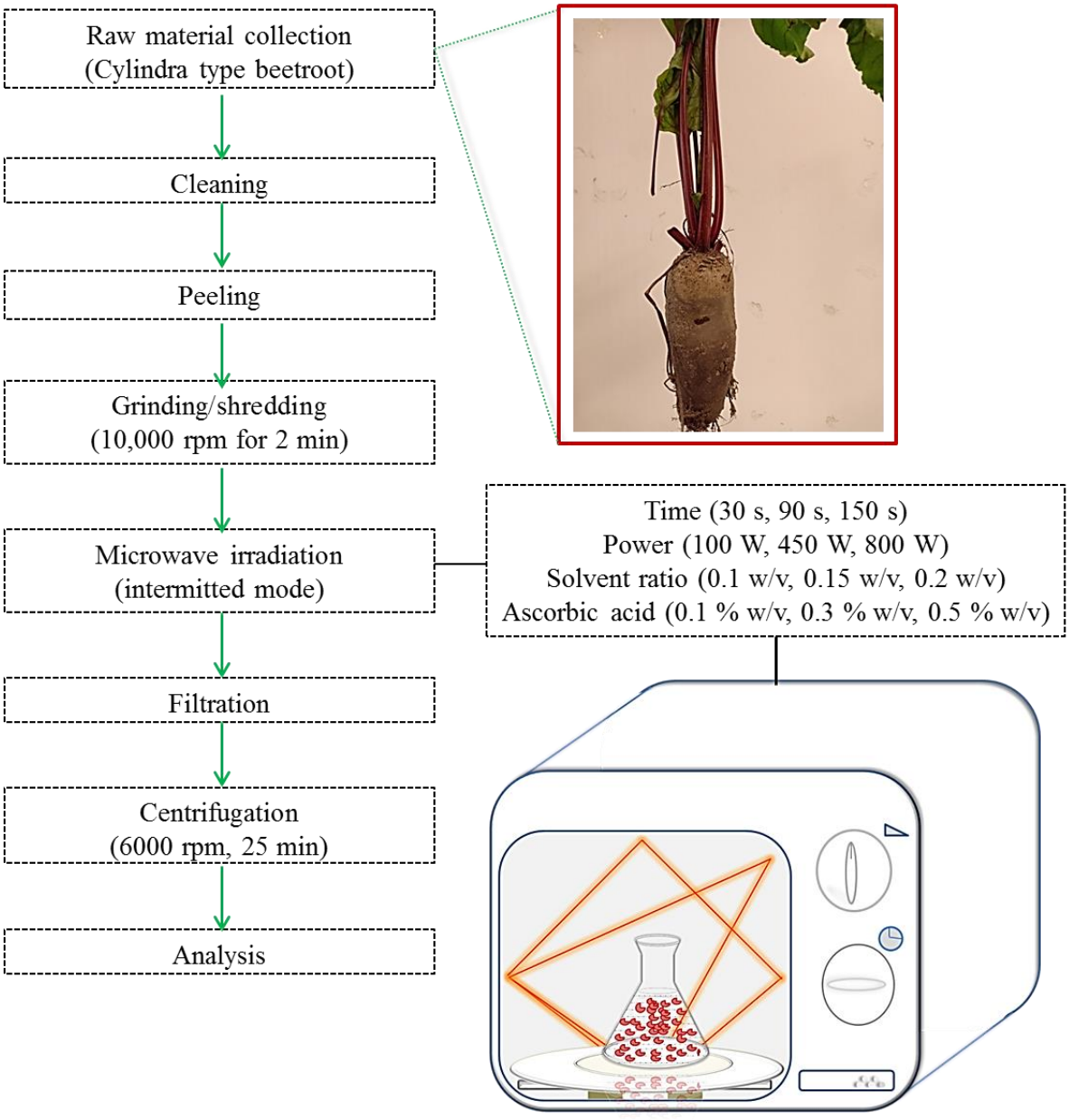


Figure 4. Flowsheet of microwave-assisted extraction

3.2 Ultrasound-assisted extraction (UAE)

The flowsheet of UAE processes is shown in Figure 5. Generally, the collected Rhonda type beetroots (*Beta Vulgaris* L.) were sorted, cleaned, and skinned properly. The only peels were processed for the experiments per the following different extraction methods;

1. 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 plant matrix per 50 mL solvent), 0.04 w/v (1 g plant matrix per 25 mL solvent), and 0.06 w/v (1 g plant 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.

2. 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.

3. 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.

4. The quantification of specific bioactive compounds was performed spectrophotometrically in which BX, BC, and TBC were measured by the second (Nilson's) method as mentioned in section (3.4.3); TPC was analyzed by Folin's method; AA was determined by FRAP (mg/g DM) and DPPH (%) methods; respectively.

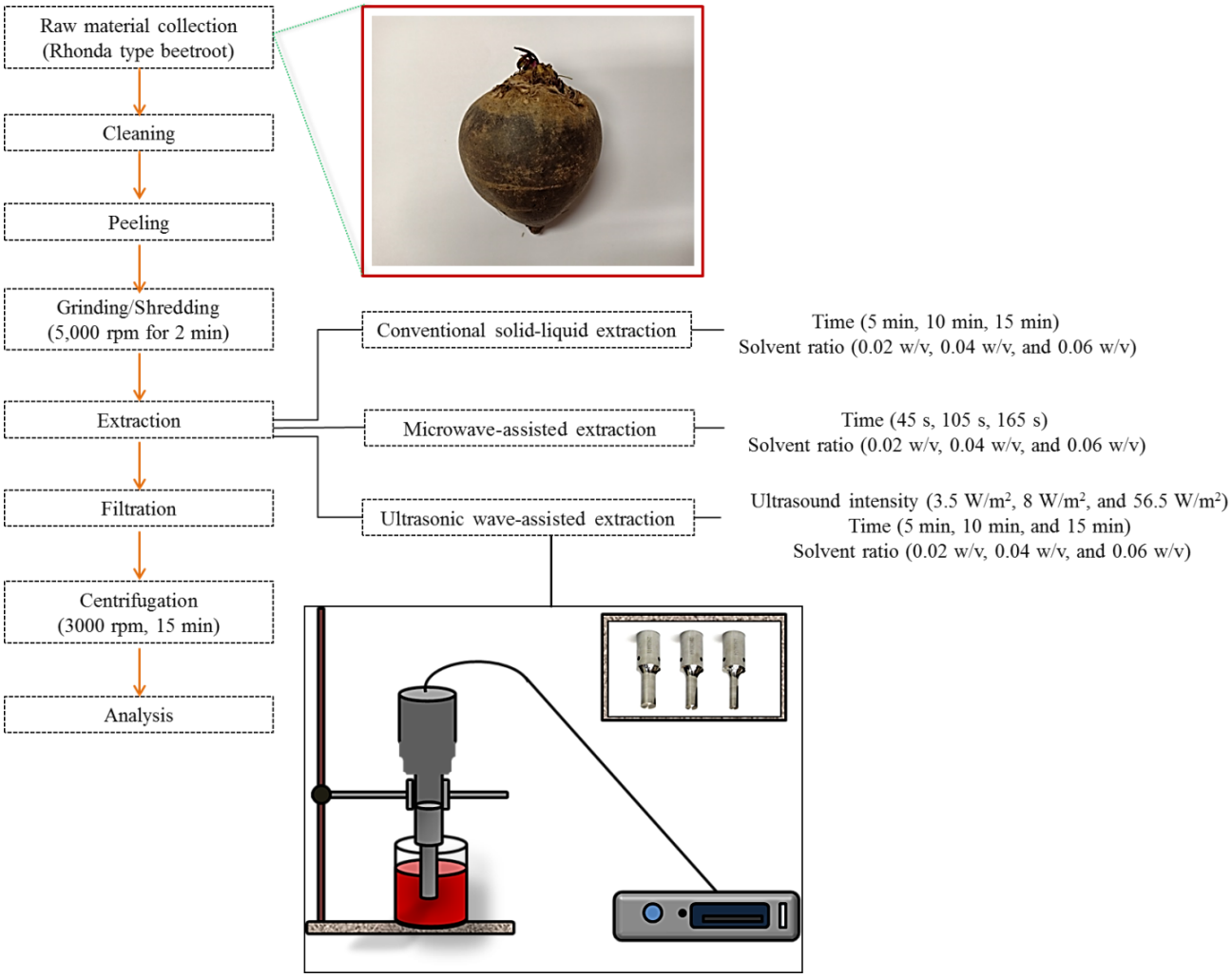


Figure 5. Flowsheet for the comparative study among UAE, MAE, and CON extractions

3.3 Membrane separation

Both nanofiltration and reverse osmosis concentrations were performed by cross-flow filtration process with DDS Filtration Equipment (LAB 20-0.72, Denmark) (Figures 6 and 7).

3.3.1 Nanofiltration process (NF)

Rhonda type beetroots (*Beta vulgaris* L.) were procured from a local market in Hungary and processed right away for extraction. Extraction was 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) at a processing temperature of 22 ºC for 60 minutes with pure water and 15 % (v/v) aqueous ethanol solvent (1:10 peel-to-solvent ratio). The crossflow filtration

process was accomplished by Polyamide Thin Film Composite (NF 200, FILMTEC^{TM} membrane) with active surface areas of 0.144 m^2 using DDS Filtration Equipment (LAB 20-0.72, Denmark). The parameters 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) (Figures 6 and 7). The colour compounds BX and BC were measured by the first colourimetric method as mentioned in section (3.4.3). TPC was analyzed by Folin's method whereas AA was determined by the FRAP method.

3.3.2 Reverse osmosis filtration process (RO)

Beetroots (*Beta Vulgaris* L.), a variety of Cylindra, were supplied from Cegléd, Hungary. Primarily the beetroots were gently cleaned to remove foreign materials and peeled. Both peel and flesh of the beetroots were separately processed for the extractions. First of all, the selected materials were grounded using GM 200 pulverizer and aqueous extraction was then attained with pure water in 1:20 solid-to-solvent ratio. The extraction was achieved by a single-batch-type mode at 40 ºC for 40 minutes. The crude extracts were stored under refrigeration until membrane separations were performed. The flowsheet of the concentration process by reverse osmosis is shown in Figures 6 and 7. RO membranes concentration was achieved by a low fouling type Trisep X20 advanced composite membrane (Microdyn) with active surface areas of 0.18 m^2. The operation was set up at TMP (40 bars) and the recirculation flow rate (400 L/h). A cooling system was set up for maintaining the temperature of the stream roughly at 27 ºC (Figures 6 and 7).

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. After separations, the analytical measurements were done for each filtrate. 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 contents of BX, BC, and TBC were measured by the second colourimetric method (Nilson's) as mentioned in section (3.4.3); TPC was analyzed by Folin's method; AA was determined by the FRAP method; respectively.

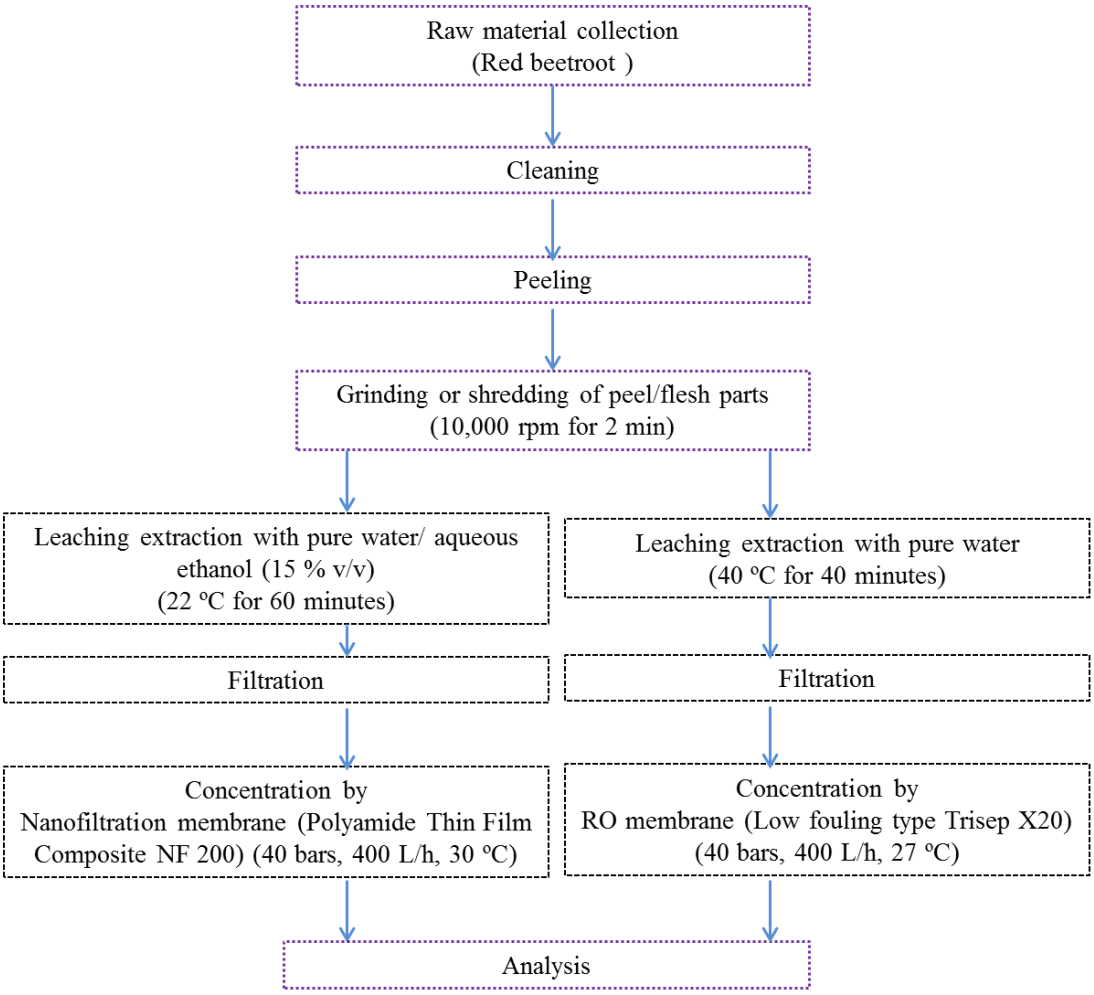


Figure 6. Flowsheet of the membrane concentration processes

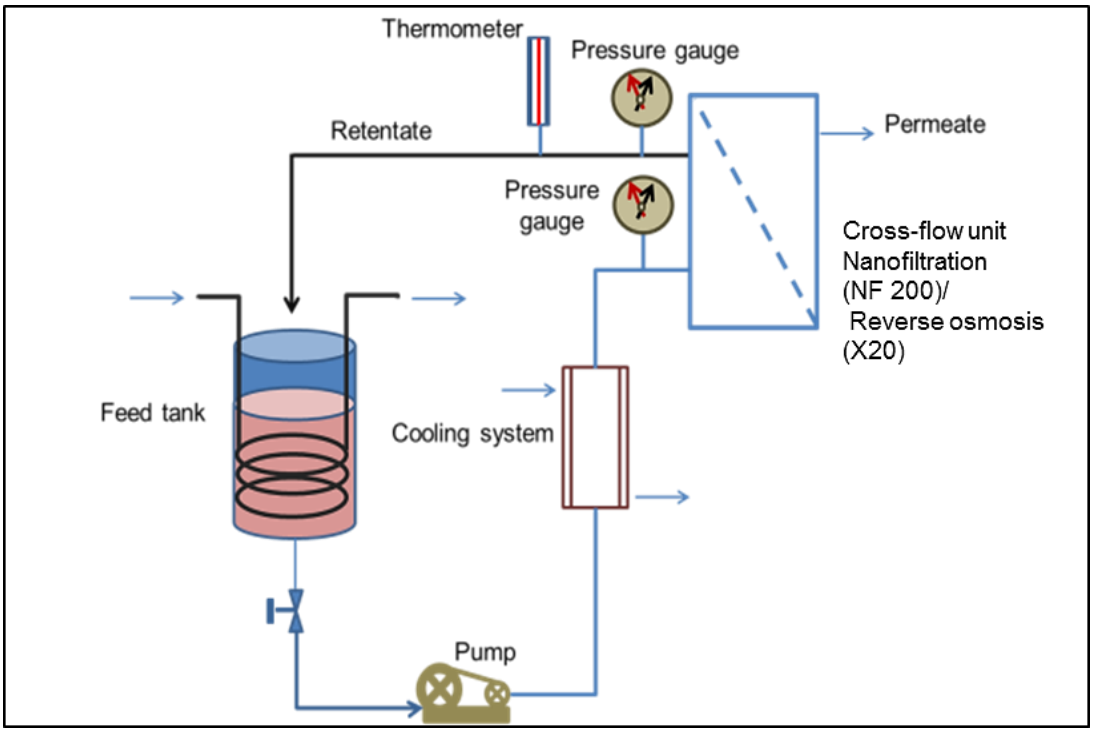


Figure 7. Scheme of cross-flow nanofiltration in batch mode

According to Darcy's law, the flux of fluid J ($m^3/(m^2 \cdot s)$) is defined by flow rate (Q) per area (A). Furthermore, the permeate flux of any fluid (J), which pass through a porous membrane, can be estimated by *TMP* (Pa) which was applied for the operation divided by the resistance of membrane, R_m (1/m), and dynamic viscosity of permeate, μ (Pa · s) (Miller et al., 2014).

$$J = \frac{TMP}{\mu \cdot R_m} \tag{10}$$

Additionally, Mulder (1997) defined volumetric flux as the measured volume of permeate in a given time interval. In this experiment, the permeate flux, J_x ($L/(m^2 \cdot h)$), of the sample was determined from the direct measurement of filtrate volume, ΔV (L), divided by the time required to collect the filtrate, Δt (h), and total active surface area of membranes, A_m (m^2) (Liu, 2011).

$$J_x = \frac{\Delta V}{A_m \cdot \Delta t} \tag{11}$$

Overall fouling resistance (R_{total}) includes membrane resistance (R_m) and fouling resistance (R_f) which can be reversible or irreversible, and internal or external fouling (Vladisavljević et al., 2003; Jiraratananon & Chanachai, 1996).

$$R_{total} = R_m + R_f \quad (12)$$

Subsequently, the values of membrane resistance (R_m) and fouling resistance (R_f) can be derived from the respective slope values of the pure water flux before (α_1) and after (α_2) the concentration process following Darcy's Law:

$$R_m = \frac{1}{\mu \cdot a_1} \quad (13)$$

$$R_f = \frac{1}{\mu \cdot a_2} - R_m \quad (14)$$

In addition, according to the research of Bánvölgyi et al. (2009), volume reduction ratio (VRR) was approved by feed volume V_0 (m^3) and volume of retentate V_R (m^3) or volume of permeate V_P (m^3) following the Eq. (15);

$$VRR = \frac{V_O}{V_R} = \frac{V_O}{V_O - V_P} \quad (15)$$

Retention (%) of a membrane can be approached from the concentration of permeate C_p and retentate C_R as follow (Bánvölgyi et al., 2009);

$$Retention\ (\%) = \left(1 - \frac{C_P}{C_R}\right) \times 100 \quad (16)$$

3.4 Analytical measurements

3.4.1 Colour (L^*, a^*, b^*) measurement

CIE (International Commission on Illumination) 1976 L^*, a^*, b^* is the uniform colour scale mostly applied in the visualization of the appearance of foods with the interpretation of colour tonation based on the respective colour coordinates: L^* for lightness (the closer to 100, the lighter in colour), a^* for redness or greenness (the higher in positive value, the more redness), and b^* for blueness or yellowness (the higher in the positive value, the more yellowish). The angle of Hue denotes the measurement of the degree between the redness and the yellowness of the sample whereas saturation or colour intensity is expressed by Chroma (C^*_{ab}) (Chandran et al., 2014). Colour patterns of the sample extracts were visualized by CHROMA METER CR-400 and calibration was performed with a calibration tile before the measurements. Total colour difference (ΔE^*_{ab}) was calculated by the following equation (17) (Gokhale & Lele, 2011):

$$\Delta E^*_{ab} = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*} \quad (17)$$

Where, ΔL^* means differences in the lightness of the sample and the standard, Δa^* means the differences in redness or greenness, Δb^* refers to the differences in yellowness or blueness. Chroma (C^*_{ab}) and Hue^o were calculated from the respective a^* and b^* values (Antigo et al., 2018):

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2} \quad (18)$$

$$Hue^o = tan^{-1}(\frac{b^*}{a^*}) \quad (19)$$

3.4.2 Browning Index (BI)

The following equation was adopted from Ding et al. (2014) to visualize the browning assessment of extracts based on the measured colour tonality (L^*, a^*, b^*) values accordingly.

$$BI = [\frac{100\ (x - 0.31)}{0.17}] \quad (20)$$

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 0.3012b^*)} \quad (21)$$

3.4.3 Betalain colour compounds measurement

Quantification of betalains was completed spectrophotometrically with GENESYS-5 UV-visible spectrophotometer (MILTON ROY, U.S.A) via two different methods: In the first method, absorbances were measured at 470 nm for the betaxanthin compound and 538 nm for the betacyanin compound. The quantification was done according to equation (22) (Nemzer et al., 2011; Chong et al., 2014).

$$\text{Betalain} = \frac{\text{Abs} \cdot \text{MW} \cdot \text{DF} \cdot 1000}{\text{Ɛ} \cdot \text{L}}\ (\text{mg}.L^{-1}) \quad (22)$$

Where Abs is the absorbance; MW is the molecular weight (g/mol); DF is the dilution factor; Ɛ is the molar extinction coefficient (L/(mol · cm)) and L is the path length (cm). Molecular weights and molar extinction coefficients for each betaxanthin and betacyanin are as follow: MW = 308 g/mol and 550 g/mol; Ɛ = 48,000 L/(mol · cm) and 60,000 L/(mol · cm).

The second one followed Nilson's method. The sample extracts were diluted with McIlvain buffer solution (pH=6.5) with a suitable dilution factor before reading the absorbances. The respective betalain compounds were evaluated according to Beer-Lambert law (equation 23) (Costa et al., 2017; Kovačević et al., 2015):

$$c = \frac{Abs\ x\ DF}{\varepsilon\ x\ l} \tag{23}$$

Where; *c* is the molar concentration, *Abs* is the actual absorbance values, *DF* is the dilution factor, ε is the molar attenuation coefficient ($E_{1\%}^{1cm}$ = 1120 for betacyanin and $E_{1\%}^{1cm}$ = 750 for betaxanthin), and *l* is the path length (1 cm). Following are the equations for the calculation of the respective absorbance values:

$$X_{BX} = 1.095\ (A_{538} - A_{600}) \tag{24}$$

$$Y_{BC} = A_{476} - A_{538} - X_{BX}/3.1 \tag{25}$$

$$Z\ (Impurities) = A_{538} - X_{BX} \tag{26}$$

$$TBC = X_{BX} + Y_{BC} - Z \tag{27}$$

3.4.4 Colour retention and the concentration ratio

Percentage of colour retention (R %), and the concentration ratio (CR) were estimated from the initial concentration of betalains right after extraction processes (C_0) and the concentration of betalains at a specified time (C_t) per the below equations (Das et al., 2019);

$$R\ \% = \frac{C_t}{C_0} \times 100 \tag{28}$$

$$CR = \frac{C_t}{C_0} \tag{29}$$

3.4.5 Degradation kinetics

The thermolability of beetroot microwave extracts was induced by heat at various temperature ranges (30-70 °C) in the absence of light. Herein, the degradation of betalains was tracked by the kinetic parameter of the first-order reaction with the reference of Antigo et al. (2018);

$$\frac{dC_f}{dt} = -kC_i \tag{30}$$

Where: C_f is the final concentration; C_i is the initial concentration; k is the first-order degradation constant (h^{-1}); t is the heating time (h). Meanwhile, the half-life ($t_{1/2}$) of TBC, BX, and BC was also investigated according to the equation (31) (Antigo et al., 2018; Das et al., 2019);

$$t_{1/2} = -\frac{\ln 2}{k} \tag{31}$$

Temperature dependency of betalains deterioration is determined by activation energy (E_a) using the Arrhenius equation, which is very useful in the estimation of bioactive compounds deterioration with time and temperature during and after the processing of foods (Chandran et al., 2014; Das et al., 2019; Chew et al., 2019);

$$k = A \cdot exp\left(-\frac{E_a}{RT}\right) \tag{32}$$

$$Ln\,(k) = -\frac{E_a}{R}\left(\frac{1}{T}\right) + \ln\,(A) \tag{33}$$

Where: k is the reaction rate constant (h^{-1}); A is the pre-exponential factor; E_a is the activation energy (kJ/mol); R is the universal gas constant ($8.3145 \cdot 10^{-3}$ kJ/K mol); T is the absolute temperature (K). In addition, temperature quotient or coefficient (Q_{10}) is the measurement of the rate of change in biochemical reactions as a consequence of temperature elevation by 10 ºC that can be calculated by the division of respective degradation constants at specific temperatures (Guneşer, 2016);

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \tag{34}$$

3.4.6 Total phenolic compounds (Folin-Ciocalteu method)

Visualization of total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method (Singleton & Rossi, 1965). In summary, the sample solution (20 µL) was mixed with 1250 µL of Folin reagent and 230 µL of the methanol-distilled water solution and waited for one minute before the addition of 1000 µL of 0.7 M sodium carbonate solution due to the proper intimation of Folin reagent and the sample. Absorbance measurement at 760 nm was accomplished after the sample mixture was incubated at 50 ºC for 5 minutes. Gallic acid was used as the standard.

$$TPC = \frac{Abs \cdot TS \cdot DF}{S \cdot a_3}\left[\frac{mg\ GAE}{L}\right] \quad (35)$$

Whereby, *Abs* is the measured absorbance; *TS* is the total amount of sample solution (µL); *S* is the amount of sample (µL); a_3 is the slope of the calibration curve; *DF* is the dilution factor.

3.4.7 Total flavonoid content (Aluminium chloride assay)

The measurement of the total flavonoid content (TFC) of the microwave extracts was accomplished by aluminium chloride assay based on the method of Ardekani et al. (2011). Quercetin was used as the standard for calibration. Firstly, sample 1 mL was diluted with 4 mL of distilled water and then mixed with 0.3 mL of 5 % $NaNO_2$ and waited for 5 min. Later on, 0.3 mL of 10 % $AlCl_3$ was added and allowed to react for one minute. 2 mL of NaOH (1 M) was then put in the mixture and made it up to 10 mL with distilled water. The absorbance was read at 510 nm and the resulted total flavonoid content was expressed as quercetin assay;

$$TFC = \frac{Abs \cdot TS \cdot DF}{S \cdot a_4}\left[\frac{mg\ QUE}{L}\right] \quad (36)$$

Whereby, *Abs* is the measured absorbance; *TS* is the total amount of sample solution (µL); *DF* is the dilution factor; *S* is the actual amount of the sample (µL); a_4 is the slope of the calibration curve.

3.4.8 Antioxidant Assays

3.4.8.1 FRAP Method

The ferric reduction antioxidant power (FRAP) method is applicable to quantify AA in the bio-extracts by a simple redox reaction in which the reduction of ferric ions to ferrous ions with intensive colour changes serves as an indicator. The number of electron donations may depend on the nature and specific property of the measured antioxidant (Benzie & Devaki 2018). FRAP reagent was prepared with acetate buffer solution (250 µL), 30 mM ferric chloride solution (25 µL), and 10 mM TPTZ solution (25 µL). FRAP reagent (1500 µL) was mixed with (30 µL) distilled water followed by a sample solution (20 µL) and the mixture was allowed to stand in the dark at room temperature. Reading the absorbance at 593 nm was performed after exactly 5 minutes of incubation. The calibration was realized with ascorbic acid instead of the sample solution and expressed as ascorbic acid equivalent. The calculation was done using the equation (37):

$$AA = \frac{Abs \cdot TS \cdot DF}{S \cdot a_5}\left[\frac{mg\ ASE}{L}\right] \quad (37)$$

where *Abs* is the absorbance; *TS* is the total amount of sample solution (µL); *S* is the amount of sample (µL); a_5 is the slope of the calibration curve; *DF* is the dilution factor.

3.4.8.2 DPPH Method

To examine the radical scavenging activity of the extracts, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay adopted from Ravichandran et al. (2013) was performed. The stock solution was prepared with 22 mg of DPPH dissolved in 50 mL of pure methanol. To acquire $6 \cdot 10^{-5}$ M of DPPH solution, 6 mL of stock solution was diluted with 100 mL of methanol and used as the control and working solution. Extract sample 0.1 mL was mixed with 3.9 mL DPPH working solution and vortexed before incubation in dark for at least 30 minutes. Optical density (OD) was then detected at 515 nm and (%) radical scavenging activity was predicted as follows:

$$DPPH\ (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \cdot 100 \quad (38)$$

3.4.8.3 ABTS Method

ABTS assay was performed following Ilyasov et al. (2020) with ABTS (2,2'-Azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid)) free radical by allowing it to react with antioxidants composed in the extracts for the speculation of how much percentage the sample has of radical scavenging activity. In brief, ABTS (7 mM) was dissolved in potassium persulfate (2.45 mM) and the mixture was allowed to stand in the dark for a minimum of 16 hrs to produce $ABTS^{\cdot+}$. The mixture was then diluted with phosphate buffer saline (PBS) (pH 7.4) to get an absorbance of 0.7±0.02 at 734 nm. Trolox standard solution (0-5 mM) or an extract was allowed to react with the diluted solution mixture in 1:10. Before reading the absorbance, the combined mixture was incubated for 5 min. The percentage of AA in the samples was calculated as follows:

$$ABTS\ (\%) = \left[\frac{(A_{control} - A_{sample})}{A_{control}}\right] \cdot 100 \quad (39)$$

4 RESULTS AND DISCUSSION

4.1 Microwave-assisted extraction (MAE)

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 material and methods section (3.1). The significance test of model fit was performed by the central composite design of Design-Expert software version 11.0.3, based on analysis of variance (ANOVA). RSM with CCD has been developed to approach the optimum process condition through the interaction between the different variables and the experimental outcomes. 2^k factorial design which is an orthogonal design is applied for fitting the multiple linear regression model. Experimental runs were randomized to avoid the effects of extraneous factors which might present. Data distribution was transformed by the square root function.

4.1.1 RSM of beetroot peel-PW extracts

The experimental outcomes of the recovered betalains, total phenolics, and their respective antioxidant activities from the peel of Cylindra type beetroot (*Beta vulgaris* L.) with PW solvent via MAE were denoted in Table 4. Twenty treatments (runs) were conducted according to CCD including replication of the centre points. The model fixations for all responses were performed by the 2FI model function. The influence of each factor on the response was investigated by holding the other process variables constant. The results were depicted in Appendix-Figure 1 in terms of 3D surface graphs built by the RSM model. Generally, microwave power has the most significant effect on the dependent variables except DPPH, which is influenced mainly by solvent ratio. The supreme amounts of bioactive compounds recovered in run 20 at 800 W of microwave power for 150 s of irradiation time with 0.2 w/v solvent ratio are as follows; TBC (12.31±0.14 mg/g DM), TPC (21.94±0.54 mg GAE/g DM), radical scavenging activities by FRAP method (35.68±0.77 mg ASE/g DM), and DPPH method (94 %), respectively. Table 5 represents the ANOVA for each response speculated from twenty runs. The regression equations for obtaining respective compounds at any given parameters under the study range were given in actual form as;

$$\text{Sqrt (TBC)} = 1.472-0.0003\cdot A-0.002\cdot B+3.368\cdot C+0.00001\cdot A\cdot B$$

$$\text{Sqrt (TPC)} = 2.443-0.003\cdot A-0.004\cdot B-0.769\cdot C+0.00002\cdot A\cdot B+0.016\cdot A\cdot C$$

Sqrt (AA) = 3.29-0.004·A-0.005·B-2.747·C+0.00003·A·B+0.02·A·C

Sqrt (DPPH) = 2.412-0.0008·A-0.004·B+21.733·C+0.00003·A·B

Table 4. Experimental outcomes of extracted compounds from beetroot (Cylindra) peel by PW solvent

Run	A:Power (W)	B:Time (s)	C:Solvent ratio (w/v)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)	AA (%)
1	450	90	0.15	7.12	9.43	12.93	44
2	800	30	0.2	5.84	6.84	10.31	41
3	450	150	0.15	3.63	4.98	8.04	22
4	450	90	0.15	3.39	3.69	7.29	21
5	100	150	0.1	4.41	6.08	9.22	46
6	450	90	0.15	3.86	4.23	8.16	28
7	800	150	0.1	4.89	6.10	9.82	46
8	100	150	0.2	4.24	6.28	7.45	38
9	800	30	0.1	7.12	7.82	12.65	37
10	450	30	0.15	8.16	11.29	17.77	43
11	800	90	0.15	3.53	4.31	6.70	37
12	100	30	0.2	3.17	3.32	7.04	27
13	450	90	0.15	4.29	5.51	8.65	30
14	450	90	0.15	4.59	6.28	9.42	46
15	450	90	0.1	3.07	3.55	6.21	27
16	450	90	0.15	4.38	6.64	10.27	35
17	100	30	0.1	3.14	4.03	6.98	21
18	100	90	0.15	3.79	5.08	7.41	33
19	800	150	0.2	5.58	8.43	12.44	50
20	450	90	0.2	12.31	21.94	35.68	94

Meanwhile, the operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds with the desirability of 0.843 was; microwave power (800 W), operation time (150 s), and solvent ratio (0.2 w/v) (Figure 8). 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 DPPH (78 %), respectively. The predicted values and actual values which can be correlated by the coded and actual equations built by the model were depicted in Figure 9.

Table 5. ANOVA for beetroot peel-PW extract

Source	Estimated coefficient				F Value			
	TBC	TPC	FRAP	DPPH	TBC	TPC	FRAP	DPPH
Intercept	2.2	2.53	3.18	6.08	9.92***	10.88***	9.49***	14.63***
A	0.3414	0.455	0.5704	0.5713	17.17***	17.61***	17.15**	9.51**
B	0.2343	0.3247	0.3886	0.5026	8.09*	8.96**	7.96*	7.36*
C	0.1684	0.3186	0.3209	1.09	4.18	8.63*	5.43*	34.4***
AB	0.2947	0.4524	0.5236	0.5578	10.24**	13.92**	11.56**	7.25*
AC		0.2777	0.3564			5.25*	5.36*	
Residual (SS)	1.02	1.65	2.66	5.15				
Lack of Fit (SS)	0.6426	1.14	1.91	3.44				
Multiple R^2	0.7257	0.7953	0.7722	0.796				
Adjusted R^2	0.6525	0.7221	0.6908	0.7416				
Predicted R^2	0.4842	0.4247	0.1524	0.5334				
Std. Dev.	0.2605	0.3429	0.435597	0.5859				
Mean	2.2	2.53	3.184218	6.08				
C.V. %	11.84	13.57	13.67989	9.63				

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05. Variables: A=Power, B=Time, C=Solvent ratio

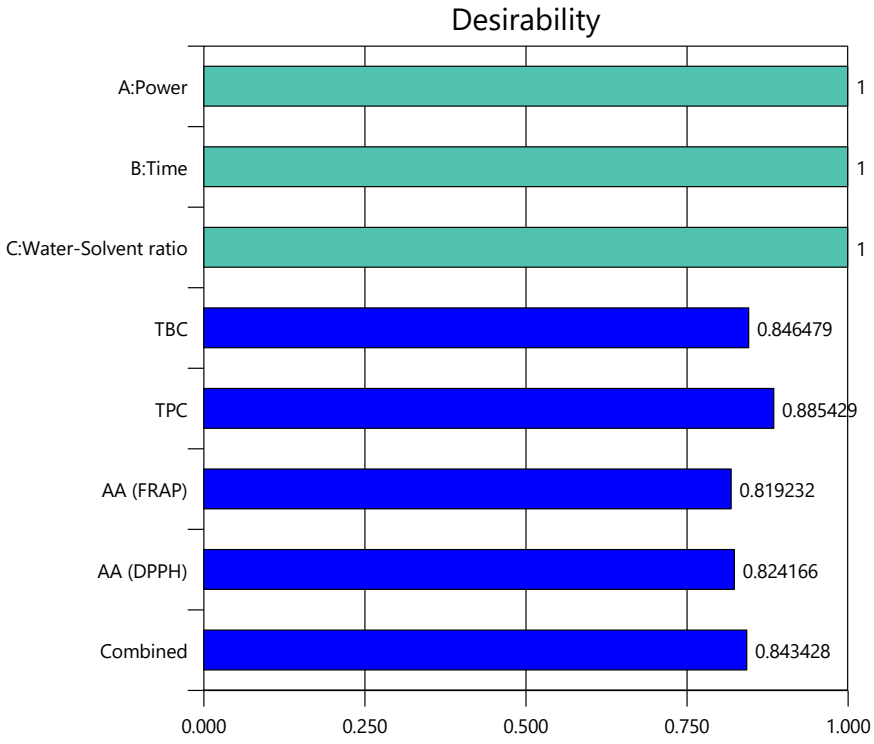


Figure 8. The desirability test based on the optimized values of targeted compounds in beetroot peel-PW extracts

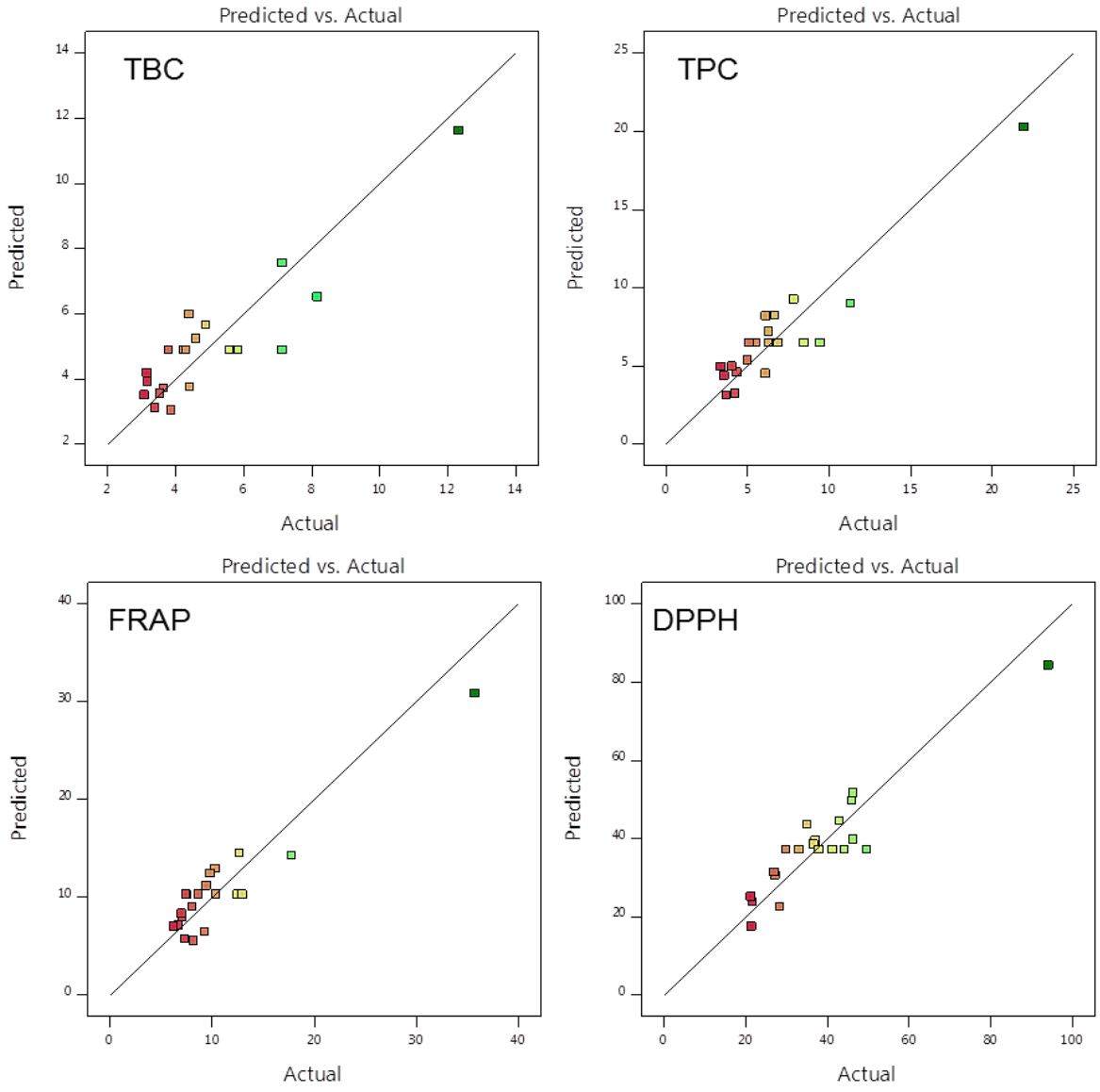


Figure 9. Correlation between the predicted values and actual values calculated by the model according to the regression equations (beetroot peel-PW extracts)

4.1.2 RSM of beetroot peel-AW extracts

Table 6 represents the experimental values of the recovered betalains, total polyphenol, and their respective antioxidant activities from the beetroot peel with AW solvent via MAE. Among the thirty runs, the utmost amounts of betalains and phenolic compounds: TBC (7.09±0.19 mg/g DM), and TPC (309.67±0.0 mg GAE/g DM) were scavenged in run 5 while the highest radical scavenging activity examined by FRAP method (231.72±4.65 mg ASE/g DM) was observed in sample 18. Thereby (89 %) of radical scavenging activity measured by the DPPH method was found in run 21. TBC, TPC, and DPPH were fixed by the quadratic model whereas FRAP was fixed by the linear model function. The ANOVA of each independent variable was shown in Table 7. As shown in the table, the overall models for six responses are significant with p-values less than 0.0001, and lack of fit is not significant for all responses. Appendix-Figure 2 demonstrates the 3D graph surfaces pointing out the correlational effects of the factors and responses on the extractability of targeted bioactive compounds from beetroot peel.

Table 6. Experimental outcomes of extracted compounds beetroot (Cylindra) peel by AW solvent

Run	A:Power (W)	B:Time (s)	C:Solvent ratio (w/v)	D:Acid (% w/v)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)	AA (%)
1	100	30	0.2	0.5	3.57	153.32	112.95	57
2	100	150	0.1	0.5	3.33	236.81	216.55	79
3	450	90	0.15	0.3	2.81	111.32	66.84	69
4	450	90	0.15	0.5	3.40	173.05	140.06	71
5	800	150	0.1	0.5	7.09	309.67	226.23	64
6	450	90	0.15	0.3	3.40	101.20	74.43	65
7	450	90	0.15	0.3	3.59	97.15	74.59	70
8	800	30	0.1	0.5	2.13	214.04	205.47	85
9	450	90	0.1	0.3	2.15	262.61	94.45	82
10	800	90	0.15	0.3	5.54	93.10	66.48	61
11	800	30	0.2	0.1	2.35	6.83	4.41	31
12	450	90	0.15	0.3	3.66	91.08	86.06	70
13	800	30	0.2	0.5	2.28	126.75	112.95	74
14	450	90	0.15	0.1	3.48	24.39	6.31	44
15	100	150	0.2	0.1	1.85	15.10	2.68	29
16	100	30	0.1	0.1	1.36	42.05	34.64	87
17	800	30	0.1	0.1	1.85	41.90	20.98	86
18	100	30	0.1	0.5	1.89	247.43	231.72	86
19	800	150	0.2	0.5	6.41	107.02	101.34	45

20	450	90	0.15	0.3	3.04	63.76	76.31	75
21	450	30	0.15	0.3	0.93	21.25	40.59	89
22	100	150	0.1	0.1	1.42	34.76	12.89	87
23	800	150	0.1	0.1	3.05	34.91	6.58	36
24	450	90	0.15	0.3	2.63	77.92	73.15	77
25	100	90	0.15	0.3	2.63	156.86	69.92	85
26	450	90	0.2	0.3	1.84	115.37	56.80	77
27	100	30	0.2	0.1	1.02	9.87	1.57	21
28	800	150	0.2	0.1	4.06	22.01	6.09	51
29	100	150	0.2	0.5	1.62	120.68	104.56	81
30	450	150	0.15	0.3	2.76	82.98	75.59	77

Table 7. ANOVA for beetroot peel-AW extract

Source	Estimated coefficient				F Value			
	TBC	**TPC**	**FRAP**	**DPPH**	**TBC**	**TPC**	**FRAP**	**DPPH**
Intercept	1.72	9.83	8	8.64	10.13***	45.5***	131.42***	8.93***
A	0.2479	-0.1807	-0.0879	-0.23	21.89***	0.3589	0.159	1.87
B	0.2228	0.3603	-0.1108	-0.2003	17.68***	1.43	0.2529	1.42
C	0.0129	-1.9	-1.62	-0.8382	0.059	39.85***	53.88***	24.88***
D	0.1731	4.3	4.78	0.6974	10.68**	203.52***	471.39***	17.22***
AB	0.1804	-	-	-0.4133	10.31**	-	-	5.38*
AC	-	-	-	0.3717	-	-	-	4.35*
BC	-	-	-	0.4246	-	-	-	5.68*
CD	-	-	-	0.492	-	-	-	7.62*
A^2	0.2869	-	-	-	5.61*	-	-	-
B^2	-0.3889	-3.58	-	-	10.31**	26.93***	-	-
C^2	-	3.04	-	-	-	19.4***	-	-
D^2	-	-	-	-0.918	-	-	-	11.94**
Residual (SS)	1.11	37.65	21.84	10.17				
Lack of Fit (SS)	1.04	33.36	21.21	9.84				
Multiple R^2	0.76	0.92	0.95	0.80				
Adjusted R^2	0.69	0.90	0.95	0.71				
Predicted R^2	0.53	0.86	0.93	0.28				
Std. Dev.	0.22	1.28	0.93	0.71				
Mean	1.66	9.51	8.00	8.09				
C.V. %	13.56	13.46	11.68	8.82				

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05. Variables: A=Power, B=Time, C=Solvent ratio, D=Acid %

The actual equations for obtaining respective compounds at any given parameters within the study range were given as follows;

Sqrt (TBC) = 0.715-0.002·A+0.019·B+0.257·C+0.866·D+0.00001·A·B+0.000002·A^2-0.0001·B^2

Sqrt (TPC) = 28.056-0.00052·A+0.185·B-402.402·C+21.511·D-0.00099·B^2+1214.42·C^2

Sqrt (AA) = 5.961-0.0003·A-0.002·B-32.346·C+23.918·D

Sqrt (DPPH) = 13.397-0.002·A-0.016·B-53.823·C+9.877·D-0.00002·A·B+0.021·A·C+0.142·B·C+ 49.202·C·D-22.95·D^2

The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds with the desirability of 0.88 was microwave power (800 W), operation time (81.815 s), solvent ratio (0.1 w/v), and acid (0.5 % w/v). Under this processing condition, the outcomes are TBC (5.79 mg/g DM), TPC (356.56 mg GAE/g DM), AA by FRAP method (205.06 mg ASE/g DM), and DPPH method (67 %), respectively (Figure 10). Figure 11 represents the predicted values and actual values which can be correlated by the coded and actual equations built by the model.

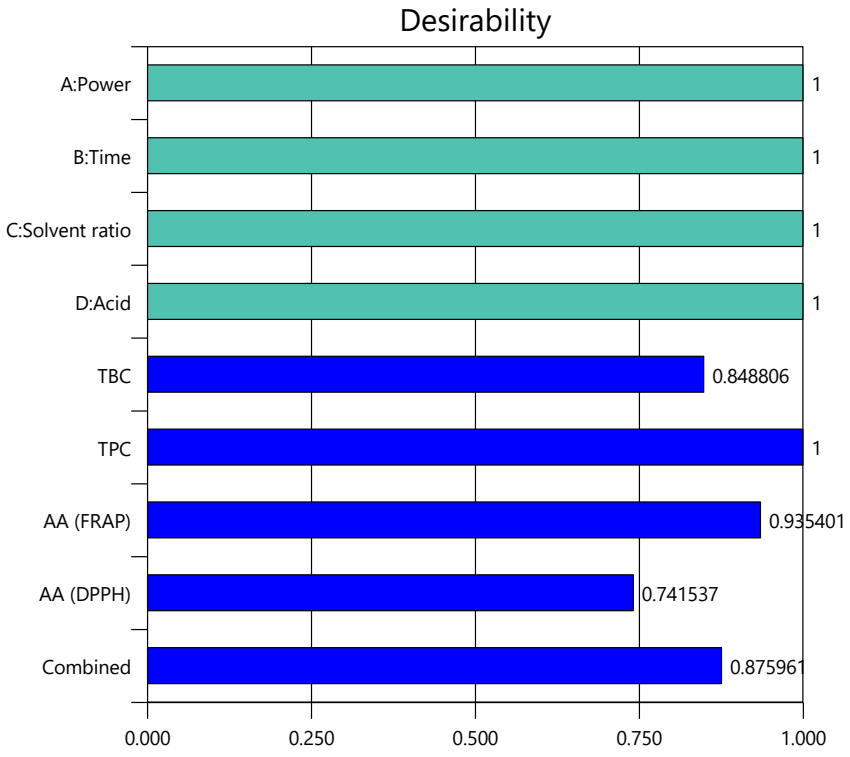


Figure 10. The desirability test based on the optimized values of targeted compounds in beetroot peel-AW extracts

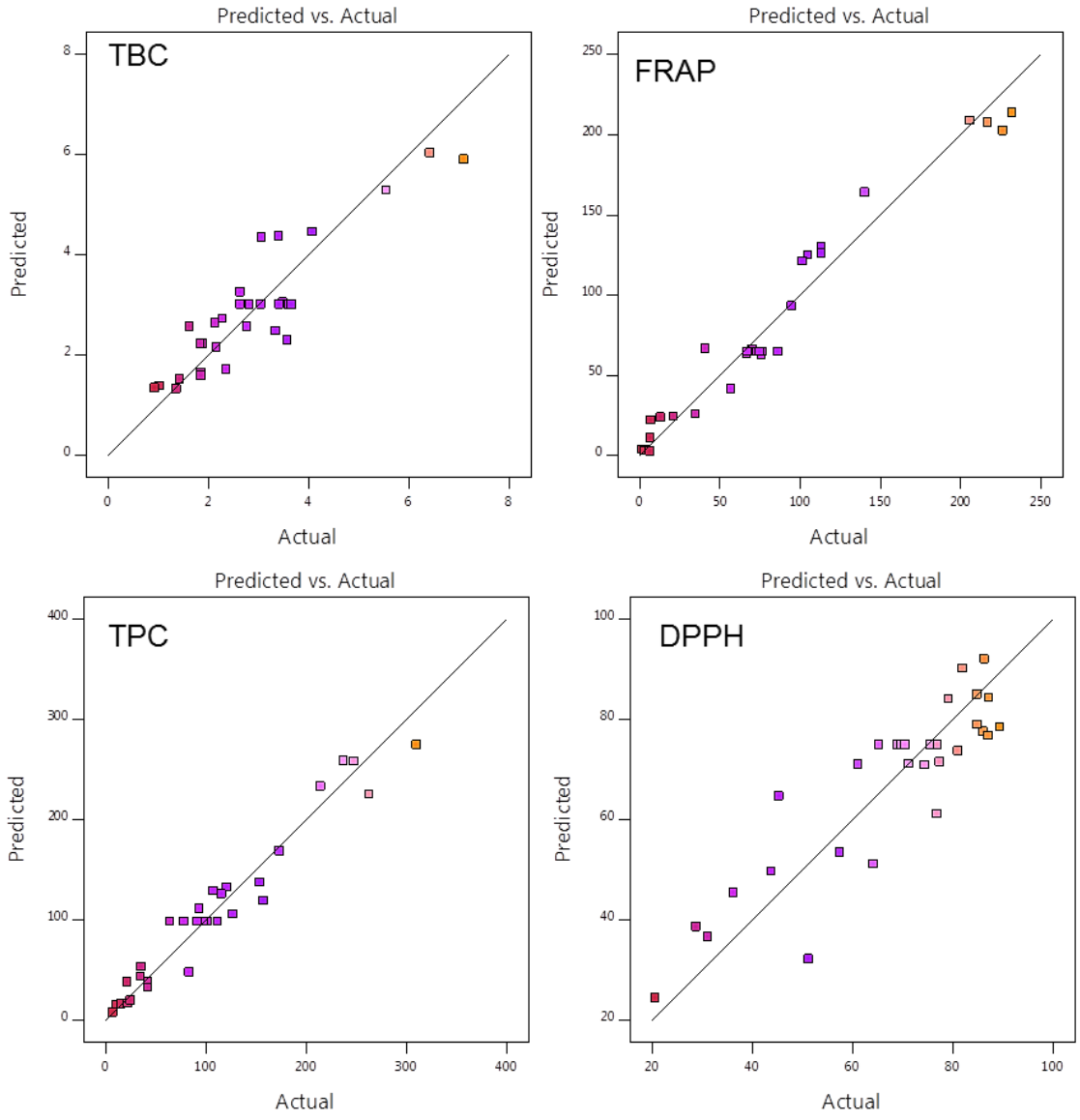


Figure 11. Correlation between the predicted values and actual values calculated by the model according to the regression equations (beetroot peel-AW extracts)

4.1.3 RSM of beetroot peel-EW extracts

The recovery of total betalain colour compounds, total phenolic compounds, and their respective antioxidant activities from the beetroot peel was performed with EW solvent via MAE. According to the variation of independent parameters, the changes in experimental outcomes were investigated which is shown in Table 8 with twenty runs in total. Amongst, TBC (11.29±0.13 mg/g DM), TPC (10.74±0.59 mg GAE/g DM), and AA (17.03±0.59 mg ASE/g DM) by FRAP were the greatest amounts recovered in run 11. AA measured by DPPH was observed to be maximum in run 5 which is 60 %. According to the analysis of the variance of each factor as depicted in Table 9, the

high F value and low p-values pointed out the significant level of the model. Lack of fit is not significant in all cases. The models of TPC, FRAP, and DPPH were fitted by the quadratic function whereas TBC was fitted by the linear model function and the results were depicted by 3D graphs (Appendix-Figure 3).

Table 8. Experimental outcomes of extracted compounds beetroot (Cylindra) peel by EW solvent

Run	A:Power (W)	B:Time (s)	C:Solvent ratio (w/v)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)	AA (%)
1	450	90	0.15	7.99	9.40	10.04	42
2	800	30	0.2	7.06	8.35	7.28	42
3	450	150	0.15	10.00	10.64	13.97	37
4	450	90	0.15	8.19	9.20	9.67	47
5	100	150	0.1	6.34	6.29	6.48	60
6	450	90	0.15	7.34	8.51	8.85	46
7	800	150	0.1	9.13	9.74	12.86	53
8	100	150	0.2	7.38	8.04	7.12	52
9	800	30	0.1	4.48	9.17	4.27	27
10	450	30	0.15	4.53	7.28	4.38	37
11	800	90	0.15	11.29	10.74	17.03	42
12	100	30	0.2	3.69	7.36	3.31	59
13	450	90	0.15	6.76	9.37	10.07	48
14	450	90	0.15	6.99	9.69	9.14	44
15	450	90	0.1	5.32	8.24	6.54	29
16	450	90	0.15	6.91	9.48	8.55	43
17	100	30	0.1	2.66	5.11	2.75	18
18	100	90	0.15	3.57	8.57	4.06	54
19	800	150	0.2	10.37	10.19	16.16	31
20	450	90	0.2	5.79	8.79	7.12	44

The followings are the actual regression equations for the estimation of respective compounds at any given parameters within the study range;

$$\text{Sqrt (TBC)} = 1.064+0.0011\cdot A+0.007\cdot B+2.658\cdot C$$

$$\text{Sqrt (TPC)} = 0.058+0.0016\cdot A+0.0022\cdot B+28.937\cdot C-0.006\cdot A\cdot C-82.005\cdot C^2$$

$$\text{Sqrt (AA)} = -2.733+0.002\cdot A+0.01\cdot B+51.613\cdot C-162.325\cdot C^2$$

$$\text{Sqrt (DPPH)} =-0.356+0.003\cdot A+0.05\cdot B+44.329\cdot C-0.024\cdot A\cdot C-0.29\cdot B\cdot C$$

Table 9. ANOVA for beetroot peel-EW extract

Source	Estimated coefficient				F Value			
	TBC	**TPC**	**FRAP**	**DPPH**	**TBC**	**TPC**	**FRAP**	**DPPH**
Intercept	2.57	3.04	3.03	6.49	22.5***	22.22***	30.39***	11.78***
A	0.3702	0.2261	0.5793	-0.3396	28.05***	53.96***	49.37***	5.78*
B	0.4184	0.1328	0.6222	0.4194	35.83***	18.62***	56.95***	8.82*
C	0.1329	0.0804	0.1458	0.3652	3.61*	6.83*	3.13*	6.69*
AC	-	-0.1061	-	-0.4246	-	9.5**	-	7.23*
BC	-	-	-	-0.8702	-	-	-	30.37***
C^2	-	-0.205	-0.4058	-	-	22.19**	12.11**	-
Residual (SS)	0.78	0.13	1.02	2.79				
Lack of Fit (SS)	0.72	0.11	0.97	2.67				
Multiple R^2	0.81	0.89	0.89	0.81				
Adjusted R^2	0.77	0.85	0.86	0.74				
Predicted R^2	0.70	0.76	0.78	0.50				
Std. Dev.	0.22	0.10	0.26	0.45				
Mean	2.57	2.94	2.83	6.49				
C.V. %	8.61	3.31	9.21	6.89				

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05. Variables: A=Power, B=Time, C=Solvent ratio

The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds with the desirability of 0.93 was microwave power (800 W), operation time (150 s), and solvent ratio (0.12 w/v) (Figure 12). Under this processing condition, the outcomes are TBC (10.78 mg/g DM), TPC (11.25 mg GAE/g DM), and AA by FRAP method (16.27 mg ASE/g DM) and DPPH method (50 %), respectively. The correlations between the predicted values and actual were depicted in Figure 13.

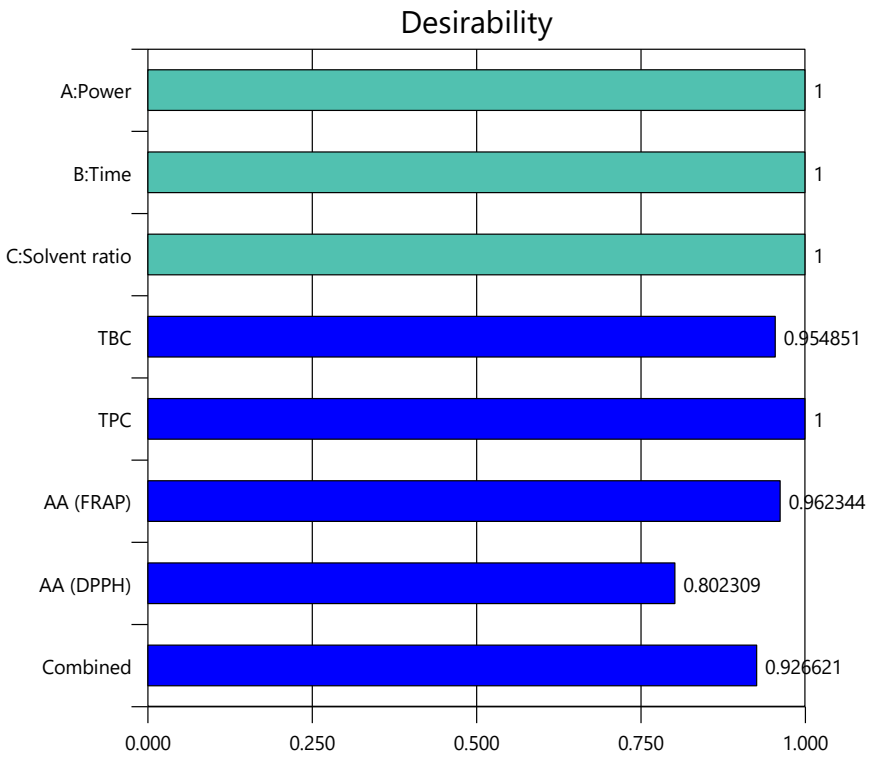


Figure 12. The desirability test based on the optimized values of targeted compounds in beetroot peel-EW extracts

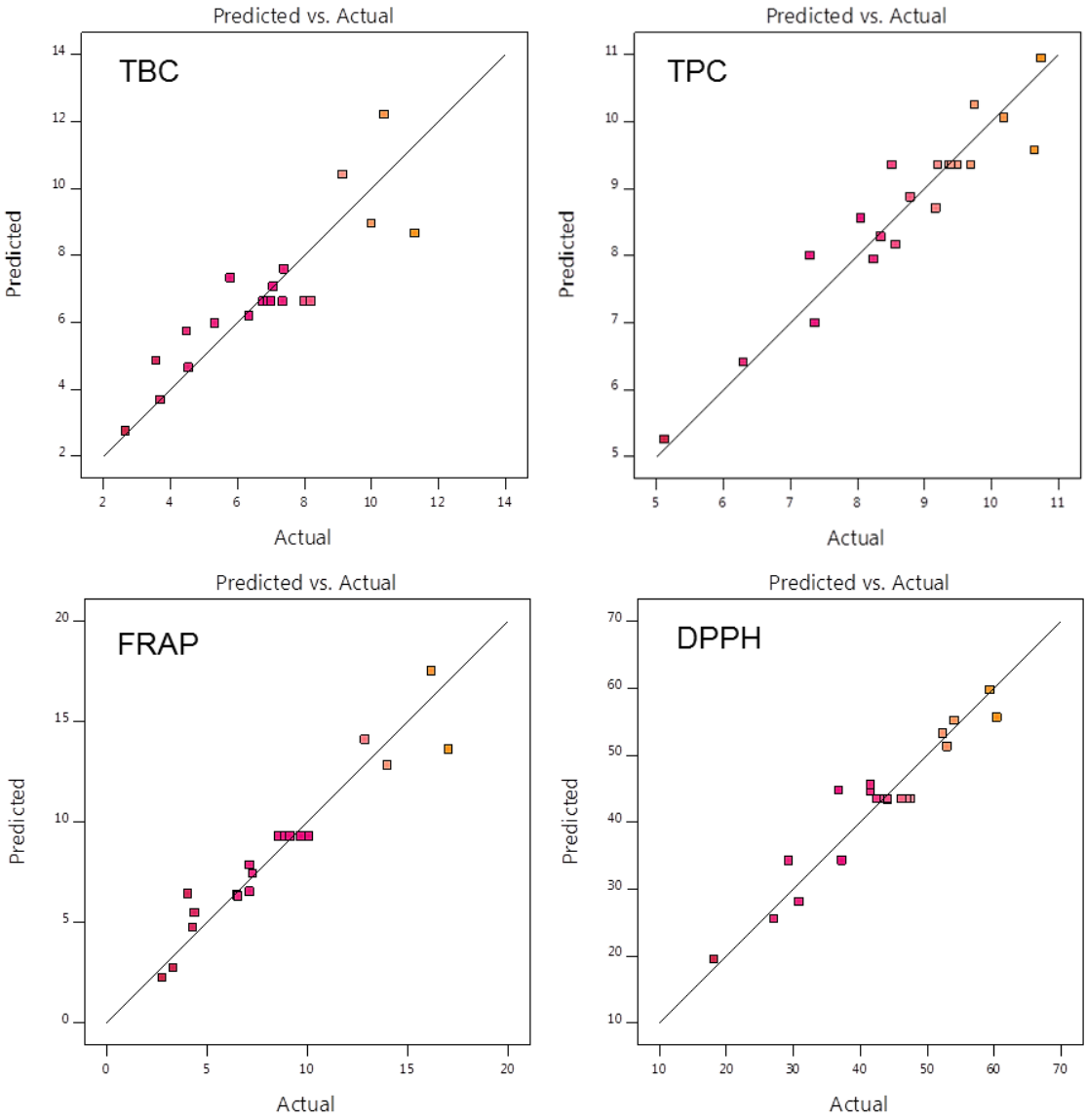


Figure 13. Correlation between the predicted values and actual values calculated by the model according to the regression equations (beetroot peel-EW extracts)

4.1.4 RSM of beetroot peel-AEW extracts

The scavenged amounts of betalains, polyphenols, and their respective antioxidant activities from the beetroot peel with AEW solvent via MAE were presented in Table 10. The variation in the responses due to the process variables was investigated with thirty experimental runs in total. Among them, the maximum amounts of TBC (12.72±0.3 mg/g DM), TPC (375.2±0.59 mg GAE/g DM), AA (266.65±1.8 mg ASE/g DM) by FRAP, and AA (88 %) were obtained in runs 1, 9 and 24, respectively. The high F values and low p-values (<0.0001) proved that the models are significant

for all responses. Along the line, lack of fit is not significant in all cases. The reduced models were fitted accordingly by the linear function for TBC and the 2FI function for TPC and AA (Table 11).

Table 10. Experimental outcomes of extracted compounds beetroot (Cylindra) peel by AEW solvent

Run	A:Power (W)	B:Time (s)	C:Solvent ratio (w/v)	D:Acid (% w/v)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)	AA (%)
1	800	150	0.1	0.1	12.72	54.76	39.18	45
2	800	150	0.2	0.1	11.81	46.15	16.99	8
3	100	150	0.2	0.1	7.05	34.57	7.68	63
4	450	90	0.15	0.1	8.19	50.20	9.84	52
5	450	90	0.15	0.3	8.37	162.90	99.24	41
6	800	150	0.2	0.5	10.65	147.55	140.58	11
7	100	30	0.1	0.1	4.53	51.37	29.02	77
8	800	30	0.2	0.1	4.80	38.01	7.07	44
9	100	150	0.1	0.5	5.48	375.02	260.12	71
10	450	90	0.15	0.3	7.85	139.25	60.38	49
11	450	30	0.15	0.3	4.07	160.72	91.83	63
12	800	150	0.1	0.5	9.55	289.60	233.45	62
13	800	30	0.1	0.1	5.21	68.42	41.12	74
14	450	90	0.15	0.5	7.16	235.78	193.85	56
15	450	90	0.15	0.3	7.76	138.52	104.81	53
16	100	90	0.15	0.3	5.03	120.53	93.36	69
17	800	30	0.2	0.5	5.92	139.93	123.89	56
18	450	90	0.15	0.3	7.26	120.53	92.87	59
19	450	90	0.1	0.3	4.92	180.79	146.93	79
20	800	90	0.15	0.3	9.36	121.94	93.60	50
21	450	90	0.2	0.3	6.42	89.20	63.79	54
22	450	90	0.15	0.3	6.98	110.46	88.36	60
23	100	30	0.2	0.5	2.69	139.09	119.27	77
24	100	30	0.1	0.5	2.29	294.40	266.65	88
25	450	90	0.15	0.3	6.20	104.44	89.25	68
26	100	30	0.2	0.1	2.61	20.32	2.50	40
27	800	30	0.1	0.5	3.88	291.58	265.20	84
28	100	150	0.2	0.5	3.70	149.18	117.24	72
29	100	150	0.1	0.1	3.52	54.19	10.64	81
30	450	150	0.15	0.3	6.70	121.75	77.03	61

Table 11. Analysis of variance for beetroot peel-AEW extract

Source	Estimated coefficient				F Value			
	TBC	**TPC**	**FRAP**	**DPPH**	**TBC**	**TPC**	**FRAP**	**DPPH**
Intercept	2.48	11.04	9.08	7.55	17.34***	109.44***	124.91***	19.89***
A	0.4086	0.0731	0.3782	-0.8703	35.39***	0.1397	3.9	30.92***
B	0.3863	0.1426	-0.0559	-0.5956	31.63***	0.5312	0.0853	14.48***
C	0.0392	-1.86	-1.87	-0.9849	0.3249	90.43***	95.05***	39.6***
D	-0.0979	4.07	4.84	0.3562	2.03	433.5***	638.28***	5.18*
AB	-	-	-	-0.7404	-	-	-	19.89***
AC	-	-	-	-0.5049	-	-	-	9.25**
BC	-	-	0.423	-	-	-	4.34*	-
CD	-	-0.9862	-0.5687	-	-	22.59***	7.84*	-
Residual (SS)	2.12	16.54	15.18	10.14				
Lack of Fit (SS)	2.02	12.04	11.45	8.13				
Multiple R^2	0.74	0.96	0.97	0.84				
Adjusted R^2	0.69	0.95	0.96	0.80				
Predicted R^2	0.59	0.93	0.95	0.67				
Std. Dev.	0.29	0.83	0.81	0.66				
Mean	2.48	11.04	9.08	7.55				
C.V. %	11.75	7.52	8.94	8.80				

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05. Variables: A=Power, B=Time, C=Solvent ratio, D=Acid %

The actual regression equations for the estimation of specific compounds at any given parameters under the investigation were given as;

Sqrt (TBC) = 1.405+0.0012·A+0.0064·B+0.783·C-0.489·D

Sqrt (TPC) = 5.767+0.00021·A+0.0024·B-7.624·C+35.161·D-98.621·C·D

Sqrt (FRAP) = 6.37+0.001·A-0.022·B-32.97·C+32.722·D+0.141·B·C-56.872·C·D

Sqrt (DPPH) = 8.603+0.005·A+0.006·B-6.715·C+1.781·D-0.00004·A·B-0.029·A·C

The interaction between the process variables and the experimental results of the betalains, phenolics, and antioxidant activities were presented in 3D surface graphs (Appendix-Figure 4). The operating condition optimized predominantly by the model depending on the maximum yields of targeted compounds with the desirability of 0.837 was microwave power (799.85 W), operation time (126.92 s), solvent ratio (0.1 w/v), and acid (0.5 % w/v) (Figure 14). Under this processing condition, the outcomes are TBC (8.94 mg/g DM), TPC (328.43 mg GAE/g DM), AA by FRAP method (270.33 mg ASE/g DM), and DPPH (59 %), respectively. The predicted values and actual

values which can be correlated by the coded and actual equations built by the model were demonstrated in Figure 15.

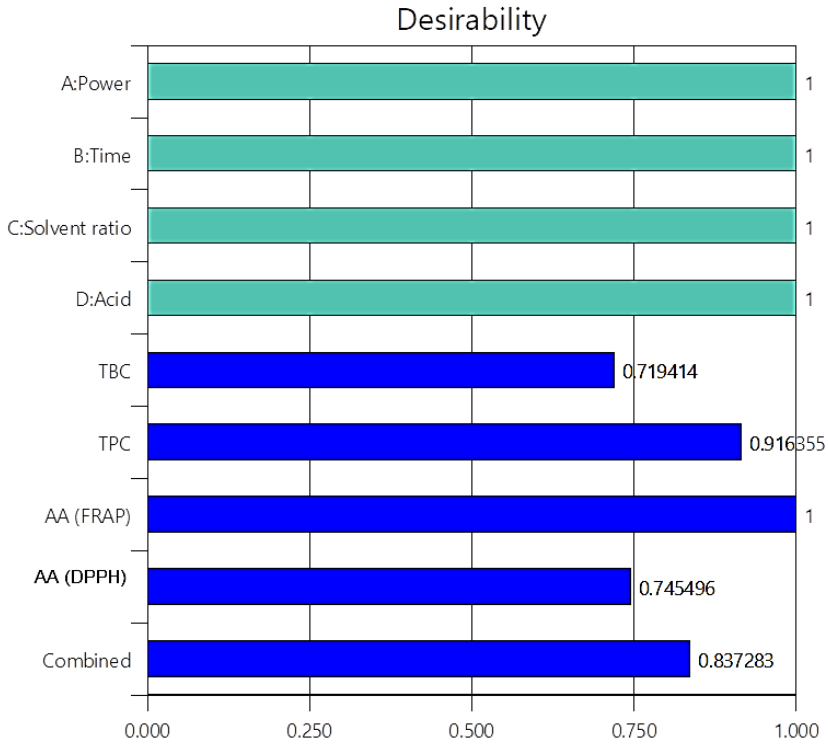


Figure 14. Desirability test based on the optimized values of targeted compounds in beetroot peel-AEW extracts

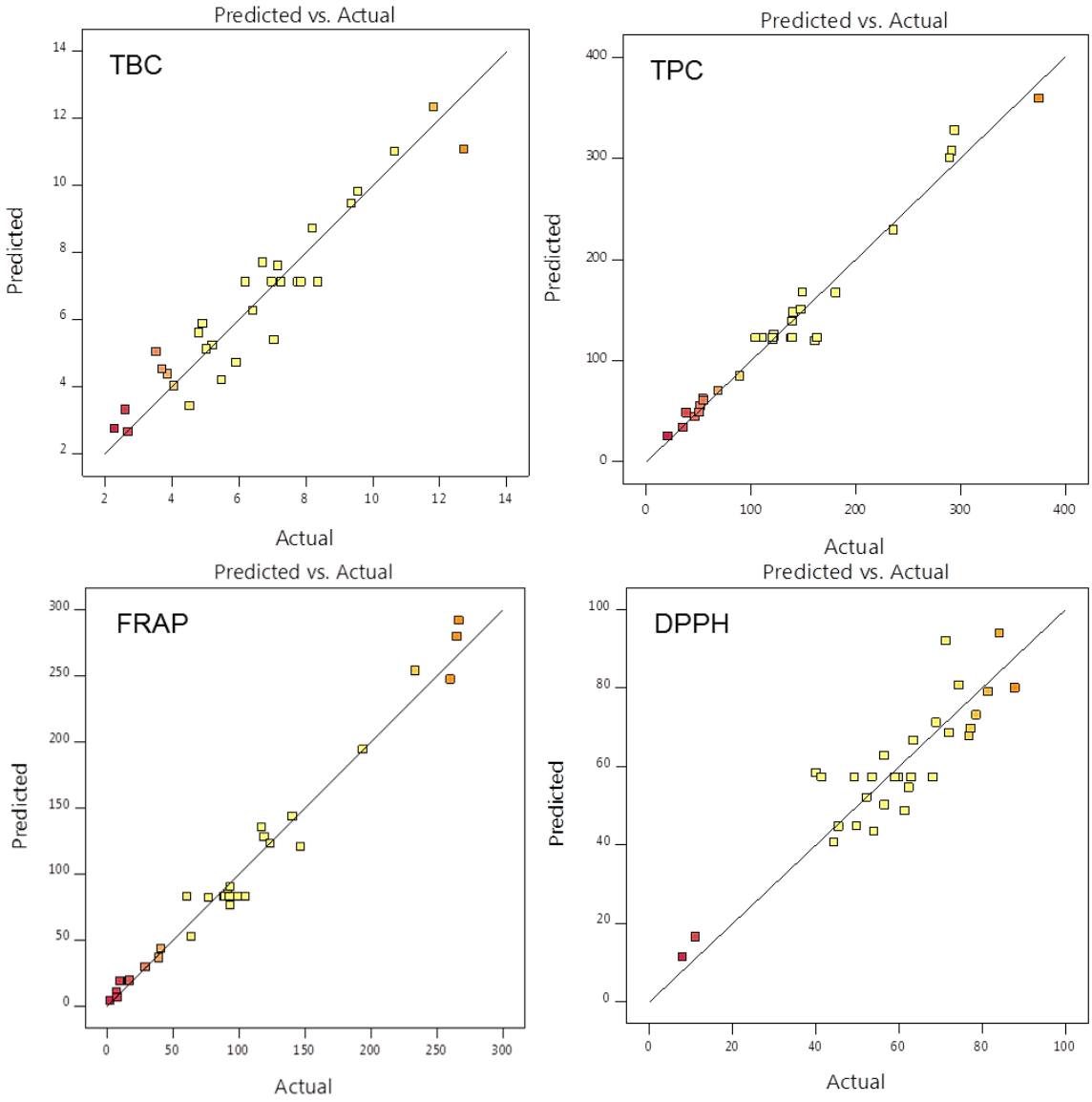


Figure 15. Correlation between the predicted values and actual values calculated by the model according to the regression equations (beetroot peel-AEW extracts)

4.1.5 Optimization of the extraction efficiency of different types of solvents

In terms of the maximum recovery amounts of targeted compounds which are the experimental values, the operation parameters are optimized as summarized in Table 12. As can be seen in the table, the same parameters can be set up to adjust the optimization level of the targeted compounds. From an overall point of view, TBC (12.55±0.11 mg/g DM) was scavenged upmost in the extract of AEW solvent when the processing variables are set up at 800 W, 150 s, 0.1 w/v solvent ratio, and 0.1 % of acid content. Meanwhile, TPC (375.2±0.59 mg GAE/g DM) was

scavenged in AEW extract with the following variables; microwave power (100 W), irradiation time (150 s), 0.1 w/v solvent ratio, and 0.5 % of acid content. Similarly, AA (266.65±1.8 mg ASE/g DM) was maximum under the same process condition except for acid content which is 0.1 % whereas 94 % of AA was optimized at 800 W, 150 s, and 0.2 w/v peel-to-solvent ratio of PW.

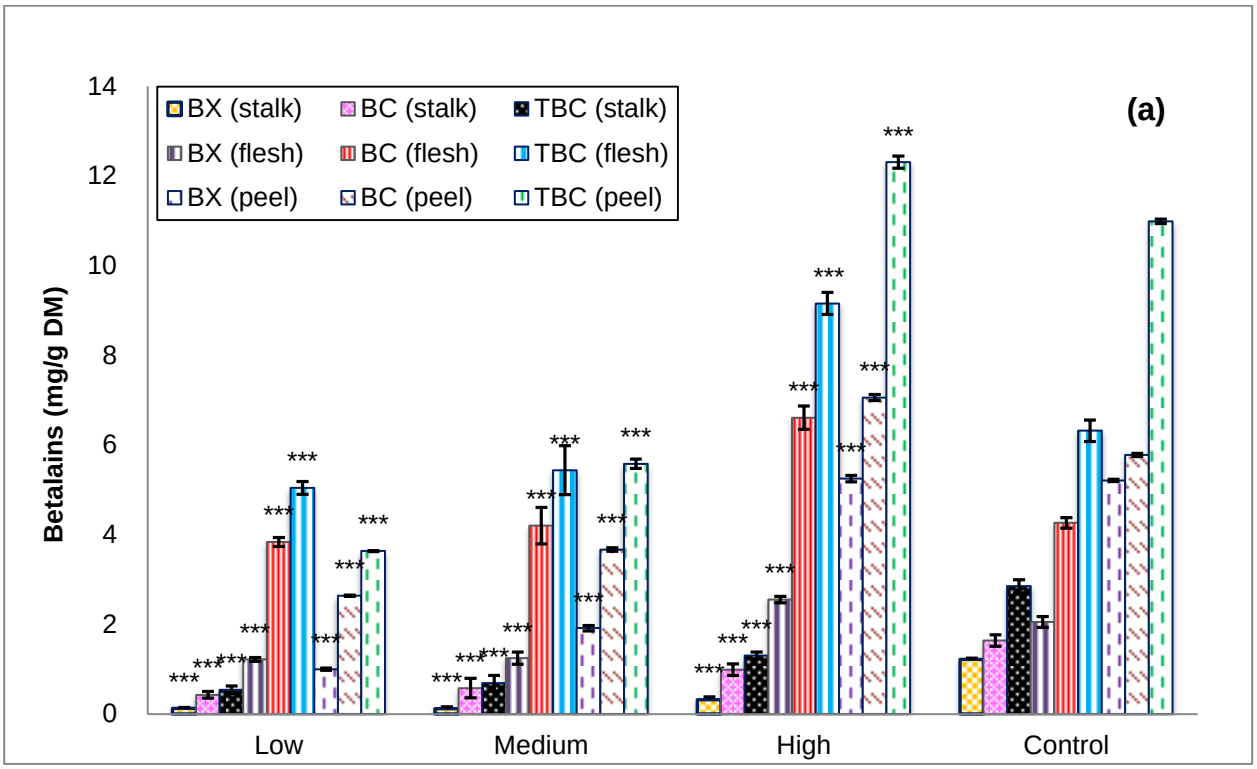
Overall, betalains were not affected by the variation in the solvent property per se whilst the phenolics seemed to be strongly influenced by the acidification process exhibiting significant improvement in amounts. This might be due to their localization area in the plant cell structure. Since phenolic compounds are existing in the plant cell wall surrounded by the protein that can be denatured by acid destroying its hydrogen bond followed by the ease of trapped compounds' leakage. Zhao et al. (2018) and Flórez et al. (2015) claimed that power output applied for microwave heating influents on phenolic compounds withal the advantage of short irradiation time due to accelerated diffusion of the solvent into the plant matrix to leach out of the compounds. Additionally, the solvent property can be turnable by adjusting its polarity due to the absorptivity of microwave radiation can be intensified by doing so (Figura & Teixeira, 2007). Synergistic effects of processing time and temperature or microwave power, solvent concentration and matrix-to-solvent ratio on mass transfer rate differ for specific plant secondary compounds thus process condition adjustment should be focused on targeted compounds to contribute the radiations uniformly. Intimation time between the plant matrix and microwave radiation has high significant implications in some cases (Hayat et al., 2009; Li et al., 2012).

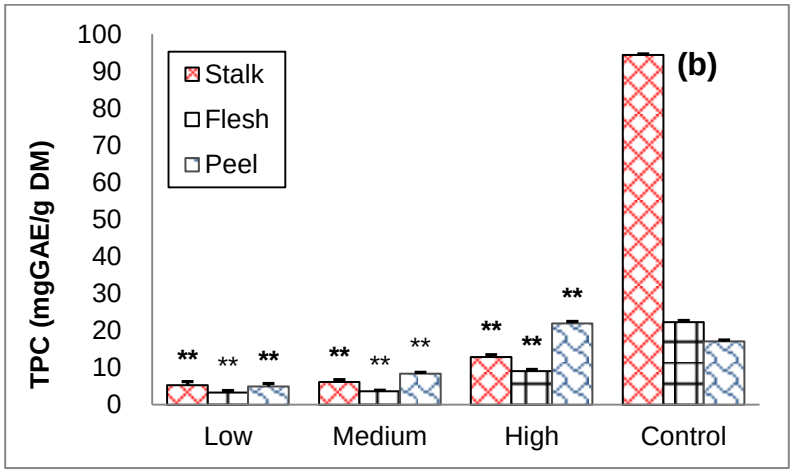
Table 12. Summary for the maximum recovery of betalains, polyphenols, and antioxidants via different solvents

Sample	Solvent	Power (W)	Time (s)	Solvent ratio (w/v)	Acid (%)	TBC (mg/g DM)	TPC (mg GAE/g DM)	FRAP (mg ASE/g DM)	DPPH (%)
A	PW	800	150	0.2	-	12.31±0.14	21.94±0.54	35.68±0.77	94
B	AW	100	30	0.1	0.5	7.09±0.19	309.67±0.0	231.72±4.65	
		450	30	0.15	0.3	-	-	-	89
C	EW	800	90	0.15	-	11.29±0.13	10.74±0.59	17.03±0.59	
		100	150	0.1		-	-	-	60
D	AEW	800	150	0.1	0.1	12.72±0.3	-	-	
		100	150	0.1	0.5	-	375.2±0.59	-	
		100	30	0.1	0.5	-	-	266.65±1.8	88

4.1.6 Comparison of stalk, flesh, and peel-PW extracts

For the comparison of the contents of the bioactive compound in the different parts of the beetroot, 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, medium level, and high level as explained in the materials and method section (3.1.1). The control sample extracts were prepared conventionally from the stalk, flesh, and peel of the beetroots with pure water for one hour at 70 ºC. Among the different processing conditions, the maximum processing level brought the highest amount of the betalains which was observed to be superior to the control except for stalk which is not obvious in the differences. This proved the fact that enough microwave exposure to plant matrix may guarantee the extraction efficacy to bring out the targeted bioactive compounds. Accordingly, the microwave absorptivity of the solvent is theoretically determined by its dielectric constant and polarity; of which the greater dielectric loss, the better the absorption of microwave (Seoane et al., 2017; Zin et al., 2020a). In MAE, the temperature can strongly affect the solubility of the matrix and the solvent by upgrading their intimation. Apart from that, the larger surface area of the matrix is preferable for better absorption of microwave to leach out trapped compounds (Destandau et al., 2013). Since polar intermolecular reaction occurs in the medium of solvent, the transformation of microwave exposure of reactants depends upon the absorptivity of the solvent whether it is protic or aprotic. Under the high level of MAE processing, betalain content is the topmost in the peel; 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=3.93±0.16 mg/g DM, BX=1.52±0.04 mg/g DM, TBC=5.44±0.15 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 total betalains amounts in the controls are as follows; 10.99±0.05 mg/g DM (peel), 3.76±0.14 mg/g DM (flesh), and 2.85±0.15 mg/g DM (stalk), respectively (Figure 16). This finding is in accordance with Slatnar and co-workers (2015) who reported the compositions of bioactive compounds in different parts of beetroot.


(a)
BX (stalk)
BC (stalk)
TBC (stalk)
BX (flesh)
BC (flesh)
TBC (flesh)
BX (peel)
BC (peel)
TBC (peel)
Betalains (mg/g DM)
0
2
4
6
8
10
12
14
Low
Medium
High
Control


(b)
Stalk
Flesh
Peel
TPC (mgGAE/g DM)
0
10
20
30
40
50
60
70
80
90
100
Low
Medium
High
Control

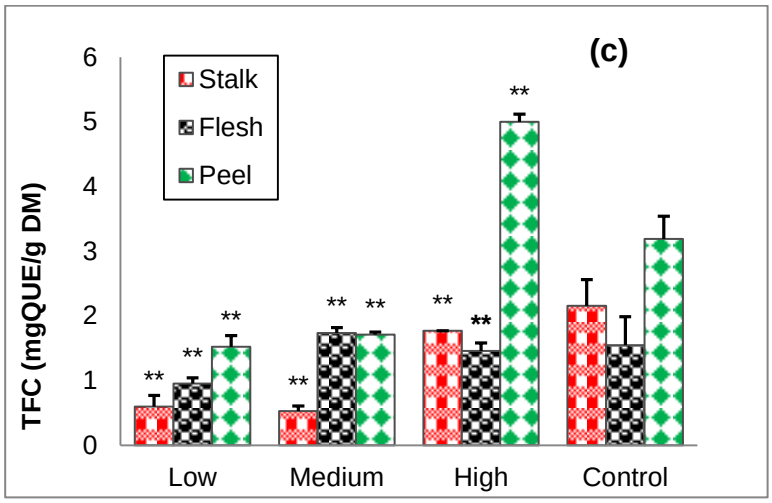


Figure 16. Comparison of the extractable amounts of (a) betalain compounds; (b) total phenolic compounds content (TPC); and (c) total flavonoids content (TFC) with pure water from the different parts of beetroot (peel, flesh and stalk) under three-level process conditions of MAE ("" denotes p-value less than 0.01; "***" denotes p-value less than 0.001)**

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) (Figure 16). 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. Furthermore, antiradical scavenging activities of the stalk, flesh and peel-water extracts were investigated by FRAP, DPPH, and ABTS methods and the results were demonstrated in Figure 17. Herein, the extracts exhibited the topmost level of antioxidant activities which are under high-level process condition of MAE in all cases and were also quite comparable to the control sample extracts. Amongst, 35.68±0.77 mg ASE/g DM (FRAP) is the maximum radical scavenging activity discovered in the peel-water extracts which amount was 1.3 times exceeded the control, and 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. Similarly, ABTS performance 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 %).

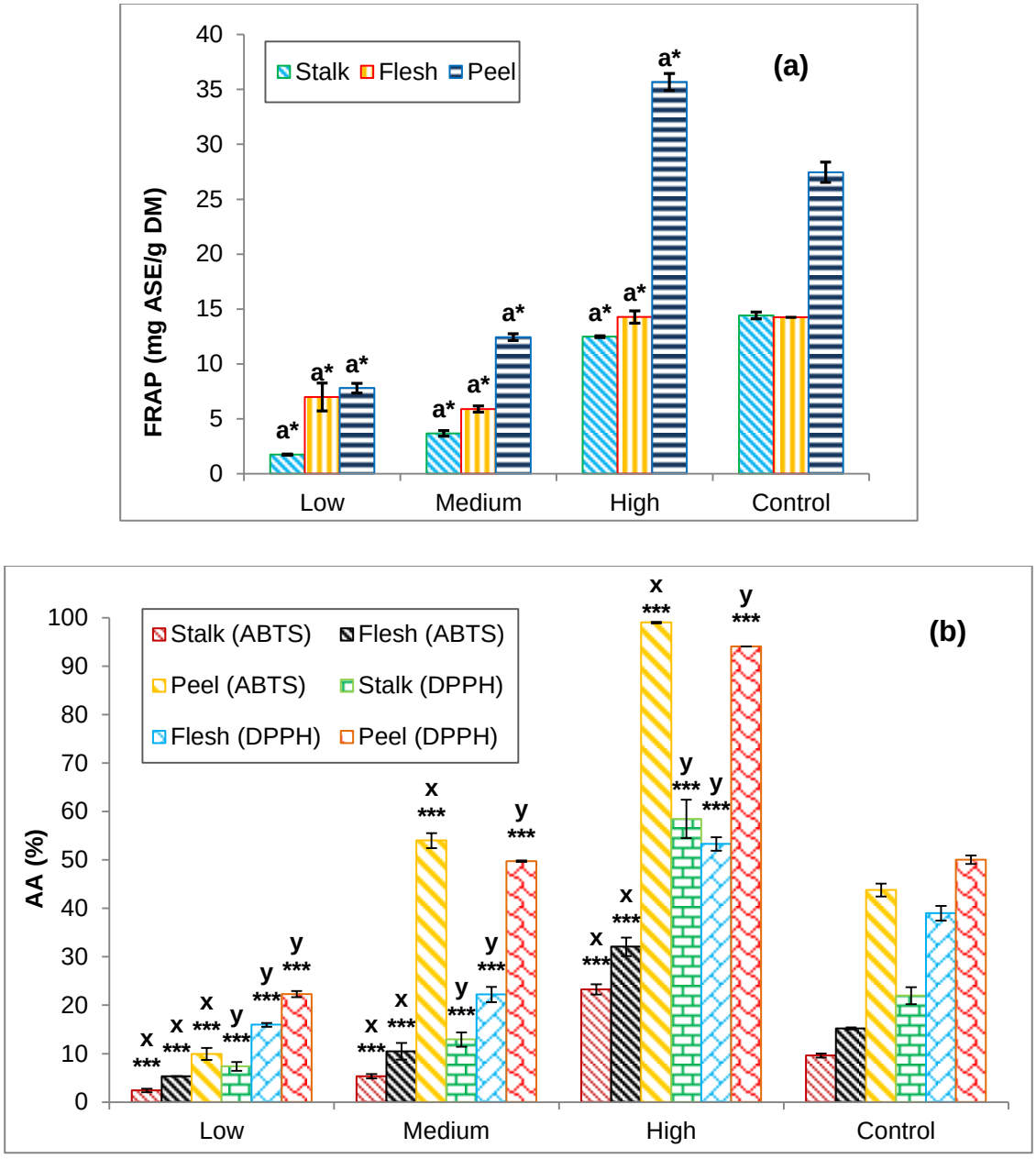


Figure 17. Comparison between the antioxidant activity assays of beetroot peel, stalk, and flesh extracts (same letters represent there are no significant differences; "*" represents p-value<0.05; "" represents p-value<0.01; "***" represents p-value<0.001)**

The results of the colour pattern (L^*, a^*, b^*) measurement of the microwave extracts were represented in Table 13 against the controls. Generally, the trends of the lightness (L^*) values, b^* and Hue angle declined with the elevated level of processing conditions while the trends of a^*, Chroma (C^*_{ab}), the total colour difference (ΔE^*_{ab}), and browning index (BI) behaved contrastingly. The lower L^* value expresses the deeper colour which is the lowest in the control samples of all kinds of extracts although the total colour difference (ΔE^*_{ab}) was found to be maximum in there. Higher chroma values are said to be clearer or brighter in colour which is observed to be superior in the microwave extracts as to the controls. Likewise, hue angle determines the ratio between the yellowness and the redness which is the maximum in the microwave extracts. Among the stalk, flesh and peel extracts, the values of yellowness or blueness (a^*), redness or greenness (b^*), and Chroma or saturation were found to be utmost in the peel extract.

Table 13. Colour pattern (L^*, a^*, b^*) measurements of beetroot (stalk, flesh, and peel) extracts via microwave

Materials	Run	L^*	a^*	b^*	C^*_{ab}	Hº	ΔE^*_{ab}	BI
Stalk	1	$77.37\pm0.05^{\alpha}$	$2.51\pm0.09^{\alpha}$	$4.98\pm0.04^{\alpha}$	$5.58\pm0.07^{\alpha}$	$63.21\pm0.61^{\alpha}$	$6.47\pm0.05^{\alpha}$	$75.33\pm0.49^{\alpha}$
	2	$59.89\pm0.11^{\alpha}$	$22.73\pm0.19^{\alpha}$	$1.62\pm0.01^{\alpha}$	$22.79\pm0.19^{\alpha}$	$4.07\pm0.04^{\alpha}$	$31.02\pm0.08^{\alpha}$	$87.22\pm0.12^{\alpha}$
	3	$59.73\pm0.28^{\alpha}$	$29.04\pm0.13^{\alpha}$	$1.57\pm0.04^{\alpha}$	$29.08\pm0.13^{\alpha}$	$3.1\pm0.08^{\alpha}$	$35.94\pm0.12^{\alpha}$	$107.52\pm0.66^{\alpha}$
	Control	18.95±0.05	18.54±0.01	21.8±0.27	28.62±0.2	49.62±0.35	82.9±0.1	73.34±0.31
Flesh	1	$42.78\pm0.27^{\beta}$	$47.15\pm0.11^{\beta}$	$0.57\pm0.04^{\beta}$	$47.16\pm0.11^{\beta}$	$0.69\pm0.04^{\beta}$	$60.64\pm0.1^{\beta}$	$2.96\pm0.08^{\beta}$
	2	$33.39\pm0.36^{\beta}$	$54.78\pm0.31^{\beta}$	$15.28\pm0.13^{\beta}$	$56.88\pm0.27^{\beta}$	$15.59\pm0.2^{\beta}$	$74.07\pm0.03^{\beta}$	$25.86\pm0.17^{\beta}$
	3	$27.91\pm0.07^{\beta}$	$48.25\pm0.19^{\beta}$	$21.57\pm0.16^{\beta}$	$52.85\pm0.24^{\beta}$	$24.09\pm0.08^{\beta}$	$74.83\pm0.17^{\beta}$	$32.47\pm0.1^{\beta}$
	Control	10.56±0.13	34±0.19	14.5±0.11	36.96±0.22	23.1±0.05	94±0.14	163.6±1.44
Peel	1	$45.48\pm0.06^{\theta}$	$56.36\pm0.41^{\theta}$	$9.13\pm0.16^{\theta}$	$57.09\pm0.43^{\theta}$	$9.2\pm0.1^{\theta}$	$67.09\pm0.37^{\theta}$	$66.46\pm0.23^{\theta}$
	2	$40.45\pm0.12^{\theta}$	$58.09\pm0.23^{\theta}$	$17.27\pm0.03^{\theta}$	$60.61\pm0.21^{\theta}$	$16.56\pm0.09^{\theta}$	$72.76\pm0.12^{\theta}$	$96.7\pm0.47^{\theta}$
	3	$27.13\pm0.15^{\theta}$	$48.75\pm0.57^{\theta}$	$22.01\pm0.34^{\theta}$	$53.49\pm0.41^{\theta}$	$24.3\pm0.55^{\theta}$	$75.83\pm0.25^{\theta}$	$104.21\pm0.39^{\theta}$
	Control	9.45±0.19	3.71±0.12	1.3±0.03	3.93±0.12	19.36±0.36	87.96±0.2	27.87±1.37

Superscript same letters represent no significant differences (α = p-value<0.05; β , θ = p-value<0.0001)

4.1.7 The efficiency of different types of solvents on bioactive compounds extraction

In MAE, solvent mixtures are recommended to facilitate the solvation of the solute by increasing the polarity of the solvent along with balancing dielectric losses for better absorption of the microwave by the treating object (Zin et al., 2020a). Table 14 defines the comparison of the bioactive compounds extractability of the pure water, acidified-water, ethanol-water, and acidified ethanol-water solvents from the flesh and peel parts of the Cylindra type beetroot. Generally, the recovery of bioactive compounds with different types of solvents was found to be most effective under the extreme processing conditions of MAE (800 W for 150 s). Among the peel-solvent extracts, the maximum amount 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 observed in the peel-water extract (sample A). Whilst, TPC (156.11±11.9 mg GAE/g DM) and antioxidant activity (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 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 samples B; BX (3.09±0.29 mg/g DM), TPC (232.14±3.32 mg GAE/g DM), and DPPH (86 %) in sample D, individually.

Since water has a high dielectric constant, although dissipation energy is lower than the other solvents such as alcoholic group and acetone, it can strongly absorb microwave energy and lead to the superheating effect (Proestos & Komaitis, 2008). Such occurrence can lead to the loss of the solvent by evaporation as well as solution spill over the open vessel treatment followed by an imbalance in the solvent and solid ratio. In our study, such a situation occurred after 150 s of treatment at 800 W when the pretests were performed. In the literature, ethanol solvent (99.9 %) proved to be efficient for extracting betalains from beetroot peels with MAE (Singh et al., 2017). Conversely, pure water could produce more amount of compounds than 15 % aqueous ethanol in our case which might be due to the changes in polarity of the solvents as high polarity solvents are preferable in MAE (Zin et al., 2020a). Aside from that, the presence of a suitable amount of water in the solvent can improve the swelling of the plant matrix increasing the contact surface area of the matrix with the solvent therein (Hayat et al., 2009). Besides, the reduction in viscosity of the solvent and so its surface tension could have helped with the desorption of the active compounds by breaking through the swelling membrane which means the higher the amount of water, the better the

solvent property (Pinela et al., 2016). Additionally, elevated temperature can assist to decrease the viscosity and surface tension of the solvent and facilitate the leaching process. Herein, the solid to solvent ratio has to be in appropriate condition to avoid the solid packing or else the swelling of the matrix can halt the proper stirring or mixing and so do the transfer of the compounds from the host to the solvent. In this study, high power (800 W) with a long exposing time (150 s) was preferable for the maximum output of the specific compounds. This is because the higher power ensures enough contact of the microwave with the matrix to expedite the bringing out of the compounds trapped in the cell wall (Hayat et al., 2009). So, high temperature or power and short irradiation time are always beneficial combinations for the MAE of plant materials (Jokíc et al., 2012).

Table 14. Comparison of the bioactive compounds extractability of the PW, AW, EW, and AEW solvents

Specific compounds	Run	PW		AW		EW		AEW	
		Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel
BX (mg/g DM)	Low	0.72±0.03	1±0.02	0.98±0.11	0.46±0.01	0.52±0.18	0.91±0.03	0.94±0.05	1.31±0.03
	Medium	0.74±0.08	1.15±0.04	2.25±0.08	0.82±0.05	1.05±0.01	2.61±0.05	0.85±0.05	2.95±0.1
	High	1.52±0.04	5.25±0.07	2.56±0.06	2.12±0.04	1.71±0.03	4.79±0.01	3.09±0.29	3.96±0.05
BC (mg/g DM)	Low	2.28±0.06	2.64±0.0	2.65±0.12	1.34±0.02	1.43±0.41	1.77±0.05	1.96±0.11	3.22±0.04
	Medium	2.5±0.24	2.64±0.01	5.52±0.22	1.81±0.1	2.57±0.06	4.31±0.06	3.2±0.1	4.82±0.15
	High	3.93±0.16	7.06±0.07	7.21±0.12	4.18±0.09	3.42±0.06	5.58±0.03	6.67±0.49	6.7±0.11
TBC (mg/g DM)	Low	3.00±0.08	3.63±0.01	3.62±0.23	1.8±0.02	1.93±0.59	2.7±0.07	2.85±0.21	4.53±0.07
	Medium	3.23±0.32	3.79±0.08	7.76±0.29	2.63±0.14	3.61±0.07	6.91±0.12	4.04±0.15	7.76±0.25
	High	5.44±0.15	12.31±0.14	9.77±0.15	6.3±0.13	5.13±0.09	10.37±0.04	9.76±0.7	10.65±0.16
TPC (mg GAE/g DM)	Low	2.13±0.0	4.98±0.71	80.87±6.39	42.05±0.61	3.14±1.19	2.23±0.3	85.13±9.1	69.86±7.07
	Medium	3.14±0.37	8.43±0.3	166.56±2.79	63.76±2.86	7.04±0.63	10.27±0.37	163.75±15.36	139.25±2.93
	High	9.12±0.73	21.94±0.54	221.18±0.43	107.02±1.07	11.5±1.07	17.34±0.05	232.14±3.32	156.11±11.9
FRAP (mg ASE/g DM)	Low	6.99±1.26	7.8±0.44	89.49±1.49	34.64±4.15	3.18±0.04	2.75±0.04	125.98±4.39	29.02±2.83
	Medium	5.89±0.28	12.44±0.31	166.25±2.43	76.31±1.38	5.63±0.02	8.85±0.23	232.7±0.39	60.38±0.28
	High	14.26±0.56	35.68±0.77	221.58±3.1	101.34±2.24	9.04±0.11	16.16±0.01	378.95±3.02	140.58±1.03
DPPH (%)	Low	15.94±0.42	22.3±0.66	80.94±2.04	88.02±0.15	32.11±0.85	36.64±1.65	85.62±1.97	12.52±1.08
	Medium	22.24±1.58	49.74±0.18	53.48±3.27	75.39±0.43	58.73±1.7	43.42±0.52	71.4±0.48	44.23±0.5
	High	53.29±1.42	94.07±0.00	18.44±3.35	42.7±0.74	70.21±4.84	45.22±2.65	29.54±2.78	41.91±0.11

4.1.8 Effects of applied solvents on the colour properties of beetroot (Cylindra) peel extracts

In order to differentiate the effects of applied solvents on the colour properties of beetroot peel extracts, the scavenged amounts of total betalains in the different extracts and their respective colour patterns (L^*, a^*, b^*) were compared. First of all, the estimated amount of total betalains is per the following descending order; 4.53±0.07 mg/g DM (D), 3.63±0.01 mg/g DM (A), 2.7±0.07 mg/g DM (C), and 1.8±0.02 mg/g DM (B) at the minimum processing point. Subsequently, total betalains recovered in the corresponding extracts under the centre point of processing condition are 7.76±0.25 mg/g DM (D), 6.91±0.12 mg/g DM (C), 3.79±0.08 mg/g DM (A), and 2.63±0.14 mg/g DM (B), respectively. The interaction of 800 W microwave power with 150 s of irradiation time which is the highest point of processing condition was observed to be the most efficacious with the following total betalains outcomes: 12.31±0.14 mg/g DM (A); 10.65±0.16 mg/g DM (D); 10.37±0.04 mg/g DM (C); 6.3±0.13 mg/g DM (B). Meanwhile, under the maximum level of process condition, PW could bring the highest numbers of specific betalains: 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), which is 49%, 16%, and 13% greater as compared to AW, EW and AEW solvents. Since solvent properties directly impact desired outcomes, the behaviour of the solvent must have been manipulated by microwave treatment. In addition, the biosynthesis reaction of compounds present in the matrix can be prohibited by acidification of the extraction medium. For example, enzymatic decolourization of betanin can be retarded by ascorbic acid, which disturbs oxidative activity of polyphenol oxidases or ß-gluconolactone and inhibits ß-glucosidase (Strack et al., 2003). Thus far, the effect of ascorbic acid (0.01-0.04 mM) on two-step microwave treatment for betalain extraction which enhanced pigment recovery was investigated by Cardoso-Ugarte et al. (2014). Acidified extraction medium of pH 3.5 adjusted by ascorbic acid upgraded the yield of betanin to the highest as well (López et al., 2009). However, within the study range, the utilized amount of ascorbic acid, as well as aqueous ethanol, did not show any influence on the expected products as pure water only ensured the topmost betalains recovery. Due to its active hydrophilic property, water seems to be more operative to extract these desired compounds than alcoholic solvents in some cases (Bastos & Gonçalves, 2017). In contrast, almost double amounts of BX and BC were recovered conventionally with 15 % (v/v) aqueous ethanol at 22 ºC in 1 hr of extraction time from beetroot (Rhonda variety) peel as to pure water (Zin et al., 2020b). With high water affinity, betalains compound extraction can simply be accomplished by pure water though changes in polarity of the solvent are also beneficial to accomplish the extraction

performance, which can further be deduced by the accompany of water-soluble proteins (Strack et al., 2003; Delgado-Vargas et al., 2010). According to Chong et al. (2014), water encourages the implementation of the desired compound extraction with less impurity than alcoholic solvents. Under the scope of waste valorization, it can be mentioned that the scavenged betalain amounts from the peel of beetroot are satisfactory according to the extracted results from other sources such as *Opuntia* fruit peel (2.02 mg/100g FW), dragon fruit peel (9 mg/L) and white-fleshed red pitaya peel (1.66 mg/g of dry extract) (Melgar et al., 2019; Thirugnanasambandham & Sivakumar, 2017; Ferreres et al., 2017).

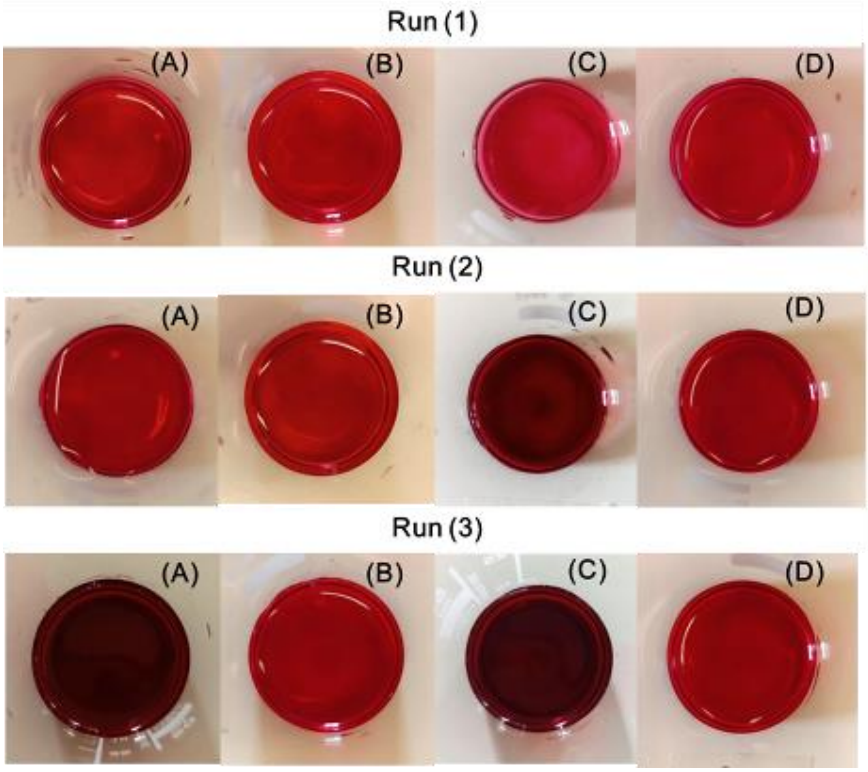


Figure 18. Crude microwave extracts of betalains with different types of solvents under three process conditions

The colour tonality (L^*, a^*, b^*) measurement of beetroot microwave extracts represented the lightness and redness of the extracts together with the clarity and the intensity of vivid colour. Herein, the visual colour of crude extracts was observed to be remarkably influenced by the behaviour of solvents as can be seen in Figure 18. Continuously, the differences in colour patterns (lightness, yellowness, and redness) of respective extracts were visualized and listed in Table 15. Herein, the examined values of ΔE^*_{ab}, which stands for the difference between the displayed colour

and the standard of the respective input content, were within the range of 61.3±0.01 and 80.21±0.02 (sample B). The greater C_{ab}^{*} and Hue^{o} values typify the more redness of the sample whereas the lower L* value represents the deeper colour intensity. As enlisted in the table, C_{ab}^{*} values ranged between 48.06±0.14 (sample C) and 64.27±0.2 (sample B) whilst Hue^{o} values were between 5.88±0.12 and 26.34±0.07 (sample B). Compared to acidified solvents, PW and EW solvents contributed lower L* values of 27.13±0.15 (A) and 24.48±0.1 (C) respectively under the highest process condition of MAE. In the extracts of AW and AEW, higher C_{ab}^{*} values (61.37±0.04 and 57.49±0.19) and Hue^{o} values (26.34±0.07 and 24.61±0.07) were exhibited. The lowest, as well as the highest amount of BI, was observed in the sample extract of B (65.77±0.01 and 112.69±0.03).

Table 15. Differences in colour patterns of the beetroot peel extracts based on the variations in process conditions

Extracts	Run	L^*	a^*	b^*	C^*_{ab}	Hue^o	ΔE^*_{ab}	BI
A	Low	45.48±0.06$^{\delta}$	56.36±0.41$^{\delta}$	9.13±0.16$^{\delta}$	57.09±0.43$^{\delta}$	9.2±0.1$^{\delta}$	67.09±0.37$^{\delta}$	75.33±0.49$^{\delta}$
	Medium	40.45±0.12$^{\delta}$	58.09±0.23$^{\delta}$	17.27±0.03$^{\delta}$	60.61±0.21$^{\delta}$	16.56±0.09$^{\delta}$	72.76±0.12$^{\delta}$	87.22±0.12$^{\delta}$
	High	27.13±0.15$^{\delta}$	48.75±0.57$^{\delta}$	22.01±0.34$^{\delta}$	53.49±0.41$^{\delta}$	24.3±0.55$^{\delta}$	75.83±0.25$^{\delta}$	107.52±0.66$^{\delta}$
B	Low	48.71±0.08$^{\partial}$	51.99±0.06$^{\partial}$	5.35±0.11$^{\partial}$	52.26±0.07$^{\partial}$	5.88±0.12$^{\partial}$	61.3±0.01$^{\partial}$	65.77±0.01$^{\partial}$
	Medium	40.65±0.22$^{\partial}$	58.92±0.23$^{\partial}$	25.67±0.12$^{\partial}$	64.27±0.2$^{\partial}$	23.55±0.14$^{\partial}$	75.69±0.15$^{\partial}$	90.24±0.33$^{\partial}$
	High	29.15±0.02$^{\partial}$	55±0.03$^{\partial}$	27.23±0.08$^{\partial}$	61.37±0.04$^{\partial}$	26.34±0.07$^{\partial}$	80.21±0.02$^{\partial}$	112.69±0.03$^{\partial}$
C	Low	46.01±1.04$^{\phi}$	50.76±0.65$^{\phi}$	12.27±0.77$^{\phi}$	52.55±0.82$^{\phi}$	13.58±0.65$^{\phi}$	62.71±1.26$^{\phi}$	69.38±2.26$^{\phi}$
	Medium	25.9±0.06$^{\phi}$	43.93±0.12$^{\phi}$	19.5±0.09$^{\phi}$	48.06±0.14$^{\phi}$	23.93±0.08$^{\phi}$	73.04±0.05$^{\phi}$	102.47±0.06$^{\phi}$
	High	24.48±0.1$^{\phi}$	44.25±0.24$^{\phi}$	18.68±0.17$^{\phi}$	48.09±0.29$^{\phi}$	22.89±0.07$^{\phi}$	74.13±0.12$^{\phi}$	107.38±0.22$^{\phi}$
D	Low	35.06±0.03$^{\omega}$	57.66±0.05$^{\omega}$	26±0.04$^{\omega}$	63.25±0.03$^{\omega}$	24.27±0.05$^{\omega}$	78.01±0.04$^{\omega}$	100.23±0.11$^{\omega}$
	Medium	28.62±0.13$^{\omega}$	53.54±0.2$^{\omega}$	25.39±0.24$^{\omega}$	59.26±0.28$^{\omega}$	25.37±0.12$^{\omega}$	78.97±0.13$^{\omega}$	111.47±0.05$^{\omega}$
	High	30.71±0.07$^{\omega}$	52.26±0.14$^{\omega}$	23.94±0.14$^{\omega}$	57.49±0.19$^{\omega}$	24.61±0.07$^{\omega}$	76.26±0.1$^{\omega}$	103.16±0.08$^{\omega}$

Superscript same letters represent no significant differences (δ, ∂, ϕ, and ω = p-value<0.0001)

4.1.9 Extraction yield percentage

In this study, three different MAE processes noted as low, medium, and high were done to compare the betalain extractability of various solvents through microwave irradiation. Amongst, the examined percentages of extraction yield were topmost under high process conditions with the following descending order: 9.14 % (sample A); 8.23 % (sample B); 7.31 % (sample C); and 6.91 % (sample D) manifesting the advantage of pure water solvent (Figure 19). Concerning the statistical approach for the significant differences among the treatments of all samples, Tukey's HSD test was performed. Additionally, the extraction yield % of the peel, stalk and flesh extracts at the different levels of processing conditions were represented in Figure 20 in which the yield % of the respective samples inclined with the higher level of processing condition and the greatest amount of yield was exhibited in the microwave extracted sample at 800 W for 150 s which are as follow; 9.14 % (peel): 11.37 % (stalk): 14.99 % (flesh). The control samples revealed the respective percentages of extraction yield as 3.62 % in the peel, 3.74 % in the stalk, and 8.53 % in the flesh extracts.

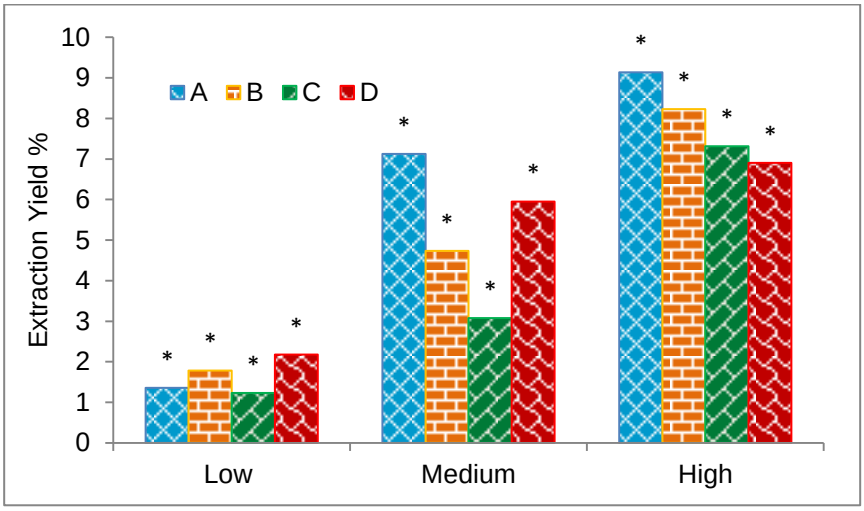


Figure 19. Respective yield % of each sample extract with different solvents at varied conditions of MAE ("*" denotes there are no significant differences among the treatments for each sample with p-values<0.01)

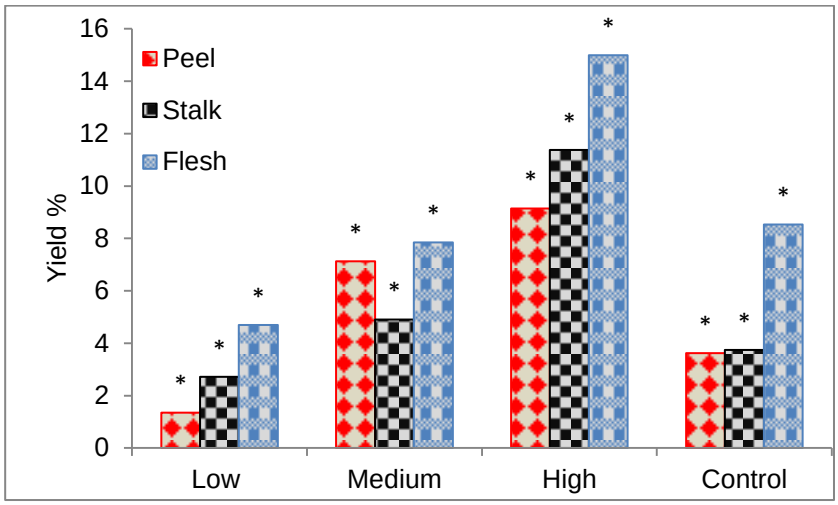


Figure 20. Respective yield % of water extracts of peel, stalk, and flesh ("*" denotes there are no significant differences among the treatments for each sample with p-values<0.01)

4.1.10 Stability of betalain compounds

Degradation kinetics of betalains extracted under medium process condition of MAE was investigated over different temperatures (30 ºC, 40 ºC, 50 ºC, 60 ºC, and 70 ºC) for the time range of 0-5 hrs with the closed vessel in the dark. The resulted variations in half-life ($t_{1/2}$), degradation constant (k), regression coefficient (R), temperature quotient (Q_{10}), and activation energy (E_a) with solvent characteristics used for MAE were listed in Table 16. The pattern of the betalains deterioration over the temperature range followed the first-order kinetic model with R values between 0.8 and 0.9 generally (Appendix-Figures 5-8). In PW and EW extracts, the rate of BC degradation is faster than BX although BC tends to degrade less than BX in AW and AEW extracts thereby revealing the efficacy of ascorbic acid in assisting faster degradation of BC.

The limit of time required to reduce betalains to half of their initial amount was expressed by half-life values. In general, the half-life values of BC declined constantly with increasing temperature in all types of extracts which are 30 hrs to 1 h (A), 39 hrs to 3 hrs (B), 34 hrs to 1 h (C), and 53 hrs to 2 hrs (D). Though the trends of BX's half-life fluctuate throughout the heat treatments (30-70 ºC) with half-life values between 30 hrs and 6 hrs (A), 14 hrs and 4 hrs (B), 32 hrs and 11 hrs (C), and 16 hrs and 3 hrs (D), respectively. In the case of BC, the changes in half-life with time are more obvious despite the lower half-life values regarding BX. The trends of samples B and D behaved the same way, in which, the half-life values of BC were triple of BX after 5hrs of heat

treatment at 30 ºC with the following k values: 0.02 h^{-1} (BC) and 0.05 h^{-1} (BX); 0.01 h^{-1} (BC) and 0.04 h^{-1} (BX) in the samples B and D, specifically. Moreover, in samples A and C, the half-life values of BC and BX were alike with a degradation value (k) of around 0.02 h^{-1} at temperature (30 ºC) howbeit with a drastic change in both of them beyond 40 ºC. Overall, half-life values of BX exceeded BC in samples A and C which is the reverse in B and D samples as the half-life values of BC surpassed BX implying more tolerance of BC to degradation in an acidic medium. Aside from that, along with elevated heat treatments, TBC exhibited the continuous decline of half-life values of all sample extracts together with their enhanced k values such as 21 hrs-2 hrs (0.02 h^{-1}-0.3 h^{-1}), 67 hrs-4 hrs (0.01 h^{-1}-0.19 h^{-1}), 32 hrs-3 hrs (0.02 h^{-1}-0.22 h^{-1}), and 31 hrs-2 hrs (0.02 h^{-1}-0.3 h^{-1}), respectively which is in accordance with the observation of Chew et al. (2019).

Figure 21 depicts the variation of k values of respective betalain compounds with elevated temperatures. Here, highly temperature dependency of BC stability in all sample extracts was demonstrated with its drastic changes in k values with a temperature notably over 50 ºC compared to BX as well as TBC. Moreover, k values of BX rose constantly up to 60 ºC but decreased again at 70 ºC. The rational explanation here is the degradation limit of BX which is maximum at 60 ºC as well as its decompartmentalization following degradation, which in turn affected the overall betalains. Howbeit, in the studies of Sharma et al. (2021) and Mikołajczyk-Bator & Pawlak (2016), BX was mentioned as more strongly temperature-dependent than BC. In the study of Minguel (2018), it was mentioned that heat treatment with exposure to oxygen can lead to dehydrogenation and decarboxylation reactions of betalains with yellow-orange colour changes. Besides, in the medium of pH>7, the hydrolysis of betanin to betalamic acid and cyclo-dopa-5-O-glucoside can occur with the improvement of the yellowish-brown colour (Minguel, 2018). Skalicky and co-workers (2020) claimed the absolute stability of betalains during storage at 22 ºC for 6 hrs. At any specific temperature, somehow, the asymptotic degradation of the colour occurred after a certain time of heat treatment (Chandran et al., 2014).

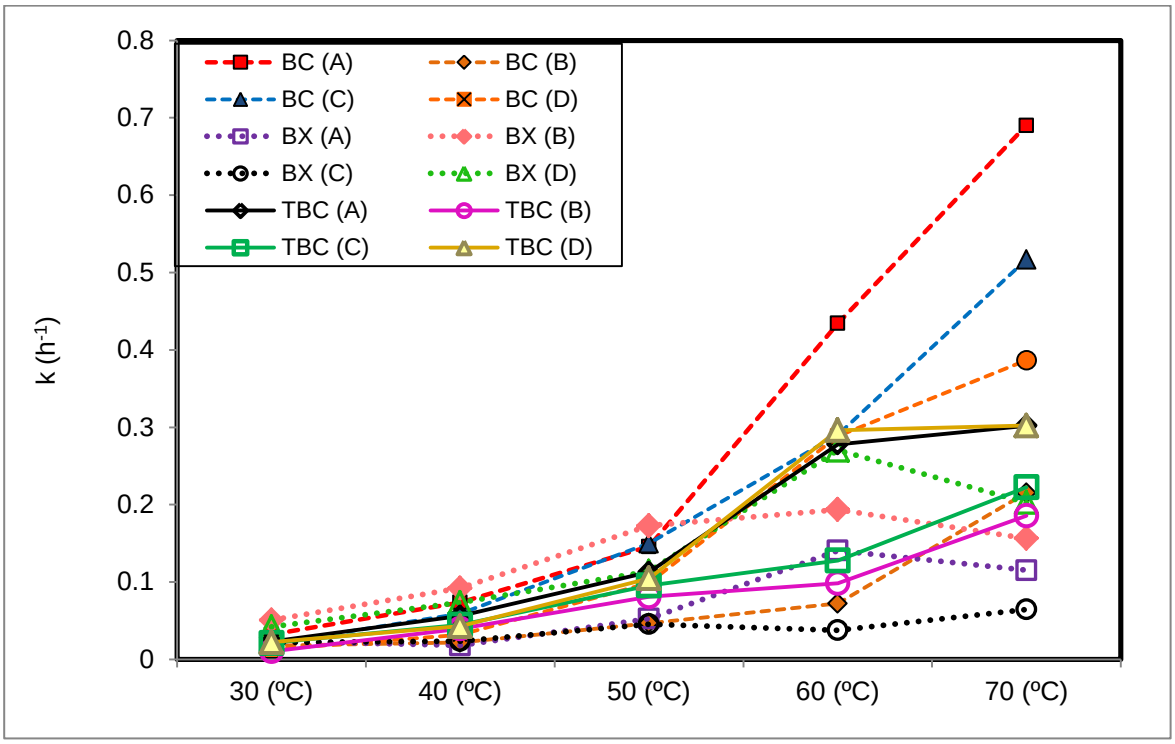


Figure 21. k values variation with elevated temperature within heat treatments for betalain stability test

The temperature-dependent compartmentalization of betalains was defined by temperature quotient (Q_{10}) which is estimated from the ratio of corresponding k values. Among the samples, the Q_{10} value of BC is maximum (3.11) at 40-50 ºC in sample (D) and the minimum amount of 1.22 is observed in sample (B) at the same temperature range. In the case of BX, the highest Q_{10} value is 2.91 which is at 40-50 ºC in sample (A) while the lowest value 0.66 is found in sample (D) at 60-70 ºC. For TBC, 3.83 is the maximum Q_{10} value (30-40 ºC) and 1.02 is the minimum one (60-70 ºC), those were found in samples B and D. This means for every increase of 10 ºC, the degradation rate will elevate approximately three times under 50 ºC and will not differ significantly beyond that temperature. Generally, the more the temperature dependence for betalain deterioration, the more its Q_{10} values are enhanced. In the study of degradation of betalains in milk, temperature elevation (10 °C) induced an approximately 1.5-fold higher reaction rate (Güneşer, 2016).

The activation energy (E_a) of BC, BX and TBC were calculated from the slope value of k versus per temperature graphs (Figure 22). E_a of BC exceeded BX in all sample types in which E_a of BC was 68.26 kJ/mol (A), 53.06 kJ/mol (B), 70.06 kJ/mol (C), and 78.04 kJ/mol (D) whereas E_a of BX was 45.26 kJ/mol (A), 26.42 kJ/mol (B), 22.73 kJ/mol (C) and 38.70 kJ/mol (D), respectively.

Consequently, E_a of TBC in each sample was 58.48 kJ/mol (A), 58.20 kJ/mol (B), 49.70 kJ/mol (C), and 62.05 kJ/mol (D). According to these results, it can be noted that E_a required for deterioration of BC and TBC is the greatest in acidic medium while it is maximum in pure water for BX. In the literature, the examined E_a of BC was 49.21 kJ/mol whilst of BX was 38.99 kJ/mol at 70-120 ºC (Chew et al., 2019); E_a of total betalains in milk was 42.45 kJ/mol at 70-90 ºC (Güneşer, 2016); E_a of water extracts was observed to be 68.76-119.75 kJ/mol whereas E_a of ethanol extracts was in the range of 22.97-125.34 kJ/mol during the storage at 4-30 ºC based on pH variation (Das et al., 2019).

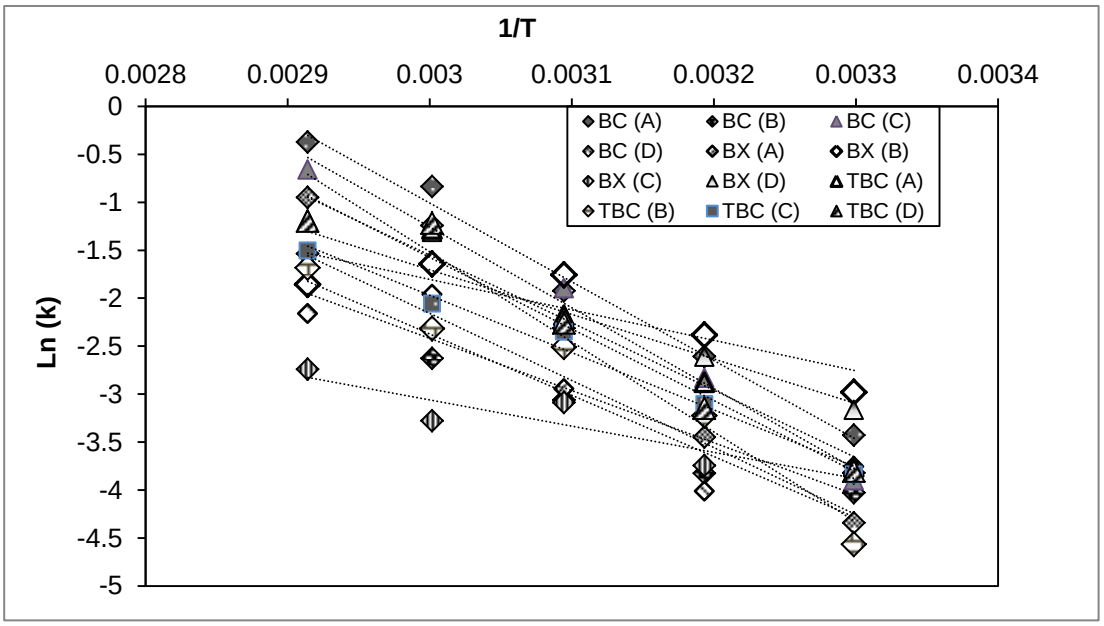


Figure 22. Variation in k values of respective colour compounds with per temperature

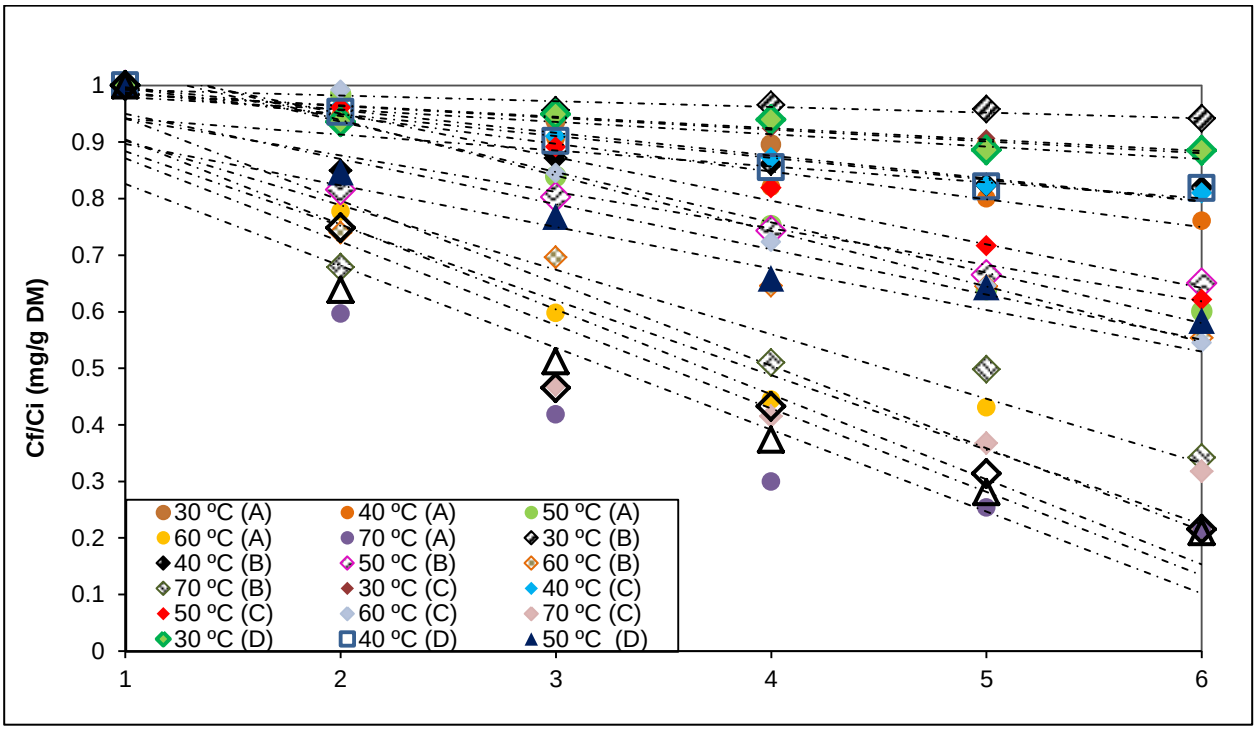


Figure 23. Concentration ratios of BC, BX, and TBC according to the varied heat treatment time (0-5 hrs)

Concentration ratios (CR) of betalains throughout the heat treatments were enlisted in Figure 23. After 5 hrs of heating, the CR of BC decreases from 0.85 to 0.03 in sample A, 0.91 to 0.29 in sample B, 0.89 to 0.07 in sample C, and 0.93 to 0.14 in sample D with increasing temperature whereas the CR of BX reduces from 0.89 to 0.56, 0.77 to 0.4, 0.87 to 0.71, and 0.81 to 0.33 in samples A, B, C and D respectively. Those CR values are additionally proving the better stability of BX compared to BC. Continuously, TBC reduces from 0.88 to 0.22 (sample A), 0.94 to 0.34 (sample B), 0.89 to 0.32 (sample C), and 0.89 to 0.21 (sample D). Overall, CR was directly affected by raising the temperature withal prolonged treatment time, as the trends of CR started to decline remarkably above 50 ºC. In samples B and D, the fluctuation of CR trends can be explained by the colour restoration with ascorbic acid function beyond its degradation limit as earlier discussed. This phenomenon is conspicuous below the heating temperature of 60 ºC as experienced by Chew et al. (2019). Likewise, ascorbic acid could retard the function of enzymatic decolouration (Strack et al., 2003).

Table 16. Temperature variation's effect on the half-life, degradation constant, regression, temperature quotient, and activation energy

Extracts	Temp (°C)	Half-life ($t_{1/2}$, h)			Degradation constant (k, h^{-1})			Regression (R)			Temperature quotient (Q_{10})			Activation energy (E_a, kJ/mol)		
		BC	BX	TBC	BC	BX	TBC	BC	BX	TBC	BC	BX	TBC	BC	BX	TBC
A	30	29.75	29.62	21.39	0.03	0.02	0.02	0.87	0.95	0.97	2.28^{a}	0.77^{a}	2.41^{a}	68.26^{θ}	45.26^{θ}	58.48^{θ}
	40	9.39	38.3	12.33	0.07	0.018	0.06	0.99	0.97	0.98	1.98^{b}	2.91^{b}	2.01^{b}			
	50	4.75	13.15	6.14	0.15	0.05	0.11	0.98	0.92	0.97	2.98^{c}	2.67^{c}	2.46^{c}			
	60	1.60	4.92	2.49	0.43	0.14	0.28	0.97	0.76	0.94	1.59^{d}	0.82^{d}	1.09^{d}			
	70	1.00	6.01	2.29	0.69	0.12	0.30	0.99	0.99	0.95						
B	30	38.94	13.64	66.65	0.02	0.05	0.01	0.79	0.88	0.81	1.22^{a}	1.8^{a}	3.83^{a}	53.06^{θ}	26.42^{θ}	58.20^{θ}
	40	31.80	7.52	17.42	0.02	0.09	0.04	0.95	0.90	0.91	2.15^{b}	1.87^{b}	2.04^{b}			
	50	14.78	4.01	8.55	0.05	0.17	0.08	0.91	0.93	0.93	1.54^{c}	1.12^{c}	1.21^{c}			
	60	9.59	3.58	7.04	0.07	0.19	0.10	0.85	0.89	0.85	2.98^{d}	0.81^{d}	1.89^{d}			
	70	3.22	4.43	3.73	0.22	0.16	0.19	0.91	0.84	0.93						
C	30	34.15	31.65	32.24	0.02	0.02	0.02	0.89	0.86	0.94	2.91^{a}	1.08^{a}	2.08^{a}	70.06^{θ}	22.73^{θ}	49.70^{θ}
	40	11.75	29.37	15.47	0.06	0.02	0.04	0.99	0.96	0.99	2.54^{b}	1.93^{b}	2.13^{b}			
	50	4.62	15.20	7.25	0.15	0.05	0.10	0.98	0.89	0.96	1.94^{c}	0.83^{c}	1.34^{c}			
	60	2.39	18.39	5.42	0.29	0.04	0.13	0.99	0.88	0.97	1.78^{d}	1.71^{d}	1.74^{d}			
	70	1.34	10.73	3.11	0.52	0.06	0.22	0.96	0.96	0.92						
D	30	53.32	16.43	31.08	0.01	0.04	0.02	0.96	0.96	0.83	2.45^{a}	1.74^{a}	1.92^{a}	78.04^{θ}	38.70^{θ}	62.05^{θ}
	40	21.80	9.42	16.16	0.03	0.07	0.04	0.99	0.98	0.96	3.11^{b}	1.55^{b}	2.44^{b}			
	50	7.02	6.09	6.62	0.10	0.11	0.10	0.99	0.90	0.96	2.92^{c}	2.71^{c}	2.83^{c}			
	60	2.40	2.26	2.34	0.29	0.27	0.30	0.98	0.97	0.98	1.34^{d}	0.75^{d}	1.02^{d}			
	70	1.79	3.42	2.29	0.39	0.20	0.30	0.99	0.85	0.99						

The superscript letters a, b, c and d mean the Q_{10} between temperatures 30 ºC and 40 ºC; 40 ºC and 50 ºC; 50 ºC and 60 ºC; 60 ºC and 70 ºC, respectively. The superscript letter θ represents the overall E_a from 30 ºC to 70 ºC.

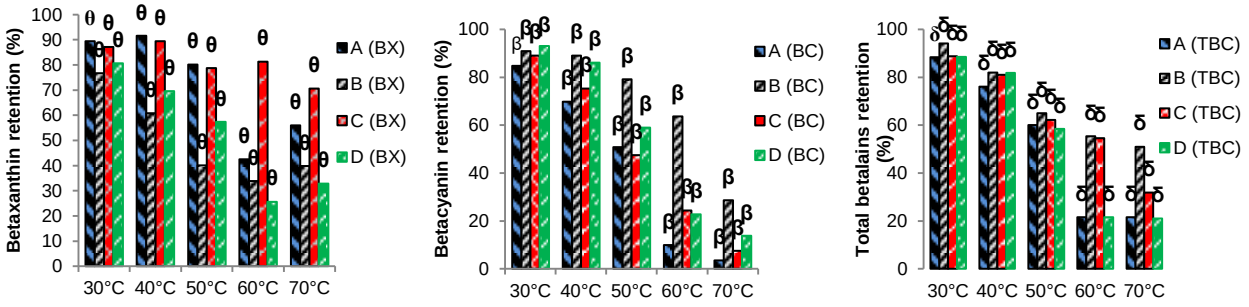


Figure 24. Respective retention percentage of betalains after 5 hrs of heat treatment at various temperatures ("θ" denotes p-values less than 0.05 whilst "β" and "δ" denote p-values less than 0.0001)

The retention percentage (R %) of the respective betalains extracts after heat treatment of 5 hrs at 30-70 ºC were compared in Figure 24. Based on the changes in the temperature from 30 ºC to 70 ºC, R % of BC in different sample extracts varied drastically in the following ranges; 85 %-3 % in sample A, 91 %-29 % in sample B, 89 %-7 % in sample C, and 93 %-14 % in sample D. Amongst, the highest BC retention evoked in sample D followed by the sample B, the possible scenario here is the efficacy of ascorbic acid in both AW and AEW extracts which could have fastened BC's stability. Likewise, R % of TBC was the supreme in sample B which is 94 %-51 % followed by 89 %-32 % in sample C, 89 %-21 % in sample D and 88 %-22 % in sample A. On the other hand, R % of BX varied slightly with temperature changes; from 89 % to 56 % (sample A), from 77 % to 40 % (sample B), from 87 % to 71 % (sample C), and from 81 % to 33 % (sample D). Despite the minor fluctuation in some cases, R % of BX was the topmost in the extract of PW (sample A) proving its better stability regardless of ascorbic acid unlike BC. Deduced colour retention which may affect the diversification of betacyanin to D-glucoside cycle-DOPA and betalamic acid; and the generation of BX and methyl derivative of arginine BX was observed with prolonged heating at an elevated temperature (Bazaria & Kumar, 2018; Das et al., 2019). In advance, deduction in the yield of BX could be explained by thermal degradation/isomerization of indicaxanthin (Cardoso-Ugarte et al., 2014; Sharma et al., 2021). In the study of Elbandy & Abdelfadeil (2008), the stability of the red colour betalain has been improved with 0.05 % of ascorbic acid. Similarly, metal-induced bleaching of betalains in *Rivina humilis* L. berry juice was studied by Khan & Giridhar (2014), in which, selenium (40 µg/mL) together with ascorbic acid (0.25 g/100 mL) could improve the half-life of BC. Mikołajczyk-Bator & Czapski (2017) pointed out the correlational changes of heat-induced betalain pigments along with different pH in which the

loss of violet colour was more intense within a pH range of 4-9; however, the yellow colour giving pigments increased with pH from 6.5 to 7. Probably, the improvement of yellow pigment is due to neo-derivatives of BC diversification via isomerization, deglycosylation, dehydrogenation, hydrolysis, deamination, and decarboxylation (Otalora et al., 2020). Apart from that, naturally originated co-pigments such as flavonoids, polyphenols, and alkaloids in pale yellowish colour can lessen the deterioration of food colour compounds as well (Rein, 2005).

4.2 Ultrasonic wave-assisted extraction (UAE)

4.2.1 UAE of betalains, polyphenols, and antioxidants from beetroot (Rhonda) peel

The extraction ability of plant compounds from different sources of food wastes has been improved with novel extraction technology. Accordingly, ultrasonic waves and microwaves have been used to assist the extraction of bioactive compounds from the Rhonda type beetroot peel regarded as food waste in our study. Cavitation created by low-frequency acoustic waves (18-40 kHz) can denature the cell wall and so allow the solvent convenient to penetrate and leach out of the desired compounds (Teixeira et al., 2014). To point out the influence of ultrasonic probes (20 kHz, 400 W) with different power intensities on recoveries of betalain colour compounds, total phenolic compounds and respective radical scavenging activities, the following parameters were set up; power intensities (3.5 W/cm^2, 8 W/cm^2, and 56.5 W/cm^2), extraction time (5 min, 10 min, and 15 min), and solvent ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v). As enlisted in Table 17, the recovery amounts of specific examined compounds are not quite differed from each other among the treatments and varied randomly. Howbeit, under 5 min of extraction time with 0.06 w/v solvent ratio and power intensity of ultrasound probe (8 W/cm^2), the greatest amounts of TBC (19.09±0.35 mg/g DM), TPC (13.13±1.71 mg GAE/g DM), and AA (24.11±0.1 mg ASE/g DM) were scavenged.

Table 18 represents the efficacy of 10 min UAE for betalains, phenolics and antioxidants extraction from beetroot peel (Rhonda) type in which it was observed that the power intensity (3.5 W/cm^2) together with 0.02 w/v solvent ratio has brought the maximum amounts of TBC (18.07±0.36 mg/g DM), TPC (15.59±2.92 mg GAE/g DM), and total antioxidants (21.39±0.3 mg ASE/g DM), respectively.

Table 17. UAE (5 min) of betalains, phenolics, and antioxidants from beetroot (Rhonda) peel

Power (W/cm^2)	Solvent ratio (w/v)	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
3.5	0.02	10.36±0.57*	4.82±0.2*	15.12±0.72*	8.19±1.27*	18.66±0.5*
8	0.02	10.46±0.21*	4.62±0.17*	15.03±0.31*	11.69±2.41*	14.75±0.62*
56.5	0.02	11.5±0.27*	4.55±0.26*	16.01±0.48*	12.47±1.79*	18.46±0.69*
3.5	0.04	8.11±0.23*	5.13±0.09*	13.22±0.32*	8.01±0.55*	12±0.21*
8	0.04	8.93±0.18*	3.96±0.09*	12.86±0.25*	8.85±0.51*	16.21±0.9*
56.5	0.04	10.73±0.02*	4.34±0.05*	15.05±0.07*	8.58±0.38*	16.58±0.17*
3.5	0.06	6.94±0.28*	4.38±0.12*	11.3±0.39*	12.01±0.37*	12.57±0.17*
8	0.06	13.51±0.24*	5.59±0.13*	19.09±0.35*	13.01±0.64*	24.11±0.1*
56.5	0.06	9.51±0.43*	4.13±0.08*	13.62±0.5*	9.77±0.53*	10.9±0.3*

*There are no significant differences at a 99.99 % significant level among the treatments according to single-factor ANOVA.

Table 18. UAE (10 min) of betalains, phenolics, and antioxidants from beetroot (Rhonda) peel

Power (W/cm^2)	Solvent ratio (w/v)	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
3.5	0.02	12.47±0.3*	5.65±0.06*	18.07±0.36*	15.59±2.92*	21.39±0.3*
8	0.02	11.81±0.33*	4.93±0.2*	16.7±0.52*	12.65±1.32*	20.71±0.67*
56.5	0.02	10.96±0.31*	5.12±0.06*	16.03±0.27*	11.64±0.53*	16.78±1.16*
3.5	0.04	8.9±0.3*	5.1±0.05*	13.97±0.34*	14.74±0.13*	13.27±0.27*
8	0.04	10.3±0.37*	4.27±0.27*	14.54±0.63*	10.33±0.8*	18.37±0.03*
56.5	0.04	11.44±0.23*	4.48±0.19*	15.89±0.37*	13.4±0.6*	17.71±0.48*
3.5	0.06	10.12±0.24*	5.77±0.18*	15.87±0.42*	9.64±0.71*	14.06±0.0*
8	0.06	10.34±0.03*	4.28±0.07*	14.6±0.08*	9.64±0.45*	18.32±0.25*
56.5	0.06	10.34±0.38*	4.4±0.09*	14.72±0.45*	9.93±0.07*	15.76±0.31*

*There are no significant differences at a 99.99 % significant level among the treatments according to single-factor ANOVA.

Additionally, 15 min of UAE withal 8 (W/cm^2) power intensity and 0.02 w/v solvent ratio boosted the extractability of bioactive compounds from beetroot peel to the highest with the amounts of TBC (19.67±0.02 mg/g DM) and TPC (19.16±0.65 mg GAE/g DM) whilst AA (24.39±0.18 mg

ASE/g DM) was found to be highest in the extract of 56.5 (W/cm^2) with the same solvent ratio (Table 19).

Table 19. UAE (15 min) of betalains, phenolics, and antioxidants from beetroot (Rhonda) peel

Power (W/cm^2)	Solvent ratio (w/v)	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
3.5	0.02	10.52±0.42*	4.65±0.17*	15.12±0.57*	14.02±0.95*	19.55±0.36*
8	0.02	13.93±0.09*	5.79±0.11*	19.67±0.02*	19.16±0.65*	21.35±0.74*
56.5	0.02	12.43±0.78*	4.66±0.26*	17.04±1.04*	12.77±2.12*	24.39±0.18*
3.5	0.04	7.03±0.25*	2.53±0.14*	9.54±0.33*	9.11±0.93*	13.05±0.07*
8	0.04	7.86±0.25*	5.46±0.14*	13.3±0.39*	15.59±0.53*	20.34±0.52*
56.5	0.04	12.41±0.34*	4.81±0.04*	17.2±0.39*	13.52±0.99*	19.02±0.3*
3.5	0.06	7.81±0.09*	3.16±0.04*	10.95±0.13*	12.02±0.35*	16.09±0.1*
8	0.06	9.91±0.22*	4.5±0.09*	14.38±0.3*	12.23±0.8*	17.81±0.0*
56.5	0.06	11.23±0.44*	4.76±0.17*	15.98±0.61*	12.08±0.09*	15.41±0.05*

*There are no significant differences at a 99.99 % significant level among the treatments according to single-factor ANOVA.

Figure 25 demonstrates the colour of crude beetroot peel extracts obtained via UAE for 5-15 min. From the overall point of view, the dependency of outcomes on the solid-to-solvent ratio in UAE varied with the sonication time accordingly. It is controversial to conclude the combined effect of solvent ratio and power intensity of ultrasound probe on the extractability of betalains, phenolics, and overall antioxidant ability under the study range. However, the ultrasound probe 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) via UAE of 15 min. 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), 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, 8 W/cm^2, and 0.06 w/v has encouraged the maximum scavenging % of 61.17±0.91. The preference for the longer extraction period might be due to enough time to improve the heat convention throughout the solvent medium was needed. Which in turn, might be the solution to why the lower solvent ratio was more operative. In terms of ultrasonic intensity, it comes to the assumption that the thermal energy emitted by the power ultrasonic probe had triggered the specific properties of the targeted compounds. Meanwhile,

the heat transfer coefficient has been manipulated by acoustic cavitation, acoustic streaming, and particles oscillations that, in turn, affected the intrusion of the cell wall to dissolve out the plant compounds (Legay et al., 2011).

Figure 25. Beetroot peel extracts after 5 min, 10 min, and 15 min of UAE

Ahmed and co-workers (2020) explored the temperature influence on the extraction behaviour of bioactive compounds under sonication. The cavitation phenomenon is subjected to ultrasonic temperature followed by intensive cell rupture. Incorporating the sonication of power (165 W) and frequency (25 kHz), BC (4.2 mg/g) and BX (2.8 mg/g) were produced from red beetroot after 90 min of heat treatment at 52 ºC and 37 ºC withal 25 % ethanol solvent (Da Silva et al., 2018). Under the optimization, BC (96.477 mg/100g FW) was delivered from quinoa hulls at an amplitude of 70 %, the cycle of 0.6 and an extraction time of 9.2 s while the amount of BX was maximized (201.01 mg of betalains/100 g FW) at amplitude = 90 %; cycle = 0.7 and extraction time = 40 s (Laqui-Vilca et al., 2018). In terms of beetroot (Beta vulgaris L.) wastes, UAE could extract 35 % to 86 % of betalains from beetroot pomace (Sutradhar et al., 2021) whereas BC (1.28±0.02 mg/g) and BX (5.31±0.09 mg/g) were scavenged from the beetroot stem under the processing condition of temperature (53 ºC), power (89 W), time (35 min), and solvent-solid ratio 1:19 g/mL (Maran & Priya, 2016). In contrast, Ramli et al. (2014) denoted that UAE had reduced the extraction yield, BC, and TPC but increased TFC exhibiting the strongest scavenging activity in the extracts of red dragon fruit peel. In this case, perhaps the nature of raw materials mattered in the extraction efficiency of ultrasound.

4.2.2 Leaching of betalains, polyphenols, and antioxidants from beetroot (Rhonda) peel

For the prospered parallel study with UAE and MAE, the conventional extraction (CON) of specific compounds from beetroot peel was accomplished with extraction time (5, 10, 15 min) and peel-to-solvent ratio (0.02, 0.04, 0.06 w/v) at 30 ºC and the examined results of the spectrophotometric analysis are listed in Table 20. Among the different processing conditions, the

extraction time (15 min) withal peel-to-solvent ratio (0.02 w/v) ascertained the topmost amounts of BC (9.54±0.14 mg/g DM), BX (5.62±0.09 mg/g DM), and TBC (15.11±0.23 mg/g DM). However, the recovered TPC amount was maximum which is 8.64±0.53 mg GAE/g DM at 10 min of extraction time with the same solvent ratio together with the highest antioxidant activity of 12.26±0.24 mg ASE/g DM.

Table 20. CON of colour compounds, total polyphenols, and antioxidants from beetroot (Rhonda) peel

Time (min)	Solvent ratio (w/v)	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	FRAP (mg ASE/g DM)
5	0.02	8.84±0.24*	4.48±0.24*	13.28±0.48*	7.14±0.53*	11±0.58*
10	0.02	7.82±0.8*	4.24±0.38*	12.02±1.18*	8.64±0.53*	12.26±0.24*
15	0.02	9.54±0.14*	5.62±0.09*	15.11±0.23*	6.95±0.27*	12±0.15*
5	0.04	5.84±0.12*	4.5±0.16*	10.32±0.28*	5.45±0.53*	5.79±0.24*
10	0.04	6.36±0.11*	4.57±0.07*	10.9±0.15*	8.45±0.27*	9.97±0.4*
15	0.04	7.76±0.24*	5.02±0.07*	12.76±0.3*	5.92±0.93*	8.1±0.13*
5	0.06	5.19±0.21*	4.13±0.21*	9.31±0.42*	4.55±0.38*	8.13±0.02*
10	0.06	5.43±0.11*	4.36±0.14*	9.77±0.24*	8.08±0.62*	7.7±0.2*
15	0.06	5.06±0.05*	4.38±0.08*	9.42±0.12*	6.51±0.35*	8.34±0.19*

*There are no significant differences at a 99.99 % significant level among the treatments according to single-factor ANOVA.

According to the investigation of De Azeredo and co-workers (2009), a betalain extraction yield of 67.12 % was achieved with the following process conditions: pH, 3; solvent-to-beetroot ratio, 5:1; temperature, 70 ºC; and grinding time, 2 min. Likewise, the extraction of betalains and phytochemicals from beetroot pomace had been conducted under varying process conditions, in which, pomace-to-solvent ratio (1:15), temperature (50 ºC), processing time (10 min) and pH (2.5) was observed to be the optimum conditions (Kushwaha et al., 2018). According to Silvia Lazar et al. (2021), the maximum amount of betalains (1.44 mg/g DW) and phenolics (2.74 mg/g DM) were traditionally scavenged from beetroot peel at 0.8 % citric acid, 60.23 % aqueous ethanol, 40 ºC extraction temperature, and 32.5 min of processing time. In the finding of Sanchez-Gonzalez and co-workers (2013), the maximum amount of betalains (92 mg/100 g of fruit) was separated by (1:4) methanol to water ratio at 10 min and 15 ºC of processing time and temperature, respectively.

4.2.3 MAE of betalains, polyphenols, and antioxidants from beetroot (Rhonda) peel

MAE is proposed as an effective alternative way of recovering bioactive compounds from agro-industry wastes with superior benefits such as higher reproducibility within a short treatment period, the possibility of zero solvent, minimal energy consumption, and simple manipulation as to classical solid-liquid based heating methods (for example soxhlet, rotary, maceration, heat refluxing, and supercritical water extraction) and even ultrasonic extraction (Zin et al., 2020a). Heating mode, duty cycle, power or heat energy, and temperature in an advanced microwave set-up are basic principles in the MAE of plant compounds. In this contribution, the novel extraction of betalains, phenolics, and respective antioxidants from beetroot peel was achieved by MAE with 800 W (50 % duty cycle). The independent variables are peel-to-solvent ratio (0.02, 0.04, 0.06 w/v) and irradiation time (45, 105, 165 s). The examined results of the spectrophotometric analysis are listed in Table 21. In general, the microwave irradiation time of 165 s and the solvent ratio of 0.06 w/v encouraged the greatest extractability of TBC (16.26±0.65 mg/g DM), TPC (19.49±0.47 mg GAE/g DM), and AA (20.24±0.04 mg ASE/g DM).

Table 21. MAE of colour compounds, total polyphenols, and antioxidants from beetroot (Rhonda) peel

Time (s)	Solvent ratio (w/v)	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
45	0.02	8.12±0.24*	3.62±0.22*	11.7±0.46*	5.18±0.21*	13.08±0.55*
105	0.02	8.13±0.52*	5.45±0.49*	13.45±1*	11.93±1.53*	19.18±0.5*
165	0.02	3.69±0.07*	3.71±0.15*	7.35±0.2*	9.58±0.77*	12.49±0.73*
45	0.04	7.52±0.23*	3.47±0.13*	10.98±0.36*	7.32±0.13*	12.95±0.24*
105	0.04	7.63±0.29*	5.67±0.21*	13.28±0.5*	9.49±0.13*	18.07±0.27*
165	0.04	6.07±0.3*	7.7±0.41*	13.75±0.71*	12.92±0.64*	15.59±0.12*
45	0.06	5.35±0.82*	2.74±044*	8.07±1.26*	8.27±0.25*	13.38±0.15*
105	0.06	5.49±0.11*	6.42±0.37*	12.1±0.25*	12.35±0.43*	14.9±0.1*
165	0.06	5.81±0.22*	10.47±0.43*	16.26±0.65*	19.49±0.47*	20.24±0.04*

*There are no significant differences at a 99.99 % significant level among the treatments according to single-factor ANOVA.

4.2.4 Comparison between ultrasonic-, microwave-assisted extraction, and leaching process

The effectiveness of solvent ratio on extraction ability of plant compounds differed based on the extraction techniques, which is pointed out by UAE, MAE, and CON. In which, the extractions were achieved with solvent ratio (0.02 , 0.04, 0.06 w/v) via UAE (56.5 W/cm^2 power intensity for 15 min); MAE (800 W for 165 s); leaching (30 ºC for 15 min). As demonstrated in Figure 26, each extraction technique behaved differently in the extraction of plant compounds according to the solvent ratio. In the case of betalains and antioxidants (FRAP method) recovery, the trends of UAE and leaching looked alike exhibiting the superiority of lower solvent ratio whilst the MAE favoured the higher solvent ratio for better extractability. Meanwhile, leaching and MAE have shown improvements in antiradical scavenging activity (DPPH method) with the enhanced solvent ratio whereas the middle solvent ratio (0.04 w/v) revealed the maximum scavenging activity in the sample of UAE. The extracted amount of phenolic compounds was not varied significantly with the solvent ratio in either leaching or UAE processes although MAE has shown the influence of expanding solvent ratio on phenolic compounds recovery.

Among the extraction techniques, BC was recovered least with MAE (5.81±0.22 mg/g DM) followed by CON (9.54±0.14 mg/g DM) and UAE (13.93±0.09 mg/g DM; 8 W/cm^2, 0.02 w/v, and 15 min). In contrast, BX amount was extracted most in MAE extract (10.47±0.43 mg/g DM at 165 s

with 0.06 w/v), then 5.62±0.09 mg/g DM in CON and 4.81±0.04 mg/g DM in UAE, individually. Moreover, TBC amount (19.67±0.02 mg/g DM; 8 W/cm^2, 0.02 w/v, and 15 min) was topmost in UAE extract, afterwards, in MAE extract (16.26±0.04 mg/g DM), and it is the least in leaching 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 highest recovery 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 leaching (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 %, UAE exhibited 44.88±1.68 %, and CON expressed 24.96±1.36 % respectively.

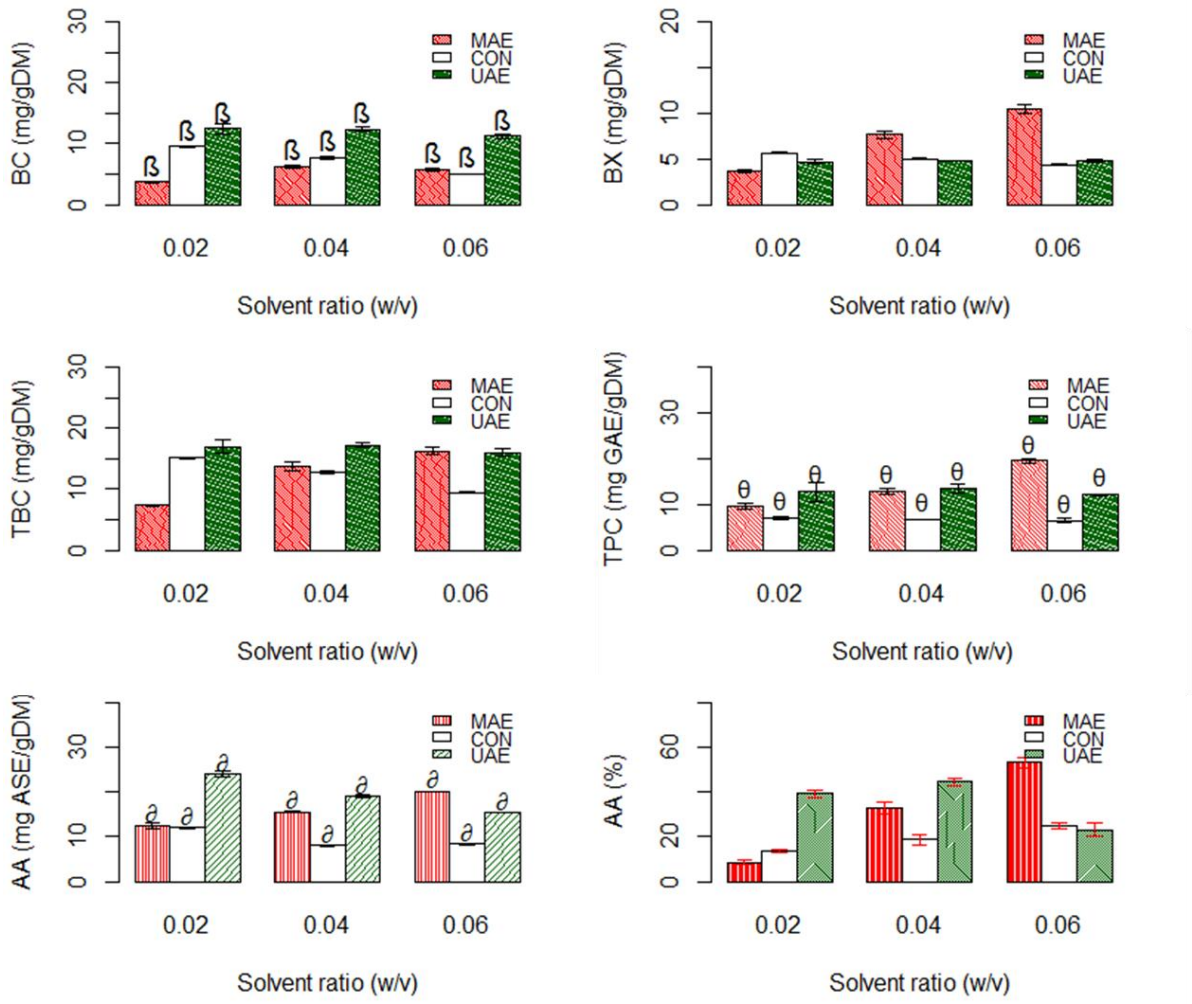


Figure 26. MAE (800 W for 165 s); leaching (30 ºC for 15 min); UAE (56.5 W/cm^2 for 15 min); same symbols represent "no significant differences at p=<0.01" by Tukey's HSD test

Under the three different plants' matrix-to-solvent ratios (0.02 w/v, 0.04 w/v, and 0.06 w/v), the investigated yield percentages in the extracts of the CON (30 ºC for 15 min), UAE (56.5 W/cm^2 for 15 min), and MAE (800 W for 165 s) were summarized in Figure 27. As can be seen in the figure, the highest yield (%) 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. Comparing the results of the BC extraction efficiencies among the techniques, UAE gave decisively higher yield values than MAE which are 46.9±4.8 mg/g DM in UAE and 39.6±1.8 mg/g DM in MAE. Though, regarding the extraction time, MAE was a faster method. On the contrary, Melgar and co-workers (2019) proved that the higher extraction level of bioactive compounds was achieved by ultrasonic extraction despite yield percentage. Similarly, UAE was pointed out as the better choice in the investigations of Rocchetti et al. (2018) and Melgar et al. (2019), though, the discrepancy in applied frequency and power wattage should be taken into account. In advance, MAE has brought 1.7 times greater yield percentage of phenolic from pomegranate (Kaderides et al., 2019) and operation time was two times shorter than UAE (Wang et al., 2010). Alongside, 5 min of microwave irradiation could bring an equivalent amount of phenolic and flavonoid compounds with an hour of ultrasonic treatment time (Gharekhani et al., 2012). It is somehow undeniable that both MAE and UAE have the advantages of internal or external mass transfer compared to classical methods.

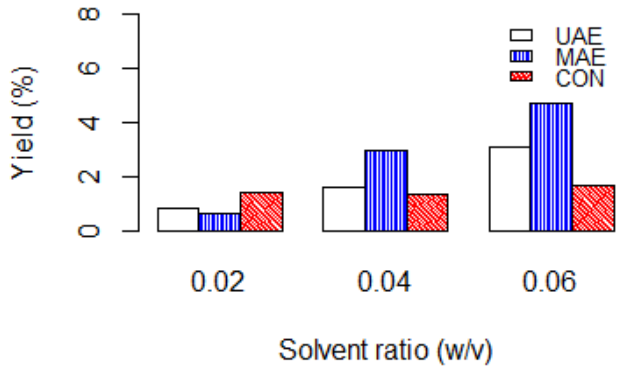


Figure 27. Yield percentages of respective extraction method

4.3 Membrane concentration

4.3.1 Nanofiltration process (NF)

Extraction processes were determined based on our previous experiment in which optimization of the process conditions was focused on achieving the highest betalain compounds, except for the peel-to-solvent ratio (Zin et al., 2020c). Sample analysis was in triplicate for the calculation of mean values and standard deviations. The measured amounts of total soluble solids (TSS in Brix %) before and after filtrations were 1.3 and 6 (water extract, WE) and 7.5 and 8.6 (ethanol-water extracts, EWE). Analysis of variance showed that a significant level (within and between groups) of all desired compounds was 99.99+% (p-value<0.001). Table 22 represents the outcomes of betalains, phenolic, and antiradical activity from the spectrophotometric analysis. The amount of those respective compounds detected in crude WE were as follows: 3.57±0.1 mg/g DM (BX), 5.86±0.05 mg/g DM (BC), 16.29±0.21 mg GAE/g DM (TPC), and 15.33±1.67 mg ASE/g DM (AA) but, their contents in EWE were a bit nether than the former: 3.06±0.06 mg/g DM (BX), 4.81±0.1 mg/g DM (BC), 15.92±1.78 mg GAE/g DM (TPC), and 12.23±0.2 mg ASE/g DM (AA). Since extraction processes were executed at low temperature and short extraction time with mild solvent concentration, the efficiency of the solvent was not significant enough to acquire greater outputs (Sawicki et al., 2016). As active hydrophilic compounds, water seems to be more operative to extract these desired compounds than alcoholic solvents in some cases (Bastos & Gonçalves, 2017). With high water affinity, betalains and phenolic compounds extraction can mostly be fulfilled by pure water; however, changes in polarity of the solvent by combining with alcohols are also beneficial to accomplish the extraction performance. This can assist in overcoming the disturbance of water-soluble proteins at some point which leads to enhancing the recovery of targeted compounds via membrane separation (Delgado-Vargas et al., 2010; Strack et al., 2003).

The amounts of betacyanin compounds were higher in both extracts than the amounts of betaxanthin since the first compound is more stable in processing than the second one (Bastos & Gonçalves, 2017). Based on the investigation of Nemzer et al. (2011), the ratio of the violet colour compound to that of yellow fundamentally differs along with the varieties of beetroot and processing conditions, albeit the amount of the former compound was always superior compared to the latter one. To achieve homogeneity of raw materials, ground peels were blended prior to extraction; however, the contrast in the amount of these compounds might be attributed to the genotype of raw

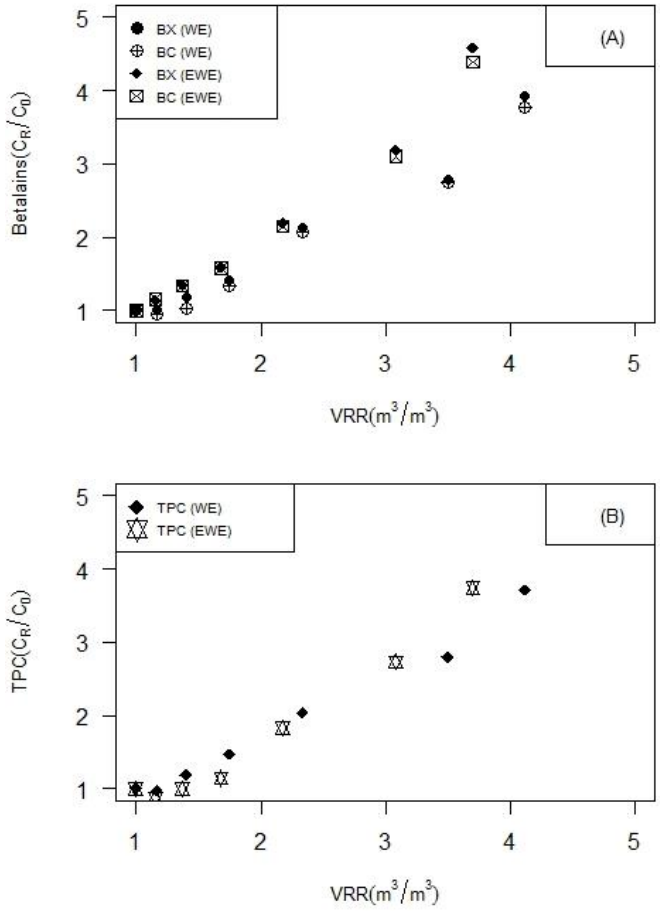
material and the season of harvesting (Chong et al., 2014; Sawicki et al., 2016; Stintzing et al., 2005).

Table 22. Variation of betalains, phenolic contents, and antioxidant activity with volume

	Sample	BX (mg/g DM)	BC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
WE	Initial	3.57±0.1	5.86±0.05	16.29±0.21	15.33±1.67
	500 mL	3.78±0.08	5.67±0.01	15.89±0.82	14.58±0.37
	1000 mL	4.07±0.06	6.09±0.01	19.32±0.1	14.87±0.14
	1500 mL	4.97±0.08	7.82±0.13	23.99±1.26	18.73±1.66
	2000 mL	7.48±0.07	12.12±0.22	33.21±0.23	24.56±1.36
	2500 mL	10±0.11	16.05±0.17	45.53±1.57	29.76±0.00
	Finale	14.03±0.15	22.42±0.08	60.49±1.17	39.32±0.91
EWE	Initial	3.06±0.06	4.81±0.1	15.92±1.78	12.23±0.2
	500 mL	3.5±0.11	5.53±0.14	13.92±0.63	16.32±0.75
	1000 mL	4.12±0.1	6.44±0.18	15.84±0.68	18.7±0.5
	1500 mL	4.85±0.04	7.48±0.12	18.14±0.52	24.13±0.92
	2000 mL	6.7±0.04	10.28±0.03	29.09±0.94	27.94±0.5
	2500 mL	9.74±0.09	14.79±0.09	43.45±0.1	38.86±0.15
	Finale	13.97±0.09	20.89±0.16	59.57±2.91	45.68±1.56

Figure 28 (A) represents betalain concentrations as a function of volume reduction ratio (VRR). As depicted in the figure, all trends of concentration ratio for colour compounds were similar in both extracts. Concentration ratios of betaxanthin (BX) and betacyanin (BC) which were achieved in ethanol-water extract (EWE) were 4.5, while a maximum of 4 was attained in water extract (WE). For TPC, trends of concentration ratio (3.7) for both extracts were comparable (Figure 28 (B)). On the other hand, drastic changes in the concentration ratio of antiradical activity were not shown in water extract displaying the maximum value of 2.6 even though aqueous ethanol could be concentrated up to 3.7 (Figure 28 (C)). The concentration ratios of all desired compounds in ethanol-water retentates surpassed pure water, implying that nanofiltration is more satisfactory for the concentration of aqueous ethanol extract than pure water extract. However, a longer filtration process was required in aqueous ethanol extract, which led to lower flux than pure water. This observation was in accordance with Kim and co-workers (2002), who observed higher flux in water than in alcoholic solvents as they deduced that differences in molecular weight, dielectric constant, surface tension and viscosity of the solvents have some influence on permeate flux. According to

Chong et al. (2014), water can extract the desired compounds with less impurity than alcoholic solvents. The formation of a polymerized layer on the surface of the membrane can also bring a drop in permeate flux, considering that this layer is the effluence of retentate concentration (Jiraratananon & Chanachai, 1996). Bolton et al. (2006) added that flux decline occurs in biotech process stream filtration as a consequence of the accumulation of foulants which creates a cake layer on membranes and causes complete blocking. The amounts of betaxanthin, betacyanin, phenolic, and antioxidants retained in the final aqueous ethanol extract were 13.97±0.09 mg/g DM (BX), 20.89±0.16 mg/g DM (BC), 59.57±2.91 mg GAE/g DM (TPC), and 45.68±1.56 mg ASE/g DM (AA), respectively.



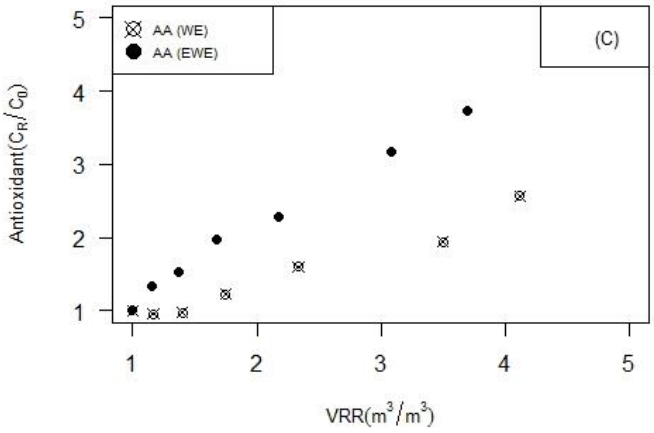


Figure 28. Changes in concentration ratios (C_R/C_0) of the respective compounds with volume reduction ratio (VRR); (A) total betalains, (B) polyphenols, (C) antioxidants

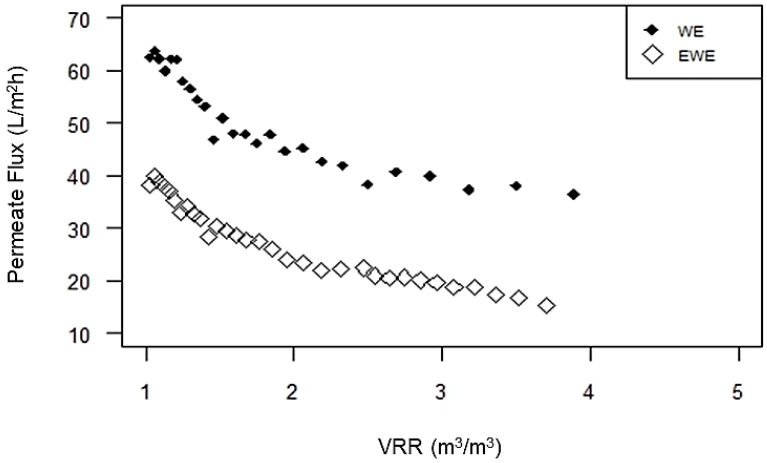


Figure 29. Variation of permeate flux values with volume reduction ratio (VRR) during the concentration process by nanomembrane type NF 200

Figures 29 typifies changes in permeate flux values with the volume reduction ratio during the concentration process. As interpreted in Figure 29, the permeate flux of ethanol-water extract was considerably lower than that of water extract. Both methods, initially, showed a rapid decrease in permeate flux; yet the flux declining rate became almost steady after approximately 12 minutes of processing time. Severe initial fouling is always exhibited in a fresh membrane when TMP is fixed for the whole operation. Comparatively, the rejection rate is elevated above the threshold flux up to a considerable accumulation of foulants on the membrane surface. Along with elevated processing time, the volume reduction ratio improved. The resistance in transport manipulates the processing time, causing permeate flux decline (Miller et al., 2014). In our work, the interference of foulants

with the membrane was found to be strong in water extract, which exhibits higher fouling resistance of membrane than aqueous ethanol and even membrane resistance to transport (Figure 30). As earlier discussed, leaching out of some hydrophilic compounds by pure water is more productive than the solvent action, which in turn might be responsible for membrane efficiency reduction. According to Al-amoudi (2010), natural organic matter like phenolic (aromatic) groups have distinct effects on membrane fouling thereby inducing reversible and irreversible permeate flux decline.

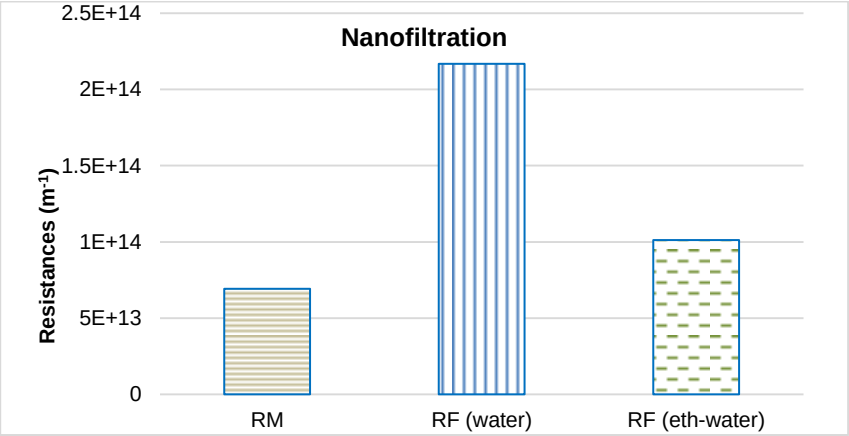


Figure 30. Fouling resistance of NF 200 membrane after concentration

Membrane retention for betalains, phenolic, and antioxidant activity was assumed to be 99 % hence they were not detectable in the permeate of either extract. From the regression analysis and the correlational test between the targeted compounds (Table 23), it can be seen that colour compounds and total phenolic compounds detected in each extract are highly correlated (R^2 greater the 0.92 in all cases) with strong antiradical activity. Besides, the significant level within and between water and ethanol-water extracts for beetroot peel was less than 0.001 for each parameter, i.e, betalains, phenolic compounds, and antioxidant activity.

Table 23. Correlation between the desired compounds and their antioxidant activity

	Variables	Correlation	Regression Statistics
WE	AA & BX[a]	0.99	R^2 = 0.9948
	AA & BC[a]	0.99	R^2 = 0.9979
	AA & TPC[a]	0.99	R^2 = 0.9925
	TPC & BX[a]	0.99	R^2 = 0.9966
	TPC & BC[a]	0.99	R^2 = 0.9957
EWE	AA & BX[a]	0.98	R^2 =0.9540
	AA & BC[a]	0.98	R^2 = 0.9571
	AA & TPC[a]	0.96	R^2 = 0.9277
	TPC & BX[a]	0.99	R^2 = 0.9877
	TPC & BC[a]	0.99	R^2 = 0.9883
	BX (WE) & BX (EWE)[a]	0.99	R^2 = 0.9944
	BC (WE) & BC (EWE)[a]	0.99	R^2 = 0.9889
	TPC (WE) & TPC (EWE)[a]	0.99	R^2 = 0.9886
	AA (WE) & AA (EWE)[a]	0.97	R^2 = 0.9421

Different superscript letters mean significant differences ($p<0.001$)

4.3.2 RO filtration process (RO)

The aqueous extraction of betalains colour compounds, polyphenols, and antioxidants was achieved by the conventional solid-liquid extraction method with a single batch extractor at 40 ºC for 40 min. Followed by, the concentration process of beetroot peel and flesh was performed with a reverse osmosis membrane (X20) with an active surface area of 0.18 m^2. Spectrophotometric analysis was conducted for the quantification of betalain compounds in initial feeds and retentates collected during the filtration process. The determined amounts of BC, BX, and TBC in each sample were shown in Table 24. As we can see in Table 24, the BC content of initial feeds for beetroot peel concentration was 4.06±0.11 mg/g DM (peel) and 1.72±0.03 mg/g DM (flesh) which values went up constantly in each sample. Those reached 15±0.17 mg/g DM and 8.98±0.11 mg/g DM in final retentates of beetroot peel and flesh extracts. The BX amounts recovered in the filtrates of beetroot peel extract were 3.02±0.15 mg/g DM (initial) and 15.06±0.13 mg/g DM (finale). In the flesh extract, the BX amount is 0.89±0.03 mg/g DM at the beginning and went up to 5.39±0.04 mg/g DM at the end of the filtration process. Meanwhile, the amount of TBC measured in the crude extract of beetroot peel is 7.22±0.06 mg/g DM which is three times greater than the one found in the flesh

extract (2.6±0.05 mg/g DM). Likewise, the finale of flesh extract revealed two times lower amounts of TBC (14.33±0.15 mg/g DM) compared to the peel concentrate (30.02±0.28 mg/g DM).

Table 24. Betalains, total phenolic compounds, and antioxidant contents in crudes and retentates of X20 type reverse osmosis membrane separation

Materials	Concentrates	BC (mg/g DM)	BX (mg/g DM)	TBC (mg/g DM)	TPC (mg GAE/g DM)	AA (mg ASE/g DM)
Peel	Initial	4.06±0.11	3.02±0.15	7.22±0.06	3.91±0.74	6.5±0.09
	500 mL	4.45±0.06	2.94±0.04	7.37±0.1	5.2±0.06	7.2±0.05
	1000 mL	4.66±0.13	2.97±0.1	7.61±0.22	5.63±0.46	8.11±0.19
	1500 mL	5.78±0.34	4.09±0.21	9.86±0.55	7.86±0.41	9.68±0.24
	2000 mL	6.94±0.1	5.04±0.18	11.96±0.27	12.26±0.53	11.97±0.28
	2500 mL	10.15±0.14	7.84±0.11	17.97±0.24	20.17±1.0	17.7±0.05
	3000 mL	12.61±0.24	9.61±0.28	22.2±0.42	23.19±1.65	21.61±0.33
	Finale	15±0.17	15.06±0.13	30.02±0.28	34.47±0.19	24.65±1.42
Flesh	Initial	1.72±0.03	0.89±0.03	2.6±0.05	1.35±0.0	1.00±0.04
	500 mL	2.00±0.04	0.99±0.05	2.98±0.08	2.13±0.0	1.28±0.1
	1000 mL	2.32±0.01	1.24±0.04	3.54±0.05	3.45±0.45	1.82±0.11
	1500 mL	2.74±0.03	1.46±0.02	4.18±0.01	4.38±0.34	3.16±0.16
	2000 mL	3.17±0.04	1.71±0.07	4.87±0.11	4.92±0.67	2.91±0.21
	2500 mL	3.98±0.13	2.21±0.09	6.17±0.21	5.57±0.17	5.31±0.08
	3000 mL	5.75±0.14	3.22±0.04	8.94±0.18	7.7±0.67	8.44±0.58
	Finale	8.98±0.11	5.39±0.04	14.33±0.15	12.74±0.42	11.6±0.1

Betalain contents in each sample that varied during RO membrane filtrations were depicted in Figure 31 in the function of VRR. As displayed in the figure, CR values of betalains in final retentates of beetroot peel were 3.69 (BC), 4.99 (BX), and 4.16 (TBC) whereas beetroot flesh filtrates exhibited CR of BC (5.22), BX (6.04), and TBC (5.52). The superiority of CR withal VRR in flesh concentrates as to peel can be explained by the existing varied ratio of dry matter, vitamins, minerals, and biomolecules in different parts of raw beetroot (Hájos & Rubóczki, 2018).

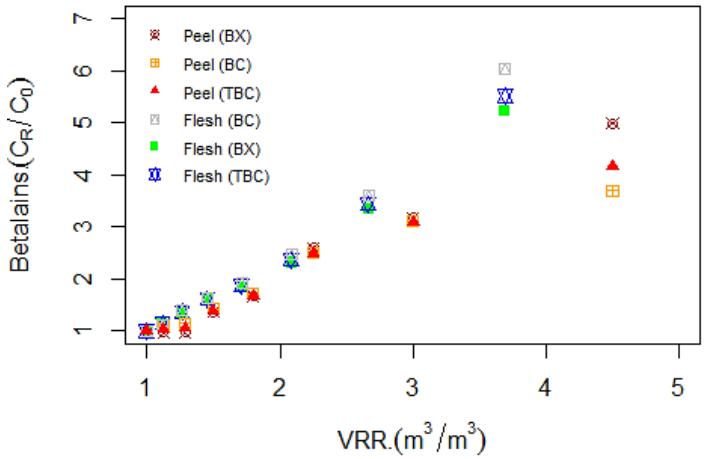


Figure 31. Variation of betalain colour compounds content during the concentration by reverse osmosis membrane (X20)

As regards the first, the superior amount of phenolic compounds was beheld in peel-water extract (3.91±0.74 mg GAE/g DM and 34.47±0.19 mg GAE/g DM) as against flesh-water extract (1.35±0.0 mg GAE/g DM and 12.74±0.42 mg GAE/g DM) in crude and final extracts (Table 24). The rationale could be that the profiles of the beetroot crude extract in terms of betalains, phenolics, and antioxidants depend upon the variety of raw materials and their sources (Hussain et al., 2018). The CR of phenolic compounds content in each extract were summarized in Figure 32; and as can be seen in the figure, the CR of beetroot flesh retentates outweighed the CR of beetroot peel filtrates which are 8.8 and 9.4 at the end of the filtration. The nature of the raw materials, as earlier discussed, might have influenced the efficiency of the membrane performance leading to the differences in VRR bearing higher CR.

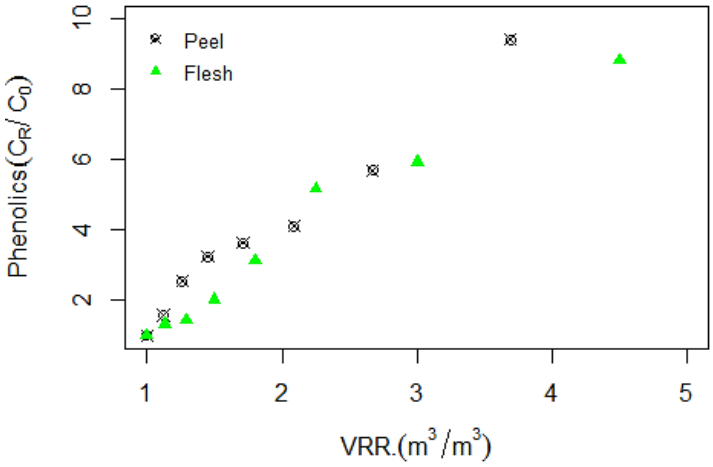


Figure 32. Total phenolic compound content with VRR during concentration by X20 reverse osmosis membrane

The antioxidant contents in beetroot peel-water and flesh-water crude extracts were recorded as 6.5±0.09 mg ASE/g DM and 1.00±0.04 mg ASE/g DM (Table 24). As we mentioned earlier, specific properties of raw materials could be responsible for these differences in value. The antioxidant activities went up in the final retentates with 24.65±1.42 mg ASE/g DM in peel extract as opposed to 11.6±0.1 mg ASE/g DM, individually. Figure 33 represents antioxidant components in each sample varied with VRR. From Figure 33, it was noticed that the CR of antioxidants in flesh-water extract went up drastically with increasing VRR in all processes. The trends of CR strayed from others for both extracts in which beetroot peel-water extract was CR = 3.8 which is significantly nether than the CR of beetroot flesh concentrates (CR = 11.6). The detected huge amount of antioxidants in the final concentrates of flesh-water extracts implies the presence of water-soluble compounds which exhibit antioxidant properties.

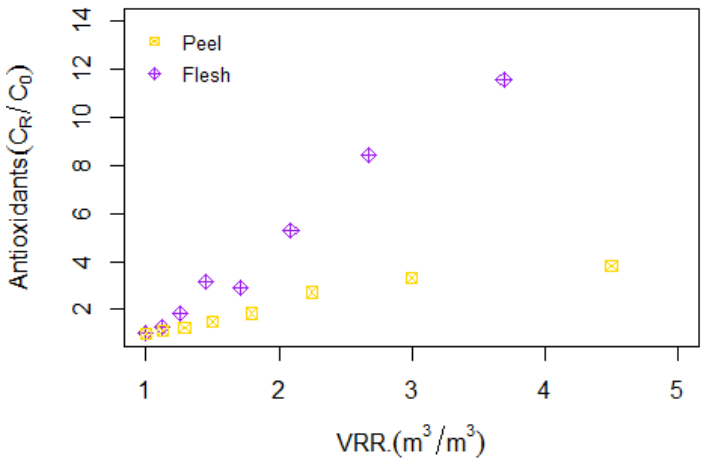


Figure 33. Variation of antioxidant capacity in each sample with VRR during concentration by X20 reverse osmosis membrane

Flux behaviour for reverse osmosis filtration of beetroot peel and flesh extracts with reverse membrane-type of X20 in the function of the volume reduction ratio is shown in Figure 34. Either water or ethanol-water extract was fed to the system at 40 bars of transmembrane pressure adjusting the stream temperature to 27 ºC. As depicted in Figure 34, the permeate flux of beetroot flesh-water extract behaved differently from peel-water by tapering off between VRR 2 and 3. The flux behaviour of peel extract shows regular deduction with increasing VRR. The initial accumulation of the compounds on and/or in the membranes led to the flux fluctuation due to membrane fouling and

concentration polarization (Couto et al., 2011). Being regarded as non-porous, fouling propensity on account of cake layer formation has to be aware in reverse osmosis membranes (Zhu & Elimelech, 1997). The accumulation of foulants, cake formation, and pore plugging on/in the membrane layers might be the responsible ones for the resistance to permeation (Miller et al., 2014).

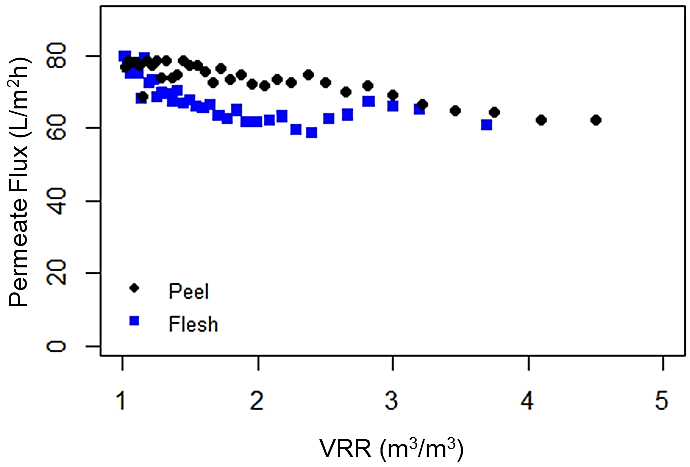


Figure 34. Permeate flux changing with VRR during the concentration process by reverse osmosis membrane (X20)

Additionally, the flux of beetroot flesh concentration reached VRR (1.5, 2, 2.5, and 3) after 7.4, 11.6, 14.3, and 15.8 min of concentration-time whilst the permeate flux of peel-water extract could reach VRR (1.5, 2, 2.5, and 3) at 6.5, 9.7, 11.9, 13.4 min. At the end of the concentration process, VRR = 4.5 was achieved after 16 min in peel extract concentration and VRR = 3.7 was reached within 17.2 min of the processing period in the concentration of flesh extract. From pure water flux measurements after the concentration process of beetroot peel and flesh extracts, fouling resistances of the membranes were calculated as demonstrated in Figure 35. A significant difference in fouling resistance was not observed between peel and flesh concentration. This experience answered the expectations as the low-fouling type membrane (X20) was chosen to fulfil this investigation. In membrane filtration, fouling starts with the interaction between the solute and the membrane material, being the chemical bonds and the Van der Waals forces are the main phenomena involved, according to the report of Hafidi et al. (2003). The extent of adsorption will be determined by several factors such as the membrane material, the feed solution, the concentration of solutes, ionic strength and pH (Mulder, 2000).

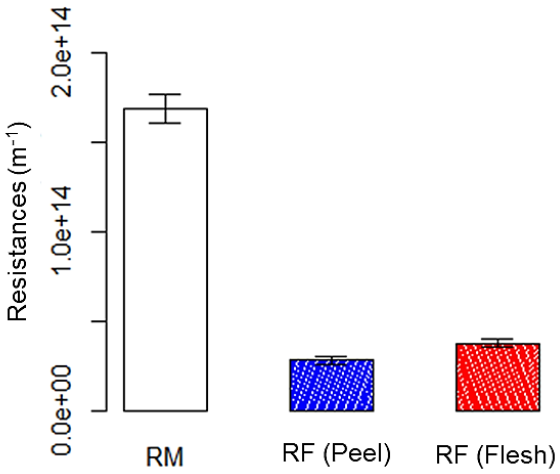


Figure 35. Fouling resistance of low fouling type X20 membrane after concentration

Retention percentages were calculated from the concentration data of final permeates and retentates. Since no betalains, phenolic compounds, and antioxidants were not determined in the permeates, it can be concluded as 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. Likewise, approximately 98 % of betalains were retained by loose reverse osmosis membrane (Mereddy et al., 2017). Figure 36 represents the colour variation of beetroot flesh and peel extracts before and after the filtration processes. The colour tonality (L^*, a^*, b^*) of beetroot peel and flesh extracts was measured to make a comparison between the crude extracts and final concentrates in terms of the lightness, redness, clarity, and intensity of the colour. The resulting differences in colour patterns of the respective samples were listed in Table 25. In beetroot-flesh extracts, all examined values of L^*, a^*, b^*, ΔE^*_{ab}, Hue^o and BI decreased after the concentration process except C^*_{ab} value. Likewise, the final peel-water concentrates typified lower values of a^*, Hue^o, ΔE^*_{ab}, and BI whereas L^*, b^*, C^*_{ab} values were found to be exceeded compared to the crude extract.

Table 25. Colour tonality (L^*, a^*, b^*) of the beetroot extracts before and after the concentration

Material	Sample	L*	a*	b*	C^*_{ab}	Hue^o	ΔE^*_{ab}	BI
Flesh	Initial$^{\beta}$	11.1±0.67	35.23±1.13	18.44±1.23	27.61±0.84	39.77±1.57	60.89±0.36	165.32±2.69
	Finale$^{\beta}$	10±0.25	18.91±1.18	14.5±0.67	37.51±0.48	23.83±1.34	57.7±0.24	119.34±7.96
Peel	Initial$^{\theta}$	3.53±0.33	11.6±0.78	4.14±0.33	19.4±0.13	12.04±0.49	66.72±0.38	163.26±6.41
	Finale$^{\theta}$	4.58±0.21	9.93±0.16	4.22±0.03	23.04±0.23	10.79±0.16	65.66±0.18	126.77±0.6

Same letters β and θ mean no significant differences among the measurements.

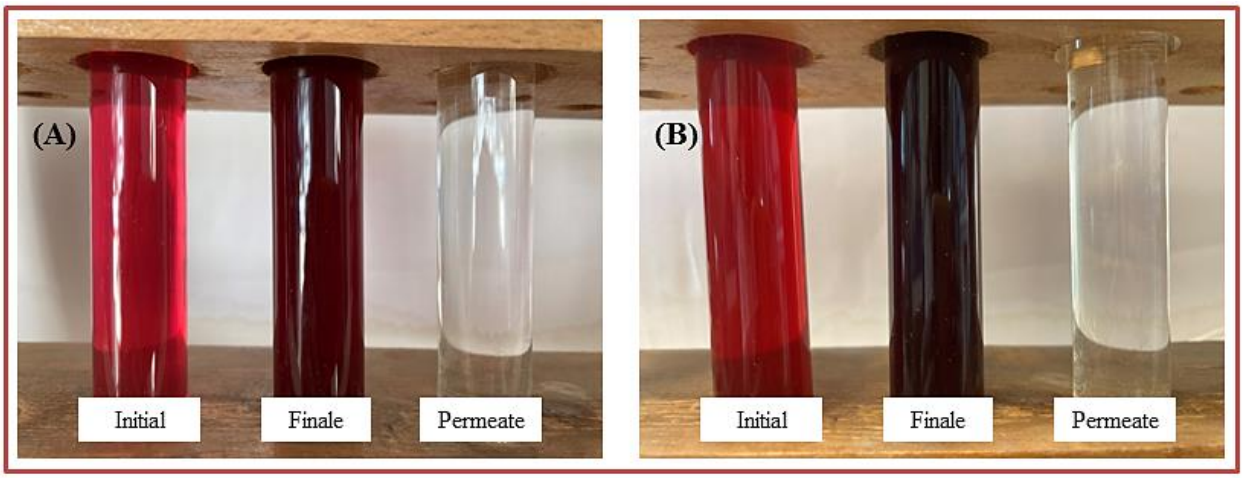


Figure 36. Crude extracts, retentates, and permeates of beetroot flesh (A) and peel (B) extracts

Table 26 represents the characteristics of the flesh- and peel-water extracts before and after the concentration. In which, the differences in moisture (%), dry matter, yield percentage, the density of the extracts, and TSS were mentioned. Significant improvements in dry matter contents were observed in the final retentates of beetroot flesh (6 times) and peel (16 times) extracts along with yield percentages after membrane concentration. The densities of the extracts did not change decisively. Albeit, TSS of crude beetroot flesh extract was improved from 0.02 to 4 % while TSS of peel extract went up to nearly 3 % than the initial feed. From the reference of Rodriguesa and co-workers (2020), the concentration of phenolic compounds, anthocyanins, vitamin C, and cyanidin-3-glucoside by 3.2, 6.5, 7, and 4.5 times by RO (R25A polyamide).

Table 26. Characteristics of the beetroot flesh and peel extracts before and after the concentration

Material	Sample	Moisture (%)	Dry matter (%)	Dry Matter (g)	Yield (%)	Density	TSS (Brix %)
Flesh	Initial	99.6	0.4	0.004	3.44	0.9998	0.02
	Finale	97.75	2.25	0.023	19.57	1.0073	4.1
Peel	Initial	99.91	0.09	0.001	0.55	0.9992	0.1
	Finale	98.5	1.5	0.015	9.11	1.0039	3

5 CONCLUSIONS AND RECOMMENDATIONS

The major purpose of this dissertation is how to boost the extractibility of bio colourants known as betalains, polyphenols, and antioxidants by emerging technologies including microwave, ultrasonic wave, and membrane. Under the investigation of MAE efficiency on bioactive compounds extraction from the beetroot peels;

- With PW solvent, the supreme amounts of 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. The recovered amounts of TBC, TPC, AA (FRAP), and AA (DPPH) are 12.31±0.14 mg/g DM, 21.94±0.54 mg GAE/g DM, 35.68±0.77 mg ASE/g DM, and 94 %, respectively. The actual values calculated from the regression equation modelled by RSM were as follows: TBC (7.87 mg/g DM), TPC (18.06 mg GAE/g DM), and AA by FRAP method (31.25 mg ASE/g DM) and DPPH method (83 %).
- Among the thirty experimental runs by AW solvent, the utmost amounts of betalains and phenolic compounds: TBC (7.09±0.19 mg/g DM), and TPC (309.67±0.0 mg GAE/g DM) were scavenged at 800 W with 0.1 w/v solvent ratio and 0.5 % acid after 150 s. Meanwhile, the highest radical scavenging activity was examined by the FRAP method (231.72±4.65 mg ASE/g DM) at 100 W, 0.1 w/v solvent ratio and 0.5 % acid after 30 s and 89 % of radical scavenging activity (DPPH) was measured at 450 W, 0.15 w/v solvent ratio, and 0.3 % acid after 30 s. From the RSM model, the amounts of targeted bioactive compounds estimated by the actual equations were 7.04 mg/g DM of TBC, 248.83 mg GAE/g DM of TPC, 213.03 mg ASE/g DM of AA, and 78 % of AA, individually.
- In the case of EW solvent extracts, the highest experimental values of detained TBC (11.29±0.13 mg/g DM), TPC (10.74±0.59 mg GAE/g DM), and antioxidants (17.03±0.59 mg ASE/g DM) by FRAP were observed in the extract of MAE at 800 W for 90 s 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. Meanwhile, the calculated scavenged amounts of the respective compounds via the RSM model were 8.84 mg/g DM (TBC), 10.97 mg QUE/g DM of TPC, and 14.87 mg ASE/g DM or 53 % of antioxidant activities.
- From thirty experimental runs with AEW solvents, the maximum amounts of TBC (12.72±0.3 mg/g DM) were recovered at the processing condition of 800 W, 150 s, 0.1 w/v solvent ratio and 0.1 % acid. TPC (375.2±0.59 mg GAE/g DM), and AA determined in the

extracts of MAE at 100 W for 30 s with 0.1 w/v solvent and 0.5 % acid were 266.65±1.8 mg ASE/g DM and 88 %. In the meantime, the RSM model estimated the scavenged amounts of TBC, TPC, and AA as 11.25 mg/g DM, 325.26 mg GAE/g DM, 270.71 mg ASE/g DM, and 83 %, respectively.

After MAE at 800 W for 150 s with PW solvent (0.2 w/v), betalain content is the topmost in the peel; 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, and TBC=1.3±0.08 mg/g DM). Meanwhile, the recovered total betalains 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. Likewise, 35.68±0.77 mg ASE/g DM (FRAP) is the maximum radical scavenging activity discovered in the peel-water extracts which amount was 1.3 times exceeded the control, and 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. Similarly, ABTS performance 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 antioxidant activity (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, 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.

To point out the influence of ultrasonic probes (20 kHz, 400 W) with different power intensities on recoveries of betalain colour compounds, total phenolic compounds, and respective radical scavenging activities, the following parameters were set up: power intensities (3.5 W/cm^2, 8 W/cm^2, and 56.5 W/cm^2); extraction time (5 min, 10 min, and 15 min); and solvent ratio (0.02 w/v, 0.04 w/v, and 0.06 w/v). The ultrasound probe 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) via UAE of 15 min. 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), 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 sonication, 8 W/cm^2 power intensity, and 0.06 w/v solvent ratio has encouraged the maximum scavenging % of 61.17±0.91.

The influence of solvent ratio on the extraction ability of plant compounds was pointed out by ultrasonic-wave-assisted extraction (UAE), microwave-assisted extraction (MAE), and conventional extraction (CON) methods. In which, the 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). From 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 % 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. From this investigation, a conclusion can be made as the mentioned emerging technologies are alternative ways of scavenging bioactive compounds from vegetable wastes.

The concentrations of beetroot juices with membranes were accomplished with two types of membrane filtrations (NF and RO). In NF, two different solvents, i.e, pure water and aqueous water, were applied for the extractions. After the filtration by NF membrane, the scavenged amounts of betalains, phenolics, and antioxidants 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. To investigate the RO membrane's effects, the extractions were carried out from beetroot peel as well as flesh. 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 betalains, phenolics, and respective antioxidants were recovered in the beetroot flesh concentrate after the filtration process. 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 to be more convenient in their practical usage.

6 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 SUMMARY

As most plant-based bioactive compounds are secondary metabolites that have pharmacological and toxicological effects on living organisms, the recovery of bio colourants and polyphenols from agri-food wastes has come to the forefront under the context of waste valorization. Fortification of foods with natural bioactive compounds for general wellbeing and combating several diseases encourages scientists to recover plant-based bioactive compounds from their matrix as novel as possible. Herein, their application in food products is not only limited to the contribution of their colour, aroma and flavouring but also are the potential to modulate the biological activity of food products. Currently, the valorization of agricultural and food wastes has been open a new arena in the food waste hierarchical pyramid. Hence, it is unambiguous to assume the recovery of bioactive compounds from agro-industrial wastes through an environmentally-benign process is promising.

The major concept of this dissertation is to investigate the influence of microwave irradiation on the extractable amounts of betalains, the tonality of coloured extracts, as well as their thermo-stability thereafter. Hundred experimental runs were achieved with the RSM modelling tool with three different operational setups such as microwave power, irradiation time, and solid-to-solvent ratio, in which; pure water, aqueous ethanol, acidified water, and acidified aqueous ethanol solvents were applied for the extraction purpose. The strategy of the betalains' stability tests was correlated with the characteristic of the applied solvents during the extractions. Apart from betalains; the content of phenolic, flavonoid, and antioxidants were also determined. The feedstocks for the extractions had been prepared from the unedible parts, i.e peel and stalk, of red beetroot (Cylindra and Rhonda types). Meanwhile, beetroot flesh was used in some cases for a better comparison. The novelty of extraction techniques was broadened by sonochemistry in terms of the UAE. The subsequent investigation was focused on the membrane technology (RO and nano) for the concentration of the traditionally extracted beetroot juice.

Within the study ranges, microwave irradiation has shown privilege by boosting the extractability of betalains, phenolics, flavonoids, and relative antioxidant activities from beetroot peel, flesh, and stalk. Besides, ultrasonic waves application typified to be crucial in assisting the extraction of the mentioned bioactive compounds to some points. The solvent characteristics played a major role in the scavenging level of the bioactive compounds, meanwhile, the stability of the betalains was intensified by acidification despite their jeopardization and decompartmentalization

behaviour. Furthermore, the membrane concentration has been found as compromising the concentration purpose of the beetroot extracts. Ultimately, the outcomes of the current investigation had led to the conclusion that the conventional extraction way could be replaced by thermal, otherwise, non-thermal emerging technologies due to the improved scavenging level of the targeted bioactive compounds which have been revealed.

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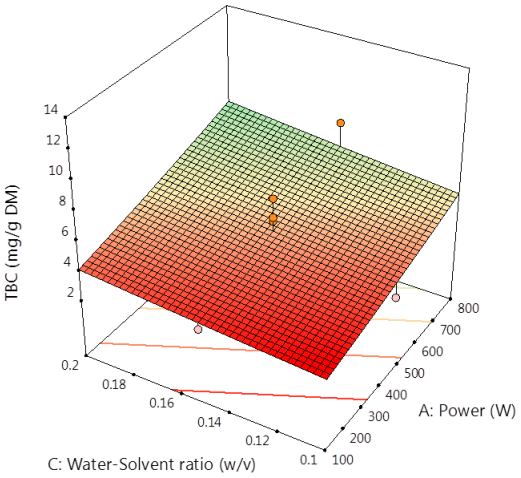
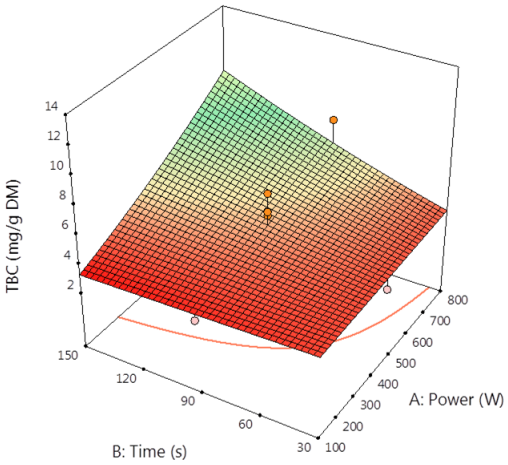
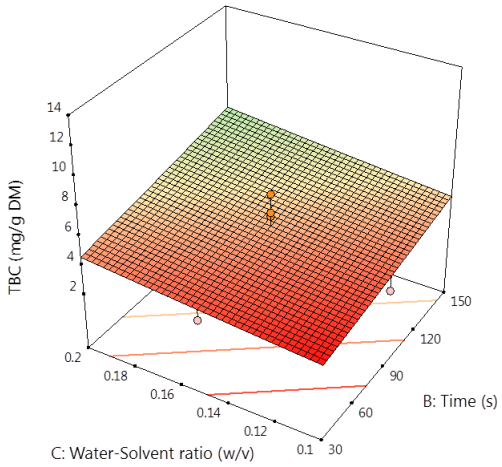
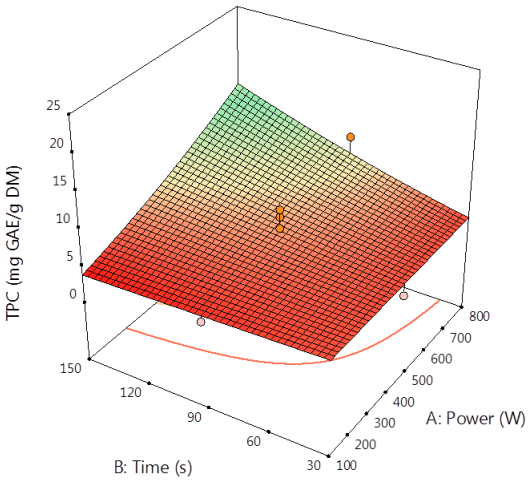
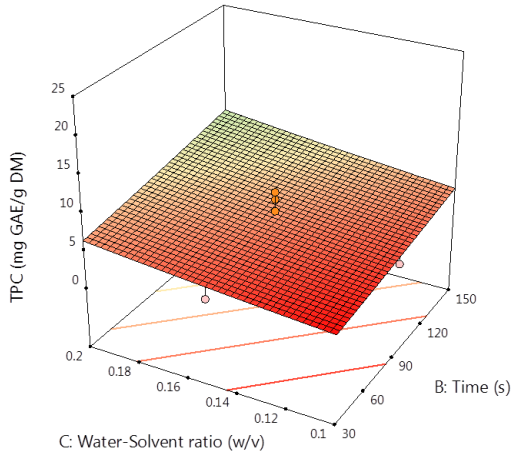
FURTHER APPENDICES (A.2)

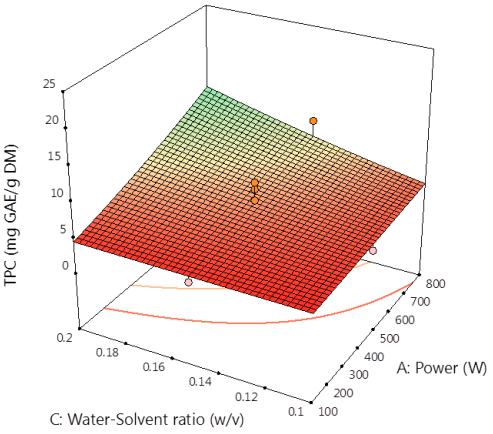


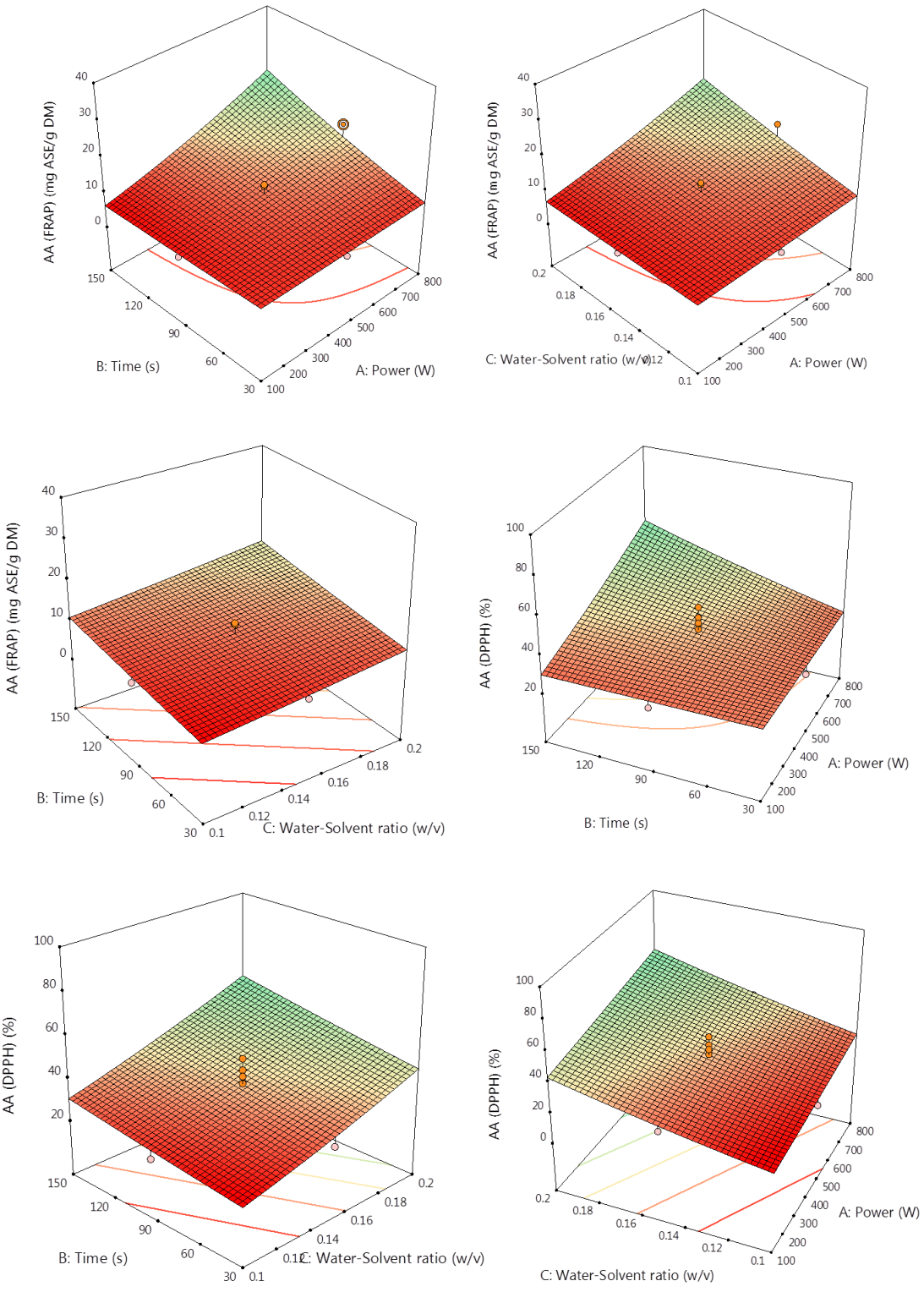




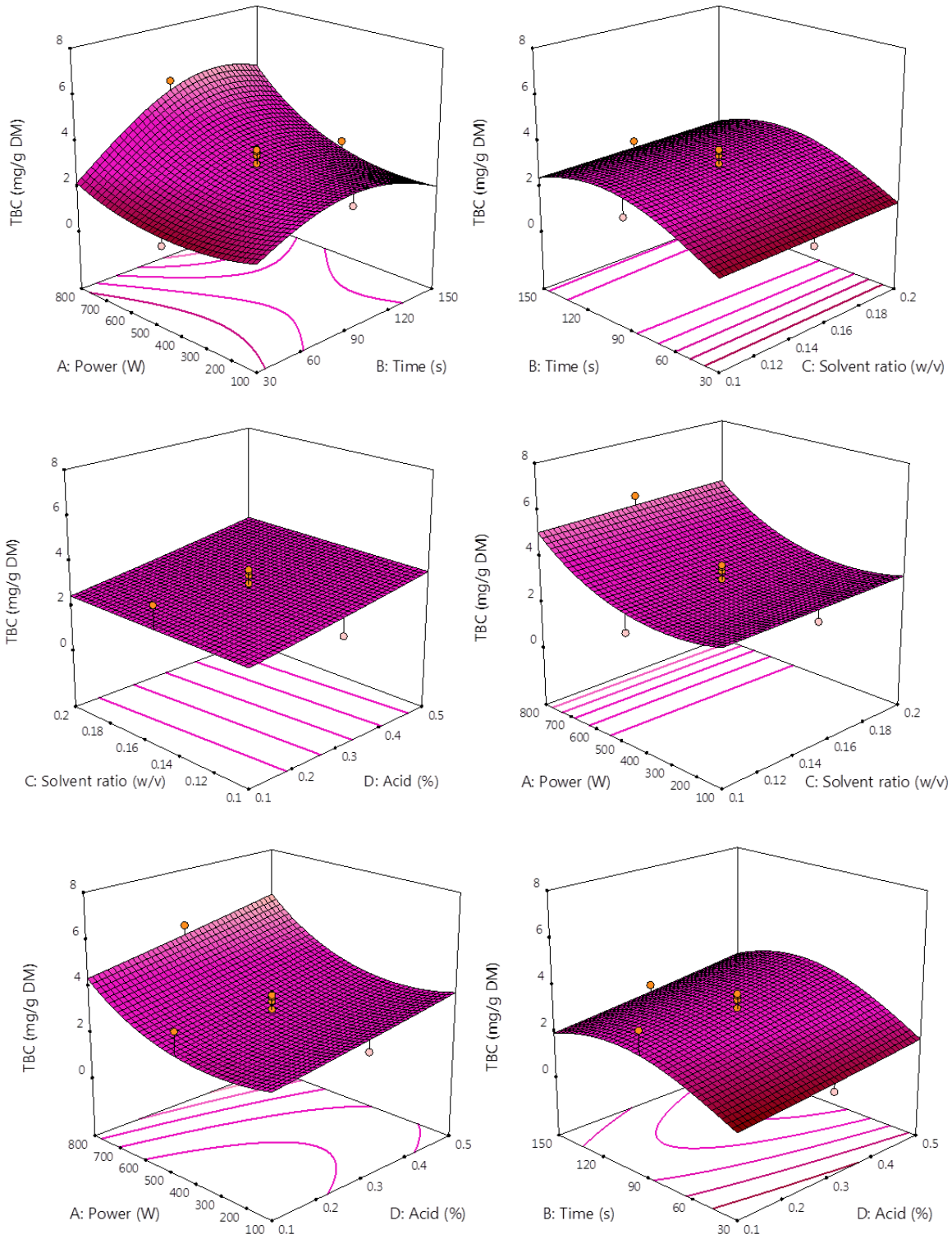


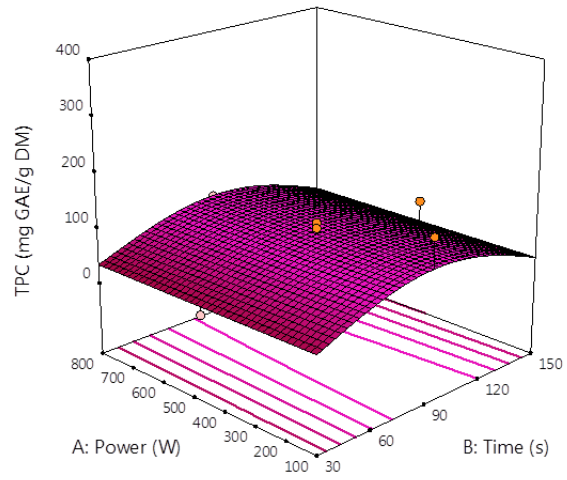


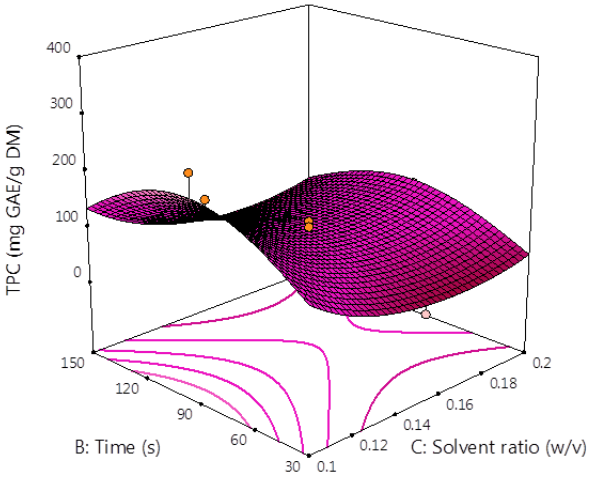


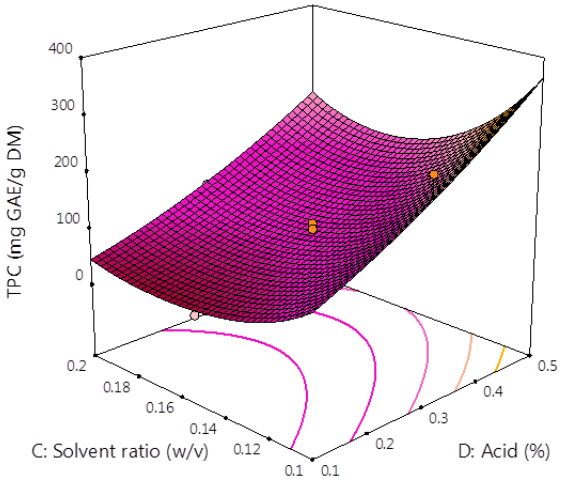


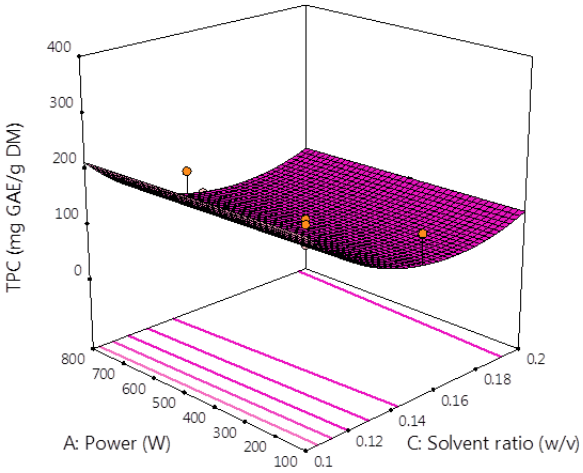
Appendix-Figure 1: 3D surface graphs for each response influenced by individual factors in the PW extracts of beetroot (Cylindra) peel (Sample A)

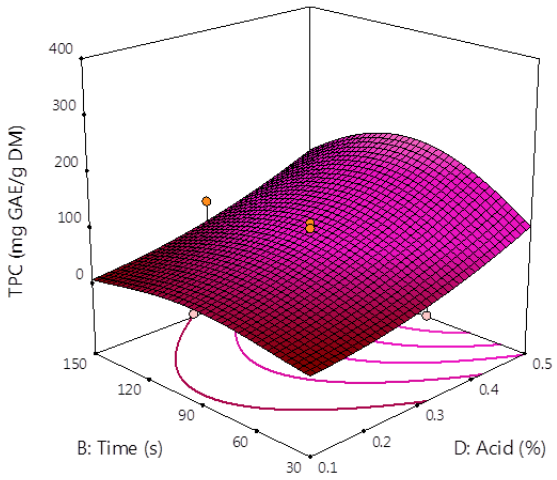

TBC (mg/g DM)
A: Power (W)
B: Time (s)
C: Solvent ratio (w/v)
D: Acid (%)

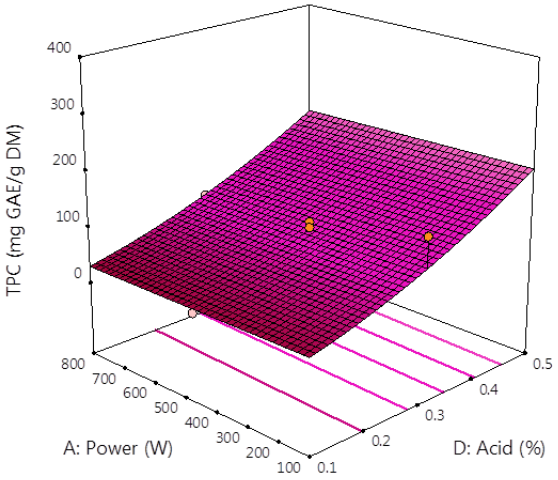

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
30
60
90
120
150
B: Time (s)

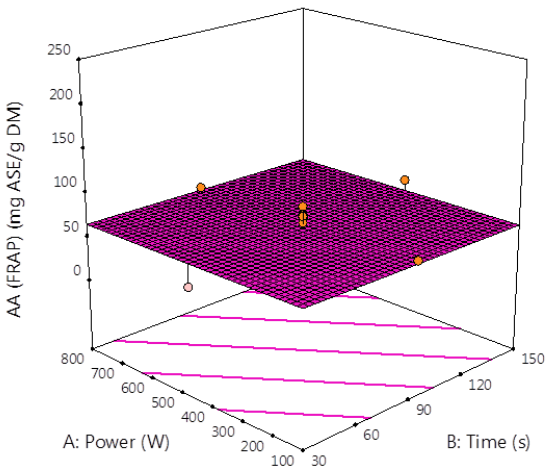

TPC (mg GAE/g DM)
400
300
200
100
0
150
120
90
60
30
B: Time (s)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

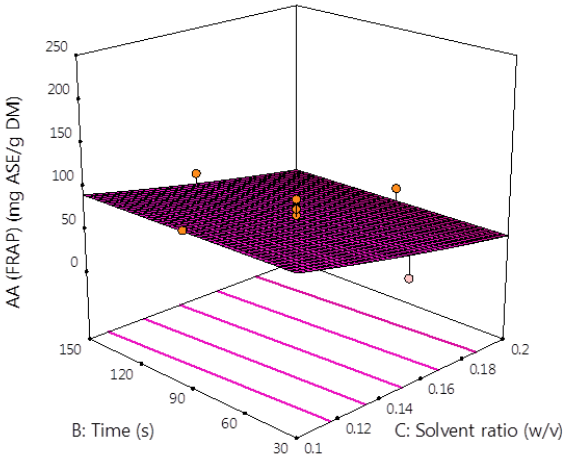

TPC (mg GAE/g DM)
400
300
200
100
0
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

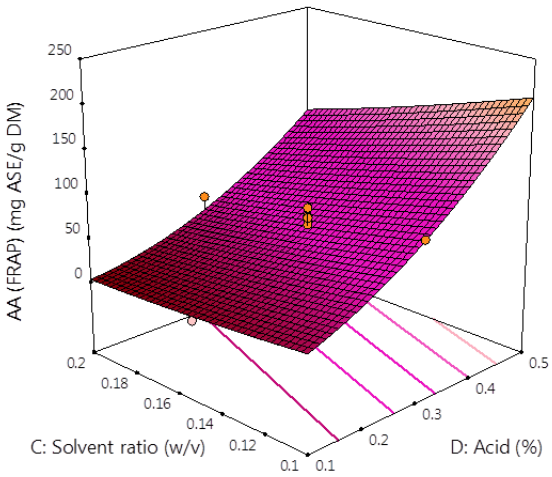

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

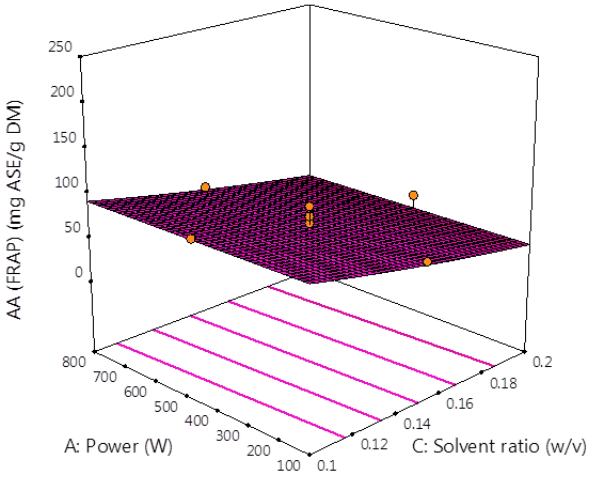

TPC (mg GAE/g DM)
400
300
200
100
0
150
120
90
60
30
B: Time (s)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

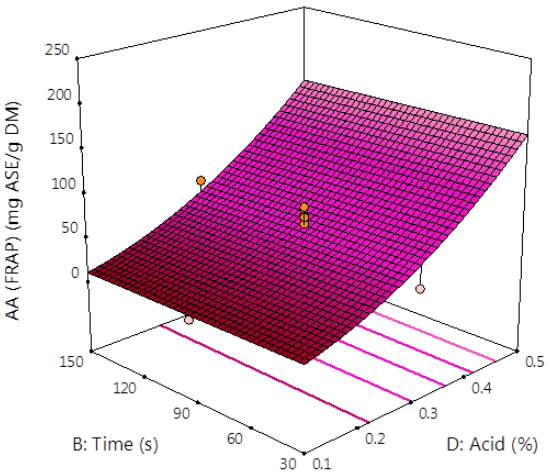

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

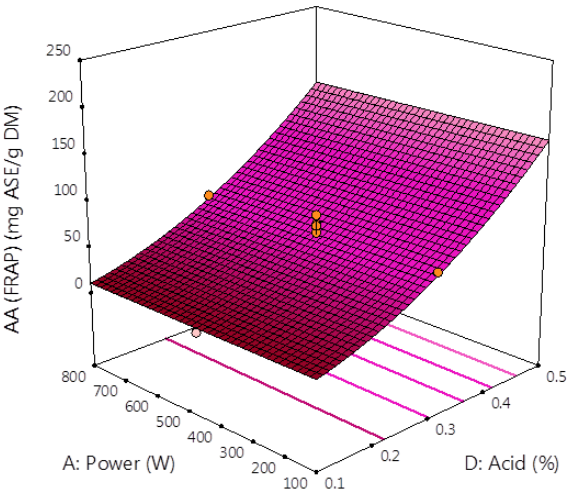

AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
150
120
90
60
30
B: Time (s)

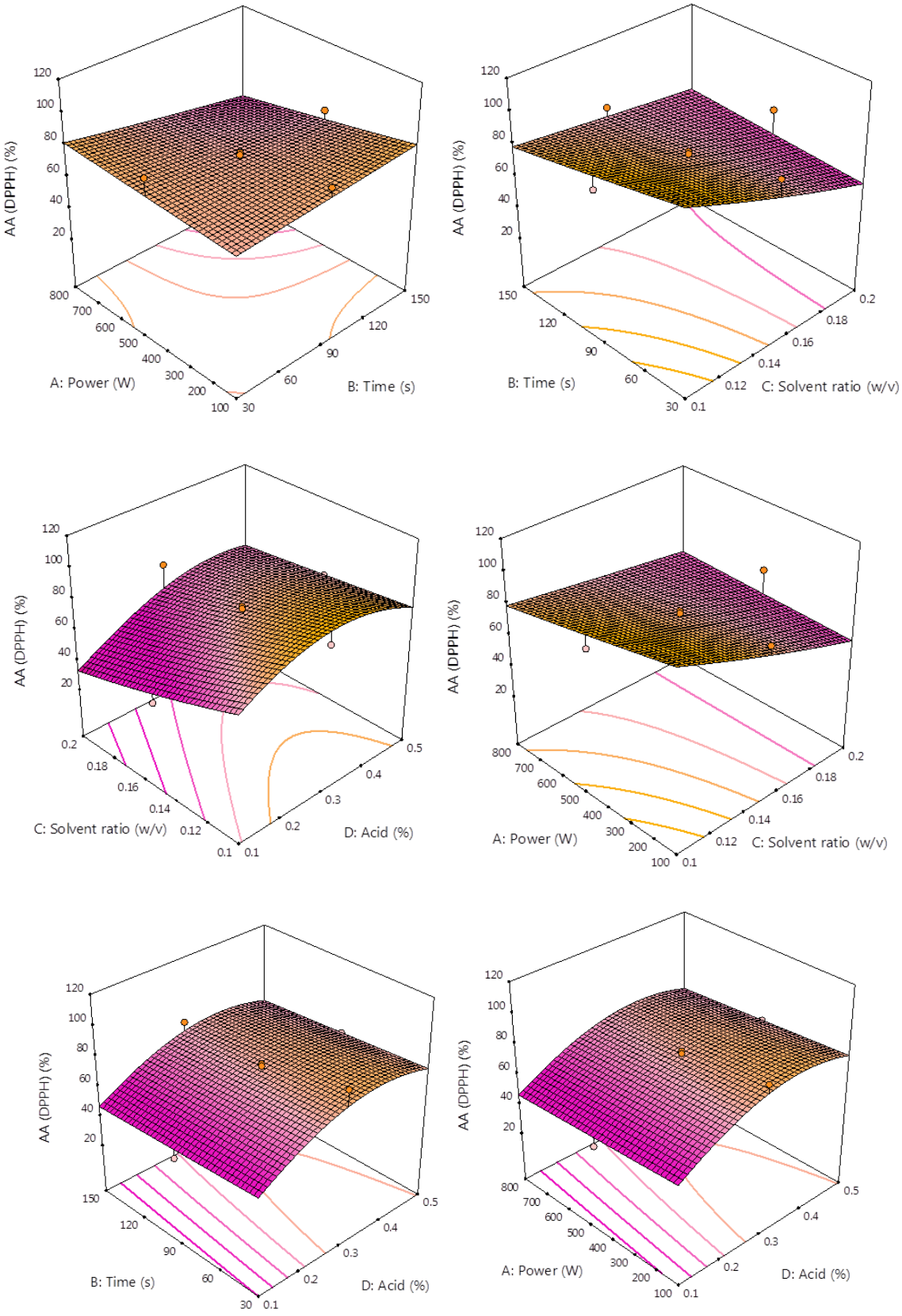

AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
150
120
90
60
30
B: Time (s)
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)


AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)

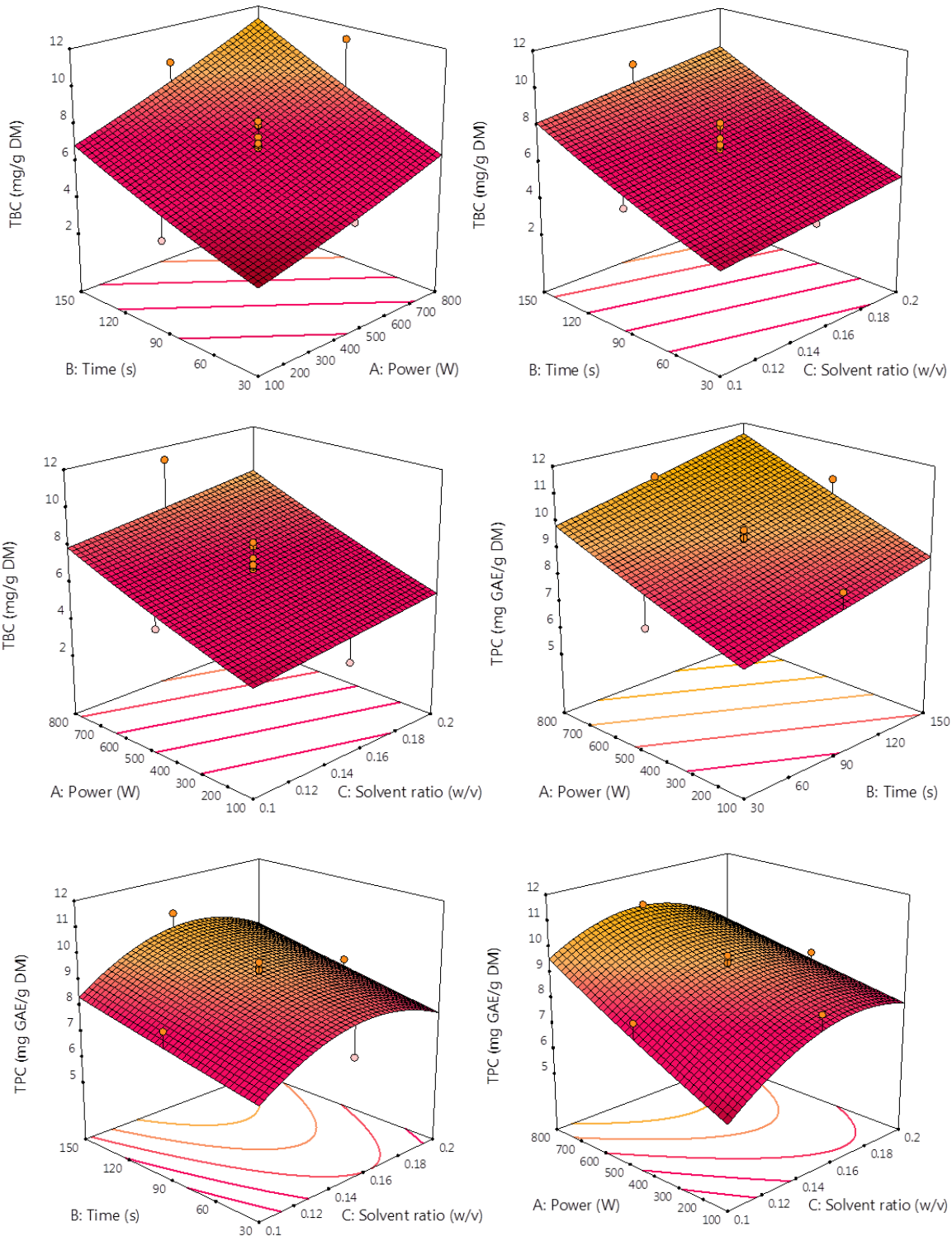

AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)

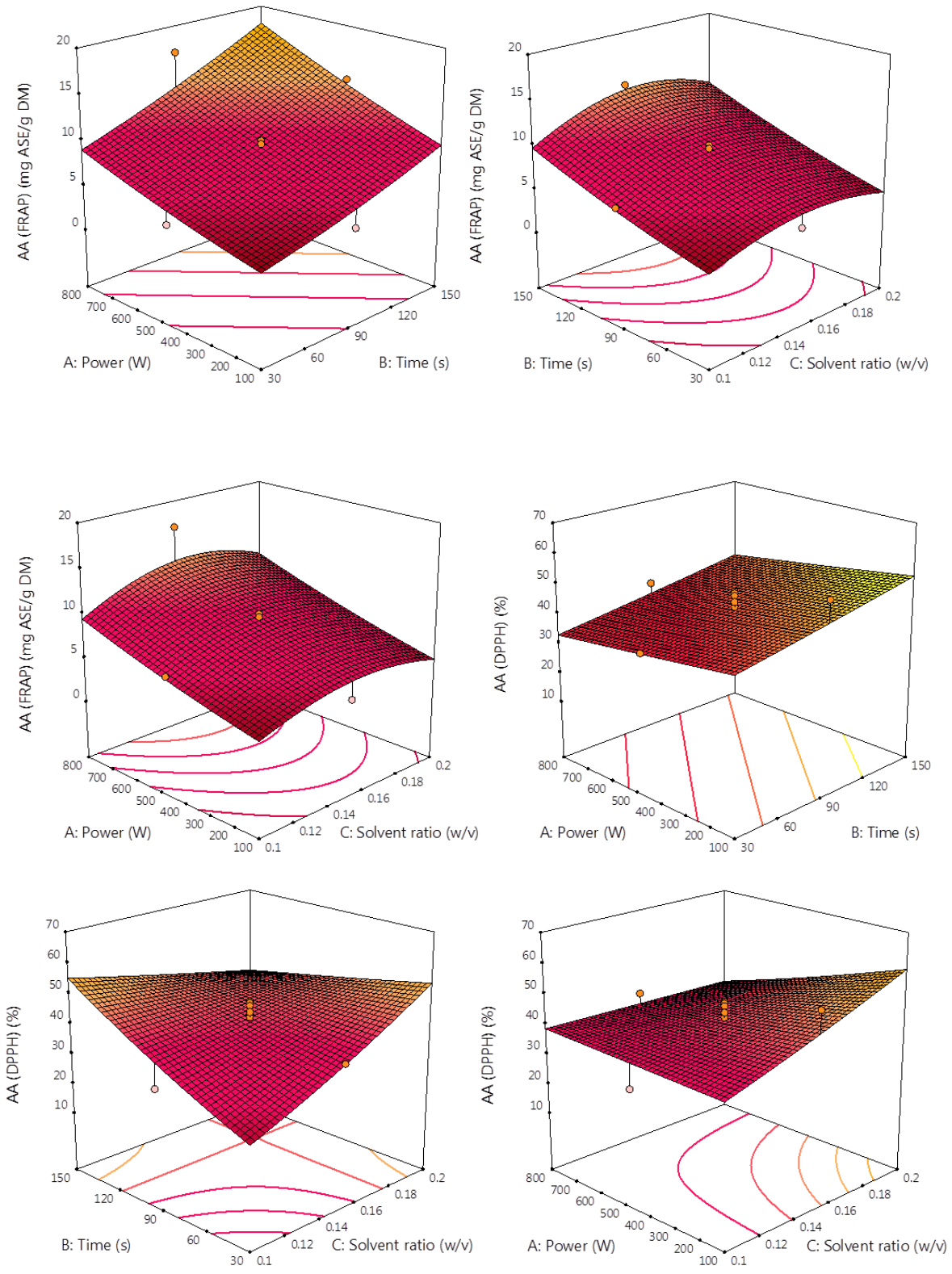

AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
150
120
90
60
30
B: Time (s)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)


AA (FRAP) (mg ASE/g DM)
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)

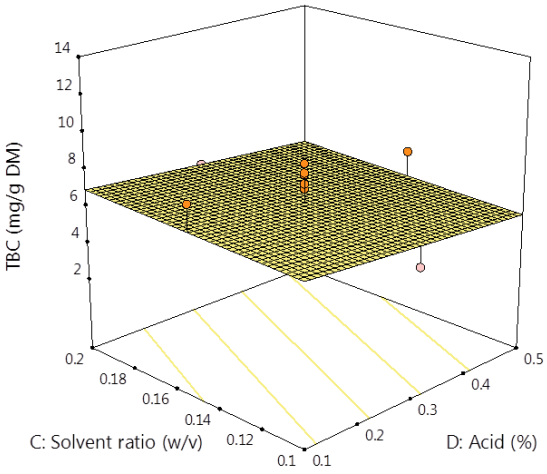


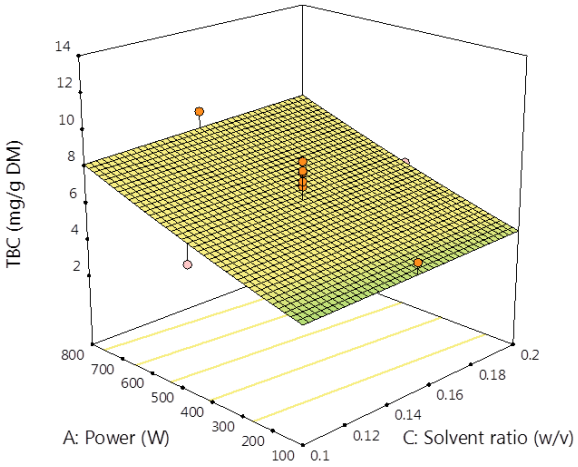
Appendix-Figure 2: 3D surface graphs for each response influenced by individual factors in the AW extracts of beetroot (Cylindra) peel (Sample B)

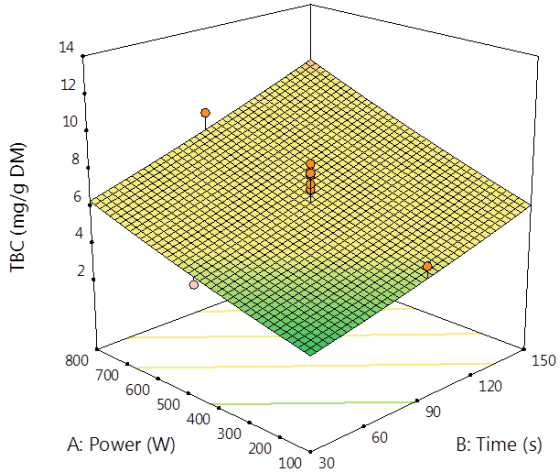

TBC (mg/g DM)
12
10
8
6
4
2
150
120
90
60
30
B: Time (s)
100
200
300
400
500
600
700
800
A: Power (W)
TBC (mg/g DM)
12
10
8
6
4
2
150
120
90
60
30
B: Time (s)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)
TBC (mg/g DM)
12
10
8
6
4
2
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)
TPC (mg GAE/g DM)
12
11
10
9
8
7
6
5
800
700
600
500
400
300
200
100
A: Power (W)
30
60
90
120
150
B: Time (s)
TPC (mg GAE/g DM)
12
11
10
9
8
7
6
5
150
120
90
60
30
B: Time (s)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)
TPC (mg GAE/g DM)
12
11
10
9
8
7
6
5
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

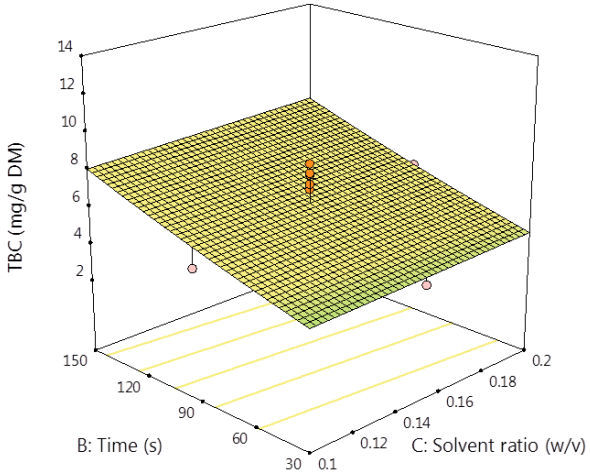


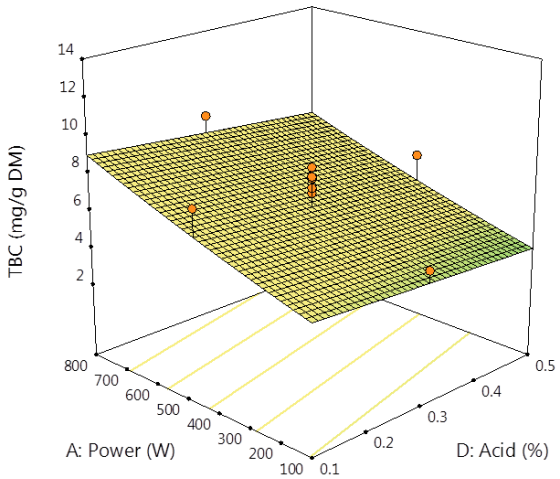
Appendix-Figure 3: 3D surface graphs for each response influenced by individual factors in the EW extracts of beetroot (Cylindra) peel (Sample C)

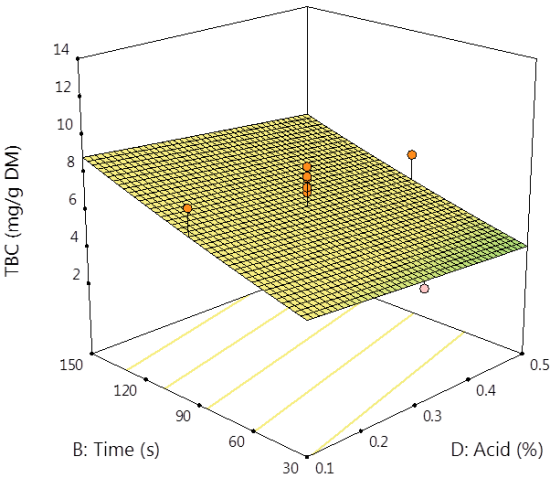

TBC (mg/g DM)
14
12
10
8
6
4
2
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

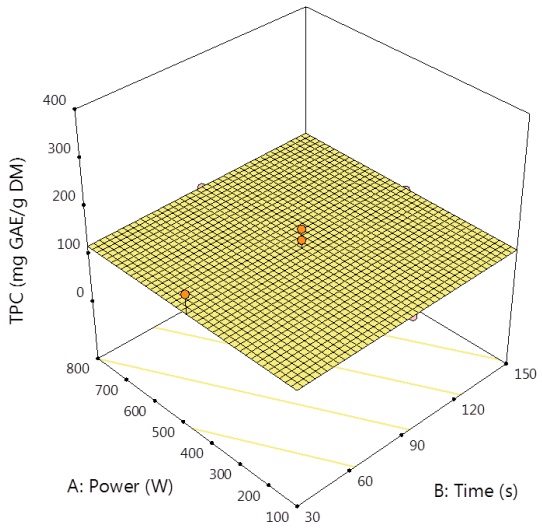

TBC (mg/g DM)
14
12
10
8
6
4
2
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

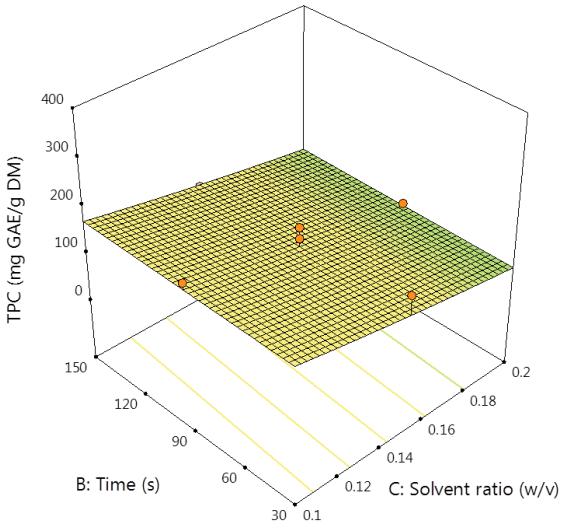

TBC (mg/g DM)
14
12
10
8
6
4
2
800
700
600
500
400
300
200
100
A: Power (W)
30
60
90
120
150
B: Time (s)

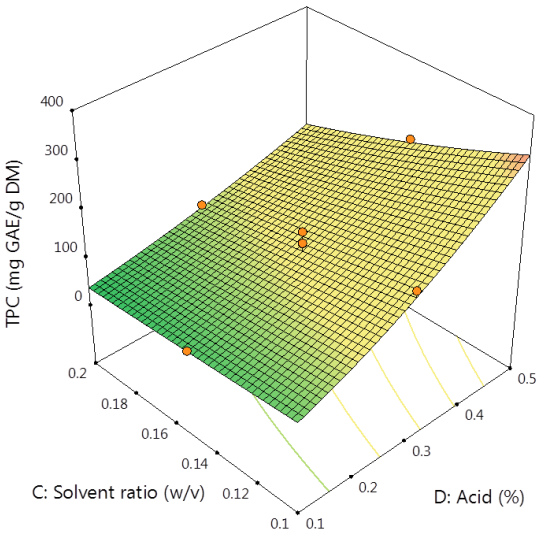

TBC (mg/g DM)
14
12
10
8
6
4
2
150
120
90
60
30
B: Time (s)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

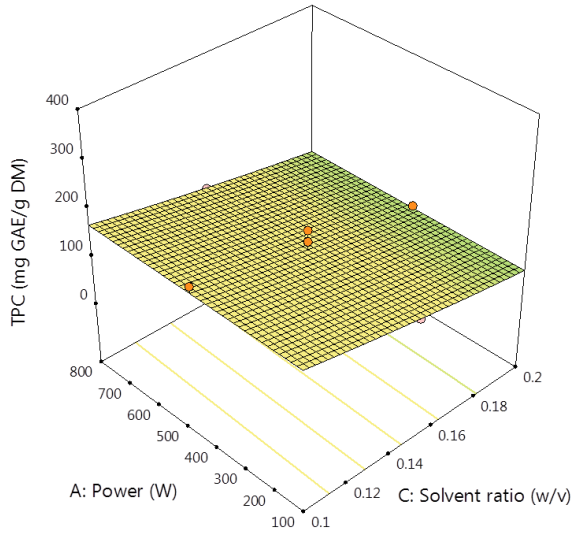

TBC (mg/g DM)
14
12
10
8
6
4
2
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

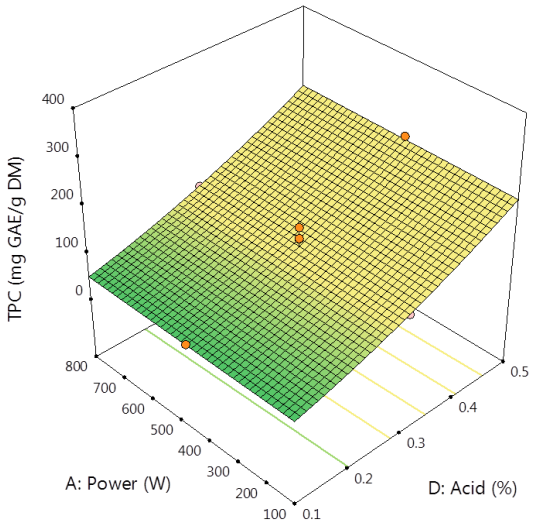

TBC (mg/g DM)
14
12
10
8
6
4
2
150
120
90
60
30
B: Time (s)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

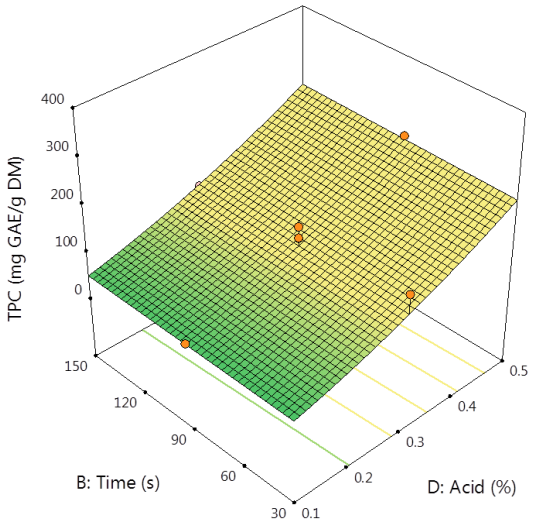

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
30
60
90
120
150
B: Time (s)

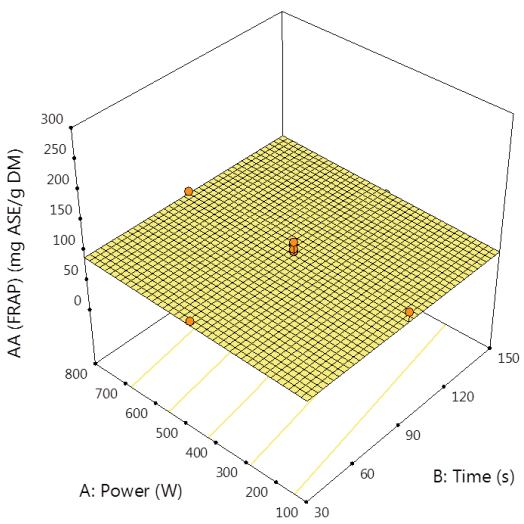

TPC (mg GAE/g DM)
400
300
200
100
0
150
120
90
60
30
B: Time (s)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

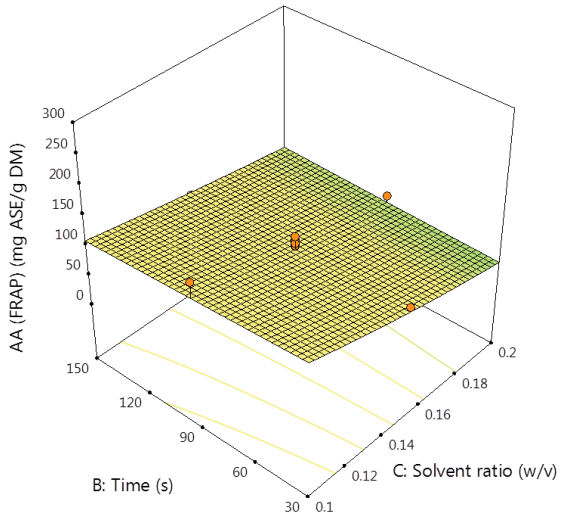

TPC (mg GAE/g DM)
400
300
200
100
0
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

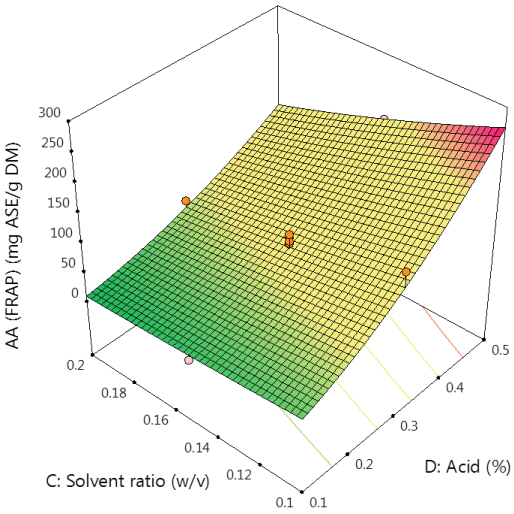

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.12
0.14
0.16
0.18
0.2
C: Solvent ratio (w/v)

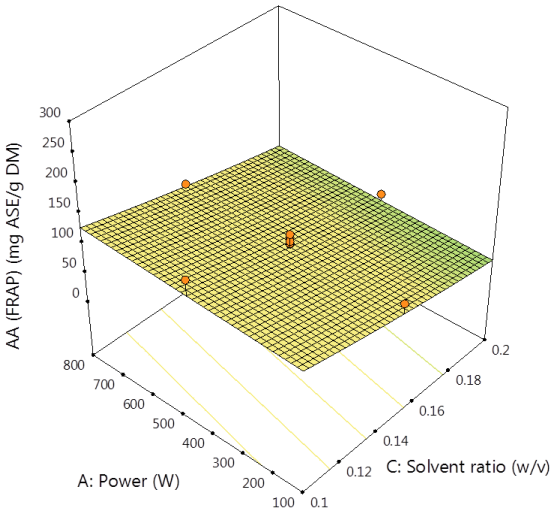

TPC (mg GAE/g DM)
400
300
200
100
0
800
700
600
500
400
300
200
100
A: Power (W)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

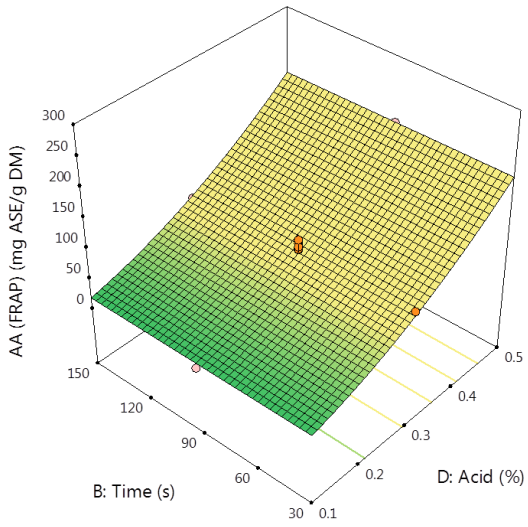

TPC (mg GAE/g DM)
400
300
200
100
0
150
120
90
60
30
B: Time (s)
0.1
0.2
0.3
0.4
0.5
D: Acid (%)

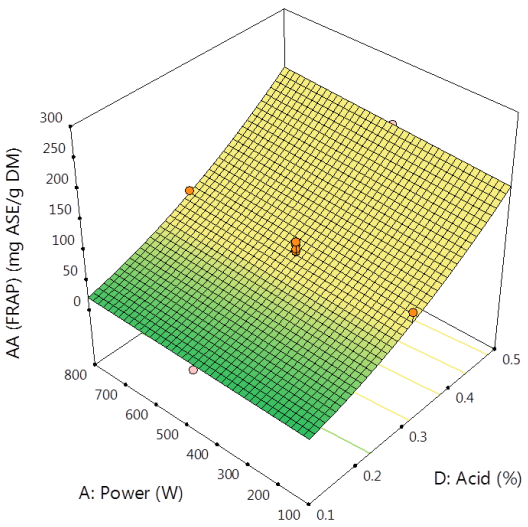

AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
150
120
90
60
30
B: Time (s)

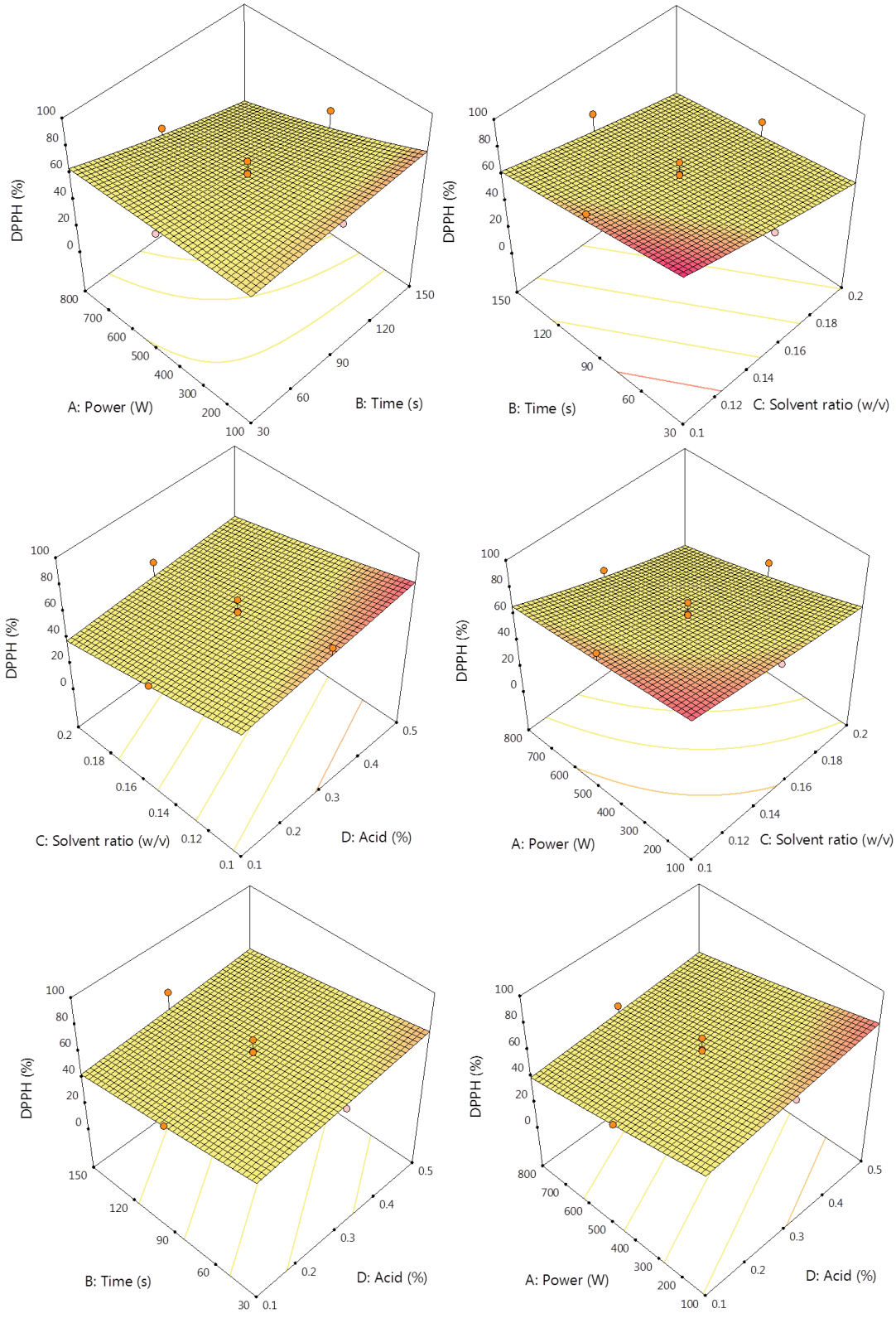

AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
150
120
90
60
30
B: Time (s)
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)


AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)

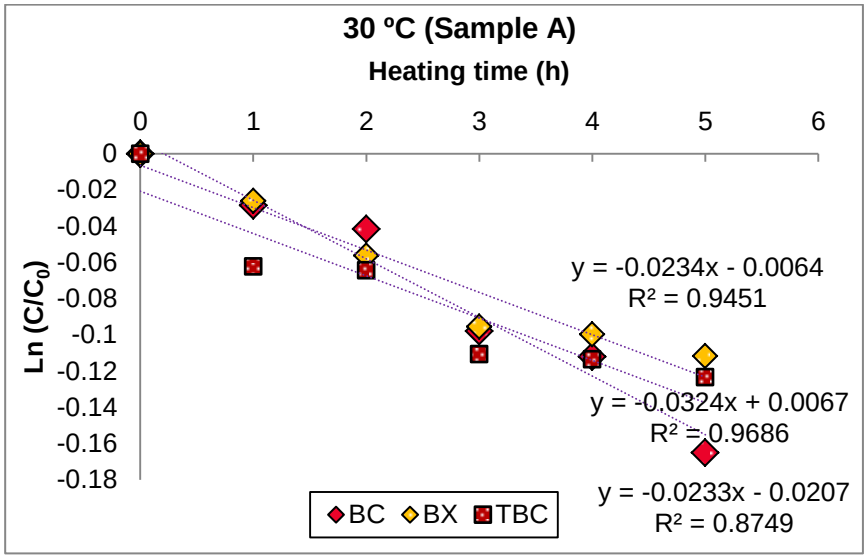

AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
0.2
0.18
0.16
0.14
0.12
0.1
C: Solvent ratio (w/v)

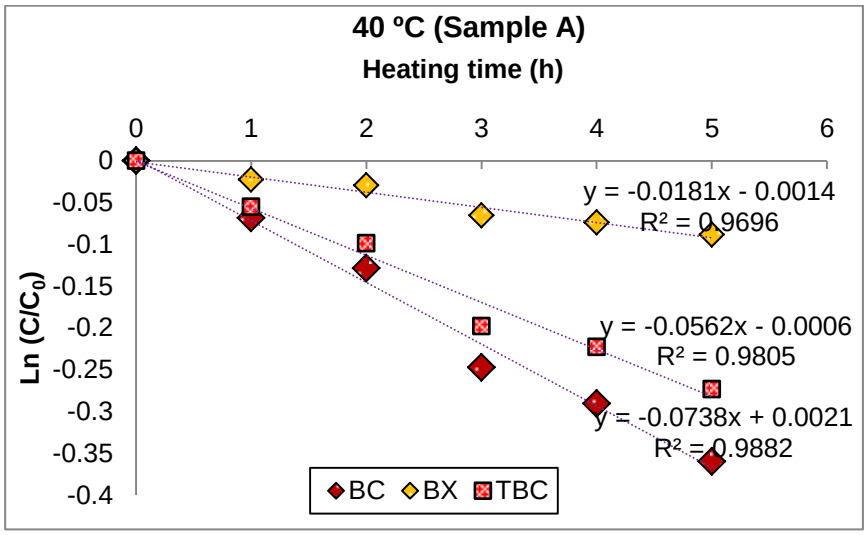

AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
150
120
90
60
30
B: Time (s)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)

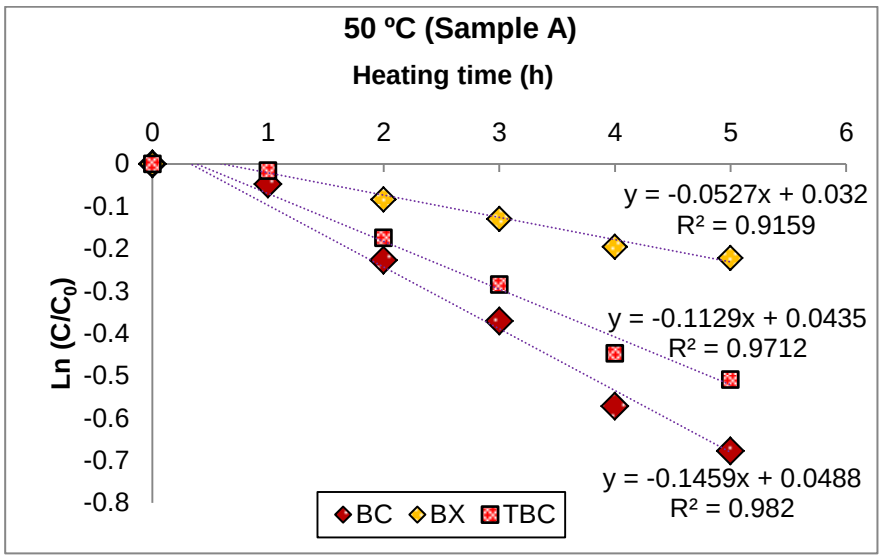

AA (FRAP) (mg ASE/g DM)
300
250
200
150
100
50
0
800
700
600
500
400
300
200
100
A: Power (W)
0.5
0.4
0.3
0.2
0.1
D: Acid (%)

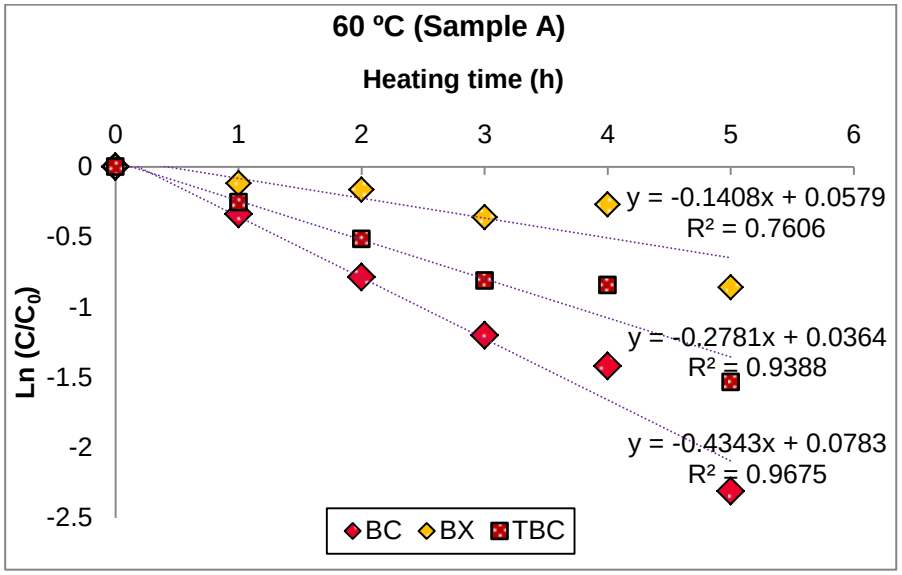


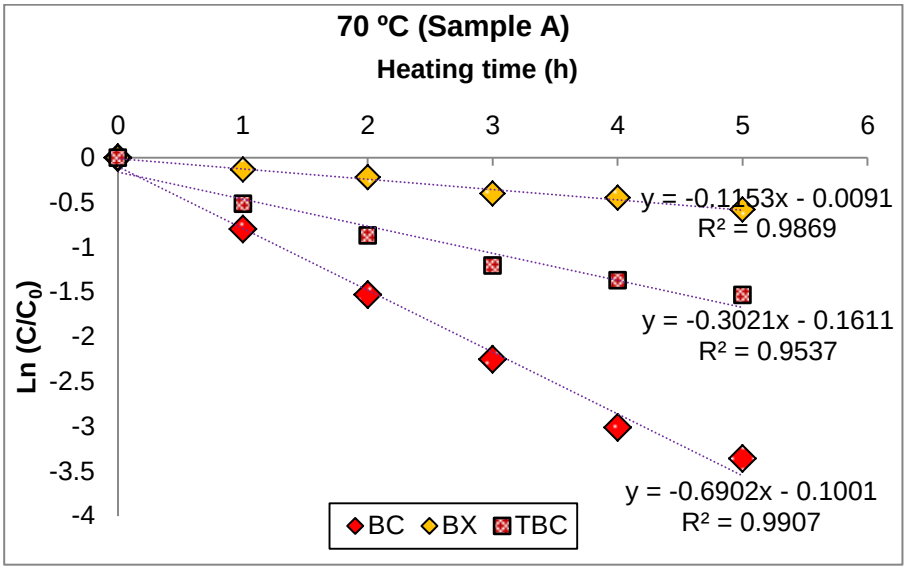
Appendix-Figure 4: 3D surface graphs for each response influenced by individual factors in the AEW extracts of beetroot (Cylindra) peel (Sample D)


30 ºC (Sample A)
Heating time (h)
Ln (C/C0)
y = -0.0234x - 0.0064
R² = 0.9451
y = -0.0324x + 0.0067
R² = 0.9686
y = -0.0233x - 0.0207
R² = 0.8749
BC
BX
TBC

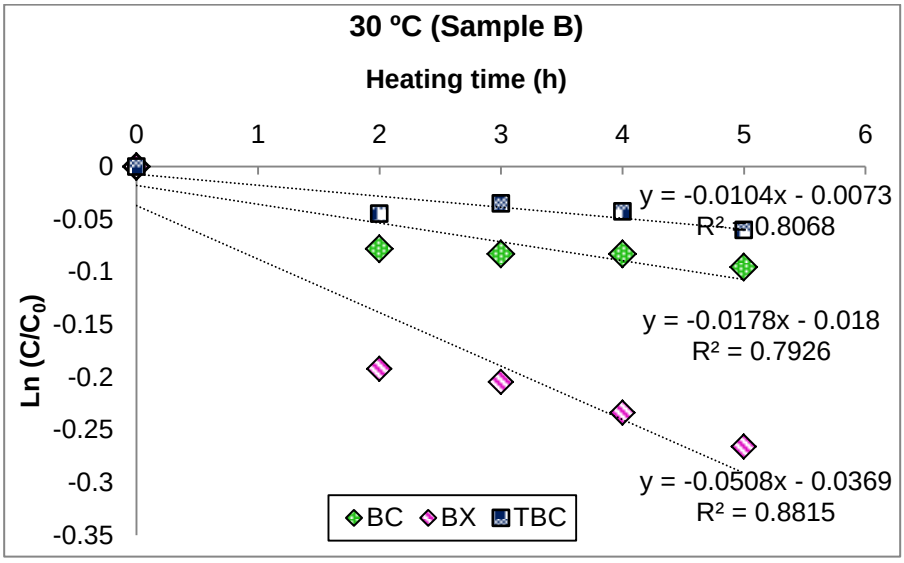

40 ºC (Sample A)
Heating time (h)
Ln (C/C0)
y = -0.0181x - 0.0014
R² = 0.9696
y = -0.0562x - 0.0006
R² = 0.9805
y = -0.0738x + 0.0021
R² = 0.9882
BC
BX
TBC

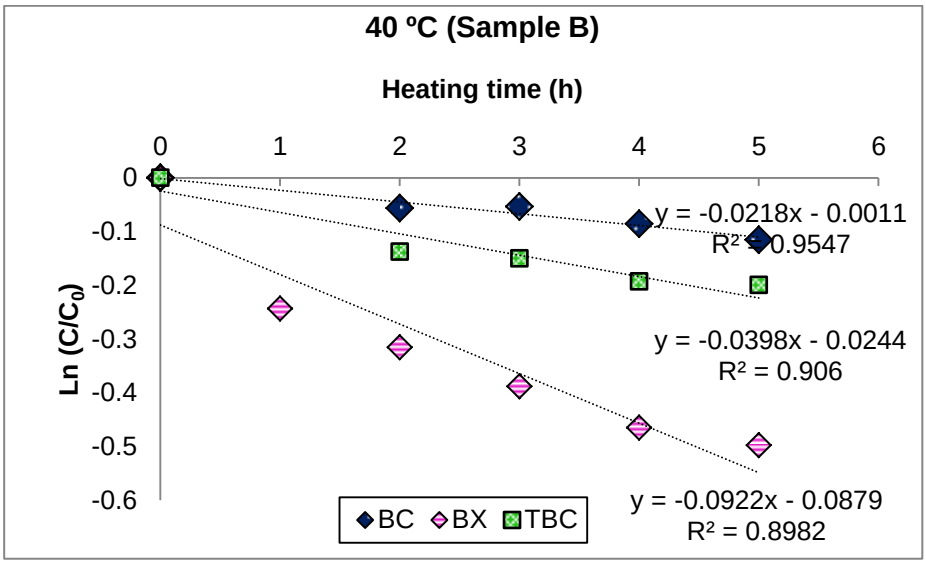

50 ºC (Sample A)
Heating time (h)
Ln (C/C0)
y = -0.0527x + 0.032
R² = 0.9159
y = -0.1129x + 0.0435
R² = 0.9712
y = -0.1459x + 0.0488
R² = 0.982
BC
BX
TBC

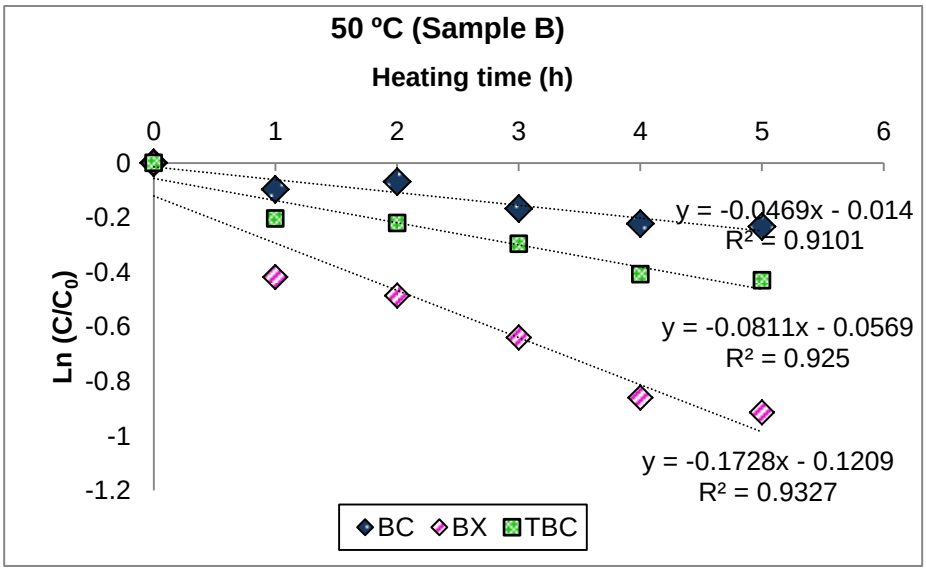


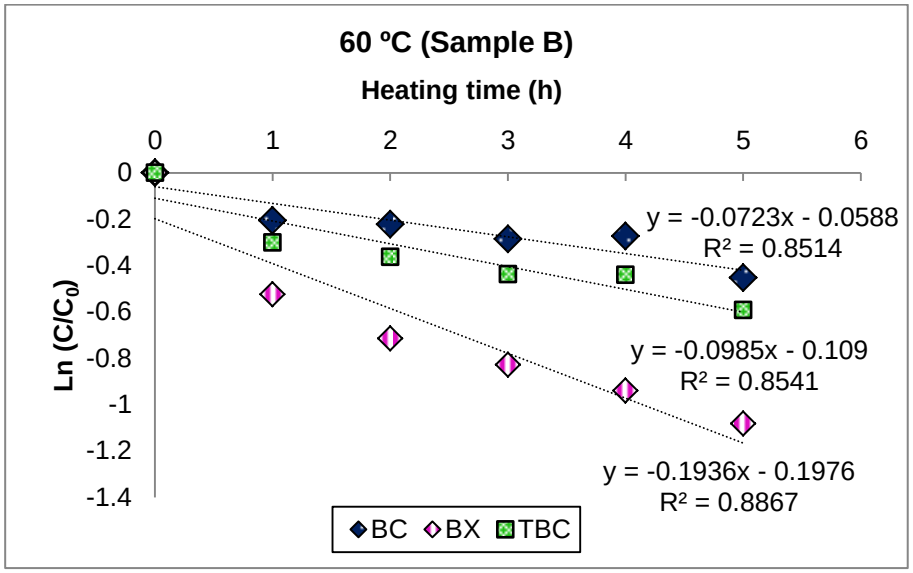


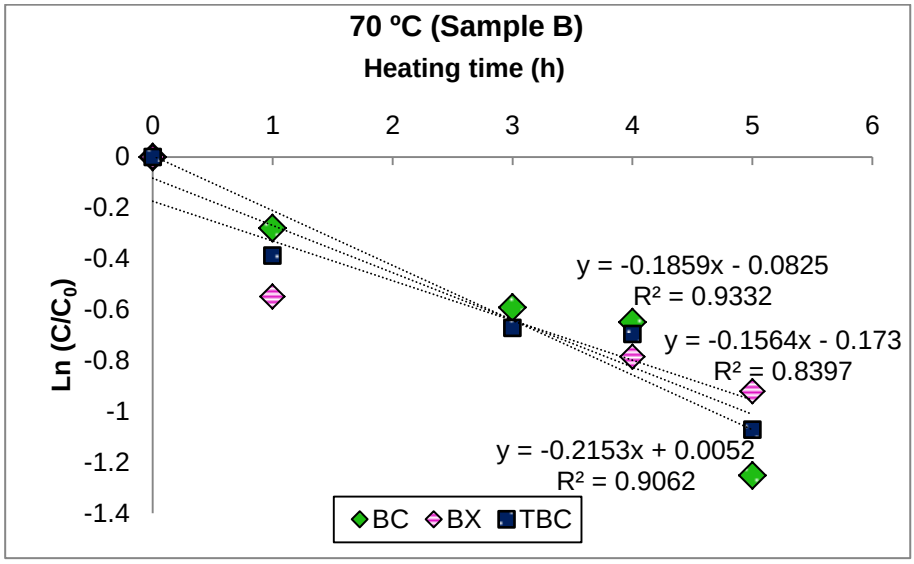
Appendix-Figure 5: Degradation kinetics of betalains in the microwave extract of beetroot (Cylindra) peel with PW solvent upon an elevated heating time (Sample A)


30 ºC (Sample B)
Heating time (h)
Ln (C/C0)
y = -0.0104x - 0.0073
R² = 0.8068
y = -0.0178x - 0.018
R² = 0.7926
y = -0.0508x - 0.0369
R² = 0.8815
BC
BX
TBC

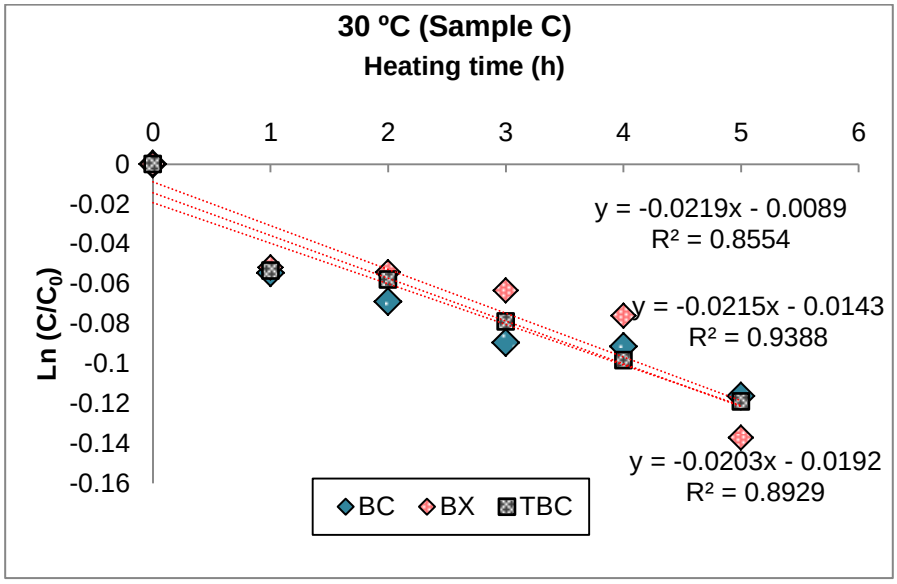

40 ºC (Sample B)
Heating time (h)
Ln (C/C0)
y = -0.0218x - 0.0011
R² = 0.9547
y = -0.0398x - 0.0244
R² = 0.906
y = -0.0922x - 0.0879
R² = 0.8982
BC
BX
TBC

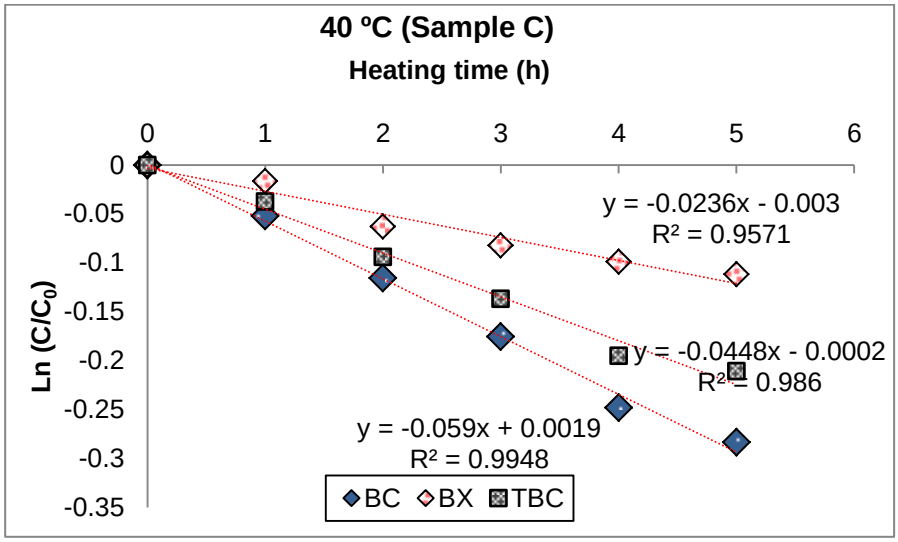

50 ºC (Sample B)
Heating time (h)
Ln (C/C0)
y = -0.0469x - 0.014
R² = 0.9101
y = -0.0811x - 0.0569
R² = 0.925
y = -0.1728x - 0.1209
R² = 0.9327
BC
BX
TBC

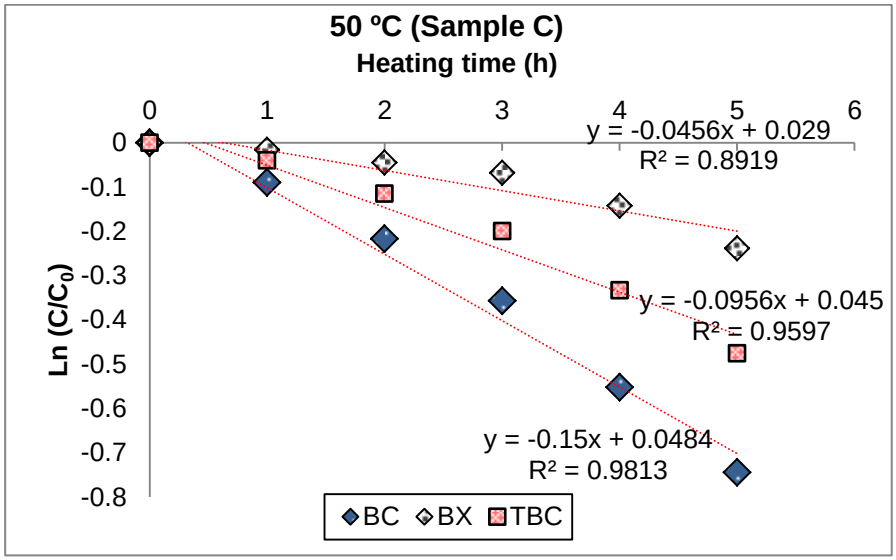


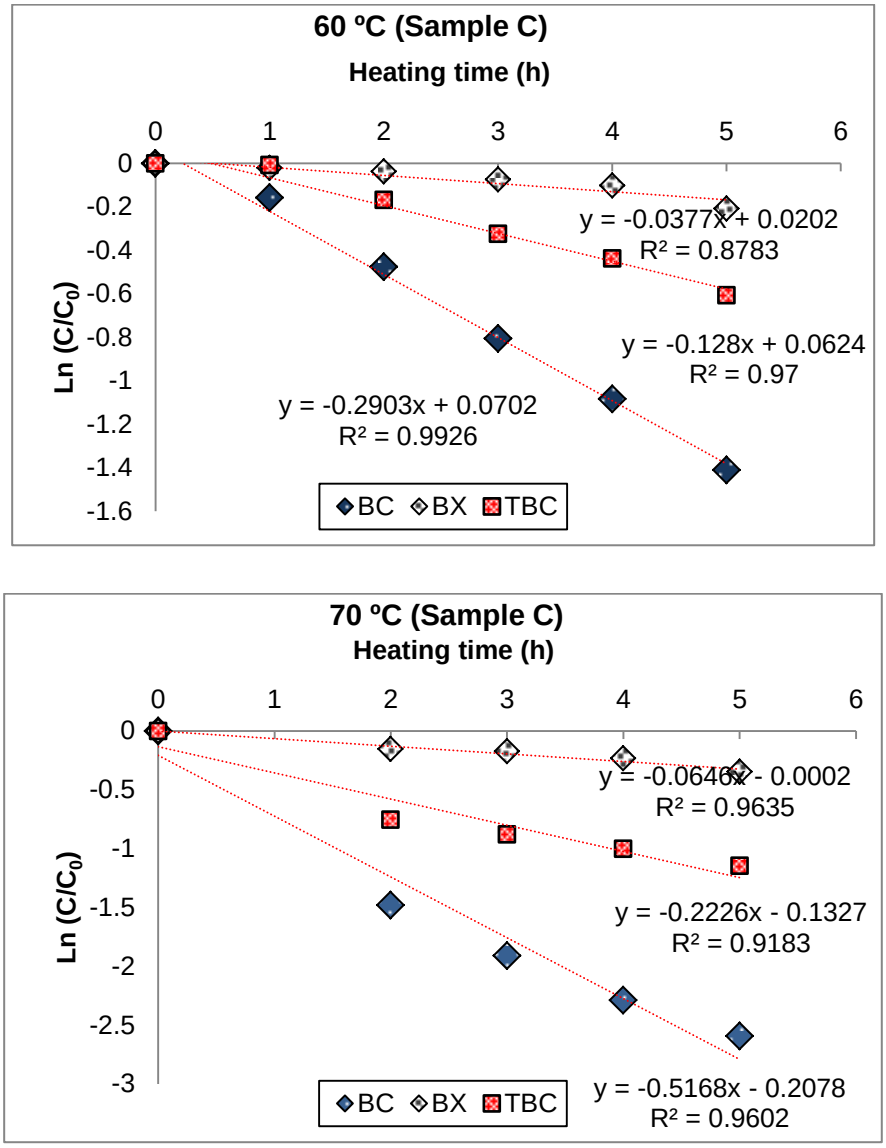


Appendix-Figure 6: Degradation kinetics of betalains in the microwave extract beetroot (Cylindra) peel with AW solvent upon an elevated heating time (Sample B)

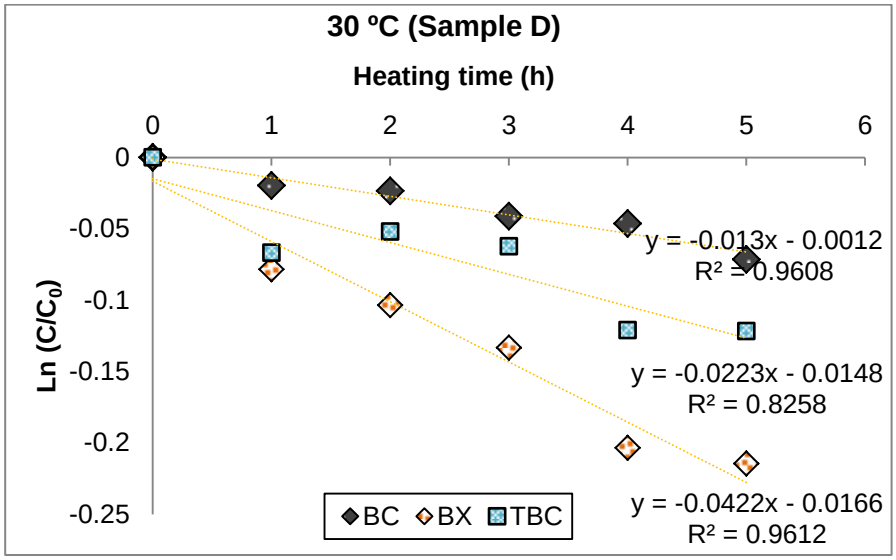

30 ºC (Sample C)
Heating time (h)
Ln (C/C0)
y = -0.0219x - 0.0089
R² = 0.8554
y = -0.0215x - 0.0143
R² = 0.9388
y = -0.0203x - 0.0192
R² = 0.8929
BC
BX
TBC

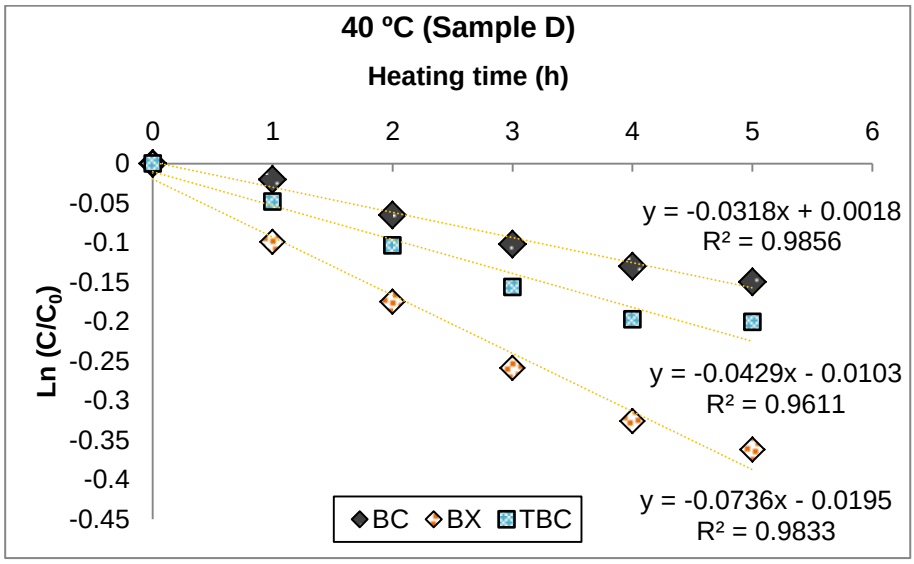

40 ºC (Sample C)
Heating time (h)
Ln (C/C0)
y = -0.0236x - 0.003
R² = 0.9571
y = -0.0448x - 0.0002
R² = 0.986
y = -0.059x + 0.0019
R² = 0.9948
BC
BX
TBC

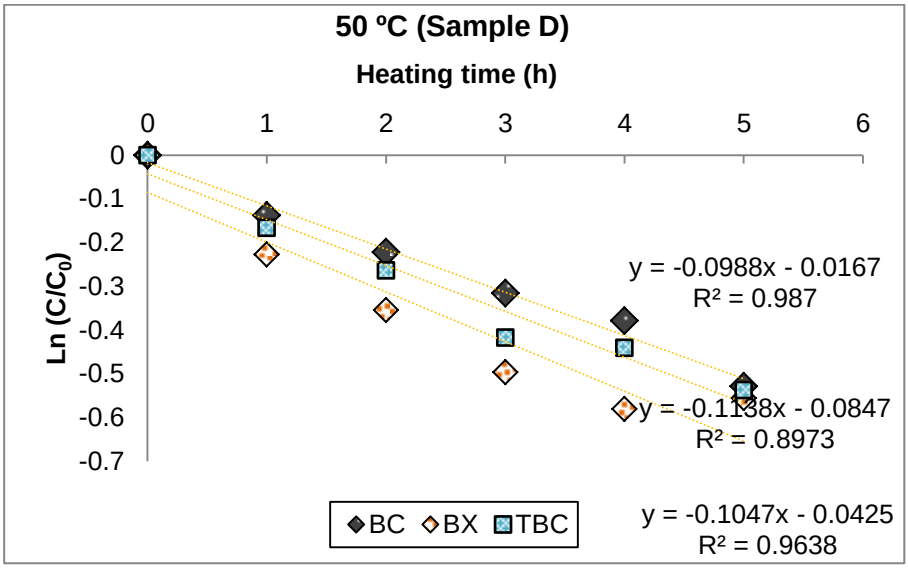

50 ºC (Sample C)
Heating time (h)
Ln (C/C0)
y = -0.0456x + 0.029
R² = 0.8919
y = -0.0956x + 0.045
R² = 0.9597
y = -0.15x + 0.0484
R² = 0.9813
BC
BX
TBC

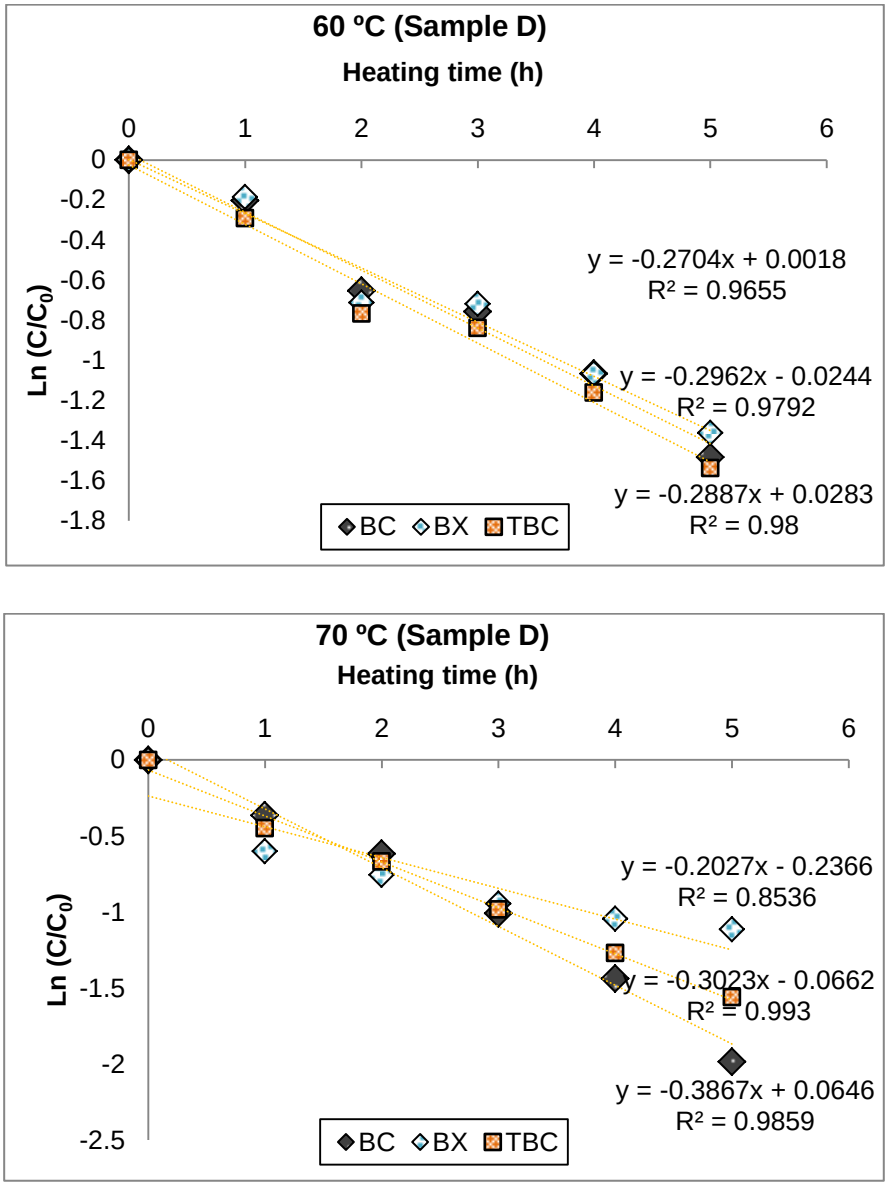


Appendix-Figure 7: Degradation kinetics of betalains in the microwave extract beetroot (Cylindra) peel with EW solvent upon an elevated heating time (Sample C)


30 ºC (Sample D)
Heating time (h)
Ln (C/C0)
y = -0.013x - 0.0012
R² = 0.9608
y = -0.0223x - 0.0148
R² = 0.8258
y = -0.0422x - 0.0166
R² = 0.9612
BC
BX
TBC


40 ºC (Sample D)
Heating time (h)
Ln (C/C0)
y = -0.0318x + 0.0018
R² = 0.9856
y = -0.0429x - 0.0103
R² = 0.9611
y = -0.0736x - 0.0195
R² = 0.9833
BC
BX
TBC


50 ºC (Sample D)
Heating time (h)
Ln (C/C0)
y = -0.0988x - 0.0167
R² = 0.987
y = -0.1138x - 0.0847
R² = 0.8973
y = -0.1047x - 0.0425
R² = 0.9638
BC
BX
TBC



Appendix-Figure 8: Degradation kinetics of betalains in the microwave extract beetroot (Cylindra) peel with AEW solvent upon an elevated heating time (Sample D)

8 ACKNOWLEDGEMENT

I would like to thank myself for not being given up easily and for enduring all the challenges that I have faced during the long PhD journey. I am still on track even if a lot of destructions like depression, anxiety, uncertainty, judgement, loneliness etc. have come along to me to bring me down all the way. I didn't fail myself and thrived instead. I am proud of myself for who I became.

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