Characterization of Cylindra Beetroot Wastes Volarized With Green Solvent by Thermal Emerging Technology (Microwave Irradiation)

Moh Moh Zin (zinlay.net.mm@gmail.com)
Hungarian University of Agricultral and Life Sciences https://orcid.org/0000-0001-5993-4308
Szilvia Bánvölgyi
Hungarian University of Agricultural and Life Science

Research Article

Keywords: beetroot wastes, microwave-assisted extraction, betalains, phenolics, flavonoids, antioxidants

Posted Date: March 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-259112/v1

CHARACTERIZATION OF CYLINDRA BEETROOT WASTES VOLARIZED WITH GREEN SOLVENT BY THERMAL EMERGING TECHNOLOGY (MICROWAVE IRRADIATION)

Moh Moh Zin[1]*, Szilvia Bánvölgyi[1]
[1]Department of Food Engineering, Hungarian University of Agricultural and Life Sciences, Ménesi Street 44, 1118 Budapest, Hungary
zinlay.net.mm@gmail.com
ORCID: 0000-0001-5993-4308

Abstract

Accordingly, with the benefits of high temperature with short time treatment, microwave irradiation has conquered novel extraction technology to volarize food wastes. Compared to other unconventional ways, microwave-assisted-extraction (MAE) is superior for its accordance with green solvents due to the mode of heating which based on the dielectric constant level of solvents. In this study, the extraction processes of bioactive compounds from waste parts of beetroot were accomplished using a home-use microwave oven. Aside from peel; stalk and flesh were utilized for extraction at three coded levels of process conditions (low, medium, and high) for comparison. Control samples were prepared at 70ºC for 1 h extraction time with 1:10 w/v solvent ratio. Spectrophotometric analysis was performed for approaching bioactive compounds existing in specific parts of the beetroot. Compared to the control, approximately 2.2 times of total betalains, phenolics, flavonoids, and the relevant antioxidants were scavenged at 800 W of microwave power together with 150 s of extraction period following the descending order: peel, flesh, and stalk. Our observation is a prove of the high extraction efficiency of green solvent since pure water solvent brought more amount of specific bioactive compounds than aqueous ethanol under the same studied process conditions.

Keywords: beetroot wastes, microwave-assisted extraction, betalains, phenolics, flavonoids, antioxidants

Statement of Novelty

In the last decades, the volarization of beetroot wastes has been explored with several techniques from extraction to their application in the food industries, however, some conflicts still persist in the process optimization as regards their sensitivity to operation conditions. Not only that, as the yields of the targeted bioactive compounds rely on the genotype, season and sowing dates, wider availability of valuable data becomes necessary for the better understanding of their behaviour. It can be expected that our observation could become one of the reliable references in the study field of the volarization of antioxidant-rich bioactive compounds from beetroot processing wastes with microwave irradiation as a novel extraction technology.

Introduction

Food additives whether compulsorily or obligatorily applied in food processing remains controversial except their nutritional values are modified. In recent days, tackling food wastes starts from the utilization of the industrial fruit and vegetable residues, peels as food-grade materials in this regard have been approached in several ways. As the foremost purpose of recovering the industrial food discharges was for ruminant feeds and landfills, somehow, the characterization of the residues was not included in the priority list. Likewise, as the disposal of food wastes from the agro-industries originated from in the handling of fruits and vegetables during processing; peeling and trimming of them play the major role to minimize the residues. Hence, those were performed in different ways under the scope of high outputs of targeted products. Therefore, some peeling methods including knife peeling, dry or wet lye peeling, frozen peeling, hot water peeling, steam peeling, high temperature peeling and chemical peeling had been explored to reduce the waste discharges from the plants which in turn leads to the assurance of total inputs usage.

Bio extracts property as antioxidants have been accepted as hygienic food additives despite their uncommon applications as preservatives [1, 2]. Antioxidants are substances with lower concentration than the oxidizable substrates [3] and their major function is the scavenging of harmful radicals such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl ions (OH), and lipid peroxyl radical (LOO) etc. They scavenge these reactive oxygen species (ROS) before they cause lethal damages of cardiovascular diseases, chronic oxidative stress diseases, ROS pathogenesis of acute central nervous system (CNS) injury, and Alzheimer's disease to the cardinal cellular components such as DNA, proteins and lipids [4]. This free radicals modulation can be enzymatic or non-enzymatic [5]. Since plant secondary metabolites are condensed with antioxidant-rich micronutrients such as vitamin C and E, which can not be produced in our bodies, it is necessary to have enough supply of antioxidants to defend against these free radicals [6]. Here, the recommended daily intake of antioxidants is 75 mg for women and 90 mg for men [7]. Vitamins A and 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 [8,9].

In the Caryophyllales plant group, Amaranthaceae and Cactaceae species are the well-known sources for bio-extracts and rich in betalains and phenolics [10]. Beetroot (*Beta vulgaris ssp. esculenta var. rubra L.*), belonging to Chenopodiaceae (Amaranthaceae) family, is a prominent vegetable ranked in the topmost list of highly nutritive vegetables fortified with antioxidants [11]. Furthermore, beetroot has some benefits for skin and it is another alternative source of carotenoids, which is well known for hair proliferation [12]. Subsequently, beetroot betalain which has occupied the food additives market is copiously available as a natural colorant in possession of strong natural red-violet colour and functionality. Acknowledging their content of minerals, proteins, carbohydrates and dietary fibres, beetroots as supplementary foods are available in powder and capsulated forms to cure chronic inflammation and oxidative stress. Aside from the whole taproot; foliage/leaf, stalk, and peel contain comparable amounts of nutrients and bioactive compounds. Like the other suppliers of dietary nitrate, beetroots are loaded with a chief source of inorganic nitrate (250 $mg \cdot kg^{-1}$ of fresh weight). Hence its application for the enhanced performance of nitric oxide in the blood circulation, along the line, reducing the nitric oxide deficiency-related diseases is one of its many benefits [13, 14]. Apart from the other bioactive compounds such as ascorbic acid, carotenoids, phenolic acids and flavonoids, the content of betalain pigment is of most important in red beetroots. Two major betalain components are red-violet betacyanins (available as betanin, E-162) and yellow betaxanthins with concentration ratio usually in the range of 1-3 with dependence mainly on different parts of the beetroot as well as varieties or genotype, cultivars, seasonal, sowing and harvesting time, environment throughout thriving, and even the supplement of water [15, 16]. Natural betalain colors are available in the market in powder form and concentrated form as well. As natural products, some challenges are still encountered in case of concentrating and drying them to overcome their deterioration during storage. For drying purposes, vacuum drying, spray drying and freeze drying have been widely investigated [17-19].

Electromagnetic waves with frequencies between 300 MHz and 300 GHz are known as microwaves in which the degree of absorptivity or transmissivity relies on the electromagnetic properties of the treated object such

as dielectric property, polarity, permittivity as well as the shape of the object [10]. With enhancements in food quality, microwave heating is versatile in food processing starting from blanching to thawing the frozen foods despite certain drawbacks of considerable cost and need for proper control to avoid uneven heating [20]. In dielectric heating of foods, the heating phenomenon originates from the transformation of electromagnetic energy to calorific energy by the friction of the ions and rotation of the diploes inherently located inside the foods [10]. Therefore, the economic advantage of microwave extraction of plant-based bioactive compounds is somehow quite reasonable compared to conventional ways considering less power usage and time consumption. In MAE, the perspective of solvent choice for extractions is mainly focused on safety, economics and environmental friendliness. Continuous increase of interest in the how to dispose the dough or cake post extraction, which mostly is a function of the solvent used during the extraction, has drawn huge attention to MAE due to their capability of solventless 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. Many researchers have described aqueous ethanol (50%) as the most suitable solvent for MAE under the range of their studies. Fundamentally, our study focused on the comparison of the effects of the combined solvent (15 % ethanol) and pure water on the extraction of the bioactive compounds under microwave irradiation. Moreover, the extractable amounts of bioactive compounds from the different parts of the beetroot under microwave treatment with the greenest solvent on earth were compared.

Materials and methods

Chemicals and reagents

Ethanol (≥99%, REANAL LABOR, Co., Hungary) was used for the extraction purpose. The applied chemicals and reagents for the analysis were collected per following lists: methanol (LACH-NER, Co., Czech Republic), citric acid (≥95%, Carl Roth GmbH + Co., Germany), gallic acid (SIGMA-ALDRICH, Co., USA, product of China), acetic acid (96%, REANAL LABOR, Co., Hungary), L-ascorbic acid (reagent grade, SIGMA-ALDRICH, Co., USA, product of China), hydrochloric acid (37%, CARLO ERBA Reagents S.A.S, France), quercetin (≥95%, SIGMA-ALDRICH, Co., USA, product of China), Trolox (97%, SIGMA-ALDRICH, Co., USA, product of Denmark), disodium hydrogen phosphate (98.5%, ACROS ORGANICS, Co., Spain), sodium hydroxide (MOLAR Chemicals KFT, Hungary), sodium carbonate (MERCK KGaA, Germany), sodium nitrite (LACH-NER, Co., Czech Republic), sodium acetate (Analar grade, VWR Chemicals, Belgium), 2,4,6-Tris(2-pyridyl)-s-triazine (≥99%, SIGMA-ALDRICH, Co., USA), Folin-Ciocalteu reagent (SIGMA-ALDRICH, Co., USA), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (SIGMA-ALDRICH, Co., USA), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (≥98%, TLC, SIGMA-ALDRICH, Co., USA, Product of Canada), potassium persulfate (REANAL LABOR, Co., Hungary), sodium chloride (VWR Chemicals, Belgium), potassium chloride (LACH-NER, Co., Czech Republic), aluminium chloride (99%, ACROS ORGANICS, Co., Germany), ferric chloride (REANAL LABOR, Co., Hungary), and potassium dihydrogen phosphate (REANAL LABOR, Co., Hungary).

Raw materials

Raw materials (Cylindra beetroots) were supplied by a local bio farm, Budapest, Hungary. Cleaning, peeling, and chopping of the beetroot parts (stalk, flesh, and peel) were completed manually. Grinding of the different portions was done using GM 200 pulverizer to improve the efficiency of MAE by enlarging the active surface area for improved contact with the solvent. For the treatment of microwave, pulse mode and cooling in between with icy water were performed to avoid superheating of the solvent plus evaporation. 30 s on 15 s off followed by 15 s on 15 s off (till the time was up) was considered based on the pretest. The different MAE extracts were analyzed by Spectronic Genesys 5 Spectrophotometer (MILTON ROY, U.S.A) for the quantification of bioactive compounds. All measurements were triplicated and averaged.

Color ($L^*a^*b^*$) measurement

CIE 1976 $L^*a^*b^*$ is the uniform color scale of upmost used in the visualization of the appearance of foods in which the interpretation of color tonation is based on the respective color coordinates: L^* for lightness (the closer to 100, the lighter in color), a^* redness or greenest (the higher in the positive value, the more redness in color), and b^* (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 color intensity is expressed by Chroma [21, 22]. CHROMA METER CR-400 was utilized for color patterns (lightness, redness, and yellowness) differentiation. Calibration was performed with calibration tile before the measurements. Total color difference (ΔE) was calculated as follows [23]:

$$\Delta E = \sqrt{\Delta L^* + \Delta a^* + \Delta b^*}.$$

Whereby, 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 or saturation and Hue angle was estimated from a^* and b^* per following equation (Antigo et al., 2017);

$$Chroma = \sqrt{a^* + b^*},$$

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

Betalain color compounds measurement (Nilson's method)

Nilson's method was applied for the quantification of betaxanthin (BX), betacyanin (BC), and total betalain colour compounds (TBC). The samples were diluted properly with McIlvaine buffer (pH=6.5) before reading the absorbance and the calculation was done by Beer-Lambert law [24, 25];

$$c = \frac{A\ x\ DF}{\varepsilon\ x\ l}$$

Where; c is molar concentration, A is real absorbance, DF is the dilution factor, ε is molar attenuation coefficient ($E_{1\%}^{1cm}$ = 1120 for betacyanin and $E_{1\%}^{1cm}$ = 750 for betaxanthin), and l is the path length (1 cm).

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

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

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

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

Total phenolic compounds (Folin-Ciocalteu method)

Visualization of total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method [26]. 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 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 and the amount of TPC was calculated as:

$$TPC = \frac{A \cdot 2500 \cdot DF}{S \cdot a}\left[\frac{mg\ GAE}{L}\right],$$

whereby *A* is the measured absorbance; *S* is the amount of sample (µL); *a* is the slope of calibration curve; *DF* is the dilution factor.

Total flavonoid compounds (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. [27]. Quercetin (100 mM to 500 mM) 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 10 % $AlCl_3$ and waited for 5 min. Later on, 0.3 mL of 5 % $NaNO_2$ 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.

$$TFC = \frac{A \cdot TS \cdot DF}{S \cdot a}\left[\frac{mg\ QUE}{L}\right],$$

whereby *A* is the measured absorbance; *TS* is the total amount of sample solution (µL); *DF* is the dilution factor; *S* is the amount of the sample; *a* is the slope of the calibration curve.

Antioxidant activity (AA) assays

FRAP Method

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 donation may depend on the nature and specific property of the measured antioxidant [6]. FRAP reagent was prepared with acetate buffer solution, ferric chloride solution, and TPTZ solution. FRAP reagent (1500 µL) was mixed with (30 µL) distilled water followed by 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 following equation;

$$AA = \frac{A \cdot 1550 \cdot DF}{S \cdot a}\left[\frac{mg\ ASE}{L}\right],$$

where *A* is the absorbance; *S* is the amount of sample (µL); *a* is the slope of the calibration curve; *DF* is the dilution factor.

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. [28] was performed. The stock solution was prepared with 22 mg of DPPH dissolved in 50 mL of pure methanol. To acquire 6×10^{-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 30 minutes. Optical density (OD) was then detected at 515 nm and (%) radical scavenging activity was predicted as follows;

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

ABTS Method

ABTS assay was performed following Ilyasov et al. [29] with ABTS 2,2'-Azino-bis (3-ethylbenzothiazoline-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 (7mM) was dissolved in potassium persulfate (2.45 mM) and the mixture was allowed to stand in the dark for minimum 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 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 of the samples was calculated as follow;

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

Statistical analysis

Statistical analysis was implemented with DOE, design of expert software (version 11.0.3). Response surface methodology (RSM) in terms of central composite design (CCD) was applied to perform the process optimization based on the resulted 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 pure water solvent was done for twenty experimental runs with three process variables and six outcomes. Those variables are microwave power, treatment time, and solid-to-solvent ratio (Table 1). The following equations which express the model written in terms of usual coded variables can be used to convert them into actual variables;

$$A = \frac{Power - 450}{350}, B = \frac{Time - 90}{60}, C = \frac{Solvent\ ratio - 1.5}{0.5}$$

Table (1) Process data for the experimental runs in terms of both natural and coded variables

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

Experimental results

RSM of peel-water extracts

The experimental values of the recovered betalains, total phenolics, and their respective antioxidant activities from the peel of Cylindra type beetroot (*Beta vulgaris L.*)with pure water solvent via MAE were denoted by the cubes built by the DOE model (Fig. 1). Herein, the highest amounts of bioactive compounds recovered at 800 W of microwave power for 150 s of irradiation time with 0.2 w/v solvent ratio are as follows; BX (231.78±2.15 mg/L), BC (172.42±2.32 mg/L), TBC (404.17±4.47 mg/L), TPC (720.48±17.75 mgGAE/L), TFC (296.67±5.77 mgQUE/L), radical scavenging activities by FRAP method (1171.72±25.23 mgASE/L), DPPH method (94 %) and ABTS method (99 %), respectively. Table 2 represents the analysis of variance for each response speculated from twenty runs which were performed according to the central composite design model. Since the model suggested 2FI function, it was used to fit the model with some modification. As shown in the table (2), 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. Among the factors, the solvent ratio is the most significant ($p<0.0001$) compared to power and time but those are also

significant to some point. Being 2FI model, the interaction between the independent variables is found to be significant as well; those are AB and AC, however, BC was not significant and so it was deduced to fit the model.

Fig. 1

The following regression equations are built by the model with coded units to predict the values of dependent variables;

$$Sqrt(BX) = 6.313+1.062*A+0.691*B+1.624*C+0.959*AB+0.627*AC$$

$$Sqrt(BC) =8.978+1.222*A+0.783*B+2.102*C+1.091*AB+0.631*AC$$

$$Sqrt(TBC) =10.91+1.764*A+1.17*B+2.669*C+1.528*AB+0.874*AC$$

$$Sqrt(TPC) =12.59+2.423*A+1.685*B+3.674*C+2.372*AB+1.681*AC$$

$$Sqrt(AA(FRAP)) =15.9+3.042*A+2.037*B+4.288*C+2.825*AB+2.161*AC$$

$$Sqrt(AA(DPPH)) =6.085+0.571*A+0.503*B+1.087*C+0.558*AB+0.359*AC$$

Where A is the applied microwave power, B is the irradiation time, and C is the peel-to-solvent ratio. According to the results of the specific correlational tests, it was found that the respective bioactive compounds were highly connected which was pointed out by R^2 values of greater than 0.9 in all cases (Table 3).

Table 2 ANOVA for reduced response surface 2FI model (Squareroot transform)

Model	Sum of Squares	df	Mean Square	F-value	p-value	Residual (SS)	Lack of Fit (SS)
BX	52.96	5	10.59	16.6	< 0.0001	8.93	5.33
BC	77.95	5	15.59	13.03	< 0.0001	16.75	9.05
TBC	140.86	5	28.17	16.21	< 0.0001	24.33	15.09
TPC	289.69	5	57.94	16.16	< 0.0001	50.18	37.74
AA (FRAP)	419.11	5	83.82	13.6	< 0.0001	86.31	64.57
AA (DPPH)	21.12	5	4.22	14.37	< 0.0001	4.12	2.41

Table 3 Correlational tests among the targeted bioactive compounds

Correlation	BX	BC	TBC	TPC	AA (FRAP)	AA (DPPH)
BX	1					
BC	0.985	1				
TBC	0.995	0.992	1			
TPC	0.994	0.968	0.986	1		
AA (FRAP)	0.987	0.953	0.979	0.994	1	
AA (DPPH)	0.977	0.962	0.968	0.964	0.95	1

Comparison of stalk, flesh, and peel-water 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 table (1).

The control sample extracts were prepared conventionally from stalk, flesh, and peel of the beetroot 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 as described in Fig. 2. Overall, total betalain content is the highest in the peel (BC=231.78±2.15 mg/L, BX=172.42±2.32 mg/L, TBC=404.17±4.47 mg/L) compared to the other parts followed by the flesh (BC=90.53±3.59 mg/L, BX=34.96±1.02 mg/L, TBC=125.41±3.37 mg/L) and the stalk (BC=13.56±1.75 mg/L, BX=4.38±0.8 mg/L, TBC=17.86±1.04 mg/L). Under high-level process condition, the scavenged amounts of betalain compounds with water solvent from the peel increased 50 % (BX), 59 % (BC), and 55 % (TBC) with reference to the control. Alternatively, the recovered betalains amounts at low and medium process conditions were remarkably lower than the control. In the same vein, flesh extracts exhibited 65 % higher amounts of TBC at the highest process condition as compared to the control. The stalk extracts, however, behaved differently due to the greater amounts of TBC (9 %) observed in the control. The results of color pattern ($L^*a^*b^*$) measurement was represented in table (3). Amongst, the trend of the lightness (L^*) values declines with the higher process conditions while the trend of total color difference (ΔE) behaved contrastingly. Moreover, hue angle (Hº) values were maximum in the highest point of process condition despite flesh extracts since a significant decrease in hue angle (Hº) values was found under the same situation. The trends of the values of chroma in stalk and flesh extracts behaved the same way as both of them increased at higher processing level whereas the peel extracts strayed from the trend and behaved randomly. Generally, the stalk extracts exhibited the lowest redness or greenness (b^*) and hue angle (Hº) values whilst the flesh extracts displayed the maximum lightness (L^*) and hue angle (Hº) values. Likewise, the values of yellowness or blueness (a^*), redness or greenness (b^*), chroma or saturation and total color difference (ΔE) were found to be maximum in the peel extract.

Table 4 Color contributions of stalk, flesh, and peel-water extracts

Material	Run	L*	a*	b*	Chroma	Hueº	ΔE
Stalk	-1	$42.78\pm0.27^{\alpha}$	$47.15\pm0.11^{\beta}$	$0.57\pm0.04^{\delta}$	$47.16\pm0.11^{\theta}$	0.69±0.04	$60.64\pm0.1^{\gamma}$
	0	$33.39\pm0.36^{\alpha}$	$54.78\pm0.31^{\beta}$	$15.28\pm0.13^{\delta}$	$56.88\pm0.27^{\theta}$	15.59±0.2	$74.07\pm0.03^{\gamma}$
	1	$27.91\pm0.07^{\alpha}$	$48.25\pm0.19^{\beta}$	$21.57\pm0.16^{\delta}$	$52.85\pm0.24^{\theta}$	24.09±0.08	$74.83\pm0.17^{\gamma}$
Flesh	-1	$77.37\pm0.05^{\alpha}$	$2.51\pm0.09^{\beta}$	$4.98\pm0.04^{\delta}$	$5.58\pm0.07^{\theta}$	63.21±0.61	$6.47\pm0.05^{\gamma}$
	0	$59.89\pm0.11^{\alpha}$	$22.73\pm0.19^{\beta}$	$1.62\pm0.01^{\delta}$	$22.79\pm0.19^{\theta}$	4.07±0.04	$31.02\pm0.08^{\gamma}$
	1	$59.73\pm0.28^{\alpha}$	$29.04\pm0.13^{\beta}$	$1.57\pm0.04^{\delta}$	$29.08\pm0.13^{\theta}$	3.1±0.08	$35.94\pm0.12^{\gamma}$
Peel	-1	$45.48\pm0.06^{\alpha}$	$56.36\pm0.41^{\beta}$	$9.13\pm0.16^{\delta}$	$57.09\pm0.43^{\theta}$	9.2±0.1	$67.09\pm0.37^{\gamma}$
	0	$40.45\pm0.12^{\alpha}$	$58.09\pm0.23^{\beta}$	$17.27\pm0.03^{\delta}$	$60.61\pm0.21^{\theta}$	16.56±0.09	$72.76\pm0.12^{\gamma}$
	1	$27.13\pm0.15^{\alpha}$	$48.75\pm0.57^{\beta}$	$22.01\pm0.34^{\delta}$	$53.49\pm0.41^{\theta}$	24.3±0.55	$75.83\pm0.25^{\gamma}$

Same superscript letters in the same column mean there are no significant differences among the extracts at 99.99% significant level.

Fig. 2

Fig. 3

TPC and TFC of the stalk, flesh, and peel extracts under different process conditions were presented in Fig. 3 (a) and (b). As depicted in Fig. 3 (a), TPC was outweighed in the control samples in flesh and stalk extracts whereas the peel behaved a bit different due to its amount was highest in the maximum process condition of MAE (720.48±17.75 mgGAE/L). Nonetheless, maximum TFC was observed in the control sample of peel extract as 330 mgQUE/L (Fig. 3 (b)). Antiradical scavenging activities of the stalk, flesh, and peel-water extracts were investigated

by FRAP, DPPH, and ABTS methods (Fig. 4). As can be seen in Fig. 4 (a), 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, 1171.72±25.23 mgASE/L (FRAP) is the maximum radical scavenging activity discovered in the peel-water extracts which amount was 2.5 times higher than the control and 3.5 and 6.5 times greater as to 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 (Fig. 4 (b)). Similarly, ABTS performance proved that the exceeded amounts of antioxidants were extracted with MAE samples which are 23 % (stalk), 32 % (flesh), and 99 % (peel) in comparison to the control samples of the stalk (10 %), flesh (15 %), and peel (44 %) (Fig. 4 (b)).

Fig. 4

The efficiency of pure water and ethanol-water solvents

Elucidation of the extractability of specific bioactive compounds from the flesh and peel of the beetroot via water and 15 % aqueous ethanol solvents were compared in table 4. The operation conditions were microwave power (100 W, 450 W, and 800 W), irradiation time (30 s, 90 s, and 150 s) and solid-to-solvent ratio (0.1 w/v, 0.15 w/v, and 0.2 w/v) (see the table 1 as well). Generally, the efficiency of water solvent and ethanol-water solvent are alike for specific compounds extractions from the flesh; howbeit, the efficiency of the water solvent significantly outweighed the ethanol-water solvent in the peel extracts (Table 4). In brief, the combined solvent could extract the individual bioactive compounds; TBC (340.51±1.28 mg/L), TPC (569.47±1.67 mgGAE/L), TFC (390 mgQUE/L), and AA (530.84±0.42 mgASE/L) from the peel of beetroot with MAE of 800 W and 150 s. The extraction ability of them was improved by 15 % of TBC, 20 % of TPC, and 54 % of AA with pure water solvent except for TFC, which was decreased by 31 %.

Table 4 Comparison of the bioactive compounds extractability of the pure water and ethanol-water solvent from beetroot pulp and peel with MAE

Specific compounds	Run	Pure water		Ethanol-water	
		Flesh	Peel	Flesh	Peel
BX (mg/L)	-1	8.35±0.29 ^{a**}	16.4±0.39^{b**}	5.95±2.12 ^{a**}	14.97±0.51 ^{b**}
	0	12.8±1.4 ^{a**}	47.12±1.49 ^{b**}	18.1±0.18 ^{a**}	64.61±0.49 ^{b**}
	1	34.96±1.02^{a**}	172.42±2.32^{b**}	39.45±0.64^{a**}	157.3±0.43^{b**}
BC (mg/L)	-1	26.27±0.69^{c**}	43.28±0.23 ^{c**}	16.43±4.7 ^{c**}	29±0.88 ^{c**}
	0	43.15±4.2 ^{c**}	90.34±1.03 ^{c**}	44.39±1.11 ^{c**}	116.41±1.41 ^{c**}
	1	90.53±3.59^{c**}	231.78±2.15^{c**}	78.8±1.38^{c**}	183.35±0.92^{c**}
TBC (mg/L)	-1	34.54±0.97^{d*}	59.68±0.16^{d*}	22.26±6.8^{d*}	44.33±1.2^{d*}
	0	55.87±5.61^{d*}	137.44±2.51^{d*}	62.36±1.29^{d*}	180.87±1.87^{d*}
	1	125.41±3.37^{d*}	404.17±4.47^{d*}	118.12±2.03^{d*}	340.51±1.28^{d*}
TPC (mgGAE/L)	-1	37.79±6.05^{e***}	81.78±11.59 ^{e***}	36.15±13.76 ^{e***}	36.63±4.92^{e***}
	0	63.48±4.28^{e***}	207.72±7.5 ^{e***}	121.66±10.82 ^{e***}	252.84±9.02^{e***}
	1	209.34±9.62^{e***}	720.48±17.75^{e***}	264.88±24.58^{e***}	569.47±1.67^{e***}

TFC (mgQUE/L)	-1	52.00±1.73^{f*}	25.5±0.71^{f*}	75±5.2^{f*}	25±7.07^{f*}
	0	93.33±1.53^{f*}	185.33±4.16^{f*}	149.33±2.89^{f*}	246.67±11.55^{f*}
	1	186.67±3.21^{f*}	296.67±5.77^{f*}	215±4.36^{f*}	390±0.00^{f*}
FRAP (mgASE/L)	-1	80.5±14.52 ^{g***}	128.08±7.27 ^{g***}	36.58±0.42^{g***}	45.2±0.59^{g***}
	0	101.79±4.83 ^{g***}	306.31±7.57 ^{g***}	97.25±0.42^{g***}	218.06±5.62^{g***}
	1	328.58±12.93^{g***}	1171.72±25.23^{g***}	208.17±2.52^{g***}	530.84±0.42^{g***}
DPPH (%)	-1	15.94±0.42^{h*}	22.3±0.66^{h*}	32.11±0.85^{h*}	36.64±1.65^{h*}
	0	22.24±1.58^{h*}	49.74±0.18^{h*}	58.73±1.7^{h*}	43.42±0.52^{h*}
	1	53.29±1.42^{h*}	94.07±0.00^{h*}	70.21±4.84^{h*}	45.22±2.65^{h*}
ABTS (%)	-1	5.3±0.06^{i*}	9.93±1.24^{i*}	3.66±1.52^{i*}	10.59±0.84^{i*}
	0	10.47±1.73^{i*}	54.01±1.55^{i*}	16.08±0.38^{i*}	47.46±0.59^{i*}
	1	32.07±1.91^{i*}	99±0.16^{i*}	35.32±6.31^{i*}	51.08±0.76^{i*}

Superscript same letters refer there are no significant differences to each other between the treatments (* = $\alpha<0.05$, ** = $\alpha<0.01$).

Discussion

Betalains, bio-colourants with high water affinity are versatile in food, pharmacy and cosmetic industries and their ease of extraction with universal solvents makes them conspicuous in the innovation trend of green chemistry. Noteworthy, their sensitiveness to pH, light and temperature changes makes them more dedicated to degradation during processing and so limited in practical usage. Subsequently, their extractability is remarkably affected by the process condition. In this study, the solvent ratio is the most significant ($p<0.0001$) factor among the process variables. Logically the MAE is influenced mostly by the solvent ratio due to microwave efficiency which mainly depends on the physical properties of the treating materials and so does the solvent behaviour. Again, time and power of microwave treatment have strong interaction with the extractability of the solvent since the surface tension and the polarity of the solvents can be manipulated by their interaction effect. In the MAE of pigments from the plant matrix, elevated temperature and long exposure time should be taken into account since they encourage the possessiveness of soft tissues and ease of degradation. Rapid pigment diffusion occurs in accordance with quick contact of microwaves with the matrix by breaking down the cell membrane. Nevertheless, care should be made for subsequent pigment compartmentalizations. The scavenged amounts of BX are about two or three times lower than BC which might be either due to their natural occurrence portion [30] or the processing conditions that have affected their degradation as well. Hence, the quantity of betaxanthin in the pressed juice and the extract was double as to betacyanin in the study of Slavov and co-workers [31]. As a consequence, the differences in the color tonation ($L^*a^*b^*$) of the extracts might be influenced by the decomposition of the betalain color compounds as well as their specific distribution ratio in various parts of the beetroot.

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 superheating effect [32]. 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, that situation was obvious after 150 s of treatment at 800 W while the pretests were performed. Ethanol solvent (99.9%) proved to be efficient for extracting betalains from beetroot peels with MAE [33]. 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 [10]. 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

[34]. Besides, reduction in viscosity of the solvent and so its surface tension could have helped with desorption of the active compounds by breaking through the swelling membrane which means the higher the amount of water, the better the solvent property [35]. 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 the 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 [34]. So, high temperature or power and short irradiation time are always beneficial combinations for the MAE of plant materials [1, 36].

Phenolic acids and flavonoids are the most common active plant-based phenolic compounds which can easily be extracted by water and alcoholic solvent [37, 38]. As plant secondary metabolites, especially aromatics, they are rich in radical scavenging activity [39]. Oxidation is one of the most responsible reactions for food degradation and thereafter make antioxidant play an important role in food processing. Peroxidases (POX) and polyphenol oxidases (PPO) are the most responsible enzymes for phenolic degradation during processing [40]. Presence of light, air, temperature, and oxidative enzymes can also affect the degradation of phenolic compounds. Phenolic compounds, being polar molecules, may ascertain greater yield with the improved polarity of the solvent [36]. Their absorptivity of microwave is high due to their permanent dipole moment with strong absorption of microwave rays which facilitate their release out of the cell wall [32]. Moreover, most of the phenolic compounds are not thermosensitive and their stability is decisively overwhelmed by their oxidizability, numbers of hydroxylic-type and methoxylic-type substituents, and heterocyclic ring system [41]. In our case, the trends of TPC and TFC in each extract were observed to incline with longer microwave exposure time at higher power. In the previous studies, extraction of phenolic compounds was maximized at 15 min of extraction time at 600 W [42] while short irradiation time (10 s) with higher power nominal (90 %) was reported as the most effective way for the phenolic compound extraction by [43]. Herein, account should be taken of the specific nature of the treated materials. Flavonoids with the general structure of 15-carbon skeleton exist abundantly in various fruits and vegetables with attributes of sensory properties to the host. The influence of process condition on the TFC recovery might be due to its glucosylation of sugar moieties as the glucosylation is responsible for the solubility, stability, bioavailability, and antioxidant properties [44]. In the study of Olumese and Oboh [45], the heating process encourages the hydrolysis of C-glycosides bonds which led to the formation of monomers. Additionally, flavonoids recovery was superior with the aqueous ethanol solvent compared to pure water which might be due to the lipophilic property of the flavonoids.

In the antioxidant activities (AA) assays, the antioxidants potential of the extracts mainly relies on the level of temperature since the extractability of the antioxidant-rich bioactive compounds are also affected by the temperature [45]. This is in accordance with our observation because the correlational tests of all expected bioactive compounds including antioxidants have claimed the characteristic of antioxidant-rich bioactive compounds with high R^2 values of over 0.9 (Table 3). Besides, it has been explored that the cultivation condition as well affects the antioxidant potential of bioactive compounds [25]. According to the DPPH assay rule, changes in coloration represent the reduction of Diphenyl picryl hydrazyl free radical to picryl hydrazine by the action of the antioxidant present in the examined sample when it transfers its hydrogen atom to the radical [46]. Shah and Modi [47] recommended DPPH assay as more efficacious, high in reproducibility and can simply be operated compared to ABTS and FRAP methods. Howbeit, in the study of Arend et al [48], it was mentioned that ABTS is more effective to measure for the antioxidant activity of the fractions and suitable for both hydrophilic and hydrophobic systems whereas DPPH assay is more applicable for hydrophobics. This is in agreement with our study as the radical scavenging percentage of each extract was found to be exceeded in the ABTS assay and more stable for DPPH assay.

Conclusion

Overall, from the peel of beetroot, 150 s of microwave exposure at 800 W power with pure water solvent could extract total betalains (404.17±4.47 mg/L), BC (231.78±2.32 mg/L), BX (172.42±2.15 mg/L), TPC (720.48±17.75 mgGAE/L) and TFC (296.67±5.77 mgQUE/L) along with their respective antiradical scavenging activities; FRAP (1171.72±25.23 mgASE/L), DPPH (94 %), and ABTS (99 %). It could be concluded that, under the examined range of power and duration, the effects of the combined solvent (15% aqueous ethanol) were not superior to pure water based on the excessive amount of recovered compounds in the water extracts. In our study, the operational mode of MAE was set up based on the pretests and the central composite design. Since peel-to-solvent ratio was mainly focused in this study, the microwave irradiation time could not set up longer than 150 s with 800 W due to witnessed solvent boiling with eventual spill over loses during the pretests even with the intermittent mode and performance of cooling in between. Therefore, further investigations for MAE of bioactive compounds from beetroot wastes are encouraged with microwave set up beyond the current study. All in all, MAE uploaded with pros is strongly recommendable in the volarization of bioactive compounds with potential upscale in food fortifications since the recovered amounts in beetroot processing wastes were noteworthy.

Acknowledgements

This study is supported by the European Union and co-financed by the European Social Fund (grant agreement no. EFOP-3.6.3-VEKOP-16-2017-00005), the Tempus Public Foundation under the Stipendium Hungaricum Scholarship Program. Cylindra beetroots were supplied by Hungarian student, Fruzsina Borda from her bio farm.

Declarations

Not applicable

Conflicts of interest

The authors declare no conflict of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable

Consent for publication

All authors have informed consent for the publication of this manuscript.

References

1. Song, J., Li, D., Liu, C., Zhang, Y.: Optimized microwave-assisted extraction of total phenolics (TP) from Ipomoea batatas leaves and its antioxidant activity. Innov. Food Sci. Emerg. Technol. 12, 282-287 (2011)
2. Martínez, L., Jongberg, S., Ros, G., Skibsted, L.H., Nieto, G.: Plant derived ingredients rich in nitrates or phenolics for protection of pork against protein oxidation. Food Res. Int (2020). https://doi.org/10.1016/j.foodres.2019.108789
3. Hatwalne, M.S.: Free radical scavengers in Anaesthesiology and critical care. Indian J. Anaesth. 56(3), 27-233 (2012)
4. Gilgun-sherki, Y., Melamed, E., Offen, D.: Antioxidant treatment in Alzheimer's disease current state. J. Mol. Neurosci. 21, 1-11 (2003)
5. Nimse, S.B., Pal, D.: Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. (2015). doi: 10.1039/c4ra13315c
6. Benzie, I.F.F., Devaki, M.: Measurement of Antioxidant Activity & Capacity: Recent Trends of Applications, First Edition. John Wiley & Sons Publishers, 77-106 (2018)
7. Szeto, Y.T., Tomlinson, B., Iris F.F. Benzie.: Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. Br. J. Nutr. 87, 55-59 (2002)
8. Moussa, Z., Judeh, Z.M.A., Ahmed, A.S.: Nonenzymatic Exogenous and Endogenous Antioxidants. Free Radic. Biol. Med. (2019). doi: http://dx.doi.org/10.5772/intechopen.87778.
9. Kavalcová, P., Bystrická, J., Tomáš, J., Karovičová, J., Kovarovič, J., Lenková, M.: The content of total polyphenols and antioxidant activity in red beetroot. Potravinarstvo. (2015). doi:10.5219/441
10. Zin, M.M., Anucha, C.B., Szilvia, B.: Recovery of phytochemicals via electromagnetic irradiation (Microwave-Assisted-Extraction): Betalain and phenolic compounds in perspective. Foods. 9(7), 918 (2020)
11. Lee, E.J., An, D., Nguyen, C.T.T., Patil, B.S., Kim, J., Yoo, K.S.: Betalain and betaine composition of greenhouse-or field-produced beetroot (*Beta vulgaris L.*) and inhibition of HepG2 cell proliferation. J. Agric. Food Chem. 62, 1324-1331 (2014)
12. Hussain, E.A., Sadiq, Z., Zia-Ul-Haq, M.: Betalains: Biomolecular Aspects, 1st ed., Springer International Publishing, AG: Cham, Switzerland (2018). https://doi.org/10.1007/978-3-319-95624-4_2
13. Clifford, T., Howatson, G., West, D.J., Stevenson, E,J.: The potential benefits of red beetroot supplementation in health and disease. Nutrients. 7, 2801-2822 (2015)
14. Yasaminshirazi, K., Hartung, J., Fleck, M., Grae-Hoenninger, S.: Bioactive compounds and total sugar contents of different open-pollinated beetroot genotypes grown organically. Molecules (2020). doi:10.3390/molecules25214884
15. Székely, D., Szalóki-Dorkó, L., Stéger-Máté, M., Szabó-Nótin, B., Ivanics, J., Monspart-Sényi, J.: Distribution of antioxidant components in roots of different red beets (Beta vulgaris L.) cultivars. Acta Aliment. 43, 164-171 (2014)
16. Sawicki, T., Bączek, N., Wiczkowski, W.: Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. J. Funct. Foods. 27, 249-261 (2016)
17. Antigo, J.L.D., Berganasci, R.D.C., Madrona, G.S: Effect of pH on the stability of red beet extract (Beta vulgaris l.) microcapsules produced by spray drying or freeze drying. Food Sci. Technol (2017). http://dx.doi.org/10.1590/1678-457X.34316
18. Bazaria, B., Kumar, P.: Optimization of spray drying parameters for beetroot juice powder using response surface methodology (RSM). J. Saudi Soc. Agric. Sci. (2018). https://doi.org/10.1016/j.jssas.2016.09.007
19. Székely, D., Illés, B., Stéger-Máté., M., Monspart-Sényi, J.: Effects of drying methods for inner parameters of red beetroot (Beta vulgaris *L.*). Acta Univ. Sapientiae, Alimentaria, 9, 60-68 (2016)
20. Shaheen, M.S., El-Massry, K.F., El-Ghorab, A.H., Anjum, F.M.: Microwave applications in thermal food processing. InTech. (2012). http://dx.doi.org/10.5772/48716

21. Akuamoah, F., Odamtten, G.T., Kortei, N.K.: Influence of gamma irradiation on the colour parameters of dry smoked shrimps (Penaeus notialis). Food Research 2(4), 350-355 (2018)
22. Chandran, J., Nisha, P., Singhal, R.S., Pandit, A.B.: Degradation of colour in beetroot (Beta vulgaris L.): a kinetics study. J Food Sci Technol (2014). doi:10.1007/s13197-012-0741-9
23. Gokhale, S.V., Lele, S.S.: Dehydration of red beetroot (Beta vulgaris) by hot air drying: Process optimization and mathematical modeling. Food Sci. Biotechnol. (2011). doi:10.1007/s10068-011-0132-4
24. Costa, A.P.D., Hermes, V.S., Rios, A.D.O., Flores, S.H.: Minimally processed beetroot waste as an alternative source to obtain functional ingredients. J. Food Sci. Technol. 54, 2050-2058 (2017)
25. Kovačević, S.Z., Tepić, A.N., Jevrić, L.R., Podunavac-Kuzmanović, S.O., Vidović, S.S., Šumić, Z.M., Ilin, Z.M.: Chemometric guidelines for selection of cultivation conditions influencing the antioxidant potential of beetroot extracts. COMPUT ELECTRON AGR 118, 332-339 (2015)
26. Zin, M.M., Márki, E., Bánvölgyi, S.: Evaluation of reverse osmosis membranes in concentration of beetroot peel extract. Period. Polytech. Chem. Eng. 64, 340-348 (2020)
27. Ardekania, M.R.S., Hajimahmoodi, M., Oveisi, M.R., Sadeghi, M., Jannat, B., Ranjbar, A.M., Gholam, N., Moridi, T.: Comparative antioxidant activity and total flavonoid content of Persian pomegranate (Punica granatum L.) cultivars. Iran. J. Pharm. Sci. 10(3), 519-524 (2011)
28. Ravichandran, K., Saw, N.M.M.T., Mohdaly, A.A.A., Gabr, A.M.M., Kastell, A., Riedel, H., Cai, Z., Knorr, D., Smetanska, I.: Impact of processing of red beet on betalain content and antioxidant activity. Food Res. Int. 50, 670-675 (2013)
29. Ilyasov, I.R., Beloborodov, V.L., Selivanova, I.A., Terekhov, R.P.: ABTS/PP decolorization assay of antioxidant capacity reaction pathways. Int. J. Mol. Sci. (2020). doi:10.3390/ijms21031131
30. Zin, M.M., Márki, E., Bánvölgyi, S.: Conventional extraction of betalain compounds from beetroot peels with aqueous ethanol solvent. Acta Aliment. 49, 163-169 (2020)
31. Slavov, A., Karagyozov, V., Denev, P., Kratchanova, M., Kratchanov, C.: Antioxidant activity of red beet juices obtained after microwave and thermal pretreatments. Czech J. Food Sci. 31(2), 139-47 (2013)
32. Proestos, C., Komaitis, M.: Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. LWT Food Sci. Technol. 41, 652-659 (2008)
33. Singh, A., Ganesapillai, M., Gnanasundaram, N.: Optimizaton of extraction of betalain pigments from beta vulgaris peels by microwave pretreatment. In IOP Conference Series: Materials Science and Engineering, Proceedings of the 14th ICSET (2017). doi:10.1088/1757-899X/263/3/032004
34. Hayat, K., Hussain, S., Abbas, S., Farooq, U., Ding, B., Xia, S., Jia, C., Zhang, X., Xia, W.: Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity. Vitro. Sep. Purif. Technol. 70, 63-70 (2009)
35. Pinela, J., Prieto, M.A., Carvalho, A.M., Barreiro, M.F., Oliveira, M.B.P.P., Barros, L., Ferreira, I.C.F.R.: Microwave-assisted extraction of phenolic acids and flavonoids and production of antioxidant ingredients from tomato: A nutraceutical-oriented optimization study. Sep. Purif. Technol. 164, 114-124 (2016)
36. Jokíc, S., Cvjetko, M., Božíc, D., Fabek, S., Toth, N., Vorkapíc-Furăc, J., Redovnikovíc, I.R.: Optimisation of microwave-assisted extraction of phenolic compounds from broccoli and its antioxidant activity. Int. J. Food Sci. Technol. 47, 2613-2619 (2012)
37. Longhi, J.G., Perez, E., José de Lima, J., Cândido, L.M.B.: In vitro evaluation of Mucuna pruriens (L.) DC antioxidant activity. Braz. J. Pharm. Sci. 47 (2011)
38. Flórez, N., Conde, E., Domínguez, H.: Microwave assisted water extraction of plant compounds. J. Chem. Technol. Biotechnol. 90, 590-607 (2015)
39. Rodriguez-Rojo, S., Visentin, A., Maestri, D., Cocero, M.J.: Assisted extraction of rosemary antioxidants with green solvents. J. Food Eng. 109, 98-103 (2012)
40. Bastos, E.L., Gonçalves, L.C.P.: Microwave-assisted extraction of betalains. Water Extraction of Bioactive Compounds: From Plants to Drug Development. In: González, H.D., Muñoz, M.J.G., (eds.) pp. 245-267. Elsevier, Amsterdam, The Netherlands (2017)
41. Liazid, A., Palma, M., Brigui, J., Barroso, C.G.: Investigation on phenolic compounds stability during

Microwave-assisted extraction. J. Chromatogr. A. 1140, 29-34 (2007)

42. Gharekhani, M., Ghorbani, M., Rasoulnejad, N.: Microwave-assisted extraction of phenolic and flavonoid compounds from Eucalyptus camaldulensis Dehn leaves as compared with ultrasound-assisted extraction. Lat. Am. Appl. Res. 42, 305-310 (2012)
43. Ballard, T.S., Mallikarjunan, P., Zhou, K., O'Keefe, S.: Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. Food Chem. 120, 1185-1192 (2010)
44. Klingel, T., Hadamjetz, M., Fischer, A., Wefers, D.: Glucozylation of flavonoids and flavonoid glucosides by mutant dextransucrose from Lactobacillus reuteri TMW 1.106. Carbohydr. Res. 483, 107741 (2019)
45. Olumese, F.E., Oboh, H.A.: Antioxidant and antioxidant capacity of raw and processed Nigerian beetroot (*Beta vulgaris*). Nig. J. Basic Appl. Sci. 24(1), 35-40 (2016)
46. Saravanakumar, A., Ganesh, M., Peng, M.M., Aziz, A.S., Jang, H.T: Comparative antioxidant and antimycobacterial activities of Opuntia ficus-indica fruit extracts from summer and rainy seasons. Front Life Sci (2015). doi: 10.1080/21553769.2015.1028655
47. Shah, P., Modi, H.A.: Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. Int. j. res. 3(6), 636-641 (2015)
48. Arend, G.D., Adorno, W.T., Rezzadori, K., Luccio, M.D., Chaves, V.C., Reginatto, F.H., Petrus, J.C.C.: Concentration of phenolic compounds from strawberry (Fragaria X Ananassa Duch) juice by nanofiltration membrane. J. Food Eng. 201, 36-41 (2017)

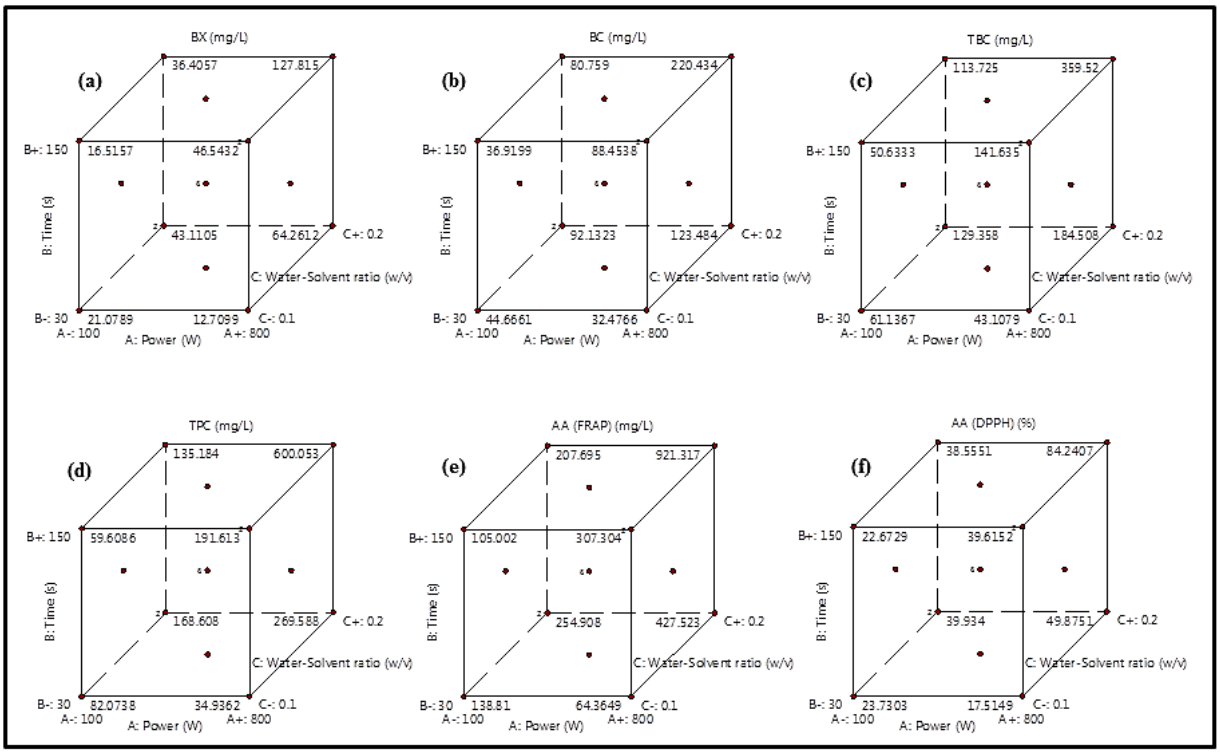

Fig. 1 Cubes representing the relationship between the process variables and the actual values of each response at the centre and the axial points

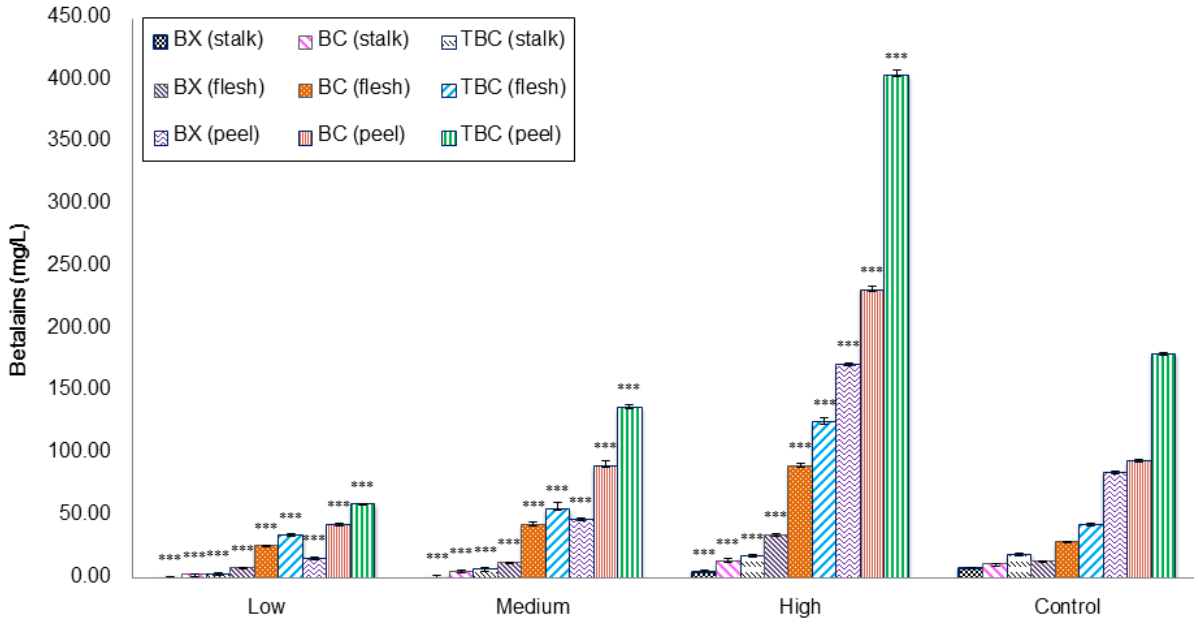

Fig. 2 Comparison of the extractable amounts of betalain compounds with pure water from the different parts of beetroot (peel, pulp and stalk) under three-level process conditions of MAE (*** denotes p-value less than 0.001)

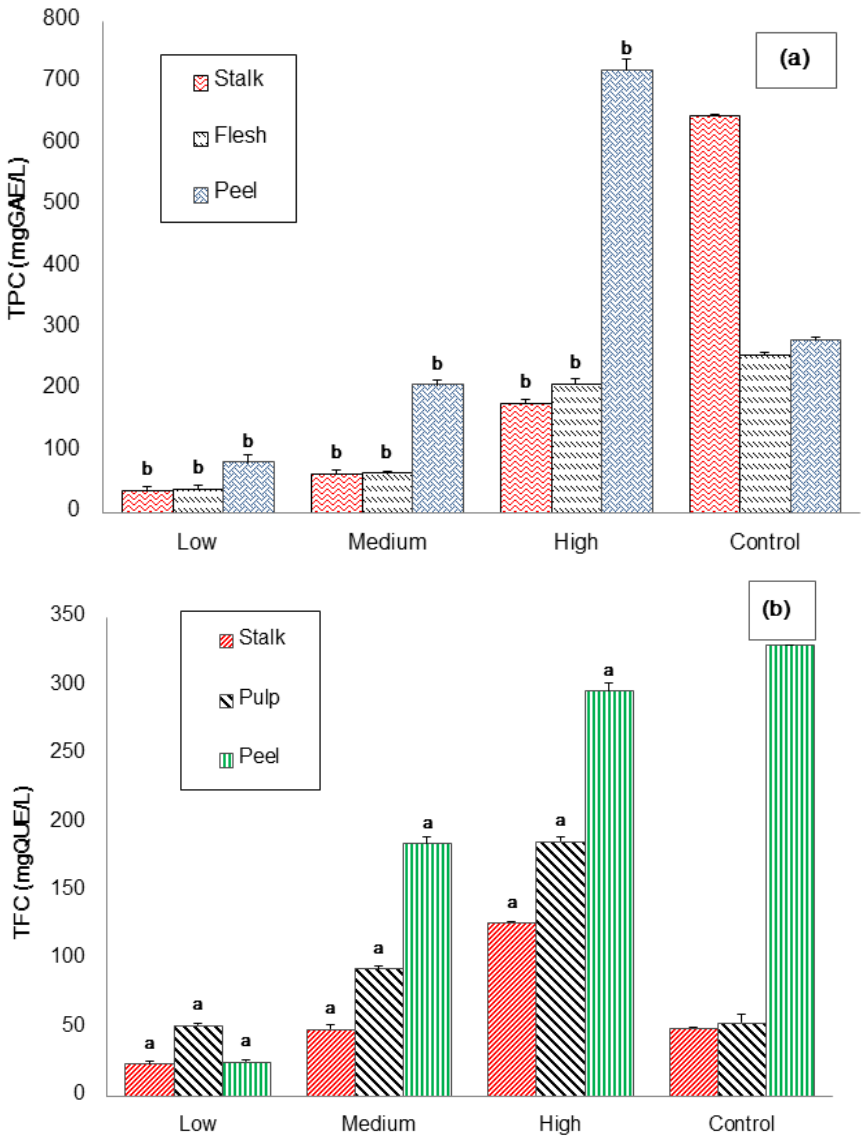

Fig. 3 Total phenolic compounds content (TPC) and total flavonoids content (TFC) of the stalk, pulp, and peel extracts under different process conditions of MAE ('a' and 'b' denote p-value less than 0.001)

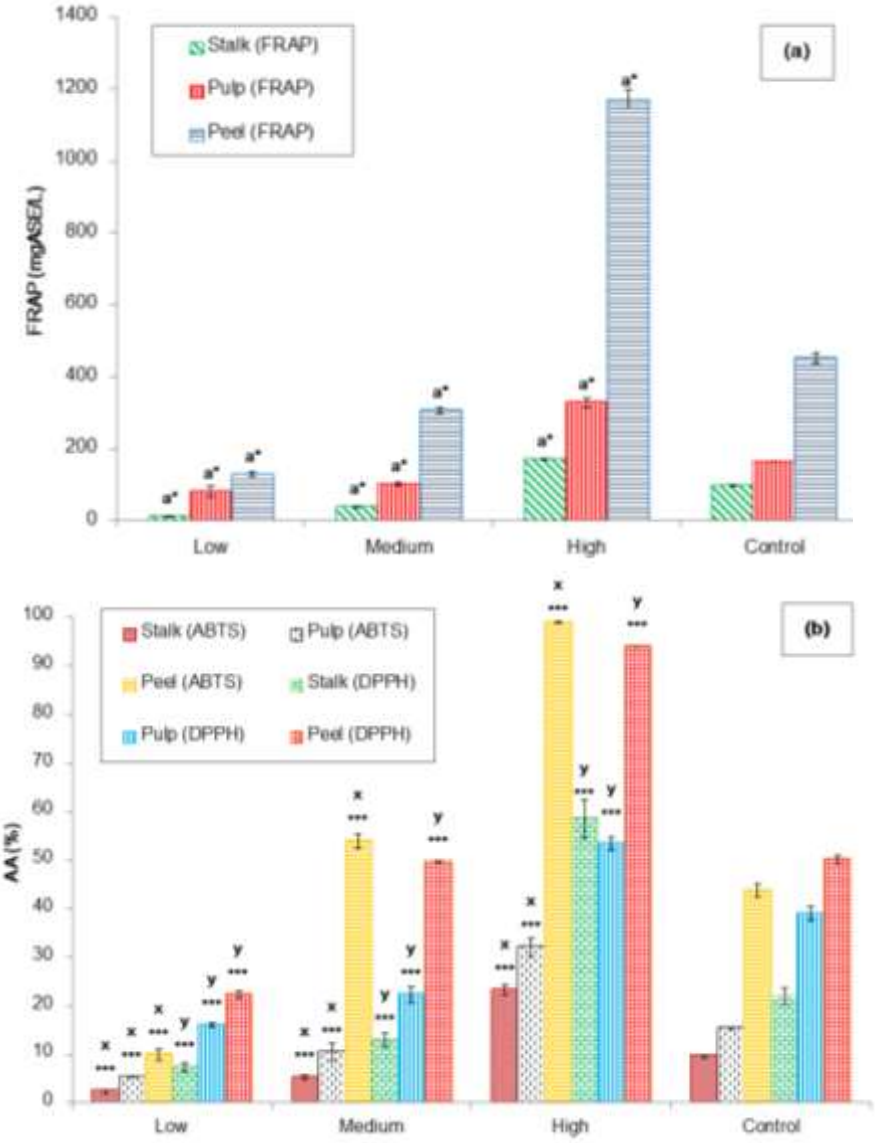

Fig. 4 Comparison between the antioxidant activity assays of beetroot peel, stalk, and flesh extracts (same letters represent there are no significant differences; * represents p-value<0.05; ** represents p-value<0.01; *** represents p-value<0.001)

Figures

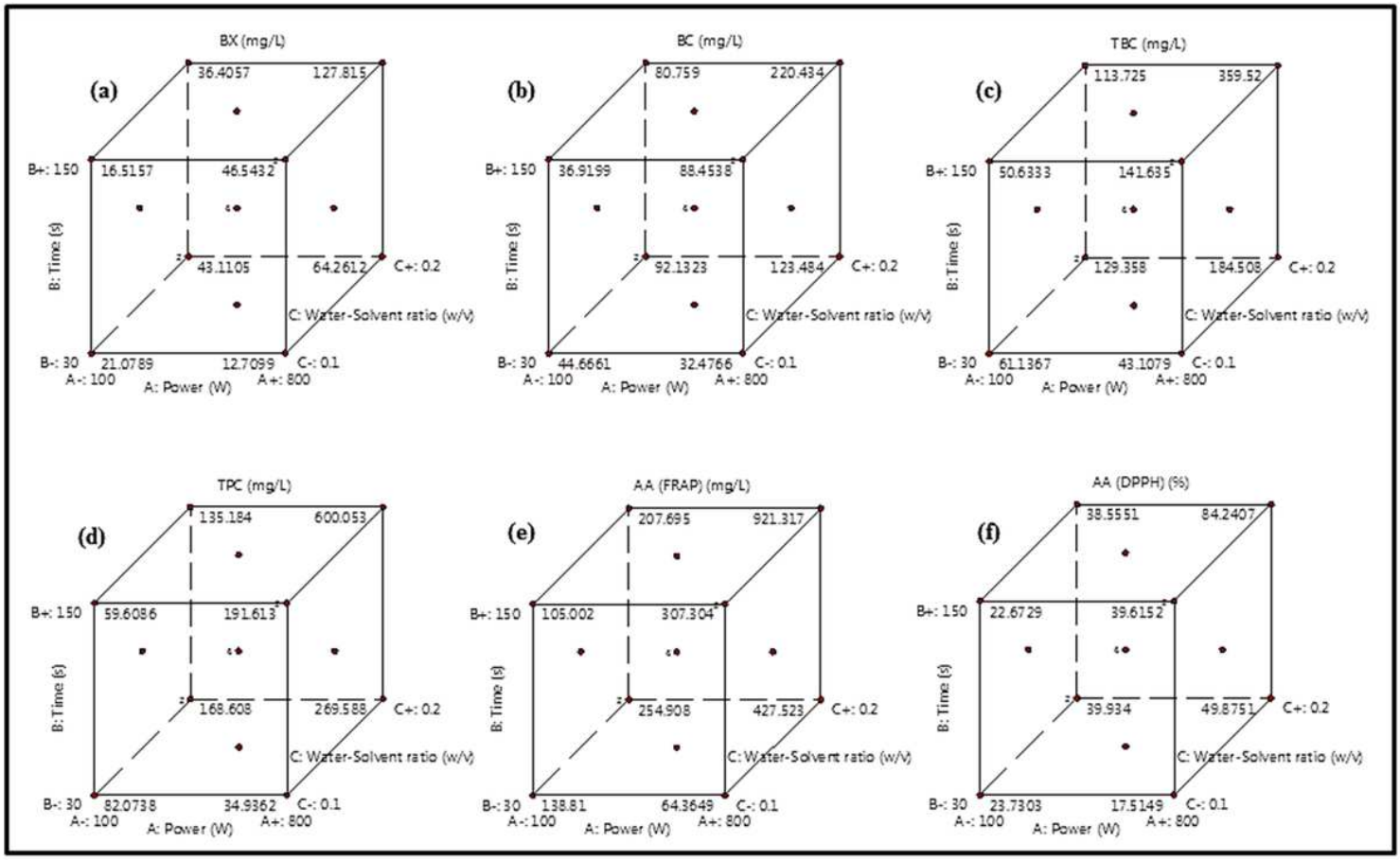

Figure 1

Cubes representing the relationship between the process variables and the actual values of each response at the centre and the axial points

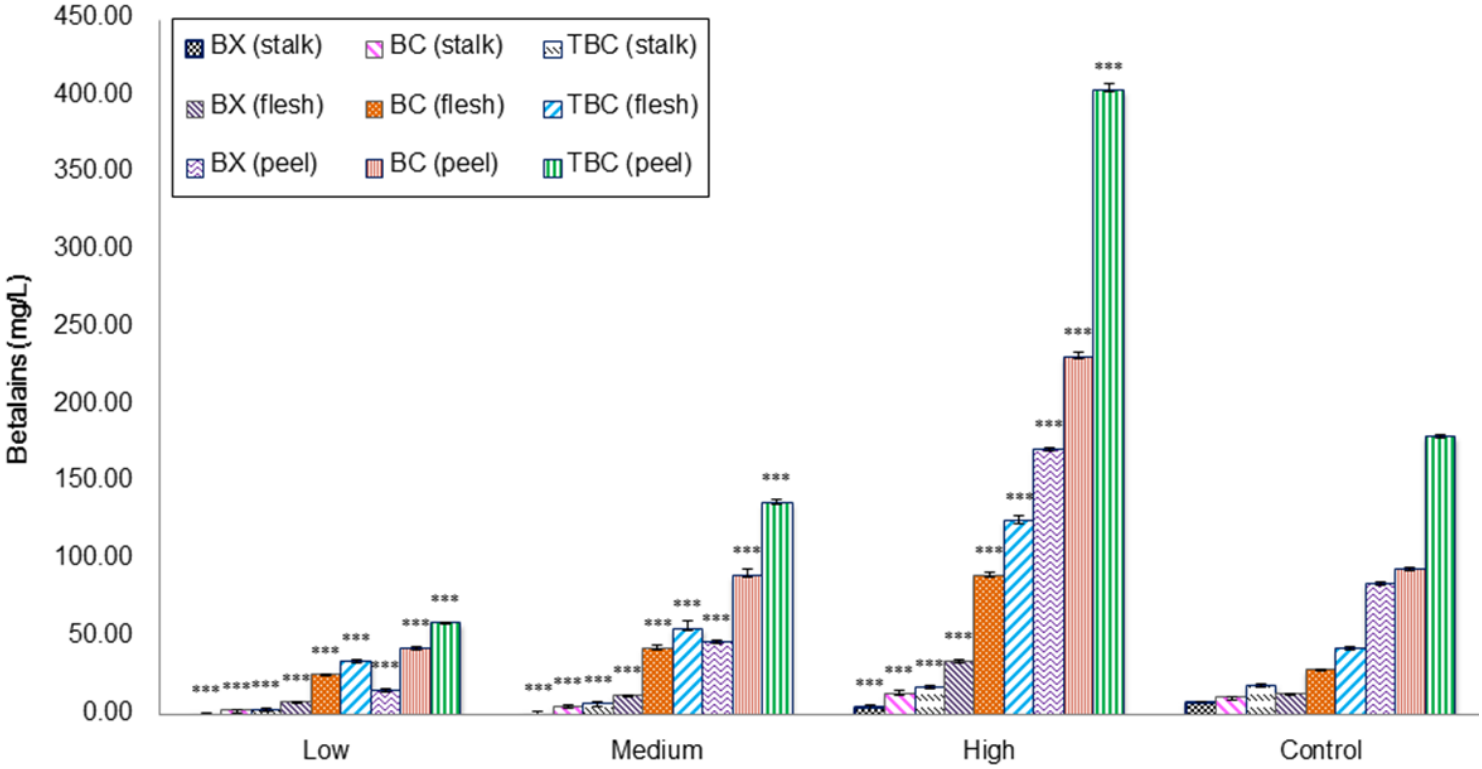

Figure 2

Comparison of the extractable amounts of betalain compounds with pure water from the different parts of beetroot (peel, pulp and stalk) under three-level process conditions of MAE (*** denotes p-value less than 0.001)

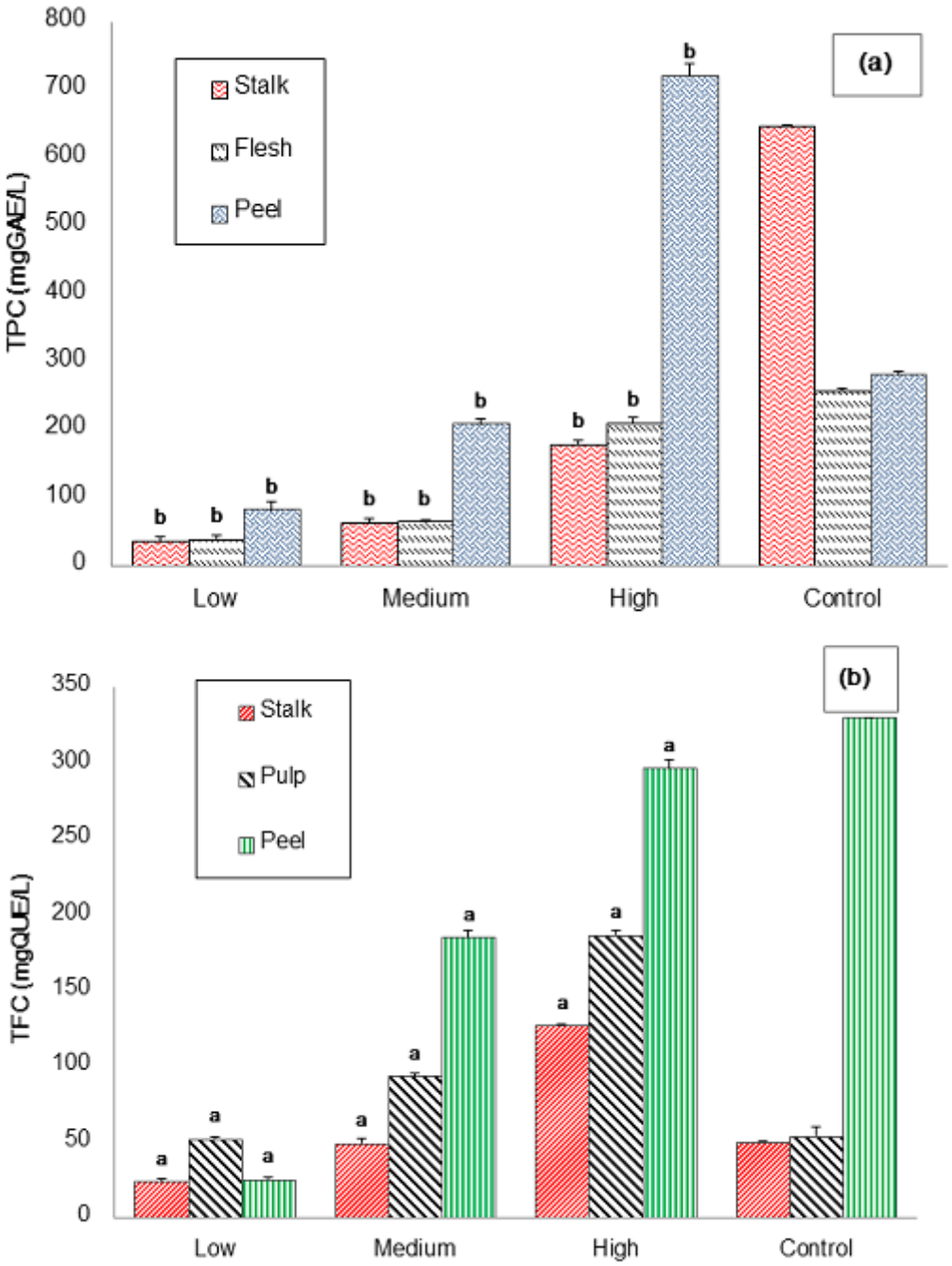

Figure 3

Total phenolic compounds content (TPC) and total flavonoids content (TFC) of the stalk, pulp, and peel extracts under different process conditions of MAE ('a' and 'b' denote p-value less than 0.001)

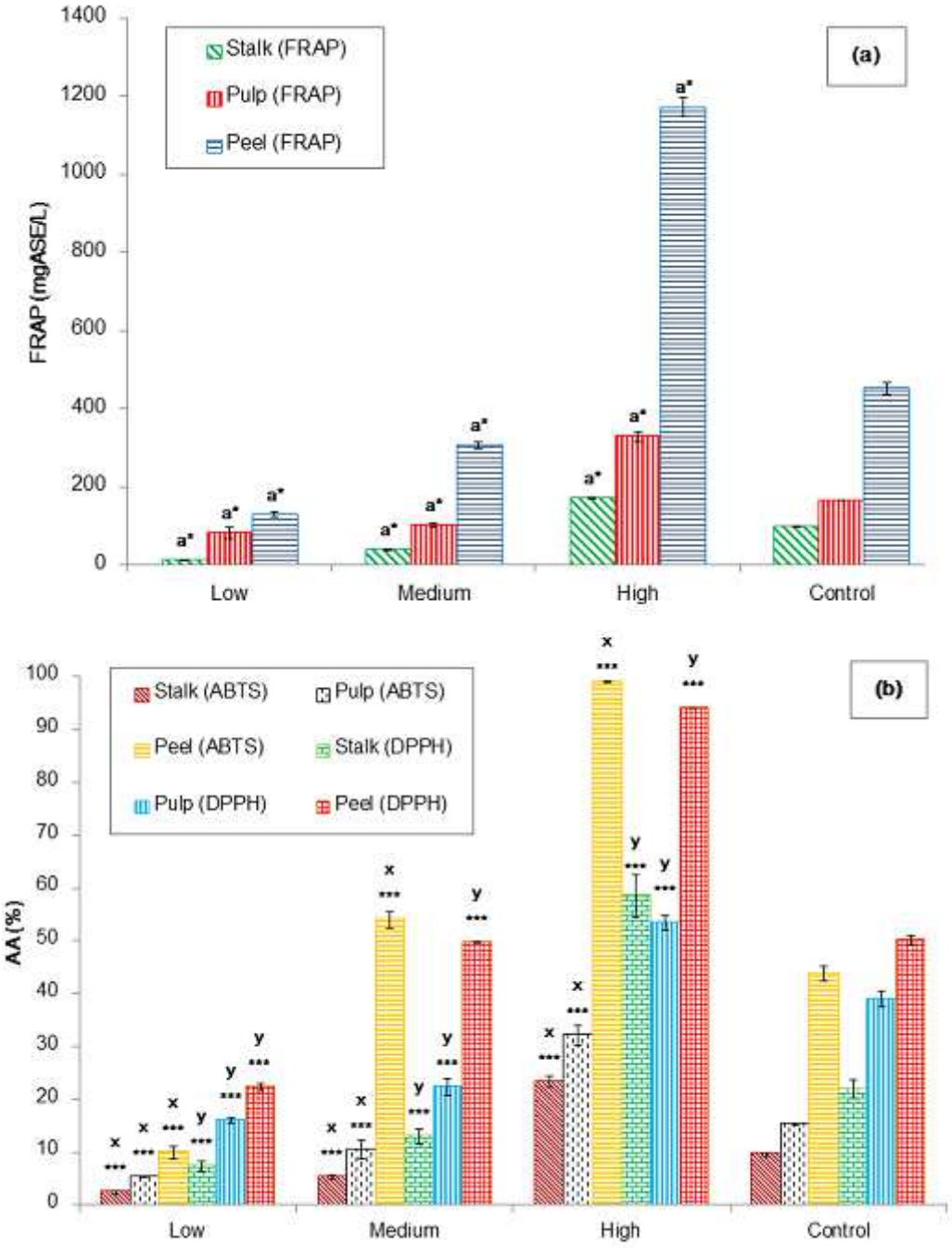

Figure 4

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)