Research Article

Extraction of Phytochemicals from Beetroot Pomace and Formulation of Phytochemical Enriched Functional Yogurt

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Abstract | Beetroot pomace is generally considered as a byproduct of the beetroot juice processing industry but is a potential source for bioactive compounds with strong antimicrobial activity and antioxidant activity. The purpose of the study was to determine the total phenolic content (TPC), antioxidant content and betalain content of beetroot pomace extract (BPE) under optimized extraction conditions. Beetroot pomace was further used to develop a flavored yogurt. The highest responses for DPPH inhibition (66.6%), betaxanthins (174 mg/L), betacyanins (265 mg/L) and TPC (2.78 mg GAE/g) were observed by extraction optimization. The antibacterial activity of the pomace extract was tested against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* by agar well diffusion method and the zone of inhibitions observed were 29.7±0.6 mm, 31±1 mm and 30±3 mm respectively. Yogurt samples fortified with extract (2% and 4%) were prepared and underwent sensory evaluation for characteristics such as color, odor, texture, acceptance of sourness and overall acceptability. The total solid content, pH and titratable acidity were determined for each of the sample along with probiotics enumeration. Significant changes in the pH were not observed, however samples fortified with 4% BPE showed the highest total solid content, titratable acidity and lactic acid bacteria count. Beetroot pomace is a promising source of betalain pigments, phenolic compounds and has strong antimicrobial potential. The effective utilization of byproducts like beetroot pomace in the formulation of functional food products can serve as a sustainable source of functional ingredient in the food industry.

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Introduction

Fruits and vegetables are rich in bioactive compounds such as high amount of antioxidants, fiber, phytochemicals, betalains, vitamins and minerals. Red beetroot (*Beta vulgaris* subsp. *Vulgaris*) is a dicotyledonous biennial plant belonging to Chenopodiaceae family and rich in antioxidants such as flavonoids, carotenoids and biologically active compounds such as vitamins (A, B, C, E and K), betalains, fibers, and inorganic nitrate and folates (Ben Haj Koubaier *et al.*, 2014; Ceclu and Nistor,

2020). Beetroot has various pharmacological activities including antimicrobial, antifungal, antioxidant, anti-inflammatory, expectorant, diuretic, anticancer, antimutagenic and anti-depressant potential (Jasmitha *et al*., 2018). Due to the medicinal benefits, beetroot as a whole and its parts (its peel, juice and pomace) have been incorporated into various products. Beetroot pomace due to its bioactive potential and color can serve as a functional food ingredient (Janiszewska-Turak *et al*., 2021). Beetroot has been used in various food products in dairy, bakery and beverage industries due to the presence of colored pigments and associated health benefits (Punia-Bangar *et al*., 2023). Beetroot pomace is a by-product of beetroot juice processing industry and it is a rich source of antioxidants, betalains and polyphenols (Ceclu and Nistor, 2020; Vulić *et al*., 2013). Betalains are nitrogen-containing water-soluble pigments that are derived from the amino acid tyrosine. Betalains are abundant in beetroot and can be divided into two groups based on absorption of wavelength; betaxanthins (yellow pigments which show maximum absorbance at the wavelength of 480 nm) and betacyanin (red pigments and their maximum absorbance occurs at 540 nm). They are commercially important as they impart a stronger color than anthocyanin and the intensity of color depends highly on the ratio between the concentrations of the red violet betacyanin to the yellow orange betaxanthins (Nemzer *et al*., 2011). The aim of this research was to optimize the extraction of betalain pigments from beetroot pomace collected as domestic waste and evaluation of antioxidant and antimicrobial potential of BPE. Moreover, a functional yogurt product was developed by incorporating beetroot betalains. The effective utilization of beetroot pomace can provide a sustainable solution in the management of food waste and formulation of functional food products.

Materials and Methods

Sampling

The fresh beetroot pomace was collected from the local juice shops of the Lahore City of Pakistan. The pomace was dried in a hot air oven (POL-EKU-APARATURA, Poland) at 38 °C till constant weight, followed by grinding into fine powder. The powdered samples were stored in Ziplock bags covered with aluminum foil at 4 °C till further use.

Ultrasonic assisted extraction

UAE was used to extract bioactive compounds from beetroot pomace powder. The frequency (20 kHz) and amplitude (40%) were taken as constant. The independent variables for the experimental runs were taken as the solvent concentration (30%, 50%, 70%), time (15, 30, 45 min) and sample to solvent ratio (1:10, 1:15, 1:20 w/v). The solvent taken for the extraction was a mixture of ethanol and 0.5 % acetic acid (Hidalgo *et al*., 2018). The sample and solvent were mixed as per the experimental design and the sonication probe (Industrial sonomechanics, USA) was immersed into the solvent mixture. RSM was used for the extraction optimization and response (independent) variables were TPC, betalain concentration and DPPH Inhibition (%).

Determination of TPC

TPC of the extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent as described by (Hiranrangsee *et al*., 2016). The results were expressed as mg of GAE/ g of sample (dry basis).

Antioxidant activity

DPPH assay was used to determine the antioxidant activity of the extract (Sadiq *et al*., 2015). The freshly prepared DPPH solution (5 ml of 40 ppm ethanolic solution) was taken in a test tube and 50 µl extract was added to this test tube, vortexed for 1 min and then kept in the dark for 30 min. The absorbance was read at 517nm and DPPH inhibition was determined by using Equation 1.

$$\text{DPPH \% inhibition} = A = \frac{AC - AS}{AC} \times 100 \quad \ldots(1)$$

Where; AC= absorbance of DPPH solution and AS = absorbance of the sample.

Determination of betalains

The extract was diluted (1:10) with phosphate buffer (pH 6.5) for the determination of betalain pigments (Ravichandran *et al*., 2013). Betacyanins and betaxanthins were measured at 538 nm and 476 nm respectively and the sum of both yielded the total betalain content. Betalain content (BC) was determined by using the Equation 2.

$$\text{BC}\left(\frac{\text{mg}}{\text{L}}\right) = \left[\frac{\text{A} \times \text{DF} \times \text{MW} \times 1000}{\varepsilon \times \text{I}}\right] \quad \ldots(2)$$

Where; A= absorbance, DF= dilution factor, l= path of length and ε= molar extinction coefficient.

Fourier transform infrared (FTIR) analysis

FTIR analysis of the extract was performed using FTIR (Agilent technologies, USA). The spectra were scanned in the range of 4000-650 cm^{-1} with a resolution of 4 cm^{-1} (Sadiq *et al.*, 2015).

Antibacterial activity

Antibacterial activity of the extract was determined against *E. coli*, *S. typhimurium*, *S. aureus* using agar well diffusion method (Khan *et al.*, 2022). Eight different concentrations of the extract were prepared (200, 100, 50, 25, 12.5, 6.5, 3, 1.5 mg/ml) in sterilized distilled water. Nutrient agar (Oxoid, UK) plates were prepared and spread with each bacterial culture (10^8 CFU/ml) separately. A well of 10 mm was made in each agar plate and 200 µl of each of the extract concentration was added, followed by incubation for 24 h at 37°C. The results were interpreted as the diameter of inhibition zone around the well.

Development and characterization of BPE enriched yogurt

Beetroot extract enriched flavored yogurt was prepared as functional product by using two different concentrations of extract (2% and 4%). One liter of pasteurized full cream milk was inoculated with starter culture (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and incubated in the dark for 5 h at 37°C, followed by refrigeration for another 5 h at 4°C (Oh *et al.*, 2016). The starter culture 4%, v/v was added into the milk at a initial inoculum size of 5×10^6 colony forming unit/ml. The yogurt samples were subjected to measurement of pH, titratable acidity and total solid contents. For enumeration of lactic acid bacteria (LAB), yogurt samples were serially diluted and spread onto the surface of de Man, Rogosa and Sharpe agar plates, followed by incubation at 37 °C for 48 h and CFU/ml were counted in each sample.

Sensory evaluation of yogurt

The yogurt samples prepared with BPE (2% and 4% extract) were subjected to sensory evaluation from a panel of 16 individuals. The characteristics to be evaluated were taste, color, odor, texture, acceptance of sourness, aftertaste and overall acceptability. Sensory attributes were measured by a 9-point hedonic scale by using a slightly modified method of Kainat *et al.* (2023). The yogurt sample without extract was used as a control.

Statistical analysis

The significant differences among mean observations were determined by using One way ANOVA and Tukey's tests, using SPSS version 23 (Hiranrangsee *et al.*, 2016).

Results and Discussion

Optimized extraction

Response surface methodology (RSM) was used to determine the optimized conditions for the extraction of bioactive compounds from beetroot pomace through ultrasonic assisted extraction (UAE). The total 17 runs were proposed by RSM for the extraction process and highest responses for 2, 2-diphenyl-1-picryl hydrazyl (DPPH) inhibition (66.6%), betaxanthins (174 mg/L), betacyanins (265 mg/L) and total phenolic content (TPC) of 2.78 mg gallic acid equivalent (GAE) per gram (dry basis) were observed during optimization (Table 1). Linear model was used to assess the influence of extraction parameters on TPC and the model was significantly fit to the experimental data ($p < 0.05$). All the extraction parameters influence the TPC, however the nature of influence was only significant ($p < 0.0001$) for sample to solvent ratio. Lack of fit was insignificant, which indicated that the data was well fitted. The coefficient of correlation (R^2) was 0.8. The other response variables such as DPPH and betalain content were not significantly influenced by the extraction parameter. UAE relies on the rupturing the cells of the vegetable/fruit by sound waves and hence ensures the increases surface contact between the solvent itself and the plant material. This ultimately leads to a higher extraction yield of the bioactive compounds (Kainat *et al.*, 2023). In this study at the optimized extraction condition (48% solvent concentration, 28 min extraction time and, 1:17 sample to solvent ratio), obtained extract was dried into powder and TPC of 37.05 mg GAE/g extract was obtained, which was significantly higher than the previous result for the TPC quantified (3.67 mg GAE/g) in beetroot juice (Vasconcellos *et al.*, 2016). UAE was reported to be an effective technique for the high yield extraction of pigments and phenolic compounds from beetroot byproducts (Fernando *et al.*, 2021).

FTIR analysis

FTIR analysis was used to detect the various functional groups present in the beetroot pomace extract (BPE) by detecting of the peaks in the infrared region. These functional groups were correlated to the various active compounds found within the extract (Figure 1).

Table 1: *Optimization of beetroot pomace extraction using response surface methodology.*

Exp no.	Time (min)	Solvent (%)	Sample to solvent ratio	TPC (mg GAE/g)	Betacyanin (mg/L)	Betaxanthins (mg/L)	DPPH inhibition (%)
1	45	70	1/15	1.94	127	43	37.6
2	30	50	1/15	2.26	108	101	66.6
3	30	30	1/20	2.77	101	56	36.5
4	45	50	1/10	1.68	96	99	39
5	30	50	1/15	2.36	265	140	40
6	30	70	1/20	2.78	28	23	60
7	15	50	1/20	2.39	83	53	38
8	30	50	1/15	1.81	108	101	18
9	30	30	1/10	1.46	81	65	10
10	45	50	1/20	2.54	37	20	43
11	15	70	1/15	2.36	30	37	50
12	15	50	1/10	1.55	65	45	43.54
13	30	50	1/15	2.06	152	92	35
14	30	70	1/10	1.53	58	40	13
15	45	30	1/15	2.24	38	27	13
16	30	50	1/15	2.11	162	174	35
17	15	30	1/15	2.54	96	52.5	25.45

Table 2: *FTIR analysis of beetroot pomace extract.*

Range (cm^{-1})	Functional groups and class of compounds	Beetrootpomace extract (cm^{-1})
3450-3250	-OH in phenols and alcohols	3293.8
2936-2913	-CH_3, -CH_2 stretch in aliphaticcompounds	2927.9
1650-1580 1618-1498	N-H, 1° Amines Benzene ring in aromaticcompounds	1619.4
1360-1150 1410-1310	-CH2X representing alkylhalides. -OH in tertiary alcohols or phenols	1367.7
1055-1020 1150-1000	Si-O-Si in silicone or organicsiloxane C-F stretch in aliphatic fluoro compounds	1026.5
700-610	Alkyne bend	667.07

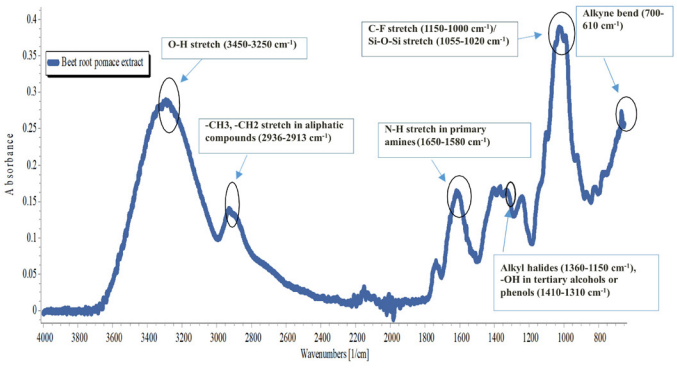

Figure 1: *Fourier Transform infrared spectrometer analysis of beetroot pomace extract.*

The peaks identified by FTIR were assigned to the corresponding functional groups (Table 2). The most intense peak was observed at 3293.8 cm^{-1} indicating the –OH group in alcohols and phenols (Sadiq *et al.*, 2015). The bands observed at 2936-2913 cm^{-1} referred to the presence of carboxylic acid in BPE (Kushwaha *et al.*, 2018). The second intense peak was observed at 1026.5 cm^{-1} which corresponds to the Si-O-Si group in silicones or organic siloxanes or the C-F stretch in aliphatic fluoro compounds (Nandiyanto *et al.*, 2019). The third intense peak was observed at 1619.4 cm^{-1} corresponding to the range 1650-1500 cm^{-1} indicating primary amines or benzene rings in aromatic compounds. Kushwaha *et al.* (2018) also reported that the absorption peaks in the range of 1000-1300 cm^{-1} corresponded to the presence of phenols and alcohols in BPE.

Antibacterial activity

All the test pathogens were found susceptible to BPE. At highest test concentration (200 mg/ml), inhibition zones of 29.67, 31 and 30 mm were observed against *E.*

coli, *Salmonella typhimurium* and *S. aureus*, respectively (Table 3). Previously, Vulić *et al.* (2013), reported antibacterial activity of BPE against both Gram +ve and -ve bacteria, with predominant activity against *S. typhimurium*. Salamatullah *et al.* (2021) also reported that beetroot pomace extract exhibited predominant antibacterial activity against Gram negative bacteria which might be due to the difference in the cell wall composition of Gram positive and negative bacteria. The phytochemicals present in the beetroot have been reported to exhibit antibacterial and antifungal properties (Salamatullah *et al.*, 2021).

Table 3: *Antibacterial activity of beetroot pomace extract.*

Concentration (mg/ml)	Diameter of inhibition zone (mm)		
	E. coli	*S. typhimurium*	*S. aureus*
200	29.67 ±0.6^{a}	31 ±1^{a}	30 ± 3^{a}
100	25.5 ±0.5^{b}	27 ±0.6^{a}	27 ±1^{a}
50	21.7 ±1.5^{a}	19.7 ±1.5^{a}	19.7 ±2^{a}
25	17.67 ±1.5^{a}	16 ±1^{ab}	13.7 ±1.5^{b}
12.5	16 ±0.6^{a}	15.3 ±1.1^{a}	15.7 ±0.5^{a}
6.25	15 ±1^{a}	14.5 ±0.7^{a}	14.7 ±1.5^{a}

Different superscript letters within a row indicate means which are significantly different ($p < 0.05$).

Beetroot extract enriched yogurt

Two different yogurt samples were prepared with varying extract concentrations: i.e., 2% and 4% and a control yogurt without extract was also prepared. pH was not significantly different among the samples but there was an increase in the total slid content with the addition of extract (Table 4). Moreover, LAB count and titratable acidity were significantly increased ($p < 0.05$) with the increasing concentration of the BPE.

Table 4: *Total solid content, pH, titratable acidity and LAB count for beetroot pomace enriched yogurt.*

Yogurt samples enriched with extract	pH	Total solid content (%)	Titratable acidity	Lactic acid bacteria count (Log CFU/ml)
Control	5.1±0.1^{a}	50±1.1^{b}	0.36±0.01^{b}	4.73±0.5^{b}
2% extract	5.2±0.3^{a}	55±1.6^{a}	1±0.1^{a}	6.92± 0.3^{a}
4% extract	5.3±0.25^{a}	57±1.8^{a}	1.08±0.04^{a}	7.5± 0.2^{a}

Different superscript letters within a column indicate means which are significantly different ($p < 0.05$).

Sensory evaluation

After the addition of BPE in yogurt, taste score was significantly decreased in comparison to control sample, however, color and odor scores were significantly high after the extract addition (Table 5). There was no significant difference in the overall acceptability score of control and extract enriched yogurt samples. Jovanović *et al.* (2021) used 3% beetroot pomace flour to develop drinking yogurt samples. However, in this study the addition of 2% BPE revealed the relatively high acceptable sensory scores. Beetroot peel powder was also reported in the formulation of functional mayonnaise with improved physical, sensory and textural attributes (Lazăr *et al.*, 2022).

Table 5: *Sensory evaluation of beetroot extract enriched yogurt.*

Sensory attributes	Yogurt with 2% extract	Yogurt with 4% extract	Control yogurt
Taste	5.6±1.9^{b}	6.1±1.9^{b}	7.94±0.25^{a}
Color	8.6±0.5^{a}	8.2±1.1^{a}	7±1.1^{b}
Odor	7.3±1.4^{a}	7.3±1.3^{a}	5.6±2.2^{b}
Texture	7.4±1.4^{a}	6.6±1.5^{a}	7.5±1.4^{a}
Aftertaste	6±1.8^{a}	6.6±1.8^{a}	6.37±0.96^{a}
Overall acceptability	6.6±1.3^{a}	6.4±1.7^{a}	6.4±1.2^{a}

Different superscript letters within a row indicate means which are significantly different ($p < 0.05$).

Conclusions and Recommendations

Beetroot pomace is a rich source of phenolic compounds and anthocyanins which are associated with antioxidant activities. The extract showed antibacterial activity against gram +ve and gram -ve bacteria. Based on the bioactive potential BPE can be used in the formulation of functional food products. Functional yogurt prepared by using the pomace extract received significantly high color and odor scores from the panelists due to the color of anthocyanins. Hence, BPE along with its color attributes and bioactive potential can be used in formulation of food and pharmaceutical products.

Novelty Statement

Beetroot pomace is a byproduct and usually exposed as a waste which can pose environmental hazards. Optimized extraction of TPC and anthocyanins can ensure the effective utilization of pomace in the formulation of food products as a coloring agent, preservative and functional component due to its antioxidant and antibacterial potential.

Author's Contribution

Hafsa Tauqir: Performed all the experiments and analyzed the data.
Muhammad Bilal Sadiq: Designed the study, interpreted the data and write the manuscript.
Imran Ahmad: Did data analysis and manuscript editing.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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