Plant Archives Vol. 18 No. 2, 2018 pp. 1623-1627 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF EXTRACTION SOLVENT TEMPERATURE ON BETALAIN CONTENT, PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND STABILITY OF BEETROOT (*BETA VULGARIS* L.) POWDER UNDER DIFFERENT STORAGE CONDITIONS

Gaurav Pandey[1], Vivek Pandey[2], Piyush Raman Pandey[3] and George Thomas[1]*

[1*]Department of Molecular and Cellular Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India.
[2]Department of Vegetable Science, Punjab Agricultural University, Ludhiana, India.
[3]KrishiVigyan Kendra, Korak, Gekutownship, Upper Siang, Arunachal Pradesh, India.

Abstract

Beetroot is a good source of natural food colorant because it contains significant amount of betalain pigment. The study target was to investigate the effect of temperature in the extraction of betalain, phenolic content and antioxidant activity as well as beetroot powder storage conditions. The extraction of betalain was performed with 5pH and 50% ethanol under different temperature combination (10, 20, 30, 40 and 50°C). Further, the stability of beetroot powder during storage was analyzed by applying three different temperatures (10, 25 and 40°C). At 30°C, the maximum recovery of betacyanin (7.74±0.38), betaxanthins (5.36±0.31), total phenolic content (4.91±0.66 mg/g) were obtained and at 40°C, maximum antioxidant activity (6.91±0.03 mM/g) was observed. The temperature of 10°C was found optimum for storage of beetroot powder up to 5 weeks with minimum loss percentage and the storage results revealed that betaxanthins is more sensitive than betacyanins.

Key words: *Betacyanin, Betaxanthin, DPPH assay, Shelf-life, Beetroot powder*.

Introduction

Beetroot (*Beta vulgaris*) belongs to Chenopodiaceae family and classified as an herbaceous biennial plant. It is a good source of natural color. The colors are of two types; the Synthetic color made by humans and the Natural color (pigments) is found in nature (Gokhale & Lele, 2014). Vegetable and fruits are a good source of natural color. Now-a-days people use synthetic color in food that is very harmful for health. Natural colors are gaining significant importance because of their health properties buthigh cost and low stability are the major limitations using natural colors in food products. Some commonly used natural colors are anthrocyanin, carmine, Carotinoid and betalain.

Betalains are water-soluble nitrogenous substances, present in vacuoles of beetroot cells and synthesized from tyrosine amino acid in to two structural groups: betacyanins (red-violets pigments) and betaxanthins (yellow-orange pigments) (Azeredo, 2009; Herbach *et al*., 2006). Beetroot (*Beta vulgaris* L.) is a major source of edible betalains and protect humans against stress related disorder by inhibiting the lipid oxidation and peroxidation (Kanner *et al*., 2001; Kaur & Kapoor, 2002; Reddy *et al*., 2005). Beetroot is also a rich source of antioxidants which protect human body from age related disease and also prevent cancer, cardiovascular diseases by removal of free radicals (Butera *et al*., 2002; Cai *et al*., 2003; Tsai *et al*., 2009). It is also a good source of in numerous vitamins A, B, C, E and K, minerals and also contains potassium, magnesium, beta-cyanine, fiber, phosphorus, folic corrosive and iron that can be found in beets and beet greens. According to Gentile *et al*. (2004), betalains show anti-inflammatory effects and antiradicals.

Stability of natural pigments is major problem to replace the synthetic color. Stability of pigments depends on several factors including temperature, pH, light

***Author for correspondence :** E-mail : georgethomas@shiats.edu.in

intensity, oxygen, sugar and enzymes activity (Delagado-vargas *et al*., 2000; Degenhardt & Winterhalter, 2001). However temperature is the most determining factor for betalain decomposition within the optimal pH. According to Herbach *et al*. (2006) thermal degradation of betalains depends on the temperature of extraction. Strack *et al*. (2003) reported that preheating of plant material for a short time is helpful to avoid the enzymatic dilapidation of pigments. Tang & Norziah (2007) reported that betalains retention was maximum in 25 to 30°C. Manchali *et al*. (2013) reported that beetroot powder was stored up to 20 week below 22°C when the pH of beetroot powder is maintained at 5.5. Based on the above mentioned factors the present study was designed to investigate the effect of temperature in betalains extraction as well as stability of beetroot powder in the storage condition.

Material and methods

Sample preparation

The seeds of Ruby queen variety were obtained from the Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore, India and sown 2 cm depth in earthen pots (20 cm diameter and 20 cm deep) containing sandy loam soil. The Ruby queen variety of beetroot is reported for better betalain extraction and store for long time without affecting their quality; this variety provides good quality as well as quantity of red color (Betalain) (Gokhale & Lele, 2014). After 8 weeks of sowing, the beetroot tubers were collected, washed with distilled water, air-dried, and stored at 4°C for further processing. The sample drying (by microwave treatment at 450 watts for 20 seconds) and betalain extraction (at 5pH and 50% ethanol) were performed according to the previously standardized procedure (Pandey *et al*., unpublished data).

Microwave-drying process

In general, for microwave-drying process, beetroot tubers were thoroughly washed with water, air dried and sliced to 1/8" thickness. The sliced sample was placed in Microwave oven (Samsung) and treated at 450 watts for 20 seconds (Cardoso-ugarte *et al*. 2014). The dried product was then ground to a-40 USS mesh powder using a hammer mill.

Extraction of betalains from beetroot powder

The beetroot powder samples (0.1 g) were dissolved in 10 ml of 50 percent ethanol and maintained the pH, 5. Samples were agitated for 10 seconds and the homogenate was centrifuged at 6000 rpm for 10 min using High speed refrigerated centrifuge (Sigma Laborzentrifugen, Germany). The supernatant was collected after centrifugation, repeated same step for 2 more times to ensure maximum extraction of betalains (Ravichandran *et al*., 2013). The supernatant was collected and further used for determination of betalains.

Determination of total betalain pigments contents

The betalains classes of betaxanthins and betacyanins in the concentrates were resolved by UV-VIS spectrophotometer type: 108 (Systronics India, Ahmedabad) at 538 nm and 480 nm, individually (Stinitzing *et al*., 2003). The obtained absorbance reading was used to calculate the betalain concentration for every sample. The betalain content (BC) was computed as:

$$BC\ (mg/L) = [(A \times DF \times MW \times 1000)/ (e \times l)]$$

where, A is the absorption, l the path length (1 cm) of the cuvette and DF the dilution factor. For measurement of betacyanins and betaxanthins, the sub-atomic weights (MW) and molar elimination coefficients (e) (MW=550 g/mol; e= 60,000 L/mol cm in H_2O) and (MW=308 g/mol; e=48,000 L/mol cm in H_2O) were used.

Antioxidant activity of beetroot powder extracts

The antioxidant activity of extracted beetroot powder samples were resolved by DPPH methodology (Lee *et al*., 2003) with modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH (Sigma-Aldrich, St. Louis, Missouri, United States) in 50 ml of methanol and kept at -20°C till further use. Six ml of stock solution was mixed with 100 ml methanol for preparation of working solution. Each of 0.1 ml samples were vortexes for 30 sec with 3.9 ml of DPPH, left for 30 min for colour development and recorded the absorbance by Spectrophotometer at 515 nm. The scavenging activity was calculated by using the following equation:

$$\text{DPPH radical-scavenging activity (\%)} = [(A\text{control} - A\text{sample})/ A\text{control}] \times 100$$

where, A is the absorbance at 515 nm.

Determination of total phenolic content

Folin-ciocalteu colorimetric process (Singh *et al*., 2002) is used for the determination of total phenolic content. In this process, 10 times diluted Folin-ciocalteu's phenol reagent (Merck Specialities Pvt. Ltd., Mumbai, India) one milliliter volume was added to 0.2 ml of beet sample. Then, 0.8 ml of 7.5% sodium carbonate (Sigma-Aldrich St. Louis, Missouri, United States) solution was added and incubated the sample at room temperature for 30 minutes. The absorbance was measured at 765 nm using spectrophotometer and results were shown as mg/g.

Shelf-life study of storage samples

Beetroot sample was dried at 50°C and packed in polyamide pouches, then stored in three different temperatures (10°C, 25°C and 40°C) relative humidity 60% for 150 days. The half-life of stored samples was measured on the basis of betalain (betacyanins and betaxanthins) content, antioxidant activity and total phenolic content.

Statistical Analysis

All analyses were performed three times and data was reported as mean±standard deviation (SD). Results were processed by Excel (Microsoft Office 2008) and SPSS (SPSS16.0 software for Windows; SPSS Inc., Chicago, USA).

Results

In present investigation the effect of different temperatures (at 10, 20, 30, 40 and 50°C) on betalain extraction showed a higher range of variation in the contents of betacyanins (3.49±0.06 to 7.74±0.22mg/gm), betaxanthins (1.24±0.18 to 5.36±0.31 mg/g), total phenols (1.28±0.14 to4.91±0.66mg/g) and antioxidant activity (3.09±0.40to 6.91±0.03mM/g). The maximum extraction yields of betacyanins were found at 30°C (7.74±0.22 mg/g) followed by 20°C (4.53±0.09mg/g) and 40°C (4.48±0.38mg/g) and minimum betacyanins was found in 50°C (3.49±0.06 mg/g). Same result were found with betaxanthins, maximum extraction were found in 30°C (5.36±0.31 mg/g) followed by 20°C (3.34±0.26 mg/g) and 40°C (2.29±0.10 mg/g), minimum extraction were found in 50°C (1.24±0.18 mg/g). The total phenolic content was found to be the maximum at 30°C (4.91±0.66 mg/gm) followed by 20°C and 40°C (4.38±0.81, 4.01±0.31 mg/g) and the minimum extraction was found at 10°C (1.28±0.14).

The maximum antioxidant activity found at 40°C (6.91± 0.03 mM/gm) followed by 30°C and 50°C (6.04±0.08 and 5.26±0.03mM/g) and minimum antioxidant activity was found in 10°C (3.09±0.40mM/g) (table 1).

Stability of beetroot powder under storage

Among the three different temperatures (*i.e.*, 10, 25 and 40°C) applied to analyze the stability of biochemical constituents present in beetroot powder under storage conditions, the minimum percentage of loss was at 10°C and maximum loss was at 40°C. At 10°C, minimum loss was found in antioxidant activity (16.49%) followed by betacyanin content (17.57%) after 150 days and the maximum loss was found in betaxanthin content (23.32%) followed by the total phenolic content (21.99%). Similar result was found at 25°C, minimum loss was found in antioxidant activity (29.37%) followed by betacyanin content (32.29%) and maximum percentage of loss found in betaxanthin content (35.82%) followed by phenolic

Table 1: Temperatures effect on extraction of betacyaninsand betaxanthins, phenolic compound and antioxidant activity of Beetroot.

Temperature (°C)	Betacyanin Content (mg/gm)	Betaxanthins Content (mg/gm)	Total Phenolic Content (mg/gm)	Antioxidant activity (mM/gm)
10	4.48±0.28	2.27±0.22	1.28±0.14	3.09±0.40
20	5.44±0.38	3.34±0.26	4.38±0.81	4.16±0.05
30	7.74±0.22	5.36±0.31	4.91±0.66	6.04±0.08
40	4.53±0.09	2.29±0.10	4.01±0.31	6.91±0.03
50	3.49±0.06	1.24±0.18	2.31±0.15	5.26±0.03

Table 2: Temperature effect of beetroot powder in storage condition

Storage period (days)	Betacyanin Content (mg/ gm)	Betaxanthins Content (mg/ gm)	Total Phenolic Content (mg/ gm)	Antioxidant activity (mM/ gm)
10°C storage temperature:				
0	7.74±0.22	5.36±0.31	4.91±0.66	6.91±0.08
30	7.58±0.25	5.22±0.19	4.81±0.34	6.83±0.15
60	7.35±0.15	5.01±0.23	4.67±0.16	6.69±0.33
90	7.09±0.08	4.76±0.32	4.46±0.29	6.41±0.09
120	6.77±0.15	4.45±0.08	4.17±0.17	6.11±0.11
150	6.38±0.11	4.11±0.05	3.83±0.12	5.77±0.15
25°C storage temperature:				
0	7.74±0.22	5.36±0.31	4.91±0.66	6.91±0.08
30	7.42±0.12	5.12±0.17	4.72±0.19	6.72±0.13
60	7.01±0.18	4.85±0.19	4.46±0.13	6.46±0.19
90	6.54±0.21	4.53±0.13	4.15±0.05	6.14±0.17
120	6.03±0.13	4.02±0.07	3.71±0.14	5.59±0.14
150	5.24±0.06	3.44±0.14	3.20±0.17	4.88±0.16
40°C storage temperature:				
0	7.74±0.22	5.36±0.31	4.91±0.66	6.91±0.08
30	7.15±0.21	5.01±0.11	4.55±0.12	6.47±0.07
60	6.51±0.17	4.53±0.09	4.01±0.05	5.89±0.14
90	5.59±0.19	3.87±0.15	3.63±0.17	5.32±0.21
120	4.41±0.12	2.93±0.18	2.57±0.13	4.43±0.12
150	3.25±0.14	1.67±0.16	1.76±0.07	2.93±0.17

Table 3: Loss percent of beetroot powder after 150 day's storage in different temperatures.

Storage Temperature (°C)	Betacyanin Content (loss %)	Betaxanthins Content (loss %)	Total Phenolic Content (loss %)	Antioxidant activity (loss %)
10	17.57	23.32	21.99	16.49
25	32.29	35.82	34.82	29.37
40	58.01	68.84	64.15	57.59

content (34.82%). The temperature treatment at 40°C was not suitable for beetroot powder storage purpose because percentage of loss was maximum (up to 68%) after 150 days (table 2, table 3).

Discussion and Conclusion

The purpose of the present investigation was to identify the suitable temperature for the maximum recovery of betacyanin, betaxanthin, phenolic content and also a maximum antioxidant activity was found. The investigation was showed that the low temperature of 10°C is not sufficient for complete extraction of betalains and at higher temperature (50°C), the betalains (betacyanins and betaxanthins) might have degraded. Herbach *et al*. (2006) reported that temperature levels are responsible for the thermal degradation of betalains and at higher temperature the degradation rate was increased. The results of present investigation also corroborate with Tang *et al*. (2007) and according to these authors, the maximum pigment (betalains) retention was found between 25 to 30°C and increasing the temperature decreased the betalain retention.

Phenolic content are important secondary metabolites, it is not involve in the reproduction and growth process of fruits in plant species but play a important role to protect against pathogen related disease. In present investigation the maximum phenolic content was found in 30°C. Kujala *et al*. (2000) reported a variation in the content of phenolic compound depending on the part of beetroot that is used for extraction, *i.e.*, in flesh, crown and peel 4.2, 11.4, 15.5 mg of GAE/g of DW respectively.

The antioxidant activity was maximum found in 40°C because increase in antioxidant activity with increasing the temperature at 40°C and above, shows that the antioxidant activity depends not only the presence of betalain but also other polyphenols which could have been increased during the thermal treatment (Dewanto *et al*., 2002; Ravichandran *et al*., 2013) by the release of the bound form of antioxidant compounds due to disruption of cell membranes and cell walls.

Stability of beetroot powder under storage

The maximum stability of beetroot powder with minimum loss percentage of its biochemical constituents was found in 10°C. This result was correlated with previously reported Devi *et al*. 2012 study. He was found maximum stability of betalain at 10 and 20°C and maximum loss percentage was found in 40, 50 and 60°C. He was also reported that betacyanin extracted from *Basella alba* was highly or moderately resistant to pH, temperature and light. According to Azeredo (2009) betalains are more stable compared to betaxanthins. Furthermore, Manchali *et al*. (2013) reported that the beetroot powder and its products (dairy products, confectionaries) are stored up to 20 weeks depending on the pH (at 5.5) and storage temperature (below 22°C). The current investigation showed that temperature has an important role in maximum recovery/extraction yield of betalain pigments as well as phenolic compounds and the temperature of 30°C was found ideal for extraction purpose. Increasing the temperature during extraction process of beetroot powder up to 40°C, the maximum antioxidant activity was recorded. The data also revealed that the beetroot powder could be stored up to 5 months at 10°C with minimum loss of biochemical constituents. During storage, the betaxanthins were found more temperature sensitive compared to betacyanins. Furthermore, this investigation is important for increasing the beetroot powder application in food, beverages, cosmetics and pharmaceutical industry.

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