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ANALYSIS OF BEETROOT (*BETA VULGARIS* L.) JUICE PROCESSING EFFECT FOR MAXIMUM BETALAIN EXTRACTABILITY AND OPTIMUM ANTIOXIDANT ACTIVITY

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Abstract

Beetroot is rich in betalain contents that are utilized as food colorants and food additives due to their health properties.The study's target was to investigate the effect of processing techniques due todirect juice process, hot-air drying and microwavingon Beetroot samples. Further, the combined affect of these processesunder the influence of variable ethanol treatments (30, 50, 70%) and pH ranges (*i.e.*, pH 3, 5, 7) were evaluated for complete betalain recovery/extractability and optimum antioxidant activity. The results revealed maximum recovery of betalain contents with direct juice procedure up to 800mg/literfollowed by microwave treatment of 450wattsfor 20 seconds up to 750mg/liter and with hot-air drying treatment up to 600mg/liter.However, the sample exposures of 150 and 750 watts microwave treatments resulted in minimum betalain extractability of 500 to 300mg/liter respectively. Similarly, the antioxidant activity increased up to 43% in microwave procedure of 450 watts for 20 seconds and 34% in hot-air drying procedure at 5pH and 50% ethanol percentage.In both processes antioxidant activity was significantly increasedas compared to control.

Keywords: Direct juice process, Hot air dry process, Microwave drying process, Betacyanins, Betaxanthins.

Introduction

Color is an important feature of food and also quality indicator. Now-a-days, the synthetic colors are extensively used for coloring food products which are very harmful from health prospects point of view (Fletcher, 2006). Vegetable and fruits are good source of natural colorants. High cost and low stability are the major limitations in using natural colors in food products (Herbach *et al*., 2006). Different types of natural colors *viz*., anthrocyanin, curcuminoids, carmine and betalains are easily available in the market (Wissgott & Bortlik, 1996; Cai *et al*., 2001).

Betalains are water-soluble nitrogenous compound, present in vacuoles of beetroot cells (Strack *et al* 2003; Stintzing & Carle, 2004; Zryd & Christinet 2004; Herbach *et al*. 2006; Moreno *et al*. 2008; Azeredo 2009). Beetroot, *Beta vulgaris* L. is a diploid (2n=18) of Chenopodiaceae family (Watson & Dallwitz 1992). Betalains is derived in to sub classes: betacyanins (red-violets pigments) and betaxanthins (yellow-orange pigments) (Delgado-Vargas *et al*. 2000). Beetroot a rich source of antioxidants that protects our body from age related diseases (Vinson *et al*. 1998). It is also a good source of invitamins A, B, C, E, K, minerals (Ca, Fe, Mg, P, K, Na, Zn, Cu, Mn, Se) and also contains beta-cyanine, fiber, folic corrosive that can be found in beets and beet greens. Betalains showed antioxidant activity, anti-inflammatory effects and antiradical properties (Tesoriere *et al*. 2004; Gentile *et al*. 2004). Antioxidant activity of beetroot pigments is involved in prevention of disease like cancer, cardiovascular disease by removing of free radicals (Clifford *et al.* 2015).

Natural pigments are mainly extracted through solid-liquid extraction procedures. In case of betalain, the source materials are ground and extracted pigments with water and the use of ethanol or methanol solutions can further enhance betalain yield. Different extraction processes are used in betalain extraction (Eskilsson & Bjorklund 2000). Microwave process is good for betalain extraction and reported to reduce the processing time,

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energy demand, solvent use and increase in pigment yield (Hemwimon *et al*. 2007; Chen *et al*. 2008; Proestos & Komaitis 2008). Maximizing the betalain yield during extraction and processing is prerequisite to cost-effective market availability and extensive use of betalains as food colourants in powder form. Betalains are susceptible to pH, temperature, light, water activity, oxygen, metal particles, and enzymatic activities (Stintzing & Carle 2006). However, temperature is themost determining factor for betalain decomposition within the optimal pH. Thus, processing changes the content of betalains, consequently colour of food and the antioxidant activity; these factors restrict betalains uses as food colorants. The physical factors like temperature, pH and solvents including ethanol, methanol, and acetone play important role in betalain extraction. The aim of this investigation was to assess the distinctive procedure of betalain extraction as direct juice process, hot air drying, microwave procedure to investigate the antioxidant activity of extracted betalain and also investigate the importance of pH, ethanol percent in extraction process.

Materials and Methods

Plant material

The fresh Beetroots were purchased from the local vegetable market, Allahabad, India and stored at 4^0C. The experiments were generally performed immediately after procurement.

Juice extracts process

The stored Beetroots were washed, pre-cooked for 5 minutes over steamto soften the beetroot tissues. The steam exposed softened beetroot samples were peeled, cut in slices and the juice extraction was performed in a domestic juice extractor. The extracted juice was filtered in a cloth strainer, and then centrifuged at 4000 rpm for 15 min, collected the supernatant, micro filteredusing an A/G Tech™ membrane with a mean pore diameter of 0.1 m. and stored for betalain content analysis by UV-VIS spectrophotometer.

Air-drying process

In general, for the air-drying, beetroots were thoroughly washed with water, dried and sliced to 1/8"thickness. The sliced product was placed on a stainless steel mesh belt and dried on a Microtech Medicraft air dryer (Medicraft Electro Medicals Pvt Ltd, Lucknow and India) at 60^0C and 40% relative humidity. The sample was driedupto 6 h and maximum product temperature applied was 60^0C. Moisture content in final product moisture was ranged between 3–5.5% on all tested samples. Dried product was then ground using a hammer millinto a -40 USS mesh powder.

Microwave-drying process

Microwave irradiation was done using Microwave oven (Samsung). Ten grams of each sample was placed over microwavable dishes and treated at 150, 450 and 750 watts for 20 seconds. The dried product was then ground to a -40 USS mesh powder using a hammer mill.

All the samples after the respective treatments were cooled to roomtemperature. The samples weretaken intriplicates for further extraction studies.

Extraction of betalains from Beetroot juice

Beetroot juice (0.5 ml) was diluted in 10 ml of 30, 50 and 70 percent ethanol and maintained the pH at 3, 5 and 7. Samples were agitated for 10 seconds and then homogenate was centrifuged at 6000 rpm for 10 min. The supernatant was collected after centrifugation and repeated same step for 2 more times to ensure maximum extraction of betalains. Finally the supernatant was collectedand further used for determination of betalains.

Extraction of betalains from Beetroot powder

Beetroot powder samples (0.1g) were dissolved in 10 ml of 30, 50 and 70percent ethanol and maintained the pH at 3, 5 and 7. 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. The supernatant wascollectedand further used for determination of betalains.

Each sample after the particular treatment was cooled and stored on room temperature. The samples were taken for further extraction studies. Each experiment was performed in triplicate.

Determination of total betalain content

The betalain classes of betacyanins and betaxanthins in the concentrates were resolved by UV-VIS spectrophotometer [type 108, Systronics (India) Limited, Ahmedabad] at 538 nm and 480 nm respectively (Stintzing *et al*. 2003). The obtained absorbance reading was used to calculate the concentration of betalains 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 pathlength (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 extracts

The antioxidant activity of extracted betalain samples were resolved by DPPH methodology (Lee *et al*. 2003) with modifications. The antioxidant activity of beetroot at different treatment was evaluated by DPPH technique because it takes very short time compared with other methods (Duh *et al*. 1999 and Chang *et al*. 2002). The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH (Sigma-Aldrich, St. Louis, Missouri, United States) in 50ml of methanol and kept at -20^{0}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 vortex for 30 secwith 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:

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

Where, A is the absorbance at 515 nm.

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 16.0 software.

Results and Discussion

The betalain extractions, as well as the antioxidant activity at different treatment conditions were investigated and found the juice process showed maximum betalain extractability and the microwave process was ideal for retaining maximum antioxidant activity. Furthermore, the betalain extractability and antioxidant activity were influenced by 5pH followed by 3pH in all treatment combinations. Similarly, in present investigation 50% ethanol showed maximum extractability of betalain and antioxidant activity followed by 30% ethanol.

Determination of betalain content

In direct juice process, maximum betalain was found with the combination of 50% ethanol at 5pH, where betacyanins and betaxanthins concentration was found 782.60±24.68 and 506.46±18.94 mg/liter, followed by the combination of 30% ethanol at 5pH and 50% ethanol at 3pH (Table1). In 2007, Tang & Narziah were also found maximum betalain extraction at 5 and 6pH in their study.

The air dry treatment, in 5pH betalains extraction was maximum and in 7pH the betalain extraction was minimum (table 1). The notable result was shown with the combination of 5pH and 50% ethanol, with this combination betacyanins and betaxanthins was found 580.23±15.48 and 389.70±15.10mg/liter followed by 70% ethanol with 5pH (505.16±6.7010mg/liter). Delgado-Vargas *et al*. (2000) reported that 20 to 50% ethanol had a significant association in extraction of betalains.

In microwave process, the impressive result was shown with the combination of 450 watts with 50% ethanol at 5pH (table.1). In this combination betacyanins and betaxanthins were found 736.73±31.01 and 494.06±20.41 mg/liter followed by 5pH and 30% ethanol combination 656±22.06 and 445.60±9.35.This result was correlated with previously reported result in which betalain

Table 1: Combine effect of ethanol and pH on extraction of betacyaninsand betaxanthins from Beet root through diverse procedure (E- ethanol percent, P- pH).

Treatment	Juice extracts process (mg/liter)		Air-drying process (mg/liter)		Microwave-drying process (150 Watt) (mg/liter)		Microwave-drying process (450 Watt) (mg/liter)		Microwave-drying process (750 Watt) (mg/liter)	
	Betacyanins	Betaxanthins	Betacyanins	Betaxanthins	Betacyanins	Betaxanthins	Betacyanins	Betaxanthins	Betacyanins	Betaxanthins
Control	521.23±17.32	297.58±12.15	483.79±18.21	265.54±12.67	434.35±11.97	243.67±21.25	543.28±15.31	305.39±8.25	215.82±12.78	131.12±7.25
P3 × E30	440±15.65	286.40±17.92	410.73±26.50	252.16±14.67	406.40±15.82	261.86±26.04	454.93±41.01	307.26±31.95	261.43±16.80	167.26±9.00
P3 × E50	577.16±39.28	375.73±15.00	405.13±15.79	267.83±34.72	461.40±26.32	308.20±18.44	566.26±27.13	372.63±20.23	356.80±36.25	232.16±24.14
P3 × E70	390.73±8.23	263.06±27.21	376.50±19.77	233.10±22.43	369.70±34.07	240.03±9.70	402.33±8.83	247.36±9.92	249.16±31.13	159.90±17.92
P5 × E30	588.73±10.46	392.60±38.36	443.36±22.23	265.70±8.70	450.83±24.62	300.13±14.23	656.93±22.06	445.60±9.35	197.56±10.36	137.16±35.42
P5 × E50	782.60±24.68	506.46±18.94	580.23±15.48	389.70±15.10	504.50±18.49	332.93±9.56	736.73±11.01	494.06±20.41	301.80±19.90	195.80±17.87
P5 × E70	477.66±14.20	306.10±20.30	505.16±6.70	331.30±6.87	423.70±11.20	285.86±8.63	580.53±35.33	394.96±12.37	189.80±26.99	115.96±24.68
P7 × E30	389.10±36.21	240±12.94	441.03±21.41	290.36±23.03	397.23±32.33	259.26±23.05	462.03±26.57	300.86±9.46	194.83±15.80	125.96±16.11
P7 × E50	434.26±30.80	263.56±8.43	393.10±8.47	245.03±33.50	439.63±22.77	287.03±6.38	524.86±8.65	358.10±15.48	250.50±29.69	150.16±21.36
P7 × E70	377.53±16.56	250.66±13.10	334.73±17.13	210.06±31.93	326.60±25.15	212.26±14.42	446.06±16	297.76±10.70	159.56±36.99	87.433±13.12

Table 2: Combined effect of ethanol and pH on antioxidant activity (DPPH assay)of Beetroot through diverse procedure (E-ethanol percent, P- pH).

Treatments	Juice extracts process (mM/10ml)	Air-drying process (mM/10ml)	Microwave drying-process (150 Watt) (mM/10ml)	Microwave-drying process (450 Watt) (mM/10ml)	Microwave-drying process (750 Watt) (mM/10ml)
Control	13.06±1.10	14.10±1.10	20.26±0.45	21.96±0.83	18.46±0.85
P3 × E30	11.50±1.10	15.00±0.70	17.40±1.22	26.33±1.26	19.86±0.35
P3 × E50	13.16±0.76	18.16±0.96	19.86±1.61	25.63±1.46	23.36±0.35
P3 × E70	12.26±1.10	13.53±0.92	14.80±0.80	23.90±0.40	23.16±1.02
P5 × E30	12.26±0.66	16.33±0.61	22.30±0.79	25.83±0.90	22.16±0.61
P5 × E50	15.00±1.30	21.26±0.75	33.96±0.80	37.63±1.62	26.60±1.44
P5 × E70	14.03±0.55	17.60±1.05	27.40±0.78	34.46±0.66	24.20±0.62
P7 × E30	13.00±0.40	14.36±1.19	14.33±0.85	23.33±1.30	18.33±0.55
P7 × E50	14.20±0.65	14.40±0.65	16.36±0.72	26.40±0.70	21.96±0.83
P7 × E70	11.33±0.85	15.56±0.75	14.40±1.24	25.16±0.80	16.73±0.66

degradation was depends on temperature and time, if temperature was high betalain degradation rate was increased (Ravichandran *et al.* 2013).

DPPH- radical- scavenging activity

In microwave process, the maximum antioxidant activity (37.63±1.62 mM/10ml) was found with the combination of 450 watts with 50% ethanol at 5pH (table 2). It shows that the antioxidant activity is not just depend on the presence of betalain but some polyphenols also contributes, which could have been increased during the treatment (Ravichandran *et al.* 2013).Generally, the antioxidant activity decreases after processing of food and vegetables (Stintzing *et al.* 2005), while in the present outcome, the antioxidant activity shows increasing trend after processing.

In air dry process, the valuable antioxidant activity was found with the combination of 50% ethanol at 5pH. The antioxidant activity increased in this event up to 34% (21.26±0.75 mM/10ml) while in case of microwave process it was increased up to 43% (table. 2). The reason behind maximum antioxidant activity found from microwave process is that, here three different thermal treatment at different ethanol percentage and pH were given while in air dry process temperature was constant (Ravichandran *et al.* 2013 and Latorre *et al.* 2013).

In juice process, the antioxidant activity showed a slight increase (14%, 15.00±1.30 mM/10ml) comparatively because no thermal treatment has been given to samples (table 2).

The study recommends an overall enhancement in betalain extractability and retention of antioxidant activity with treatment combinations of 5pH, 50% ethanol and 450 watt microwave process for 20 seconds. However, all treatment combinations of 7pH showed detrimental effect on betalain extraction and antioxidant activity. Similarly the treatment with 70% ethanol also showed minimum betalain extractability and antioxidant activity. The endogenous enzymes like β-galactosidase, polyphenol oxidases and peroxidases are responsible for the betalain degradation and color losses (Escribano 2002). Betalains extraction influenced by factors like pigment content, moisture content, pH, light, temperature and oxygen are also responsible to effect the stability of betalain, which needs to be considered to ensure optimum pigment and color retention in foods (Herbach *et al.* 2006). Dewanto *et al.* (2002) was found that due to thermal processing, there was enhanced nutritional value of tomato and total antioxidant activity. Adefegha & Oboha (2009) also found that cooking causes a significant increment in antioxidant activity in tropical green leafy vegetables. On the basis of this outcome it is suggested that the thermal process assume imperative part to increase the antioxidant activity.

Conclusion

The current investigation revealed significant impact of processing Beetrootjuice samples for efficient betalain extraction and enhanced antioxidant activity. The direct juice process as well as optimized microwave exposure, ethanol treatment and pH have increased betalain extraction and antioxidant activity to a desired level. The higher antioxidant activities of Beetroot extract after microwave treatment have scope for improvement of a biosynthetic procedure to obtain additives with a potential for application in food, cosmetics and pharmaceutical industry.

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