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Original article

Nutritional Differences Found In Different Stages Of Beetroot And Its Importance In Value-Added Products

Running title- Different stages of beetroot and value-added products

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ABSTRACT

The present investigation was designed to investigate the effect of different maturity stages (60, 80 and 100 days) of beetroot on its nutrients and also evaluate the nutritional composition of value-added products prepared by using different stages of beetroot. In this study, the beetroot was organically grown at 60, 80 and 100 days of maturity. The different stages of beetroot were used to developed value-added products i.e. traditional products, baked products, dessert, and preserved products at different percentage of proportion. All the developed value-added products were sensory evaluated by using 9-point hedonic scale and most acceptable one were selected for further nutritional analysis. The fresh beetroot of different stages and all value-added products of different stages were nutritionally analyzed with the standard method of analysis and AOAC method. Our data show that beetroot and its value-added products at 60 days of maturity had significantly ($p<0.05$) highest content of

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folate, choline, betanin, and betaine but the content of carbohydrates, saponins, and oxalic acid were significantly highest at 100 days of maturity. The content of vitamin C was firstly increased from 60 to 80 days after that it was decreased significantly from 60 to 100 days. This study provides comparative data to evaluate the effect of maturity stages of beetroot on its nutrients and according to the need of nutrient, we can supplement beetroot or its value-added products.

Keywords: Beetroot, maturity stages, 9-point hedonic scale, AOAC, value-added products, betanin, betaine.

1. INTRODUCTION

It was well-documented that health benefits of high fruit and vegetables led to growing interest is called "functional food" and their application is useful in disease prevention.[1] So that, people tend to consume more fruits and vegetable-based products which has high levels of bioactive phytonutrients.[2] Beetroot is one of the few vegetables that contain pigments which are bioactive called betalains. [1] Betanin is the most liberal compound found in betalain, which make 75-95% of total betalains. Betaine has exceptionally high free-radical scavenging activity. Beetroot also contain some anti-nutrients such as oxalate and saponins. [3] According to some scientific studies on food the chemical composition of fruits and vegetables are changes due to maturity stages. [4] To best of our knowledge there is no study available who addresses the effect of different maturity stages on chemical composition of beetroot and nutritional differences found in value added products prepared by using different stages of beetroot.

So keeping in above view the present study is planned to assess the effect of different maturity stages of beetroot on its nutritional composition and also evaluate the nutritional composition of value added products prepared by using different stages of beetroot.

2. MATERIALS AND METHODS

2.1. Sample

The beetroots which were used in this study were organically grown in Ghala farm, Lucknow at 60, 80 and 100 days of maturity at the month of November to March for every year at the period of 3 years.

2.2. Products Formulation

After that all the three stages of beetroots were used to prepare the value added products by using standard method of cooking. Beetroot sabji were prepared from different stages of beetroots by incorporating beetroot with potatoes at different percentage i.e. 60% beetroot and 40% potato (Type-A), 80% beetroot and 20 % potato (Type-B) and 100% beetroot and 0% potato (Type-C) but in control sabji only 100% potato was used. All these sabjies were prepared by applying traditional Indian method of cooking with stir fry. However, various types of beetroot cutlets were also prepared from different maturity stages

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of beetroot. The beetroot cutlets were also prepared by incorporating beetroot with potatoes, cheese and bengal gram flour i.e. Type-A (20% beetroot + 25% potato + 20% cheese+ 10% bengal gram flour), Type-B (30% beetroot + 15% potato + 20% cheese + 10% bengal gram flour) , Type-C (40% beetroot + 5% potato+ 20% cheese + 10% bengal gram flour) and control cutlets (0% beetroot + 45% potato+20% cheese+10% bengal gram flour) were prepared by using deep frying method. The different types of beetroot salad were prepared from different maturity stages of beetroots. All the beetroot salad were prepared from 100% beetroot by applying different method of cooking i.e. raw beetroot (Type-A), steamed beetroot (Type-B) and boiled beetroot (Type-C) and the plain salad was prepared by carrot as a control salad. In baked product three different types of beetroot cakes and beetroot biscuits were prepared by incorporating beetroot powder. The beetroot cakes were prepared by 30% (Type-A), 40% (Type-B) and 50% (Type-C) of beetroot powder at different stages of maturity and control cake was prepared without beetroot powder. But beetroot biscuits were prepared by incorporation of beetroot powder at 5 (Type-A), 10 (Type-B), 15 percent (Type-C) and control biscuit were prepared without beetroot powder. However, the beetroot powders were prepared from freeze dryer (Lyophilizer) and they were dried at -20 ^{0}C at 1.0±0.3 mmHg vacuum pressure for 48 hours freeze drying and ground by hammer mill. The preserved products beetroot jam and beetroot pickle were also prepared from different stages of beetroots. The beetroot jams were developed by 60% (Type-A), 80% (Type-B) and100% (Type-C) of beetroot in apples by using standardized basic formula for preparation method but control jam was prepared by using only 100% apple. The three different types of beetroot pickles were developed by 100% of beetroot by using different method of cooking i.e. raw beetroot (Type-A), steamed beetroot (Type-B) and boiled beetroot (Type-C). However, pickle prepared with raw lemon was considered as control pickle.

Organoleptic evaluation of all developed products

All the developed value added products were evaluated for their organoleptic characteristics i.e. color, flavor, texture, taste and overall acceptability by applying 9-point Hedonic Scale by semi- trained panel of 10 judges from BBDU and Era's Lucknow medical university.

Analysis

The fresh beetroot of different stages of maturity (60, 80 and 100 days) and their value added products were analyzed for nutrients (carbohydrates, folic acid, choline and vitamin C), pigments (betanin and betaine) and anti-nutrients (Saponins and oxalic acid).

i. Nutrients

The content of total carbohydrates was estimated by using AOAC (2000) method. The folate content was also estimated by applying standard method of AOAC, 2000. In this method reagents buffer solution, 0.1 M HCl, α-amylase, M NaOH: NaOH pellets, rat serum and basal media were used. After doing heat treatment and tri-enzyme treatment the sample extract was used to determine the total folates in food and it can be kept for 1 month. Then

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microbiological assay was done for estimation of folate and the result was calculated by the regression line of the standard curve responses by using 4th-degree polynomial plots. However, the choline estimation was done by using AOAC official method 999.14. In this method acid hydrolysis and enzymatic determination was done. After that the estimate the choline concentration in test sample was calculated by applying following equation.

Choline hydroxide, mg/100 g = A/S V/1000 100/W

Where,

A = net absorbance of sample

S = slope of standard graph

V = volume in mL of hydrolysate (50 mL)

W = weight in g of sample; 100 = conversion to 100 g basis; 1000 = conversion of g to mg.

The Vitamin C content was also estimated by using the standard method of analysis given by AOAC (2000). In this method some reagents which were used for estimation of vitamin C were as follows: metaphosphoric acetic acid solution, ascorbic acid standard solution and indophenol standard solution. After that extraction and estimation was done and finally, the amount of vitamin C (mg/100 g) was calculated as follows: [5]

$$\text{Vitamin C (mg/100g)} = \frac{Y - B \times V \times 100}{X - B \times W}$$

Where,

Y = Volume of dye solution used for sample aliquot

B = Volume of dye solution used against blank

X = Volume of dye solution used against standard

V = Volume of aliquot made

W = Weight of sample (g)

ii. Pigments

a. Betanin

The betanin content in fresh beetroot and its value added products was purified by using Column Chromatography methods and analysis of purified betanin was done by using Spectrophotometric and Spectra deconvolution method (Goncalves et al., 2012). [6]

b. Betaine

The Betaine content in fresh beetroot and its value added products was estimated by applying HPLC method (Bessieres et al., 1999). In this method the reagents Liquid nitrogen,

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betaine, MilliQ grade water, AG1 8X resin, 200–400 mesh, OH– form, Sodium heptane sulfonate, Na_2SO_4 and H_2SO_4 were used for analysis of betaine. Then peak area of the absorbance plotted on 0.05-4 mM of concentration and measuring the absorbance peak area of extract at the unknown concentration of betaine was done. [7]

iii. Antinutrients

a. Saponins

The Saponins content was estimated by applying modified method of Gestetner et al. (1966). The saponins was analyzed by using some reagents such as Standard saponin solution, 1 N H2SO4 in dioxane, Acetic acid 10%, Conc. H2SO4, Sodium sulfate, Alumina (aluminium oxide, acetic acid), Benzene, Diethyl ether and Methanol Solution (3%) in benzene. The extraction, isolation and estimatio were done for analysis of saponins content. [8]

b. Oxalic acid

The Oxalic acid content was determined by the standard method of analysis A.O.A.C (1980). The reagent which were used for the analysis of saponins where Potassium permanganate solution, Acetate buffer solution pH 4.5, 2.5 g anhydrous calcium chloride, acetic acid, calcium oxalate powder and Tungstophosphoric acid. All the samples were homogenized for 15 minutes at room temperature and the slurry was used for further analysis. Then precipitation of oxalic acid was done and determination of oxalic acid was done by titration method.[9]

Statistical analysis

The obtained data were statistically analysis for one-way analysis of variance (ANOVA) and also finding the correlations among different obtained parameters. If the values were less than 0.05 obtained data were considered statistically significant.

3. Results

Acceptance is a positive attitude which measured the actual composition of food items, which evaluate how much an individual likes or dislikes that products.[10] On organoleptic evaluation of all value added developed products of different stages of beetroot by nine-point hedonic scale in term of their colour, appearance, aroma, texture, taste and overall acceptability. According to the result in traditional products i.e in various types of beetroot sabji it was found that the Type-A was most acceptable products then Type-B and Type-C in all sensory attributes. There was significanrt effect of stages of maturity was also observed in beetroot sabji in all sensory values. It was found that the acceptability of all beetroot sabji was decreased with increasing the maturity stages of beetroot. The different types of beetroot cutlets were prepared by using different stages of fresh beetroot. The cutlet prepared by 20 percent of chopped beetroot was found to the best in term all organoleptic characteristics. In beetroot salad Type-C was most acceptable product in all stages of maturity.

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The baked products were prepared by using different percentage of beetroot powder at different concentration. After evaluating the sensory observations of various types of beetroot cakes at stage-I, stage-II and stage-III it was found that the Type-B was the most acceptable product in all sensory score as its all sensory score were lying at Avg+CD, while Type-C was inferior in comparison to Type-A and Type-B in all sensory values. The beetroot biscuit prepared with incorporation 5 per cent of beetroot powder of different stages of maturity having highest scores in all sensory values. After evaluating the sensory characteristic of various types of beetroot jam of stage-I, II and III the Type-A was most acceptable products as its average sensory score were lying above the Avg line, while other types were lying below the Avg line. In beetroot pickle Type-C pickles of all stages were the most acceptable products which was lies between Avg and Avg + CD.

Analysis

On chemical analysis of fresh beetroot at different stages of maturity i.e. 60, 80 and 100 days of maturity after sowing are given in Table 1. The result regarding effect of different stages of maturity of beetroot shows that the content of carbohydrates , saponins and oxalic acid were significantly ($p<0.05$) increased with the advancement of maturity stages from 60 to 100 days of maturity. The beetroot harvested at 60 days after sowing had significantly ($p<0.05$) highest content of folate, choline, betanin and betaine than those harvested at 80 days and 100 days after sowing. But the content of vitamin C was firstly increased from stage-I (60 days) to stage-II (80 days) after that it was decreased significantly ($p<0.05$) from stage-I (60 days) to stage-III (100 days).

On chemical analysis of most acceptable developed products prepared from beetroot at different stages of maturity the obtained data are presented here at Table 2 to Table 5. The present data shows that on chemical analysis of traditional products (beetroot sabji , beetroot cutlet and beetroot salad), baked products (beetroot biscuits and beetroot cake and preserved products (beetroot jam and beetroot pickles) also shows significantly ($p<0.05$) highest content of carbohydrates, saponins and oxalic acid at Stage-III (100 days of maturity). However, the content of folate, choline, betanin and betaine values were significantly ($p<0.05$) highest in all value added products of beetroot at Stage-I (60 days of maturity) and vitamin C content was firstly increased from stage-I to stage-II then it was decreased significantly.

Table 1: Nutritional Composition of fresh beetroot at different stages of maturity

Stages	Carbohydrates (g/100 g)	Folate (mg/100g)	Choline (mg/100g)	Vit. C (mg/100g)	Betanin (mg/100g)	Betaine (mg/100g)	Saponins (mg/100g)	Oxalic Acid (mg/100g)
Stage I (60	30.9±0.0	80.3±0.5	6.0±0.0	8.9±0.0	181.3±1.1	127.6±0.5	8.4±0.5	29.1±0.1

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days)								
Stage II (80 days)	32.2±0.2	70.3±0.3	5.9±0.0	9.0±0.0	150.6±0.5	124.6±0.5	11.6±0.5	33.3±1.1
Stage III (100 days)	36.2±0.1	64.5±0.4	3.5±0.4	8.0±0.0	110.3±0.5	110.0±0.0	15.6±0.5	36.6±0.5
p-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values are mean ± SE of three independent determinations
NS- Non significant at 5% level

Table 2: Nutritional Composition of traditional products at different stages of maturity

Beetroot Sabji								
Stages	**Carbohydrates (g/100 g)**	**Folate (mg/100 g)**	**Choline (mg/100 g)**	**Vit. C (mg/100 g)**	**Betanin (mg/100 g)**	**Betaine (mg/100 g)**	**Saponins (mg/100 g)**	**Oxalic Acid (mg/100 g)**
Stage-I (60 days)	18.2±0.1	23.7±0.3	4.1±0.0	4.9±0.4	98.9±0.3	79.0±0.2	3.1±0.1	17.3±0.4
Stage-II (80 days)	19.3±0.1	22.4±0.2	3.3±0.1	5.8±0.4	96.1±0.1	77.4±0.3	4.4±0.1	18.0±0.5
Stage-III	20.4±0.1	21.3±0.0	2.6±0.1	5.3±0.0	95.2±0.2	76.3±0.1	5.5±0.2	19.3±0.1

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(100 days)								
p<0.05	<.001	<.001	<.001	0.062 NS	<.001	<.001	<.001	0.005
Beetroot Cutlet								
Stage -I (60 days)	24.9±0.0	70.2±0.1	4.03±0.0	5.2±0.0	98.9±0.3	79.0±0.2	2.4±0.0	15.6±0.5
Stage -II (80 days)	25.4±0.1	69.0±0.2	3.05±0.0	6.2±0.0	96.1±0.1	77.4±0.3	3.5±0.0	17.4±0.1
Stage -III (100 days)	26.1±0.0	68.3±0.2	2.03±0.0	5.2±0.0	95.2±0.2	76.3±0.1	4.3±0.2	18.5±0.0
p<0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Beetroot Salad								
Stage -I (60 days)	14.3±0.0	68.3±0.1	4.4±0.1	7.8±0.3	167.1±0.1	125.0±0.0	10.2±0.2	28.2±0.0
Stage -II (80 days)	15.3±0.0	68.5±0.4	4.3±0.0	7.6±0.6	147.6±0.3	123.1±0.1	8.6±1.1	29.1±0.0
Stage -III (100 days)	17.2±0.0	61.8±0.3	3.2±0.0	7.6±0.6	108.6±0.5	107.6±0.5	13.0±1.0	29.1±0.0
P<0.05	<.001	<.001	<.001	0.809 NS	<.001	<.001	0.003	<.001

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Values are mean ± SE of three independent determinations
NS- Non significant at 5% level

Table 3: Nutritional Composition of baked products at different stages of maturity

Beetroot Cake								
Stages	**Carbohydrates (g/100 g)**	**Folate (mg/100 g)**	**Choline (mg/100 g)**	**Vitamin C (mg/100 g)**	**Betanin (mg/100 g)**	**Betaine (mg/100 g)**	**Saponins (mg/100 g)**	**Oxalic Acid (mg/100 g)**
Stage-I (60 days)	57.8±0.0	9.2±0.2	6.6±0.1	6.7±0.0	86.3±1.1	79.0±0.2	6.5±0.2	8.6±0.4
Stage-II (80 days)	84.8±0.5	7.1±0.0	5.3±0.1	5.2±0.1	96.1±0.1	77.4±0.3	8.2±0.0	12.9±0.1
Stage-III (100 days)	86.3±0.1	4.7±0.4	3.4±0.0	4.0±0.1	95.2±0.2	76.3±0.1	9.9±0.1	13.5±0.6
P<0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Beetroot Biscuits								
Stage-I (60 days)	76.1±0.0	4.6±0.0	0.2±0.0	3.8±0.0	60.1±0.1	6.7±0.0	0.4±0.0	1.8±0.0
Stage-II (80 days)	77.0±0.0	3.0±0.0	0.2±0.0	4.2±0.2	40.0±0.0	5.0±0.0	0.5±0.0	2.2±0.0
Stage-III (100	79.7±0.6	2.0±0.0	0.1±0.0	3.1±0.0	37.7±0.5	4.0±0.0	0.7±0.0	3.0±0.0

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days)								
P<0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Table 4: Nutritional Composition of preserved products at different stages of maturity

Beetroot Jam								
Stages	**Carbohydrates (g/100 g)**	**Folate (mg/100 g)**	**Choline (mg/100 g)**	**Vitamin C (mg/100 g)**	**Betanin (mg/100 g)**	**Betaine (mg/100 g)**	**Saponins (mg/100 g)**	**Oxalic Acid (mg/100 g)**
Stage-I (60 days)	80.7±0.6	8.6±0.4	5.0±0.0	7.5±0.0	65.6±2.0	17.3±0.5	0.2±0.0	1.5±0.0
Stage-II (80 days)	83.5±0.8	15.5±0.5	3.2±0.0	9.1±0.1	49.0±1.0	15.7±0.1	0.5±0.0	3.8±0.1
Stage-III (100 days)	84.9±0.1	14.4±0.2	2.3±0.0	8.9±0.0	47.7±0.2	14.4±0.1	0.9±0.0	5.7±0.1
P<0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Beetroot Pickles								
Stages	**Carbohydrates (g/100 g)**	**Folate (mg/100 g)**	**Choline (mg/100 g)**	**Vitamin C (mg/100 g)**	**Betanin (mg/100 g)**	**Betaine (mg/100 g)**	**Saponins (mg/100 g)**	**Oxalic Acid (mg/100 g)**
Stage-I (60	24.7±0.0	9.7±0.0	0.5±0.0	8.9±0.0	99.6±0.5	20.6±0.0	0.3±0.0	2.4±0.0

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days)								
Stage -II (80 days)	26.4±0.3	8.1±0.2	0.3±0.0	9.9±0.1	85.0±1.0	19.1±0.0	0.2±0.0	3.3±0.1
Stage -III (100 days)	28.1±0.1	6.6±0.0	0.1±0.0	8.2±0.0	81.3±0.5	17.1±0.0	0.3±0.0	5.3±0.0
P<0.05	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

4. DISCUSSION

In this present study we have documented the nutritional changes in beetroot at different stages of maturity was found. The content of carbohydrates, saponins and oxalic acid were increased with the advancement of maturity stages of beetroot from stage-I (60 days) to stage-III (100 days). The content of carbohydrates may be increased in beetroot with maturity stages due to increasing the activity of amylase enzyme by advancement of maturity stages. Similar finding have been reported by Biondo et al. (2013) they found that with advancement of maturity stages of beetroot leaves the content of carbohydrates was increased.[11] According to the Osipitan et al., 2015 and Wobeto et al. 2007 the saponins content was also present in abundance in cassava root and it was increased with maturity of the plant.[12] The present findings are agreed with previous study done by Freiding and Goldman (2011) who reported that the content of oxalic acid was increased in table beet and its leaf with advancement of maturity.[13] Musa et al. (2011) reported that anti-nutrients contents were increased with maturity of the plant it may be due to increasing the secondary plant substances or secondary metabolites in plant tissues and organs during aging.[14] Jesus et al., 2009 also reported that content of folic acid was decreased with ripping of the vegetable.[15] Almost similar finding have also in other vegetable too. According to Kumari (2016) in okra vegetable the content of vitamin C was increased initially by increased the maturity stages but later on with advancement of maturity stages it was reduced. However, the pigment anthocyanin present in okra vegetable was also decreased with increased the maturity stages.[16]

The present study showed that the chemical constituents in the beetroot was changed during its different maturity stages and however the greatest amount of folate, choline, betanin and betaine was found at stage-I (60 days). So the value added products prepared from this stage are more benefical for the prevention of many disease i.e. due to folate content it is helpful for the prevention of anemia, coronary artery disease, hypertension, stroke, diabetes,

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cancer, kidney diseases, infertility etc.[17] While highest amount of choline present in this stage make more benifical for Memory Development, Heart Disease, the study done by ATTICA indicated that the subject whose diet were rich in choline and betaine had lowest percentage of several inflammatory markers and it also benifical for prevention of breast cancer.[18] Betaine is a lipotrope it help in reduction of accumulation of fat in the liver. Studies shows that on healthy and diabetic animals feed a high-fat diet leads to hepatic steatosis that is prevented by ingestion of betaine or choline.[19] However, betanin having antioxidant property it prevents LDL oxidation and DNA damage.[20] But the content of vitamin C was highest at 80 days of maturity (stage-II). While value added products prepared from beetroot of 100 days of maturity have highest value of carbohydrates and anti-nutrients (oxalic acid and saponins). Though excess amount of carbohydrates consumption is not beneficial for health. While higher consumption of oxalic acid causes reduction in bioavailability of minerals and kidney stone.[21]

5. CONCLUSION

Overall it can be concluded that beetroot was very nutritious vegetable it was quite rich source of carbohydrates, vitamin C, folate, choline and antioxidants such as betaine and betanin and also have anti-nutrients (saponins and oxalic acid). The nutritional changes found in beetroot at different stages of maturity. According to need of nutrient we can supplement the beetroot or its value added products of different stages of maturity.

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