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# Standardization of process condition in batch thermal pasteurization and its effect on antioxidant, pigment and microbial inactivation of Ready to Drink (RTD) beetroot (*Beta vulgaris* L.) juice

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*Betalains*
*Color*

**Abstract**

Thermal pasteurization is still one of the most effective methods for inactivating undesirable microorganisms in liquid foods. Pasteurization produces safer liquid foods with longer shelf-life. The batch thermal pasteurization process was standardized with different total heating time (*fh*) [($T_1$: Control-Un treated) ($T_2$: 96°C, 540 s) ($T_3$: 96°C, 720 s) ($T_4$: 96°C, 900 s)]. Decimal reduction time ($D_{96}$-*value*) of 1.5 min and *Z-value* of 10°C used to calculate maximum P-values. The effects of in-pack pasteurization on betalain pigment (Betacyanin and Betaxanthin), antioxidant (% of DPPH scavenging activity), CIE color ($L^*$ $a^*$ $b^*$ values), native micro flora and other physicochemical quality parameters were also evaluated during prolonged storage at ambient temperature (27-30 ± 2°C). The processing and storage of beetroot juice had a decisive impact and significant ($p < 0.05$) degradation in the betalain (betaxanthin and betacyanin) content, color and antioxidant activity during storage.



## Introduction

The escalating trepidation of consumers about their health and new lifestyles that are fascinating them towards increased fresh vegetables, fruits and vegetable-fruit juice blends consumption (Sloan, 2005). Vegetables juices provide antioxidant compounds and a complex mixture of other natural substances that promote antioxidant capacity which leads to health benefits (Arnao *et al*., 2001). The presence of bioactive compounds in fruits and vegetables has recently been considered to be of nutritional importance in the prevention of chronic diseases, such as cardiovascular disease, various types of cancers, diabetes and neurological diseases (Willet, 1994; Kalt *et al.*, 1999). Beet root (*Beta vulgaris* L.) ranks among the 10 most powerful vegetables with respect to its antioxidant capacity ascribed to a total phenolic content of 50–60 µmol/g dry weight (Vinson *et al*., 1998; Kahkonen *et al.*, 1999). Beet root is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which comprise two main groups, the red betacyanins and the yellow betaxanthins. They are free radical scavengers and prevent active oxygen-induced and free radical-mediated oxidation of biological molecules (Pedreno and Escribano, 2001). The antioxidant activity of betalains pigment preventing the cancer (Cao *et al*., 1996; Kapadia *et al*., 1996). According to Nilsson (1970), the Betacyanin and Betaxanthin contents of red beetroots vary depending on the cultivar (Von Elbe, 1975), although some new varieties produce higher betalain contents.

Conventional thermal pasteurization is the most common method for extending the shelf life of vegetable and fruit juices, by inactivating microorganisms and enzymes, which relies on a mathematical calculation to ensure the safety of the products. Theoretically this is a combination of the time-temperature profile and the microbial destruction/inactivation. Thermal process design is normally adopted to maximize microbial inactivation with minimal collateral degradation to product quality (Gould, 1995). At a pH below 4.5, the risk of growth and toxin production by *Clostridium botulinum* is extremely low and for products with pH values between 4.0 and 4.5, processes are aimed at controlling the survival and growth of spore forming organisms such as *Bacillus coagulans*, *Bacillus polymyxa* and *Bacillus macerans.* A heat process of 9-15 min at 96.0°C is regarded as adequate for this purpose, when the pH is between 4.0 and 4.3 (Ramesh, 1995). The need to standardize the processing conditions arises when the behaviour of the different components is considered because the rate of a chemical reaction generally doubles for a 10°C rise or 2 minutes extend where as rates of bacterial destruction increases ten-fold under similar conditions. The major constraint on optimising procedures is that the desired degree of sterility must be achieved (Holdsworth, 1985).

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However, more processing time concomitant losses in terms of flavor, color, sensory and nutritional qualities occur when foods are heat treated. Therefore standardizing the processing conditions of beetroot juice by a thermal processing such as in-pack pasteurization is totally justified (Goodman *et al*., 2002). There are many studies on betalain purification, antioxidant and antimicrobial activities of beet root pomace extracts. However, there is no systematic study for thermal pasteurization of Ready-to-Drink (RTD) beetroot juice and its effect on physico-chemical quality parameters. Therefore the main objectives of this work were to standardization of thermal pasteurization process and determination of P-value of Ready-to-Drink (RTD) beetroot (*Beta vulgaris* L.) juice in multilayer laminated pouches and to study the effects of in-pack pasteurization on betalain pigment (Betacyanin and Betaxanthin), antioxidant activity, color, native micro flora and other physicochemical quality parameters of beetroot juice.

## Materials and Methods

*Chemicals and Raw materials*

All Analytical chemicals were purchased from Sigma Aldrich Chemicals Pvt. Ltd. (Bangalore-India). Fresh beetroots were purchased from local market and used immediately for the experiment. The beetroot was washed thoroughly to remove the soil.

*Beetroot (Beta vulgaris L.) juice preparation*

Fresh beetroot (*Beta vulgaris* L.) juice was obtained after washing the beetroot 3-4 times with running tap water to remove the surface soil and then it was peeled out and sliced, the slices were grinded in a wet grinder and then pulped by using hydraulic press (D.K. Barry & Co (P) Ltd, New Delhi-India) and the extracted juice was again filtered using a four layer cheese cloth to remove remaining pomace. The fresh beetroot juice total soluble solids (°Brix) was adjusted (12°Brix) with sucrose followed by acidified (pH 4.2) with DL-Malic acid and then 200 mL juice was filled in pre-fabricated multilayer laminated pouches consisting of 12 µm Polyethylene terephthalate / 9 µm Aluminium foil / 15 µm Nylon / 80 µm Cast. Polypropylene (Total thickness 116 µm) of 200 ml capacity with a dimension of 15 x 20 cm at sterile conditions and then pouch was hermetically sealed using impulse sealing machine (Model: HP Impulse Sealer, M/s Sunray Industries Mysore, India). Then the pouches were divided into four parts and the following treatments were given to the samples; $T_1$: Control, $T_2$: In-pack-thermal pasteurization for 540 seconds, $T_3$: In-pack-thermal pasteurization for 720 seconds, and $T_4$: In-pack-thermal pasteurization for 900 seconds.

*Thermal pasteurization*

Conventional in-pack thermal pasteurization was carried out using steam jacketed kettle with the help of steel basket with proper closure. The process condition like temperature was fixed as 96°C (product temperature) and total heating time (*fh*) was given 540 s, 720 s and 900 s for $T_2$, $T_3$ and $T_4$, respectively. Heat penetration of the product was monitored through copper-constantan thermocouples fixed at the geometrical centre of the pouch and also a reference thermocouple was also placed in the steam jacketed kettle to maintain and monitor kettle temperature. Thermocouple outputs were connected to a data logger (Model: CTF 9004, M/s. Ellab, Denmark). The temperature of the beetroot juice and steam jacketed kettle was measured from the thermo-electro-motive-force at regular intervals of 60 seconds. Once the treatment time was over, samples immediately removed and placed in cold water 2-3 minutes for cooling. The total heating time (*fh*) and P value was calculated for all treatments. The thermal processed pouches were tested for sterility then the samples were used for further analysis.

*Process standardization and P-value determination*

The thermal pasteurization was conducted for three levels of total heating time (*fh*) (540, 720 and 900 seconds) with standard product temperature (96°C). The total heating time (*fh*) was standardized with respect to an inactivation effect of the thermal pasteurization on beetroot juice native micro flora. Red beetroot contains betalain substance that had been shown to have therapeutic effect on the body. It is more important to preserve the activity of the betalain components when producing the product (Wolf *et al*., 2009). Therefore P-value was determined with a 6D process of inactivation for *Bacillus coagulans* has been used for this study to complete inactivation of native micro flora and also to minimize the degradation of betalain compounds. Generally the heavy load of *Bacillus coagulans* in acid/acidified juices is $10^5$-$10^6$ spores. The $D_{96}$ Value of the *Bacillus coagulans* spores at 96°C can be up to 1.5 min and *Z value* is 10°C (Peng *et al*., 2012). According to 6D Concept of inactivation the minimum processing time was 9 mins ($D_{96}$ Value X 6D: 1.5 X 6 = 9 mins). The *Z value*, $D_{96}$ *value* and reference temperature was feed into Ellab Val suit Pro software prior to the processing, during processing the time-temperature profile monitored and recorded and P-value was calculated with help

of Ellab Val suit Pro software for thermal pasteurized juices.

*Methods of analysis*

Parameters of the samples were analysed as described below and all the experiments were carried out in triplicate.

*Total soluble solids (°Brix)*

The soluble solids (°Brix) were measured using a hand Refractometer (RF.5580 Euromex Brix hand Refractometer). Measurements were performed at 25.0 ± 2°C. The refractometer prism was cleaned with distilled water after each analysis.

*pH*

The pH was determined with a pH 700 Digital meter at 25.0 ± 2°C (Model: Eutech Instruments, Singapore). The pH meter was standardized using pH buffer of 4.0, 7.0 and 10.2.

*Acidity*

The titratable acidity (TA) was determined by titrating 1 mL of each sample (diluted to 20 mL final volume with deionized water) using 0.1 mol $L^{-1}$ NaOH. Results were expressed as percentage of malic acid 100 $mL^{-1}$ sample (Araujo *et al*., 2011).

*CIE Color ($L^*$ $a^*$ and $b^*$)*

The CIE Color ($L^*$ $a^*$ and $b^*$) values were measured using a Hunter Lab Scan Spectrophotometric colorimeter controlled by a computer that calculates color ordinates from the reflectance spectrum. (Hunter Lab Color Flex EZ 45/0° color spectrophotometer, USA). The results were expressed in accordance with the CIELAB system with reference to illuminate $D_{65}$ and with a visual angle of 10°. The samples were placed in an optical glass tray, using the white plate of the colorimeter as the background (Standard white plate no. CFEZ0503 X = 79.05, Y = 84.00, Z = 87.76). This background was used to standardize the measurements. The measurements were made through a diaphragm 30 mm.

*Betalain (Betacyanin and Betaxanthin) pigment analysis*

The betalain content (Betacyanin and Betaxanthin) was quantified by a method described by Nilsson (1970) and Mobhammer *et al*. (2006) with few modifications. The beetroot juice was diluted (30 times) with deionized water and the absorbance of the diluted juice was read at 538 nm and 480 nm using a spectrophotometer (Model: UV- Spectrophotometer, Spectronic® Genesys™ 2 Instruments, USA). The betalain content was calculated using an equation proposed by Cai and Corke (1999). The betacyanin and betaxanthin evaluated as equivalents to betanin and indicaxanthin respectively. Betacyanin content was calculated as:

$$\text{Betacyanin [mg/l]} = A.\ DF.\ Mw.\ 100/\ \varepsilon.\ L$$

wherein A is the absorption value at betanin λ max (538 nm) corrected by the absorption at 700 nm, DF is a dilution factor, Mw is the betanin molecular weight (550 g $mol^{-1}$), ε is the betanin molar extinction coefficient (60,000 L $mol^{-1}$ $cm^{-1}$) and L is the path length (1.0 cm) of the cuvette. Betaxanthin content was calculated as:

$$\text{Betaxanthin [mg/l]} = A.\ DF.\ Mw.100/\ \varepsilon.\ L$$

Where A is the absorption value at indicaxanthin λ max (480 nm) corrected by the absorption at 700 nm, Mw is the indicaxanthin molecular weight (308 g $mol^{-1}$), ε is the indicaxanthin molar extinction coefficient (48,000 L $mol^{-1}$ $cm^{-1}$).

*Antioxidant (% of DPPH radical scavenging) activity*

The percentage of 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity of the beetroot juice was determined by a method proposed by Canadanovic-Brunet *et al.* (2011) with few modifications. The hydrogen atom or electron donation abilities of the juice were measured from the bleaching of a purple-coloured methanol solution of stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). Briefly, 0.1 ml of samples or 0.1 ml of methanol (control) were mixed with 2.9 ml of 0.004% DPPH solution (10 mg in 250 ml of methanol prepared freshly) and methanol used as a blank. The mixture was vortexed thoroughly for 1 min and left at 37°C temperature for 30 min in dark, and then the spectrophotometer absorbance was read against blank at 517 nm (Model : UV Spectrophotometer, Spectronic® Genesys™ 2 Instruments, USA).

The capability to scavenge the DPPH radicals, DPPH scavenging activity (SADPPH), was calculated using the following equation:

$$SADPPH\ (\%) = (A_{control} - A_{sample})/A_{control} \times 100$$

Where:

$A_{control}$: absorbance of the control reaction (containing all reagents except the juice)

$A_{sample}$: absorbance in the presence of the juice

*Sensory Quality determination*

Sensory quality was determined using 9 point

Hedonic scale rating method (Like extremely-9; Dislike extremely-1) (Ranganna, 1999). For sensory taste, odor and Over all Acceptability (OAA) 20 semi-trained panellist were selected. The samples (Treated juices and throughout the storage samples) were presented in a glass with a capacity of 100 ml. For characteristics, (Odor, Taste and Over all Acceptability) the judge rated the preferred samples in comparison control T1 (untreated).

*Micro flora analysis*

Microbial analysis method was followed by Kathiravan *et al.* (2013). For the microbial counts, samples were serially diluted, plated in total count agar (PCA) for total plate (aerobic) counts, and in acidified Potato dextrose agar (PDA) for mold and yeast counts. Plates were incubated at 30°C for 48 h or 5 days for Total Plate Counts and Molds and Yeast respectively. Violet Red Bile Agar used for Coliforms detections.

*Data analysis*

All the experiments were carried out in triplicate. The data were analysed statistically to find out mean, standard deviations and significance (Snedecor and Cochran, 1988).

## Result and Discussion

*Process standardization and p-value determination*

The aim of process standardization is to reduce the thermal stress without affecting the quality attributes of the product, by the optimum total heating time (*fh*). The temperature of the product was preset to 96°C and total heating time (*fh*) was given three different timings like 540 s, 720 s and 900 s for $T_2$, $T_3$ and $T_4$, respectively. The total heating time (*fh*) with respect to 6D concept of inactivation was calculated as 540 seconds (9 mins) of minimum heating time and the heating time was increased upto 900 seconds. The first batch of beetroot juice ($T_2$) was thermal pasteurized at 96°C for a total heating time (*fh*) of 540 seconds, and 6.52 P value was achieved, however the native micro flora was not completely inactivated in $T_2$ batch juice. The second and third batch of beetroot juice ($T_3$ and $T_4$) also pasteurized at 96°C for a total heating time (*fh*) of 720 and 900 seconds respectively. The P-values of $T_3$ and $T_4$ batch was 11.16 and 15.89, respectively. The native micro flora of the $T_3$ and $T_4$ batch beetroot juice was totally inactivated. According to the microbial safety the $T_3$ batch (total heating time (*fh*): 720 s) was completely inactivated native micro flora, and maximum retention of betalain content (Betacyanin and Betaxanthin) and antioxidant when compare to $T_4$ (total heating time (*fh*): 900 s), for this reason the $T_3$ batch process condition (96°C for total heating time (*fh*): 720 s) was standardized to develop Ready-to-drink acidified vegetable juices with minimal degradation of pigment/color and antioxidant activity (Figure 3). Further the standardized $T_3$ batch was analysed throughout the ambient storage to find out the thermal pasteurization effect on antioxidant, pigment and other physico-chemical quality parameters.

*Effect of thermal pasteurization and storage on total soluble solids (°Brix), pH and total titratable acidity*

Table 1 results revealed the thermal pasteurization effect and storage time on the total soluble solids (°Brix) of Control ($T_1$ batch: Untreated) and standardized $T_3$ batch (96°C for total heating time (*fh*): 720 s) beetroot juice. The total soluble solids of thermally pasteurized beetroot juice were higher than the control beetroot juice. According to Tandon *et al.* (2003) the higher soluble solids of pasteurised juice are due to water evaporation during thermal pasteurising in the steam jacketed kettle. The total soluble solids remained almost invariable and no significant ($p > 0.05$) changes were observed during the storage. Tandon *et al*. (2003) also reported no significant changes in the total soluble solids during storage. Bull *et al*. (2004) also reported that the °Brix of thermally processed orange juice did not change significantly during storage time.

pH is one of the main quality characteristics that describes the stability of bioactive compounds in fruit juice (Sanchez-Moreno *et al*., 2006). The pH of thermal pasteurized beetroot juice was higher than that of control (Table 1). The increase in pH may take place due to the ascorbic acid degradation by thermally pasteurized juices. The pH of the beetroot juice found that no significant ($p > 0.05$) changes were observed throughout the storage. It leads to maintain good quality of juices. A similar study that described the pH of thermally processed Valencia and Navel orange juice found no significant modifications throughout the storage (Bull *et al*., 2004). Rivas *et al.* (2006) also reported no pH variations in thermally treated juice (blended orange and carrot juice) during storage. Yeom *et al.* (2000) also did not observe significant changes in heated orange juice during storage.

The total titratable acidity of beetroot juice was decreased slightly after thermal pasteurization and did not found any changes throughout the storage of juice. The decrease in total titratable acidity related to the increase found in pH (Table 1). The results compromise with Bull *et al.* (2004) who studied the

Table 1. Effect of thermal pasteurization and storage on physico-chemical and Hunter Color ($L^*$, $a^*$ and $b^*$) Values

| Storage Days | °Brix | pH | Acidity (% of malic acid ) | Hunter Color Values | | |
|---|---|---|---|---|---|---|
| | | | | L* | a* | b* |
| **Control ($T_1$)** | 12 ± 0 | 4.21 ± 0.00 | 0.1114 ± 0.0003 | 0.543 ± 0.005 | 1.956 ± 0.015 | 0.563 ± 0.005 |
| **0** | 13 ± 0 | 4.37 ± 0.00 | 0.1080 ± 0.0007 | 3.386 ± 0.005 | 4.706 ± 0.011 | 1.856 ± 0.005 |
| **15** | 13 ± 0 | 4.37 ± 0.00 | 0.1079 ± 0.0010 | 3.576 ± 0.005 | 4.910 ± 0.010 | 2.066 ± 0.115 |
| **30** | 13 ± 0 | 4.37 ± 0.00 | 0.1085 ± 0.0011 | 3.713 ± 0.005 | 5.126 ± 0.011 | 2.130 ± 0.010 |
| **45** | 13 ± 0 | 4.44 ± 0.00 | 0.1009 ± 0.0007 | 3.836 ± 0.011 | 5.416 ± 0.005 | 2.213 ± 0.005 |
| **60** | 13 ± 0 | 4.44 ± 0.00 | 0.1009 ± 0.0007 | 3.890 ± 0.000 | 5.550 ± 0.010 | 2.323 ± 0.005 |
| **75** | 13 ± 0 | 4.45 ± 0.00 | 0.1008 ± 0.0008 | 3.910 ± 0.010 | 5.626 ± 0.011 | 2.346 ± 0.005 |
| **90** | 13 ± 0 | 4.45 ± 0.00 | 0.1009 ± 0.0007 | 4.023 ± 0.023 | 5.683 ± 0.142 | 2.420 ± 0.000 |
| **105** | 13 ± 0 | 4.44 ± 0.00 | 0.1008 ± 0.0008 | 4.653 ± 0.005 | 5.826 ± 0.005 | 2.533 ± 0.005 |
| **120** | 13 ± 0 | 4.44 ± 0.00 | 0.1009 ± 0.0007 | 4.730 ± 0.010 | 5.923 ± 0.015 | 2.626 ± 0.020 |
| **135** | 13 ± 0 | 4.45 ± 0.00 | 0.1008 ± 0.0008 | 4.823 ± 0.005 | 5.953 ± 0.005 | 2.810 ± 0.051 |
| **150** | 13 ± 0 | 4.44 ± 0.00 | 0.1009 ± 0.0007 | 4.910 ± 0.000 | 6.016 ± 0.011 | 2.803 ± 0.005 |
| **165** | 13 ± 0 | 4.44 ± 0.00 | 0.1008 ± 0.0008 | 5.020 ± 0.020 | 6.183 ± 0.057 | 2.836 ± 0.023 |
| **180** | 13 ± 0 | 4.45 ± 0.00 | 0.1008 ± 0.0008 | 5.123 ± 0.005 | 6.250 ± 0.062 | 2.923 ± 0.005 |

Mean ± SD of three determinations

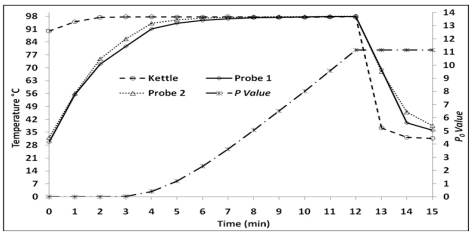


Figure 1. Heat penetration study and Pasteurization value ($P_0$ *value*) of thermally pasteurized ($T_3$) of beetroot juice

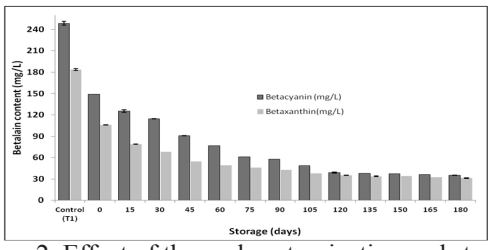


Figure 2. Effect of thermal pasteurization and storage on betalain pigment (Betaxanthin and Betacyanin) of beetroot juice

thermally processed Valencia and Navel orange juice found no significant modifications of total titratable acidity throughout the storage time.

*Effect of thermal pasteurization and storage on CIE Color ($L^*$ $a^*$ and $b^*$)*

The color degradation of beetroot juice by thermal pasteurization was investigated using CIE color parameters ($L^*$ $a^*$ and $b^*$). The major causes of color change in beetroot juice due to betalain pigment (Betaxanthin and Betacyanin). Table 1 represents the CIE Color values of the thermally pasteurized beetroot juice and control ($T_1$ batch: Untreated). The control juice sample had a redness ($a^*$), yellowness ($b^*$) and luminosity ($L^*$) values were 1.956 ± 0.015, 0.563 ± 0.005 and 0.543 ± 0.005, respectively (Table 1). The redness ($a^*$), yellowness ($b^*$) and luminosity ($L^*$) value of beetroot juice was significantly increased ($p < 0.05$) after thermal processing and throughout the storage. The increase in the Luminosity ($L^*$) values was evidently indicate that the degradation of betalain pigment in beetroot juice. Redness ($a^*$) values are also higher than the control juice sample. It is due to the degradation of betacyanin pigment by influences of heat, while degradation the betacyanin changes to yellowish-brown from deep violet-red color. Chandran *et al.* (2012) also found the betacyanin changes to yellowish-brown from deep violet-red color during heat treatment. The yellowness ($b^*$) of the beetroot juice was found to be not as much of change as redness ($a^*$). According to Gokhale and Lele (2011) the yellow pigments of beet root, betaxanthins, are more stable than the betacyanins (red pigments). Zhang *et al.* (1997) also found a color degradation in the heat pasteurized juice samples.

*Effect of thermal pasteurization and storage on betalain pigment (Betaxanthin and Betacyanin)*

Figure 2 shows the effect of thermal pasteurization and storage on beetroot juice betalain pigment (Betaxanthin and Betacyanin). Betalain is an important pigment component it possesses antioxidant ability and provides the protection against free radicals. It is also considered an indicator quality of juices, the higher degradation of betalain content leads to consumer dissatisfaction. The control ($T_1$) beetroot juice had a 183.58 ± 1.27 and 248.69 ± 2.86 mg/L betaxanthin and betacyanin respectively. The thermal pasteurization leads to significant ($p < 0.05$) degradation in the betaxanthin and betacyanin content (Figure 2). After thermal pasteurization the degradation was found to be 42.28 and 39.9% for betaxanthin and betacyanin respectively. Further it was linearly reduced 64.13 and 67.42% for betaxanthin and betacyanin respectively at 105$^{th}$ day storage. After 105$^{th}$ day storage it was gradually reduced. Similar results were reported by Herbach *et al*. (2004) who observed that during heating at 85°C the betalain pigment degrade and forms yellow-red in

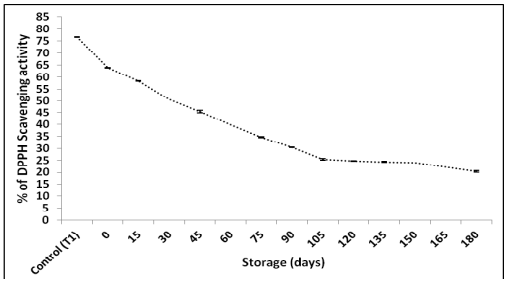


Figure 3. Effect of thermal pasteurization and storage on antioxidants activity (% of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging activity) of beetroot juice

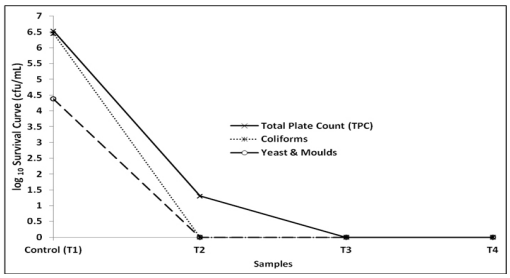


Figure 4. Micro flora inactivation of Control ($T_1$) and thermally pasteurized ($T_2$, $T_3$ & $T_4$) of beetroot juice.

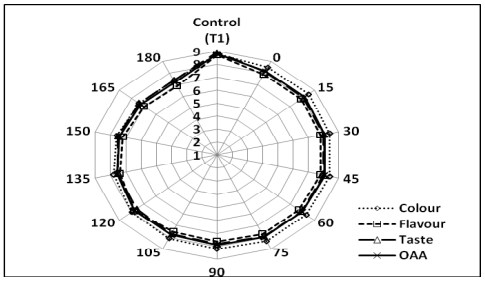


Figure 5. Effect of thermal pasteurization and storage on sensory analysis of beetroot juice

colour. Similarly Fan yung and Khotivari (1975) also observed that degradation of betalain pigment during heating.

*Effect of thermal pasteurization and storage on antioxidants activity*

Red beetroot had a superior antioxidant activity and had been ranked as one of the 10 leading vegetables having antioxidant effect (Kujaka *et al*., 2000). Imino and hydroxyl groups as well as phenolics substance contribute the antioxidant activity in beetroot juice (Wu *et al*., 2006). The antioxidant activity of control (T1) beet root juice had a 76.71 ± 0.17%, after thermal treatment it was reduced to 63.87 ± 0.12%, but during the storage time the antioxidant activity of the beetroot juice was Significant ($p < 0.05$) reduced (Figure 3). Initially it was reduced drastically and then it become stabilised. Our results were in accordance with Elez-Martinez *et al.* (2006a) who observed that thermally treated orange juice had a significant decrease in antioxidant activity. There is no comparison available in literature sources concerning the thermal pasteurization effect on antioxidant activity of Ready-to-Drink beetroot juice.

*Effect of thermal pasteurization and storage on micro flora and sensory analysis*

The native micro flora of the $T_2$ treatment (at 96°C for a total heating time (*fh*) of 540 seconds) was not completely inactivated after treatment; it has a 1.30 log CFU/mL of total plate count, where as the control ($T_1$) had a microbial load of 6.53, 6.44 and 4.38 log CFU/mL for total plate count, coliforms, yeast and moulds, respectively (Figure 4). The $T_3$ and $T_4$ treatment was completely inactivated the native micro flora of beetroot juice and throughout the storage time also not detected any microbial count. Our results were in accordance Fontanet *et al*. (2013) who studied the thermal processed grape juice; he also found that the microbial counts were drastically trimmed in thermally processed grape juice.

The sensory score was based on the 9 point hedonic scale rating given by the panellist, the overall acceptability (OAA) scores of the control beetroot juice was higher rating (8.8) (Figure 5). During storage time sensory score was significantly ($p < 0.05$) reduced. The color is the main factor to trim down the panellist score of the beetroot juice. It is due to the thermal pasteurization effect; however the beet root juice was accepted after 180$^{th}$ day ambient (27-30°C) storage. Dunn and Pearlman (1987); Min and Zhang (2003) also found that thermally processed juice had a significant decreased in the sensory score.

## Conclusion

Standardization of process condition and quality degradation of beetroot juice due to thermal pasteurization was studied. The standardized $T_2$ batch with total heating time (*fh*) of 720 s thermal in-pack pasteurization leads to the minimal degradation of color, betalain content (Betacyanin and Betaxanthin), antioxidant activity and complete inactivation of micro flora of beetroot juice. The color, betalain content (Betacyanin and Betaxanthin) antioxidant activity and sensory of the beetroot juice was significantly ($p < 0.05$) reduced during 180 days ambient (27-30°C) storage, but still the quality of the juice was adequate upto 180 days. We concluded that thermal pasteurization of 96 °C for a total heating time (*fh*) of 720 seconds with P-values of 11.16 would be a good method to produce microbiologically stable beetroot juice with the retention of quality attributes.

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