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Original Article

The effects of different short-term Storage Modalities on the Antioxidant Capacity of Beetroot Juice: Short term refrigeration of Beetroot Juice Better Preserves its Antioxidant Capacity

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ABSTRACT

Beetroot contains phytochemicals that have anti-inflammatory and antioxidant properties. The nutritional quality of consumed beetroot is, however, affected by post-harvest processing. To determine the effect of different short-term storage modalities on beetroot antioxidant activity. Beetroot samples were freshly sourced and randomly divided into 3 groups: Group A was used to prepare fresh beetroot juice on the day of analysis; Group B was used to prepare beetroot juice that was then refrigerated for one week before analysis while Group C was kept whole at room temperature for one week and then used to prepare the beetroot juice. Beetroot juice was prepared by mixing one-part of beetroot with one-part of clean water and blending the mixture. It was then freeze-dried, and the resultant powder used to measure the residual antioxidant activity and for phytochemical analysis to determine the presence of flavonoids. At analysis, all the three samples contained the same biochemical compounds and had no notable gross differences in smell, viscosity, and consistency. The juice prepared from beets stored whole for a week was of a more intense colour compared to that of the refrigerated beetroot juice. At lower concentrations of the extracts, refrigerated juice had lower antioxidant activity while that stored as a whole had the highest. Refrigerated juice had the highest antioxidant activity (IC50 of 10,457.11 mg/ml) while beetroot stored had the lowest activity (IC50 of 210,069.4 g/ml). Short term refrigeration of beetroot juice better preserves its antioxidant capacity.

1. Introduction

Red beetroot (Beta vulgaris rubra) is a root vegetable that was originally grown in the temperate climates of Asia, North Africa and Europe. Its cultivation is now however more widely spread to include other parts of tropical Africa, including Kenya, and the Americas (1,2). Beetroot is a highly nutritious vegetable that contains considerable amounts of both macro- and micro nutrients including starch, proteins and essential vitamins. Furthermore, beetroot is rich in nitrate which is reduced in the body to nitrites and nitric oxide (NO) which play an important role in the regulation body immunity, vascular homeostasis and metabolism (3). Other metabolically important flavonoids in beetroot include saponins, phenolics, carotenoids and betalains (4,5). These are phytochemicals with vital anti-inflammatory, antioxidant and chemo-preventive activities (6,7). Moreover, because of its rich colour mainly attributed to the betalain pigment, beetroot is increasingly being used as an organic source of food colouring. The nutritional quality of consumed beetroot is however affected by many factors including growing conditions, harvesting time and post-harvest processing (2,4).

Various methods have been used to preserve beetroot with variable effects on the intensity of the colour of the stored product and its antioxidant capacity. Fresh beetroot, for instance, is of a more intense colour than processed juices and is thought to be of superior quality following customer-

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satisfaction surveys. The colour intensity however declines with time when the juice is stored at room temperature and is accompanied by a concurrent decline in the inherent antioxidant activity (4). The effect of refrigeration on the colour intensity and the antioxidant capacity of beetroot juice is however hardly investigated. Other processes that have been associated with a reduction of the colour intensity of beetroot include short-term refrigeration (8), pasteurization and fermentation (4,9). Similarly, the antioxidant capacity of beetroot has been reported to be negatively affected by among others post harvesting processing and storage procedures like blending with other juices, boiling and fermentation (10,11). Other process like high-pressure treatment and vacuum and microwave drying techniques have, conversely, been shown to enhance the antioxidant capacity of beetroot (4). They are, however, expensive and not routinely available for everyday use in many families.

Over the past decades, both commercial and subsistence beetroot farming have gained momentum in Kenya. It is widely sold in supermarkets and by roadside vendors as a vegetable for use in making salads, soups and juices. There are several ways of storing the beetroot either as a whole or refrigerating the already prepared juice. The effect of these storage modalities on the oxidant capacity and colour intensity of beetroot juice has hardly been investigated. This study therefore aimed at determining the effect of different storage modalities of differently stored beetroot juices.

2. Materials and Methods

2.1. Sample collection and preparation

Beetroot samples for this experiment were sourced fresh from the same farm in Kericho County, Kenya. The collected samples were then randomly divided in to 3 groups: Group A was used to prepare a fresh beetroot juice; Group B was used to prepare beetroot juice that was then stored in the fridge for one week before analysis while Group C was kept whole in room temperature and under dry conditions for one week and then used to prepare the beetroot juice on the day of analysis.

2.2. Extraction of Beetroot Juice

The beetroot juice was prepared by mixing one part of the whole beetroot with one part of clean water and blending the mixture using a kitchen blender. Each extract thus prepared was then freeze-dried for three days and the powder thus gotten used for measuring the residual antioxidant activity and for phytochemical analysis to determine the presence of saponins, tannins, alkaloids, flavonoids and steroids.

2.3. Phytochemical analysis

The qualitative phytochemical screening was performed as previously described by (12) and Williams (2009). These methods employ the principle of chemical reactions of certain compounds with the target phytochemical in a given plant extract with a positive reaction being either a particular colour change or the formation of a known precipitate. The extracts that were tested were observed based on their colour change and precipitate formation as outlined below:

Saponins: The crude solvent extracts were mixed with 5ml of water and vigorously shaken. The formation of stable foam would indicate the presence of saponins.

Tannins (ferric chloride test): In this test, 0.5 ml of 5% ferric chloride solution were added to 0.5 ml of the sample solutions. Formation of a dark green colour would indicate the presence of tannins.

Alkaloids (Mayer's test): One ml of Mayer's reagent (potassium mercuric iodine) was added to 1ml of the test solutions and observed for a white precipitate, which would be the positive indicator for presence of alkaloids.

Flavonoids: Three drops of ammonia solution were added to 1ml of the crude extract followed by 0.5ml of concentrated hydrochloric acid. The formation of pale brown coloration was an indicator of the presence of flavonoids.

Steroids: One millilitre of chloroform was added to 1 ml of the sample solution then concentrated sulphuric acid was carefully added drop-wise by the side of the test tube to form a lower layer. A red brown ring was formed at the interface indicating the presence of steroids.

2.4. Measurement of Anti-oxidant Activity

The antioxidant activity of beetroot extracts and the control was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. This procedure was done according to Nithianantham et al., (2011) and Locatelli et al., (2009) with few modifications as described below. Twenty-five milligrams of each sample was diluted with methanol in 50 ml volumetric flasks. Then, 0.1MM of DPPH was prepared by adding 3.94 mg of DPPH in 100 ml methanol and then stored in the dark to minimize degradation. A 2.8 ml of DPPH solution was mixed with 200 µl of beetroot extract at various concentration ranging from 7.8 µg/ml to 500 µg/ml for both extracts and ascorbic acid standard. The samples were kept in a dark room for about 30 minutes and optical density was measured at wavelengths of 517nm using Cecil-Elect Spectrophotometer. The optical density was recorded and the percentage inhibition was calculated using the formula given below:

$$\text{Percentage Inhibition (\%)} = \frac{Asorbance\ of\ DPPH - Absorbance\ of\ sample}{Absorbance\ of\ DPPH} \times 100$$

2.5. Determination of inhibitory concentration (IC50)

The concentration required to inhibit 50% of DPPH radical (IC50) was determined using the regression line of probit according to log10 of extract concentration (15).

2.6. Data Analysis and Management

The collected data were entered in to excel sheets and used to generate tables and graphs for pictorial presentation

3. Results

3.1. Qualitative Phytochemical Analysis and Anti-Oxidant Activity

The beetroot juice prepared from beets directly from the farm and stored whole for a week was of a more intense colour compared to that of the refrigerated beetroot juice. There were no other notable gross differences in the appearance of the various juices including smell, viscosity and consistency.

Phytochemical screening of all the three samples analysed (Direct from farm, refrigerated juice and stored as a whole) tested positive for saponins, tannins, flavonoids, steroids and terpenoids. Among these phytochemicals, flavonoids, saponins and tannins are important secondary metabolites and may be compounds responsible for medicinal value of beetroot. The extracts had different radical scavenging activity with the refrigerated juice showing the lowest antioxidant activity at lower concentrations while the beetroot stored as a whole had the highest antioxidant activity (Table 1 and Graph 1). At higher extract concentrations, however, the antioxidant activity was lowest for the sample obtained from the beetroot stored as a whole. All through, ascorbic acid as a control had the highest antioxidant activity.

Table 1. Anti-oxidant activity of aqueous beetroot extracts stored at different conditions

Extract Concentration (mg/ml)	Ascorbic acid (% of inhibition)	Stored as a whole (% of inhibition)	Direct from farm (% of inhibition)	Refrigerated juice (% of inhibition)
7.8	15.29 ± 0.68	5.12 ± 0.89	4.16 ± 0.50	3.36 ± 0.75
15.25	28.68 ± 0.67	5.36 ± 0.58	5.11 ± 0.25	4.13 ± 0.08
31.25	56.17 ± 0.82	5.47 ± 0.70	5.29 ± 0.28	4.35 ± 0.23
62.5	59.27 ± 0.45	5.50 ± 0.60	5.43 ± 0.28	5.92 ± 0.76
125	96.55 ± 0.13	6.06 ± 1.22	6.77 ± 0.50	6.11 ± 0.81
250	97.18 ± 0.29	7.59 ± 0.76	7.78 ± 0.55	7.38 ± 0.52
500	97.39 ± 0.05	9.09 ± 1.63	10.31 ± 1.38	10.55 ± 1.58

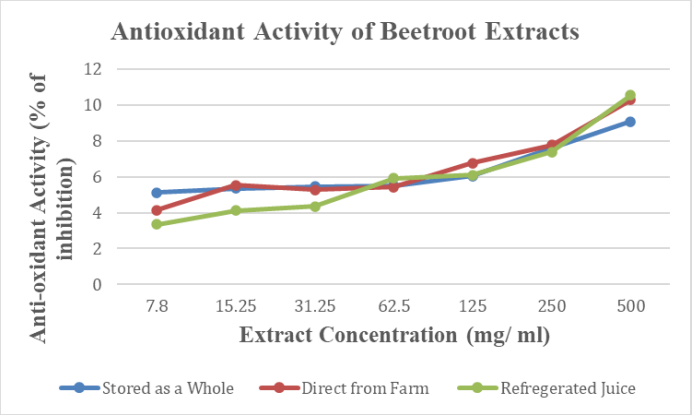

Figure 1. Anti-oxidant activity of aqueous beetroot extracts stored at different conditions

3.2. The IC50 Values of Beetroot Extracts Required to Inhibit 50% of DPPH

In the present study, beetroot juice stored as a whole had the lowest IC50 value of (210,069.4 g/ml), followed by direct from farm (157.9 g/ml) and refrigerated beetroot juice (10.5 g/ml). All beetroot juice extracts showed a significant DPPH scavenging activity when compared with positive control (ascorbic acid) which had an IC50 value of 0.0000295 g/ml.

Table 2. IC_{50} values of the of aqueous beetroot extracts in g/ml)

Test sample	IC_{50} (g/ml)
Ascorbic acid	0.0000295
Direct from farm	157.9
Refrigerated	10.5
Stored as a whole	210,069.4

Discussion

Red beetroot (Beta vulgaris rubra) is a root vegetable that is rich in inorganic nitrate (1). When beetroot is consumed, the nitrate is reduced in the body to nitrites and nitric oxide (NO) which play an important role in the regulation body immunity, vascular homeostasis and metabolism. Other metabolically important components of beetroot include vitamins, phenolics, carotenoids and betalains (5). These are phytochemicals with vital anti-inflammatory, antioxidant and antimicrobial activities (7). The quality of beetroot is, nevertheless, affected by many factors including growing conditions, harvesting time and post-harvest processing.

Fresh beetroot is of a more intense colour than processed juices. Similar to our findings, previous research has documented that refrigeration for shorter periods has been associated with less marked influence on the intensity of the

colour of beetroot juice (8,16). This has been attributed to the ability of betalains, responsible for the red colour of beetroot, to remain stable in as low temperatures as -300C (17). Furthermore, freeze-drying of beetroot during juice extraction process has been shown to release a greater amount of the antioxidants (including betalains) by causing tissue damage that releases these compounds from the cellular vacuoles they are contained in (10). Other processing methods including pasteurization and fermentation have been shown to significantly reduce the intensity of the red colour of beetroot (4,9). This occurrence has been attributed to the heat-related disintegration of betalains resulting in lower amounts of betaxanthins and betacyanins in beetroot juice since these heat sensitive compounds start disintegrating at temperatures above 500C (8,18). The decline in colour intensity increases with a longer duration of storage and is accompanied by a concurrent decline in the antioxidant activity of beetroot (4,8). Prolonged fermentation on the other hand has been shown to reduce the overall content of the flavonoids contained in beetroot (19).

The bioactive flavonoids investigated in our study were present in all the samples analysed. These compounds included flavonoids that provide vital antioxidant, anti-inflammatory and anti-cancer properties (5,20,21). Similarly, terpenoids are essential plant oils that serve as precursors for steroidogenesis besides having anti-pathogenic properties (22,23). Other bioactive compounds found during our analysis included saponins and tannins, which have been found to have the ability to lower cholesterol, act as antimicrobials and as anti-inflammatory agents and modulate the functioning of intracellular enzymes (1,24). Various studies have documented the respective quantities of the various flavonoids contained in beetroot (19,25). The actual quantity is, however, known to vary depending on the specific cultivar grown, the region of cultivation and the harvesting period (26–28). It was, nevertheless, beyond the scope of the current study to specify and quantify the flavonoids contained in the analysed specimens and the effect of the various storage modalities on the same.

The different storage conditions of the respective beetroot juices had a noticeable influence on the antioxidant capacity of the juices with the juice made from beetroot stored as a whole having the lowest antioxidant potential while the refrigerated juice had the highest. This was reflected by the refrigerated beetroot juice having the lowest IC50 values required to inhibit 50% of DPPH. Previous researchers have documented that a higher dry matter content found in freshly prepared beetroot juice has been attributed to the lower antioxidant activity compared to processed juices (4). This might explain the lower antioxidant potency of the freshly prepared juice. Similarly, relative increase in water content in refrigerated watermelon juice has been credited with a reduction in the concentration of bioactive compounds which might explain the lower antioxidant activity of refrigerated juice at lower concentrations (29). For all the three samples, however, the antioxidant potential was further amplified by increasing the extract concentration. This is similar to past findings that have shown that increasing the concentration of plant extracts enhances the proportion of the active ingredient with pharmaceutical value in the plant extract under investigation (30).

The variances in the antioxidant capacity of the differently stored beetroot might be explained by the influence of the different storage modalities on the quantities of the various bioactive flavonoids found in beetroot. This is due to the fact that the quantity of antioxidants in a given beetroot sample is determined by various factors including the storage condition and the processing methods employed to preserve the beetroot (Bianchi et al., 2021; Koss-Mikołajczyk et al., 2019). Such an eventuality results from the transformation of these flavonoids from one form to another during processing and their variable release under different storage conditions, which has been attributed to the various changes in the content of the bioactive compounds found in beetroot (17). In this regard, variable temperature storage conditions result in marked differences in the oxidative potential of differently processed beetroot. For instance, cold storage of beetroot products (up to -800C) has been shown to maintain the stability of the constituent flavonoids and might explain the enhanced antioxidant activity of the refrigerated juice. Storage of beetroot juice at room temperature has on the other hand, been shown to result in a significant decline in antioxidant activity occasioned by disintegration of the nitrates found in beetroot juice that occurs within 24 hours of storage (31). In contrast, polymerization of bioactive beetroot monomers during heat processing, for instance, and as occurs during high-pressure treatment and vacuum and microwave drying techniques, has been attributed to a higher antioxidant capacity of beetroot (4). Such techniques have been thought to damage the tissues of the beetroot resulting in a more efficacious extraction of antioxidants. Furthermore, heat-processed beetroot retains a higher antioxidant activity compared to freshly prepared beetroot despite a considerable reduction in the content of total betalain. The same process, nonetheless, results in the heat-related release of other bioactive compounds including vitamins and polyphenols that are thought to be variably affected by these processing aspects and have a synergistic antioxidant activity to the betalains (4,32).

The effects of other processing and storage methods have similarly been investigated and shown to have a different effect on the antioxidant activity of beetroot. Blending of beetroot juice with other juices for instance, and which is associated in a significant lowering of the beetroot juice pH, significantly reduces its antioxidant capacity (4). Short term fermentation of beetroot, although known to cause the release of more bioactive compounds, has been shown to significantly reduce the antioxidant capacity of beetroot (9,10,33). Transformation of these active compounds from, one form to another during fermentation, has been attributed to the various changes in the potency of the bioactive compounds found in beetroot. Prolonging fermentation beyond 14 days yields a further significant reduction in the quantity of bioactive flavonoids and phenolic acid via enhanced disintegration of the released compounds (11,19).

4. Conclusion

Short-term refrigeration of beetroot juice results is a better preservation of its antioxidant activity.

Appendix

Availability of data and materials: This study was part of an academic study and the entire thesis will be availed at the University of Nairobi online repository once the examination process is complete

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Authors' contribution: J.M., A.M, and M.O. conceptualized the work, wrote the manuscripts and prove read the work prior to submission. J.M., and V.K. performed the experiment, collected and analyzed the data.

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