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The effects of freezing and reheating on anthocyanin retention in beet root (*Beta vulgaris)* curry

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Abstract

This research focuses on the impact of freezing and reheating on the retention of anthocyanin in the beetroot curry, which is a commonly consumed food by Sri Lankans. Anthocyanin is a flavonoid compound which is known for its high antioxidant properties and vibrant colours. Anthocyanins offer a range of significant health benefits, including antimicrobial and anticancer properties, anti-aging effects, and potential roles in enhancing cardiovascular and cognitive health. Additionally, they contribute to the management of obesity and the prevention of type 2 diabetes. The stability of anthocyanin can be influenced by pH, temperature, light, enzymes, oxygen, etc. In this study, three samples were made; freshly prepared beetroot curry, a sample which was frozen at 4℃ overnight without reheating and a freshly prepared curry which was kept at room temperature and then reheated using a gas stove. Acidified ethanol (2% Acetic acid, 18% distilled water and 80% ethanol) was used to extract and stabilize the pigment. The concentration of anthocyanins was measured using UV visible spectrophotometry and cyanidin-3-glucoside was used as the standard. The results indicated that freshly prepared curry samples had the highest concentration of anthocyanin followed by the frozen sample and reheated samples had the lowest concentration of anthocyanin present. These findings show that multiple cycles of reheating and prolonged freezing can lead to a significant loss in anthocyanins which are sensitive to heat. This paper provides insight into the effect of different temperatures on the quantity of anthocyanins.

Keywords: Anthocyanin, *Beta vulgaris*, Freezing, Reheating

1. Introduction

Recently researchers have been conducting studies on fruits and vegetables with vibrant colors, due to their antioxidant properties etc. These vibrant colours are mostly attributed to compounds such as carotenoids and flavonoids, including anthocyanins.[1] Anthocyanins are water-soluble pigments that mainly fall under the flavonoid groups. They are highly found in purple kale, red lettuce, berries, grapes, pomegranates, beetroots, red onions etc. There are six major anthocyanin groups, cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin.[2] These groups show a variety of colours in different pH, temperature and light.[3] Cyanidin-3-glucoside is considered the most found anthocyanin in plants.[4] The basic structure of anthocyanin includes the flavylium cation which consists of two benzene rings and a pyrylium ring. Which is responsible for the range of colours expressed by the pigment by absorbing different wavelengths of UV visible light and the carbon rings provide the core structure of the pigment.[5] Therefore, the UV visible spectrophometry can be used to quantify the anthocyanins.

Anthocyanins acquire high antioxidant properties due to the presence of flavylium cation. They reduce or inhibit oxidative stress by scavenging the free radicals through the direct method by transferring a single electron or donating an H atom. Anthocyanins also activate or stimulate the synthesis of certain enzymes through indirect methods like catalase, NADPH oxidase, superoxide dismutase (SOD) etc.[6] Anthocyanins also act as an anti-inflammatory.

Studies have shown that anthocyanins help reduce pain and inflammation in arthritis.[7] Anthocyanins facilitate the prevention of leukaemia and ovarian cancers because they have powerful cancer-fighting abilities.[8] Anthocyanins improve heart health by lowering LDL and improving HDL cholesterol levels.[9] Studies have proven that consuming anthocyanin-rich food may reduce heart risk by up to 9.[10] Anthocyanins have the potential of slowing the progression of Alzheimer's.[11]

However, anthocyanins are sensitive to extreme factors such as pH, temperature, light, oxygen levels, co-pigments, enzymes, etc. Anthocyanins show different colours in different pH. Anthocyanins are found purple in neutral environments, red in acidic and blue in alkaline nature.[12] Scientific studies show that storing anthocyanin in the range of 2-4℃ can help retain its beneficial properties. Still, prolonged storage can lead to a significant loss due to the formation of ice crystals which cause damage to the cell wall and membrane, thereby upsurging the degradation rate. At elevated temperatures, anthocyanin pigments undergo degradation through various chemical reactions. Heat can break the bond between the anthocyanidin core and its sugar moiety, forming less stable anthocyanidins. Additionally, water molecules may attack electron-deficient sites on the anthocyanin structure, leading to destabilization and loss of colour. High temperatures also promote polymerization, where anthocyanins interact with other compounds to form larger, less soluble molecules, and can cause the breakdown of the pigment’s three-ring structure, producing simpler compounds like coumarin glycosides. These reactions collectively reduce the colour intensity, stability, and bioactivity of anthocyanins, highlighting the importance of temperature control during processing and storage.[12] Co-pigmentation is a specific phenomenon found in anthocyanin and, not other non-polyphenolic or polyphenol compounds.[13] It is a process where the pigments form a complex with a metal ion or a colourless compound to create or change the colour intensity. It is pH and temperature-dependent, therefore high pH and high-temperature lead to a disruption of anthocyanin structure and cause loss of colour intensity and antioxidant capacity.[14] Researchers have said that the stability of anthocyanins is highly affected due to exposure to fluorescence light.[15]

In Sri Lankan cuisine, beetroot curry is a common dish in every household. However, due to current busy lifestyles, meal preparation has significantly increased, and freezing and reheating pre-cooked curries has become very common among urban locals.The following statistical facts indicate the importance of consuming beetroot curry in Sri Lanka which helps to live a healthy life, by improving heart health, fighting against cancer, improving brain health, reducing obesity and diabetes etc.The percentage of deaths associated with cardiovascular disease increase from 24.92% to 28.05% in the years 2011 to 2019.[16] According to the World Health Organization, the adult obesity percentage in Sri Lanka has increased from 16.8% to 25.2%.[17] The prevalence of type 2 diabetes was highest in the years 2011 to 2021 (17.5%) than in the 1990s and 2000s.

2. Methodology

2.1 Beetroot Curry Preparation. Nine large beetroots were taken, cleaned and cut into small cubes to prepare the curry. Relevant spices were added while cooking. Once prepared it was divided into three portions which weighed 80g each and were labelled as fresh, frozen, and reheated samples. The frozen sample was kept in the refrigerator at 4℃ overnight, and the freshly prepared sample which was reheated using the gas stove at 45℃ the next day.

2.2 Crude extraction. Each sample was thoroughly mixed in 80mL of distilled water at room temperature. A cheesecloth was used to filter the mixture twice to obtain the crude extract. Each sample was divided into six falcon tubes and labelled. The top was secured with filter paper, and the falcon tubes were stored in the freezer at -20℃ overnight. Then, the tubes were kept in the freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, German) for four days.

2.3 Stabilization of the anthocyanin present in the crude extraction. All three completely dried samples were obtained and dissolved in a minimum volume of distilled water. The solutions were then incorporated with acidified ethanol (2% Acetic acid, 18% distilled water and 80% ethanol) in a 1:2 ratio and occasionally stirred under a fume hood for two hours.[18] Each sample was evaporated to dryness in the rotary evaporator (Heidolph Instruments ,Germany) at 40°C for one hour at low pressure .[19] Once the ethanol was evaporated and the volumes were diminished, they were transferred into falcon tubes and stored in the freezer at -20°C. Once the samples were frozen the lids of the falcon tubes were replaced with filter paper and kept in the freeze dryer for forty-eight hours. The dried samples were used for further assays.

2.4 Analyzing the retention of anthocyanin pigments present in all three samples. The mass of the dried samples was measured using an analytical balance. All three samples were dissolved in a minimum amount of distilled water and made into the stock solution. A serial dilution was made for each sample for screening purposes. The spectrophotometer (Brandtech Scientific, USA) was used to measure the absorbance values of samples at 520 nm to quantify anthocyanin amounts .[20]

3. Results and Discussion

The fresh beetroot curry sample had the highest mean anthocyanin concentration (0.0395 ± 0.0142 µg/mL) followed by the frozen sample (0.0285±0.0071 µg/mL). In contrast, the reheated curry sample had the lowest (0.0268±0.0053 µg/mL). This can be demonstrated by the following graph in Figure 1.

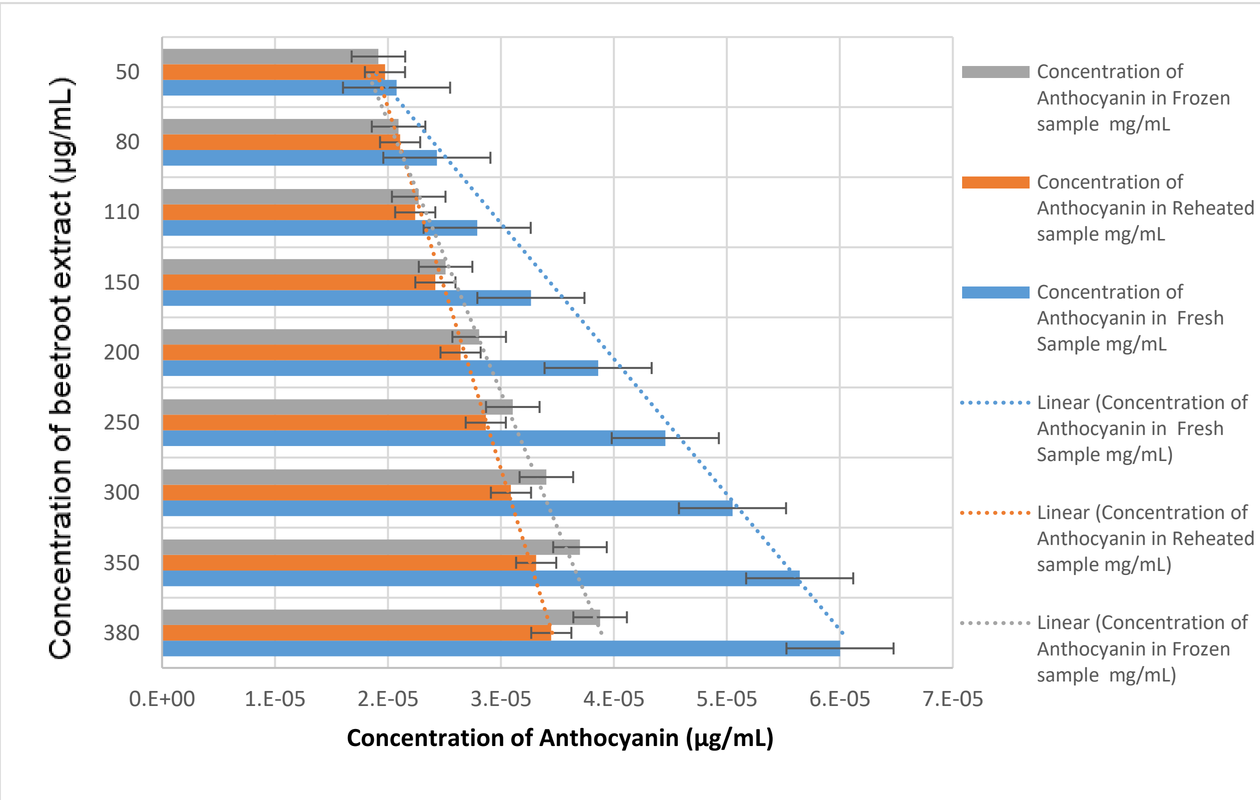

Figure 1. The concentration differences of anthocyanin present in the fresh, frozen and reheated curry samples. The graphical data is represented using the mean±SD of three separate experiments.

The finding of the anthocyanin concentration present in the freshly prepared, frozen and reheated beetroot curry sample has significant importance as anthocyanin is considered one of the main flavonoid components with high health-beneficial properties.

3.1 The effect of reheating and freezing on anthocyanin retention. This study was mainly carried out since Sri Lankans consume beetroot more often daily. The curries are preserved in the refrigerator and reheated multiple times in the Sri Lankan household. This study focuses on the effect of temperature on the retention of the anthocyanin pigment. As mentioned, the freshly prepared curry had the highest concentration of anthocyanins (0.0395 ± 0.0142 μg/mL) followed by the frozen sample (0.0285±0.00711 μg/mL) while the reheated curry sample had the lowest value (0.0268±0.00533 μg/mL). A study has been conducted on purified crude extracts and thermally processed food which proved that an increase in temperature will increase the degradation of the anthocyanin pigments while storing in low temperatures such as 2-4℃ will preserve the pigment and reduce the degradation although prolonged storage in refrigerators can induce the degradation.[21] This can be seen in the results obtained above. Studies have shown that anthocyanins degrade at high temperatures following a first-order reaction kinetics model, meaning the degradation rate depends on the amount of anthocyanin remaining.[22] Heat exposure causes significant structural changes, affecting anthocyanins' stability, colour, and antioxidant properties. Two main degradation pathways have been identified. The first involves water molecules breaking the bonds between anthocyanins and their sugar groups (deglycosylation), transforming them into unstable aglycone forms called anthocyanidins, which then degrade further into smaller compounds like methanol, chalcones, diketones, benzoic acid, and aldehyde derivatives. The second pathway involves the opening of the flavylium ring, converting anthocyanins into chalcones, which subsequently form coumarin glycoside derivatives.[23] Both processes lead to a loss of vibrant red and purple hues of anthocyanins, reduced antioxidant activity, and diminished health benefits. These findings highlight the need to carefully control heat during cooking and reheating to preserve the colour, nutritional value, and functional properties of anthocyanin-rich foods. Strategies such as shorter cooking times, maintaining an acidic environment, or using encapsulation techniques could help minimize degradation and retain the benefits of anthocyanins.

Both freezing and reheating can damage the cell wall and membrane at different levels, which provides more exposure of the anthocyanin pigments to oxygen. This causes oxidative stress and facilitates some enzymes like peroxidase and polyphenolic oxidases to act on the pigments leading to further degradation. Freezing leads to such conditions due to the formation of ice crystals within the cells, which disrupts the cell wall and membrane. The initial disruption of the cell wall and membrane was done during the cooking process, which led to the extraction of anthocyanins and other flavonoid compounds in the beetroot. The addition of spices and salt while preparing the curry leads to pH changes, which also impacts the retention of the anthocyanin pigments. Flavylium cations are more stable in acidic pH, but while cooking, freezing, or reheating the pH shifts to neutral, or alkaline which causes instability to the cation leading to degradation.[24] According to the graph obtained, it clearly shows that freezing leads to the loss of anthocyanin, but it is comparatively less than the reheating process. Therefore, reheating at a low temperature might reduce the loss of anthocyanins

3.2 Future Insights. This experiment focuses on providing critical knowledge about food preparation techniques and food storage methods by emphasizing the need for evidence-based guidelines to expand the advantages of foods rich in anthocyanin pigments by understanding how reheating and freezing impact anthocyanins' degradation and stability. Various food preservation techniques can be practiced by the food industries such as cryogenic freezing or vacuum-sealed packaging which can improve the retention of anthocyanins and other flavonoid components. Nanoliposomes made with anthocyanins, primarily containing cyanidin-3-O-glucoside and peonidin-3-O-glucoside, were created using lecithin and cholesterol in a ratio of 5:98.

These nanoliposomes successfully retained 85.60% of the anthocyanins after being stored at 25°C for 16 days, demonstrating their effectiveness in preserving anthocyanins over time.[25]

4. Conclusion

It is evident that the freshly prepared beetroot curry sample had the highest retention of anthocyanins present, followed by the frozen sample while the reheated sample had the least. This study proves that repetitive heating and prolonged freezing can cause vast amounts of degradation and loss in the functional properties of anthocyanins. Modern innovations have been introduced for stabilizing anthocyanins during freezing and heating, which adds valuable perceptions to the existing knowledge in the field.

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