

**Original Article****Vitamin C Content in Fresh and Frozen Berries**Andrea C. Soto¹, Samira Ziaei^{*1}

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Received: August 2024

Accepted: February 2026

ABSTRACT

Berries are renowned for their high vitamin C content, a potent antioxidant linked to numerous health benefits. However, the impact of freezing on this essential nutrient remains a subject of interest. This study aimed to compare vitamin C levels in fresh and frozen strawberries, blueberries, and raspberries using spectrophotometry and iodine titration. Our findings revealed significantly higher vitamin C concentrations in frozen berries compared to fresh samples. Specifically, vitamin C levels were 29% and 12% greater in frozen berries as determined by titration and spectrophotometry, respectively. These results suggest that freezing effectively preserves vitamin C, making frozen berries a reliable and convenient source of this critical nutrient. Our data highlight the potential of frozen berries as an excellent source of vitamin C. By offering convenience and comparable or even superior nutritional value to fresh berries, frozen options can contribute to increased consumption of this essential nutrient.

Keywords: Vitamin C, Fresh Berries, Frozen Berries, Ascorbic Acid, Antioxidant**Highlights**

- Vitamin C levels were evaluated in fresh and frozen strawberries, blueberries, and raspberries.
- Frozen berries showed 29% higher vitamin C by iodine titration and 12% higher levels by spectrophotometry.
- Freezing effectively preserved vitamin C, supporting frozen berries as a reliable dietary source.

Introduction

Vitamin C, ascorbic acid, is an essential water-soluble vitamin that is a very potent antioxidant. It cannot be manufactured by the human body and is mainly obtained via dietary intake of fresh fruits and vegetables. In humans, the gene for gulonolactone oxidase, the terminal enzyme in the ascorbic acid synthesis pathway, has undergone mutations that make it non-functional (1). The Recommended Dietary Allowance (RDA) for vitamin C of 75 mg for women and 90 mg for men is, for the first time, based on the vitamin's role as an antioxidant as well as protection from deficiency (2,3). The vitamin C content of fruit and vegetables is highly variable and depends on the food type and ripeness, season, time of harvest, transport, storage, and method of preparation (4). Amongst the fruits, fresh strawberries are

considered to have the highest levels of vitamin C with ranges between 5 to 50 mg/100 g fresh weight (fw) (5). Raspberries have a similar content of ascorbic acid as strawberries, 5-40 mg/100 mg fw (5), and blackberries 5-30 mg/100 mg fw (6), about three-times more ascorbate than blueberries (7).

An increasing body of research indicates that berries provide antioxidants, primarily vitamin C, and anticancer protection via free radical scavenging DNA damage preventions, apoptosis induction, and cancer growth and proliferation inhibition (8). Several cancer cell lines including A431 (epidermal), Panc-1 (pancreatic), HeLa (cervical), HT29 (colon), and MCF-7 (breast) were shown to undergo cell death in response to vitamin C exposure (9). Vitamin C has been found to support chemotherapy and radiotherapy, improving treatment effects and patients' quality of life by reducing adverse side

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effects (10). The antioxidant activities of vitamin C are associated with its ability to neutralize reactive oxygen species and protect cells from free radical damage, formed via cell metabolism, which are linked to tissue damage and diseases (11,12).

Vitamin C has several other health benefits apart from its antioxidant properties. These include cardiovascular disease and obesity management, strengthening the immune system, skin health, and enhancing iron uptake. In several clinical studies, low levels of vitamin C (assessed by dietary intake or plasma analysis) have been associated with a number of conditions, including high blood pressure, endothelial dysfunction, heart disease, atherosclerosis, and stroke (13–15). Several reports indicate that vitamin C supplementation induces weight loss (16) and improved lipid and carbohydrate metabolism in type-II diabetic patients (17). Vitamin C has been shown to stimulate neutrophil migration to the site of infection, enhance phagocytosis and oxidant generation, and microbial killing (18). At the same time, it protects host tissue from excessive damage by enhancing neutrophil apoptosis and clearance by macrophages, and decreasing neutrophil necrosis and NETosis (19). Ascorbic acid is required for collagen synthesis (20). Studies have demonstrated that vitamin C can protect against damage from UVA and UVB rays, correct pigmentation problems, and improve inflammatory skin conditions (21,22). Vitamin C intake from food or supplements can increase the bioavailability of iron by improving the absorption of the non-heme iron (23).

While vitamin C offers numerous benefits, it's important to consume it through a balanced diet. While vitamin C supplementation can be helpful, whole foods are preferred for optimal nutrient intake. This study investigated the vitamin C content of fresh and frozen berries to address the limited information available on the impact of freezing on this essential nutrient. In this study, we measured the vitamin C content of fresh and frozen berries via spectrophotometry and titration. Our results show that the vitamin C content was higher in frozen berries. These findings indicate that the freezing process can effectively preserve vitamin C in berries, mitigating the losses that occur during storage and transportation. Our data highlights the potential of frozen berries as a convenient and reliable source of vitamin C.

Materials and Methods

Materials

Fresh strawberries, blueberries, and raspberries were purchased from a local supermarket (ShopRite, Lodi, NJ). The cultivation conditions of the purchased

fresh berries were not controlled for this study, as they were obtained from a commercial source.

Upon receipt, the fresh berries were divided into two groups. One group was designated for immediate fresh analysis, while the other group was frozen. Fresh berries for immediate analysis were stored at 4°C for no more than 24 hours prior to analysis. The fresh berries designated for freezing were flash-frozen using a blast freezer (Individual Quick Freezing method at -30°C). This process involved spreading the berries in a single layer on trays to ensure individual freezing. Frozen berries were stored at -20°C for 24 hours prior to analysis.

All berries (both fresh and frozen) were washed thoroughly under cold running water. For each type of berry (strawberry, blueberry, raspberry), 100 g portions were weighed and blended with 50 mL distilled water to form a homogeneous slurry. The slurry was then passed through cheesecloth to obtain the berry extract for vitamin C analysis.

Spectrophotometry Procedure

A stock ascorbic acid solution was prepared at a concentration of 0.5 mg/mL by dissolving 2g of vitamin C tablets (Sigma-Aldrich) in distilled water. Berry samples were diluted 1:5 with distilled water prior to analysis. Spectrophotometric measurements were conducted at 243 nm, a wavelength chosen for optimal vitamin C absorbance.

Iodine Titration Procedure

A 0.01 M iodine stock solution was prepared by dissolving iodine and potassium iodide in distilled water. A 1% starch solution was prepared to serve as a colorimetric indicator. To determine vitamin C content, 25 mL of the berry sample solution and 10 drops of starch indicator were added to an Erlenmeyer flask. The solution was titrated with the iodine solution until the blue starch-iodine complex disappeared. Vitamin C content was calculated using the formula: Vitamin C content = $(V * F * 100) / A$, where V is the titration volume, F is Tillman's factor, and A is the sample volume after titrant addition. Tillman's factor was determined by dividing the mass of vitamin C used in a standard titration by the corresponding titrant volume.

Statistical analyses

Data were presented as means ± standard error of the mean. Student's t-test was performed to evaluate differences between fresh and frozen berries. Values were considered statistically significant at $P < 0.05$.

Results

Figure 1 shows that the titrated samples were reddish, which was expected given the acidic nature of ascorbic acid. As depicted in Figure 2, frozen berries had a 29% greater vitamin C concentration than fresh berries. Specifically, fresh berries contained 4.9 mg/mL of vitamin C, while frozen berries contained 6.3 mg/mL.

Spectrophotometry

To corroborate the titration results, vitamin C levels were quantified spectrophotometrically. As illustrated in Figure 3, frozen berries exhibited a 12% higher vitamin C concentration compared to fresh berries. Absorbance values were 1.29 for fresh berries and 1.46 for frozen berries.

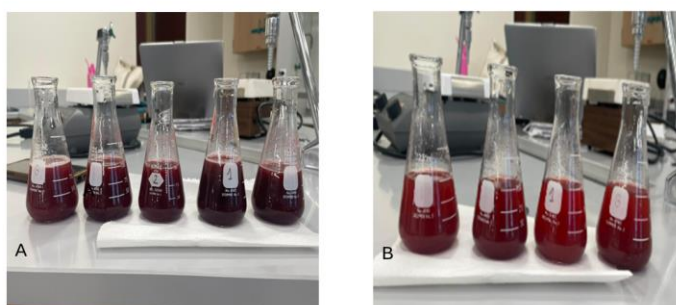


Figure 1. Comparison of titrations after endpoint. A) Frozen Berries. B) Fresh Berries

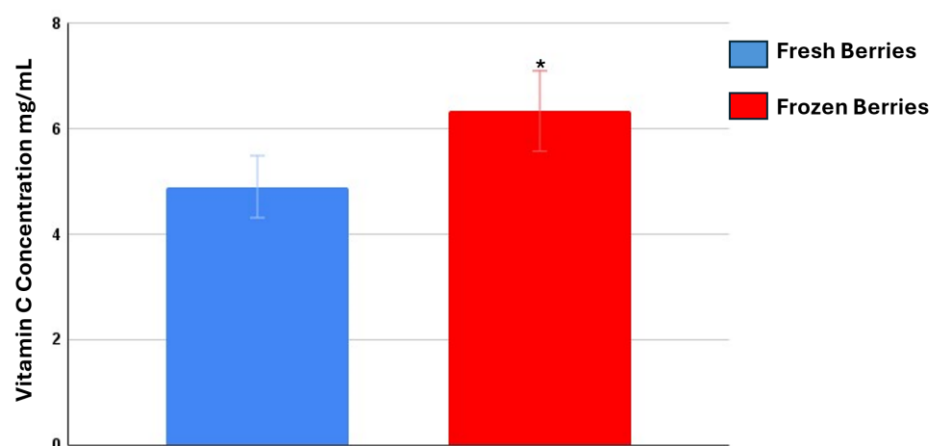


Figure 2. Vitamin C determinations via titration. Values shown are $\pm$ SEM of three independent experiments. * $P < 0.002$. Abbreviation: SEM, standard error of the mean.

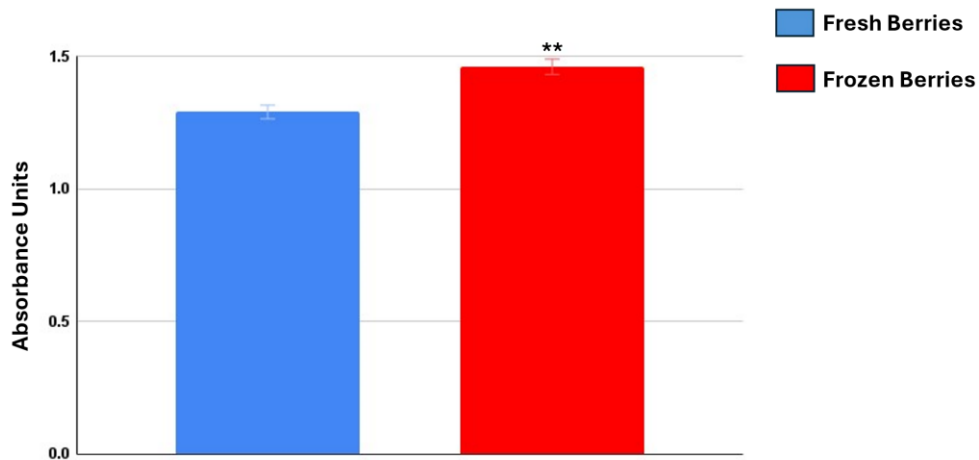


Figure 3. Vitamin C determinations via spectrophotometry. Values shown are $\pm$ SEM of three independent experiments. $**P < 0.0007$. Abbreviation: SEM, standard error of the mean.

Discussion

Vitamin C: A Potent Antioxidant

Vitamin C, or ascorbic acid, is a potent antioxidant essential for human health. Its antioxidant properties stem from its ability to directly neutralize reactive oxygen species, such as peroxyl radicals (24). By reducing vitamin E radicals back to their active form, vitamin C indirectly protects cell membranes from oxidative damage (25). This dual action protects cellular components, including lipids, proteins, and DNA, from oxidative stress.

Vitamin C: Essential Nutrient and Its Role in Health

As an essential nutrient, vitamin C is required for collagen synthesis, a process crucial for skin, bone, and connective tissue health (26). Vitamin C deficiency leads to scurvy, which is characterized by fatigue, weakness, and impaired wound healing (27). Obesity is often associated with lower vitamin C levels due to factors like dietary patterns, increased oxidative stress, and potentially reduced absorption. Obese patients often consume highly processed, energy-dense foods which contain inadequate amounts of micronutrients (28). Consequently, individuals with obesity may require higher vitamin C intake to maintain optimal health.

Berries: Nutrient-Rich Sources of Vitamin C

Berries are excellent sources of vitamin C, contributing to their reputation as health-promoting foods. However, the nutrient content of berries can fluctuate due to factors such as harvesting, processing, temperature conditions, and storage. Our findings

suggest that the higher vitamin C content in frozen berries compared to fresh ones may be attributed to the freezing process effectively preserving the vitamin, mitigating losses that occur during storage and transportation of fresh berries. While fresh berries are often preferred, their perishability can lead to significant vitamin C loss.

The higher vitamin C content in frozen berries has significant implications for public health. By encouraging the consumption of frozen berries, we can improve dietary vitamin C intake and potentially reduce the risk of vitamin C deficiency. Furthermore, frozen berries are a reliable and convenient source of this essential nutrient that is available year-round.

Future Research Directions

To further explore the impact of freezing on other nutrients and compounds in berries, additional research is warranted. This includes conducting a comparative study on berries frozen using flash-freezing (as in the current study), slow-freezing, and cryo-freezing, analyzing the retention of anthocyanins, dietary fiber, and minerals in each method. Studies could also examine how freezing affects the levels of ellagic acid, another potent antioxidant found in berries, and phenolic compounds in addition to vitamin C.

Employing additional analytical techniques, such as high-performance liquid chromatography (HPLC), to quantify specific individual phenolic compounds (e.g., quercetin, resveratrol) and carotenoids (e.g., lutein, zeaxanthin) in fresh versus frozen berries. This would provide a more detailed profile of how these

specific compounds are affected by the freezing process.

Broader Health Implications

The higher vitamin C content found in frozen berries suggests that choosing frozen over fresh berries can lead to increased dietary intake of this essential nutrient. Frozen berries provide a reliable and convenient year-round source of vitamin C, helping consumers meet their daily recommended dietary allowance. As vitamin C is a potent antioxidant, higher intake from frozen berries can contribute to enhanced antioxidant protection against reactive oxygen species and free radical damage, which are linked to tissue damage and various diseases. By encouraging the consumption of frozen berries, the risk of vitamin C deficiency can potentially be reduced, especially since many individuals may not consume adequate amounts through fresh produce alone due to factors like storage losses. Adequate vitamin C intake is associated with numerous health benefits beyond its antioxidant properties, including cardiovascular health, obesity management, immune system strengthening, skin health, and enhanced iron uptake. Therefore, consistent consumption of vitamin C-rich frozen berries can contribute to these broader health outcomes. Furthermore, frozen berries offer a convenient option that may be more accessible and affordable than fresh berries at certain times of the year, allowing consumers to maintain a consistent intake of vitamin C regardless of fresh berry seasonality or perishability.

Practical Applications

Applications in the Food Industry

Our findings and those of other studies (29,30) indicate that freezing contributes to prevention of damage to fruits and maintains elevated levels of vitamin C. Another study found no significant differences in vitamin C content in twenty-one kinds of fruits, fresh and frozen, (31). Food manufacturers can leverage this information for product development and marketing, creating and promoting products like frozen smoothie mixes, yogurts, or fortified frozen desserts that highlight frozen berries as a superior source of vitamin C. This emphasizes the "vitamin C preserved" aspect. Furthermore, the industry can refine its freezing and storage protocols for berries to optimize vitamin C retention, reinforcing the importance of flash-freezing (Individual Quick Freezing) and maintaining consistent freezer temperatures. Companies can also confidently highlight the vitamin C content of their frozen berry products on nutritional labels and in

marketing materials, providing consumers with accurate and appealing health information. Ingredient sourcing can also be optimized, as food service providers and manufacturers of processed foods can prioritize the use of frozen berries in recipes where vitamin C content is a desired nutritional benefit, rather than relying solely on fresh berries that may have experienced significant loss during transit and storage. Finally, the food industry, in collaboration with health organizations, can launch educational campaigns to inform consumers about the nutritional benefits of frozen berries, dispelling any misconceptions that fresh is always nutritionally superior.

Recommendations for Consumers Regarding Berry Consumption

Consumers are encouraged to regularly incorporate frozen berries into their diet as a convenient, year-round, and highly nutritious source of vitamin C. When purchasing frozen berries, consumers should look for indications of flash-freezing or IQF (Individual Quick Freezing) methods, as this study specifically used this technique and showed positive results. Frozen berries are often more affordable than fresh ones, especially out of season, offering good nutritional value for their cost. They are also highly versatile and can be added directly to smoothies, oatmeal, yogurt, baked goods, or even thawed for use in salads and desserts. While not as consistently high in vitamin C as frozen, fresh berries still offer benefits, and consumers should be advised to consume them quickly after purchase and store them properly (e.g., at 4°C for no more than 24 hours as per the study's protocol) to minimize vitamin C loss. It is important to reiterate that while frozen berries are excellent for vitamin C, a balanced diet including a variety of fruits and vegetables (both fresh and frozen) is crucial for overall nutrient intake.

Acknowledgement

The authors wish to thank Kacey Cravello for technical assistance.

Financial disclosure

The authors report no conflicts of interest in this work.

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