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ANALYSIS OF NITRITE CONTENT IN FRESH-CUT VEGETABLES UNDER DIFFERENT STORAGE CONDITIONS

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Abstract: *This study quantitatively assessed the nitrite content (calculated by sodium nitrite) in four commonly consumed fresh-cut vegetables using spectrophotometric analysis. The investigation focused on the variations in nitrite levels in tender ginger, old ginger, chili, and garlic under varying storage temperatures and durations. Results indicated that nitrite concentrations were higher in vegetables stored at ambient temperature compared to those stored at lower temperatures. Notably, the nitrite content in both tender and old ginger exhibited a rapid increase at ambient temperature, reaching approximately 160 mg/kg, significantly exceeding the safe consumption threshold for humans. Over time, nitrite levels initially rose and subsequently declined, whereas no substantial changes were observed in chili and garlic during storage. Furthermore, no significant difference was detected between the nitrite content and the baseline values in vegetable samples with intact tissue structures under identical storage conditions. In conclusion, this study provides a theoretical foundation for mitigating the risk associated with nitrite consumption in fresh-cut vegetables, thereby contributing to the protection of human health.*

Keywords: *fresh-cut vegetables, ginger, nitrite, nitrate, storage conditions*

1. Introduction

Fresh vegetables are rich in nitrogen, primarily in the form of nitrate. Under certain conditions, nitrate and nitrite can interconvert, and the concentration of nitrite is influenced by storage conditions and processing methods. While nitrate itself is non-toxic, nitrite is toxic, and high nitrate levels in vegetables may have significant health risks to humans [1], [2]. Nitrate can be converted into nitrite through the action of nitrate reductase or microbial activity. Nitrite, when combined with amines present in food and the human body, can form nitrosamines, compounds known to induce gastric, intestinal, esophageal, and other types of cancers. Additionally, excessive maternal intake of nitrite may result in fetal malformations [3], [4]. It is evident that fresh vegetables absorb substantial amounts of nitrogen from their environment, primarily as nitrate, leading to nitrate accumulation within the plant [5], [6]. The levels of nitrate and nitrite in vegetables vary across different species and fluctuate during cultivation, harvesting, storage, and processing, with varying degrees of change [7], [8]. Current research indicates that people are increasingly concerned about nitrite levels exceeding permitted limits in preserved meats, pickled vegetables, and leftovers, while there is limited investigation into the dynamic changes in nitrite content in fresh vegetables stored in everyday settings [9-11]. Studies have shown that after various vegetables are prepared into dishes, the nitrite content of most of leafy vegetables increases over time, and low-temperature storage can delay the rate of nitrite production [12-14].

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Some scholars have conducted related research on nitrite content in fresh vegetables. For example, Deng et al [15] found that sprouts and leafy vegetables had higher nitrite content [15]. Li et al [16] studied the changes in nitrite during vegetable storage and compared normal temperature and low-temperature storage methods, finding that the changes in leafy vegetables were more pronounced [16]. Therefore, examining nitrite levels in fresh vegetables is of significant importance. Given the practical context of daily life, where tender ginger, old ginger, chili, and garlic are frequently used as freshly cut seasonings in meal preparation and may be pre-cut and stored in restaurants for consumer use over a period of time, this study selects these four commonly used fresh vegetables as test materials. The study aims to determine the nitrite content over time under different storage conditions. The objective is to develop better preventive measures, mitigate risks, reduce nitrite intake, and safeguard consumer health by analyzing the patterns of nitrate and nitrite changes in fresh-cut vegetables. Additionally, the study explores the effects of different storage methods and durations on nitrite content.

2. Materials and methods

2.1 Reagents and materials

All the reagents were analytical grade unless otherwise stated. Sodium nitrite (purity ≥ 99%) and sodium nitrate (purity ≥ 99%) were obtained from the ANPEL Scientific Instrument Co., Ltd (Shanghai, China). The ultrapure water was produced by an Ultra-pure water system Advanced-1-18 (Nanjing, China). Four kinds of fresh vegetables were randomly collected from the market in Dali City: tender ginger (growth cycle of 4 months), old ginger (growth cycle of 10 months), chilli, and garlic.

2.2 Methods

2.2.1 Sample pretreatment

Initially, the soil from the fresh vegetables was removed by potable water, and the samples were air-dried at room temperature. Subsequently, the tender ginger, old ginger, chili, and garlic were each crushed using a homogenizer. The homogenized samples were then divided into three parts and stored at three different temperatures: room temperature (20 °C - 25 °C), cold storage (0 °C - 4 °C), and frozen (-18 °C), for one month. A portion of each sample was retained, and the entire fresh vegetable sample (not broken for sample preparation) was placed under refrigeration (0 °C - 4 °C) for the same duration. The nitrite content is determined at the same time every day for three weeks. The curve is observed, and the variation curve is explored.

2.2.2 Nitrite detection method

Weigh approximately 5 grams of a homogeneous sample and place it into a 250 mL conical flask equipped with a stopper. Add 12.5 mL of a 50 g/L saturated borax solution, followed by approximately 150 mL of water heated to approximately 70 °C. Mix the contents thoroughly and heat the mixture in a boiling water bath for 15 minutes. Allow the mixture to cool to room temperature, then quantitatively transfer the extract into a 200 mL volumetric flask. Add 5 mL of a 106 g/L potassium ferrocyanide solution and 5 mL of a 220 g/L zinc acetate solution. After completing these additions, let the mixture stand for 30 minutes, then filter it. Discard the initial 30 mL of the filtrate and collect the remaining filtrate for further use. Transfer 40 mL of the collected filtrate into a 50 mL colorimetric tube with a stopper. Prepare a series of standard solutions by transferring 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, and 2.5 mL of a sodium nitrite standard reserve solution (500 mg/L)

into separate 50 mL colorimetric tubes with stoppers. To both the standard and sample tubes, add 2 mL of a 4 g/L sulfanilic acid solution, and shake well. Allow the mixtures to stand for 5 minutes, then add 1 mL of a 2 g/L naphthyl ethylenediamine hydrochloride solution to each tube. Fill each tube to the mark with water, mix thoroughly, and let them stand for 15 minutes. The absorbance of the solution was measured with a 1 cm colorimetric tube at 538 nm wavelength by an ultraviolet spectrophotometer model TU1901, which was obtained from the Beijing Puyang General Instrument Co., LTD (Beijing, China), and the zero point was adjusted with a reagent blank solution. Draw the standard curve for comparison. Draw a standard curve to calculate the results (Fig. 1).

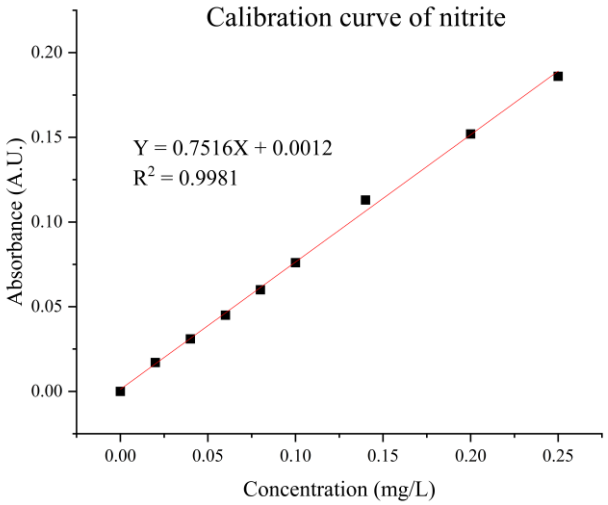

Fig. 1 Calibration curve of nitrite

2.2.3 Calculation of nitrite (sodium nitrite) content

The formula for calculating sodium nitrite is as follows:

$$X_1 = m_1/(m_2 \times V_1/V_0) \quad (1)$$

where, X_1 is the content of sodium nitrite in the sample (mg/kg), m_1 is the mass of sodium nitrite in the sample solution for determination (g), m_2 is the mass of the sample (g), V_1 is the volume of the sample solution for determination (mL), and V_0 is the total volume of the sample treatment solution (mL).

2.2.4 Nitrate detection method

Approximately 10 g of a homogeneous sample should be weighed and placed into a 250 mL conical flask. Subsequently, add 100 mL of water, 5 mL of ammonia buffer solution (pH =9.6), and 2 g of powdered activated carbon. Subject the mixture to oscillation at a reciprocating speed of 200 times per minute for 30 minutes. Quantitatively transfer the contents to a 250 mL volumetric flask, then add 2 mL of a 150 g/L potassium ferrocyanide solution and 2 mL of a 300 g/L zinc sulfate solution. Mix thoroughly, fill to the mark with water, shake well, allow to stand for 5 minutes, and filter the supernatant using quantitative filter paper. The resulting filtrate is reserved for further analysis. Conduct blank experiments concurrently. Transfer 10 mL of the filtrate into a 50 mL volumetric flask, dilute to the mark with water, and mix thoroughly. Measure the absorbance at 219 nm using a 1 cm quartz cuvette with an ultraviolet spectrophotometer model TU1901.

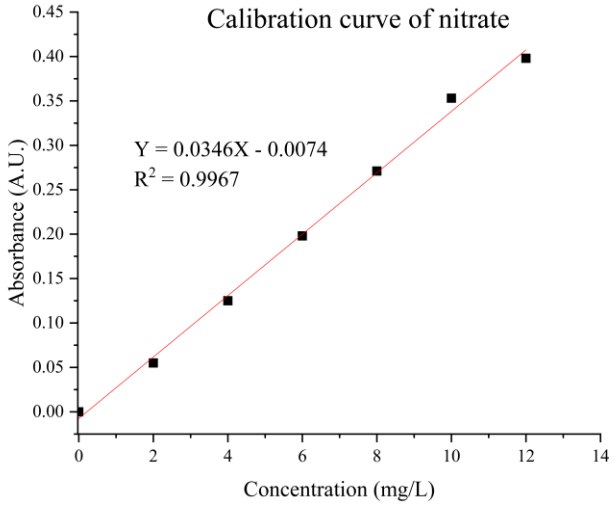

Fig. 2 Calibration curve of nitrate

which was obtained from the Beijing Puyang General Instrument Co., LTD (Beijing, China). Nitrate standard curve working solution: Absorb 0 mL, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1.0 mL and 1.2 mL of a nitrate standard reserve solution (500 mg/L) into separate 50 mL colorimetric tubes with stoppers, add water to the scale. Draw the standard curve for comparison (Fig. 2).

2.2.5 Calculation of nitrate (nitrate radical) content

The formula for calculating nitrate radical is as follows:

$$X_2 = (\rho \times V_2 \times V_4)/(m_3 \times V_3) \quad (2)$$

where: X_2 is the content of nitrate in the sample (mg/kg), m_3 is the mass of the sample (g), $\rho$is the mass concentration of nitrate in the sample solution obtained from the working curve (mg/L), V_2 is the constant volume of the extraction solution (mL), V_3 is the volume of the filtrate sucked (mL), and V_4 is the constant volume of the solution to be tested (mL).

2.3 Data processing

The test data is processed by Microsoft Excel 2019 and plotted by Origin 2018 software.

3. Results

3.1 Changes of nitrite content in tender ginger

The nitrite content in tender ginger initially increased and then decreased with prolonged storage, both at room temperature and under cold storage conditions (Fig. 3). Initially, nitrite levels in tender ginger were negligible, as the nitrate present had not yet been converted.

Over time, nitrate reductase activity and microbial processes led to the partial transformation of nitrate into nitrite, causing an increase in nitrite levels.

On the first day of storage at room temperature (24 hours), nitrite levels rose significantly, exceeding the safe threshold for human consumption and peaking at approximately 159 mg/kg on the fourth day. This finding is consistent with previous studies [17]. A "nitrite peak" was observed on the fourth day, after which levels began to decline gradually. During the initial four days, nitrite levels exhibited substantial fluctuations, which stabilized in the later stages of decline.

At this point, the tender ginger showed signs of wilting

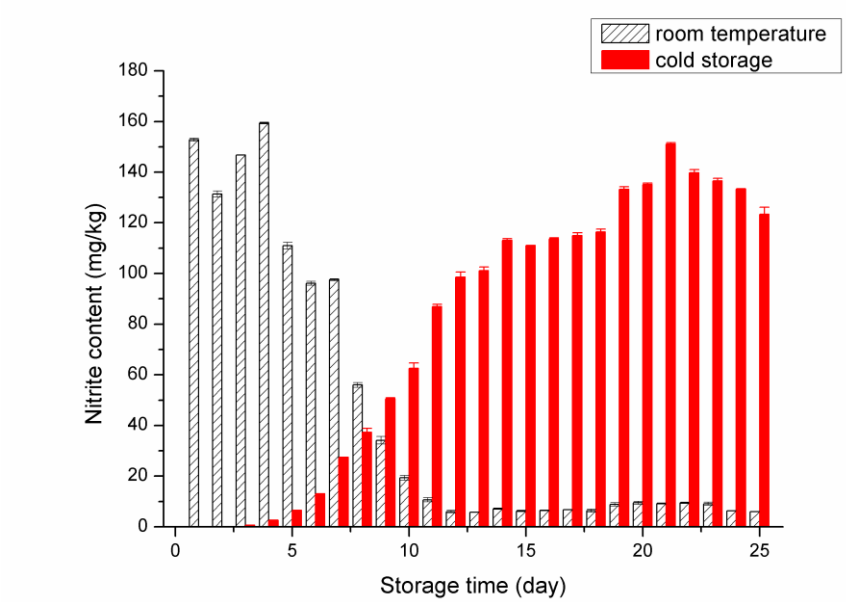

Fig. 3 Change of nitrite content in tender ginger under different storage conditions

and decay, those ginger with unpleasant odors are not suitable for consumption. The trend in nitrite levels during cold storage displayed that observed at room temperature, with an initial increase followed by a decrease. However, as shown in Fig. 3, the increase in nitrite levels was slower under cold storage conditions, with the maximum concentration of approximately 150 mg/kg not being reached until 21 days.

Experimental results indicate that nitrite levels during cold storage remained lower than those at room temperature, deeming consumption safe within 1-3 days. It is evident that both room temperature and cold storage significantly influence nitrite content changes in tender ginger, with cold storage delaying the rate of nitrite accumulation.

3.2 Change of nitrite content in old ginger

The temporal variation in nitrite content for both old and tender ginger at different temperatures followed a similar pattern, with an initial increase followed by a decrease over time (Fig. 4). At room temperature, the nitrite concentration in old ginger rose rapidly, exhibiting a notable "nitrite peak" on the fourth day of storage, similar to tender ginger, followed by a decline from the fifth day onward. Under cold storage conditions, the nitrite content in old ginger increased gradually and stabilized in the later stages, although the fluctuation range was minimal and still exceeded the standard limit value. In a frozen environment, the nitrite content for both tender and old ginger remained below 1 mg/kg, likely due to the inhibition of microbial growth.

3.3 Changes of nitrite content in chilli and garlic

During both ambient and cold storage conditions, the nitrite content in chili and garlic remained relatively stable at initial levels over time, with values falling below the detection limit (1 mg/kg). The minimal impact of temperature on nitrite content suggests either an absence of nitrate-reducing bacteria and reductase in these vegetables or a species-specific variation in nitrite levels. Despite the high nitrate content in these vegetables, nitrite conversion is unlikely to occur without the presence of corresponding reducing bacteria, indicating that

microbial activity is the dominant factor [21], [22]. The temporal variation in nitrite content for both old and tender ginger at different temperatures followed a similar pattern, with an initial increase followed by a decrease over time (Fig. 4). At room temperature, the nitrite concentration in old ginger rose rapidly, exhibiting a notable "nitrite peak" on the fourth day of storage, similar to tender ginger, followed by a decline from the fifth day onward.

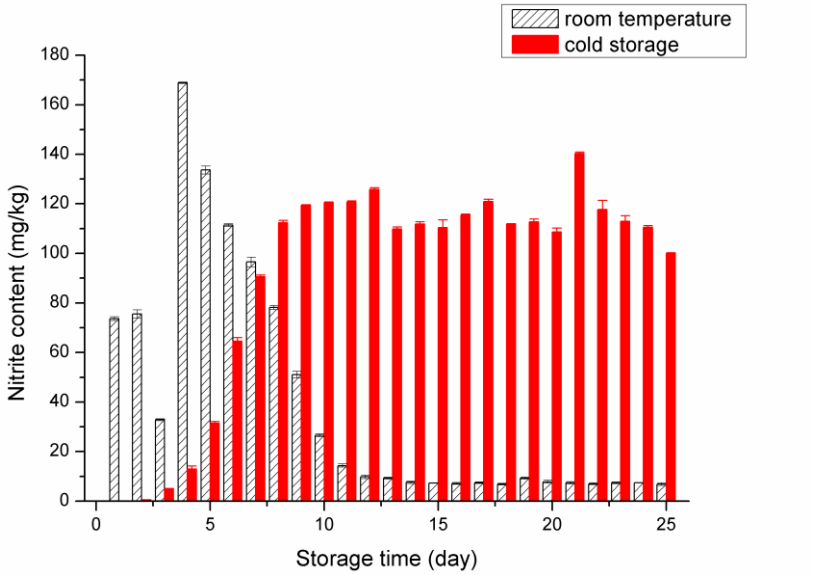

Fig. 4 Change of nitrite content in old ginger under different storage conditions

Under cold storage conditions, the nitrite content in old ginger increased gradually and stabilized in the later stages, although the fluctuation range was minimal and still exceeded the standard limit value. In a frozen environment, the nitrite content for both tender and old ginger remained below 1 mg/kg, likely due to the inhibition of microbial growth.

3.3 Changes of nitrite content in chilli and garlic

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3.4 Effect of frozen and intact preservation on nitrite content

Experimental results demonstrated that when four types of chopped vegetables were stored in a frozen environment, their nitrite levels remained largely unchanged and below the detection limit (1 mg/kg). Similarly, no significant change in nitrite content was observed in whole plants with intact tissue structures after cold storage. These findings indicate that the optimal storage conditions, in descending order, are: frozen > cold storage > room temperature. The results also showed

that the preservation effect of whole plants was better than that of chopped or damaged tissues. This suggests that the larger surface area of chopped vegetables exposed to air facilitates contact with reducing bacteria, thereby promoting microbial proliferation.

3.5 Determination of nitrate content in four kinds of fresh vegetables

Fig. 5 illustrates that among the four fresh vegetables analyzed, garlic and chili exhibited the highest nitrate content, followed by tender ginger, with old ginger displaying the lowest levels. This observation underscores the fact that vegetables are inherently nitrate-rich crops [23], [24]. Several factors contribute to this phenomenon: (1) Environmental conditions, such as water, soil, atmosphere, and temperature, significantly influence nitrate production in vegetables; (2) The processes of nitrogen fixation and microbial activity within the vegetables themselves can lead to nitrate accumulation; (3) The rapid advancement of modern industry has led to the extensive use of chemical fertilizers to enhance crop yields.

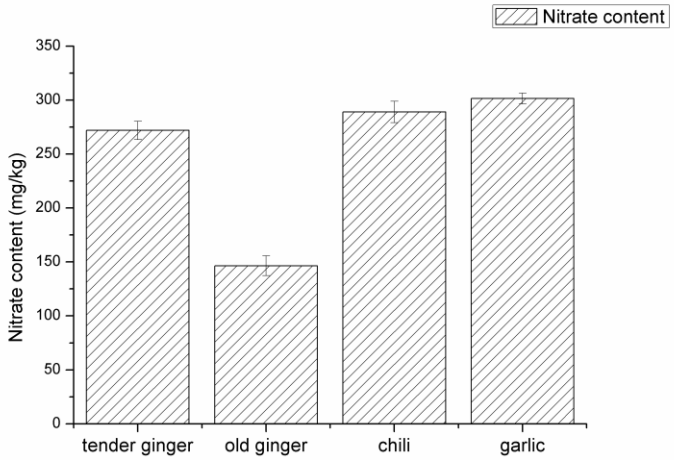

Fig. 5 Comparison of nitrate content in fresh-cut vegetables

Several factors contribute to this phenomenon: (1) Environmental conditions, such as water, soil, atmosphere, and temperature, significantly influence nitrate production in vegetables; (2) The processes of nitrogen fixation and microbial activity within the vegetables themselves can lead to nitrate accumulation; (3) The rapid advancement of modern industry has led to the extensive use of chemical fertilizers to enhance crop yields.

These fertilizers are absorbed and metabolized by plants, resulting in increased nitrate content and subsequent accumulation in vegetables [25].

4. Discussion

The accumulation of nitrite is closely associated with the elevated nitrate levels in vegetables. The potential causes for nitrite accumulation are as follows:

4.1 Analysis of the change mechanism of nitrite

The conversion of nitrate to nitrite in both types of ginger occurs rapidly, primarily driven by microbial activity. Previous research has identified numerous nitrate-reducing bacteria within ginger plants, predominantly Gram-negative bacteria, including members of the Enterobacteriaceae and *Flavobacterium genera*.

These Gram-negative bacteria are the predominant endophytic bacteria in ginger, capable of reducing nitrate and resulting in nitrite accumulation [18]. In the initial stages, the abundant nutrients in ginger facilitate the active growth and rapid proliferation of microorganisms, leading to an increase in nitrite content, which can reach a peak level. Over time, as microbial respiratory metabolism accelerates, oxygen becomes depleted, inhibiting bacterial colony reproduction and reducing the rate of nitrate conversion. For instance, Enterobacteriaceae (*Klebsiella psychrotrophica* and *Klebsiella ascorbata*) have been isolated from ginger stored at room temperature and contribute to significant nitrite transformation and accumulation within a short period [19]. The growth of these microorganisms correlates with the observed trend in nitrite levels. As time progresses, nutrient depletion, decreased enzyme activity, and gradual microbial death result in a decline in nitrite production. In cold environments, the proliferation of these microorganisms is inhibited, resulting in a slower increase in nitrite levels.

Consequently, microbial reproduction is identified as the primary factor influencing changes in nitrite concentration [20].

4.2 Enzymatic action

Nitrite accumulation in vegetable samples primarily originates from the continuous dissolution of nitrate, which can be catalytically reduced to nitrite by nitrate reductase [26]. In ginger, nitrate is catalytically converted into nitrite by specific reductases, leading to a significant increase in nitrite content during prolonged storage. This phenomenon is attributed to changes in temperature, humidity, and pH, which enhance reductase activity and accelerate the reduction of nitrate to nitrite. When vegetables wilt and rot, certain enzymes within them become inactivated, acidity increases, oxygen concentration decreases, and antioxidant loss inhibits nitrite production, while nitrite itself is also decomposed [27], [28]. It is possible that ginger contains unique enzymes, or that the enzyme activity in ginger is more pronounced than that in chili and garlic, resulting in more pronounced nitrite dynamics under the same storage conditions. Low temperatures not only reduce the metabolic rate of vegetables but also inhibit enzyme activity, thereby slowing the conversion of nitrate to nitrite.

4.3 Microbial Activity

The metabolic processes of microorganisms are highly complex. Nitrate-reducing bacteria play a significant role in converting substantial amounts of nitrate to nitrite. As storage time progresses, the reduction of nitrate intensifies, leading to increased nitrite content [29]. During the initial stages of storage, vegetables provide ample nutrients that create favorable conditions for microbial activity, resulting in a rapid rise in nitrite levels. However, as storage continues, factors such as moisture loss, increased acidity, and a decline in organic matter become detrimental to microbial growth and reproduction, causing a subsequent decrease in nitrite content.

This process is characterized by an initial increase followed by a decrease in nitrite levels. The type and quantity of microorganisms are directly correlated with nitrite production [30]. During storage, the conversion of nitrate to nitrite in chili and garlic remains minimal despite high nitrate levels, whereas in two varieties of ginger, the conversion is significantly higher.

This discrepancy may be attributed to the predominance of nitrate-reducing bacteria in ginger, which proliferate extensively and exhibit diverse species at room temperature, resulting in nitrite

accumulation. Additionally, the reduction ability of endogenous reducing bacteria in different types of ginger varies.

The change in nitrite content between tender and old ginger is distinct, potentially related to differences in microbial strains and living environments.

4.4 Different pretreatment methods and storage temperature

When fresh vegetables are mechanically mashed and stored, the destruction of cell tissue releases a large number of reductases from the cytoplasm, enhancing their activity [31]. This condition makes vegetables more susceptible to bacterial and fungal contamination. Research indicates that nitrate-reducing bacteria, including certain Gram-negative bacteria, Enterobacteriaceae, Kluyvera species, and other miscellaneous bacteria, contribute to dynamic changes in nitrite levels [32], [33]. While the nitrite content in chili and garlic remains relatively stable and consistently below 1 mg/kg, both tender and old ginger harbor numerous endogenous reducing bacteria. The proliferation of these bacteria can lead to a rapid increase in nitrite content in both types of ginger over a short period. Furthermore, the variation in nitrite content between pretreated ginger and intact ginger is notably distinct, potentially attributable to differences in surface area exposed to air. Temperature also plays a significant role as an influencing factor. A room temperature environment is conducive to the growth and reproduction of most microorganisms. In contrast, nitrite content increases more slowly under cryopreservation conditions. This slower increase is likely due to cryopreservation's inhibitory effects on microbial reproduction, enzyme activity, and the respiration rate of the sample [34], [35]. Consequently, the conversion of nitrate to nitrite is indirectly suppressed, allowing cryopreservation to extend the shelf life of vegetables and delay the rate of nitrite formation.

5. Conclusions

In this study, the variations in nitrite content among four common vegetables under various storage conditions were initially investigated using spectrophotometry. The findings indicated that nitrite levels in fresh vegetables increased over time across different temperatures, with distinct variations observed among the different types of vegetables. Furthermore, it was found that frozen storage conditions could extend the shelf life of vegetables compared to room temperature environments, and the intact preservation effect of whole plants was better than that of chopped or damaged tissue.

Recommendations:

(1) For Consumers: It is advisable for consumers to select fresh and intact vegetables and avoid chopping or grinding them and leaving them exposed to air at room temperature for extended periods.

(2) For the Catering Industry: The management of raw materials should be rigorously regulated to minimize mechanical damage to vegetables during transportation and processing. It is essential to prevent microbial contamination, promptly remove spoiled vegetables, and ensure appropriate preservation and processing based on the specific characteristics of each type of vegetable. Furthermore, it is imperative to optimize nutrient retention while effectively controlling nitrite content.

(3) For the Plantation Industry: It is essential to control nitrate accumulation at the source by optimizing soil conditions, avoiding the use of contaminated water for irrigation, and

applying nitrogen fertilizers judiciously during vegetable cultivation.

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