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Increasing the shelf life of tomato fruits using physical, chemical and genetic modification methods

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Abstract

The tomato is one of the most consumed vegetables and is rich in numerous beneficial and nutritious compounds. As climacteric fruits, tomatoes undergo significant metabolic changes during their growth and ripening. During fruit ripening, irreversible changes occur in the color, taste and appearance of the fruit. Shortly after ripening, the fruit begins to lose its shape and structural integrity. Approximately 50% of ripe tomatoes do not reach consumers. The primary cause of this loss is excessive fruit softening, which compromises the integrity of tomatoes during harvesting and transportation, making them susceptible to fungal and bacterial infections. Generally, fruit softening results from increased enzymatic activity that breaks down the fruit cell wall. Currently, chemical, physical and biotechnological methods are employed to extend tomato shelf life. These methods help reduce or inhibit the enzymatic activity responsible for fruit softening. The review provides a concise overview of these preservation methods. We focus on enhancing fruit preservation through plant genome modifications using modern biotechnological techniques, such as RNA interference (RNAi) and CRISPR/Cas9. Additionally, we will briefly discuss the advantages and limitations of these genetic engineering approaches.

Keywords

genetic modification; shelf life; *Solanum lycopersicum*; tomato

Introduction

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop after potato. According to FAO data, 186 million tons of tomatoes were harvested in 2024. China is the leading producer of tomatoes. Tomatoes are widely consumed and served as a model plant for studying fruit development and functional genomics. The complete tomato genome has been sequenced, with epigenetic and RNA-seq data accessible through the Sol Genomics Network.

The shelf life and structural integrity of tomatoes significantly influence consumer purchasing decisions (1). Extending the shelf life of tomatoes can enhance their resistance to fungal and bacterial infections, thereby increasing consumer demand. Currently, various chemical, physical and biotechnological methods are employed to extend the shelf life of tomatoes (2-4). Physical methods for tomato storage primarily focus on modifying environmental conditions during storage. These techniques

include low-temperature storage, controlled atmospheres and advanced packaging technologies. A key factor in fruit ripening is the increased activity of enzymes that degrade cell wall carbohydrates. Storing fruits at low temperatures reduces enzymatic activity, thereby preserving their structural integrity for a longer period (5-7). Studies have shown that regulating the O_2 / CO_2 ration in tomato storage air extends shelf life more effectively than low-temperature storage alone (8). Pre-packaging treatment with hot water (5 min at 54°C) has been found to significantly extend shelf life compared to modified atmosphere packaging (MAP) (9-11).

Chemical methods for extending the shelf life of tomatoes involve the use of natural or synthetic compounds. Antioxidants such as ascorbic acid and tocopherols help reduce oxidative stress and delay fruit degradation (12). Treating the tomato surface with a chitosan-allyl isothiocyanate (AIT) solution has been shown to extend shelf life by inhibiting microbial growth on the fruit skin (13). Genetic modification techniques are increasingly being used to enhance tomato fruit preservation. Genetic engineering enables the targeted manipulation of genes involved in fruit ripening, decay and shelf-life regulation. Fruit shelf life has been successfully extended by modifying the expression or nucleotide sequence of genes that regulate fruit ripening. (14, 15).

This review article explores various tomato storage methods, including physical, chemical and biotechnological approaches. We discuss the underlying mechanisms, effectiveness in preserving fruit quality and potential advantages and limitations of each approach. Additionally, we examine modern genome editing technologies, particularly the CRISPR/Cas9 system and their applications in tomato preservation.

Increasing the Shelf Life of Tomato Fruits using Physical Methods

Effect of changes in storage temperature on tomato fruit

Temperature management is crucial for extending the storage life and maintaining the quality of tomato fruits. Cold storage is the primary physical method used to delay or reduce biotic and abiotic diseases in fresh fruits and vegetables (16). Higher temperatures accelerate metabolic activities such as respiration, ethylene production and enzymatic reactions, leading to faster ripening, softening and decay. In contrast, lower temperatures slow these processes, thereby extending the shelf life of tomatoes. Increasing the storage temperature from 18-20°C to 26°C reduced the average shelf life of tomatoes by 4 ± 1 days and increased fungal susceptibility by 11% ± 5% across most genotypes (17). Tomatoes ripen best at temperatures between 18°C and 21°C. However, low temperatures (5-12° C) are commonly used for storage to delay ripening, reduce post-harvest losses and increase shelf life (17). Additionally, edible coating creates a protective barrier on the tomato surface, preventing moisture loss, microbial contamination and oxidative reactions. Ozone treatment is also employed as a complementary method to enhance fruit preservation.

Increasing the Shelf Life of Tomato Fruits using Chemical Methods

Chemical preservation methods effectively extend the shelf life of tomatoes by inhibiting microbial growth, reducing oxidative stress and delaying physiological deterioration. Ozone (O_3) treatment has gained popularity as a promising method for preserving tomato fruits. As a powerful oxidizing agent and a natural antimicrobial agent, ozone effectively inhibits microbial growth, reduces spoilage and extends the shelf life. Research has explored the application of ozone treatment, its mechanisms of action and its impact on fruit quality and safety. Studies suggest that submerging tomatoes in O_3-saturated water or in water bubbled with O_3 is more effective for removing pesticide residues. In addition to removal, ozone treatment also facilitates pesticide residue degradation (18). To maintain fruit quality and extend shelf life up to 12 days at room temperature, the optimal packaging method involves combining ozone treatment with perforated polyethylene packaging (19).

Edible coatings have emerged as a promising preservation technique for extending the shelf life of fruits. These coatings protect food products from light and ultraviolet radiation while also serving as a mechanical barrier with physical and biological properties. The formation of a semipermeable protective layer on the fruit surface alters the gaseous exchange of O_2 and CO_2, thereby reducing respiration rates and suppressing ethylene biosynthesis. This process ultimately delaying the ripening-related changes, preserving fruit quality for an extended period (20).

Improving the Storage of Tomato Fruits using Biotechnological Methods

Fruit ripening is regulated by three main factors: ethylene, ripening-associated transcription factors and DNA methylation. Tomatoes are considered the genetic model for studying climacteric fruit ripening (21). Biotechnological advancements offer innovative strategies to extend the shelf life of tomatoes, enhance quality attributes and reduce post-harvest losses. These approaches include genetic modification, gene expression regulation and molecular breeding to improve fruit storage characteristics.

Delaying ripening through genetic modifications

Genetic modification techniques can be employed to alter the expression of genes involved in tomato fruit ripening, particularly those encoding ethylene biosynthesis enzymes, ethylene receptors and cell wall degrading enzymes. Suppressing or delaying the production and perception of these components can effectively extend the shelf life of tomatoes. Ethylene is key regulator of ripening, influences the expression of genes and transcription factors that drive this process. Delaying fruit ripening can be achieved by inhibiting ethylene biosynthesis or blocking ethylene hormone receptors (22-24). Several enzymes contribute to fruit softening, including polygalacturonase (PG), β-galactosidase (β-gal) and N-glycoprotein-modifying enzymes such as α-mannosidase (α-Man) and β-D-N-acetyl hexosaminidase (β-Hex) (25). Suppressing the activity of α-

Man and β-Hex using RNA interference (RNAi) has been shown to produce tomato lines with extended storage life (14). Genetic modification can also target genes involved in softening, color change and flavor development, allowing for extended storage while preserving desirable quality traits. Currently, RNAi and CRISPR/Cas technologies are the primary tools used to silence or completely knock out genes involved in tomato ripening (15).

Use of RNAi in tomato fruit preservation

RNA interference (RNAi) is a biotechnological method used to selectively silence genes involved in fruit ripening or quality degradation. RNAi-based approaches can delay ripening and extend storage life by targeting and suppressing the expression of genes associated with softening, decay other undesirable traits. Studies have shown that silencing multiple genes through RNAi can significantly increase the shelf life of tomatoes. Ripening and senescence in plants are primarily regulated by type 2C protein phosphatases (PP2Cs). In tomato transgenic lines carrying the SlPP2C RNA interference (RNAi), delayed senescence and ripening were observed in leaves, flowers and fruits (26). Ethylene, a key ripening hormone, plays a crucial role in initiating, regulating and synchronizing the expression of genes involved in the ripening process. Silencing the aminocyclopropane-1-carboxylate (ACC) synthase (ACS) gene using RNAi delayed fruit ripening and extended storage time up to 45 days (27). Additionally, RNAi -mediated suppression of genes responsible for ethylene synthesis (ACS2, ACS4, ACO1 and ACO3) and ripening-related genes (RIN, TAGL1, FUL1, FUL2, LoxC and PE) in SlCMB1-RNAi tomato fruits resulted extended shelf life and delayed deterioration (28).

Use of CRISPR/Cas in tomato fruit preservation

The CRISPR/Cas9 is a genome-editing tool derived from the adaptive immune system of bacteria or archaea, which protects against invasive viruses or phages. Due to its simplicity low cost and high efficiency precision, CRISPR/Cas has become the most widely used genome-editing technique in molecular biology laboratories worldwide (29, 30). CRISPR (clustered regularly interspaced short palindromic repeats) and its associated Cas9 protein provide a precise and efficient method for modifying the genome of any living organism. The CRISPR/Cas-9 system consists of two key components: guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins. The genome editing mechanism follows three main phases: recognition, cleavage and repair (31). The engineered single guided RNA (sgRNA) recognized the target sequence through complementary base pairing, while the Cas-9 nuclease indices double-strand breaks three base pairs upstream to the protospacer adjacent motif (PAM). The breaks are then repaired by either homology-directed repair (HDR) or non-homologous end joining (NHEJ) in the cell.

CRISPR/Cas9 has been extensively used to characterized and edit various tomato traits, including: plant architecture and flower development (leaf, stem, flower, male sterility, fruit and parthenocarpy), fruit ripening, quality and nutrition (lycopene, carotenoid, GABA, total soluble solids, anthocyanin, shelf-life), disease resistance (late blight, TYLCV and powdery mildew), abiotic stress tolerance (heat, drought and salinity), C-N metabolism and herbicide resistance (32). CRISPR/Cas9 has been widely applied to enhance tomato fruit quality and shelf life. Knocked out of SBP-CNR and NAC-NOR transcription factors led to delayed fruit ripening in some plants, while other exhibited partially ripening (21). However, when the ALC gene was mutated using CRISPR/Cas9, long-shelf-life tomato lines were produced, but the overall ripening period remain unchanged (15). One of the key genes associated with fruit firmness is polygalacturonase (PG). CRISPR/Cas9-mediated mutation of PG in tomatoes resulted in fruits that retained their firmness longer under natural conditions compared to the control plants (33).

Increase fruit storage through gene overexpression

The shelf life of tomato fruit can be extended by increasing the activity of transcription factors or genes responsible for the synthesis of key enzymes involved in fruit ripening and senescence. Gene overexpression can be achieved through genetic engineering, transgenic approaches or gene editing technologies such as CRISPR-Cas. By introducing additional copies of specific genes or modifying their regulatory regions, researchers can enhance their expression levels, thereby improving fruit storage characteristics. Overexpression of SlMYB75 gene in tomato plants resulted in prolonged fruit storage and increased resistance to *Botrytis cinerea* (34). MADS-box genes, which encode transcription factors, play essential roles in various plant biological processes in tomatoes. The SlFYFL gene, a meber of MADS-box family, was isolated and overexpressed, leading to delayed leaf senescence and fruit ripening, improved storability and elongated sepals. Additionally, carotenoid accumulation was reduced and ethylene content, ethylene biosynthesis and responsive genes were downregulated in transgenic tomato fruits (35). Furthermore, the overexpression of the SlMSI1 gene in tomatoes has been shown to suppress genes linked to ripening, effectively promoting extended fruit storage (36).

Benefits of Tomato Genetic Modification Techniques

Genetic modification methods offer significant advantages over traditional selection techniques for improving plant characteristics. These methods are faster, more precise and specifically target desired traits without encountering unrelated ones. They allow for the introduction of desirable traits in plants in a shorter timeframe compared to traditional selection methods, which involve repeated cycles of crossbreeding and selection. They also enable researchers to make precise modifications at the molecular level, targeting specific genes or gene regions associated with the desired traits. This precision reduces the likelihood of introducing unintended changes in the plant's genetic makeup.

Currently, there are several gene-editing methods available, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRSIPR/Cas. ZFNs and TALENs were among the first genome editing technologies. The efficiency of gene modification using ZFNs ranges from 1% to 10% (37). However, this method has low specificity, leading to a higher likelihood off-target mutations

(38). TALEN technology is approximately 30% effective in creating DNA mutations, with a low off-target mutation rate (39). In both ZFN and TALEN methods, the target DNA sequence is identified using a synthetically constructed protein.

CRISPR/Cas method is the most recently discovered genome editing method and is widely used today due to its simplicity and low cost. The genome of many organisms has been successfully edited by this method (40). The percentage of gene editing is as high as 75%-85% in CRISPR/Cas (41). By increasing the number of gRNAs, mutations can be introduced at multiple locations simultaneously. Since gRNAs are nucleotide sequences, they can be easily synthesized. When CSIPR/Cas genome editing is performed, experimental efficiency and side effects on other DNA fragments are eliminated during gRNA designing.

Using the CRISPR/Cas method for obtaining transgenic plants can reduce some concerns regarding the release of genetic constructs into the environment. This method allows for the modification of the plant genome without the need for antibiotic resistance genes, reducing the potential risks associated with their presence.

Drawback of Tomato Genetic Modification Techniques

However, genetic modification techniques also have certain limitations. For example, TALENs are unable to modify methylated DNA regions and the main limitation of ZFNs and TALENs is the formation of the endonuclease (*Fok*I). The PAM sequence is important for DNA fragment editing by CRISPR/Cas9. The PAM sequence consists of a 2-5 bp nucleotide sequence and is found in many locations in the genome. Since gRNAs are designed to target DNA containing this PAM sequence, the editing of any DNA fragment by CRISPR/Cas9 is somewhat limited (42). It is also necessary to pay attention to reducing the probability of off-target effects in genome editing using these methods (43). While these methods make it easier to reduce or completely stop the expression of genes, mainly by editing the genomes of plants, introducing a new gene into the plant genome or replacing a piece of DNA with a new one is somewhat difficult due to the low frequency of HDR in plants (44).

Countries have varying regulations on genetically modified plants. The European Union imposes strict restrictions, whereas the United States permits their cultivation and consumption. Asian countries have different views on this (45). The acceptance of transgenic plants by humans, particularly in the case of edible plants, is a significant concern. There are apprehensions about consuming plants that contain genetic constructs used for selection purposes, as well as those that include antibiotic resistance genes.

Conclusion

The quality of tomato fruit in the market is evaluated by its appearance. Since the softening of fruits is the result of the activity of enzymes in their skin, fruit storage is mainly carried out by reducing the activity of these enzymes using various methods such as chemical, physical and biotechnological. Since the use of chemical and physical methods requires special equipment and conditions, the use of biotechnological methods is currently developing. Using biotechnological methods, the activity of the genes responsible for the softening of the fruit shelf life is reduced or stopped. These modern biotechnological methods are especially convenient for farmers who do not have the necessary conditions for storage.

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Authors' contributions

The manuscript was written by AAM and MSA. Word literature was gathered and analysed by ANY, NSO, BOM, LKK, ZHB and SOK. MSA read and edited the manuscript critically and created a subsection. ZTB and IYA rigorously revised and approved the article.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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