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Article

Stability of antioxidative, peroxidative, and stress genes of food materials in different packaging materials under refrigeration

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Abstract: Traditional packaging materials for food include glass, metal, paper, paperboard, and plastic. The stability of food antioxidants depends on storage temperature, storage time, and type of packaging material. After stress exposure, gene expression is tightly controlled and reversible using various molecular pathways that are very dependent on specific stress and organism type. The current study examined the stability of lipid peroxidative, stress genes, and antioxidants in fruits and vegetables stored in various packaging materials under refrigeration. A plastic container, a brown paper bag, and a ziplock bag were utilized to store tomatoes and lemons under refrigeration (5–6 ℃), and the parameters were examined on Day 0 (control), Day 1, and Day 15. The values of the parameters were estimated via various technical methods, while the expressions of genes were determined using the process of polymerase chain reaction and visualized using agarose gel electrophoresis. The stability of antioxidants, lipid peroxidative, and electrolyte levels of the tomatoes and lemons stored in the various packaging materials were found to be significantly altered on Day 15 compared with those of the control. In addition, the expressions of stress genes of the tomatoes stored under refrigeration in the various packaging materials, especially the plastic container, were significantly downregulated on Day 15 compared with those of the control. In conclusion, no packaging materials are suitable for retaining the levels of antioxidants, lipid peroxidative, and stress genes of food materials under refrigeration.

Keywords: beta-carotene; electrolytes; GSH; lipid peroxidation; packaging materials; stress genes; vitamin C

1. Introduction

Foodborne diseases contribute to serious health issues for most of the world's population [1]. The shelf life of packaged foods can be increased by maintaining or improving their condition in the packaging material [2]. To avoid the entry and growth of fungi, pathogens, bacteria, etc., in food during preservation, various packaging materials, such as plastic containers, paper bags, ziplock bags, etc., have been used to protect food and extend its shelf life [3]. Nanocomposites based on biopolymers can improve the mechanical, thermal, and barrier qualities of food packaging. These environmentally friendly materials offer antibacterial, antioxidant, and UV protection properties by combining biopolymer matrices with nanofillers, such as clay, metal oxides, and carbon structures. They promote environmentally friendly packaging options, guarantee food safety, and increase food shelf life [1, 4]. However, the plastic containers used in these experiments are made up of polypropylene (PP), and ziplock bags are made up of low-density polyethylene (LDPE) [5, 6].

Vitamin C, termed ascorbic acid, is a water-soluble antioxidant that is available as a dietary supplement. In situations where the production of free radicals is elevated,

it is vital to maintain a balance between the production and intake of antioxidants. Vitamin C is classified as a butanolide organic compound and possesses excellent antioxidant properties [7]. It serves as an ideal antioxidant to effectively shield the cells of most aerobic organisms from the harmful effects of reactive molecules, cellular metabolism, and exposure to pollutants and toxins [8]. By donating electrons to free radicals and fulfilling the electron requirement for the formation of stable radicals, vitamin C can effectively prevent the oxidation of other molecules. The antioxidant properties of vitamin C can help protect other nutrients in food from oxidation. For example, it can prevent the deterioration of other vitamins (e.g., vitamin A and vitamin E) that are also susceptible to oxidation [9]. By conserving the overall nutritional quality of food, the introduction of vitamin C in packaged food materials can contribute to promoting healthier and more nutritious food choices. It is important to note that the effectiveness of vitamin C incorporation in packaged food materials may vary depending on the specific packaging design, storage condition, and nature of the packaged food [10].

Electrolytes carry an electric charge and minerals in blood and other body fluids and are important for basic life functioning. Preserving electrical neutrality in cells and generating and conducting action potentials in nerves and muscles are also essential functions of electrolytes. The process is required to control the *Pseudomonas sp.* pathogen during essential disinfection processes in food production and processing and to maintain the nutritional benefits and sensory qualities of food to inhibit microbial contamination. Electrolyzed water has been recognized as possessing high disinfection efficacy among chlorine sanitizers in the food industry. Analyzing the levels of sodium and potassium in food materials is essential to guarantee the safety and quality of packaged food products, particularly under refrigeration. Sodium and potassium are electrolytes that play a crucial role in various physiological processes in the human body. However, excessive levels of these elements can be harmful to health, especially for individuals with certain medical conditions, such as hypertension [1].

Lipid oxidation degrades the effect of food quality. The color of food products is the most important thing for fresh food. Microorganisms present in food due to hygiene and storage conditions may lead to foodborne illness and induce food deterioration. Several low-cost and effective antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) are used to control the oxidative deterioration and extend the shelf life of food and added during food processing or preparation. Lipid oxidation is a complex chemical process that occurs when fats and oils are exposed to oxygen, leading to the degradation of lipids and the formation of various volatile and non-volatile compounds. Thiobarbituric acid (TBA) reacts with lipid oxidation products, particularly malondialdehyde (MDA), to form a pink-colored complex. The intensity of this color is directly proportional to the concentration of MDA, making it a convenient and widely accepted method for assessing lipid oxidation in food samples [11].

Carotenoids are free antioxidants and play a vital role in cancer prevention. Vitamin A is a source of beta-carotene and is also found in a large variety of fruits and vegetables, such as carrots, broccoli, lemon, tomato, carrots, grapefruits, sweet potatoes, etc. [12]. Ripe tomato fruits contain minor levels of beta-carotene (pro-

vitamin A), the orange cyclization product of red linear carotene lycopene (a dietary antioxidant), and a large amount of lycopene. Lycopene is transformed into beta-carotene via the action of lycopene beta-cyclase. Antioxidants have two types, which are enzymatic antioxidants and non-enzymatic antioxidants. The most widely used quantification procedures to determine the total antioxidant activity (TAA) are spectrophotometric, chromatography, fluorescence, light emission, and electrophoretic techniques. The determination of beta-carotene in food materials is essential to ensure the quality and safety of packaged foods. Packaged food materials are often subjected to various environmental conditions, including refrigeration, which can potentially impact the stability and release of beta-carotene [13].

Glutathione (GSH), a vital antioxidant, holds significant importance in humans, animals, and plants. It exists in two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). Composed of amino acids, such as glutamic acid, glycine, and cysteine, GSH functions as a tripeptide. While exogenous GSH can be obtained through the consumption of various fruits and vegetables, it does not directly increase innate GSH levels. However, it plays a vital role in stimulating the production of endogenous GSH within cells. The estimation process of the level of GSH in packaged food materials involves scientific techniques and analytical methods. Numerous factors, such as packaging material composition, storage temperature, and storage duration, can affect the stability of GSH levels. Therefore, controlled laboratory experiments or studies have been conducted to simulate refrigerated conditions and assess a packaging material's performance [14].

In the realm of molecular biology, stress-responsive genes emerge as key players in the battle against oxidative stress. DNA methylation may alter gene expression and also modify DNA methylation exposure to stress. In response to exposure to stress, gene expression regulation is tightly controlled and reversible. In a plant's cell wall, pectin is the most abundant macromolecule in both the middle lamella and primary walls. Pectin methylesterases (PMEs) exist in plants as multigene families, where different PME genes exhibit different expression specificities. The PME gene has emerged as a stress-responsive gene in plants. Its expression and activity are influenced by different stress factors and affect plant responses to biotic and abiotic stresses. Under stress environments, the expression of PMEs can be induced or repressed, depending on the specific stress and plant species. The altered expression of PMEs in response to stress is believed to modulate pectin remodeling, which affects cell wall integrity, cell expansion, and signaling pathways involved in stress tolerance. Chalcone isomerase (CHI) is an enzyme that plays an important role in plant metabolism and defense mechanisms. It is involved in the biosynthesis of flavonoids, a diverse class of secondary metabolites with various functions in plants. Flavonoids serve as pigments, as attractants for pollinators, and most importantly, as antioxidants and defense compounds against environmental stress [15, 16]. This study aimed to assess the impact of packaging material by analyzing the levels of antioxidants, lipid peroxidative, and stress genes of food materials stored under refrigeration in various packaging materials and to determine which packaging material offered the longest shelf life for the food materials.

2. Materials and methods

2.1. Study design and sample collection

Ripened tomatoes and lemons were purchased from local grocery stores in Bangalore, Karnataka, India. The collected fruits and vegetables were packed in three different packaging materials, which were a plastic container, a brown paper bag, and a ziplock bag, and kept in a refrigerator at 5–6 °C. The samples were examined prior to the experiment as control (Day 0), after one day (Day 1), and after the 15th day (Day 15), as shown in **Figures 1–3**.

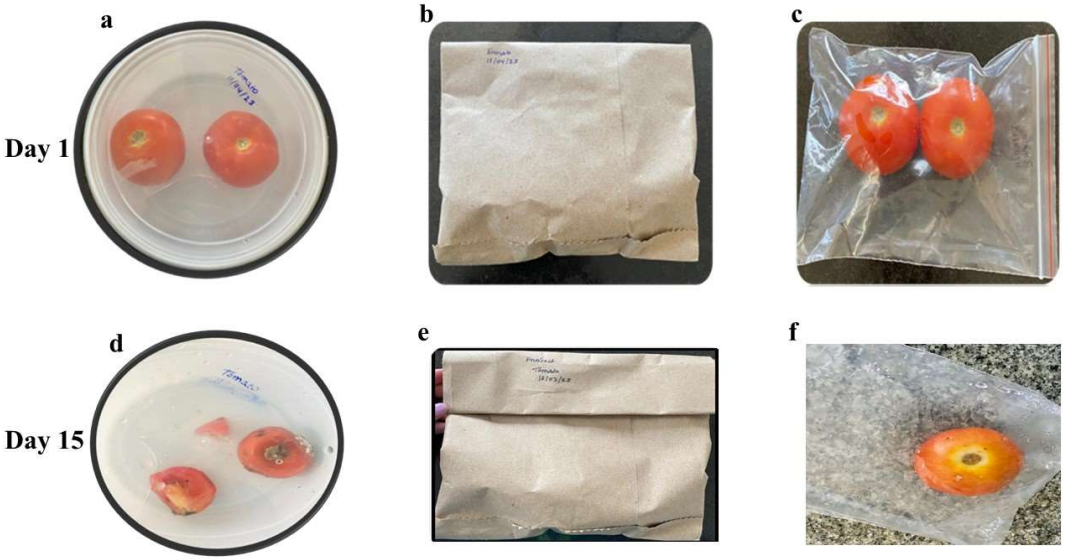

Figure 1. Tomato stored in plastic containers, brown paper, zip lock for Day 1 and Day 15 under refrigerated conditions.

Note: Tomato stored in (a) plastic containers; (b) brown paper; (c) zip lock; for Day 1 and (d) plastic containers; (e) brown paper; (f) zip lock; for Day 15 under refrigerated conditions.

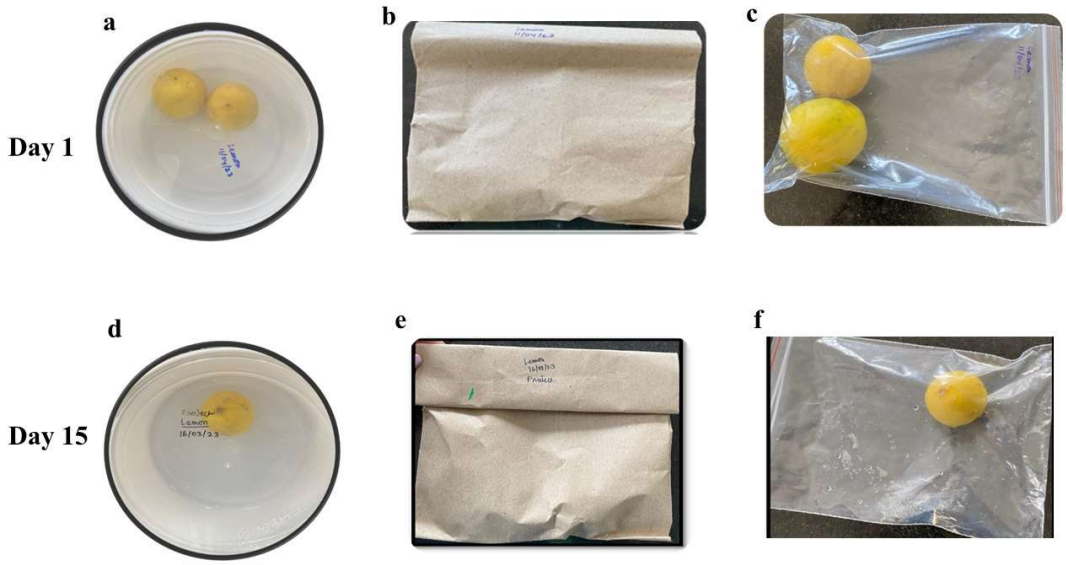

Figure 2. Lemon stored in plastic containers, brown paper, zip lock for Day 1 and Day 15 under refrigerated conditions.

Note: Lemon stored in (a) plastic containers; (b) brown paper; (c) zip lock; for Day 1 and (d) plastic containers; (e) brown paper; (f) zip lock for Day 15 under refrigerated conditions.

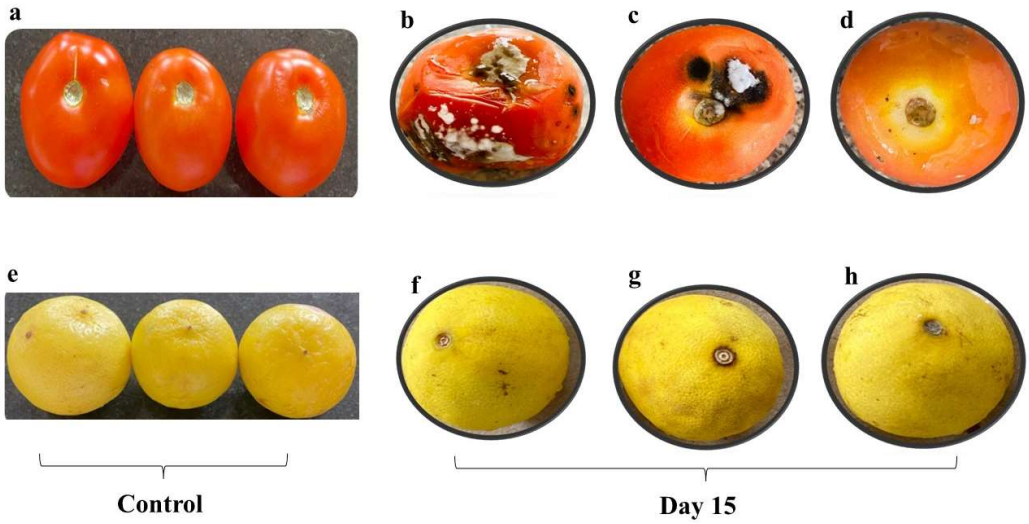

Figure 3. Tomato and lemon stored in control, plastic containers, brown paper, and zip lock under refrigerated conditions (Day 15).

Note: Tomato (a) control; (b) plastic containers; (c) brown paper; (d) zip lock; kept under refrigerated conditions (Day 15) and lemon (e) control; (f) plastic containers; (g) brown paper; (h) zip lock kept under refrigerated conditions (Day 15).

2.2. Estimation of vitamin C

To determine the vitamin C content in the samples, the following procedure was performed. A solution was prepared by mixing a food sample with 20 ml of oxalic acid, 0.2 ml of 0.01% methylene blue, and 1 ml of acetate, and the solution was then filtered. The filtered sample solution was then combined with a few drops of bromine. Excess bromine was eliminated from the sample solution by adding a few drops of a thiourea solution. The sample solution and the calibration standards (5, 10, 15, 20, and 25 µg/ml) were each mixed with 1 ml of a 2,4-dinitrophenylhydrazine solution. The 2,4-dinitrophenylhydrazine solution would cause a coupling process. The standards and the sample solution were maintained at 37 ℃ for 3 hours. The solutions were then cooled in an ice bath, followed by the addition of 5 ml of H_2SO_4. Consequently, colored solutions were produced, where their absorbance at a certain wavelength was measured using a colorimeter.

2.3. Estimation of beta-carotene

A representative quantity of 1 g of a sample was precisely weighed in a glass test tube before extraction. After adding 5 ml of cold acetone, the tube was centrifuged for 10 min at 1370× g, vortexed at high speed, and kept at 4 ± 1 ℃ with periodic shaking for 15 min. After the supernatant was collected and placed into a different test tube, the solution was extracted once more by adding 5 ml of acetone and centrifuged as earlier. The two supernatants were combined and run through a Whatman No. 42 filter paper. The extract's absorbance was measured in a UV-Vis spectrophotometer at a wavelength of 449 nm. The same method used for acetone extraction was also used to prepare other extracts of diethyl ether, acetonitrile, and methanol. Samples of raw lemon and tomato were prepared. Stock solutions at 1 mg/ml were stored at 4 ℃ to prepare a 32µg/ml working standard. To spike the samples, various dilutions were created using this working standard. The working standard was added to 1.0 g of the samples to achieve final beta-carotene concentrations of 16.0, 8.0, 4.0, 2.0, 1.0, 0.5,

0.25, 0.125, 0.062, 0.031, and 0.015 μg/g. The extraction process was then carried out as previously described. Using back extrapolation techniques, calibration curves were created by comparing optical density values with the corresponding concentrations. The beta-carotene content of the examined samples was measured using these curves.

2.4. Estimation of GSH

The estimation of GSH was executed using a modification of the method provided by Cohn and Lyle [17]. A pH8.0 phosphate-EDTA buffer at 1 mg/ml was added to 0.5 ml of the supernatant. A total of 100 μL of the diluted supernatant, along with 1.8 ml of the phosphate-EDTA buffer and 100 μL of an o-phthalaldehyde solution, were included in the final assay mixture (2.0 ml). The solution was moved to a quartz cuvette following careful mixing and a 15-minute incubation period at room temperature. Activation at 350 nm was used to determine fluorescence at 420 nm [18].

2.5. Estimation of TBA

A 4.0 mM TBA solution was prepared by dissolving 57.66 mg of TBA in 100 mL of glacial acetic acid. To prepare MDA calibration standards, 31.35 mg of MDA was dissolved in 100 mL of glacial acetic acid to create a 1mM stock solution. Standards with concentrations ranging from 0.1 to 1.0 mM were then prepared. A total of 1 g of each food sample was mixed with 5 mL of either 100% glacial acetic acid or 50% acetic acid containing 0.01% butylated hydroxytoluene and agitated for 1 hour before filtered and centrifuged as needed. A mixture of 1 mL of the MDA solution and 1 mL of the TBA solution was heated for 60 min at 95 ℃ and then cooled down, and the analysis process involved measuring the mixture's absorbance at 532 nm. This process was repeated for fast food samples, such as samosa, potato chips, fried bread, and Shami kebab, where TBARS was calculated using calibration curves and the sample weight [19].

2.6. Analysis of electrolyte levels

Flame photometry measures the concentration of sodium, potassium, calcium, and lithium in a solution by quantifying the element itself. Standard solutions must contain specific concentrations of these elements, such as 1 mg/100 mL for Na and K and 10 mg/100 mL for Ca and Li. The unit of parts per million (ppm) is used for very small quantities, representing grams of solute per million grams of solution. For example, 1 ppm NaCl/KCl means 1 mg of KCl per liter of the solution. A 1000 ppm standard solution contains 1000 mg of an element in 1000 mL of a solution. A 100mL solution requires a calculated amount of salt to contain 250 mg of the element [20].

2.7. DNA extraction from tomato and lemon samples

We isolated the DNA from the lemon and tomato samples using a BioLit plant DNA extraction minikit as per the manufacturer's guidelines (Sisco Research Laboratories Pvt. Ltd, Mumbai, MH, India).

2.8. Polymerase chain reaction

The polymerase chain reaction (PCR) process begins with the initialization step, where a reaction chamber is heated to 94–96 ℃ (or 98 ℃ for heat-stable polymerase) for 1–9 min. In the denaturation step, DNA strands are separated at 94 ℃. In the annealing step, the temperature is lowered to 50–65 ℃ for primers to bind, which initiates DNA synthesis. The extension step occurs at 72 ℃, where Taq polymerase adds complementary dNTPs to form new DNA strands. A final extension step at 70–74 ℃ for 5–15 min ensures all DNA is fully synthesized. The reaction can be stored at 4–15 ℃ for short-term use.

2.9. Oligonucleotide primers

The oligonucleotide primers used in this work were PME and CHI, where the PME's forward primer sequence is 5'-AATTCCGAGAGTTGGGAATCTT-3', the reverse sequence is 5'-'TGCTCCGAGCGACTTCTTTT-3', and its PCR product's length is 461 bp. The CHI's forward primer sequence is 5'-GCGGGGACAGCAAAACAGAA-3', the reverse sequence is 5'-CGCCTTCTCCTTCAGAGCAA-3', and its PCR product's length is 165 bp.

2.10. Statistical analysis

Results are expressed as mean ± SD. The differences between groups were examined using the Student's *t*-test, where a *p*-value of less than 0.05 was taken as significant.

3. Results and discussion

3.1. Estimation of vitamin C of food materials stored in different packaging materials under refrigeration

As presented in **Figure 4a,b**, the concentration levels of vitamin C were significantly lower on Day 15 in the food materials stored in the plastic container under refrigeration when compared with those of the control, whereas the food materials stored in the other two packaging materials under refrigeration did not have that significantly different values on Day 15 than those of the control. This may be due to the tighter packaging of the plastic container than those of the other two packaging materials. In addition to giving information on product features, nutritional status, and ingredient information, packaging plays a crucial role in the food processing business by confining food and shielding it from physical and chemical harm. Fruit juices are frequently packaged using a variety of materials, including glasses, metal cans, polypropylene containers, and high-density polyethylene containers [21]. The juices are technologically packaged in these materials to aid with preservation. However, metal cans are costly, and closing them requires specialized equipment [22], but they have been shown to affect ascorbic acid retention better than other packaging materials [23]. There is little evidence available about how packaging affects a food product's loss of carotenoids during storage, and the pieces of evidence are occasionally contradictory or understudied, such as light transmission, defects in a seal's hermiticity, oxygen permeability through a packing material, oxygen dissolved in a

product, oxygen availability in a package's headspace, and storage temperature and duration [24]. For instance, commercial fried potato chips have been shown to have an average shelf life of 90 days when packaged in biaxially-oriented polypropylene (BOPP) or metalized BOPP (met-BOPP) packs using atmospheric air (21% oxygen).

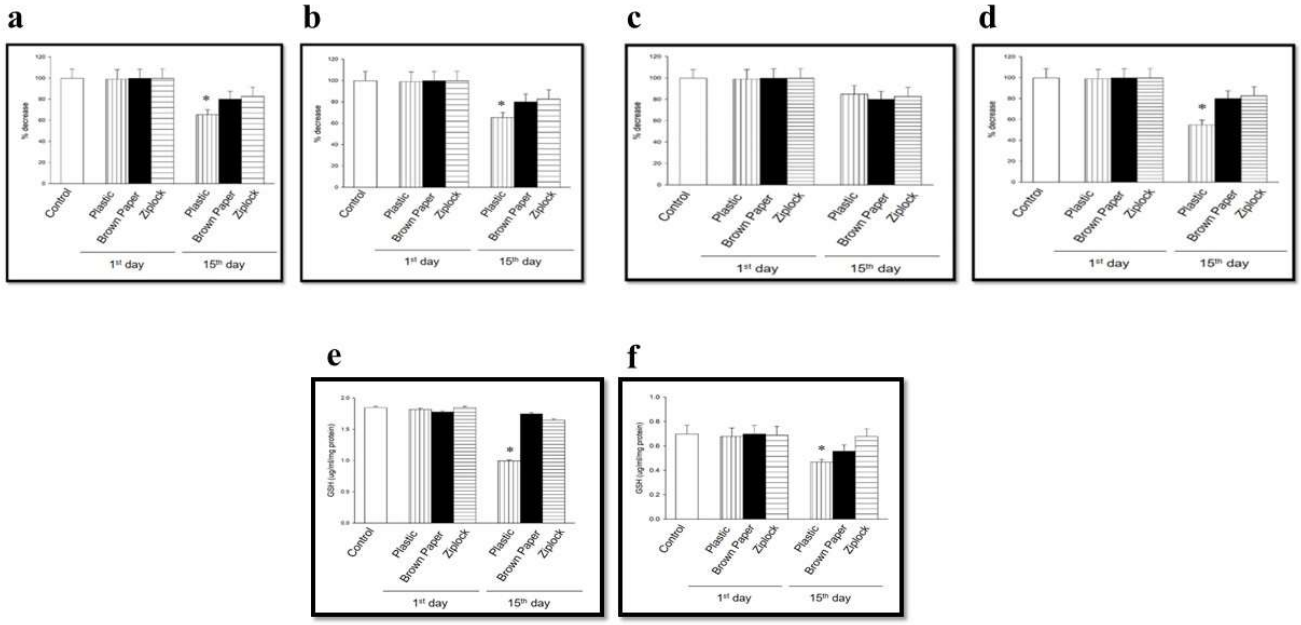

Figure 4. Estimation of non-enzymatic antioxidants in different packaging materials at refrigerated conditions.

Note: Represents the non-enzymatic antioxidants in different packaging materials at refrigerated conditions. (a) Represents the vitamin C levels in tomato; (b) shows the vitamin C levels in lemon; (c) investigates the beta-carotene levels in tomato; (d) shows the beta-carotene levels in lemon; (e) represents the GSH levels in tomato; (f) shows the GSH levels in lemon. The bar graph represents mean ± SD. with triplicates * $p < 0.05$ vs control: Student's *t*-test.

3.2. Estimation of beta-carotene of food materials stored in different packaging materials under refrigeration

Figure 4c,d show that the concentration levels of beta-carotene were significantly lower on Day 15 in the food materials stored in the ziplock bag under refrigeration when compared with those of the control. However, the concentration levels of beta-carotene in the food materials stored in the other two packaging materials were not significantly different on Day 15 than those of the control. This may be due to the tighter packaging when using the plastic container than when using the other two packaging materials.

3.3. Estimation of GSH of food materials stored in different packaging materials under refrigeration

Figure 4e,f display the levels of GSH of tomatoes and lemons stored in the various packaging materials. The levels of GSH were significantly lower on Day 15 in the food materials stored in the plastic container when compared with those of the control. The levels of GSH of the food materials stored in both the brown paper bag and ziplock bag were not significantly different on Day 15 when compared with those of the control. This may be due to the tighter packaging when using the plastic container. To elute glutathione S-transferase-fused proteins using glutathione-agarose beads, reduced L-glutathione is typically added to an elution solution. For GSH

analyses, this has been utilized to create a standard curve. Food packaging prevents chemical contamination and extends shelf life, which benefits customers by making food products safer and easier to handle and transport. Food packaging has been made from a variety of materials, including paper, paper composite, metal, glass, and plastic. However, the transfer of hazardous elements from packing materials into food is of rising concern due to consumers' heightened health awareness [25]. According to several publications, the observed rise in the values of reactive oxygen species (ROS) may be explained by the penetration of sharp-edged graphene particles into biological membranes, which damages intracellular organelles and increases the generation of free radicals by the damaged cells [26, 27]. Surprisingly, Caco-2 cells exposed to graphene derivatives have not been studied for other typical oxidative stress measures, such as the GSH level. The GSH content can be decreased using graphene oxide and reduced graphene oxide. By eliminating ROS, GSH, which is the primary low-molecular-weight thiol-containing peptide found in a majority of live cells, aids in the prevention of oxidative stress. Therefore, the cellular antioxidant defense can somewhat offset the production of ROS.

3.4. Estimation of TBA of food materials stored in different packaging materials under refrigeration

Lipid peroxidative products are often estimated using MDA as the substrate. In our study, as shown in **Figure 5a,b**, we observed that the levels of TBA were highly elevated on Day 15 in the food materials stored in the plastic container when compared with those of the control. There were no significant differences on Day 1 in the levels of TBA in the food materials stored in the different packaging materials compared with those of the control.

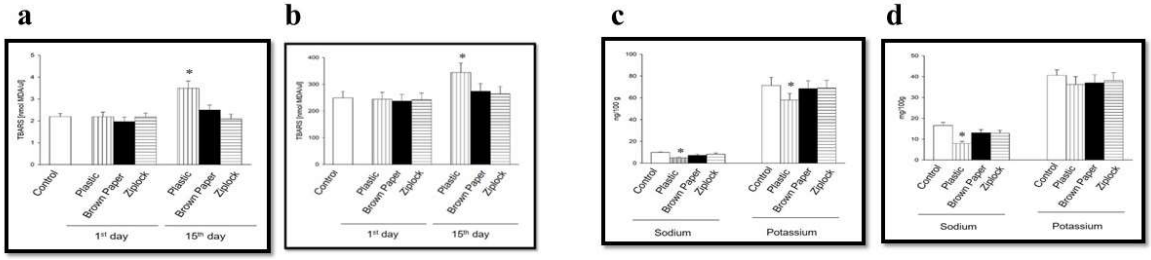

Figure 5. Determination of lipid peroxidative and electrolytes levels in different packaging materials at refrigerated conditions.

Note: Represents the lipid peroxidative and electrolyte levels in different packaging materials at refrigerated conditions. (a) Examine the TBA levels in tomato; (b) investigate the TBA levels in lemon; (c) represent the electrolytes such as sodium and potassium levels in tomato; (d) show the electrolyte such as sodium and potassium levels in lemon. The bar graph represents mean ± SD. with triplicates * $p < 0.05$ vs control: Student's *t*-test.

Membrane lipid peroxidation is caused by ROS (superoxide anion, hydrogen peroxide, and hydroxyl radicals), which are greatly stimulated by calcium. Apoptotic cell death is brought on by the calcium-dependent rise in membrane lipid peroxidation [28]. For instance, storage conditions, including temperature, light, and oxygen concentration, encourage lipid oxidation in meat, which speeds up metmyoglobin formation and the chemical breakdown of protein by producing free radicals [29]. By breaking down and denaturing meat protein, protein oxidation produces harmful substances, with the meat's odor adversely affected, as well as the meat's texture and

capability to retain water [30]. Preserving or improving oxidative stability is crucial for preserving the quality of meat during storage. Likewise, the packaging material is very important for storing food materials under refrigeration.

3.5. Analysis of electrolyte levels of food materials stored in different packaging materials under refrigeration

As presented in **Figure 5c,d**, the levels of sodium and potassium were elevated on Day 15 in the food materials stored in the plastic container under refrigeration compared with those of the control. The electrolyte levels on Day 1 were not affected by the various packaging materials compared with those of the control. Both sodium and potassium levels on Day 15 were not significantly affected in the food materials stored in both the brown paper bag and zip-lock bag compared with those of the control. Since packaging using plastics is tighter compared with packaging using the other two materials, the effects of plastic packaging on food preservation can be more pronounced. Electrolytes are crucial for various bodily processes, making their measurement in blood one of the commonly performed tests. Electrolyte analysis, including sodium and potassium levels, is not only conducted in traditional clinical chemistry labs but is increasingly used in point-of-care testing. Potassium imbalances can lead to heart arrhythmias. A serum potassium level below 3.6 mmol/L indicates hypokalemia, which manifests as muscle weakness, fatigue, and twitching. Severe cases may result in hypokalemic paralysis, which can be either sporadic or hereditary. Conversely, a serum potassium level above 5.5 mmol/L indicates hyperkalemia, which can also cause arrhythmias. Symptoms of hyperkalemia may include muscle weakness, cramps, myoglobinuria, and rhabdomyolysis [31–33].

3.6. PCR cycle

The PCR cycles were as follows for both genes: initialization was done at 94 ℃ for 5 min, denaturation at 94 ℃ for 30 seconds, annealing at 59 ℃ for 30 seconds; elongation at 72 ℃ for 60 seconds, additional elongation at 72 ℃ for 10 min, and cooling at 4 ℃. A total of 35 cycles were run (**Figure 6**).

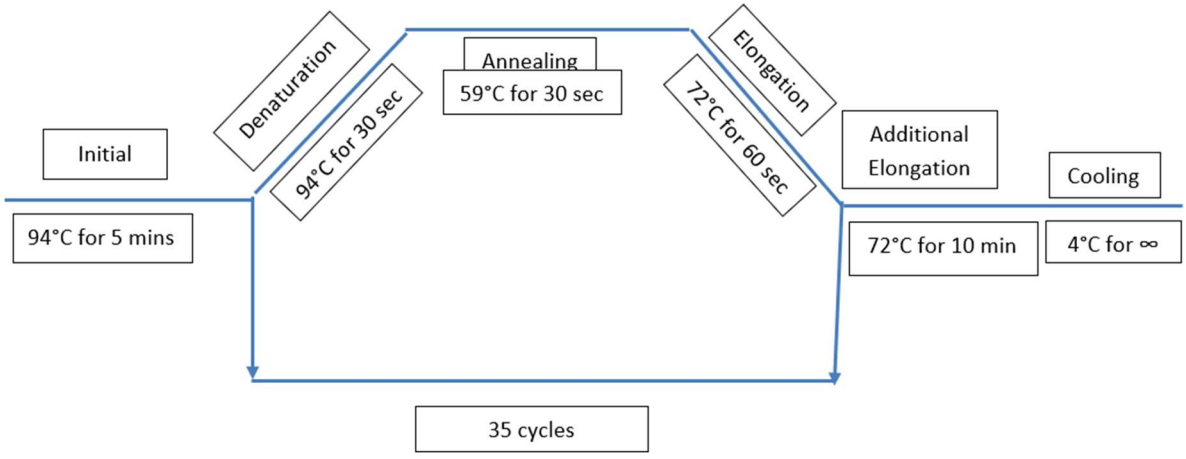

Figure 6. Specific PCR cycle for gene expression.

3.7. Expressions of stress genes of food materials stored in different packaging materials under refrigeration

Multigene groups of pectin methylesterases (PMEs; E.C. number 3.1.1.11) encode isoforms with distinct action patterns by removing methyl esters to affect cell wall characteristics. Changes in cell wall machinery can clarify how plant cell walls react to certain environmental stimuli through cell-wall-modifying proteins. This clarifies the role PMEs play in altering the content and structure of cell walls. In response to abiotic stress, PMEs may mediate the fine-scale modification of apoplastic calcium ion (Ca^{2+}) content for the pectic network's formation and disintegration [34,35]. Extreme environmental factors, such as a variety of biotic and abiotic stresses, provide difficulties for plants and harm their growth and development. Since plants are sessile creatures and cannot relocate to more hospitable areas, they have evolved an astounding array of methods to lessen their negative environmental effects. One of the most harmful stresses to plants is global climate change, which causes ambient temperatures to rise. By the end of the 21st century, the United Nations' Intergovernmental Panel on Climate Change predicts that these temperatures will be 2–5 ℃ higher than they are now. It has been demonstrated that plant cells have complex systems to respond to a wide range of stressors, including the heat shock response (HSR), which can improve crop yield even under extreme climate change conditions [36,37]. In our study, as shown in **Figure 7a**, we observed that the PME stress gene was significantly downregulated on Day 15 in the food materials stored under refrigeration in the plastic container and the brown paper bag (Lane 4 and Lane 5, respectively) when compared with those of the control (Lane 3). This correlates with the results from other research studies.

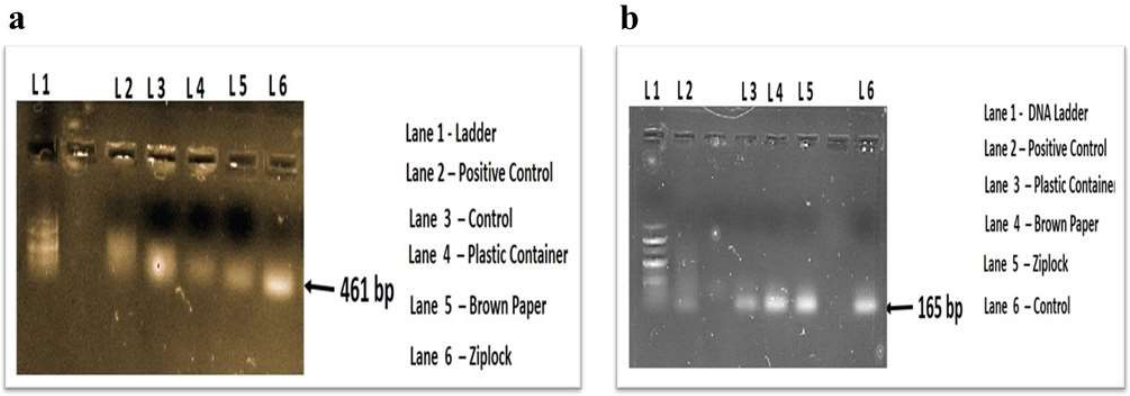

Figure 7. Expressions of the stress genes in different packaging materials at refrigerated conditions.

Note: Expressions of the stress genes in different packaging materials at refrigerated conditions in tomato, Agarose gel electrophoresis (0.67%) (a) PME-lane details: Lane 1: DNA ladder, Lane 2: Positive control, Lane 3: Control, Lane 4: Plastic container, Lane 5: Brown paper, Lane 6: Ziplock (b) CHI-lane details: Lane 1: DNA ladder, Lane 2: Positive control, Lane 3: Plastic container, Lane 4: brown paper, Lane 5: Ziplock, Lane 6: Control.

One of the key enzymes in the flavonoid biosynthesis pathway that can change chalcone into (2S)-naringenin is chalcone isomerase (CHI; E.C. 5.5. 1.6). The precursor of apigenin, luteolin (flavones), and eriodictyol (flavanone), CHI catalyzes the heterocycle C closure to produce naringenin. *Ginkgo biloba* L2 has been used to clone chalcone isomerase. The production of naringenin from 6′-hydroxy chalcone could be catalyzed by the recombinant GbCHI protein, according to the *in vitro*

enzyme activity measured using high-performance liquid chromatography. CHI is a crucial gene for controlling the accumulation of flavonoids in ginkgo leaves, as evidenced by the positive correlation between the expression of CHI activity and changes in the transcription level of the CHI gene. The GbCHI activity has also been found to positively correlate with the total levels of flavonoids in ginkgo leaves. Petunia CHI gene overexpression in tomatoes has shown an increase of about 78-fold in fruit peel flavonols, mainly due to rutin accumulation [38–40]. In our study, as shown in **Figure 7b**, we observed that the CHI stress gene was significantly downregulated on Day 15 in tomatoes stored under refrigeration in the plastic container (Lane 3) when compared with that of control (Lane 6). In contrast, CHI expressions were not downregulated on Day 15 in tomatoes stored under refrigeration in the brown paper bag and ziplock bag. This correlates with the results from other research studies. A comparative study of the results of the parameters obtained from this study and previous studies is shown in **Table 1**.

Table 1. Comparative study of results of all parameters from previous studies and current study.

Sl. No.	Parameter	Current study' result	Previous study's result	Reference
1	Vitamin C	Reduced	Reduced	[41]
2	Beta-carotene	Reduced	Increased	[42]
3	GSH	Reduced	Reduced	[43]
4	TBA	Increased	Reduced	[43]
5	Electrolyte levels	Reduced	Increased	[44]
6	Stress genes	Downregulated	Upregulated	[45,46]

4. Conclusion

It can be concluded that the stability of antioxidants, lipid peroxidative, and electrolyte levels of the food materials stored in the various packaging materials under refrigeration were significantly altered. In addition, the expressions of stress genes were downregulated considerably for the food materials stored under refrigeration in the various packaging materials, particularly the plastic container. Our study highlights the importance of selecting the appropriate packaging material to preserve the nutritional and biochemical quality of fresh produce during storage.

Author contributions: Conceptualization, SR; methodology, NM (Nitya Mankal) and NM (Nabanita Mandal); writing—original draft preparation, NM (Nitya Mankal) and NM (Nabanita Mandal); writing—review and editing, NM (Nitya Mankal) and SR; visualization, NM (Nitya Mankal) and NM (Nabanita Mandal); supervision, SR; project administration, NM (Nitya Mankal) and SR. All authors have read and agreed to the published version of the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Abbreviations

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
PG	Propyl gallate
TBA	Thiobarbituric acid
MDA	Malondialdehyde
TAA	Total antioxidant activity
GSH	Glutathione
PME	Pectin methylesterases
CHI	Chalcone isomerase
APX	Ascorbate peroxidase
CHS	Chalcone synthase
ROS	Reactive oxygen species
ppm	Parts per million
OS	Oxidative stress

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