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BIO-PRESERVATION OF APPLE – A REVIEW

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Abstract– Apple is one of the most popular fruits grown worldwide. A raw apple is 86% of water and 14% carbohydrates with negligible content of fat and protein. The majority of apples are eaten fresh, but a little portion is processed to make juices, jellies, canned slices, and other products. Its trees and fruits are prone to fungal, bacterial, and pest problems, which can be controlled by several organic and non-organic means. This review includes studies on the bio preservation of apple fruits and related products. In this review, data have been collected from various databases, e.g., PubMed, and Google Scholar. This article includes studies that used bio methods for preserving apples and their products alone or in combination with other physical or mechanical methods. Bio-preservation is an effective method for the preservation of apples and their products. In addition to this, they reduce the harmful effects caused by chemical preservatives when they are used in food commodities. Studies prove that Bio-preservation is an effective means for increasing the shelf-life of apples.

INTRODUCTION

The use of biological systems-microorganisms and the products of their metabolism, enzymes-to increase food safety and prolong the shelf life of food and feed is known as "Bio-preservation"(Lücke, 2024). Another name for this technique is "living preservation"(Hussein, 2022). Bio-preservation is the process of extending food's shelf life while upholding safety regulations by using bacteria or their metabolic byproducts. Among the main bio-preservatives are microbial substances like bacteriocins, bacteriophages, and antifungal agents; plant extracts like flavonoids and essential oils; and substances derived from animals like lysozyme, chitosan, and lactoferrin (Ranathunga *et al*., 2023). The use of natural food additives has grown in the modern era. Peptides, enzymes, bacteriocins, bacteriophages, plant extracts, essential oils, and fermented compounds are examples of natural antimicrobial substances that can be utilised in place of chemical antimicrobials. Terpenes, flavonoids, aldehydes, and phenolic chemicals that have antibacterial and antioxidant properties can be found in plant extracts and essential oils (Amiri *et al*., 2021).

The subject of replacing chemical preservatives has come up as a result of the growing consumer demand for less processed and more natural food items while maintaining the products' quality, safety, and shelf life. To combat fungal spoiling and meet consumer and regulatory requirements, bacteria, fungus, and their metabolites are natural alternatives of interest for usage in food as bioprotective instruments (Leyva Salas *et al.,* 2017).

After bananas, oranges, and grapes, apples (*Malus pumila*) rank fourth in terms of global production and are the most important temperate fruit in terms of commerce. The world's largest producer of apples is China. More than 4,000 years ago, apples first appeared in the Middle East. The majority of apples are eaten fresh, but a little portion are processed to make juices, jellies, canned slices, and other products (National Horticulture Board, 2024). Numerous biotic and abiotic illnesses that affect apples during storage result in large financial losses. In addition to lowering the apples' market value and compromising their quality and shelf life, these diseases call for proactive disease control and preservation approaches. Numerous species from

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several genera, such as *Penicillium, Botrytis, Alternaria, Gleosporium, Mucor, Rhizopus, Fusarium, Monilinia,* and *Aspergillus*, are responsible for fungal diseases that impact apples (Khadiri *et al*., 2024).

METHODOLOGY

For this review, data have been collected from various databases, e.g., PubMed, and Google Scholar. This article includes studies that used bio methods for preserving apples and its products alone or in combination with other physical or mechanical methods.

Bio Preservation of Apple

Compared to harvest point (HP) samples, cold-stored (CS) samples have greater fungal richness, evenness, and variety. Significant changes in the fungal composition between the CS and HP samples are revealed by beta diversity analysis. During cold storage, the abundance of *Acremonium, Monascus, Archaeorhizomyces*, and *Yarrowia* decreased. In contrast, *Penicillium, Aspergillus, Mucor, Botrytis, Mycosphaerella, Rhodotorula,* and *Wallemia* considerably increased in CS samples (Shen *et al*., 2018).

Application of lactic Acid Bacteria

Certain strains of Lactic Acid Bacteria (LAB) can help in the control of fire blight disease, as a source of biological agents (BCA) in the plants of apples (Roselló *et al*., 2013). In a study of lactobacillus strains isolated from naturally fermented green olives, they decreased the *C. pelliculosa* population from 5 to 2.5 log CFU/mL after 48h of inoculation in apple juice (Abouloifa *et al*., 2021). *Lactobacillus acidophilus* NCDC 343's purified bacteriocin exhibits antagonistic activity against pathogens that cause food spoiling. Bacteriocin immobilized in calcium alginate beads is more effective than chemical preservatives and has great promise in reducing the bacteria population in fruit juices. In apple and pomegranate juice, bacteriocin treatment reduced the bacterial burden by 14% and 25.4%, respectively. Because bacteriocins are safe and harmless, it can be advised to use them to preserve fruit juice (Rani *et al*., 2016). To choose LAB with exceptional inhibitory activity against Salmonella linked to fresh-cut apples, the mathematical modelling approach is crucial (Luo *et al*., 2015).

Application of other Bacteria

The biocontrol agent *Pantoeaagglomerans* strain E325 (E325), an antagonist to the bacterial plant pathogen *Erwinia amylovora* that causes fire blight, a devastating disease of apples, has been studied through microencapsulation and controlled release, yielded very encouraging results (Kim *et al*., 2012). The development of *Aureobasidium pullulans* LW14 and the metabolism of all examined sugars (glucose, sucrose, and fructose) are successfully inhibited when cyanobacterium (*Arthrospira platensis*) biomass is introduced to fresh apple juice at a concentration of 1.15% w/v (Wajda *et al*., 2020).

Application of Bacteriocin

One of the bacteria that cause fruit juices to deteriorate the most is *Alicyclobacillus acidoterrestris*. In a study on the bacteria, 16 of 21 strains of A. acidoterrestris were found to be sensitive when bacteriocin RC20975 was applied to apple juice (Pei *et al*., 2017). After treating apple juice with combination of Enterocin AS-48 and high-intensity pulsed-electric field (HIPEF) to combat the cider-spoilage, exopolysaccharide-producing strain *Lactobacillus diolivorans 29*, samples were free of detectable lactobacilli for at least 15 days at both 4 and 22 degrees Celsius (Martínez Viedma *et al*., 2009).

Application of Yeasts

The combined impact of hot air (at 38°C for 96 hours) and *Pichia guilliermondii* (at 1×108 CFU ml^{-1}) treatments on the three main postharvest diseases of Red Fuji (Malus pumila var. domestica) apple fruit: *Botrytis cinerea, Penicillium expansum*, and *Colletotrichum gloeosporioides* resulted in a total suppression of apple fruit wound infection (Zhao and Yin, 2018). The microenvironment of apple wounds is more resistant to oxidative stress when glycine betaine is pretreated with the yeast antagonist *Candida oleophila* (Sui *et al*., 2012).

Application of Aloe vera gel

Granny Smith apples treated with aloe vera gel can delay quality deterioration. Granny Smith's increased weight loss and loss of green colour can be significantly controlled by aloe vera gel treatments. Granny Smith apple fruit treated with Aloe vera gel (5 and 10%) had greater soluble solids content and percentage titratable acidity (Ergun and Satici, 2012; Song *et al*., 2013). The application of carboxymethylcellulose (CMC) and aloe vera gel (AVG) coating formulated with anti-browning agents significantly affected the quality and shelf-

life of 'Royal Delicious' apple wedges by limiting weight loss, enzymatic browning, and inhibiting the growth of microorganisms during storage at 5 ± 2 °C (Kumar *et al*., 2018). A combination of carboxymethyl cellulose and aloe vera may be utilized as a nontoxic, antibacterial, and anti-browning agent to extend the shelf life and provide nutritional value to fresh-cut apples (Tosif *et al*., 2023).

Application of Chitosan

Chitin is the source of chitosan, a copolymer of glucosamine and N-acetyl glucosamine. The cell walls of certain algae, bacteria, insects, fungi, and invertebrate animals contain chitin (Pellis *et al*., 2022). The quality preservation of cold-stored apple fruits is positively impacted by coatings containing chitosan (CH) or chitosan-olive oil processing wastes (CH-OOW), particularly the 2% chitosan-olive leaf extract. Similarly, anthocyanins, total phenolic, flavonoids, carotenoids, chlorophylls, and antioxidant activities were all successfully delayed by CH-OOW (Khalifa *et al*., 2017).

A modified bio-polymer shell of chitosan enriched with extracts from Zanthoxylum can be employed in place of the peel to preserve fresh-cut apples. During the storage term, this treatment slows down the overall post-harvest quality fall of the preserved apple samples(Yang *et al*., 2022). The addition of apple peel polyphenols (APP) to chitosan (CS) greatly improves the film's physical characteristics by increasing its thickness, density, solubility, opacity, and swelling ratio, while moisture content and water vapour permeability decreased, indicating that the film's water barrier property was improved. Compared to the CS control film, the CS-APP film with 1% APP has tensile strength and elongation at break of 16.48 MPa and 13.33%, respectively. While the CS-based APP film's antioxidant and antibacterial properties were markedly enhanced, the produced films' thermal stability was reduced. The CS-APP2 film with APP concentration added may be a viable substitute for synthetic materials and help prolong the shelf life of food (Riaz *et al*., 2018).

A study combined Walnut green husk extract (WE) and chitosan/guar gum (CS/GG) film matrix to create a bioactive food packaging film. This interaction between the two molecules creates an intermolecular hydrogen bond that improves the film's mechanical, water-resistant, and barrier qualities. The film's antioxidant capacity is greatly increased when the WE content (0-4 wt %) rises. In addition to dramatically reducing the hardness, weight loss, and total soluble solids (TSS) of fresh-cut apples during storage, CG-WE film can amazingly prevent the browning of fresh-cut apples(Jiang *et al*., 2022). By generating a regulated environment and functioning as a semi-permeable barrier for gases, vapour, and water, edible films and coatings extend the shelf life and enhance the quality of food by offering protection against mechanical and physical harm. Naturally occurring substances including polysaccharides, proteins, and lipids, or a combination of these, are used to make edible films and coatings. Additionally, these films and coatings provide the opportunity to include various functional components, including flavouring, colouring, antioxidants, antimicrobials, and nutraceuticals. Living microorganisms can also be included in films and coatings. When banana peel extract (BPE) was added to chitosan, BPE impacted the mechanical, structural, and physical characteristics of the film. Better functionality was demonstrated by films that contained 4% BPE (Zhang *et al*., 2020).

Carboxymethyl chitosan (CMCS) can form coatings and has antimicrobial properties. Fresh-cut apples refrigerated for preservation showed improved quality (weight loss, browning index, firmness), nutritional value (ascorbic acid and total phenolic content), and microbial safety during storage when a CMCS coating containing flavonoids from guava (*Psidium guajava* L. cv. Carmine) leaf (GLF) was added (Wang *et al*., 2023). When Chitosan, liquorice ethanolic extract (LE), and chitosan-liquorice extract complex (CHLE) were tested for their ability to prevent blue mould and prolong apple shelf life, the fruits were kept at 25 °C after being covered with chitosan (1.0%), LE (62.5 mg/ml), and CHLE coating. The trials showed that apples coated with CHLE had the lowest levels of water loss (3.8%), TSS (14.53%), and hardness (5.6 kg/cm^2). Additionally, after storage, this coating considerably reduced the growth of *penicillium expansum* (Madanipour *et al*., 2019).

Application of Alginate

Alginate, a polymeric saccharide that is generally obtained from the cell walls of brown seaweed (Brownlee *et al*., 2005), when applied to the minimally processed 'Gala' apples (on wedges), coating reduced weight loss during storage maintained firmness, and delayed browning

(Olivas, 2007). When combined with high-pressure homogenisation (HPH), *zanthoxylum bungeanum* leaves (ZBLs) can also improve apple preservation (Li *et al*., 2024).

Application of Essential oils

Natural products such as eucalyptus essential oil (ER), rosemary EO, their mixture and eucalyptol can be used in vapor form on fresh apples as natural fungicides, as they are able to prevent the growth of *P. expansum* in apples (Xylia *et al*., 2021). *Fructus Ligustri Lucidi* essential oil (FEO), a natural bacteriostatic agent, shows good compatibility with zein, a corn protein and exhibits anti-browning properties in fresh-cut apple (Chen *et al*., 2025). When the quantity of cinnamon essential oil (CEO) is less than 4%, it exhibits good film-forming qualities with chitosan (CS). Both the direct inhibitory activity of CEO against pathogens in vitro and the induced resistance caused by CS on the fruits are responsible for the composite coating's efficacy in postharvest apple disease (Zhang *et al*., 2023).

When apple juice was tested for the ability to reduce rotting bacteria and yeasts using a variety of techniques, including mild heat (54 °C for 10 minutes) or pulsed electric field (25 pulses; 25 kV/cm; 3.35 kJ/cm per pulse) treatments and essential oil contents (carvacrol, citral, and (+)-limonene). Carvacrol and mild heat worked best together, inactivating 99.99% of Saccharomyces cerevisiae CECT 1172 cells and 99% of Leuconostoc fallax 74 cells (Chueca *et al*., 2015).

The quality metrics (°Brix, pH, and titratable acidity) that define unsweetened juices are not compromised by the useful combinations of citrus lemon essential oil (CLEO) or citrus reticulata essential oil (CREO) with mild heat treatment (MHT) to suppress the autochthonous spoilage bacteria. These findings suggest that using MHT in conjunction with CLEO or CREO at concentrations below the sensory rejection threshold (RT) is a practical method of reducing autochthonous spoilage bacteria in fresh fruit juices (de Souza Pedrosa *et al*., 2019). Apple cider prepared with modest heat may be safer if cinnamon or clove essential oil is used (Knight and McKellar, 2007). Modern produce wax coating technology has a lot of promise to improve food safety and give produce commodities better defence against germs and fungi. novel method by employing whey protein concentrate to nano-encapsulate cinnamon bark essential oil and incorporating the nano-encapsulates with food-grade wax frequently used on product surfaces (Arcot *et al*., 2024).

Application of various extracts

The application of liquorice aqueous extract may delay apple fruit blue mould deterioration without having a major impact on the fruit's qualities. This might be regarded as a novel, environmentally beneficial method of fruit preservation (Soleimani *et al*., 2021). A study sought to determine whether extracts of cinnamon, pimento, and laurel had antifungal properties both in vitro and against apple postharvest grey mould. The most effective extract against apple grey mold was cinnamon extract; nevertheless, larger amounts of the extracts are needed to effectively inhibit B. cinerea in apples while they are being stored (Sernaite *et al*., 2020). When oregano extract (OX) was used as a post-harvest therapy to significantly reduce conjugated triene and superficial scald, it was more noticeable when soluble chitosan oligosaccharide (COS) was used to stimulate antioxidant enzyme responses (Sarkar *et al*., 2018). When apple slices are kept in Methylcellulose/glutaraldehyde/Noni (*Morindacitrifolia*) Leaf Extract (MGN) active films at room temperature, the minimum reduction in vitamin C, reducing sugar content, percentage weight loss, pH, and total phenolic content indicates that MGN is a cost-effective and practical substitute for conventional single-use plastic packaging in the cut fruit industry (Eelager *et al*., 2024). To stop *Salmonella typhimurium* DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus* from contaminating food, roselle calyx ethanol extract and protocatechuic acid may be effective food additives (Chao and Yin, 2009).

Application of Flavanones

In order to preserve fresh apple juice, steppogenin may be used as an anti-browning agent. When combined with ascorbic acid, its anti-browning properties are significantly enhanced (Tao *et al*., 2017).

Application of Edible gums

Research done by Moreira and colleagues, After extended storage, the microbiological deterioration of fresh-cut apples can be delayed and the sensory attribute scores can be kept above the rejection limits by applying a pulsed light treatment (12 J/cm^2) in

conjunction with an edible coating enriched with apple fibre that is based on gellan gum (0.5% w/v) (María R. Moreira *et al.*, 2015). In the study of Zheng *et al.* (2023) Pullulan polysaccharide (PP)/xanthan gum (XG) composite films (PP/XG/GSE or PXG) used for apple preservation benefited from the addition of grape seed extract (GSE) by becoming more antibacterial, antioxidant, mechanical, UV-resistant, and water-resistant (Zheng *et al.*, 2023).

Application of non-nutritional components

The effectiveness of phytic acid as a browning inhibitor for apple juice was examined in a study. The 0.1 mM Phytic acid inhibited the polyphenol oxidase (PPO) from the apple juice by 99.2 (Du *et al*., 2012). Longan shell tannins (LSTs) are exceptional tyrosinase inhibitors, according to the study of Chai *et al.* (2024) by preventing phenolic metabolism, boosting the antioxidant system, and lowering lipid peroxidation, LSTs can postpone the browning of fresh-cut apples (Chai *et al.*, 2024).

Others

Five distinct coating formulations of fenugreek and flaxseed polysaccharide were prepared as part of the experimental plan and then applied to the apple fruit. Improved organoleptic qualities resulted from the coated apple fruits' considerable ($p \leq .05$) decreased weight loss, pH, total sugars, total soluble solids, and maximal retention of ascorbic acid, firmness, acidity, and antioxidant concentrations, in comparison to non-coated (Rashid *et al.*, 2023). Apples (Rich Red) covered with a composite coating material consisting of â-glucan stearic acid ester (SABG) and wheat straw arabinoxylan (AXE) at concentrations ranging from 1% to 2%. The application of AX-SABG dramatically decreased weight loss, respiration, colour deterioration, fruit softening, and ripening index, and were also successful in preserving sensory qualities, preventing microbiological spoiling, and preventing scent loss during storage (Ali *et al.*, 2020). Combining cold plasma (CP) treatment with honey coating (HC) results in the maximum polyphenol oxidase (PPO, 60%) and respiration inhibition in fresh-cut apple slices, which lowers browning and improves quality while also letting down microbial growth and ensuring safety (Du *et al.*, 2024). Packaging properties were positively impacted by bioactive food packaging consisting of starch, citric pectin, and functionalised with Acca sellowiana waste by-product (feijoa peel flour, FPF) (Sganzerla *et al.*, 2020). Vanillic acid kills *E. coli* O157:H7 in unpasteurised apple juice in a concentration, pH, and time-dependent manner (Kwan *et al.*, 2006). To inactivate Listeria and maintain apple quality, phenolic branched-chain fatty acid (PBC-FA) may be applied as an antimicrobial coating (Ryu *et al.*, 2024). The primary drawback of curcumin, a naturally occurring antioxidant, is its poor solubility in water. In the Wu *et al.* (2018) investigation, β-cyclodextrin was used to encapsulate curcumin to create the βCD/CUR combination. GL-βCD-CUR films, which are gelatin films containing βCD/CUR complexes, were created. Red Fuji (*Malus pumila* mill) apple juice packed in GL-βCD-CUR films showed a gradual decrease in L values (Wu *et al.*, 2018).

CONCLUSION

Bio-preservation is an effective method for the preservation of apples and its products. In addition to this, they reduce the harmful effects caused by chemical preservatives when are used in food commodities. These studies prove that Bio-preservation an effective means for increasing the shelf-life of apples.

Conflict of interest - The authors have no conflicts of interest to declare.

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