Middle East Journal of Applied Sciences
Volume: 14 | Issue: 04| Oct.– Dec. | 2024

EISSN: 2706 -7947 ISSN: 2077- 4613
DOI: 10.36632/mejas/2024.14.4.48
Journal homepage: www.curresweb.com
Pages: 671-689

# Edible Coating for Shelf-Life Enhancement and Deterioration Prevention of Fresh-Cut Apple

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**Received:** 11 August 2024 **Accepted:** 10 Oct. 2024 **Published:** 30 Oct. 2024

## ABSTRACT

Bay leaf (*Laurus nobilis*) has been used for many years as a food flavoring and in traditional medicine due to its antidiabetic, anti-diarrhea, antiviral, and anti-inflammatory properties. This work's objective was to assess the impact of bay leaf extract (BLE) at different concentrations (0, 4, 8, 12, and 15 mg/g) on the properties of edible coatings made of whey protein isolate (WPI) for shelf-life extension of apple slices. Fresh apple slice quality parameter such as weight loss, browning index, pH, and texture profile were evaluated. Antioxidant activity (Total phenolic content, total flavonoid content, and radical scavenging activity) and antimicrobial activity against different microbes (*Salmonella Typhamirum, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Candida albicans, and Aspergillus flavus*) of BLE were investigated. Also, shelf life was studied by measuring antioxidant properties and also through microbial analysis for coated apple slices during 15 days of cold storage at 4°C. The results indicate that the quality properties of fresh-cut apple slices were improved by the addition of BLE. The BLE showed higher antioxidant properties such as total phenolic content (105.59 mg GAE/g) and free radical scavenger (67.13%). The addition of BLE especially in high concentrations of whey protein improved the antioxidant properties of fresh apple slices during storage periods. Adding BLE to coating solution could be a promising novel and effective natural antimicrobial agent ($p< 0.05$) when used concentration up to 12 mg/g.

***Keywords:*** Bay leaves, edible coating, antioxidant, antimicrobial, shelf life, fresh apple slices

## 1. Introduction

Fruits and vegetables are prone to microbial growth, which results in a short shelf life, due to their high metabolic activity. Food manufacturing has used many preservation techniques over the year to extend the shelf life and preserve the freshness of perishable foods such as fruits and vegetables (Kumar *et al.,* 2023). The uncontrolled use of food packaging obtained from petroleum-based plastics has created a serious environmental problem (Ludwicka *et al.,* 2020; Motelica *et al.,* 2020). Therefore, the food packaging industry needs to shift towards the development packaging from biodegradable natural polymers. The incorporation of bioactive plant extracts rich in antioxidants with the film material to create edible coating materials has been proposed (Flórez *et al.,* 2022).

Edible films and coatings are not intended to replace artificial packaging materials but rather to lower the price and volume of current packaging (Esmaeili *et al.,* 2020; Lopez-Polo *et al.,* 2023; Wang *et al.,* 2015). Natural, healthy and convenient to prepare food that is free from artificial additives is prioritized by customers, including fresh-cut, packed, and ready-to-eat products. Therefore, natural biodegradable films containing bioactive substances like phenolics and flavonoids can be used to achieve this goal (Díaz-Montes and Castro-Muñoz 2021; Čulina *et al.,* 2021) Additionally, films that have been supplemented with plant extracts can also serve as a vehicle for other functional ingredients such as anti-browning, antibacterial, and texture-enhancing compounds to further enhance shelf life (Al-Moghazy *et al.,* 2023; Kumar *et al.,* 2018).

*Laurus nobilis*, commonly referred to as bay laurel, is an aromatic evergreen tree or large shrub that is native to the Mediterranean region. Research on ethnobotany and historical sources have documented

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its use in treating a variety of diseases, from inflammation and infections to dyspepsia and arthritis. This wide range of applications is believed to be caused by the presence of a unique combination of bioactive compounds (Bektaş *et al.,* 2023; Khodja *et al.,* 2023; El Faqer *et al.,* 2022). Bay leaves are an important component of many industrial applications, including those in food, cosmetics, and pharmaceuticals. Several biological activities, including those related to healing wounds, antioxidants, antivirals, antibacteria, immunostimulants, anticholinergic, antifungal, insect repellent, anticonvulsant, antimutagenic, analgesic, and anti-inflammatory properties, are found in bay leaf (Batool *et al.,* 2020).

Milk protein constitutes 80% casein and 20% whey protein, which have further been characterized into different components. Milk proteins, either in total or their components, can be used for the formation of edible food coatings. Several studies were used whey protein as edible food coatings (Table 1). Whey proteins (WP) have been employed in the food industry due their high nutritional value as well as gelling, emulsifying, and foaming properties (Asdagh *et al.,* 2021). Additionally, they have bioactive peptides that have biological effects like immunomodulation, anticancer, hypocholesterolemic, antioxidant, and antibacterial activity (Vasconcelos *et al.,* 2021). WP edible films have sufficient mechanical, sensory, and visual qualities as well as moisture and oxygen permeability resistance (Tulipano, 2020). Milk proteins (whey, casein, and caseinates) have proven to be excellent components in edible coating preparation (Shendurse *et al.,* 2018).

**Table 1:** Summarizing recent studies on alternative whey protein isolate-based coatings and novel methodologies.

| Composition of coatings/films | Edible coating/ films | Application | References |
|---|---|---|---|
| **WPI + pullulan (WPI–Pul)** | Coating | fresh roasted chestnuts | Gounga, *et al.,* (2008). |
| **WPI + glycerol + antimicrobial compounds {lactic acid, natamycin, or chitooligosaccharides (COS)}** | Coatings | Cheese | Ramos, *et al.,* (2012). |
| **Whey protein and WPI** | Films | Potato pellets chips | Angor, *et al.,* (2014). |
| **WPI + essential oils (lemon and lemongrass)** | Coatings | Fresh cut pears | Galus, *et al.,* (2021). |
| **WPI + jojoba oil** | Coatings | Fresh-cut root parsley | Galus, *et al.,* (2022). |
| **Clove oil, glycerol monosterate, xanthan gum, and whey protein isolate** | Coatings | Tomatoes | Kumar and Saini (2021). |

Whey protein isolate (WPI) and whey protein concentrate (WPC) are the two major forms of whey protein involved in the formation of edible films and coatings. An edible whey film is a dry, highly interacting polymer network with a three-dimensional gel-type structure. Films/coatings made from whey proteins are colorless, odorless, flexible, and transparent with outstanding mechanical and barrier properties compared to polysaccharides and other-protein polymers (Kandasamy *et al.,* 2021 and Poonia and Petkoska 2023).

This study aimed to determine the impact of bay leaf extract (BLE) addition as a natural antioxidant to whey protein isolate (WPI) edible coatings on the quality, antioxidant properties, antimicrobial activity, and shelf life of fresh-cut apple slices during 15 days of cold storage at 4 °C.

## 2. Materials and Methods

### 2.1. Materials and chemicals

Samples of red apples were purchased from the local market in Giza, Egypt (January 2023). Apples were sorted for uniformity in size, color, and the absence of damages or fungus. Bay leaves were provided by an Egyptian farm 6 October city, Egypt January 2023. Folin-Ciocalteau reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid, and sodium carbonate were purchased from Sigma-Aldrich (Steinhein, Germany). All other reagents used in HPLC analysis were of HPLC grade.

**2.2. Source of microbial strains**

The bacteria used in the antimicrobial evaluation were *Listeria monocytogenes* ATCC 5980, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* B-3711, *Aspergillus flavus* ATCC 9643, and *Candida albicans* ATCC 10231, which were all obtained from the Dairy Department (Microbiological lab, National Research Centre, Giza, Egypt).

**2.3. Methods**

**2.3.1. Preparation and Ultrasound-assisted extraction of Bay leaves**

Bay leaves (BLs, 500 g) were dried in an oven at 40°C for 8 h followed by grinding using a laboratory mixer MIENTA super blender (Model BL-721) and then stored in polyethylene bags at -18°C until analysis. Bay leaf (BL) powder was extracted at a ratio of 1:15 with ethanol (70%), using Ultrasonic apparatus (Sonics VCX-750 Vibra-Cell Ultrasonic USA). After that, the extract from the BLs was collected, and the procedure was repeated twice. After filtration, the extracted material was centrifuged (SIGMA 3-18ks Laborzentrifugen, Germany) at 4,000 rpm for 10 min. Using a rotary evaporator (Heidolph Instruments GmbH & Co., Bacic Hei VAP-HL Shwabach, Germany), the supernatant was concentrated under vacuum at 40 °C. Lastly, until analyzed, the crude extract from BLs was stored at -18°C.

**2.3.2. Phytochemical screening and biological activities of bay leaves extract (BLE)**
**Screening and quantifying phynolic and flavonoid compounds**

Phenolic and flavonoid compounds in BLE were identified by HPLC at network Chromatography Central Lab, National Research Centre in Cairo, Egypt, estimated as µg/g. Chromatographic analysis was carried out using the Agilent 1260 series (Agilent Technologies/Hewlett Packard, Wald bronn, Germany). The analysis was carried-out using a on a C 18: Shodex Colum (250mm x 4.6 mm). Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which had a flow rate of 0.90 ml/min. The linear gradient was sequentially programmed into the mobile phase as follows: 0 minutes (82% A), 0-5 min (80% A), 5-8 min (60% A), 8 min (12% A), 12 min (15% A), 15 min (16% A), and 16 min (20% A). At 280 nm, the multi-wavelength detector was observed. For every one of the sample solutions, 5 µL of injection volume was used. The column temperature was kept at 40°C. The analytes were identified by comparing the retention times and spike samples with polyphenol standards with subsequent quantification of phenolic and flavonoid compounds.

**2.3.3. Antioxidant activity of bay leaves and coating apple slices extract**

The total phenolic content was determined by the Folin-Ciocalteu method according to Boukroufa *et al.,* (2015). The total flavonoid content was quantified using the aluminum chloride method according to Khatiwora *et al.,* (2010), and the antioxidant activity was examined using the DPPH assay according to Brand-Williams *et al.*, (1995).

**2.3.4. Preparation and characterization of WP-BLE Coating solution**

Under constant stirring, distilled water was used to dissolve WPI (8% w/w). The WPI solution was then heated in a water bath for 30 minutes at 90 °C while being stirred until complete dissolution to improve the flexibility of the WPI-Coating formulation, glycerol (10% w/w) of the biopolymer was added and stirred for 30 min. BLE was incorporated into the WPI solution at varying concentrations of 0, 4, 8, 12, and 15 mg/ g of WPI.

**2.3.5. Antimicrobial activities of bay leaves extract (BLE) and WPI/BLE coating solutions**

The most contaminating microbes in foods were used for the evaluation of the antimicrobial activity of BLE and WPI/ BLE coating solutions. An aliquot of 20 µL of each overnight prepared microbial culture suspension (*S. Typhamirum, E. coli, S. aureus, B. cereus, L. monocytogenes, C. albicans, and A.flavus*) were spread on nutrient agar culture medium plates using sterile cotton swabs for bacteria, and using malt extract agar culture medium plates for yeast and fungi. All media plates were rested for 15 min to saturate with pathogen strains. After that, a cork borer of 6 mm was used to cut the agar media and performed different wells in agar. Different wells were filled with 100 µL BLE (100 mg/ g dissolved by DMSO) and different WPI/BLE coating solution concentrations (0, 4, 8, 12, and 15 mg /g of WPI).

The plates were incubated for 18 h at 37°C for bacteria and 48 h at 25°C for yeast and fungi. The appearance of a clear inhibition zone and its diameter were recorded after incubation time (El-Sayed and El-Sayed, 2021).

### 2.3.6. Food application for WPI/ BLE coating solution

The apples were chosen for their uniform hue (red epidermis) and lack of flaws. Five kilograms of apples were rinsed in potable water. Then, apples were sanitized in cold water (10°C) with sodium hypochlorite solution 100 mg/L for 20 min, to prevent contamination during the process. The apples were manually cut into slices of about 5 mm thickness using a vegetable and fruit slicer, and then immediately submerged in a solution of 50 mg/L hypochlorite at 4°C for 10 min. Apple slices were dipped in the WPI/BLE coating solution after the excess liquid was drained on absorbent paper. Control Samples of apple slices were interspersed in distilled water without being coated and samples were air dried at 25°C for 3h.

For the storage period, 100 g of each apple slice treatment was packaged in trays and stored at 4°C, 90% RH. Fresh apple slices' physical, chemical, and microbiological qualities were assessed after 0, 3, 7, 12, and 15 days of storage (Fig. 1) (Duong *et al.,* 2023). Six fresh apple slice trials were conducted on a laboratory scale: uncoated (AU), coated with WPI alone (AWPI), and coated with WPI/BLE at various doses (4, 8, 12, and 15 mg/g), as indicated by the codes A1, A2, A3, and A4, respectively.

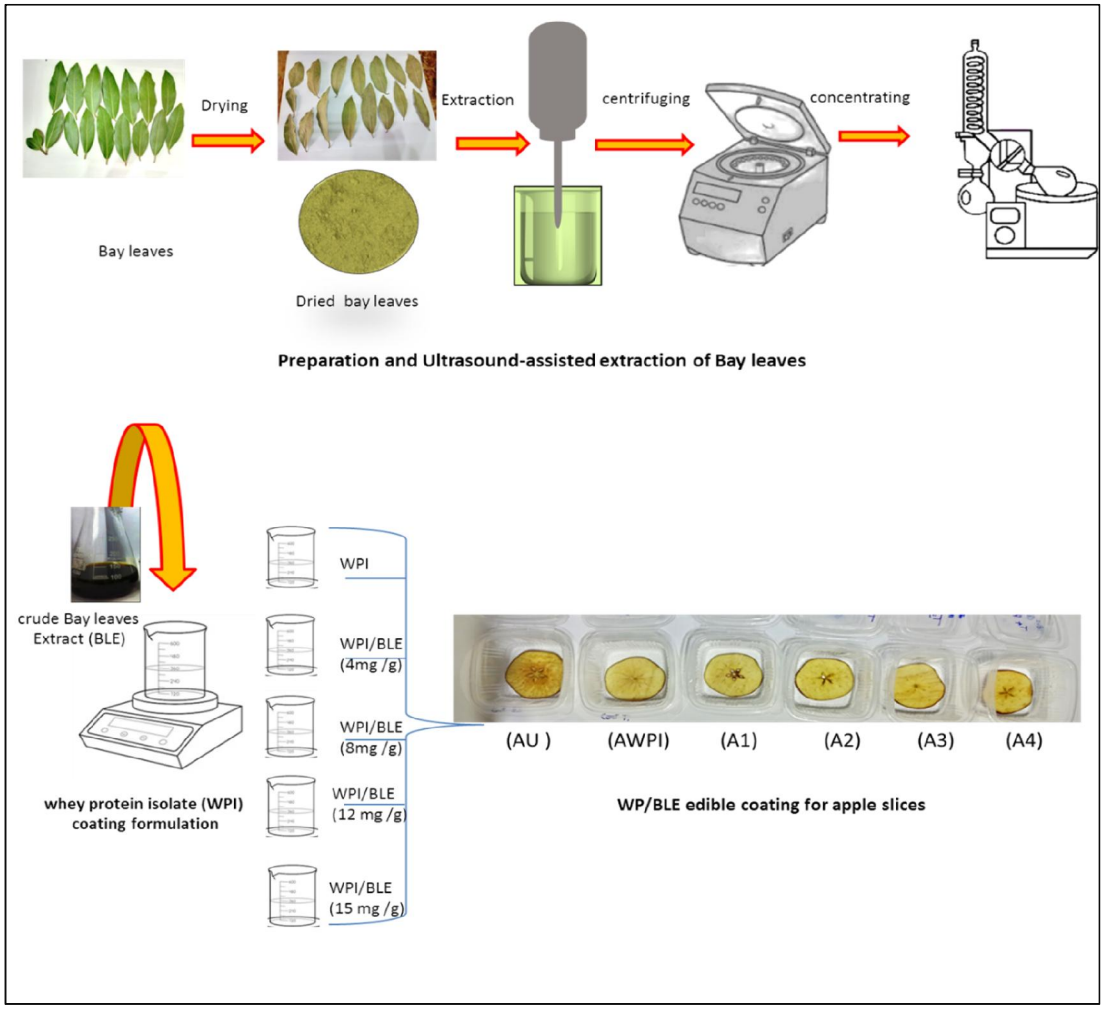



**Fig. 1:** Schematic application of Whey protein isolate (WPI) edible coating for apple slices with bay leaf extract (BLE); Apple uncoated (AU), Apple slices coated with WPI (AWPI), and apple slices coated with WPI/BLE at different ratios (A1, A2, A3, and A4) during storage period at 4±1 °C.

### 2.4. Fruit quality and shelf life

#### 2.4.1. Weight loss of coated apple slices

The weight loss during storage was determined by calculating the difference between the initial and final weights of the apple slices outside the storage bag (Ruelas-Chacon *et al.,* 2017).. The value was expressed as a relative percentage and calculated as follows:

Weight loss (%) = (wi − wt)/wi x 100
Where wi is the initial weight and wt is the weight measured during storage.

#### 2.4.2. Browning Index and pH of coated apple slices

The Browning Index (BI) and pH for coated apple slice samples were estimated at different storage periods. The BI was determined according to (Cohen *et al.,* 1998). The pH of samples was measured according to method 943.02 using a pH meter (Adwa, Ad1000&temereture meter, Romania, Europe) (AOAC, 2007).

#### 2.4.3. Texture analysis of coated apple slices

Apple slice samples were subjected to texture profile analysis (TPA). Using a texture analyzer (Texture Pro-CT V1.6 Build, Brookfield, Engineering Labs, Inc.) with TA-MTP 4 R probe, 50% compression, and a test speed of 1.0 mm/s. Consistency, hardness, cohesion, adhesiveness, springiness, gumminess, and chewiness were among the texture characteristics measured; the texture parameter of samples of apples was averaged from three replications (Bourne, 2002).

#### 2.4.4. Microbial Analysis of BLE and coated apple slices

Briefly, 10 g of each sample was mixed with 90 mL of sterilized distilled saline solution (0.9 g NaCl/100 mL) in a 100 mL Erlenmeyer flask and followed by 10-fold serial dilution. One mL of each of the appropriate serial dilutions was plated by standard microbiological media to count different microbes as follows; the plate count agar medium was used for determined aerobic total bacterial counts and the plates's incubated at 37 °C for 48 h (Wehr *et al.,* 2004). The plate of violet red bile glucose agar medium was used to enumerate coliforms with incubation at 37 °C for 24 h (Omar and Wilson, 2002). The *E. coli* counts were detected by EMB (eosin methylene blue agar). *E. coli* appear on EMB agar as (a blue-black colony with a greenish metallic sheen) incubated at 37 °C for 24 h (Wehr *et al.,* 2004). The psychrotrophic bacteria were detected by plate count agar medium with incubation at 5 °C for 7 days (Omar and Wilson, 2002). Mold and yeast counts were assessed by spreading 0.1 ml of a suitable dilution on rose bengal chloramphenicol agar plates with aerobic incubation at 25 °C for 4 days (Bengal, 1995). The results were expressed as log total microbial counts of colonies per dilution (log CFU/mL). All microbiological determinations were performed in triplicate.

### 2.5. Statistical Analysis

Statistical analysis (Means ± SD) of three replicates for each experiment are statistically analyzed using one-way analysis of variance (ANOVA), $P<0.05$ was used to indicate significance. Statistical software (Assist at Version 7.7, Brazil) was used for all statistical analyses (Silva & Azevedo, 2009).

## 3. Results and Discussion

### 3.1. Screening and identification phytochemical: profiling of phenolic and flavonoid compounds of bay leaves extract

Phytochemical profiling of ethanolic bay leaves extract by HPLC analysis is presented in Table 2. As can be observed, catechin was a dominant component detected (1458.06 mg/g). Other significant components of the investigated phenolic acid are methyl gallate (984.87 mg/g) and ellagic acid (874.34mg/g). The three most prevalent phenolic compounds identified in bay leaves are gallic acid (388.79 mg/g) and chlorogenic acid (556.98 mg/g). This result agreed with those found by (Dall'Acqua *et al.,* 2006; Lu *et al.,* 2011 and Awada *et al.,* 2023).. Flavonoids such as quercetin 82.76 (µg/g), naringenin (820.11 µg/g), vanillin (28.62 µg/g), kaempferol (14.61 µg/g) and hesperetin (27.85 µg/g) were also found. High levels of antioxidant activity were previously shown by Bennett *et al.* (2013), who also found that the glycosides based on kaempferol and quercetin dominated the profile of the bay leaf extract found by HPLC chromatography. The hydroxyl groups attached to the ring structure of

flavonoids are responsible for their antioxidant properties; these groups also serve as reducing agents, hydrogen donors, metal chelators, and radical scavengers Carocho & Ferreira (2013).

**Table 2:** Phytochemical profiling of ethanolic bay leaves extract by HPLC analysis

| Components | Conc. (µg/g) |
|---|---|
| **Gallic acid** | 388.79 |
| **Chlorogenic acid** | 556.98 |
| **Catechin** | 1458.06 |
| **Methyl gallate** | 984.87 |
| **Pyro catechol** | 173.21 |
| **Rutin** | 274.30 |
| **Ellagic acid** | 874.34 |
| **Coumaric acid** | 30.09 |
| **Vanillin** | 28.62 |
| **Ferulic acid** | 147.89 |
| **Naringenin** | 820.11 |
| **Daidzein** | 8.63 |
| **Querectin** | 82.76 |
| **Cinnamic acid** | 5.25 |
| **Kaempferol** | 14.61 |
| **Hesperetin** | 27.85 |

### 3.2. Antioxidant activity of bay leaf extract

Total phenolic content (TPC), total flavonoid (TFC), and free radical savaging activity (RSA/DPPH) for BLE are shown in Figure. 2. The obtained data revealed that the TPC and TFC for leaves contained 105.59 (mg GAE/g) and 51.77 mg/g (mg catechin/g), respectively. On the other hand, the BLE found high antiradical activity was assayed by DPPH (67.13%), The high antiradical activity may be due to phenolic and flavonoid compounds that have been previously identified and quantified. The increased radical-scavenging activity of the separated compounds was explained by the enhanced H-donating capacity of the free hydroxyl groups. Bay leaves could be regarded as an excellent source of phenolic antioxidants because of the significance of their bioactive chemicals (Khodja *et al.,* 2022).

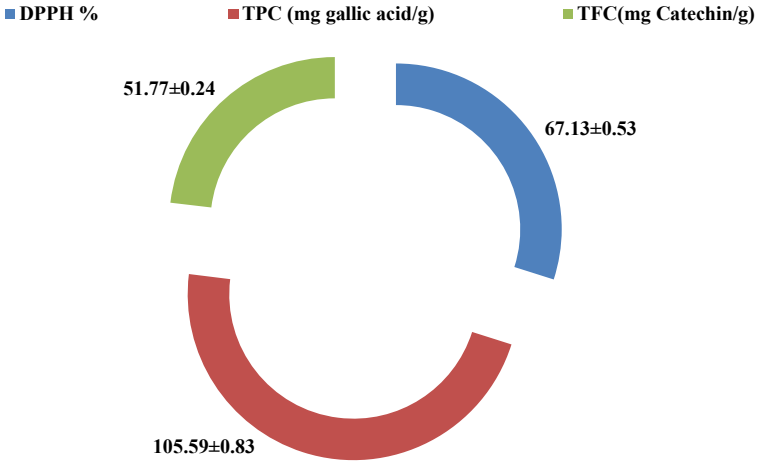



**Fig. 2:** Total phenolic content (TPC) total flavonoid (TFC) and free radical scavenging activity (DPPH %) of bay leaf extract (BLE).

### 3.3. Antimicrobial activity of bay leaf extract and coating solution

The results of the antimicrobial activity of coating solution concentration are presented in Table (3). The data indicated antimicrobial action against different microbes at the concentration 12 mg/g of WPI/BLE coating solution, in which the diameter of the inhibition zone ranged between 3 to 10 mm. However, the low concentration of WPI/BLE coating solution did not donate antimicrobial activities

against tested microbes. The antimicrobial action was enhanced at the concentration 15 mg/g, which is indicated by the inhibition zone diameter that increased to record between 5 and 15 mm. In addition, the BLE at the concentration of 100 mg/g had inhibition action ranging between 11 and 22 mm depending on the microbial strain. So, adding BLE as a natural antimicrobial agent to the coating solution was effective but in a high concentration of up to 12 mg/g. These results may be related to the WPI solution having active sites that bind to phenols and other active ingredients and slow down their release which is responsible for antimicrobial activities (Tenney *et al.,* 2017). Moreover, WPI managed the active extract's release into the food at a slow diffusion rate. These characteristics assisted in maintaining the active extract concentration at the necessary level throughout the various foods' storage periods (Kristo *et al.,* 2008). Additionally, BLE had antimicrobial agents related to the presence of phytochemical compounds evaluated by HPLC (as previously mentioned in 3.1) and confirmed by other studies (Siriken *et al.,* 2018; Batool *et al*., 2020; Sharma *et al.*, 2021).

**Table 3:** Antibacterial and antifungal activity of BLE and different coating solutions

| Microbial strains | 100 mg/ mL BLE | WPI coating solution | A1 | A2 | A3 | A4 |
|---|---|---|---|---|---|---|
| | | | Diameter of inhibition zone (mm) | | | |
| ***S. Typhamirum*** | $11^{Ea}$±0.05 | N.D | N.D | N.D | $3^{Cc}$±0.06 | $5^{Db}$±0.06 |
| ***E. coli*** | $15^{Ca}$±0.11 | N.D | N.D | N.D | $8^{ABc}$±0.05 | $10^{Bb}$±0.11 |
| ***S. aureus*** | $20^{Aa}$±0.10 | N.D | N.D | N.D | $9^{Ac}$±0.10 | $15^{Ab}$±0.17 |
| ***B. cereus*** | $18^{Ba}$±0.18 | N.D | N.D | N.D | $10^{Ac}$±0.15 | $13^{ABb}$±0.10 |
| ***L. monocytogenes*** | $10^{Ea}$±0.08 | N.D | N.D | N.D | $5^{BC}$±0.06 | $7^{Cb}$±0.09 |
| ***C. albicans*** | $22^{Aa}$±0.20 | N.D | N.D | N.D | $8^{ABc}$±0.10 | $13^{ABb}$±0.10 |
| ***A. flavus*** | $14^{Da}$±0.28 | N.D | N.D | N.D | $5^{Bc}$±0.07 | $10^{Bb}$±0.15 |

-N.D (not detected). n=3
-Means within the same row (lowercase letters) and column (uppercase letters), for each microorganism, with the same letter, do not statistically differ from each other (P > 0.05) ±SD. n=3
- Whey protein isolate (WPI), bay leaf extract (BLE) at level: 4 (A1), 8 (A2), 12 (A3) and 15 (A4) mg/g WPI/BL

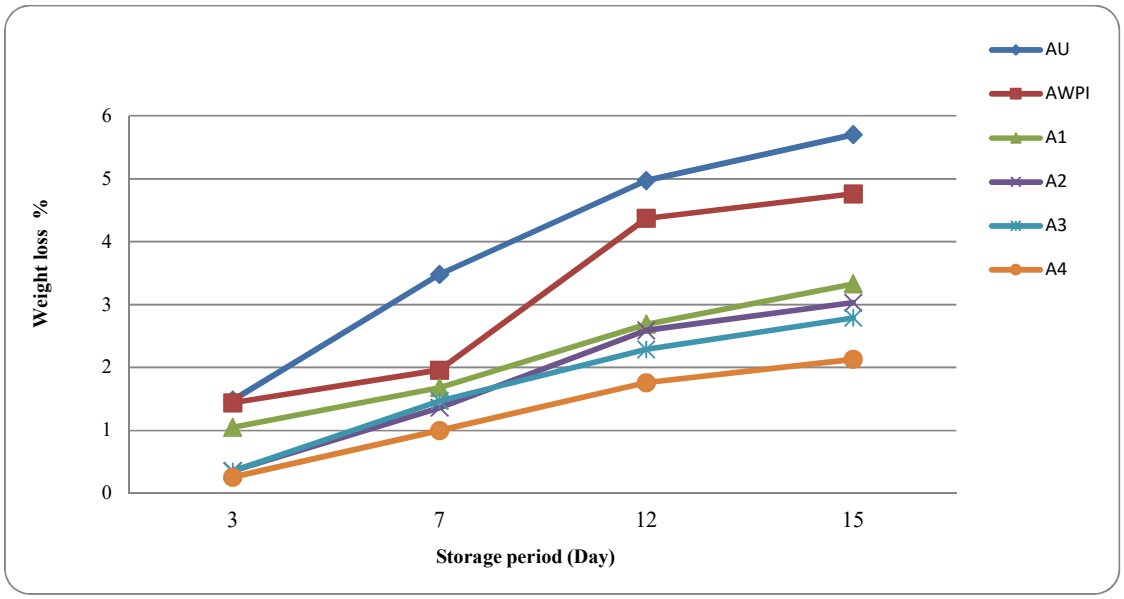
### 3.4. Fruit quality and shelf life

#### 3.4.1. Weight loss of coated apple slice samples

Weight loss is considered an important factor that involves quality of the fruits. It is an indicator of freshness and mostly increases during storage of the minimally fresh-cut fruits produced as mentioned by Antunes *et al.* (2010) and Rincon *et al.* (2019). The weight losses of non-coated and coated fresh-cut apples during 15 days of storage at 4 ±1 °C were compared. The obtained results are clearly illustrated in Fig. (3).

Results indicated that both A3 and A4 reduced the rate of weight loss of the sliced apples to a greater extent compared to AU, AWP1, and other samples after 15 days of storage. This is due to the porosity of the BLE-coated apple slices decreased within 15 days of storage. The variance in the porosity of apple slices may be due to the binding of BLE molecules to the surface of the apple slices. These findings agree with those of Ahmad *et al.* (2021). Who reported that edible coating can be a good barrier for fresh-cut fruit during storage periods and extend their shelf life.

Also, as mentioned by Saberi *et al*. (2018), the lowest weight loss was obtained in samples treated with coating solutions containing whey protein which could be also related to their lower water activity and thus maintained stability of coatings with the lowest decadence for fruits. Also, Eom *et al.* (2018) state that, the structure and composition of the film affect the moisture loss rates of the fruits, due to the closed structures possessing strong interactions between polymeric molecules, which limits moisture loss.




Samples of uncoated (AU) and coated apple slices with whey protein (WPI) plus bay leaf extract (BLE)at level: 4 (A1), 8 (A2), 12 (A3) and 15 (A4) mg/g WPI/BLE.

**Fig. 3:** Effect of storage period for 15 days at 4±1°C on weight loss of apple slices before and after coating with edible whey protein plus leaf extract at different level (0, 4, 8, 12 and 15 mg/g).

### 3.4.2. Browning Index (BI) of coating apples slices

When slicing apples, tissue cells during apple processing and storage are damaged, and color development and browning appear as the action of polyphenol oxidase (enzymatic browning) Rrocha and Morais (2001) revealed that during the first several days of storage, apple cv. Jonagold observed significant color changes, probably as a result of enzymatic browning caused by tissue degradation and the increased interaction between enzymes and substrates.

This phenomenon can be delayed by applying coatings with solutions containing high concentrations of antioxidants. The browning index of uncoated apple slices (AU), coated with WPI only, and coated with WPI/BLE at various ratios during storage days 7 to 15 at 4 °C is shown in Figures (4 and 5).

In contrast to all coated slices, the browning index increased significantly in all samples. The (BI) of apple slices steadily increased in AU ($p > 0.05$). Throughout the 15-day research, there were no discernible differences between the AWP, A1 samples, and the A2 and A3 samples in terms of BI values ($p > 0.05$). Compared to remaining uncoated and coated samples, A4 showed less development in the brown index over the days of storage. This outcome was consistent with research by Kumar *et al.* (2018) that demonstrated edible coatings reduced the enzymatic reactions of coated apple slices and therefore, changes in the browning index. According to Perez-Gago *et al.,* (2006), while employing composite edible coatings made with whey protein isolate (WPI) and whey protein concentrate (WPC) as coatings, apple slices during a 7-day storage time at 5 ºC.

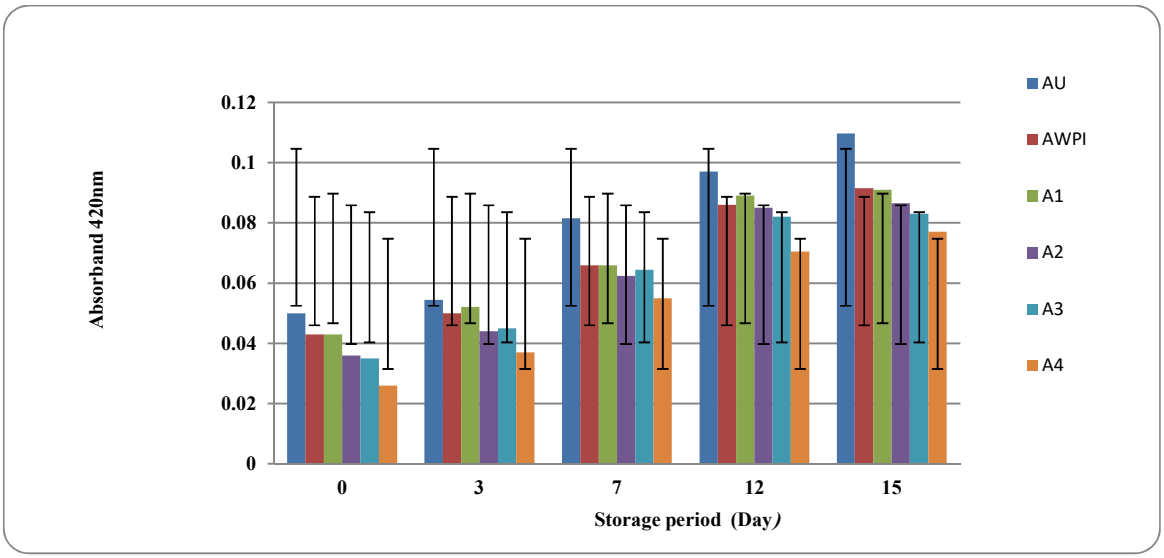



**Fig. 4:** Effect of storage period for 15 days at 4±1°C on browning index (BI) at absorbance 420 nm of apple slices before and after coating with edible whey protein plus leaf extract at different level (0, 4, 8, 12 and 15 mg/g).

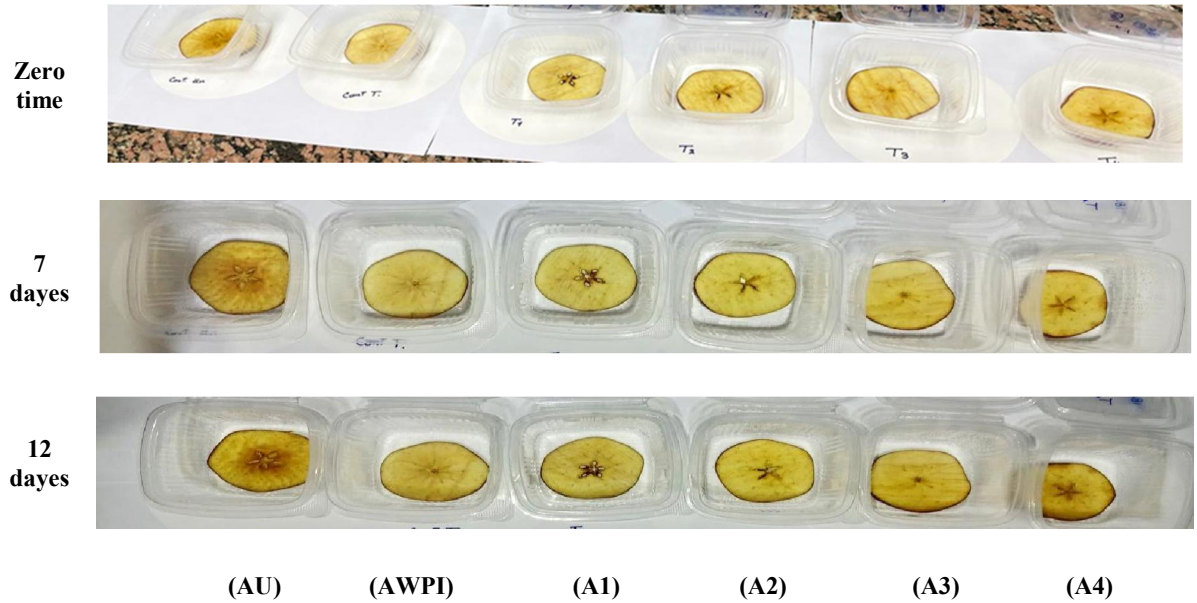



**Fig. 5:** Apple slices with before and after coating with whey protein plus bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1 °C.

### 3.4.3. pH of coated apple slices

Throughout the storage period, the pH of freshly cut apples treated with various concentrations of whey protein and the natural antioxidant in bay leaves was evaluated (Fig. 6). The pH values decreased significantly ($P<0.05$) for all the samples during storage. This percentage of decrease in pH for coated samples is higher than in control samples. Whereas, the percentage of decreases in the control samples were 3.96% and 4.06, while the coated samples were 3.25, 4.51, 4.18, and 4.21% respectively, during and until the end of the storage period. Lower changes in PH may be reflected in the reduced respiration rate (Rocha and Morais, 2003; Soares and Fonseca, 2008; Cofelice *et al.,* 2019). pH depends on the presence of acidic compounds. Acid content in fruits tends to decrease over time probably due to the organic acids oxidation which occurs with fruit ripening (Islam *et al.,* 2013), because the presence of

acidic chemicals affects pH. Fruits tend to lose acid with time, most likely as a result of organic acids oxidizing during fruit ripening (Suriati *et al*., 2020; Sikora and Świeca, 2018).

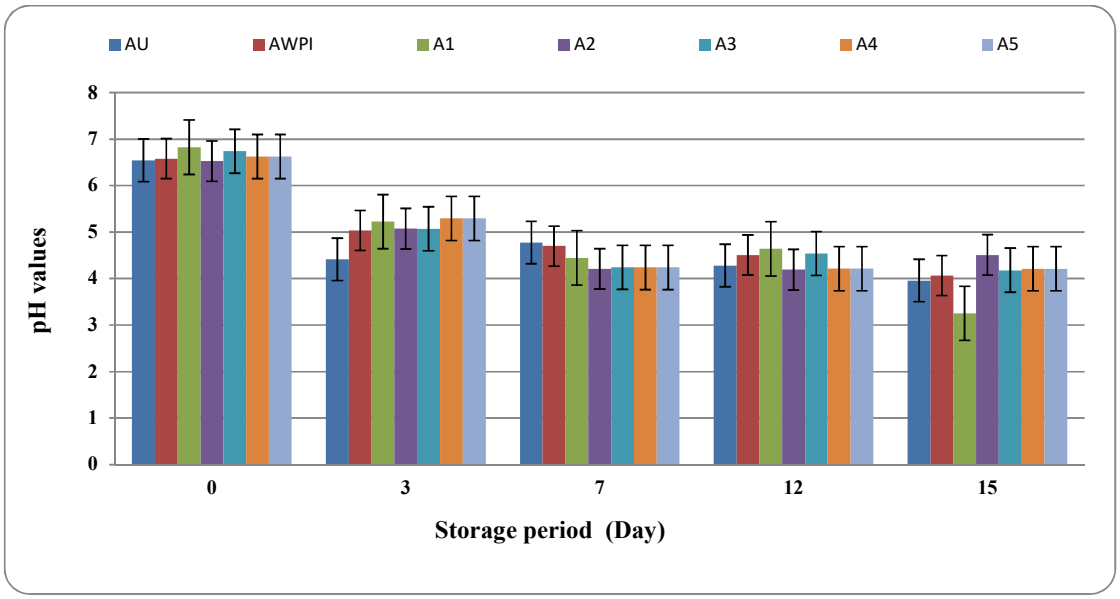



**Fig. 6:** pH of apple slices before and after coating with whey protein edible coatings with added bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1°C.

**3.4.3. Texture analysis of coated apple slices**

Texture has been utilized as a measure of fruit quality in both fresh and processed fruits. The consistency of fruits is determined by cellular structures, chemical components, moisture levels, and the composition of cell walls (Olivas *et al.,* 2003). The results for the different textural characteristics (chewiness, hardness, adhesiveness, fracturability, cohesiveness, springiness, and Gumminess) can be found in Table 4 regarding the rheological properties of apple slices. Apple slices showed increased hardness before and after the storage period, as per texture analysis. An increase in hardness was observed regardless during storage; it is thought that WPI prevented softening of the structure during storage (Mustafa *et al.,* 2018). Chewiness values increased during storage, probably due to the loss of water to a critical point at which a compact structure is formed, causing an increase in chewiness beyond this point.

Adhesiveness, which is easily observed with samples in the collected findings, is the propensity of the food to stick to the teeth as it is chewed. The amount of pectic materials coated with film at the surface of slices determines the cohesiveness, which showed stability throughout the storage period.

The higher cohesiveness of the A4 sample could be related to the adhesive nature of biopolymers (Dhanapal *et al.,* 2012). Cohesiveness during storage was observed in the samples due to the formation of a compact structure because of moisture loss.

It was noted from Table 4 that the majority of the apple samples showed higher values for springiness. According to the analysis, coated slice apple samples, especially A4, exhibited the highest degrees of hardness, chewability, and fractureability before and after storage. The stability of these properties is essential for coating and storage success.

**Table 4:** Texture analyses of apple slices with no coating or after coating with whey protein edible coatings with added bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1 °C)

| Treatments | Texture parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chewiness g.cm | Hardness N | Adhesiveness g.cm | Fracturability N | Cohesiveness | Springiness mm | Gumminess N |
| **At zero time** | | | | | | | |
| **AU** | 23.00 | 4.99 | 3.00 | 4.26 | 0.22 | 0.93 | 1.10 |
| **AWPI** | 17.33 | 6.06 | 3.60 | 4.91 | 0.24 | 1.04 | 1.61 |
| **A1** | 9.66 | 6.59 | 3.66 | 4.94 | 0.27 | 1.06 | 1.66 |
| **A2** | 15.66 | 6.91 | 3.66 | 5.11 | 0.27 | 1.18 | 1.87 |
| **A3** | 25.66 | 6.94 | 4.00 | 5.52 | 0.30 | 1.25 | 1.99 |
| **A4** | 31.66 | 7.75 | 4.20 | 5.69 | 0.30 | 1.32 | 2.33 |
| **After 15 days** | | | | | | | |
| **AU** | 14.00 | 5.96 | 1.00 | 5.10 | 0.23 | 0.95 | 1.27 |
| **AWPI** | 20.33 | 6.33 | 1.00 | 5.73 | 0.25 | 1.00 | 1.56 |
| **A1** | 20.00 | 7.17 | 1.00 | 6.00 | 0.28 | 1.01 | 1.64 |
| **A2** | 30.33 | 7.46 | 1.00 | 6.07 | 0.30 | 1.14 | 1.70 |
| **A3** | 29.00 | 7.98 | 1.00 | 7.17 | 0.30 | 1.21 | 1.88 |
| **A4** | 47.00 | 7.92 | 1.00 | 7.72 | 0.38 | 1.29 | 2.30 |

(AU)apple slices uncoated;(AWPI) Apple slices coated with WPI; (A1, A2, A3,and A4) apple slices coated with WPI/BLE at different ratios at zero and After 15 days of storage at 4±1°C.

### 3.5. Antioxidant Activity of coated apple slices during storage

#### 3.5.1. Total phenolic contents (TPC) of coated apple slices

The total phenolic compounds (TPC) of different apple slices during storage periods of 0-7-15 days at a temperature of 4±1 °C were measured. Uncoated apple slices (AU) showed a loss in the content of phenolic compounds, at about 8.67 %, after 7 days of storage, and the percentage of loss reached 27.39 % after the 15-day storage time. The decrease in TPC in the storage period might be due to the down of cell structure released phenolic to be exposure to enzymatic oxidation (Peña-Ortiz *et al.,* 2023). At the end of the storage period, the TPC loss of apple samples A3 and A4 was much lower than that of the control and AWP1 (Figure 7), at 8.82, 8.41, 27.39, and 17.54%, respectively. TPC loss during storage did not differ significantly ($p < 0.05$) between A1 and A2 samples (Figure 7). After 15 days of storage, the control sample had the highest TPC loss. The results showed that coating apple slices with bay leaf extract was the most effective way to prevent the loss of polyphenolic compounds from apple tissues. Even though the overall number of phenolic compounds in apple slices decreased gradually as they were stored. Because the edible coating containing BLE decreased the degradation of apple slices during storage.

#### 3.5.2. Total flavonoid contents TFC of coated apple slices

A similar trend in prevention loss of TFC in apple slices coated with bay leaf was observed in Figure 8. The apple slices coated with 15 mg/g bay leaf showed the least amount of loss in total flavonoid content at the end of storage, followed by A3 and A2 with losses of 5.83, 7.64, and 9.99%, respectively. The lowest TFC loss among coated samples with whey protein was observed for the AWP1 sample, then control 12.91 and 16.81%, respectively, at the end of the storage period. This result is in agreement with Awada *et al.* (2023).

#### 3.5.3. Free radical scavenging activity of coated apple slices

The free radical scavenging activity of apples, as measured by DPPH, showed a significant correlation between TPC and TFC concentration, and these findings imply that phenol compounds are reliable predictors of antioxidant activity.

The loss of DPPH radical-scavenging activity of cut apple samples (Figure 9) decreases gradually with the increasing concentration of bay leaf from 4 to 15 mg/g at storage periods. An increase in antioxidant activity (radical scavenging activity) was parallel to an increase in phenolics and flavonoid content in the sample (Tometri *et al.,* 2020).

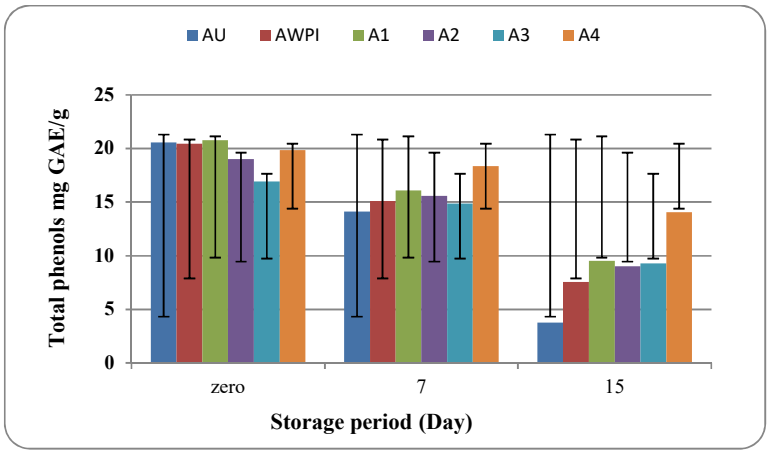



**Fig.7:** Effect of storage periods at 4±1°C on Total phenolic (mg GAE/g) of apple slices before and after coating with whey protein and different level of bay leaf extract at (0, 4, 8, 12, and 15 mg/g).

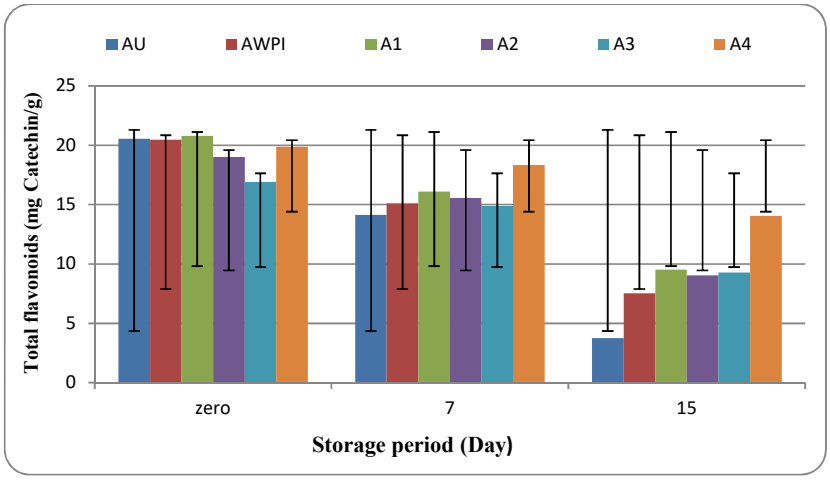



**Fig. 8:** Total flavonoids (mg catechin/g) of apple slices with no coating or after coating with whey protein edible coatings with added bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1°C.

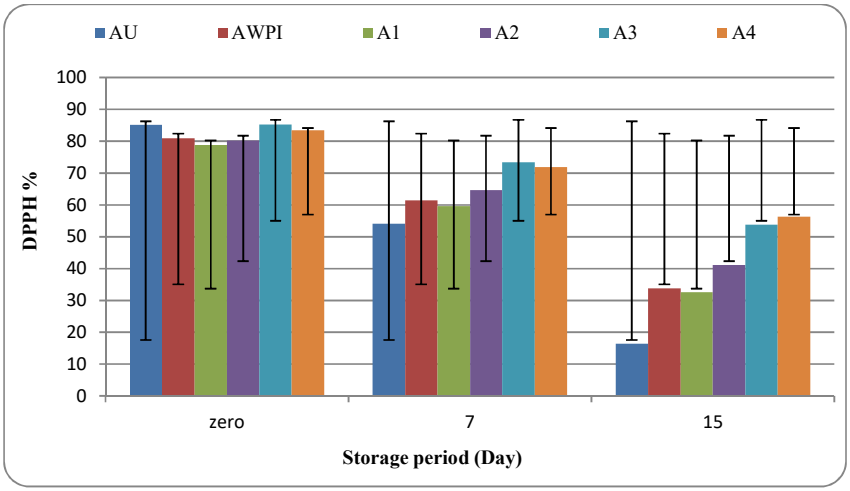



**Fig. 9:** DPPH% of apple slices with no coating or after coating with whey protein edible coatings with added bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1°C.

Due to the hydroxyl groups attached to their ring structure, which also operate as reducing agents, hydrogen donors, metal chelators, and radical scavengers, flavonoids have antioxidant properties.

The findings demonstrated that the addition of whey protein and 15 mg/g of bay leaf extract, which functions as an antioxidant, prevented the migration of phenolic compounds from coated films into the fruit's surface and loss of TPC, TFC, and DPPH (Pająk *et al.,* 2017). The reduction of radical scavenging activity during storage may be related to oxygen-catalyzed reactions and browning reactions. Marquez *et al.* (2017) showed that coating with WPI decreases the loss of radical scavenging activity and total phenolic content in fresh-cut fruits.

**3.5.4. Microbial Analysis of coated apple slices**

The results of observing the number of apple slices total coliform are given in **(**Table 5). In the first 3 days of storage, the analysis did not detect coliform count. During storage time, the coliforms were enumerated in all apple slice samples at 7 days of storage, but the more enumerated counts were detected in the AU sample followed by the AWPI sample. The other coated samples (A1, A2, and A3) had coliform counts ranging between 1.00 and 1.10 log CFU/g without significant differences between them at 7 days. Moreover, the samples coated with WPI/BLE (A4) did not detect coliform during the storage period and little count was recorded at 15 days of storage. So, the results found the samples coated with WPI/BLE A3 and A4 were the most suitable coating formula to reduce the coliform amplification than others.

The *E. coli* counts on the first day were not detected and were gradually observed during the storage period in uncoated firstly on the third day of storage with a count of 1.90 log CFU/g, but in coated samples with AWPI, A1, and A2 were detected at day 12 of storage with count 2.44, 2.17 and 1.59 log CFU/g, respectively. In addition, the *E. coli* count was detected in the A3 sample only at day 15 of storage with a count of 1.88 log CFU/g but did not detect *E. coli* count for apple slice samples coated with WPI/BLE (A4) during storage time. Therefore, the WPI/BLE 15mg/g was the suitable coating formula to control the *E. coli* progress.

The results of aerobic bacterial counts during storage are illustrated in Table (5). The results showed that the initial counts of aerobic bacteria in all samples ranged between 3.80 and 1.98 log CFU/g and significantly enhanced with the storage time, especially for AU and AWPI samples. The results showed that adding 15mg/g BLE to WPI coating had a significant antimicrobial effect, and the counts in these samples during the storage period remained in the same log cycles until the end of storage time (the initial count of 1.98 and at the end of storage reached 1.59 log CFU/g)., at the end of storage, the aerobic bacteria counts were recorded at 3.40, 3.90, and 3.20 log CFU/g for A1, A2, and A3, respectively without significant differences. So, the addition of BLE with a concentration of up to 12 mg/g can maintain the shelf life of apple slices.

On the other hand, mold and yeast counts were detected during cold storage in the same table. In fresh, all samples are free from mold and yeast counts. After that, small counts in AU and AWPI samples were recorded at 1.94 and 2.13 log CFU/g, respectively, and increased to reach 5.75 and 4.29 log CFU/g at 12 days, respectively. Also, noticed small counts in other samples coated with WPI/BLE (4, 8, and 12 mg/g) at 7 days, which counts recorded 1.50, 2.00, and 1.30 log CFU/g, respectively, and increased with the time to recorded 3.00, 2.90 and 2.70 log CFU/g for A1, A2, and A3, respectively at the end of storage (day 15). Also, the apple slice samples coated with WPI/BLE 15mg/g (A4) had less mold, and yeast counts reached 1.24 log CFU/g at the end of storage. So, the addition of BLE with a concentration of up to 8 mg/g can reduce the mold and yeast counts progress during storage than others.

Additionally, the psychrophilic counts were recognized during storage in Table (5). The initial psychrophilic counts of samples were detected first in the AU sample on day three of storage with a count of 3.00 log CFU/g and increased in samples with time. The lowest number of psychotropics at the end of storage was observed in the sample coated with WPI/BLE 15 mg/g (A4). The data did not observe significant differences between samples A1, A2, and A3, which the psychrophilic counts. So, the coating of the sample with BLE up to 12mg/g had positive effects on apple slices due to the antimicrobial effect.

**Table 5:** Microbial Analysis of apple slices with no coating or after coating with whey protein edible coatings with added bay leaf extract (0, 4, 8, 12, and 15 mg/g) after 15-days of storage at 4±1°C).

| Variants (log CFU/g) | Storage days | Apple slices samples | | | | | |
|---|---|---|---|---|---|---|---|
| | | **AU** | **AWPI** | **A1** | **A2** | **A3** | **A4** |
| **Total coliform groups** | **0** | N.D | N.D | N.D | N.D | N.D | N.D |
| | **3** | N.D | N.D | N.D | N.D | N.D | N.D |
| | **7** | $2.10^{Ca}$±0.07 | $2.00^{Ba}$±0.09 | $1.00^{Cb}$±0.10 | $1.10^{Bb}$±0.11 | $1.00^{Bb}$±0.10 | N.D |
| | **12** | $3.29^{Ba}$±0.09 | $2.30^{Bb}$±0.06 | $2.80^{Bb}$±0.05 | $2.00^{Ab}$±0.05 | $2.10^{Ab}$±0.06 | N.D |
| | **15** | $4.20^{Aa}$±0.11 | $3.95^{Ab}$±0.13 | $3.98^{Ab}$±0.11 | $2.28^{Ac}$±0.10 | $2.00^{Ac}$±0.09 | $1.00^{Ad}$±0.09 |
| ***E. coli*** | **0** | N.D | N.D | N.D | N.D | N.D | N.D |
| | **3** | $1.90^{Ca}$±0.06 | N.D | N.D | N.D | N.D | N.D |
| | **7** | $2.18^{Ba}$±0.08 | $1.06^{Bb}$±0.10 | N.D | N.D | N.D | N.D |
| | **12** | $3.20^{Aa}$±0.07 | $2.44^{Ab}$±0.11 | $2.17^{Ab}$±0.14 | $1.59^{Bc}$±0.10 | N.D | N.D |
| | **15** | $3.88^{Aa}$±0.11 | $2.90^{Ab}$±0.09 | $2.30^{Ab}$±0.09 | $2.40^{Ab}$±0.10 | $1.88^{Ac}$±0.05 | N.D |
| **Aerobic bacterial counts** | **0** | $3.80^{Ca}$±0.10 | $2.55^{Cb}$±0.13 | $2.60^{Bb}$±0.16 | $2.33^{Bb}$±0.11 | $2.44^{Bb}$±0.10 | $1.98^{Ac}$±0.17 |
| | **3** | $4.28^{Ba}$±0.16 | $3.19^{Cb}$±0.10 | $2.40^{Bc}$±0.11 | $2.84^{Bc}$±0.09 | $2.31^{Bc}$±0.07 | $1.94^{Ad}$±0.09 |
| | **7** | $4.55^{Ba}$±0.8 | $4.20^{Ba}$±0.10 | $3.00^{Ab}$±0.05 | $2.30^{Bc}$±0.05 | $2.00^{Bc}$±0.09 | $1.44^{Ad}$±0.10 |
| | **12** | $5.29^{Aa}$±0.09 | $4.80^{Bb}$±0.07 | $3.28^{Ab}$±0.07 | $3.45^{Ab}$±0.10 | $2.94^{ABc}$±0.05 | $1.35^{Ad}$±0.8 |
| | **15** | $5.75^{Aa}$±0.12 | $5.25^{Aa}$±0.11 | $3.40^{Ab}$±0.08 | $3.90^{Ab}$±0.06 | $3.20^{Ab}$±0.05 | $1.59^{Ac}$±0.10 |
| **Mold and yeast** | **0** | N.D | N.D | N.D | N.D | N.D | N.D |
| | **3** | $1.94^{Da}$±0.06 | $2.13^{Ba}$±0.09 | N.D | N.D | N.D | N.D |
| | **7** | $2.18^{Ca}$±0.10 | $2.40^{Ba}$±0.15 | $1.50^{Cb}$±0.10 | $2.00^{Aab}$±0.06 | $1.30^{Bb}$±0.09 | N.D |
| | **12** | $3.88^{Ba}$±0.11 | $2.80^{Bb}$±0.09 | $2.15^{Bb}$±0.6 | $2.34^{Ab}$±0.07 | $2.00^{Ab}$±0.10 | $1.00^{Bc}$±0.05 |
| | **15** | $5.75^{Aa}$±0.13 | $4.29^{Ab}$±0.11 | $3.00^{Ac}$±0.09 | $2.90^{Acd}$±0.10 | $2.70^{Ad}$±0.07 | $1.24^{Ae}$±0.08 |
| **Psychrophilic bacteria** | **0** | N.D | N.D | N.D | N.D | N.D | N.D |
| | **3** | $3.00^{Ca}$±0.05 | N.D | N.D | N.D | N.D | N.D |
| | **7** | $4.29^{Ba}$±0.10 | $2.80^{Cb}$±0.9 | $2.30^{Bb}$±0.7 | $2.00^{Bb}$±0.09 | $2.10^{Bb}$±0.09 | N.D |
| | **12** | $5.30^{Aa}$±0.11 | $3.40^{Bb}$±0.10 | $3.00^{Ab}$±0.08 | $2.90^{ABb}$±0.08 | $3.00^{Ab}$±0.10 | $1.85^{Bc}$±0.11 |
| | **15** | $5.89^{Aa}$±0.09 | $4.50^{Ab}$±0.8 | $3.70^{Ac}$±0.10 | $3.15^{Ac}$±0.11 | $3.12^{Ac}$±0.10 | $2.11^{Ad}$±0.10 |

-N.D (not detected).
Each value represents mean ± standard deviation of the three replicates(n = 3).
-Means within the same row (lowercase letters) and column (uppercase letters), for each microorganism, with the same letter, do not statistically differ from each other (P > 0.05). ±S.D.
-(AU) apple slices uncoated;(AWPI) Apple slices coated with WPI; (A1, A2, A3,and A4) apple slices coated with WPI/BLE at different ratios during storage period at 4±1°C.

The microbiological evaluation in the present work revealed that the use of WPI with BLE with 12 or 15 mg/g concentration to prepare edible coating was effective in delaying the apple slices' damage or spoilage during the 15 days of cold storage. These are related to the phenolic components with biological activities and other antimicrobial compounds present in BLE as mentioned before (in 3.1 and 3.3). The plant extract's antibacterial properties are due to the phytochemical compounds like phenolic and flavonoid substances.

These substances serve as a defense line against attacks from natural enemies and may increase microbial infection resistance (Gebrechristos *et al.,* 2020). Furthermore, visible spoilage was observed for uncoated apple slices at seven days of storage with enumerated counts of mold and yeast reaching 2.18 CFU/g. For coated apple slices, spoilage was visibly observed with samples A1 and A2 at 12 days of storage. In addition, with other samples (A3 and A4), spoilage was initially visible at 15 days of storage (at this time, the microbial counts test ended). These results were confirmed by other studies that indicated the leaves of plant extracts have active compounds such as tannins, flavonoids, saponins, alkaloids, and essential oilsThese compounds have antimicrobial impact and are used as preservative agents for foods (Alloh *et al.,* 2024; El-Sayed *et al.,* 2024; Mursyida *et al.,* 2021; Algabri *et al.,* 2018). Generally, the extracts from bay leaves are nontoxic have antimicrobial and anti-biofilm qualities, and could be integrated into the coating materials for foods as a preservative agent (Karimou *et al.,* 2024; Sharma *et al.,* 2021).

## 4. Conclusion

The use of whey protein isolate was very effective as an edible coating on fresh-cut apples. The incorporation of bay leaf extract led to maintain the high quality of apple slices during refrigerated storage, including TPC, TFC, and antioxidant activity. Whey protein isolates characterized with its edible coating, especially those enriched with antioxidant agents may successfully compete with bay leaf extract for the ability to inhibit weight loss, decrease brown index, antioxidant activity, and microbial growth, and extend the shelf life of fresh apple slices during 15 days of storage. Even though these results are encouraging, more research will be needed to improve the current use of bay leaf and whey protein in the preservation of fresh fruit slices.

**Declaration of competing interest**

No conflict of interest exists.

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