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## Effects of Edible Coatings Enriched with Chamomile Essential Oil on Postharvest Quality and Shelf Life of Mushrooms (Agaricus bisporus)



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#### Abstract

White button mushrooms (Agaricus bisporus) are particularly perishable due to their excessive moisture content and absence of a natural protective barrier, making them prone to browning, softening, and microbial deterioration. This study investigated the effects of different edible coating materials xanthan gum, carboxymethyl cellulose, and potato starch each incorporated with chamomile (Matricaria chamomilla) essential oil at two concentrations (0.5% and 1% w/v), on the shelf life and quality of fresh mushrooms during 20 days of cold storage. The purpose was to analyze the influence of both coating type and essential oil concentration on the physicochemical, microbiological, and sensory properties of the mushrooms, and to classify treatments based on preservation efficacy. Results indicated that all coated samples displayed considerably enhanced quality characteristics relative to uncoated controls during the storage period. Among the coating types, potato starch produced the most beneficial outcomes, followed by xanthan gum and carboxymethyl cellulose. Mushrooms treated with 1% chamomile essential oil demonstrated greater moisture retention, higher total soluble solids, improved firmness, reduced cap opening and surface browning, and superior sensory scores. Additionally, these samples maintained higher levels of total phenolic content, DPPH radical scavenging capability, and important enzyme activities. Microbial growth was significantly inhibited in proportion to the essential oil concentration, demonstrating robust antibacterial action. These findings imply that bioactive coatings, particularly those based on potato starch mixed with chamomile essential oil, constitute a promising technique for increasing the shelf life and preserving the quality of button mushrooms during storage.

**Keywords:** Agaricus bisporus; Edible coatings; Chamomile essential oil; Shelf life extension; Xanthan gum; Carboxymethyl cellulose; Potato starch; Cold storage; Microbial spoilage; Physicochemical attributes; Sensory evaluation; Bioactive coating

#### 1. Introduction

Edible mushrooms are globally valued for their nutritional, medicinal, and economic importance. However, they are highly perishable and susceptible to rapid quality deterioration after harvest due to mechanical damage, microbial contamination, and discoloration, largely resulting from insufficient preservation techniques. [1] Among these, button mushrooms (*Agaricus bisporus*) are widely consumed but particularly prone to spoilage due to their high respiration rate, lack of protective barriers, and sensitivity to environmental conditions. [2, 3] Fresh mushrooms are rich in nutrients such as carbohydrates, proteins, dietary fiber, vita mins, and phenolic compounds, with moisture content ranging from 81.8% to 94.8%. [4] Despite their nutritional value, storage leads to rapid declines in texture, color, flavor, and nutritional content, driven by metabolic changes involving enzymatic activity, microbial growth, and the accumulation of reactive oxygen species (ROS) like H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>, which cause oxidative damage and accelerate senescence. [5-10]

Understanding these spoilage mechanisms is essential for developing effective preservation strategies. Common causes of spoilage include bacterial growth, mold formation, and fungal contamination especially by Aspergillus, Penicillium, and Fusarium species. [11] Both intrinsic factors (such as water activity and respiration rate) and extrinsic storage conditions (temperature, humidity, and handling) influence mushroom quality post-harvest. Given their high-water content and near-neutral pH, mushrooms offer an ideal environment for microbial growth. [12] As

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a result, there is growing urgency to implement advanced preservation techniques to maintain quality and extend shelf life. Due to their high moisture content and the absence of a hard outer protective layer, button mushrooms have a short shelf life of 3–4 days. This leads to a reduction in marketability, caused by enzymatic reactions and microbial attacks, which result in browning, softening, and senescence. [13] Traditional packaging methods typically involve placing mushrooms in plastic containers, covering them with PVC film, and refrigerating them. However, the high moisture released by the mushrooms, combined with the low water vapor permeability of the film, often causes condensation to form inside the packaging, which is visible under the film on containers in markets.

In recent years, there has been a growing consumer demand for convenient, healthy, and ready-to-eat products. [15, 16] Fresh fruits are particularly appealing due to their freshness, nutritional value, safety, and overall eating experience, driving the expansion of this market segment. To extend the shelf life of fresh and fresh-cut fruits while maintaining their quality, several innovative technologies have been developed. These include active packaging, natural preservatives, and various physical treatments. [17] To improve the shelf life and quality of mushrooms, it is crucial to adopt advanced packaging technologies, like nano-packaging, biodegradable packaging, edible coatings, modified atmosphere packaging (MAP), and active packaging. It highlights their effects on enzyme activity, antimicrobial and antioxidant properties, respiration rates, and changes in texture, color, and nutritional value, emphasizing how these packaging solutions help preserve mushroom quality. [18]

Edible coatings are thin layers of consumable materials applied to the surface of food and agricultural products, providing an adaptable solution that creates a physical barrier. This barrier controls the movement of moisture, oxygen, carbon dioxide, flavorings, and dissolved compounds, thereby delaying enzymatic processes and microbial contamination, ultimately extending shelf life. [19] Coatings made from polysaccharides **Louis** *et al.*, [12] have been found effective in prolonging the shelf life of edible mushrooms. These coatings can be further enhanced with bioactive compounds, such as plant extracts, essential oils, probiotics, and antimicrobial agents, to improve their efficacy. [19] Edible coatings have gained attention as a sustainable and consumer-friendly method for extending the shelf life of fruits and reducing postharvest losses. [20] Catering to the increasing demand for natural, minimally processed foods, these coatings protect fruits by inhibiting microbial growth, enzymatic browning, and overall quality deterioration. Their effectiveness depends on the composition and application method, and incorporating active agents such as antimicrobials, antioxidants, or enzymes can further enhance their ability to preserve fruit quality and prevent spoilage.

Chamomile (*Matricaria chamomilla L.* or *Matricaria recutita L.*), an annual herbaceous plant of the Asteraceae family, is among the most extensively cultivated and utilized medicinal and essential oil crops worldwide. It is grown in various countries including Germany, Hungary, France, Russia, India, Turkey, Egypt, and Algeria. [21] The essential oil (EO) of chamomile is extracted primarily through steam distillation of its inflorescences, and is highly valued in both traditional and modern medicine for its therapeutic properties. [22] Chamomile EO is rich in sesquiterpenes, particularly chamazulene,  $\alpha$ -bisabolol, and (E)- $\beta$ -farnesene, as well as oxygenated derivatives such as bisabolol oxide A, bisabolol oxide B, and bisabolone oxide A, which are recognized as its main bioactive constituents. [23-26] Furthermore, the EO exhibits strong antioxidant activity, largely attributed to its dark blue component, chamazulene. [27-29] (Recent studies have demonstrated that chamazulene and  $\alpha$ -bisabolol oxide A exhibit the highest in vitro radical scavenging activity among the oil's constituents, with (E)- $\beta$ -farnesene also contributing significantly to its overall antioxidant potential. [30-33]

One effective strategy for preventing microbial and physicochemical spoilage reactions is the use of natural, cost-effective compounds. [34] Edible coatings enriched with bioactive compounds have shown great promise in improving the quality and extending the shelf life of edible mushrooms. As noted by **Shonte** *et al.*, [18] these coatings not only preserve freshness but also provide beneficial properties, such as high air and moisture permeability. These functional attributes enable the coatings to serve as effective carriers for active ingredients, helping to delay spoilage and maintain the overall postharvest quality of mushrooms. [35] emphasized the effectiveness of edible coatings infused with essential oils such as oregano, thyme, and cinnamon in extending product freshness by inhibiting microbial growth. These natural additives not only enhance antimicrobial activity but also influence the coatings' physicochemical properties, including viscosity, color, and transparency. The type and concentration of essential oils play a critical role in determining the overall performance and quality of the coating.

The postharvest quality and shelf life of Agaricus bisporus mushrooms are highly susceptible to deterioration due to factors such as water loss, enzymatic browning, and microbial spoilage. Recent advancements in edible coating technologies have shown promising results in mitigating these effects through natural, biodegradable formulations. **Gholami** *et al.* [36] demonstrated that alginate-based coatings enriched with phlorotannins extracted from Sargassum ilicifolium effectively reduced weight loss, delayed browning, and preserved vitamin C and total phenolic content during 12 days of refrigerated storage. Similarly, **Moradi** *et al.* [37] reported that bitter almond gum–fish gelatin conjugate coatings, particularly at a 2% concentration, maintained mushroom firmness, minimized browning, and enhanced sensory quality, with spray-coated samples outperforming untreated controls. In another study, **Najabi** *et al.* [38] found that salep gum-based coatings enriched with probiotics not only extended

shelf life by maintaining antioxidant levels and texture, but also served as a delivery system for viable probiotic cultures. **Yazicioglu** [39] explored guar gum coatings incorporating waste leek powder and sunflower oil, which contributed to reduced weight loss, shape preservation, and improved color retention over seven days of storage. Additionally, **Cavusoglu** *et al.* [40] emphasized the efficacy of sodium alginate and gum-based coatings in minimizing shrinkage, browning, and quality degradation, thereby extending the marketability of button mushrooms. Collectively, these studies underscore the potential of biopolymer-based edible coatings in preserving mushroom quality and enhancing their functional value during cold storage.

The present study aimed to evaluate the effects of various bioactive coating formulations, enriched with chamomile essential oil, on the shelf life and postharvest quality of white button mushrooms (Agaricus bisporus). This investigation proposes a novel technological approach to enhance storage stability and maintain the physicochemical and microbiological quality of mushrooms during the postharvest period.

#### 2. Materials and methods

#### 2.1. Raw materials and chemicals

Button mushrooms (*Agaricus bisporus*) were supplied from mushroom V farm in Tanta, Gharbia Governorate and pre-cooled at 4 °C overnight in a refrigerator. The mushrooms were white and uniform in size, with no mechanical damage and decay. Xanthan gum, carboxymethyl cellulose were purchased from Mifad Company, Badr city, Egypt. Potato starch was obtained from Leader Factory for chips, Tanta city. Preliminary treatment of potato starch (PS) was achieved using the method of **Elabd.** [41] All chemicals used were food grade and bought from El-Gomhoria Company for chemical and drugs, Egypt.

#### 2.2. Extraction of the essential oils

The dried chamomile flowers and stems were ground into a coarse powder using a mortar and pestle. The powdered material was homogenized, and 500 g was subjected to hydro-distillation using a Clevenger-type apparatus for 3 hours. The resulting essential oil was dried using anhydrous sodium sulfate. This extraction process was repeated five times. The oil was then stored in amber bottles and kept in a refrigerator at 4°C until further analysis. [42]

## 2.3. Physical and Chemical properties of essential

## 2.3.1. Determination of Solubility

The solubility of the essential oil in 80% ethanol was determined by titrating a known volume (1ml) of the oil with 80% ethanol until a homogeneous solution was achieved. The result is expressed as volume per volume (v/v) according to the method described by **Yadav.** [43]

## 2.3.2. Specific Gravity

The specific gravity of the oil was determined using a pycnometer (1 ml capacity) as described by **Guenther.** [44]

#### 2.3.3. Refractive Index

The refractive index was determined using Abbe refractometer model 60. According to the procedure in AOAC (2019) [45]

## 2.3.4. Acid Number

The acid number was determined according to the method described in AOAC. [45] A known weight of the oil (1g) was dissolved in a neutral ethanol (10 ml) and directly titrated by ethanolic potassium hydroxide (0.1 N) using phenolphthalein as an indicator.

$$Acid number = \frac{V*N*56.1}{W}$$

Where V= volume in millileters of KOH solution, N= normality of KOH solution, W= weight of the oil in grams

### 2.3.5. The Saponification Number

The saponification number of the various substances encountered in the present investigation was determined according to the following procedure which was essentially similar to the standard procedure previously reported by **Guenther** [44] used phenolphthalein as an indicator which proved to be a about 1.5 gram of the oil

was accurately weighed in a 150ml flask. The oil was treated with a known volume (10 ml or 20 ml) of ethanolic potassium hydroxide (about 0.5 N). The mixture was heated in a water –bath at 100 c for one hour, using an air-cooled condenser. At the end of this period, the excess potassium hydroxide was back titrated with hydrochloric acid (0.15 N) using the indicator. A blank determination was carried out using the same quantity of ethanolic potassium hydroxide. The saponification number was calculated from the following equation:

**Saponification Number** = 
$$\frac{(A-B) X N X 56.1}{W}$$

A= HCl for blank (ml), B= HCl for sample (ml), N= Normality of HCl, W= weight of sample (g)

The saponification number represents the ester number of the essential oil when the number of the sample is small.

#### 2.3.6. The Ester Number

According to **Gunther (1950),** [46] the ester number is the difference between the saponification number and acid number.

**Ester number** = Saponification number - Acid number

#### 2.4. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of Essential Oils (EOs)

GC/MS analysis of chamomile and ginger essential oils was done at the Faculty of Agriculture, Alexandria University. One ml of hexane was added to each EO sample in ratio 1:10ml and mixed well. Then, one  $\mu L$  was directly injected to GC/MS for the analysis of chemical components of EOs, GC Ultra ISQ apparatus with automatic sampler 7683B series-injector and split/split less injection system. The GC was fitted with MS capillary column (TG-1MS, 30m, 0.32mm I.D., 0.25 $\mu$ m film thickness). The temperature of program was as follows: Temperature of injector 250°C, Pressure 146.99 Kilopascal (kPa), temperature of MS detector 280°C, temperature of oven 40°C for 4min, then gradient 4°C /min to 260°C, 4min hold time, 63min was the finaltime and transfer line temperature was 200°C. Helium was used as carrier gas at kPa pressure with flow 1ml/min, linear velocity 30cm/s. The mass spectrometer had a vacuum compensation on, solvent delay time 4min to avoid the solvent peak, split ratio 1:10 and electronic pressure control on. Scan time was 29-650 m/z. Ionization energy was set at 70eV. The components of the EOs were identified by matching their mass spectral fragmentation patterns with those reported in computerized MS- data bank spectral libraries NIST98 and WILEY 138. [47]

#### 2.5. Preparation of Coating Formulations

Mushroom samples were divided into seven treatment groups, including a **control group** (non-coated) and six coating treatments as follows:

- **XG1**: Xanthan gum 0.5% + 0.5% chamomile essential oil
- **XG2**: Xanthan gum 0.5% + 1% chamomile essential oil
- **CMC1**: Carboxymethyl cellulose 1.5% + 0.5% chamomile essential oil
- CMC2: Carboxymethyl cellulose 1.5% + 1% chamomile essential oil
- **PS1**: Potato starch 3%+ 0.5% chamomile essential oil
- **PS2**: Potato starch 3%+ 1% chamomile essential oil

The xanthan gum-based coatings were prepared according to the method described by. [48] The carboxymethyl cellulose and potato starch coatings were prepared following the procedure of **El-Abd and Badawy (2017).** [49] In all coating formulations, 0.1% (v/v) Tween 80 was added as an emulsifier agent to enhance the dispersion and solubility of chamomile essential oil within the coating matrix. [50]

## 2.6. Sample Preparation and Treatment

Fresh mushrooms were selected for uniformity in shape, size, color, and with no physical damage. Prior to treatment, the mushrooms were washed with distilled water and air-dried. The prepared samples were then immersed in the respective treatment solutions for 3 minutes. After immersion, the mushrooms were removed and placed in sterile baskets to allow excess solution to drain. The control group consisted of mushrooms treated identically but immersed only in distilled water. Subsequently, all samples were placed in perforated plastic containers and stored under controlled conditions at  $4 \pm 1^{\circ}$ C and 70-75% relative humidity for 20 days. Sampling was conducted on day 0 and subsequently every 4 days throughout the storage period. All treatments were applied in triplicate for experimental consistency.

## 2.7. Determining physicochemical parameters of mushrooms during storage

### 2.7.1. Weight Loss

Weight loss was calculated as the percentage reduction from the initial fruit weight, following the procedure outlined by **AOAC.** [51]

#### 2.7.2. Firmness

Firmness of the whole fruit was measured using a hand dynamometer (Model FDP 1000) fitted with a 2 mm plunger. The force was initially recorded in gram-force (gf) and then converted to Newtons using the standard conversion factor (1 gf = 0.00980665 N).

#### 2.7.3. Total Soluble Solids (TSS)

TSS content in fruit juice was determined using a manual refractometer (Model R R 12, Nr 05116, range 0–35% at 20°C, made in Poland) at room temperature, based on the refractometric method described by **AOAC.** [51]

## 2.7.4. Cap Opening Percentage

The percentage of opened caps, indicating the developmental progression toward an umbrella-like shape and subsequent membrane detachment, was assessed according to the method described by **Nasiri** *et al.* [52] This parameter was used as an indicator of morphological maturity in button mushrooms.

## 2.7.5. Determination of Color Indices and Browning Degree

The surface color characteristics of coated and uncoated mushroom samples were evaluated in terms of lightness (L\*), redness/greenness (a\*), yellowness/blueness (b\*), total color difference ( $\Delta E$ ), and browning index (BI). According to **Qu** *et al.*, [53] the L\* value indicates luminosity,  $\Delta E$  reflects overall color variation compared to a reference color, while the **BI** expresses the purity and intensity of the brown color formed during storage. Color measurements (L\*, a\*, b\*) were performed using a color reader (CR-20, KONICA MINOLTA, Inc., Japan) at room temperature on three randomly selected mushroom samples per treatment. Each sample was measured in triplicate to ensure accuracy.

#### 2.7.5.1. Total Color Difference (△E)

The total color difference ( $\Delta E$ ) was calculated in comparison to the ideal fresh mushroom cap color ( $L_0$ \* = 97,  $a_0$ \* = -2,  $b_0$ \* = 0) using the following formula:

$$\Delta E = [(L^* - 97)^2 + (a^* - (-2))^2 + (b^*)^2]^{1/2}$$

#### 2.7.5.2. Browning Index (BI)

The browning index (BI) was determined to assess the degree of browning according to the method of Cavusoglu et al.: [40]

$$BI = 100 (x - 0.31)/0.17$$

Where  $x = (a^* + 1.75 L^*) / (5.645 L^* + a^* - 3.012 b^*)$ 

## 2.7.6. Determination enzyme activities (Polyphenol Oxidase (PPO) and Peroxidase (POD) Activity)

Enzyme extracts were prepared from mushroom tissue to evaluate PPO and POD activity. The extraction buffer consisted of 0.2 M sodium phosphate buffer (pH 6.5), supplemented with 4% (w/v) polyvinyl polypyrrolidone (PVPP) and 1% (v/v) Triton X-100. Ten grams of homogenized mushroom tissue were mixed with 20 mL of this extraction solution. The mixture was then centrifuged at 4 °C for 10 minutes using a LISA centrifuge (France). The resulting supernatant was collected and used for subsequent analysis of PPO and POD activities, expressed as absorbance change per minute per gram of fresh weight (abs  $min^{-1} g^{-1}$ ), following the procedures outlined by **Eldib** *et al.* and Moosavi-Nasab *et al.* [54, 55]

## 2.7.7. Extraction of total phenolic compounds (TPC)

According to **Salem** *et al.*, [56] samples (10 g) were soaked in 100 ml of ethanol (80%), overnight in a shaker at room temperature, The extracts were filtrated through Whatman No.1 filter paper. The residues were reextracted three times under the same conditions. The combined filtrates were evaporated under vacuum in a rotary evaporator below 40°C. The extracts were obtained after evaporation of organic solvents and stored at -20°C until further analysis.

#### 2.7.7.1. Determination of total phenolic compounds (TPC)

The total phenolic content of each extract was measured with the Folin–Ciocalteau method as described by the Folin Ciocalteau method according to **Singleton** *et al.*, [57] The procedure consisted of diluting an aliquot of the extract (0.1ml) with water to 5 ml in a 10 ml volumetric flask, and then 0.5 ml of Folin–Ciocalteau reagent was added. After 3 min, one ml of saturated sodium carbonate solution was added. The content was mixed, and the mixture was made up to 10 mL with water. After one hour at room temperature, absorption was measured in 1 cm cells at 760 nm using a Spectro-photometer (PEAK INSTRUMENTS C-7200). A blank was prepared using water and reagents only. A standard curve was carried out using gallic acid (GAE), and the results were expressed as mg GAE/100 g, of dry mass plant extract. GAE served as a standard compound and was used for the preparation of calibration in the range of 0-10 ppm.

#### 2.7.7.2. Total Antioxidant Activity (TAA)

The total antioxidant activity of the extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A volume of  $100~\mu L$  of ethanolic extract was mixed with  $2000~\mu L$  of 0.1 mmol DPPH solution. The mixture was immediately stirred and then incubated in the dark at room temperature for 60 minutes to allow the reaction to stabilize. The absorbance of the solution was then measured at 517 nm using a s Spectrophotometer (PEAK INSTRUMENTS C-7200). Antioxidant capacity was expressed as the percentage of DPPH inhibition, [58] calculated using the following equation:

% 
$$\mathbf{DPPH}_{SC} = (A_{cont} - A_{samp}) / A_{cont} \times 100$$

Where: %  $DPPH_{SC}$  is the percentage of free radical scavenging activity,  $A_{samp}$  is the absorbance of the sample with DPPH,  $A_{cont}$  is the absorbance of the DPPH control solution.

#### 2.7.7.3. Determination of Malondialdehyde (MDA) Content

Malondialdehyde (MDA) content was measured following the method of **Huang** *et al.*, [59] with slight modifications. Briefly, 1 g of button mushroom was homogenized with 7 mL of 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at  $3,500 \times g$  for 30 minutes at 4°C. Subsequently, 2 mL of the resulting supernatant was mixed with 2 mL of 0.67% (w/v) thiobarbituric acid (TBA) solution. The mixture was incubated in a water bath at 80°C for 30 minutes, then cooled to room temperature. After cooling, the mixture was centrifuged again at  $3,500 \times g$  for 30 minutes at 4°C. The absorbance of the supernatant was recorded at 450, 523, and 600 nm using a Spectrophotometer (PEAK INSTRUMENTS C-7200). MDA content was expressed as nmol  $g^{-1}$  fresh weight (FW).

## 2.8. Microbiological Analysis

Microbiological quality of the mushroom samples was evaluated by determining the counts of mesophilic and psychrophilic bacteria, as well as yeasts and molds, following the method described by **Gao** *et al.* [60] Ten grams of mushrooms were aseptically mixed with 0.1% peptone water and homogenized using a Stomacher for 2 minutes. Mesophilic bacteria were enumerated by plating on Plate Count Agar (PCA) and incubating at 35 °C for 2 days, while psychrophilic bacteria were incubated at 4 °C for 7 days on the same medium. Yeast and mold counts were determined by plating on Potato Dextrose Agar (PDA) and incubating at 28°C for 5–7 days.

## 2.9. Sensory evaluation

The sensory evaluation of Agaricus bisporus mushrooms was carried out on days 0, 4, 8, 12, 16, and 20. Various sensory attributes were assessed, including odour, gill colour, gill surface, cap uniformity, and the presence of dark spots on the cap. A 10-point hedonic scale was used for evaluation, where a score of 10 indicated the strongest off-odour, and 0 the weakest; 10 represented the highest cap uniformity, and 0 the lowest; 10 denoted the greatest number of dark spots, while 0 indicated minimal spotting; and for colour acceptability, 10 corresponded to excellent appearance, while 0 reflected very poor colour quality. [36]

#### 2.10. Statistical analysis

Experiments were conducted in triplicates. Data were analyzed using SPSS (version 16; IBM, USA). Analysis of variance (ANOVA) followed by Duncan's multiple range test was used to distinguish the treatments at p < 0.05.

## **3.** Results and Discussion:

## 3.1. Characteristics of chamomile essential oil

## 3.1.1. Physical properties of chamomile essential oil

The physicochemical properties of chamomile essential oil are summarized in **Table 1**. The results show that the specific gravity, refractive index, and acid value of chamomile essential oil were 0.89, 1.43, and 0.92 mg KOH/g oil, respectively. Additionally, the oil was found to be soluble in 80% alcohol, indicating its compatibility with various alcohol-based formulations. These values indicate the purity and versatility of chamomile essential oil for use in food preservation, cosmetics, and pharmaceuticals. The specific gravity and refractive index align with other essential oils, while the low acid value highlights the oil's quality and stability. These findings are in agreement with those reported by **Lopez and Blazquez** and **Mohamed** *et al.* [61, 62]

The data presented in **Table 1** show that the saponification value (190 mg KOH/g oil) and ester value (175 mg KOH/g oil) align with the typical range for essential oils, confirming the oil's suitability for industrial applications, particularly in formulations that require stability. The oil yield of 1.1% further demonstrates the efficiency of the extraction process. These findings are consistent with those reported by **Mohamed** *et al.* and **Salah**. [62, 63] Minor differences in the refractive index and acid value compared to previous studies may be due to factors such as geographical location, seasonal variations, and environmental conditions, as suggested by **Rizk** *et al.*, (2020).

properties	Chamomile
Specific gravity	0.89
Refractive index	1.43
Solubility in alcohol (80%)	Soluble
Acid value (mg KOH/g oil)	0.92
Saponification value (mg KOH/g oil)	190
Ester value (mg KOH/g oil)	175
Yield of extracted oil (ml)	1.1 %

Table 1: physicochemical properties of chamomile essential oil

## 3.1.2. Volatile Composition of Chamomile Essential Oil

The volatile constituents of chamomile essential oil were identified and quantified using Gas Chromatographic (GC) analysis, as presented in **Table 2**. A total of 17 compounds were detected, each contributing to the oil's aromatic profile and biological functionality. These components are primarily composed of terpenes, sesquiterpenes, and oxygenated derivatives, which play crucial roles in flavor, aroma, and potential food preservation activities.

The most abundant compound identified was **Bisabolol oxide A** (35.0%), followed by **Bisabolol oxide B** (16.5%), and **Trans \beta-farnesene** (7.75%). These three major components are known for their **anti-inflammatory**, **antioxidant**, and **antimicrobial** activities, making chamomile essential oil a promising natural additive for enhancing **shelf life and safety** in food products. **Bisabolol oxide A and B** are oxygenated sesquiterpenes known for their **soothing and antimicrobial** effects. In the food industry, these compounds can help **inhibit spoilage microorganisms** and extend the freshness of perishable items, particularly when used in active packaging or natural preservatives.

Trans  $\beta$ -farnesene (7.75%) and Cis  $\beta$ -farnesene (6.10%) are sesquiterpenes with known insect-repellent and antimicrobial properties. These attributes are especially valuable for food storage systems, where reducing microbial load and repelling pests are critical.

Chamazulene (2.7%), recognized for its deep blue color and strong antioxidant potential, not only contributes to the aesthetic and aroma of the essential oil but also helps stabilize lipid-rich food matrices by preventing oxidative rancidity.

Citronellal (1.52%) and Artemisia ketone (1.44%) possess refreshing citrus-like aromas and bacterio-static effects, which enhance the flavor profile of food products while also acting as natural preservatives.

 $\beta$ -Ocimene (1.5%) and  $\gamma$ -terpinene (0.69%) are monoterpenes with known antioxidant and flavor-enhancing effects, which contribute to both organoleptic quality and stability in processed foods.

Minor components like **Sabinene**, **Bicycloelemene**, **Germacrene-D**, and **Caryophyllene oxide**, although present in lower percentages, add complexity to the oil's sensory characteristics and synergistically boost its biological activity.

The overall composition of chamomile essential oil reflects a balanced mixture of bioactive volatiles that are **both aromatic and functional**, supporting its application in the **food industry** as A **natural flavoring agent**,

an antimicrobial preservative, a natural antioxidant, a component of edible coatings or active packaging, for controlled release and prolonged freshness.

The GC analysis confirms that chamomile essential oil possesses a rich profile of volatile compounds with significant **technological and functional benefits** for the food sector. These findings reinforce the potential of incorporating chamomile oil in **clean-label**, **health-conscious food formulations**, aligning with the growing demand for **natural and sustainable food preservation methods**. These results are in agreements with those obtained by **Ljiljana** *et al.*, **Abutaleb** *et al.*, **El-Gohary et al. and Elsaid** *et al.* [30, 64-66]

<b>Table 2</b> : Volatile components of Chan	omile essential oil by Gas	Chromatographic (	GC)	analysis
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Compound	Rt (min)	%	M. formula	M.W
Sabinene	11.125	0.18	$C_{10} H_{16}$	136.23
β-Ocimene	13.597	1.5	$C_{10} H_{16}$	13.597
γ-terpinene	13.963	0.69	$C_{10}H_{16}$	136.23
Artemisia ketone	14.255	1.44	$C_{10}H_{16}O$	152.23
Citronellal	16.476	1.52	$C_{10} H_{18} O$	154.25
CisbetaFarnesene	26.396	6.10	$C_{15}H_{24}$	204.19
Bicycloe lemene	26.603	0.739	$C_{15}H_{24}$	204.00
Caryophyllene oxide	<b>29</b> .678	0.65	C <sub>15</sub> H <sub>24</sub> O	222.37
Lanceol, cis	31.764	0.09	C <sub>15</sub> H <sub>24</sub> O	222.37
Bisabolol oxide B	33.216	16.5	$C_{15} H_{26} O_2$	238.54
Trans β-farnesene	34.379	7.75	$C_{15}H_{24}$	204.19
Germacrene-D	34.819	0.122	$C_{15}H_{24}$	204.19
α-farnesene	36.319	1.399	$C_{15}H_{24}$	204.19
Chamazulene	36.649	2.7	C <sub>14</sub> H <sub>16</sub>	184.27
Bisabolol oxide A	37.692	35.0	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238.54
Geranyl isovalerate	46.66	0.23	$C_{15} H_{26} O_2$	238.54
Hexacosane	52.51	0.66	$C_{26}H_{54}$	380.00

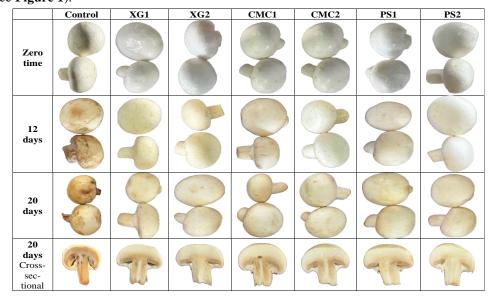
Rt = Retention time

M. formula = Molecular formula

M.W. = Molecular weight

# **3.2.** Visual Changes in Agaricus bisporus Mushrooms During Storage Under Different Coating Treatments Over a 20-Day Period

The coatings include xanthan gum (XG), carboxymethyl cellulose (CMC), and potato starch (PS), incorporated with different concentrations (0.5 and 1%) of chamomile essential oil. Progressive discoloration, shrinkage, and surface browning can be observed over time, with variations depending on the type and concentration of the coating applied. Cross-sectional views at day 20 are also shown to illustrate internal quality changes in the mushroom tissues (see **Figure 1**).



**Figure 1**: Visual appearance of *Agaricus bisporus* mushrooms coated with different edible coatings compared to the uncoated mushrooms at 0, 4, 8, 12, 16 and 20 days of storage

# 3.3. Changes in physiochemical characteristics of mushrooms treated with different coatings incorporated with chamomile essential oil

#### 3.3.1. Weight Loss

The data presented in **Figure 2** demonstrate the effects of different edible coatings xanthan gum (XG), carboxymethyl cellulose (CMC), and potato starch (PS) incorporated with chamomile essential oil at two concentrations (0.5% and 1.0%) on the weight loss (%) of mushroom samples stored at  $4\pm1^{\circ}$ C and 70-75% relative humidity for 20 days. Weight loss increased progressively across all treatments throughout the storage period, which aligns with the high respiration rate and water content of mushrooms, making them particularly susceptible to dehydration and microbial deterioration. [67] The uncoated control samples showed the most severe weight loss, reaching 38.68% by day 20, significantly higher than all coated treatments (p < 0.05). This is attributed to the absence of a protective barrier, resulting in faster moisture evaporation and spoilage.

Among all treatments, PS2 showed the lowest weight loss (24.82%), closely followed by XG2 (24.86%). These treatments were statistically superior to both their lower concentration counterparts and the control group. The enhanced performance can be attributed to the higher concentration of chamomile essential oil, which improve d the coatings moisture barrier and antimicrobial properties. These findings are consistent with **Rajabi et al.**, (2022), who demonstrated that active coatings containing walnut and lemon peel essential oils significantly extended the shelf life of mushrooms by forming bioactive, moisture-retentive films. The PS1 and XG1 treatments also reduced weight loss significantly compared to the control (25.78 and 25.51%, 38.68 respectively), although their effectiveness was slightly lower than that of their 1.0% counterparts. This result supports the dose-dependent efficacy of essential oils, as highlighted by **Nasiri et al.**, [68] who emphasized the stronger antimicrobial and water barrier effects at higher concentrations.

Coatings based on CMC (CMC1 and CMC2) exhibited intermediate effectiveness, with weight losses of 26.78 and 26.30%, respectively. The slightly higher weight loss in these samples may be attributed to the lower film-forming ability and higher water solubility of CMC compared to PS and XG, which may weaken the coatings protective barrier during prolonged storage.

Based on the recorded values, it is clear that the coatings can be ranked based on their effectiveness in reducing weight loss by day 20 as follows: PS2 > XG2 > PS1 > CMC2 > CMC1 > XG1 > Control. This ranking highlights the superior performance of potato starch and xanthan gum coatings at higher essential oil concentrations in preserving mushroom quality. These results are consistent with **Jiang** *et al.*, [69] provided compelling evidence that chitosan-oil coatings significantly limit water loss in shiitake mushrooms during cold storage. Echoing this, **Yazicioglu** [39] demonstrated that guar gum-based coatings enriched with leek powder and sunflower oil can successfully minimize moisture loss and shrinkage in Agaricus bisporus. and In a broader context, **Nourozi & Sayyari** [70] affirmed the positive impact of Aloe vera and basil oil coatings in reducing weight loss and browning in apricots. Equivalent outcomes have been reported in earlier studies strawberries, [71] and mushrooms, [72] where the use of Aloe vera gel either by itself or blended with basil oil enhanced preservation and extended product shelf life.

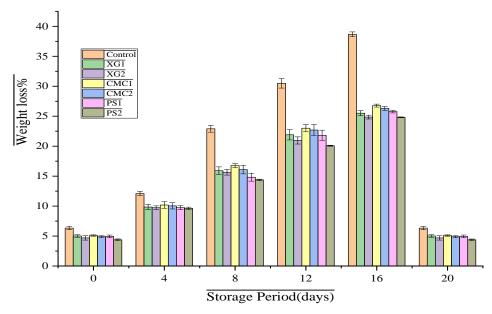


Figure 2: Effects of various coatings enriched with chamomile essential oil on weight loss% in mushroom samples stored at  $4\pm1$ °C for 20 days

Control: uncoated samples- XG1: mushrooms coated with xanthan gum +0.5% chamomile essential oil - XG2: mushrooms coated with xanthan gum +1% chamomile essential oil - CMC1: mushrooms coated with carboxymethyl cellulose +0.5% chamomile essential oil - CMC2: mushrooms coated with carboxymethyl cellulose +1% chamomile essential oil - PS1: mushrooms coated with potato starch +0.5% chamomile essential oil - PS2: mushrooms coated with potato starch +1% chamomile essential oil. Each value is an average of three determinations  $\pm$  standard deviation. in a column; means with the same small superscript letters are not significantly different at 5% level. in a raw means with the same capital superscript letters are not significantly different at 5% level.

#### 3.3.2. Firmness

Firmness is a critical parameter in determining the postharvest quality and marketability of mushrooms. Mushrooms are highly perishable due to their high respiration rate and delicate cell structure. According to **Ni** et al., [73] the decline in firmness is largely due to cell wall degradation, loss of turgor, and enzymatic breakdown of cellular components during storage.

This study in **Figure 3** demonstrated that edible coatings significantly slow the loss of firmness in mush-rooms during refrigerated storage. Among the tested biopolymers, potato starch infused with 1.0% chamomile essential oil (PS2) was the most effective in maintaining firmness (3.65 N at Day 20), followed by xanthan gum with 1.0% CEO (XG2) and PS1 (0.5% CEO). The lowest firmness retention among coated samples was observed in CMC1, although still superior to the uncoated control. These results suggest that potato starch-based coatings provide superior structural retention, likely due to their effective barrier properties. [12, 37]

The enhancement of firmness with increasing CEO concentration (from 0.5 to 1.0%) was consistent across all coating types, supporting the antimicrobial and antioxidant role of essential oils in slowing enzymatic degradation of cell walls. [74] The protective effects of edible coatings align with previous findings on plant-based films, such as gumghatti with chamomile oil, [75] Aloe vera and rosehip essential oil on stone fruits, [76-78] and tragacanth gum with Satureja khuzistanica essential oil on mushrooms. [68] Overall, these results reinforce that edible coatings especially those based on potato starch and enriched with higher CEO concentrations are effective in preserving mushroom firmness and extending postharvest shelf life by mitigating microbial and enzymatic activity during storage. [73]

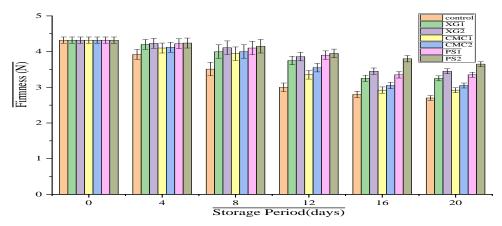


Figure 3: Effects of various coatings enriched with chamomile essential oil on Firmness (N) in mushroom samples stored at  $4\pm1$  °C for 20 days

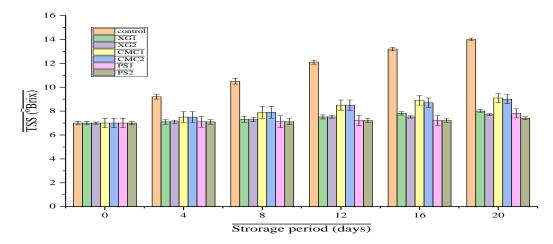
## 3.3.3. Total soluble solids (TSS)

Total Soluble Solids (TSS) serve as a key indicator of maturity, ripening, and overall biochemical changes in fruits and vegetables during storage. In this study **Figure 4**, the TSS values of mushrooms increased across all treatments during storage, with the **control group exhibiting the most substantial rise** from 7.00°Brix to 14.0°Brix over 20 days. This sharp increase indicates rapid dehydration and accelerated metabolic activity, consistent with earlier findings that attribute rising TSS to moisture loss, respiration, and the enzymatic breakdown of complex carbohydrates into simple sugars. [79, 80]

All edible coatings were effective in mitigating the increase in TSS, demonstrating their ability to **preserve** internal moisture and reduce the intensity of metabolic degradation. Among them, **potato starch coatings** (**PS1** and **PS2**) showed the smallest increase in TSS, ending at 7.81°Brix and 7.41°Brix, respectively, at Day 20. This suggests **PS coatings are the most efficient in creating a moisture barrier**, thereby minimizing concentration of solutes. Similarly, **xanthan gum coatings** (**XG1** and **XG2**) effectively slowed the rise in TSS, with final values of

8.01°Brix and 7.71°Brix, outperforming **carboxymethyl cellulose coatings** (CMC1 and CMC2), which reached 9.10°Brix and 9.00°Brix, respectively.

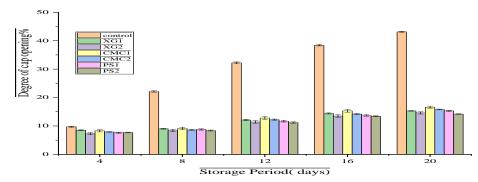
The trend that **1.0% CEO coatings consistently outperformed 0.5% CEO variants**, though not dramatically, further supports the role of chamomile essential oil in **suppressing microbial activity and reducing metabolic reactions.** [38] These findings align with previous studies: Elabd and Gomaa [50] reported a similar TSS retention using pectin-guar gum with cinnamon oil on persimmons, and Nasiri *et al.*, [52] noted TSS stability in mushrooms coated with tragacanth gum containing thyme and marjoram essential oils. Furthermore, Golmohammadi *et al.*, [81] showed that gelatin coatings with cinnamon oil significantly limited TSS rise in Agaricus bisporus.



**Figure 4**: Effects of various coatings enriched with chamomile essential oil on TSS (°Brix) in mushroom samples stored at  $4\pm1$ °C for 20 days

## 3.3.4. Degree of cap opening

Cap opening is a key physiological and visual indicator of mushroom aging and postharvest quality. A greater percentage of cap opening signifies advanced maturation and reduced marketability. In this study Figure 5, a gradual increase in cap opening was observed across all treatments, with the control group exhibiting the most pronounced increase (43.1%) by Day 20, indicating accelerated senescence due to dehydration and lack of barrier protection. Edible coatings significantly retarded cap opening, with the most effective being potato starch with 1.0% chamomile essential oil (PS2), which limited cap opening to just 14.2%. This was followed by XG2 (14.6%) and CMC2 (15.8%), suggesting that increased CEO concentration enhances the protective capacity of the coatings. The superior performance of PS coatings can be attributed to their strong moisture barrier and potential for partial occlusion of surface pores, which reduces water vapor and gas exchange (Wang et al., 2015). This moisture retention delays the weakening of protein water interactions responsible for veil rupture and cap separation. Furthermore, the gas permeability of coatings played a critical role. Lower oxygen permeability in coatings likely contributed to delaying cap opening by slowing respiration and metabolic degradation, in line with the findings of Gholami et al., (2020). The differences observed between 0.5% and 1.0% CEO coatings, though modest, reinforce the benefit of higher essential oil concentration in prolonging shelf life. These results are consistent with previous studies, Golmohammadi et al., [81] reported reduced cap expansion using BCNCs-gelatin coatings with cinnamon oil, Nasiri et al., [52] observed similar effects using tragacanth gum with thyme and marjoram essential oils, and Zhu et al., [2] demonstrated delayed veil rupture in Pholiota nameko using sodium alginate coatings with thymol, Lcysteine, and nisin.



**Figure 5**: Effects of various coatings enriched with chamomile essential oil on degree of cap opening% in mushroom samples stored at 4±1°C for 20 days

## 3.3.5. Effects of coatings on color indices and browning of mushrooms ( $L^*$ , $a^*$ , $b^*$ , $\Delta E$ , BI)

## 3.3.5.1. Effects of coatings on color indices and browning of mushrooms $(L^*, a^*, b^*)$

**Figure 6** illustrates the influence of various edible coatings enriched with chamomile essential oil on the lightness (L\*) values of mushroom samples stored at  $4\pm1^{\circ}$ C for 20 days. As L\* values reflect the degree of lightness, a reduction over time indicates progressive darkening, often associated with enzymatic browning, oxidation, and dehydration.

The control group (uncoated mushrooms) exhibited the most pronounced decline in lightness (**L\* values**), decreasing from 94.50 on day 0 to 63.40 by day 20. This sharp reduction reflects significant browning and deterioration in quality, largely due to the absence of a protective barrier to prevent moisture loss and oxidative reactions. These findings align with those of **Mohammadi** *et al.*, [71] and **Nasiri** *et al.*, [68] who observed similar discoloration trends in uncoated mushrooms during cold storage.

Among the coated treatments, PS2 was the most effective in preserving lightness, with L\* values declining only to 88.40 by day 20. This demonstrates the potent antioxidant and anti-browning properties of chamomile essential oil at higher concentrations, consistent with results reported by **Kumar** *et al.*, [82] and **Golmohammadi** *et al.* [81] XG2 also performed well, maintaining an L\* value of 86.90 at the end of the storage period. The incorporation of essential oils into xanthan gum matrices appears to enhance both light-barrier properties and enzymatic browning inhibition, corroborating the findings of **Plesoianu and Nour.** [83] Similarly, PS1 and CMC2 preserved L\* values of 84.70 and 84.80, respectively, underscoring the beneficial impact of higher essential oil concentrations on reducing discoloration. In contrast, XG1, CMC1, and the control group were the least effective in maintaining lightness. Their reduced performance is likely attributable to lower concentrations of chamomile oil and the comparatively weaker barrier properties of their polymer matrices. These outcomes are consistent with prior studies by **Rajabi** *et al.*, [84] and **Rizk** *et al.*, [85] which emphasize the critical influence of both polymer type and active compound concentration on coating effectiveness.

Although all treatments experienced a gradual decrease in L\* values over time, coatings enriched with higher levels of chamomile essential oil demonstrated a significantly slower rate of discoloration As discussed earlier Chamomile essential oil contains volatile bioactive compounds with strong antioxidant properties, such as Bisabolol oxides, Trans- $\beta$ -farnesene, and Chamazulene. These enhance sensory qualities and prevent oxidative spoilage, making the oil an effective natural preservative for extending shelf life and ensuring food safety (**Table 2**). This observation reinforces previous findings that highlight the role of essential oils in inhibiting polyphenol oxidase activity and preserving visual quality. [52]

A higher positive **a\* value** indicates increased redness, while lower values indicate less red or more green coloration. The data in **Figure 6** show the control group exhibited the most significant increase in a\* values, rising from 0.86 on day 0 to 6.40 by day 20. This marked color change indicates rapid surface browning and degradation, likely driven by enzymatic reactions and microbial activity. These findings are consistent with the observations of **Nasiri** et al., [68] and **Mohammadi** et al., [72] who reported considerable discoloration in uncoated mushrooms under similar refrigerated conditions. In contrast, coated mushroom samples showed a much slower rise in a\* values, suggesting better surface color preservation and delayed onset of redness. Among all treatments, the PS2 coating exhibited the greatest effectiveness, limiting the a\* value to just 3.05 by day 20. This result highlights the potent antioxidant activity of chamomile essential oil combined with the effective barrier function of the potato starch matrix, echoing the findings of **Kumar** et al., [82] and **Golmohammadi** et al. [81]

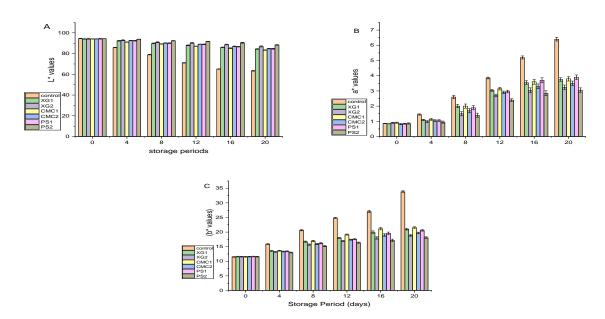
XG2 followed closely, with a final a\* value of 3.24. This can be attributed to the combined effects of xanthan gum's reduced oxygen permeability and the essential oil's antimicrobial and anti-browning properties, as also reported by **Plesoianu and Nour. [83]** Moderate protective effects were observed in PS1, CMC2, and CMC1, which resulted in final a\* values of 3.90, 3.50, and 3.80, respectively. These outcomes support the concentration-dependent role of chamomile essential oil in enhancing coating performance. This trend corroborates **Rajabi** *et al.*, [84] who emphasized that higher essential oil content improves a coating's ability to prevent oxidative discoloration and maintain product appearance during storage. Among the coated samples, XG1 containing a lower concentration of chamomile oil was the least effective, with a final a\* value of 3.75. Nevertheless, this still represented a significant improvement over the uncoated control.

Overall, while all treatments experienced some increase in redness over time, coatings enriched with higher concentrations of chamomile essential oil notably slowed the rate of discoloration. PS2 demonstrated the best performance, highlighting the synergistic interaction between biopolymer films and natural essential oils in mitigating oxidative and enzymatic deterioration. These findings reinforce the value of bio-based edible coatings enhanced with natural essential oils in extending mushroom shelf life. Such coatings not only serve as multifunctional barriers that preserve visual freshness but also provide antimicrobial and antioxidant protection, as supported by **Ganje** *et al.* [86] and **Rizk** *et al.* [85]

A higher b\* value indicates increased yellowness, while a lower value indicates less yellow or a shift towards blue. The results in Figure 6 demonstrate that the control mushrooms, which lacked any form of protective

coating, exhibited the most significant increase in b\* values from 11.50 on day 0 to 33.85 by day 20. This sharp rise reflects substantial spoilage and accelerated yellowing, likely due to enhanced enzymatic activity and oxidative degradation in the absence of a physical barrier. These findings align with those of **Nasiri** et al., [68] and **Mohammadi** et al., [72] who reported similar discoloration trends in uncoated mushrooms during refrigerated storage.

In contrast, mushrooms treated with the XG1 coating showed a more moderate increase in b\* value, reaching 21.00 by day 20. This improvement in color retention is likely attributed to the antioxidant and antimicrobial properties of chamomile essential oil. The XG2 formulation, containing a higher concentration of chamomile oil, yielded even better results, with b\* values peaking at only 18.90, underscoring the significance of oil concentration in enhancing color stability, as also reported by **Plesoianu and Nour [83]** and **Rajabi** *et al.*, **[84]**. Similarly, CMC-based coatings demonstrated improved effectiveness compared to the control. CMC1 and CMC2 exhibited b\* values of 21.55 and 19.70, respectively, with the superior performance of CMC2 likely due to its higher chamomile oil content. These outcomes are consistent with findings from **Ganje** *et al.*, **[86]**, which highlighted the efficacy of essential oil-enriched polysaccharide coatings in delaying yellowing. Likewise, PS1 coated mushrooms showed reduced yellowing, with b\* values rising to 20.63. However, the PS2 treatment combining potato starch with 1.0% chamomile oil proved to be the most effective, limiting the b\* value to just 18.08 by day 20. This minimal discoloration underscores the superior protective effect of PS2, likely resulting from the synergistic interaction between the biopolymer matrix and the essential oil, as supported by **Kumar** *et al.*, **[82]** and **Golmohammadi** *et al.*, **[81]**.



**Figure 6**: Effects of various coatings enriched with chamomile essential oil on color (L\*, a\*, b\* values) in mushroom samples stored at 4±1°C for 20 days

## 3.3.5.2. The total color difference ( $\Delta E$ )

 $\Delta E$  is a crucial indicator of overall color change, with higher values signifying more significant degradation of color. The data in **Figure 7** clearly show that control samples experienced the most significant color degradation, with  $\Delta E$  increasing from 9.76 on day 4 to 38.69 by day 20. This rapid discoloration is attributed to the lack of a protective coating, leading to faster spoilage and loss of visual quality, as is typical in untreated produce. [86]

In comparison, XG1-coated mushrooms had a more moderate  $\Delta E$  increase, reaching 13.79 by day 20. Though XG1 helped slow color change, it was less effective than coatings with higher chamomile oil concentrations. XG2, with a higher essential oil content, performed better, showing a  $\Delta E$  of 10.62 at day 20. These results are consistent with **Kumar** *et al.* [82], who reported that greater essential oil concentrations enhance coating efficacy by improving protective barriers.

CMC1 also reduced color change ( $\Delta E = 14.95$ ), but was still less effective than PS2 and XG2, suggesting the polysaccharide type plays a role in coating performance. CMC2, with increased chamomile oil, showed improved results ( $\Delta E = 12.67$ ), likely due to its enhanced protective capacity. PS1-treated mushrooms demonstrated a  $\Delta E$  of 13.48 by day 20, confirming the effectiveness of the potato starch–chamomile oil combination. However, PS2 yielded the best results, with the lowest  $\Delta E$  of 9.02, indicating minimal discoloration. This superior performance is likely due to the synergistic effect of the potato starch matrix and the higher chamomile oil content. [81]

These findings highlight the critical roles of both coating composition and chamomile essential oil concentration in maintaining appearance and extending mushroom shelf life.

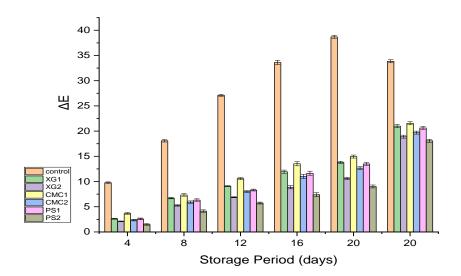


Figure 7: Effects of various coatings enriched with chamomile essential oil on  $\Delta E$  in mushroom samples stored at  $4\pm1$  °C for 20 days

## 3.3.5.3. Brown index values (BI)

The data in **Figure 8** illustrates the effects of different coatings enriched with chamomile essential oil on the browning index (BI) of mushroom samples stored at  $4\pm1^{\circ}$ C for 20 days. The browning index serves as a crucial indicator of color deterioration, with higher values correlating to increased browning and a subsequent decline in the quality of the mushrooms.

The control samples showed the highest increase in browning index, rising from 13.34 on day 0 to 60.77 by day 20. This sharp increase reflects significant quality deterioration, largely due to the lack of a protective coating. Without any barrier, the mushrooms were more prone to oxidation, enzymatic activity, and moisture loss all of which accelerated browning. These findings are consistent with earlier studies, which have demonstrated that uncoated mushrooms are more vulnerable to oxidative damage and enzymatic browning. [82]

XG1-coated mushrooms showed a moderate reduction in browning, with a browning index of 31.32 by day 20. While it performed better than the control, its effectiveness was limited compared to other coatings. In contrast, XG2, which contained a higher concentration of chamomile essential oil, was more effective, reducing the browning index to 26.77 by day 20. This suggests that increased chamomile oil enhances the coating's protective effect, likely due to its antioxidant and anti-enzymatic properties (**Golmohammadi** *et al.*, **2024**). Similarly, CMC1 reduced browning to some extent, with a browning index of 32.66 on day 20 better than the control but still less effective than PS2 and XG2. A more notable reduction was observed with CMC2, which achieved a browning index of 28.95, likely due to its higher chamomile oil content, which contributed to stronger browning inhibition. [81, 82] Mushrooms coated with PS1 showed a noticeable decrease in browning, reaching a browning index of 30.71 by day 20. Although PS1 was effective in reducing browning, it was outperformed by both PS2 and XG2. Among all treatments, PS2 with its higher concentration of chamomile essential oil demonstrated the greatest efficacy, maintaining the lowest browning index of 24.95 by day 20. These results indicate that PS2 was the most successful in preserving the mushrooms color and overall quality during storage. [86]

Browning increased over time across all treatments; however, coatings especially those with higher concentrations of chamomile essential oil significantly reduced browning compared to the uncoated control. These results highlight the importance of both coating type and chamomile oil concentration in maintaining the visual quality and shelf life of mushrooms during storage. [81, 82]

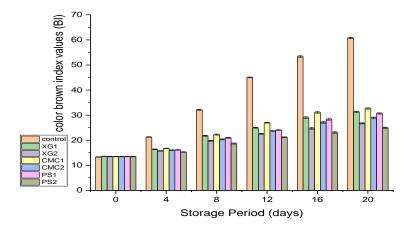


Figure 8: Effects of various coatings enriched with chamomile essential oil on color brown index values (BI) in mushroom samples stored at  $4\pm1$ °C for 20 days

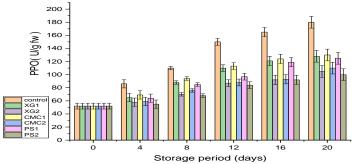
## 3.3.6. Effects of coating on enzyme activities (PPO) and (POD)

Enzymatic browning and oxidative degradation are fundamental processes that contribute to the postharvest deterioration of mushrooms. Polyphenol oxidase (PPO) and peroxidase (POD) are the principal enzymes involved in these oxidative processes. Both enzymes catalyze the oxidation of phenolic compounds to quinones, which are then polymerized to form brown pigments, a key visual indicator of quality loss in mushrooms. [87] This degradation not only compromises the visual appeal of mushrooms but also affects their nutritional properties and overall market value.

#### The control group in

Figure 9, lacking any protective edible coating, showed the highest PPO activity (180 U/g fw) by day 20, indicating accelerated enzymatic browning and substantial loss in commercial quality. In contrast, all edible coatings effectively suppressed PPO activity, thereby mitigating browning. Among all treatments, potato starch with 1.0% chamomile essential oil (PS2) demonstrated the lowest PPO activity (100 U/g fw), followed by XG2 (105 U/g fw) and CMC2 (110 U/g fw). This suggests that PS2 provided the most efficient enzymatic inhibition, possibly due to its excellent barrier properties, reducing oxygen availability needed for enzymatic oxidation. Furthermore, as discussed earlier, the volatile compounds present in chamomile essential oil contains powerful bioactive compounds such as Bisabolol oxides, Trans- $\beta$ -farnesene, and Chamazulene that offer strong antioxidant effects. These compounds enhance sensory appeal and help prevent oxidative rancidity, making the oil a valuable natural additive for improving food safety and extending shelf life (Table 2).

The trend of increasing CEO concentration (from 0.5% to 1.0%) resulting in reduced PPO activity was consistently observed across all coating types (PS, XG, and CMC). This supports the idea that higher levels of CEO enhance the protective effect by stabilizing phenolic compounds and inhibiting oxidative enzymes, as also noted by similar studies involving essential oil-enriched coatings on perishable produce. [50, 52] In agreement with the findings of Golmohammadi et al., [81] and Zhu et al., [2], who demonstrated reduced browning through antioxidant-rich coatings, the current results emphasize the effectiveness of biopolymer-based coatings in controlling enzymatic browning in mushrooms. PS2 emerged as the most promising formulation for preserving visual and nutritional quality during cold storage.



**Figure 9**: Effects of various coatings enriched with chamomile essential oil on PPO (U/g fw) in mushroom samples stored at 4±1 °C for 20 days

**Figure 10** shows changes in peroxidase (POD) activity, an enzyme linked to oxidative stress and deterioration in mushrooms. Higher POD levels suggest greater oxidative damage and faster spoilage. Control (uncoated mushrooms) showed the highest POD increased POD activity rose from 0.5 U/g fw (day 0) to 4.8 U/g fw (day 20). This confirmed significant oxidative degradation and poorer quality retention compared to coated samples. Coatings slowed POD activity increase (better preservation) XG1 reached 3.6 U/g fw at day 20, while XG2 had a lower increase (2.9 U/g fw). Higher CEO concentration 1% in XG2 resulted in better enzymatic control. CMC1 had 3.7 U/g fw, while CMC2 had 3.2 U/g fw. CMC2 performed better than CMC1, meaning chamomile essential oil (CEO) concentration 1% in CMC2 were more effective. Potato Starch Coatings (PS1, PS2) Provided the best protection PS1 (0.5% CEO) had 3.5 U/g fw, while PS2 (1.0% CEO) had the lowest value (2.8 U/g fw). PS2 was the most effective treatment, significantly reducing oxidative stress and extending shelf life. So uncoated mushrooms had the fastest enzymatic degradation, confirming the importance of coatings in extending shelf life. Increasing chamonil essenial oil concentration (from 0.5% to 1.0%) improved preservation, with PS2 (1.0% potato starch) being the most effective. Xanthan gum (XG2) and CMC2 were also effective, but slightly less than PS2.

#### From

Figure 9 and Figure 10, the PS2 coating, made from potato starch and 1.0% chamomile essential oil (CEO), was the most effective in preserving mushroom quality over 20 days. It significantly reduced the activity of browning-related enzymes (PPO and POD), delayed spoilage, and maintained sensory quality. The effectiveness was due to the combination of the starch barrier and the strong antioxidant and antimicrobial properties of chamomile oil. Higher concentrations of CEO (1.0%) provided 15–20% better enzyme inhibition compared to lower amounts. This is mainly due to active compounds in CEO such as bisabolol oxides, trans-β-farnesene, and chamazulene which help prevent browning, spoilage, and improve food safety and shelf life (Table 2). This finding aligns with previous research showing that coatings prevent oxidation in harvested fruits and vegetables by inhibiting the activities of enzymes like PPO and POD. [14, 88] And Lara et al., [89] which observed similar effects with xanthan gum coatings on fresh-cut lotus root, highlighting the potential of coatings to control enzymatic processes that cause browning. Additional studies using alternative coating materials, such as bitter almond gum-fish gelatin conjugates [90] and Phlorotannins from Sargassum ilicifolium seaweed combined with alginate, [91] further support the findings.

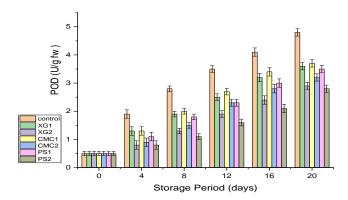


Figure 10: Effects of different various coatings enriched with chamomile essential oil on POD (U/g fw ) in mushroom samples stored at  $4\pm1$ °C for 20 days

## 3.3.7. Effect of coatings on Total phenolic contents (TPC)

Figure 11 shows the analysis of total phenolic compound retention in different coatings. Total phenolic content (mg gallic acid/100g) is a key indicator of antioxidant capacity in mushrooms, higher phenolic retention means better quality and antioxidant properties over storage time. Phenolic compounds are natural antioxidants found in plants that contribute to the color, shelf life, and health benefits of fruits and vegetables. However, their exposure to oxygen triggers degradation through polyphenol oxidase (PPO), leading to browning. [92] The study showed that edible coatings especially those containing chamomile essential oil help reduce oxygen exposure, thereby preserving phenolic content. In mushrooms, which are rich in phenolics and flavonoids, refrigerated storage led to a decline in phenolic levels, with the sharpest decrease seen in uncoated control samples. [93]

All treatments showed a decline in total phenolic content over 20 days. The uncoated mushrooms lost the most phenolics (from  $32.80 \text{ mg}/100\text{g} \rightarrow 14.65 \text{ mg}/100\text{g}$ ). Coated mushrooms retained more phenolics, with PS2

performing the best (ending at 23.40 mg/100g). Coated samples retained higher total phenolic content (TPC) in the mushrooms, with preservation levels increasing in proportion to the concentration of chamomile essential oil in the coating solution throughout the storage period. Uncoated mushrooms showed the sharpest decline, while coated samples retain more phenolics. The best overall PS2 was the highest phenolic retention after 20 days (23.40 mg/100g) and slowest degradation rate compared to other treatments. Thicker barrier from potato starch slowed moisture loss and oxidation, and higher chamomile oil (1.0%) provides stronger antioxidant protection. XG2 was the second-highest phenolic content at day 20 (21.32 mg/100g) Provided effective protection but slightly less than PS2. After day 8 of storage, the loss of total phenolic content (TPC) accelerated, likely due to oxidative and enzymatic activity. However, this loss was reduced in coated mushrooms, suggesting that the edible coatings helped inhibit browning-related enzyme reactions during storage. [94] Similar effects were observed by **Guo** *et al.*, [14] in shiitake mushrooms treated with chitosan and polysaccharide-based coatings. In this study, coatings that included bitter almond gum showed improved phenolic retention, with higher concentrations correlating with greater preservation. These findings are consistent with **Moradi** *et al.*, [90] who reported that bitter almond gum fish gelatin coatings at concentrations of 1–3% effectively preserved mushroom quality during 20 days of cold storage.

Higher chamomile oil (1.0%) significantly improved phenolic retention, especially in potato starch (26% better). XG2 also performed well with 17% improvement over XG1. CMC2 had the least improvement (only 3%), indicating that CMC alone is not as effective in preserving phenolics. Phenolic loss is highest in uncoated mushrooms (55.3%). While coatings help slow degradation, with PS2 losing only 27.8%.

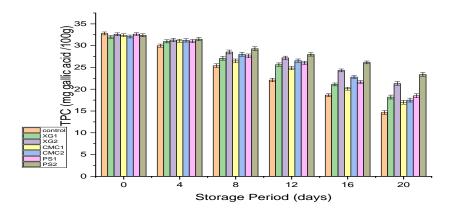


Figure 11: Effects of various coatings enriched with chamomile essential oil on Total phenolic compound contents (mg gallic acid /100g) in mushroom samples stored at 4±1°C for 20 days

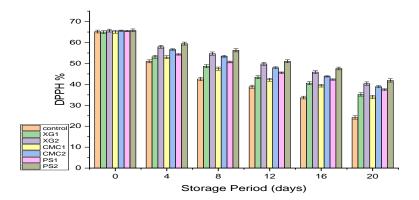
## 3.3.8. Effect of coatings on antioxidant activity DPPH

The **Figure 12** presents data on the effect of different coatings on the antioxidant activity (DPPH %) of mushroom samples stored at  $4\pm1^{\circ}$ C for 20 days. DPPH (2,2-diphenyl-1-picrylhydrazyl) % is a measure of antioxidant activity. A higher DPPH % means stronger antioxidant activity, which helps prevent oxidation and spoilage. The DPPH radical scavenging activity showed a decline during the refrigeration period (Fig.14). This reduction was more pronounced in the control samples compared to coated mushrooms, indicating that the coating may help preserve phenolic and flavonoid compounds along with their associated antioxidant activity. [14]

Antioxidant activity decreases over time for all treatments, indicating degradation of bioactive compounds during storage. And all coatings help in slowing down the decline of antioxidant activity. Control (uncoated samples) showed the steepest decline (from  $65.25\% \rightarrow 24.20\%$ ). Coated mushrooms retain more antioxidant activity, with PS2 performing the best (ending at 41.90%). Control loses antioxidant activity the fastest (62.9% decline). Coated mushrooms retain more antioxidant power than uncoated ones. Higher chamomile essential oil concentration (1.0%) enhances antioxidant retention.

Potato starch formed a strong protective barrier, reducing oxygen exposure. 1.0% chamomile essential oil boosts antioxidant stability. Better moisture retention prevents enzymatic oxidation. XG2 (1% chamomile essential oil) retained more antioxidant activity (40.40% at day 20) compared to XG1 (35.20%). Suggests that a higher concentration of chamomile essential oil essential oil performed better (38.95% at day 20) than CMC1 (34.00%). Indicates that increasing the essential oil concentration enhances preservation. PS2 (1% chamomile essential oil) maintained the highest antioxidant activity (41.90%) by day 20. PS1 (0.5% oil) also performs well (37.50%), but lower than PS2. Suggests that potato starch with essential oil is most effective in delaying antioxidant degradation. As discussed earlier, the volatile compounds present in chamomile essential oil contains powerful bioactive compounds such as Bisabolol

oxides, Trans-β-farnesene, and Chamazulene that offer strong antioxidant effects. These compounds enhance sensory appeal and help prevent oxidative rancidity, making the oil a valuable natural additive for improving food safety and extending shelf life (**Table 2**). The findings support previous research showing that edible coatings such as gum ghatti with essential oils [75] and formulations using Arabic gum or bitter almond gum—fish gelatin conjugates can effectively extend shelf life and preserve antioxidant activity in fresh produce.



**Figure 12**: Effects of various coatings enriched with chamomile essential oil on DPPH % in mushroom samples stored at 4±1°C for 20 days

#### 3.3.9. Effect of coatings on Malondialdehyde content (MDA)

MDA is a byproduct of lipid peroxidation and serves as a marker of oxidative stress. Elevated MDA levels indicate increased oxidative damage and potential deterioration in food quality. The data in **Figure 13** highlight the significant impact of various coatings on the reduction of lipid peroxidation (as measured by MDA content) in mushrooms stored for 20 days. The control group, without any coating, showed a marked increase in MDA levels from 0.280 nmol/g on day 0 to 5.40 nmol/g on day 20, reflecting rapid lipid degradation and poor-quality during storage, which aligns with previous findings by **Kumar** *et al.* **[82]** This suggests that uncoated mushrooms are highly susceptible to oxidative stress and spoilage.

The study demonstrated that the effectiveness of edible coatings in reducing lipid peroxidation, as measured by malondialdehyde (MDA) levels, varied depending on both the type of polysaccharide used and the concentration of chamomile essential oil (CEO). Among the xanthan gum-based coatings, XG2 containing a higher concentration of CEO was the most effective, reducing MDA levels to 2.70 nmol/g by day 20. This was superior to XG1 (3.30 nmol/g), which had a lower CEO content, aligning with **Golmohammadi** *et al.*, [81] and supporting the view that CEO concentration directly enhances antioxidant efficacy. [82, 86]

Similarly, carboxymethyl cellulose (CMC) coatings showed a dose dependent effect: CMC2, with a higher CEO content, reduced MDA to 2.95 nmol/g, compared to 3.60 nmol/g in CMC1. Also, Potato starch-based coatings exhibited the strongest antioxidant performance. The PS2 treatment, with the highest CEO concentration, achieved the lowest MDA level (2.60 nmol/g), outperforming PS1 (3.15 nmol/g). These findings underscore the synergistic role of potato starch and chamomile oil in minimizing lipid oxidation and preserving postharvest quality, consistent with earlier work. [81, 86]

So, coatings enriched with higher concentrations of chamomile essential oil particularly PS2, XG2, and CMC2 were most effective in reducing oxidative degradation and extending the shelf life of mushrooms. The potent antioxidant compounds in chamomile oil, including Bisabolol oxides, Trans- $\beta$ -farnesene, and Chamazulene (**Table 2**), not only inhibit lipid peroxidation but also enhance sensory quality and food safety, reinforcing the oil's value as a natural preservative.

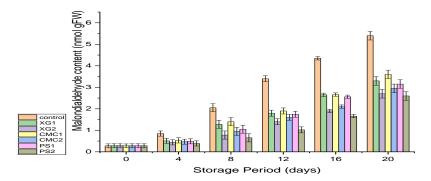


Figure 13: Effects of various coatings enriched with chamomile essential oil on Malondialdehyde content (nmol gFW) in mushroom samples stored at  $4\pm1$ °C for 20 days

## 3.4. Effect of coatings on microbiological quality (Psychrophilic, Mesophilic, Y&M)

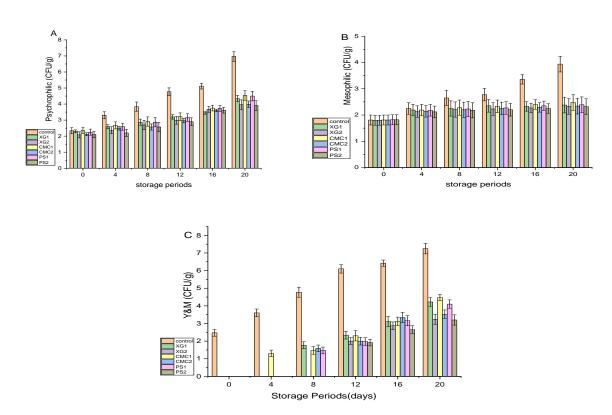
The initial microbial load of fresh mushrooms, measured at 1.81 log10 CFU/g for mesophilic bacteria, 2.34 log10 CFU/g for psychrophilic bacteria, and 2.47 log10 CFU/g for yeasts and molds, increased significantly over 20 days of refrigeration.

The control (uncoated mushrooms) experienced the highest increase in microbial populations, particularly in **psychrophilic bacteria Figure 14**, which rose from 2.34 CFU/g at day 0 to 6.97 CFU/g by day 20. In contrast, coated mushrooms showed significantly lower microbial counts (p < 0.05) at every time point. Among the coatings, PS2 demonstrated the best antimicrobial effect, with the lowest psychrophilic count (3.91 CFU/g) by day 20. XG2 and CMC2 showed similar effectiveness, with microbial counts of 3.96 CFU/g and 3.99 CFU/g, respectively, and were statistically comparable to PS2 but significantly lower than the control. Other coatings, such as PS1, XG1, and CMC, exhibited moderate inhibition of microbial growth, but their protective effects were less pronounced than those of PS2, XG2, and CMC2. By day 8, the control group had significantly higher psychrophilic counts (3.84 CFU/g) compared to the coated samples, which ranged from 2.57 to 2.91 CFU/g. The coatings, especially those with chamomile essential oil, effectively slowed the microbial growth, with the control group showing the highest microbial count at the end of the 20-day storage period. The coatings' ability to preserve membrane integrity and protect mushrooms from microbial contamination is attributed to their barrier properties, likely enhanced by the antimicrobial effects of chamomile essential oil.

The effects of different coatings on mesophilic bacterial growth in mushrooms Figure 14 were evaluated over 20 days of storage at 4±1°C, as presented in Table 19. Mesophilic bacteria are key spoilage organisms, and their growth negatively impacts the shelf life and safety of stored mushrooms. The uncoated mushrooms showed the fastest bacterial growth, increasing from 1.81 CFU/g at day 0 to 3.94 CFU/g by day 20, indicating rapid spoilage. This confirms that uncoated mushrooms are highly susceptible to mesophilic contamination. Coated mushrooms showed significantly lower bacterial counts compared to the control (p < 0.05), suggesting that the coatings act as a barrier against microbial contamination. Higher concentrations (1%) of chamomile essential oil (CEO) were more effective than 0.5% in reducing bacterial growth. Among the coatings, PS2 consistently exhibited the lowest mesophilic growth (2.32 CFU/g at day 20), making it the most effective formulation. XG2 and CMC2 also performed well, but PS2 was slightly superior, particularly in the later stages of storage. The presence of chamomile essential oil significantly reduced bacterial growth in a dose-dependent manner (1% > 0.5%), and the coatings with essential oil kept bacterial counts below 2.5 CFU/g by day 20, a substantial reduction compared to the control. The antimicrobial properties of chamomile oil, attributed to compounds like α-bisabolol and chamazulene, appear to disrupt bacterial cell walls and metabolic processes, extending the antimicrobial effect when combined with coatings. The coatings' effectiveness became more pronounced after day 8, suggesting that their protective benefits increase with prolonged storage time.

Yeast and mold (Y&M) contamination is a primary cause of spoilage in mushrooms. Fig. 14C highlights the effects of various edible coatings combined with chamomile essential oil (CEO) on Y&M growth during 20 days of storage at 4±1°C. Uncoated mushrooms exhibited the highest Y&M growth, increasing from 2.47 CFU/g at day 0 to 7.25 CFU/g by day 20, indicating that they are highly susceptible to fungal contamination. Coated mushrooms showed a significant reduction (40-60%) in fungal growth compared to the uncoated control, with all treatments proving effective in inhibiting Y&M development. The combination of coatings and higher concentrations (1%) of CEO was notably more effective than lower concentrations (0.5%). Among the treatments, PS2 demonstrated the most substantial antifungal activity, with the lowest Y&M growth at 3.20 CFU/g by day 20. XG2 also performed well, with no detectable Y&M growth until day 12. Coatings with 1% CEO consistently outperformed those with 0.5% CEO, particularly in PS2 and XG2 formulations.

Overall, the results show that the coatings, especially when combined with higher concentrations of chamomile oil, significantly reduced Y&M contamination, with PS2 providing the most effective long-lasting protection against fungal growth. The enhanced antifungal effect of CEO (1%) supports its use in improving the shelf life and safety of mushrooms during storage. As discussed earlier, the volatile compounds present in chamomile essential oil effectiveness in food preservation is due to its rich composition of bioactive compounds with antimicrobial, insect-repellent, and bacteriostatic properties. These include Bisabolol oxides,  $\beta$ -farnesenes, Citronellal, and Artemisia ketone. (Table 2), which help reduce microbial contamination and repel pests making the oil highly valuable for maintaining quality and safety in food storage systems and inhibit fungal growth. Disrupts bacterial and fungal cell walls and membranes, reducing spore formation. Synergistic effects with coatings help prolong antifungal activity over time. This supports previous findings by Rajabi et al. [84] and Ganje et al. [86] they found the inclusion of chamomile essential oil still provided notable antimicrobial benefits. Jiang et al., [69] who reported that essential oil-enriched coatings can effectively suppress spoilage microorganisms and enzymatic browning, thereby contributing to improved visual and microbial quality during storage. Similarly, Thakur et al., [95] found that carboxymethyl cellulose-based coatings reduced microbial loads on mushrooms in a concentration-dependent manner. This antimicrobial activity has been linked to higher phenolic content and Maillard reaction intermediates in denser coatings. [96, 97]



**Figure 14**: Effects of various coatings enriched with chamomile essential oil on Psychrophilic Mesophilic and Y&M (CFU/g) in mushroom samples stored at 4±1°C for 20 days

## 3.5. Effect of coatings on Sensory evaluation

**Figure 15** presents how different coatings affected sensory properties (off-odor, gill color, gill uniformity, cap uniformity, and dark zones) of mushroom samples stored at  $4\pm1^{\circ}$ C for 20 days. This study suggested that the essential oil-enriched coatings effectively maintained the mushrooms' appearance, aroma, texture, and overall acceptability during refrigerated storage.

The coatings, especially PS2 effectively delayed the formation of off-odor, with the treated mushrooms showing much lower off-odor scores compared to the control. Coated mushrooms retained better color, with PS2 and XG2 showing the best results in preventing browning. The coatings, particularly PS2 and XG2, helped maintain better gill and cap uniformity, reducing shrinkage and softening. These coatings provided structural integrity, which helped preserve the visual appeal and firmness of the mushrooms. These effects are largely attributed to the inhibition of polyphenol oxidase (PPO) and suppression of spoilage organisms, both critical for maintaining visual and sensory quality. [36, 98] Control samples had the darkest spots (8.12 at day 20), indicating severe spoilage. Coated

samples had significantly fewer dark spots and prevented dark spot formation, with PS2 (3.00) and XG2 (3.53) performing the best. Higher chamomile concentrations (1%) provided greater protection. PS2 was the most effective at preventing dark zone formation, followed by XG2 and CMC2.

So the best coatings for Sensory Quality is PS2, the second-best is XG2, CMC2 was also effective. Coatings with 0.5% chamomile (PS1, XG1, CMC1) were less effective but still better than control. Uncoated mushrooms (control) had the worst sensory deterioration. Chamomile oil played a key role in inhibiting polyphenol oxidase activity, which is responsible for browning. As discussed earlier, the volatile compounds present in chamomile essential oil contains powerful bioactive compounds such as Bisabolol oxides, Trans- $\beta$ -farnesene, and Chamazulene that offer strong antioxidant effects. These compounds enhance sensory appeal and help prevent oxidative rancidity, making the oil a valuable natural additive for improving food safety and extending shelf life (**Table 2**). These results are consistent with previous research. **Gholami et al.**, **Moradi et al.**, and **Huang et al.** which highlighted the role of coatings in extending the shelf life of fresh produce by limiting microbial contamination and oxidative degradation. [36, 59, 90]

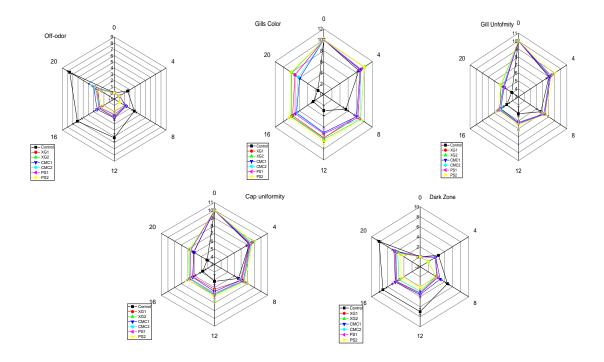


Figure 15: Effects of different coatings on Sensory properties of mushroom samples stored at 4±1°C for 20 days

## 4. Conclusion

This study confirms the effectiveness of edible coatings based on xanthan gum (XG), carboxymethyl cellulose (CMC), and potato starch (PS), in preserving the quality and extending the shelf life of button mushrooms during cold storage. These coatings significantly reduced weight loss, microbial growth, and enzymatic activity, thereby minimizing browning and spoilage while maintaining essential sensory attributes such as color, texture, odor, and appearance. The findings highlight the potential of natural, bioactive coatings as a sustainable approach to reducing postharvest losses in the mushroom industry. The incorporation of chamomile essential oil, with its notable antimicrobial and antioxidant properties, further supports the use of plant-based compounds in food preservation. Future research could investigate advanced enhancements such as nanotechnology or controlled-release systems to broaden the applications of these coatings across various types of perishable produce.

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#### 6. Institutional Review Board Statement

Not applicable

## 7. Data Availability Statement

Data are presented in the manuscript.

#### 8. Conflicts of Interest

The authors declare no conflict of interest

#### 9. References

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