Research Article

Gelatin-Based Edible Coating Combined with *Mentha pulegium* Essential Oil as Bioactive Packaging for Strawberries

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The effect of gelatin-based edible coating incorporated with *Mentha pulegium* essential oil (MEO) on physicochemical (pH, titratable acidity (TA), weight loss, total soluble solids (TSS), and total phenolic content (TPC)), microbiological (total aerobic mesophilic flora (TAMF) and yeasts and moulds (YM)), and sensorial (color and firmness) characteristics of strawberries stored under refrigeration was studied. Strawberries were coated with gelatin alone (4%) and/or gelatin combined with two concentrations (0.5 and 1%) of MEO and stored at 4°C for 13 days. Gelatin coating and MEO combination significantly inhibited total flora and moulds and yeasts with comparison to control (uncoated strawberries) and had better hygienic quality at the end of storage. The effect was MEO concentration dependent. Our results also showed that the bioactive coating used in this investigation slowed down changes in pH, TA, weight loss, TSS, firmness, TPC, and color of strawberries. Gelatin coating incorporated with MEO at 1% protected at least 60% of strawberries from deterioration after 13 days of storage and could be used as bioactive packaging to prolong the shelf life and an alternative of pesticides use.

1. Introduction

The Moroccan agricultural sector contributes around 12% of GDP and remains until today the largest employment provider in the country [1]. Loukkos and Gharb (Northwest of Morocco) are the most important agricultural regions. Red berries occupy the first place of cultivated products providing 169000 tons in 2017 [2].

Strawberries (*Fragaria*) are perennial plants belonging to the Rosaceae family. They are rich in antioxidants, polyphenols, and other beneficial nutrients [3]. However, strawberries are perishable and maintaining their freshness and quality after harvest is mostly related to the handling, transport, and storage methods that are used [4].

Most of the postharvest fruits and vegetables losses are caused by fungal and bacterial diseases. *Botrytis cinerea* is

a serious problem for strawberry growers. The disease can develop during storage if the fruit has been contaminated during harvesting or handling [5]. Also, *Rhizopus* soft rot caused by *Rhizopus stolonifer* can be problematic in small fruits. Cooling and fruit preservation by chemicals like fungicides are traditional techniques that could reduce the incidence of these fungi. However, the problem of resistance development in microbial strains questions their effectiveness especially when the regulation is stricter.

The use of biopolymers-based edible coatings for food preservation has been largely used in the last decades to improve the shelf life of fruits and preserve their freshness [6]. Edible coatings can be defined as a thin layer of edible material that coats the food and limits the migration of water vapor, oxygen, and CO₂, among others [7]. Biopolymers usually applied for food preservation can be of different

natures like protein, lipids, and polysaccharides. The chemical and physical characteristics of the biopolymer used determine their efficacy to be used for food shelf life prolongation. These coatings can also become bioactive by the incorporation of biomolecules with antimicrobial and antioxidant properties.

Essential oils have been largely used to preserve the quality of food due to their antimicrobial and antioxidant activities [8–13]. MEO is considered as one of the most effective essential oils against a large spectrum of bacterial and fungal strains [14, 15]. Several biopolymers-based coating incorporated with EOs have been used for strawberries preservation [16, 17]. Due to their mechanical resistance, barrier properties, and sensorial acceptability, gelatin has been largely used to prolong the shelf life of foods such as meat and fish [18–20]. However, to the best of our knowledge, the effect of gelatin coating on strawberry preservation is limited and has been used in only a few studies [21].

In this sense, our work aims at evaluating the combined effect of gelatin-based coating and MEO on physicochemical, microbiological, and sensorial characteristics of strawberries during storage under refrigeration.

2. Materials and Methods

2.1. Raw Material. Fresh strawberries (Sabrina) were collected from the experimental center of the National Institute of Agronomic Research (Larache, Morocco) during the season of 2018. They were transported to the laboratory. Noninjured fruit were sorted for their uniformity in size, firmness, and color.

2.2. Essential Oil Isolation. Essential oil used in this investigation was obtained by hydrodistillation from plants of *M. pulegium* collected in the northeastern part of Morocco (Oujda). The taxonomic identities of the plants were confirmed by comparing voucher specimens with those of known identity previously deposited in the National Herbarium of the Scientific Institute of Rabat.

2.3. Gelatin Coating Formation. Twelve grams of gelatin powder (HiMedia Laboratories Pvt. Ltd, India) was dissolved in 300 mL of sterile distilled water and stirred at 45°C for 10 min. Sorbitol (Loba Chemie Pvt. Ltd, India) (w/w of gelatin) was added to increase mixture stability. 1.5 and 3 mL of MEO were added to prepare bioactive gelatin coating at 0.5 and 1%, respectively. TWEEN® 80 was used as an emulsifier.

After mixture homogenization, washed strawberries were dipped into the prepared solution for about 90 seconds, they were filtered thereafter using a filter funnel and reset into labeled plastic punnets. Four treatments were prepared:

- T1: control (uncoated strawberries)
- T2: gelatin coating without MEO
- T3: gelatin coating with 0.5% MEO
- T4: gelatin coating with 1% MEO

The punnets were packed with a plastic wrap and stored in at 4°C. The physicochemical, microbiological, and sensorial parameters were tested on the first day and after 3, 7, 10, and 13 days.

2.4. Microbiological Parameters. The count of total aerobic mesophilic flora (TAMF) and yeasts and moulds (YM) were performed according to Jay et al. [22] after 0, 3, 7, and 10 days of storage.

2.5. Physicochemical and Sensorial Parameters. pH, total soluble solids (TSS), and titratable acidity (TA) were measured as described in official methods of analysis [23].

The maturity index (MI) was calculated as the ratio of TSS and TA. Fruit firmness was measured using a TA1 texture analyzer (Ametek Lloyd Instruments Ltd, UK), by setting crosshead speed at 1 mm/s and penetration depth at 10 mm. Firmness, expressed in *N*, is the textural parameter measured on the resulting force-distance curve.

Fruit color was measured using CR-300 Chroma Meter (Konica Minolta, CR-300, Osaka, Japan), and *L** (lightness), *a** (redness), and *b** (yellowness) values were obtained. The chroma value *C* was calculated using the following equation:

$$C = (a^2 + b^2)^{1/2}. \quad (1)$$

The difference between the initial and final weight was considered as the total weight loss. Strawberry samples were weighed at the beginning (i.e., 0 days) and at the end of each storage interval. The results were expressed as weight loss percentage. Total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent (Solvachim Laboratoires, Casablanca) according to the method described by Siriwoharn et al. [24] using gallic acid (Loba Chemie Pvt. Ltd, India) as standard. The calibration curve was plotted by mixing 0.5 mL of 10, 50, 100, 200, and 400 ppm gallic acid solution with 7.5 mL of distilled water and 0.5 mL of the Folin–Ciocalteu reagent. 0.5 mL of the strawberry sample was added to 7.5 mL of distilled water and 0.5 mL of the Folin–Ciocalteu reagent. 0.5 mL of distilled water was used for blank preparation. Afterwards, all tubes were mixed with a vortex and held for 10 min at room temperature. Then, 3 mL of sodium carbonate (20%) solution was added to each test tube. Tubes were stirred and placed in a water bath at 40°C for 20 min and then cooled directly in an ice bath for 3 min. The absorbance of all samples was measured using a 755 nm UV spectrophotometer (JP Selecta S.A., Spain). TPC was expressed in mg gallic acid equivalent (GAE)/g dry weight.

Visual decay was determined by a visual inspection at 0, 3, 7, 10, and 13 storage days and was estimated as the number of infected fruits divided by the total number of fruits, expressed in percentage (%).

2.6. Statistical Analysis. All measurements were done in triplicates. Data analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests to evaluate significant differences among the

FIGURE 1: Total aerobic mesophilic flora (TAMF) (a) and yeast and mould (YM) (b) counts of coated and uncoated strawberries during storage at 4°C for 10 days. Vertical bars represent the standard deviation ($n = 3$).

samples at 95% confidence interval using SPSS computer program, version 20.0. The level of significance was $p \leq 0.05$.

3. Results and Discussion

3.1. Microbiological Characteristics of Control and Gelatin and/or Gelatin + MEO Coated Strawberries. The effect of gelatin and/or gelatin + MEO coating at two concentrations (0.5 and 1%) on the evolution of TAMF and YM during storage at 4°C is shown in Figures 1(a) and 1(b).

Initial microbial load of fresh strawberries in this study was 2.3 log-UFC/g and 2.2 log-UFC/g for TAMF and YM, respectively. Our values are similar to those obtained by Shahbazi [25]. Microbial load was increased significantly ($p \leq 0.05$) with increasing storage time in all treatments. Gelatin coating alone (T2) retarded slowly (<0.7 log-UFC/g) the microbial growth after 10 days of storage. Gelatin coating incorporated with MEO (1%) (T4) delayed significantly ($p \leq 0.05$) the microbial growth by 1.74 log-UFC/g and 0.82 log-UFC/g for TAMF and YM, respectively, after 10 days. At the end of storage, TAMF and YM were in the order of 3.63 and 3.89 log CFU/g, respectively, for gelatin + MEO (1%) (T4) coated strawberries, which is under the limit of acceptance established for the shelf life of fruit-based products [26] and indicate a good hygienic quality of the fruits after 10 days of storage. The inhibition magnitude was EO concentration dependent. This is due to antibacterial and antifungal activities of the MEO largely demonstrated [15, 27]. Similar behavior was observed by Raquel et al. [28]

who found that YM and psychrophils flora decrease following the application of antimicrobial coating. Shahbazi [25] also showed that the use of chitosan coating with *Mentha spicata* EO was significantly effective against total bacteria, YM, and extend the shelf life of fresh strawberries at least up to 12 days. In our case, the antimicrobial activity of *M. pulegium* may be attributed to the oxygenated monoterpenes such as pulegone and piperitone generally found in *M. pulegium* EOs [15].

3.2. Physicochemical and Sensorial Characteristics of Control and Gelatin and/or Gelatin + MEO Coated Strawberries

3.2.1. pH, Titratable Acidity (TA), Total Soluble Solids (TSS), and Maturity Index (MI). Variations of pH, TA, TSS, and MI during storage at 4°C are shown in Table 1. TA and TSS content decreased while pH increased significantly ($p \leq 0.05$) during the storage period for all treatments. Gelatin + MEO (1%) (T4) coating showed the lowest increase in pH (3.98) after 13 days, which indicates that the use of this coating slows down changes in pH values.

Table 1 shows also that gelatin-based coating (T2, T3, and T4) reduced considerably the decrease of TA after 13 days of storage in comparison with the control (T1). Our results are similar to those obtained by Shin et al. and Petriccione et al. [29, 30] who reported that TA decreased with increasing storage period of strawberries. The same authors studying the effect of chitosan coating on strawberries have noticed that TA decrease was more pronounced

TABLE 1: pH, total soluble solids (TSS), titratable acidity (TA), and maturity index (MI) of coated and uncoated strawberries during storage at 4°C for 13 days.

Storage time (days)		0	3	7	10	13
pH	T1	3.80 ± 0.04 ^{Aa}	3.89 ± 0.02 ^{Ab}	3.93 ± 0.01 ^{Ab}	4.19 ± 0.01 ^{Ac}	4.08 ± 0.02 ^{Ad}
	T2	3.63 ± 0.03 ^{Ba}	3.72 ± 0.07 ^{Ba}	4.09 ± 0.01 ^{Bbc}	4.13 ± 0.01 ^{Bb}	4.01 ± 0.01 ^{Bc}
	T3	3.76 ± 0.02 ^{Aa}	3.80 ± 0.15 ^{ABbb}	3.84 ± 0.01 ^{Cb}	3.97 ± 0.01 ^{Cc}	4.03 ± 0.02 ^{Bd}
	T4	3.62 ± 0.02 ^{Ba}	3.76 ± 0.02 ^{Bb}	3.79 ± 0.01 ^{Db}	3.88 ± 0.01 ^{Dc}	3.98 ± 0.01 ^{Cd}
TSS (brix)	T1	8.53 ± 0.15 ^{Aa}	7.47 ± 0.25 ^{Ab}	6.63 ± 0.15 ^{ABc}	5.17 ± 0.12 ^{Ad}	4.97 ± 0.12 ^{Ad}
	T2	8.03 ± 0.15 ^{ABb}	6.83 ± 0.06 ^{Bb}	6.80 ± 0.17 ^{Ab}	5.20 ± 0.10 ^{Ac}	5.30 ± 0.17 ^{Ac}
	T3	8.33 ± 0.15 ^{Ba}	6.43 ± 0.12 ^{Cb}	6.37 ± 0.06 ^{Bb}	5.53 ± 0.58 ^{Bc}	5.07 ± 0.11 ^{Ad}
	T4	7.96 ± 0.15 ^{Ba}	6.77 ± 0.06 ^{BCb}	6.57 ± 0.06 ^{ABbc}	6.47 ± 0.12 ^{Cc}	6.10 ± 0.10 ^{Bd}
TA (%)	T1	0.57 ± 0.02 ^{ABa}	0.45 ± 0.01 ^{Ab}	0.25 ± 0.02 ^{ACd}	0.22 ± 0.01 ^{Ac}	0.27 ± 0.01 ^{Ad}
	T2	0.53 ± 0.01 ^{Aa}	0.47 ± 0.02 ^{Ab}	0.31 ± 0.01 ^{Bc}	0.23 ± 0.01 ^{Ad}	0.37 ± 0.01 ^{Be}
	T3	0.58 ± 0.01 ^{Ba}	0.39 ± 0.03 ^{Bb}	0.40 ± 0.01 ^{Cb}	0.34 ± 0.02 ^{Bc}	0.31 ± 0.01 ^{Cc}
	T4	0.56 ± 0.01 ^{ABa}	0.49 ± 0.01 ^{Ab}	0.47 ± 0.01 ^{Dbc}	0.44 ± 0.01 ^{Cc}	0.37 ± 0.02 ^{Bd}
Maturity index (%)	T1	14.93 ± 0.29 ^{Aa}	16.51 ± 0.22 ^{Aad}	26.93 ± 2.48 ^{Ab}	23.29 ± 0.25 ^{Ac}	18.49 ± 0.56 ^{Ad}
	T2	15.07 ± 0.54 ^{Aa}	14.45 ± 0.82 ^{ABa}	21.83 ± 0.54 ^{Bb}	24.48 ± 0.72 ^{Ac}	14.29 ± 0.92 ^{Ba}
	T3	14.26 ± 0.48 ^{Aa}	16.46 ± 1.47 ^{Aa}	15.87 ± 0.25 ^{Ca}	18.98 ± 0.89 ^{Bb}	16.28 ± 0.65 ^{Ba}
	T4	14.26 ± 0.63 ^{Aa}	13.56 ± 0.37 ^{Ba}	13.99 ± 0.38 ^{Ca}	11.83 ± 0.11 ^{Cb}	16.28 ± 0.97 ^{Bc}

The values are given as mean ± standard deviation ($n=3$). Different capital letters within a column indicate significant differences among treatments ($p \leq 0.05$). Different small letters within a row indicate significant differences due to storage time ($p \leq 0.05$). T1: control; T2: gelatin coating only; T3: gelatin + MEO (0.5%) coating; T4: gelatin + MEO (1%) coating.

in uncoated samples (control). Previous studies have suggested that the higher acidity loss in uncoated fruits is due to the use of organic acids as substrates for respiratory metabolism during storage [31]. In this sense, Gol et al. [16] reported that coatings can slow down respiration rate and may therefore reduce the use of organic acids which leads to the retention of TA in coated fruits.

The decrease of TSS content in gelatin + MEO (1%) coating (T4) was delayed significantly ($p \leq 0.05$) in comparison to the control (T1) at the end of storage (day 13). Our results agree with those obtained by Amal et al. [32] who reported that incorporation of thymol EO in soy protein or gluten films was the most effective treatments in maintaining TSS during storage and concluded that the application of coatings on the surface of the strawberry reduced respiration rate, hence reducing the loss of TSS. Finally, MI calculated as TSS/TA ratio, the most important parameter for evaluating strawberries quality, shows that uncoated strawberries (T1) exhibited a significant bigger increase ($p \leq 0.05$) with comparison to the coated ones (T2, T3, and T4), reflecting reduced ripening compared to uncoated strawberries.

3.2.2. Weight Loss and Total Phenolic Content. Figures 2 and 3 show the effect of gelatin and/or gelatin + MEO coating at 0.5 and 1% on the weight loss and TPC of strawberries during the storage at 4°C, respectively.

Weight loss increased throughout the storage period for the control (T1) and gelatin coated (T2, T3, and T4) strawberries with significant differences ($p \leq 0.05$). Uncoated fruits (T1) had the highest weight loss (24%) after 13 days while gelatin + MEO (1%) (T4) had the lowest percentage of weight loss (3%) being under the limit for strawberries acceptance (6%) (Figure 2). Gelatin alone (T2) also reduces weight loss but not as effective as gelatin + MEO (1%) (T4) treatment. Our results are in accordance with

FIGURE 2: Weight loss of coated and uncoated strawberries during storage at 4°C for 13 days. Vertical bars represent the standard deviation ($n=3$).

Wang et al. [33] demonstrating that coatings reduce respiration rate and water loss. Fakhouri et al. and Poverenov et al. [21, 34] report that among the substances used to coat and prolong the shelf life of fruits, gelatin is a feasible alternative to avoid the product's weight loss after harvest. The evolution of TPC in all treatments is summarized in Figure 3.

Total phenolic content (TPC) obtained in this study are in the range of those obtained by Hoda et al. [35] but superior of Li et al. [36] (80 mg/100 g). Anttonen et al. [37] reported that TPC varies according to many parameters such as variety, level of fertilization, or date of planting. Figure 3 shows also that TPC tends to decrease through the storage

FIGURE 3: Total phenolic content (TPC) of coated and uncoated strawberries during storage at 4°C for 10 days. Vertical bars represent the standard deviation ($n = 3$). Bars with different small letters indicate significant differences due to storage time ($p \leq 0.05$).

TABLE 2: Firmness and color of coated and uncoated strawberries during storage at 4°C for 13 days.

Storage time (days)		0	3	7	10	13
Firmness (N)	T1	2.14 ± 0.56 ^{Aab}	3.07 ± 0.62 ^{Aa}	3.05 ± 0.25 ^{Aa}	2.52 ± 0.23 ^{Aab}	1.29 ± 0.86 ^{Ab}
	T2	2.87 ± 0.81 ^{Aab}	3.87 ± 0.99 ^{Aab}	4.86 ± 0.57 ^{Aa}	3.60 ± 0.25 ^{Bab}	2.16 ± 1.57 ^{Ab}
	T3	2.93 ± 0.92 ^{Aa}	4.01 ± 1.38 ^{Aa}	4.65 ± 2.07 ^{Aa}	4.77 ± 0.27 ^{Ca}	2.95 ± 1.17 ^{Aa}
	T4	2.24 ± 0.23 ^{Aa}	3.01 ± 0.40 ^{Aa}	3.41 ± 1.13 ^{Aa}	2.77 ± 0.62 ^{ABa}	2.91 ± 0.92 ^{Aa}
<i>L*</i> value	T1	46.16 ± 0.92 ^{Aa}	42.13 ± 2.25 ^{Aab}	43.68 ± 1.78 ^{Aab}	40.93 ± 2.58 ^{Abc}	37.11 ± 0.99 ^{Ac}
	T2	44.04 ± 0.61 ^{Aa}	42.06 ± 1.66 ^{Aab}	42.37 ± 1.60 ^{Aab}	41.39 ± 3.01 ^{Aab}	38.77 ± 1.88 ^{ABb}
	T3	45.98 ± 0.59 ^{Aa}	42.50 ± 1.44 ^{Aab}	42.42 ± 2.08 ^{Aab}	42.90 ± 0.51 ^{Aab}	40.06 ± 1.79 ^{ABb}
	T4	39.04 ± 1.03 ^{Ba}	46.33 ± 1.63 ^{Ab}	40.15 ± 0.79 ^{Aa}	40.93 ± 2.58 ^{Aa}	41.39 ± 1.48 ^{Ba}
Chroma value	T1	30.31 ± 8.37 ^{Aa}	24.22 ± 1.87 ^{Aab}	21.36 ± 2.10 ^{Aab}	19.47 ± 1.11 ^{Aab}	17.56 ± 3.52 ^{Ab}
	T2	24.69 ± 0.33 ^{Aa}	20.09 ± 2.25 ^{Aab}	16.98 ± 1.65 ^{Ab}	17.66 ± 2.09 ^{Ab}	21.19 ± 1.72 ^{Aab}
	T3	23.77 ± 2.78 ^{Aa}	18.88 ± 2.24 ^{Aa}	19.46 ± 1.65 ^{Aa}	18.26 ± 1.14 ^{Aa}	20.09 ± 3.95 ^{Aa}
	T4	31.77 ± 4.62 ^{Aa}	22.31 ± 2.89 ^{Ab}	19.43 ± 2.20 ^{Ab}	17.19 ± 0.74 ^{Ab}	20.93 ± 2.61 ^{Ab}

The values are given as mean ± standard deviation ($n = 3$). Different capital letters within a column indicate significant differences among treatments ($p \leq 0.05$). Different small letters within a row indicate significant differences due to storage time ($p \leq 0.05$). T1: control; T2: gelatin coating only; T3: gelatin + MEO (0.5%) coating; T4: gelatin + MEO (1%) coating; *L**, lightness.

period also in coated (T2, T3, and T4) and uncoated (T1) strawberries. The decrease in TPC through storage could be attributed to the degradation of anthocyanins during the senescence period [38]. Gelatin coating alone (T2) slightly reduces TPC loss. Li et al. [36], using starch-based coating for strawberries preservation, noted that coating reduces the losses of TPC. The same result was obtained by Gol et al. [16] using chitosan-based coating. Surprisingly, gelatin + MEO (1%) (T4) coating was the most effective treatment that limits losses in TPC after 10 days of storage. In a similar trend, Oms-Oliu et al. [39] concluded that coating treatment with chitosan, starch, and cinnamon EO promotes enzymatic activity of phenylalanine ammonia lyase responsible for the accumulation of total phenols in strawberries.

3.2.3. Firmness, Color, and Decay Rate. Firmness is one of the most important quality parameter for strawberries

acceptance. In our study, gelatin-based coatings did not affect significantly firmness of the strawberry fruit during cold storage, and the firmness of the fruits was maintained (Table 2). Our results agree with those obtained by Poverenov et al. [34] for red bell peppers using chitosan/gelatin coating. Amal et al. [32] and Petriccione et al. [30] also showed that strawberries lose their firmness during the storage due to cell walls degradation during storage. The same authors pointed out the positive effect of coating to limit firmness loss. The color parameter is usually used to evaluate the quality and ripening degree of fruits. The results obtained in this study are shown in Table 2, and color changes were estimated by *L* and chroma values.

Despite that all treatments (from T1 to T4) show significant decrease in the estimated values, gelatin coating clearly slows down these changes. Similar trends were reported by Petriccione et al. [30] and Hernández-Muñoz et al. [40]. Velickova et al. [41] reported that coatings protect

FIGURE 4: Visual decay of coated and uncoated strawberries during storage at 4°C for 13 days. Vertical bars represent the standard deviation ($n = 3$).

fruits from advanced atmospheric dehydration and consequently delayed their senescence.

Visual decay of uncoated (T1) and coated (T2, T3, and T4) strawberries during storage time at 4°C is shown in Figure 4. As it can be observed, gelatin + MEO (1%) (T4) was the most effective treatment protecting at least 60% of strawberries from decay after 13 days of cold storage while gelatin alone (T2) was the lowest effective treatment. Our results are in accordance with those obtained by Li et al. [36] using a polysaccharide-based coating for strawberries preservation. The high protection level in visual decay for T4 treatment can be attributed to antimicrobial activity of MEO, as previously demonstrated with microbes being the first responsible for strawberries decay.

4. Conclusions

This study shows the effectiveness of coating-based gelatin + MEO (1%) for postharvest strawberries preservation up to 13 days by delaying microbial growth. Physicochemical and sensorial properties (weight loss, TA, TSS, firmness, color, and TPC) of coated strawberries were always better than those of the control. Gelatin coating alone was not as effective as gelatin incorporated with MEO. The use of this combination would be a good alternative to extend shelf life of strawberries and to limit the use of pesticides in post-harvest treatments.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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