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ORIGINAL ARTICLES

Coating based on Montmorillonite, essential oils, and amaranth to preserve mango

Evelyn Erika Pillco Ramos[1]*, Maria Cecilia Pacco-Huamani[1], Sandriane Pizato[2], Rosalinda Arévalo Pinedo[3], William Renzo Cortez-Vega[2] and Grethel Teresa Choque Delgado[1]

[1]Departamento Académico de Ingeniería de Industrias Alimentarias, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú

[2]Departamento de Engenharia Agrícola e de Solos, Faculdade de Ciências Agrárias, Universidade Federal do Amazonas, Manaus, AM, Brasil

[3]Departamento de Engenharia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Federal da Grande Dourados, Dourados, MS, Brasil

**epillcor@unsa.edu.pe*

Abstract

This study aimed to evaluate the coating based on Amaranthus flour (AF), montmorillonite, and three essential oils (clove, muña, and matico) to extend the shelf life of minimally processed mango. The mango cubes were divided into four different treatments. T1- control (uncoated mango), T2 (0.3% w/v of clove), T3 (0.3% w/v of muña), and T4 (0.3% w/v of matico). All treatments had 0.6% w/v Amaranth flour and 0.02% w/v montmorillonite (MMT) and were subjected to 5°C for 12 days. Water activity (Aw), pH, Total soluble solids, acidity, weight loss, color, texture, and antimicrobial activity were evaluated for each treatment. Matico treatment maintained pH and had the lowest count of yeast and mold forming units on mango (3.47 log UFC g-1). On the last day of storage, all coating treatments showed less weight loss and favorable results than the control. The matico treatment showed higher efficiency for mango preservation.

Keywords: *amaranthus caudatus, buddleja globose, minthostachys mollis, montmorillonite, syzygium aromaticum.*

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1. Introduction

Mango (*Mangifera indica* L) belongs to the *Anacardiaceae* family and it is one of the most popular tropical fruits with high nutritional value and good organoleptic characteristics[1]. This fruit is climacteric and has a short shelf life of 4-8 days, however, this could last longer if post-harvest handling and storage conditions are adequate[2]. Mango export requires refrigeration or freezing due to its metabolic changes and fast maturation process[3]. Consequently, alterations in fruit occur in the sensory attributes such as color and texture[4]. Additionally, the hydrolysis of starch into sugars causes softening in mango[3].

Currently, there are new technologies to preserve fruits and increase their shelf life[5]. Edible films and coatings have been shown to have a good gas barrier effect. However, these preservation methods lack antimicrobial agents against molds and yeasts that could improve their preservation properties[6,7]. Coatings act as a protective layer that delays the ripening and respiration of fruits[8].

Coatings could maintain the fruit's quality and extend its shelf life due to the addition of biodegradable polymers and essential oils. Montmorillonite is an easily adsorbed nanoclay, with a porous structure, and a uniform surface that could improve the permeability capacity coating[9]. Kiwicha (*Amaranthus caudatus* L.) is an Andean grain and has high nutritional value due to its essential amino acids, vitamins, and dietary fiber[10]. In addition, its starch has water-soluble, gelling, and thickening characteristics[11]. On the other hand, essential oils have antioxidant and antimicrobial properties. Clove essential oil (*Syzygium aromaticum*) presents bioactive antioxidants such as eugenol and its derivatives. Eugenol is a phenolic compound with antifungal and antioxidant properties[12]. Muña (*Minthostachys mollis)* is a shrub native to the high Andean area of South America[13]. It also presents monoterpenes of the menthone and pulegone type[14]. Matico (*Buddleja globosa*) is a shrub native to Chile, Peru, and Argentina. Its main component is flavonoids[15].

Coating studies based on native essential oils applied to fruits are scarce. This manuscript aimed to evaluate three different Andean essential oils in coating based on Amaranth and nanoclay to extend the shelf life of minimally processed mango.

2. Materials and Methods

2.1 Materials

Mangoes from the Tommy Atkins variety were purchased from a local market in Dourados-MS, Brazil. The fruits were

selected according to their color and size. The matico oil was acquired from the ORUM Company in Tacna, Peru. The muña oil was bought from the Nua Company in Tarapoto, Peru. Finally, clove oil was obtained from the local market of Dourados-MS, Brazil. Amaranth flour was obtained from a milling process and previously selected raw grains from Arequipa, Peru.

2.2 Mango processing

Mango stems were removed with a stainless steel knife. Then, the mango was sanitized in a sodium hypochlorite solution at $2gL^{-1}$ for 3 minutes and the water was drained into sieves for 3 minutes. The mango peel was removed and the pulp was cut into pieces of 2 x 2 x 2.5cm.

2.3 Development of coatings

To 500 mL of heated water, 500 mL of a mixture of distilled water and amaranth flour (0.6%) was added with slow homogenization. The mixture was heated at 80 °C for 20 minutes with constant magnetic stirring. Subsequently, 0.02% of montmorillonite (MMT) was added with constant magnetic stirring for 20 minutes until reaching 60 °C. At that temperature, 0.1% of glycerol was added, and stirring continued for another 20 minutes. Finally, 0.3% of essential oils were added at different solubilization temperatures (Clove at 40°C, Muña and matico at 35°C). The mango pieces were divided into four treatments: T1 - Control (uncoated); T2 - clove oil (0.3%); T3 - muña oil (0.3%); and T4 - matico oil (0.3%). All treatments contained 0.6% of Amaranth flour, 0.1% of glycerol, and 0.02% of MMT. The mango pieces were immersed in the different coatings for 5 minutes and they drained in sieves for another 5 minutes. Then, they were packaged in polyethylene terephthalate (PET) with a lid (Sandpack). Finally, they were refrigerated at 5 °C for 12 days.

2.4 Characterization of coatings

Physical, chemical, and microbiological analyses were performed in triplicate for days 0, 1, 3, 5, 7, 9, and 12 of storage.

2.4.1 pH, Total Soluble Solids (TSS), and Titratable acidity

To determine the pH, 5 g of sample was mixed in 50 ml of distilled water and it was measured in a Quimis benchtop pH meter. The analysis was carried out according to the official method[16] with some modifications. The total soluble solids content was examined using an Abbé bench refractometer and the results were expressed in °Brix. The titratable acidity was determined by titrating 5 ml of the sample, previously homogenized with 50 mL of distilled water, and 4 drops of phenolphthalein using 0.1 mol/L NaOH.

2.4.2 Weight loss

To determine weight loss, mango pieces refrigerated at 5±1°C were weighed on an analytical balance on days 0, 1, 3, 5, 7, 9, and 12 of storage. The weight loss was obtained from the difference in the initial and final weight of the mango multiplied by 100 in each analysis. Results were expressed as a percentage of weight loss.

2.4.3 Color

Color measurements were performed with a Konica Minolta colorimeter (Model CR-400/CR-410, Osaka, Japan) previously calibrated, using the CIE L*a*b system (Commission Internationale de LÉclairage).

2.4.4 Texture

The mango pieces were cut and sheared uniaxially. Its texture was determined with a texturometer (TA-XT Plus, Godalming, UK). The cutting of the samples was at 2 mm/s uniaxially. Shearing of the sample was carried out with a blade at a distance traveled of 35 mm. The cutting force was expressed in Newton (N)[17].

2.4.5 Antimicrobial activity

Microbiological analyses were performed for molds and yeasts, *Salmonella spp*., and *Escherichia coli* (ATCC 25922), following the methods described in APHA - American Public Health Association[18].

2.5 Statistical analysis

The results were statistically evaluated using the Analysis of Variance (ANOVA) and the Tukey test at 5% significance. It used the STATISTIX ® 10.0 program.

3. Results and Discussions

3.1 pH, Total Soluble Solids (TSS), and Titratable acidity

Table 1 shows the pH values for the mango pieces coated for 12 days at 5 ± 1°C. Treatments T1 and T2 decreased their pH significantly during storage time compared to treatments T3 and T4, which maintained their values. At the end of the storage period, T3 (0.3% Muña) showed a significant difference in pH (3.5) compared to the other treatments.

Other studies showed similar pH values. Sánchez Aldana et al.[19] made edible coatings based on a pectic extract of Mexican lemon and essential oil applied to Haden mango. They obtained pH values of 3.2 on the 15th day of storage. Moreover, Ploy and Rungsinee[20] showed pH values between 3-4 in mango coated with nanocomposites based on hydroxypropylmethylcellulose and Thai essential oils during 15 days of storage. The decrease in pH in the coated mango during storage would be caused by carbonic acid due to the fermentation of sugar in the fruit during its ripening[21].

Regarding TSS values in mango pieces coated with Amaranth flour and different essential oils, all treatments significantly increased TSS during the 12 days of storage except treatment 2 (0.3% clove). The control treatment showed a significant increase in TSS compared to the coating treatments at the end of storage. Studies carried out by Passafiume et al.[22] showed a similar behavior in mango pieces coated with neem oil during the storage period. They reported 13.9 °Brix on the last day of storage. Moreover, Gupta et al.[23] showed an increase in TSS of 18-21°Brix in mango coated with polysaccharides during storage. The increase in TSS in mango pieces during storage is due to the transformation of organic matter into sugars, acids, and minerals[24].

Table 1. pH, Total soluble solids, and Titratable acidity values of mango coated with essential oil of clove, muña, and matico at 5 ± 1°C during 12 days of storage.

	Days	Treatments			
		T1	T2	T3	T4
pH	0	3.53 ± 0.14^{aB}	3.75 ± 0.00^{aA}	3.58 ± 0.04^{aAB}	3.59 ± 0.06^{aAB}
	1	3.47 ± 0.08^{abB}	3.73 ± 0.03^{aA}	3.56 ± 0.01^{aB}	3.55 ± 0.09^{aB}
	3	3.38 ± 0.04^{abcB}	3.73 ± 0.04^{aA}	3.54 ± 0.10^{abAB}	3.52 ± 0.13^{aAB}
	5	3.13 ± 0.01^{dB}	3.35 ± 0.02^{bcAB}	3.43 ± 0.02^{bcAB}	3.51 ± 0.27^{aA}
	7	3.29 ± 0.01^{bcdA}	3.30 ± 0.03^{bcA}	3.41 ± 0.01^{cA}	3.37 ± 0.18^{aA}
	9	3.33 ± 0.05^{bcA}	3.43 ± 0.04^{bA}	3.43 ± 0.03^{bcA}	3.42 ± 0.04^{aA}
	12	3.23 ± 0.02^{cdB}	3.27 ± 0.12^{cB}	3.53 ± 0.01^{abcA}	3.30 ± 0.02^{aB}
Total soluble solids (°Brix)	0	10.25 ± 0.35^{cA}	10.17 ± 0.29^{aA}	9.75 ± 0.35^{bA}	9.83 ± 0.29^{bA}
	1	10.67 ± 0.76^{bcA}	10.25 ± 0.29^{aA}	9.83 ± 0.29^{abA}	9.88 ± 0.25^{bA}
	3	11.00 ± 0.58^{bcA}	10.33 ± 0.29^{aA}	10.00 ± 0.41^{abA}	10.13 ± 0.63^{bA}
	5	11.75 ± 0.35^{bA}	10.50 ± 0.41^{aB}	10.25 ± 0.35^{abB}	10.17 ± 0.29^{bB}
	7	13.17 ± 0.29^{aA}	10.67 ± 0.29^{aB}	10.50 ± 0.50^{abB}	10.33 ± 0.29^{abB}
	9	13.67 ± 0.29^{aA}	10.75 ± 0.65^{aB}	10.63 ± 0.48^{abB}	10.67 ± 0.29^{abB}
	12	14.00 ± 0.50^{aA}	10.83 ± 0.29^{aB}	10.88 ± 0.25^{aB}	11.38 ± 0.48^{aB}
Titratable Acidity (g/100ml citric acid)	0	0.41 ± 0.05^{aA}	0.36 ± 0.02^{aA}	0.41 ± 0.04^{aA}	0.40 ± 0.09^{aA}
	1	0.37 ± 0.02^{abA}	0.34 ± 0.03^{aA}	0.39 ± 0.05^{abA}	0.36 ± 0.09^{abA}
	3	0.35 ± 0.07^{abA}	0.34 ± 0.05^{aA}	0.32 ± 0.04^{abcA}	0.33 ± 0.01^{abA}
	5	0.33 ± 0.05^{abA}	0.34 ± 0.01^{aA}	0.30 ± 0.00^{bcA}	0.30 ± 0.01^{abA}
	7	0.31 ± 0.01^{abA}	0.32 ± 0.03^{aA}	0.29 ± 0.00^{cA}	0.29 ± 0.01^{abA}
	9	0.26 ± 0.08^{abA}	0.29 ± 0.03^{aA}	0.28 ± 0.05^{cA}	0.24 ± 0.07^{bA}
	12	0.19 ± 0.03^{bB}	0.28 ± 0.02^{aA}	0.28 ± 0.01^{cA}	0.23 ± 0.02^{bAB}

Averages of three repetitions ± standard deviation, followed by the lowercase letter in the column (difference between storage days) and the row (differences between treatments) by Tukey test ($p > 0.05$). T1: control (uncoated); T2: 0.3% clove essential oil; T3: 0.3% muña essential oil; T4: 0.3% matico essential oil.

During the 12 days of storage, all treatments showed a decrease in titratable acidity (Table 1). For T1, T3 and T4, its decrease was significant. At the end of storage, the coating treatments showed acidity values of 0.23 - 0.28 and they were significantly different from the control treatment. The decrease in titratable acidity was also reported by Ali et al.[25], they applied a coating based on tragacanth gum to the mango and showed values between 0.1 – 0.6% on the last day of storage. In addition, Coelho et al.[26] applied biodegradable coating with 10% gelatin and rice flour on mango and obtained 0.75 $g.100^{-1}$ of citric acid during 10 days of storage. This result would be associated with the degree of ripeness of the mango.

3.2 Weight loss

Figure 1 shows the weight loss values of the minimally processed mango for a storage period of 12 days at 5 ± 1°C. All treatments showed a significant increase in weight loss during storage days. The control treatment obtained greater weight loss compared to the coating treatments that presented values of 8.21-8.58%. Other studies have presented weight loss results, such as those by Yu et al.[24] evaluated the coating of chitosan with cinnamon essential oil on mangoes. Weight loss ranged from 13.76 - 18.24% during 12 days of storage. Moreover, Santacruz and Hurel[27] reported an 8.4% weight loss in mangoes coated with cassava starch, cinnamaldehyde, and thymol after 14 days of storage. Fruit weight loss is caused by damage to the plasma membrane under cold conditions. This is due to the production of malondialdehyde, a byproduct of lipid peroxidation and oxidative stress that damages the cell membrane and causes electrolyte leakage[28]. Our study shows lower values of weight loss compared to the studies mentioned above. This coating could reduce cell membrane damage and protect the fruit from chilling injury.

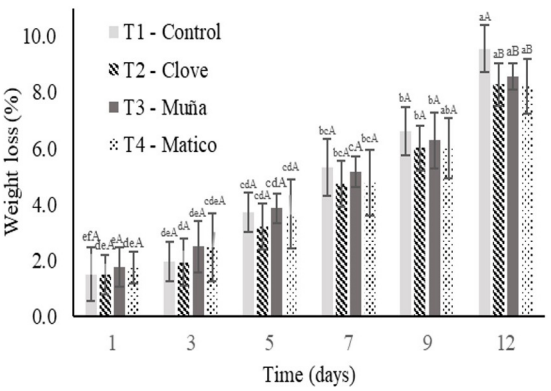

Figure 1. Weight loss of mango coating based on amaranth flour, montmorillonite, clove, muña, and matico essential oil in different concentrations at 5±1 ºC, for 12 days.

3.3 Color

Our results showed a decrease in L*, C, and h color levels. This could be due to the presence of the polyphenol oxidase enzyme that causes the oxidation of phenol and produces the browning of the fruit during cold storage[28].

Table 2 shows the color values L*, C*, and h. Regarding L*, all treatments decreased significantly during the 12 days of storage. However, T1 and T2 show a greater decrease compared to T3 and T4 at the end of storage.

Similar studies reported a progressive and significant decrease in L* values during storage days. Passafiume et al. [22] applied edible coating based on neem oil on cut mango. After 9 days of storage, L* values of 65 – 70 were reported. On the other hand, Lieu and Dang[29] used a 2.5% pectin/nano-chitosan coating applied to mango and showed a decrease in L* during the 15 days of storage. Moreover, Marín et al. [30] evaluated edible coatings with carboxymethylcellulose, aloe vera powder, whey protein isolate, potassium ascorbate, and antioxidants (citric acid and acetyl-N-cysteine). After 14 days of storage, L* values decreased from 72 to 66 on the last day of storage for the Tommy Atkins-type mango.

Regarding C values, all treatments showed a significant decrease during the 12 days of storage. The treatment values on the last day were 24 – 41. However, studies carried out by Mshora et al.[31] presented C* values of 65 – 75 on the last day of storage for mango coated with chitosan. In addition, Sarria et al.[32] carried out studies on mango with edible avocado oil coating where the C* value presented values of 45 – 67.

Finally, Treatments 3 and 4 showed a significant decrease in h values during storage time (81-86). Studies carried out by Mshora et al.[31] obtained lower h values (65 - 78) in mango coated with chitosan during 28 days of storage. On the other hand, Hernández-Guerrero et al.[33] coated mango with banana, soursop, and mango starch for 15 days of storage. They presented h values of 90 -100 the first 10 days, however, this value decreased to 70 on day 15 in all their treatments.

3.4 Texture

Figure 2 shows the texture values, T1 had a significant increase during the 12 days of storage. A study carried out by Almeida et al.[34] also demonstrated an increase in the texture of the control sample over the days of storage on minimally processed pineapple. The authors related this increase to the loss of mass (moisture) of the control sample during the 12 days of storage, whereas treatments with gum tragacanth and tea tree essential oil managed to maintain the texture of the pineapples for longer. On the other hand, our coating treatments showed a significant decrease during storage days. On the last day, their values were 16, 19, and 23 for muña, matico and clove, respectively. Studies carried out by Wang et al.[35] on dip coating with chitosan, sodium alginate, and cinnamon essential oil on mango also showed decreasing texture values during 10 days of storage. Their values were 25.47 and 17.62 (N). Moreover, Chuacharoen and Sabliov[36] presented decreasing texture values during the storage of mango coated with zein nanoparticles loaded with curcumin. On the ninth day, 6.91 (N/cm2) was reported.

3.5 Antimicrobial activity

Figure 3 shows the growth of molds and yeasts in fresh-cut mangoes. The presence of *Escherichia coli* ($<10^2$ CFU.g^{-1}) and *Salmonella sp.* (absence in 25 g) was not detected in fresh-cut mango pieces. It confirms the efficiency of good manufacturing practices and the effectiveness of the organic chlorine used to disinfect the fruits. Similar results were found in other studies with minimally processed fruits coated with gums. They presented low *E. coli* counts and the absence of *Salmonella*[37,38].

Table 2. Color obtained from mango coated with essential oil of clove, muña, and matico at 5±1°C for 12 days of storage.

	Days	Treatments			
		T1	T2	T3	T4
L*	0	64.13 ± 0.46^{aB}	56.97± 0.93^{aC}	67.98 ± 0.01^{aA}	70.97± 1.40^{aA}
	1	56.60± 0.53^{bB}	55.02± 0.82^{bB}	67.05± 0.94^{abA}	67.85 ± 0.45^{aA}
	3	54.99± 0.37^{cB}	53.65± 0.57^{bcB}	65.91 ± 0.58^{bcA}	66.50 ± 1.00^{aA}
	5	53.38 ± 0.21^{dB}	52.28± 0.32^{cB}	64.77± 0.25^{cA}	65.14± 1.55^{aA}
	7	51.78 ± 0.64^{eB}	47.86± 0.47^{dC}	62.11± 0.91^{dA}	62.03± 0.58^{bA}
	9	50.06 ± 0.62^{fC}	46.04± 0.05^{dD}	58.26 ± 0.27^{eB}	59.79± 0.57^{bcA}
	12	48.25 ± 0.10^{gB}	43.51 ± 1.07^{eC}	58.14± 0.45^{eA}	57.86 ± 0.75^{cA}
C	1	49.58 ± 0.39^{bA}	43.33 ± 0.65^{bB}	48.43 ± 1.09^{aA}	50.97 ± 1.66^{aA}
	3	43.28 ± 0.58^{cA}	35.91 ± 0.46^{cB}	45.72 ± 1.43^{abA}	44.59 ± 1.29^{bA}
	5	36.98 ± 0.77^{dB}	28.47 ± 0.27^{dC}	43.01 ± 1.32^{bcA}	38.21 ± 0.93^{dB}
	7	32.49 ± 0.13^{eC}	34.22 ± 0.06^{cC}	41.40 ± 0.61^{cdA}	38.07 ± 1.46^{dB}
	9	31.53 ± 0.56^{efB}	24.88 ± 1.92^{eC}	39.42 ± 0.45^{deA}	39.11 ± 0.42^{cdA}
	12	30.37 ± 0.53^{fC}	24.18 ± 0.02^{eD}	37.98 ± 0.20^{eB}	41.83 ± 0.20^{bcA}
h	1	84.06 ± 0.01^{cB}	84.04 ± 1.08^{cB}	89.16 ± 0.53^{aA}	88.16 ± 0.91^{aA}
	3	84.64 ± 0.52^{bcB}	84.87 ± 0.86^{cB}	88.37 ± 0.71^{abA}	87.82 ± 0.55^{aA}
	5	85.20 ± 0.74^{abcB}	85.70 ± 0.65^{bcB}	87.72 ± 0.40^{abA}	87.48 ± 0.21^{aA}
	7	87.07 ± 0.30^{aA}	87.47 ± 0.65^{abA}	87.26 ± 0.97^{abA}	88.49 ± 0.86^{aA}
	9	86.22 ± 0.89^{abB}	88.09 ± 0.28^{abA}	86.50 ± 0.98^{bAB}	86.90 ± 0.18^{aAB}
	12	85.11 ± 0.80^{abcB}	89.15 ± 0.91^{aA}	86.39 ± 0.45^{bB}	81.49 ± 1.07^{bC}

Averages of three repetitions ± standard deviation, followed by the lowercase letter in the column (difference between storage days) and the row (differences between treatments) by Tukey test ($p > 0.05$). T1: control (uncoated); T2: 0.3% clove essential oil; T3: 0.3% muña essential oil; T4: 0.3% matico essential oil.

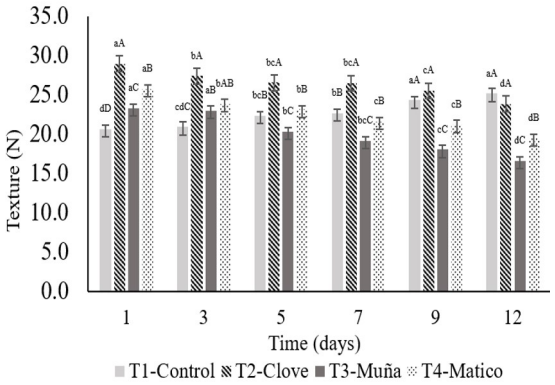

Figure 2. Texture (N) from coated mango with amaranth flour, montmorillonite, and essential oil of clove, muña, and matico at 5±1°C for 12 days of storage.

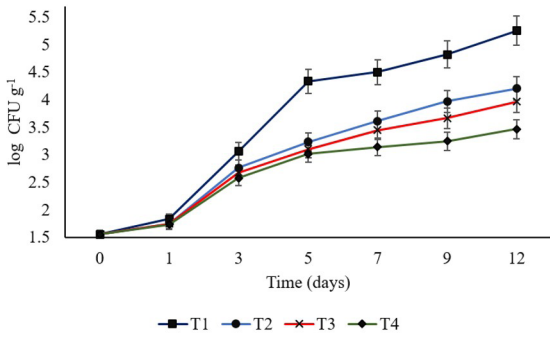

Figure 3. Count of molds and yeasts in minimally processed mangoes coated with Amaranth flour, montmorillonite, and different essential oils. T1 – control treatment; T2 – clove essential oil; T3 – muña essential oil; T4 – matico essential oil.

Rojas-Graü et al.[39] affirm that the excessive growth of molds and yeasts can compromise the sensorial acceptance of fruits. The acceptable microbial load was established at 10^6 CFU.g^{-1} as numbers exceeding this limit can lead to the appearance of toxic substances. Our results show that there was a growth of molds and yeasts over the days of storage for all treatments evaluated. The control treatment (T1) showed the greatest increase. From day 5 onwards, the difference between the other treatments was significant and on the 12th day, the minimally processed uncoated mangoes showed a growth of 5.26 log CFU.g^{-1}. Treatments T2 and T3 (4.21 and 3.97 log CFU.g^{-1} respectively) showed similar behavior during the days of analysis. At the end of 12 days of cold storage, they did not notice any significant difference. Treatment T4, which contained matico essential oil, was the one with the lowest mold and yeast counts during refrigerated storage. This differs statistically from the other treatments evaluated (3.47 log UFC g^{-1}).

The lowest mold and yeast counts were for treatments T2, T3, and T4. It is due to essential oils that have antimicrobial properties, especially matico essential oil. The mechanism of antimicrobial action of essential oils could be related to the ability of phenolic compounds to disrupt the cytoplasmic membrane of microorganisms. The hydrophobic components of essential oils penetrate the plasma membrane and it produces inhibition of the cell's functional activity. Moreover, it produces the separation of lipids from the cell and mitochondrial membrane and causes cell death[40].

Barbosa et al.[41] obtained a similar microbial inhibition. They used polymeric capsules of rosewood and cinnamon essential oil. Nandhavathy et al.[42] determined the efficiency of biocomposite films made from pomegranate peel fibers incorporated with thyme and clove essential oil. Thyme oil was more effective than clove oil, with an inhibition percentage of up to 66%. Cai et al.[43] also had promising results, they used starch films containing thyme essential oil microcapsules. These films slowed down the mango ripening and reduced the loss of vitamin C. The starch film with the addition of thyme essential oil inhibited the appearance of spoilage microorganisms. These results are similar to the present study and essential oils delayed the growth of molds and yeasts in fresh-cut mangoes.

4. Conclusions

This study showed the effectiveness of coatings based on biopolymers and native essential oils to extend the shelf life of minimally processed mangoes. Coated treatments using essential oils presented prominent results in physicochemical and less growth for mold and yeast. Matico treatment stands out for having the lowest antimicrobial activity in freshly cut mangoes. In addition to essential oils, elicitors such as γ-aminobutyric acid are suggested to improve tolerance against abiotic stress and regulate the physiological processes of the fruit. Coatings or films with those components would have a synergistic effect and would prolong the shelf life and preserve the quality of the fruit.

5. Author's Contribution

- **Conceptualization** – Evelyn Erika Pillco Ramos; Grethel Teresa Choque Delgado; William Renzo Cortez Vega
- **Data curation** – Evelyn Erika Pillco Ramos; Maria Cecilia Pacco Huamani
- **Formal analysis** – Evelyn Erika Pillco Ramos; Maria Cecilia Pacco Huamani
- **Funding acquisition** – Evelyn Erika Pillco Ramos
- **Investigation** – Evelyn Erika Pillco Ramos
- **Methodology** – William Renzo Cortez Veja; Maria Cecilia Pacco Huamani
- **Project administration** – Grethel Teresa Choque Delgado
- **Resources** – William Renzo Cortez Veja; Sandriane Pizato; Rosalinda Arévalo Pinedo; Evelyn Erika Pillco Ramos
- **Software** – NA
- **Supervision** – Grethel Teresa Choque Delgado
- **Validation** – Evelyn Erika Pillco Ramos
- **Visualization** – Evelyn Erika Pillco Ramos; Grethel Teresa Choque Delgado

- **Writing – original draft** – Evelyn Erika Pillco Ramos; Grethel Teresa Choque Delgado
- **Writing – review & editing** – Grethel Teresa Choque Delgado

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