

Using Cannabidiol as a potential postharvest treatment to maintain quality and extend the shelf life of strawberries

Haley Inselberg*, Maria Cecilia do Nascimento Nunes

Food Quality Laboratory, Department of Cell Biology, Microbiology and Molecular Biology, University of South Florida, 4202 E. Fowler Avenue, Tampa, FL 33620, USA

ARTICLE INFO

Keywords:

Strawberry
Cannabidiol
CBD
Natural antimicrobial
Quality
Shelf life

ABSTRACT

Cannabis has been used in ancient medicine to treat a wide array of medical issues. Specifically, Cannabidiol (CBD), a non-psychoactive component of cannabis, has been linked to containing antimicrobial properties. However, research surrounding the potential use of CBD as an antimicrobial agent is still preliminary. This study aims to examine the potential of CBD oil as a postharvest treatment used by consumers at home to reduce microbial growth and extend the shelf life of strawberries. CBD oil was applied to fresh fruit after harvest followed by storage at 1 °C for 8 days and 10 °C for 8 days. Strawberries were evaluated for visual quality and microbial load before and during storage. Results from this study showed that CBD oil was effective at maintaining the visual appearance of strawberries, above the minimum threshold of a visual rating score of 3, compared to the fruit that was not treated. It was also found that CBD oil was effective at reducing the microbial load on treated strawberries compared to fruit that was not treated. This research shows that CBD oil has the potential to be used by consumers at home as an effective antimicrobial treatment and to extend strawberry shelf life.

1. Introduction

Cannabidiol (CBD) is a non-psychoactive cannabinoid of cannabis that has many beneficial properties. The *Cannabis sativa* plant contains over 80 biological compounds, but CBD and THC (delta-9-tetrahydrocannabinol) are the most known (Mermelstein, 2019). Both compounds have gained popularity due to their ability to treat certain diseases, such as epilepsy (Głodowska and Łyszcz, 2017). These cannabinoids have also been found to possess antibacterial properties and have been shown to inhibit the growth of certain bacteria such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Cassano et al., 2013; Fathordoobady et al., 2019).

Cannabis has a stigma associated with it in modern society, but it has been used for centuries as a common prescription for illness in ancient medicine (Głodowska and Łyszcz, 2017). The first recorded use of cannabis as a medicinal treatment was in ancient China around the year 4000 BCE (Fathordoobady et al., 2019). It was used to treat rheumatic pain, intestinal disorders, issues with the female reproductive system, malaria, and various other health concerns (Głodowska and Łyszcz, 2017). In modern times, cannabis has been demonstrated to efficiently treat a multitude of different diseases such as epilepsy, Parkinson's, multiple sclerosis, Tourette's syndrome, and other neurological diseases

(Głodowska and Łyszcz, 2017). In addition to treating neurological disorders, cannabis has shown potent antimicrobial, insecticidal, and preservative properties (Nafis et al., 2019). In particular, the oil extracted from the plant contains potent antioxidants, which can help with disease prevention and a healthy lifestyle (Yu et al., 2005). Several have studied the use of CBD against antimicrobial activity in human diseases, but there's still a lack of research on its effect against plant pathogens (Głodowska and Łyszcz, 2017).

With so many benefits associated with CBD consumption, it is no surprise that the food industry has begun to incorporate this non-psychoactive component into food products. The fast increase in public popularity has been met with slow action from the Food and Drug Administration (FDA) to regulate cannabis products. This lack of regulation has resulted in products being marketed to the public as treatments for medical conditions with no proof and no guarantee of high quality (U.S. Food and Drug Administration, 2020). Currently, the FDA has only approved one CBD prescription drug product, Epidiolex, which has been found to safely and effectively treat epilepsy (Mermelstein, 2019; FDA, 2020). Due to this lack of federal monitoring, there are still many uncertainties surrounding the use of CBD as a food additive and the science and benefits behind using CBD in food formulations. While marijuana is still illegal on a federal level, hemp products (including

* Corresponding author.

E-mail address: hinselbe@gmail.com (H. Inselberg).

CBD) are legal in all US states (Mermelstein, 2019). This legalization has made CBD infused products a popular and growing alternative to the use of THC containing products in the United States. With the legalization and acceptability of using CBD, more research can be conducted to determine the benefits of adding CBD to food products. Additionally, the scope of legality means that CBD-containing products will be subject to the same standards of quality and safety as food products (Mermelstein, 2019).

The use of CBD as an antimicrobial agent is still new and has not been fully investigated (Apendino et al., 2008). All five of the major cannabinoids found in cannabis have antimicrobial properties, and studies have shown that they are effective at eliminating methicillin-resistant *Staphylococcus aureus* (MRSA) (Apendino et al., 2008; Fathordoobady et al., 2019)). CBD has also shown to be effective at inhibiting the growth of both gram-positive and gram-negative bacteria, as well as certain pathogenic fungi like *Aspergillus niger*, which can cause rot in fruits and vegetables, and *Candida albicans*, which can cause infection in humans (Głodowska and Lyszcz, 2017; Fathordoobady et al., 2019). However, such findings are difficult to confirm as studies have not been replicated and have been done generally. Thus, more research is needed to access CBD's true potential against pathogens (Apendino et al., 2008). Nevertheless, the use of CBD shows promising potential, and due to the rise in antibiotic resistance, it is important to access its use as a potential alternative to current antimicrobials. It is rare for resistance to occur between bacteria and antimicrobial agents in plants (Apendino et al., 2008; Lopez-Romero et al., 2015). Therefore, CBD could be a promising solution to the problem of treating antibiotic resistant bacteria. CBD has many characteristics that make it a good candidate as an antimicrobial. However, as of now, no products containing CBD are being used to extend the shelf life of food products while there is growing consumer demand for environmentally safe and natural products (Benelli et al., 2018).

The main objective of this study was to assess the potential use of CBD as a postharvest treatment applied onto the surface of strawberries by consumers at home to delay microbial growth and maintain quality, thus extending fruit shelf life. This was done by using CBD oil as an edible coating to protect the strawberries from deterioration. Edible coatings have been shown to protect some fruits and vegetables from perishing too quickly and succumbing to decay. Currently, edible coatings made of pectin, pullulan, and chitosan have been used on strawberries to extend shelf life (Treviño-Garza et al., 2015). These edible coatings have been shown to maintain firmness, delay microbial growth, and maintain the color, flavor, and texture of coated fruits and vegetables (Salehi, 2020). Additionally, the coated strawberries experienced an increased shelf life compared to the strawberries that were not coated (Treviño-Garza et al., 2015). In our experiments, CBD oil was applied as a coating on strawberries' fruit and calyx. The oils used in this experiment were coconut oil and MCT oil. MCT oil contains only medium chain triglycerides (MCT), while coconut oil contains long chain triglycerides (LCT) (Wang et al., 2018). MCT oil is derived from CBD oil and is highly processed to remove the LCT so that only MCT remain (Wang et al., 2018). MCT oil also remains a liquid at room temperature, while coconut oil remains more solid. These oils have also shown to have antimicrobial potential and have been able to inhibit the growth of *Staphylococcus aureus* (Widjaningrum et al., 2019). MCT oil is commonly used in the cannabis industry as a medium to mix with CBD isolate.

Initially, the study was conducted as a preliminary experiment using store-bought strawberries, and only one storage temperature was used. However, after obtaining promising results from this preliminary experiment, more inclusive experiments were conducted. In the second experiment, strawberries were obtained fresh from the field, treated immediately with CBD coating, stored at optimum, and non-optimal temperature conditions for 8 or 9 d and evaluated for microbial load and physical appearance (i.e., color, firmness, shriveling, decay). To our knowledge, this is the first study that uses CBD oil as a treatment to reduce microbial growth and extend strawberry shelf life.

2. Materials and methods

2.1. Experiment 1

2.1.1. CBD treatments

Strawberries were purchased at a local supermarket and brought to the USF-Food Quality Laboratory in Tampa, Florida, USA. Since the fruit were purchased from the store and not picked fresh from the field, the specific cultivar of strawberry used in this first experiment was unable to be determined. After sorting the fruit, a ready-made sample of CBD oil containing 33 mg mL⁻¹ CBD isolate mixed with MCT oil (CBDistillery, Denver, Colorado, USA) was applied to the strawberries. The oil was gently massaged into the strawberries, and fruit was then placed into clamshells. Additionally, a set of strawberries treated with 1 mL of sterile water was also subjected to the same treatment, as well as a set of untreated control berries (i.e., no CBD oil and no sterile water). For visual analysis, a total of 35 strawberries were used in this experiment, with five strawberries in each clamshell and each treatment having three replicates. The treatments were as follows: untreated strawberries, CBD with MCT oil treated strawberries, and water treated strawberries.

2.1.2. Storage conditions

The replicated treatments were held at the same temperature over the same amount of time in humidity-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 10.0 ± 0.3 °C at 85 % RH. Quality of the fruit was evaluated on days 0, 2, and 4. For destructive analysis (yeast/mold testing) a total of 70 fruits were used. After performing qualitative analysis (visual, firmness, etc.), three replicate samples of five fruit each per treatment (CBD with MCT oil, sterile water, and untreated) were used for microbial testing on days 0 and 4.

2.2. Experiment 2

2.2.1. CBD treatments

'Florida Radiance' strawberries were harvested two times during the 2019 production season, on February 5 and February 26, from Fancy Farms in Plant City, Florida, USA. This cultivar of strawberry was chosen because it is the leading strawberry cultivar grown in central Florida (Wu et al., 2015; Whitaker et al., 2016). Three flats of strawberries (i.e., a total of 36 clamshells containing approximately 0.453 kg of fruit each; total fruit weight ≈ 43 kg) were brought to the laboratory within about one hour of harvest and fruit used from these flats were sorted and used in this experiment. Immediately upon arrival, strawberries were sorted and selected from the initial sample for uniformity of color and freedom from defects. For visual analysis, a total of 180 strawberries were used. Strawberries were sorted into clamshells containing ten fruit each, with three replicates per treatment. The treatments were as follows: untreated held at 1 °C and 10 °C, MCT oil held at 1 °C and 10 °C, and CBD isolate with MCT oil held at 1 °C and 10 °C.

CBD isolate containing 99.6 % CBD (from Extract Labs American Hemp, Boulder, Colorado, USA) was used to make CBD oil. To make the oil, 1 g of CBD isolate was mixed with 20 mL of pure refined lab grade coconut oil (© 2020 Thermo Fisher Scientific Inc.) or MCT oil (Medium-Chain Triglyceride; Bulk Apothecary, Aurora, Ohio) in a 50 mL falcon tube to yield a solution with 50 mg mL⁻¹. Each strawberry was lubricated with 0.5 mL of CBD oil, with 0.25 mL applied on each side of the strawberry. For the MCT oil and coconut oil treatments, 0.5 mL of only the MCT oil or coconut oil was applied on the surface of the fruit and calyx. The oil was gently massaged into the strawberries, and fruit was then placed into clamshells. For the first harvest, coconut oil was used, and for the second harvest, MCT oil was used. Coconut oil was replaced by MCT oil because coconut oil was shown to leave a waxy-like layer on the fruit held at colder temperature. The control strawberries were untreated (i.e., no coconut or MCT of CBD oils).

2.2.2. Storage conditions

Thirty strawberries were used for initial quality evaluations. Three replicate samples of 10 fruit each per treatment (CBD oil, MCT oil, MCT or coconut oil only, and untreated) were carefully distributed to three clamshells (capacity ≈ 0.453 kg) and used for non-destructive analysis (subjective appearance). The treatments were replicated and held at the same temperature over the same amount of time in humidity-controlled chambers set at 1.0 ± 0.5 °C and 10.0 ± 0.3 °C at 85 % RH. Quality of the fruit was evaluated on days 0, 4, and 8. For destructive analysis (yeast/mold testing), after performing visual analysis, three replicate samples of 10 fruit each per treatment (CBD with MCT oil, MCT oil or Coconut oil only, untreated) were used for microbial testing on days 0 and 8.

2.2.3. Temperature and relative humidity monitoring

For both experiments, the temperature and relative humidity (RH) inside the chambers was monitored throughout the study using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 0.35 °C. The RH was monitored using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 2.5 % from 10 to 90 % RH.

2.2.4. Sensory quality evaluation

For both experiments, sensory quality evaluation of strawberry was performed always by the same trained personnel using a previously published protocol which in the absence of a formal trained sensory panel was shown to be a reliable way to assess sensory quality of strawberry fruit (Nunes, 2015; Kelly et al., 2016). Color, shriveling and decay severity were determined subjectively using a 1–5 visual rating scale where 5 = excellent, 3 = acceptable, and 1 = poor (Nunes, 2015; Kelly et al., 2016). Firmness was determined based on the whole fruit resistance to slightly applied finger pressure and recorded using a 1–5 tactile rating. A score of 3 was considered the limit of acceptability for sale (Table 1).

2.2.5. Microbiology

For both experiments, the microbial testing was done using 3 M Petrifilm Rapid Yeast and Mold count plate, 3 M Rapid Aerobic count plate, and 3 M Total Coliform count plate (AOAC ISO 16140). The plates were enumerated for a 10-fold dilution following standard dilution methods (3M, 2020a,b,c). To prepare the sample, 11 g of strawberry was mixed with 99 mL of Butterfield's buffer and homogenized for 30 s using a Stomacher 400 Circulator (Seward Laboratory Systems Inc., Port Saint Lucie, U.S.A.) (M Food Safety, 2020a,b,c; 3M, 2020a,b,c). At a minimum, two serial dilutions were performed to ensure plates would be in a countable range. The samples were plated following the steps outlined by 3 M Food Safety (3M, 2020a,b,c) and tested on days 0 and 4 from each replicate, for a total of 16 tests. The 3 M Petrifilm Rapid Yeast and Mold count plates were incubated for 48 h at 25 °C ± 1 °C, the 3 M

Petrifilm Rapid Aerobic count plates were incubated for 24 h at 32 °C ± 1 °C and, the 3 M Petrifilm Coliform count plates were incubated for 24 h at 30 °C ± 1 °C. Petrifilm plates for yeast and mold that had a count of 20–150 colony forming units (CFU) were counted, and plates with a count of 30–300 CFU were counted for APC and coliforms (3M, 2020a,b,c).

2.2.6. Statistical analysis

For both experiments, the Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data. The data was treated by two-way analysis of variance (ANOVA) with harvests, coating treatments, storage temperatures and time as main effects. Significant differences between coating treatments, storage temperatures and time were detected using Tukey's Studentized Range (HSD) test at the 5% level of significance.

3. Results

3.1. Experiment 1

3.1.1. Aerobic plate count

There was a significant difference in aerobic plate count (APC) between the treatments on day 0 and 4 (Fig. 1). On day 4, the CBD oil treatment was significantly more effective at reducing the APC (microbial load of 2.30 log CFU mL⁻¹) when compared to the water treated strawberries, which had a count of 3.98 log CFU mL⁻¹. However, on day 4, the APC count for the untreated and CBD oil-treated berries was very similar, with CBD at day 4 containing 2.30 log CFU mL⁻¹ and the control having 2.65 log CFU mL⁻¹. There was a significant increase in the microbial load from day 0 to day 4 for the control and water treated strawberries. The control increased from 2.04 log CFU mL⁻¹ on day 0– 2.65 log CFU mL⁻¹ on day 4. The APC on water treated strawberries increased from 2.14 log CFU mL⁻¹ on day 0– 3.98 log CFU mL⁻¹ on day 4. There was no significant increase in APC for the CBD treated strawberries from day 0 to day 4.

3.1.2. Total coliforms

There was no significant difference in total coliform counts between the water and CBD treatments on day 0, with each of them having 1.43 log CFU mL⁻¹. However, the total coliforms in the control was significantly lower on day 0 compared to the water and CBD treatments, with a count of 1 log CFU mL⁻¹ (Fig. 1). On day 4, the CBD oil treatment was more effective at reducing total coliforms when compared to the control and water treated strawberries. However, the coliform count for the CBD oil-treated strawberries (2.20 log CFU mL⁻¹) was only significant when compared to the water treated strawberries (2.59 log CFU mL⁻¹). The microbial growth was lower on the CBD treated strawberries compared to the control on day 4, with the CBD treated berries with a load of 2.20 log CFU mL⁻¹ and the control treated berries with 2.32 log CFU mL⁻¹.

Table 1
Visual quality scores and descriptors for strawberry.^a

	Scores and description				
	1	2	3 ^b	4	5
	Very poor	Poor	Acceptable	Good	Excellent
Color	Very dark purplish-red; extremely overripe or senescent	Overripe; very dark red	Fully red	Fully light red	Three-quarter to fully light red
Shriveling	Extremely wilted and dry	Severe shriveling, fruit is shriveled and calyx is wilted and dry	Shriveling evident, fruit and calyx show evident signs of moisture loss	Minor signs of shriveling, calyx slightly wilted	Field-fresh fruit, and calyx appear very fresh and turgid
Decay	100 %, characteristic sporulation, the fruit is either partial or completely rotten	75 %, moderate to heavy mycelium growth	50 %, spots with decay and some mycelium growth	25 %, slight brown discoloration of the tissues, probable decay	0%, no visible changes in the tissues
Firmness	Extremely soft and deteriorated	Soft and leaky	Minor signs of softness	Firm but less turgid	Very firm and turgid

^a Modified from Kelly et al., 2016.

^b Score of 3 was the minimum acceptable quality before strawberries become unmarketable.

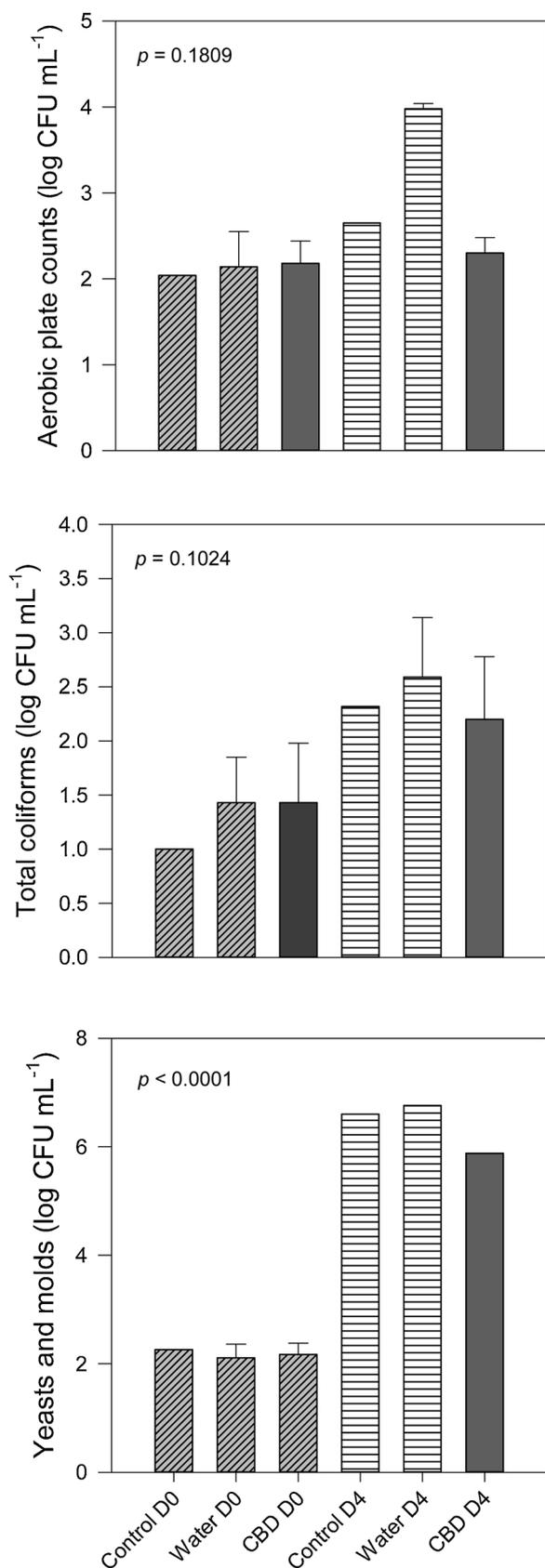


Fig. 1. Aerobic plate counts (APC), total coliforms, and yeast and molds count for store-bought strawberries untreated (control) or treated with water or cannabidiol (CBD) after storage for 4 d at 10 °C and 85 % RH. Different letters above each bar represent statistically significant averages.

However, the control treated strawberries had a significantly lower microbial load on day 0 (1 log CFU mL⁻¹) compared to the CBD treated berries (1.43 log CFU mL⁻¹), which could have had a impact on the microbial load on day 4. The results for the coliform counts were similar to the results seen for the APC counts. For this reason, the coliform testing was not repeated in the second experiment, and only APC data was obtained.

3.1.3. Yeast and mold

There was no significant difference between treatments on day 0 for yeast and mold growth. A significant difference was seen in the yeast and mold results between treatments on day 4 (Fig. 1). On day 4, the CBD oil treatment, with a microbial load of 5.88 log CFU mL⁻¹, was significantly better at reducing the yeast and mold counts when compared to the control (6.60 log CFU mL⁻¹) or water-treated strawberries (6.78 log CFU mL⁻¹). There was also a significant increase in yeasts and molds for all treatments from day 0 to day 4. The control treatments increased from 2.26 log CFU mL⁻¹ on day 0–6.60 log CFU mL⁻¹ on day 4. Yeasts and molds in the water treated strawberries increased from 2.11 log CFU mL⁻¹ on day 0–6.76 log CFU mL⁻¹ on day 4. In the CBD treated berries yeasts and molds increased from 2.17 log CFU mL⁻¹ on day 0–5.88 log CFU mL⁻¹ on day 4.

3.1.4. Color

There was no significant difference in the color of strawberries between treatments on days 0 and 2 (Fig. 2). However, on day 4, the CBD-treated strawberries had significantly higher visual color ratings than the untreated and water-treated strawberries. Additionally, by day 4, the CBD-treated strawberries maintained the color ratings above the minimum acceptable threshold (above a rating of 3). In contrast, the color of both the untreated and water-treated strawberries fell below this threshold (Fig. 2). Overall, the CBD treatment reduced visual color deterioration in strawberries.

3.1.5. Firmness

There was no significance between treatments on day 0, but a significant difference was observed between treatments on days 2 and 4 (Fig. 2). The CBD oil-treated strawberries were significantly firmer than both the untreated and water-treated fruit. The CBD oil-treated strawberries also maintained their firmness above the minimum acceptable threshold by day 4, while firmness of both the untreated and water-treated strawberries fell below this threshold (ratings below 3). Overall, the CBD treatment was effective at maintaining firmness of the strawberries.

3.1.6. Shriveling

There was no significant difference in shriveling between treatments on day 0, but on days 2 and 4, a significant difference was observed between treatments (Fig. 2). The CBD oil-treated strawberries were significantly less shriveled than both the untreated and water-treated strawberries. The CBD oil-treated strawberries also had shriveling above the minimum acceptable threshold by day 4, while shriveling on both the untreated and water-treated strawberries fell below this threshold (ratings below 3). The water-treated strawberries also experienced substantially more shriveling compared to both the untreated and CBD oil-treated strawberries (Fig. 2). Overall, the CBD treatment was effective at reducing the shriveling of the strawberries.

3.1.7. Decay

There was no significant difference in the decay rating between treatments on days 0 and 2 (Fig. 3). However, on day 4, the CBD oil-treated strawberries had significantly less decay than the untreated and water-treated strawberries. On day 4, all the treatments were able to maintain the decay above the minimum acceptable threshold. Still, the rating for the CBD oil-treated strawberries was better compared to the untreated and water-treated strawberries (Fig. 2).

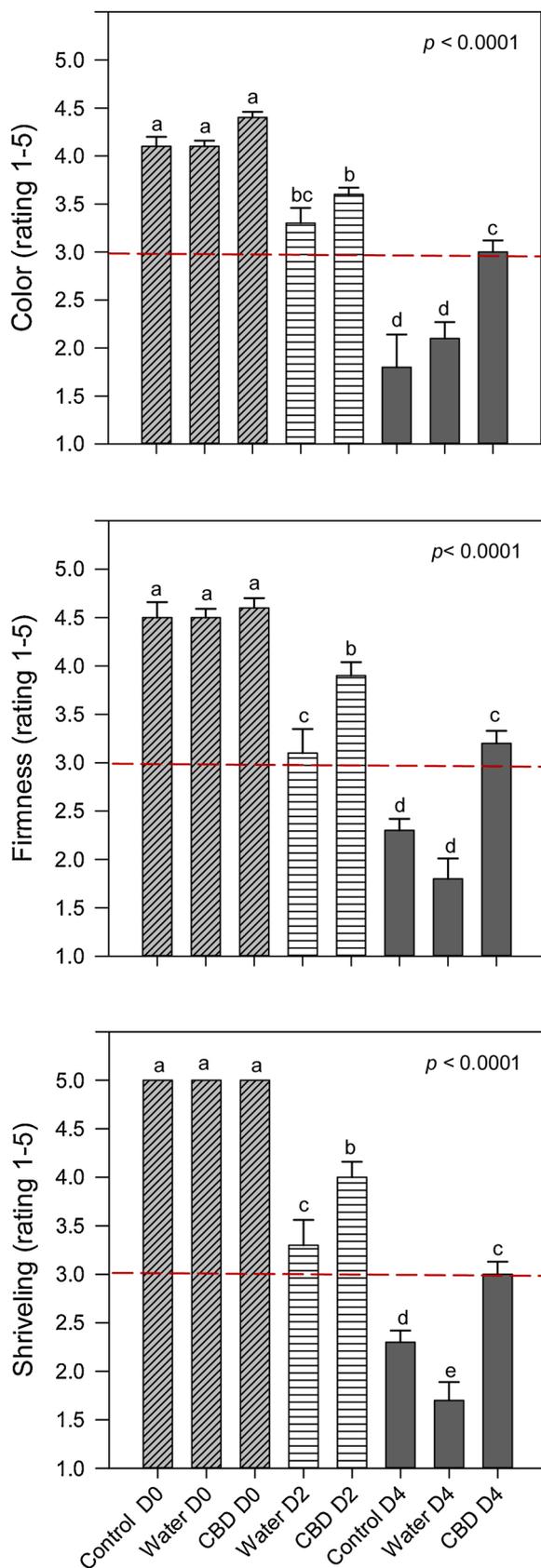


Fig. 2. Color, firmness, and shriveling ratings for store-bought strawberries untreated (control) or treated with water or cannabidiol (CBD) after storage for 2 or 4 d at 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). Different letters above each bar represent statistically significant averages.

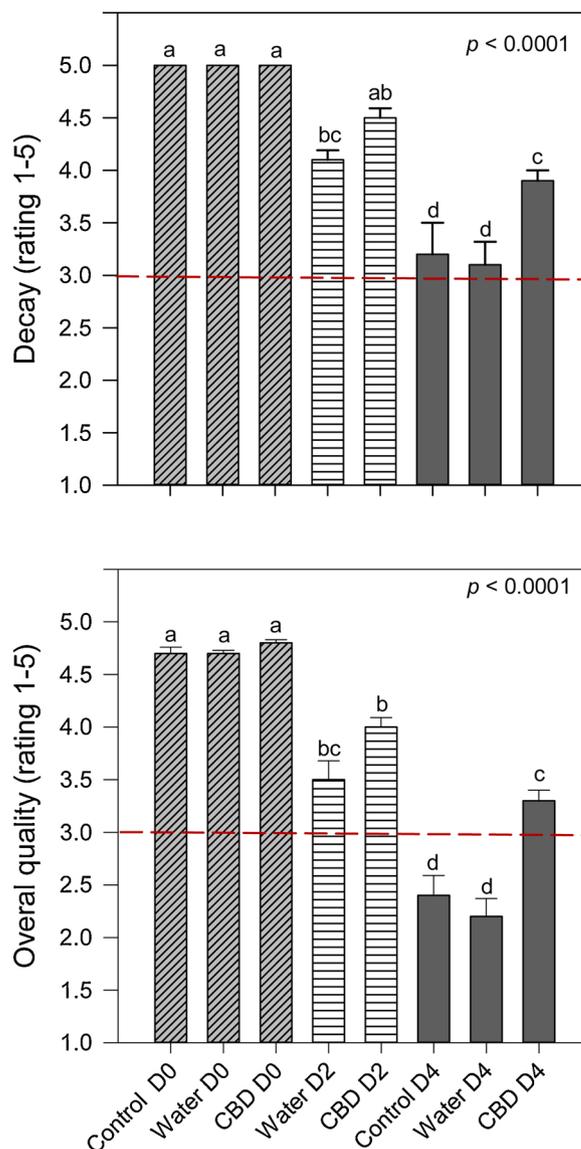


Fig. 3. Decay and overall subjective quality ratings for store-bought strawberries untreated (control) or treated with water or cannabidiol (CBD) after storage for 2 or 4 d at 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). Different letters above each bar represent statistically significant averages.

3.1.8. Overall quality

There was no significant difference in overall quality ratings between treatments on days 0 and 2, but a significant difference was observed between treatments on day 4 (Fig. 3). By day 4, the CBD oil-treated strawberries had overall appearance ratings above the minimum acceptable threshold (ratings above 3), while both the untreated and water-treated strawberries fell below this threshold and had significantly lower overall visual ratings. Overall, the CBD treatment was effective at maintaining the overall quality of the strawberries for 4 d.

In general, these results showed that applying CBD oil to strawberries has the potential to reduce microbial counts on the surface of the berries when compared to treating them with only water or not treating them at all. The physical quality of the strawberries was also better in the fruit treated with CBD oil and these fruits were able to be maintained above the minimum quality threshold after the hold time at both temperatures. However, an additional experiment needed to be conducted to more clearly support these findings. The second experiment further examines these results by testing the APC and yeast/mold count of fresh farm

picked strawberries, instead of store-bought strawberries, and using two different storage temperatures.

3.2. Experiment 2

3.2.1. Aerobic plate count

For harvest 1, there was no significance seen between treatments at 1 °C and at 10 °C (Fig. 4). The initial value for APC on day 0 was 2.10 log CFU mL⁻¹. On day 8 at 1 °C, the untreated strawberries had a microbial load of 3.67 log CFU mL⁻¹ and the CBD and the oil treated strawberries had a load of 3.90 log CFU mL⁻¹. On day 8 at 10 °C, the untreated strawberries had a microbial load of 3.67 log CFU mL⁻¹, the CBD treated strawberries 3.92 log CFU mL⁻¹, and the oil treated strawberries 3.77 log CFU mL⁻¹.

For harvest 2, the initial APC value for day 0 was 1.88 log CFU mL⁻¹. There was a statistically significant difference seen between the CBD (3.76 log CFU/mL) and oil (4.45 log CFU/mL) treated strawberries on day 9 at 10 °C. Overall, the CBD oil treatment (3.76 log CFU/mL) seemed to be more effective at reducing the APC growth when compared to the untreated strawberries (3.98 log CFU/mL) at 10 °C. At 1 °C on day 9, the CBD treated (1.74 log CFU/mL) strawberries were more effective at reducing the microbial growth compared to the untreated (1.75 log CFU/mL) and oil (1.82 log CFU/mL) treated strawberries. In harvest 2, both the CBD and MCT oil treatments were effective in reducing the APC in fruit stored at 1 °C, but at 10 °C, the CBD oil was more effective than the MCT oil at reducing the APC on strawberries (Fig. 4).

3.2.2. Yeast and mold

Although in both harvests, there was no significant difference between most of the treatments, the CBD treatment seemed more efficient at reducing yeast and mold growth compared to untreated samples and slightly more effective than the oil (Fig. 5). In harvest 1, the initial count for the strawberries on day 0 was 2.27 log CFU mL⁻¹. At 1 °C on day 8, the CBD treatment had the lowest microbial count (3.23 log CFU mL⁻¹) when compared to the untreated (3.41 log CFU mL⁻¹) and the oil treated (3.41 log CFU mL⁻¹) strawberries. This was also the same at 10 °C, with CBD treated strawberries (3.41 log CFU mL⁻¹) having a lower microbial count compare to untreated (3.87 log CFU mL⁻¹) and oil treated (3.49 log CFU mL⁻¹) strawberries. Yeast and molds in the CBD and oil treated strawberries were significantly lower at 10 °C on day 8 compared to the untreated strawberries.

For harvest 2, the yeast and mold value on day 0 was 2.44 log CFU mL⁻¹ (Fig. 4). On day 9 at 1 °C, the CBD treated strawberries (2.25 log

CFU mL⁻¹) had significantly lower growth than the untreated (3.62 log CFU mL⁻¹) and the oil treated (3.45 log CFU mL⁻¹) strawberries. At 10 °C on day 9, both the CBD (3.94 log CFU mL⁻¹) and the oil treated (3.82 log CFU mL⁻¹) strawberries had significantly lower yeast and mold growth than the untreated (5.88 log CFU mL⁻¹) strawberries.

Overall, it seems that the application of CBD on strawberry surfaces was more effective than the other treatments in reducing or delaying microbial growth, particularly when strawberries were exposed to 10 °C compared to 1 °C. This observation agrees with some reported findings that showed CBD being a strong antimicrobial agent (Appendino et al., 2008).

3.2.3. Color

In the first harvest, CBD-treated strawberries had the best color ratings at both temperatures on days 4 and 8, whereas the untreated strawberries had the worst color ratings overall (Fig. 6). The CBD and oil treatments were better at maintaining strawberry color quality when the fruit was stored at 10 °C for 4 and 8 d. In the second harvest, after 4 d, there was no significant difference between treatments. On day 8, the color of CBD-treated strawberries stored at 1 °C obtained the best color ratings compared to the other treatments. At 10 °C, the CBD-treated strawberries showed the best color ratings after 4 or 8 d. Overall, the CBD treatments reduced visual color deterioration in strawberries, especially at 10 °C (Fig. 6).

3.2.4. Firmness

In the first harvest, strawberries treated with CBD and oil and stored for 4 d at 1 °C were significantly firmer than the untreated strawberries (Fig. 7). After 8 d at 1 °C, the CBD treatment was significantly better at maintaining the firmness of strawberries than the untreated or oil treatments. In the second harvest, CBD-treated strawberries were significantly firmer than those untreated or treated with oil strawberries on day 4 at 10 °C and were firmer than the untreated fruit after 8 d. Overall, the CBD treatment was effective at maintaining the firmness of the strawberries (Fig. 7).

3.2.5. Shriveling

In the first harvest, strawberries from the CBD and oil treatments stored at 1 °C for 4 and 8 d were significantly less shriveled than the untreated strawberries (Fig. 8). At 10 °C, the CBD-treated berries had significantly less shriveling than the oil or untreated strawberries after 4 and 8 d of storage. In the second harvest, untreated strawberries stored for 8 d at 1 or 10 °C showed the most shriveling, whereas CBD-treated

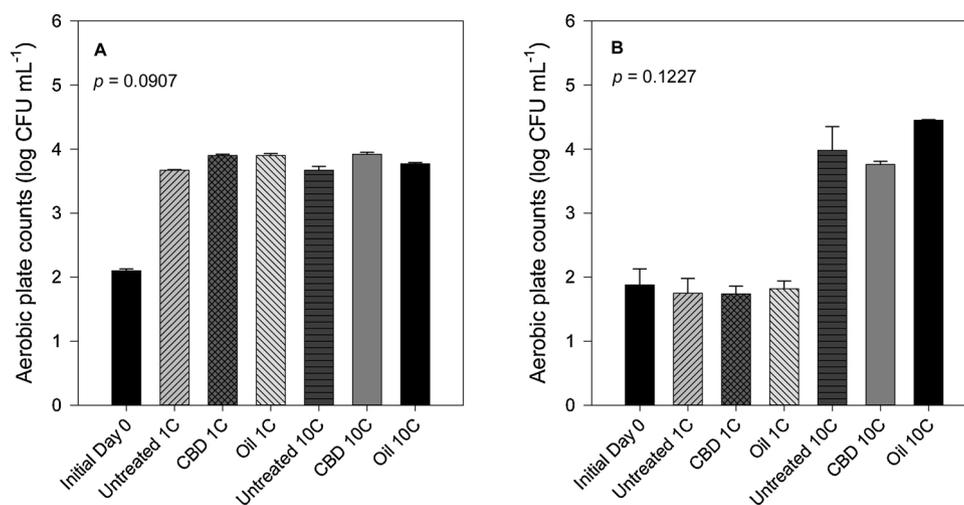


Fig. 4. Aerobic plate counts (APC) for 'Florida Radiance' strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A = Harvest 1), or MCT oil (B = Harvest 2) after storage for 8 d (A) or 9 d (B) at 1 °C and 10 °C and 85 % RH. A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

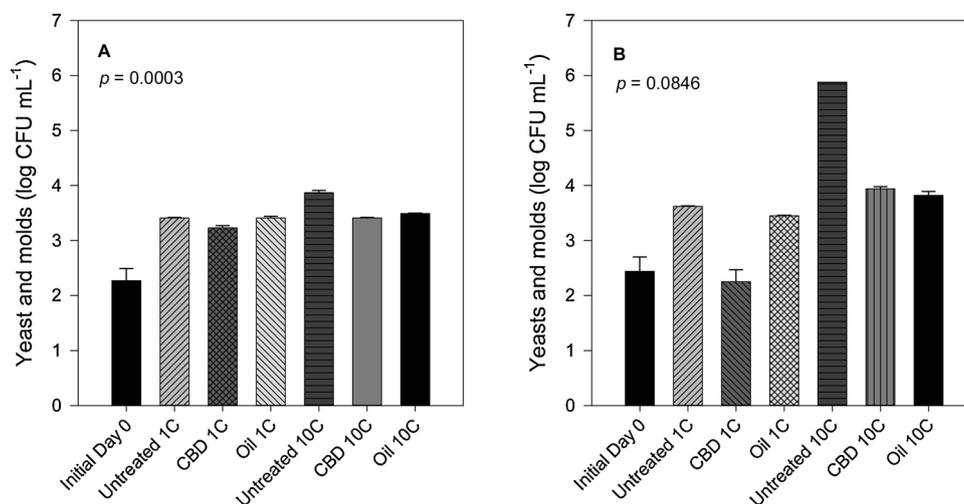


Fig. 5. Yeast and molds for 'Florida Radiance' strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

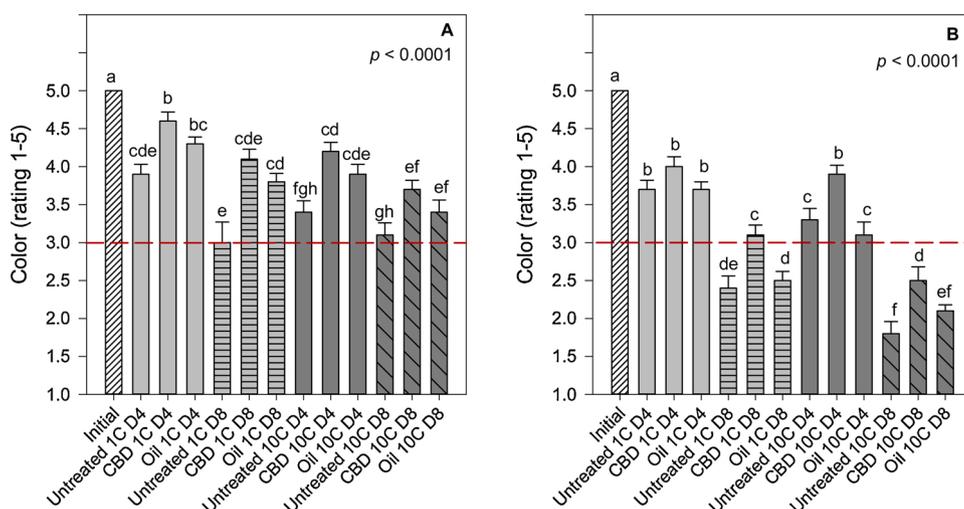


Fig. 6. Color ratings for 'Florida Radiance' strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

fruit stored at 1 or 10 °C showed the least shriveling after 4 d of storage. Overall, the CBD treatment seems to be effective at reducing shriveling in strawberries.

3.2.6. Decay

Decay never attained objectionable levels during storage (ratings above 3.0), regardless of the treatment or time of harvest (Fig. 9). Nonetheless, in the first harvest, CBD-treated strawberries stored for 8 d at 10 °C showed significantly less decay compared to oil-treated or the untreated strawberries. In the second, CBD-treated strawberries stored for 8 d at 10 °C showed significantly less decay compared to oil-treated or the untreated strawberries. Overall, CBD was better at preventing decay in strawberries at 10 °C than the untreated and oil treatments (Fig. 9).

3.2.7. Overall quality

In the first harvest, CBD and oil-treated strawberries held at 1 °C for 4 d showed significantly better overall quality than the untreated strawberries (Fig. 10). On day 8, CBD-treated strawberries stored at 1 °C had significantly better quality than the oil and untreated berries. CBD-

treated strawberries stored for 4 and 8 d at 10 °C also had significantly better overall quality than the coconut oil and untreated strawberries. In harvest 2, CBD-treated strawberries stored at 10 °C had significantly better quality than the oil and untreated fruit. The CBD treatments appear to be better at maintaining the overall quality of fruit stored at 10 °C than at 1 °C.

4. Discussion

Coconut oil used in the second experiment (first harvest) did not seem to be the right medium to dissolve the CBD isolate, because although the oil remains solid at room temperature, it solidified during storage at cold temperatures and thus the results were not so conclusive. The coconut oil created a hard shell on the strawberries, which impacted the evaluation of microbial content and visual ratings. The MCT oil was a much more favorable medium to dissolve the CBD isolate because this oil is highly processed and remains liquid at room temperature. MCT oil is flavorless and is commonly used in the industry to make CBD oil. MCT oil also contains antimicrobial properties that could work in unison with the CBD isolate to reduce microbial growth and extend shelf life.

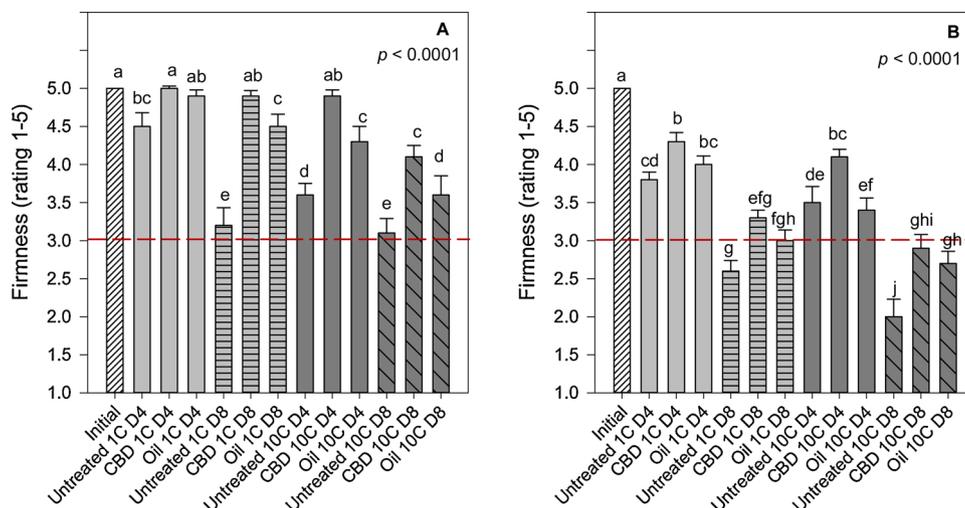


Fig. 7. Firmness ratings for ‘Florida Radiance’ strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

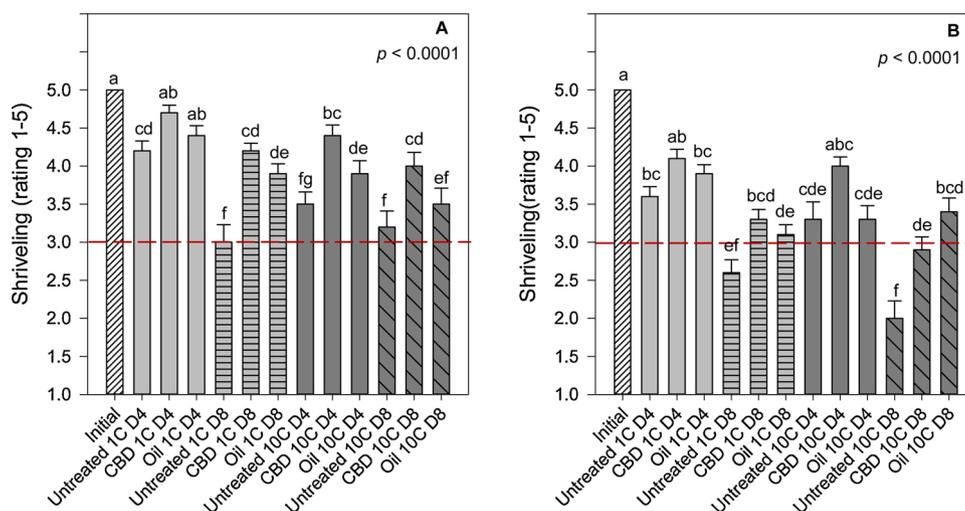


Fig. 8. Shriveling ratings for ‘Florida Radiance’ strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

Additionally, the oil covering the entire surface of the strawberry can act as a protective barrier to help reduce moisture loss and prevent shriveling. The CBD isolate was completely dissolved into the oil and left only a slightly oily sheen on the strawberries after application. During storage, the strawberries still retained this shine, but to a lesser degree as time passed. Overall, MCT oil left the strawberries with a visual appearance that was not as drastically altered and would still be acceptable to consumers. In contrast, the coconut oil application would be seen on the strawberries and would impact consumer acceptability.

This mixture of MCT oil and CBD isolate could potentially be used by consumers at home as a treatment or wash to extend the shelf life of strawberries. The application method of coating the strawberries for consumers to easily and effectively apply the CBD to the fruit needs further investigation. In this experiment, the strawberries were individually coated, and the oil was massaged into the strawberries. This method of application would be very tedious for consumers, and a more time-efficient method would need to be developed. The reasoning for using this application method was to ensure that the fruit would be entirely covered with the oil and thus to provide precise results. At home, treatments for strawberries that could potentially be used to

apply CBD oil could be a spray or a soak, which would be more suitable for at-home use. A soaking treatment would require a larger volume of CBD oil and so could potentially be economically unfeasible. CBD oil is costly and, even in bulk quantities, it would be difficult to make a product that the average consumer could afford. A premixed spray of CBD oil would be most economical and would still allow consumers to spray the strawberries in an acceptable amount of time individually. Besides, a spray application would be able to cover a large amount of surface area and would waste less product than a soaking treatment would, since the CBD oil that is not sprayed from the bottle can be used for later treatment.

5. Conclusions

Results from these experiments provide for the first-time evidence that CBD oil has the potential to reduce microbial load and maintain the visual quality of strawberries, which would lengthen their shelf life. Overall, CBD oil seems to be most effective at reducing microbial growth and maintaining quality when the fruit is stored at 10 °C than at 1°C. Though results showed that CBD oil has a great potential to be used as an

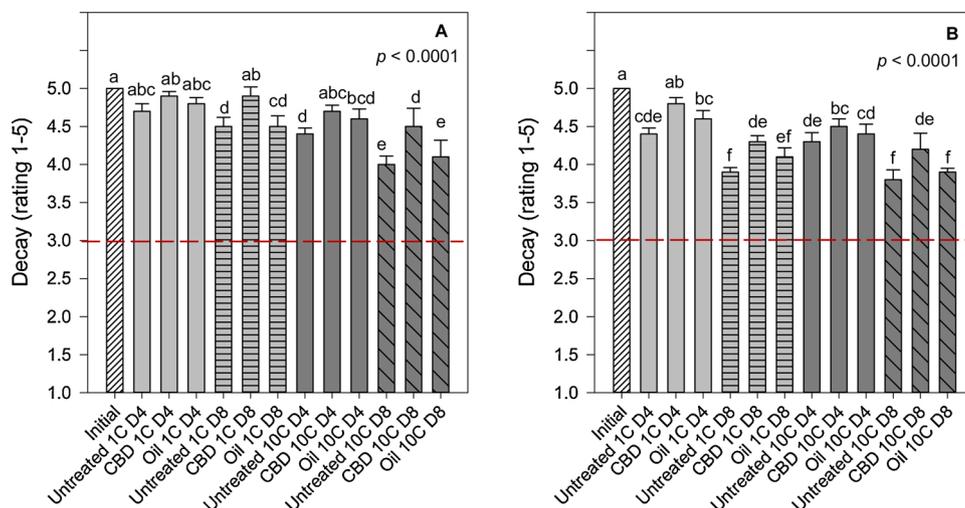


Fig. 9. Decay ratings for 'Florida Radiance' strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

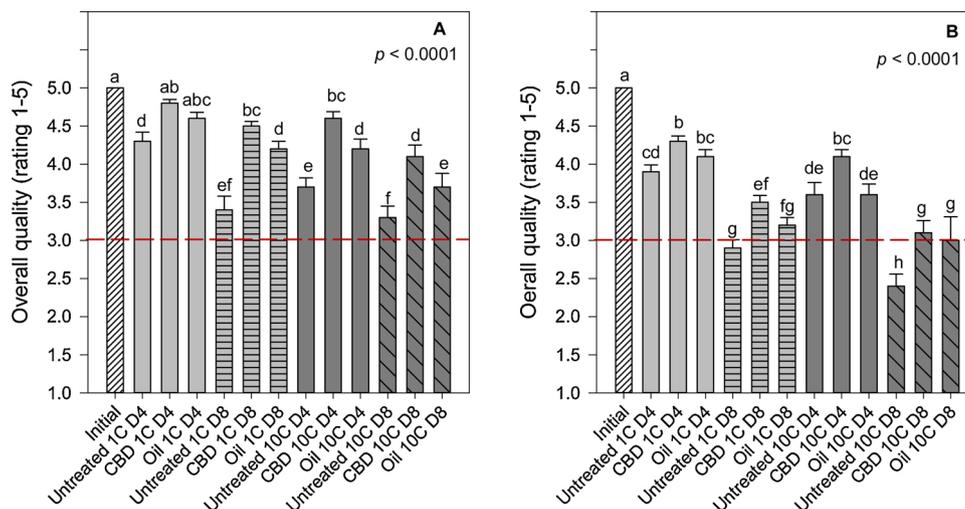


Fig. 10. Overall quality ratings for 'Florida Radiance' strawberries untreated (control) or treated with cannabidiol (CBD), coconut oil (A), or MCT oil (B) after storage for 8 d at 1 °C and 10 °C and 85 % RH. 5 = Excellent; 3 = Acceptable; 1 = Very poor. Dash lines represent the maximum acceptable for sale (rating of 3). A = Harvest 1 on February 5, 2019; B = Harvest 2 on February 26, 2020. Different letters above each bar represent statistically significant averages.

antimicrobial in food applications, there is still a significant amount of research that needs to be done to examine the extent of this potential. For example, further research should be conducted to determine the amount of CBD isolate that needs to be used to obtain optimum results, the ideal concentration of CBD isolate, and what type of oil that should be used to treat the strawberries. It would also be essential to test the effect of CBD on known food pathogens.

Further experiments should examine how the growth of specific strains of bacteria isolated on a plate and treated with CBD isolate would be impacted. Besides, more research needs to be conducted on how a CBD oil treatment would affect the sensory, chemical, physical, and microbial characteristics of strawberries and, eventually, other fruits and vegetables. Finally, since CBD oil can also be expensive, the effort would need to be put into finding CBD oils of high quality, but still affordable to consumers and eventually for commercial use.

CRedit authorship contribution statement

Haley Inselberg: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing -

original draft, Visualization, Project administration. **Maria Cecilia do Nascimento Nunes:** Methodology, Validation, Formal analysis, Resources, Data curation, Writing - review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

Acknowledgments

The strawberries used in the second experiment were donated by Fancy Farms Inc (Plant City, Florida). The CBD isolate was donated by Extract Labs (Boulder, Colorado).

References

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., Smith, E., Rahman, M.M., 2008. Antibacterial cannabinoids from *Cannabis sativa*: a structure-activity study. *J. Nat. Prod.* 71, 1427-1430. <https://doi.org/10.1021/mp8002673>.

- Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Santini, G., Fiorini, D., Sut, S., Dall'Acqua, S., Canale, A., Maggi, F., 2018. The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Ind. Crops Prod.* 122, 308–315. <https://doi.org/10.1016/j.indcrop.2018.05.032>.
- Cassano, R., Trombino, S., Ferrarelli, T., Nicoletta, F.P., Mauro, M.V., Giraldi, C., Picci, N., 2013. Hemp fiber (*Cannabis sativa* L.) derivatives with antibacterial and chelating properties. *Cellulose* 20 (1), 547–557. <https://doi.org/10.1007/s10570-012-9804-3>.
- Fathordoobady, F., Singh, A., Kitts, D.D., Singh, A.P., 2019. Hemp (*Cannabis sativa* L.) extract: anti-microbial properties, methods of extraction, and potential oral delivery. *Food Rev. Int.* 35 (7), 664–684. <https://doi.org/10.1080/87559129.2019.1600539>.
- Glodowska, M., Łyszcz, M., 2017. *Cannabis sativa* L. And its antimicrobial properties – a review. In: Leśny, J., Chojnicki, B., Panfil, M., Nyčkowski, J. (Eds.), *Badania I Rozwój Młodych Naukowców w Polsce Agronomia I ochrona roślin. Młodzi Naukowcy, Poznań, Poland*, pp. 77–82.
- Kelly, K., Whitaker, V.M., Nunes, M.C.N., 2016. Physicochemical characterization and postharvest performance of the new Sensation® 'Florida127' strawberry compared to commercial standards. *Sci. Hort.* 211, 283–294. <https://doi.org/10.1016/j.scienta.2016.09.012>.
- Lopez-Romero, J.C., González-Ríos, H., Borges, A., Simões, M., 2015. Antibacterial effects and mode of action of selected essential oils components against *Escherichia coli* and *Staphylococcus aureus*. *Evid. Based Complement. Altern. Med.* 795435, 1–9. <https://doi.org/10.1155/2015/795435>.
- M Food Safety Guide to dilution preparation, 2020a Available online <https://multimedia.3m.com/mws/media/2411390/3m-petrefilm-plates-guide-to-dilution-preparation-flyer.pdf> (accessed in April 2020).
- M Food Safety Interpretation Guide, 2020b. Available online: <https://multimedia.3m.com/mws/media/9020460/3m-petrefilm-rapid-ym-count-plate-interpretation-guide.pdf> (accessed in April 2020).
- M Food Safety Sample preparation, 2020c. Available online: <https://multimedia.3m.com/mws/media/2411100/3m-petrefilm-plates-sample-preparation-flyer.pdf> (accessed in April 2020).
- Mermelstein, N.H., 2019. Setting standards for Cannabis edibles. *Food Technol. Mag.* 73 (11). Available online: <https://www.ift.org/news-and-publications/food-technology-magazine/issues/2019/november/columns/setting-standards-for-cannabis-edibles> (assessed in August 11, 2020).
- Nafis, A., Kasrati, A., Jamali, C.A., Mezrioui, N., Setzer, W., Abbad, A., Hassani, L., 2019. Antioxidant activity and evidence for synergism of *Cannabis sativa* (L.) essential oil with antimicrobial standards. *Ind. Crops Prod.* 137, 396–400. <https://doi.org/10.1016/j.indcrop.2019.05.032>.
- Nunes, M.C.N., 2015. Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. *Postharvest Biol. Technol.* 107, 43–54. <https://doi.org/10.1016/j.postharvbio.2015.05.001>.
- Treviño-Garza, M.Z., García, S., del Socorro, Flores-González, M., Arévalo-Niño, K., 2015. Edible active coatings based on pectin, pullulan, and chitosan increase quality and shelf life of strawberries (*Fragaria ananassa*). *J. Food Sci.* 80, M1823–M1830. <https://doi.org/10.1111/1750-3841.12938>.
- US Food and Drug Administration, 2020. Available online: <https://www.fda.gov/consumers/consumer-updates/what-you-need-know-and-what-were-working-find-out-about-products-containing-cannabis-or-cannabis> (accessed on March 2020).
- Wang, Y., Liu, Z., Han, Y., Xu, J., Huang, W., Li, Z., 2018. Medium Chain Triglycerides enhances exercise endurance through the increased mitochondrial biogenesis and metabolism. *PLoS One* 13 (2). <https://doi.org/10.1371/journal.pone.0191182>.
- Widianingrum, D.C., Noviandi, C.T., Salasia, S.I., 2019. Antibacterial and immunomodulator activities of virgin coconut oil (VCO) against *Staphylococcus aureus*. *Heliyon* 5 (10). <https://doi.org/10.1016/j.heliyon.2019.e02612>.
- Wu, F., Guan, Z., Whitaker, V., 2015. Optimizing yield distribution under biological and economic constraints: florida strawberries as a model for perishable commodities. *Agric. Syst.* 141, 113–120.
- Yu, L.L., Zhou, K.K., Parry, J., 2005. Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chem.* 91 (4), 723–729. <https://doi.org/10.1016/j.foodchem.2004.06.044>.