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EFFECTS OF LEMON OR CINNAMON ESSENTIAL OIL VAPOR ON PHYSICOCHEMICAL PROPERTIES OF STRAWBERRIES DURING STORAGE

A Thesis

Presented to

The Faculty of the Department of Nutrition, Food Science and Packaging

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Elise Christine Anita Freche

December 2021

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by

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ABSTRACT

EFFECTS OF LEMON OR CINNAMON ESSENTIAL OIL VAPOR ON PHYSICOCHEMICAL PROPERTIES OF STRAWBERRIES DURING STORAGE

by Elise Christine Anita Freche

Essential oils are currently being investigated for their potential role as food preservatives. They contain a mixture of bioactive compounds that would help increase the shelf life of food and might help preserve fruits without using synthetic chemicals. Although much research has been conducted on their antimicrobial properties, their effect on the physicochemical properties and organoleptic characteristics needs more investigation. In this study, the effects of lemon or cinnamon essential oil vapor on the physicochemical properties of strawberries were analyzed, focusing on the weight loss, color, firmness, acidity status, sugar content and visible decay. Strawberries were treated for 12 hours with lemon or cinnamon oil vapor before being stored for 5 days at 22°C or 18 days at 4°C. Lemon and cinnamon essential oil vapor treatments both show promising results, with the ability to blunt weight loss during the first days of storage ($P < 0.05$), without significantly altering the acidity status of strawberries, nor their soluble solid concentration. Lemon essential oil vapor delayed darkening ($P < 0.05$), but reduced firmness ($P < 0.05$). Cinnamon essential oil vapor increased the concentration of reducing sugars ($P < 0.05$). Cinnamon treatment also decreased visible decay by 16.7% after four days of storage, although the difference was not significant. These results show the potential benefits of using lemon or cinnamon essential oil as natural preservatives in food systems

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Introduction

Food waste is a prevalent issue in the United States as approximately one-third of the food produced for human consumption is wasted along the production and supply chain (Gustavsson et al., 2011). For example, consumers discard large amounts of food, especially aging fruit (Buzby et al., 2014). Moreover, in North America, more than 60 percent of food waste occurs at the consumer level (Lipinski et al., 2013).

Consumer interest in natural and minimally processed food is increasing as the food industry works on meeting consumer demand by extending the shelf life of fruit while avoiding synthetic preservatives. For example, the FreshGlow Company "Freshpaper" is a product that uses plant extracts on a filter paper to extend fruit freshness at home (FreshGlow, 2021). Essential oils are also promising candidates for improving the longevity of fruit. These oils are extracts obtained from plants, and most are generally recognized as safe (GRAS) (Substances Generally Recognized as Safe, 2021), meaning that they are considered safe for human consumption. Moreover, the use of essential oils could satisfy increased desire by consumers for natural food. In this review, we will focus on the effects of lemon essential oils and cinnamon essential oils on the quality of fruit from microbial, physicochemical, and sensory perspectives. Lemon and cinnamon essential oils are part of the GRAS list and are popular essential oils in the United States (Elgendy et al., 2017; Manion & Widder, 2017). Moreover, there is extensive research on their properties (Al-Jabri & Hossain, 2016; Dimić et al., 2014; Fisher & Phillips, 2006; Umagiliyage et al., 2017; Vitoratos et al., 2013). Strawberries were selected as a model fruit because their shelf life is

less than one week (United State Department of Health & Human Services [HHS], 2019), which makes them a suitable candidate for research.

Food Loss and Food Waste in The United States

Food loss refers to all the food that is lost due to food processing, cooking, natural shrinkage, mold or pest, spillage, and spoilage before it reaches its intended form for consumption (United Nations Environment Programme [UNEP], n.d.). Food waste is the loss of any food ready for consumption discarded prior to being consumed (UNEP, n.d.). Food loss occurs due to decisions made by food producers in the supply chain while food waste arise from actions made by retailers, food service providers and consumers (Food and Agriculture Organization of the United Nations [FAO], 2021). Food waste happens at the consumers level in part because fruits and vegetables have reached the end of their shelf life.

In the United States, more than one-third of food available for human consumption is not consumed (Buzby et al., 2014) with more than 40% of food loss and waste occurring at retail and consumer levels (Gustavsson, 2011). Such loss and waste also represent a waste of land and financial resources (United States Department of Agriculture [USDA], n.d.). Reducing food loss and waste could also help reduce food insecurity (USDA, n.d.). From these perspectives, it is essential to find ways to reduce food loss and waste.

Fruit Preservation

Shelf life is defined as “the length of time a product may be stored without becoming unsuitable for use or consumption” (Tanner, 2016, p. 3). Many factors influence the shelf life of a food product: its environment (exposure to light, heat, and moisture); the transmission of gases; mechanical stresses; and contamination by microorganisms (bacteria, fungi) (Matar et

al., 2018; Tanner, 2016). Shelf life can vary greatly based on the food item (HHS, 2021). When refrigerated, fruits and vegetables can last from a few days (e.g., shelf life of strawberries is less than a week), to a few months (e.g., apples can last six to eight weeks) (HHS, 2019).

Fruits and vegetables continue to be physiologically active after being harvested and stored as they are still breathing, transpiring, and undergoing senescence (i.e., aging). These physiological processes affect the quality of fruits and vegetables. However, such processes are manageable to some extent by controlling the environment, including maintaining an optimal gas composition, temperature, and humidity (Florkowski et al., 2014; Tanner, 2016).

Agricultural products need careful post-harvest handling to maintain quality and shelf life. Chemical fungicides are commonly used during product transportation since fruits and vegetables are highly susceptible to fungi (Sakudo, 2017). Although fungicides are highly effective, these chemicals could negatively impact human health and the environment (Pennati et al., 2006). For example, azole compounds found in fungicides are suspected to create craniofacial malformations in vertebrates (Pennati et al., 2006).

Other current methods of disinfection involve thermal treatments (autoclaving, ohmic heating, steam pasteurization, etc.), non-thermal technology disinfection methods (ozone, radiation, etc.), active packaging (moisture-absorbing, antimicrobial-releasing packaging, and edible coating with natural active compounds). These methods can vary in their effectiveness, cost, and potential toxicity (Brandelli et al., 2017; Mogoşanu et al., 2017; Sakudo, 2017; H. Q. Zhang et al., 2011).

Focus on Strawberries

The strawberry fruit is one of the most consumed berries worldwide. The United States is one of the major producers of strawberries, along with China, Mexico, Turkey, and Egypt (Padmanabhan et al., 2016). Strawberries are one of the favorite fresh fruits in the United States (Pan et al., 2014), and California produces nearly 90% of them (California Strawberry Commission, 2021).

The strawberry is a plant that belongs to the family Rosaceae and genus Fragaria (Padmanabhan et al., 2016). Strawberries are non-climacteric fruits, meaning that they do not continue ripening after harvest. However, strawberries continue to breathe after harvest, resulting in fruit aging (Azam et al., 2019). Strawberries are rich in flavonoids, fibers, vitamins, and potassium and as it ripens, sucrose, fructose, and glucose are produced (Azam et al., 2019; Petrasch et al., 2019).

Strawberries are a highly perishable fruit and are thus susceptible to postharvest losses (Padmanabhan et al., 2016). Most of these losses are due to fungal infection resulting in disease and a loss of product quality (Petrasch et al., 2019). The main contributor to spoilage is *Botrytis cinerea*, the fungi responsible for gray mold disease (Amiri et al., 2018). Other fungi are *Rhizopus stolonifer, Mucor spp., Colletotrichum spp., Penicillium spp*., which are responsible for soft rot disease and Penicillium fruit rot. Some losses are also due to bacterial growth on the strawberries. Pathogenic bacteria that have been found on strawberry fruits are *Escherichia coli, Shiga toxin-producing E. coli*, *Salmonella, Listeria, Campylobacter, Cronobacter,* and *Hepatitis A* (Ceuppens et al., 2015; Dziedzinska et al., 2018). Fungi and bacteria that infect strawberries are also commonly present on other fruits and vegetables.

For example, *Botrytis cinerea* is the primary reason for fruit rejections by shippers and consumers worldwide (Petrasch et al., 2019). In addition, *Salmonella* and *E. coli* are very common sources of disease outbreaks from fruits.

Essential Oils are Suitable Candidates for the Preservation of Fruit

Essential oils are volatile and aromatic compounds extracted from plants. They are usually obtained via distillation, which involves separating the liquid from the solids, converting it into vapor for chemical separation, and then condensing it back into liquid form (Ríos, 2016). Essential oils are of great interest to the cosmetic, pharmaceutical, agricultural, and food industries because of their antimicrobial and antioxidant properties (Dimić et al., 2014; Liu et al., 2016; Tzortzakis, 2007; Wang et al., 2013). Essential oils are excellent candidates as natural products to preserve the post-harvest qualities of fruits and vegetables since these oils show antimicrobial properties and are included on the GRAS list.

Antifungal and Antibacterial Properties of Lemon Essential Oil

Lemon essential oil is typically extracted from the lemon peels and leaves (Martínez et al., 2008). Lemon essential oil contains at least 35 components that can be grouped into the following categories: sesquiterpene hydrocarbons, carbonyl compounds, esters, and alcohols (Allegrone et al., 2006; Gök et al., 2014). Limonene is the main component of lemon essential oil, representing more than 50% of the total composition (Al-Jabri & Hossain, 2016; Gök et al., 2014). Lemon essential oil is particularly interesting since it is on the GRAS list and is thus considered safe to ingest (Substances Generally Recognized as Safe, 2021). Many researchers are thus currently working on the application of lemon essential oil in different types of food, including fish and fruit juice (Alfonzo et al., 2017; Yen et al., 2018).

Lemon essential oil has antifungal properties. This oil delays the growth of several fungi including *Botrytis cinerea* that are responsible for fruit decay (Table 1) (Dimić et al., 2014; Umagiliyage et al., 2017; Vitoratos et al., 2013). These effects happen when the oil is applied directly by contact or even as a vapor (Dimić et al., 2014). The growth of *Botrytis cinerea* in strawberries is thus reduced and can be completely stopped at a low concentration of 0.05 μL/mL (Vitoratos et al., 2013). This feature makes lemon essential oil an excellent candidate for preserving fruit since *Botrytis cinerea* is one of the most common fungi infecting fruit and is a major source of fruit loss.

The antibacterial power of lemon oil is present but weak when applied to several strains of bacteria (see Table 2) (Al-Jabri & Hossain, 2016; Fisher & Phillips, 2006). For example, the oil had very limited inhibitory effect on *Listeria monocytogenes* when there was direct contact. However, sweet lemon essential oil was shown to have antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* at 2 mg/ml concentration (Al-Jabri & Hossain, 2016). Limonene, the main component of lemon essential oil, exhibits antibacterial activity against *E. coli* and *S. aureus*, and this activity is proportional to the concentration of molecules (Lan et al., 2020). In addition, limonene delays decay in mangoes and blueberries (Lan et al., 2020; Umagiliyage et al., 2017). More extensive studies are needed to understand the potential applications for these oils in food.

Table 1

Results Related to the Antifungal Activity of Lemon Essential Oil (Includes Limonene)

Pathogens	Approach	Substrate	Concentration	Results	Reference
Aspergillus Parasiticus	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%): 77	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%): 100	
			4.17 µL/mL	Inhibition (%): 100	
			5.83 µL/mL	Inhibition (%): 100	
Cladosporium cladosporioides	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%): 85	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%): 100	
			4.17 µL/mL	Inhibition (%): 100	
			5.83 µL/mL	Inhibition (%): 100	
Eurotium herbariorum	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%): 100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%): 100	
			4.17 µL/mL	Inhibition (%): 100	
			5.83 µL/mL	Inhibition (%): 100	
Penicillium chrysogenum	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%): 100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%): 100	
			4.17 µL/mL	Inhibition (%): 100	
			5.83 µL/mL	Inhibition (%): 100	
Aspergillus carbonarius	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%): 100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%): 100	
			4.17 µL/mL	Inhibition (%): 100	
			5.83 µL/mL	Inhibition (%): 100	
Aspergillus Parasiticus	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):100	

Pathogens	Approach	Substrate	Concentration	Results	Reference
			4.17 µL/mL	Inhibition (%):100	
			5.83 µL/mL	Inhibition (%):100	
Cladosporium cladosporioides	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):100	
			4.17 µL/mL	Inhibition (%):100	
			5.83 µL/mL	Inhibition (%):100	
Eurotium herbariorum	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):100	
			4.17 µL/mL	Inhibition (%):100	
			5.83 µL/mL	Inhibition (%):100	
Penicillium chrysogenum	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):100	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):100	
			4.17 µL/mL	Inhibition (%):100	
			5.83 µL/mL	Inhibition (%):100	
Aspergillus carbonarius	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):61	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):100	
			4.17 µL/mL	Inhibition (%):100	
			5.83 µL/mL	Inhibition (%):100	
Botrytis cinerea	Mixed in agar	*In vitro*	5µL/mL	Radial growth (%): 83	Vitoratos et al., 2013
			8µL/mL	Radial growth (%): 68	
			11µL/mL	Radial growth (%): 49	
			13µL/mL	Radial growth (%): 37	
			15µL/mL	Radial growth (%): 10	
			18µL/mL	Radial growth (%): 0	
Botrytis cinerea	Mixed in agar	*In vitro*	5µL/mL	spore germination (%): 81	Vitoratos et al., 2013
			8µL/mL	spore germination (%): 70	

Pathogens	Approach	Substrate	Concentration	Results	Reference
				13μL/mL	spore germination (%): 51
			18μL/mL	spore germination (%): 32	
Botrytis cinerea	Vapor	*In vivo*	0 μL/mL	Infected fruit (%): 100	Vitoratos et al., 2013
			0.025μL/mL	Infected fruit (%): 32	
			0.035μL/mL	Infected fruit (%): 25	
			0.05 μL/mL	Infected fruit (%): 0	
Botrytis cinerea	Liposome	*In vitro*	0 μmol/L	Count (49days) (Log_{10} CFU/mL): 5	Umagiliyage et al., 2017
			10 μmol/L	Count: 1	
			50 μmol/L	Count: 0	
Penicillium chrysogenum	Liposome	*In vitro*	0 μmol/L	Count: 5	Umagiliyage et al., 2017
			10 μmol/L	Count: 2.6	
			50 μmol/L	Count: 2	

Table 2

Results Related to the Antibacterial Activity of Lemon Essential Oil

Pathogens	Approach	Substrate	Concentration	Results	Reference
Escherichia coli O157	Mixed in agar	*In vitro*	0.1mL/ 2cm diameter disc	Inhibition (mm): 21	Fisher & Philips, 2006
Campylobacter jejuni				Inhibition (mm): 18	
Listeria monocytogenes				Inhibition (mm): 41	
Bacillus cereus				Inhibition (mm): 29	
Staphylococcus aureus				Inhibition (mm): 23	
Escherichia coli O157	Vapor	*In vitro*	0.1mL/ 2cm diameter (lid)	Inhibition (mm): 0	Fisher & Philips, 2006
Campylobacter jejuni				Inhibition (mm): 0	
Listeria monocytogenes				Inhibition (mm): 0	
Bacillus cereus				Inhibition (mm): 0	
Staphylococcus aureus				Inhibition (mm): 0	
Escherichia coli O157	Mixed in agar	*In vitro*	N/A	MIC* (% v/v): 1	Fisher & Philips, 2006
Campylobacter jejuni			N/A	MIC* (% v/v): 4	
Listeria monocytogenes			N/A	MIC* (% v/v): 0.25	
Bacillus cereus			N/A	MIC* (% v/v): 1	
Staphylococcus aureus			N/A	MIC* (% v/v): 4	
Escherichia Coli	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): 15	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): 5	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): 5	
Pseudomonas aeruginosa	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): nd**	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): 27	
			0.5 mg/mL	Inhibition (mm): nd**	

Pathogens	Approach	Substrate	Concentration	Results	Reference
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): nd**	
Staphylococcus aureus	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): nd**	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): nd**	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): 30	
Proteus vulgaris	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): nd**	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): nd**	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): nd**	
Escherichia Coli	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): 35	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): nd**	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): 4	
Pseudomonas aeruginosa	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): 6	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): 29	
			0.5 mg/mL	Inhibition (mm): nd**	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): 6	
Staphylococcus aureus	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): 20	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): 15	
			1 mg/mL	Inhibition (mm): nd**	

Pathogens	Approach	Substrate	Concentration	Results	Reference
				2 mg/mL	Inhibition (mm): nd**
Proteus vulgaris	Mixed in agar	*In vitro*	0 mg/mL	Inhibition (mm): 28	Al-Jabri & Hossain, 2016
			0.25 mg/mL	Inhibition (mm): nd**	
			0.5 mg/mL	Inhibition (mm): nd**	
			1 mg/mL	Inhibition (mm): nd**	
			2 mg/mL	Inhibition (mm): nd**	
Escherichia Coli	Edible Film (polyvinyl alcohol+chitosan)	*In vitro*	0 (PVA)	Inhibition (mm): 0	Lan et al., 2020
			0 (PVA+CS***)	Inhibition (mm): 2.15	
			2.50%	Inhibition (mm): 4.7	
			5%	Inhibition (mm): 5.06	
			7.50%	Inhibition (mm): 5.12	
			10%	Inhibition (mm): 6.61	
Staphylococcus aureus	Edible Film (PVA+CS)	*In vitro*	0 (PVA)	Inhibition (mm): 0	Lan et al., 2020
			0 (PVA+CS)	Inhibition (mm): 3.43	
			2.5% (PVA+CS)	Inhibition (mm): 4	
			5% (PVA+CS)	Inhibition (mm): 5.15	
			7.5% (PVA+CS)	Inhibition (mm): 5.28	
			10% (PVA+CS)	Inhibition (mm): 6.88	
Escherichia coli	Liposome	*In vitro*	0 µmol/L	Count (49days) (Log_{10} CFU/mL): 5	Umagiliyage et al., 2017
			10 µmol/L	Count: 4	
			50 µmol/L	Count: 3.5	
Listeria monocytogenes	Liposome	*In vitro*	0 µmol/L	Count: 5	Umagiliyage et al., 2017
			10 µmol/L	Count: 4	
			50 µmol/L	Count: 2	

Note. *MIC: minimal inhibitory concentration; ** nd: Not detectable; ***PVA+ CS: polyvinyl alcohol + Chitosan

Antifungal and Antibacterial Properties of Cinnamon Essential Oil

Cinnamon essential oil is extracted from the *Cinnamomum cassia Presl* or *Cinnamomum verum Presl* plants. The oil is typically extracted from the barks, twigs, and leaves of the trees via hydro-distillation (Huang et al., 2019). Hydrodistillation is an extraction technique that uses water or steam to extract bioactive compounds (Silva et al., 2005). The predominant compounds present in cinnamon essential oils are copaene (34%), eugenol (32%), cinnamaldehyde (27%), monoterpenes (0.01 to 38%), and pinene (5%) (Huang et al., 2019).

Cinnamon essential oil can inhibit the growth of fungi both by direct contact and by vapor (Table 3) (Dimić et al., 2014; Ventura-Aguilar et al., 2018; Wang et al., 2013; Xing et al., 2010). The antifungal effect of cinnamon essential oil is weak on certain fungi such as *Aspergillus Parasiticus, Cladosporium cladosporioides, Eurotium herbarioru, Penicillium chrysogenum,* and *Aspergillus carbonarius*, with an inhibitory effect of less than 20%. However, there is a significant antifungal effect against two fungi: *Colletotrichum fragariae,* and *Botrytis cinerea*. Antifungal activity against *Colletotrichum fragariae* was tested in strawberries and exhibited a decay of 30% less than that of the control at 3 days (Ventura-Aguilar et al., 2018). The antifungal activity against *Botrytis cinerea* was tested on inoculated pears, both in a microemulsion (1 mg/mL) and as vapor (31 μg/L). Microemulsion and vapor showed decay rates of 45% and 28%, respectively, less than that for the control at 4 days (Wang et al., 2013). The low concentration of cinnamon oil as a vapor to limit *Botrytis cinerea* contamination makes it an excellent candidate for preserving fruit.

The antibacterial power of cinnamon essential oils has been tested in *Escherichia coli, Salmonella enterica,* and *Staphylococcus aureus* (Table 4) (Pokatong & Decyree, 2019; Todd

Table 3

Results Related to the Antifungal Activity of Cinnamon Essential Oil

Pathogens	Approach	Substrate	Concentration	Results	Reference
Colletotrichum fragariae	Chitosan Coating	*In vivo* - strawberry (20°C)	0%	Incidence (3days) (%): 80%	Ventura-Aguilar et al., 2018
			0.025%	Incidence (%): 50%	
Botrytis cinerea	Microemulsion	*In vivo* - pears - 20°c	0 µg/L	Decay (%): 83	Wang et al., 2013
			250 µg/L	Decay (%): 75	
			500 µg/L	Decay (%): 45	
			1000 µg/L	Decay (%): 38	
Botrytis cinerea	Microemulsion	*In vivo* - pears - 20°c	0 µg/L	Lesion (4 days) (mm): 7.5	Wang et al., 2013
			250 µg/L	Lesion (mm): 7	
			500 µg/L	Lesion (mm): 4	
			1000 µg/L	Lesion (mm): 3	
Botrytis cinerea	Microemulsion vapor	*In vivo* - pears - 20°c	0 µg/L	Decay (4 days) (%): 35	Wang et al., 2013
			7.9 µg/L	Decay (%): 5	
			15.87 µg/L	Decay (%): 15	
			31.74 µg/L	Decay (%): 7	
Botrytis cinerea	Microemulsion vapor	*In vivo* - pears - 20°c	0 µg/L	Lesion (4 days) (mm): 3	Wang et al., 2013
			7.9 µg/L	Lesion (mm): 0	
			15.87 µg/L	Lesion (mm): 1.5	
			31.74 µg/L	Lesion (mm): 1	
Aspergillus Parasiticus	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%):7	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):10	
			4.17 µL/mL	Inhibition (%):10	
			5.83 µL/mL	Inhibition (%):13	
Cladosporium cladosporioides	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%):15.8	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):15	
			4.17 µL/mL	Inhibition (%):19.2	
			5.83 µL/mL	Inhibition (%):19.2	

Pathogens	Approach	Substrate	Concentration	Results	Reference
Eurotium herbariorum	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%):5.3	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):5.8	
			4.17 µL/mL	Inhibition (%):6	
			5.83 µL/mL	Inhibition (%):8.9	
Penicillium chrysogenum	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%):12	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):18.5	
			4.17 µL/mL	Inhibition (%):18.5	
			5.83 µL/mL	Inhibition (%):18.5	
Aspergillus carbonarius	Mixed in agar	*In vitro*	1.25 µL/mL	Inhibition (%):3.5	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):3.5	
			4.17 µL/mL	Inhibition (%):3.5	
			5.83 µL/mL	Inhibition (%):5	
Aspergillus Parasiticus	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):2.7	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):7.9	
			4.17 µL/mL	Inhibition (%):9.2	
			5.83 µL/mL	Inhibition (%):17.2	
Cladosporium cladosporioides	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):5.8	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):11.7	
			4.17 µL/mL	Inhibition (%):13.3	
			5.83 µL/mL	Inhibition (%):17.8	
Eurotium herbariorum	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):11.4	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):15.8	
			4.17 µL/mL	Inhibition (%):15.8	
			5.83 µL/mL	Inhibition (%):22.8	
Penicillium chrysogenum	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):0.9	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):2.8	
			4.17 µL/mL	Inhibition (%):8.3	
			5.83 µL/mL	Inhibition (%):12	

Pathogens	Approach	Substrate	Concentration	Results	Reference
Aspergillus carbonarius	Vapor	*In vitro*	1.25 µL/mL	Inhibition (%):0.4	Dimić et al., 2014
			2.5 µL/mL	Inhibition (%):0.4	
			4.17 µL/mL	Inhibition (%):0.4	
			5.83 µL/mL	Inhibition (%):5	
Aspergillus flavus	Mixed in agar	*In vitro*	0%	Inhibition (mm) = 0	Xing et al., 2010
			0.1%	Inhibition (mm) = 14.2	
			0.25%	Inhibition (mm) = 15.1	
			0.50%	Inhibition (mm) = 17.5	
			1%	Inhibition (mm) = 20.3	
			2%	Inhibition (mm) = 25.8	
			3%	Inhibition (mm) = 32.3	
Penicillium expansum	Mixed in agar	*In vitro*	0%	Inhibition (mm) = 0	
			0.1%	Inhibition (mm) = 13.9	Xing et al., 2010
			0.25%	Inhibition (mm) = 14.9	
			0.50%	Inhibition (mm) = 16.6	
			1%	Inhibition (mm) = 19.3	
			2%	Inhibition (mm) = 23.6	
			3%	Inhibition (mm) = 27	
Rhizopus nigricans	Mixed in agar	*In vitro*	0%	Inhibition (mm) = 0	Xing et al., 2010
			0.1%	Inhibition (mm) = 11.9	
			0.25%	Inhibition (mm) = 13.8	
			0.50%	Inhibition (mm) = 15	
			1%	Inhibition (mm) = 16.5	
			2%	Inhibition (mm) = 20.5	
			3%	Inhibition (mm) = 25.3	
Aspergillus flavus	Mixed in agar	*In vitro*	N/A	MIC* (%) = 0.15	Xing et al., 2010
Penicillium expansum	Mixed in agar	*In vitro*	N/A	MIC* (%) = 0.15	Xing et al., 2010
Rhizopus nigricans	Mixed in agar	*In vitro*	N/A	MIC* (%) = 0.65	Xing et al., 2010

Note. * MIC = Minimal inhibitory concentration

Table 4

Results Related to the Antibacterial Activity of Cinnamon Essential

Pathogens	Approach	Substrate	Concentration	Results	Reference
Salmonella enterica	PBS*** (1 min, 4°c)	*In vivo*- Iceberg	0%	Count (3 days) (Log_{10} CFU/g): 4.5	Todd et al., 2013
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (2min, 4°c)	*In vivo*- Iceberg	0%	Count: 4.5	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (1 min, 8°c)	*In vivo*- Iceberg	0%	Count: 4.5	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (2min, 8°c)	*In vivo*- Iceberg	0%	Count: 4.5	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (1 min, 4°c)	*In vivo*- Romaine	0%	Count: 4.3	
			0.5% v/v	Count: 1	
Salmonella enterica	PBS*** (2min, 4°c)	*In vivo*- Romaine	0%	Count: 4.7	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (1 min, 8°c)	*In vivo*- Romaine	0%	Count: 4.7	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (2min, 8°c)	*In vivo*- Romaine	0%	Count: 4.7	
			0.5% v/v	Count not detected	
Salmonella enterica	PBS*** (1 min, 4°c)	*In vivo*- Spinach	0%	Count: 4.7	
			0.5% v/v	Count: 1	
Salmonella enterica	PBS*** (2min, 4°c)	*In vivo*- Spinach	0%	Count: 4.8	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (1 min, 8°c)	*In vivo*- Spinach	0%	Count: 4.8	
			0.5% v/v	Count: not detected	
Salmonella enterica	PBS*** (2min, 8°c)	*In vivo*- Spinach	0%	Count: 4.7	
			0.5% v/v	Count: not detected	

Pathogens	Approach	Substrate	Concentration	Results	Reference
General Bacterial count	Edible film	*In vivo* - Strawberry	0	Total count (Log_{10} CFU/g) = 4.82	Pokatong & Decyree, 2019
			0.3% v/v	Total count: 4.75	
			0.5% v/v	Total count: 4.73	
General Bacterial count	Edible film	*In vivo* - Strawberry - cold temp	0	Total count: 4.69	
			0.3% v/v	Total count: 4.69	
			0.5% v/v	Total count: 4.79	
Escherichia coli	Mixed in agar	*In Vitro*	N/A	MIC*: 1 mg/mL	Y. Zhang et al., 2016
			N/A	MBC**: 4mg/mL	
Staphylococcus aureus	Mixed in agar	*In Vitro*	N/A	MIC*: 1 mg/mL	
			N/A	MBC**: 2mg/mL	

Note. * MIC = minimal inhibitory concentration; ** MBC = minimal bactericide concentration; *** PBS = phosphate buffered saline

et al., 2013; Y. Zhang et al., 2016), and is considerably effective against these pathogens, both *in vivo* and *in vitro*.

Physicochemical Characteristics

Physicochemical characteristics of fruits include dozens of parameters (Barret et al., 2010); however, this review will focus on five characteristics related to organoleptic experience: moisture, acidity, sugar content, texture, and color.

Moisture in fruits and vegetables is paramount because it is linked to desirable appearance and texture (Barret et al., 2010; Pădureț et al., 2017). However, the current literature regarding the effects of lemon and cinnamon essential oil on moisture is mixed. For example, no difference was found between strawberries preserved in chitosan coating without lemon essential oil compared to strawberries preserved in coating with essential oils (Perdones et al., 2012). However, mangoes coated with chitosan film incorporated with limonene had a lower weight loss than those coated with chitosan film without limonene (Lan et al., 2020). Tzortzakis (2007) showed that strawberries exposed to air enriched in cinnamon essential oil had a greater weight loss compared to those exposed to ambient air. Many factors can influence the moisture content of strawberries, including the following: specific cultivar of fruit, respiration rate, type of treatments applied, evaporation through the skin, environment, and storage procedure (Ali et al., 2011; Shehata et al., 2020). Moreover, these factors can differ from one experiment to another and might explain the differences in obtained results.

Fruit acidity is an important determinant of consumer-perceived fruit quality (Wang et al., 2013) and can be determined by measuring the pH and the titratable acidity of the fruit

subject. The pH is correlated with the concentration of hydrogen ions present, while the titratable acidity is directly correlated with the content of organic acid present in the fruit (Shehata et al., 2020). Strawberries have, on average, a pH of 3.4 (Brown, 2019), but this can vary depending on the cultivar. The primary organic acids present in strawberries are citric acid and malic acid, with citric acid content being the highest (Hwang et al., 2019). These acids are closely linked to the acidic taste that is sometimes attributed to strawberries. However, research on the effect of essential oil on fruit acidity is limited. Lemon essential oils incorporated in a matrix slightly decreased the pH of strawberries after 14 days (control: 3.95 vs. lemon oil 3.7) (Perdones et al., 2012). However, several studies indicated no difference in the strawberries treated with cinnamon essential oil and the control, and the pH and concentration of citric acid did not vary significantly (Pokatong & Decyree, 2019; Tzortzakis, 2007). Cinnamon essential oil has been shown to slightly decrease the pH of guava fruit after 21 days of storage, with a pH of 4.05 versus 4.26 for the control (Etemadipoor et al., 2019). Since concentration of citric acid did not change significantly at 28 days of storage in the presence of cinnamon essential oil, it remains difficult to clearly ascertain the effect of essential oils on the acidity of fruit. The acidity varies depending on the hydrolysis rate of the polysaccharides in the fruit, the presence of microorganisms and the treatment application methodology. Additional studies are warranted to better understand the effect of essential oils on the acidity status of fruit.

Soluble sugars can also be used to assess the quality of fruit as the ratio of soluble sugar with titratable acid is a good indicator of sweetness (Murti et al., 2012). Generally, the soluble solid content values of all strawberry samples gradually decreased with increasing

storage period (Shehata et al., 2020) which means that strawberries become less sweet as they age. The main soluble sugars in strawberries are fructose, glucose, and sucrose (Ornelas-Paz et al., 2013). During storage, sucrose decreases significantly, suspectedly due to its being required for respiration (Blanch et al., 2012). However, there are very few studies on the impact of lemon essential oil on the soluble sugar in fruit. Perdones et al. (2012) and Shehata et al. (2020) studied lemon oil incorporated into an edible coating. The lack of control with regular product coating prevents us from concluding the effect of lemon essential oils as part of a matrix on the soluble solid content. However, essential oil of cinnamon decreased the total soluble solids loss in fruit when the fruit was exposed for several days (Etemadipoor et al., 2019; Tzortzakis, 2007).

Reducing sugars have either a free aldehyde group or a free ketone group that is readily converted to a carboxylic acid by mild oxidants, and thus react as reducing agents. The primary reducing sugars in strawberries are glucose and fructose. The status of these sugars can vary depending on several factors including the breakdown of polysaccharides and fibers into smaller sugars which increases the concentration of reducing sugars. However, reducing sugars can also decrease due to their reaction with the increased oxidized substances in fruits (Fuqi & Xuxiang, 2017). Further research is needed to understand if essential oil can affect the status of these sugars.

The texture of fruit is related to the alteration, disintegration, and flow of the food subjected to a force. Pectin, a complex biopolymer, has a major role in fruit firmness in many fruits including strawberries (Posé et al., 2012). Firm fruit is easier to store, more attractive to consumers and less susceptible to rotting (Drobek et al., 2020). Firmness is also considered

to be an essential factor related to consumer acceptance (Barret et al., 2010; Drobek et al., 2012). The texture of strawberry is also highly influenced by endogenous enzyme activities during ripening. For example, as the cellulase activity increases, cellulose and other polysaccharides are decomposed, resulting in additional softening of the product texture (Abeles & Takeda, 1990). Polygalacturonases and pectin-methyl-esterases can deteriorate pectin (Azodanlou et al., 2004). All these changes in the chemical structure of the cell wall of strawberries allow the fruit to become softer. Perdones et al. (2012) noticed that lemon essential oils had no effect on the firmness of strawberries when integrated into an edible coating. In contrast, cinnamon essential oil affects the firmness of several fruits, including strawberries. The firmness of the strawberry decreased more in the presence of cinnamon oil vapor compared to the control, with a force of 0.8 kg/cm^2 and 0.7 kg/cm^2, respectively (Tzortzakis, 2007). In guava, the firmness decreased at a slower rate when the fruit was treated with cinnamon essential oil, with firmness of 60 Newtons after 14 days for the treated fruit versus 47 Newtons for the control (Etemadipoor et al., 2019). The effect of oils on firmness seems to vary depending on the oil and the fruit. This could be due to the oil degrading the cell's membrane or affecting the activity of microorganisms.

Color is a critical quality factor in fruits. For example, the shape, gloss, and color of the fruit are the first characteristics that appeal to consumers when they are selecting fruit (Barrett et al., 2010). Color primarily comes from the natural pigments present in fruits and can be sorted into several categories: chlorophylls (green), carotenoids (yellow, orange, and red), anthocyanins (red, blue), flavonoids (yellow), and betalains (red). The color of the fruit reveals important information on ripeness and freshness as well as the flavor quality. For

example, in strawberries, there is an increase in anthocyanins during the ripening process (Ornelas-Paz et al., 2013). Because consumers tend to associate expected colors with specific fruit items (Crisosto et al., 2003), maintaining the bright red color of strawberries is crucial. Lemon essential oils do not seem to affect the color of the strawberries when incorporated in an edible coating, compared to the edible coating without essential oil (Perdones et al., 2012). The effect of cinnamon oil on the discoloration of fruits and vegetables was briefly noted in several studies with regard to product content and consumer acceptance (Todd et al., 2013; Tzortzakis, 2007). However, Wang et al. (2013) demonstrated that cinnamon oil does not affect the color of the fruit. This result was later confirmed by Etemadipoor et al. (2019) who found that cinnamon oil did not significantly modify the color of guavas. More research is needed to find out if essential oils of lemon or cinnamon can alter the color of strawberries by interacting with pigments.

Sensory Characteristics

Sensory evaluation is necessary to assess the quality of fruit since it provides direct information about how the consumer relates to the product. The primary strawberry quality attributes are firmness, color, aroma and taste (including acidity, sweetness, sourness, astringency) (Azodanlou et al., 2004; Perdones et al., 2012; Santos et al., 2018). Aroma and sweetness are the most important attributes for consumer acceptance (Azodanlou et al., 2003).

The effect of lemon essential oils on the sensory characteristics of fruit has shown divergent results. For example, Perdones et al. (2012) determined that lemon oil as part of an edible coating was negatively affecting the aroma and flavor of strawberries, while a study

conducted by Shehata et al. (2020) described the opposite. In both studies, lemon essential oil was incorporated into an edible emulsion concentration of 3% or less, and both studies included trained-panelist tests. However, additional research needs to be conducted to understand the effect of lemon essential oils on the sensory characteristics of fruit, particularly regarding consumer preferences.

Similarly, the effects of cinnamon essential oils on the sensory quality of fruits are inconclusive. For example, the effect of this oil incorporated in an edible coating has been studied in apples (Santos et al., 2018). The results showed that the incorporation of the essential oil at a low concentration can negatively affect both the flavor and the aroma of fruit. The score was lower for aroma and flavor and higher for color. Additional studies are warranted, particularly regarding the effect of cinnamon essential oils on sensory quality and acceptability.

Conclusion

Essential oils are extracts from plants, and these oils have been extensively used in the pharmaceutical and cosmetic industries. Researchers are currently studying the potential application of these extracts in the agricultural and food industries. Essential oils appear to be a viable candidate for the extensive preservation of fruit to decrease food loss and waste. Extensive research has been conducted on the antimicrobial properties of essential oils including the effects on foodborne pathogens and fungi that are involved in the loss of quality in fruits. For example, cinnamon essential oil is highly efficient at reducing bacterial growth, and both lemon and cinnamon essential oils have shown acceptable results as antifungal agents, especially against the major fungus, *Botrytis cinerea*. However, only a few

studies have described the impact of cinnamon essential oils and lemon essential oils on the physicochemical characteristics and sensory properties of fruits. The research results for these two essential oils are promising with very few alterations in the physicochemical and sensorial characteristics of the fruit. However, the data is limited and inconsistent. Understanding the effect of lemon essential oils and cinnamon essential oils on the physicochemical properties and sensory characteristics of fruits is the necessary next step. Moreover, enhancement of product quality and safety with a healthier preservative is not sufficient in and of itself; it must also preserve the quality-related characteristics of the food item. Essential oils show promising potential for their use as fruit preservatives in the food industry.

Chapter 2: Journal Article

EFFECTS OF LEMON OR CINNAMON ESSENTIAL OIL VAPOR ON PHYSICOCHEMICAL PROPERTIES OF STRAWBERRIES DURING STORAGE

Abstract

Physicochemical properties of strawberries (*Fragaria x ananassa*) were evaluated after a 12-hour treatment with lemon essential oil (*Citrus x limon*) or cinnamon essential oil (*Cinnamomum cassia*) vapor during storage at 22°C for four days in an accelerated shelf life study and 4°C for 18 days in a validation study. Weight loss was blunted in fruit treated with oil vapor during the first days of storage ($P < 0.05$). Lemon essential oil delayed fruit darkening ($P < 0.05$) but reduced firmness of strawberries ($P < 0.05$). Strawberries treated with cinnamon essential oil had a higher concentration of reducing sugars ($P < 0.05$), and a decrease of 16.7% visible decay, although the difference was not significant. Oil vapor treatment did not alter pH, organic acid content, or soluble solid content during storage as compared to controls. Since lemon and cinnamon essential oils have well documented antimicrobial properties, they may be suitable to naturally preserve fruit.

Keywords: Shelf life; Quality; Fruit; Fresh Produce; Natural Preservatives

1. Introduction

Strawberries (*Fragaria* × *ananassa*) are one of the most consumed fruits globally while being one of the most perishable food items. They are commonly consumed fresh and are favored for their rich taste and nutritional values (Shehata et al., 2020). However, they are very susceptible to microbial infections, especially to fungal pathogens. They are often thrown away due to loss of quality and fruit decay (Vitoratos, Bilalis, Karkanis, & Efthimiadou, 2013). Although many synthetic preservatives are effective against strawberry pathogens, consumers gained interest in natural and minimally processed food, inciting the food industry to consider using natural products as preservatives (Fisher & Phillips, 2006). Therefore, organic compounds have gained in popularity for their potential application in food and are being investigated. Essential oils are attractive candidates to improve the quality of fruits during storage. They are extracts obtained from plants, and most of them are on the FDA GRAS list. Lemon essential oil and cinnamon essential oil have antimicrobial properties, meaning they can prohibit the growth of many bacteria and fungi at low concentrations, such as *Escherichia coli, Salmonella,* and *Botrytis cinerea* (Al-Jabri & Hossain, 2016; Dimić, Kocić-Tanackov, Mojović, & Pejin, 2014; Fisher & Phillips, 2006; Umagiliyage, Becerra-Mora, Kohli, Fisher, & Choudhary, 2017; Vitoratos, et al., 2013). Since their bioactive compounds vaporize easily, it allows sanitation via fumigation (Tzortzakis, 2007). However, the use of essential oil for preserving fruit quality still requires research. While many studies have shown their antimicrobial effect, only a few studies have shown the effects of lemon and cinnamon essential oils on the quality-related properties of the fruit, especially strawberries. Therefore, this research aimed to study the effects of

essential oil vapor from lemon and cinnamon on physicochemical attributes of strawberries, including visible decay, weight loss, texture, color, titratable acidity, pH soluble solids, and reducing sugars. The hypothesis is that strawberries treated with lemon or cinnamon essential oils vapor will have a decrease in weight loss and visible decay during storage, and other parameters will not be negatively affected. The analysis presented in this study will convey valuable information about the benefits of essential oils as fruit preservatives. Indeed, it brings new information about the use of essential oil vapor treatment to preserve fruit, and potentially decreasing fruit loss and waste along the food supply chain.

2. Material and Methods

2.1. Strawberry Samples and Essential Oils

Strawberries were purchased from a local grocery store. Sixty-three were selected for their absence of defects, and uniformity in weight, shape, and color. Organic essential oils of lemon (*Citrus x limon*; originated from Argentina) and cinnamon (*Cinnamomum cassia*; originated from China) were obtained from Plant Therapy Essential Oils Corporate (Twin Falls, US). Purity of oils and extraction methods were verified by the suppliers.

2.2. Treatment with Essential Oils

Fifteen strawberries were placed inside a polystyrene container (Volume: 14.37 L) for each group: cinnamon treatment, lemon treatment and untreated strawberries. Essential oil of lemon or cinnamon was added to the container at a concentration of 100 ppm via seven Whatman No.1 filter papers (diameter: 7 cm). Filter papers without oil were placed in the control group. Volatile compounds were allowed to vaporize in the container for 12 hours at a temperature of 4°C. After treatment, strawberries were moved to aluminum foil baking

pans and stored at 22°C with 50% humidity for four days. Decay, weight loss, color, texture, pH, titratable acidity, soluble solids, and reducing sugar were measured each day for four sequential days. Three replications were used for each analysis.

A similar protocol was replicated for a validation study, where two groups of 9 strawberries were analyzed: cinnamon essential oil vapor treatment and untreated strawberries. They were stored at 4°C with 85% humidity for 18 days after treatment. Weight loss, color and soluble solids were measured with nondestructive testing strategies at day zero, three, seven, fourteen and eighteen. Additionally, texture, pH, titratable acidity and reducing sugars were measured on the last day of the experiment.

2.3. Physicochemical Properties

2.3.1. Decay

Decay was visually evaluated following the exposure to oils. It was determined by evaluating the presence of mold on strawberries, using a scale from 0 to 4, where 0 refers to no decay; 1 refers to 1-25% decay (probable decay); 2 to refers 26-50% decay (moderate); 3 refers to 51-75% decay (moderate to severe); and 4 refers to 76-100% decay (extreme) (Kahramanoğlu, 2019). It was expressed in percentage, based on the methodology used by Cao et al. (2010):

$$(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4) \times 100/(4 \times N)$$

where N is the total number of fruits measured and N_1, N_2, N_3, and N_4 were the number of fruits that had different level of decay.

2.3.2. Weight Loss

After treatment with oils, the strawberries were individually weighed and labeled before being placed for storage. During the experiment, they were weighed every day using an analytical balance (OHAUS, United States). In the validation study, strawberries were weighed at day zero, three, seven, 14 and 18 days. Weight loss was calculated using the following formula:

$$WL\ (\%) = \frac{(FW - IW)}{IW} \times 100$$

where FW is the final weight and IW is the initial weight.

2.3.3. Surface Color

Surface color was determined by using ColorFlex EZ Spectrophotometer (Hunterlab, Reston, Virginia, USA). Areas selected for color measurements were free from obvious defects that may affect the uniform color readings. The results were expressed in the CIELAB color space, where L* value, a* value and b* value refer respectively to lightness, red/green coordinate, and yellow/blue coordinate. Color was analyzed using lightness, chroma and hue. Chroma and hue were calculated with the following formula:

$$Chroma = \sqrt{(a*^2 + b*^2)}$$

$$Hue = tan^{-1}\left(\frac{b*}{a*}\right)$$

2.3.4. Firmness

Strawberry fruit firmness was measured using a QTS25 texture analyzer (Brookfield, Middleboro, MA, USA) with a cylindrical probe of 13 mm. Strawberries were cut in half and

were placed on their flat surface. The force required to compress five millimeters of strawberry fruit was measured in Newtons.

2.3.5. pH and Titratable Acidity

Five grams of strawberry fruit was diluted in 15 mL of distilled water and homogenized at 16,000 rpm for one minute with Fisherbrand™ 850 Homogenizer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, U.S). Homogenized solution was used to measure pH, using a Mettler Toledo FG2/EL2 pH meter (Columbus, OH, USA).

Titratable acidity was measured by potentiometric titration. Five grams of sample was diluted in 15 mL distilled water and homogenized. The juice sample (10 mL) was titrated with 0.05N NaOH. Titration was monitored using a pH meter and was stopped when the solution reached a pH between 8.25 to 8.40. Results are expressed in grams of citric acid per 100 grams of strawberry. The following formula was used:

$$TA = \frac{V \times N \times Eqv.Wt.C.A.}{W \times 1000} \times 100$$

where V is the volume of NaOH used (mL), N is standardized NaOH normality, Eqv. Wt. C.A. is the equivalent weight of citric acid (64.4), and W is the weight of the sample.

2.3.6. Sugar Content

Soluble solid content was determined using a digital handheld refractometer (Tiaoyeer, China). Strawberries were squeezed to extract the juice and placed on the refractometer. The results were expressed in percentage. During the validation study, soluble solids were measured using a non-destructive Brix Meter, PAL-HIKARI 4 (Atago, Bellevue, WA, USA).

Measurements of reducing sugars were based on the Somogyi-Nelson Methodology (Somogyi, 1952). Five grams of sample was diluted in 15 mL distilled water and homogenized. After centrifuging (10,000 rpm for 5 minutes), 2 mL sample was used to analyze reducing sugar content. A cuvette-based spectrophotometer UV-2600 (Shimadzu Scientific Instruments (SSI), Columbia, Maryland, USA) was used to read the absorbance at 520 nm (Nelson, 1944). Polymethyl methacrylate cuvettes were used (BrandTech Scientific Inc., Essex, CT, USA). A standard curve with a concentration range of zero to 50 µg/mL was prepared with glucose (Sigma-Aldrich, USA) to calculate the reducing sugar content in the samples.

2.4. Statistical Analysis

Data were presented as means with standard deviations. Data were analyzed using the IBM® SPSS® software 26.0. Weight loss, color, firmness, pH, titratable acidity, soluble solids, reducing sugar and pH were analyzed by One-way ANOVA, comparing treatments for each day, as well as comparing each treatment in time. Decay was analyzed using a mixed ANOVA using time and treatment as factors. Post-hoc comparisons of means were performed by Bonferroni's tests. Results were considered statistically significant when $P \leq 0.05$.

Validation study data were analyzed using an independent t-test for each day of data. Results were adjusted for multiple comparisons with the Bonferroni correction for weight loss, color, and soluble solids.

A post hoc power analysis was performed to determine the sensitivity to detect type II errors in the treatment effects on visible decay, using the G*Power software, version 3.1.9.2 (Faul, Erdfelder, Lang, & Buchner, 2007).

3. Results and Discussion

3.1. Weight Loss

Weight loss is strongly linked to perishability in fruits (Dhital, Mora, Watson, Kohli, & Choudhary, 2018) and is correlated with respiration rate, evaporation of water through the skin, type of treatments, environment, and storage procedure (Ali, Abrar, Sultan, Din, & Niaz, 2011; Shehata et al., 2020). Weight loss significantly increased over time for treated and untreated strawberries ($P < 0.05$), which is consistent with results reported by similar studies on strawberries (Ali et al., 2011; Shehata et al., 2020; Ventura-Aguilar, Bautista-Baños, Flores-García, & Zavaleta-Avejar, 2018). Strawberries that received lemon or cinnamon treatment had lower weight loss during the first day of storage than control; this gap disappeared after the first day, as shown in Fig. 1. This delayed onset of weight loss may be due to the antimicrobial and antioxidant activity of lemon and cinnamon oils (Hsouna, Halima, Smaoui, & Hamdi, 2017; Montero, Souza, Oliveira, & Rosa, 2021; Vitoratos et al., 2013; Xing, Li, Xu, Yun, & Lu, 2010; Zhang, Liu, Wang, Jiang, & Quek, 2016). Previous research on essential oil vapor has shown its benefits in reducing weight loss in fruit (Sumalan et al., 2020). Increasing treatment concentration or fumigating the strawberries for a longer time might be beneficial for decreasing weight loss in strawberries (Tzortzakis, 2007).

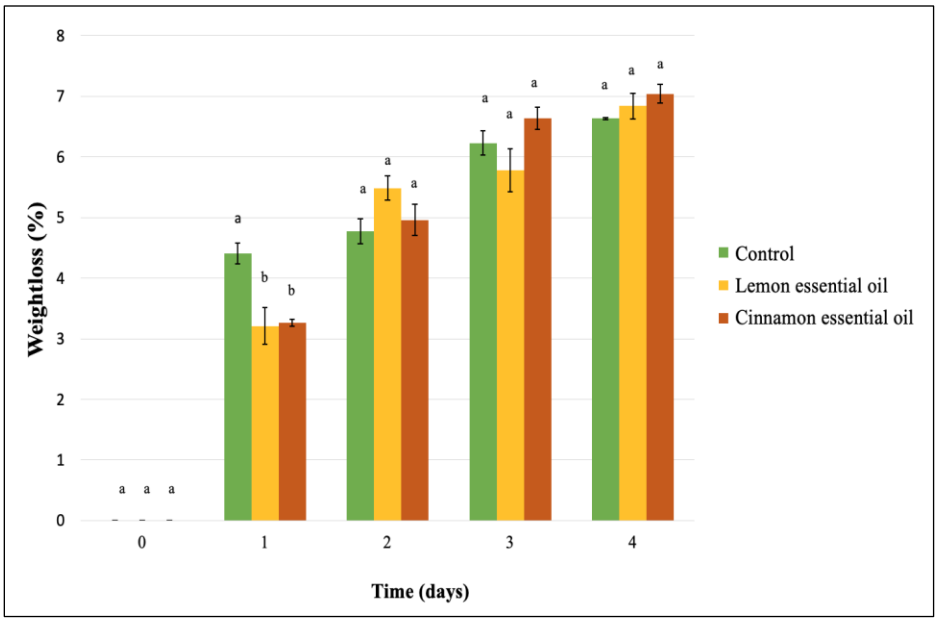

Fig. 1. Weight loss (%) in strawberries stored at 22°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at P < 0.05 after Bonferroni's correction.

3.2. Color

Color is one of the main factors that appeal to consumers when selecting fruit (Barrett, Beaulieu, & Shewfelt, 2010). The surface color of strawberries was defined by measuring the lightness, the hue (i.e., the tint), and the chroma (i.e., the saturation), as shown in Fig. 2. Lightness and chroma decreased in time for all strawberries, regardless of the treatment ($P < 0.05$). Hue remained constant through the experiment. Similar studies found comparable results with a loss of luminosity and color vividness in time (Perdones, Sanchez-Gonzalez, Chiralt, & Vargas, 2012; Shehata et al., 2020; Wang et al., 2013). Loss of bright glossy red color in strawberries comes inevitably as storage time increases due to the degradation of anthocyanins, loss of water, and oxidation reactions (Holzwarth, Korhummel, Kammerer, & Carle, 2012). However, strawberries treated with lemon essential oil tended to have a lighter

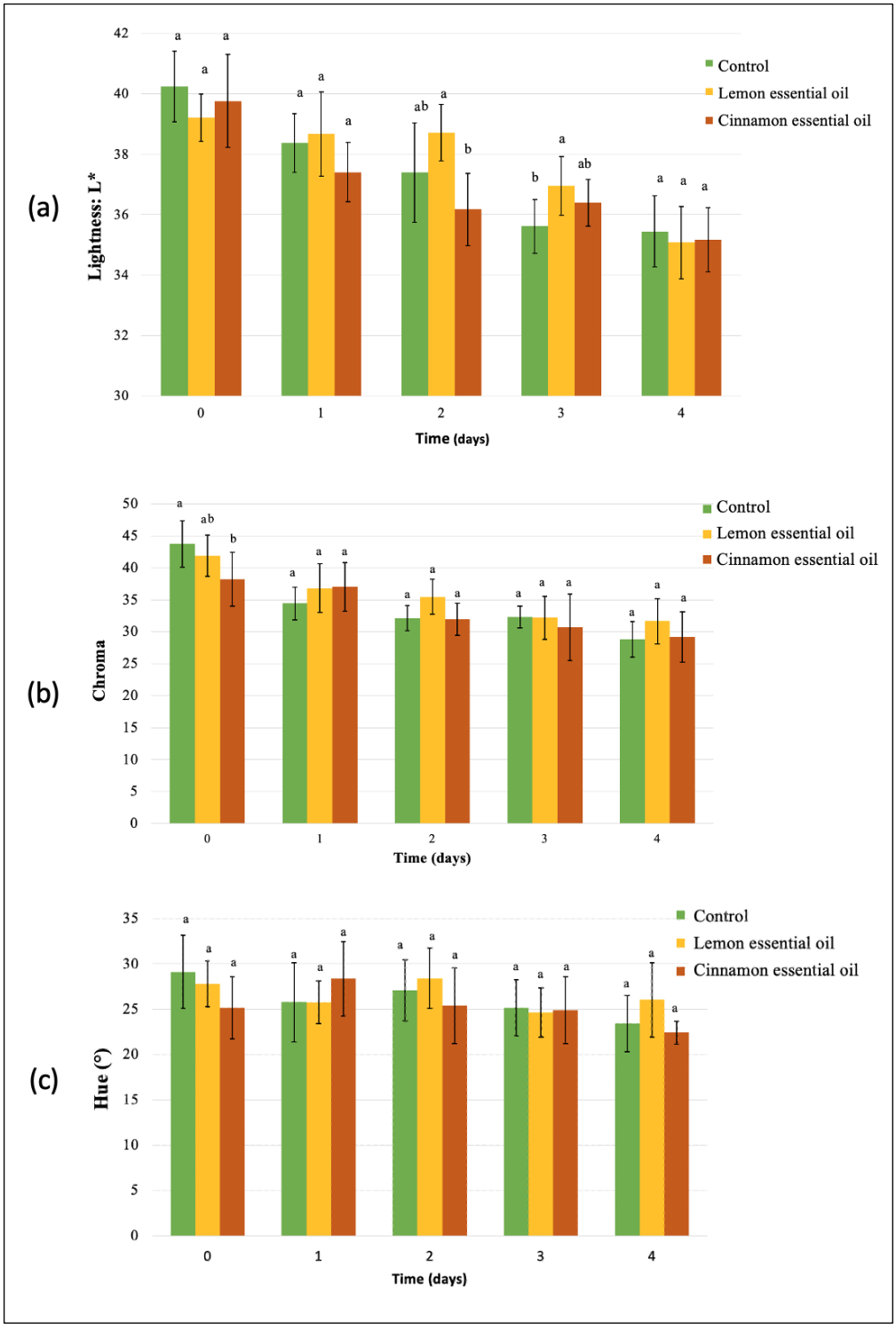

Fig. 2. Luminosity (a), chroma (b), and hue (c) of strawberries stored at 22°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at $P < 0.05$ after Bonferroni's correction.

color than the control after two days of storage, with a significant difference on the third day of storage. This difference did not subsist on day four. Lemon essential oil compounds might have participated in preserving pigments at the surface of strawberries for longer. Perdones et al. (2012) reported that strawberries treated with lemon essential oil chitosan coating also had a slightly lighter color than control, as well as a redder tint.

Strawberries treated with cinnamon essential oil vapor had no significant difference with control, except for chroma. It was lower right after treatment, meaning the strawberry shows less saturated color. This difference disappears early during storage time. Similar studies on cinnamon essential oil incorporated in edible coatings or vapor phase showed no effects of the oil on strawberry color (Tzortzakis, 2007; Wang et al., 2013).

3.3. Firmness

Firmness is an essential factor in consumer acceptance (Barrett et al., 2010; Drobek, Frąc, Zdunek, & Cybulska, 2020). Strawberry texture is influenced by endogenous enzyme activities altering the chemical structure of the cell wall, resulting in softening of the fruit during ripening and storage (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2004). Firmness decreased in time for all strawberries ($P < 0.05$), as illustrated in Fig. 3. On average, a force of 8.0 Newtons was needed to go through five millimeters of strawberry for the strawberries treated with lemon essential oil compared to 11.7 for the control on day two. This difference continued to increase on storage day three and four. Strawberries treated with lemon essential oil vapor became significantly softer starting the second day of storage. This loss of firmness might have been due to an increased breakdown of polysaccharides, fibers,

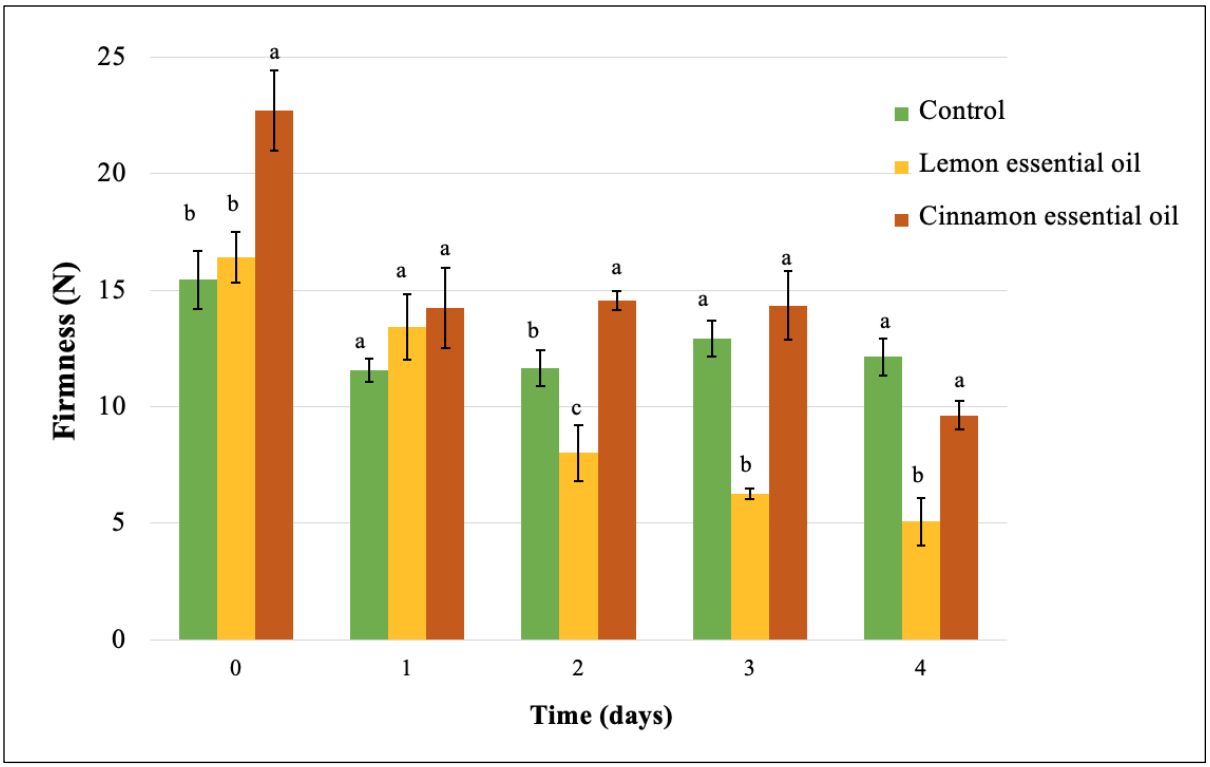

Fig. 3. Firmness of strawberries stored at 22°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at P < 0.05 after Bonferroni's correction.

and cell membranes due to contact with lemon essential oil acidic compounds (Cho & Buescher, 2012; Howard, Burma, & Wagner, 1994).

Cinnamon essential oil vapor significantly increased the firmness of strawberries right after treatment (day zero), with a force of 22.7 Newtons compared to 15.5 for the control ($P < 0.05$). However, this difference disappeared after one day of storage. Afterwards, strawberries treated with cinnamon essential oils had a similar firmness than the untreated strawberries. These results were consistent with similar studies reporting that cinnamon essential oil treatment as vapor or in microemulsion did not affect the firmness of strawberries (Tzortzakis, 2007; Wang et al., 2013).

3.4 Acidity Status

pH and titratable acidity are indicators of acidity in strawberries (Murti, Kim, & Yeoung, 2012). pH is correlated with the concentration of hydrogen ions present, while titratable acidity is directly correlated with the content of organic acid present in the fruit (Shehata et al., 2020). Primary acids are citric acid and malic acid; these acids are closely linked to the acidic taste to strawberries (Hwang, Kim, & Shin, 2019). Organic acids can fluctuate based on microbial growth and metabolic activity (i.e., respiration) (Perdones et al., 2012). The effects of lemon essential oil vapor and cinnamon essential oil vapor on pH and titratable acidity are shown in Fig. 4 (a) and (b). Lemon or cinnamon essential oil vapor treatment did not affect the pH and the concentration of citric acid in strawberries.

There were considerable variations of pH between individual strawberries within each group. Strawberries were selected based on their appearance (e.g., redness, texture profiles), and their physical aspect was not consistent with their acidity (Scott, Williams, Wallace, & Du, 2021). It might explain why no difference was observed while previous studies have found a slight decrease in pH in the presence of lemon or cinnamon treatment compared to untreated strawberries (Perdones et al., 2012; Tzortzakis, 2007). Titratable acidity results were consistent with those reported by Perdones et al. (2012) and Tzortzakis (2007).

3.5. Sugar Content

Soluble solid content corresponds to the soluble constituents in the fruit. They are mainly sugars, including fructose, glucose, and sucrose (Ornelas-Paz et al., 2013). During the ripening process, the concentration of soluble solids fluctuates depending on strawberry metabolism, time of harvest, and the cultivar of the strawberry (Azam, Ejaz, Rehman, Khan,

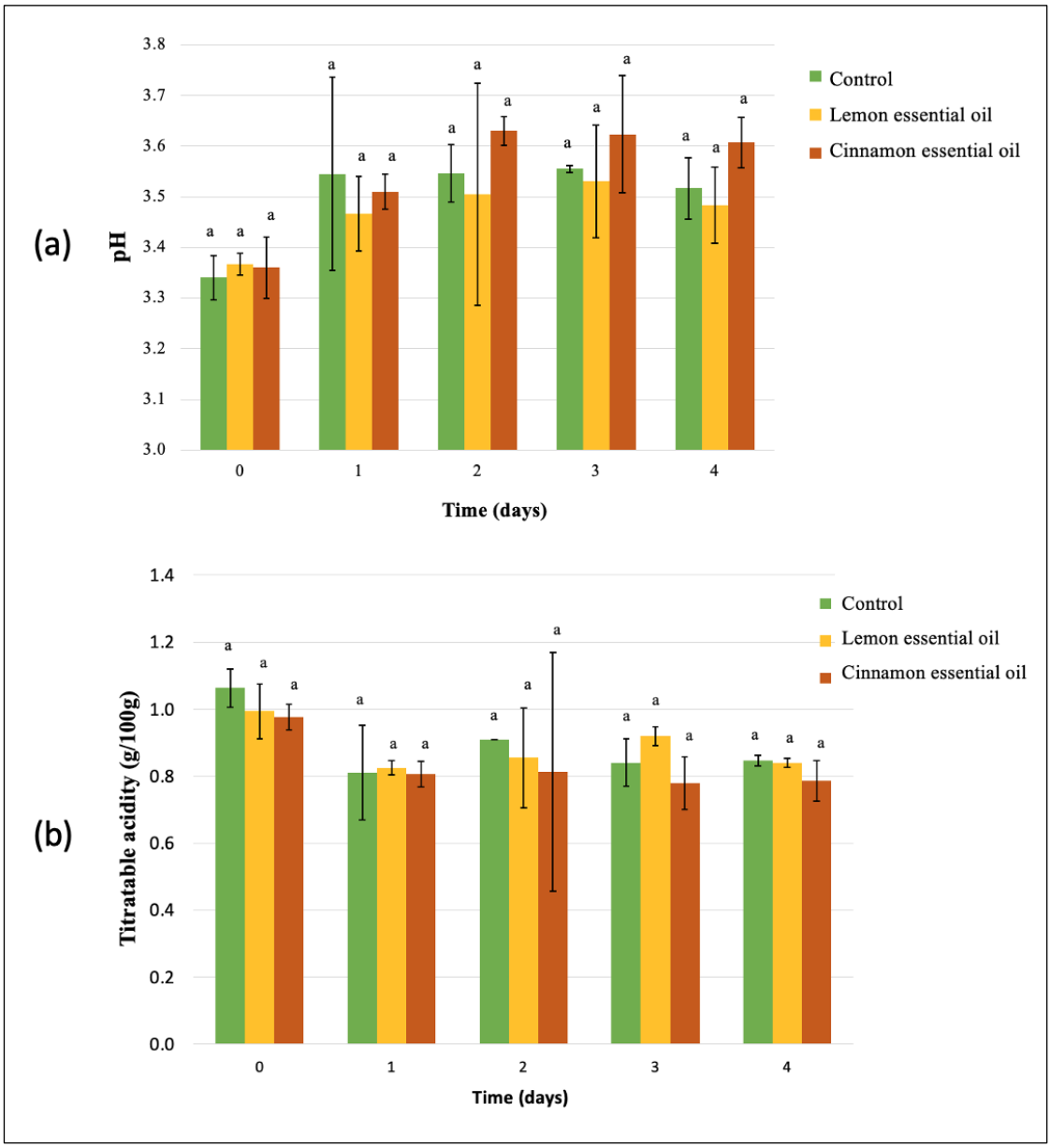

Fig. 4. pH and titratable acidity of strawberries stored at 22°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at $P < 0.05$ after Bonferroni's correction.

& Qadri, 2019; Hasing, & Whitaker, 2014; Nunes, Brecht, Morais, & Sargent, 2006). In this study, the soluble solids concentration in strawberries did not differ between treated and untreated strawberries, as shown in Fig. 5 (a).

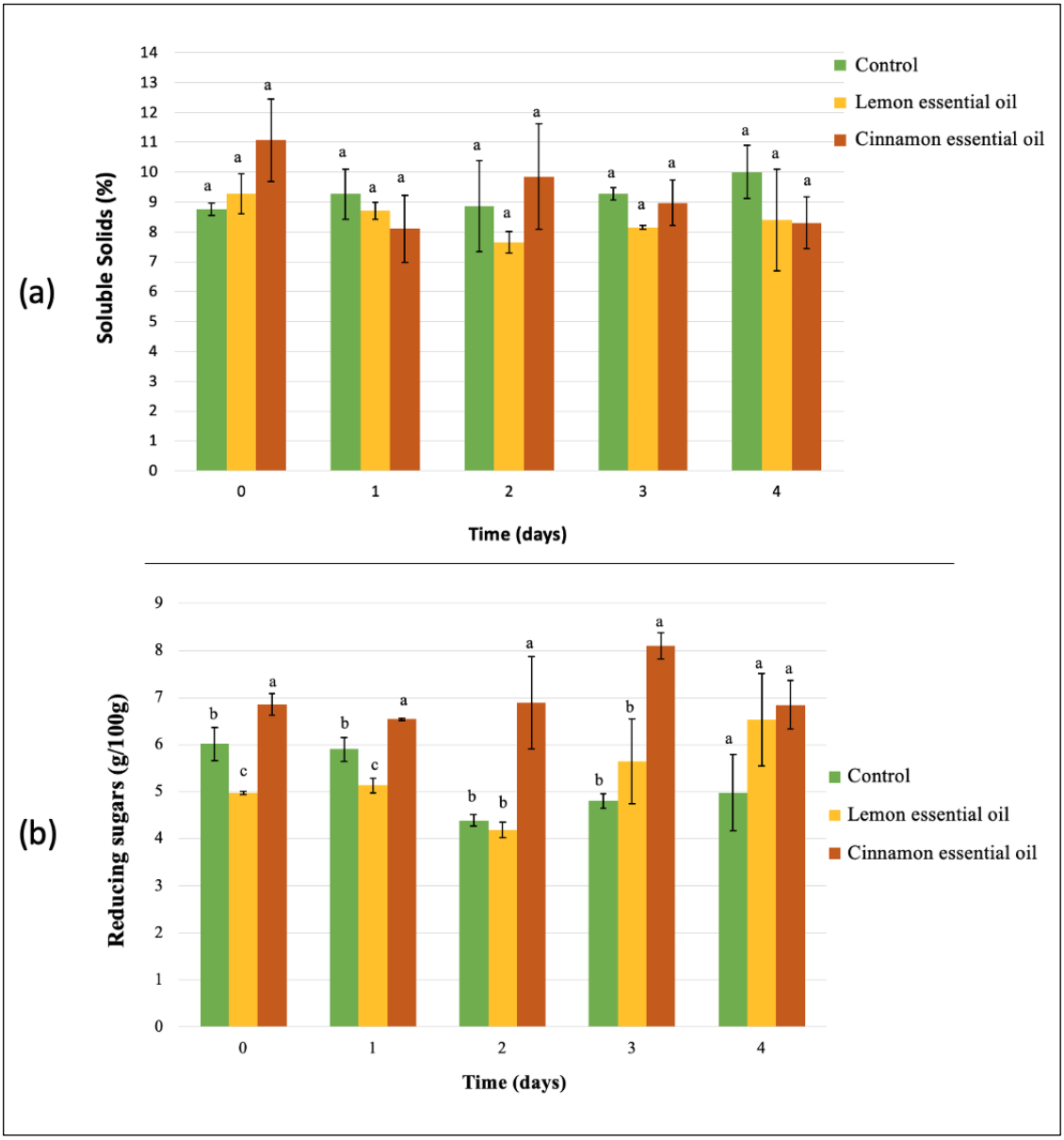

Fig. 5. Concentration of soluble solids and reducing sugars of strawberries stored at 22°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at $P < 0.05$ after Bonferroni's correction.

Reducing sugars have a free aldehyde or ketone group that is readily converted to a carboxylic acid by mild oxidants. Prevalent reducing sugars in strawberries are glucose and fructose. Several factors affect the concentration of reducing sugars in strawberries: during

the ripening process, polysaccharides are hydrolyzed to smaller sugars and increased the amount of reducing sugars (Azam et al., 2019). However, microorganisms and strawberry cells can use these sugars and therefore decreasing their concentration. Also, these sugars could be oxidized/metabolized to other compounds (Fuqi & Xuxiang, 2017). There is currently no literature found on the effects of lemon or cinnamon essential oil on reducing sugars. Fig. 5 (b) shows the reducing sugar concentration in strawberries for each treatment when stored at 22°C. The concentration of reducing sugars is lower in strawberries treated with lemon essential oil vapor compared to untreated strawberries during the first and second day of storage, which did not persist afterwards. Strawberries treated with cinnamon essential oil vapor showed a higher concentration of reducing sugar right after treatment, until day four. It is possible that cinnamon essential oil affects microbial growth and slows the ripening of strawberries by maintaining a reducing environment, leading to a higher amount of reducing sugars.

3.6. Decay

During this experiment, decay was visually assessed for each storage day, as shown in Fig. 6. Decay increased in time for treated and untreated strawberries ($P < 0.05$). Lemon essential oil has been shown to have antifungal properties against some pathogens, including *Botrytis cinerea*, the primary source of decay in strawberries (Dimić et al., 2014; Umagiliyage et al., 2017; Vitoratos et al., 2013). However, no differences were observed between untreated strawberries and strawberries treated with lemon essential oil vapor. One possible reason is that species of fungi less sensitive to the oil could grow enough to be

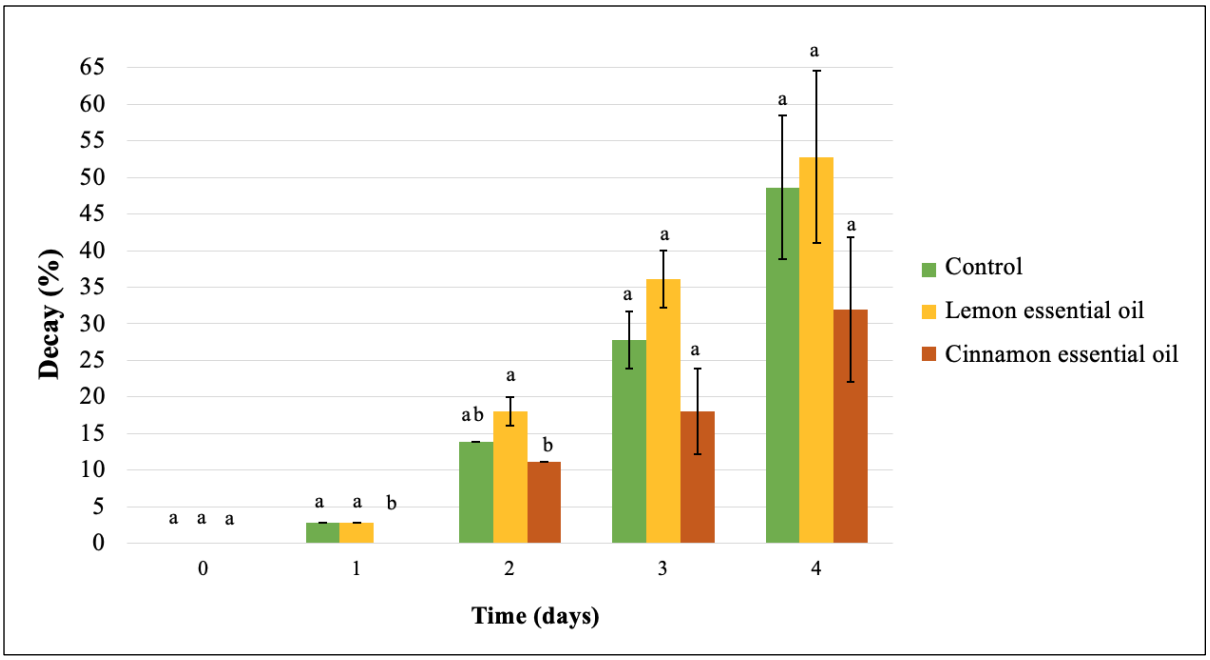

Fig. 6. Decay percentage in strawberries stored at 22°C.

visible. Moreover, lemon essential oil significantly decreased the firmness of strawberries, which might have made the fruit surface more suitable for microbial growth.

Strawberries treated with cinnamon essential oil vapor did not show significant difference overall. As shown in Fig. 1, there was a tendency for treated strawberries to have less decay than untreated strawberries, with a percentage difference of 16.7% between control and cinnamon essential oil on the fourth day, although the difference was not significant. Cinnamon essential oil has been shown to have antimicrobial effects (Dimić et al., 2014; Ventura-Aguilar et al., 2018; Wang, et al., 2013; Xing et al., 2010). Tzortzakis (2007) also reported that strawberries exposed to cinnamon essential oil vapor for several days had a slower decay than the control. The lack of significant results in this study could be explained by the fact that the strawberry sample size was slightly low, decreasing the statistical power

of the decay analysis. Further investigation with increased sample sizes would help establish the effects of cinnamon essential oil regarding the growth of visible mold.

3.7. Validation Study

A validation study was conducted at 4°C with 85% humidity for 18 days to test the cinnamon group in a practical and real storage condition. Percentage weight loss increased over time for the control and cinnamon essential oil vapor treatment ($P < 0.05$), as shown in the supplementary figure. Strawberries treated with cinnamon essential oil vapor had less weight loss than untreated strawberries until day seven. Afterward, no difference was found between treatments and control.

Overall, strawberry firmness and color did not differ significantly when treated with cinnamon essential oil vapor compared to untreated. Cinnamon essential oil vapor treatment had a different effect immediately after treatment compared to the accelerated study, with a small decrease in lightness ($P < 0.05$), and no change in chroma. These minor variations in effects might be due to the sample size used in the experiment. Lightness and chroma decreased in time for all strawberry groups, comparably to the accelerated shelf life study. A minor decrease of hue was seen for all strawberry groups when stored at 4°C. The same tendency was detected in the accelerated shelf life experiment, but the difference was not significant. Perdones et al. (2012) noticed a decrease of strawberry hue in time. Variation in hue might be depending on the type of cultivar and method of storage. Overall, the findings noted in the validation study were similar to the accelerated shelf life study.

pH and titratable acidity showed similar results between cold storage and accelerated study. pH ranged from 3.3 to 3.8, and titratable acidity ranged from 0.7 to 1 gram per 100

grams of strawberry, which is consistent with the current literature (Ali et al., 2011; Brown, 2019; Ornelas-Paz et al., 2013). Soluble solids concentration increased by 4% between the first and third day. Since the microbial growth is slowed by low temperature (Ali et al., 2011), the ripening process may increase soluble solids in the strawberries. Soluble solids then decreased concurrently to the formation of mold on the surface of strawberries (data not presented). No difference was found between the control group and strawberries treated with cinnamon essential oil vapor.

Overall, there was a higher concentration of reducing sugars in strawberries, with a concentration of 5.5 - 10.5 grams of sugar per 100 grams of strawberries in the validation study compared to 4 - 8 grams of reducing sugar per 100 grams of strawberry in the accelerated study. Moreover, there was no difference between the control and the strawberries treated with cinnamon vapor in the validation study, contrarily to the accelerated study. This difference might be due to the decreased growth of microorganisms during cold storage, which leads to less consumption of reducing sugars. Since cinnamon essential oil has been shown to decrease microbial growth, the difference between treated and untreated strawberries does not appear in cold environments. More research is needed to confirm the potential connection between cinnamon essential oil treatment and increased reducing sugar levels.

This study has some strengths and limitations. It was the first study to investigate the effects of lemon essential oil in vapor state on strawberries. Moreover, it included a validation study confirming the effects found during the accelerated shelf life research. One limitation was the small sample size of strawberries during this experiment, which decreased

the statistical power of this research. It might also explain the large standard deviations found for some results.

4. Conclusion

Lemon and cinnamon essential oils are part of the FDA GRAS list and possess antimicrobial properties, making them promising candidates as natural food preservatives (Al-Jabri & Hossain, 2016; Dimić et al., 2014; Fisher & Phillips, 2006; Umagiliyage et al., 2017; Vitoratos et al., 2013). Essential oils can carry bioactive compounds in their vapor phase, making them convenient fumigants for fruit preservation (Tzortzakis, 2007). Investigating the effects of such oils on the physicochemical parameters and of food items is crucial to determine their effectiveness. This study aimed to examine the effects of lemon or cinnamon essential oil vapor on several physicochemical characteristics of strawberries, including weight loss, color, firmness, sugar content, and acidity status and visible decay. Lemon and cinnamon essential oils decreased water loss of strawberries at the beginning of storage. Lemon essential oil tended to delay the darkening process related to fruit aging but started decreasing firmness after two days of storage at 22°C; cinnamon oil altered firmness and color immediately after treatment, but the effects did not subsist during storage. Cinnamon essential oils increased the reducing sugars concentration in strawberries stored at 22°C during storage until the fourth day compared to untreated strawberries, but this effect was not present at cold temperature. Lemon and cinnamon essential oil treatments did not alter the acidity profile of strawberries, nor their soluble solid content. They did not significantly decrease visible decay in time, even though cinnamon essential oil treatment showed a tendency to delay it.

Overall, this research brings new light to the potential application and limitations of essential oil vapor treatment for fruit fumigation, with promising results about their effects on the physicochemical parameters of strawberries. Further research needs to be conducted to discern the optimum dosage and the effects of these oils on the organoleptic characteristics of the fruit.

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Supplementary Material for

Effects of Lemon or Cinnamon Essential Oil Vapor on Physicochemical Properties of Strawberries during Storage.

Supplementary material content:

Number of pages: 2

Number of figures: 2 (Fig. S1 and S2)

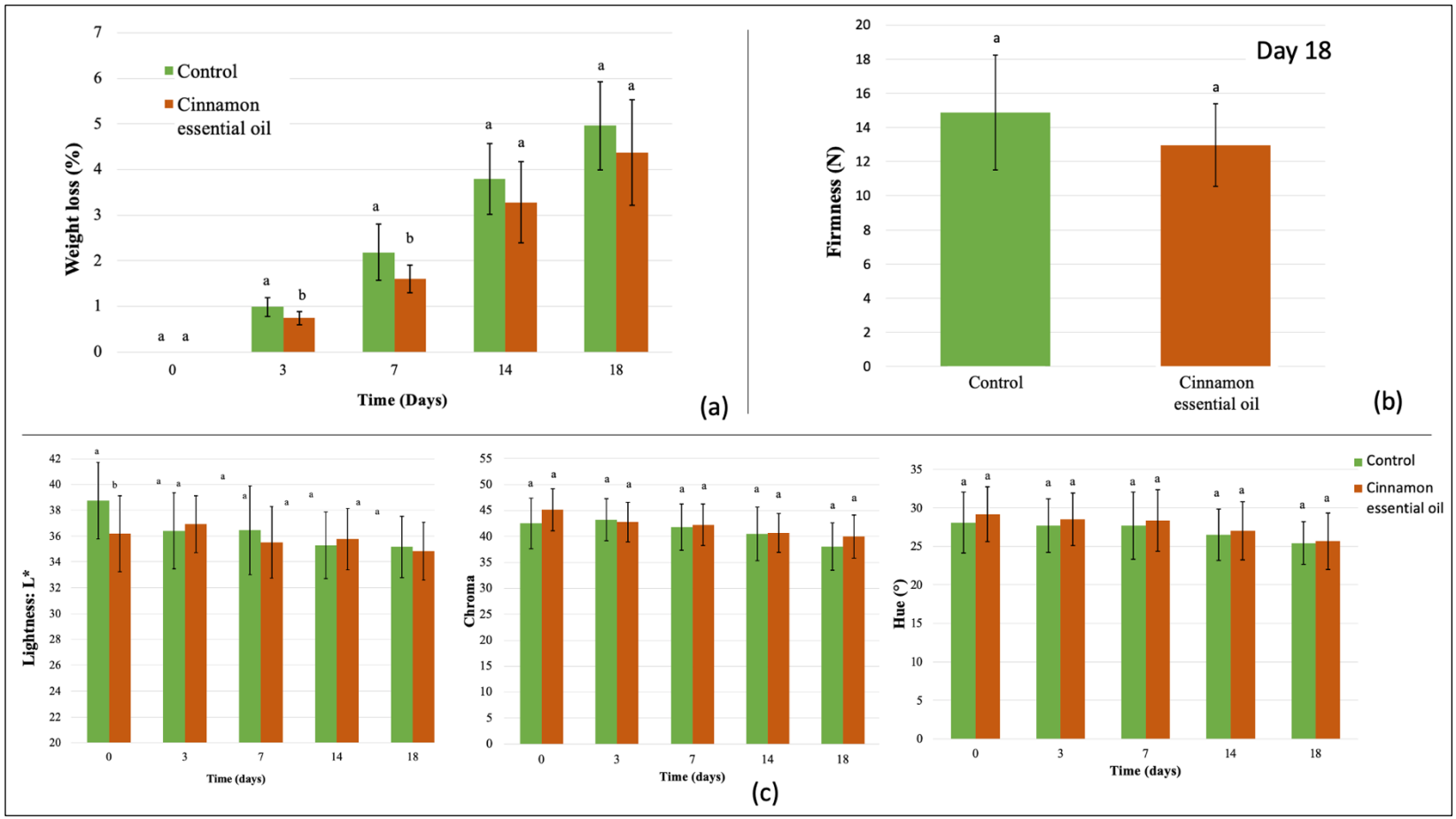

Fig. S1. Weight loss, firmness and color of strawberries stored at 4°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at $P < 0.05$ after Bonferroni's correction.

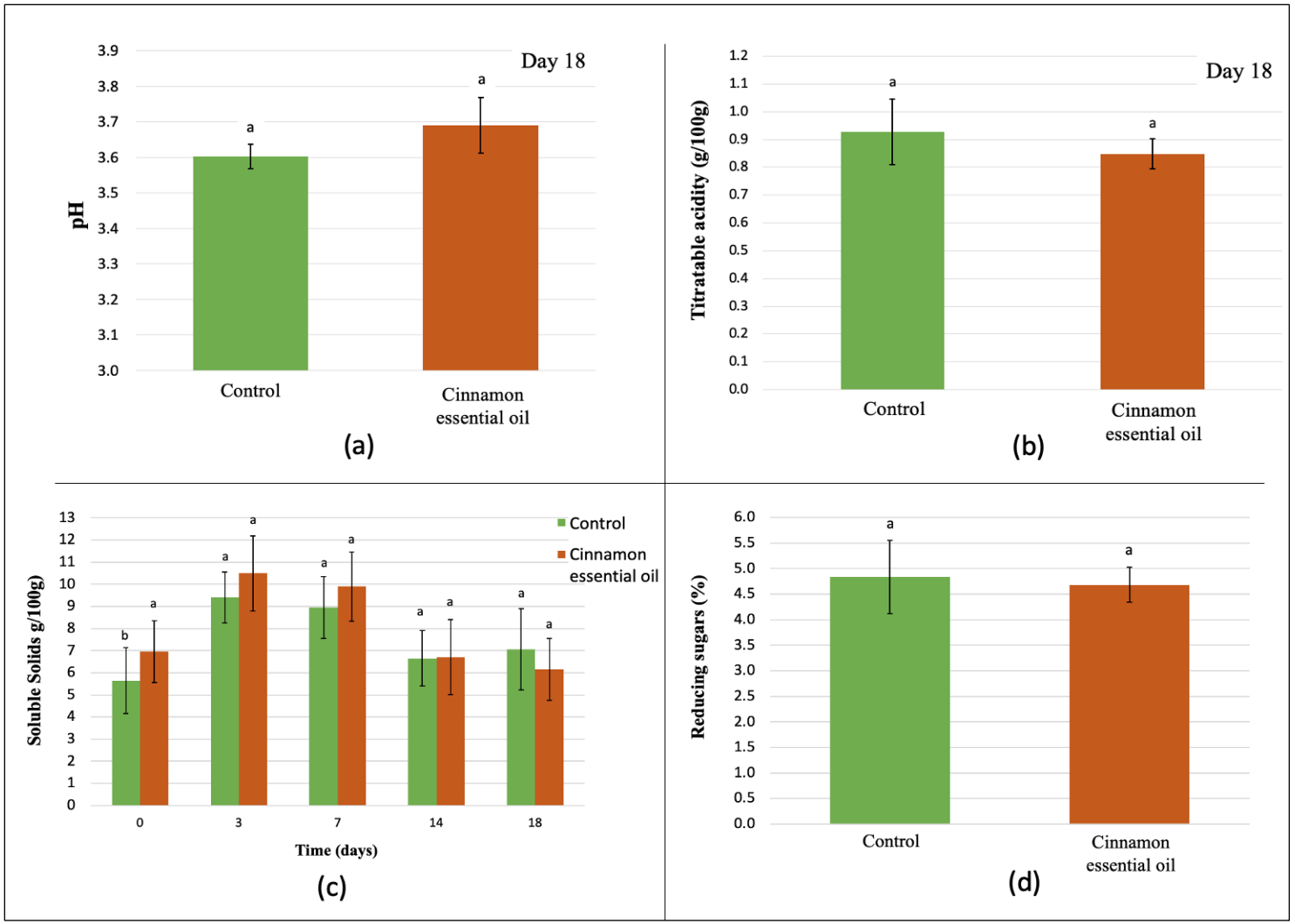

Fig. S2. Acidity status and sugar content of strawberries stored at 4°C. n =3/treatment group/day. Columns represent mean and bars represent standard deviations. Within each day, bars with uncommon letters (a, b) indicate significant differences between treatments at $P < 0.05$ after Bonferroni's correction.

Chapter 3: Summary and Recommendations

Summary

Fruits are particularly susceptible to waste since they are sensitive to contamination by bacteria and fungi. Current preservation methods are being challenged since consumers are asking for products grown and stored without synthetic chemicals (Serrano et al., 2005). Therefore, new postharvest technologies are being investigated for their use as natural preservatives. Essential oils are good candidates since they are environmentally safe and possess bioactive compounds capable of limiting microbial growth (Al-Jabri & Hossain, 2016; Dimić et al., 2014; Fisher & Phillips, 2006; Tzortzakis, 2007; Umagiliyage et al., 2017; Vitoratos et al., 2013). As seen in chapter one, lemon essential oil and cinnamon essential oil have shown antimicrobial properties against several pathogens, including *Escherichia coli*, *Salmonella*, and *Botrytis cinerea*, which are primary pathogens present in fruits, especially in strawberries. However, there is currently limited research about their effects on the physicochemical components and organoleptic properties of food. To be considered an effective food preservative, essential oils need to limit microbial growth while conserving attributes related to fruit quality during storage.

This study aimed to investigate the effects of lemon essential oil vapor and cinnamon essential oil vapor on quality-related parameters using instrumental methods, such as decay, weight loss, color, firmness, sugar content, and acidity status of strawberries. Lemon and cinnamon essential oil vapor treatment showed promising results in maintaining quality in fruits: they did not alter the acidity status, nor the soluble solid content of strawberries. They decreased water loss at the beginning of storage, a factor strongly related to perishability in

fruit. Lemon essential oil vapor treatment significantly delayed the darkening of strawberries but decreased their firmness after a few days of storage. Cinnamon essential oil vapor treatment altered firmness and color immediately after treatment, but this difference did not persist during storage. It also increased the reducing sugar content when strawberries are stored at 22°C, but these effects were not visible at cold temperature. Lemon and cinnamon essential oil vapor treatment did not decrease the visible decay in this study, even though cinnamon treatment showed a tendency to decrease it. More research is needed to understand the effects of lemon and cinnamon vapor treatment on fruits. This study was the first to assess the effects of lemon essential oil vapor on fruit physicochemical parameters. Overall, this research suggests that essential oil of lemon or cinnamon in a vapor phase might be used to preserve fruit during storage.

Recommendations

Essential oils have drawn attention for their antimicrobial properties and are currently investigated for their use as a natural food preservative. This thesis researched the effects of lemon or cinnamon essential oil vapor treatment on the physicochemical properties of strawberries. Although it conveyed information about these oils as potential preservatives, more research would help increase the scientific knowledge about their potential application in the food system. Investigation on other quality-related parameters could be further researched, such as nutrient values and antioxidants properties. Studying the effects of essential oil treatment on an increased variety of fruit and vegetables would also bring information about the range of performance of essential oils.

Another crucial path of investigation concerns the effects of oils in a realistic setting. For example, essential oils could be tested during the stages of distribution. It could include analyzing oil treatment on fruits during transportation between producer and retailers, during sales to consumers, and finally, on the consumer level, oil could be tested in a setting like home storage, in or out of the fridge.

Studying different application methods would also be helpful; it could increase the extent of the application of essential oils. Promising research has been conducted on essential oils incorporated in edible coatings and modified atmosphere packaging (Etemadipoor et al., 2019; Perumal et al., 2021; Shehata et al., 2020). Moreover, research on plant-based natural preservatives used at home by consumers has already been marketed (FreshGlow, 2021). Essential oils are popular in households; applied research on natural home products for food preservation could also be investigated.

Finally, additional study about consumer acceptance of fruit treated with essential oils is needed. Consumer acceptance is a crucial step in marketing food preservatives. Instrumental research can give objectives and precise results on the effects of oil; however, it is essential to know what purchasers think about it. Essential oils might alter the smell or taste of food items, increasing or decreasing consumer acceptance. Moreover, we observed some effects of lemon essential oil on firmness; however, we do not know if this change will affect the consumer approval of the food. This information is crucial to understand the potential commercial use of essential oils as food preservatives.

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