

**CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE**

**Faculty of Tropical AgriSciences**



**Faculty of Tropical  
AgriSciences**

**Effect of various formulations of natural  
wax-based edible coatings on physical  
properties of avocado**

MASTER'S THESIS

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## **Declaration**

I hereby declare that I have done this thesis entitled Effect of various formulations of natural wax-based edible coatings on physical properties of avocado independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 25<sup>nd</sup> of April 2024

.....  
Bc. Viktorie Vodičková

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## **Abstract**

One of the most significant tropical fruits in terms of economy is the avocado, which is mostly imported into the US and Europe. Avocados are climacteric, perishable fruits. As a result, long-distance transportation may incur significant losses, which may lead to financial losses. Fruit quality and shelf life may be preserved with the use of edible coatings. A thin barrier that allows gasses, moisture, and solutes to pass through is created on the fruit's surface when an edible coating is placed. One of the most crucial elements in preserving the fruit's freshness and prolonging its shelf life is moisture loss. In order to avoid fruit weight loss, this study compared the application of several natural waxes as an edible covering. Two concentrations of shellac 5.5% and 10%, carnauba wax 10.16% were compared. The dipping, spraying, and spreading techniques for application were also contrasted. Avocados were kept at 6 °C and 20 °C. The weight loss at 6 °C was the highest in control samples. However, there was no significant difference compared to the treatments, with shellac 10% being the lowest but very close to other treatments. At 20 °C the differences were more significant, with shellac 5.5% as being the best treatment in terms of weight loss at higher temperatures. The results did not verify the difference between the coatings. Total colour difference  $\Delta E$  was calculated and compared between samples. Shellac 10% had best results with having the lowest colour change progression over time.

**Key words:** edible coating, shelf life, edible wax, *Persea americana*, storage, physical attributes

## Abstrakt

Jedním z hospodářsky nejvýznamnějších tropických plodů je avokádo, které se dováží především do USA a Evropy. Avokádo je klimatické ovoce, které rychle podléhá zkáze. V důsledku toho mohou při dálkové přepravě vznikat značné ztráty, které mohou vést k finančním ztrátám. Kvalitu a trvanlivost ovoce lze zachovat použitím jedlých obalů. Po nanesení jedlého povlaku se na povrchu ovoce vytvoří tenká bariéra, která propouští plyny, vlhkost a rozpuštěné látky. Jedním z nejdůležitějších prvků pro zachování čerstvosti ovoce a prodloužení jeho trvanlivosti je ztráta vlhkosti. Aby se zabránilo ztrátě hmotnosti ovoce, porovnávala tato studie použití několika přírodních vosků jako jedlého povlaku. Byly porovnávány dvě koncentrace šelaku 5,5 % a 10 %, karnaubský vosk 10,16 %. Rovněž byly porovnávány techniky nanášení namáčením, postříkem a roztíráním. Avokáda byla uchovávána při teplotách 6 °C a 20 °C. Úbytek hmotnosti při 6 °C byl nejvyšší u kontrolních vzorků. V porovnání s ošetřením však nebyl zjištěn významný rozdíl, přičemž šelak 10 % byl nejnižší, ale velmi blízký ostatním ošetřením. Při 20 °C byly rozdíly výraznější, přičemž šelak 5,5 % byl z hlediska úbytku hmotnosti při vyšších teplotách nejlepší. Výsledky neověřily rozdíl mezi jednotlivými nátěry. Byl vypočten celkový barevný rozdíl  $\Delta E$  a porovnán mezi vzorky. Nejlepších výsledků dosáhl šelak 10 % s nejnižší progresí změny barvy v čase.

**Klíčová slova:** jedlý povlak, trvanlivost, jedlý vosk, *Persea americana*, skladování, fyzikální vlastnosti

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# 1. Introduction

One of the most nutrient-dense fruit is the avocado (*Persea americana*), a tropical or subtropical fruit that is native to South America. It is highly prized for its distinctive texture, exquisite taste, and aroma, as well as for its nutritional profile and other health advantages. Because of all of this, avocado has gained notoriety on a global scale, and in recent years, consumption of the fruit has significantly increased (Hurtado-Fernández et al. 2018). More focus should be placed on lowering postharvest losses in addition to raising output in order to meet the ever-growing demand for fresh goods. One of the potential strategies for ensuring food security and safety is postharvest activities. Edible coating of fresh food appears to be a useful strategy in this situation to address concerns related to produce quality and safety (Armghan Khalid et al. 2022).

Avocados experience fast biochemical changes, which are evident in both their look and content, much like other climacteric fruits. At the time of sale, the avocado should be firm and glossy, with a healthy appearance and no microbes. Proper postharvest handling can preserve these qualities under certain conditions for extended periods of time. On the other hand, the development of microorganisms and losses due to the accelerated decomposition process pose a threat to avocado fruits and can negatively impact the product's presentation and sensory quality (Choque-Quispe et al. 2022).

Edible coatings are a green technology used on a variety of items to regulate oxidation, gas exchange, and moisture transfer. Edible coatings have the potential to alter the internal gas composition in the same way as modified environment storage, while also offering an extra layer of protection (Dhall 2013). It is important to properly set the quality criteria and to continuously monitor the quality parameters of fruits and vegetables coated with edible films during the storage period. Fruits with edible film coatings need to be monitored with their colour change, firmness loss, and weight loss (Armghan Khalid et al. 2022).

## 2. Literature Review

### 2.1. Avocado (*Persea americana*)

The avocado tree belongs to the broad plant family known as the Lauraceae, also known as the laurels, which is typically found in tropical or subtropical climates (Bergh & Ellstrand n.d.). It also belongs to the *Persea* genus, which is divided into three subgenera that collectively contain more than 150 species: *Persea* (which only contains two species, *P. americana* and *P. schiedeana*), *Eriodaphne* (which contains about 70 species, including *P. caerulea*, *P. indica*, and *P. americana*, whose fruit is the common avocado, is the *Persea* species that has received the most attention and relevance. The avocado tree, *Persea americana* Mill., is one of the few representatives of the genus *Persea* that is important to commerce. In Mexico, the Aztecs called this fruit Ahuacatl, from whence the terms "avocado," "aguacate" (in Spanish), "avocat" (in French), and "abacate" (in Portuguese) originated. Because of their fruit's resemblance to testicles, the Aztecs named avocados huacatl, or "aphrodisiac." In addition to being known by several other names, including vegetable butter, butter pear, alligator pear, and midshipman's butter, the fruit is also known as palta in Chile, Ecuador, and Peru (Yahia & Woolf 2011). Although butter pear and alligator pear are two of its other names, avocado is the most popular English term for it (Chen 2009). The avocado is a fruit that originated in Central America and Mexico and has been a staple food for at least 9,000 years. Three distinct ecological races—Mexican, Guatemalan, and West Indian (or Antillean)—can be distinguished within *P. americana*. In terms of leaves, fruits, flowering time, and other traits, each race exhibits typical traits. Therefore, hybridization easily takes place anywhere trees of various races are growing close together. Because of this, the majority of commercial avocado cultivars are interracial hybrids with varying degrees of hybridization that were created from unintentional seedlings. As seen in Fig. 3, avocado cultivars range greatly from one another in terms of, for example, size, shape, and colour (Whiley 2002).

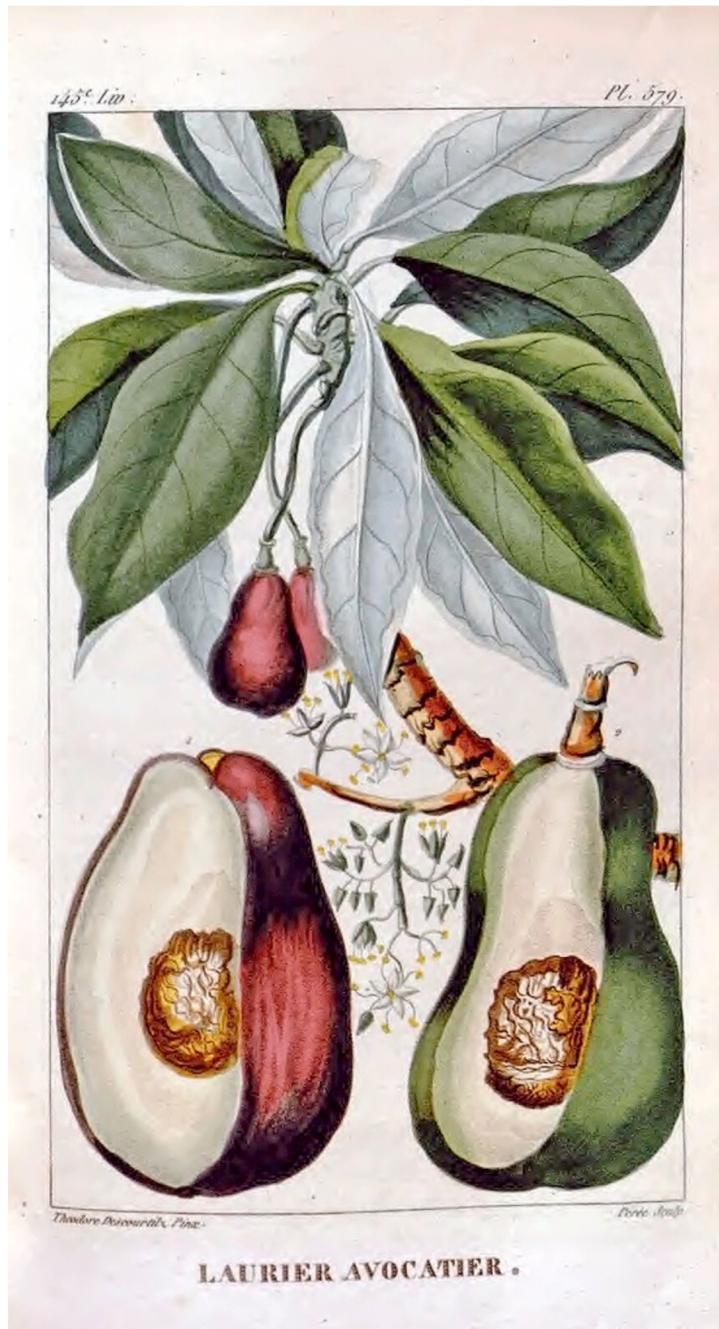


Figure 1. Picture of inflorescence, flower, fruit, and seed of *Persea americana*.  
Source: Swallowtail Garden (2014).





Figure 2. *Persea americana* tree. Source: Forest and Kim Starr (2022).



Figure 3. External appearance of different avocado cultivars. From left to right, top: Gwen, Hass, Reed; bottom: Ettinger, Fuerte, Pinkerton. Source: Hurtado-Fernández (et al. 2018).

### 2.1.1. Taxonomy

Long acknowledged are three distinct ecological races of avocado, and the races differ in terms of fruit maturity and oil content. Typically, they have been referred to as the Mexican, Guatemalan, and West Indian kinds or races, after their presumable places of origin (Yahia & Woolf 2011). *Persea americana* Mill. var. *americana* (*P. gratissima* Gaertn.) is a tropical fruit with a large variably shaped fruit and low oil content found in the West Indies (WI); *P. americana* Mill. var. *drymifolia* Blake (*P. drymifolia* Schlecht. & Cham.) is a semitropical fruit with a smaller, elongated fruit with thin skin and a higher oil content found in the Mexican (MX) variety; and *P. nubicena* var. *guatemalensis* L. Wms. is a subtropical fruit with half round fruit and an intermediate oil content found in Guatemala (G). Many features that are important to commerce are different between the races (Defilippi et al. 2009).

**TABLE 1** Comparison of the Three Different Horticultural Races of Avocado Fruit

	Trait	Race		
		Guatemalan (G)	Mexican (M)	West Indian (WI)
Tree	Climate	Subtropical	Semitropical	Tropical
	Cold tolerance	Intermediate	Most	Least
	Salinity tolerance	Intermediate	Least	Most
	Leaf anise	Absent	Present	Absent
	Young leaf color	Green with red tinge	Green	Pale yellow
	Mature leaf color	Dark green	Dark green	Pale green
Fruit	Blooming season	March to April	January to February	February to March
	Bloom to fruit maturity	10 – 18 months	5 – 7 months	6 – 8 months
	Size	Small to large	Tiny to medium	Medium to very large
	Shape	Mostly round	Mostly elongate	Variable
	Color	Green	Often dark	Green or reddish
	Skin thickness	Thick	Very thin	Medium
	Skin surface	Rough	Waxy bloom	Shiny
	Skin peelability	Rigid	Membranous	Leathery
	Seed size	Small	Large	Variable
	Seed cavity	Tight	Loose	Variable
	Seed surface	Smooth	Smooth	Rough
	Oil content	High	Highest	Low
	Pulp flavor	Rich	Anise-like, rich	Sweeter, milder

Figure 4. Comparison of the Three Different Horticultural Races of Avocado Fruit. Source: Hurtado-Fernández (et al. 2018).

Guatemalan race, this fruit verges the greatest in horticultural quality among the three races, as Table 1 indicates. The pulp within is shielded by the thicker skin. Typically, the seed is smaller and fits tightly in the cavity (Chanderbali et al. 2013).

The Mexican variety, as shown in Table 1, has thin skin that is easily ruptured by a fingernail, making it less safe to export or handle. The seed is also big and frequently uncomfortably loose in the cavity. For instance, the variety Topa Topa appears tight, but the seed is loose inside the coat and just the seed coat occupies the hole. Additionally, most of the fruits are smaller than what is desired economically (Ayala Silva & Ledesma 2014). The fruit of the Mexican race, which evolved in the highlands of Mexico and Central America, is characterized by its petite size, with a skin that is smooth and weighing between 75 and 300 grams. The majority of Mexican race fruits have green skin, with the exception of the naturally occurring "criollo," which has black skin. The pulp is green in colour and contains a very high oil content—up to 30% by fresh weight. Of the three races, Mexican cultivars are the most cold resistant since they are best suited to the chilly temperatures of the tropics and subtropics. Mexican race mature trees can tolerate temperatures as low as -4 °C without suffering any harm.

The purpose of the West Indian race, which is tropical and suitable for more tropical regions like Florida for example, is similar to that of the Mexican race in that it is well-adapted (it produces Florida cultivars for the early season), and its hybrids with Guatemalans combine good Guatemalan quality with good West Indian adaptation to tropical climates, bridging the two harvesting seasons (Chanderbali et al. 2013).

### **2.1.2. Botanical Description of *Persea americana***

The avocado tree is evergreen, despite the fact that its leaves have an unusually short lifespan of just 12 months. Its roots are shallow, have poor water uptake, and have poor hydraulic conductivity, and it grows quickly, reaching heights of up to 20 m. Despite the trees producing a lot of flowers, often less than 0.1% of these flowers develop fruit. The fruit is a drupe, or fleshy fruit with seed within, with a highly oily pulp that is frequently eaten. It has a thin, greenish skin and a flavour similar to walnuts. B vitamins, which are abundant in avocados, aid in the body's defence against illnesses and infections. Three main climacteric elements can affect blooming and fruit set: the incidence of frost in the winter, the presence of low mean temperatures, and the occurrence of extremely



high temperatures during fruit set. The incidence of frost throughout the winter, the presence of low mean temperatures, and the occurrence of extremely high temperatures during fruit set are three major climacteric elements that might affect blooming and fruit set (Hurtado-Fernández et al. 2018).

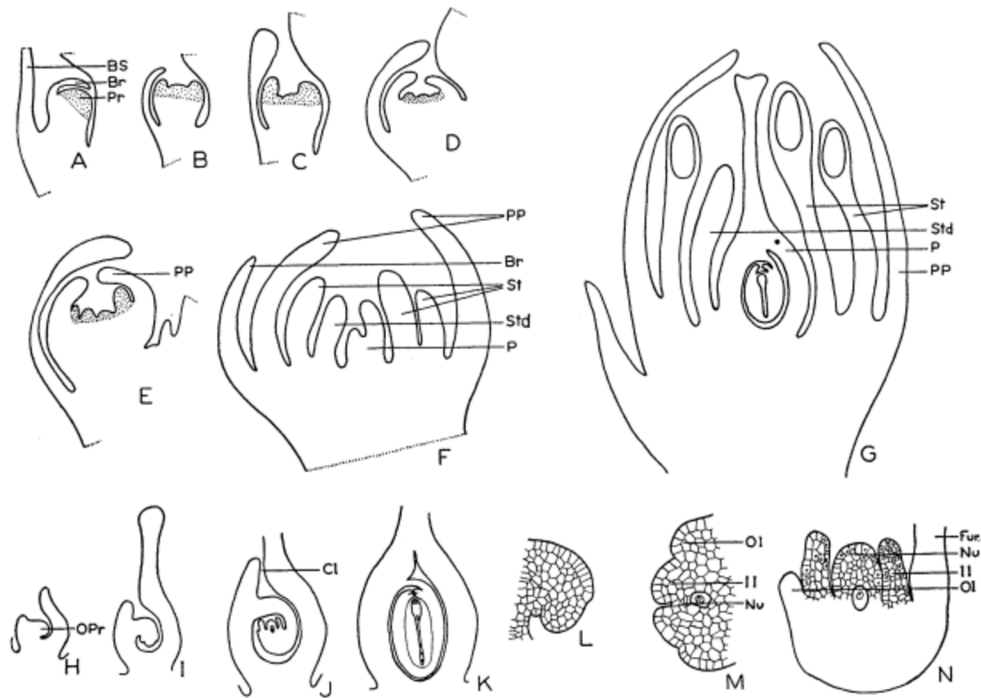


Figure 5. *Persea americana*. A-G, differentiation of flower parts. A, flower primordium in axil of bud scale. B, initiation of perianth primordia. C-E, initiation of stamens and staminodes. F, section through young flower shortly after initiation of pistil. G, young flower with all parts differentiated. H-N, differentiation of ovule and integuments. H, initiation of ovule in young pistil. I-K, later developmental stages of ovary and ovule. L, differentiation of inner integument in young ovule. M, differentiation of outer integument in young ovule. N, older stage of ovule with both integuments. BS, bud scale; Br, bract; P, pistil; PP, perianth part; Pr, bud primordium; St, stamen; Std, staminode; OPr, ovule primordium; Cl, cleft in pistil; OI, outer integument; II, inner integument; Fux, funiculus; Nu, nucellus. Source: Schroeder (1952).

### 2.1.3. Use of *Persea americana*

In many regions of the world, this plant has long been used traditionally to cure a variety of medical conditions. In many tropical and subtropical nations, traditional medicine makes considerable use of the root, bark, fruit, seed, and leaf for the treatment

of a variety of illnesses (Tcheghebe et al. 2016). *Persea americana* has been reported in numerous studies to be used by people from tropical countries to treat a variety of health issues, including typhoid fever, malaria, high blood pressure, rheumatism, diarrhoea, dysentery caused by helminths and amoebas, toothaches, intestinal worms, diabetes, skin rashes, and infectious processes caused by fungi and bacteria. It has also been used to lower high blood pressure, stimulate uterine contractions, and lessen painful menstrual periods. Air purification is another benefit of having this tree planted around the house. It has been shown that consuming this plant extract up to a level of 500 mg/kg body weight is safe (Tcheghebe et al. 2016). In traditional medicine, it is used as an aphrodisiac and to treat tumours, hypertension, inflammation, sore throats, haemorrhage vermifuges, and dysentery (Falodun et al. 2013). The extract of *Persea americana* leaves has been shown in several rat experiments to have hypotensive, antioxidant, and hypocholesterolemic effects. Numerous elements of *Persea americana* leaf extract have previously been identified by chemical analysis. These consist of reducing sugars, flavonoids, alkaloids, coumarins, saponins, and triterpene glycosides (Lima et al. 2012).

#### 2.1.4. Biochemical characterization

The avocado fruit (*P. americana*) is a berry made up of a sizable central seed and pericarp, which is made up of the outer layer around the seed (endocarp), the edible portion (mesocarp), and the skin (exocarp) (see Fig. 4) (Meyer et al. 2011).

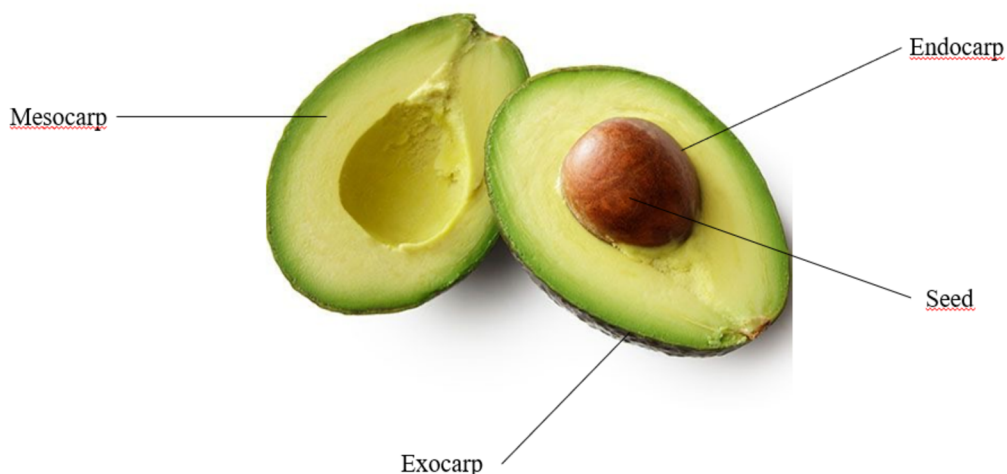


Figure 6. The different parts of the avocado fruit. Source: Meyer (et al. 2011).

The growth of avocado fruit is the result of intricate metabolic processes that are influenced by the environment, including temperature, the availability of water and nutrients to the plant, the quality and quantity of light, and several plant hormones (Cowan et al. 2001).

The single sigmoid curve that defines avocado growth can be used to distinguish between the three primary phases of this process. The ovary development, fertilization, and fruit formation are all parts of the first phase (lag phase), which is also characterized by slow growth and 90% abscission. A significant number of cell divisions, seed germination, and early embryo development occur during the second phase (growth) (Crane et al. 2013). The third stage, known as the "physiological maturation phase," is characterized by the seed maturing and a reduction in cell proliferation. This tropical fruit has been noted to be highly uncommon since the mesocarp's cells continue to divide slowly throughout the remainder of development and maturation in addition to occurring fast during the early stages of growth. As a result, both cell division and cell expansion contribute to avocado growth (Dabas et al. n.d.). One of the most remarkable characteristics of avocados, when compared with other fruits, is that they do not ripen on the tree; they start ripening after harvesting, which usually occurs after fruits reach the physiological maturity (defined as the stage in which avocados could continue the developmental process even if detached from the tree) (Hartz & Smith 2009).

According to (Prasanna et al. 2007), ripening is a "highly coordinated, genetically programmed, and an irreversible phenomenon" in which the avocado goes through significant chemical and physiological changes like pulp softening, textural changes, colour changes (due to the synthesis of pigments and the loss of chlorophyll), the formation of aroma volatiles, an increase in flavour, and changes in sugar and acid concentrations. In addition, ethylene production and fruit respiration both significantly increase when the avocado ripens (Seymour & Tucker 1993), which explains why this tropical fruit is included in the category of climacteric fruits. Three stages of avocado respiration may be identified: pre climacteric (lowest respiration), climacteric (highest respiration), and post climacteric (reduction in respiration). The majority of the alterations indicated above occur in the pre climacteric and climacteric periods. Ethylene is a phytohormone that plays a significant part in the ripening process since it triggers the process by increasing production at the start of ripening. A number of authors have also

claimed that ethylene directly affects the upkeep of this mechanism (Hiwasa-Tanase & Ezura 2014).

The fiber from avocado seeds may have technical and biological applications. The seed is one of the greatest sources of dietary fiber and is rich in antioxidants and potassium. Because the seed contains tannins and polyphenolic chemicals, it has been shown to have larger levels of phenolic and a more strong in vitro antioxidant potential than the edible sections (pulp) and the typical synthetic antioxidants like Trolox. Numerous kinds of natural compounds, including saponin, phytosterols, triterpenes, fatty acids, furanoic acids, flavonol dimers, and proanthocyanidins, have been found by some phytochemical research conducted on avocado seeds. A few of them have to do with larvicidal, antifungal, and antibacterial properties (Barbosa-Martín et al. 2016).

The amount of nutrients in the fruit's edible pulp (also known as the mesocarp) varies greatly depending on the type, level of ripeness, and growing conditions. Avocado is made up of an extremely intricate matrix of different chemicals. It is highly valued for being a superior source of fatty acids, vitamins, and energy (see Fig. 5) (Unlu et al. 2005).

General nutritional composition	Energy	160 kcal
	Water	73.23 g
	Protein	2.00 g
	Total lipids	14.66 g
	Carbohydrates	8.53 g
	Total dietary fiber	6.70 g
	Sugars	0.66 g
Fatty acids	Saturated fatty acid	2.13 g
	Monounsaturated fatty acids	9.80 g
	Unsaturated fatty acids	1.82 g
Vitamins	Vitamin C (ascorbic acid)	10.00 mg
	Thiamine (B <sub>1</sub> )	0.07 mg
	Riboflavin (B <sub>2</sub> )	0.13 mg
	Niacin (B <sub>3</sub> )	1.74 mg
	Pyridoxine (B <sub>6</sub> )	0.26 mg
	Folate (DFE)*	89 µg
	Vitamin A (RAE)*	7 µg
	Vitamin E (α-tocopherol)	2.07 mg
Minerals	Vitamin K (phylloquinone)	21 µg
	Calcium (Ca)	12 mg
	Iron (Fe)	0.55 mg
	Magnesium (Mg)	29 mg
	Phosphorus (P)	52 mg
	Potassium (K)	485 mg
	Sodium (Na)	7 mg
Zinc (Zn)	0.64 mg	

\*DFE, dietary folate equivalents; RAE, retinol activity equivalents.

Figure 7. Nutritional Content of 100 g of Avocado Fruit. Source: Unlu (et al. 2005).

Because fat is one of the avocado's primary ingredients, it is not unexpected that the fruit is also referred to as the "butter fruit." In general, as an avocado ripens, its oil content (Ozdemir & Topuz 2004). The predominant fatty acids are monounsaturated, and oleic acid stands out as one of the most distinctive among them. Even though they are less prevalent, linoleic (polyunsaturated) and palmitic (saturated) acids are also significant components of the avocado fruit (Villa-Rodríguez et al. 2011). Lipids are one of the most researched chemical groups in avocado due to their abundance and the fact that some of the main health advantages of avocado have been linked to its high monounsaturated fatty acid content. Regarding the remaining ingredients, the avocado has a greater protein content than other fruits, with values around 2%, compared to the average protein content of 1% for most fruits (Plaza et al. 2009). Avocado is also a very

significant source of phenolic compounds, seven-carbon sugars, D-mannoheptulose, and perseitol, as well as vitamins (particularly vitamins E and C), pigments (anthocyanins, chlorophylls, and carotenoids), sterols, and other nutrients (Meyer & Terry 2010).

All of these nutritive and non-nutritive components of avocado fruit are what give it some of its organoleptic qualities, and they may also improve human health (due to the potential health benefits associated with some of the metabolites found in this matrix) (Devalaraja et al. 2011). Over the years, many researchers have focused on the link between eating avocados and better health, discovering that some of the many substances found in avocados are closely related to several positive health effects for people, including the maintenance of normal serum cholesterol, control of weight, prevention of cancer, and diabetes (Dreher & Davenport 2013). Studies show that the presence of fatty acids, dietary fiber, D-mannoheptulose, perseitol, potassium, magnesium, vitamins C, E, K, and B group, carotenoids, phenolics, phytosterols, or terpenoids in this fruit is the primary cause of all these benefits (Ding et al. 2007).

## **2.2. Post-harvest handling of avocado**

The climacteric fruit *Persea americana* undergoes physiological alterations upon harvest. Even though they are still hard and green, they are picked after they have reached what is known as physiological maturity in order to prevent fruit damage (Melado-Herreros et al. 2021). Avocados are matured in chambers with controlled temperature and ethylene application after they arrive at warehouses, until they are at an edible maturity. The most widely sold avocado cultivar globally is Hass. Fruit during postharvest management is diverse and unpredictable due to the prolonged flowering time, poor fruit set percentage, and incapacity to mature on the tree. Variable postharvest ripening or ripening heterogeneity affects the markets for Hass avocados (Hernández et al. 2016).

After being harvested, avocado fruit still goes through a life cycle and starts to ripen. At 25, fruit ripens over the course of five to seven days. The respiration of avocados occurs in three distinct climacteric stages: the pre-climacteric minimum respiration, the maximum climacteric respiration, and the post-climacteric stage, which is equivalent to a reduction in ventilation. The fruit undergoes several alterations during the pre-

climacteric and climacteric phases (Kassim et al. 2013). Avocados can age more quickly due to increased respiration rate, which lowers their quality. Lowering the temperature, raising the carbon dioxide level, and lowering the oxygen concentration within reasonable bounds are essential to minimizing respiration in avocado postharvest treatment. After a product is harvested, postharvest handling is done on it until it is eaten or goes through additional processing. The goal of postharvest management is to increase shelf life while preserving avocado quality in terms of texture, flavour, nutrition, and safety (Cahyono et al. 2019).

### **2.3. Factors affecting the safety and quality of avocado**

The fruit *Persea americana* is perishing. Temperature, humidity, and gas composition are factors that can prolong shelf life. Another crucial time of year is harvest season. The quality and flavour of the ripe fruit will decline if it is harvested before it is fully developed (Elik et al. 2019). The sensory qualities, nutritional qualities, chemical composition, mechanical qualities, functional qualities, and flaws of avocado products may all be used to determine their quality. Colour, texture, flavour, and scent are all significant factors that buyers consider when selecting high-quality avocados. Irregular postharvest handling and insufficient technology lead to product losses or damage. It is important postharvest management once you harvest the avocados from the tree. Postharvest losses can be avoided, and losses reduced by using proper postharvest management techniques. Avocados can be cleaned, sorted, stored, packaged, and transported as the first step in the postharvest handling process (Puspitasari et al. 2023).

#### **2.3.1. Temperature**

Like many other fruits, avocados are affected by the temperature they are exposed to throughout the ripening process in terms of how long it takes for the fruit to soften and how good it becomes after that. In the field and during preconditioning (ethylene ripening) or storage, the fruits are frequently kept for brief intervals at quite high temperatures after harvest, with uncertain consequences on the quality of the fruit that ripens later (Dantas et al. 2018).

It can be necessary to store harvested avocados in distribution centers or for long-distance export for two to four weeks under typical air conditions. Avocados should normally be stored between 4 and 6 degrees Celsius, as this is the temperature range at which ripening slows down without causing the damaging effects of chilling damage that occur when avocados are kept at lower temperatures (Arpaia et al. 2018). On the other hand, other studies suggest that fruits spoiled in a refrigerator have a browning of the mesocarp, inappropriate softening, and poor flavour. Furthermore, as a result of cold storage, the cell membrane displays separations between the two-layer proteins and the phospholipids. However, small manufacturers that keep their products in household settings (8–10 °C) without access to industrial refrigeration find that this deterioration accelerates more quickly (Ortiz-Viedma et al. 2018).

There are several types of damage to agricultural goods, such as mechanical, chemical, biological, physical, and microbiological damage, depending on the cause (Puspitasari et al. 2023). Damage brought on by the material's metabolic processes or the activity of its enzymes, which cause deterioration and damage, is referred to as physiological damage. Avocados can avoid suffering physiological harm by being cooled down. In order to slow down the pace of respiration in the material and hence slow down the damage process, cooling is done to reduce the product's temperature (Sukmawaty et al. 2019). 10 °C is the operating temperature of a cool box used for low-temperature storage. Character alterations preserved in a cold box are less than those caused by room temperature storage and polyethylene packing. Alternative methods for treating postharvest fruit include storing it at low temperatures, keeping it in a controlled environment, or wrapping it in plastic (Cahyono et al. 2019).

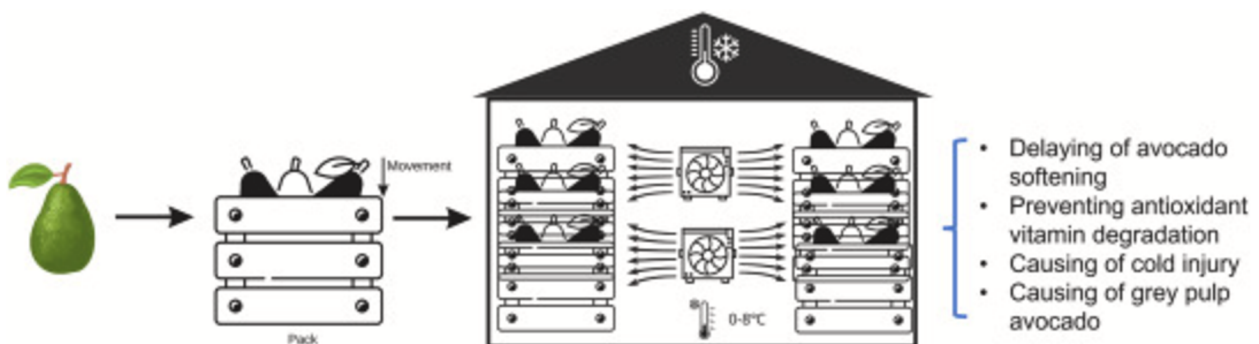


Figure 8. Temperature control by cold storage method. Source: Lieu (et al. 2024).



Preharvest factor	Postharvest effect on fruit quality	Management
Climate or environment: temperature, wind and rainfall	Increased disease incidence, chilling injury	Pruning to expose the fruit to direct sunlight
Rootstock or scion	Susceptibility to physiological disorders during the cold chain, postharvest decay	Choose less susceptible rootstock or scion
Pruning practices	Poor fruit storability	Strike a balance between vegetative and reproductive growth and correct timing is important
Pest and disease management	Changes in fruit composition influences the ripening behavior and decay development (anthracnose)	Maintain a clean orchard and correct application of chemical is important
Plant nutrition (N/Ca)	Development of physiological disorders (mesocarp discoloration or gray pulp) and rots	Manage vegetative growth and avoid excessive nitrogen during fruit development
Plant growth regulators	Poor storability	Manage vegetative growth
Irrigation	Influences polyphenol oxidase levels thus mesocarp discoloration	Avoid water stress during fruit growth and development

Figure 9. Preharvest factors that affects the postharvest quality of avocados. Source: Sivakumar (et al. 2014).

### 2.3.2. Humidity

Avocados have a high water content of 80%, which is gradually lost by breathing (Sivakumar et al. 2014). Avocados, like other fruits, can shrivel, thus it's crucial to maintain a high humidity level. Consequently, in order to avoid weight loss and skin desiccation, avocados should be kept between 90 and 95 percent relative humidity. Water

loss through stomata, stem scars, and the cuticle is directly linked to weight loss; the quantity of water lost relies on the thickness and content of the cuticle, which changes depending on the cultivar and maturity stage. On the other hand, excessive humidity can encourage the growth of rot, particularly if the fruit sweats or condenses moisture on it for extended periods of time when the temperature is changing while being transported (Sivakumar et al. 2014).

### **2.3.3. Gas composition**

A system capable of continuously regulating and maintaining the desired gas composition—that is, decreased O<sub>2</sub> and/or increased CO<sub>2</sub>—during the storage and/or transit phase is known as controlled atmosphere storage, or CA (Bill et al. 2014). Research has demonstrated that the combination of low temperature storage and controlled atmosphere (CA) may effectively postpone fruit ripening and increase its shelf life (Smock 1979). According to reports, certain fruit species experience less chilling injuries in environments with low oxygen concentrations. This may be because the rapid oxidation processes linked to certain chilling injuries are inhibited (Morris 1990). Avocado fruit that has been chilled is prone to discoloration of the flesh in the early stages of development, followed by a dark browning of the pulp (mesocarp) later on. The oxidation of phenols by polyphenol oxidase is the cause of this discoloration in avocados (Truter et al. 1992).

Although CA storage is mostly utilized for the long-term preservation of fruits, such apples, it is also being employed more frequently for fruit shipment by sea. In general, CO<sub>2</sub> causes a delay in many fruit reactions to ethylene. It was observed that the rates of respiration and ethylene synthesis were lowered in CA storage due to the increased CO<sub>2</sub> and reduced O<sub>2</sub> (Cukrov et al. 2019). Because of this phenomena, CA may have an impact on the fresh produce's postharvest physiology based on the O<sub>2</sub>/CO<sub>2</sub> balance. In low O<sub>2</sub>/elevated CO<sub>2</sub> atmospheres, avocados have a reduction in the severity of chilling damage, a physiological disease. It is necessary to determine the precise optimal amounts of CO<sub>2</sub> in low oxygen environments since levels above 5% may negatively impact the quality of "Hass" avocado fruit (Toivonen & DeEll 2001).

Avocados may typically be stored for five to six weeks with an O<sub>2</sub> concentration of 2-4 percent and a CO<sub>2</sub> content of 3-10%. Systems that are either static (SCA) or

dynamic (DCA) can be used to preserve CA storage, claim Burdon and Lallu. Toivonen and DeEll described DCA as the situation in which the respiring fruits in the store change their gas composition frequently. The production of ethanol is one of the metabolic processes that alters when the O<sub>2</sub> level drops below a threshold level (Pesis et al. 2002). Additionally, the stress that results from this reduction causes the chloroplasts to illuminate. The O<sub>2</sub> level in the storage is managed by DCA by the monitoring of either ethanol generation or chlorophyll fluorescence. Until the conclusion of the storage period, the O<sub>2</sub> concentration in the SCA system is kept at a set concentration. Burdon and Lallu report that whereas the fruit kept in SCA storage took seven days to mature, the DCA-stored "Hass" avocados produced in New Zealand ripened in four days, much like the fruit that was stored and ripened in air (Flores et al. 2004).

#### **2.3.4. Diseases and pests**

Avocado fruit is highly susceptible to infestation by insects or decaying matter. Fresh fruit interacting with contaminated microorganisms via water, soil, damaged fruit, and postharvest industrial operations is the main source of postharvest losses. The majority of avocado losses are caused by rotting (Goliáš 2011). Among the most well-known illnesses are stem end rot and anthracnose. The highest prevalence of anthracnose is found in regions with high humidity and temperatures. Fruit, leaves, flowers, and twigs can all get infected. Fruits experience postharvest degradation during transportation owing to cold storage and shelf life at the retail outlet. The most frequent postharvest fungal disease in avocados is called anthracnose (*Colletotrichum gloeosporioides* Penz and Sacc). Through direct penetration, *C. gloeosporioides* remains dormant and infects the fruit latently. Upon reaching the climacteric apex, the fruit breaks its latency. Phenotypic signs of anthracnose in mature fruit are deep, dark brown to black decay lesions; when high humidity is present, the lesions develop noticeable pinkish-orange mottling on them (Sivakumar et al. 2021). Stem-end rot is the second most prevalent illness. Though it can damage any portion of the fruit, the illness most commonly attacks the stem end (Morales, L. 2001). If 1-2 cm of the stem is left on after harvest, infection can be minimized. Additional avocado illnesses and associated pathogens are shown in Table 1.

There are a number of ways to prevent disease, such as using fungicides or hot water and vapour heat therapy. Diseases are not the only things that result in large productivity losses. Due to their damage to the fruit, branches, stem, leaves, and other vegetation, pests have an expensive effect on avocado producers. When fruit is exported, quarantine limitations may also be imposed due to pests (Dorantes 2004). It is also very important to disinfect all the instruments in the spirit.

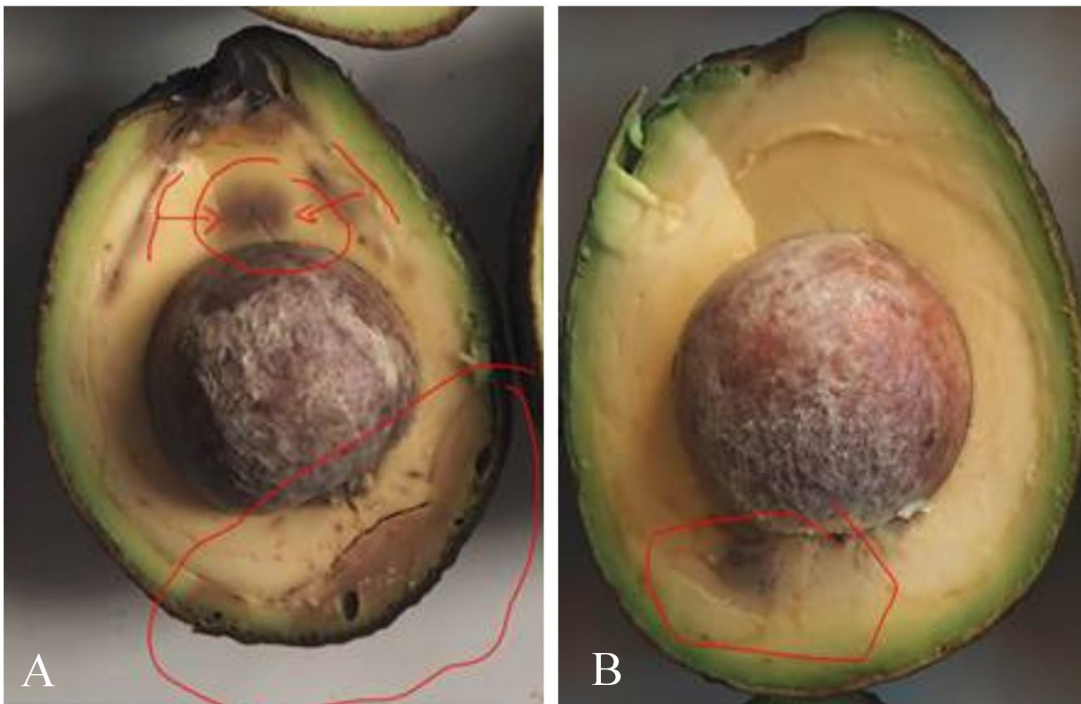


Figure 10. A) Lower anthracnose, upper bruising from handling. B) The bruise is often like this at the stalk. Source: Author.



Figure 11. This is stem end rot. Source: Ondřej Nedoma (2023).

The most significant avocado disease worldwide is root rot. One of the most dangerous fungus on the planet, *Phytophthora*, kills trees. The disease's initial sign appears when the afflicted trees' foliage starts to deteriorate and turn yellow. Until the branches are all that are left, the leaves begin to fall. A recovery may occasionally be seen during the dry season if the land is treated with a fungicide and fertilizer high in nitrogen. Because of this, the farmers do not uproot and disinfect the trees as advised, which allows the illness to spread (Vidales-Fernández 1999).

The second most common pest in Florida is avocado blight, often known as "roña." Fruit (in all stages), leaves, and young branches are all targeted by the *Sphaceloma perseae* fungus. At first, the infected fruits have round or irregularly shaped brown lesions with a corky look. These lesions can cover a significant portion of the fruit or the entire fruit as they expand and unite, and they can also cause fissuring in the branches and leaves. When the infestation is severe, the insect causes minute, individual dark brown stains on leaves that are smaller than 3 mm in diameter. The nervations in the leaves are also deformed. The lesions are elongated and slightly apparent in nervations or green

branch barks. The fruit is still healthy despite the damages that do not affect the exocarp (Gallegos Espinosa 1983).



Figure 12. Fruits affected by avocado blight or "roña". Source: Gallegos Espinosa (1983).

Table 1. Diseases of avocado. Source: Author.

<b>Diseases</b>	<b>Causal organisms (pathogens)</b>	<b>References</b>
Anthracnose	<i>Colletotrichum gloeosporioides</i> , <i>C. acutatum</i>	(Barboza et al. 2023)
Stem-end rot	<i>Colletotrichum</i> , <i>Botryodiplodia</i> , <i>Dothiorella</i> , <i>Phomopsis</i> and <i>Lasiodiplodia</i> genera	(Barboza et al. 2023)
Root rot	<i>Phytophthora cinnamomi</i>	(Barboza et al. 2023)
Avocado blight	<i>Sphaceloma perseae</i>	(Dorantes 2004)
Thrips	<i>s Liothrips perseae</i> Watson, <i>Scirtothrips aceri</i> Moulton, <i>Frankiniella cephalica</i> , <i>Heliethrips haemorrhoidalis</i>	(Dorantes 2004)
Small seed weevil	<i>Conotrachelus perseae</i> Barber	(Dorantes 2004)
Large seed weevil	<i>Heilipus lauri</i> Boheman	(Dorantes 2004)
Seed moth	<i>Stenomoma catenifer</i> , <i>Walsingham</i>	(Gallegos Espinosa 1983)
Red or brown mites	<i>Oligonychus</i>	(Gallegos Espinosa 1983)
White fly	<i>Trialeurodes floridensis</i> Quaintance, <i>Tetraleurodes</i> sp., <i>Paraleyrodes perseae</i> Quaintance	(Dorantes 2004)
Dog worm or swallow wing butterfly	<i>Papilio garamas garamas</i> Hübner, <i>Papilio victorinus merelius Rothschild and Jordan</i> , <i>Papilio crespontes</i> Cramerg	(Dorantes 2004)



## 2.4. Edible coating

The food business has drawn the attention of several researchers to the use of edible films or coatings to preserve fruit quality. The primary characteristic of edible coatings is their biodegradability, as they are composed of biopolymers such as proteins, lipids, and carbohydrates (Maftoon Azad 2006). A modified internal environment is created in the fruit by the edible coatings, which also function as a surface barrier to impede gas flow. But the characteristics of various edible coatings composed of various materials vary.

A food coating that is safe to eat is called an edible coating. The coatings technique must be suitable for use on food, particularly horticulture items, biodegradable, and composed of biological components like cellulose and starch (Careli-Gondim et al. 2020). The purpose of applying edible coatings is to coat the surface of the material in a way that limits the rate of liquid diffusion and reduces the transfer of water vapor, so protecting against mechanical damage. When fruits are covered with edible coatings, a semi-permeable layer forms around them, creating a changed environment. This alters the fruit's interior atmosphere, which helps to lessen the incidence and severity of decay when compared to control fruits. It's possible that the fruit's covering altered the environment, making the skin's pH lower and less conducive to the growth of *C. gloeosporioides*, which causes rotting. Additionally, because of the semi-permeable modified environment layer on the fruit surface, the edible coatings slow down the rate of respiration, moisture loss, senescence, and weight loss (Sivakumar et al. 2021). Edible coatings can be applied in a variety of methods, for as via spraying, dipping, drying, wrapping, brushing, or basting (Fitch-Vargas et al. 2019). The fruit surface film coating modifies the environment by lowering respiration rate, delaying ripening, increasing resistance to anthracnose, prolonging shelf life, etc (Pham 2023).



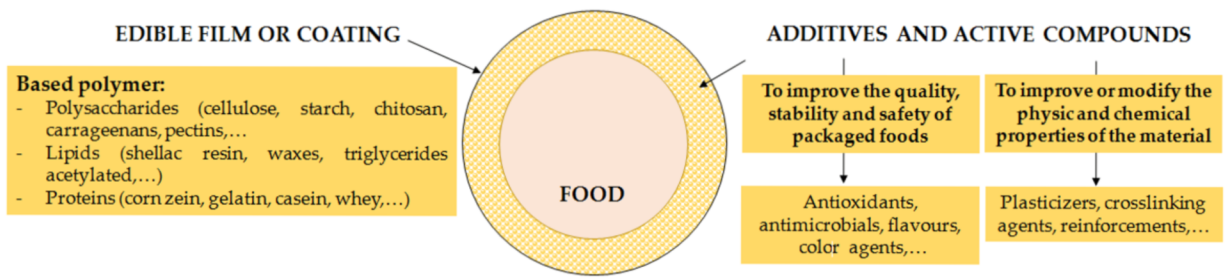


Figure 13. Edible films' and coatings' compositions. Source: Valdés (et al. 2017).

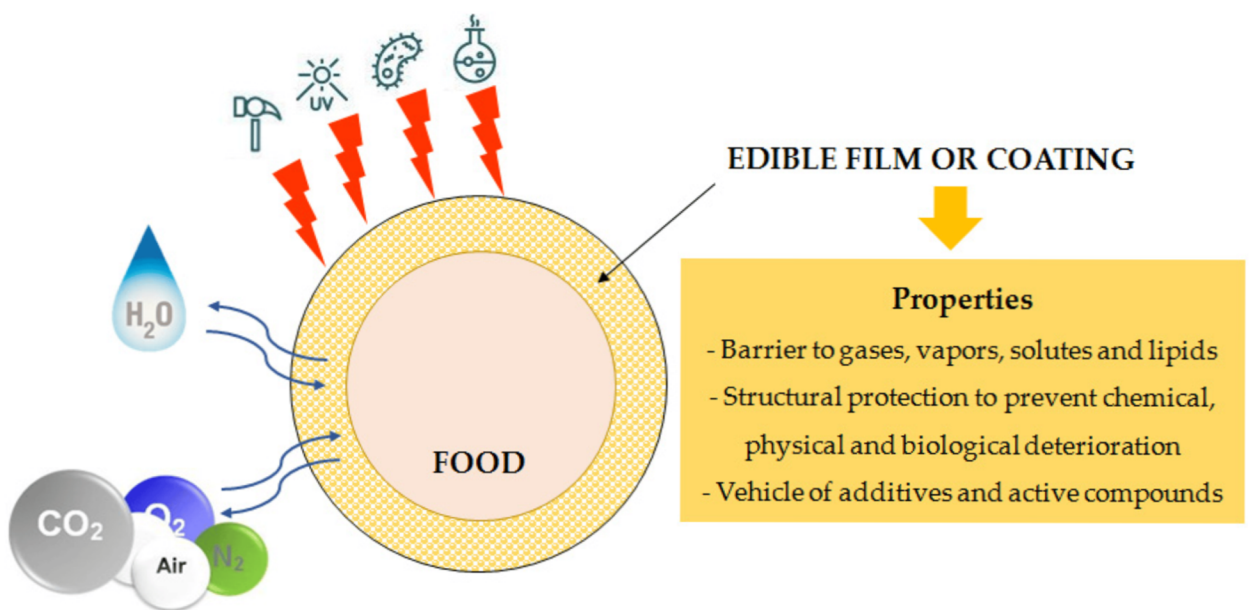


Figure 14. Main functions of edible films and coatings in food packaging applications. Source: Valdés (et al. 2017).

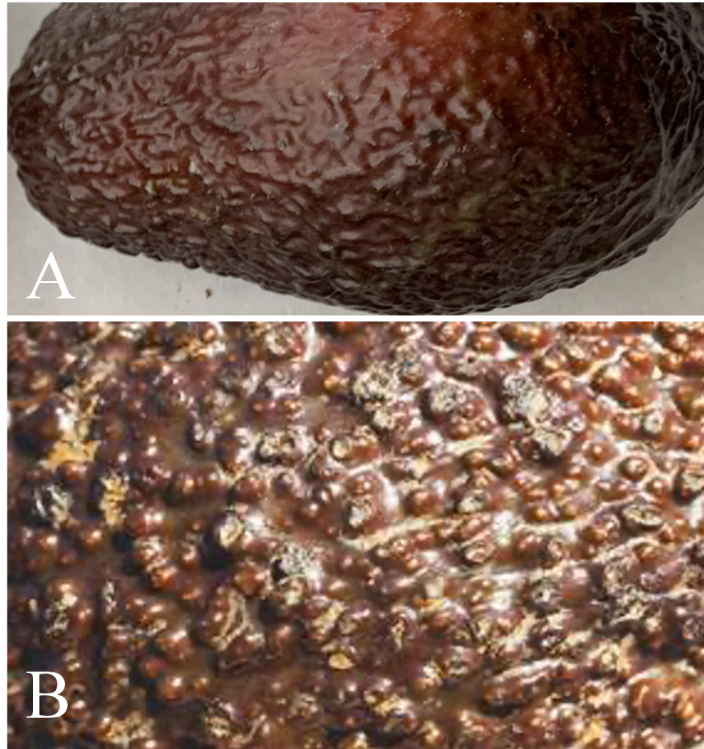


Figure 15. A) Avocado without any application. B) Too thick a layer of carnauba/steaming after application. Source: Author.

#### 2.4.1. History of edible coatings

The history of employing edible films and coatings may appear recent, spanning only the previous fifty years, but it actually goes back to the 12th and 13th centuries. In the 12th century, wax was applied to oranges and lemons in China to prevent water loss during storage and transit. The first edible film creation in Japan dates back to the early 15th century, and it was created by using soy milk proteins that were boiled in pans and then further air dried. These films are known as "Yuba" films. In order to safeguard various meat items, the first US patent for gelatin films was obtained during the 19th century (Pavlath & Orts 2009). By reducing gas transfer through edible coatings, sucrose and sugar derivatives were utilized as a protective coating on nuts to stop oxidative rancidity. In order to prevent dehydration during transit, fruits and vegetables were coated with lipids and commercially waxed in the 1930s while allowing for natural respiration (Dangaran et al. 2009). The increasing demand for textile items made from agricultural resources containing protein during World Wars I and II in the early 20th century drove

up the development of marketed protein-based wool alternatives. These products included blankets and uniforms for troops (Petersen et al. 1999). The creation of commercial edible films ready for various food packaging systems is driven by customers' growing quality needs, which include fresh and safe food ingredients and healthy packaging options (Erkmen & Barazi 2018).

#### **2.4.2. Legislation of edible coatings**

The edible coating needs to adhere to all regulatory criteria because it is a component of the food's edible element. The laws that apply to the coating may differ from one nation to the next (Armghan Khalid et al. 2022). According to European Directive (95/2/EC), edible coatings are those that fall under the categories of food products, food additives, food components, food contact substances, or food packaging materials (Khayelihle 2018). According to European legislation, all additives must be approved and included in the EU positive list along with usage recommendations. Every element needs to meet technological requirements, be safe, and not mislead customers. EC Regulation 1333/2008 specifies the need for additives. There are definitions for processes, labeling, and usage. According to the European Parliament (2008), some of the substances that are permitted are xanthan gum (E415), pectin (E440), shellac (E904), beeswax (E901), carnauba wax 10.16 % (E903), and others. Every component that comes into touch with food must adhere to acceptable manufacturing procedures, not change the food's color, flavor, texture, or odor, and not have any negative effects on the food or jeopardize public health (Armghan Khalid et al. 2022).

#### **2.4.3. Types of edible coatings**

Three primary categories are used to classify edible coatings. Coatings based on polysaccharides, such as starch, chitosan, cellulose, alginate, pectin, and gums, coatings based on proteins, such as zein, whey protein, wheat gluten, casein, soy protein, egg albumin, and gelatin, coatings based on lipids, such as waxes and fatty acids, and composite coatings, are created by mixing multiple materials or substances (Kurek et al. 2017).

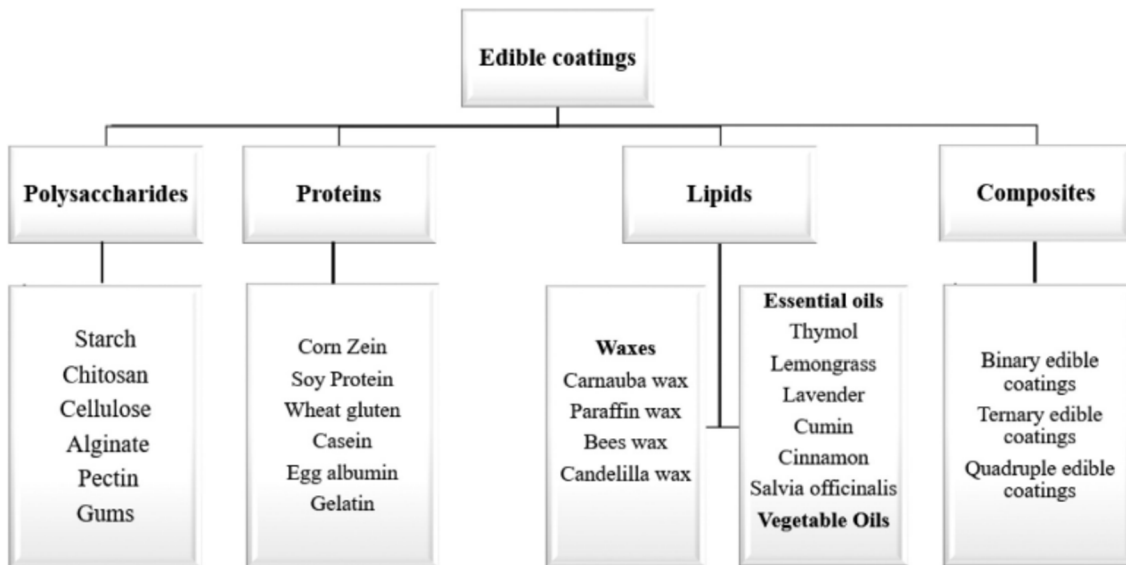


Figure 16. Types and subtypes of edible coatings. Source: Armghan Khalid (et al. 2022).

### Polysaccharides-based edible coating

One kind of naturally occurring polymer that is utilized to create coatings is polysaccharides. Some of the basic materials used in polysaccharide-based coatings for food preservation include starch, gums, and chitosan. Utilizing these coatings offers a number of benefits, including increased availability and low cost. Certain polysaccharides, including alginate and carrageenan, are very hygroscopic and have thick film properties, even while other polysaccharides have lesser water vapor barrier capabilities. The edible coating made of polysaccharides possesses both antioxidant and antibacterial properties. Fruits and vegetables are effectively preserved, and their quality is also improved. These molecules cannot function as a moisture barrier because of their hydrophilic nature (Galgano 2015).

### Protein-based edible coating

Plants and animals provide the protein used in edible coatings. Zein from maize, soy protein, gluten from wheat, and so forth are examples of plant-based protein coating materials; animal-based protein sources include whey protein, casein from milk, egg albumin, and gelatin. Its ability to form a barrier against mechanical strength, high oxygen

permeability, and organoleptic and aroma retention is significantly higher than that of other materials. However, because of its hydrophilic properties—which could be reinforced by the addition of hydrophobic materials like lipids—it does not possess a moisture barrier (Mohanty et al. 2020).

### **Lipid-based edible coating**

Lipid-based edible coatings include mineral, vegetable oil, wax, and acetylated monoglycerides, which give fresh produce a glossy, lustrous appearance. Lipid coatings can help reduce the effects of oxygen, water, light, and other external factors on product quality during storage and also slow down the pace at which water evaporates from the food item since they are hydrophobic. Additionally, they protect against chilling damage, which primarily happens during cold storage (Dhall 2013).

### **Methods of edible coating applications**

There are several techniques for applying coatings. One of the most popular techniques is dipping, which involves letting fruits and vegetables air dry after they've been immersed in the coating mixture for a while. An alternative method involves applying a coating solution on fruits and vegetables by forcing it via a spray machine. Different coating techniques enable the proper application to various product while taking into account its distinct physical qualities, such as size and form, as well as the coating solution's properties, such as viscosity. Fruits and vegetables are surrounded by edible coverings that act as a physical barrier between them and their surrounding environment (Garcia & Davidov-Pardo 2020). A prolonged shelf life is the outcome of the slowing down of certain physiological processes, which delays the ripening process. Furthermore, coatings can help to limit microbiological contamination, maintain chemical composition, and lessen the possibility of mechanical damage during the transportation and storage of products (Dhall 2016). The application technique affects how effective the coating is. According to Debeaufort and Voilley (2009), the size, shape, and intended fruit thickness all influence the coating application technique. Additionally, according to Andrade et al. (2012) the coating's density, viscosity, and surface tension. The most popular application

technique is dipping and spraying. Another technique that might be employed is spreading.

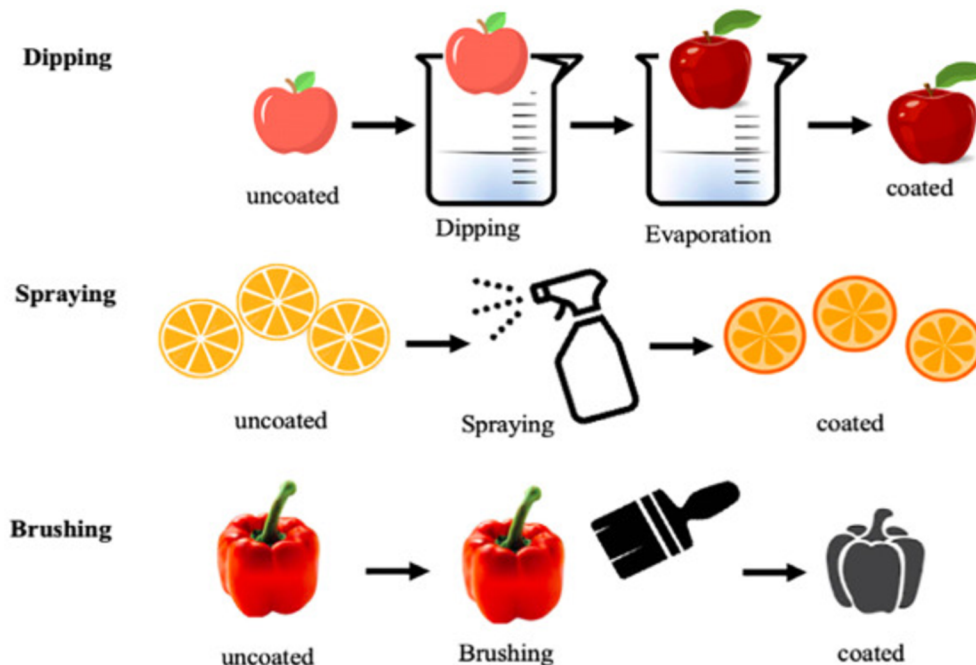


Figure 17. Methods of applying edible coatings. Source: Jafarzadeh et al. (2021).

### Dipping method

Among other culinary products, the dipping procedure has been used to produce coatings onto fruits, vegetables, and meat (Lu et al. 2010). Given that thick coating layers may be formed by dipping techniques, parameters including the density, viscosity, and surface tension of the coating solution are crucial in determining the film thickness (Tavassoli-Kafrani et al. 2016). By immediately immersing the product into the aqueous coating mixture and then allowing it to air-dry, this approach creates a membranous layer over the product surface. Three phases may be distinguished in this process:

**Immersion and residence:** To make sure there is sufficient interaction between the substrate and the coating solution for full wetting, the substrate is submerged into the precursor solution at a steady pace, followed by dwelling.

**Deposition:** Through deposition, a thin coating of the precursor solution is created on the food's surface. The surplus liquid flows off the surface and is taken out.



**Evaporation:** The thin film is created when the surplus solvent evaporates from the fluid (Schneller et al. 2013).

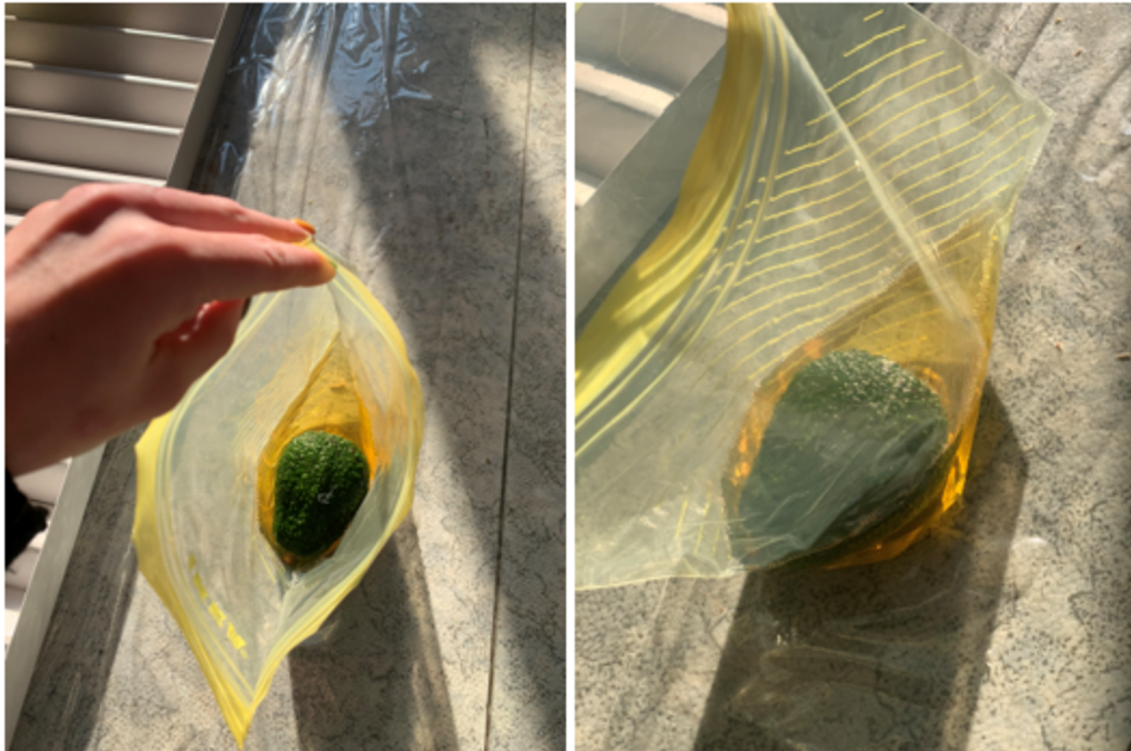


Figure 18. Dipping method with *Persea americana*. Source: Author.

### **Spraying method**

The most common technique for applying low-viscosity coatings is spraying. The dipping approach is preferred over spraying for high viscosity coatings (Dhanapal et al. 2012). A thin coating layer can develop on the surface by adjusting the viscosity of the solution. Using this technique, the coating's thickness may be efficiently controlled. According to Andrade et al. (2012), this technique may be utilized to apply a coating in numerous layers or across greater surfaces. The spray gun, nozzles, temperature, polymer solution, flow rate, and humidity all have an impact on the coating's quality. Time, temperature, and drying techniques also have an impact on the coating. There are several applications for this technique. One method is the traditional spray technique, which yields a thin layer. The size distribution of the droplets reaches up to 20 micrometers. Another is electrospraying, which creates homogeneous particles with a size of less than a nanometer (Skurtys et al. 2010).

## **Spreading**

Spreading is another name for brushing. This technique, which goes by the name "brushing," entails carefully applying a suspension to the material surface that has to be dried further. For the manufacture of films with dimensions greater than those created by casting processes, this technique is regarded as a legitimate substitute. Hot air circulation keeps the film drying on the support itself while a blade linked to the bottom portion of the spreading device regulates the thickness of the coating suspension. This process may be used to make protein-based films and polysaccharides (Sahuquillo-Arce et al. 2013). In this regard, contact angle measurements are frequently employed to assess the extent to which a given liquid spreads over or wettability of a surface. Numerous variables influence spreading, including the features of the substrate (such as its geometry and surface roughness), the system parameters (such as temperature and relative humidity), and the characteristics of the liquid (viscosity, surface tension, and density) (Kumar & Prabhu 2007).



### **3. Aims of the Thesis**

The aim of this work was to compare the effect of various natural waxes and the modes of their application as an edible coating on the physiological aspects of the fruit such as weight loss, colour change of the peel during storage, avocado firmness at different parts of the fruit and its overall shelf life. Weight loss was measured and processed over time during storage and expressed as a percentual weight difference. Colour characteristics of the peel was described as change over time of experiments compared to the first day. The fruit firmness was constructed in terms of measured pressure change of the fruit over time. An additional aim of this thesis was to create an avocado colour palette graphically visualizing its colour changes.

## **Material and Methods**

### **3.1. Plant material**

The avocados were Hass varieties from Peru, Israel and Morocco. The avocados from Peru was shipped to Holland by a ship and kept in the container for one day at six degrees Celsius. The avocados from Israel was shipped to Marseille and kept in the container for 2 days at six degrees Celsius. The avocados from Morocco was shipped to Holland and kept in the container for one day at six degrees Celsius. The plant material and the waxes were obtained from a company called Titbit, a business that supplies fresh produce to supermarkets in the Czech Republic, Hungary, Poland, and the Slovak Republic. All the untreated avocados were then shipped to the Czech Republic and kept in Titbit's storage facilities for 3-4 days at eight degrees Celsius. Every avocado was kept in the same storage environment. The avocados were in decent shape. The fruit was mostly unblemished. Transport or harvesting were the causes of these. The avocados used for the experiment weren't all the same size, colour, and ripening stage. The first avocados from Peru were more pre-ripe and were from the harvest from the end of the season with twenty-six percent dry matter. The avocados from Israel and Morocco were more light green or medium green and were from the second harvest.

### **3.2. Storage experiment**

We did two experiments with avocados.

1. The first experiment was cold storage shelf life test with avocados from Peru. The cold storage shelf life test was tested at six degrees Celsius. We divided the avocados into four categories of ten avocados each. The avocados were sprayed with three waxes (shellac 5.5 %, shellac 10 %, carnauba wax 10.16 %) and we used different methods. The first method of application we used was spraying method. We sprayed the avocado on all sides with a thin layer and then we let the wax dry. The second method of application we used was dipping when we put the avocado into the plastic bag with wax for few seconds and then we pulled it out and let it dry. The third method of application we used was brushing when we

carefully applied a suspension to the material surface and then let the wax dry. This experiment lasted five days.

2. The second experiment was market display shelf life test with avocados from Israel and Morocco. This market display shelf life test was tested at twenty degrees Celsius. We divided the avocados into eight categories of thirteen avocados each. The avocados were sprayed with three waxes (shellac 5.5 %, shellac 10 %, carnauba wax 10.16 %) and we only used the spraying application method. Each day we measured the weight loss of the avocados, measured the colour characteristics of the peel and every third day we measured the fruit firmness. This experiment lasted ten days. We did four repetitions.



Figure 19. Storing avocados in a cool environment at six degrees Celsius. Source: Author.



Figure 20. Storing avocados in a market display shelf life test at twenty degrees Celsius. Source: Author.

### 3.3. Coatings and films preparation

The experiment used three different types of waxes: carnauba wax 10.16 % dry matter content, 10% dry matter content, and 5.5% dry matter content of shellac wax (Fig. 22). The avocados were divided in the first experiment into four categories of ten pieces each. Ten samples from three categories were sprayed with each wax. The last category was a control without any wax. In the second experiment, the avocados were divided into eight categories of thirteen pieces each. Thirteen samples from seven categories were sprayed with each wax. The last category was a control. In order to improve alignment between the findings, the samples were first characterized. After that, they were set on an iron framework. Next came the real application with the waxes. Spray application was used (Fig. 21). All surfaces were coated, and the coating was given time to dry, especially the carnauba wax 10.16 %, because this wax needed more time to dry. The fruit was flipped over and the coating was applied from the opposite side once again from all sides when the liquid wax dried. A single spray from all directions was adequate for all waxes other than carnauba wax 10.16 %. A heavier coat was applied to the carnauba wax 10.16 %. In one location, the treatment was sprayed twice. Following coating application, the samples were allowed to air dry. The samples were weighed once they had dried. Eight more specimens in the experiment received the 5.5% shellac

treatment, although it was done in a different way. Four specimens were treated by spreading, and four by dipping (Fig. 22). Fruit was dipped into a bag filled with wax during the dipping procedure, and the fruit was subsequently dried. A brush was used for the brushing. The sample was covered with a coating and then allowed to dry. The completed samples were kept in a room temperature. They were kept there for seven days at twenty degrees Celsius.

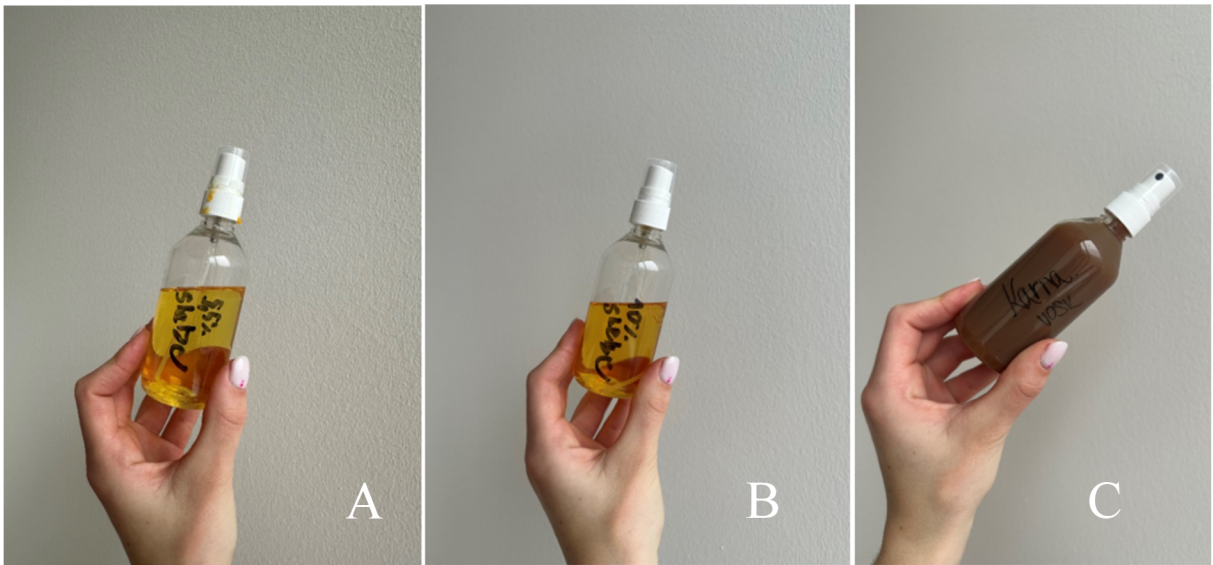


Figure 21. Applied coatings A) shellac 5.5 %, C) shellac 10 %, C) carnauba wax 10.16%. Source: Author.

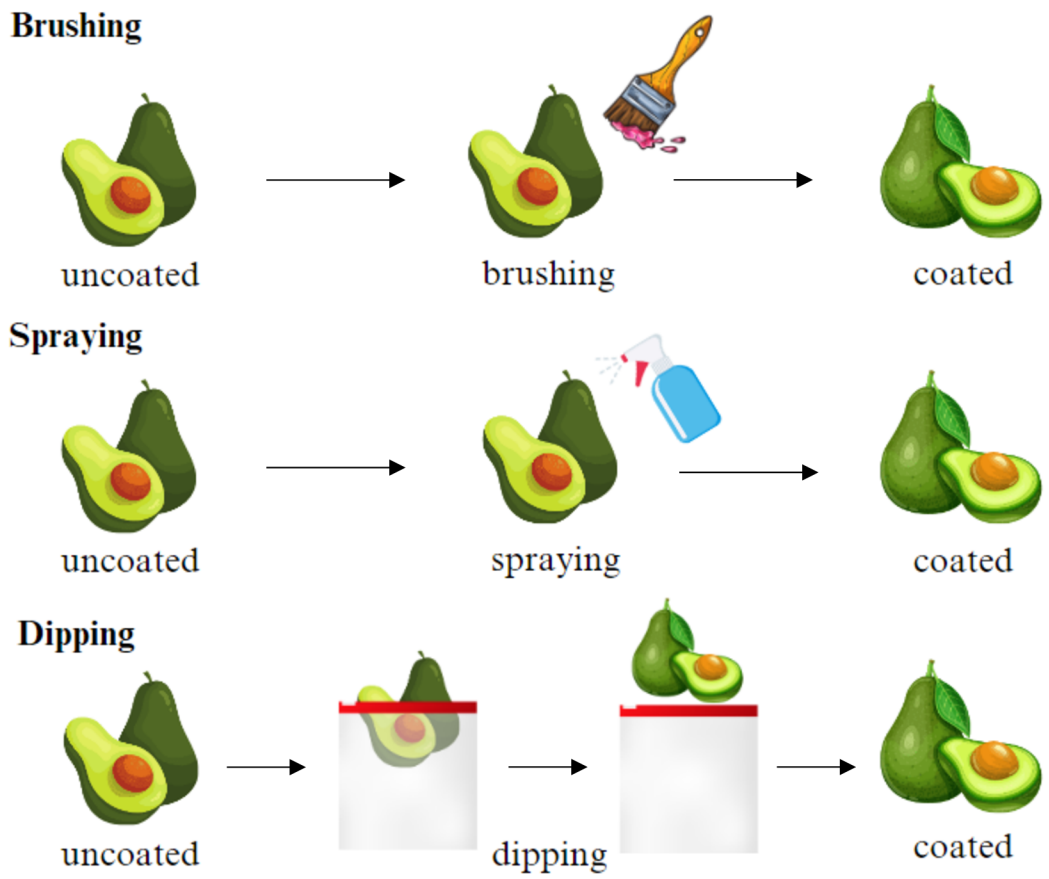


Figure 22. Application methods of edible coatings. Source: Author.

### 3.4. Weight loss

The primary cause of weight loss during storage is moisture loss, which results from the fruit's cellular activity, physiological processes, and the water vapor pressure difference between it and its surroundings. Although they might be impacted by the temperature and relative humidity of the surrounding air, this loss can be reduced by the use of barriers like films, which would stop transpiration, and the interchange of gases in the fruit with the environment (Choque-Quispe et al. 2022).

A digital scale (RADWAG Wagi Elektroniczne, model PS 1200.R2) was used to measure weight reduction. The observation was conducted over 7 days. The samples were weighed, and the findings were recorded at around the same time each day. To avoid any confusion, each fruit was tagged with numbers and with the name of the wax. After that, the data were analysed and contrasted with both the control samples and one another. Using the following formula, weight loss was computed as a percentage of the starting weight:

$$\frac{(\text{average of day 0} - \text{average of day } n^{\text{th}})}{\text{average of day 0}} \cdot 100.$$

The mass loss of each fruit was estimated by weighing it and taking into account the variation between its original mass and the mass attained at each sample interval during the course of the seven-day analysis.



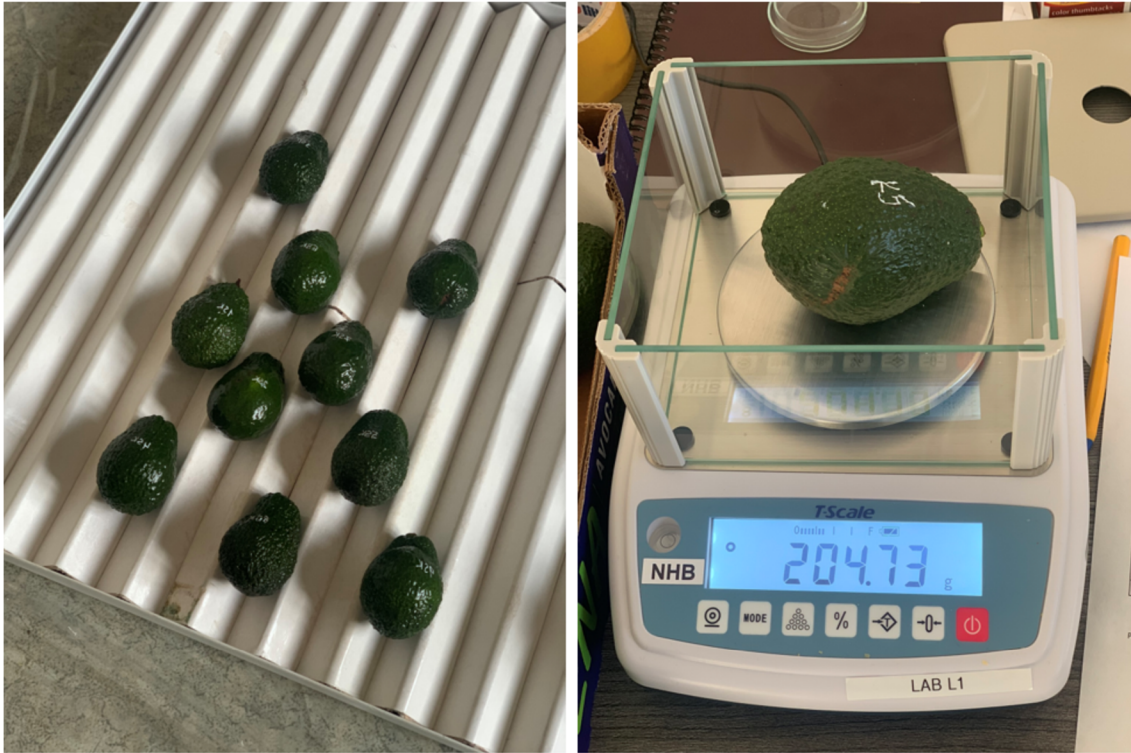


Figure 23. Measure the weight reduction of avocado on a digital scale. Source: Author.



### 3.5. Colour characteristics of the peel

A colorimeter (Konica Minolta, CM-600d, Osaka, Japan) operating in CIE mode D65 was used to detect the instrumental color characteristics of the fruit's peel and pulp.  $L^*$  (lightness), which ranges from dark/opaque to white,  $a^*$  (green/red),  $b^*$  (blue/yellow), which conveys color and ranges from blue ( $270^\circ$ ) to green ( $180^\circ$ ) to yellow ( $90^\circ$ ) and purple-red ( $0^\circ$ ), were the parameters that were studied. As a color's lightness, it may be defined as the amount of light you wish to add to it. A colour's lightness can be expressed as follows: 0% represents no light at all (black), 50% represents half of the light (white), and 100% represents entire lightness (black). The colorimeter was warmed up for 10 minutes and was calibrated with a white standard. The colorimeter's readings were all taken straight from it. We took twelve readings from the skin and pulp of each fruit at various locations from the eight categories on the surface of the fruit. To monitor the fruits' skin appearance, pictures were taken every day.

The colour of the sample from each tested group on the first measurement day was used as the reference value to generate the  $\Delta E$  index, which is provided by the following equation.  $\Delta E$  was used to assess the impact of various pretreatments on the total combined colour of avocado:

$$\Delta E = \sqrt{(L - L_{base})^2 + (a - a_{base})^2 + (b - b_{base})^2}$$

According to Kucerova et al. (2018), the overall colour difference  $\Delta E$  as calculated by  $\Delta E$  index can be analytically categorized as: not noticed (0–0.5), mildly noticeable (0.5–1.5), noticeable (1.5–3.0), very visible (3.0–6.0), and large (>6.0).

Colour index CI was also calculated and evaluated from the measured data. It was used to determine the colour change between the four tested groups over the tested time period. The calculation of CI is as followed:

$$CI = \frac{a \cdot 1000}{L \cdot b}$$

According to Choque-Quispe et al. (2022) the colour index represents colour in single numerical data and its ranges represent specific colour:

- -40 to -20 colours range from blue violet to deep green,
- -20 to -2 colour range from deep green to yellowish green,
- -2 to +2 colours represent greenish yellow,
- +2 to +20 colours range from pale yellow to deep orange,
- +20 to +40 colour range from deep orange to deep red.

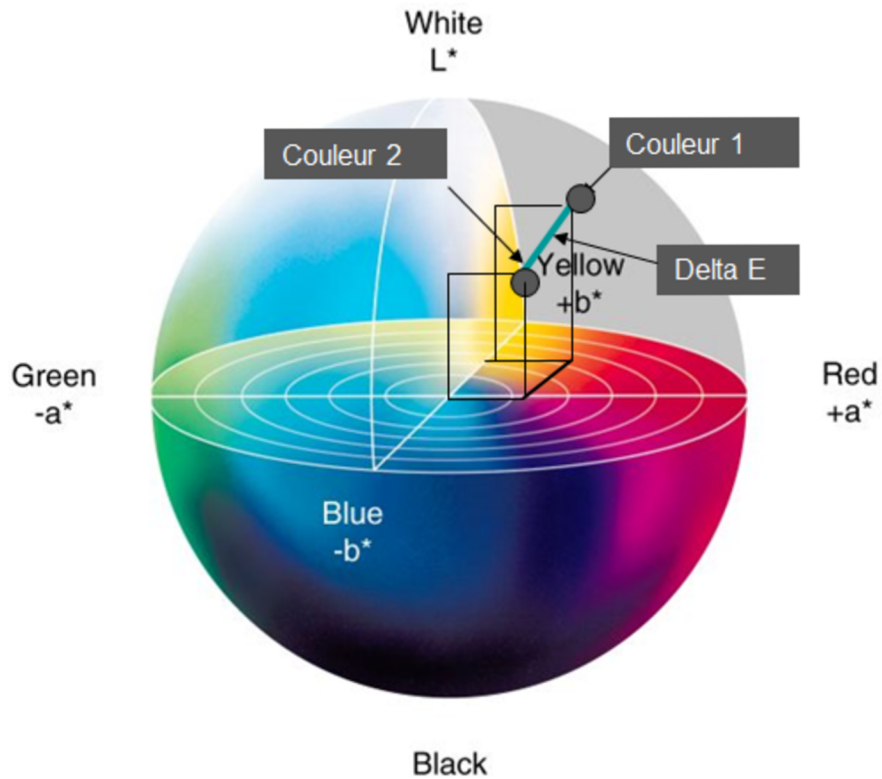


Figure 24. Delta E colour difference. Source: (Couto et al. 2023)

### 3.6. The fruit firmness

Each fruit's level of firmness was evaluated independently. With the use of a manual digital Fruit Hardness Tester Instron, model 34SC-2, measurements were taken in the equatorial area at two evenly spaced spots. An 8 mm diameter probe was manually pierced (10 mm depth). In Newtons (N), the findings were expressed. The experiment was monitored for 15 days.  $F_s$  strength was measured with a penetrometer in units of N using an 8 mm diameter punch on both sides of the fruit. The punch was pushed into the fruit at least 0.5 mm. A distinct peak was produced on the instrument after impaction into the stone.

The value in N before impacting the fruit stone was used for the calculation. From the measured values, the N units were converted to pressure in MPa before statistical evaluation according to the equation below.

$$A = \pi \cdot r^2 \qquad \delta_{ps} = \frac{F_s}{A}$$

$R$ [mm]	radius of the punch
$A$ [mm <sup>2</sup> ]	area of the punch
$F_s$ [N]	force required to penetrate the pulp
$\delta_{ps}$ [Mpa]	compressive stress

The firmness of an avocado determines its ripeness. The values in are obtained by penetrometer and the calculated results expressed in MPa can tell when the fruit is ripe and ready to be consumed:

- $\delta_{ps} > 10$  avocado is unripe,
- $10 > \delta_{ps} > 8$  avocado is beginning to ripe,
- $\delta_{ps} < 5.5$  avocado is losing it's bad bitter taste and is starting to be edible,
- $\delta_{ps} = 3$  avocado is ready to be eaten,
- $\delta_{ps} < 1$  avocado is overripe.



Figure 25. Measurement of the firmness of avocado on Instron penetrometer.

Source: Author.

## **4. Results**

### **4.1. Weight loss**

In the first part of the experiment, we investigated whether there would be any difference between the edible coatings and the control in terms of weight loss. Cold storage experiment was made first. Three different edible coating options (shellac 5.5%, shellac 10%, carnauba wax 10.16%) were applied on avocado peels. We coated the avocados using spraying, brushing, and dipping methods. During coating application, it was discovered that spraying is the most effective method for evenly distributing and conserving edible coatings compared to brushing and dipping. After this result, it was decided to use spraying in further experiments and observations.

The weight loss of the avocados during cold storage can be seen in

Table 2. According to the results displayed in Table 3, there wasn't any statistical significance between the results. Also, the relative values of all groups are very similar to each other. However, the shellac 10% inclines towards the least weight loss, followed by carnauba wax 10.16 % and shellac 5%. Control had the biggest weight loss out of all groups.

In the second part of the weight loss experiment, we observed weight loss in market display storage over 4 days. The results can be seen in Table 4. The weight loss differences between tested groups in the market display were larger compared to the differences in cold storage. The control group experienced the largest amount of weight loss. The best results of treatments were with shellac 5.5% coating, followed by carnauba wax 10.16%, and then shellac 10% with the worst coating results. Although we did not find any statistical significance in the results obtained, there is an indication, that treatments become more relevant in higher temperatures.

Factorial analysis of variance (ANOVA) was used to analyze the data, and mean comparisons were made for each answer at  $p \leq 0.05$ . Microsoft Excel was used to generate the results.

Table 2. Results of relative weight loss of avocados to the first day in the cold storage shelf life experiment.

Day	Treatment			
	control	shellac 5.5 %	shellac 10 %	carnauba wax 10.16 %
1	0.00 %	0.00 %	0.00 %	0.00 %
2	0.32 %	0.46 %	0.35 %	0.35 %
3	0.76 %	0.84 %	0.71 %	0.74 %
4	1.19 %	1.29 %	1.03 %	1.08 %
5	1.67 %	1.62 %	1.32 %	1.39 %

Table 3. Statistical evaluation of weight loss in the cold storage shelf life experiment.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	7.7683E-06	3	2.5894E-06	0.07184883	0.97419348	3.23887152
Within Groups	0.00057664	16	3.604E-05			
Total	0.00058441	19				

Table 4. Results of relative weight loss to the first day in the market display shelf life experiment.

Day	Treatment			
	control	shellac 5.5 %	shellac 10 %	carnauba wax 10.16 %
1	2.681	1.197	1.589	0.475
2	3.406	1.474	1.889	1.904
3	5.173	2.534	3.332	3.105
4	7.034	3.796	5.585	4.580

Table 5. Statistical evaluation of weight loss in the market display shelf life experiment.

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	0.00060834	3	0.00020278	0.6635271	0.59024816	3.49029482
Within Groups	0.00366731	12	0.00030561			
Total	0.00427565	15				

## 4.2. Firmness

One important trait that has a direct impact on marketability and consumer acceptance of avocados is their firmness. The purpose of our study was to find out how well different edible coatings maintained the firmness of avocados over time. Our data showed a complex trend in avocado firmness, with some unexpected changes found, which was contrary to early predictions. Although the general perception is that avocados become softer with age, our research showed certain cases of avocados becoming harder. Nonetheless, the substantial standard deviations in our findings emphasize the natural fluctuations in avocado ripening dynamics. Gaining a thorough understanding of how avocados soften was challenging due to rapid softening rates and limited sample availability.

There is an assumption that the hardness will decrease, which was also expected, but our data (Table 6) did not confirm this. Rather, our results suggest that the hardness was increasing, but due to the large standard deviations, it is not possible to come to a clear conclusion, so the results are affected by the fact that there would probably need to be more samples to test.

Unfortunately we did not have enough samples available. Avocados soften over time, do not firm, the error is due to poor sampling - it was not possible to take a sufficient number of pieces for the experiment. Soft samples were cut first to save samples for tests. And this is how it was injured - the softening of the flesh was distorted in the pieces that ripened more slowly. Further, there was insufficient time to set up additional trials to allow for larger sampling for strength determination. The samples ripened very quickly and went into soft pressure and changed colour immediately overnight. By increasing the number of samples it might help to reduce the error in randomly choosing more ripe avocado in one group compared to the other. As the results in day one say, the average firmness of shellac 5.5 % for example was much softer at 0.309 MPa than control sample at day one at 1.599 MPa with large standard deviations in the same order of magnitude as the averages.

The results also hint that at day five all avocados were harder than day one even for control sample. That is very unexpected behaviour and the sample number might not

be enough to eliminate errors like choosing more ripe avocado, penetrating its weakest spot etc.

Table 6. The firmness of avocado in MPa

	control	shellac 10 %	shellac 5.5 %	carnauba wax 10.16 %
Day 1	1.599 ± 1.796	0.873 ± 0.932	0.309 ± 0.199	0.641 ± 0.868
Day 3	0.754 ± 0.883	1.917 ± 1.819	0.750 ± 0.000	1.594 ± 1.513
Day 5	2.344 ± 1.668	3.211 ± 2.972	1.408 ± 1.720	1.884 ± 1.736



### 4.3. Colour change of the peel

To determine the colour, avocados were selected on the colourimeter as shown in Table 7. Six avocados were chosen, and each piece was measured 10 times on the surface of the fruit. The projections are given in Table 7. With average  $L$  noticeably declining, we can see that the avocados are getting darker. As the value approaches zero, the colour of the skin darkens. The measured  $a$  values for the avocado peel samples show different levels of redness. Red is more prevalent in samples with larger positive  $a$  values, whereas red is more subdued in samples with values near zero. Overall, the study points to a spectrum of redness in avocado peel samples, some of which have strong red tones and others that have more subdued variations. These results provide light on the colour properties of avocado peels and demonstrate the variation in red pigmentation among samples. The measurements of the  $b$  values for the avocado peel samples show different levels of yellowness: samples with higher positive  $b$  values show more yellow present, while samples with values closer to zero show a relatively weaker yellow hue. Overall, the analysis indicates a spectrum of yellowness in the avocado peel, with some samples showing more subtle variations and others displaying pronounced yellow tones. These results shed light on the colour characteristics of the avocado peel and highlight the variability in yellow pigmentation across different samples.



Figure 26. A) Light green, B) Medium green, C+D) Breaking, E) Pre-ripe, F) Dark ripe. Source: Author.

Table 7. Average values of avocado colouring stages (from A - first stage to F - last stage)

avocado coloring stage according to the color palette	average $L$			average $a$			average $b$		
A	43.733	±	3.573	-9.365	±	0.778	27.793	±	4.038
B	31.613	±	2.953	0.023	±	0.994	9.003	±	3.438
C	31.358	±	0.593	-1.465	±	0.443	9.413	±	1.028
D	29.800	±	0.655	0.725	±	0.585	7.078	±	0.988
E	25.915	±	0.920	3.528	±	0.268	5.003	±	0.386
F	26.268	±	0.148	1.533	±	0.153	1.020	±	0.200

Experiment was made two times separately. We calculated  $\Delta E$  and  $CI$  from measured values  $L$ ,  $a$  and  $b$ . Results of the first experiment can be seen in Table 8. Results of the second experiment can be seen in Table 11. Statistical evaluation of  $CI$  is in Table 9 (ANOVA) and Table 10 (Tukey's test) for experiment 1 and in Table 12 (ANOVA) Table 13 (Tukey's test) for experiment 2.

It was observed that the luminosity  $L$  declined over the days, meaning that avocados were getting darker with each next day of measurement. The fastest and most significant decline was with control samples. The samples with treatments had a slower decline which correlates with avocados getting less dark with time compared to the control. The smallest decline was with the shellac 10 % in the first experiment and with carnauba wax 10.16 % in the second experiment. The  $L$  values for control were the lowest at the end of both experiments.  $L$  values of treatments at the end of measurement were higher than control and had similar values compared to each other. The avocados without treatment went darker overall and had a faster decline. Treated avocados ended up less dark, implying that they could last longer if tested by color only.

The values of chroma  $a$  slightly increased, meaning the colour change went towards red on the spectrum. However, the standard deviation was relatively high and all values of all groups were very similar. Avocados with control ended up with  $a$  values higher than with treatment although not with large difference. In summary all of the values didn't change significantly compared to each other and they all increased only by few units. The differences from each other when compared don't imply such significance as in  $L$  values.

Chroma  $b$  slightly decreased over the days, meaning the change went towards blue on the spectrum. The overall change was also similar between all tested groups. The largest decline of  $b$  was in the control group. The differences from each other when compared don't imply such significance as in  $L$  values.

The colour mix in total leans towards darker green-brownish colour over the days with  $a$  increasing and  $b$  decreasing on the spectrum. The avocados went from lighter appearance towards darker at the end. The control group had the greatest change in all  $L$ ,  $a$ ,  $b$  values in both experiments.

$\Delta E$  was calculated from average values of  $L$ ,  $a$ ,  $b$ . With this number we can calculate colour difference compared to first day of measurement within each tested group. All tested groups ended up with  $\Delta E$  value larger than 3, meaning that all groups ended up being *very different* in terms of colour change. Results also show that control group had the biggest colour changes of all tested groups. The changes of control group were significantly higher with each day compared to treated groups. Shellac 10 % had the best results from all groups and ended up as a best treatment in terms of smallest colour change over the experiment 1. In experiment 2, carnauba wax 10.16 % had slightly better result than shellac 10 %, although the results are very similar.

Experiment 2 showed smaller colour change due to the shorter experiment (only 4 days compared to 6 days in experiment 1). The behavioral changes were similar in both experiments though.

We also measured the color index, which put all the parameters into single numerical expression which determines the color of an avocado. First the colours of the samples were on a spectrum from -20 to -2 meaning they started as deep green to yellowish green.  $CI$  values went all up over the tested days and ended up at a spectrum from +2 to +20 meaning the colours changed towards pale yellow to deep orange on the  $CI$  spectrum. Only the control group in experiment one ended at value 27.51 on spectrum from deep orange to deep red. The  $CI$  in treated samples had similar increase in value over the tested days, implying that they changed overall colour similarly. The  $CI$  in control group had the greatest increase and changed colour the most of all tested groups.

$CI$  was statistically evaluated with ANOVA and Tukey's test, showing statistical significance. In experiment 1 there was statistical significance between control group and treated groups. In experiment 2 there was only significance in control vs. shellac 5.5 %.

In experiment 2 all of the values ended up lower than in experiment 1 due to the shorter testing length (6 days of observing in experiment 1 compared to 4 days in experiment 2). The results in both experiments showed similar behavior though.

Factorial analysis of variance (ANOVA) and Tukey's test was used to analyze the data, and mean comparisons were made for each answer at  $p \leq 0.05$ . Microsoft Excel was used to generate the results.

Table 8. Colour of Hass variety avocado in market display shelf life test.

Day	<i>L</i>			<i>a</i>			<i>b</i>			<i>CI</i>			<i>ΔE</i>
	$\bar{x}$	$\pm$	SD	$\bar{x}$	$\pm$	SD	$\bar{x}$	$\pm$	SD	$\bar{x}$	$\pm$	SD	$\bar{x}$
<b>control</b>													
1	34.25		3.24	-3.59		0.60	13.57		1.00	-7.72		2.12	
2	30.44		3.94	-1.76		0.74	10.36		1.15	-5.59		1.52	5.31
3	29.77		3.87	-1.00		0.72	9.39		1.14	-3.59		1.60	6.66
4	27.37		3.93	-0.40		0.65	8.95		1.02	-1.62		1.50	8.89
5	23.50		3.85	2.22		0.77	7.15		1.15	13.19		1.62	13.81
6	20.75		3.70	2.94		0.69	5.14		1.10	27.51		1.49	17.20
<b>carnauba wax 10.16 %</b>													
1	30.80		3.98	-3.75		0.88	9.54		1.17	-12.78		2.53	
2	29.80		3.90	-2.65		0.75	8.86		1.20	-10.04		2.10	1.63
3	28.87		3.11	-1.88		0.64	7.43		1.02	-8.75		2.15	3.42
4	28.68		3.54	-0.43		0.63	8.32		1.06	-1.81		1.52	4.13
5	26.81		3.94	1.85		0.73	6.49		1.20	10.67		2.09	7.53
6	26.14		3.17	2.15		0.62	6.14		1.01	13.43		1.94	8.26
<b>shellac 5.5 %</b>													
1	30.13		3.75	-2.23		0.66	9.35		0.99	-7.93		1.57	
2	29.65		4.01	-1.35		0.72	7.36		1.05	-6.19		2.01	2.23
3	28.29		3.79	-0.19		0.66	6.47		1.09	-1.03		1.34	3.98
4	27.83		3.74	0.49		0.67	5.88		1.13	2.97		2.21	4.97
5	27.10		2.88	1.93		0.58	6.46		0.83	11.05		2.54	5.91
6	26.98		3.83	2.24		0.66	5.90		1.09	14.05		1.34	6.47
<b>shellac 10 %</b>													
1	30.01		3.72	-1.35		0.65	8.92		0.99	-5.03		1.48	
2	29.09		3.19	-0.65		0.79	9.02		0.96	-2.49		2.79	1.16
3	28.87		4.04	-0.12		0.73	7.03		1.17	-0.59		1.78	2.52
4	27.46		2.96	0.78		0.65	6.39		0.74	4.45		2.32	4.18
5	28.35		3.58	1.19		0.84	5.30		1.07	7.95		2.85	4.72
6	27.60		3.99	2.34		0.73	7.04		1.16	12.06		1.99	4.79

Where:  $\bar{x}$ , arithmetic mean; SD, standard deviation

Table 9. Statistical evaluation of colour index (CI) - ANOVA - experiment 1

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	23456.2505	3	7818.75015	18.9984016	2.9272E-12	2.60636533
Within Groups	2509618.41	6098	411.547787			
Total	2533074.66	6101				

Table 10. Statistical evaluation of colour index (CI) - Tukey's test - experiment 1

comparison	<i>q</i> <i>CV</i> difference	3.63 1.885 <i>CV</i>	significant?
control vs shellac 5.5 %	-2.180	1.885	yes
control vs shellac 10 %	-5.478	1.885	yes
control vs carnauba wax 10.16 %	-2.987	1.885	yes
shellac 5.5 % vs shellac 10 %	-0.807	1.885	no
shellac 5.5 % vs carnauba wax 10.16	-0.807	1.885	no
10 % vs carnauba wax 10.16 %	2.491	1.885	yes

Table 11. Colour of Hass variety avocado in cold storage shelf life test.

Day	<i>L</i>			<i>a</i>			<i>b</i>			<i>CI</i>			<i>ΔE</i>
	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$	±	SD	$\bar{x}$
<b>control</b>													
1	32.65		4.16	-2.59		0.72	11.48		1.14	-6.90		1.54	
2	29.44		3.81	-0.90		0.85	10.77		1.07	-2.84		2.94	3.70
3	27.46		3.44	0.79		0.59	9.44		0.98	3.06		1.71	6.52
4	24.66		3.86	2.15		0.73	8.75		1.07	9.98		1.86	9.68
<b>carnauba wax 10.16 %</b>													
1	31.27		3.90	-2.85		0.68	11.74		1.14	-7.77		1.62	
2	30.55		3.28	-1.75		0.52	10.77		0.83	-5.33		1.65	1.64
3	29.53		3.89	0.53		0.67	8.65		0.99	2.08		1.77	4.90
4	28.96		2.56	1.94		0.75	7.88		1.09	8.48		3.01	6.57
<b>shellac 5.5 %</b>													
1	30.44		3.36	-2.65		0.69	12.97		1.11	-6.72		2.71	
2	29.84		3.08	-1.75		0.73	11.85		0.91	-4.96		2.92	1.56
3	28.27		3.72	0.23		0.86	10.74		1.24	0.77		2.72	4.25
4	27.85		4.19	2.75		0.72	8.95		1.16	11.01		2.14	7.21
<b>shellac 10 %</b>													
1	31.68		3.69	-2.30		0.70	11.96		1.09	-6.06		1.69	
2	30.44		3.59	-1.84		0.53	9.03		0.81	-6.70		2.13	3.22
3	29.84		2.65	0.46		0.51	8.39		0.90	1.82		1.82	4.87
4	28.36		3.10	1.07		0.67	7.29		0.79	5.16		2.63	6.65

Where:  $\bar{x}$ , arithmetic mean; SD, standard deviation

Table 12. Statistical evaluation of colour index (CI) - ANOVA - experiment 2

ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	6949.50514	3	2316.50171	4.39540406	0.00429083	2.60713722
Within Groups	2100734.25	3986	527.028161			
Total	2107683.75	3989				

Table 13. Statistical evaluation of colour index (CI) - Tukey's test - experiment 2

comparison	difference	q	CV	significant?
		3.63		
		CV	2.639	
control vs shellac 5.5 %	-3.607		2.639	yes
control vs shellac 10 %	-1.861		2.639	no
control vs carnauba wax 10.16 %	-0.979		2.639	no
shellac 5.5 % vs shellac 10 %	2.628		2.639	no
shellac 5.5 % vs carnauba wax 10.16 %	2.628		2.639	no
shellac 10 % vs carnauba wax 10.16 %	0.881		2.639	yes



## 5. Discussion

Avocado is highly perishable fruit (Rico-Londoño et al. 2021). Using edible coatings that obstruct the flow of gasses, solutes, and moisture is one way to prolong shelf life while preserving the quality of the fruit. One of the most important things in extending fruit storage and preserving fruit quality is water loss. Weight loss, no matter how slight, can lead to postharvest degradation (a decline in food quality, composition, and appearance) and consequent financial loss (do Nascimento Nunes & Emond 2007).

### *Weight loss*

According to Maftoonazad & Ramaswamy (2008). Fruits weight loss ranged from 8.6 % to 13.8 % of their initial weight during storage, depending on the temperature and type of coating. The main reason for this weight loss was moisture evaporating from the fruit through transportation. Although the mentioned study used different coating (pectin solution mixed with sorbitol and beeswax) there was a positive impact of using a treatment on weight loss over time. The weight loss pace was significantly slower with coated avocados in all tested temperatures. The most substantial difference was in temperature of 20 °C, at which the weight loss of approximately 13 % occurred in 5 days for control and in 10 days for treatment. At this temperature the treated avocado could be stored double the time. In storing temperature of 15 °C the avocado reached its weight loss around 10 % in 11 days for control and in 14 days for treatment. Overall, the steepness of weight loss was described in the work with rate constant  $k$  in linear approximation. The  $k$  values had smaller differences between untreated and treated samples in lower temperatures indicating that treatment is becoming less significant with lower temperatures. Compared to this work, in which there was no significant impact for treated avocados in cold storage. This can be due to much shorter experiment length of only 5 days and small amount of samples overall. This work showed a similar trend of coating significance in higher temperatures as in (Maftoonazad & Ramaswamy 2008). To achieve statistical significance, the experiment would require a larger sample size. Similar positive impact of treatments on weight loss showed in study of Choque-Quispe et al. (2022), although authors used again different treatments than in this work. In cited work in 14 days the control samples lost on average 6.63 % its former weight compared to 5.33% with treatment 1 and 5.19 % with treatment 2.

Also, since the weight loss is mostly bound to moisture loss (Choque-Quispe et al. 2022), there is a possible correlation between humidity of storing space and weight loss of an avocado. Humidity control is bound to temperature control, even more so in enclosed spaces (i.e. avocado in a bag stored in a fridge) therefore temperature control has direct impact on humidity therefore on weight loss.

### ***Firmness***

In researched studies by Choque-Quispe et al. (2022) the firmness was measured on treated and untreated avocados over multiple days. In all cases the results show that firmness decreases over time during avocados ripening process. This shows relation between ripening and decreasing firmness.

In study by Maftoonazad & Ramaswamy (2008) the firmness was tested on untreated avocados and treated avocados with pectin, sorbitol and beeswax mix at three different temperatures (10 °C, 15 °C, 20 °C). Length of experiment ranged from 7 to 34 days based on temperature. The experiment started at 11 MPa meaning all of the avocados were unripe at the start. Results show three things. First that the ripening process slows down at lower temperatures implying that the temperature has huge impact. Second, the treated avocado showed better results, slightly decelerating the ripening process and helping to maintain its firmness longer. Third, that the lower the temperature, the bigger was difference in firmness between control and treated samples.

Second study by Maftoonazad & Ramaswamy (2005) which used methyl-cellulose as treatment observed firmness changes only at one temperature of 20 °C. The experiment started with much softer, implying more ripe avocados and lasted only 6 days for control and 10 days for treatment. The results showed a similar trend as in (Maftoonazad & Ramaswamy 2008) meaning that treatment helps with slowing down the ripening process and retains its firmness for a longer time than the control sample. Similar results were also obtained in the study by Choque-Quispe et al. (2022) although with relatively small differences between control and treatment samples. In their results, the avocados ripeness of all tested groups was constant for about 6 days and started to differ only after that, with control being the fastest in terms of losing its firmness. When comparing the studies to this work, they show very similar results and trends, unlike this work, which unexpectedly showed an increase in its firmness. Even

subjectively by touch the avocados were getting harder over the days. This can be due to several limitations in this study.

First, the overall length of the experiment was much shorter and based on the research, some avocados can show significant change even only after 6 days or more, which could be this case. Another limitation for evaluating the outcome of treatments are measured results with severely large standard deviation, which was the same order as calculated average values. The reason for such deviations might be not choosing always the exact same penetration spot at the avocado fruit when using the penetrometer. Another drawback was very small portion of avocados tested. The reason for this is in order to perform the firmness test, the avocado peel has to be damaged, which invalidates the sample for further tests conducted in this work. After damaging the peel the avocado is not fully coated and there is no point to perform other tests like colour change or weight loss.

To achieve better results it would require to have significantly larger number of avocados to eliminate as many errors as possible. Since the avocados were getting subjectively harder by touch over the days for all of the groups including the control. This might suggest that due to poor sample number a lot of errors could have happened. That includes choosing a too ripe avocado day one and less ripe at day three for example or penetrating weaker and riper spot on one sample compared to another.

### ***Colour change of the peel***

The work showed that colour change was affected expressed in  $\Delta E$  during the ripening process by the usage of treatments. The colour change  $\Delta E$  of untreated control group was much bigger than in treated samples. Untreated samples became darker and changed colour more on the colour index and  $\Delta E$  scale over time compared to treated samples. Treated samples ended up greener and lighter at the end of experiment. All treatments had very similar impact in terms of results with shellac 10 % showing the most promising results. Unfortunately, researched studies compared to this one didn't use same exact coating but at least showed comparable behaviour.

Similar results were also reached in researched studies (Choque-Quispe et al. 2022), (Jeong et al. 2003) in which there was proven positive impact of coatings on colour change over time. Study of Choque-Quispe et al. (2022) showed bigger differences in colour changes expressed in  $\Delta E$  values between control and treated avocados when

compared to control and treatment differences in Choque-Quispe et al. (2022). This study Maftoonazad & Ramaswamy (2005) which used methyl-cellulose based showed similar results as this work. At the time of the 6<sup>th</sup> day,  $\Delta E$  averaged around 5 for treatment and 17 for control. Compared to this work which reached values of  $\Delta E$  17.20 for control, 8.26 carnauba wax 10.16 %, 6.47 for shellac 5.5 %, 4.79 for shellac 10 %. This study Maftoonazad & Ramaswamy (2008) which used pectin solution with sorbitol and beeswax showed also similar trend in terms of colour change behaviour. Total colour difference was more impacted by treatments resulting in slower change a darkening over time. Also the lower the storing temperature, the slower was colour change. The bigger the temperature, the bigger was the colour difference between control and treated samples implying that there is also relevance of storing temperature on overall colour change.

## **6. Conclusions**

The use of edible wax treatments on avocados showed promising effects on several aspects of fruit quality, including weight loss, colour change. The results did not verify neither positive nor negative impact of treatments on their firmness due to poor sample count and huge measurement errors. Throughout the experiments, we tested three different wax treatments (shellac 5.5 %, shellac 10 % and carnauba wax 10.16 %) and compared them to the untreated control group. Application methods were evaluated with spraying being the best in terms of even distribution difficulty of application and coating material consumption and to see how they affected the avocados condition during storage.

This work showed that the presence or lack of coating and storage circumstances affected a number of quality changes of avocados that were kept in storage. Fruit ripening might be impeded by coating, which would also affect all other pertinent factors such as colour changes, weight loss, and chemical changes. Expectation of positive impact of coatings on firmness changes wasn't verified due to unprecise results and scarce number of tested samples.

Avocado quality features were negatively impacted by elevated temperatures, particularly over extended periods of time. Fruits become softer and darker as they were stored. Higher temperatures increased negatively impacted and accelerated all weight loss and colour change, hence the presence of coating at lower temperatures provided better storage options. The relevance of coatings increases with higher temperatures, and they are less significant at lower temperatures. Because of this, the type of coating and storage temperature have to be considered when doing any storage research.

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# Appendices

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## Appendix 1: ANOVA for the firmness of avocado.

### ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2,14652618	3	0,71550873	1,04678138	0,42317306	4,06618055
Within Groups	5,4682572	8	0,68353215			
Total	7,61478338	11				

## Appendix 2: Raw data 1. The firmness of avocado

Date	Number of sample	Variety	Firmness (N)	A	$\delta ps$ (Mpa)
1.3.	1	control	301,4	50,266	5,996
1.3.	3	10%	114,1	50,266	2,270
1.3.	6	carnauba wax 10.16 %	141,3	50,266	2,811
1.3.	2	carnauba wax 10.16 %	3,95	50,266	0,079
1.3.	4	control	12,85	50,266	0,256
1.3.	2	control	4,065	50,266	0,081
1.3.	2	5,5,%	25,54	50,266	0,508
1.3.	2	10%	8,69	50,266	0,173
5.3.	4	10%	187,8	50,266	3,736
5.3.	6	control	104,5	50,266	2,079
5.3.	7	carnauba wax 10.16 %	156,2	50,266	3,107
5.3.	1	control	4,515	50,266	0,090
5.3.	3	control	4,7	50,266	0,094
5.3.	2	5,5,%	37,7	50,266	0,750
5.3.	10	10%	4,89	50,266	0,097
5.3.	1	carnauba wax 10.16 %	4,09	50,266	0,081
8.3.	3	5,5,%	7,6	50,266	0,151
8.3.	5	10%	12	50,266	0,239
8.3.	7	carnauba wax 10.16 %	5,4	50,266	0,107
8.3.	3	carnauba wax 10.16 %	11,75	50,266	0,234
8.3.	2	control	13,9	50,266	0,277
8.3.	1	5,5,%	4,25	50,266	0,085
8.3.	4	control	150,4	50,266	2,992
8.3.	2	carnauba wax 10.16 %	5,228	50,266	0,104

### Appendix 3: Raw data 2. The firmness of avocado

Date	Number of sample	Variety	Firmness (N)	A	$\delta ps$ (Mpa)
13.3.	3	control	29,8	50,266	0,593
13.3.	2	carnauba wax 10.16 %	6,938	50,266	0,138
13.3.	1	carnauba wax 10.16 %	5,27	50,266	0,105
13.3.	9	carnauba wax 10.16 %	3,622	50,266	0,072
13.3.	4	5,5,%	5,5	50,266	0,109
13.3.	1	10%	8,8	50,266	0,175
13.3.	5	control	4,04	50,266	0,080
13.3.	8	control	130,2	50,266	2,590
15.3.	6	control	12,1	50,266	0,241
15.3.	7	carnauba wax 10.16 %	223,6	50,266	4,448
15.3.	11	5,5,%	200,5	50,266	3,989
15.3.	8	10%	310,795	50,266	6,183
15.3.	13	carnauba wax 10.16 %	186,8	50,266	3,716
15.3.	8	control	265,5	50,266	5,282
15.3.	1	carnauba wax 10.16 %	135,5	50,266	2,696
15.3.	2	control	147,2	50,266	2,928

## Appendix 4: Colour palette of Hass avocado.



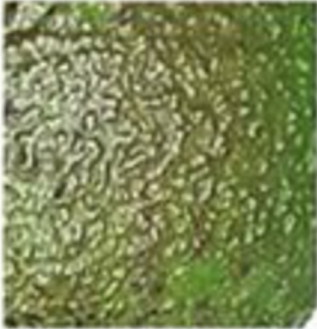

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#8AC377 Hue 105, Saturation 39, Brightness 76

#377D22 Hue 106, Saturation 73, Brightness 49

#7CB362 Hue 101, Saturation 45, Brightness 70

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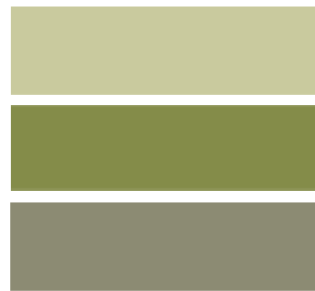

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#94966E Hue 63, Saturation 27, Brightness 59

#5E7736 Hue 83, Saturation 55, Brightness 47

#C1BF96 Hue 57, Saturation 22, Brightness 76

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#C9CA9E Hue 61, Saturation 22, Brightness 79

#848C49 Hue 67, Saturation 48, Brightness 55

#8C8B73 Hue 58, Saturation 18, Brightness 55

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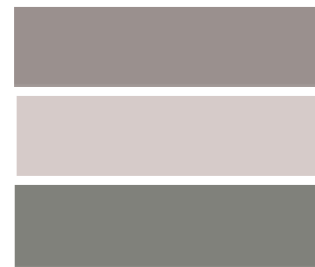

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#312615 Hue 36, Saturation 57, Brightness 19

#908D6E Hue 55, Saturation 24, Brightness 56

#75735E Hue 55, Saturation 20, Brightness 46

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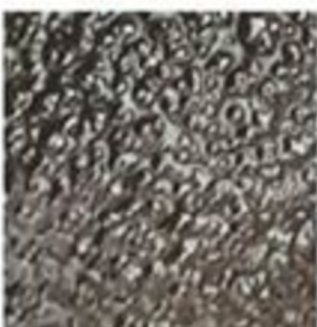

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#9A908E Hue 10, Saturation 8, Brightness 60

#D6CBC9 Hue 9, Saturation 6, Brightness 84

#80817B Hue 70, Saturation 5, Brightness 51

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#3E3935 Hue 27, Saturation 15, Brightness 24

#58534D Hue 33, Saturation 13, Brightness 35

#675D53 Hue 30, Saturation 19, Brightness 40

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