



Treball Final de Grau

Developing of edible coatings for preservation of fresh-cut fruits

Isabel Maria Vidal Carreras

June 2020



UNIVERSITAT DE
BARCELONA

Aquesta obra està subjecta a la llicència de:
Reconeixement–NoComercial–SenseObraDerivada



<http://creativecommons.org/licenses/by-nc-nd/3.0/es/>

*Gràcies als meus avis, als meus amics y
companys que m'han donat suport incondicional.
Gràcies al meu tutor José per implicar-se tant.*

CONTENTS

SUMMARY	i
RESUMEN	iii
1. INTRODUCTION	1
2. OBJECTIVES	3
3. SELECTION OF FRUITS TO PROTECT FROM DETERIORATION	5
4. SELCTION OF DETERIORATION MECHANISM TO AVOID IN SELECTED FRUITS	9
4.1 ETHYLENE PRODUCTION	10
4.1.1 Ethylene production in fresh-cut apples	14
4.1.2 Ethylene production in fresh-cut mango	15
4.2 BROWNING	16
4.3 WATER LOSS	20
4.4 MICROBIAL ACTIVITY	21
5. DEVELOPING OF THE PRODUCTS	23
5.1 PRODUCTS FOR MELON	24
5.1.1 Bibliographic study for melon	24
5.1.2 Selection of ingredients to avoid deterioration of fresh-cut melon.	27
5.1.3 Products for application of the ingredients to preserve melon.	28
5.2 PRODUCTS FOR APPLES	29
5.2.1 Bibliographic study for apples	30
5.2.2 Selection of ingredients to avoid deterioration of fresh-cut apples.	33
5.2.3 Products for application of the ingredients to preserve apples	33
5.3 PRODUCTS FOR MANGOS	35
5.1.1 Bibliographic study for mango	35

5.1.2 Selection of ingredients to avoid deterioration of fresh-cut mango.	38
5.1.3 Products for application of the ingredients to preserve mango.	39
6. BASIC DESIGN OF A MANUFACTURING PLANT FOR THE PRODUCTS DEVELOPED	41
6.1 ESTIMATED ANNUAL PRODUCTION DEVELOPED	41
6.2 GENERAL ASPECTS OF RAW MATERIALS	47
6.3 APPLICATION OF EQUIPMENT	49
7. CONCLUSIONS	51
REFERENCES AND NOTES	55
ACRONYMS	59

SUMMARY

. The consumption of minimally processed fruit has increased in recent years and with it the interest in developing edible coatings that preserve the quality of the fruit. This work has focused on the search for ingredients that improve the quality of melon, apple and cut mango, based on consumer preferences in Spain.

The mechanisms of deterioration that most affect the selected fruits have been studied and it has been determined that cut melon is affected by water loss and microbial infections, cut apple by darkening of the surface and cut mango by ethylene production. The selected ingredients will form solutions in which the selected fruits will be immersed and will fulfil a certain function.

In the case of cut melons, a solution of 2% (w/w) chitosan with 1% (w/w) calcium chloride and 0.1% (w/w) vanillin will be applied to reduce water loss and microbial infection.

Two solutions will be applied to coat the cut apple, the first to incorporate the active ingredients and the second to protect the fruit from the environment. The first solution will have a concentration of 3% (w/w) of citric acid, 4% of ascorbic acid and 1.5% of N-acetyl cysteine (NAC) which will inhibit the darkening of the surface. The second solution will be composed of the combination of 2% (w/w) alginate with 1.5% (w/w) glycerol.

The cut handle will also be immersed in two solutions. The first solution is composed of 0.5% (w/w) melatonin and 0.05% (w/w) 1-Methylcyclopropene (1-MCP) with the function of inhibiting ethylene production, and the second solution is composed of the combination of 2% (w/w) carboxymethyl cellulose (CMC) and 0.5% (w/w) chitosan.

Finally, by means of an analysis of the consumption of the selected cut fruits in the metropolitan area of Barcelona, the quantities of solutions (products) to be produced in a year have been established and the necessary equipment to produce them has been chosen.

Keywords: Minimally processed food, edible coating, fruit quality, fresh-cut fruit, shelf life, antimicrobial, browning, ethylene biosynthesis, water loss.

RESUMEN

El consumo de fruta mínimamente procesada ha ido aumentando en los últimos años y con ello el interés en desarrollar recubrimientos comestibles que conserven la calidad de la fruta. Este trabajo se ha enfocado en la búsqueda de los ingredientes que mejoren la calidad del melón, la manzana y el mango cortado, en base a las preferencias de los consumidores en España.

Se han estudiado los mecanismos de deterioro que más afectan a las frutas escogidas y se ha determinado que el melón cortado se ve afectado por la pérdida de agua y las infecciones microbianas, la manzana cortada por el oscurecimiento de la superficie y el mango cortado por la producción de etileno. Los ingredientes seleccionados van a formar soluciones en las que se van a sumergir las frutas escogidas y van a cumplir con una función determinada.

En el caso del melón cortado se va a aplicar una solución de 2% (w/w) de quitosano con 1% (w/w) de cloruro cálcico y 0.1% (w/w) de vainillina para disminuir la pérdida de agua y la infección microbiana.

Para recubrir la manzana cortada se van a aplicar dos soluciones, la primera para incorporar los ingredientes activos y la segunda para proteger la fruta del medio. La primera solución va a tener una concentración de 3% (w/w) de ácido cítrico, 4% de ácido ascórbico y 1.5% de N-acetil cisteína (NAC) que inhibirán el oscurecimiento de la superficie. La segunda solución estará compuesta por la combinación de 2% (w/w) de alginato con 1.5% (w/w) de glicerol.

El mango cortado estará sumergido también en dos soluciones. La primera estará compuesta por 0.5% (w/w) de melatonina y 0.05% (w/w) de 1-Metilciclopropeno (1-MCP) con la función de inhibir la producción de etileno, y la segunda solución está compuesta por la combinación de 2% (w/w) de carboximetilcelulosa (CMC) y 0.5% (w/w) de quitosano.

Finalmente, mediante un análisis del consumo de las frutas cortadas escogidas en el área metropolitana de Barcelona se han establecido las cantidades de soluciones (productos) a producir en un año y se han escogido los equipos necesarios para producirlas.

Palabras clave: mínimamente procesados, recubrimiento comestible, calidad de la fruta, fruta recién cortada, vida útil, antimicrobianos, oscurecimiento, biosíntesis de etileno, pérdida de agua

1. INTRODUCTION

The demand for healthy products has been increasing over the years along with the feeling of preserving the environment by consuming preferably biodegradable products. In this context, the consumption of fresh-cut fruits fits with the consumer's preferences. For these reasons, interest in the research activity in edible packaging has been increasing today.(Hassan et al., 2018)

The economic importance of fresh-cut fruit industry is increasing as a result of the growing consumption of ready-to-use food. Sales of these products have been facilitated by product development, processing innovation and new packaging designs.

On a commercial scale, minimally processed fruits are successfully sold in countries with developed technologies and markets. In developing countries, the market for minimally processed fruit is only a nascent stage. However, considering the growing demand for these new products, a considerable increase in this market segment is expected.(Yousuf, Qadri, y Srivastava, 2018)

The International Fresh Produce Association (IFPA) defines fresh-cut products as fruits that have been peeled or cut that is packaged to provide consumers with high nutrition and flavor while preserving its properties. The difference between intact fruit and fresh-cut fruit lies in the physiology and conditions for handling and storage. The processing of fresh-cut fruits modifies the tissues of the fruit causing a decrease in the shelf life of the product. It is for this reason that these pre-cut products need very special attention due to microbiological factors affecting safety. Knowledge of the nature of the fresh-cut fruit in terms of handling before and after harvest, processing, packaging and finally storage is necessary to ensure integrity and nutritional value.(Lamikanra, 2002)

Extending the shelf life of fresh-cut fruit is very important as it can represent an economic benefit for food companies. The application of edible coatings on fruits increases the shelf life of fresh-cut fruits.

The mechanisms of deterioration to which fresh-cut fruits are exposed is one of the areas on which research is focused to prolong the shelf life of fruits. Water loss, softening, surface browning, ethylene production, microbial infection and respiratory tract prolongation are some of the damages caused by minimal processing of the fruit.

The application of edible films and coatings is one of the solutions. Edible films and coatings act as a barrier to the environment surrounding the surface of the fruit, thereby reducing water loss, oxidation reaction rates and respiration rates. These coatings are often used as carriers of antimicrobial and antibrowning components because in most fresh or processed foods, microbial infection occurs in the outer layer of the food. Moreover, packaging plays also a key role in controlling microbial growth.

Antimicrobial films and coatings have been developed to reduce, delay or inhibit microbial growth on food surfaces. The direct application of antimicrobial agents has been shown to be less effective than the application of edible antimicrobial films or coatings. (Hassan et al. 2018)

This work will focus on the application of products to preserve the selected fresh-cut fruits from the most important mechanisms of deterioration to which they are exposed.

2. OBJECTIVES

The objective of this work is the development of products that applied to freshly cut fruits prepared for consumption retard their deterioration facilitating their commercialization.

The completion of this work involves the following tasks:

- Selection of fruits to protect from deterioration. The market will be analyzed, and a bibliographic search will be carried out to select those fruits that are most frequently sold prepared for immediate consumption.

- Selection of deterioration mechanisms to avoid in selected fruits. This selection will be made through a bibliographic study and will be determinant in selection of active ingredients of the product.

- Developing of the products to be applied on freshly cut fruits. Developing of the products will consists fundamentally in selecting the active ingredients needed for avoiding the deterioration of the freshly cut fruits and establishing the concentration of the aqueous solutions to be applied on the fruits.

- Basic design of a manufacturing plant for the products developed. Annual production of the developed products will be estimated and raw materials for manufacturing them will be selected. Batch sizes needed for production will be established and, for them, equipment will be selected.

3. SELECTION OF FRUITS TO PROTECT FROM DETERIORATION.

In this section, fruits to be protected by edible coatings are selected. A market study has been made and a bibliographic search has been carried out to select the fruits that are most frequently marketed prepared for immediate consumption.

At the time of consume fresh fruit, it should be considered if it is a season's fruit or not. It is clear that today you can buy apples and oranges in summer and melon and watermelon in winter, even if it is not the harvest season.

Personally, I would like to point out in the consumer's preferences when it comes to buying fruit. It is preferable to eat seasonal fruit rather than all kinds of fruit at any time of year for various reasons, for example, seasonal fruits are more natural than fruit grown in greenhouses. You can also think about the planet and stop eating fruit out of season could mean a significant reduction in resources and energy used for its cultivation and subsequent marketing.

Having said that, we must consider the country we are living in, as the same fruit is not consumed in Asia as in Europe or America. For this reason, this work will basically focus on the fruit that is marketed in Europe, specifically in Spain.

The most common fresh cut fruits you can find in supermarkets, in a fruit shop or in markets are apple, melon, pineapple, watermelon and orange. However, there is a wide variety of fresh and cut fruits available at the market.

On the one hand, according to what has been observed, there are several markets that offer the sale of fresh cut fruit, as well as the Boqueria market in Barcelona. This market is a food market that offers high quality fresh products and offers you the possibility to find products from all over the world. In this market you can find several fruit stands that offer a wide variety of fruit, and in relation to fresh-cut fruit, you can see the commercialization of fruits such as: Pineapple,

Orange, Watermelon, blueberries, blackberries, kiwi, papaya, peach, coconut, melon, apple, grapes, cherries and strawberries.



Figure 1: Fruit stand in the Boqueria Market, Barcelona.

We can also find these ready-to-eat products in supermarkets like Carrefour, where you can buy freshly cut fruit such as, pineapple, kiwi, melon, watermelon, papaya, strawberries and coconut among others.



Figure 2: Sale of fresh-cut fruit in Carrefour.

On the other hand, according to the bibliographical sources consulted, several researches on edible coatings and their effects have been found.

Edible coatings have demonstrated favorable results in preserving the physiological effect and physicochemical properties of fruits such as pome fruits, citrus fruits, stone fruits, tropical and exotic fruits, berries, melon and tomatoes.

In pome fruits, the storage life of the apple and pear is much reduced if not properly cared for. The application of an edible coating improves the quality and storage life of these fruits. (Maringgal et al. 2020)

Citrus fruits are one of the world's major crops, with a total production of 18.9 million tons in 2017. The main problem for these fruits is post-harvest losses. Several studies have focused on the research of methods to preserve the quality of the fruit.

Stone fruits are characteristic for their nutritional and organoleptic value, as well as their antioxidant activity. In the stone fruits market, we can find nectarines, cherries, plums, peaches and mangos. These fruits deteriorate easily after harvesting and their quality decreases rapidly over time. Storage at low temperatures is not enough to preserve the quality of these fruits. For this reason, various studies have been carried out on the effect of applying edible coatings to stone fruit stored at low temperatures. (Maringgal et al. 2020)

Interest in consuming new flavors has led society to consume exotic and tropical fruits. As with pome fruits, the deterioration of these fruits takes place in the storage stage due to changes in the physiology of these fruits. Edible coatings have proven to be a promising solution for the preservation and quality conservation of fresh-cut tropical and exotic fruits.

These edible coatings have been tested on pitayas, soursop, pineapples, papayas, bananas, guavas and also mangos. Once coated, the fruits have demonstrated significant changes in physical-chemical properties during storage.

Avocados suffer a great loss of water due to the loss of humidity caused by transpiration. In recent years the use of edible coatings for avocados has increased. (Maringgal et al. 2020)

Blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry are berry fruits that are used in the human diet because of their high content of antioxidants.

Several researchers have evaluated the effect of edible coatings on berry fruits. One of them concluded that a strong edible coating, which remains intact during the vibration to which these fruits are exposed during transport, is vital. (Dhital et al. 2017)

In the fresh-cut fruit sector, the most consumed fruit is melon, representing almost 22% of the market. Fresh-cut melon easily deteriorates due to softening, flavor degradation, weight loss, juice leakage and microbial spoilage. To avoid this deterioration, it is necessary to apply edible coatings.

The tomato is a fruit that progressively deteriorates after harvest and has a relatively short shelf life. This fruit is exposed to diseases caused by pathogenic fungi, therefore, to reduce these symptoms, different edible layers are applied. (Maringgal et al. 2020)

Although edible coatings can be applied to many fruits, this work will focus on the main fruits most consumed in the markets and are well received by consumers, which are apples and melon. According to the interest in tropical fruits, mango has turned out to be one of the fruits that has better adapted to the market. Thus, the fresh-cut fruits on which this work will focus are melon, apple and mango.

These fruits have been chosen because they are representative of the group to which they belong. The apple is a type of pome fruit as well as the pear, so they have similar properties and deterioration mechanisms. Edible coatings applied to apples may be applied to pears. In the same way with mangoes, they are representative of all stone fruits and some tropical fruits like papaya. The results obtained for mango could be applied to peaches, due to their similarity.

Finally, since melon is one of the most consumed fresh-cut fruits in the market, researches on edible coatings on the surface of melon are getting relevance, because its application could mean an increase in the economic benefit for processed food industry.

4. SELECTION OF DETERIORATION MECHANISMS TO AVOID IN SELECTED FRUITS.

There are many factors involved in the epidemiology of product-related diseases. The risks to which fresh-cut products are exposed can be divided into two categories.

In one category, there are the factors and conditions that contaminate fresh products during harvest. Agronomic practices, sanitary control and/or poor personal hygiene are one of the causes of these risk factors.

In the other category, there are the risk factors related to the microbiological risk to food during the cutting operation in the cutting plant. The main microbiological risks come from the fact that there is no killing step (for example cooking of the food) in the elimination of possible human pathogens, the increase of storage days (10-14 days) may be sufficient for the growth of pathogens and a modified atmosphere suppresses the growth of spoilage organisms. (Lamikanra, 2002)

As mentioned above, processed products such as freshly cut fruit are exposed to deterioration mechanisms that lead to poorer fruit quality and therefore reduced shelf life. Overall, the main deterioration mechanisms to which most fruits are exposed are loss of water, weight loss, discoloration, off-flavor, microbial infection, surface browning, loss of firmness, and early ripening.

The fruits that have been chosen suffer from such deterioration. Apple, mango and melon suffer from loss of firmness, loss of water, microbial infections, an increase in the rate of respiration and an increase in ethylene production.

Fresh-cut apples have a short shelf-life because of their rapidly deterioration, water loss and the main problem for its quality preservation, cut-surface browning. Browning is the most visually evident mechanism of deterioration that affects to fresh-cut apples. For this reason, the appearance of freshly cut apple is damaged and if there is no edible coating applied to prevent

its degradation there would be great losses in the sale of this fruit. (Koushesh Saba y Sogvar 2016). Also, fresh-cut apples increase their ethylene production and respiration rate due to cuts in their processing and consequently damage their quality.

Mango is a commercial fruit growing produced in the tropical regions in the world. It is highly consumed for its delicious taste, pleasantness, and rich source of nutrients and chemicals (Vitamin C and Vitamin E). Mango fruit contains different antioxidant and nutritional properties. However, the quality and shelf life of fresh-cut mango is limited because of a high respiration rate, ethylene production and excessive ripening. Important issues that fresh-cut mango suffers is browning on its surface and softening. (Ntsoane et al. 2019)

As previously mentioned, melon is one of the fruits that is produced most as a whole fruit and as fresh cut fruit, covering 22% of the production of this sector. The interest in this fruit is due to its sweet taste, its nutritional value and its vitamin C content among other reasons. However, freshly cut melon deteriorates easily and quickly, being the loss of water the main cause of its deterioration. This loss of water leads to a high-water content in its surface, facilitating microbial invasion, which is the second reason why the quality of this fruit is damaged. (Ortiz-Duarte et al. 2019)

The mechanisms of deterioration on which this work is going to focus will be those that mainly affect the chosen fruits. Thus, the deterioration mechanism that most affects freshly cut apples is browning on their surface. In the case of freshly cut mangoes, the objective will be the respiration rate and the production of ethylene, and finally, freshly cut melons are mainly affected by the loss of water followed by microbial infection.

4.1 Ethylene Production

Ethylene (C₂H₄) is an organic chemical compound widely used in the chemical industry. It is mainly used to produce polyethylene. However, ethylene is also a gaseous natural plant hormone and is used in agriculture to force the ripening of fruits.

Ethylene is produced in plant tissues and consists of two biosynthesis systems. In system-1, ethylene acts as an auto-inhibitor during vegetative growth. In system-1, ethylene production is low and contributes to the basal levels of the plant and non-climacteric fruit. In system-2,

ethylene acts as an autocatalytic agent and takes place during climacteric ripening. In this system there is an increase in ethylene production.(Cortellino et al. 2015)

To understand the role of ethylene production in fruits, it is necessary to know the concept of climacteric and non-climacteric fruit. Initially, these two differed in the presence or absence of an increase in the respiratory rate of the fruit during ripening, although to date, this difference lies more in the presence or absence of evidence of autocatalytic ethylene production.

Therefore, a climacteric fruit is one that can produce a large quantity of ethylene, promoting its ripening, and a non-climacteric fruit is one that can only produce a small basal amount of ethylene during ripening. (Cortellino et al. 2015)

Climacteric fruits can continue ripening once harvested, while non-climacteric fruits stop ripening once the fruit is separated from the plant. Climacteric fruits are apples, pears, peaches, nectarines, kiwis, papayas, bananas, figs, mangos, blueberries, tomatoes and melons among other fruits. Non-climacteric fruits include nuts, grapes, cherries, raspberries and citrus fruits.

Ethylene is biosynthesized from the amino acid L-methionine, which together with the enzyme methionine S-adenosyl transferase produces S-Adenosyl Methionine (SAM), forming an intermediate, 1-aminocyclopropyl-1-carboxylic acid (ACC), by the action of the enzyme aminocyclopropane-1-carboxylic acid synthase (ACS). Subsequently, this intermediate is oxidized by the action of oxygen and the enzyme aminocyclopropanecarboxylate oxidase (ACO), which uses ascorbic acid as a cofactor, giving as products ethylene, hydrocyanic acid and carbon dioxide.(Martin-Beloso and Fortuny, 2011)

The main ethylene regulatory enzymes are the enzyme aminocyclopropane-1-carboxylic acid synthase (ACS) and the enzyme aminocyclopropanecarboxylate oxidase (ACO). Wound response ethylene increases the activity of ACS, and with it the production of ethylene.

The Figure 3 represents the Yang cycle in which ethylene biosynthesis occurs in one of its stages.(Martin-Beloso and Fortuny, 2011)

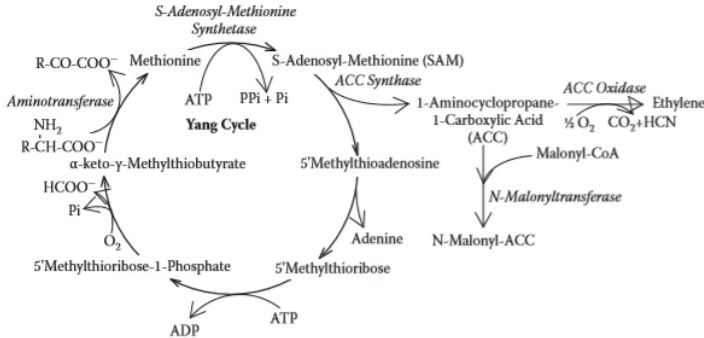


Figure 3: Ethylene synthesis pathway and Yang cycle. (Martin-Bellosso and Fortuny, 2011).

The synthesis of ethylene requires O_2 , thus, an increase in the respiration rate is related to the increase of ethylene production. The Figure 4 shows the evolution of the respiration rate over time for samples of bananas and kiwis from a study according to (Lamikanra, 2002). This study also compares the respiration rate of whole fruit versus fresh-cut fruit. In the graph the respiration rate of fresh-cut fruit is higher than the whole piece of fruit.

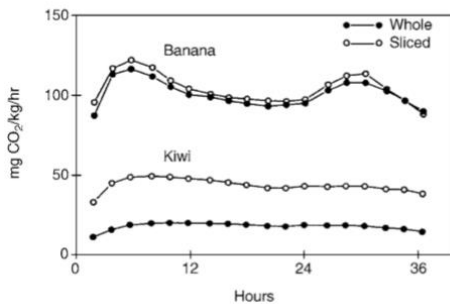


Figure 4: Respiration rates of sliced and whole banana and kiwifruit held at 20°C. (Lamikanra, 2002).

In order to have a biological effect, ethylene must bind to a receptor. If the receptor is blocked, then ethylene will have no effect. CO_2 , 1-MCP, silver ions are inhibitors that can block the ethylene receptor: although at present only 1-MCP is approved for use on fresh-cut fruits.

It has been shown that ethylene receptors can act as biological clocks that regulate the start of fruit ripening. These receptors are degraded by the presence of ethylene. For this reason, since more ethylene is produced in immature fruits, there are more connections with receptors. Once the binding is done, the receptors are degraded and cause an earlier ripening of the fruit. (Martin-Belloso and Fortuny, 2011)

If the production of ethylene wants to be inhibited or reduced, one of the objectives is to inhibit or reduce the enzyme responsible for its production, the enzyme 1-aminocyclopropyl-1-carboxylic acid synthase (ACS) together with aminocyclopropanecarboxylate oxidase (ACO).

For fresh-cut fruit, ethylene causes discoloration, softening, ripening and senescence, reducing the shelf life of the fruit. Inhibition or reduction of ethylene synthesis or action can prolong the shelf life of fresh-cut fruit. In this way, many techniques have emerged to reduce the production and effects of ethylene.

It has been shown that at temperatures above 35°C ethylene synthesis is disrupted. At temperatures below 2.5°C ethylene synthesis is slowed down due to the reduction in ACO activity. The Figure 5 shows the increase in ethylene production over time of freshly cut tomatoes and whole tomatoes as a function of temperature. It can be seen that at lower temperatures, ethylene production decreases, while at higher temperatures ethylene production increases.

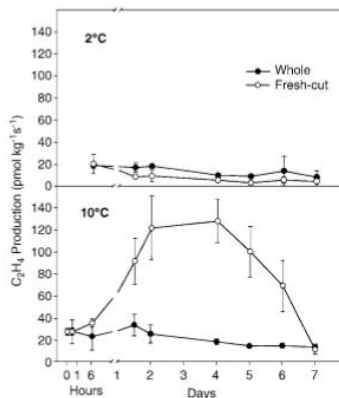


Figure 5: Ethylene production of whole and fresh cut 'Durinta' tomato. (Martin-Belloso and Fortuny., 2011).

At low O₂ and high CO₂ levels, ethylene production is inhibited.

It has also been shown that ethanol vapor inhibits ethylene production in apples and cut mangoes, although it can cause bad taste in the fruit. In the case of mangoes, ethanol production was reduced and the microbial population on the surface of the slice was also reduced.

1-MCP also inhibited the production of ethylene in fresh-cut apples in conjunction with the reduction in browning of “Baeburn” apples. This also occurred in the case of “Galia” melon, where the application of 1-MCP resulted in the reduction of softening. (Martin-Belloso and Fortuny., 2011)

4.1.1 Ethylene production in fresh-cut apples

Plant stress is a state in which the plant grows in non-ideal conditions. The stress of a plant can be caused by any unfavorable condition that affects the metabolism, physiology, physicochemical properties, reproduction...Peeling and cutting are measures that cause stress on the plant or fruit. In fruits, this stress can cause increased ethylene production and respiratory activity. In climacteric fruits the effect of the wound is remarkable since the production of ethylene promotes ripening and softening. As mentioned above, ethylene produces harmful effects on the quality of the fruit; therefore, the main objective is to decrease its production in order to extend the fruit's shelf life. Senescence is the aging process of plants and is another cause of stress suffered by plants

As the apple is a climacteric fruit, it is more sensitive to ethylene. It has been seen that atmospheres with low O₂ and high CO₂ levels can reduce ethylene production and respiration rate, however, they did not stop senescence completely and were not enough to prolong the storage time of the fresh fruit. (Cortellino et al. 2015)

Knowing that oxygen has an important role in the biosynthesis of ethylene, helping the conversion of ACC to ethylene, it has been proven that under anaerobic or low O₂ conditions, it is capable of inhibiting ethylene production. At low concentrations, CO₂ acts as a cofactor of the enzyme ACO, and therefore contributes to the biosynthesis of ethylene. However, CO₂ at high concentrations may inhibit the synthesis of the enzyme ACO

A study was conducted on fresh cut "Golden Delicious" apples to completely inhibit ethylene production. It was concluded that ethylene production in fresh-cut Golden Delicious apples is

completely inhibited by the presence of N_2 in storage. However, for those apples that were stored in the presence of air, ethylene production increased significantly after processing and packaging, with ethylene concentrations up to 100 times higher than slices of apples packed with N_2 , for 10 days and storage.

Other tests were also carried out with N_2O . Nitrous oxide was shown to inhibit ethylene production and action. In preclimacteric treated fruits, N_2O prolonged the latency period and reduced the ethylene production rate. In fruits treated in the climacteric stage, it considerably inhibited the autocatalytic evolution of ethylene.

4.1.2 Ethylene production in fresh-cut mango

As the mango is a climacteric fruit, the production of ethylene would advance its state of maturity. Mango ripening is strongly related to the amount of ethylene in the fruit. As a consequence of the presence of ethylene, the activity of the enzymes related to ripening begins, initiating the synthesis of carotenoids and converting starch into sugar. Another consequence of ripening is the change in the cell wall. Disassembly, solubilization and finally desertification of the polysaccharides that form the cell wall take place. It has been shown that the modulation of ethylene biosynthesis contributes to the softening process of this fruit by modifying the cell wall. (Liu et al. 2020)

The study by (Plotto et al. 2006) applied ethanol vapor to the whole mango to reduce ethylene production in the subsequent mango pieces. The ethanol vapor was found to be effective in reducing ethylene production, however, it altered the flavor of the mango by reducing its taste. If the ethylene vapor concentration is low enough, the negative effects on flavor are reduced, however, it is discarded as an efficient inhibitor of ethylene production, although it has been shown to be effective in inhibiting microbial infection at the surface. As stated above, ethylene production is not the main problem of fresh-cut mangoes, however some ethylene inhibitors such as 1-MCP also reduced more serious aspects affecting fresh-cut mangoes such as browning and softening (Vilas-Boas and Kader, 2007).

In the industry of minimally processed fruit, wounds have general consequences on all fresh cut fruits. The production of ethylene is one of these properties, as it is more associated with the cut and not as a characteristic parameter of each fruit. It is true that each fruit has its own way of responding to ethylene production, but the difference lies more in the type of fruit. Climacteric fruits are, as mentioned above, more susceptible to ethylene, since they can continue to ripen in the presence of ethylene. As apple, melon and mango are climacteric fruits, ethylene production affects them in a similar way so the products used to minimize ethylene production can be applied to all of them.

In this way, the objective will be a general minimization in the three fruits chosen from the ethylene production. In the case of the mango, as the main affected to the production of ethylene in front of the other selected fruits, it is going to look for a product that applied on the cut fruit, delays the ripening and its effects as the production of ethylene and decreases softening. As mentioned, ethylene production is related to ACS and ACO enzyme activity and to the presence of oxygen. Thus, a product that decreases or inhibits the action of ACS and ACO together with a MAP that has low O₂ levels will be needed.

4.2 **Browning**

One of the factors that have influence on the commercialization of processed food is its appearance. For fruits that suffer from browning, as well as apples, it is a problem. The decomposition of the membranes inside the fruit tissue cells and the mixing of polyphenol with polyphenol oxidase (PPO) cause oxidative browning.

PPO is an enzyme found in plants. It is commonly found in cytoplasmic organelles such as chloroplasts, while its phenolic substrates are mainly found in vacuoles, but also in the apoplast compartment and the cell wall. PPO catalyzes two reactions in the presence of oxygen:

A) The hydroxylation of monophenols to diphenols. This reaction is slow and results in colorless products.

B) The oxidation of diphenols to quinones. The reaction is rapid and results in colored products.

The Figure 6 shows these two reactions that causes oxidative browning. (Cortellino et al. 2015)

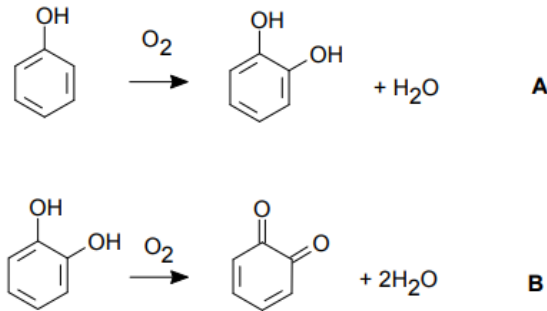


Figure 6: A) Oxidation of monophenol to diphenol and B) Oxidation of diphenol to a quinone. (Cortellino et al., 2015).

The reactions that take place after the quinones cause an accumulation of melanin, which is the brown pigment associated with the darkening of the fruit and the development of compounds depending on the specific structure of the polyphenolic substrate.(Cortellino et al. 2015)

The rate of enzymatic browning in fruits is determined by factors such as the concentration of active PPO, the concentration of phenolic compounds, pH, temperature and the amount of oxygen present.

An enzymatic browning reaction requires essential elements like the presence of active PPO, oxygen and phenolic substrates. Prevention of browning is possible, through elimination of substrates or inhibition of enzymes.

PPO needs oxygen to initiate the browning reaction at the site of injury. Therefore, if no oxygen is present, degradation of the fruit in this respect can be prevented. MAPs with low levels of O₂ and high levels of CO₂ help to avoid browning in fresh-cut fruit. However, these methods are not enough to effectively inhibit browning in fresh-cut fruits such as apples,

bananas, pears... due to their phenolic content. For this reason, the MAP system must be combined with antioxidants to delay browning. (Cortellino et al., 2015)

Another important factor is the time between slicing and washing. The Figure 7 reflects differences in absorbance at 440 nm of fresh-cut apples from two different cultivars as a function of time after slicing. Also, in the Figure 7 there are two ways for cutting apples. On one hand, slices cut in air and 30 seconds later were dipped into water. On the other hand, slices cut in water. (Lamikanra, 2002)

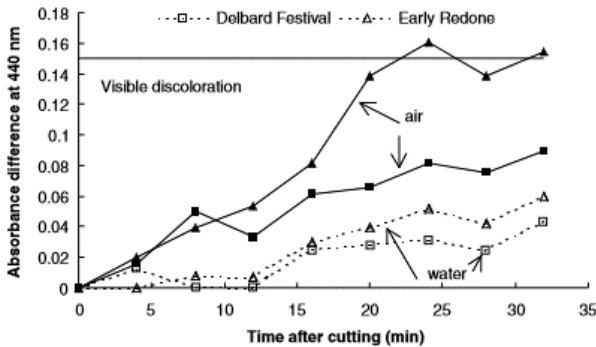


Figure 7: Absorbance of apple slices cut in air and cut under water. (Lamikanra., 2002).

While the browning of the slices cut in the air presented a peak in absorbance, the slices cut in the water did not present that peak. In addition, the apple slices cut in water did not become brown for a few hours stored at 8°C. The prevention of browning in slices cut in water is because of to the instantaneous washing of the cell sap released by the cut. In the apple slices cut in the air and quickly immersed in water, the exudate was immediately spread on the inner layers of the tissues before washing. As the interval between cutting and washing becomes longer, the slices become browner during storage. (Lamikanra, 2002)

To quantify the degree of browning it is used the CIE system. The Commission Internationale de l'Eclairage (CIE) established the parameters L^* a^* b^* as indicators. L^* indicates lightness, a^*

is the red/green coordinate and b^* is the yellow/blue coordinate. ΔL^* , Δa^* and Δb^* may be positive or negative numbers.

ΔL^* is the difference between L^* sample and L^* standard. ΔL^* is the difference in lightness and darkness, positive number means more light and negative number means more dark.

Δa^* is the difference between a^* sample and a^* standard. Δa^* is the difference between red and green, positive number means red and negative number means green.

Δb^* is the difference between b^* sample and b^* standard. Δb^* is the difference between yellow and blue, positive number means yellow and negative number means blue.

The color difference is given by ΔE^* :

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

The higher the ΔE^* value, the more color difference exists.

According to a study, (Cortellino et al., 2015) it was shown that N_2 packaging combined with the use of O_2 impermeable packaging offered better results compared to fruit packed only with a MAP system with low O_2 levels and high CO_2 levels. It was also found that packs with low O_2 , low CO_2 and high N_2 were more efficient than packs with only 100% N_2 .

An experiment with N_2O in the MAP system (90% N_2O , 5% CO_2 , 5% O_2) proved to be efficient in limiting color change when combined with immersion of the cut fruit in a bath of ascorbic and citric acid. (Lamikanra, 2002)

In the cultivation of different types of apples, it has been found that phenolic compounds and polyphenol oxidase PPO activity showed that browning correlated with the amount of degraded phenolics. It was also observed that cellular changes in PPO were produced during controlled atmosphere storage.

In fresh-cut mangoes, the combination of anti-browning agents is more related to maintaining mango quality than the role of modified atmosphere packaging. It has been shown that a combination of anti-browning agents is more effective than individual applications. (Lamikanra, 2002)

In summary, browning is the discoloration suffered by the fruit as a result of the action of the enzyme polyphenol oxidase PPO. This discoloration generally affects all selected cut fruits but the one that suffers it most quickly and its effect is most visible is the fresh-cut apple.

Measures can be taken in the modified atmosphere surrounding the cut fruit. As with ethylene production, a MAP with low O₂ reduces surface darkening, although it is not enough. To reduce or inhibit browning it is necessary to apply a product that is capable of inhibiting PPO activity. One can also consider the application of a product that inhibits the post-PPO reaction by attacking the quinones to prevent their subsequent conversion to dark pigments. The use of O₂ impermeable edible packaging or film is required.

4.3 Water Loss

The outer skins and peels are responsible for protecting the internal fruit tissue from microbial invasion. One of the most important factors that determines water loss in fruits is the resistance of the periderm to transpiration. However, when a fruit is cut or peeled, this protective barrier is eluded, allowing internal juices to filter through and reach the surface of the slice, accelerating microbiological growth. Two issues are important for water loss: reducing tissue volume, in other words, increasing the surface/volume ratio, and removing the protective tissues of the periderm. Both issues cause an increase in water loss.

After harvest, products only use internal moisture; water lost through transpiration cannot be replaced. Small changes in water content can have a large impact on product quality, causing losses that can occur within a few hours. Water losses of 5% in the apple prevent it from being marketed. (Lamikanra, 2002)

Water loss cause weight loss, shriveled appearance, a decrease in nutritional value, loss of aroma, loss of flavor and an increase in chilling injury.

To prevent water loss, different measures can be taken: the use of appropriate packaging materials, the use of sharp knives to cut the fruit. Water loss occurs through the skin or abscission cuts, due to differences in relative humidity between the internal and surrounding atmosphere. For this reason, fresh-cut products should be stored under high relative humidity

environments, as a complement to optimum storage temperature. Thus, the temperature of storage should be as low as possible without injuring the product and relative humidity should be as high as possible (more than 80%) (Martin-Belloso and Fortuny., 2011)

The handling of the fruit, the peeling process, the wounds caused by cutting and storage of the fruit over time, as mentioned above, cause several deteriorations in the melon that impoverish its quality. Melon is one of the most susceptible fruits to water loss due to its high-water content and high relative humidity.

In addition, the loss of firmness of the fruit is another of the deteriorations related to water loss, reducing the useful life of freshly cut melon. The destruction of surface cells and the stress of tissue injuries occur during melon processing operations causing the release of enzymes such as peptic enzymes that cause changes in firmness.

The reactions that occur on the surface of the melon cause sensory damage such as loss of firmness, discoloration, negative changes in the taste of the melon. Therefore, storage causes a rapid deterioration of the melon if no product is applied to avoid or delay such effects.

For this reason, applying an edible coating with a waterproof layer on the surface of the freshly cut melon would help to contain the water and thus increase its shelf life. In this way, it is necessary to have a product that is impermeable to the atmosphere that surrounds the fruit in order to reduce the loss of water, the reduction of moisture contained in the melon and finally maintain its firmness.(Raybaudimassilia, Mosquedamelgar and Martin-Belloso 2008)

4.4 Microbial Activity

It has become necessary to prevent outbreaks of food-borne diseases associated with fresh-cut products because they are usually consumed uncooked without any decontamination treatment.

For different reasons minimally processed fruits are more susceptible to microbial infection. The principal reasons are the operations of peeling and cutting. These operations remove the barrier that protect the fruit from the exterior bacteria. Other reasons that increase the susceptibility of fresh-cut fruits to be attacked by pathogenic microorganism are contamination

during the processing phases in the plant and the growth and fermentation of sugar by yeasts from damaged fruit tissues.(Yousuf, Qadri and Srivastava, 2018)

The bacteria that frequently attack pre-cut fruits are *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* spp. (Kang and Song, 2019)

It is vitally important to be aware of the dangers of eating bad fruit. For years, there have been several outbreaks of diseases caused by fruits in poor condition, causing hundreds and thousands of infections and even several deaths. Outbreaks of diseases transmitted by freshly cut fruit are low, however, the severity can be high. For example, in May 2011, Germany suffered an outbreak of Shigatoxin disease caused by *E. coli* bacteria (STEC) serotype O104:H4. The outbreak caused 3785 infections and 45 deaths.(Goodburn and Wallace 2013)

The bacterium *Listeria monocytogenes* is the cause of listeriosis and is one of the pathogens that causes very violent food infections with a mortality rate of 20 to 30%. It is capable of proliferating between 1°C and 45°C. *Listeria monocytogenes* is one of the most dangerous food-borne toxicants. *Salmonella* spp was the cause of classical swine fever between 1855 and 1884, among other diseases.

A series of Good Agricultural Practices (GAP) have been adopted. Codex Alimentarius, Code of Good Practice for Fresh Fruits and Vegetables (2010) is an internationally accepted code that sets out requirements for the safe production of fresh produce. There is also a safety guide for the food production industry where you can find information from the Refrigerated Foods Association and risks of pathogens in ready-to-eat fruits among others.(Goodburn and Wallace, 2013).

To count microbial populations in freshly cut fruits, studies are conducted on the development of populations of mesophilic bacteria, psychotropic bacteria, yeasts and molds.

As it has been said, microbial infection affects all cut fruits, but this work is going to focus on the microbial infection suffered by the melon, since its main problem is the loss of water, the surface of the melon pieces show a high content of humidity, providing a medium that encourages the growth of bacteria and microbial infection. Therefore, an edible coating that provides impermeability to the environment surrounding the freshly cut melon and the application of an antibacterial carrier product will be necessary.

5. DEVELOPING OF THE PRODUCTS.

Once the mechanisms of deterioration that mainly affect the chosen fruits have been explained, it is necessary to specify the functions that the products being looked for will have to satisfy. In the case of mango we are going to look for a product that improves the general aspects prioritizing the reduction of ethylene, but also that is capable of decreasing the rate of respiration, and improving general aspects such as prevention of microbial infections, softening, loss of water, maintain firmness and reduction of browning on the surface. In the case of fresh-cut apples, it will be necessary to apply a product with anti-browning properties. Finally, for fresh-cut melons, it will be necessary to apply a product that is impermeable to water and carries antimicrobial agents.

The nature of the ingredients to be chosen for the edible coating has a very important role, (Martin-Belloso and Fortuny, 2011) .

Edible coatings are normally made from ingredients made from polysaccharides as well as starch and its derivatives such as cellulose, alginates, peptics and gellans. It is common to apply lipids in the form of an emulsion or in the form of a solution with the coating, to provide a waterproof barrier to prevent events such as water loss (Yang y Paulson 2000), (Garcia, Martino and Zaritzky, 2000)

In this way, an edible coating can be made by combining a polysaccharide base as the main compound that characterizes the coating, providing a structural support matrix that acts as a selective barrier to gases such as oxygen and carbon dioxide together with a lipid compound that acts as a barrier to water vapor with the addition of antibrowning and antimicrobial agents (Vargas et al., 2009)

Therefore, the selection of ingredients to be used in coatings is entirely related to the function to be performed by the edible coating, the nature of the ingredients and the method of application (Debeaufort, Quezada-Gallo, and Voilley, 1998)

Since the deterioration mechanisms to be avoided are different for each fruit, the ingredients will be chosen for each one of them. This selection will be made according to a bibliographic study that will analyze several studies with the aim of keeping the ingredient that presents the best results to fight each mechanism of deterioration depending on the fruit to be treated.

5.1 Products for melon

As mentioned, fresh-cut melon requires the application of a product that is impermeable to reduce water loss and loss of relative humidity. In addition, whether it is the same product or a combination of several, the application of antimicrobials is necessary because a large amount of water on the surface gives rise to the growth of bacteria. The bibliographic study will be carried out on the next section, where the results of each study will be compared and then the ingredient that best suits the needs of the fresh-cut melon will be chosen.

5.1.1 Bibliographic study for melon

For melon, different studies have been found research which substances manage to improve the properties of melon after minimal processing and storage.

The study by (Ortiz-Duarte et al., 2019) presents chitosan combined with silver. Chitosan is a biopolymer used in the industry for the synthesis of coatings and for its antimicrobial properties. This study, (Ortiz-Duarte et al., 2019) observed that the antimicrobial activity of chitosan increased with the presence of metal ions and that the metal with the highest antimicrobial activity was Ag⁺. It was observed that the growth of mesophilic bacteria was reduced by the application of the Ag-chitosan coating.

The study by (Silveira et al., 2015) treated the cut melon samples with vanillin and cinnamic acid, as they are natural antimicrobials.

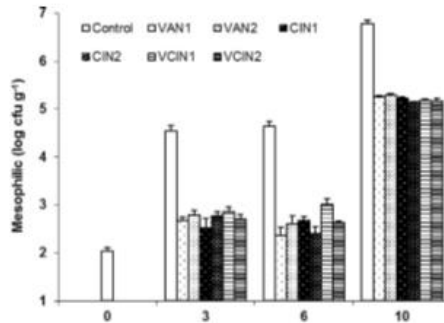


Figure 8: Psychotropic growth (\log_{10} cfu g^{-1}) of fresh-cut cantaloupe melon treated or not with antimicrobials and stored 10 days at 5°C. (Silveira et al., 2015).

The **¡Error! No se encuentra el origen de la referencia.** (Silveira et al., 2015) demonstrated that the application of a solution of vanillin, cinnamic acid and the use of cinnamic acid vapor in MAP at low concentrations was an effective method to inhibit the growth of bacteria, in particular, to inhibit the growth of mesophilic and psychotropic bacteria that give way to senescence.

(Oms-Oliu, Soliva-Fortuny and Martín-Belloso, 2008) compared the effects of polysaccharide derivatives, alginate, gellan and peptic in edible coatings on freshly cut melon. The application of alginate in the edible coating proved to be an antimicrobial carrier by reducing the growth of bacteria on the surface of the freshly cut melon.

The study by (Chong, Lai, and Yang, 2015) showed that samples treated with the combination of chitosan and calcium chloride showed a significant reduction in the amount of psychotropic substances. It has also been observed that calcium chloride has a more effective control on yeasts and molds than chitosan.

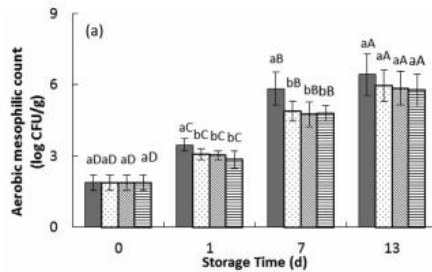


Figure 9: Effect of different treatments on microbial survivals of honeydew melon aerobic mesophilic count. (Chong, Lai and Yang, 2015).

The Figure 9 showed that coated samples had a smaller mesophilic and psychotropic population than the control sample.(Chong, Lai and Yang, 2015)

On the other hand, regarding water loss, it has been found that the combination of lipids and/or proteins with a polysaccharide base waterproofed the edible coating according to (Hassan et al., 2018). The study of (Oms-Oliu, Soliva-Fortuny and Martín-Belloso, 2008) added that the samples coated with alginate, gellan and peptic showed less dehydration compared to the uncoated samples (control) as a consequence of the reduction of the air-filled surface tissue. Thus, it was concluded that alginate was a carrier of antimicrobials in addition to reducing dehydration of fresh-cut melon. The study by (Chong, Lai and Yang, 2015) analyzed the effects of the combination of chitosan with calcium chloride (CaCl_2) on fresh-cut melon samples.

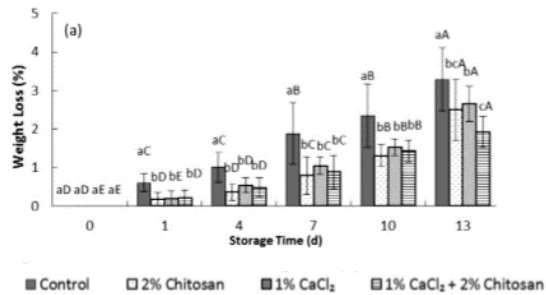


Figure 10: Effect of different treatments on weight loss of fresh-cut honeydew melon. (Chong, Lai and Yang, 2015).

According to Figure 10, control samples showed increased water loss. As the storage time passed, the water loss increased, however, the coated samples showed less water loss. When coatings were applied on the surface of the melon, the passage of moisture from the interior to the surface is reduced, thus decreasing water loss and respiration rate. The combination of chitosan with calcium chloride was the coating which reduced the weight loss the most. (Chong, Lai and Yang, 2015)

5.1.2 Selection of ingredients to avoid deterioration of fresh-cut melon

The coatings applied must meet several requirements. The ingredients that shall constitute the coatings must be stable substances at high relative humidity, be GRAS substances, provide a barrier to water vapor, a barrier to oxygen and carbon dioxide, have sensory properties that do not modify the original taste of the fruit, adhere to the fruit and finally be of reasonable cost.

Among the several ingredients proposed to reduce the growth of bacteria in fresh-cut melon, we will choose chitosan because it is used in the synthesis of films and coating. As said before, the ingredients that make up the coatings must be GRAS substances. Chemicals classified as

food additives or substances generally recognized as safe that have low toxicity and minimal impact on the environment are called GRAS. Thus, although the use of the Ag⁺ metal ion has been shown to increase the antimicrobial capacity of chitosan, its use will be discarded as it does not fit the GRAS product definition that our product should follow. Vanillin, being a polyphenol and a GRAS substance, has proven to be a good natural antimicrobial, therefore, to make prevention more effective against the growth of possible bacteria on the surface of the melon, chitosan can be combined with vanillin. In this way, the combination of chitosan with vanillin will be responsible for protecting the melon against microbial infections.

To prevent water loss, the chosen ingredient will be calcium chloride. As observed in the (Chong, Lai and Yang, 2015) the combination of chitosan with calcium chloride is the one that managed to reduce water loss the most. Therefore, our product will be the combination of chitosan, vanillin and calcium chloride.

As the concentrations that have been used in the article (Chong, Lai and Yang, 2015) have had good results, the same concentration will be used for the chitosan and the calcium chloride, this way according to the (Silveira et al., 2015) the same concentration will be used for the vanillin. Therefore, there will be a combination of 1% (w/w) of calcium chloride, 2% (w/w) of chitosan and 0.1% (w/w) of vanillin.

5.1.3 Products for application of active ingredients to preserve melon

This work has focused on the application of an edible coating instead of an edible film. One of the differences between these two is their method of application, while edible coatings are defined as a thin layer of edible material formed by a coating on the food and their method of application is in liquid form, usually by immersion of the product in a solution, edible films are defined as a thin perforated layer made of edible material and applied in the form of solid sheets which in the form of a coating are put on the food (McHugh and Senesi, 2000)

A more complete and detailed definition is that of (Juan and Aldemar, 2010) which defines edible coatings as thin layers of a biopolymer material (being either a protein or a polysaccharide in the form of a hydrocolloid solution or as an emulsion with lipids) that are

applied on the surface of a food behaving as a barrier that prevents the exchange of gases with its surroundings improving and extending the life of the coated food.

Following the previous sources, the chitosan solution formed by 1% (w/w) of CaCl_2 , 2% (w/w) of chitosan and 0.1% (w/w) of vanillin will be applied in solution. The combination of these ingredients will form the solution called chitosan product. The ingredients chosen, such as chitosan and vanillin, are soluble substances and therefore will not present problems when making the solution. The calcium chloride is a chemical compound with a water solubility of 74.5 g /L at 20 ° C should not present problems when making the mixture in solution.

According to the article (Chong, Lai and Yang, 2015) and (Silveira et al., 2015) the melon samples will be immersed in the chitosan product for a certain time. The immersion of the samples will have to be done once the melon has been peeled and cut. Prior to the handling operations, the samples are to be disinfected in a 200 ppm NaClO solution (Oms-Oliu, Soliva-Fortuny and Martín-Belloso, 2008). The melon samples shall be immersed in the chitosan, CaCl_2 and vanillin solution for 10 minutes. Afterwards, the samples will be taken to remove the excess solution and will be left to dry drop by drop in the air and at room temperature (20°C) for 1 hour. Finally they will be stored in cold (4°C) in a maximum of 10 days (Chong, Lai and Yang, 2015)

5.2 Products for apples

As mentioned above, the apple is one of the fruits that fits best among consumers, both whole and cut and therefore has been chosen as the fruit that will represent the pome fruits. The apple requires a product or a combination of several, which can delay the browning on its surface as the main objective. The following section will deal with the bibliographic study of possible ingredients that reduce browning on the surface of freshly cut apples to improve their quality of life. The following section will deal with the bibliographic study of possible ingredients that reduce browning on the surface of freshly cut apples to improve their quality of life.

5.2.1 Bibliographic study for apples

For the apple, different articles have been found that study the effects of ingredients such as ascorbic acid, citric acid, NAC, edible coatings made from carboxymethyl cellulose and coatings made from alginate and gellan gum with anti-browning such as N-acetylcysteine and glutathione. All these ingredients and coatings are intended to inhibit browning of fresh-cut apples.

(Yan et al., 2017) studied the effect of the combination of ascorbic acid with ethanol on fresh-cut apples. Ascorbic acid is an organic acid with antioxidant properties and ethanol has disinfectant properties and is used to delay senescence in fruits.

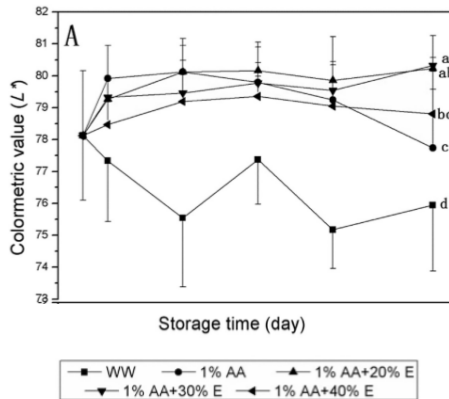


Figure 11: Effect of chemical treatments on the color parameter L^* . (Yan et al., 2017).

The Figure 11 observed that the concentration of both ingredients that had better results inhibiting browning of apples was 1% of ascorbic acid together with 30% of ethanol meaning 10 g /L of ascorbic acid with 0.3 L /L of ethanol.

(Gao et al., 2018) evaluated the anti-browning effect of exogenous γ -aminobutyric acid (GABA) on fresh-cut apples. The study analyzed the values of browning index (IB) and L* parameters.

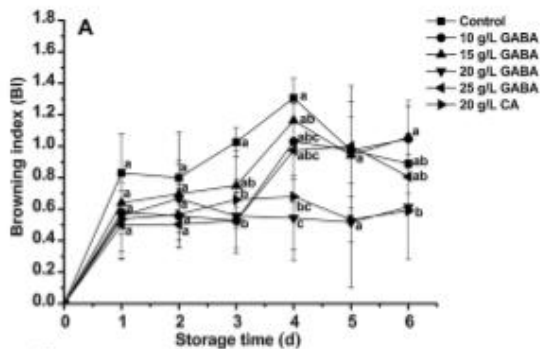


Figure 12: Influence of different GABA concentrations and soaking times of fresh-cut apples during storage for 6 d at 4°C on browning index. (Gao et al., 2018).

They observed, Figure 12, that the GABA solution with a concentration of 20 g /L had the lowest IB values during apple storage, therefore it was the one that most effectively inhibited the effects of browning. In addition, the solution with 20 g /L of GABA also inhibited the activity of the PPO enzyme.

According to another investigation (Fan, Sokorai and Phillips, 2018) three anti-browning substances were combined in solution (citric acid, ascorbic acid, calcium ascorbate and NAC) at different concentrations and their effects on fresh-cut apple samples were evaluated. N-acetyl cysteine (NAC) is a substance that is part of the Sulphur-containing amino acids that are able to inhibit browning by preventing polymerization instead of inhibiting the activity of polyphenols.

The results obtained showed that after 21 days of storage at 4°C, the optimal combination was 3% citric acid, 3-4% ascorbic acid and 1.5-2.0% NAC.

It is also common to use edible coatings with a polysaccharide base such as alginate or gellan, along with the addition of active components such as N-acetyl cysteine and glutathione, this is the edible coating that applied in their research to study their anti-browning effects on samples of apple pieces.

(Rojas-Graü et al., 2007) studied the effect of N-acetyl cysteine and glutathione as anti-browning agents applied on an edible coating based on alginate and gellan. The coatings were applied to samples of fresh-cut apples into solution. In addition, to add resistance to water migration, sunflower oil was added as a lipid providing a water vapor barrier to the coating. The results obtained from this study showed that both N-acetyl cysteine and glutathione caused decreases in browning of apple samples.

Another polymeric base was studied in edible coatings for freshly cut apples. This is the case of (Koushesh Saba y Sogvar 2016) which studied the effects on apple browning of an edible coating based on carboxymethyl (CMC) cellulose combined with CaCl_2 and ascorbic acid (AA).

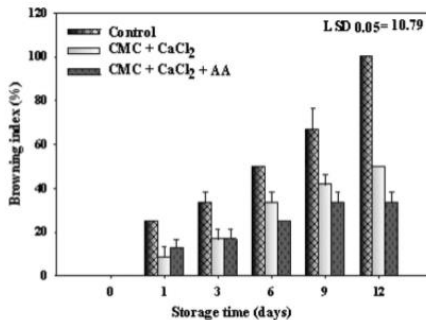


Figure 13: Browning index of fresh-cut apples in fruit either uncoated or coated with CMC alone or in combination with AA. Fruit were stored at 4°C for up to 12 days. (Koushesh Saba and Sogvar, 2016).

The results of the browning index (IB) in Figure 13 showed that the solution with a lower index after 12 days of storage at 4°C was the combination of CMC, 0.5% (w /w) of CaCl₂ and 2% (w /w) of ascorbic acid. It was also observed that this concentration was the one that managed to further reduce the activity of the PPO enzyme, thus correctly inhibiting the browning reaction. (Koushesh Saba and Sogvar, 2016).

5.2.2 Selection of ingredients to avoid deterioration of fresh-cut apples

As stated at the beginning of the chapter, an edible coating is composed of a polymeric base, which may be a polysaccharide base, and the addition of active ingredients which perform a function on the food. In the case of freshly cut apples it is necessary to inhibit browning on the surface. Of the ingredients listed above, the use of ethanol on the edible coating may cause sensory changes such as bad taste and odor. For this reason, the use of ethanol on the edible coating will be dispensed with. Despite the fact that the compound GABA has had good results in the study of (Gao et al., 2018) and that it is a GRAS substance, its use has not been very common and is currently under investigation.

From the ingredients proposed to inhibit the darkening of the surface of freshly cut apples, an edible coating based on alginate will be chosen, since it is an anionic polysaccharide widely used in the industry for various reasons: for its colloidal properties, for its gelling properties, for being a GRAS compound. Industrially, the alginate-calcium gels are used as thickeners, emulsion stabilizers, gelling agents, to improve the texture of the food... (Rojas-Graü et al., 2007).

Alginate will be combined with anti-browning ingredients, as demonstrated by the combination of ascorbic acid, citric acid and N-acetyl cysteine, which have been shown to be components capable of effectively inhibiting browning on apples.

According to the results observed in (Fan, Sokorai and Phillips, 2018) the dose that showed to have better results was 3% of citric acid, 3-4% of ascorbic acid and 1.5-2.0% of NAC,

therefore the dose to be chosen will be the following: 3% (w /w) of acid citric, 4% of ascorbic acid and 1.5% of NAC.

5.2.3 Products for application of the ingredients to preserve apples

As mentioned in the previous section, the ingredients chosen to inhibit browning of fresh-cut apples are a polymeric base of 2% (w /w) alginate together with the combination of 3% (w /w) citric acid, 4% (w /w) ascorbic acid and 1.5% (w /w) NAC as anti-browning ingredients.

The edible coating to be applied to the apples shall consist in two solutions, one composed of the anti-browning ingredients of citric acid, ascorbic acid and NAC and the other with the polymeric base of alginate.

The first solution will be constituted by the anti-browning ingredients with the concentrations agreed in the previous section. The percentages are in w/w for the citric acid we will have 3% in weight formed by 3 g of citric acid for each 100 g of solution and in the same way for the other ingredients. The combination of 3% (w/w) of citric acid, 4% (w/w) of ascorbic acid and 1.5% (w/w) of NAC will form the solution called acid product.

The second solution is prepared from 2% alginate (w/ w) and will be diluted in distilled water, following the study of (Rojas-Graü et al., 2007). Alginate is a compound that at room temperature is in solid state, therefore, 2 grams of this compound will be added for every 100 g of distilled water. It is quite common to add plasticizers to the solutions that constitute edible coatings. Plasticizers are liquid substances that increase the viscosity of the solution by improving the flexibility of the coating. The most common plasticizers are glycerin and glycerol. One of the reasons why glycerol is usually added to edible coatings is to improve the adhesion of the coating to the surface of the fruit, thus improving the mechanical properties of the polymer base, making it more resistant. In this way, glycerol will be chosen as a plasticizer substance to our alginate solution since glycerol is soluble in water. Following the study of (Rojas-Graü et al., 2007) a 1.5% (w/w) glycerol will be added to the solution. The combination of 2% (w/w) of alginate and 1.5% (w/w) of glycerol will form the solution called alginate product.

Once the compositions of the two solutions have been established, the method of application and the order will be specified. First, the apple samples are to be immersed in the acid product that contains 3% (w/w) of citric acid, 4% (w/w) of ascorbic acid and 1.5% (w/w) of

NAC, for 4 minutes, then take the samples out in the air for 2 minutes and drop the excess of the first solution. Then immerse the samples in alginate product that contains 2% (w/w) of alginate and 1.5% (w/w) of glycerol, for 4 minutes and leave it to dry for a further two minutes in the air so that the excess solution falls drop by drop. Finally, take the samples to be stored at a temperature of 5°C.

5.3 Products for Mangos

Finally, the mango has been chosen to represent all tropical fruits. As explained above, in the case of mango we will look for a product or combination of some products that are able to generally improve the quality of this fruit. Mainly we are looking for it to delay the ripening process, so that it acts on the main parameters that are affected by this deterioration, inhibiting the production of ethylene and decreasing the rate of respiration. In addition, the application of an edible coating that provides antibacterial compounds to prevent the growth of pathogens is sought. In this way we are going to proceed with the bibliographic study of the possible ingredients to be applied on fresh-cut mango.

5.3.1 Bibliographic study for mangos

As mentioned above, ethylene production increases due to injuries during processing of fresh-cut fruit and its accumulation is harmful to fruit quality. Several studies (Vilas-Boas and Kader, 2007), (Watkins, 2006), (Lee et al., 2012) have shown that 1-Methylcyclopropene (1-MCP) is a compound that inhibits the action of ethylene, in addition to reducing respiration rate.

As it has been exposed in the chapter of deterioration mechanisms, the production of ethylene and the increase in respiration rate are caused by the processing operations and are responsible for the initiation of the senescence and therefore the end of the useful life of that food, therefore its production must be inhibited or reduced. 1-MCP is a compound very used in

the industry of processed foods to inhibit the production of ethylene. It was also found that the addition of 2% of CaCl_2 to the 1-MCP solution positively increased its inhibitory effects.

Articles such as (Chien, Sheu and Yang, 2007) analyzed the effects of chitosan on mango pieces. It was observed that chitosan, as seen previously in melon, delayed water loss and inhibited the growth of microorganisms. The article from (Plotto et al., 2006) proposed the application of ethanol steam to analyze its effects on mango. Conflicting results were observed on its effects inhibiting the ripening of mango and concluded that ethanol can cause bad taste to the fruit, besides not assuring its efficacy inhibiting the production of ethylene, although at low concentrations it could be used as a microbial control.

The article from (Salinas-Roca et al., 2018) studied the effects of different polysaccharide bases on mango pieces. Edible coatings based on alginate, pectin, carboxymethyl cellulose and chitosan were applied. It was observed that all coated samples showed a decrease in the respiration rate as they provided a barrier with low oxygen permeability. The results regarding the respiration rate, all coatings managed to decrease the respiration rate. All the coatings except the MCP managed to reduce the growth of psychrophilic bacteria, molds and yeasts. The samples coated with chitosan showed the lowest levels of molds and yeasts compared to the samples coated with alginate, pectin and carboxy methyl, as mentioned above, this behavior is normal considering that the nature of chitosan is to be an antimicrobial compound.

Another polymeric base was analyzed by (Chiumarelli et al., 2011) that used an edible coating based on manioc starch or sodium alginate with the addition of citric acid with and without glycerol as a gelling agent. Results showed that samples immersed in citric acid and coated with manioc starch without the addition of glycerol had the lowest respiration rate compared to the other treatments reaching values up to 41% less respiratory activity.

(Liu et al., 2020) studied the application of a solution with melatonin on fresh-cut mango samples. Melatonin is a hormone found in plants among other sites and is synthesized from the amino acid tryptophan. In plants, melatonin activates the antioxidant system and participates in various physiological processes, as well as ripening and senescence. It is considered a GRAS compound and is effective against aging.

In fruits such as bananas, melatonin applied by immersion inhibited ethylene production, delayed ripening and maintained the quality of this fruit (Hu et al., 2017).

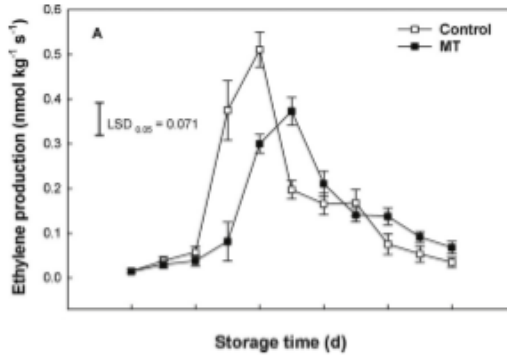


Figure 14: Ethylene production in 'Guifei' mango fruit during storage at 25 °C following treatment with 0.5 mM MT or water (control). (Hu et al., 2017).

In general, mango samples did not produce high levels of ethylene, however, it was observed on Figure 14 that treated samples delayed ethylene production compared to control samples.

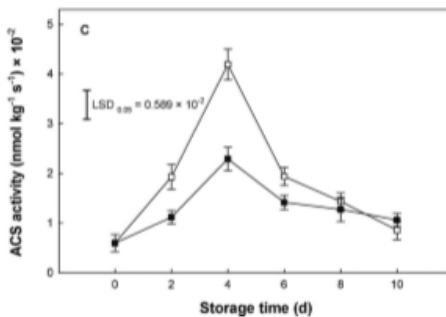


Figure 15: ACS activities in 'Guifei' mango fruit during storage at 25 °C following treatment with 0.5 mM MT or water (control). (Hu et al., 2017).

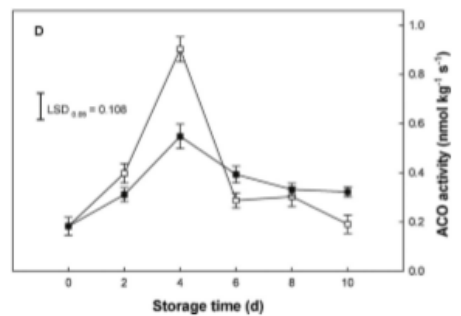


Figure 16: ACO activities in 'Guifei' mango fruit during storage at 25 °C following treatment with 0.5 mM MT or water (control). (Hu et al., 2017).

Figure 15 and Figure 16 showed that ACC content was increasing with storage until the fourth day when it began to decrease. In the melatonin-treated samples the ACC content was effectively suppressed by repressing the activity of ACC oxidase and ACS synthase giving lower values of these enzymes compared to the control samples.

5.3.2 Selection of ingredients to avoid deterioration of fresh-cut mango

Although ingredients are sought for freshly cut mangoes that can improve the quality of the fruit and increase its shelf life, at the beginning of the chapter the emphasis was on reducing the rate of respiration and inhibiting ethylene production. Based on these requirements and functions, one of the ingredients to be chosen to inhibit ethylene production will be melatonin. The melatonin solution studied by (Liu et al., 2020) has been shown to inhibit ethylene production as well as to reduce the activity of the enzymes ACS and ACO involved in the reaction that produces ethylene, therefore it is a viable choice to increase the shelf life of freshly cut mangoes.

As 1-MCP is an industrially common compound in processed foods for its inhibitory effect on the production of ethylene, it will be selected to be combined with melatonin in a solution. In this way and following the references of the articles mentioned, the concentrations to be chosen are the following: 0.05% (w/w) of 1-MCP together with 0.5%(w/w) of melatonin.

In addition to melatonin and 1-MCP, cassava starch will be chosen as the polymer base for the edible coating of freshly cut mangoes, as it has been shown to effectively reduce the respiration rate and consequently increase the shelf life of mangoes.

The application of ethanol vapor has been ruled out because, as explained in chapter 4, ethanol is capable of inhibiting the production of ethylene but one of its side effects is to irretrievably damage the natural flavor of the mango and thus adversely affect its quality, preventing its subsequent marketing.

The polymeric bases used in the articles have shown good results in achieving good adhesion of the active ingredients on the fruit, providing a barrier with the environment surrounding the fruit reducing the exchange of gases along with the loss of water by moisture difference.

The application of cassava starch in the polymer base will be discarded because microorganisms are involved in its fermentation process that would deteriorate the quality of the solution. Discarding the option of starch, the use of carboxymethyl cellulose will be preferred as the polymer base of the solution applied to the mango, since the use of this polymer has had good results in the above-mentioned studies, in addition to fulfilling without disadvantages its function of being a barrier between the fruit and its surroundings and containing the active ingredients according to (Chiumarelli et al., 2011)

In addition, it is preferable the carboxymethyl cellulose instead of starch because when preparing the aqueous solution, the carboxymethyl cellulose has a much higher water solubility than starch, therefore, it would not be necessary to jacket the reactor (cheaper reactor).

5.3.3 Products for applying the active ingredients to preserve mango

Once the ingredients and their concentrations to be used to coat the handle parts are clear, the method of application of the coating will be established. Mango samples will be dipped in two solutions which will form the edible coating. The first solution will be made by the active ingredients that will be responsible of the inhibition of ethylene production and the second solution will be made by the polymer base that will act as a barrier between the fruit and its surroundings.

The mango samples will be dipped into the first solution containing 0.5% Melatonin and 0.05% 1-MCP. The combination of these ingredients will form the solution called melatonin product. This solution is very diluted because the concentrations of solute are low, therefore, it can be assumed that its preparation will be simple and fast to perform. The 1-methylcyclopropene (1-MCP) is a compound that mainly inhibits the action of ethylene which, the longer the exposure time, the lower the concentration necessary to obtain the same inhibiting effect.

The second solution, called carboxymethylcellulose product will be composed of 2% carboxymethylcellulose and 0.5% chitosan. Carboxymethylcellulose (CMC) is a derivative of cellulose that is soluble in water, so when preparing the solution there should be no problems of precipitation or insolubility. Nevertheless, the mixture will be made at 70°C (as in the case of alginate) to ensure total solubility. To avoid the growth of bacteria, chitosan will be used which, as seen above, is an antimicrobial compound.

Thus, the mango samples will be immersed in the melatonin product consisting in 0.05% (w/w) of 1-MCP and 0.5% (w/w) of melatonin for 3 minutes and allowed to air dry for 2 minutes to remove the excess solution drop by drop. The samples will then be immersed in the carboxymethylcellulose product consisting in 2% (w/w) carboxymethylcellulose (CMC) and 0.5% (w/w) chitosan solution for another 3 minutes and then air-dried for 2 minutes. Finally, the samples will be stored at 5°C.

6. BASIC DESIGN OF A MANUFACTURING PLANT FOR THE PRODUCTS DEVELOPED

Once the ingredients that will form the coatings for each fruit have been determined and the concentration for each of them has been established, the amount of product to be produced for each fruit will be decided.

6.1 Estimated annual production

In order to choose the amount of solution to be produced for each fruit, two factors must be considered. On the one hand, the current production of cut fruit, which is directly related to the consumption of the area where it is sold, and on the other hand, a relationship has to be established between the amount of solution needed to cover the entire production of cut fruit.

The study focused on the commercial fruit sector distributed by "Mercabarna" throughout the metropolitan area of Barcelona, where 36 municipalities are located, and 3 239 337 people live. Therefore, all the data that will be provided and the subsequent assumptions will be based on the consumption of cut fruit in the metropolitan area of Barcelona.

The tons of melon commercialized in Catalonia between 2016 and 2019 have been kept in about 35 000 t, being the "Piel de Sapo" type melon the most commercialized one, reaching 70% of the total sold melons.

The tons of apple sold are smaller and have remained at around 28 000 t each year between 2016 and 2019, with yellow apple being the most consumed, accounting for almost 50% of sales.

Being the representation of tropical fruit, mango has tripled its consumption in Spain in the last five years, reaching 3000 t of mango sold in 2019 approximately throughout Catalonia

The research institute of the Catalan government carried out a study on Trends and New Processed Vegetable Products and found that 10% of the fruit sold was freshly cut. Assuming the values from these sources, it is possible to estimate the production of freshly cut melon per year and consequently determine the tons of edible cover to be manufactured.

As it is difficult to cover more than 20 / 25% of the trade of a sector in the industry, it will be assumed that the first year of production of cut and coated melon, apple and mango will cover 10% of the trade. Therefore, if in all the trade of Mercabarna have been sold 35 000 t of melon, 28 000 t of apple and 3000 t of mango and 10% of these sales have been for the production of cut fruit, the total of cut fruit that has been sold is 3500 t of melon, 2800 t of apple and 300 t of mango. Considering that this work will take care of 10% of the sector, the amount of fruit that will be processed, coated and sold later will be 350 t of melon, 280 t of apple and 30 t of mango.

Both for freshly cut melon and freshly cut apple, the use of one type of melon and one type of apple will be preferred, as the aim is to produce coatings for each of them and to avoid mixing different types of melons and apples, the application of the edible coating will be preferred over the "Piel de Sapo" type melon (70%) and the yellow apple (50%) as they are the most sold on the market.

Therefore, the quantities to be processed are 245 t of "Piel de Sapo" type melon, 140 t of yellow apple and since only one type of mango has been sold, 30 t of mango will be processed.

Once the fruit production per year is oriented, a relationship must be established between the amount of coating applied and the fruit to be treated. In order to make this selection, some essential parameters must be established such as the type of cut for the chosen fruit and the net weight to be covered (once the stone or pips have been peeled and removed)

Among the different types of cuts that are used in the culinary world is the julienne type cut, which consists of strips of 6 cm long and a width and thickness of 2 mm, the brunoise type cut that consists of cubes of 1 or 2 cm side, the Macedonian type cut of 4 cm side, the irregular Mirepoix cuts, the pearl-type cut that takes the shape of a spoon (like a sphere), the biais-type cut consisting of thin slices at an angle and the Vichy cut (slices approximately 2 mm thick).

The fruit chosen is to be sold with the aim of being consumed on the spot, therefore, the type of cut and size will determine the shape of the package. As has been seen in the various markets mentioned in chapter 2, the most common packaging in which minimally processed fruit is sold is plastic and in the form of a cup or also in the form of a tub, of an individual size that

can be held in one hand. In order to optimize the space of the package, the shape and size of the fruit are important factors, for this reason, it will be decided to cut the fruit in the form of cubes of 2 cm side, as it offers comfort when eating the pieces, both for the apple, the melon and the mango. To get an idea of the percentage of weight of the fruit that is used after peeling and removing the pips or stone, an experimental approach will be made as follows:

A Piel de Sapo melon, a yellow apple and a mango have been bought. The whole fruit has been cleaned with water and weighed before and after processing (peeling and removing the stone or pips). Finally, the sample has been cut into a cube of approximately 2 cm side.

The results are shown in the Table 1:

Table 1: Experimental results on the weight of melon, apple and mango.

	Melon	Apple	Mango
Whole piece weight (g)	595	129	635
Weight of peeled and stone less fruit (g)	380	96	511
Quantity of usable fruit	64%	74%	80%
Piece weight (g)	8	7	9

It should be considered that $\frac{1}{4}$ melon, $\frac{1}{2}$ apple and a whole mango were weighed. Since the objective is to know the relationship between the amounts that is taken from each fruit, it does not affect whether the whole fruit or part of it has been weighed. With these data, the % of usable fruit was calculated (the relation between the weight of the peeled and stone less fruit with the weight of the whole fruit) so it was observed that 64% of the melon, 74% of the apple and 80% of the mango were used, so the tonne per year of fruit that are really sold minimally processed are 150 t of cut melon, 100 t of cut apple and 25 t of mango.

The ingredients applied to the 150 t of melon will constitute 1% of the weight (except for 1-MCP). Therefore, the calculation of the amount of ingredients that the melon will have is made in the following way:

$$150 \text{ t} * \frac{1}{100} = 1.5 \text{ t of ingredients}$$

Once you have the amount of ingredients that the melon will contain, you will calculate the equivalent amount of chitosan product needed:

$$1.5 \text{ t of ingredients} * \frac{100 \text{ g of chitosan product}}{3.1 \text{ g of ingredients}} = 48.39 \text{ t of chitosan product}$$

Thus, as an estimate, it will be assumed that 50 t of chitosan product will be produced to coat 150 t of cut and peeled melon.

The Table 2 brings together the results for the productions of the five products developed:

Table 2: Determination of the amount of solution to be produced in a year

	Chitosan product	Acid product	Alginate product	Melatonin product	Carboxymethylcellulose product
Composition (w/w)	1% CaCl ₂ + 2% Chitosan + 0.1% Vanillin	3% Citric acid + 4% ascorbic acid + 1.5% NAC	2% Alginate + 1.5% Glycerol	0.5% Melatonin + 0.05% 1-MCP	2% Carboxymethyl cellulose (CMC) + 0.5% Chitosan
Quantity of fruit to be coated	150 t melon	100 t apple	100 t apple	25 t mango	25 t mango
Quantity of total ingredients (w/w)	3.1%	8.5%	3.5%	0.55%	2.5%
Ingredients concentration	1%	1%	1%	0.5%	1%
Quantity of ingredients (tons)	1.5	1	1	0.025	0.25
Quantity of solution (tons)	50	12	30	5	10

According to the table, the quantities to be produced of each solution have been determined: 50 t of chitosan product, 12 t of acid product, 30 t of alginate product, 5 t of melatonin product and 10 t of carboxymethylcellulose product.

The production of solutions is to be done in batches with a set load. In this way, it must be established how many reactors and what size will be available. Considering that the productions are small, only one reactor will be bought, capable of producing 500 kg of product, therefore, all the solutions would be made in the same reactor.

Establishing that the reactor load (batch size BS) is 500 kg (1 batch), the batches to be produced per year will be: 100 batches of chitosan product, 24 batches of acid product, 60 batches of alginate product, 10 batches of melatonin product and 20 batches of carboxymethylcellulose product. In one year, 214 batches of 500 kg each will be produced. The plant will only fabricate the products, package them, store them and sell them to the melon, apple and mango cut fruit industry. Each lot will be stored and distributed in 20L containers, so each lot will have 25 containers.

To determine the time needed to produce a batch, the following times will be differentiated: time of preparation of the raw materials, time of loading the materials in the reactor, time of agitation, time of emptying and time needed to clean the equipment.

In the case of the alginate product and the carboxymethylcellulose product, the mixture must be heated to 70°C, as these are polymeric substances, this temperature ensures that the bases dissolve correctly and more easily, therefore, the heating of the mixture will begin when the materials are first thrown into the reactor. In this way, the mixture is filled with one third of distilled water, the heating process begins, the raw materials are added, and the necessary remaining water is added. Once the mixture has been heated and the stirring time has passed, alginate product and the carboxymethylcellulose product will need additional time to cool down and once at room temperature (30°C) the reactor will begin to empty. In the case of the remaining products, chitosan product, acid product and melatonin product, it is not necessary to heat the mixture and therefore the charging time will be changed to the stirring time.

The reactor will be gravity drained, with a discharge outlet in the center of the lower zone. From the outlet, the containers are going to be filled with the solutions, so the time it takes to

pack will be the time it takes to empty the reactor. Finally, the time it takes to clean the reactor with pressurized water must be considered. The sum of all these times is the batch time (BT)

Knowing the time required for producing a batch and the quantity of batches to be produced gives the plant's occupancy time. The Table 3 gathers the times that have been established for each stage:

Table 3: production time estimation.

	Chitosan product	Acid product	Alginate product	Melatonin product	Carboxymethylcellulose product
Preparation time (min)	15	15	15	15	15
Load time (min)	20	20	20	20	20
Heating time (min)	0	0	30	0	30
Agitation time (min)	30	30	60	30	60
Cooling time (min)	0	0	45	0	45
Emptying time (min)	60	60	60	60	60
Cleaning time (min)	30	30	30	30	30
BT (min)	155	155	240	155	240
Number of batches	100	24	60	10	20
Time of occupation (h)	258	62	240	26	80

According to the results of the plant, the annual production of chitosan product will take 258 hours, acid product will take 62 hours, alginate product will take 240 hours, melatonin product will take 26 hours and finally carboxymethylcellulose product will take 80 hours. In total, it will

take 666 hours for the annual production of the five solutions. If the working day is 8 hours a day from Monday to Friday, there are 220 working days left in the year, which gives a total of 1760 working hours of which 1094 the plant will not produce batches. Thus, the chosen reactor will be enough for producing the starting productions and would permit to cover future increases in demand.

6.2 General aspects of raw materials.

The raw materials needed to form the solutions will be purchased in solid form. In the industry market there are different ways to buy chemicals on a large scale, for example, you can find calcium chloride, ascorbic acid, citric acid, N-acetyl cysteine, glycerol, alginate and carboxymethylcellulose in 25 kg sacks. Depending on the annual production of each solution, if purchased in 25 kg sacks, the necessary ingredients will need to be purchased several times a year.

In the case of vanillin, melatonin and 1-methyl-cyclopene are produced in smaller quantities, therefore, they go by the kilo. The opposite of these three ingredients is chitosan, which is a product that is marketed in larger quantities, from 25 kg to 100 kg of chitosan.

Distilled water will be available for the formation of the solutions to be produced.

On the other hand, the purchased raw materials must be treated and stored in a specific way, according to the indications of the supplier of each substance. The general and most common aspects to consider are that the products to be used should be stored in a dry and cold place avoiding direct light.

The Table 4 is based on an estimate of how the raw materials will be purchased, calculated in kg per sack and considering the production of each product per year, the bags per year to be purchased have been determined.

Table 4: Estimation of the sacks per year to buy of each raw material

Substances	Kg/ sack	Kg /batch	Total batches	kg/ year	Sacks/ year
CaCl ₂	25	5	100	500	20
Ascorbic acid	25	20	24	480	20
Citric acid	25	15	24	360	15
N-acetyl cysteine (NAC)	25	7.5	24	180	8
Alginate	25	10	60	600	24
Carboxymethyl cellulose (CMC)	25	10	20	200	8
Glycerol	25	7.5	60	450	18
Chitosan1	25	10	100	100	40
Chitosan2	25	2.5	20	50	2
Vanillin	1	0.5	100	50	50
1-Methylcyclopropeno (1-MCP)	1	0.25	10	2.5	3
Melatonin	1	2.5	10	25	25

6.3 Selection of equipment

The reactor to be used must meet several requirements and have characteristics in line with production needs. Thus, as the load chosen is 500 kg, considering that the density of the solutions is practically that of water (1kg/L) the nominal volume of the reactor will be 500L. For safety reasons, the reactor will be filled up to 80/85% of its total capacity, therefore the real volume will be 600L.

To homogenize the mixture, an agitator is needed. The use of baffles will not be necessary as they are simple solutions and do not require high agitation.

As mentioned above, for alginate product and carboxymethylcellulose product, mixing needs to be done at 70°C, so the reactor must be jacketed. In the industry there are many varieties of jackets and different ways to heat, the preferred option to heat the mixture to 70°C and then cool it down to 30°C is the use of a half-round jacket. The half pipe jacket is used in the industry when heating and cooling with fluids is required to have a better transfer area. The fluids that will be used to heat and cool the solutions will be steam from a boiler for heating and liquid water from a cooling tower for cooling the mixture. Steam has been chosen to heat the solution, as the latent heat from the phase change brings more heat to the solution and therefore heats up faster.

The reactor should have two inlets, one for solids and one for liquids. The ingredients needed to form the solution will be purchased in solid form, so the reactor will need to have a manhole through which the solid raw materials will be introduced. To make the products, distilled water is needed, therefore, apart from the manhole, the reactor must have a pipe inlet through which the distilled water will be introduced. As mentioned above, an outlet is required which will be located at the bottom and in the center where the tank will be emptied by gravity.

Considering all the characteristics that the reactor must comply with, a 600L stainless steel 316 reactor with agitator and jacket manufactured by TEMEC has been chosen.



Figure 17: 600L volume reactor made of 316 stainless steel by TEMEC.



Figure 18: 600L volume reactor made of 316 stainless steel by TEMEC.

7. CONCLUSIONS

Products for inhibit the deterioration of fresh-cut fruits have been developed.

Through a market and bibliographic study carried out the main fruits marketed as freshly cut have been detected. From these, melon, apple and cut mango have been chosen as a representation of the main types of fruits: melons, pome fruits and stone fruits.

Main deterioration mechanisms of these types of fruits have been studied in order to develop products inhibiting them. Main deterioration mechanism for melons are water loss and microbial infection (since the high-water content on the surface caused by cutting wounds gives rise to ideal environments for the growth of bacteria). In the case of cut apples, the deterioration mechanism to be avoided is browning on the surface (apart from being the most visually noticeable). For mangos, it has been determined that the deterioration mechanism to be avoided is the production of ethylene.

The products to be applied on the chosen fruits are solutions containing the active ingredients that fulfill the necessary functions for each fruit. Thus, the chitosan product composed of 2% (w/w) of chitosan with 1% (w/w) of calcium chloride and 0.1% (w/w) of vanillin in which the cut melon samples are to be immersed, has proven to prevent water loss, since a barrier is formed that makes the fruit waterproof and chitosan is an antimicrobial compound.

The acid product of 3% (w/w) of citric acid, 4% of ascorbic acid and 1.5% (NAC) of in which the cut apple samples are first immersed has been shown to inhibit the reactions responsible for browning on its surface, as well as the alginate product applied to the apple samples which is composed of 2% (w/w) of alginate with 1.5% (w/w) of glycerol. In the case of mango, the melatonin product composed of 0.5% (w/w) of melatonin and 0.05% (w/w) of 1-Methylcyclopropene (1-MCP) has been shown to effectively inhibit ethylene production and the carboxymethylcellulose product composed of 2% (w/w) of carboxymethyl cellulose (CMC) and 0.5% (w/w) of chitosan has fixed the ingredients well in addition to protecting the fruit from its environment.

Market for fresh-cut fruits in the Barcelona metropolitan area has been estimated in 150 t of cut melon, 100 t of cut apple and 25 t of cut mango.

Finally, assuming 10% of this market production of developed products has been estimated in 50 t of chitosan product, 12 t of acid product, 30 t of alginate product, 5 t of melatonin product and 10 t of carboxymethylcellulose product. This production could be made in 500 kg batches and a 600L reactor with agitator has been chosen to make the solutions.

REFERENCES AND NOTES

- Chien, Po-Jung, Fuu Sheu and Feng-Hsu Yang., 2007. «Effects of Edible Chitosan Coating on Quality and Shelf Life of Sliced Mango Fruit». *Journal of Food Engineering* 78 (1): 225-29. <https://doi.org/10.1016/j.jfoodeng.2005.09.022>.
- Chiumarelli, Marcela, Cristhiane C. Ferrari, Claire I.G.L. Sarantópoulos and Miriam D. Hubinger., 2011. «Fresh Cut 'Tommy Atkins' Mango Pre-Treated with Citric Acid and Coated with Cassava (*Manihot Esculenta* Crantz) Starch or Sodium Alginate». *Innovative Food Science & Emerging Technologies* 12 (3): 381-87. <https://doi.org/10.1016/j.ifset.2011.02.006>.
- Chong, Jia Xin, Shaojuan Lai and Hongshun Yang., 2015. «Chitosan Combined with Calcium Chloride Impacts Fresh-Cut Honeydew Melon by Stabilising Nanostructures of Sodium-Carbonate-Soluble Pectin». *Food Control* 53 (july): 195-205. <https://doi.org/10.1016/j.foodcont.2014.12.035>.
- Cortellino, Giovanna, Serena Gobbi, Giulia Bianchi and Anna Rizzolo., 2015. «Modified Atmosphere Packaging for Shelf Life Extension of Fresh-Cut Apples». *Trends in Food Science & Technology* 46 (2): 320-30. <https://doi.org/10.1016/j.tifs.2015.06.002>.
- Debeaufort, Frédéric, Jesús-Alberto Quezada-Gallo and Andrée Voilley., 1998. «Edible Films and Coatings: Tomorrow's Packagings: A Review». *Critical Reviews in Food Science and Nutrition* 38 (4): 299-313. <https://doi.org/10.1080/10408699891274219>.
- Dhital, Rajiv, Prabesh Joshi, Nathalie Becerra-Mora, Arosha Umagiliyage, Tan Chai, Punit Kohli and Ruplal Choudhary., 2017. «Integrity of Edible Nano-Coatings and Its Effects on Quality of Strawberries Subjected to Simulated in-Transit Vibrations». *LWT* 80 (july): 257-64. <https://doi.org/10.1016/j.lwt.2017.02.033>.
- Fan, Xuetong, Kimberly Sokorai and John Phillips., 2018. «Development of Antibrowning and Antimicrobial Formulations to Minimize *Listeria Monocytogenes* Contamination and Inhibit Browning of Fresh-Cut "Granny Smith" Apples». *Postharvest Biology and Technology* 143 (september): 43-49. <https://doi.org/10.1016/j.postharvbio.2018.04.009>.
- Gao, Haiyan, Shiyuan Wu, Qing Zeng, Peizhong Li and Wenqiang Guan., 2018. «Effects of Exogenous γ -Aminobutyric Acid Treatment on Browning and Food-Borne Pathogens in Fresh-Cut Apples». *Postharvest Biology and Technology* 146 (december): 1-8. <https://doi.org/10.1016/j.postharvbio.2018.08.007>.
- Garcia, M.A., M.N. Martino and N.E. Zaritzky., 2000. «Lipid Addition to Improve Barrier Properties of Edible Starch-Based Films and Coatings». *Journal of Food Science* 65 (6): 941-44. <https://doi.org/10.1111/j.1365-2621.2000.tb09397.x>.

- Goodburn, Caroline and Carol A. Wallace., 2013. «The Microbiological Efficacy of Decontamination Methodologies for Fresh Produce: A Review». *Food Control* 32 (2): 418-27. <https://doi.org/10.1016/j.foodcont.2012.12.012>.
- Hassan, Bilal, Shahzad Ali Shahid Chatha, Abdullah Ijaz Hussain, Khalid Mahmood Zia and Naseem Akhtar., 2018. «Recent Advances on Polysaccharides, Lipids and Protein Based Edible Films and Coatings: A Review». *International Journal of Biological Macromolecules* 109 (april): 1095-1107. <https://doi.org/10.1016/j.ijbiomac.2017.11.097>.
- Hu, Wei, Hai Yang, Weiwei Tie, Yan Yan, Zehong Ding, Yang Liu, Chunlai Wu, et al., 2017. «Natural Variation in Banana Varieties Highlights the Role of Melatonin in Postharvest Ripening and Quality». *Journal of Agricultural and Food Chemistry* 65 (46): 9987-94. <https://doi.org/10.1021/acs.jafc.7b03354>.
- Juan, C and H Aldemar., 2010. «Películas y recubrimientos comestibles: importancia y tendencias recientes en la cadena hortofrutícola», 26.
- Kang, Ji-Hoon and Kyung Bin Song., 2019. «Antibacterial Activity of the Noni Fruit Extract against *Listeria Monocytogenes* and Its Applicability as a Natural Sanitizer for the Washing of Fresh-Cut Produce». *Food Microbiology* 84 (december): 103260. <https://doi.org/10.1016/j.fm.2019.103260>.
- Koushesh Saba, Mahmoud and Ommol Banin Sogvar., 2016. «Combination of Carboxymethyl Cellulose-Based Coatings with Calcium and Ascorbic Acid Impacts in Browning and Quality of Fresh-Cut Apples». *LWT - Food Science and Technology* 66 (march): 165-71. <https://doi.org/10.1016/j.lwt.2015.10.022>.
- Lamiknra, Olusola., 2002 «Fresh-Cut Fruits and Vegetables», 482.
- Lee, Jin Su, Donald J. Huber, Christopher B. Watkins and Brandon M. Hurr., 2012. «Influence of Wounding and Aging on 1-MCP Sorption and Metabolism in Fresh-Cut Tissue and Cell-Free Homogenates from Apple Fruit». *Postharvest Biology and Technology* 67 (may): 52-58. <https://doi.org/10.1016/j.postharvbio.2011.12.013>.
- Liu, Shuaimin, Hua Huang, Donald J. Huber, Yonggui Pan, Xuequn Shi and Zhengke Zhang., 2020. «Delay of Ripening and Softening in 'Guifei' Mango Fruit by Postharvest Application of Melatonin». *Postharvest Biology and Technology* 163 (may): 111136. <https://doi.org/10.1016/j.postharvbio.2020.111136>.
- Maringgal, Bernard, Norhashila Hashim, Intan Syafinaz Mohamed Amin Tawakkal and Mahmud Tengku Muda Mohamed., 2020. «Recent Advance in Edible Coating and Its Effect on Fresh/Fresh-Cut Fruits Quality». *Trends in Food Science & Technology* 96 (febrero): 253-67. <https://doi.org/10.1016/j.tifs.2019.12.024>.
- Martin-Belloso, Olga and Robert Soliva Fortuny., 2011 «Advances in Fresh-Cut Fruits and Vegetables Processing», 402.
- McHugh, T.H. and E. Senesi., 2000. «Apple Wraps: A Novel Method to Improve the Quality and Extend the Shelf Life of Fresh-Cut Apples». *Journal of Food Science* 65 (3): 480-85. <https://doi.org/10.1111/j.1365-2621.2000.tb16032.x>.
- Ntsoane, Makgafele Lucia, Manuela Zude-Sasse, Pramod Mahajan and Dharini Sivakumar. 2019. «Quality Assesment and Postharvest Technology of Mango: A Review of Its Current Status and Future Perspectives». *Scientia Horticulturae* 249 (april): 77-85. <https://doi.org/10.1016/j.scienta.2019.01.033>.
- Oms-Oliu, G., R. Soliva-Fortuny and O. Martín-Belloso., 2008. «Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon».

-
- LWT - Food Science and Technology* 41 (10): 1862-70.
<https://doi.org/10.1016/j.lwt.2008.01.007>.
- Ortiz-Duarte, Génesis, Laura Eugenia Pérez-Cabrera, Francisco Artés-Hernández and Ginés Benito Martínez-Hernández., 2019. «Ag-Chitosan Nanocomposites in Edible Coatings Affect the Quality of Fresh-Cut Melon». *Postharvest Biology and Technology* 147 (january): 174-84. <https://doi.org/10.1016/j.postharvbio.2018.09.021>.
- Plotto, A., J. Bai, J.A. Narciso, J.K. Brecht and E.A. Baldwin., 2006. «Ethanol Vapor Prior to Processing Extends Fresh-Cut Mango Storage by Decreasing Spoilage, but Does Not Always Delay Ripening». *Postharvest Biology and Technology* 39 (2): 134-45.
<https://doi.org/10.1016/j.postharvbio.2005.09.009>.
- Raybaudimassilia, R, J Mosquedamelgar and O Martinbeloso., 2008. «Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon». *International Journal of Food Microbiology* 121 (3): 313-27.
<https://doi.org/10.1016/j.ijfoodmicro.2007.11.010>.
- Rojas-Graü, M.A., M.S. Tapia, F.J. Rodríguez, A.J. Carmona and O. Martin-Belloso., 2007. «Alginate and Gellan-Based Edible Coatings as Carriers of Antibrowning Agents Applied on Fresh-Cut Fuji Apples». *Food Hydrocolloids* 21 (1): 118-27.
<https://doi.org/10.1016/j.foodhyd.2006.03.001>.
- Salinas-Roca, Blanca, Ariadna Guerreiro, Jorge Welti-Chanes, Maria D. C. Antunes and Olga Martín-Belloso., 2018. «Improving Quality of Fresh-Cut Mango Using Polysaccharide-Based Edible Coatings». *International Journal of Food Science & Technology* 53 (4): 938-45. <https://doi.org/10.1111/ijfs.13666>.
- Silveira, Ana C., Gláucia C. Moreira, Francisco Artés and Encarna Aguayo. 2015. «Vanillin and Cinnamic Acid in Aqueous Solutions or in Active Modified Packaging Preserve the Quality of Fresh-Cut Cantaloupe Melon». *Scientia Horticulturae* 192 (august): 271-78.
<https://doi.org/10.1016/j.scienta.2015.06.029>.
- Vargas, Maria, Clara Pastor, Ana Albers, Amparo Chiralt and Chelo González-Martínez., 2008 «Development of Edible Coatings for Fresh Fruits and Vegetables: Possibilities and Limitations», 9.
- Vilas-Boas, Eduardo V. de B. and Adel A. Kader., 2007. «Effect of 1-Methylcyclopropene (1-MCP) on Softening of Fresh-Cut Kiwifruit, Mango and Persimmon Slices». *Postharvest Biology and Technology* 43 (2): 238-44.
<https://doi.org/10.1016/j.postharvbio.2006.09.010>.
- Watkins, Chris B., 2006. «The Use of 1-Methylcyclopropene (1-MCP) on Fruits and Vegetables». *Biotechnology Advances* 24 (4): 389-409.
<https://doi.org/10.1016/j.biotechadv.2006.01.005>.
- Yan, Shoulei, Yaguang Luo, Bin Zhou and David T. Ingram., 2017. «Dual Effectiveness of Ascorbic Acid and Ethanol Combined Treatment to Inhibit Browning and Inactivate Pathogens on Fresh-Cut Apples». *LWT* 80 (july): 311-20.
<https://doi.org/10.1016/j.lwt.2017.02.021>.
- Yang, L and A T Paulson., 2000. «Mechanical and Water Vapour Barrier Properties of Edible Gellan @lms». *Food Research International*, 8.
- Yousuf, Basharat, Ovais Shafiq Qadri and Abhaya Kumar Srivastava., 2018. «Recent Developments in Shelf-Life Extension of Fresh-Cut Fruits and Vegetables by

Application of Different Edible Coatings: A Review». *LWT* 89 (march): 198-209.
<https://doi.org/10.1016/j.lwt.2017.10.051>.

ACRONYMS

ACC: 1-aminocyclopropane-1-carboxylic acid

ACS: Aminocyclopropane-1-carboxylic acid synthase

ACO: Aminocyclopropanecarboxylate oxidase

PPO: Polyphenol Oxidase

MAP: Modified atmosphere packaging

GAP: Good Agricultural Practices

GRAS: Generally Recognize As Safe

NAC: N-Acetyl Cysteine

CMC: Carboxymethylcellulose

BT: Batch Time

BS: Batch Size

GABA: Exogenous γ -aminobutyric acid

APPENDICES

APPENDIX 1: SHORT DESCRIPTIVE TITLE

APPENDIX 2: SHORT DESCRIPTIVE TITLE

