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Master's Degree in Sustainable Agriculture

Role and effectiveness of 1-MCP (Fysium®) in maintaining commercial quality of apples.

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Glossary

0. ABSTRACT

Nowadays, markets require the highest possible levels of apple quality. The aesthetic and the shelf-life properties of apples determine their economic value. Apples must be firm, crisp, with a proper size, gloss and with enticing colours. These features must be maintained by apples after several weeks of shelf-life or after long transports under non-optimal storage conditions. Huge amounts of products are wasted every year because they don't match these required characteristics.

This research aims to investigate a way to reduce these losses using 1-Methylcyclopropene (1-MCP) conveyed to apples in the form of Fysium®, a commercial product developed by *Janssen PMP*®. This product is applied in very little amounts (at ppm level) after apple harvest and before their storage. It allows to reduce apples production of ethylene thus implying a slowdown or, sometimes even a stop in the post-harvest ripening process and, therefore, a prolongation of postharvest life.

The orchards selected for this analysis were in the North-Eastern part of Italy, mainly in the region Trentino-Alto Adige. The data were collected in 2021.

The parameters considered for this analysis were cultivars, time of storage after the treatment, apples starch, apples ethylene content and apples firmness.

Two main results have been analysed and discussed:

1) Fysium® seems to have worked properly in all the varieties of apples.
2) Fysium® effectiveness seems to be related to the initial conditions (temperature of apples, temperature of storage chambers) and to the ripening state of apples at the time of the treatment.

It was ascertained that the storage techniques, based mainly on different atmosphere composition, when applied after the treatment contribute in a complementary way to the final quality of apples.

This research is a first step in a possible wider study to determine how it is achievable to foresee the final state of apple quality as a function of the maturity level reached at harvest, and of the storage room environmental parameters at the time of the treatment.

1. INTRODUCTION

1.1. Premise

Nowadays everyone talks, writes, and spreads the use of the word sustainability in all the possible fields where it can be inserted. I asked myself multiple times what is the meaning of sustainability and how it can be implemented in the real world without remaining an abstract concept which follows a trend. In these last two years, studying sustainable agriculture, I understood that sustainability could be reached in a multitude of ways and that its principle implies the use of resources at rates that do not exceed the capacity of the earth to replace them. Specifically, sustainable agriculture is the production of food, fibres, or other plant or animal products using farming techniques that are unaffecting the environment, public health, human communities, and animal welfare. According to Foodprint (n.d.) this form of agriculture aims to produce healthful food without compromising future generations' ability to do the same. This concept, translated, means to produce food in a less impacting way, increasing efficiency and avoiding wastes of resources. This is very difficult to be reached in practice in a world where population is continuously increasing while agricultural land per capita is decreasing year by year. According to global cropland area per capita was reduced over the period between 1961 and 2016 from about 0.45 hectare per capita in 1961 to 0.21 hectare per capita in 2016 (FAO, 2020).

One of the ways that I really see as a concrete and sustainable solution in reaching the objective of increasing food availability, according with the second Sustainable Development Goal (SDG) of zero hunger is to minimize losses of already produced food. We have, indeed, 1.4 billion hectares of land, that is 28 percent of the world's agricultural area, used annually to produce food that is lost or wasted. (FAO, 2023). This is a huge issue considering that we will need in the next years more and more land to support the growing population demand of food and hopefully to reduce malnutrition. Another important problem is food wastage's carbon footprint which is estimated at 3.3 billion tonnes of CO_2 equivalent of GHG released into the atmosphere per year. Also,

from an economic point of view the direct consequences of food wastage (excluding fish and seafood) run to the tune of $750 billion annually (FAO, 2023).

Therefore, it is of primary importance to invest in new technologies and methods that can lengthen products shelf-life and to improve post-harvest storage management.

Another factor that is contributing to reducing yields of most of crops is climate change with increasing temperatures, longer drought periods and more frequent extreme events as floods, high speed winds and hailstorms. The Intergovernmental Panel on Climate Change (IPCC) already in the models of 2014 in the AR5 reported an estimated reduction between 0 and 80 percent in the estimated average crop yields due to climate change over the 21st century. Properly for these forecasts of constriction in production and increase in pre-harvest losses it becomes even more important to reduce losses that happen after the harvesting phase. Amongst all the possible crops, I decided to investigate how to reduce post-harvest losses in apples. The reason why is not only driven by the fact that I live in an area where apples cultivation is widespread, but derives mainly by the importance of this fruit cultivation worldwide, in the past decades.

According to FAO (2022) in 2019 apples accounted for 10% of total fruit and vegetable production worldwide, being the fourth most produced fruit after tomatoes, bananas, and watermelons. The production increased from 59,130,404 tonnes in 2000 to 86,442,716 tonnes in 2020 while in the same period the area harvested with apples decreased from 5,462,740 ha to 4,622,366 ha. These data describe an increasing trend in yield through these years. According to FAO (2022) with an average production of more than 32 M tonnes per year between 2000 and 2020. China represents the main producer of apples worldwide, followed by US with 4.5 M, Turkey with 2.7 M, Poland with 2.6 M, Iran with 2.5 M and Italy at the sixth position with 2.2 M. These data demonstrate that apple is between the most consumed fruit in the world, and it is well known that its consumption, due to its low decay index and due to its high demand, happens throughout the year. Apples are a climacteric fruit species, so they can start ripening after harvest has already happened. Thanks to all these intrinsic features and

their direct consequences we can observe how apples can be a valid solution that can contribute in reducing hunger in the world, in line with the second Sustainable Development Goal (SDG) that aims to achieve zero hunger by the year 2030.

1.1. Post-harvest technologies: goals

The United Nations (UN) General Assembly declared 2021 as the international year of fruits and vegetables. This was done to increase awareness of the nutritional and health benefits of these products and their contribution to a balanced and healthy lifestyle. Another objective was, according to the UN, to draw attention on the need of reducing losses and wastes in the sector, while generating social, economic, and environmental benefits in the same direction with the Sustainable Development Goals (SDG).

A critical phase in fruits and vegetables losses is the *post-harvest* period. This is the time that passes between the harvesting and the retail sale of products. In developing countries more than 40% of the food losses occur at post-harvest and processing levels (Gustavsson, 2011). Therefore, it becomes more and more important to work on this phase to reduce losses and their multiple consequences as hunger, excessive land-use, resources waste and so on. The adoption of incorrect post-harvest strategies results in the loss of the product itself as well as of all the energy inputs. The energy inputs accounted for within the cropping systems included plant materials, fertilizer, pesticide, human labor, tractor diesel, irrigation pump electricity and diesel, the transport of fertilizer and pesticide, and the embodied energy of tractors and irrigation pumps. (Hall, 2011).

The main objectives of post-harvest management are to maintain the product quality through time, generate a value-added product and generate new market opportunities.

1.2. Respiration and transpiration

Most fresh fruits and vegetables are classified as perishable unless they have been processed in some way (Dauthy, 1995). This implies that after they are harvested, they pass through enzymic changes, chemical changes, physical changes, and biological

changes that affect their nutritional quality in processes of deterioration. Each fruit species has a different degree of perishability that directly influences its shelf life and its decay index. This index shows the maximum storage time possible for a variety that should coincide with its average potential life expressed in weeks. Apples, thanks to their low decay index, can be easily stored for 8-16 weeks, this time varies depending on the cultivar and can be lengthened through optimal storage conditions.

In fresh products, respiration and transpiration are among the processes that heavily influence the ripening phase, which is the final stage of development that occurs when the fruit has ceased growing, and it is ripe. After this phase, it begins the ageing phase, also called senescence, here the product is brought to a non-edible state.

The extrinsic factors controlling these processes (and so the rate of deterioration) are mainly temperature, water activity, gas atmosphere and light exposure.

Respiration is a basic reaction of all plant material, both in the field and after harvest (Food and Agriculture Organization of the United Nations, 1989). Fresh products cannot replace carbohydrates or water during post-harvest. So, in this phase they use stored starch or sugar until the reserves are exhausted (Food and Agriculture Organization of the United Nations, 1989). Post-harvest respiration of fruit causes various consequences. First, it is observed the loss of energy reserves, then a second effect is the decreasing of fruit nutritional value. There is also a loss in the weight of fruit, and so in the net commercial value. Secondary effects directly linked to respiration are the reduction of oxygen in the structures used for fruit storage and the increase of CO_2 released from the fruit into the storage chambers. Furthermore, respiration induces a temperature increase in the external environment (Becker & Fricke, 2002). Internal factors that regulate respiration rate in cultivars are genotype, development phase, respiration substrate, tissue water content, surface/volume ratio and pre-harvest factors. The first actions that can be taken to control it are selecting varieties with low respiration rate and planning the perfect time to harvest the fruit. External factors that increase fresh fruit respiration rate are mechanical damages, high temperatures, high O_2 and ethylene concentrations and low CO_2 concentrations (Becker & Fricke, 2002).

Apples are fruits with a very low respiratory activity which falls in the range between 5 and 10 mg CO_2/(kg*h).

Most fresh products contain from 65 to 95 percent of water when harvested (Food and Agriculture Organization of the United Nations, 1989). Transpiration is the loss of water, in the form of vapour, from the product surface when it is exposed to the air. The process is driven by a difference in water vapor pressure between the product surface and the environment (Becker & Fricke, 2002).

Fresh products continue to lose water after harvest, but unlike the growing plant they can no longer replace the lost water from the soil and so, they must use their reserves. The main consequences of this process are shrinkage and loss of weight (Food and Agriculture Organization of the United Nations, 1989) that result in loss of value in the market and, above all, waste of food. This reduces also external quality, firmness, and volume of the fruit. When harvested products lose 5 or 10 percent of their fresh weight, they begin to wilt and soon become unusable (Food and Agriculture Organization of the United Nations, 1989).

As for the case of respiration, transpiration rate depends on a series of internal and external factors. The first internal factor is indeed the same and consists in the genotype of the species, so it depends on the cultivar. The second factor is directly related to the first one and it is the tissue type. Then, transpiration depends on integrity and sanitary conditions of the product. Also, in this case it is very important to harvest at the proper time to have fresh products at the right state of development. Each species has a different transpiration coefficient that depends on these factors so this process can cause more problems to some species and less to others. For instance, lettuce and celery have very high transpiration coefficients, while potatoes and onions have very low values of this constant. Apples are among the products that have a very low transpiration coefficient due to their intrinsic and extrinsic characteristics. Their coefficient is about 4 mg/(kg*s*bar) which is very low compared for instance with grapes coefficient (12) or with lemons coefficient (19) or with lettuce coefficient (740).

External factors affecting transpiration are relative humidity (indirectly proportional), temperature (directly proportional), and air movement (causes increase in transpiration rate) (Becker & Fricke, 2002).
To conclude the most important thing to do to increase shelf life of fruit is to keep the rate of water loss as low as possible.

1.4. Climacteric and non-climacteric ripening

There are two types of fruit that behave differently during ripening phase: climacteric and non-climacteric fruit ripening. Non-climacteric fruit ripening refers to those fruits which ripen only while still attached to the parent plant (Kou et al., 2021). In this kind of fruit, the nutritional quality will be maximum if it is harvested when ripening has finished because their sugar and acid content cannot increase further. In this category of fruit respiration slows down during growth and after harvest. Maturation and ripening are a gradual process (Kou et al., 2021). Grapes, berries, and lemons are part of this category. In non-climacteric strawberries ethylene does not appear to play a major role in initiating or stimulating ripening of immature fruits (Kou et al., 2021).
Climacteric fruit ripening, on the other hand, refers to those fruits that can be harvested when they are mature but before ripening has started. In these categories the start of ripening is accompanied by a rapid rise in respiration and ethylene synthesis rate, called the respiratory climacteric (Kou et al., 2021). After this phase respiration rate decreases and fruit has usually reached a satisfying nutritional quality. Apples belong properly to the climacteric fruit ripening category, so ethylene plays an important role in their ripening process during the post-harvest phase.
For commercial purposes there is, as we can notice, a great advantage in climacteric fruit ripening, this derives by the fact that it is possible to delay or speed up ripening after harvesting the fruit. This means that ripening rate can be controlled allowing to carefully plan transport and distribution of fruit even a long time after harvest.

1.5. Role of ethylene

Ethylene is an olefine. This molecule, with the molecular formula C_2H_4, is a gas which is produced in plant and fruit tissues, and it is known to be a very important factor in starting fruit ripening (Kou et al., 2021). It is often used for inducing ripening of climacteric fruits as mangoes and bananas after their transportation in refrigerated containers at the immature stage. During post-harvest phase natural release of ethylene by fruits can cause serious problems because it can speed up ripening and senescence. In fruit species, such as the apple, it has been established that low ethylene production is correlated with long storage life (Pech et al., 2008). Ethylene induces degradation of chlorophyll, causing the yellowing of fresh products. This effect can be positive for products as apricots but should be avoided for products that require a green color to testify their freshness as lettuce and other vegetables. Mechanical damages that cause injuries to fruit can speed up ethylene production. The same happens when there are moulds attacks. In both these situations ethylene release can shorten the shelf life of the product during transport or storage. This is why attention should be taken in handling fruit and damaged or decayed products should not be stored.

1.6. 1-Methylcyclopropene molecule

1.6.1. Active compound

One of the possible solutions used for having better fruit and apples storage is the molecule 1-Methylcyclopropene, or 1-MCP, its molecular formula is C_4H_6. It is obtained through a synthesis in which methallyl chloride reacts with phenyllithium to form a cyclopropene with a side methyl group as a substituent. This molecule is often used because it has a conformation similar to that of ethylene, which as we anticipated in the previous chapter, is an essential molecule involved in starting and speeding up the process of fruit ripening. For this reason, having a molecule with the same conformation is essential, 1-MCP can indeed link to the same ethylene receptors in an irreversible way. This allows to interfere with the activation and release of the ripening signals, and,

blocking the transmission of these signals, it can delay the onset of ripening and senescence. The scheme of this process is showed in *Figure 1*.

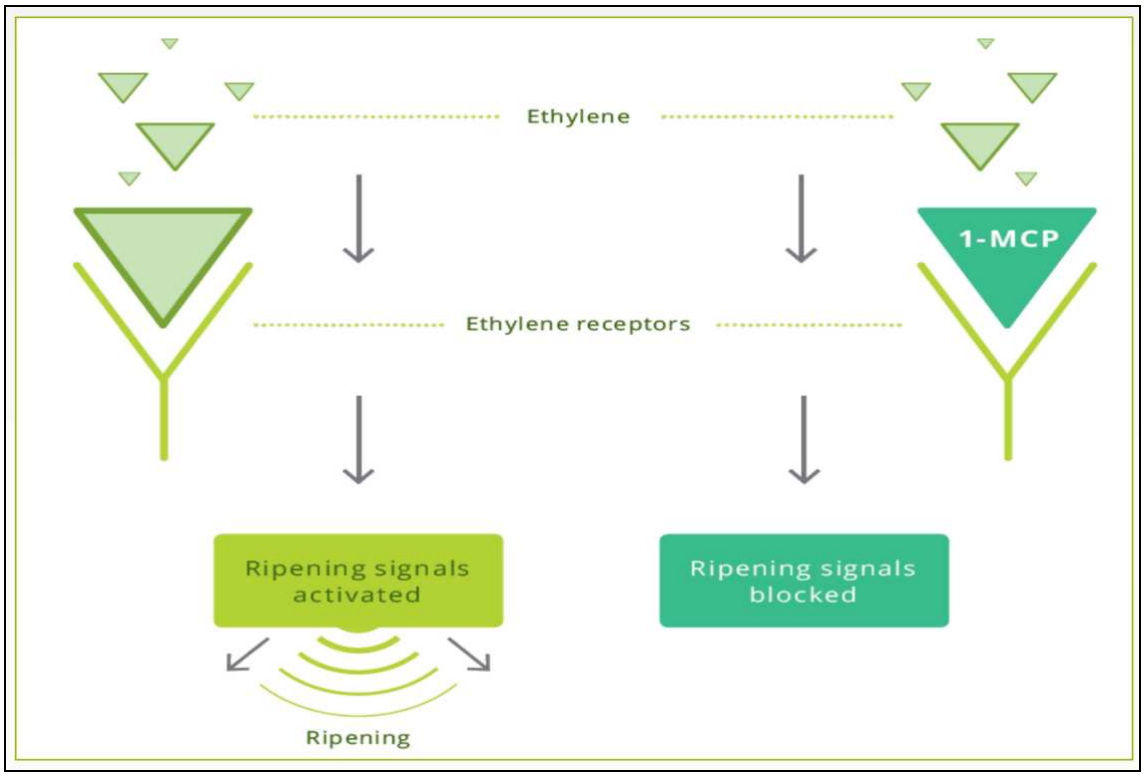

Figure 1 Process of 1-MCP reaction with ethylene receptors (Janssen PMP®, 2018).

1.6.2. History of 1-MCP

The discovery of 1-MCP was due thanks to Edward Sisler for this reason synthesized a cyclic diolefin with an attached diazo group, the diazo-cyclopentadiene (DACP), a gaseous molecule which was very reactive at low concentrations in inhibiting ethylene reaction. The main problem was, anyway, that it required exposure at fluorescent light to be effective. Furthermore this molecule was highly explosive and toxic and it had to be stored dissolved in hexane at -80 ºC (Blankenship S.M. & Sisler E.C, 1993) After this discovery Sisler with his colleague Sylvia Blankenship had the intuition that just one component of the many released from DACP breakdown was the active principle

responsible for the ethylene inhibition, this was properly the 1-MCP. At this point Sisler freely provided other researchers with samples of this compound. Some of them reconfirmed the efficacy of this gas in preventing the adverse effects deriving from ethylene action. Given the good efficacy data in ornamentals Sisler sought companies working with the ornamentals industry to license 1-MCP and to finance its further development and registration. Then, Staby persuaded Sisler and Blankenship to license 1-MCP for food as well as ornamental crops and worked with them in accomplishing the product's EPA registration (Reid & Staby, 2008). Data of that period confirmed the low animal toxicity of the product and its use at low rates. This helped a lot in spreading its use for ornamentals at first and then for fruit and in particular apples storage. Nowadays, a lot of protocols for the use of 1-MCP have been developed and the effectiveness of this gas is witnessed in many scientific studies worldwide.

1.7. Fysium

1.7.1. Patent & commercial product

One of the protocols developed for the diffusion of the molecule 1-MCP was developed by *Janssen PMP®* (Protection and Material Protection), a division of *Janssen Pharmaceutica NV*, a society with it headquarter in Beerse, Belgium. The commercial name of the product is Fysium® and it is a system, covered by a patent, for the generation of the active principle 1-MCP *in situ*. This technology includes a generator and a cartridge (*Figure 2*). Inside the cartridge are present three components (A, B & C) in specific amounts. When these are linked together, they react to form the gas, which is produced when the cartridge is inserted in the generator. At this moment the process can start. To have the system working correctly apples must be exposed to the gas while they are maintained in closed areas as storage rooms, greenhouses, containers, cold rooms or in structures used for food storage under controlled atmosphere. The product is not suited for an open-space usage, and treatment areas must be sealed up to keep the gas inside, otherwise the treatment could lose efficacy. The packaging of the Fysium®

Generator also includes a Fysium® release tube which is an essential part of the treatment as it is used to connect the technology with the refrigerated chamber.

Thanks to the active principle 1-MCP, Fysium® technology confers several advantages to the apples during transport and storage phase. It helps in postponing the expiration date, maintaining firmness, reducing respiration, keeping acidity, delaying ripening and ageing. It also reduces incidence of greasiness of apples peel. From an economic point of view, Fysium® allows companies to schedule the deliveries of apples and to have a greater homogeneity of the product. Furthermore, losses are reduced significantly as it will be shown in the data in the next chapters.

Figure 2 Fysium® technology composed by generator (below) and cartridge (above) to generate 1-MCP in situ (Janssen PMP®, 2018).

1.8. Smartfresh™

A second protocol developed before than Fysium®, and very diffused in the field of post-harvest storage is Smartfresh™. It is an *Agrofresh* product which is very similar to Fysium® for certain aspects, while it differs in the way it is applied and, in the services, offered by the agency that are included within the treatment. The first thing to be noticed is that the active principle comes from the same active ingredient which is 1-Methylciclopropene. In Smartfresh™ it constitutes the 3.3% of the total components. Smartfresh™ is a powder that, when mixed with water in a specific generating system releases the volatile active ingredient (1-MCP). Smartfresh™ can be used immediately after harvest, prior to storage, prior to shipment and/or just prior to sale. Smartfresh™ is effective under both cool (below 13°C) and warm (above 13°C) temperature conditions. To have the best results out of its usage in controlling senescence, products should be treated as soon as possible after harvest. As for Fysium® fruits must be treated in storage chambers or closed rooms which preferably have a controlled atmosphere, and which are sealed up. Leakage, indeed, causes a decrease in the effectiveness of the treatment.

The application of Smartfresh™ is different from the one of Fysium®. It implies the use of generators that are provided by the firm in two sizes (large and small). The choice of the size depends on the amount of Smartfresh™ powder that needs to be used that depends on the amount of fruit that must be treated. The large generator, anyway, is chosen when the necessary powder is greater than 18 grams. So, after the chamber is filled with fruit, and the room has been checked (no air losses, proper bins positioning), the proper generator must be placed on a stable surface of the chamber. The optimal position would be within the air flow of the internal refrigeration system, so that the gas would be spread over the entire chamber. At this point the generator can be started, and the proper amount of water can be added for the reaction to start (8 liters are necessary for the big sized generator, 0.8 liters for the small one). The water can be inside the range of temperature that goes from 20°C to 40°C. At this moment must be removed the appropriate size Smartfresh™ water soluble pouches from the protective foil packets

and the pouches must be dropped into the water contained in the generator. It is needed, then, to leave immediately the storage area and to seal the door up to contain the 1-MCP vapor and ensure the maximum efficacy out of its application. The release of the gas into the area will start several minutes after the water-soluble pouch is added to the generator. As for the Fysium® process a sign must be hanged on the outside walls of the chamber to warn workers and visitors not to open the door in any case for the following 24 hours. Otherwise, the treatment would be compromised. Meanwhile, inside the chamber, the air must circulate by the means of the internal air movement system for at least one hour. At the end of the treatment period the internal ventilating system should work for further 30 minutes with the doors of the storage chamber opened to change the air inside and to allow to operators to enter in it. This period is called Restriction Enter Period (REI).

1.9. Storage practices

There are, nowadays, a series of practices and precautions known and applied to reach an optimal fruit storage. One of these is the pre-refrigeration of fruit. This is a set of techniques applied to achieve a rapid cooling before transport and storage. It allows to reduce post-harvest losses related to high emissions of water vapour, to lower sensitivity to parasitic attacks, to increase resistance to mechanical injuries, and to maintain a higher quality and greater commercial potential in foreign markets.

Other advantages achieved with this process are an extended marketing period, the possibility to postpone harvesting time and to save refrigerated space and energy for storage. Among the mostly used cooling methods are water, ice, and air. Water and ice are good options because of their high conductivity and heat capacity. Air is less effective from this point of view but, on the other hand, is much used for the better ventilation of fruit. Also vacuum can be a way to improve storage efficacy because with a lower pressure also the evaporation point is lowered and so, less water is lost.

Even the method of applying mediums can differ: the pre-cooling can be done in a cold room, with a cold wall, or with forced air into storage containers. Hydro-refrigeration

can be done through sprinkling or flooding systems. When ice is used, the fruit or the product can be completely immersed in it and this is called *body icing* or ice can be put only as a cover on the fruit and this is called *top icing (Elansari et al., 2019)*.

Another important parameter to be kept into consideration for optimal fruit storage is temperature, which usually falls into a range between -1° and 4° (Fenton et al., 2019). Other more sophisticated and advanced storage practices are applied, some of them will be showed and analysed in the next chapters of this analysis. Another widespread way to better control fruit post-harvest storage is the adoption of controlled atmosphere inside the storage chambers. Oxygen (O_2) and carbon dioxide (CO_2) are the two compounds most controlled because they directly affect respiration. As anticipated to reduce respiration rate both O_2 and CO_2 should be kept at very low levels that for fruit usually fall inside the range between 1 and 15 percent. They cannot be completely removed by the chambers because this would imply the death of fruit once respiration has ceased. The controlled atmosphere consists also in the management of the percentages of relative humidity for keeping a very low transpiration rate (Yahia et al., 2019).

1.10. Storage room management

It is of primary importance for the effectiveness of the treatment to have the products stored in a suitable environment. That is the reason why in this section are gathered some essential information to producers or to entities dealing with storage to have the best conditions that allow an effective treatment to be applied.

First, correct filling of the storage chamber ensures correct air distribution. The uniformity of the chamber filling is essential for a correct distribution of 1-MCP that is released during Fysium® treatment. The minimum space to leave between the bins in the room is 45-60 cm on the same wall where the evaporators are positioned, on the opposite wall to them and 50-70 cm between the roof of the chambers and the upper part of the bins, leaving 10-15 cm on the sides of the chamber. Efficiency is improved by closing the space between the limit bottom of the evaporators and the top of the

nearest bins. In this way the air flows in the right direction. When placing the bins, it is also necessary to make sure that its uniformity is respected, not placing any row separately from the others. The prevention of re-circulation areas and the guarantee of uniform air flows throughout the chamber can be obtained with the appropriate use of vehicle tarpaulins.

Another important aspect is the uniformity of the products, it is strongly suggested to treat apples or fruits that come from the same variety and are uniform in size, weight, ripening state and all their main characteristics. Very heterogeneous lots can respond differently to treatments with Fysium®. On ripened fruits 1-MCP may not only have zero effect but losses of effectiveness may also occur in fruits of the same lot that would respect the ideal conditions.

As anticipated in the previous chapters, the natural metabolism of fruits can be slowed down by the variation of atmospheric gases. It can happen a risk related to this variation that is the appearance of physiological disorders in apples. One of the main components that can cause this kind of problems due to the high sensitivity of various cultivars is the CO_2. 'Kidd and West' (Kidd et al. 1927) showed that low O_2 and high CO_2 atmospheres inhibited the respiration climacteric and were beneficial for long-term storage of apples (Reid & Staby, 2008). However, it was not until 1962 that Stanley and Ellen Burg demonstrated that the physiological and biochemical basis of these effects was not simply a reduction in respiration, but also an inhibition of the production and action of ethylene (Stanley P. Burg, Ellen A. Burg, 1962) (Reid & Staby, 2008). For these reasons, during the first month of storage it is recommended to keep the CO_2 concentration at less than 1%. The criticality of the potential CO_2 damages appears to be wider during these early stages of storage. In the following period, indeed, it is often possible to raise the concentration up to 2%. Cooling, like increasing CO_2, plays a fundamental role in the correct storage chamber management. The correct lowering of temperatures in a storage chamber is critical to reduce potential dehydration and damage from cold. Also, for thermal management it is necessary to investigate the best solutions based on the variety that is treated. Damages from cold occur mostly in the cases of

very hot summers. This is due to the high thermal difference that is registered when fruits enter in the storage chambers. 1-MCP is a molecule that can increase the symptoms of these damages, that is why particular attention must be taken. Often, a progressive cooling is the proper solution to decrease the effects due to this problem. Another aspect to be kept into consideration is to avoid the overfilling of the chamber, respecting its cooling capacity.

One of the main damages from cold that can occur because of a too fast cooling process is cold browning. This is an internal damage, so its symptoms can be observed some millimeters below the peel toward the central part of the pulp (Berti, 2018). At an early stage, only a slight radial browning is often observed. Later, the symptomatology becomes more pronounced and can also be recognized from the outside, through the peel. In general, the appearance of this type of damage can occur, in the presence of storage temperatures that are too low, on all varieties (Berti, 2018). A similar damage that can be confused with this, but with different causes and symptoms is the internal browning from storage in controlled atmosphere CA. CA is one of the two main storage regimes which is used to guarantee an optimal storage of apples. It is a technique that controls the atmosphere components and their percentages with the aim of slowing down fruit respiration and its consequent senescence and decay. CA storage linked to low temperatures allows to store fruit for longer periods. This is due to the lower respiratory activity of apples given by low oxygen concentrations and higher carbon dioxide concentrations. Furthermore, this techniques allows to reduce the production of ethylene which, as anticipated in the previous chapters, plays an important role in ripening of climacteric fruit. CA storage technique is often associated with the applications of ULO conditions, that means *Ultra Low Oxygen* conditions, which has O_2 values between 0.8% and 1.0%.

Damages from internal browning from storage in CA begin in the inner part of the pulp and when the intensity increases, their spread is observed towards the peel. Internal browning from CA storage is variety-specific and it is caused by excessive concentrations of CO_2 in the room atmosphere and/or a too rapid application of CA

storage parameters. The latter situation can also favor the development of externally visible damages, such as the skin necrosis; they are characterized by a partial depression of the skin and occur, for the most part, on the less colored side of the fruit (Berti, 2018). The main cause is due to an imbalance in the O_2/CO_2 ratio, in which the concentration of O_2 is too low, while that of CO_2 is excessive. Great influence on the appearance of these symptoms is attributed to the variety and to the environmental conditions, among which the respiration intensity at the time of placement of the fruits in the chamber is an important factor.

Vitrescence, often associated with late harvest, is a disorder generally reabsorbed by the fruit during cold storage. The appearance of vitrescence is facilitated by very favorable photosynthetic conditions in pre-harvest. This state leads to an increase in the production of sugars that at night, due to lower temperatures, are not entirely disposed of during the breathing process, and are, therefore, in excess The symptoms of this disorder happen in numerous areas of the pulp of the fruit (depending on gravity) with an appearance translucent and glassy (Berti, 2018). There can be some problems with using 1-MCP on fruits with this disorder. In these cases, it will be difficult to reabsorb the characteristic, it is therefore recommended to store apples with a normal refrigeration process with a gradual cooling. Furthermore, it can be helpful a time deferral in the activation of CA storage parameters.

Other storage physiological disorders that may appear on apples after harvest are related to the superficial browning of the epidermis (defined as *superficial scald*) which, however, does not extend to the underlying tissue. Depending on the stage of ripening, the areas of the fruit in which the symptoms occur and the combination with other damages, it is possible to identify different types of scald whose symptomological pictures are often not optically distinguishable from each other (Berti, 2018). These types of scald are properly called common or superficial scald, senescence scald, diffuse scald, and sun scald. The strategy to prevent the appearance of different types of scald provides for a combination of agronomic measures to be implemented in the field, as well as the choice of optimal storage conditions. Basically, it is possible to avoid the

onset of common and senescence scald by harvesting the fruits at the optimal time, which must be adapted to the duration and conditions of preservation, respecting the conditions of good shelf life of the fruits, and maintaining, during storage, low O_2 values. Storage under ULO conditions (O_2 0.8-1.0 %) allows the appearance of common scald to be contained (Berti, 2018). The diffuse scald can be counteracted only with a gradual cooling of the fruits and postponing the treatments with 1-MCP, while the appearance of sun scald can be prevented with a careful varietal choice and with the use of anti-hail nets that reduce the risk of solar burns and, consequently, also the possible onset of scald alone during storage. DCA, or *dynamic controlled atmosphere*, is another storage techniques that guarantees great results against different types of apples browning due to scald. This technique consists in varying oxygen percentages in the storage room, reaching very low values (0.3-0.5%) during the stress periods that can vary between 8 and 18 days according to the different cultivars and to other parameters such as ripening state, temperature of the product and atmosphere values. In this way the storage time of fruits can be lengthened without compromising qualitative and nutritional aspects and without making use of chemical agents. It must be taken into consideration the fact that fruits under hypoxia conditions change their metabolism, and they favor the formation of ethanol. If this molecule reaches too high levels inside the fruit this can compromise its nutritional properties, so this is why the atmosphere is managed dynamically and it is essential to keep under control the levels of O_2 and CO_2 in the management of the process. This technique is often associated with ILOS (*Initial Low Oxygen Stress*) storage technique which involves the controlled accumulation of alcohol inside the fruit to control scald. This latter system is based on periodical analysis of the ethanol dissolved in the juice of apples to maintain its minimum predetermined levels throughout the whole storage period.

1.11. Waxing

To have the full potential of storage realized there is another practice that is, in certain cases, necessary to adopt, this is properly waxing. Waxing was applied in the United States since 1920 for citrus and then extended in 1950 on other kind of fruits and vegetables to overcome the long distances of distribution which at that time were travelled without refrigerated containers. Each fruit, and apple, is covered by a natural waxy cover that protects it from external agents as fungi, insects, or bacteria. Then this barrier is hydrophobic and thus it repels water keeping the inside part safe. It also decreases gaseous exchanges and evapotranspiration losses increasing consistence and slowing ageing processes.

Furthermore, this cover reflects harmful ultraviolet rays, and it keeps natural integrity of fruits after physical damages. The problem is that this natural and "primordial" film is often lost or partially removed by the industrial processes that aim to wash the epidermis of apples. To overcome this issue many producers chose to apply coating agents that artificially substitute the natural waxy film. These agents have similar properties and functions of the natural film and, furthermore, they significantly reduce the respiration process in the fruits and so, their ageing. This reduces the loss of weight, the problems of wilting, the degradation of its components. Other advantages of these agents are the inhibition of rot growth, reduction of oxidation and reduction of pitting. If coating agents are made with ethanol they can also act as disinfectants against bacteria as listeria. Then waxing is often required by some markets because it conveys to apples more brightness and attractiveness (*Figure 3*).

This film is necessary for long distance transport of fruits like for overseas shipments. Another case where these agents are very helpful is for long-time storage of products, and when the products must be kept for some time under normal environmental conditions for selling (for instance in some markets).

It is of primary importance to have at least an overview on waxing process because this thesis has the aim of understanding how to reduce food losses, and in this peculiar case apples losses. Sometimes the only way to reduce losses having at the same time high

quality products over the whole year is to combine different strategies adopting all the available technologies for an optimal storage over the long period.

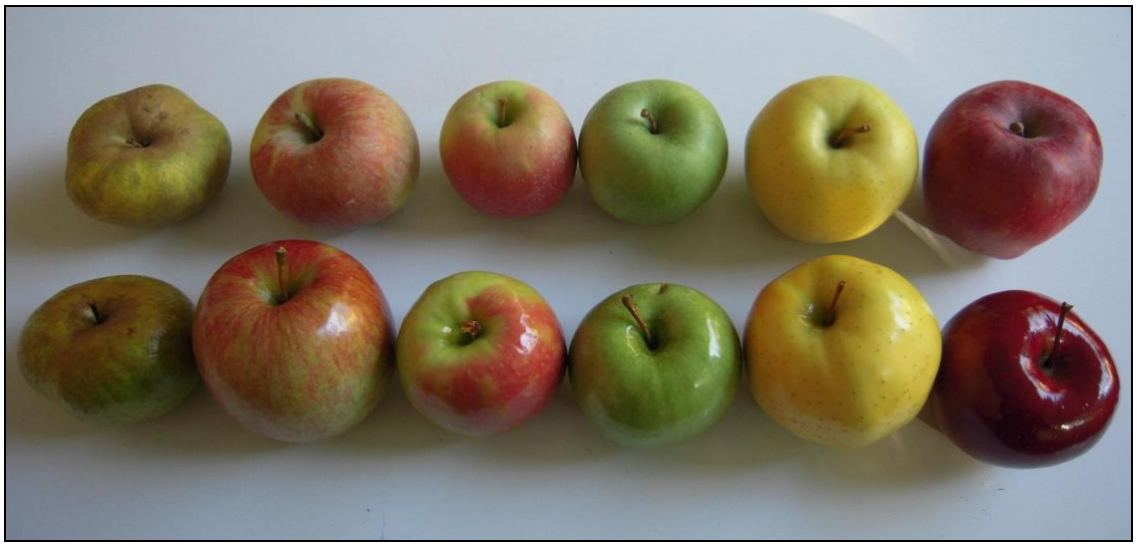

Figure 3 Difference in the aesthetic appearance of waxed (below) and non-waxed (above) apples of some of the main varieties (Retarder, 2019).

2. AIMS OF THE STUDY

The main aim of this thesis work is to investigate the effectiveness of 1-Methylcyclopropene (1-MCP) on different apples cultivars. The molecule efficacy depends on the methodology and on the means utilized. Given this premise, this investigation will give an evaluation specifically and solely on Fysium® application path. There are several factors, anyway, that contribute to the high or low effectiveness of the treatment. The main factor, as explained in the previous paragraphs, is the initial condition of the apples that are about to be treated that can be referred to as their maturity level. This "status" is determined analysing specific parameters of apples such as their starch content, their firmness, and their ethylene content after harvest, or better, just before the treatment. So, more in detail, the aim of the study is to investigate the correlation between the 1-MCP treatment applied through Fysium® and the final status of the apples while keeping into consideration the initial conditions of the apples, the conservation environment (the storage chamber) and the time of opening of the chambers. It will be of primary interest to understand which of these parameters have the greatest influence on the result.

It would be a great achievement if, in the future treatments, more attention was paid on this aspect, optimizing the process and thus, reducing post-harvest losses.

3. MATERIALS AND METHODS

3.1. Dataset sources

The data analyzed in this thesis were collected by Retarder®, the official and exclusive distributor of Fysium® in Italy. This company entered a five-year contract with *Janssen PMP*® and, thanks to this, each year it buys the product, distribute it to the Italian regions where it is required and links with the local industries and producers that need and buy the treatment. Furthermore, the company guarantees to the buyers the service of monitoring the storage chambers that are treated with Fysium® in two ways.

The 1st way is the analysis of the apples that is performed at two different moments: 7-10 or 20-25 days after the treatment (based on the variety), and at the opening of the chambers before the shipment of the product, this varies based on the demand.

There is, then, the evaluation of the air inside the storage chambers that is performed to assess if the treatment was successful and to verify if 1-MCP was present. This is performed immediately after the treatment through the collection of a sample of air in a plastic bag by the applicators. During the storage period further air samples are collected indicatively each 45 days to evaluate the ethylene concentration.

Thanks to these processes of monitoring there is a database collection year by year that can be used for statistical analysis to evaluate the effectiveness of the product and to investigate possible solutions to increase this effectiveness through a more specific management of the harvest and post-harvest phases.

Given that Fysium® technology usage in Italy is quite recent (introduced in 2018) in the first years its spread was limited by the older and more known technology Smartfresh™. This fact implies that the dataset deriving from the first period of applications are not sufficient to perform a solid statistical analysis. The first and only year with a great collection of data from the most diffused Italian cultivars is the year 2021. The year 2022 has the greatest number of data but these are still not completely collected and so they are not useful for this analysis. This is the reason why the next chapters will take into consideration the dataset deriving from the year 2021.

3.1.1. Excel spreadsheet summary of data

The data from the year 2021 were stored into an excel spreadsheet. To summarize, for each storage chamber, the data and information that were collected are:

- date of treatment
- warehouse indication
- number of the storage chamber
- volume of the storage chamber
- apple variety
- pre-treatment apples temperature (°C)
- pre-treatment storage chamber temperature (°C)
- pre-treatment starch content
- pre-treatment firmness (Kg/cm^2)
- content of 1-MCP (ppm) after the treatment
- ethylene synthesis after the treatment
- date of apples 1^{st} analysis (usually about 1 month after the treatment)
- ethylene synthesis of treated and non-treated apples at 1^{st} analysis
- firmness of treated and non-treated apples at 1^{st} analysis
- decay (shelf life at environmental T°)
- date of storage chambers opening (2^{nd} apples analysis)
- ethylene synthesis of treated and non-treated apples at 2^{nd} analysis
- firmness of treated and non-treated apples at 2^{nd} analysis

To perform the statistical analysis, in this research, the storage chambers and the warehouses weren't identified to guarantee the anonymity.

3.2. Groups selection parameters

To have a solid analysis the excel file with the collection of the totality of data was split into several spreadsheets, according to the following three parameters.

The first selection of data was performed creating a spreadsheet for each cultivar. The cultivars considered were the ones with a significant amount of data: Gala, Pink Lady, Fuji, Red Delicious and Granny Smith.

This was done because each variety reacts to the treatment in a different way and it has peculiar characteristics, thus analysing the data altogether would have brought to misleading results. This division, nevertheless, has not been considered sufficient to have homogeneous samples for the analysis.

The second parameter taken into consideration for the division of data was the starch content detected at the time of the treatment. The three classes of starch content were not selected in the same way, but according to the cultivar. Each variety has, indeed, specific starch content requirements at the pre-treatment analysis to complete the process under optimal conditions. In most of the cases, anyway, this second division led to only two classes of starch content that were: low content of starch or medium content of starch. These two classes for simplicity are referred to as S1 (for low starch content) and S2 (for medium starch content).

This means that local farmers harvested apples that had already started their ripening processes and, sometimes, the harvest was done too late, resulting in apples with non-optimal storage parameters (low starch content means that most of it already got converted into sugars as explained before in the *starch degradation* section).

The third parameter for a further selection of data was individuated in the opening time of the storage chambers. This was very important for the evaluation of the efficiency of the treatment. Only through the evaluation of data of apples stored for a similar amount of time the results could be considered comparable and reliable. The three classes of storage time considered were used for the 2nd analysis done in the same period. The class was T1 if the 2nd analysis was done within 3 months after the treatment, T2 if the 2nd analysis was done in the range between 4-6 months after the treatment and T3 if the 2nd analysis was done in the range between 7-9 months after the treatment.

Given these three parameters the selection resulted in the creation of 13 excel spreadsheets, each one containing only one variety, similar starch content and similar

time of storage under controlled atmosphere. At this point it was possible to perform a statistical analysis to evaluate whether the treatment has been effective.

The treated and non-treated samples were considered as two different samples and the analysis was done to evaluate if they came from the same population (null hypothesis) or if they came from two different population (alternative hypothesis). If they came from the same population, and so, if the null hypothesis was accepted, then the treatment would have been considered ineffective. If, on the other hand, the null hypothesis was rejected, and so the alternative hypothesis was accepted, then, the two samples derived from two different populations, and this would have meant that the treatment was effective.

The test which has been chosen was a 't-test' for two samples with unequal variances. The parameters considered for the test were ethylene synthesis (related to the content) and firmness of apples at the 1st analysis (7-10 or 20-25 days after the treatment) and at the 2nd analysis (at the opening of the storage chambers, some months after the treatment).

3.3. Ripening indicators for choosing whether to treat

3.3.1. Pre-treatment sampling

Quality control was done through the analysis of samples that were randomly taken in different points of the lot that was under inspection. Immediately after this, the producer brought the apples in the storage chamber, and the sampling procedure began. This was done to verify if it was possible to perform the treatment, because under certain conditions it would have not been effective to do it.

At least 15 apples were taken (3 per different bin) and they were inserted in bags. It was controlled that the apples taken did not have particular imperfections due to the sun or caused by insects or fungi. The bag was marked with the name of the warehouse and that of the lot to locate it in order to validate the sampling. Then the bag was marked with the wording "firmness". The same procedure was carried out with another set of 15 apples that were marked with the wording "starch".

3.3.2. Firmness calculation

The next step of the test was the calculation of the average firmness of the apples in the lot using a penetrometer. This measure was based on the applied pressure necessary to insert a piston with selected diameter for a determined depth inside the fruit. It was used a handheld penetrometer keeping the angle in which the pressure was applied at 90° and it was applied a light and uniform pressure.

To sample it was taken the bag with the wording "firmness". The values were measured at the two opposite sides of the fruit, in the case of bi-coloured fruit the firmness was measured first on the most coloured side (the one previously exposed to the sun) and then on the less coloured one. To prepare the measure it was necessary to connect the piston with the correct diameter (of 11 mm for apples) to the penetrometer and then to peel a little disk of surface (only the skin) where it is required for the measure. Then it was set to zero the penetrometer and it was applied a light and constant pressure keeping firm the apple and going in deep until the piston had reached the marked zone. Then the penetrometer could be removed, and the result could be read and registered.

This procedure was used also by the Retarder® specialized laboratory for the 1st and 2nd analysis to calculate apples firmness both of treated and untreated samples.

3.3.3. Starch degradation

Starch degradation was measured using a solution of lugol containing iodine. Iodine turns indeed into a blue-black colour if it is exposed in contact with starch. With the proceeding of ripening less starch is present and so a less expanded area of blue-black is observable and evident.

There are several models to evaluate this change in colour. The one used in this thesis work were the radial types. This test is particularly suited for apples. It can be used, anyway, only to determine the phase of ripening after harvest but not during the successive storage period because starch tends to decrease physiologically during the storage phase and not due to maturation causes.

The solution used for this measurement was prepared by dissolving 10 g of potassium iodide (KI) in 30 ml of distilled water and then adding 3 g of molecular iodine (I2). After the dissolution of the iodine (I2), distilled water was added at 10 ° - 30 ° C up to a total of 1 litre. To prepare the measurement it was taken the bag with the wording "starch" then each fruit was cut in half in the equatorial zone. Cutting surfaces must be clean. Then, the lugol solution was sprayed on the cut surface with a pipette or a sprinkler, the apples could also be immersed in a tray as an alternative way. Then there was to wait a minute to have the right time for the solution to be effective. After this time the index of starch degradation could be registered. There are various scales of reading. There is, for instance, the Washington standard scale from 1 to 6 or the Ctifl apple starch regression table from 1 to 10 (OECD Quality Guide of the fruits) this second scale of reading is shown in the *Figure 4* below and it is the one that was utilized in this analysis. If in the scale high values were observed (7 to 10) it meant that there was low starch content and most of it already turned into sugars (got degraded). If, on the other hand, the scale showed low values (less than 6), it meant that most of the starch was still present inside the apples, and apples were still in the beginning phase of ripening. The pre-treatment starch content data for each sample was the average of the 15 apples collected as explained in the paragraph 3.3.1.

It was of primary importance to be careful on the interpretation of the models because each apple variety had a different behavior towards starch production and degradation.

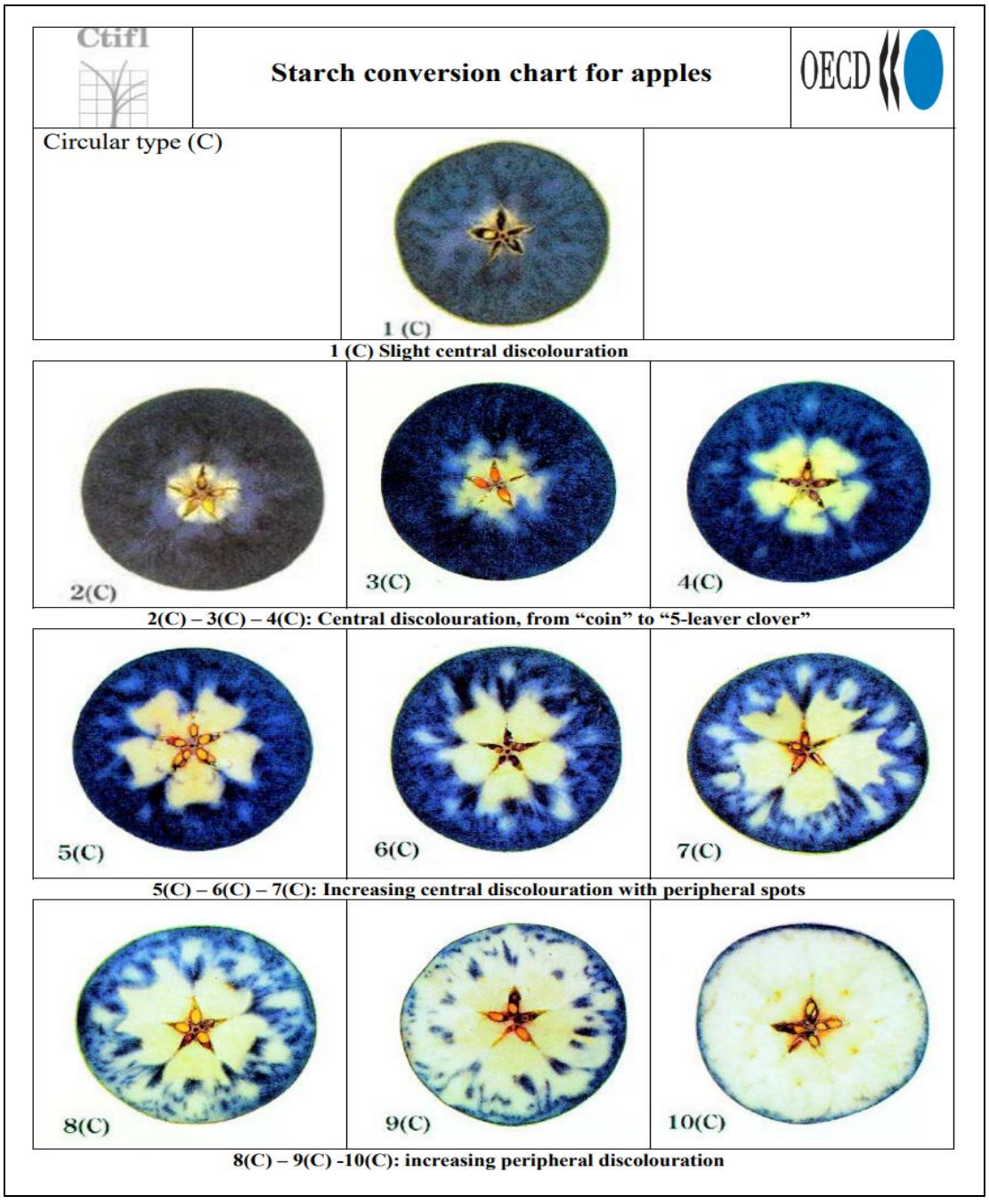

Figure 4 OECD circular type starch conversion scale for apples (https://www.oecd.org/agriculture/fruit-vegetables/publications/guidelines-on-objective-tests.pdf).

3.3.4. Conditions for starting the treatment

First, it was necessary to annotate the values obtained from the measurements of the firmness of the fruit and of the starch degradation index. Then, these values were compared with the industry standards adopted for the use of 1-MCP or, if this is not reported, they were compared with the standard scales used by the industry for the calculation of optimal ripeness for long-term storage of apples. If the firmness and the starch index of all 30 fruits analysed were within the right interval, then the lots could be successfully treated with Fysium®. If 3 or more apples did not fall inside these intervals, it was necessary to withdraw a second sample, if this still was not in the adequate interval then the lot could not have the right requirements to be treated and the Fysium® application was refused because it was probable that it would not have reached the expected results without a proper fruit ripeness. Only if the producer had understood and accepted the risks and he assumed the responsibility, Fysium® could be used. In case of immature or overripe fruit, the results of the post-treatment application test could differ from the results that would be expected if the fruits were within the ripening parameters suitable for treatment. In the *Figure 4* below it is possible to observe the suited intervals for starch index and firmness for the main apple varieties.

Another essential condition for treating was to have ethylene content tending to zero ppm inside the storage chamber. To avoid the burden of performing an air analysis for each storage chamber before the treatment, a simple method was used. Every time, before Fysium® application, the storage chamber was ventilated to guarantee the absence of ethylene inside it. Otherwise Fysium® would have been useless because the apples receptors would have been already occupied by the ethylene as it can be observed in *Figure 1* (paragraph 1.6.1.).

Cultivar	starch (Ctifl 1-10)			firmness (kg/cm2)		to treat within (days)
Gala	5	-	8	>	7	7
Golden Delicious	6	-	8	>	6.5	7
Granny Smith	4	-	7	>	7	7
Cripps Pink / Rosy Glow	5	-	8	>	7	7
Red Delicious	4	-	6	>	6.5	5
Fuji	7	-	9.5	>	7.5	7
Modì	6.5	-	9	>	8	7
Annurca	5.5	-	7.5	>	7.5	5
Ambrosia	6	-	8	>	6.5	7
Morgenduft	7	-	9	>	6.5	5
Pinova / Evelina	6	-	8	>	6.5	7
Stayman / Winesap	4	-	5.5	>	6.6	5

Figure 3 Intervals of expected starch content and firmness for the best result in the treatment within the following days (Janssen PMP®,, Fysium® user instructions, 2018).

3.4. 1-MCP applications

The desired environmental concentration of 1-MCP is 0.650 ppm. This is the quantity required for the best efficacy of the product. The amount of component A and component B to generate this concentration depended on the volume of the room. This was calculated multiplying its length for its width for its height in meters (m) to obtain a volume in (m^3). The supplier company (Janssen PMP®) calculated the exact amount of the components required to fill the cartridge for the treatment of a specific volume. For rooms with a volume greater than 2800 m^3 two Fysium® generators were used contemporarily. Fysium® was spread in the storage chambers immediately after the harvesting phase, preferably within 7 days from harvest. Nevertheless, Fysium® could also be used before storage and before shipment. For best results, it was avoided the use on apples previously treated with products for the acceleration of ripening. For an optimal use of the product, it was applied exclusively on high quality fruit that has been refrigerated suddenly after harvest. The fruits were at about 4-5°C while they are being

treated. Furthermore Fysium® was applied before the climacteric peak of respiration has been reached and before the peak of ethylene production has been reached. Once the Fysium® generator was connected through the tube with the inside of the chamber the process could start, after 15 minutes from pressing the button (pre-gassing phase) the gas was produced and released inside the chamber for 105 minutes (gassing phase). So, overall, the process took 2 full hours to get completed and then it stopped automatically at the end. After this time the generator was shut off, the cartridge removed and suddenly closed, the tube was cleaned through aeration (through a pump) and the chamber was kept closed for another 24 hours. This was necessary so the gas could penetrate the fruit and reach the ethylene receptors. During treatment the storage chamber was ventilated inside to guarantee a correct spreading of the gas in every direction, a homogeneous contact of 1-MCP with all the fruit. After a whole day (24 hours) from the ending of the treatment, before entering the chamber, it was opened and ventilated with open doors for 30 minutes. The treatment was considered not valid if the doors of the chamber were opened before the prescribed time.

3.5. Post applications management

For long-term storage, Fysium® guarantees better results when used with apples at optimal ripeness. After application, apples were stored according to standard good practices, in refrigerated or controlled atmosphere storage environments. To keep apples healthy and of high quality, the general chain maintenance rules were followed. Cold was kept at all stages and producers strictly adhered to prescribed phytosanitary practices. The temperature of the fruits was decreased from the starting 4-5°C of about 0.5°C every 3 days, until the core had reached a temperature of about 1.5°C. This process was slower in some cases, as for instance when apples needed a longer period to adapt to the cold environment because their starting temperature was higher.

So, the final temperature of the chambers after this slow reduction was kept constant at about 0.8-1.2°C, with a relative humidity always greater than 93%. Regarding the controlled atmosphere, O_2 was kept between 0.5% and 2.0% and CO_2 in the range

between 0.8-2.0%. The specific final values of O_2, CO_2, relative humidity (R.H.) and the storage conditions used for each cultivar are reported in table 1 below. (*Table 1*)

Variety	1-MCP	Storage technique	Parameters	Annotations
Gala	Yes, always	CA, controlled atmosphere with ULO	O_2: 1.2-1.5 CO_2: 1.6-2.0 T°: 0.8-1.3 R.H. > 93%	$CO_2 > O_2$ Rifts with high R.H.
Red Delicious	Yes, always	DCA, dynamic controlled atmosphere with ILOS	O_2: 0.5-0.6 CO_2: 0.8-0.9 T°: 0.8-1.2 R.H. ± 95%	For long storage (> 3 months) apply at least 3 stresses
Granny Smith	Yes, always	CA, controlled atmosphere with ULO	O_2: 1.2-1.5 CO_2: 1.0-1.2 T°: 1.0-1.5 R.H. 90 / 95%	Low oxygen long storage
Fuji	Yes, always	CA, controlled atmosphere with ULO	O_2: 1.0-1.3 CO_2: < 1.2 T°: 0.8-1.2 R.H. > 90%	Different values of the parameters if there is vitrescence
Pink Lady	Yes, always	CA, controlled atmosphere with ULO	O_2: 1.5-2.0 CO_2: 1.0-1.3 T°: 4 to 2.0-2.5 R.H. > 93%	Cool slowly, first up to 4 °C then in 14 days flat up to 2.0 - 2.5 °C, then put in air conditioning and maintain.

Table 1 Storage conditions used for the main cultivars treated with Fysium® (author re-elaboration starting from the data by *Janssen PMP®, Fysium® user instructions, 2018).*

3.6. Ethylene analyses

The pre-treatment data were collected directly by the applicators. On the other hand, the further analysis used for the monitoring of the chambers were performed by a specialized laboratory inside Retarder® validated by the Department of Agricultural, Forest and Food Sciences of the University of the Studies of Turin. The data analyzed by the Retarder® laboratory were the ethylene synthesis by apples and their firmness at the 1st and 2nd analysis.

Ethylene analyses were performed on a sample of 8-10 apples according to the maximum amount that could enter in the container (it was used always the same container for each analysis). For each chamber was analysed the same number of apples treated with Fysium® and untreated. The ppm of ethylene at the 1st and 2nd analysis referred to the amount of ethylene released by the apples inside the container in a predetermined amount of time that varied based on the variety. These values are reported in *Table 2* together with the shelf-life spent by the apples before the 1st analysis.

VARIETY	SHELF-LIFE (days)	TIME OF ETHYLENE RELEASE (h)
Gala	7-10	2
Pink Lady	7-10	2
Fuji	20-25	4
Red Delicious	7-10	2
Granny Smith	20-25	4

Table 2 Shelf-life of apples before 1st analysis and time spent inside the containers during the ethylene release analyses (Janssen PMP®, Fysium® user instructions, 2018).

3.7. Heat maps development

For evaluating the effectiveness of the treatment with Fysium® heat maps were created for each cultivar for the 1st and the 2nd analysis.

To evaluate ethylene biosynthesis difference between treated with Fysium® and untreated samples it was used the following formula:

Ethylene ratio = (Untreated - Fysium®)/Untreated

Untreated stands for the synthesis of ethylene (in ppm) of the samples that did not receive the treatment, *Fysium*® stands for the ppm of ethylene synthetized by the treated samples. The values obtained in a scale from 0 to 1 showed how much Fysium® had been effective. If the values were close to 1 it meant that Fysium® had been towards 100% of effectiveness, if they were lower the effectiveness was less and if they were towards 0 than Fysium for that specific storage chamber had low effectiveness. A scale of colors was developed to have immediately an overview of the chambers where Fysium® was effective in inhibiting the production of ethylene.

To evaluate firmness the most interesting result to be investigated was the difference in firmness between treated and non-treated samples. It was used the following formula for evaluating the firmness difference between the samples:

Firmness Ratio = (Fysium® – Untreated) / Fysium®

Fysium® stands for the average firmness (in kg/cm2) detected on the treated samples, *Untreated* stands for the average firmness (in kg/cm2) detected on the samples that did not receive the treatment. The values obtained in a scale from 0 to 1 showed the increase in firmness given by the treatment. If the value obtained was for instance 0.12, it meant that the treatment had maintained the average firmness for that storage chamber 12% higher than it would have been without its application. As for the ethylene

synthesis, also in this case a scale of colors was developed to guarantee an immediate overview of the chambers where the treatment had been effective.
For both ethylene synthesis and firmness, the scale of colors chosen went from red if the effectiveness was low (closer to 0 in both the cases) to green if the effectiveness was high (higher values, closer to 1).

3.8. Charts development

To have more clear and easily observable the effectiveness of the treatment some further charts were developed in this analysis. These were very useful to individuate immediately the storage chambers were treated and untreated samples had very close values (meaning that the treatment had low effectiveness) or very far (meaning that the treatment had a great incidence on the final result).
The parameters considered in these charts were the ethylene synthesis and the firmness of apples at the two different steps (1^{st} and 2^{nd} analysis) for treated and non-treated samples deriving from the 13 groups.
The charts showing these results are reported in the Supplementary Figures section below (*Supplementary Figure SF1* to *Supplementary Figure SF52*). The names of the samples in the charts refers to the simplifications explained before, where S refers to the starch class (S1 means low starch content, S2 means medium starch content and S3 means high starch content) , and T refers to the storage time class for the 2^{nd} analysis (T1 means the second analysis was done between 0 and 3 months, T2 for 3 to 6 months and T3 for 6 to 9 months).

4. RESULTS

4.1. T-test: firmness and ethylene biosynthesis

From the vertical analysis performed using the t-test to evaluate the effectiveness of the use of Fysium®, the 13 selected groups showed the same results. In all these groups, indeed, the samples treated with Fysium® were significantly different from the ones not treated according to the t-test results. This brought to the rejection of the null hypothesis of coincidence, and so, to the acceptance of the alternative hypothesis. This meant that the treatment was 100% effective on all the cultivars where it was applied and, on both the analyses, regardless the time passed after the treatment (considering periods longer than 7-10 days for the 1st analysis). Another important result that was observable is that the treatment worked adequately for both the starch classes S1 and S2, classified on the bases of the apples initial starch content.

It was also relevant to consider that not a single type of superficial scald was detected in any of the storage chambers treated with Fysium® along the year 2021.

4.2. Tables and charts interpretation

From the developed heat maps (*Table 2 to Table 22*) and from the charts reported in the Supplementary Figures section (*SF1* to *SF52*) are reported in this section some general observations derived from the complexity of data and regarding all the samples without any distinction based on the cultivar or on the other initial parameters.

The heat maps made very easy to observe how the treatment with Fysium® worked for almost the totality of the chambers, reaching 100% at the 2nd analysis. At the first analysis there was only one chamber with a negative value for ethylene synthesis (the 49th sample of Gala). This sample had a low pre-treatment starch content that was not optimal for the treatment success. Even in this case, anyway, at the 2nd analysis the heat map showed a positive value, meaning that Fysium® worked properly. A general observation related to the heat maps is that they made clear (observing at the gradations of colors) how Fysium® was generally more effective at the 2nd analysis. This seemed to be because, at the 1st analysis, very often the untreated samples had low values of

ethylene and high firmness. On the other hand, samples that were not treated tended to deteriorate over the long period (at the 2nd analysis), while the treated apples maintained their quality longer and it was verified that this was due to Fysium®.

The charts made immediately clear that the curves related to the samples treated with Fysium® (in orange) and the ones related to the untreated samples (in blue) were always sharply separated. This was a confirmation of the results of the t-test which validated that the two samples both for the ethylene synthesis and for the firmness were significantly different. A second consideration could be done observing the *Y* axis of the charts related to the ethylene synthesis by apples. In this case the ppm of ethylene synthetized by the apples at the 1st analysis (7-10 or 20-25 days after the treatment depending on the variety) were relatively low for most of the storage chambers (there were some outliers in the untreated samples of *Supplementary Figures SF1*, *SF9*, *SF33, and SF37*). The curves of the samples treated with Fysium® and the untreated ones were already sharply separated but the effects of the treatment sometimes could be low and difficult to appreciate as for instance in *Supplementary Figure SF45* and *SF49.*

On the other hand, considering the ppm of ethylene synthetized by the apples at the 2nd analysis (which coincide with the opening of the storage chambers) the difference between treated and untreated samples were much more prominent, as a confirm of the observations made previously on the heat maps.

In this latter group of charts, the ppm of ethylene synthetized by the treated samples were quite low (apart from 2 exceptions: 28th sample of *Figure SF14* and 20th sample of *Figure SF42*) and could be almost approximated to zero compared to the ppm of ethylene synthetized by the untreated samples. In these charts related to the ethylene synthesis at the 2nd analysis the two curves were, indeed, even more sharply separated and this fact implied that the effect of Fysium® was much greater and more visible over the long period.

Looking at the charts reporting firmness of treated and untreated samples it was possible to evaluate how in this case the curves were much more stable, and they did not contain outlier for both the 1st and the 2nd analysis. As for the ethylene charts, also in this case

the two curves were sharply separated. Anyway, a peculiar characteristic of the charts related to the firmness was that they didn't show a marked difference between the 1st and the 2nd analysis regarding the distance between treated and untreated samples. This means that Fysium® in this latter parameter seemed to be already effective after 7-10 or 20-25 days from the treatment.

4.2.1. Gala

The first cultivar analyzed through a qualitative evaluation is Gala. This variety is sensitive to internal browning, wound rot, and sun damage. It has, on the other hand, low sensitivity to scald. The application of Fysium® may accentuate the sun damages with a darkening of the color in the burned area. For this variety, the optimal range of pre-treatment starch content within applying the treatment is 5.0-6.0 and the firmness should be equal or higher than 6.8-7.0 kg/cm^2 according to the 2019 Fysium® Dossier. The sugars content for an optimal ripening state should be 11.0° BRIX or more.

The cultivar was divided into 2 groups for the statistical analysis: S1T2 and S1T3. The totality of the samples, indeed, had average values of pre-treatment starch content above 6.0 giving as a result only groups belonging to the S1 category (low starch content, according to the selection of the classes used in this research and explained in the Materials and Methods section, paragraph 3.2.). The two groups S1T2 and S1T3 showed some similar characteristics: ethylene synthetized by the treated samples was very low compared to the one synthetized by the untreated apples, this was more easily observable at the 2nd analysis (at the opening of the storage chambers) there were some peaks of ethylene synthetized by untreated samples while the treated ones tended to zero ppm, as in *Supplementary Figures SF2* and *SF6.*

Considering the heat maps the average ethylene total inhibition given by the treatment for this cultivar was 60.58% at the 1st analysis and 60.00% at the 2nd analysis (*Table 5).*

Looking at *Supplementary Figures SF3, SF4, SF7 and SF8* it was observed how firmness values tended to be more stable with less peaks for both treated and untreated samples. The differences between the 1st and the 2nd analyses didn't seem to be so

marked, and between the treated and the untreated samples the gap remained quite similar for both the analyses: it was between 0.5 and 1.5 kg/cm^2 on average. The only difference was detected in the absolute values that were quite lower at the 2nd analysis (as expected for the longer storage time).
Considering the heat maps the average firmness increase given by the treatment for this cultivar was 13.00% at the 1st analysis and 12.72% at the 2nd analysis (*Table 6*).

4.2.1.1. S1T2

In the S1T2 group the whole of the storage chambers gave as a result ethylene synthesis values tending to 0 ppm for the samples at the 2nd analysis treated with Fysium®. This fact was true also for the cases where the untreated samples had values above 60 ppm.
At the 1st analysis in 4 storage chambers, it was noticed that the ethylene synthesis by the treated samples was lower than the one of the untreated samples by less than 1 ppm (8th, 16th, 47th, and 48th sample of *Supplementary Figure SF1*). Looking for a reason of this low effectiveness of the treatment (that could be also appreciated on the heat map of *Table 4*), the pre-treatment apples temperatures were investigated. In two of these 4 samples (16th and 47th sample of *Supplementary Figure S1F1*) this result seemed to be due to the initial temperature of the apples that were, respectively, 17°C and 9.64°C (the optimal temperatures to treat with Fysium® should have been about 4.0-5.0°C). In the 47th sample also the starting temperature of the chamber was quite high (8.1°C). For the other two samples the starting temperatures of apples and chambers were inside the optimal ranges, so these results were probably due to other causes.
In another storage chamber the ethylene synthetized by the treated sample was even higher than the one of the untreated sample (17.6 ppm treated versus 14.2 ppm of the untreated one) (49th sample of *Supplementary Figure SF1)*. This latter result could be explained by the temperature of the apples that entered in that storage chamber that was on average 12.66°C. The chamber temperature was also higher than the optimal, before the treatment it was 7°C.

Considering firmness, the actual pre-treatment firmness values were always higher than 7.0 kg/cm^2 apart from one storage chamber where the detected firmness was 6.9 kg/cm^2. The differences in firmness at the 2nd analysis between treated and non-treated groups were always equal or above 0.4 kg/cm^2 apart from two chambers where it was equal to 0.3 kg/cm^2 and one storage chamber where it was equal to 0.1 kg/cm^2 (4th, 11th, and 24th samples of *Supplementary Figure SF4*). The starting temperatures of these samples were, respectively 12.7°C, 8.84°C, and 9.2°C partially explaining the lower effectiveness of the treatment (observable for these samples on *Table 5*).

This result was confirmed by the one of the 1st analysis, where differences in firmness between treated and untreated samples were equal or higher than 0.4 kg/cm^2 in all the chambers apart from two of them where they were equal to 0.2 kg/cm^2 (13th and 39th sample of *Supplementary Figure S3*). Their starting temperatures were 7.86°C and 9.66°C partially explaining also in this case the lower effectiveness of the treatment (observable for these samples on *Table 5*).

4.2.1.2. S1T3

Considering the group S1T3 similar results were confirmed at the 2nd analysis. All the storage chambers showed ethylene values for the treated samples tending to 0 ppm, and difference in firmness between the two groups of samples was always equal or higher than 0.5 kg/cm^2. At the 1st analysis the difference of synthetized ethylene between treated and untreated samples was always significant. It was noticed, as for the first group, that the values of synthetized ethylene were higher for both the samples and not always tending to zero for the treated ones. The differences in firmness were equal or higher than 0.5 kg/cm^2 in most of the cases, with only 2 chambers where they were equal to 0.3 kg/cm^2. In these two samples (59th and 64th samples of *Supplementary figure SF7*) the starting temperatures were, anyway, in the right ranges. The treatment at the 2nd analysis, indeed, became effective (*Table 4*).

From this qualitative analysis it could be observed, in general, that for Gala cultivar an initial low starch content didn't seem to be a really determining factor for the final

quality of the samples treated with Fysium®, at least in terms of ethylene synthesis at the opening of the chambers. Another consideration which is worth to mention is the fact that the synthesis of ethylene seemed to be higher at the 1st analysis for both the groups, without affecting, anyway, the firmness detected at the 2nd analysis.

GALA		ETHYLENE		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,95768569	T2	0,21875
2		0,99313433		0,33096927
3		0,81578155		0,33027523
4		0,97028172		0,52380952
5		0,72627917		0,30046948
6		0,30643774		0,99305205
7		0,11645696		0,30845771
8		0,05443108		0,7062635
9		0,51256		0,96340909
10		0,29964645		0,9399069
11		0,32173088		0,52511416
12		0,24749808		0,95932967
13		0,33514399		0,21226415
14		0,62222768		0,58490566
15		0,93664094		0,70526316
16		0,09670195		0,95683453
17		0,38135082		0,99876314
18		0,33688651		0,99552573
19		0,48847942		1
20		0,78195151		1
21		0,44430869		1
22		0,72068403		0,20192308
23		0,83048959		0,98906049
24		0,79718113		1
25		0,90554787		0,94814815
26		0,74913019		0,82608696
27		0,1786788		0,44767442
28		0,84317803		0,376

29		0,83100951		0,23880597
30		0,93917438		0,83001531
31		0,82224336		0,46212121
32		0,88429659		0,45138889
33		0,65338852		0,44144144
34		0,41093575		0,71307301
35		0,37035939		0,33613445
36		0,98639802		0,99494119
37		0,99668498		0,97848669
38		0,76502019		0,58571429
39		0,64205428		0,18285714
41		0,40293434		0,99497025
42		0,99596853		0,99457522
43		0,99532022		0,98813685
44		0,45170331		0,25396825
45		0,17505975		0,30656934
46		0,90352382		0,57560976
47		0,58059453		0,18072289
48		0,01310453		0,95572354
49		-0,2351184		0,34913793
50		0,26795856		0,2139738
51		0,79766331		0,96620016
52		0,61755819		0,41891892
53	S1	0,17245394	T3	0,35294118
54		0,66469598		0,525
55		0,9346174		0,20833333
56		0,9552332		0,48739496
57		0,49009375		0,99105424
58		0,89879025		0,13461538
59		0,4565885		0,51633987
60		0,14308047		0,78694158
61		0,96080912		0,17901235
62		0,58317418		0,15833333
63		0,9108935		0,67422096
64		0,73210863		0,96588001
65		0,3768037		0,97612893
66		0,37361296		0,33838384
67		0,67298081		0,47560976
68		0,97689623		0,36950147

69		0,50716038		0,41666667
70		0,95307722		0,08839779

Table 3 Gala heat maps for ethylene synthesis at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

GALA		FIRMNESS		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,1038961	T2	0,07462687
2		0,26315789		0,08823529
3		0,15151515		0,14516129
4		0,14473684		0,015625
5		0,15068493		0,09375
6		0,07142857		0,08955224
7		0,13235294		0,140625
8		0,13235294		0,09677419
9		0,17910448		0,09090909
10		0,18461538		0,11666667
11		0,19354839		0,05
12		0,11428571		0,05797101
13		0,02739726		0,21212121
14		0,21428571		0,14754098
15		0,21126761		0,07352941
16		0,05714286		0,11940299
17		0,16		0,15873016
18		0,09230769		0,15
19		0,18461538		0,1
20		0,14285714		0,09859155
21		0,07317073		0,11111111
22		0,06756757		0,1
23		0,14102564		0,08823529
24		0,17567568		0,04545455
25		0,12903226		0,06451613
26		0,1875		0,11666667

27		0,13235294		0,08823529
28		0,16666667		0,11111111
29		0,13157895		0,12307692
30		0,17948718		0,13559322
31		0,08219178		0,13333333
32		0,08450704		0,09836066
33		0,1025641		0,109375
34		0,10606061		0,08064516
35		0,16949153		0,19298246
36		0,08695652		0,24324324
37		0,12121212		0,13513514
38		0,06153846		0,22727273
39		0,02739726		0,10606061
41		0,13235294		0,22727273
42		0,19047619		0,09230769
43		0,28787879		0,20634921
44		0,12328767		0,15625
45		0,09090909		0,20895522
46		0,19444444		0,15625
47		0,06756757		0,12903226
48		0,1969697		0,203125
49		0,03225806		0,13333333
50		0,20289855		0,11111111
51		0,14084507		0,14925373
52		0,13513514		0,10769231
53	S1	0,05970149	T3	0,10294118
54		0,10144928		0,09677419
55		0,12162162		0,22222222
56		0,16666667		0,1875
57		0,07462687		0,13559322
58		0,12820513		0,11290323
59		0,19736842		0,15873016
60		0,04477612		0,12307692
61		0,14864865		0,13114754
62		0,08974359		0,18461538
63		0,17333333		0,18333333
64		0,12658228		0,09230769
65		0,04054054		0,19402985
66		0,09859155		0,07692308

67	0,06944444	0,14285714
68	0,13157895	0,07692308
69	0,13924051	0,10606061
70	0,1375	0,13846154

Table 4 Gala heat maps for firmness at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE GALA ETHYLENE Inhibition		
	1ST ANALYSIS	2ND ANALYSIS
S1T2	0,58898707	0,64226946
S1T3	0,6535039	0,4802642
TOTAL	0,60581755	0,60000722

Table 5 Average ethylene synthesis inhibition for Gala cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE GALA Firmness increase		
	1ST ANALYSIS	2ND ANALYSIS
S1T2	0,13593242	0,12374812
S1T3	0,11386775	0,13702224
TOTAL	0,13017642	0,12721094

Table 6 Average firmness increase for Gala cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

4.2.2. Pink Lady

Pink Lady is the second most represented cultivar of this research. It is sensitive to internal browning, wound rot, sun damage. It has low sensitivity to scald. Treatment with Fysium® alone is not able to control post-harvest rot to which the variety is sensitive, especially in the case of a rainy harvest period. For this variety, the optimal range of pre-treatment starch content within applying Fysium® is 5.0-7.0 and the firmness should be equal or higher than 7.0-7.5 kg/cm^2 according to the 2019 Fysium® Dossier. The sugars content for an optimal ripening state should be 12.0° BRIX or more.

The cultivar was divided into 3 groups for the statistical analysis: S1T1, S1T2 and S2T3. In the first two groups, then, the pre-treatment starch content was on average lower than the optimal with values relative to the scale always higher than 7.0.

In the third group the starch was within the right range of application of the treatment for all the chambers with values of the scale between 5.0 and 7.0. Then, it was expected that the third group was the one showing the best quality of apples at the 2nd analysis, keeping into consideration, anyway, the time class related to the opening of the storage chambers that was T3 for the third group (storage chamber opened 6 to 9 months after the treatment). The first observable characteristics regarded synthetized ethylene, and it was common to the 3 groups (S1T1, S1T2 and S2T3).

Considering the heat maps the average ethylene total inhibition given by the treatment for this cultivar was 73.08% at the 1st analysis and 94.68% at the 2nd analysis (*Table 9*). The best result belonged to the group S2T3 with an average ethylene reduction of 96.12% at the 2nd analysis (*Table 9*).

Looking at the charts in the supplementary figures, samples treated with Fysium® had always lower values of ethylene than untreated ones, even in the cases of very high values in the untreated apples (as in 4th sample of *Supplementary Figure SF18*).

Considering the heat maps the average firmness increase given by the treatment for this cultivar was 9.36% at the 1st analysis and 10.34% at the 2nd analysis (*Table 10*).

4.2.2.1 S1T1

In the group S1T1 ethylene synthesis values at the 2nd analysis for the treated samples were significantly different from the ones of the untreated samples. Only in one storage chamber the average ethylene value synthetized by the treated sample overcame 10 ppm. This result was compared, anyway, with the untreated sample, where it was detected the synthesis of 112 ppm of ethylene. At the 1st analysis the same result regarding ethylene synthesis was confirmed without any peculiar case.

Concerning the pre-treatment firmness, it was always higher than 7.0-7.5 kg/cm^2 (inside the optimal range for the application of the treatment). At the 2nd analysis the differences in firmness between treated and untreated samples were equal or higher than 0.4 kg/cm^2 for 20 chambers out of 21, one had a firmness difference of 0.3 kg/cm^2. This result was confirmed also at the 1st analysis with firmness differences always equal or higher than 0.4 kg/cm^2.

4.2.2.2. S1T2

In the group S1T2 the results concerning ethylene synthesis at the 2nd analysis were almost the same of the 1st group, except from one storage chamber where the synthesis was about 26.5 ppm for the treated sample (to be compared with a concentration of about 48.5 ppm for the untreated sample). These results about ethylene synthesis were confirmed also at the 1st analysis without any exception.

Concerning the pre-treatment firmness, as for the first group, it was always higher than 7.0-7.5 kg/cm^2. At the 2nd analysis the difference in firmness between treated and untreated samples was always higher than 0.4 kg/cm^2. The same results were detected at the 1st analysis with differences always higher than 0.4 kg/cm^2.

4.2.2.3. S2T3

Regarding the group S2T3 the results were similar to the previous ones with ethylene concentrations at the 2nd analysis tending to zero for 5 out of 5 treated samples.

At the 1st analysis all the chambers had ethylene values tending to zero ppm for the treated samples.

Concerning pre-treatment firmness, it was always higher than 7.0-7.5 kg/cm^2. The differences in firmness at the 2nd analysis were equal or higher than 0.4 kg/cm^2 for 6 out of 6 storage chambers. A similar result was confirmed at the 1st analysis with firmness differences equal or higher than 0.4 kg/cm^2 for 5 out of 6 chambers (only one with 0.2 kg/cm^2, the 37th sample of *Supplementary Figure SF19*, but this was probably related to the low ethylene production of the untreated sample, considering that the starting temperature of the apples was inside the optimal range).

It was interesting to notice that in this group the class of time was T3, meaning that the 2nd analysis was performed 6 to 9 months after the treatment with Fysium®. Regardless the longer storage time, the detected values of firmness at the 2nd analysis were always higher than 7.0 kg/cm^2 and, in 4 out of 5 chambers, they were equal or higher than 7.6 kg/cm^2.

This latter observation allowed to confirm that, when apples were treated under an optimal ripening state, after a proper treatment with Fysium® was done, and a correct storage was performed, their final quality could be maintained high even after several months. It was kept into consideration, anyway, that this fact could be true only for Pink Lady's cultivar where it was detected. There is the need to evaluate its validity through the analysis of data coming from other cultivars before extending this consideration to all apple's varieties.

PINK LADY		ETHYLENE		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,85610092	T1	0,96042029
2		0,51275917		0,96509555
3		0,45454545		0,97317252
4		0,95150729		0,90860412
5		0,54212356		0,97669321

6		0,91825106		0,98689852
7		0,41768263		0,98636037
8		0,9295109		0,95886511
9		0,17277574		0,96159492
10		0,95491833		0,96209208
11		0,98960217		0,98753571
12		0,84933157		0,97864889
13		0,99430692		0,96671314
14		0,98471967		0,96719127
15		0,96801701		0,93018767
16		0,62541528		0,98522486
17		0,98282803		0,93015787
18		0,99310159		0,89836133
19		0,33454766		0,9885824
20		0,79332936		0,77478862
21		0,98917609		0,98499833
22	S1	0,28345802	T2	0,99732403
23		0,97632171		0,83021853
24		0,01480638		0,98765497
25		0,94709596		0,980346
26		0,32661629		0,98741102
27		0,42830009		0,99572438
28		0,8911584		0,45403412
29		0,79314435		0,98304187
30		0,73593074		0,99286328
31		0,99335105		0,9913717
32		0,45065579		0,97992642
33	S2	0,37772926	T3	0,9871526
34		1		0,98281496
35		0,48583773		0,96036694
36		0,95119944		0,99390495
37		1		0,85629139
38		0,89953145		0,98698804

Table 7 Pink Lady heat maps for ethylene synthesis at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

PINK LADY		FIRMNESS		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,10126582	T1	0,06097561
2		0,12987013		0,07692308
3		0,16666667		0,08860759
4		0,05263158		0,12162162
5		0,11267606		0,10666667
6		0,1038961		0,08235294
7		0,11842105		0,0952381
8		0,14102564		0,12658228
9		0,14864865		0,16049383
10		0,12		0,06756757
11		0,07792208		0,03896104
12		0,06756757		0,06756757
13		0,06578947		0,02439024
14		0,05333333		0,13580247
15		0,04		0,11842105
16		0,09722222		0,12345679
17		0,1125		0,1
18		0,09459459		0,16
19		0,06493506		0,12
20		0,07407407		0,08974359
21		0,1125		0,12162162
22	S1	0,10666667	T2	0,13157895
23		0,12987013		0,08823529
24		0,08		0,17857143
25		0,1025641		0,15853659
26		0,05479452		0,11538462
27		0,09090909		0,09210526
28		0,12987013		0,08536585
29		0,05		0,11111111
30		0,09638554		0,13157895
31		0,1125		0,05405405
32		0,125		0,12
33	S2	0,09459459	T3	0,05714286
34		0,08219178		0,10526316
35		0,07594937		0,10843373
36		0,05063291		0,11688312

37		0,02469136		0,1025641
38		0,09638554		0,08860759

Table 8 Pink Lady heat maps for firmness at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE PINK LADY ETHYLENE INHIBITION		
	1ST ANALYSIS	2ND ANALYSIS
S1T1	0,77212145	0,95391366
S1T2	0,62189443	0,92544694
S2T3	0,78571631	0,96125314
TOTAL	0,73078124	0,94683216

Table 9 Average ethylene synthesis inhibition for pink lady cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE PINK LADY FIRMNESS INCREASE		
	1ST ANALYSIS	2ND ANALYSIS
S1T1	0,09788286	0,09938065
S1T2	0,09805093	0,115138373
S2T3	0,07074093	0,096482427
TOTAL	0,09364594	0,103484482

Table 10 Average firmness increase for pink lady cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

4.2.3. Fuji

Fuji is the third most represented cultivar of this research. It is sensitive to internal browning, wound rot, sun damage, and vitrescence. The problem of vitrescence can be difficult to overcome if apples are harvested after a rainy period. This variety has, on the other hand, low sensitivity to scald. For this variety, the optimal range of pre-treatment starch content within applying Fysium® is 7.0-9.0 and the firmness should be equal or higher than 7.5-8.0 kg/cm^2 according to the 2019 Fysium® Dossier. The sugars content for an optimal ripening state should be 12.0° BRIX or more.

The cultivar was divided into 3 groups for the statistical analysis: S1T1, S1T2 and S1T3. As it can be noticed there was only one class of starch, which was S1, the pre-treatment starch contents were, indeed, very low, as confirmed by the starch values of the scale that were always higher than 8.5 and in most of the cases even higher than 9 (almost all the starch was already turned into sugars before the treatment was applied). Ethylene synthetized by the samples treated with Fysium® and by the untreated ones seemed to follow a similar path to the one observed in Pink Lady for all the 3 groups (S1T1, S1T2 and S1T3).

Considering the heat maps the average ethylene total inhibition given by the treatment for this cultivar was 55.43% at the 1st analysis and 85.30% at the 2nd analysis (*Table 13)* with a difference of about 30% between the 2 analyses. There didn't seem to be any case where ethylene synthetized from treated samples was close to the values observed in the untreated one.

Considering the heat maps the average firmness increase given by the treatment for this cultivar was 8.49% at the 1st analysis and 14.00% at the 2nd analysis (*Table 14*).

4.2.3.1. S1T1

In the group S1T1, ethylene synthetized at the 2nd analysis for the treated samples was in most of the cases tending to zero ppm (in only one chamber it was about 3.4 ppm, while the untreated sample ethylene concentration was about 158.7 ppm). The

difference in ethylene synthetized by treated and untreated samples was significant for all the storage chambers also at the 1st analysis.

The detected firmness in the pre-treatment phase was always lower than the optimal values of 7.5-8.0 kg/cm^2. The differences detected at the 2nd analysis, anyway, were equal or higher than 0.6 kg/cm^2 in 8 out of the 9 storage chambers (in only one chamber it was equal to 0.4 kg/cm^2). At the 1st analysis these differences were thinner but in only two cases they were equal to 0.3 kg/cm^2 (in the 7th and 8th samples of *Supplementary Figure SF23*). In the 7th sample this seemed to be due to the pre-treatment temperature of the apples that was on average 8.57°C (far from the optimal).

4.2.3.2. S1T2

In the S1T2 group, ethylene synthesis of the treated samples at the 2nd analysis was always tending to zero ppm compared to the untreated samples. This was true also at the 1st analysis for all the chambers apart from one where the treated sample synthetized 1.7 ppm of ethylene versus 1.9 ppm synthetized by the untreated one.

The values of firmness detected in the pre-treatment phase were in most of the cases below the optimal range similarly as what was observed in the 1st group. At the 2nd analysis, anyway, the difference of firmness was 0.4 kg/cm^2 or higher for 18 out of 19 storage chambers (with only one chamber showing a difference of 0.3 kg/cm^2). At the 1st analysis in two chambers the difference in firmness was very thin (only 0.1 kg/cm^2), in one case it was equal to 0.3 kg/cm^2, but in all the other chambers it was equal or higher than 0.4 kg/cm^2. Between these cases, only the 12th sample of Supplementary Figure SF27 seemed to be related to the pre-treatment temperature of apples that was on average 7.11°C.

4.2.3.3. S1T3

In the S1T3 group, ethylene synthesis of treated samples at the 2nd analysis was tending to zero ppm for 4 out of the 5 storage chambers. Particularly, one treated sample synthetized about 2.2 ppm of ethylene, while the untreated one synthetized only 4.5

ppm with a little difference between the two concentrations. In this case the initial ripening state was far from the optimal, given that the pre-treatment starch content was 10 in a scale 1-10. This could probably be the reason why the treatment was not so effective in the inhibition of ethylene production. It was, anyway, comforting to observe that even in this storage chamber the difference in firmness at the 2nd analysis between the treated and untreated samples was 1.1 kg/cm^2. At the 1st analysis there wasn't any peculiar result with all the treated samples giving ethylene values tending to zero ppm.

The firmness detected in this group at the 2nd analysis fell in the range between 5.8-6.3 kg/cm^2 being considerably lower than in the other groups. It must be remembered, though, that this group belonged to the T3 class, so apples spent 6-9 months in the storage chambers before having their firmness detected. The treatment, anyway, seemed to have worked properly for the firmness: differences were detected, indeed, between treated and untreated samples, and these were equal or higher than 0.4 kg/cm^2 at both the 1st and the 2nd analysis.

It was noticed, in all the three groups, that the samples of Fuji had lower values of firmness compared to the ones of Pink Lady for the same storage time classes (coherently with the characteristics of the cultivars). Firmness detected in the samples of this variety had, on the other hand, similar values to the ones of Gala for the same storage time classes.

FUJI		ETHYLENE		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,9663624	T1	0,98101506
2		0,05490388		0,99541393
3		0,30571208		0,99252753
4		0,59401709		0,98255101
5		0,87866109		0,5
6		0,84585714		0,96669235
7		0,41110614		1
8		0,13428827		0,11524164

9		0,31572669		0,9787045
10		0,03820896		0,99101473
11		0,97249337		0,02590674
12		0,71751825		0,99527495
13		0,09120172		0,99303203
14		0,97448062		0,9881019
15		0,48887859		0,99726088
16		0,90609064		0,98788834
17		1		0,98441492
18		0,74752941		0,73318386
19		0,87034646		0,64130435
20	S1	0,61403509	T2	0,90226171
21		0,07692308		0,44805195
22		0,15537849		0,98950829
23		0,38543046		0,98889917
24		0,47933884		0,99688506
25		0,4045323		0,99821733
26		0,64570904		0,84818731
27		0,41819034		0,99674819
28		0,07356322		0,97308221
29		0,42036387		0,87206823
30		0,84650398		0,51344146
31		0,31650894		0,68592965
32	S1	0,73284692	T3	0,95924836
33		0,98666481		0,98535313
34		0,97653696		0,9970516

Table 11 Fuji heat maps for ethylene synthesis at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

FUJI		FIRMNESS		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,08196721	T1	0,0952381

2		0,109375		0,15625
3		0,12903226		0,28125
4		0,14754098		0,11666667
5		0,11290323		0,06349206
6		0,078125		0,22580645
7		0,0483871		0,07142857
8		0,0483871		0,109375
9		0,06666667		0,27118644
10		0,17460317		0,12068966
11		0,09090909		0,109375
12		0,01515152		0,08955224
13		0,08196721		0,15517241
14		0,09375		0,19047619
15		0,12698413		0,14754098
16		0,07692308		0,12698413
17		0,10169492		0,06349206
18		0,01666667		0,10169492
19		0,046875		0,20588235
20	S1	0,09230769	T2	0,20588235
21		0,0625		0,04918033
22		0,11764706		0,17241379
23		0,09677419		0,10526316
24		0,07692308		0,19298246
25		0,08196721		0,18333333
26		0,05882353		0,10144928
27		0,06349206		0,14754098
28		0,06557377		0,09836066
29		0,06666667		0,07142857
30		0,06349206		0,17741935
31		0,07575758		0,06349206
32	S1	0,08196721	T3	0,26984127
33		0,15873016		0,12068966
34		0,07692308		0,09836066

Table 12 Fuji heat maps for firmness at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE FUJI ETHYLENE INHIBITION		
	1ST ANALYSIS	2ND ANALYSIS
S1T1	0,45448437	0,85031608
S1T2	0,54957915	0,86106723
S1T3	0,77181232	0,82820484
TOTAL	0,55429145	0,85307242

Table 13 Average ethylene synthesis inhibition for fuji cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE FUJI ETHYLENE INHIBITION		
	1ST ANALYSIS	2ND ANALYSIS
S1T1	0,45448437	0,85031608
S1T2	0,54957915	0,86106723
S1T3	0,77181232	0,82820484
TOTAL	0,55429145	0,85307242

Table 14 Average firmness increase for fuji cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

4.2.4. Red Delicious

Red delicious is a variety sensitive to internal browning, rot of the heart, and bitter pit. It has also high sensitivity to scald that must be monitored together with mealiness.

For this variety, the optimal range of pre-treatment starch content within applying Fysium® is 4.0-6.0 and the firmness should be equal or higher than 6.8-7.5 kg/cm^2 according to the 2019 Fysium® Dossier. The sugars content for an optimal ripening state should be 10.0° BRIX or more.

The cultivar was divided into 3 groups for the statistical analysis: S1T2, S2T2 and S2T3. In the 1st group the pre-treatment starch content was low, with scale values higher than 7.2 for all the chambers. In the second and in the third group it was inside the optimal range or close to the optimal values of the scale (4.0 to 6.0).

Considering the heat maps the average ethylene total inhibition given by the treatment for this cultivar was 82.45% at the 1st analysis and 94.04% at the 2nd analysis (*Table 17*) with the best result showed by the group S2T2 at the 2nd analysis with 97.25% of average ethylene inhibition (*Table 17*). Looking at the heat maps regarding firmness, its increase given by the treatment for this cultivar was 14.19% at the 1st analysis and 17.53% at the 2nd analysis (*Table 18*). Red Delicious was, with these latter results, the cultivar which showed the best response to the treatment both in terms of ethylene inhibition and firmness increase.

4.2.4.1. S1T2

In the S1T2 group, ethylene synthesis of the treated samples at the 2nd analysis was always tending to zero ppm, being considerably different compared to the untreated samples. At the 1st analysis the same result was confirmed apart from one chamber that had ethylene values for the treated sample of 12.5 ppm compared with the untreated sample that had 63.7 ppm.

The values of firmness detected at the pre-treatment phase were in most of the cases inside the optimal range or higher. At the 2nd analysis the differences in firmness were

always equal or higher than 0.5 kg/cm^2. At the 1st analysis this result was even more marked with differences in firmness always greater than 0.8 kg/cm^2.

4.2.4.2. S2T2

In the group S2T2 the results concerning ethylene synthesis of the treated samples were far from the ones of the untreated samples for all the storage chambers. The same result about ethylene synthesis was confirmed at the 1st analysis for all the storage chambers.

The values of firmness detected at the pre-treatment phase were in most of the cases inside the optimal range or higher similarly to the 1st group. The difference in firmness at the 2nd analysis was 0.7 kg/cm^2 or higher for 8 out of 9 storage chambers (one chamber showed a difference in firmness of only 0.3 kg/cm^2). At the 1st analysis the differences in firmness were equal or higher than 0.6 kg/cm^2 for all the storage chambers apart from one case where it was equal to 0.2 kg/cm^2. In both the chambers with low differences in firmness the pre-treatment temperatures were inside the optimal ranges.

4.2.4.3. S2T3

Inside the group S2T3 in three out of 10 storage chambers the ethylene synthesis detected for the treated samples was higher than usual. They were properly 9.9 ppm (versus 86.0 ppm of the untreated sample) 13.9 ppm (versus 84.0 ppm of the untreated sample) and 27.6 ppm (versus 130.7 ppm of the untreated sample). This fact could be linked, anyway, to the T3 class of this group, considering the normal behavior of Red Delicious cultivar when it is conservated for more than 6 months. This fact was, indeed, confirmed by the results of synthetized ethylene at the 1st analysis that was always lower for both the untreated and the treated group, with the latter tending to zero ppm in all the samples.

The values of firmness detected in the pre-treatment phase were in most of the cases inside the optimal range or higher similarly to the other groups for this cultivar. The difference in firmness at the 2nd analysis was 0.5 kg/cm^2 or higher for the totality of the

storage chambers. In three chambers of this group the difference in firmness detected was considerably great (2.2 kg/cm^2, 1.9 kg/cm^2 and 1.7 kg/cm^2). The same results were detected at the 1st analysis with differences in firmness between treated and untreated samples always equal or higher than 0.5 kg/cm^2. Another general consideration for this group is that, even with higher values of synthetized ethylene, the difference in firmness was always significant, being in some cases higher than in other groups.

RED DELICIOUS		ETHYLENE		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,80253952	T2	0,87714444
2		0,97274666		0,97983167
3		0,99715882		0,9896418
4		0,92806975		0,97490296
5		0,91062045		0,98183172
6		0,55805965		0,9842087
7	S2	0,80284877	T2	1
8		0,97602131		0,98939488
9		0,97913635		0,95116158
10		0,96468905		0,93238223
11		0,97884825		0,96308115
12		0,40223214		0,99528627
13		0,89012434		0,99703
14		0,96478552		0,95000589
15		0,99246926		0,97487807
16	S2	0,7164323	T3	0,99161546
17		0,86181856		0,92012122
18		0,99946807		0,88442135
19		0,7555254		0,86023529
20		0,92076703		0,7888531
21		0,75560574		0,96408985
22		0,49395355		0,99282385
23		0,96540754		0,97374037
24		0,89366231		0,76037823

25		0,13165594		0,83451877

Table 15 Red Delicious heat maps for ethylene synthesis at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

RED DELICIOUS		FIRMNESS		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S1	0,29090909	T2	0,20689655
2		0,21428571		0,07692308
3		0,24615385		0,18644068
4		0,16923077		0,171875
5		0,24242424		0,24193548
6		0,11428571		0,18333333
7	S2	0,10144928	T2	0,04545455
8		0,02941176		0,12903226
9		0,10606061		0,125
10		0,27692308		0,15
11		0,14754098		0,20967742
12		0,08571429		0,11290323
13		0,14084507		0,10769231
14		0,19672131		0,21428571
15		0,08695652		0,11290323
16	S2	0,10769231	T3	0,35483871
17		0,12307692		0,32758621
18		0,08955224		0,20689655
19		0,125		0,08333333
20		0,14492754		0,10526316
21		0,05882353		0,15
22		0,01515152		0,390625
23		0,25396825		0,078125
24		0,109375		0,14705882
25		0,07142857		0,265625

Table 16 Red Delicious heat maps for firmness at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE RED DELICIOUS ETHYLENE INHIBITION		
	1ST ANALYSIS	2ND ANALYSIS
S1T2	0,86153248	0,96459355
S2T2	0,88346167	0,97258001
S2T3	0,74942964	0,89707975
TOTAL	0,82458585	0,94046315

Table 17 Average ethylene synthesis inhibition for red delicious cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE RED DELICIOUS FIRMNESS INCREASE		
	1ST ANALYSIS	2ND ANALYSIS
S1T2	0,21288156	0,17790069
S2T2	0,13018032	0,13410541
S2T3	0,10989959	0,21093518
TOTAL	0,14191633	0,17534818

Table 18 Average firmness increase for red delicious cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

4.2.5. Granny Smith

Granny Smith is a variety sensitive to internal browning and sun damage. It is also very sensitive to scald. For this cultivar, the optimal range of pre-treatment starch content within applying Fysium® is 4.0-5.0 and the firmness should be equal or higher than 6.8-7.5 kg/cm^2 according to the 2019 Fysium® Dossier. The sugars content for an optimal ripening state should be 10.0° BRIX or more.

The cultivar was divided into 2 groups for the statistical analysis: S2T1, and S2T2. As it can be noticed the pre-treatment starch content gave rise to only one class, which is S2, and which stands for starch contents close to the optimal. In the group S2T2, anyway, three storage chambers had too low pre-treatment starch contents, with values of the scale around 7, so they should have fallen inside the higher starch class which is S1. Due to the lack of data, they were kept inside the S2T2 group to allow to perform the statistical analysis.

Considering the heat maps the average ethylene total inhibition given by the treatment for this cultivar was 75.51% at the 1st analysis and 88.82% at the 2nd analysis (*Table 21*). This was the 2nd best result for a cultivar after Red delicious.

Looking at the heat maps regarding firmness, its increase given by the treatment for this cultivar was 8.50% at the 1st analysis and 9.68% at the 2nd analysis (*Table 22*) so, in this case firmness increase was not so solid.

4.2.5.1. S2T1

In the group S2T1 the results concerning ethylene synthesis of the treated samples were far from the ones of the untreated samples for all the storage chambers and they were always tending to zero. The same was validated by the results of the 1st analysis.

The values of firmness detected in the pre-treatment phase were in most of the cases inside the optimal range or a little higher. The differences in firmness detected at the 2nd analysis were 0.7 kg/cm^2 or higher for all the samples. At the 1st analysis these differences were thinner but always equal or higher than 0.4 kg/cm^2.

4.2.5.2. S2T2

In the second group of this cultivar, S2T2, the results concerning ethylene synthesis of the treated samples were significantly different from the ones of the untreated samples for all the storage chambers and they were always tending to zero ppm. At the 1st analysis ethylene was tending to zero ppm for both untreated and treated samples.

The values of firmness detected in the pre-treatment phase were in most of the cases inside the optimal range or higher. In this group, the differences in firmness detected at the 2nd analysis were 0.4 kg/cm^2 or higher for all the samples apart from one where it resulted to be 0.2 kg/cm^2 (the pre-treatment temperature of this sample was, anyway, inside the optimal range and did not seem to be the cause of this result). At the 1st analysis these differences were 0.4 kg/cm^2 or higher for all the storage chambers with no exceptions.

Generally, ethylene synthesis for the treated samples regardless of the storage time was always tending to zero. This is probably related to the fact that the pre-treatment ripening state of apples was optimal or close to the optimal (given from the values of starch and firmness detected before the treatment). Another reason of these low values of synthetized ethylene could be found, anyway, in the fact that in this cultivar the storage time classes were only T1 and T2, so apples were not stored for periods longer than 6 months.

GRANNY SMITH		ETHYLENE		
CHAMBERS	STARCH	1ST ANALYSIS	TIME CLASS	2ND ANALYSIS
1	S2	1	T1	0,628400796
2		0,990108249		0,998636113
3		0,318181818		0,987184495
4		0,913898917		0,997686109
5	S2	1	T2	0,999937656
6		0,688679245		0,994269496
7		0,071532847		0,990640654

8		0,809779368		0,982955434
9		0,835069444		0,99631703
10		0,923809524		0,306451613

Table 19 Granny Smith heat maps for Ethylene synthesis at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

GRANNY SMITH		FIRMNESS		
CHAMBERS	STARCH	1ST	TIME CLASS	2ND
1	S2	0,05797101	T1	0,12328767
2		0,02941176		0,10294118
3		0,1369863		0,11594203
4		0,05405405		0,15584416
5	S2	0,08219178	T2	0,02898551
6		0,06849315		0,09375
7		0,12676056		0,08823529
8		0,10144928		0,1
9		0,05633803		0,09859155
10		0,13636364		0,06060606

Table 20 Granny Smith heat maps for Firmness at 1st and 2nd analysis, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE GRANNY SMITH ETHYLENE INHIBITION		
	1ST ANALYSIS	2ND ANALYSIS
S2T1	0,805547246	0,902976878
S2T2	0,721478405	0,878428647
TOTAL	0,755105941	0,88824794

Table 21 Average ethylene synthesis inhibition for granny smith cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

AVERAGE GRANNY SMITH FIRMNESS INCREASE		
	1ST ANALYSIS	2ND ANALYSIS
S2T1	0,069605784	0,124503758
S2T2	0,095266072	0,078361402
TOTAL	0,085001957	0,096818344

Table 22 Average firmness increase for granny smith cultivar given by the treatment with Fysium®, scale of colors from red (low effectiveness) to orange/yellow (medium-high effectiveness) to green (very high effectiveness).

5. DISCUSSIONS AND CONCLUSIONS

The vertical analysis applied in the previous chapters allowed to determine the presence of two statistically different populations: apples treated with Fysium® and apples not treated for all the different cultivars and selected groups. These two populations were distinguished basing on the parameters of firmness and synthetized ethylene, both detected in two phases, at the 1st analysis (after 7-10 or 20-25 days from the date of the treatment depending on the cultivar), and at the 2nd analysis, which corresponded with the opening date of the storage chambers and so, it varied from chamber to chamber. This first conclusion was the confirmation of the effectiveness of Fysium® application in the contribution of an effective post-harvest storage of apples. 1-MCP conferred following the Fysium® patent's instructions reduced the synthesis of ethylene by apples and, in this way, it maintained apples high crunchiness and firmness. Given that the parameters that determine a great storage quality are low synthesis of ethylene and high firmness, all the methods that guaranteed to reach these results could help in obtaining an optimal storage.

The second part of the analysis, the qualitative investigation, allowed to take into consideration all the parameters that characterized the storage chamber history from the entering of the apples in the storage chamber to the opening of it when the products were ready to be conferred to the market. As for the statistical part different groups were selected and separately investigated based on homogeneous parameters (cultivar, starch content, and time of opening of the chamber as an indicator of the storage period).

This second qualitative approach allowed to analyze room by room each treatment and to verify if there were any isolated cases where Fysium® had not been much effective for some reasons. To reach this, if there were anomalies in the parameters of apples at the two analysis (such as high ethylene synthesis of treated groups or very low firmness differences between treated and untreated apples), samples conditions at the time of the treatment were evaluated. The analyzed initial conditions were apples temperature, storage rooms temperature, apples firmness, and apples starch content. This was done to verify if these initial conditions could explain the reasons of such apples behaviors that

were different from the average and from what expected (an optimal storage given by the treatment in addition to the controlled atmosphere of the rooms, with high quality apples at the end of it).

This analysis led to a second conclusion: it was validated the importance of having correct apples and storage chambers temperatures before the treatment. In many cases apples temperatures were above the optimal ranges. This implied a different behavior of the samples in response to Fysium®. The low effectiveness of the process was accentuated by high storage chambers pre-treatment temperatures. Fysium®, under these conditions, seemed to be less effective, as observed in the results section. The low effectiveness was mainly expressed at the 1st analysis but there were repercussions also on the 2nd analysis.

Regarding starch, it was detected how in some storage chambers the low pre-treatment starch content could be one of the possible reasons of a less effective treatment with Fysium®. Nevertheless, it was difficult to determine if this was the only parameter that led to this conclusion, there could be, indeed, other causes that brought to this result (as pre-treatment firmness, or other initial conditions as the previously stated ones).

Another conclusion that it is worth to mention was that Red Delicious, according to the heat maps, was the cultivar that expressed the best results of ethylene biosynthesis inhibition and firmness increase, due to Fysium® treatment.

The hope is that this thesis could bring to further future investigations. This work was, indeed, only a first approach that tried to foresee the storage chambers behavior linking it with the initial conditions of the chambers and with the pre-treatment ripening state of the apples.

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6.1. Sitography

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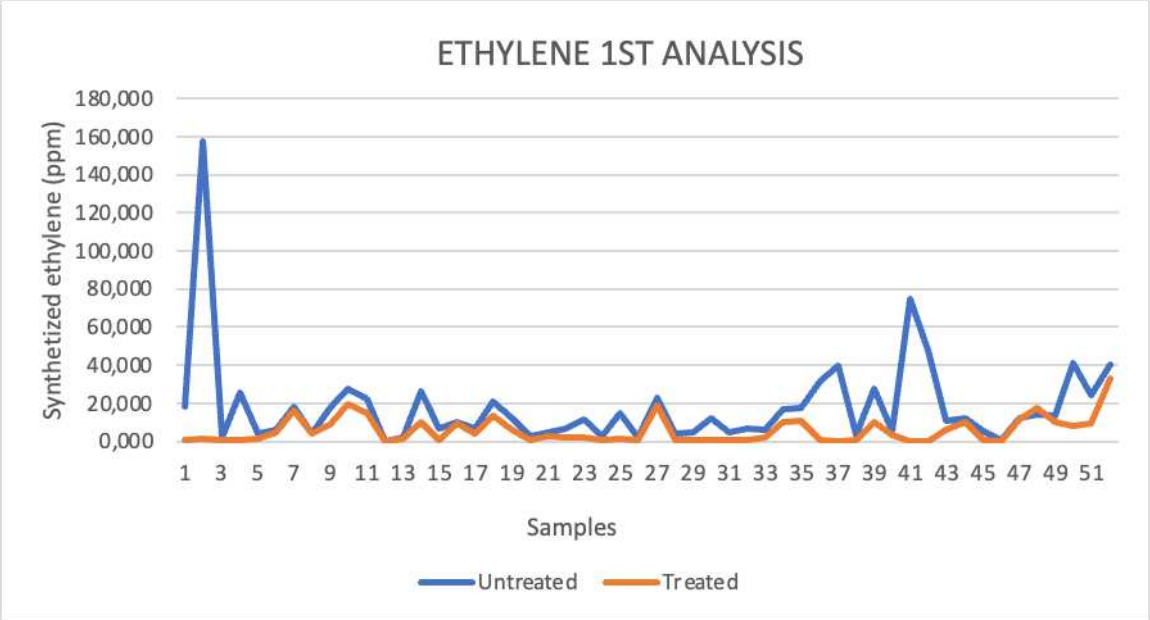
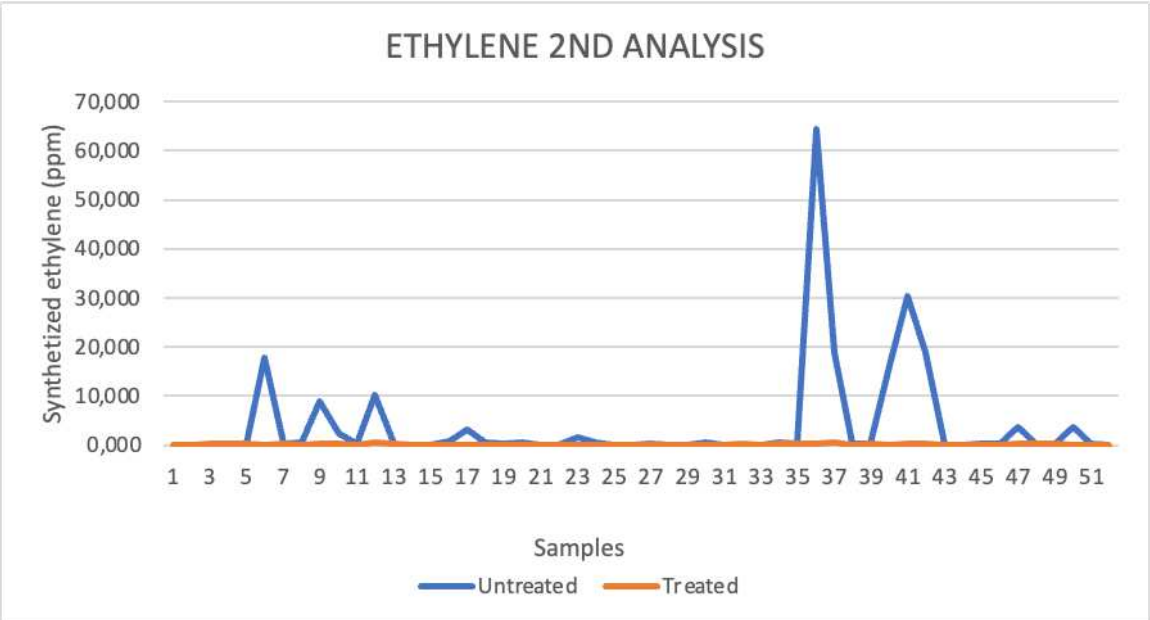
7. SUPPLEMENTARY FIGURES

7.1. Gala



SF1 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Gala S1 at harvest.



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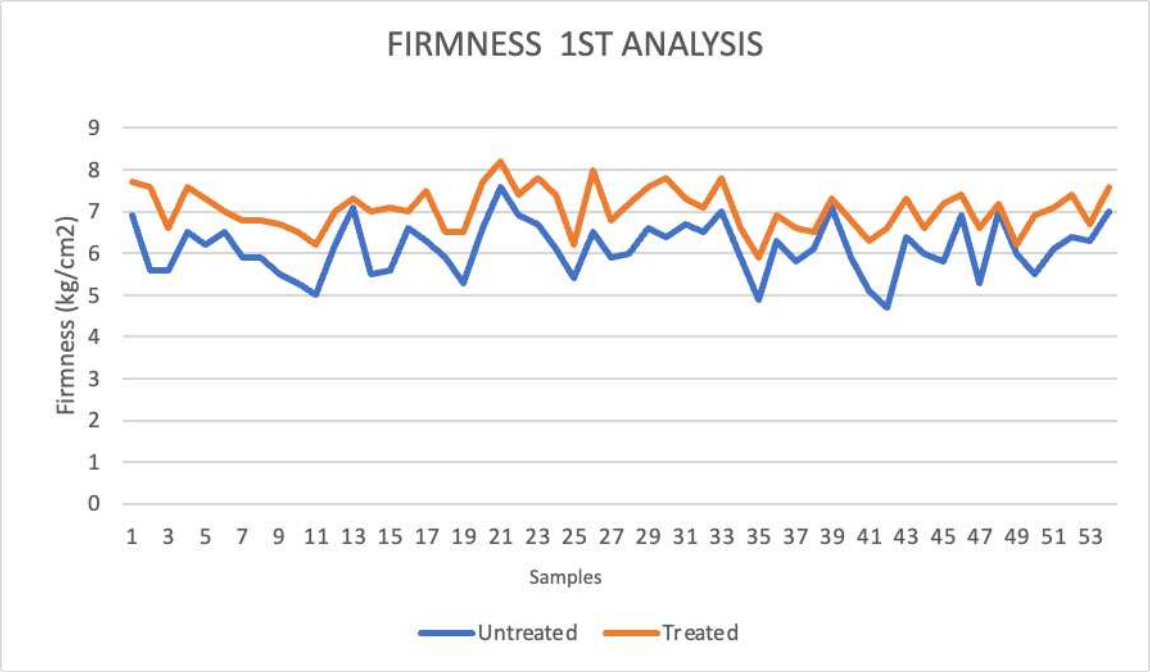
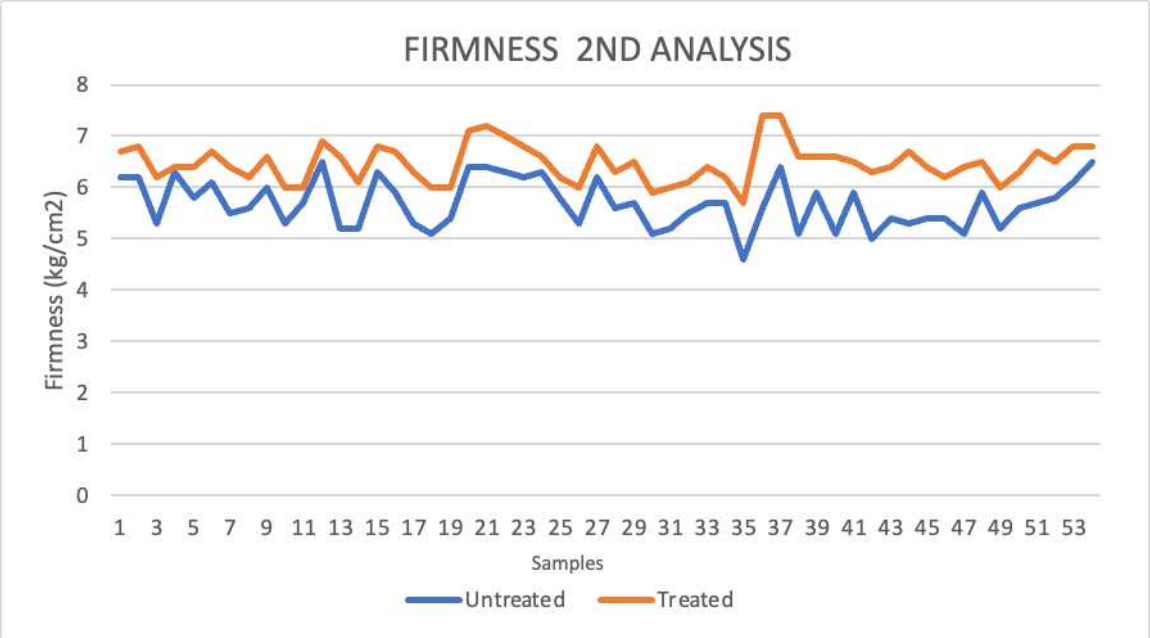
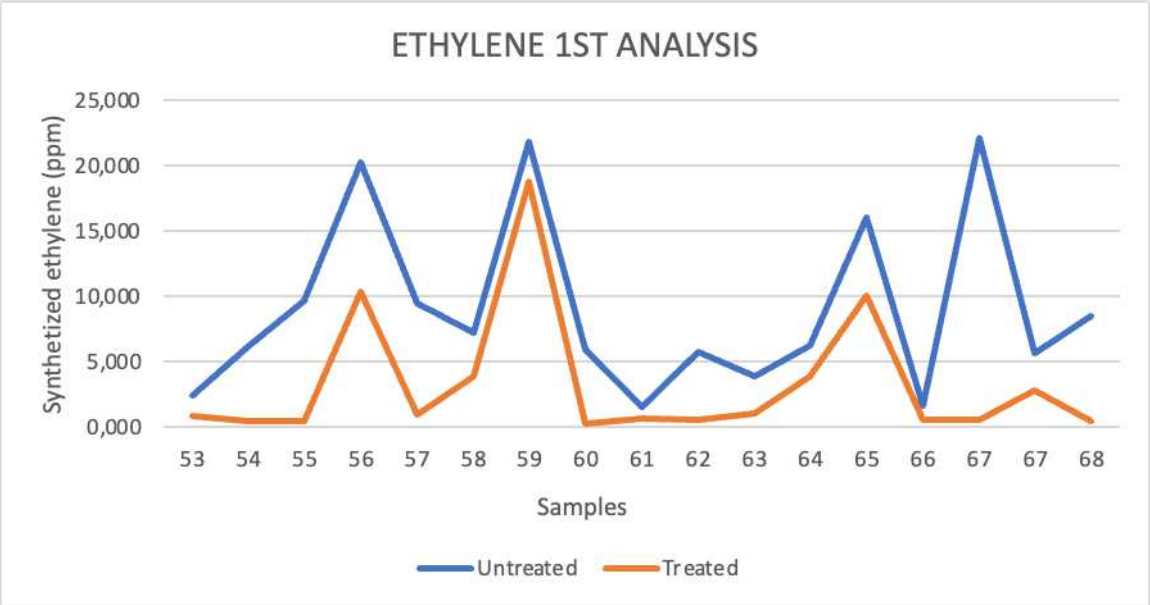


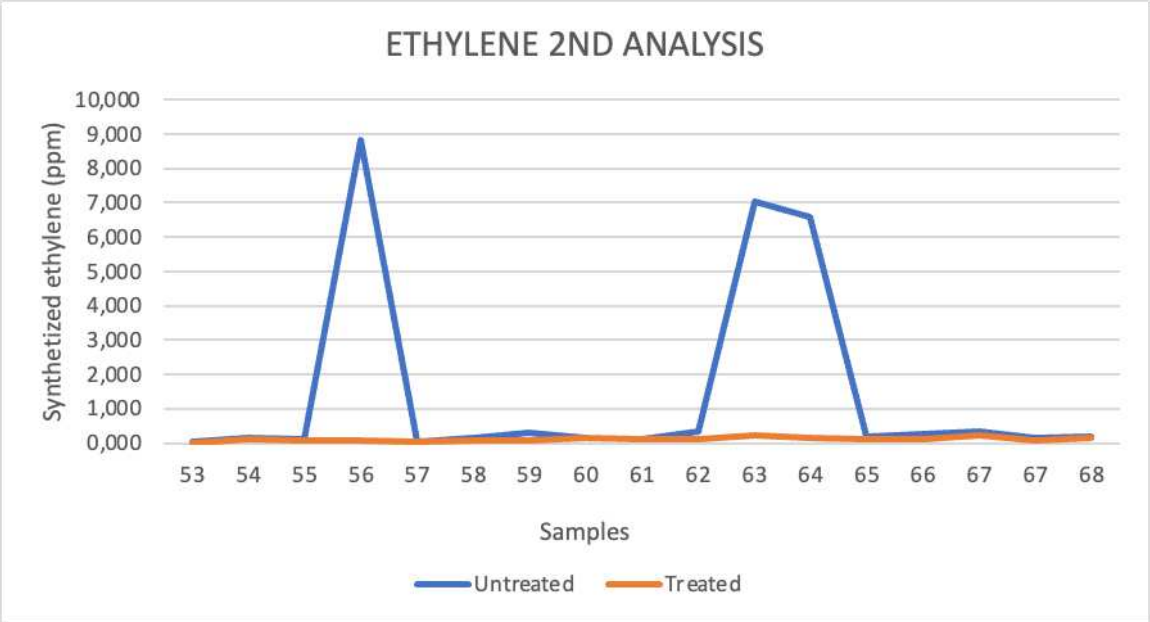
SF3 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Gala S1 at harvest.



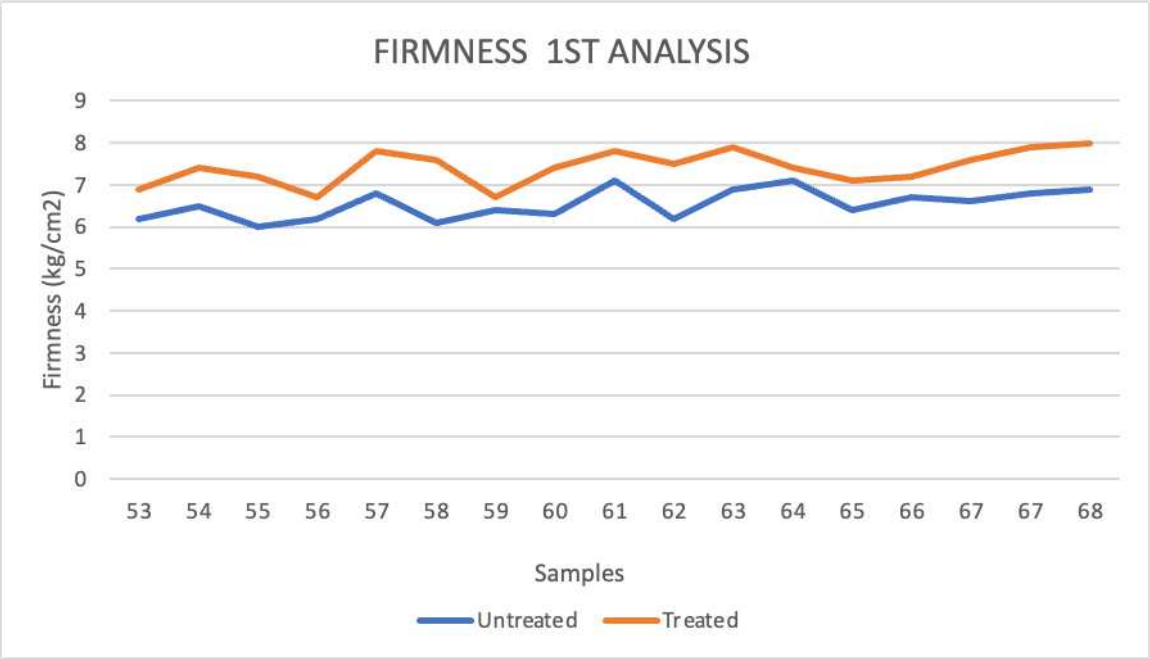
SF4 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Gala S1T2.



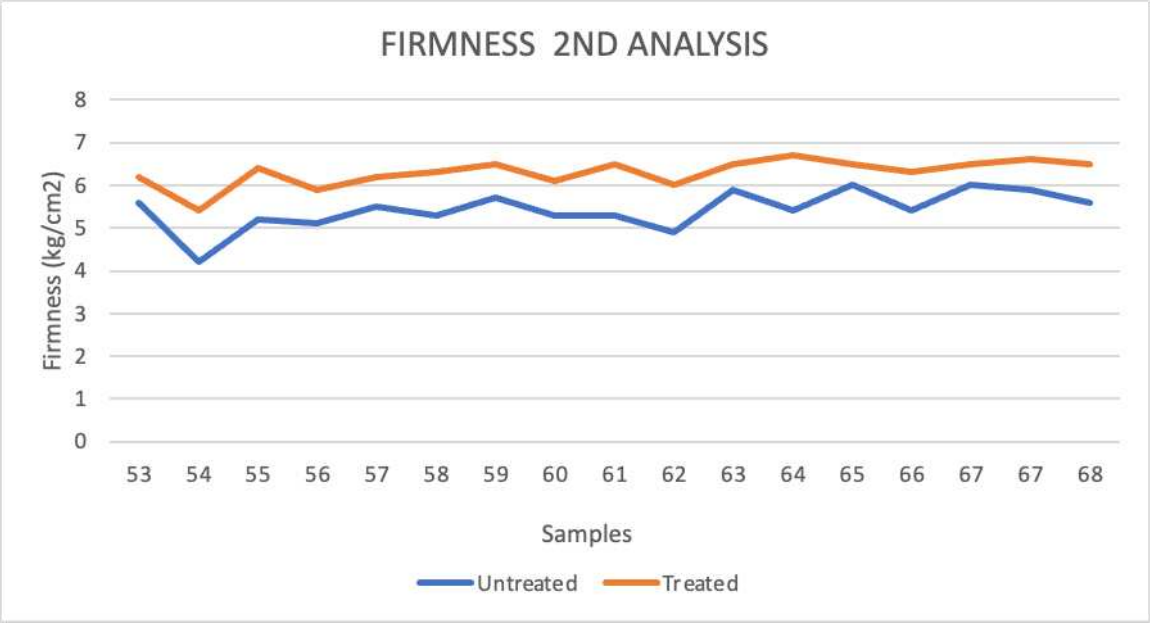
SF5 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Gala S1 at harvest.



SF6 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Gala S1T3.

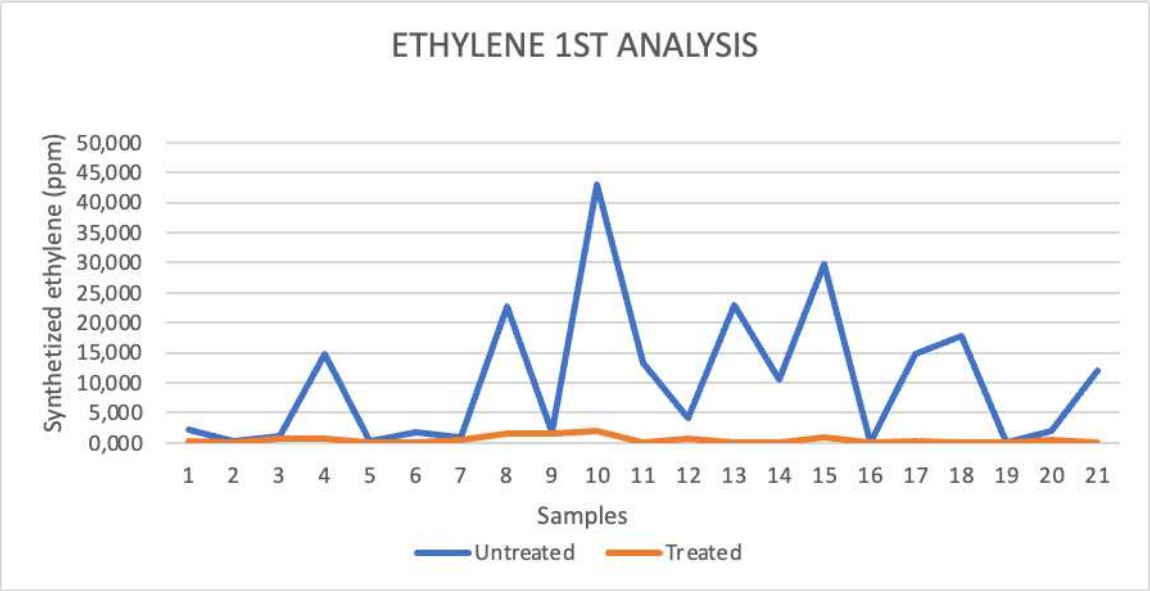


SF7 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Gala S1 at harvest.

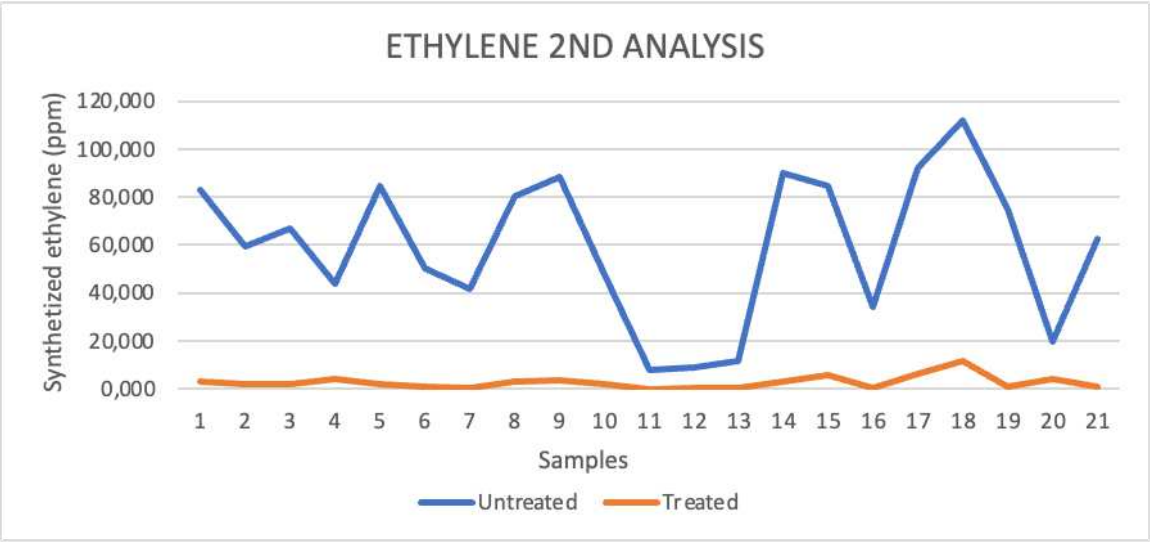


SF8 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Gala S1T3.

7.2. Pink Lady



SF9 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Pink Lady S1 at harvest.



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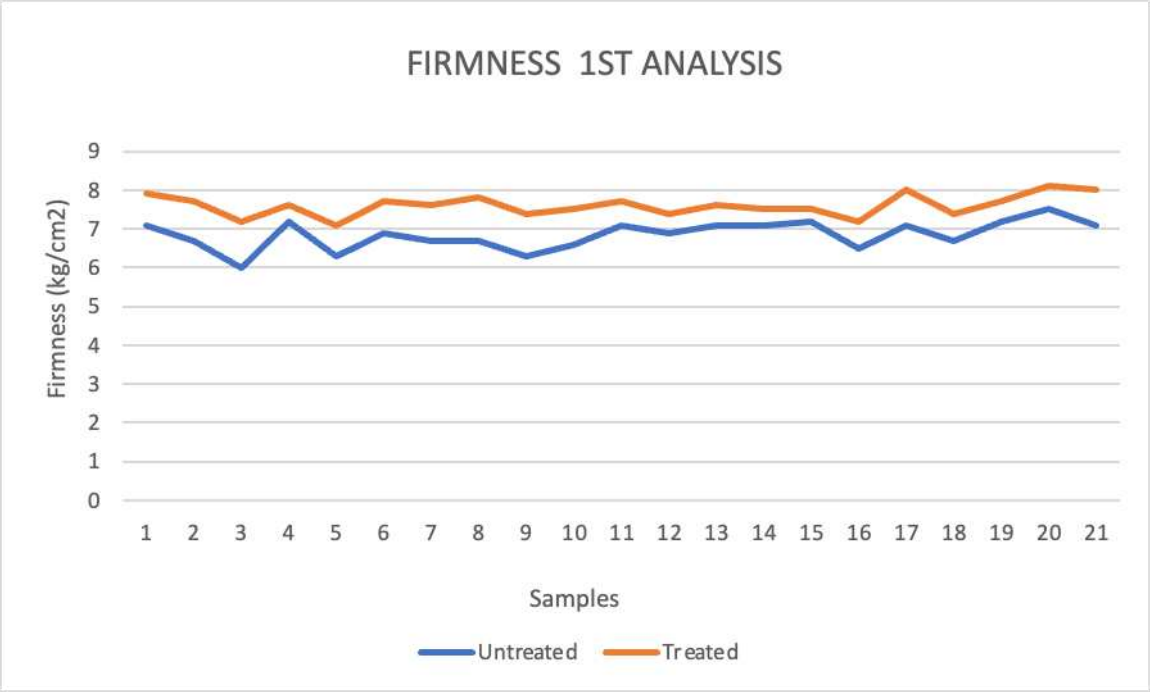
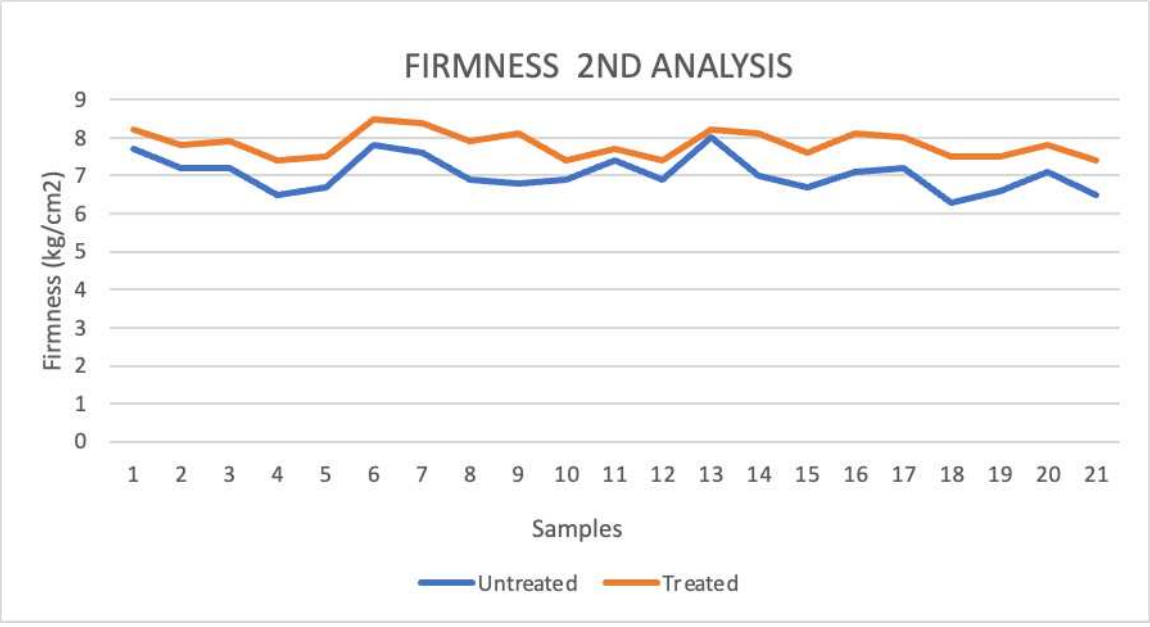
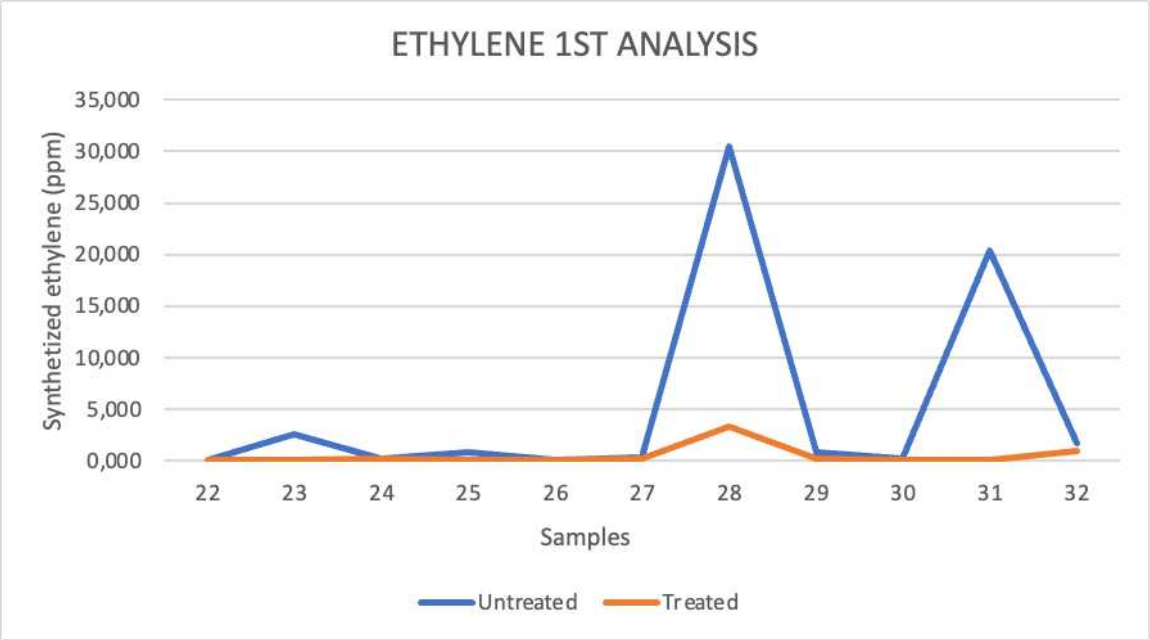


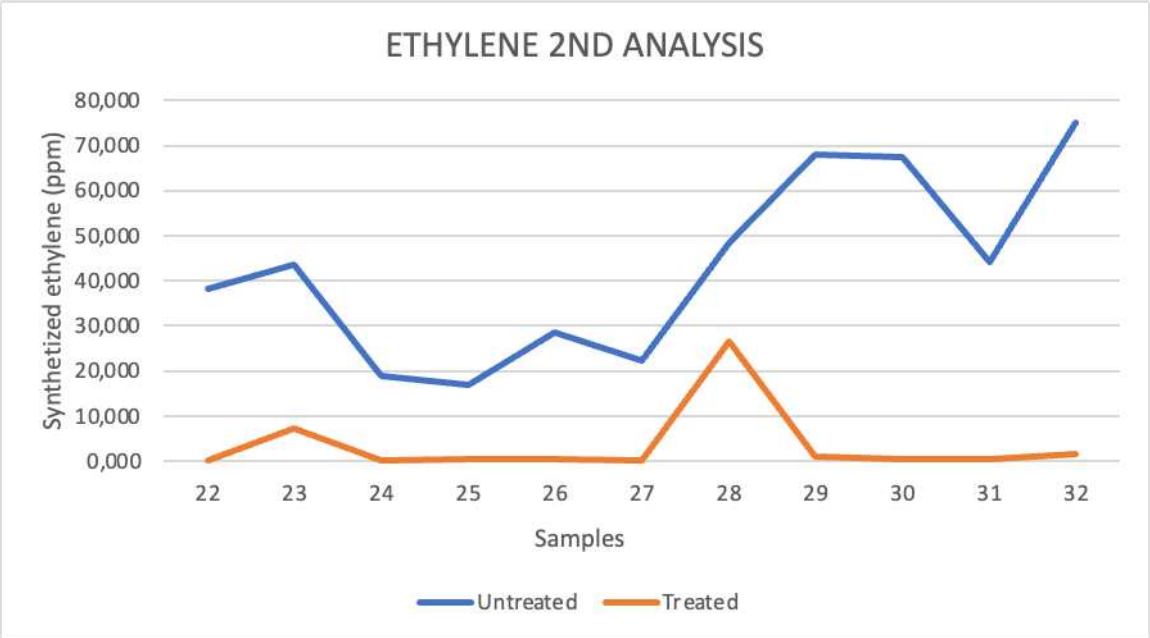
SF11 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Pink Lady S1 at harvest.



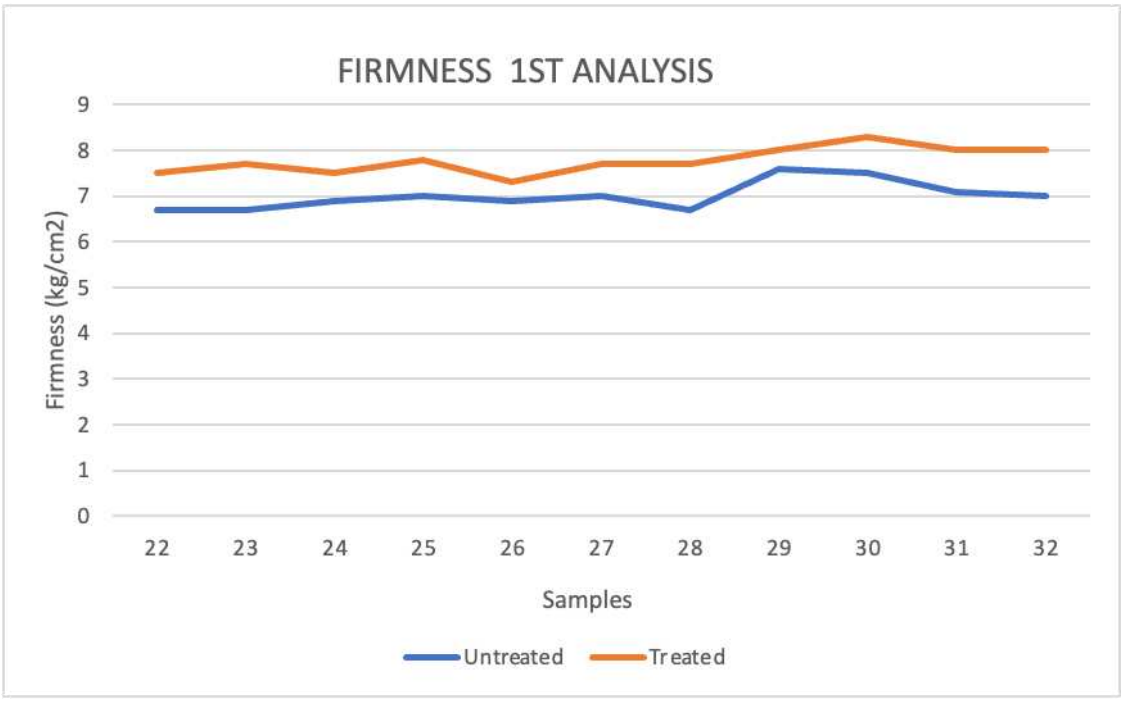
SF12 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Pink Lady S1T1.



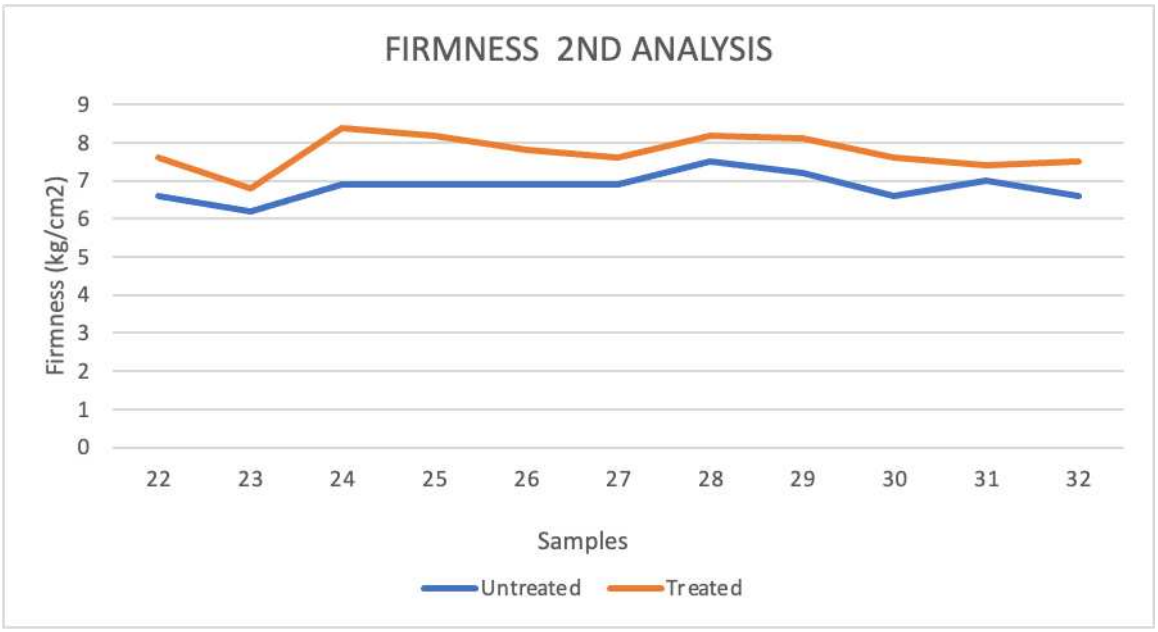
SF13 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Pink Lady S1 at harvest.



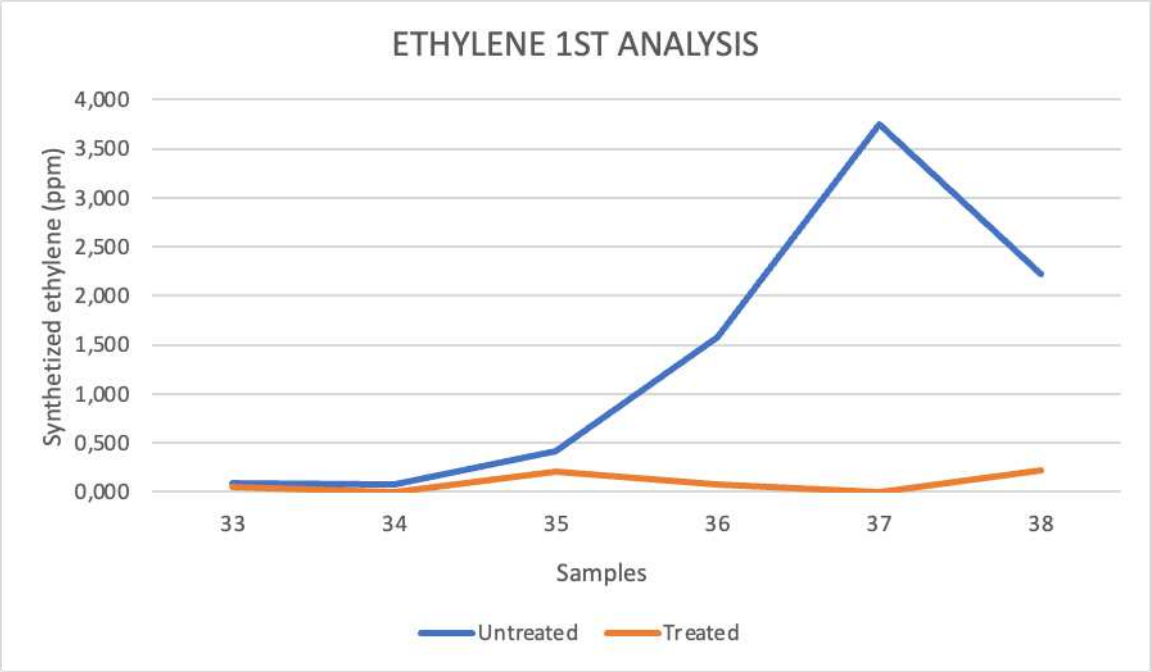
SF14 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Pink Lady S1T2.



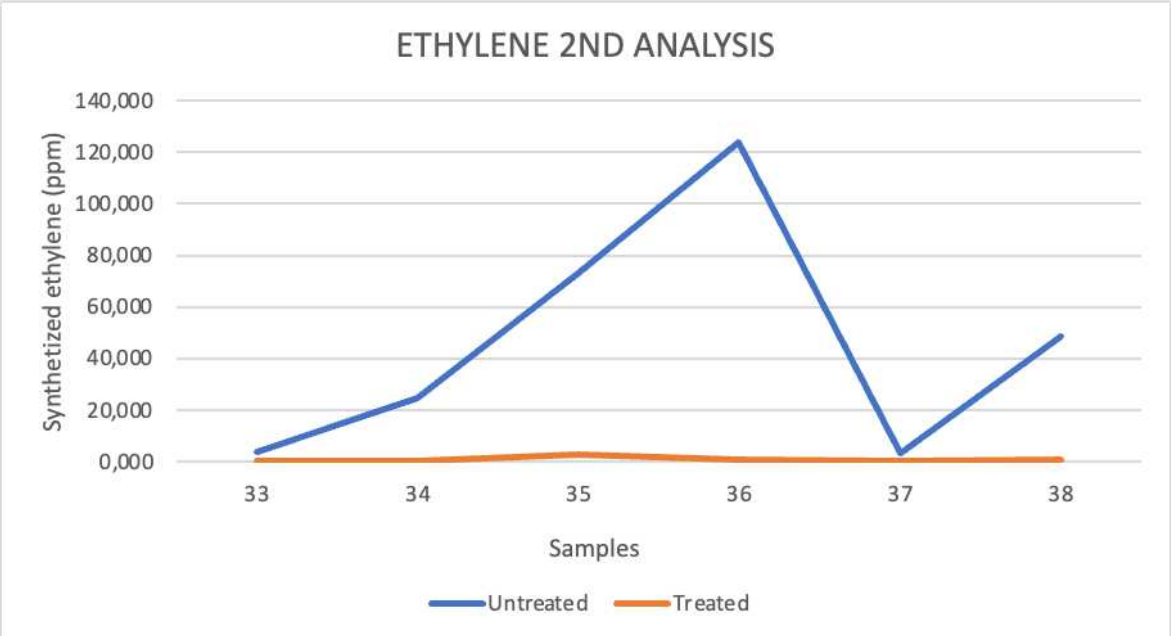
SF15 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Pink Lady S1 at harvest.



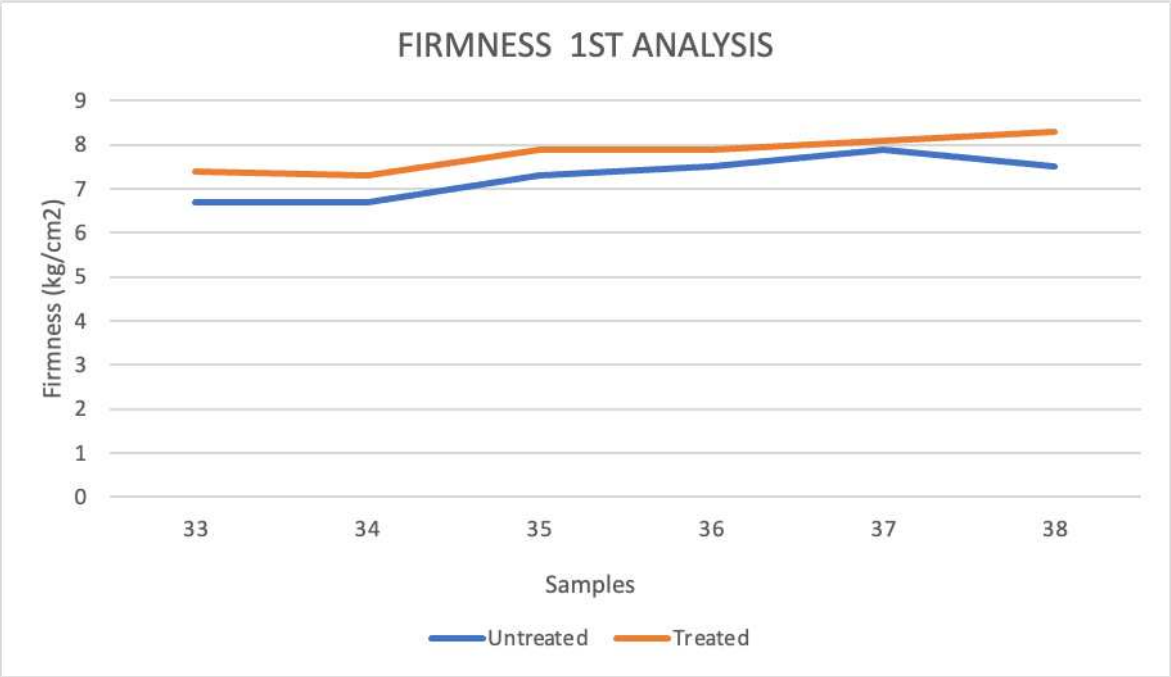
SF16 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Pink Lady S1T2.



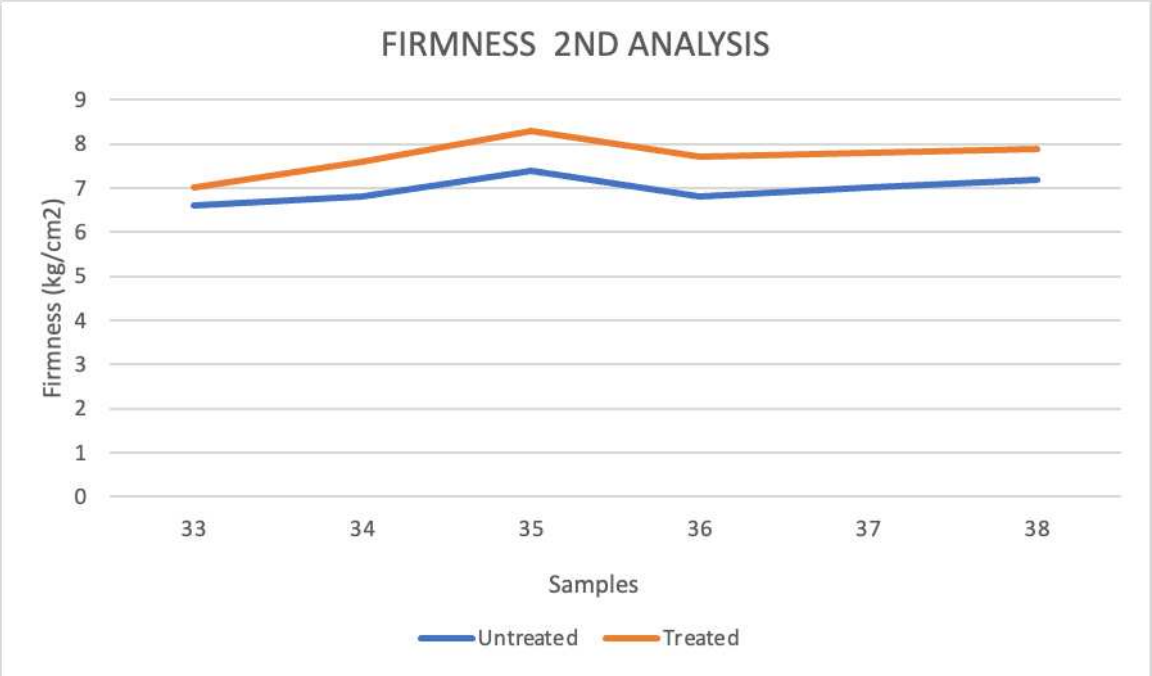
SF17 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Pink Lady S2 at harvest.



SF18 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Pink Lady S2T3.

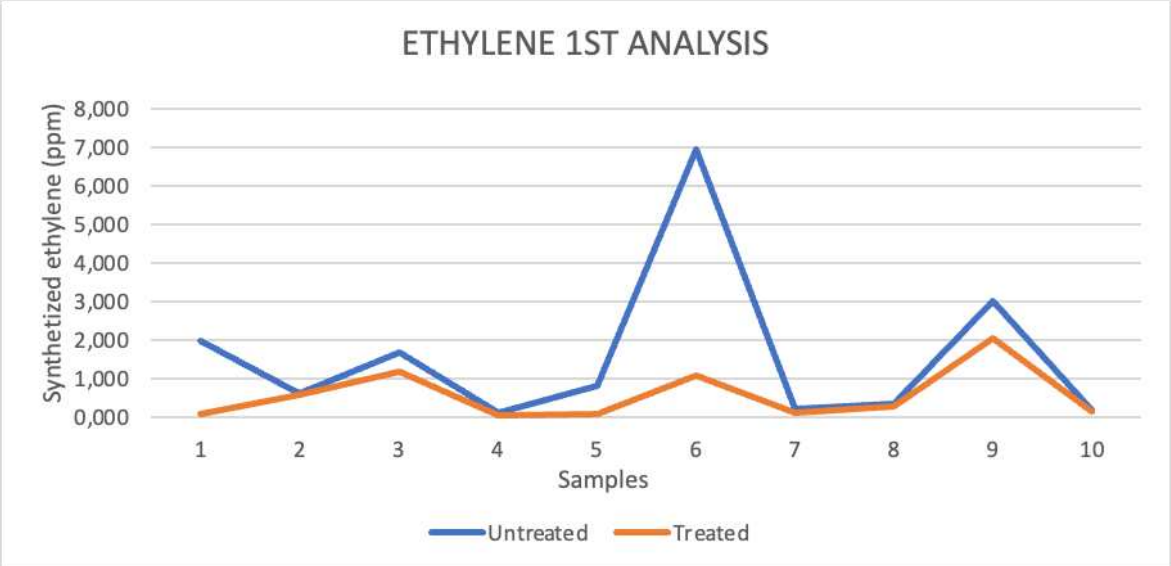


SF19 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Pink Lady S2 at harvest.

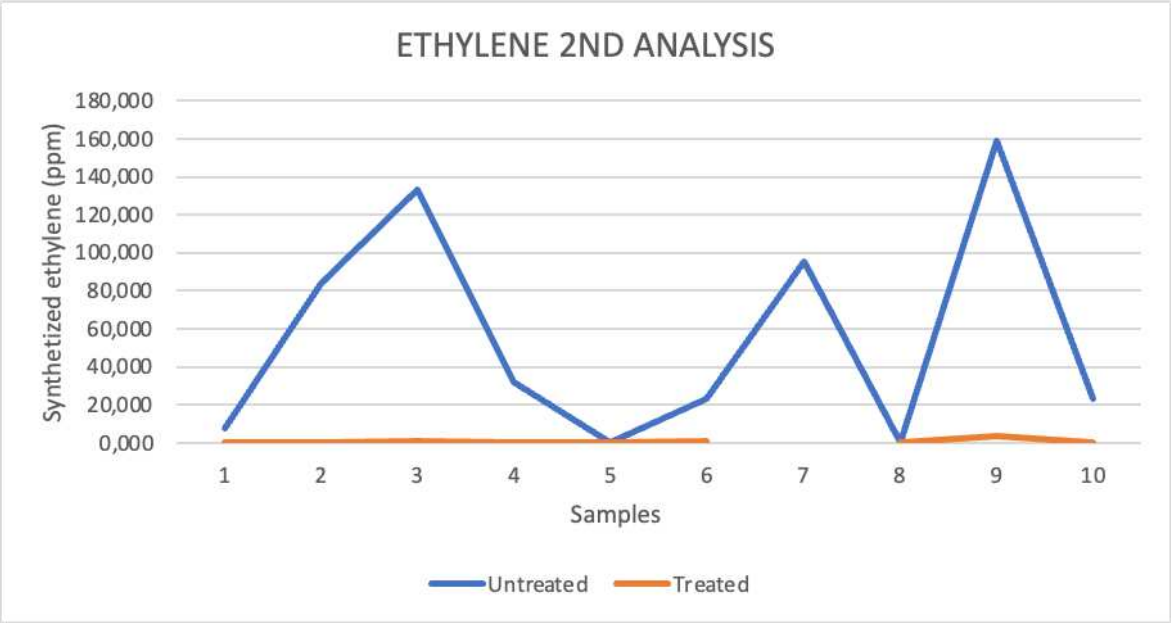


SF20 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Pink Lady S2T3.

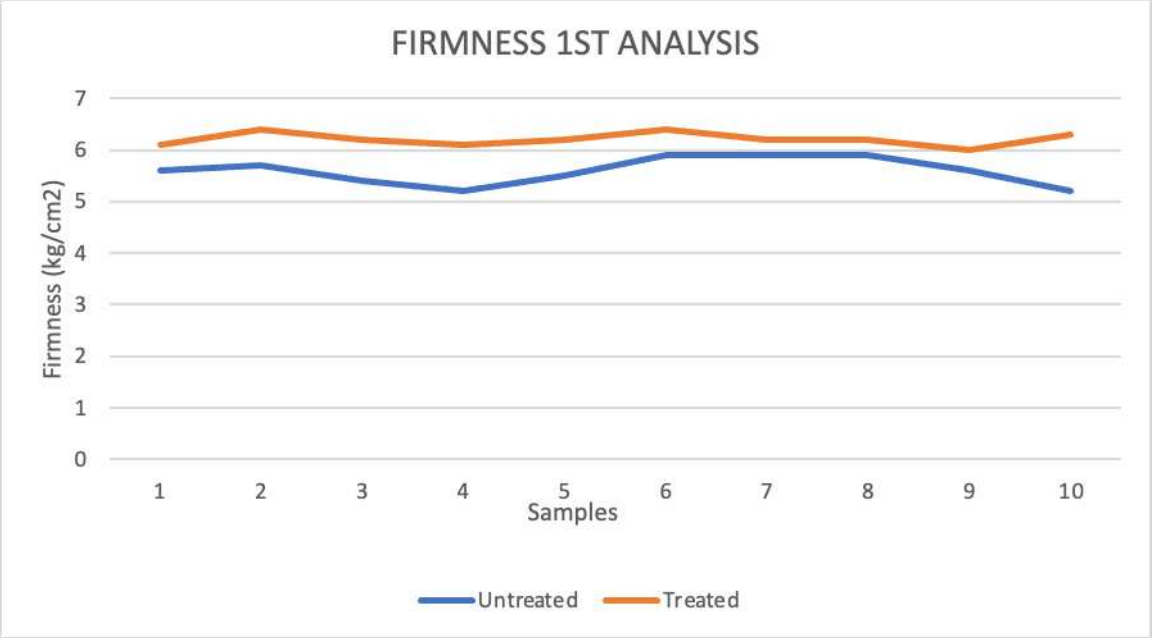
7.3. Fuji



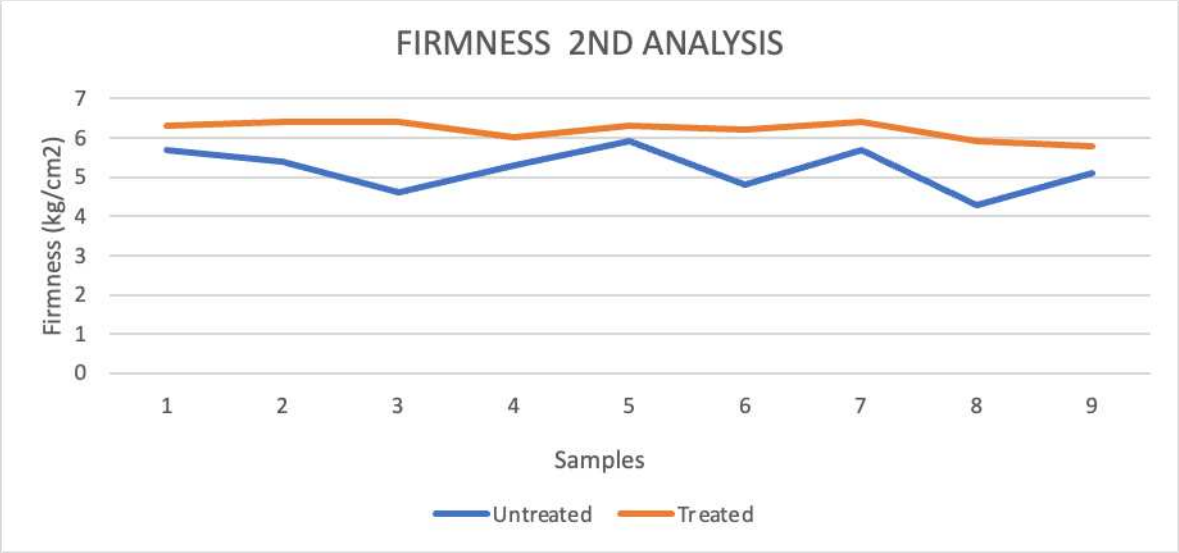
SF21 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Fuji S1 at harvest.



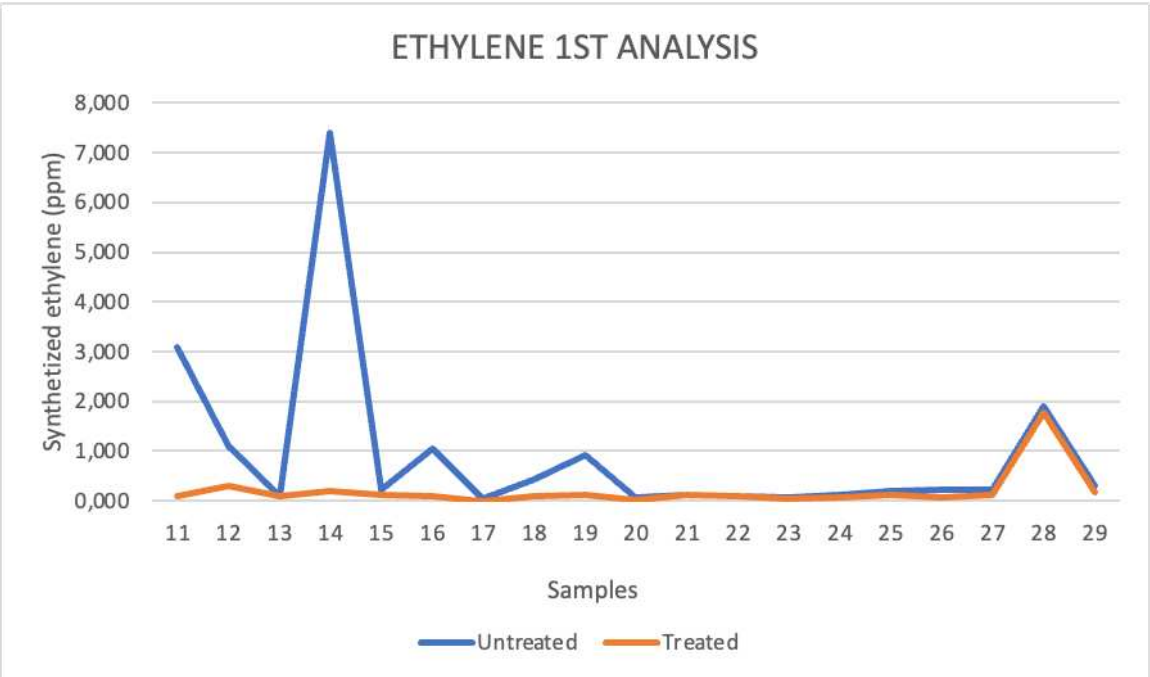
SF22 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Fuji S1T1.



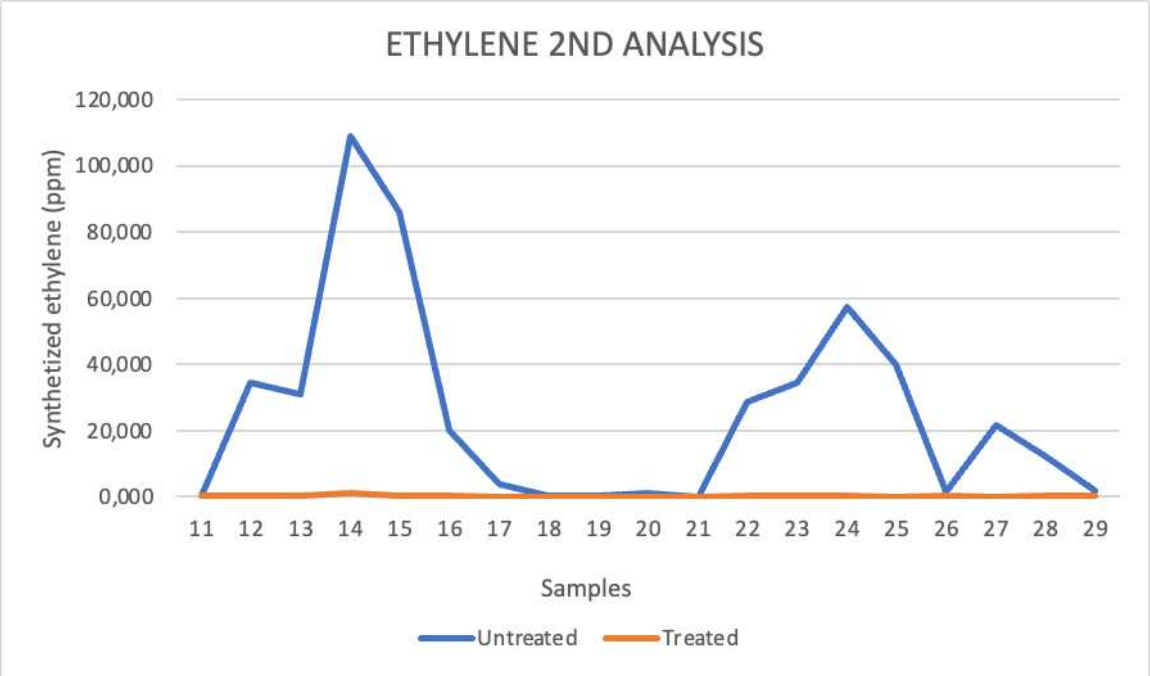
SF23 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Fuji S1 at harvest.



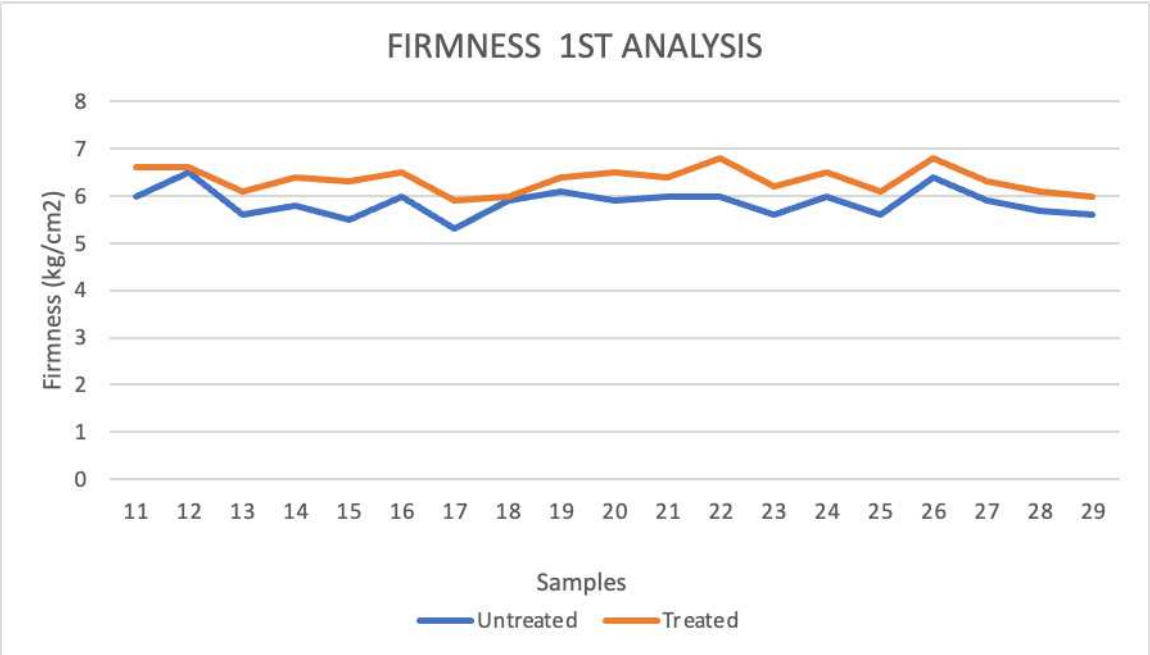
SF24 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Fuji S1T1.



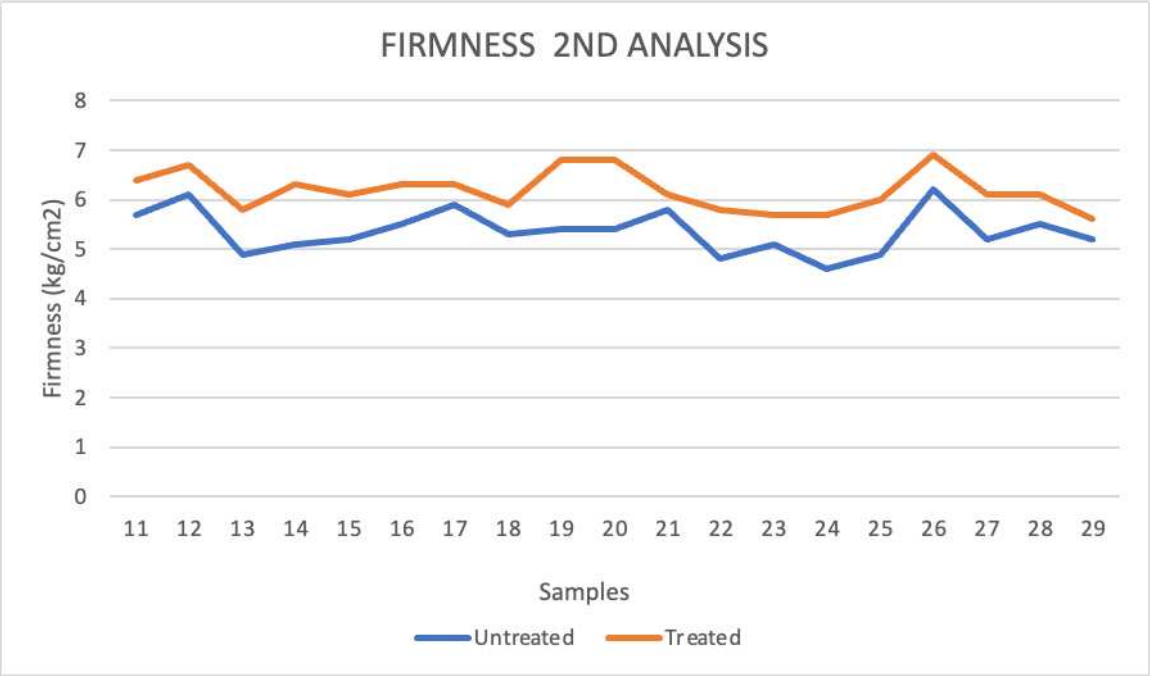
SF25 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Fuji S1 at harvest.



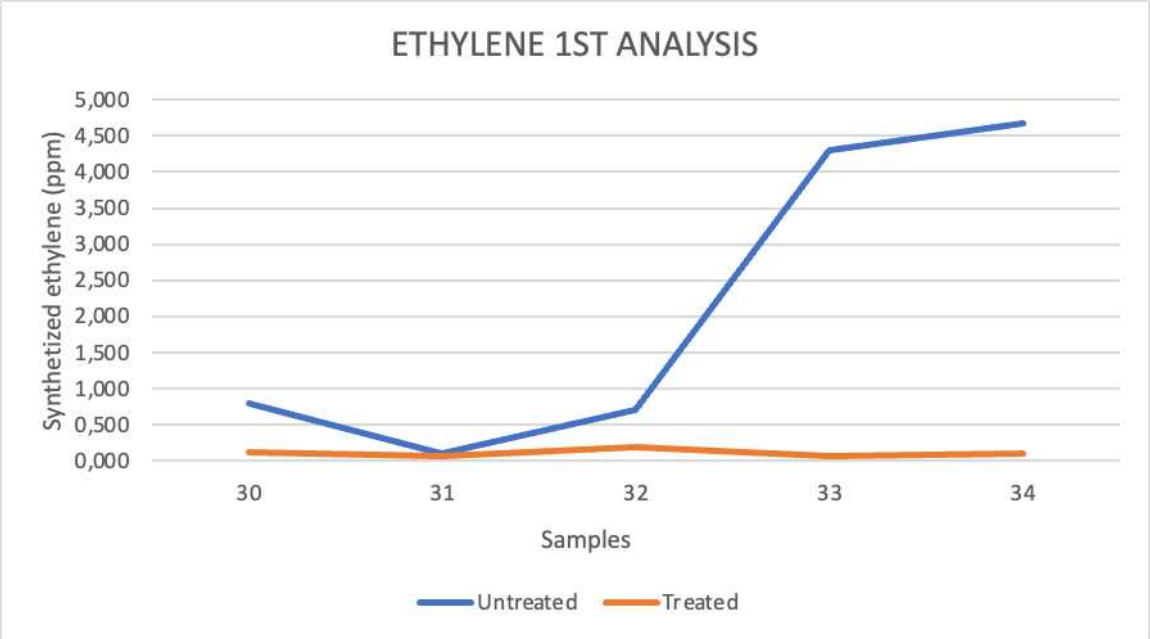
SF26 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Fuji S1T2.



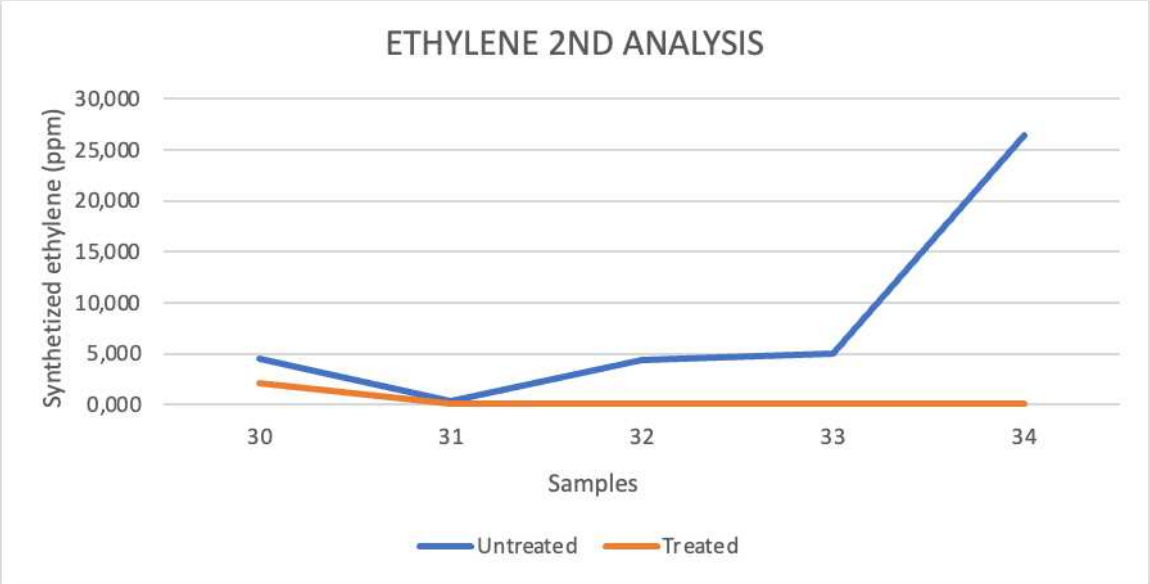
SF27 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Fuji S1 at harvest.



SF28 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Fuji S1T2.



SF29 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Fuji S1 at harvest.



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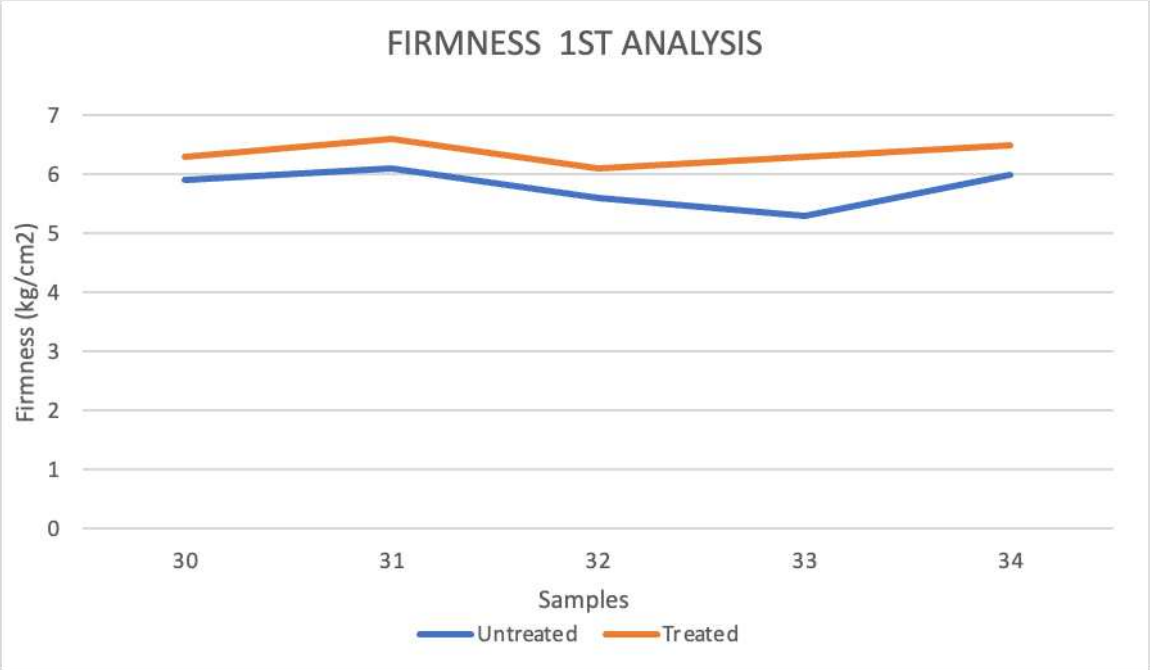
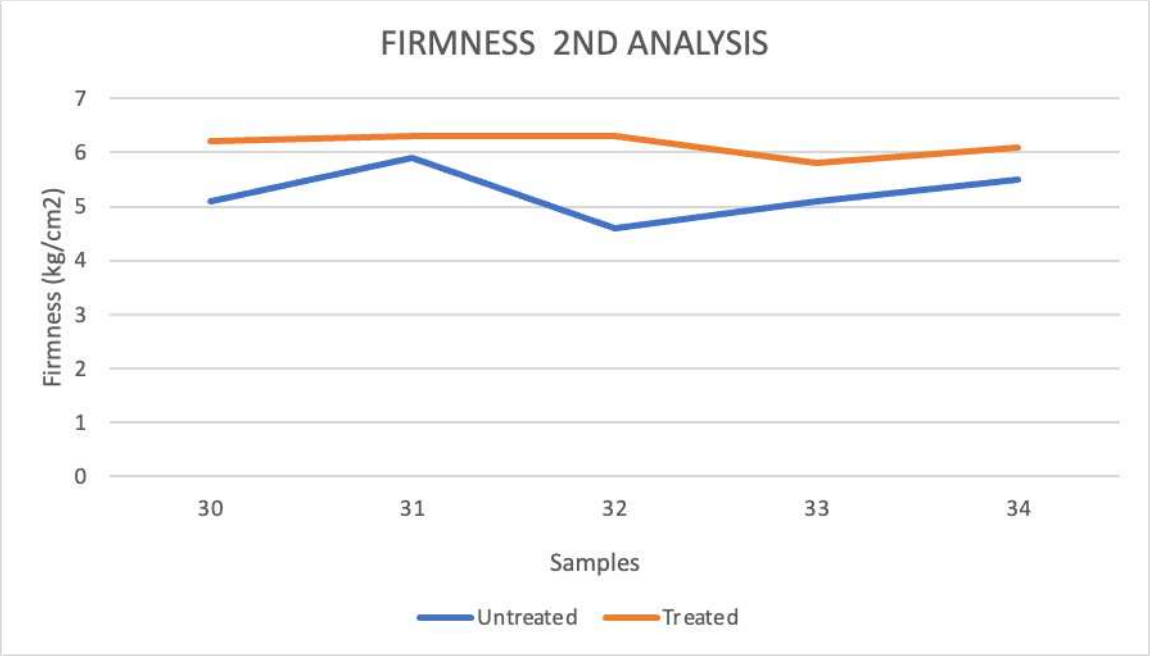
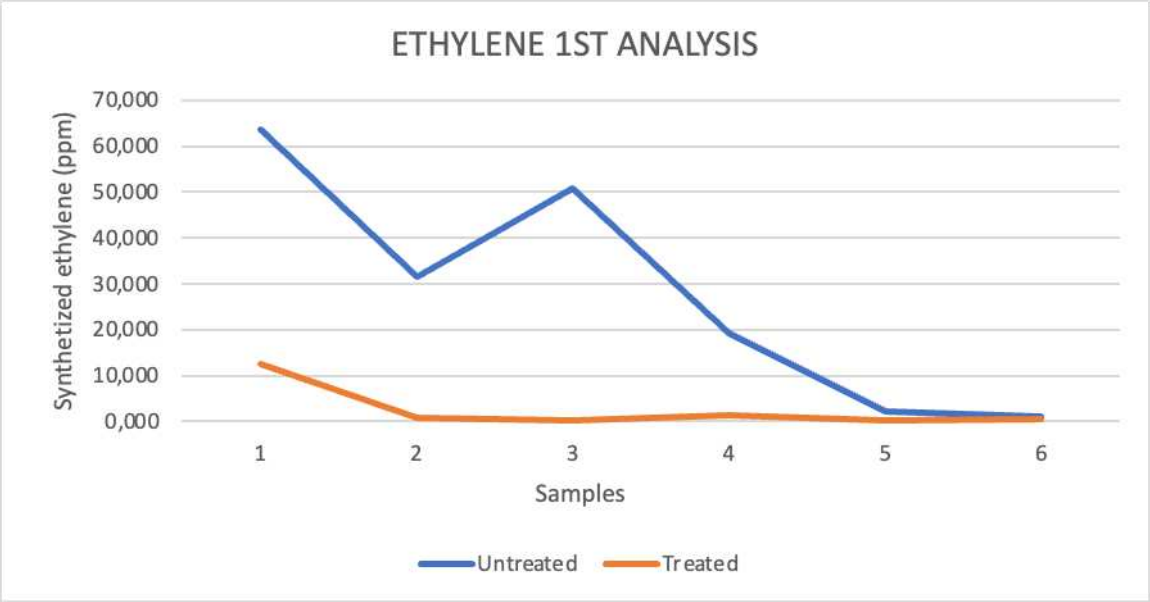


SF31 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Fuji S1 at harvest.



SF32 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Fuji S1T3.

7.4. Red Delicious



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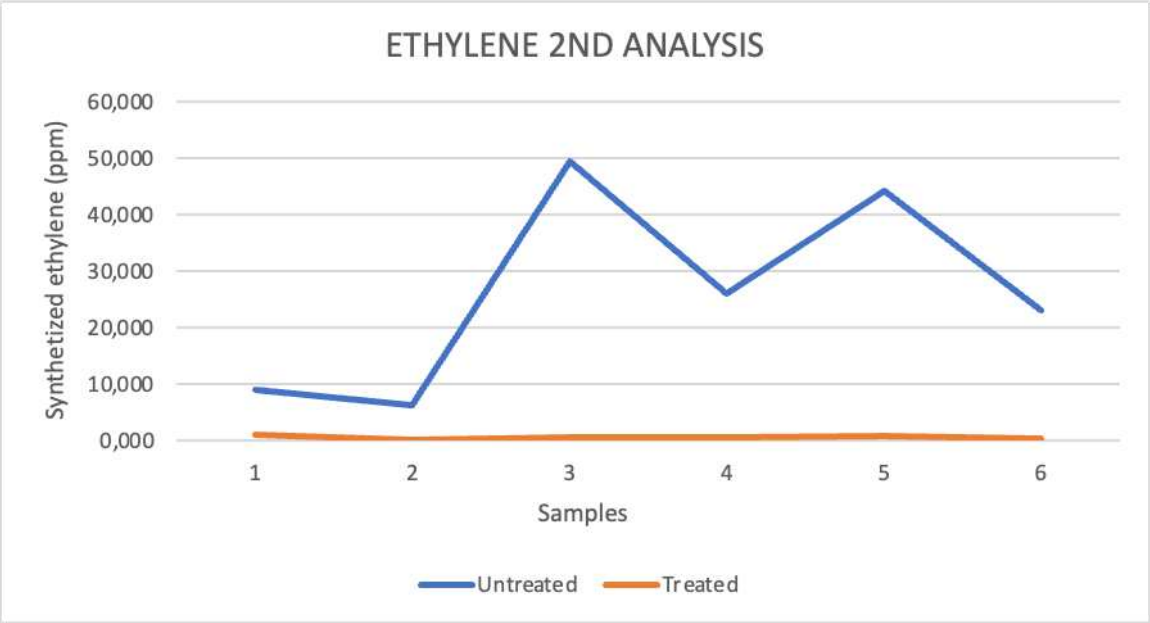
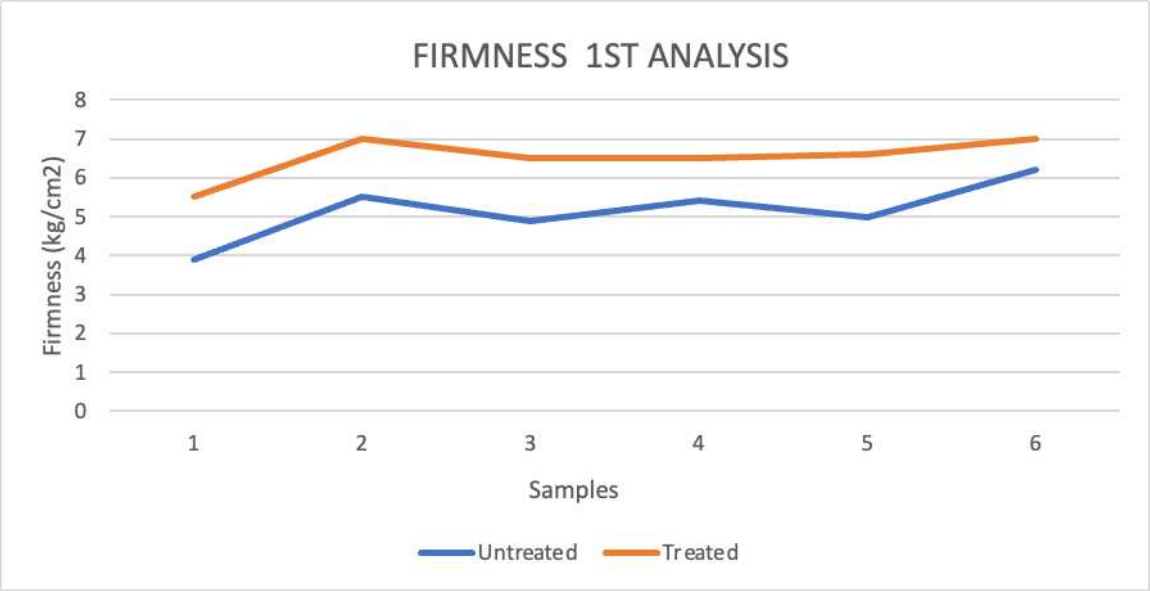
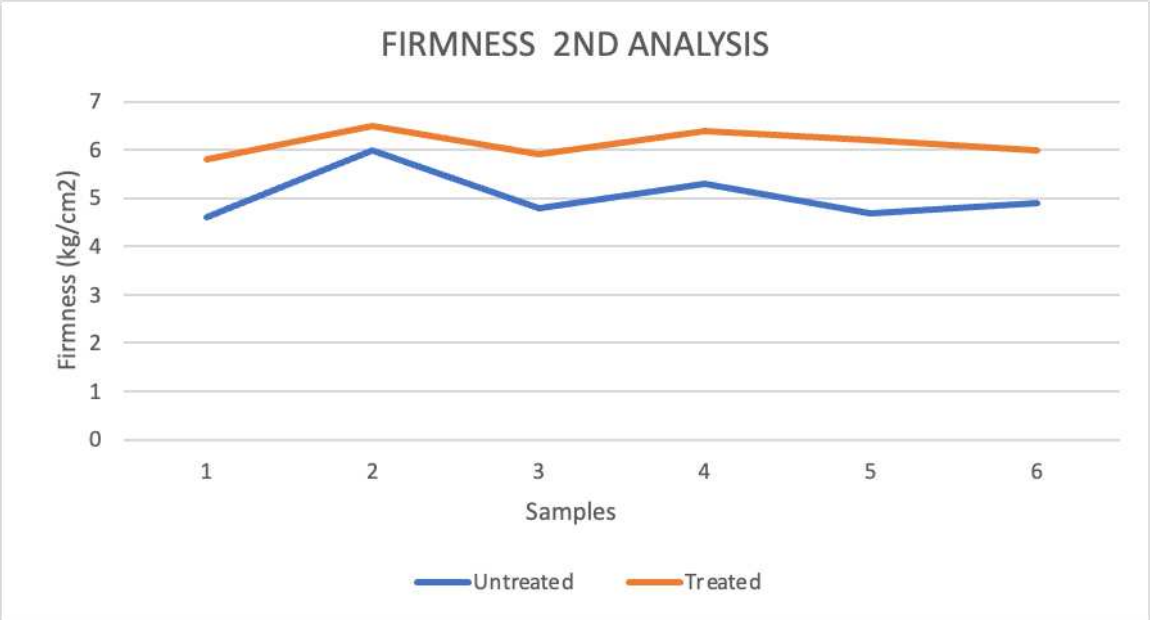


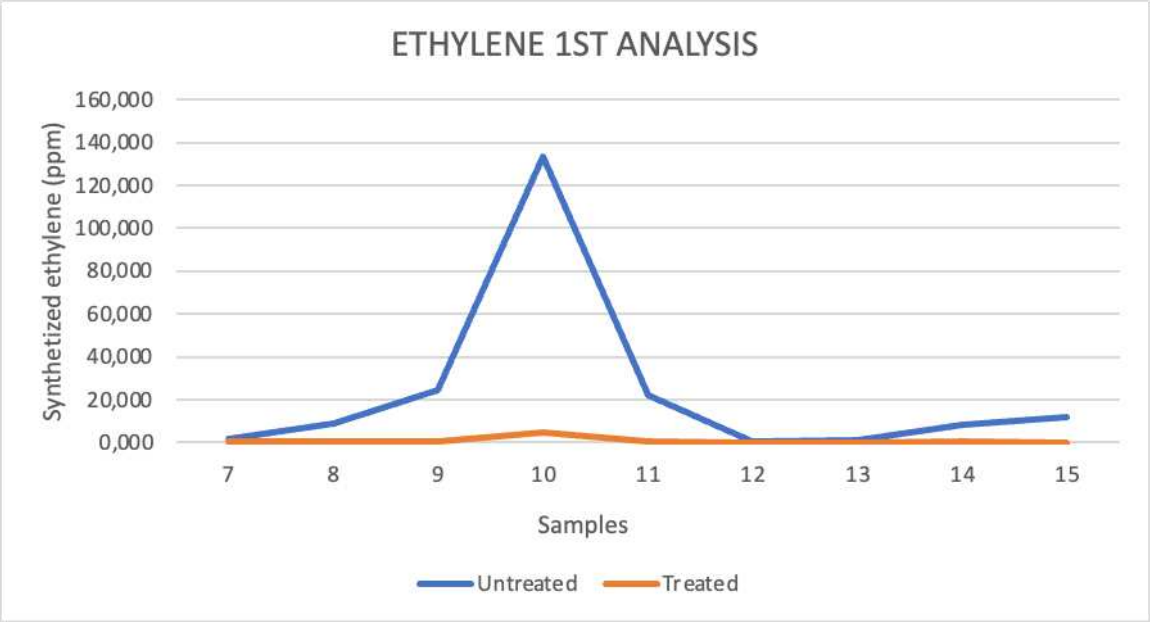
SF34 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Red Delicious S1T2.



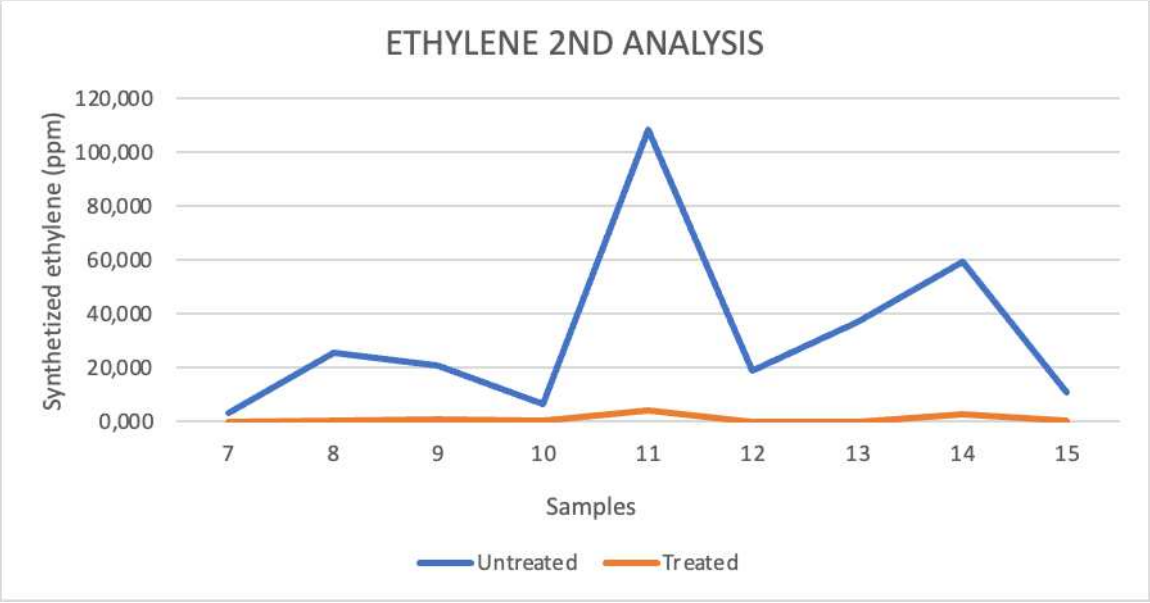
SF35 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Red Delicious S1 at harvest.



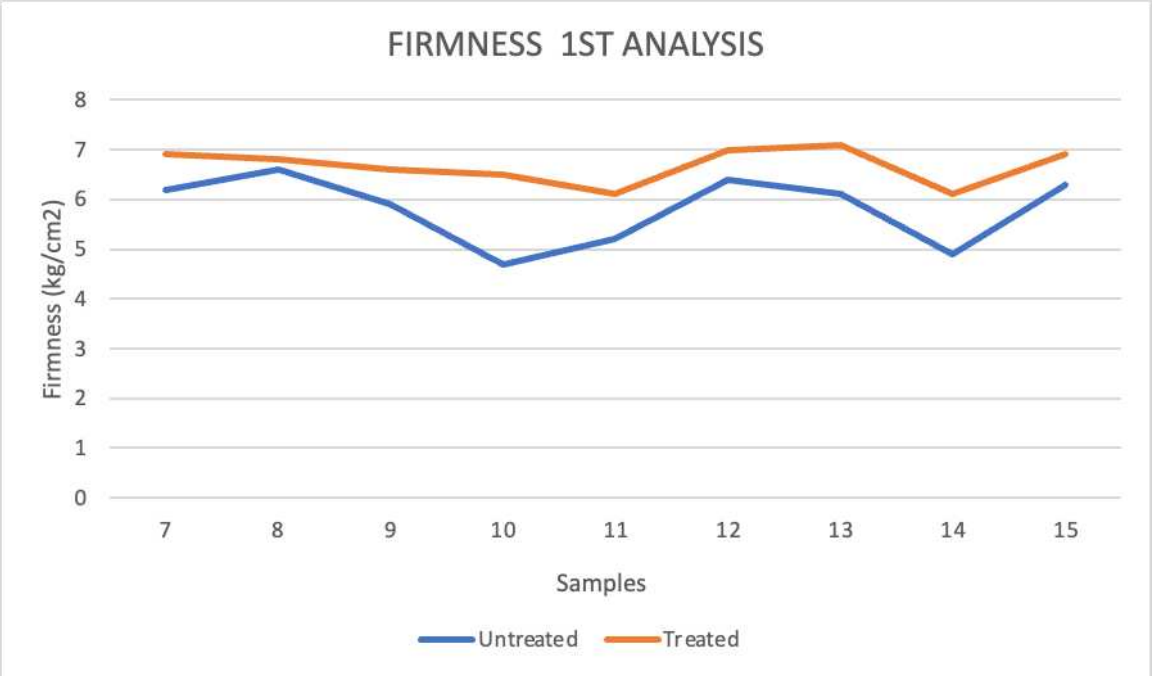
SF36 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Red Delicious S1T2.



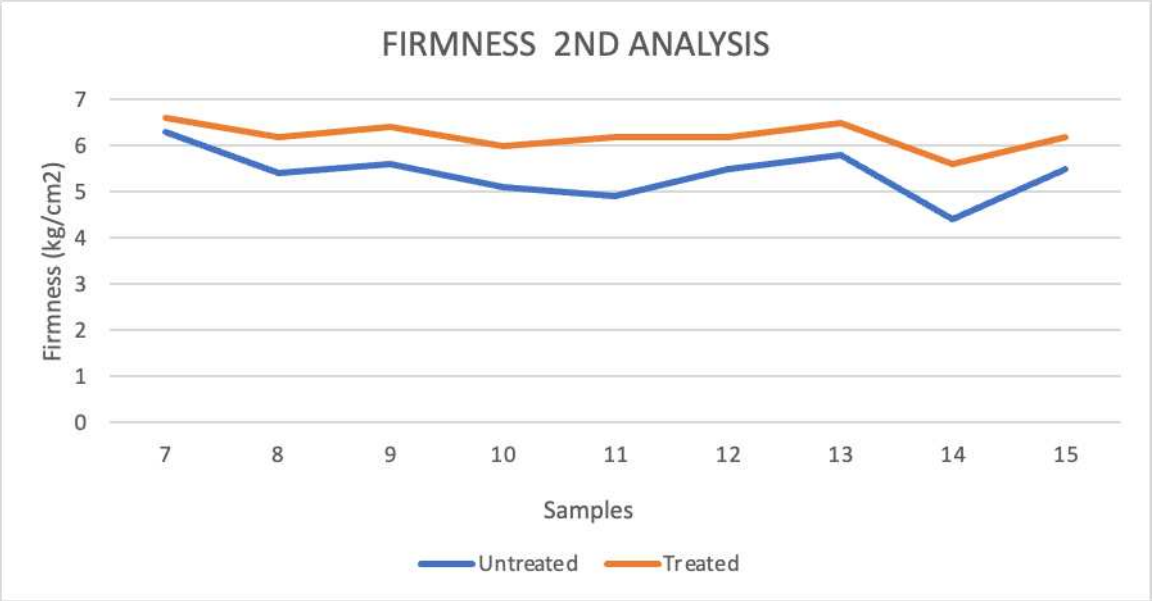
SF37 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Red Delicious S2 at harvest.



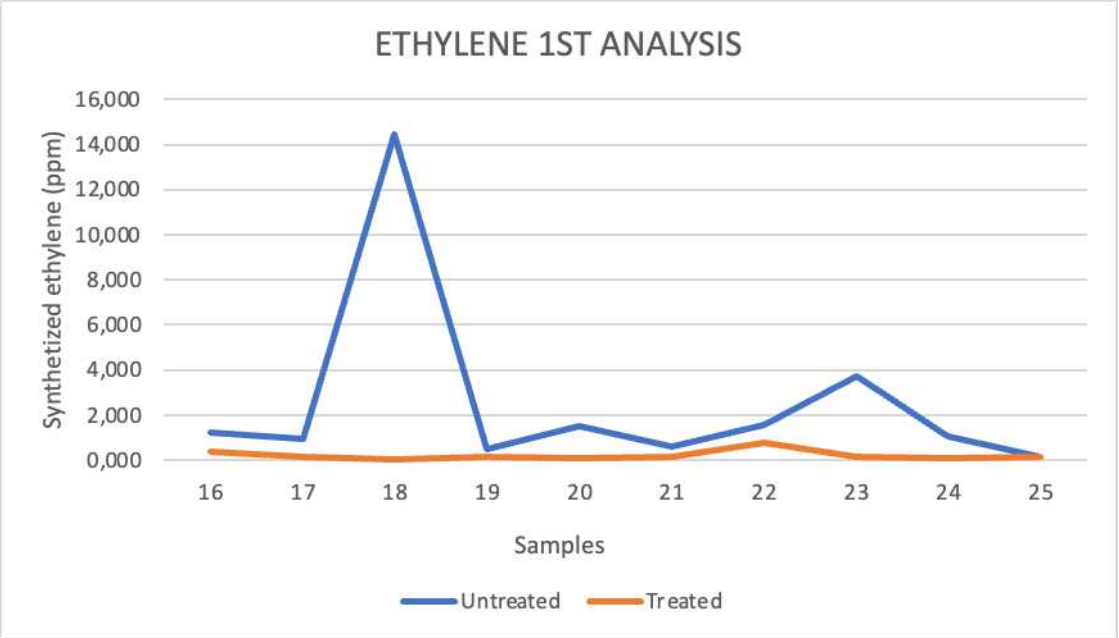
SF38 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Red Delicious S2T2.



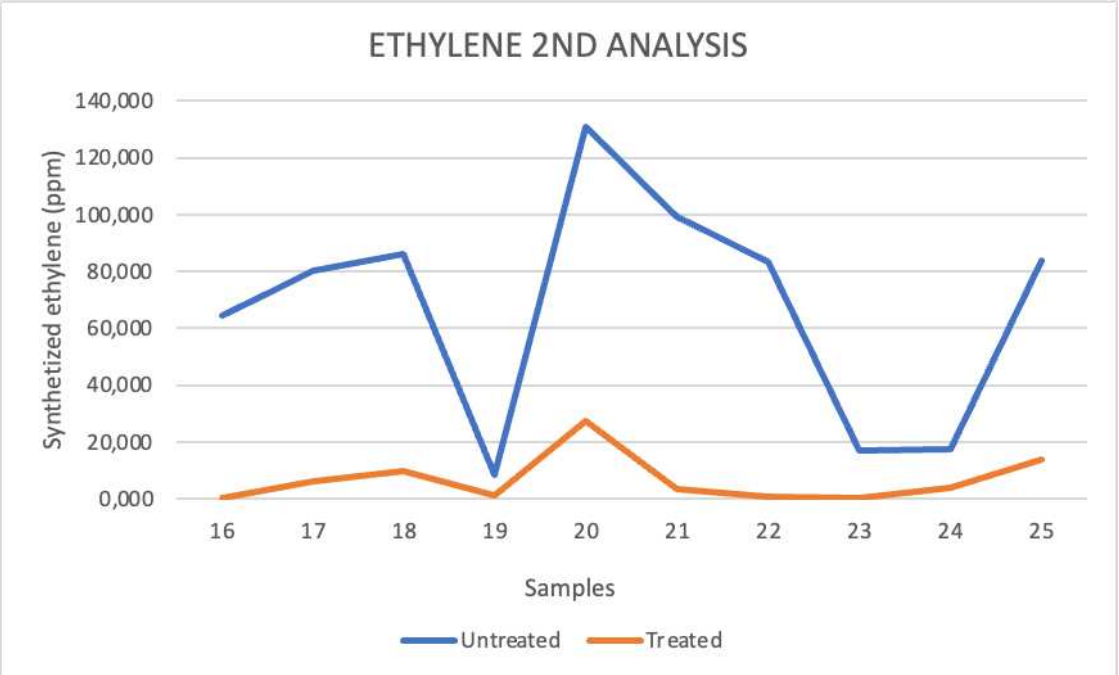
SF39 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Red Delicious S2 at harvest.



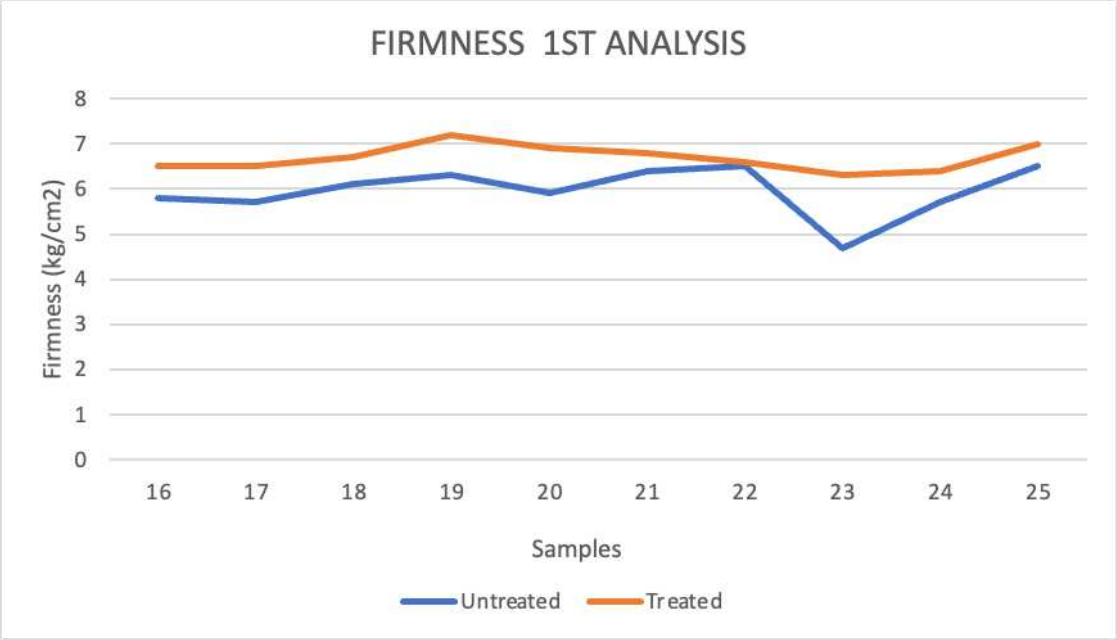
SF40 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Red Delicious S2T2.



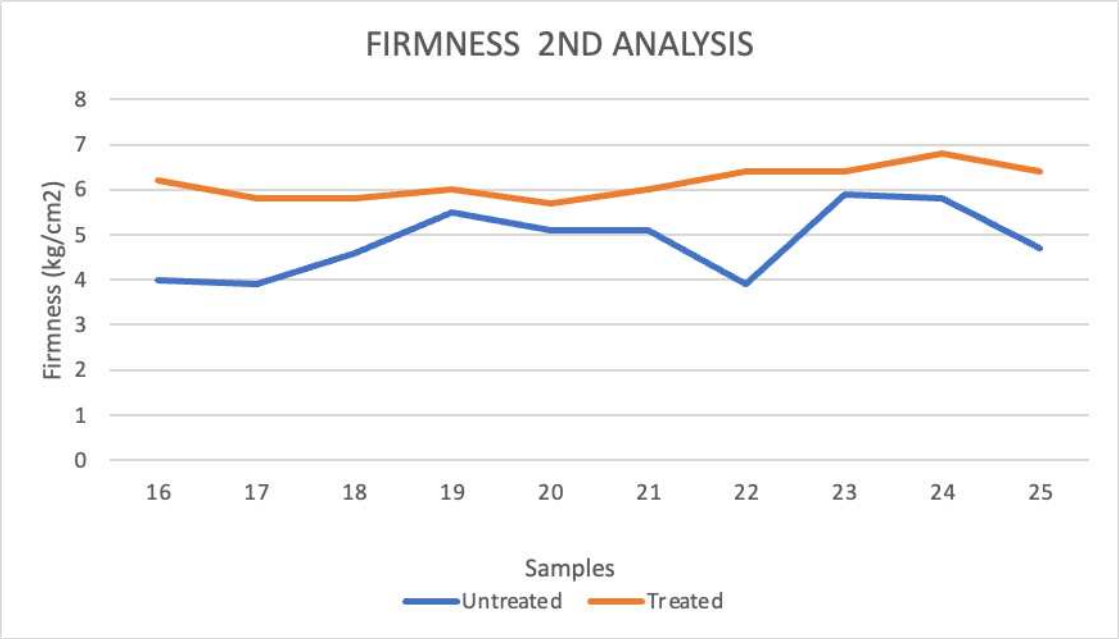
SF41 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Red Delicious S2 at harvest..



SF42 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Red Delicious S2T3.

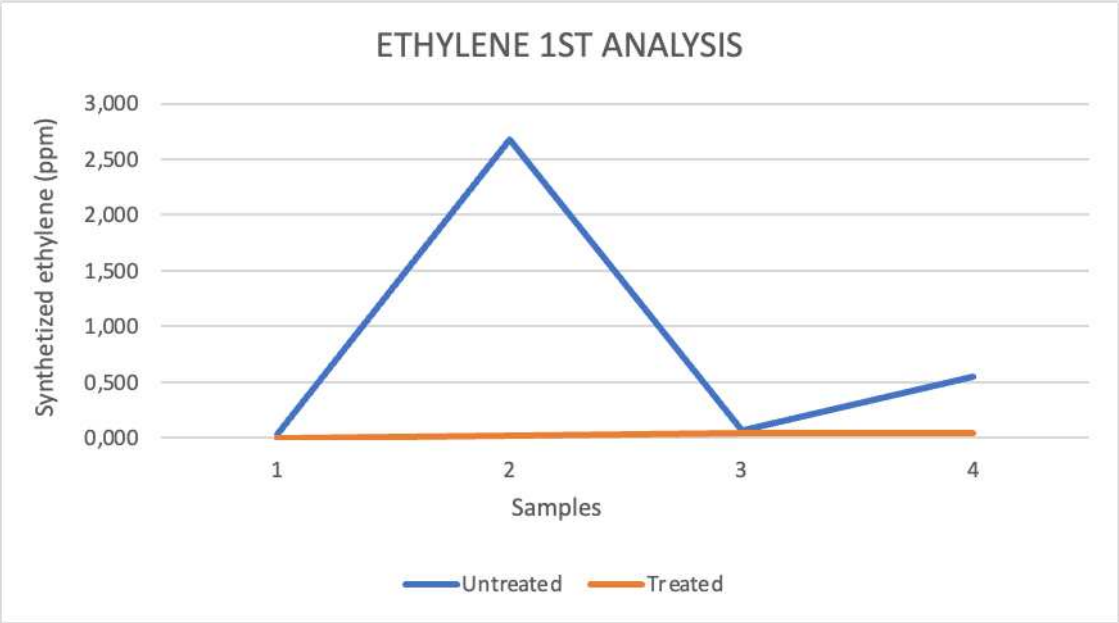


SF43 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Red Delicious S2 at harvest.

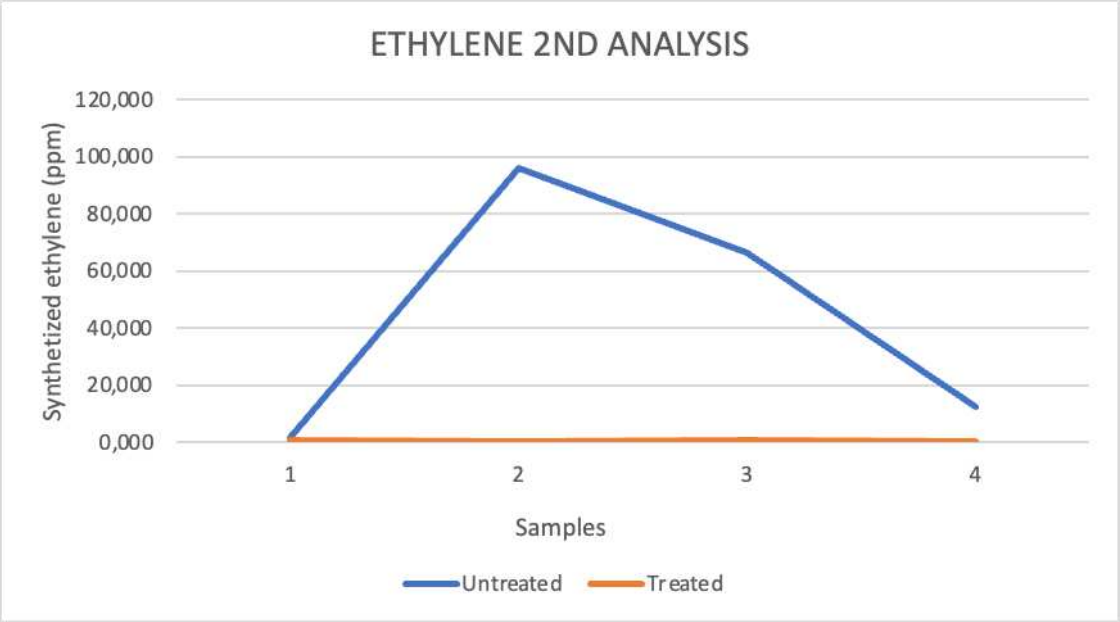


SF44 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Red Delicious S2T3.

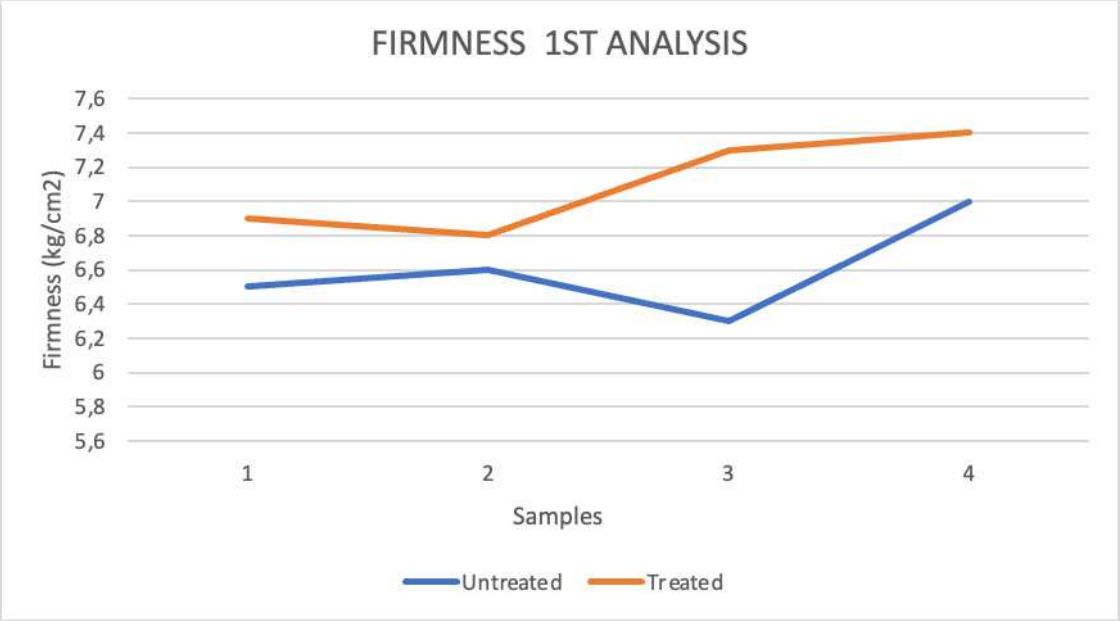
7.5. Granny Smith



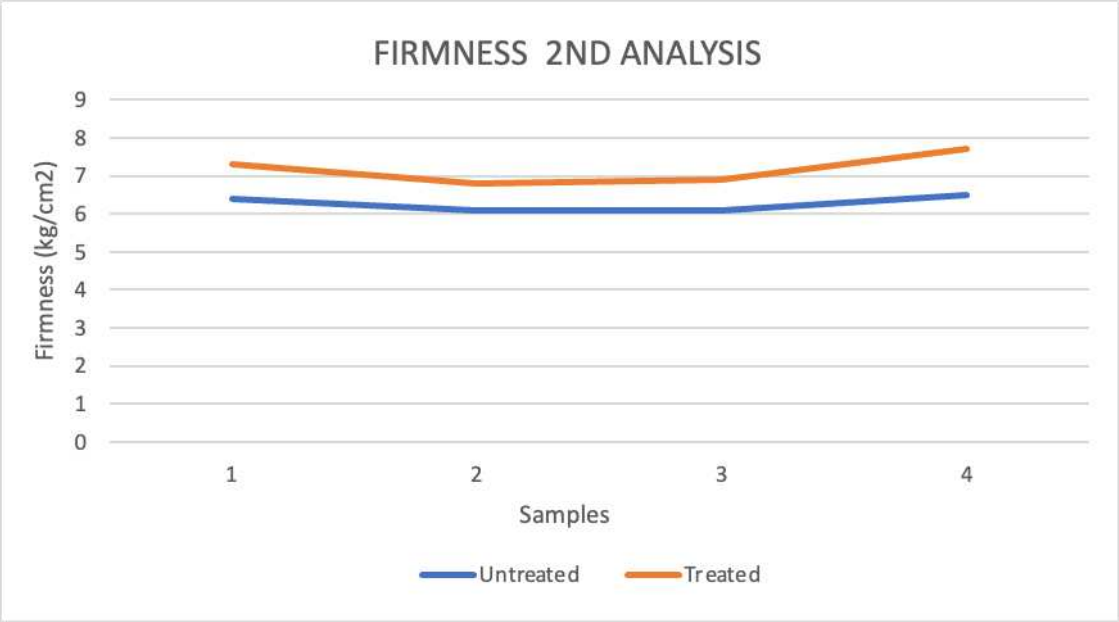
SF45 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Granny Smith S2 at harvest.



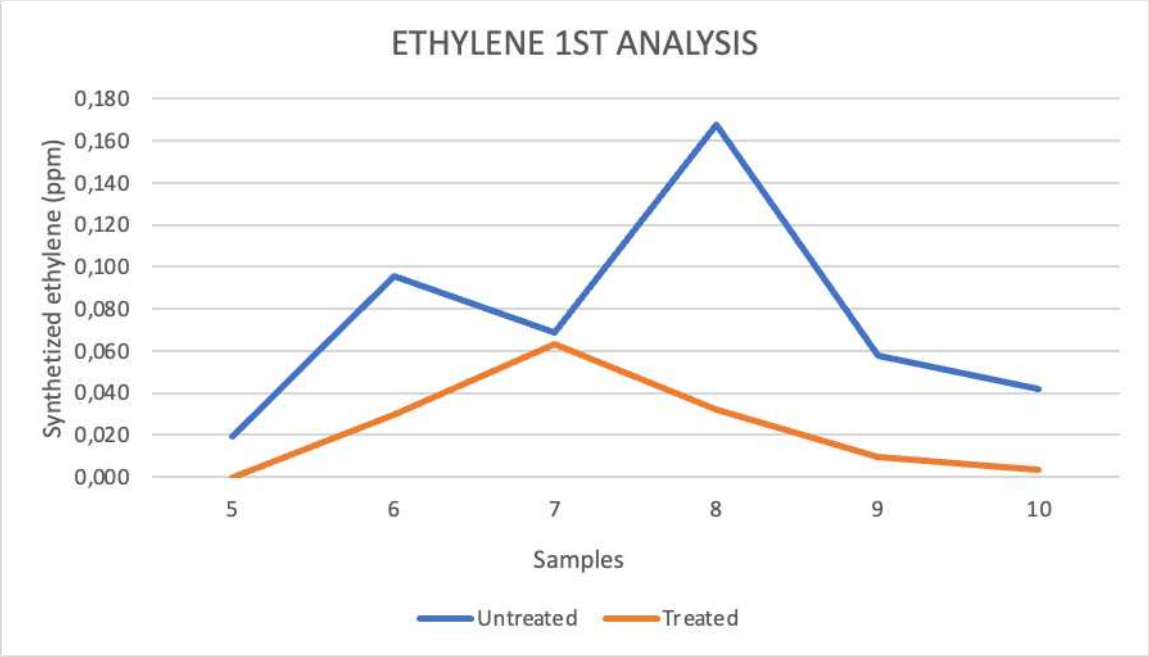
SF46 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Granny Smith S2T1.



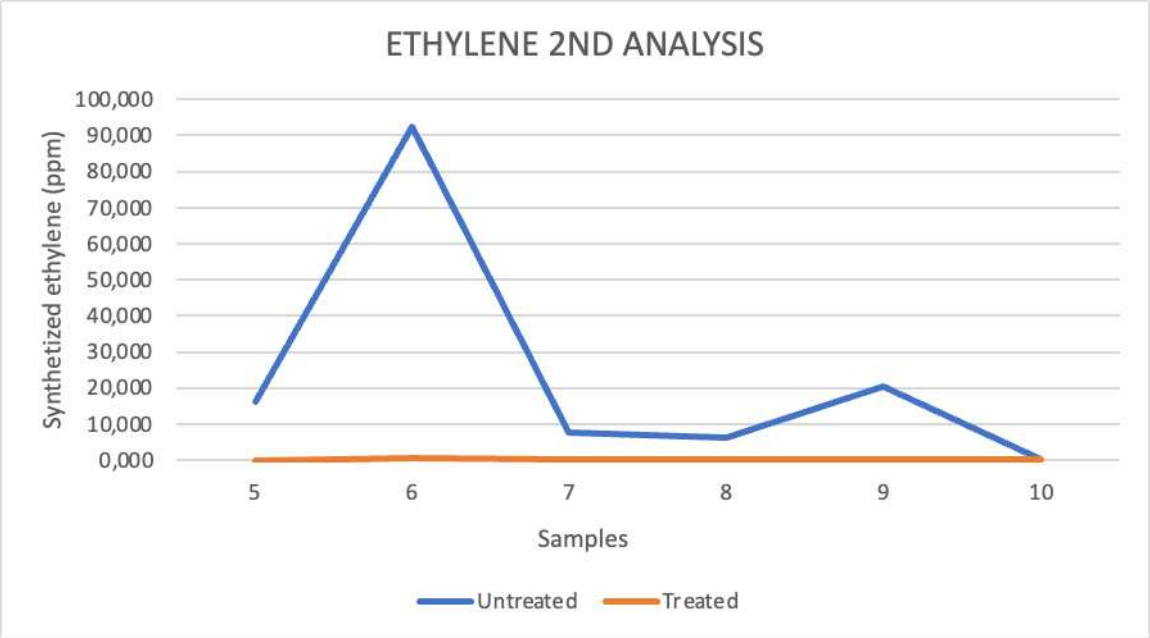
SF47 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Granny Smith S2 at harvest.



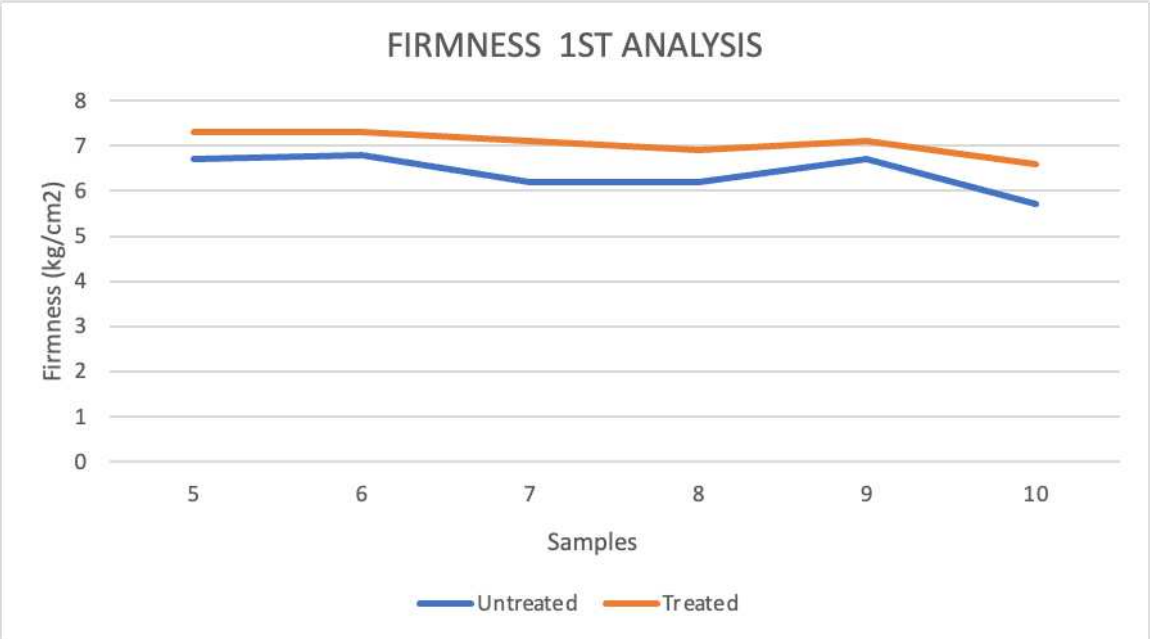
SF48 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Granny Smith S2T1.



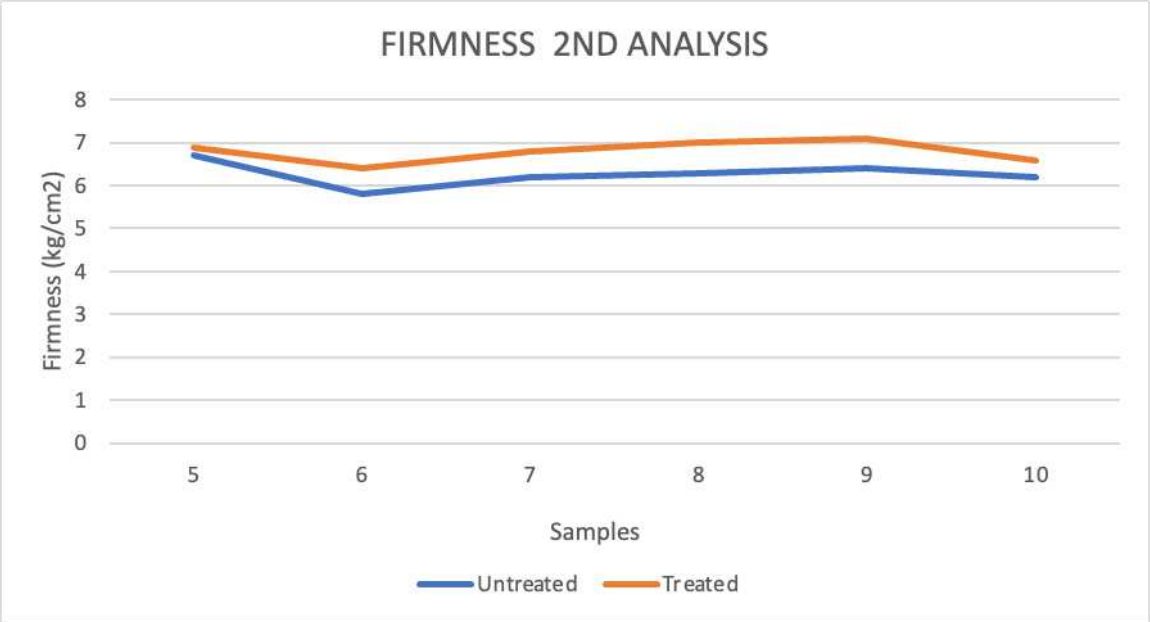
SF49 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 1st analysis for the samples Granny Smith S2 at harvest.



SF50 Ethylene synthesized by Treated with Fysium® and Untreated apples at the 2nd analysis for the samples Granny Smith S2T2.



SF51 Firmness of Treated with Fysium® and Untreated apples detected at the 1st analysis for the samples Granny Smith S2 at harvest.



SF52 Firmness of Treated with Fysium® and Untreated apples detected at the 2nd analysis for the samples Granny Smith S2T2.

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