## University of Trás-os-Montes e Alto Douro

Postharvest of *Actinidia deliciosa* cv. 'Hayward' and *Actinidia chinensis* cv. 'Jintao' - Effect of storage and shelf-life -

Master thesis in Agronomic Engineering

Ricardo José Menezes Rodrigues

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# Universidade de Trás-os-Montes e Alto Douro

Pós-colheita de *Actinidia deliciosa* cv. 'Hayward' e *Actinidia chinensis* cv. 'Jintao' - Efeito do armazenamento e tempo de prateleira -

Dissertação de mestrado em Engenharia Agronómica

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### **Abstract**

Kiwifruit production kept growing each year on the last decades in Portugal, and the main production regions are Entre-Douro e Minho region followed by Beira Litoral. Interest on kiwifruit keeps increasing due to its high nutritional value when compared to other fruits. These fruits are often stored on normal (NA) or controlled (CA) atmosphere chambers which can extend its marketability and make them available during at least 6 months. Several physicochemical changes occur during maturation and ripening of this fruit with a higher or lower intensity according to the metabolic activity demonstrated, where modified storage atmospheres intend to keep low, as well as maintaining the fruit overall quality and marketability through time. On this master thesis, physicochemical assays were done to evaluate the postharvest progression of two species of kiwifruits, Actinidia deliciosa cv. 'Hayward' and Actinidia chinensis ev. 'Jintao', where 'Hayward' was stored on CA and NA chambers while 'Jintao' was only stored in a NA chamber. The objectives are focused on the differences occurred between CA and NA for 'Hayward' kiwifruits through time and its shelflife performance, as well as the differences between 'Hayward' and 'Jintao' through time and on their shelf-life performance. The kiwifruit's weight, size, °Brix, pH, NaOH volume spent in titration, texture, skin and flesh colour, free-sugars concentrations, organic acids, antioxidant activity, vitamin C and carotenoids were all assessed during the experimental period of this work from November 2015 to March 2016. Following the objectives, a comparison was made between CA and NA for 'Hayward', the differences encountered between 'Hayward' and 'Jintao', as well as the shelf-life behaviour on a 7-day period. The predominant soluble sugars identified were fructose and glucose, and on lower concentrations sucrose and galactose, which tended to increase through time. Quinic and citric acids are predominant over malic and ascorbic acid with slight differences through time. Overall, CA provided better results than NA on preventing weight loss, keeping higher texture values, providing kiwifruits with more lightness and chroma, while no significant differences were found on °Brix, free-sugars, antioxidant activity and carotenoids; however, quinic acid was higher on NA. The main differences between cultivars were: an overall higher weight and firmness for 'Hayward'; a general decrease of lightness; 'Brix was clearly higher for 'Jintao'; free-sugars were also present on higher concentrations on 'Jintao' as well as an overall higher antioxidant activity. On the 7-day shelf-life period, no significant differences were found on weight loss, a general decrease in chroma, and firmness decreased significantly, °Brix had an increasing trend, no significant differences were obtained in free-sugars, carotenoids and antioxidant activity while ascorbic acid had significantly decreased between the start and the end of shelf-life.

**Keywords:** *Actinidia deliciosa*, *Actinidia chinensis*, storage, texture, quality, secondary metabolites

### Resumo

Nas últimas décadas, a produção de quivis em Portugal registou uma tendência crescente tendo como principais regiões de produção o Entre-Douro e Minho seguido da Beira Litoral. O interesse no consumo de quivis continua a aumentar devido ao alto valor nutritivo quando comparado com outros frutos. Os quivis são armazenados frequentemente em câmaras de atmosfera normal ou controlada, que podem estender a sua comercialização e torná-los disponíveis durante pelo menos 6 meses. Várias alterações físico-químicas ocorrem durante a maturação e amadurecimento com intensidade variável de acordo com a atividade metabólica demonstrada, em que as atmosferas modificadas de armazenamento pretendem manter baixa, bem como manter a fruta em bom estado geral de qualidade e comercialização ao longo do tempo. Nesta dissertação, análises físico-químicas foram feitas para avaliar a progressão em pós-colheita de duas espécies de quivis, Actinidia deliciosa cv. 'Hayward' e Actinidia chinensis cv. 'Jintao', onde 'Hayward' foi armazenado em atmosfera controlada e normal, enquanto 'Jintao' foi armazenado somente em atmosfera normal. Os objetivos estão focados nas diferenças ocorridas entre atmosfera controlada e normal para os quivis 'Hayward', através do tempo, e o seu desempenho em prateleira, e as diferenças entre 'Hayward' e 'Jintao' no tempo e no seu desempenho em prateleira. A massa dos quivis, tamanho, °Brix, pH, volume de NaOH utilizado, textura, cor da casca e polpa, concentrações de acúcares livres, ácidos orgânicos, atividade antioxidante, vitamina C e carotenoides foram avaliados durante o período experimental desta dissertação desde novembro de 2015 até março de 2016. Seguindo os objetivos, foi feita uma comparação entre atmosfera controlada e normal para 'Hayward', as diferenças encontradas entre 'Hayward' e 'Jintao', bem como o comportamento de vida de prateleira dessas duas situações num período de 7 dias. Os açúcares livres predominantes identificados foram a frutose e glicose, e em menores concentrações, sacarose e galactose, que tendem a aumentar com o tempo. Os ácidos quínico e cítrico são predominantes sobre o ácido málico e ascórbico com ligeiras diferenças ao longo do tempo. No geral, a atmosfera controlada proporcionou melhores resultados do que a atmosfera normal na prevenção da perda de massa, manutenção de valores de textura superiores aos de atmosfera normal, proporcionando aos quivis mais luminosidade e croma, apesar de não terem sido encontradas diferenças significativas em °Brix, açúcares livres, atividade antioxidante e carotenoides. Em atmosfera normal e entre cultivares as principais diferenças obtidas foram: valores de massa e textura geralmente mais elevados para 'Hayward'; uma diminuição geral de luminosidade; °Brix foi significativamente superior em 'Jintao'; açúcares livres presentes em concentrações superiores

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**Palavras-chave:** *Actinidia deliciosa*, *Actinidia chinensis*, refrigeração, textura, qualidade, metabolitos secundários

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### **Abbreviations list**

°C – Celsius degree; °h − Hue angle;  $\lambda_{max}$  – Wavelength where absorbance is maximum; μg – Microgram; μL – Microliter; μm – Micrometre; μM – Micromole; AA – Antioxidant activity; Abs – Absorbance; ABTS – 2.2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); Atm.-Atmosphere;C – Columella; C\* - Chroma; C<sub>18</sub> – Octadecyl carbon chain column; CA – Controlled atmosphere storage; CaCl<sub>2</sub> – Calcium chloride; CO<sub>2</sub> – Carbon dioxide; Csb – Warm-summer Mediterranean climate; CuCl<sub>2</sub> – Copper chloride; CUPRAC - Cupric reducing antioxidant capacity; cv. – Cultivar; DHA - Dehydroascorbic acid; DM – Dry matter; DPPH – 2.2-diphenyl-1-picrylhydrazyl; DTT – Dithiothreitol; DW – Dry weight; EC – Commission Regulation; EPA – United States Environmental Protection Agency; FeCl<sub>3</sub> – Iron(III) chloride; FeSO<sub>4</sub> – Iron(II) sulphate;

FRAP – Ferric-reducing antioxidant power;

FW – Fresh weight;

```
H1 – 'Hayward' producer number 1;
H2 – 'Hayward' producer number 2;
H3 – 'Hayward' producer number 3;
H4 – 'Hayward' producer number 4;
ha – Hectare;
HPLC - High Performance Liquid Chromatography;
HPLC-DAD – High Performance Liquid Chromatography with Diode-Array Detection;
IP – Inner pericarp;
IPMA – Instituto Português do Mar e da Atmosfera;
IU – International Unit;
J1 – 'Jintao' producer number 1;
K<sub>3</sub>Fe(CN)<sub>6</sub> - Potassium hexacyanoferrate;
L* - Lightness;
Max – Maximum;
mM – Millimole;
N - Newton;
NA – Normal atmosphere storage;
NaOH – Sodium hydroxide;
NH<sub>4</sub>Ac – Ammonium acetate;
nm – Nanometre;
OP – Outer pericarp;
ORAC – Oxygen radical absorbance capacity;
pH – Potential of hydrogen;
RI – Refractometric index;
rpm – Rotations per minute;
s – Second:
SL – Shelf-life;
SSC – Soluble solids content;
t - Tons;
TA – Titratable acidity;
TBA - Thiobarbituric acid;
TBARS – Thiobarbituric acid reactive substances;
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TCA – Tricarboxylic acid cycle;

TE – Trolox equivalent;

TFA - Trifluoracetic acid;

Tmax – Average maximum temperatures;

Tmin – Average minimum temperatures;

USDA – United States Department of Agriculture;

UV – Ultraviolet.

### **Chapter I - Introduction**

The production of kiwifruit in Portugal started in 1973, but only on the 1980's there was a boom, due to the good productivities and price. In 1992, the area occupied by this culture was 2000ha. The main production regions of this fruit, mostly *Actinidia deliciosa* cv. 'Hayward', are the Entre-Douro e Minho followed by Beira Litoral, and the area of this culture keeps growing each year (Franco, 2008a).

Fruits are essential for human health, and are vital for the maintenance of a good health, well-being and an active lifestyle. Kiwifruit also plays an important role in improving health in several domains, and the most evident is the improvement of gastrointestinal tract function. The quality of a fruit is associated to a series of qualitative parameters like the aspect, texture, flavour, nutritional value, which are determined by the maturity phase. The variation of these parameters is influenced by the pre- and postharvest conditions, in which the postharvest handling of the fruits purpose is to maintain the maximum quality of the product and its organoleptic, nutritional and sanitary characteristics (Singletary, 2012).

These fruits auto-catalyse ethylene during ripening at room temperature, but not at temperatures below 10°C. With this behaviour, it is possible to detect advantages that can be used to lengthen the storage time and by that, allow its commercialization throughout the year (Antunes, 2007). Normal and controlled atmosphere storage are two methods that ensure kiwifruit conservation for at least 6 months for normal atmosphere storage, and 9 months for controlled atmosphere storage (Antunes, 2008). On the other hand, kiwifruits are very sensitive to the effects of ethylene that can be harmful to its storage capacity. Besides that, before being stored, their condition should be free from wounds or infections like *Botrytis cinerea*, because these defects will sparkle the production of ethylene, even on low temperature, and then will alter and cause unwanted ripening inside the storage chambers. By the points described above, it is imperative to know the kiwifruit's condition on arrival at the storage chambers, and have special attention during their harvest, handling, transport and storage so that their commercialization is not affected (Antunes, 2007).

Physiochemically, after harvest of kiwifruits, and during storage at 0°C, the °Brix increases, while firmness decreases as Marsh *et al.* (2003) and Barboni *et al.* (2010) concluded, and those two factors are important on a quality point of view.

The most important sugars in kiwifruit are glucose, fructose and sucrose, while the most important organic acids are citric, quinic and malic (Soufleros *et al.*, 2001). These two groups of components are determinant for the flavour of the fruit (Singletary, 2012).

Interest in kiwifruit keeps increasing mainly due to its nutritional value, where vitamin C and antioxidant activity play an important role on maintaining a healthy diet and lifestyle. However, different species differ on vitamin C which may vary from 50 to 430mg/100g fresh weight, with levels on *A. chinensis* being higher than *A. deliciosa*, 100mg/100g to 85mg/100g respectively (Singletary, 2012).

On the present study two cultivars of different species will be studied, *Actinidia deliciosa* cv. 'Hayward' and *Actinidia chinensis* cv 'Jintao' on their postharvest performance under normal and controlled atmosphere storage, and their behaviour on a 7-day shelf-life experiment on the last 4 sampling dates. Three conductive lines are traced based on 10 sampling dates: 'Hayward's performance based on normal and controlled atmosphere storage from the first days of storage after harvest to 18 weeks of storage; 'Hayward' and 'Jintao' performances under normal atmosphere storage from the first days of storage after harvest to 18 weeks of storage; a 7-day shelf-life experiment of 'Hayward' from CA and NA and both cultivars comparison on the last 4 sampling dates. Various parameters were studied such as 'Brix, pH, NaOH volume spent in titration, texture, flesh and skin colour, free sugars, organic acids, antioxidant activity, vitamin C and carotenoids.

### **Chapter II - Literature review**

### 1. The kiwifruit

There are numerous identified species on the Actinidia genus, and the genetic diversity is superior in Actinidia deliciosa than A. chinensis. Although worldwide kiwifruit production is based on A. deliciosa cv. 'Hayward' and A. chinensis cv. 'Hort16A', there are other cultivars of A. deliciosa such as 'Abbott', 'Allison', 'Bruno' and 'Monty' and pollinator cultivars such as 'Matua', 'Tomuri' and 'Chieftain' (Neves, 2008a). Those two species are native only in China and in 1400 its first reference and description was done while in 1847 it was classified by the botanist Planchon. It was introduced in Europe in 1903 by a British botanist, however, it was New Zealand that elevated Actinidia from a wild plant to an industrial crop of great interest. The first productions took place in 1910 and it was from New Zealand that the investigators shared the knowledge of the Actinidia production with the rest of the world (Cacioppo, 1989c; Huang et al., 2002). The fruit is a berry, with hundreds of small, dark seeds involved in pulp. Depending from specie to specie, the fruits are spherical, elongated or cylindrical, with a great variation in pulp colour, and with or without pubescence on its skin as Neves (2008a) describes, and can be seen on figure 1. Fruits of Actinidia species are known for their high content in vitamin C and having antioxidant and immune-stimulation properties (Nishiyama et al., 2004).



Figure 1 - Different kiwifruit cultivars profiles (Wong, 2012).

Italy, New Zealand, China and Chile are responsible for 30, 21, 18 and 13% of the world kiwifruit production. However, significant plantings are taking place in France, Japan, Greece,

United States, Iran and Spain, with a world production of kiwifruit exceeding 1.2 million tons per year (Atkinson and MacRae, 2017). In the European Union, Italy, Greece, France, Spain and Portugal are the main producers of kiwifruit and the area occupied by this culture keeps growing each year. Portugal by 2013 had an area occupied by this culture of 2127ha, in 2014 was 2255ha and in 2015 was 2305ha, with a crop yield of 21306 tons, 18150t and 28331t, respectively. In commercial terms, Portugal imported 11109t and exported 12412t in 2014. The Portuguese market consumes 20000t of kiwifruit annually, and the Portuguese production lasts on the market for around 7 months due to the conservation limitations (Veloso and Oliveira, 2008; INE, 2016; FAOSTAT, 2017).

The EC Regulation no 1673/2004 of 24 September of 2004 establishes the commercialization norm applied to kiwifruit and enumerates several minimum quality characteristics. For all categories, kiwifruit must be whole, sane and without rotting, clean, free from parasites and its attacks, sufficiently firm, well formed, free from abnormal external moisture, free from odours or strange flavours. The development and condition of the kiwifruit must allow them to support the transportation and other movements that are subjected and arrive its destination in satisfactory conditions.

Soluble solids content (SSC) and firmness are used as harvest indexes. A minimum soluble solids content (SSC) of 6.2% was established in New Zealand for 'Hayward' kiwifruits for export, however for storage performance and long-term storage, the optimum SSC range is situated between 7-9% (Burdon *et al.*, 2013). At this point, firmness value is approximately 78.45N and the kiwifruit is ready to consume at a range of 4.9 to 9.8N (measured with a penetrometer with a cylindrical tip of 8mm of diameter, to a deepness of 7mm) (Antunes, 2007). A 6.5°Brix as harvest index is commonly used, otherwise if harvested with a lower °Brix, in storage it will not reach minimum °Brix of 12-14° for consumption (Antunes, 2008). On yellow-fleshed cultivars, pulp colour is one of the main attributes in marketing, it is also used as a harvest index although it is not a robust measure of predicting an optimal harvest as it can vary from one location to another and it is not consistent enough with °Brix and firmness values (Burdon *et al.*, 2014).

To have a good commercialization of the kiwifruits, it is important to be aware of their firmness as it is crucial to maintain the quality of the fruit. Firmness problems are very concerning, as it decreases during ripening and makes the fruit more vulnerable to bruises and consequently the promotion of ethylene production, inducing unwanted ripening effects, also attracting fungus and other microorganisms that harm their quality and viability of

commercialization. By respecting good postharvest techniques it is possible to preserve kiwifruits as long as 9 months only with slight quality changes (Antunes, 2007).

## 2. Plant development and edaphoclimatic preferences

The root system explores a large area of soil, over 80m<sup>3</sup>, and depending on the soil characteristics and cultural conditions it can go as deep as 4m or more. It is a climbing plant, with flexible branches, and when young these have an herbaceous aspect and fast growth. The size and position of the branches affect the number of flowers and consequently, fruit quantity and size, where the first ones and longer branches have a trend to originate more fruits and of bigger size (Neves, 2008b).

Actinidia adapts well to different soil characteristics if these have a good balance between water retention and drainage. It is to avoid soils with high clay content with drainage difficulties as the plants are more susceptible to have root asphyxiation. On the other hand, soils that have a low water retention capacity that induce a rapid hydric stress are also to avoid (Oliveira and Veloso, 2008).

The optimal range of pH level is between 5.5 and 6.8. Above a value of 7.5, the availability of micronutrients is shortened and under a value of 5.5 a soil correction must be made. Rich, fertile, well-drained and deep soils, high in organic matter are suitable for a good development of a kiwifruit orchard. Soils rich in clay and limestone are not very suitable for this culture (DISQUAL, 2004; Oliveira and Veloso, 2008).

The *Actinidia* plants are designated as subtropical, preferring a long growth season with at least 240 days free of frosts. During winter, 700 to 900 hours of temperatures below 7°C are important to break dormancy. The optimal temperatures for its growth are between 14 and 25°C, being resistant until -15°C (with a risk of suffering trunk damage), and above 25°C it copes well if its hydric necessities are guaranteed. The hydric requirements of these plants vary during its cultural cycle, and to produce 25 000kg of fruits it needs 6000m³ of water (Oliveira and Veloso, 2008). The plant is well adapted and prefers climates with high relative humidity, absence of strong winds so it does not break its branches or cause deformations and spots in the fruit. Excessive sunlight is also harmful to the leaves and fruits as it may cause the loss of fruit pubescence and also abortion phenomenon (DISQUAL, 2004).

Frosts occurring during spring have different effects than the ones that occur in autumn. Late frosts in spring are especially harmful to the aerial part of the plant destroying its young leaves or floral buds partially or entirely, and compromising the production. Early frosts in

autumn prove to be harmful to the branches and even more on kiwifruits, causing necrosis on the peduncle making it fall and also cell destruction that reduces firmness, turning the fruits improper for storage (Beutel, 1997; Oliveira and Veloso, 2008).

From the end of winter and through the beginning of spring, the root system restarts its activity and consequently, the buds start to sprout. After approximately 2 months, flowering starts and when summer starts, the growth rate of the branches decreases and coincides with the fast growth of the fruits. On this stage, floral induction also occurs and it will determine the next year's flowering and production. 'Hayward' kiwifruits are matured approximately 150 days after flowering. After losing the leaves, the plant enters in vegetative rest from the beginning of December with a minimum average temperature of 5-6°C, and it continues this phase until the end of winter with the swelling of the buds (Neves, 2008b).

## 2.1. Preharvest factors that influence postharvest performance

Determining the stage of fruit development before harvest is an important factor to consider as it will greatly influence postharvest quality. Harvesting before physiological maturity stage will not develop the full flavours and aromas of the kiwifruit, and it will not ripen nor store properly (Boukouvalas and Chouliaras, 2005).

Several preharvest factors may influence postharvest quality of the kiwifruits. Postharvest techniques only maintain the fruit's quality at which it was harvested and it will not improve it, so it is important to determine the best harvest opportunity and keep good practices during harvest, transport and storage.

Climatic, soil and cultivation conditions also play an important role on kiwifruit production as the plant condition will determine the final fruit quality and size. An increase of temperature by 3-4°C during physiological maturity stage will raise total soluble solids content. Fertilization and irrigation will also influence the photosynthetic rate and mobilization of carbohydrates on the plant and therefore on the fruits. Pruning is essential to control growth, shaded areas and crop load of the plants. A heavy crop load will decrease the total soluble solids content (Boukouvalas and Chouliaras, 2005). For this, production pruning is important to promote a balanced relation between fruitification and vegetative growth, a regular and good quality fruit production, to allow light penetration and make pollination easier and well distributed (Rodrigues, 2008).

Soil water content should be maintained during the vegetative and fruit growth, without causing much hydric stress as it may reduce final productivity up to 25%, affecting fruit size (Boukouvalas and Chouliaras, 2005).

Plant nutrition also influences postharvest quality, where nitrogen affects kiwifruit's flesh firmness. When the plant's leaves have less than 2% nitrogen, fruits have a longer postharvest life, however, when this value is higher than 2% it promotes flesh softening (Boukouvalas and Chouliaras, 2005).

Calcium, as a cell wall element, is very important on the kiwifruit's storage life, preventing the appearance of *Botrytis cinerea* and extending the storage duration slowing up its maturation progress. Preharvest applications of this element are a plus to extend storage life, and are more effective than postharvest dips in calcium solutions (Boukouvalas and Chouliaras, 2005). However, Antunes *et al.* (2007) recommends a postharvest immersion of kiwifruit in a 2% CaCl<sub>2</sub> solution to extend storage life.

## 3. Actinidia deliciosa cv. 'Hayward' and Actinidia chinensis cv. 'Jintao'

Actinidia deliciosa cv. 'Hayward' kiwifruits date back to its selection process in 1920, being commercially available in 1930. The plants have a median vigour and productivity. The harvest season is, in general, during the first two to three weeks of November on the northern hemisphere. The pulp is light green with good flavour and aroma. The longitudinal shape of the kiwifruit is ellipsoidal-ovate and transversally is elliptic. It has a good capacity of being stored under low temperatures during several months (Cacioppo, 1989a). Consumers prefer  $\geq 12.5\%$  of soluble solids on ripe kiwifruit and dislike kiwifruits that have  $\leq 11.6\%$  (Crisosto and Crisosto, 2001).

Actinidia chinensis cv. 'Jintao' is marketed as 'Jingold<sup>TM</sup>' from the Italian Kiwigold<sup>®</sup> consortium (Burdon *et al.*, 2014). 'Jintao', meaning 'Gold peach' in Chinese, originates from southeast China and it was discovered in 1981 through a breeding program. Since then, it has been further developed for commercial production until it was officially released for propagation in 2001 in Italy. Harvest dates, when compared with 'Hayward' are more anticipated from middle to late September to middle to late October, differing per locations but on average, 'Jintao' is harvested 20 to 25 days earlier than 'Hayward'. Fruits are long, cylindrical and uniform, light-brown coloured and have very few pubescence. Flesh colour is green at harvest and as the fruit ripens turns into yellow. Its edible pulp is sweet, tender and juicy with a good balance between acid and sugar content (Huang *et al.*, 2002). According to

maximum soluble solids content, on two seasons, values of 15.5% and 14.3% were obtained (Burdon *et al.*, 2014).

## 4. Nutritional and dietetic composition

Fruits in general are essential for a healthy lifestyle as a good source of vitamins, minerals and other nutrients that maintain the proper function of our organism. In Portugal there is a preference for apple (31%) following the orange (22%), pear (16%), banana (12%) and then the kiwifruit (3.5%) (COTHN, 2008).

A comparison of the nutritional quality and quantity of the two most consumed fruits in Portugal and kiwifruit is done on table 1. It is evident that kiwifruit has a higher content on almost all its constituents when comparing to the other fruits that are widely consumed and preferred by the consumers.

Water makes up more than 80% of the fruits constitution, and by USDA (2016) reports, the least caloric fruit is the orange (47kcal) followed by apple (52kcal), pear (57kcal), 'Hayward' kiwifruits (61kcal), 'SunGold' kiwifruits (63kcal) and banana (89kcal). The protein amount on the several fruits is higher in kiwifruits, from 1.02 to 1.14g per 100g of edible portions as well as on banana (1.09g/100g of edible portion), while apple and pear present lower amounts of protein, 0.26 and 0.36 g per 100g of edible portion, respectively. Total lipid content is higher for kiwifruits, between 0.28g and 0.52g per 100g of edible portion, while orange, pear and apple show closer values, 0.12g/100g, 0.14g/100g, 0.17g/100g of edible portions respectively. On carbohydrates, banana leads with 22.84g/100g, then there is 'SunGold' kiwifruits and pear with a value of 15.79g and 15.23g per 100g of edible portions respectively, while 'Hayward' kiwifruits vary between 10.90g and 14.66g while apple and orange have a lower content, 13.81g/100g and 11.75g/100g of edible portion respectively. Overall, kiwifruits are not the most or the least caloric fruit, but possess the highest values of protein and total lipid content, and the carbohydrates content is at an intermediate level, when compared to the other most consumed fruits in Portugal as seen on table 1.

Singletary (2012) obtained a content of 3.4g of dietary fibers/100g of fresh fruit which represent 10% of the recommended daily requirement for dietary fiber. Also by having insoluble fibers as lignin, there are evidences that this fruit has laxation properties and reduces the time of food transit on the intestines. Park *et al.* (2011) also indicates a high dietary fiber content on kiwifruits, without significant differences between total, soluble and insoluble dietary fibers.

Table 1 - Nutritional value of two kiwifruit cultivars, apple and orange per 100g of edible fruit.

Nutrients	Kiwifruit (Hayward) <sup>1</sup>	Kiwifruit (Hayward) <sup>2</sup>	Kiwifruit (ZESPRI SunGold) <sup>1</sup>	Apple <sup>a</sup>	Orange <sup>b 1</sup>
Water (%)	83.07	82.90	82.44	85.56	86.75
Energy (kcal)	61.00	60.00	63.00	52.00	47.00
Protein (g)	1.14	1.10	1.02	0.26	0.94
Total lipid (g)	0.52	0.50	0.28	0.17	0.12
Carbohydrate (g)	14.66	10.90	15.79	13.81	11.75
Total dietary fiber (g)	3.00	-	1.40	2.40	2.40
Total sugars (g)	8.99	-	12.30	10.39	9.35
Calcium (mg)	34.00	19.00	17.00	6.00	40.00
Iron (mg)	0.31	0.40	0.21	0.12	0.10
Magnesium (mg)	17.00	18.00	12.00	5.00	10.00
Phosphorous (mg)	34.00	28.00	25.00	11.00	14.00
Potassium (mg)	312.00	300.00	315.00	107.00	181.00
Sodium (mg)	3.00	9.00	3.00	1.00	0
Zinc (mg)	0.14	0.20	0.08	0.04	0.07
Vitamin C, total ascorbic acid (mg)	92.70	72.00	161.30	4.60	53.20
Thiamine (mg)	0.027	0.02	0	0.017	0.087
Riboflavin (mg)	0.025	0.05	0.074	0.026	0.04
Niacin (mg)	0.341	0.30	0.231	0.091	0.282
Vitamin B6 (mg)	0.063	0.02	0.079	0.041	0.06
Folate, DFE (μg)	25.00	42.00	31.00	3.00	30.00
Vitamin B12 (μg)	0	0	0.08	0	0
Vitamin A, RAE (μg)	4.00	7.00	1.00	3.00	11.00
Vitamin A (IU)	87.00	-	23.00	54.00	225.00
Fatty acids (saturated) (g)	0.029	0.10	0.065	0.028	0.015
Fatty acids (monounsaturated) (g)	0.047	0.10	0.023	0.007	0.023
Fatty acids (Polyunsaturated) (g)	0.287	0.20	0.11	0.051	0.025

<sup>&</sup>lt;sup>1</sup>(USDA, 2016);<sup>2</sup>(PortFIR, 2016); <sup>a</sup>Apples' data are based on Red Delicious, Golden Delicious, Gala, Granny Smith and Fuji cultivars, unpeeled; <sup>b</sup>Orange's data is based on all comercial cultivars, peeled.

The values in table 1 point to a similar amount of total dietary fibers for 'Hayward' kiwifruits (3.0g/100g of edible portion) and a lower content for 'SunGold' kiwifruits (1.40g/100g of edible portion). Pears also have a similar value of 3.1g, while bananas have 2.6g and then apple and orange with 2.40g per 100g of edible portions. Franco (2008b) highlights the nutritional importance of dietary fibers and explains that insoluble dietary fibers are not digested and absorb water making the faeces less solid, reducing the time that food remains in the intestines and facilitates defecation. It also can be important to reduce obesity as these fibers occupy more space and are less caloric, contributing for a lower food consumption and a higher chewing time, producing a satiety sensation earlier. Franco (2008b) and Singletary (2012) report a potential benefit on cardiovascular diseases by the protective behaviour of dietary fibers on controlling cholesterol and blood glucose.

The mineral content of kiwifruits is overall higher when compared to the other most consumed fruits in Portugal and also promotes a better functioning of our organism, on vital functions such as the cardiovascular system (Singletary, 2012). Calcium is present in higher quantity in oranges (40g/100g of edible portion), and kiwifruits come next with different values between authors for 'Hayward', 34g to 19g/100 of edible portions and 'SunGold' with 17g/100g of edible portion. Iron, magnesium, phosphorous, potassium and all minerals represented are in general significantly higher on both kiwifruit cultivars. This reveals a strong nutritional potential of this fruit, that can boost health and prevent several diseases through its high content on almost all constituents. Park *et al.* (2011) conducted a mineral content assay with four different cultivars of *Actinidia* ('Hayward', 'Daeheung', 'Bidan' and 'Haenam') and concluded that the kiwifruits had high and comparable values of minerals, and only 'Bidan' significantly differed with the highest mineral content. Potassium was significantly higher than the other minerals and 'Hayward' is rich in potassium ( $1683 \pm 21.1 \text{mg}/100g$  DW), phosphorous ( $244 \pm 10.9 \text{mg}/100g$  DW), and calcium ( $146 \pm 7.02 \text{mg}/100g$  DW).

On vitamin C, 'SunGold' kiwifruits lead with 161.30mg/100g of edible portion and 'Hayward' kiwifruits come next with 92.70mg and 72.0mg/100g of edible portions. Orange comes next with 53.20mg, banana with 8.70mg, apple with 4.60mg and pear with 4.30mg per 100g of edible portions. This vitamin is especially important on the neutralization of free radicals that can cause severe problems on our organism such as cancer and other diseases as well as the reinforcement of the immunity system (Singletary, 2012).

# 5. Volatile organic compounds

These are organic chemical compounds whose composition makes its evaporation possible under normal atmospheric conditions of temperature and pressure. They include any compound of carbon, except the ones that participate in atmospheric photochemical reactions like carbon monoxide or carbon dioxide (EPA, 2016).

The two most commercially important kiwifruit species *A. deliciosa* and *A. chinensis* had similar volatile composition, containing essentially esters, alcohols and aldehydes. This indicates that the differences in flavours are due to the proportion of these compounds, as well as the presence of exclusive compounds of a specie or another. Eucalyptol and methylsulfanyl compounds were found to be exclusive for *A. chinensis* cv. 'Hort16A', and C6 aldehydes responsible for the fresh, green and grassy notes were exclusive for *A. deliciosa* cv. 'Hayward' (Garcia *et al.*, 2012).

These compounds have more intensity on the *A. chinensis* cv. 'Hort16A' when comparing to *A. deliciosa* cv. 'Hayward' which has a lower intensity. A grassy odour was higher on 'Hayward' than 'Hort16A' (Jaeger *et al.*, 2003).

During storage of the kiwifruits, the content on aldehydes decreased with ripening, while esters rose. This explains the flavour change from 'green' and 'grassy' to 'fruity'. During a long-term storage of kiwifruits, it is detected an aromatic decrease especially to the sulphurous compounds of the *A. chinensis* ev. 'Gold' (Garcia *et al.*, 2012).

Jaeger et al. (2003) studied the influence of flavour and odour in consumer acceptance towards new genotypes as A. chinensis 'Hort16A' and 'C2', and found a segment of consumers that preferred these two genotypes of yellow-fleshed, sweet and fruity flavoured kiwifruits, when compared to A. deliciosa cv. 'Hayward' as green-fleshed, and a sweet-acid taste. The authors describe the flavour of 'Hort16A' as being sweeter and less acid than 'Hayward' but with a higher odour than blackcurrant, aromatic melon and cotton candy. For astringency, 'Hayward' and 'Hort16A' had the highest and the lowest scores respectively. Besides flavour and odour, the average dimension, succulence and flavour intensity of the kiwifruits of each cultivar influenced consumer preference, which is targeted as objects of future studies. These authors suggest that future cultivars should be neither bland nor less succulent.

It should be noted that consumers have varied preferences, and with the genotypes of *A*. *deliciosa* that are characterized by its green pulp and bitter-sweet taste, the consumers will also have at their disposal *A. chinensis* genotypes that have yellow pulp and are sweeter with a fruity flavour (Jaeger *et al.*, 2003).

# 6. Cold storage – Normal and controlled atmosphere

There are several methods for storing kiwifruits or other fruits. Depending on their destination, fruits can go directly from harvest to the stores, or can go to storage facilities and be available to the markets during several months. These storage facilities may work with different atmospheres, temperatures and humidity and the combination of these factors is done accordingly to the fruit stored and its destination.

Conservation of kiwifruits in different temperatures may reveal different metabolisms when compared with kiwifruits stored at 0°C, and other stored above 0°C. This also results in different sensory characteristics, which are detected by taste, where kiwifruits stored at 4°C are more acid and less sweet than those stored at 0°C and 10°C that are sweeter and less acid. These differences occur since there is a decrease on the level of soluble solids or an increase of the malic acid. Meanwhile, kiwifruits stored at 4 and 10°C have similar soluble solids content but with differences on the acids content. Kiwifruits stored at 4°C are 1-2% poorer on soluble solids content than those cooled at 0°C, over seven weeks of air storage.(Marsh *et al.*, 2003).

Storage with temperature above 0°C can affect the flavour of the kiwifruits, and an increase of 3-4°C during the storage period will result on the decrease of sweetness and a higher perception of acid flavour, as well as 'off-flavours' such as woody/stalky and metallic flavour. However, there are differences on the ripening behaviour of these fruits according to the climate in which they are grown with regard to acidity, and hence it is more advantageous to opt for alternative conservation strategies in warmer climates than New Zealand (Marsh *et al.*, 2003).

Kiwifruits have an excellent capacity for storage, being able to withstand 4-8 weeks on fresh conditions without additional cooling. However, to have a good marketability, refrigeration is necessary. Under cold NA storage, it lasts 4-5 months with 0±0.5°C and 90-95% relative humidity. Controlled atmosphere storage provides better results when compared to normal atmosphere, and also several factors determine the success of storage such as ethylene concentrations, atmosphere combination, air temperature and humidity (Özer *et al.*, 1999).

Different atmosphere combinations have different effects on the kiwifruits stored on their physical and biochemical characteristics. Benefits of CA were assessed by Li *et al.* (2017) regarding firmness retention of 'Hayward' kiwifruits subjected to different gases concentrations  $(2\%O_2 + 2\%CO_2)$  and  $2\%O_2 + 5\%CO_2$ , obtaining a better firmness retention on the  $5\%CO_2$  experiment, and in addition, firmness rates were maintained a couple of weeks more when transferred to air-storage. Antunes (2008) reported that the most adequate CA combination is  $2\%O_2 + 5\%CO_2$ , and on these conditions, kiwifruits can be stored for 9 months, at  $0^{\circ}$ C and 90-

95% relative humidity, on an ethylene-free environment. Özer *et al.* (1999) concluded that the best combination of oxygen and carbon dioxide are  $5\%O_2 + 5\%CO_2$  or  $2\%O_2 + 5\%CO_2$ , but other combinations also gave good results,  $3\%O_2 + 3\%CO_2$  and  $5\%O_2 + 3\%CO_2$ . Besides manipulating these gases concentrations, an air temperature of  $0\pm0.5$ °C and 90-95% relative humidity was maintained during storage.

Ethylene can be problematic in inducing ripening on storage chambers and mechanisms of removing this gas are available and can be helpful on preventing unwanted ripening effects. Kiwifruits are sensitive to ethylene and ethylene removal techniques as potassium permanganate, ozone generators or catalytic oxidation can be used. Preventive techniques as not storing fruit with mechanical damage or with pathological agents as *Botrytis cinerea*, together with temperature monitoring is a good way to prevent ethylene production (Antunes, 2008).

# 6.1. Physiological and microbiological disorders

During kiwifruit's storage, some problems can occur from microbiological infections to a loss of firmness inducing ethylene production and causing unwanted ripening effect. There are physiological disorders that can occur during storage such as: low temperature breakdown when kiwifruits are exposed to a rapid pre-cooling turning the external pericarp watery, as well as a freezing damage spotted as flesh translucency starting at the stem end and progressing to the blossom; a hard-core happens when the kiwifruits are exposed to ethylene and carbon dioxide on levels above 8% and the core fails to ripen, while the rest of the fruit does; pericarp granulation mostly happens at the stylar end of the fruit but can extend to the sides of the fruit, on kiwifruits on prolonged storage or after ripening at 20°C; white core inclusions are caused by the presence of ethylene on CA storage and the symptoms are visible after 3 weeks of storage on 0°C (Crisosto *et al.*, 1996; Antunes, 2008).

The most common microbiological disorder is the one caused by *Botrytis cinerea*, inducing the rot of kiwis. There is also *Sclerotinia sclerotiorum* which is as capable as *B. cinerea* of developing under temperatures below 0°C, causing rots on the fruits stored, however does not have a big impact on fruit losses as *B. cinerea* does (Antunes, 2008). A sharp control on ethylene concentration must be done so its production and action are manipulated to reach the optimum quality of the fruit. With a temperature above 10°C, kiwifruits produce ethylene and behave as climacteric fruits, meanwhile below 10°C kiwifruits do not produce ethylene but are highly sensitive to its action, even on low temperatures. This induces a rapid ripening effect

that can make kiwifruit's firmness decrease from 78.45 to 19.61N in 5 days at 20°C and in 8 days at 5°C, and this ripening effect continues even when storage at 0°C (Antunes, 2008).

# 7. Postharvest performance

# 7.1. Physical and chemical parameters

After harvest, fruits experience several physicochemical modifications where kiwifruits are no exception. The following factors are part of the postharvest performance of kiwifruit.

# 7.2. Dimensions and weight

The average weight of *A. deliciosa* cv. 'Hayward' kiwifruits is between 80-120g (Cacioppo, 1989b). According to the European commission regulation number 1673/2004, kiwifruits are calibrated for their weight and categorized: for category 'Extra' a minimum weight of 90g, category I is 70g minimum and category II is 65g minimum.

A. chinensis cv. 'Jintao' has long, cylindrical and uniform fruits with an average length of 6.3 to 7.5cm and an average diameter of 3.7 to 4.2cm. The kiwifruit's weight ranges between 65 and 120g with an average weight of 85g (Huang *et al.*, 2002).

Jaeger *et al.* (2003) registered for 4 genotypes of *A. deliciosa* ('Hayward', 'Tomua', 'D1', 'D2') a similar size but when compared to other 3 genotypes of *A. chinensis* ('C1', 'C2' and 'Hort16A'), these differed greatly, with 'C2' being half the size of the 'Hayward', 'C1' being 25-40% bigger and the 'Hort16A' between 'C2' and 'Hayward'.

In cold storage, the weight of cv. 'Hayward' kiwifruits decreased about 3.5%, after 7 weeks of storage at 0°C in one chamber, and at 0°C with ozone treatment in another. Jourdain *et al.* (1982) cited by Barboni *et al.* (2010) also observed a decrease of 1.7% on the same conditions. The weight continued to decrease until 25 weeks of storage. Between 25 weeks and 29 weeks, this decrease of weight stabilized at 91.0g in cold storage and 93.2g in the ozone treatment chamber. At the end, on the storage at 0°C there was a loss of 12.5% of fruit weight and on the ozone treatment there was a 12% fruit weight loss. Therefore there was no significant difference between the storage method (Barboni *et al.*, 2010).

'Hayward' kiwifruits showed a dry matter content varying between 14 and 17% of fresh weight at harvest. On a consumer perspective, to exist a significant difference on the overall liking of the kiwifruit, there must be at least a 4% variation of dry matter between fruits. In the experiment of Burdon *et al.* (2004), 'Hayward' kiwifruits were separated on 8 different values of dry matter since <14% to >20% on 1% intervals. The authors also state that there was a higher preference for high dry matter fruits according to sensory analysis, as higher dry matter

fruits are also richer on soluble solids content. Fruits with a dry matter content below 11% would be more difficult for the consumer to appreciate, whereas a fruit with a dry matter content above 16% would be more likely to be appreciated by the consumer.

At harvest and on a commercial maturity stage, 'Hayward' kiwifruits showed a dry matter content of 17.4% (Jabbar and East, 2016). Dry matter of 'Hayward' kiwifruits varies according to the storage conditions, where at 4°C and 10°C there was a decrease on this parameter as well as on soluble solids content, which are highly correlated, and on a storage condition of 0°C, those parameters did not vary (Marsh *et al.*, 2003).

A high or low content of dry matter, combined with higher or lower firmness, affects the perception of flavour. When there is a high content of dry matter and moderate firmness, this makes a sweeter and more aromatic kiwifruit with higher acceptance from the consumers, and when there is a very soft kiwifruit the aromas turn unacceptable due to a high content on esters which cause a sickly and sweet-vomit notes (Garcia *et al.*, 2012).

### 7.3. Texture

This is a basic qualitative characteristic of any fruit as it assesses the resistance to deformation. At a commercial level, there is a loss of kiwifruit quality which is mostly due to a loss of firmness. One of the most important quality attributes is tissue firmness, that can influence greatly the shelf-life capability of a fruit and their marketability (Tavarini *et al.*, 2009). During storage and subsequent ripening of *A. arguta* and *A. purpurea* kiwifruits, a loss of firmness was observed as a result of the polygalacturonase enzyme activity (Krupa *et al.*, 2011).

Epidermis's pubescence of the fruits from *A. deliciosa* genotypes is higher than the fruits of the *A. chinensis* genotypes, and although 'Hayward' was considered a more pubescent fruit, it was more firm and difficult to break down (Jaeger *et al.*, 2003).

By storing the kiwifruits at different temperatures it is expected a different softening behaviour where kiwifruits stored at 10°C and 4°C had a faster softening than the kiwifruits stored at 0°C, during 7 weeks of storage (Marsh *et al.*, 2003).

Li *et al.* (2016) evaluated texture on *A. deliciosa* cv. 'Hayward' and *A. chinensis* cv. 'Gold3', larger than 'Hayward', and its flesh turns from green to yellow when ripe. At harvest, 'Hayward' had an average flesh firmness of 83.9N and 'Gold3' an average firmness of 42.9N. After 16 weeks of storage, 0°C for 'Hayward' and 1°C for 'Gold3', their firmness decreased to 13.1N and 8.6N, respectively. At harvest and on a commercial maturity stage, 'Hayward'

kiwifruits presented a value of 59.5N (Jabbar and East, 2016). Pranamornkith *et al.* (2012) in their study with *A. chinensis* cv. 'Hort16A', experienced a decrease of firmness from 50N to 13N during 3 weeks of storage with various ethylene treatments.

For the cultivars of *Actinidia arguta* 'Weiki' and '74-49' on commercial maturity stage (8°-10°Brix), the firmness values varied from 19.9 to 33.4N, but at the optimal maturity stage, these values decreased to values between 2.56 and 3.56N. These kiwifruits were subjected to cold storage in normal atmosphere, and there was a rapid decrease of firmness until the end of the storage period of 42 days, to values between 1.80 and 0.90N (Krupa *et al.*, 2011).

On two different methods of storage during 29 weeks, one at 0°C and the other with ozone treatment also at 0°C with a relative humidity of 90-95%, firmness decreased until 17 weeks then stagnated at 29 weeks and no significant differences were observed between storage methods (Barboni *et al.*, 2010).

Another study, with cultivars of *A. arguta*, *A. deliciosa* and *A. eriantha*, showed values of firmness at the optimal maturity stage at harvest of 3.36 to 3.93N. Kiwifruits from the 'Ananasnaya' cultivar obtained the highest firmness values (4.94N), on the same conditions (Leontowicz *et al.*, 2016).

# 7.4. Chromatic parameters

Differences in fruit flesh colour were spotted between cultivars, on which the *A. chinensis* genotypes were yellow, while the *A. delicios* a genotypes were pale green (Jaeger, et al., 2003). Among the *A. deliciosa* genotypes there is one which is named 'Goldy' that is not green as the other genotypes, it is pale yellow as it cannot accumulate chlorophyll on its fruit tissues (Montefiori *et al.*, 2009).

The skin of the 'Jintao' kiwifruit is light brown, while the flesh colour goes from green and yellow when the fruit is harvested, to a more yellow and orange during ripening to full maturity (Huang *et al.*, 2002).

Studying flesh colour of kiwifruits from nearly all genotypes of *A. deliciosa*, a green outer and inner pericarp was detected. In fruits from genotypes of *A. chinensis* the flesh colour can vary from green to bright yellow. On 200 examined genotypes of ripe fruit from *A. chinensis*, 2.2% had fruit with green flesh (hue angle,  $h^{\circ}>110^{\circ}$ ), 57.3% had fruit with a yellow-green flesh ( $h^{\circ}$  100°-110°) and 40.5% with yellow flesh ( $h^{\circ}<100^{\circ}$ ) (Montefiori *et al.*, 2009).

During storage and ripening, fruits from A. deliciosa and A. chinensis show variations in their internal colour, more evident on A. chinensis ('Hort16A', 'Jinfeng', 'Wuzhi No.3')

genotypes than on the *A. deliciosa* 'Hayward' genotype. At commercial harvest stage, all four cultivars had a pericarp tissue with a hue angle of h°>110° which was green. 'Hayward' kiwifruits showed a slight decrease on the hue angle since the last months of the fruit development, to the commercial maturity stage and to 45 days after that, and remained green during all stages of development (Montefiori *et al.*, 2009).

The colour variation of the pericarp was more evident on two of three *A. chinensis* studied cultivars. 'Wuzhi No.3' had a different behaviour on the pericarp colour change, from green-fleshed to bright green-fleshed pericarp, when compared to the other two *A. chinensis* genotypes, 'Hort16A' and 'Jinfeng'. These two yellow-fleshed cultivars had a rapid change on their internal colour around 130-140 days after full bloom, with a decrease of the hue angle from >110° to <98° during 7 weeks of analysis, which corresponds on a change of appearance from bright green to bright yellow. In New Zealand, the time of harvest for *A. chinensis* cv. 'Hort16A' was based on the flesh mean hue angle measured by chromameter when it is <103°, which is when it starts to lose its green colour, becoming yellow (Montefiori *et al.*, 2009).

All these changes in flesh colour also corresponded to fruit softening and an increase in soluble solids. Chroma (C\*) and lightness (L\*) also varied, being more evident in *A. chinensis* cultivars than in 'Hayward' (Montefiori *et al.*, 2009).

However, changes in colour occurred mainly due to the disappearance of chlorophyll and not due to an increase in carotenoid content. Hence, the carotenoids are responsible for the flesh's yellow colour due to the absence of chlorophyll. 'Hayward' cultivar has the ability to retain its chlorophyll content from an unripe stage to fully ripe, while *A. chinensis* yellow-fleshed cultivars have this compound present at an unripe fruit stage but goes to a not significant level by the time it is fully ripe (Montefiori *et al.*, 2009).

# 7.5. Routine analysis

#### 7.5.1. Refractometric index

At harvest and on a commercial maturity stage, 'Hayward' kiwifruits had a value of soluble solids, depending on the grower, from 9.7%, 11% and 11.2% (Jabbar and East, 2016). At commercial maturity, several cultivars from New Zealand and China studied by Ma *et al.* (2017) had an average soluble solids content of 15.58%, varying from 12.27 to 20.37%.

Based on the study of Barboni *et al.* (2010), 'Hayward' kiwifruits stored under different conditions, (at 0°C and ozone treatment), analysed 11 times during 7 months, starting at 6°Brix approximately, after 7 weeks the 'Brix increased to 12, and after 21 weeks it stagnated at

15°Brix. No significant difference was found due to the storage method. Lloret *et al.* (1990) and Tavarini *et al.* (2008) cited by Barboni *et al.* (2010) recommend a minimum sweetness level between 12° and 14°Brix, which was attained after 18 weeks of storage.

At harvest and during 4 months of cool storage (4°C), 'Jintao' kiwifruits had a soluble solids content of 12% and 18% respectively (Huang *et al.*, 2002).

Kiwifruits from two cultivars of *A. arguta* ('Weiki' and 'D14'), had their RI values ranging between 11.7 and 13.9% for optimal maturity phase respectively, while for commercial maturity phase these values were between 9.9 and 8.2% for 'Weiki' and 'D14' respectively. An increase of these values was also identified during ripening and storage of the fruits and after 7 days of storage for 'Weiki' and 14 days of storage for 'D14', these fruits had a similar level of soluble solids content than those which were left to ripe on the vine. The main increase of soluble solids occurred during the first to the third week of storage (Krupa *et al.*, 2011).

# 7.5.2. pH

The pH of 'Hayward' kiwifruits harvested from 3 different orchards, at a commercial maturity stage had a pH ranging from 3.21 to 3.24 (Marsh *et al.*, 2003). Barboni *et al.* (2010) concluded that for 'Hayward' kiwifruits, pH rose until 21 weeks of storage on two different methods (ozone treatment and cold storage) with no significant difference between storage methods.

After harvest and at a commercial establishment, yellow-fleshed kiwifruits pH levels were at 3.6, and the green-fleshed ones were at 3.5 (Sun-Waterhouse *et al.*, 2013).

# 7.5.3. Titratable acidity

Storage at different temperatures (0, 4, 10°C) of 'Hayward' kiwifruits does not affect its titratable acidity, and at harvest, the values range from 1.25 to 1.48% (Marsh *et al.*, 2003). These values are also in line with Ma *et al.* (2017) that had TA varying between 0.56 and 1.77% with an average of 1.20%. 'Hort16A' cultivar showed a value of titratable acidity of 1.34 mg/g FW which was highly correlated with glucose concentration. Citric and malic acid were also correlated with TA. However, pH and TA showed a high negative correlation (Cheng *et al.*, 2004), as expected.

Titratable acidity can influence flavour of kiwifruit and varies according to the acids concentrations (Ma *et al.*, 2017). With a variation of 1g/kg on malate concentration, it should have a 0.1% increase in titratable acidity, however, this happened on kiwifruits from one single orchard, but not for a combination of 2 orchards. A decrease in other acid components of the

fruit may have been the origin for the changes on titratable acidity to be balanced. No differences were found on quinate concentrations regarding the fruit's storage conditions and from harvest to consumption. However, it can occur variations on the organic acids concentration without any change on titratable acidity (Marsh *et al.*, 2003).

Three cultivars 'Weiki', '74-49' (*A. arguta*) and 'D14' (*A. arguta* x *A. purpurea*) were assessed at the optimal and commercial maturity stage, on which the titratable acidity varied between 1.25% and 1.03%. However, during a storage of 42 days there was a constant decrease on the TA value between 17% ('47-49') and 40% ('Weiki' and 'D14') depending on the cultivars (Krupa *et al.*, 2011).

# 8. Chemical analysis

### 8.1. Free sugars

Analysing 4 cultivars of kiwifruit using edible portions without peel and expressing the results per 100g of edible portion, *A. deliciosa* cv. 'Hayward', *A. chinensis* cv. 'Hort16A', and other two, *A. deliciosa* cv. 'Sweet Green' and *A. chinensis* cv. 'SunGold', all these had fructose and glucose as prominent sugars, that ranged between 4.13 to 5.20g/100g (glucose), 4.68 to 5.68g/100g (fructose) for 'Hayward' and 'Hort16A', respectively, and 4.79 to 5.28g/100g (glucose), 5.34 to 5.8g/100g (fructose) for 'Sweet Green' and 'SunGold', respectively. Sucrose was undetected in 'Hayward' and 'Hort16A' and had low values in 'Sweet Green' and 'SunGold' as 1.94 and 1.22g/100g respectively (Sivakumaran *et al.*, 2016).

The major soluble sugars present on 'Hayward' kiwifruits at harvest were fructose (18.1g/l), followed by glucose (15.9g/l) and sucrose (6.4g/l). These concentrations increased until 15 weeks of storage and then stagnated. This did not happen for sucrose that increased until 23 weeks in an ozone treatment chamber and 21 weeks on cold storage chamber (0°C), and then stagnated. No other differences were found due to the storage method until the end of 29 weeks of storage (Barboni *et al.*, 2010). As the ripening of the fruit progresses, its content on starch is converted into soluble sugars (Montefiori *et al.*, 2009).

### 8.2. Organic acids

'Hayward' is known for its acidity and it is very important to pay attention to it during the maturation control and harvest. MacRae *et al.* (1989), cited by Marsh *et al.* (2003), concluded that kiwifruits had 0.9 to 2.5% total acidity at harvest, with 40 to 50% as citrate, 40 to 50% as quinate and 10% as malate and that there were at least 3 tissue zones with different values of acidity, and the balance of these acids varied within the zones. The proportion of

citrate was higher in the inner pericarp, quinate was higher in the outer pericarp, and the columella had the lowest total acid content (about half the other zones) where citrate was dominant.

At harvest, 'Hayward' kiwifruits had citric acid varying between 13.2 and 14.9g/L FW, quinic acid between 13.0 and 14.7g/L FW, malic acid between 6.1 and 6.6g/L FW and ascorbic acid had a value of 0.3g/L FW, determined using the juice of the whole fruit (Barboni *et al.*, 2010). However, Ma *et al.* (2017) used kiwifruit pulp to determine organic acids and found that citric acid (approximately 1.1 to 1.25g/100g FW for 'Hayward' and approx. 0.9g/100g FW for 'Jintao') was predominant, followed by quinic (approx. 0.3 to 0.6g/100g FW for 'Hayward' and approx. 0.38g/100g FW for 'Jintao'), malic (approx. 0.4 to 0.45g/100g FW for 'Hayward' and approx. 0.45g/100g FW for 'Jintao') and tartaric (approx. 0.2g/100g FW for 'Hayward' and approx. 0.18g/100g FW for 'Jintao') acids.

The total acidity on two different storage methods (cold storage and ozone treatment) decreased until 21 weeks of storage on both, which shows that the total acidity was not significantly affected by the storage method. In a 0°C cold storage, citric and quinic acids concentration decreased until 7 weeks of storage then stagnated to 29 weeks, except malic acid that continued to decrease until the end of 29 weeks of storage. The authors also reported that cold storage provided better conservation for organic acids than ozone storage (Barboni *et al.*, 2010).

The level of acidity did not vary in 'Hayward' kiwifruits stored at different temperatures (0°C, 4°C and 10°C). However, the acids concentration (malate and citrate) varied in storage conditions, but no significant differences were found on quinate. Malate concentrations were higher on fruit stored at 4°C (2.53g/kg) compared to 0°C (1.50g/kg) and fruit stored at 10°C (2.42g/kg). Between fruits stored at 0 or 4°C, no significant differences were found on citrate level, however, fruits stored at 10°C had a higher level of this acid. Quinate had similar values at the three different temperatures 0°C (7.2g/kg), 4°C (6.8g/kg) and 10°C (7.7g/kg). Citrate content was the highest on the total acids content (Marsh *et al.*, 2003).

The most important organic acids in kiwifruit are citric, quinic and malic (Soufleros *et al.*, 2001). Organic acids were similar in four different cultivars: 'Hayward', 'Hort16A' and other two, 'Sweet Green' and 'SunGold', between 2.0 and 2.4g/100g. Citric acid had the following values: 0.97 and 0.52g/100g for 'Hayward' and 'Hort16A', and 0.87 and 0.9g/100g for 'Sweet Green' and 'SunGold'. Malic acid had the following values: 0.26 and 0.21g/100g for 'Hayward' and 'Hort16A', and 0.61 and 0.62g/100g for 'Sweet Green' and 'SunGold'.

Quinic acid had the following values: 0.78 and 1.35g/100g for 'Hayward' and 'Hort16A', and 0.89 and 0.71g/100g for 'Sweet Green' and 'SunGold'. These determinations were based on edible portions, flesh and seeds without peel (Sivakumaran *et al.*, 2016).

# 8.3. Antioxidant activity

Antioxidants are substances that retard the oxidation speed, through one or more mechanisms, such as the inhibition of free radicals and metals complexation (Pietta, 2000).

ABTS and DPPH are both spectrophotometric methods used to measure the radical scavenging ability of antioxidants present on extracts. ABTS assay can be used to measure the antioxidant activity of compounds of hydrophilic (phenolic compounds and ascorbic acid) or lipophilic (carotenoids) nature, through the scavenge of the ABTS radical generated by a chemical, electrochemical or enzymatic reaction. DPPH method consists in evaluating the antioxidant capacity through the scavenge of the free radical DPPH, with a purple colour with a maximum wave length of 517nm. When the antioxidants act, DPPH is reduced and decolorates, turning yellow and the % of antioxidant activity is measured by the amount of DPPH consumed by the antioxidants (Borges *et al.*, 2011; Bursal and Gülçin, 2011).

ABTS and CUPRAC are two electron transfer assays, resulting in similar results (Park et al., 2014). Spectrophotometric CUPRAC assay is conducted by mixing Cu(II)-neocuproine (Nc) chelate, a chromogenic oxidizing agent, with an antioxidant solution. After 30 minutes, as a result of a redox reaction, Cu(I)-chelate colour absorbance is read at 450nm. Although being less extensive than FRAP method, it is useful as it works at physiological pH (pH 7), while FRAP works at 3.6. It can also determine both hydrophilic and lipophilic antioxidants as Cu(II)-Nc is soluble in both aqueous and organic environments (Xu et al., 2017).

The ferric-reducing antioxidant power (FRAP) measures the reducing capacity of antioxidants of Fe<sup>3+</sup> to Fe<sup>2+</sup> under a pH=3.6, with a blue colour. The ability of antioxidants is positively related with an increase in absorbance. This assay is suitable for antioxidants that complete the reaction rapidly, within 4 to 6 minutes, such as ascorbic and uric acids. However, compounds such as thiols and proteins that act by radical scavenging (H transfer) are not detected by this method (Xu *et al.*, 2017).

'Hayward' kiwifruits are known for its antioxidant potential and several assays were done to determine it. By ABTS, DPPH and CUPRAC, the antioxidant potential of ethylene treated kiwifruits was in the range of 14.09, 9.33 and 19.72μM TE/DW, respectively, using edible portions without peel (Gorinstein *et al.*, 2009).

Measured by an oxygen radical absorbance capacity (ORAC) method, 'Hayward' had its antioxidant capacity ranging from 6.0 to 9.2μM TE/FW when determined by ORAC, while 'Hort16A' had a higher antioxidant capacity of 12.1μM TE/FW by the ORAC method (Skinner *et al.*, 2011). ORAC method measures the degree of inhibition of peroxy-radical-induced oxidation, as Trolox equivalent, including both hydrophilic and lipophilic antioxidants (Haytowitz and Bhagwat, 2010). Another study using a peeled and edible portion of 'Hayward' kiwifruits in a commercial maturity stage, had for the ABTS, DPPH, CUPRAC and FRAP assays the following values: 22.9, 8.49, 23 and 11 μM TE/DW (Park *et al.*, 2011).

Lipid peroxidation assay method uses a Fenton-like system (Co(II) + H<sub>2</sub>O<sub>2</sub>), to induce lipid peroxidation (Pisoschi and Negulescu, 2012). A hydroxyl radical generally induces lipid peroxidation, creating a chain of oxidative reactions in the lipid bilayer of cell membranes that becomes vulnerable to free radicals' attacks. Vitamin C and E can contribute to inhibit lipid peroxidation as tocopheroxyl radical is stable enough to allow its reduction by these vitamins. Vitamin E is particularly effective in inhibiting lipid peroxidation, preventing the accumulation of products from this process that are associated with numerous diseases. Its action as antioxidant is due to the donation of an hydrogen atom to peroxyl radicals (Morales *et al.*, 2013). The lipid peroxidation of green and gold kiwifruits was at approximately 45 and 60% respectively, showing a strong inhibition rate of lipid oxidation, with gold kiwifruits having stronger effects than green kiwifruits. The results were obtained using an L-α-phosphatidyl ethanolamine solution from egg yolk (Iwasawa *et al.*, 2011).

The total antioxidant capacity is influenced by several types of antioxidants and it is not clear which ones are responsible for its variation. However, when determining the existent correlation between the antioxidant capacity and the main antioxidant substances (Ascorbic acid and polyphenols), it is possible to conclude that antioxidant capacity of the *Actinidia* fruits is influenced by the content of these substances. The kiwifruit is known for its high antioxidant capacity but it varies much between *Actinidia* species and cultivars, like on the wild cultivars such as *A. eriantha* and *A. latifolia* that had a higher antioxidant capacity and a higher radical scavenging when comparing to the widely known 'Hayward' (Du *et al.*, 2008)

# 8.4. Vitamin C

The content on vitamin C on kiwifruits is variable, depending on the cultivar, production factors, climate, maturity phase and postharvest conditions. There is a general decrease of vitamin C through time and storage (Barberis *et al.*, 2012).

There are two enzymes responsible for the metabolic oxidation of ascorbic acid: ascorbate oxidase and ascorbate peroxidase. During tissue growth, ascorbate oxidase catalyses ascorbic acid, while ascorbate peroxidase acts on the modification of ascorbic acid content during ripening, storage or wounding. Due to its antioxidant capacity, ascorbic acid is important on maintaining the oxidative stability of the fruit. The loss of this component limits the nutritional quality, so it is an important asset for the fruit's quality and its derivatives (Barberis *et al.*, 2012).

There are several methods to determine the content of vitamin C of a fruit, like titration and chromatography, which assess this parameter accurately. Immediately after cutting a ripe 'Hayward' kiwifruit, the ascorbic acid content was 65mg/100g FW (Barberis *et al.*, 2012). Du *et al.* (2008) also obtained a similar vitamin C content on 'Hayward' kiwifruit, 63.41mg ascorbic acid per 100 grams of fresh weight. Two other cultivars of *A. chinensis* ('Hongyang', 'Xixuan'), had its vitamin C content varying from 73.97 to 42.27mg ascorbic acid/100g FW, respectively (Du *et al.*, 2008). These values are in line with Ma *et al.* (2017) that obtained 54.86 to 159.08mg/100g FW on several cultivars with an average of 87.19mg/100g FW.

A comparison study among 'Hayward', 'Hort16A' and other two, 'Sweet Green' and 'SunGold', found different values on vitamin C content: 85mg/100g and 109mg/100g for 'Hayward' and 'Hort16A', and 150mg/100g and 161mg/100g for 'Sweet Green' and 'SunGold' (Sivakumaran *et al.*, 2016). Ma *et al.* (2017) showed that 'Jintao' (148.82mg/100g FW) has a higher vitamin C content than 'Hayward' (around 55 to 80mg/100g FW) and another yellow-pulp kiwifruit 'Hort16A' (approximately 60mg/100g FW).

Cultivars from *A. arguta*, had their ascorbic acid content varying from 61.6 to 120mg/100g FW. Comparing two harvest dates, one at a commercial stage and the other at an optimal stage, there were no significant differences on the ascorbic acid content. However, this content decreased during storage (Krupa *et al.*, 2011).

There was a decrease in the ascorbic acid content during storage of 'Hayward' kiwifruits in 3 different situations of kiwifruit slices. The cold storage reduced significantly the degradation rate until the 4<sup>th</sup> day in comparison to the other situations. By interrupting the cold-chain, the content on ascorbic acid is rapidly affected in case the refrigeration conditions are not replaced. Even if these conditions were not rapidly replaced, the kiwifruit slices were negatively affected by it, losing more than 50% until the 3<sup>rd</sup> day, comparing with the control samples. The authors concluded that the more ripe is the fruit, the less is its ascorbic acid content which happens concurrently as the degradation of the fruit tissues (Barberis *et al.*, 2012).

The *A. eriantha* 'Bidan' kiwifruit cultivar has the highest antioxidant activity comparing to others, but it is less popular. It also has the highest content on Vitamin C and is less sweet than 'Hayward' (Leontowicz *et al.*, 2016).

#### 8.5. Carotenoids

Fruits contain various amounts of pigments, including chlorophylls and carotenoids, responsible for the variation in colour of these fruits. Also, these pigments have health benefits as  $\beta$ -carotene and lycopene having provitamin A activity and antioxidant potential. A diet rich in antioxidants may prevent heart diseases and certain types of cancer, while carotenoids as  $\beta$ -carotene, lycopene, lutein and zeaxanthin are good free radical scavengers. Lutein is also associated to the protection of age-related macular degeneration and cataract formation (Nishiyama *et al.*, 2005; Mditshwa *et al.*, 2017).

Qualitatively, the carotenoids detected were the same in ripe and unripe kiwifruits, as well as few qualitative differences between *A. deliciosa* and *A. chinensis* were found, only lutein, neoxanthin and violaxanthin were present in higher amounts in *A. chinensis* kiwifruits (Montefiori *et al.*, 2009). The colour differences between cultivars of *A. deliciosa* or *A. chinensis* are mainly due to the presence or absence of chlorophylls, not to a much higher number of carotenoids on one cultivar or another. 'Hayward' kiwifruits have the ability of retaining chlorophylls from an unripe to a fully ripe stage, while in *A. chinensis* kiwifruits, chlorophylls concentration decreased until insignificant levels until fully ripe. So, changes in kiwifruit flesh colour are not mainly due to changes in carotenoids content, however these are responsible for the yellow colour in the absence of chlorophyll (McGhie and Ainge, 2002; Montefiori *et al.*, 2009; Leontowicz *et al.*, 2016).

Montefiori *et al.* (2009) and D'Evoli *et al.* (2015) found that lutein was the most abundant carotenoids of the carotenoids detected in kiwifruits from *A. deliciosa* cv. 'Hayward' (1.11µg/g FW), and three *A. chinensis* cultivars: 'Hort16A' (0.88µg/g FW), 'Jinfeng' (0.34µg/g FW) and 'Wuzhi No.3' (1.02µg/g FW). With a concentration of 40%, and on a smaller concentration there was violaxanthin, neoxanthin and  $\beta$ -carotene. During ripening, the total carotenoid content tended to decrease but did not disappear completely, and the ones that were present on the beginning of ripening were still present at a full maturity stage, with few variations from *A. deliciosa* and *A. chinensis* cultivars. D'Evoli *et al.* (2015) reported a content of lutein in 'Hayward' of 0.2mg/100g and an average  $\beta$ -carotene content of 0.06mg/100g.

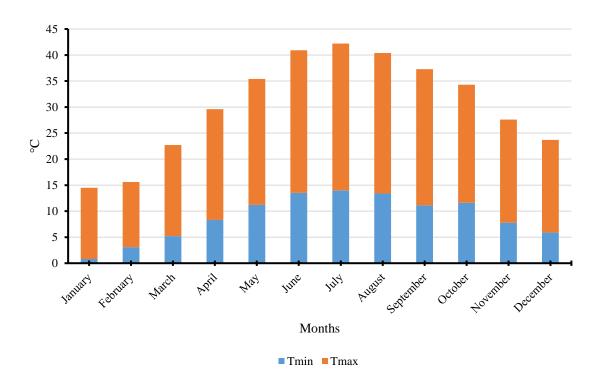
# **Chapter III - Materials and methods**

#### 1. Kiwifruit cultivars

Two cultivars of different *Actinidia* species were studied, *Actinidia deliciosa* cv. 'Hayward' and *Actinidia chinensis* cv. 'Jintao'. Further on, to address each cultivar it will be used only its cultivar name, 'Hayward' and 'Jintao'. The fruits came from 5 different producers from the 'Minho' region.

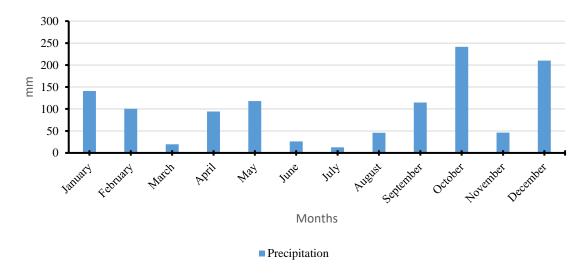
# 2. Climate conditions

Kiwifruits came from producers of different areas, all being part of the 'Minho' region, Viana do Castelo, Felgueiras, Guimarães and Póvoa de Lanhoso. Figure 2 and figure 3 show the averages of minimum and maximum temperatures and total precipitation in mm per month for Braga, part of the Minho region for the year 2015. The study refers to kiwifruits after storage.



**Figure 2-** Average minimum temperatures (Tmin) and average maximum temperatures (Tmax) per month, in °C, for the year 2015 in Braga, part of the Minho region (IPMA, 2017a).

The climate on Braga region is Csb according to Koppen classification, which corresponds to a temperate climate (Figure 2) (IPMA, 2017a). The annual average precipitation is 1465.7mm on the period 1971/2000, and it mostly occurs in the winter months (Figure 3) (IPMA, 2017b).



**Figure 3-** Average precipitation per month, in mm, for the year 2015 in Braga, part of the Minho region (IPMA, 2017a).

# 3. Harvest, storage conditions and fruit sampling

'Hayward' kiwifruits were harvested during the first two weeks of November 2015, while 'Jintao' kiwifruits were harvested on the third week of October 2015. There were 4 producers for 'Hayward' and 1 producer for 'Jintao' kiwifruits (Table 2).

**Table 2** – Kiwifruits harvest dates and their storage conditions, its atmosphere, programmed temperature, humidity, O<sub>2</sub> and CO<sub>2</sub> levels.

Producer	Harvest dates	Atmosphere	Temperature	Humidity	O <sub>2</sub>	CO <sub>2</sub>
H1	04/11/2015 to 19/11/2015	Controlled	0°C	≥ 90%	≤ 2%	4.5%
H2	02/11/2015 to 12/11/2015	Controlled	0°C	≥ 90%	≤ 2%	4.5%
Н3	02/11/2015 to 14/11/2015	Normal	-0.5°C	≥ 90%	-	-
Н4	10/11/2015 to 14/11/2015	Normal	-0.5°C	≥ 90%	-	-
<b>J</b> 1	19/10/2015	Normal	-0.5°C	≥ 90%	-	-

In controlled atmosphere (CA) chambers the programmed temperature was  $0^{\circ}$ C and a humidity level of  $\geq$ 90%, with controlled  $O_2$  and  $CO_2$  concentrations that were programmed for  $\leq$  2% and 4.5% respectively. The kiwifruits that were kept on these chambers were the ones from the producers H1 and H2.

In normal atmosphere (NA) chambers were stored the kiwifruits from producers H3, H4 and J1, and on these chambers the programmed temperature was  $-0.5^{\circ}$ C as well as a humidity level of  $\geq 90\%$ . The conditions of the cooling chambers were controlled and registered computationally every day, hour by hour. At least once a week, the pulp temperature of the fruits was measured to be able to control its temperature due to variations of the temperature

inside the chambers. The chambers were equipped with a system that alerted and sent warning messages if the conditions were abnormal and not corresponded to the pre-established conditions on gases, and temperature.

Kiwifruits were already harvested and were collected from cold-storage chambers of 'Frutas Douro ao Minho' in Guimarães and then were brought to 'Universidade de Trás-os-Montes e Alto Douro' in Vila Real to be analysed. On each sampling date, 10 kiwifruits from each producer were brought and analysed on the same day, having a total of 50 kiwifruits analysed per sampling date. The number was doubled when shelf-life experiment was done.

**Table 3** – Sampling dates and 1-week shelf-life sampling dates for all kiwifruits.

Sampling dates	1 week-shelf life
11/11/2015	-
18/11/2015	-
02/12/2015	-
16/12/2015	-
06/01/2016	-
20/01/2016	-
03/02/2016	10/02/2016
17/02/2016	24/02/2016
02/03/2016	09/03/2016
16/03/2016	23/03/2016

In table 3, starting on 11<sup>th</sup> November 2015, the analysis of the kiwifruits samples took place every 2 weeks, having a total of 10 sampling dates. However, to have a better understanding of the shelf-life behaviour of the kiwifruits, on the 3<sup>rd</sup> of February 2016, 50 more kiwifruits were brought, also 10 kiwifruits from each producer on the same conditions and kept at air conditions at approximately 18°C to simulate and evaluate its shelf-life performance by the end of 1 week. The last sampling date was on the 16<sup>th</sup> of March 2016, when it completed the 10 sampling dates since the 11<sup>th</sup> of November 2015.

All kiwifruits on arrival at the laboratory were subjected to weight and dimensions measurements as well as skin colour. Kiwifruits subjected to shelf-life experiment were also measured for weight, dimensions and skin colour. To further analyse the kiwifruits, texture (peeled and unpeeled kiwifruits), flesh colour, °Brix, pH, NaOH volume spent in titration, were also assessed on arrival at the laboratory, and the kiwifruits subjected to shelf-life experiment were only analysed for these parameters when 1 week was completed. The samples separation

was done by producer but the statistical treatment was done by storage atmosphere, and by cultivar.

Approximately half of each peeled kiwifruit was put aside, also separated per producer and were frozen on individual bags referring to its sampling date and producer. Then they were lyophilized to assess free sugars concentrations, organic acids, antioxidant activity, vitamin C and carotenoids, on 5 sampling dates: 11/11/2015, 06/01/2016, 03/02/2016, 16/03/2016 and 23/03/2016. For the shelf-life experiment, 16/03/2016 corresponds to 'Day 0' and 23/03/2016 to 'Day 7', for these analyses. The results are based on the average of 3 repetitions per producer for each sampling date.

### 4. Routine analysis

On each sampling date 50 kiwifruits were analysed, 10 per producer. The analysis includes dimensions and weight, skin and pulp colour, texture, °Brix, pH and NaOH volume spent in titration, which will be described next.

For shelf-life evaluation, another 50 kiwifruits were brought, also 10 per producer but the only assessments done on arrival were non-destructive: dimensions, weight and skin colour. By the end of 1 week, dimensions, weight and skin colour were assessed again, using the same kiwifruits, and then analysed for pulp colour, texture, °Brix, pH and NaOH volume spent in titration.

### 4.1. Dimensions and weight

Length, width and thickness dimensions were taken with a calliper rule and the weight was measured with a KERN weighing machine with a maximum capacity of 2200g. Each fruit was measured for its length, width, thickness and weight, all individually.

Throughout the sampling dates, a part of approximately 250g from the 10 peeled kiwifruits of each producer was frozen, lyophilized and then were reduced to powder. The powder was packaged and separated per producer and sampling date.

# 4.2. Refractometric index, pH and NaOH volume spent in titration

Juice from each kiwifruit was extracted by a centrifugal juice extractor Tefal Elea, around 50mL at room temperature and then, two to three drops of juice were used for RI determination and the remaining for pH and NaOH volume spent in titration assessments.

The equipment used for the refractometric index (RI) measurement was an 'Atago PR-101' refractometer calibrated for °Brix, the pH level was measured using a 'Jenway 3310'. All fruits were individually analysed for °Brix and pH.

The NaOH volume spent in titration (mL) to neutralize free hydrogen protons of the solution was assessed by a 'Schott titroline easy' titrator to a final pH of 8.2. The solution was composed by 10mL of juice from 2 kiwifruits and 10mL of distilled water titrated with NaOH 0.1M.

#### 5. Texture

Peeled and unpeeled texture of the kiwifruits was analysed by a texture analyser TAXT Plus of the Stable Micro System. The test was conducted with a load cell of 50kg, 2mm/s test velocity to a distance of 25mm through the kiwifruit, with a cylindrical probe with 6mm of diameter (P6) and a contact area of 28.27mm<sup>2</sup>. This test allows the assessment of the resistance of the fruit, peeled and unpeeled, to an external strength stress. On each sampling date, all 50 fruits were individually analysed for its unpeeled and peeled firmness.

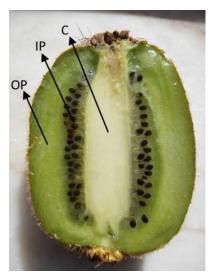
The tests' results were divided on different parameters and given the following designations on results and its discussion:

- 'Force on initial penetration'(N) which corresponds to the force needed on the first penetration which can be the skin (unpeeled kiwifruit) or flesh (peeled kiwifruit);
- 'Force at 25mm' (N) corresponds to the force done at the end of the penetration test;
- 'Work on the whole test' (N.mm) represents the amount of work done during the whole length of the test;
- 'Work on initial penetration' (N.mm) corresponds to the amount of work done until the skin or flesh is penetrated;
- 'Gradient for the initial penetration' (N/mm) represents the slope until the skin or flesh is penetrated;
- 'Force at 10mm' (N) relates to the force done at 10mm deep into the flesh of the kiwifruit.

# 6. Chromatic parameters

The equipment used to assess colour was a Minolta Chroma Meter CR300 using a CIELab system and the chromatic parameters assessed were L\*(Lightness), flat coordinates a\*(green/red) and b\*(yellow/blue), and calculated angular coordinates for C\* (chroma) and h° (hue angle). The skin colour as well as the flesh colour were assessed. For the skin colour, 4 samples on opposite sides of the fruit's epidermis were taken on each fruit. For the flesh colour, 5 shots on a longitudinal internal profile of the kiwifruits, being 2 of them on the outer pericarp (OP), other 2 on the inner pericarp (IP) and the last one on the columella (C) (Figure 4). Fruit

condition is divided in unpeeled and peeled, referring to the epidermis and flesh colour, and this procedure was used equally for both cultivars.



**Figure 4 -** 'Hayward' kiwifruit longitudinal profile with each colour assessment area: Outer pericarp (OP), inner pericarp (IP) and columella (C).

# 7. Free sugars

The method determines the individual free-sugars in HPLC-DAD on a  $C_{18}$  column after derivatization with benzoyl-chloride (Sigma-Aldrich, Tauferkichen, Germany) and visualized on a wave-length of 270nm. The method with some modifications was adapted from Daniel *et al.* (1981).

At first, a sample extraction was made for the free-sugars determination and weighed 100mg of dry extract and added 5mL of ethanol at 80% and left at 20°C for 2 hours. Then, it was removed 1mL to plastic vials with screw and cover of 2mL and centrifuged at 13000rpm, at 4°C for 10 minutes. It was removed 100µL from the previous procedure to plastic vials of 2mL with screw and cover and left to dry with forced air until its complete evaporation.

It was added  $500\mu L$  of derivatization reagent (10% Benzoyl in pyridine – 5mL of Benzoyl in 45mL of pyridine) and left at  $37^{\circ}C$  for 16 hours.

Thereafter, it was added 1mL of diethyl ether, thoroughly mixed on the vortex and centrifuged at 13000rpm, 4°C for 20 minutes. Then it was removed  $750\mu L$  of supernatant to plastic vials of 2mL with screw and cover. Past this, it was dried with forced air until its complete evaporation. Then it was suspended with  $750\mu L$  of methanol at 100% and introduced on glass HPLC vials and injected on HPLC.

The standards of the sugars were prepared, on this case they were glucose, galactose, fructose and sucrose by the desired concentration. For each standard, it was removed  $50\mu L$  and

put on 2mL vials to dry with forced air until its complete evaporation. It was then added  $500\mu L$  of derivatization reagent and continued the process as it was a normal sample, as detailed before.

For this method, there are conditions that must be followed for the HPLC-DAD run. It was needed a  $C_{18}$  column (250 x 4.5 mm), 2 solvents A and B (A: ultra-pure water with 0.1% of trifluoracetic acid (TFA) and B: acetonitrile with 0.1% of TFA). Run time was 35 minutes with binary gradient (0 minutes - 80% of solvent B; 7 minutes- 90% of solvent B; 12 minutes - 90 % of solvent B; 20 minutes - 100 % of solvent B; 25 minutes - 100 % of solvent B; 30 minutes - 80 % of solvent B; 35 minutes - 80 % of solvent B) and wave lengths ranging from 200 to 600nm (230, 270 and 520nm).

The flow was 1mL per minute with an injection volume of  $20\mu L$ . The reading of the results was at 270nm.

The calculation of the content of free-sugars on the samples was made by the retention times of the external individual standards and using the calibration curves previously made with the same standards. The results of the free-sugars on the samples are expressed as mg per gram of fresh weight (FW), based on the average of 3 repetitions per producer for each sampling date.

# 8. Organic acids

The method allows the quantification of the organic acids in HPLC-DAD with a  $C_{18}$  column and visualized at two wave lengths, 210 or 214nm. The method was adapted with some modifications from Phillips *et al.* (2010).

The extraction phase started with weighing 1g of dry extract to containers of 10mL with screw cap, added 10mL of ultra-pure water and stirred in the vortex. It was then taken to the sonicator for 5 minutes and stirred again in the vortex. The samples were then put to centrifuge for 15 minutes at 4000rpm at room temperature. The supernatant was then filtered and the precipitate was rejected. Approximately 1mL was removed to HPLC glass vials of 2mL being filtered with Spartan 13/0.2µm filters. It was then injected 20µL into the HPLC.

The run conditions start with a column  $C_{18}$  (250 x 4.5mm) on the HPLC-DAD equipment. The solvent on the isocratic gradient was the dihydrogenophosphate of potassium (6.8g/L) on which the pH level was adjusted to 2.1 by adding ortophosphoric acid at 85% ( $\rho$ 20 = 1.71g/mL). The run length was 20 minutes with a flow of 0.8mL/ minute, at 20°C and the wave lengths were between 200-600nm (230, 270, 520) visualized on the chromatogram at 210nm.

The standards used were tartaric acid, quinic acid, malic acid, citric acid, shikimic acid, acetic acid, succinic acid and fumaric acid and were prepared to the desired concentration and was used the 5g/L concentration. They were then transferred to HPLC glass vials of 2mL and injected  $20\mu L$  on the HPLC. Then, calibration curves with the different concentrations of the respective standards were made, along with the mathematic equations of the lines that were needed to calculate the concentration of the organic acids.

The output order of the commercial standards, no matter the run conditions and column used were the following: tartaric acid, quinic acid, malic acid, shikimic acid, acetic acid, citric acid, succinic acid and fumaric acid.

The identification of the organic acids was done by comparison with the retention times of the individual external standards, as well as by the respective spectrums on the chromatogram. The calculation of the average content of the different organic acids present on the samples was done according to their retention times and using the calibration curves previously done with the standards.

The results of the organic acids on the samples were expressed in mg per gram of FW, based on the average of 3 repetitions per producer for each sampling date.

# 9. Antioxidant activity

Antioxidant activity was determined by 4 different methods, DPPH, FRAP, Lipid peroxidation and CUPRAC and the results were based on the average of 3 repetitions per producer for each sampling date.

### 9.1. **DPPH**

The 2.2-diphenyl-1-picrylhydrazyl (DPPH) method determines non-enzymatic antioxidant activity and was adapted from Brand-Williams *et al.* (1995), Sánchez-Moreno *et al.* (1998) and Siddhraju and Becker (2003).

A freshly prepared DPPH solution was made by mixing 4mg of 2.2-diphenyl-1-picrylhydrazyl radical with 100mL of 95% ethanol. To each well of the microplate was added 285µL of DPPH solution and 15µL of sample and mixed thoroughly. A blank preparation (DPPH solution and solvent extraction instead of sample) was made to be used in the formula below.

The microplates with the mixtures were left in a dark room by room temperature for 30 minutes. Thereafter, the absorbance was read at 517nm in a microplate reader.

The results (% of antioxidant activity (AA), or % of DPPH radical scavenging capacity) were calculated using the following formula: %AA=[(Abs<sub>blank</sub> – Abs<sub>sample</sub>) / Abs<sub>blank</sub>] x 100.

### 9.2. FRAP

The ferric-reducing antioxidant power (FRAP) method determines non-enzymatic antioxidant activity, ion reduction of  $Fe^{3+}$  to  $Fe^{2+}$  and was adapted from Gülcin *et al.* (2006) and Hinneburg *et al.* (2006).

To each microplate well,  $15\mu L$  of extract was added to  $25\mu L$  sodium phosphate (pH 6.6; 0.2M). Then,  $50\mu L$  of 1% aqueous potassium hexacyanoferrate [K<sub>3</sub>Fe(CN)<sub>6</sub>] solution was added and incubated at  $50^{\circ}$ C for 30 minutes. After incubating,  $25\mu L$  of 10% trichloroacetic acid was added and mixed thoroughly.

Then,  $100\mu L$  of ultra-pure water and  $25\mu L$  of 0.1% of aqueous FeCl<sub>3</sub> were added and mixed thoroughly.

The absorbance was read at 700nm against blank in a microplate reader. An increase in absorbance values means an increase of antioxidant activity by reduction of Fe<sup>3+</sup> in Fe<sup>2+</sup>.

The results were expressed as % of antioxidant activity using a blank. The blank had the extraction solvent only and the results were calculated using the following formula:  $AA = [Abs_{sample} - Abs_{blank}] / Abs_{sample} x 100$ .

# 9.3. Lipid peroxidation

The method determines antioxidant activity by inhibition of lipid peroxidation assay by thiobarbituric acid-reactive species (TBARS) in a 96-well microplate, based on Chang *et al.* (2001), Daker *et al.* (2007) and Adithya *et al.* (2013).

An egg yolk was prepared in a phosphate buffer solution on 10% proportion. It was removed 1mL and add 1mL of the buffer solution and centrifuge at 4000rpm for 3 minutes. On a 12mL test-tube, it was added 0.5mL of egg emulsion,  $50\mu$ L of FeSO<sub>4</sub> 70mM and 100mL of sample. Then, it was incubated at  $37^{\circ}$ C for 30 minutes.

Thereafter, 1mL of acetic acid at 20%, 50µL of trichloroacetic acid at 28% and 1mL of thiobarbituric acid (TBA) at 0.8% in phosphate buffer were added to the preparations and incubated at 95°C for 60 minutes. Then, it was centrifuged at 4000rpm for 5 minutes and pipetted to Elisa plaques and read at 532nm.

The results were expressed as inhibition % and were calculated using the following formula: % of inhibition =  $[1-(Abs_{sample}/Abs_{blank})] \times 100$ .

### 9.4. CUPRAC

Cupric reducing antioxidant capacity (CUPRAC) determines non-enzymatic antioxidant activity and L-ascorbic acid with modifications based on Apak *et al.* (2004). This method is based on the changes in absorption characteristics of neocuproine complex when it is reduced by an antioxidant.

The following solutions were prepared: cuprous chloride (10 mM in water), neocuproine (7.5 mM in 96% ethanol) and a buffer solution of ammonium acetate (1 mM, pH 7.0, in water). To each well of a 96-wells microplate,  $50\mu L$  of  $CuCl_2$  and  $50\mu L$  of neocuproine were added. Then,  $50\mu L$  of  $NH_4Ac$  was added.

Thereafter,  $25\mu L$  of sample and  $25\mu L$  of water was added and mixed thoroughly in a vortex. The final volume should be  $200\mu L$ . The mixture was then incubated in dark and room temperature for 30 minutes.

After 30 minutes, the absorbance values were read at 450nm against blank (all reagents except  $CuCl_2$ ) using a microplate reader. Trolox was used as standard for calibration curve and the results were expressed as  $\mu M$  of trolox equivalents per gram of FW ( $\mu M$  TE/g FW) for antioxidant activity and L-ascorbic acid.

# 10. Vitamin C

The method determines the content on vitamin C by HPLC (High performance liquid chromatography), adapted with some modifications from Hernández *et al.* (2006).

At first, an extraction from the samples was made, on dark containers it was weighed 0.2g of sample, dry weight, and added 5mL of solvent (3% metaphosphoric acid + 8% acetic acid) and 1mM of tert-butylhydroquinone (16.62mg/100mL). The mixture was then homogenised (ultra turrax) and centrifuged at 4000rpm and 4°C for 2 minutes. Finally, the extracts were filtered for HPLC vials and stored immediately at -4°C and injected on the HPLC on the same day of the extraction.

The HPLC run conditions start with a  $C_{18}$  column (Dimension: 250 x 4.6mm, 5 $\mu$ m), a mobile phase of 0.2% ortophosphoric acid, an isocratic gradient, a flux of 1.2mL/minute, a column temperature of 25°C, an injection volume of 20 $\mu$ L, an UV detection of 245nm (between 200 and 700nm). The quantification was done by comparison of the retention time of the commercial standards of vitamin C (ascorbic acid) and the respective external calibration curves.

To determine dehydroascorbic acid (DHA), after the sample extraction, 200µL of dithiolthreitol was added (DTT) to 2mL of extract and left to rest on dark and 30°C for 15 minutes. This procedure was followed on the HPLC for the total ascorbic acid. This step was to decompose the total content of vitamin C in dehydroascorbic acid and ascorbic acid which both added result on the total vitamin C content. The results were expressed in mg per gram of FW of vitamin C and based on the average of 3 repetitions per producer for each sampling date.

### 11. Carotenoids

The carotenoid determination method by spectrophotometry was based on Scott (2001) and Liu *et al.* (2011).

For the extraction, it was weighed 200mg of dry extract and then added 10mL of solvent which varies accordingly to the pigment to be determined (Table 4). The mixture was then agitated and left to stand in the dark for 20 minutes at room temperature ( $\pm 22^{\circ}$ C). Past the time, it was centrifuged for 5 minutes at 4000rpm and then the extracts were filtered through a Whatman No.4 filter paper. The absorbance was read in a UV spectrophotometer at  $\lambda_{max}$  (Table 4) against blank (only the respective solvent in a cuvette). The concentration of the carotenoid is then calculated by the following Lambert-Beer formula: []  $\mu g/mL = Abs \times 10^4 / A^{1\%}$ . The results were expressed as mg of carotenoid per gram of FW and based on the average of 3 repetitions per producer for each sampling date.

**Table 4** - Data on extinction coefficients ( $A^{1\%}$ ), maximum wavelength ( $\lambda_{max}$ ) and solvents used on extraction, of a selection of carotenoids.

Pigment	Extinction coefficients (A <sup>1%</sup> )	λ <sub>max</sub> (nm)	Solvent used in extraction
α-carotene	2170	445	Hexane
β-carotene	2592	450	Hexane
<b>β-cryptoxanthin</b>	2460	450	Hexane
Lycopene	3450	470	Hexane
Zeaxanthin	2480	450	Hexane
Lutein	2550	445	Ethanol

### 12. Results and statistical analysis

There are 3 different situations on which the results were evaluated by the dependent variable assessed, and its discussion will be sequentially processed by each variable for the 3 situations. The first situation focused on the postharvest comparison of 'Hayward' through time

on 2 different storage atmospheres, controlled (CA) and normal (NA); the second, on postharvest comparison of 'Hayward' and 'Jintao', stored on NA chambers; and the third situation focused on the final 4 sampling dates with a shelf-life experiment done to evaluate 'Hayward' behaviour from CA and NA, as well as 'Hayward' with 'Jintao' comparison over the same period.

For the statistical treatment of 'Hayward' and 'Jintao' on NA storage, only one producer of 'Hayward' from NA was used to compare with the only producer of 'Jintao'.

The results were presented without the producer variable and instead, storage atmosphere and cultivar origins of variation were used. The analysis was done in terms of average with an analysis of variance and Tukey averages separation test, on which the differences of average values are identified by letters. The results are expressed in tables.

# **Chapter IV - Results and discussion**

# 1. Postharvest weight and size

# 1.1. 'Hayward' kiwifruits from CA and NA storage

'Hayward' kiwifruits used on this study had an average weight value of 97.30g, and an average size of 64.85mm in length, 53.08mm in width and 48.22mm in thickness. Table 5 presents physical changes on weight and size, on CA and NA storage with significant differences between both storage methods.

**Table 5** – Mean values of weight (g), length (mm), width (mm) and thickness (mm) of 'Hayward' kiwifruits in CA and NA storage on different sampling dates.

	-	Weight	Length	Width	Thickness
Atmosphere	CA	104.04a	66.97a	54.10a	48.91a
	NA	90.56b	62.73b	52.05b	47.53b
Sampling dates	11/11/15	113.92ab	70.99a	55.49a	50.25a
(CA)	18/11/15	104.66abc	66.54abc	54.70ab	49.19abcd
	02/12/15	101.32abcde	66.24abc	54.03abc	48.49abcde
	16/12/15	107.05abc	67.50ab	54.82ab	49.80ab
	06/01/16	100.47abcde	64.75abcde	53.61abcd	47.99abcde
	20/01/16	98.37abcdef	65.74abcd	52.71abcd	48.39abcde
	03/02/16	77.98fg	67.50ab	53.10abcd	48.45abcde
	17/02/16	78.20fg	65.48abcd	53.07abcd	48.15abcde
	02/03/16	117.34a	69.20a	55.88a	50.20a
	16/03/16	97.84abcdefg	65.79abcd	53.54abcd	48.17abcde
Sampling dates	11/11/15	107.74abc	68.58a	54.43abc	49.11abcd
(NA)	18/11/15	95.93bcdefg	65.47abcd	51.52abcd	47.67abcde
	02/12/15	107.34abc	67.17ab	55.44a	49.67abc
	16/12/15	103.99abc	67.71ab	54.12abc	48.45abcde
	06/01/16	92.60cdefg	61.90bcdef	53.85abcd	48.41abcde
	20/01/16	82.58defg	61.51bcdef	50.16cd	46.90bcde
	03/02/16	103.73abcd	57.65f	50.21cd	47.10abcde
	17/02/16	95.70bcdefg	59.15def	50.81bcd	45.50e
	02/03/16	82.13fg	60.07cdef	50.55bcd	46.50cde
	16/03/16	76.68g	58.12ef	49.47d	46.05de
ANOVA (p-values	s)				
Atmosphere		<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dates*A	tmosphere	<0.0001*	<0.0001*	0.0048*	0.0154*

Levels not connected by the same letter are significantly different, at the same column.

There was a general decreasing trend in weight and size from the beginning to the end of the experiment, with a significant difference on all parameters (Table 5). Although storage facilities control temperature, humidity and gases concentrations to reduce the fruit metabolism and prevent water loss, there was a general trend for the fruit's weight to decrease along the 18 weeks of the experiment. Barboni *et al.* (2010) registered a value of 3.5% weight loss in 7 weeks in both ozone treatment chamber and 0°C air conditions, and by 29 weeks of storage the authors observed a total decrease in weight of 12.5% for cold storage and 12% for ozone treatment chamber and therefore, no significant difference was found regarding storage methods. Burdon and Clark (2001) also reported a decrease in weight through storage time.

Significant differences were obtained between CA and NA, through time and their combination, with CA providing better results on preventing general weight loss and size decrease. During storage, kiwifruits respire and take O<sub>2</sub> while giving off CO<sub>2</sub>, and in CA the O<sub>2</sub> concentration tends to decrease while CO<sub>2</sub> increases, and in a long-term storage this is important so that the fruit ripening is postponed. This happens as there is less O<sub>2</sub> available for the fruits respiration, reducing their metabolic activity and achieving a longer conservation with good marketability, as the fruits do not ripe as quickly as in a NA storage chamber. Associated with fruit ripening is ethylene production, and CO<sub>2</sub> concentration is kept higher in CA so that a lower ethylene concentration is met, also slowing down the biological processes associated with ripening (Anaparti, 2017).

The ability to store fruits is related to its respiration rate which is an expression of their metabolic activity. So, by manipulating  $O_2$  and  $CO_2$  concentrations inside the storage chambers, it is possible to extend the fruits' storage life and CA is more efficient on this aspect. NA also induces weight loss by moisture loss, keeping relative humidity high enough not to condensate on the fruits, and lower enough not to remove moisture from them but does not influence gas concentrations which prevent an increase on respiration rate (UNIDO, 2003).

# 1.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

In terms of weight and size of the studied cultivars, 'Hayward' exceeded 'Jintao' in weight from an average of 90.56g to 85.70g respectively, and size with 'Hayward' being generally bigger than 'Jintao', with significant differences. These results are in line with Huang *et al.* (2002) and Jaeger *et al.* (2003). 'Hayward' and 'Jintao' had a general decrease in weight through storage period. Along with the loss in weight, size also decreased through time on all 3 size measurements (Table 6).

Table 6 - Mean values of weight (g), length (mm), width (mm) and thickness (mm) of 'Hayward' and 'Jintao'

kiwifruits, on different sampling dates after NA storage.

	,	Weight	Length	Width	Thickness	
Cultivar	'Hayward'	90.56a	62.73	52.05a	47.53a	
	'Jintao'	85.70b	62.69	47.92b	45.64b	
Sampling dates	11/11/15	107.74ab	68.58ab	54.43ab	49.11abc	
('Hayward')	18/11/15	95.93bcd	65.47abc	51.52bcdef	47.67abcde	
	02/12/15	107.74ab	67.17ab	55.44a	49.67ab	
	16/12/15	103.99abc	67.71ab	54.12abc	48.45abcd	
	06/01/16	92.60cde	61.90cdefg	53.85abcd	48.41abcd	
	20/01/16	82.58defg	61.51cdefg	50.16efg	46.90cdef	
	03/02/16	77.98fgh	57.65fg	50.21efg	47.10cde	
	17/02/16	78.20efgh	59.15defg	50.81cdef	45.49efgh	
	02/03/16	82.13defg	60.07defg	50.55def	46.50def	
	16/03/16	76.68fgh	58.12efg	49.46efgh	46.05defg	
Sampling dates	11/11/15	86.67cdef	63.19bcdefg	48.41fghi	46.03defg	
('Jintao')	18/11/15	92.97bcdef	64.08bcde	49.47efgh	47.09abcdef	
	02/12/15	116.13a	71.21a	53.40abcde	50.33a	
	16/12/15	97.70abcd	66.28abc	49.76defgh	47.89abcde	
	06/01/16	91.20bcdef	63.54bcdef	49.60defgh	46.67bcdefg	
	20/01/16	93.74bcdef	65.11abcd	49.18efghi	47.49abcdef	
	03/02/16	75.82efgh	60.23cdefg	45.75hij	44.02fghi	
	17/02/16	65.63gh	56.73fg	44.33ij	42.51hi	
	02/03/16	63.29h	56.41g	43.26j	41.04i	
	16/03/16	73.87fgh	60.14cdefg	46.06ghij	43.37ghi	
ANOVA(p-values	)				_	
Cultivar		0.0022*	0.9404	<0.0001*	<0.0001*	
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*	
Sampling dates*C	Cultivar	<0.0001*	0.0002*	0.0020*	<0.0001*	

Levels not connected by the same letter are significantly different, at the same column.

A significant difference on width and thickness between both cultivars was achieved, 'Hayward' was wider and thicker than 'Jintao' kiwifruits, although no significant difference was obtained in length (Table 6). This resulted in a smaller and more egg-shaped 'Jintao' kiwifruit than 'Hayward'. Both cultivars were cold-stored in NA and an overall decrease in weight was observed, with a consequent decrease in size, which is in line with Burdon and Clark (2001) and Barboni *et al.* (2010) reports.

'Hayward' kiwifruits used on the present study had an overall weight superior to 'Jintao' kiwifruits, and had a similar behaviour on weight loss through time and a consequent variation in size. As both cultivars were stored in NA, a similar metabolic activity was shared between them, with only a low temperature and high humidity level playing a role in the conservation of the fruits through time. The presence or absence of pubescence on the fruits' skin is also important to retard water loss and 'Jintao' is an almost hairless cultivar and can be more susceptible to water loss during storage than 'Hayward'.

### 1.3. Kiwifruits behaviour after one week shelf-life

There were significant differences between kiwifruits from CA and NA on weight and size, and shelf-life had no significant difference between them. Table 7 demonstrates differences occurred from the beginning to end of the 7-day shelf-life experiment.

**Table 7 -** Mean values of weight (g), length (mm), width (mm) and thickness (mm) of 'Hayward' kiwifruits from CA and NA, after shelf-life.

CA a	nd NA, after shell-life.	***		**** 1.1	753 4 3
		Weight	Length	Width	Thickness
Atmosphere	CA	100.72a	65.77a	53.84a	48.09a
	NA	78.32b	58.42b	50.45b	45.36b
Shelf-life	03/02/2016	87.68	63.42	51.20	46.31ab
	03/02/2016 + 7 days	85.82	61.38	51.18	46.46ab
	17/02/2016	87.36	62.58	52.19	46.49ab
	17/02/2016 + 7 days	85.10	61.14	51.72	45.74ab
	02/03/2016	95.78	63.24	53.42	47.76a
	02/03/2016 + 7 days	93.42	61.91	52.56	47.09b
	16/03/2016	91.65	62.16	52.69	47.31ab
	16/03/2016 + 7 days	89.35	60.92	52.20	46.63ab
ANOVA (p-va	alues)				
Atmosphere		<0.0001*	<0.0001*	<0.0001*	<0.0001*
Shelf-life		0.0568	0.3610	0.2087	0.0407*
Atmosphere*	Shelf-life	0.3673	0.7829	0.1905	0.4549

Levels not connected by the same letter are significantly different, at the same column.

A general slight decrease in weight and size was observed from the initial and final day of each one-week shelf-life experiment with no significant differences (Table 7). Özer *et al.* (1999) reported that prolonged storage and shelf-life periods increased weight loss, with an

increase in respiration rate and decrease of firmness. Providing conditions to reduce respiration rate and avoid mechanical injuries is important to ensure a prolonged shelf-life period and marketability. Kiwifruits from CA and NA on the last 4 sampling dates represented on table 7 shown highly significant differences in weight and size between atmospheres, and were subjected to one-week shelf-life, with no significant differences from the initial and final day of each one-week shelf-life experiment.

**Table 8** - Mean values of weight (g), length (mm), width (mm) and thickness (mm) of 'Hayward' and 'Jintao' kiwifruits from NA, after shelf-life.

Kiwiiruits from NA, after sneif-	Weight	Length	Width	Thickness
Cultivar 'Hayward'	78.32a	58.42	50.45a	45.36a
'Jintao'	69.20b	57.60	44.75b	42.60b
Shelf-life ('Hayward') 03/02/2016	77.07bcd	58.02	49.74abc	45.22abc
03/02/2016 + 7 days	75.26cde	56.76	49.75abc	45.25abc
17/02/2016	77.85bc	58.67	51.44a	45.07bc
17/02/2016 + 7 days	75.59bcde	57.35	50.78ab	44.33bcd
02/03/2016	86.70a	60.35	52.02a	46.95a
02/03/2016 + 7  days	84.39ab	59.70	50.68ab	46.09ab
16/03/2016	75.88bcd	59.07	49.85abc	45.42abc
16/03/2016 + 7 days	73.82cdef	57.43	49.39abc	44.58bc
Shelf-life ('Jintao') 03/02/2016	75.15bcdef	58.64	45.98cd	43.58cde
03/02/2016 + 7  days	73.47cdefg	57.74	46.05cd	43.70cde
17/02/2016	66.70defg	57.38	43.69d	42.25de
17/02/2016 + 7 days	64.96efg	56.40	43.39d	42.17de
02/03/2016	64.06fg	56.40	43.61d	41.79e
02/03/2016 + 7 days	62.23g	55.62	42.96d	41.34e
16/03/2016	74.76bcdef	59.95	46.79bcd	43.77cde
16/03/2016 + 7 days	72.28cdefg	58.72	45.49cd	42.24de
ANOVA (p-values)				
Cultivar	<0.0001*	0.0952	<0.0001*	<0.0001*
Shelf-life	0.1596	0.2258	0.8140	0.0119*
Shelf-life*Cultivar	<0.0001*	0.0164*	0.0057*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

No significant differences were found on weight and size during shelf-life. However, after being removed from the storage atmospheres and put to air conditions, a higher metabolic activity associated with a higher respiration rate and consequent water loss was expected on

this shelf-life trial, and an average of 2g were lost from the beginning to the end, although without significant differences. In table 8, a similar behaviour between 'Hayward' and 'Jintao' was reached on weight and size, with significant differences between cultivars. From the first to the last day of shelf-life, a general slight decrease in weight was obtained (Table 8), followed by the kiwifruits slight shrink, with no significant differences. However, 'Hayward' was still superior in both weight and size.

### 2. Texture

# 2.1. 'Hayward' kiwifruits from CA and NA storage

Table 9 presents texture measurements done on 'Hayward' kiwifruits from CA and NA. There were significant differences on all measurements regarding the storage atmosphere that the kiwifruits came from. It was also clear that CA provided a harder and more resistant kiwifruits than NA, being able to maintain these values longer through storage, although with a slight decrease but not as high as NA.

A general decrease in texture values done by the P6 penetration test was observed with significant differences, through sampling dates (Table 9). The force on initial penetration at the first sampling date was clearly higher (61.27N) when compared to the last sampling date (27.66N), and CA (42.63N) provided higher figures than NA (35.10N), resulting in an overall harder kiwifruit. Consequently, the work done on CA kiwifruits on the whole test and on the initial penetration was also superior compared to NA. There was no significant difference between atmosphere on 'Gradient for the initial penetration' which resulted in a similar skin behaviour between kiwifruits. On 'Force at 10mm', CA fruits had a harder flesh than NA fruits, with 13.32N and 9.57N, respectively. Also, the 'Force at 25mm' was higher on CA (74.65N) than NA (50.08N).

Peeled and unpeeled texture of kiwifruits (Table 9) was also measured with significant differences on all parameters except on the 'Force at 25mm'. The absence or presence of peel induces significant differences, with lower force values for peeled kiwifruits (27.37N) than unpeeled (50.36N), as the peel plays an important role on protecting the fruit from external mechanical interferences.

Analysing the combination of sampling dates and atmosphere (Table 9.1), there was a significant difference between CA and NA on all measurements. Both CA and NA kiwifruits had similar starting force values, but through time, these values tended do decrease. This

decrease was sharper for NA kiwifruits than CA, while CA showed a better preservation of these values due to a lower metabolic activity of the fruit while NA did not.

**Table 9** – Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per sampling dates, storage atmospheres and fruit condition of 'Hayward' kiwifruits.

•		Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Sampling	11/11/15	61.27a	102.06a	1209.96a	1170.02a	4.10ab	31.02a
dates	18/11/15	58.37a	90.78b	1040.17b	978.12b	4.16ab	24.61b
	02/12/15	45.94b	70.70c	715.22c	599.89c	4.68a	14.19c
	16/12/15	42.68b	68.23c	652.99c	544.30c	4.42ab	10.84d
	06/01/16	32.08cd	57.57d	511.94d	428.12d	3.37b	6.97e
	20/01/16	31.66cd	52.88de	476.42d	379.47d	3.71ab	6.62e
	03/02/16	30.25cde	50.80def	449.65de	373.47de	3.58b	5.70ef
	17/02/16	32.72c	49.68ef	450.08d	355.19de	3.61ab	5.88ef
	02/03/16	26.02e	37.80g	312.04f	229.43f	3.58ab	3.84g
	16/03/16	27.66de	43.13fg	371.19ef	287.38ef	3.48b	4.73fg
Atmosphere	CA	42.63a	74.65a	737.48a	667.67a	3.78	13.32a
	NA	35.10b	50.08b	500.45b	401.41b	3.91	9.57b
Fruit	Unpeeled	50.36a	61.54	641.09a	510.86b	4.86a	12.75a
condition	Peeled	27.37b	63.18	596.85b	558.21a	2.83b	10.14b
ANOVA (p-v	alues)						
Sampling dat	es	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0009*	<0.0001*
Atmosphere		<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.4013	<0.0001*
Fruit condition	n	<0.0001*	0.1356	<0.0001*	0.0002*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

On 'Force on initial penetration' CA had superior values than NA, which meant that these fruits had a stronger skin and flesh, which can also be seen on 'Work on initial penetration'. It also means that CA provided better conditions to preserve firmness for longer than NA, with the decrease in firmness postponed when compared with NA that provoked a faster firmness decrease on the fruits.

**Table 9.1 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per sampling dates and storage atmosphere, regardless of the condition of 'Hayward' kiwifruits.

		Force on initial penetration	Force on 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Date	11/11/15	62.54a	106.45a	1251.65a	1210.56a	4.28abc	32.41a
(CA)	18/11/15	63.84a	103.58a	1202.47a	1169.75a	4.14abc	30.48a
	02/12/15	48.18cd	79.13b	812.51b	734.71bc	4.11abc	16.07bc
	16/12/15	47.74cd	77.29bc	763.03bc	632.76cd	5.29c	14.05cd
	06/01/16	34.59fgh	69.34bcd	611.87fg	536.63de	3.31c	7.74ef
	20/01/16	38.61ef	71.24bcd	647.37cd	540.99de	4.18abc	8.43e
	03/02/16	35.85efgh	67.49bcd	607.74de	545.17de	3.36c	7.17efg
	17/02/16	36.89efg	65.65cd	593.27de	538.02de	2.88c	6.87efgh
	02/03/16	25.63ij	45.81e	373.08gh	323.16fgh	2.86c	4.09hi
	16/03/16	32.43fghi	60.52d	511.85ef	444.93efg	3.36abc	5.87efghi
Date	11/11/15	60.00ab	97.67a	1168.27a	1129.48a	3.92abc	29.63a
(NA)	18/11/15	52.90bc	77.98bc	877.86b	786.48b	4.18abc	18.75b
	02/12/15	43.70de	62.28d	617.93de	465.06ef	5.25ab	12.32d
	16/12/15	37.62ef	59.17d	542.96de	455.84efg	3.55a	7.64efg
	06/01/16	29.57ghij	45.80e	412.01de	319.61gh	3.43bc	6.21efghi
	20/01/16	24.71ij	34.52ef	305.46ghi	217.94hi	3.24c	4.81ghi
	03/02/16	24.64ij	34.11ef	291.57ghi	201.77hi	3.35c	4.23hi
	17/02/16	28.55hij	33.72ef	306.92ghi	172.36i	4.35abc	3.59fghi
	02/03/16	26.41ij	29.78f	251.00hi	135.70i	4.30abc	3.59i
	16/03/16	22.88j	25.74f	230.52i	129.83i	3.59abc	4.89i
ANOV values) Sampli dates*	•	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

There were some significant differences between time and unpeeled and peeled texture of the fruits (Table 9.2), mainly a decrease from the first half of the sampling dates to the second half. Although storage methods manage to keep metabolic activity low, there were still changes occurring as can be seen on the overall decrease in firmness and force values assayed.

**Table 9.2 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per sampling dates and fruit condition, regardless of storage atmospheres influence, of 'Hayward' kiwifruits.

		Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Date (Unpeeled)	11/11/15	71.98	96.45ab	1228.41	1187.38a	3.88cdefg	35.22a
(Unpeeled)	18/11/15	69.46	89.07b	1064.37	983.33b	4.44bcde	27.67b
	02/12/15	58.43	67.71cd	730.15	523.04de	6.41a	16.07d
	16/12/15	55.23	67.61cd	668.24	493.87def	5.90ab	11.61e
	06/01/16	44.50	53.97efg	516.33	393.82efghi	4.24bcdef	7.59fg
	20/01/16	44.35	51.76efg	489.22	327.21ghijk	5.23abc	7.23fg
	03/02/16	42.37	51.50efg	480.68	351.18fghijk	4.65abcd	6.12ghi
	17/02/16	44.05	52.03efg	489.87	344.04ghijk	4.74abcd	6.46gh
	02/03/16	36.18	41.63gh	348.25	238.15jk	4.55bcd	4.14hi
	16/03/16	37.01	43.70fgh	395.33	266.59ijk	4.58abcd	5.33ghi
Date	11/11/15	50.56	107.66a	1191.50	1152.66a	4.32bcdef	26.81b
(Peeled)	18/11/15	47.28	92.49b	1015.96	972.90b	3.88cdefg	21.56c
	02/12/15	33.45	73.70c	700.28	676.74c	2.95defg	12.31e
	16/12/15	30.14	68.85cd	637.75	594.73cd	2.94defg	10.08ef
	06/01/16	19.66	61.17de	507.55	462.42defg	2.50fg	6.35ghi
	20/01/16	18.97	54.00ef	463.61	431.73efgh	2.19g	6.01ghi
	03/02/16	18.13	50.10efg	418.63	395.76efghi	2.06g	5.27ghi
	17/02/16	21.38	47.34fg	410.29	366.33fghij	2.49fg	5.30ghi
	02/03/16	15.87	33.97h	275.83	220.71k	2.61efg	3.54i
	16/03/16	18.31	42.56fgh	347.04	308.17hijk	2.38g	4.13hi
ANOVA (p	-values)						
Sampling d		0.3932	0.0048*	0.9344	0.0198*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

However, in table 9.3 there were significant differences between CA and NA in 'Force at 25mm' and 'Gradient for the initial penetration', with the core of the CA (78.15N) fruits being firmer than those from NA (48.22N). Although the columella or core of the kiwifruits from CA registered higher values than NA, no significant differences were obtained in the pulp firmness as 'Force at 10mm' values show. Other authors reported that at harvest, 'Hayward'

obtained an average flesh firmness of 83.9N and after 16 weeks of 0°C storage it decreased to 13.1N (Li *et al.*, 2016).

**Table 9.3** - Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per atmosphere and fruit condition, regardless of the sampling dates, of 'Hayward' kiwifruits.

		Force on initial	Force at 25mm	Work on the whole	Work on initial	Gradient for the initial	Force at 10mm
		penetration		test	penetration	penetration	
Atm. (Fruit	CA* Unpeeled	54.56	71.15b	752.62	646.68	4.36b	14.69
condition)	CA* Peeled	30.70	78.15a	722.34	688.65	3.20c	11.95
	NA*Unpeeled	46.15	51.93c	529.55	375.04	5.36a	10.80
	NA*Peeled	24.05	48.22c	471.35	427.78	2.47d	8.33
ANOVA	(p-values)						
Atm.*Fru	it condition	0.2168	<0.0001*	0.2086	0.6754	<0.0001*	0.6070

Levels not connected by the same letter are significantly different, at the same column.

Another study reported that at harvest and on a commercial maturity stage 'Hayward' presented a value of 59.5N peeled firmness (Jabbar and East, 2016). Flesh firmness by the 'Force at 10mm' on the present study progressed from an average of 26.81N on the first sampling date to 4.13N after 18 weeks (Table 9.2) and from 107.66N to 42.56N at 'Force at 25mm' on the same period.

Softening is accelerated when the fruits come out of the storage chambers due to a higher metabolic activity and conditions which induce water loss. Various factors play a role on this complex phenomenon where cell turgor pressure is fundamental and loss of water during storage, however, cell wall biochemical changes and the activity of enzymes such as amylase, β-galactosidase and polygalacturonase also promote fruit softening during time (Bonghi *et al.*, 1996; Garcia *et al.*, 1998; Li *et al.*, 2016). However, these mechanisms remain unclear, although enzyme-catalysed changes to wall structure and composition are a major factor on reducing fruit firmness over time. Still, on Tavarini *et al.* (2009) study, polygalacturonase plays an important role on decreasing fruit firmness over time, as this enzyme has an increased activity associated with a decrease of fruit firmness, and it is also proven that the softening process occurs before respiratory ethylene is produced or present, and this only occurs when the fruit has softened considerably. The breakdown of polysaccharides (starch) also promote fruit softening turning fruits more susceptible to mechanical injuries (Jayashiva, 2012). Besides having a role in the softening of the kiwifruits, these enzymes are not the only responsible for the fruit softening, Tavarini *et al.* (2009) also explains that a late harvest results in kiwifruits

with lower flesh firmness after being stored at 0°C for 2 months and put to air conditions with 25°C. Some preharvest factors influence fruit firmness such as fertilization, paying attention to nitrogen, potassium and calcium nutrients where nitrogen and potassium affect negatively the postharvest fruit firmness and calcium affects it positively (Boukouvalas and Chouliaras, 2005; Mditshwa *et al.*, 2017). However, it is important to balance these nutrients levels with a low nitrogen:calcium ratio for a proper fruit firmness and postharvest quality as Mditshwa *et al.* (2017) concluded.

Storage conditions also play an important role, and CA storage is capable of further extend fruit's storability than NA. Fruits stored on NA conditions with 0% CO<sub>2</sub>: 21% O<sub>2</sub> after 90 days of storage showed higher quality losses than CA with different combinations as 5:2, 3:3, 5:5 and 3:5, even at the end of 180 days of storage. Also, on the first 15 days of shelf-life period, CA proved to have positive effects on fruit quality. According to the author, the combinations recommended were 5:5 or 5:2 and the kiwifruits were able to withstand a shelf-life period no longer than 15 days (Özer *et al.*, 1999). From both atmospheres with highly significant differences, CA provided better results on maintaining texture throughout the sampling dates, resulting in an extended period of marketability for kiwifruits from this type of storage. The decreasing trend, visible on both CA and NA, was sharper for NA kiwifruits than CA kiwifruits.

## 2.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

Table 10 presents textural parameters for 'Hayward' and 'Jintao'. 'Hayward' showed higher values than 'Jintao'. There were also significant differences through time for both cultivars with a general decrease. Also, the presence of skin allows more resistance to the force of penetration and compression.

From the first sampling date to the last (Table 10), there was a general decrease in texture values regardless of the cultivars. 'Hayward' demonstrated higher overall texture values compared to 'Jintao'. The average 'Force on initial penetration' for 'Hayward' was 35.08N and 'Jintao' 24.45N. The 'Force at 25mm' was also superior for 'Hayward' (48.53N) than 'Jintao' (31.66N) translating in a harder core, as well as the 'Work on initial penetration' with 376.67 and 181.45N.mm, respectively, translating in a stronger skin for 'Hayward'. The 'Force at 10mm' applied for 'Hayward' (9.01N) was also higher than 'Jintao' (5.81N).

On this situation, unpeeled and peeled firmness had the same behaviour as for 'Hayward' in CA and NA, but with 'Jintao' offering an overall lower value on texture than

'Hayward' with significant differences. When combining sampling dates and cultivar (Table 10.1), significant differences appear, although with the same decreasing behaviour through time. By the last sampling dates, 'Hayward's' texture values approximate to the third and subsequent sampling dates of 'Jintao's' which translated in a more rapid loss of firmness for 'Jintao'. The work required to penetrate the kiwifruit from both cultivars also decreases through time, as well as flesh firmness as can be seen on 'Force at 10mm' with an initial value of 29.71N for 'Hayward' and a final value of 3.93N. By 16/12/2015, the significant differences on 'Force at 10mm' attenuated and got similar between cultivars.

**Table 10 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per sampling dates, cultivars and fruit condition, of 'Hayward' and 'Jintao' kiwifruits after NA storage.

	, g	Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial	Force at 10mm
						penetration	
Sampling dates	11/11/15	52.21a	77.98a	918.79a	787.20a	4.85a	24.05a
	18/11/15	44.69b	63.31b	672.75b	527.70b	4.82a	14.02b
	02/12/15	31.90c	43.59c	392.42c	288.55c	3.93ab	8.28c
	16/12/15	27.31cd	36.75cd	311.65cd	220.08cd	3.39b	3.92d
	06/01/16	21.78d	30.41de	257.71de	166.64d	3.07b	3.86d
	20/01/16	23.03d	34.19de	276.27de	213.87cd	2.71b	4.91d
	03/02/16	22.12d	28.58de	233.31de	155.16d	3.02b	3.60d
	17/02/16	26.91cd	30.86de	269.56de	157.31d	3.66ab	3.86d
	02/03/16	22.31d	26.24e	215.44e	131.80d	3.01b	4.27d
	16/03/16	25.38d	29.04de	243.42de	142.27d	3.41b	3.31d
Cultivar	'Hayward'	35.08a	48.53a	478.82a	376.67a	3.97a	9.01a
	'Jintao'	24.45b	31.66b	279.45b	181.45b	3.20b	5.81b
Fruit	Unpeeled	40.31a	42.73a	407.02a	237.64b	5.31a	8.41a
condition	Peeled	19.22b	37.46b	351.25b	320.47a	1.86b	6.41b
ANOVA (p	-values)						
Sampling d	ates	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Fruit condi	tion	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

**Table 10.1 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per sampling dates and cultivars, regardless of fruit condition of 'Hayward' and 'Jintao' kiwifruits after NA storage.

		Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Date	11/11/15	61.40a	98.89a	1168.18a	1138.67a	3.95abcde	29.71a
('Hayward	1')18/11/15	50.99b	78.14b	853.88b	751.31b	4.11abcd	16.15bc
	02/12/15	36.77cde	49.37cd	473.37de	361.72cd	4.17abcd	10.13de
	16/12/15	30.71defg	42.25de	374.46defg	280.19de	3.58bcdef	5.03fg
	06/01/16	25.42fghij	34.82efg	323.35fgh	205.57ef	3.75abcdef	5.33fg
	20/01/16	29.96defg	44.19cde	384.21def	299.44cde	3.43cdef	5.58fg
	03/02/16	26.39fghi	33.87efg	288.30fghij	195.87ef	3.67abcdef	4.62fg
	17/02/16	34.23cdef	38.98def	357.26efg	202.14ef	4.98abc	5.77efg
	02/03/16	28.06efgh	32.93efgh	279.74fghijk	163.93ef	4.16abcd	3.81fg
	16/03/16	26.85fghi	31.87efgh	285.41fghij	167.83ef	3.93abcde	3.93fg
Date	11/11/15	43.03bc	57.07c	669.41c	435.73c	5.74a	18.38b
('Jintao')	18/11/15	38.39cd	48.49cd	491.61d	304.09cde	5.54ab	11.89cd
	02/12/15	27.03fghi	37.81def	311.47fghi	215.37def	3.68abcdef	6.43ef
	16/12/15	23.92ghij	31.25efgh	248.85ghijk	159.96ef	3.21cdef	2.81fg
	06/01/16	18.13ij	26.00fgh	192.08hijk	127.70f	2.40def	2.40fg
	20/01/16	16.11j	24.18gh	168.33jk	128.30f	1.99ef	4.23fg
	03/02/16	17.86ij	23.29gh	178.31ijk	114.44f	2.38def	2.58fg
	17/02/16	19.60hij	22.74gh	181.87ijk	112.49f	2.34def	1.96g
	02/03/16	16.56j	19.56h	151.14k	99.68f	1.86f	4.73fg
	16/03/16	23.91ghij	26.20fgh	201.43hijk	116.72f	2.89def	2.68fg
ANOVA	(p-values)						
Sampling dates*Cu	ltivar	0.0036*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

These results show a similar trend with the obtained by Li *et al.* (2016) which concluded that 'Hayward' had an average flesh firmness of 83.9N and *A. chinensis* cv. 'Gold3' an average of 42.9N. After 16 weeks of storage, 0°C for 'Hayward' and 1°C for 'Gold3', firmness values decreased to 13.1N and 8.6N, respectively. Pranamornkith *et al.*, (2012) reported a decrease in firmness values on *A. chinensis* cv. 'Hort16A' from 50N to 13N during 3 weeks of storage with various ethylene treatments.

**Table 10.2** - Mean values force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per cultivars and fruit condition, regardless of the sampling dates of 'Hayward' and 'Jintao' kiwifruits after NA storage.

	Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
<b>Cultivar (Fruit condition)</b>						
'Hayward'*Unpeeled	46.66a	51.29	512.29	346.16	5.62	10.21
'Hayward'*Peeled	23.49c	45.77	445.35	407.18	2.33	7.80
'Jintao'*Unpeeled	33.96b	34.17	301.75	129.13	5.0	6.61
'Jintao'*Peeled	14.95d	29.15	257.15	233.77	1.39	5.01
ANOVA (p-values)						
Cultivar* Fruit condition	0.0147*	0.8336	0.3474	0.0948	0.3757	0.3034

In table 10.2 no significant differences were found on the combination of cultivars and unpeeled and peeled texture, except on 'Force at initial penetration' meaning a stronger skin and flesh for 'Hayward' (46.66N for skin and 23.49N for flesh) than 'Jintao' (33.96N for skin and 14.95N for flesh). *A. chinensis* cultivars tend to be less firm and easier to break down than those of *A. deliciosa* as Jaeger *et al.* (2003) reported and confirmed by the present work.

A similar decreasing trend throughout the sampling dates on texture values on both cultivars was registered, with 'Hayward' obtaining higher values than 'Jintao', with highly significant differences. With this, 'Hayward' can have a prolonged marketability when compared to 'Jintao'.

#### 2.3. Kiwifruits behaviour after one week shelf-life

Table 11 represents the effect of CA and NA storage atmospheres, and a 7-day shelf-life exposure on peeled and unpeeled force and firmness of 'Hayward' kiwifruits. There were significant differences on all the studied texture parameters, regarding the storage atmosphere, presence or absence of skin and at day 0 and day 7 of the shelf-life exposure (Table 11). This comparison was based on different kiwifruits from day 0 and day 7.

The 'Force on initial penetration' on the whole length of the test was higher on kiwifruits that came from CA storage (31.21N) than NA storage (23.30N), encountering a more difficult skin to break down, and the 'Force at 25mm' of CA kiwifruits (55.05N) was higher NA kiwifruits (27.43N), showing a stronger core. CA fruits are on their whole, more resistant than

NA with 458.89N.mm to 227.20N.mm, respectively. CA kiwifruits required more work to have their skin ruptured than NA fruits, with 397.67N.mm to 134.48N.mm, respectively.

**Table 11 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per atmosphere, fruit condition, shelf-life and the combination of atmosphere and fruit condition regardless of shelf-life, of 'Hayward' kiwifruits.

		Force on initial	Force at 25mm	Work on the whole test	Work on initial	Gradient for the initial	Force at 10mm
		penetration			penetration	penetration	
Atmosphere	CA	31.21a	55.05a	458.89a	397.67a	3.06b	5.21a
	NA	23.30b	27.43b	227.20b	134.48b	3.47a	3.43b
Fruit	Unpeeled	37.75a	43.90a	377.13a	260.32	4.38a	4.71a
condition	Peeled	16.76b	38.58b	308.97b	271.84	2.14b	3.92b
Shelf-life	Day 0	29.16a	45.35a	395.74a	311.37a	3.51a	5.04a
	Day 7	25.35b	37.12b	290.36b	220.79b	3.02b	3.60b
Atm. (Fruit condition)	CA* Unpeeled	42.15	53.81a	488.09	390.39	3.72b	5.55
	CA*Peeled	20.27	56.29a	429.70	404.96	2.40c	4.88
NA	*Unpeeled	33.35	33.98b	266.17	130.24	5.04a	3.88
	NA*Peeled	13.25	20.87c	188.24	138.72	1.89d	2.97
ANOVA (p-v Atmosphere	alues)	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0026*	<0.0001*
Fruit condition	on	<0.0001*	<0.0001*	<0.0001*	0.2791	<0.0001*	<0.0001*
Shelf-life		<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0003*	<0.0001*
Atmosphere* condition	Fruit	0.2323	<0.0001*	0.3214	0.7749	<0.0001*	0.3598

Levels not connected by the same letter are significantly different, at the same column.

CA fruits had a lower 'Gradient for the initial penetration' as their storage method managed to keep a lower metabolic activity and respiration rate, a lower loss of water and the maintenance of cells' turgescence, providing a more plastic behaviour to the fruits' skin. On the contrary, NA kiwifruits had a higher gradient which means their skin had a more elastic behaviour due to a higher metabolic activity, higher loss of water and enzymes activity, with the replacement of water molecules by air. On 'Force at 10mm', CA fruits had a harder flesh than NA fruits, with 5.21N and 3.43N, respectively.

The fruits' skin provided a higher value of texture on the several studied parameters with significant differences, except on 'Work on initial penetration' due to the absence of skin, and the amount of force accumulated was similar also translating in a similar initial flesh tissue behaviour.

**Table 11.1** - Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per atmosphere and shelf-life, fruit condition and shelf-life, and the combination of atmosphere and fruit condition and shelf-life, of 'Hayward' kiwifruits.

	Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Atmosphere (Shelf-life)	•			-	•	
CA*Day0	32.70	59.87	521.48a	462.82a	3.12b	6.00
CA*Day7	29.73	50.24	396.31b	332.53b	3.00b	4.42
NA*Day0	25.62	30.84	270.00c	159.91c	3.90a	4.08
NA*Day7	20.98	24.01	184.41d	109.05d	3.03b	2.78
Fruit condition (Shelf-life)						
Unpeeled*Day0	39.90	47.21	428.53	299.99	4.63	5.51
Unpeeled*Day7	35.60	40.58	325.74	220.64	4.14	3.92
Peeled*Day0	18.42	43.49	362.95	322.74	2.39	4.56
Peeled*Day7	15.10	33.67	254.98	220.93	1.90	3.29
Atmosphere (Fruit condition) (Shelf-life)						
CA*Unpeeled*Day0	43.86	58.02	548.49	458.02	3.62c	6.44
CA*Unpeeled*Day7	40.45	49.60	427.70	322.76	3.82bc	4.66
CA*Peeled*Day0	21.54	61.71	494.48	467.62	2.61d	5.56
CA*Peeled*Day7	19.01	50.87	364.91	342.30	2.18de	4.19
NA*Unpeeled*Day0	35.94	36.41	308.57	141.95	5.63a	4.59
NA*Unpeeled*Day7	30.76	31.56	223.77	118.53	4.45b	3.17
NA*Peeled*Day0	15.30	25.27	231.42	177.87	2.16de	3.56
NA*Peeled*Day7	11.20	16.47	145.05	99.57	1.62e	2.38
ANOVA (p-values)						
Atmosphere* Shelf-life	0.2615	0.2057	0.0448*	0.0002*	0.0054*	0.2891
Fruit condition* Shelf- life	0.5106	0.1514	0.7928	0.2913	0.9817	0.2176
Atmosphere* Fruit condition* Shelf-life	0.9441	0.7304	0.8546	0.1281	0.0193*	0.7373

Shelf-life also had significant differences and a decrease on texture values on all studied parameters, as 'Force on initial penetration' value shows with 29.16 to 25.35N for day 0 and day 7. This is an important parameter to consider when storing kiwifruits on the commercial chain to prevent losses of firmness and harm their marketability. At this stage, kiwifruits on day 0 had a minimum of 12 weeks and a maximum of 18 weeks of storage in CA and NA units, and

Li et al. (2016) after 16 weeks of storage obtained a kiwifruit firmness of 13.1N for 'Hayward' and 8.6N for A. chinensis cv. 'Gold3', Pranamornkith et al. (2012) also obtained similar values.

In table 11, when combining storage atmosphere and unpeeled and peeled measurements, there were significant differences on 'Force at 25mm' and 'Gradient for the initial penetration' meaning that although there were no significant differences between peeled and unpeeled force at 25mm of CA kiwifruits, there were significant differences between NA kiwifruits peeled and unpeeled force, with CA kiwifruits peeled force (56.29N) higher than NA kiwifruits unpeeled force (33.98N). On this case, CA kiwifruits' core required more force to be surpassed than the NA kiwifruits' core. On 'Gradient for the initial penetration' as mentioned before, CA kiwifruits' skin shown a less elastic behaviour than NA kiwifruits but, when comparing flesh of CA and NA fruits, CA firmness was superior.

When combining all other factors (Table 11.1), no significant differences were found meaning that shelf-life had no significant differences on the texture of the fruits, although texture having a trend to decrease through time. The overall softening of the kiwifruit accompanies its maturation and ripening which is crucial for its shelf-life durability.

Significant differences were found between CA and NA on the last 4 sampling dates corresponding to the shelf-life experiment, with CA being superior to NA, and comparing the overall texture values of the initial (Day 0) and final day (Day 7), there were highly significant differences, however combining shelf-life with storage atmospheres resulted in no significant differences. So, for marketability, a 7-day period had a highly significant difference on the kiwifruits texture, however the storage atmosphere did not interfere on this variation.

In table 12, significant differences on texture between cultivars and day 0 and day 7 of shelf-life were found. As on the previous situation of 'Hayward' and 'Jintao' comparison without shelf-life, 'Hayward' possessed an overall higher texture values than 'Jintao', however more significant differences between day 0 and day 7 were seen on 'Hayward' rather than 'Jintao'. The 'Force at 25mm' showed a significant difference between day 0 and day 7 for 'Hayward' (34.41 to 29.03N) and not for 'Jintao' (22.95 to 22.19N), resulting in a stronger core for 'Hayward' but with a significant decrease through time, which did not occur for 'Jintao'. 'Force on initial penetration' also registered a general decrease from 24.18N to 21.11N from day 0 to day 7 respectively, but the 'Force at 10mm' had no significant difference in the same time interval.

**Table 12-** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N), per cultivar, fruit condition, shelf-life and the combination of cultivar and fruit condition regardless of shelf-life, and the combination of cultivar and shelf-life regardless of fruit condition, of 'Hayward' and 'Jintao' kiwifruits.

	dwiiruits.	Force on initial penetration	Force at 25mm	Work on the whole test	Work on initial penetration	Gradient for the initial penetration	Force at 10mm
Cultivar 'H	layward'	26.87a	31.72a	263.57a	161.06a	3.78a	3.92a
	'Jintao'	18.43b	22.57b	175.16b	113.14b	2.21b	2.95b
	U <b>npeeled</b>	32.38a	33.18a	259.08a	131.14	4.48a	4.47a
condition	Peeled	12.92b	21.11b	179.66b	143.06	1.51b	2.41b
Shelf-life	Day 0	24.18a	28.68a	240.43a	146.64a	3.28a	3.76
	Day 7	21.11b	25.61b	198.30b	127.56b	2.71b	3.12
Cultivar (Fruit condition)							
'Hayward'*l	U <b>npeeled</b>	37.88a	38.86a	307.58	153.66	5.55a	4.43a
'Hayward	'*Peeled	15.85c	24.59b	219.57	168.46	2.00c	3.41a
'Jintao'*l	U <b>npeeled</b>	26.89b	27.50b	210.58	108.62	3.41b	4.50a
'Jintao	'*Peeled	9.98d	17.63c	139.74	117.66	1.02d	1.40b
Cultivar (Shelf-li	fe)						
'Haywar	d'*Day 0	28.88	34.41a	302.68a	182.44a	4.19	4.53
'Haywar	d'*Day 7	24.85	29.03b	224.47b	139.67b	3.36	3.32
'Jinta	o'*Day 0	19.48	22.95c	178.19c	110.83b	2.36	2.99
'Jinta	o'*Day 7	17.38	22.19c	172.14c	115.45b	2.06	2.91
ANOVA (p-value	es)						
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0216*
Fruit condition		<0.0001*	<0.0001*	<0.0001*	0.1513	<0.0001*	<0.0001*
Shelf-life		0.0006*	0.0025*	<0.0001*	0.0219*	0.0002*	0.1274
Cultivar* Fruit c	ondition	0.0039*	0.0296*	0.3268	0.7282	0.0001*	0.0140*
Cultivar*Shelf-li	fe	0.2729	0.0223*	<0.0001*	0.0045*	0.0826	0.1788

The combination of all factors (Table 12.1) resulted in a significant difference only on the 'Work on initial penetration', with a general decrease in firmness from day 0 to day 7, comparing cultivars and their fruit condition. A more significant decrease in firmness values was registered in 'Hayward', however, 'Jintao' still had lower values of firmness on all cases and did not evidenced significant changes through time.

Overall, 'Hayward' registered a more significant decrease of texture on shelf-life, than 'Jintao', although demonstrating an overall stronger skin and flesh more resistant to external forces. For marketability, 'Hayward' although possessing a higher decrease in texture values than 'Jintao' was still more resistant to external force than 'Jintao'.

**Table 12.1 -** Mean values of force on initial penetration (N), force at 25mm (N), work on the whole test (N.mm), work on initial penetration (N.mm), gradient for the initial penetration (N/mm) and force at 10mm (N) on the combination of cultivar, fruit condition and shelf-life of 'Hayward' and 'Jintao' kiwifruits.

	Force on	Force at	Work on	Work on	<b>Gradient for</b>	Force at
	initial	25mm	the whole	initial	the initial	10mm
	penetration		test	penetration	penetration	
<b>Cultivar (Fruit condition)</b>						
(Shelf-life)						
'Hayward'*Unpeeled*Day 0	39.96	40.45	344.31	158.60ab	6.17	5.09
'Hayward'*Unpeeled*Day 7	35.80	37.26	270.84	148.73bc	4.93	3.78
'Hayward'*Peeled* Day 0	17.81	28.37	261.05	206.29a	2.20	3.98
'Hayward'*Peeled* Day 7	13.90	20.80	178.10	130.62bc	1.80	2.85
'Jintao'*Unpeeled* Day 0	28.76	28.76	220.89	112.95bc	3.58	4.50
'Jintao'*Unpeeled* Day 7	25.00	26.24	200.27	104.29c	3.24	4.51
'Jintao'*Peeled* Day 0	10.20	17.13	135.49	108.71bc	1.15	1.48
'Jintao'*Peeled* Day 7	9.76	18.13	144.00	126.60bc	0.89	1.32
ANOVA (p-values)						
Cultivar* Fruit condition *Shelf-life	0.3844	0.0503	0.2703	0.0056*	0.2057	0.8380

Levels not connected by the same letter are significantly different, at the same column.

#### 3. Skin and flesh colour

# 3.1. 'Hayward' kiwifruits from CA and NA storage

Lightness (L\*) and chroma (C\*) of kiwifruits tended to decrease, while hue angle (h°) remained stable along the sampling dates. However, a significant difference was observed in h° and C\* between atmospheres. L\* had no significant difference between atmospheres. However, kiwifruits from CA tended to be brighter and more colourful than NA (Table 13).

Time significantly affected all chromatic parameters on the different situations analysed, on the fruit's unpeeled or peeled condition (Table 13). By combining storage atmosphere and fruit condition chromatic measurements, there were also significant differences on all situations. According to L\*, CA unpeeled kiwifruits (42.89) had a lower value than NA unpeeled fruits (43.90), and the pulp of CA (57.34) had a higher L\* value than NA (56.22) meaning a brighter pulp for CA. Chroma was higher for fruits from CA than NA, with

significant differences within their pulps, with CA (26.32) being more colourful than NA (24.52). No significant differences were found on the fruits pulp on h°, contemplating the outer and inner pericarps as well as columella. Huang *et al.* (2002) for 'Hayward' flesh obtained a lightness value of 62.00, a C\* value of 32.87 and a h° of 158.59°, although brighter and more colourful but with a similar hue angle with the results of the present study.

**Table 13 -** Mean values of chromatic parameters, L\*(Lightness), flat coordinates (a\*; b\*) and angular coordinates (C\*(Chroma); h°(hue angle)) per sampling date regardless of atmosphere and fruit condition, atmosphere regardless of sampling dates and fruit condition, fruit condition regardless of sampling dates and atmosphere and atmosphere combination with fruit condition regardless of atmosphere of 'Hayward' kiwifruits.

114 1141	d' kiwifruits.	L*	a*	b*	C*	h°
Sampling dates	11/11/15	53.75a	-4.81e	27.31a	28.74a	85.02ab
	18/11/15	53.03a	-4.98e	27.15a	28.71a	82.39b
	02/12/15	52.41a	-4.60e	26.31ab	27.71ab	86.24a
	16/12/15	50.46b	-3.78d	25.37bc	26.62bc	84.68ab
	06/01/16	49.34bc	-3.15cd	25.15c	26.38c	83.23ab
	20/01/16	48.58c	-2.75bc	23.23de	24.39de	82.63b
	03/02/16	48.74bc	-2.74bc	22.98de	24.11de	82.86ab
	17/02/16	48.69bc	-2.76bc	23.79d	24.86d	82.67b
	02/03/16	48.16c	-1.70a	22.48e	23.58e	85.79ab
	16/03/16	47.71c	-2.35ab	22.98de	24.00de	83.59ab
Atmosphere	CA	50.12	-3.45	25.08a	26.38a	83.04b
	NA	50.06	-3.27	24.27b	25.44b	84.78a
Fruit condition	Unpeeled	43.40b	3.41a	26.11a	26.40a	10.54b
	Peeled	56.78a	-10.13b	23.24b	25.42b	157.28a
Atmosphere	CA*Unpeeled	42.89d	3.68a	26.13a	26.43a	9.07c
(Fruit condition)	CA*Peeled	57.34a	-10.58d	24.02b	26.32a	157.01a
	NA*Unpeeled	43.90c	3.13b	26.09a	26.37a	12.02b
	NA*Peeled	56.22b	-9.68c	22.45c	24.52b	157.54a
ANOVA (p-value	es)					
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0007*
Atmosphere		0.8223	0.0779	<0.0001*	<0.0001*	0.0005*
Fruit condition		0.0000*	0.0000*	<0.0001*	<0.0001*	0.0000*
Atmosphere*Fru	it condition	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0160*

Levels not connected by the same letter are significantly different, at the same column.

On 'Hayward', the presence of chlorophylls on the fruit tissues are the reason for the green colour of the pulp. Along with the chemical changes such as sugars, acids and softening, chloroplasts are also converted into chromoplasts which results in a loss of chlorophyll and accumulation of carotenoids (Montefiori *et al.*, 2009).

A slight drop of the h° on the pulp through 3 months of storage was also obtained by Montefiori *et al.* (2009), appearing green on all stages of development with a value of h°>110° measuring the outer pericarp. The author also explained that these changes were accompanied by the fruit softening and rise on soluble solids, however the green colour is maintained on 'Hayward' during all stages of development due to the presence of chlorophylls, not due to changes in carotenoids which are responsible for the yellow colour, and the maintenance of chlorophylls on 'Hayward' may be due to an insufficient enzymatic activity of chlorophylls degradation, or to the retention of chloroplast ultrastructure on ripening which prevents chlorophyll breakdown.

Overall, time had highly significant differences on all chromatic parameters, with a general loss of lightness, and the storage atmosphere caused significant differences on b\* value, with CA kiwifruits obtaining a higher value and closer to yellow. Also, C\* had highly significant differences between atmosphere, with CA providing kiwifruits with a higher colour saturation.

## 3.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

Both cultivars had similar lightness values but had significant differences on the other chromatic parameters. Time also influenced lightness with a general decrease as well as colourfulness. Between each cultivar fruit condition, significant differences were also reported (Table 14).

There was a general decrease on lightness through time for both cultivars, with no significant differences between them, however 'Jintao' tended to be more colourful than 'Hayward' presenting a higher C\* value, 26.65 to 25.44, respectively. For 'Jintao', on a ripe stage, Huang *et al.* (2002) obtained a lightness value of 61.9; C\* value of 27.86 and h° of 172.16°, which are in line with the results obtained on the present study, and for 'Hayward' a lightness of 62.00, a C\* value of 32.87 and a h° of 158.59°.

Through time, unpeeled 'Hayward' was brighter and more colourful than 'Jintao's', however 'Hayward's' pulp was less bright and colourful than 'Jintao's'. The hue angle for both cultivars was situated on the green tone. It would be expected for 'Jintao' to have a more yellow

colour, but as these fruits were analysed on a mature phase but not fully ripe, so, on this stage presented a greener pulp. It is known that *A. chinensis* fruit pulp can vary between green and yellow at a riper stage, initially being green which turns into more yellow along with the fruit ripening (Montefiori *et al.*, 2009).

**Table 14 -** Mean values of chromatic parameters, L\*(Lightness), flat coordinates (a\*- value of red and green colours; b\*- value of yellow and blue colours) and angular coordinates (C\*(Chroma); h°(hue angle)) per sampling date regardless of cultivar and fruit condition, cultivar regardless of sampling dates and fruit condition, fruit condition regardless of sampling dates and cultivar and the combination of fruit condition and cultivar regardless of sampling dates between 'Havward' and 'Jintao' after NA storage.

condition and cultivar re	L*	a*	<b>b</b> *	C*	h°
Sampling dates 11/11/15	53.95a	-2.89d	28.16a	29.33a	92.95a
18/11/15	53.33a	-1.89c	26.52b	27.79ab	87.69a
02/12/15	53.17ab	-1.92c	25.80bc	26.84bc	91.07a
16/12/15	50.96bc	-1.45bc	25.19bcd	26.17cd	92.02a
06/01/16	50.28cd	-0.83ab	25.42bcd	26.33bcd	87.53a
20/01/16	49.95cde	-0.75ab	24.33cde	25.31cde	87.72a
03/02/16	49.27cde	-0.78ab	23.31e	24.34e	87.99a
17/02/16	48.76cde	-0.76ab	24.05de	24.96de	87.89a
02/03/16	47.78e	-0.38a	23.45e	24.30e	91.54a
16/03/16	48.46de	-0.63a	24.25de	25.11de	88.34a
Cultivar 'Hayward'	50.64	-3.42b	24.37b	25.44b	85.84b
'Jintao'	50.54	0.96a	25.72a	26.65a	93.11a
Fruit condition Unpeeled	43.76b	5.02a	25.81a	26.52a	15.97b
Peeled	57.42a	-7.48b	24.29b	25.58b	162.98a
Cultivar (Fruit 'Hayward'* condition) Unpeeled	45.07c	2.46b	26.98a	27.15a	13.95d
condition) Unpeeled 'Hayward'* Peeled	56.21b	-9.30d	21.76c	23.74c	157.73b
'Jintao'*Unpeeled		7.59a	24.64b	25.88b	18.00c
'Jintao'*Peeled	58.63a	-5.67c	26.81a	27.42a	168.22a
ANOVA (p-values)					
Sampling dates	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0061*
Cultivar	0.7779	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Fruit condition	<0.0001*	0.0000*	<0.0001*	<0.0001*	0.0000*
Cultivar*Fruit condition	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

A. chinensis cultivars at the first stages of development have chlorophyll present on the tissues which provides them with the green colour, and progressively the chlorophyll

concentration decreases, losing the green colour and turning yellow due to the presence of carotenoids, mainly lutein, xanthophylls and  $\beta$ -carotene (Montefiori *et al.*, 2009).

Both cultivars suffered a loss of lightness through time with no significant differences between them, however all other chromatic parameters revealed highly significant differences between them, with a\* being closer to green for 'Hayward' and almost neutral for 'Jintao', however the b\* value was closer to yellow for 'Jintao'. A higher C\* value was registered for 'Jintao'.

## 3.3. Kiwifruits behaviour after one week shelf-life

All chromatic parameters analysed had significant differences between cultivars, as well as for 'Hayward' fruits from CA or NA. The 3 different pulp positions also had highly significant differences between atmospheres (Table 15). As on the previous situations, 'Hayward' pulp from CA was brighter and more colourful than NA as well as 'Jintao' pulp was brighter and more colourful than 'Hayward's', with significant differences. On shelf-life, regardless of cultivar, atmosphere and pulp position, significant differences were obtained with an increase in lightness (56.04 to 57.10), also the flat coordinate a\* increased, leading to a lighter green, and b\* was not influenced by shelf-life as well as C\*. The effect of shelf-life on each cultivar resulted in significant differences between them except on h°. In lightness, 'Hayward' and 'Jintao' demonstrated an opposite behaviour, with a decrease for 'Hayward' (55.60 to 54.54) and an increase for 'Jintao' (56.48 to 59.66), from day 0 to day 7. 'Hayward' pulp from day 0 to day 7 registered a decrease in a\* resulting in a lighter green while 'Jintao' also registered a decrease on this coordinate and an increase on b\* resulting in a less green and more yellow kiwifruit.

Differences were also observed on the pulp positions, regardless of cultivar, with columella registering the highest lightness value (67.34) as it is predominantly white, followed by the outer pericarp (54.10) that can be predominantly green or yellow depending on the cultivar, and the inner pericarp (48.26) with the lowest value mainly due to the presence of dark seeds.

The main effects of shelf-life on 'Hayward' approximated to a less saturated and bright green, with a decrease in lightness, chroma and a\*. On the other hand, 'Jintao' demonstrated an opposite behaviour with an increase in lightness and chroma, and an approximation to the yellow colour with an increase in b\* while losing the green colour as the increase in a\* suggests.

The values and behaviours observed are also in line with Huang *et al.* (2002) and Montefiori *et al.* (2009).

**Table 15 -** Mean values of chromatic parameters, L\*(Lightness), flat coordinates (a\*- value of red and green colours; b\*- value of yellow and blue colours) and angular coordinates (C\*(Chroma); h°(hue angle)) per cultivar, atmosphere, shelf-life, pulp position, and cultivar and shelf-life regardless of pulp position between 'Hayward' from CA and NA, and 'Jintao' kiwifruits, after shelf-life.

		L*	a*	b*	C*	h°
Cultivar	'Hayward'	55.07b	-8.24b	20.14b	21.83b	158.40b
	'Jintao'	58.07a	-5.96a	28.47a	29.14a	168.48a
Atmosphere	e CA	57.66a	-7.51b	25.11a	26.39a	163.19b
	NA	55.48b	-6.68a	23.50b	24.58b	163.69a
Shelf-life	Day 0	56.04b	-7.29b	24.29	25.51	163.22b
	Day 7	57.10a	-6.91a	24.33	25.46	163.66a
Pulp positio	n OP	54.10b	-8.96c	25.90b	27.67b	160.67c
	IP	48.26c	-4.69a	19.24c	19.89c	165.76a
	C	67.34a	-7.65b	27.78a	28.90a	163.89b
Cultivar (Shelf-life)	'Hayward'*Day 0	55.60b	-8.55c	20.82c	22.58c	158.30
(Shen-me)	'Hayward'*Day 7	54.54c	-7.93b	19.46d	21.08d	158.50
	'Jintao'*Day 0	56.48b	-6.03a	27.75b	28.44b	168.14
	'Jintao'*Day 7	59.66a	-5.89a	29.19a	29.84a	168.83
ANOVA (p-	values)					
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0000*
Atmosphere	2	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0007*
Shelf-life		0.0090*	0.0003*	0.8673	0.8388	0.0107*
Pulp positio	n	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Cultivar*Sh	elf-life	<0.0001*	0.0237*	<0.0001*	<0.0001*	0.1486

Levels not connected by the same letter are significantly different, at the same column. (OP- Outer pericarp; IP- Inner pericarp; C- Columella)

**Table 15.1 -** Mean values of chromatic parameters, L\*(Lightness), flat coordinates (a\*- value of red and green colours; b\*- value of yellow and blue colours) and angular coordinates (C\*(Chroma); h°(hue angle)) per cultivar and pulp positions, and shelf-life on each pulp position.

		$\mathbf{L}^*$	a*	b*	<b>C</b> *	h°
Cultivar (Pulp position)	'Hayward'*OP	52.18c	-11.52e	22.72c	25.49c	153.23e
1 tup position)	'Hayward'*IP	45.20d	-5.25b	15.81e	16.70e	161.93c
	'Hayward'*C	67.83a	-7.95d	21.89d	23.31d	160.03d
	'Jintao'*OP	56.02b	-6.39c	29.07b	29.84b	168.11b
	'Jintao'*IP	51.32c	-4.13a	22.67cd	23.08d	169.59a
	'Jintao'*C	66.85a	-7.35d	33.67a	34.50a	167.76b
ANOVA (p-valu	es)					
Cultivar*Pulp position		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column. (OP- Outer pericarp; IP- Inner pericarp; C- Columella)

#### 4. Refractometric index, pH and NaOH volume spent in titration

# 4.1. 'Hayward' kiwifruits from CA and NA storage

The °Brix level had an increasing trend through time and there was no significant difference between storage atmospheres, however, pH and NaOH volume spent in titration were significantly different between CA and NA (Table 16).

In table 16, the first date (11/11/2015) corresponds to the beginning of CA and NA storage, and had an average °Brix value of 7.13° and 7.60° respectively. At harvest and on a commercial maturity stage, °Brix levels ranged from 9.7° to 11.2° as Jabbar and East, (2016) analysed. Also, after 5 weeks of storage, the average °Brix rose from 7.36° to 10.77° and after 18 weeks to 12.98°, almost double of the starting value. There was a slight decrease through time on pH levels on both storage methods, and there was a significant difference between CA and NA. NA fruits had a more significant difference through time on pH levels than CA. There was a significant difference between CA and NA on NaOH volume spent in titration, with a higher value for CA (22.87mL) than NA (22.20mL), and decreased on both storage methods during the storage time.

Barboni *et al.* (2010) studied the influence of different storage methods on kiwifruits physicochemical parameters and during 7 months of storage there was no significant difference regarding the storage method. °Brix values rose from 6° to 12° during 7 weeks and after 21 weeks stagnated at 15°. Lloret *et al.* (1990), Tavarini *et al.* (2008) cited by Barboni *et al.* (2010)

recommend a minimum sugar level between  $12^\circ$  and  $14^\circ$ Brix which was attained after 8 weeks of storage.

**Table 16** - Mean values of °Brix, pH and NaOH volume spent in titration (mL) of 'Hayward' kiwifruits after CA and NA on different sampling dates.

	n different samp	°Brix	pН	NaOH Volume	
Atmosphere	CA	11.12	3.32b	22.87a	
	NA	11.03	3.37a	22.20b	
Sampling dates	11/11/15	7.13h	3.30cde	23.49abc	
(CA)	18/11/15	7.66h	3.35abcde	22.89abcd	
	02/12/15	9.58g	3.37abcde	24.07a	
	16/12/15	10.96ef	3.37abcde	22.95abcd	
	06/01/16	12.29abcd	3.34bcde	23.64ab	
	20/01/16	12.34abcd	3.35abcde	22.43abcd	
	03/02/16	12.66abcd	3.28e	22.95abcd	
	17/02/16	12.71abcd	3.33bcde	22.66abcd	
	02/03/16	12.88ab	3.25e	21.57bcd	
	16/03/16	13.04a	3.28de	22.05abcd	
Sampling dates	11/11/15	7.60h	3.43abcd	22.68abcd	
(NA)	18/11/15	8.70g	3.49a	22.85abcd	
	02/12/15	9.55g	3.46ab	22.50abcd	
	16/12/15	10.57f	3.45abc	21.01d	
	06/01/16	12.84abc	3.36abcde	23.59abc	
	20/01/16	11.83de	3.36abcde	21.19cd	
	03/02/16	12.01bcd	3.36abcde	21.40bcd	
	17/02/16	12.43abcd	3.30cde	22.80abcd	
	02/03/16	11.87cde	3.26e	22.48abcd	
	16/03/16	12.93ab	3.24e	21.53bcd	
ANOVA (p-values	s)				
Atmosphere		0.2889	0.0002*	0.0022*	
Sampling dates*A	atmosphere	<0.0001*	0.0165*	0.0676	
Sampling dates		<0.0001*	<0.0001*	0.0002*	

Levels not connected by the same letter are significantly different, at the same column.

An increase of pH level was obtained by Barboni *et al.* (2010) throughout 21 weeks of storage from 3 to 3.4, and Marsh *et al.* (2003) had values at harvest ranging from 3.21 to 3.24,

and no difference was found between storage methods. Marsh *et al.* (2003) reported that storage at different temperatures (0, 4, 10°C) of 'Hayward' kiwifruits does not affect its titratable acidity, and Barboni *et al.* (2010) reported that total acidity decreased during 21 weeks of cold storage. Cheng *et al.* (2004) concluded that pH and TA have a high negative correlation.

Storage atmosphere had no influence on the °Brix variation, but on pH and NaOH volume in titration there were significant differences, with a higher pH value for NA than CA and a higher NaOH volume spent for CA than NA. Through time, °Brix increased regardless of storage atmosphere.

## 4.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

Comparing both cultivars, 'Jintao' exceeded 'Hayward' on 'Brix as demonstrated on table 17, with 11.03° to 15.04° for 'Hayward' and 'Jintao' respectively. Besides 'Jintao' being higher in 'Brix, also had a higher pH level than 'Hayward'. NaOH volume spent in titration had a value of 22.20mL for 'Hayward' and 17.27mL for 'Jintao'.

A general increase in °Brix was observed on both cultivars under normal atmosphere (Table 17). A clear difference between cultivars is the starting and ending of °Brix, while 'Hayward' started with a value of 7.6°Brix on the first sampling date, and after 18 weeks of storage reached 12.93°Brix, 'Jintao' started with a 12.58°Brix value and ended with 16.33°Brix. Analysing pH levels, these had a decreasing trend throughout storage on 'Hayward' but not very evident on 'Jintao', while NaOH volume spent had some variations and decreased little.

At the beginning of the experiment, both studied species had a strong difference on °Brix level, with 'Jintao' starting with 12.58°Brix, and 'Hayward' only starting with 7.6. These results were similar, although slightly higher in comparison to Huang *et al.* (2002) study, that at harvest for 'Jintao' reported 12% soluble solids and 6.5% for 'Hayward', and after 4 months in cool storage (4°C) obtained 18% for 'Jintao' and 12% for 'Hayward'.

Highly significant differences were found between cultivar on °Brix, pH and NaOH volume spent in titration, with 'Jintao' obtaining a higher 'Brix value than 'Hayward', a higher pH value than 'Hayward' and a lower NaOH volume spent than 'Hayward', resulting in a sweeter and less acid kiwifruit when compared to 'Hayward'. A highly significant difference was obtained through time regardless of the cultivar, with an increase on 'Brix and a slight decrease on pH values.

**Table 17** - Mean values of °Brix, pH and NaOH volume spent in titration (mL) of 'Hayward' and 'Jintao' kiwifruits from NA on different sampling dates.

from NA on different sampling dates.								
		°Brix	pН	NaOH volume				
Cultivar	'Hayward'	11.03b	3.37b	22.20a				
	'Jintao'	15.04a	3.46a	17.27b				
Sampling dates	11/11/15	7.60h	3.43bcd	22.68a				
('Hayward')	18/11/15	8.70gh	3.49abc	22.85a				
	02/12/15	9.55fg	3.46abc	22.50a				
	16/12/15	10.57f	3.45bcd	21.01abcd				
	06/01/16	12.84de	3.36bcde	23.59a				
	20/01/16	11.83e	3.36bcde	21.19abcd				
	03/02/16	12.01e	3.36bcde	21.40abc				
	17/02/16	12.43de	3.30de	22.80a				
	02/03/16	11.87e	3.26e	22.48a				
	16/03/16	12.93de	3.24e	21.53ab				
Sampling dates ('Jintao')	11/11/15	12.58de	3.50abc	18.06def				
( Jintao )	18/11/15	13.42cd	3.47abcd	17.30ef				
	02/12/15	14.50bc	3.64a	19.08bcde				
	16/12/15	14.79abc	3.50abc	15.76ef				
	06/01/16	15.51ab	3.53ab	16.77ef				
	20/01/16	15.79ab	3.50abc	15.18f				
	03/02/16	15.57ab	3.36bcde	17.08ef				
	17/02/16	15.68ab	3.31cde	17.60ef				
	02/03/16	16.24a	3.36bcde	18.24cdef				
	16/03/16	16.33a	3.41bcde	17.58ef				
ANOVA (p-valu	ies)							
Cultivar		<0.0001*	<0.0001*	<0.0001*				
Sampling dates	*Cultivar	<0.0001*	0.0266*	0.2252				
Sampling dates		<0.0001*	<0.0001*	0.0002*				

Levels not connected by the same letter are significantly different, at the same column.

## 4.3. Kiwifruits behaviour after one week shelf-life

No significant differences were obtained between sampling dates, however between atmospheres and through time, CA kiwifruits increased initially on °Brix and then stagnated, while NA remained stable, with lower values compared to CA (Table 18). At these 4 final

sampling dates, only the atmosphere interfered on the results, with a higher °Brix, lower pH and NaOH volume spent in titration for CA. At this point, shelf-life did not influence the results.

**Table 18 -** Mean values of °Brix, pH and NaOH volume spent in titration (mL) between CA and NA of 'Hayward' kiwifruits after shelf-life.

		°Brix	pН	NaOH volume
Atmosphere	CA	12.83a	3.26b	23.00a
	NA	12.40b	3.34a	25.58b
Shelf-life (CA)	10/02/2016	12.38ab	3.29abc	23.18
	24/02/2016	12.98a	3.24c	23.68
	09/03/2016	12.93a	3.23c	22.63
	23/03/2016	13.02a	3.28bc	22.50
Shelf-life (NA)	10/02/2016	12.53ab	3.38ab	21.04
	24/02/2016	12.47b	3.28abc	21.78
	09/03/2016	12.61ab	3.40a	22.07
	23/03/2016	11.98ab	3.30abc	21.46
ANOVA (p-valu	es)			
Shelf-life		0.3251	0.0503	0.5407
Atmosphere		0.0039*	<0.0001*	0.0005*
Atmosphere*Sh	elf-life	0.0424*	0.0268*	0.4390

Levels not connected by the same letter are significantly different, at the same column.

'Jintao' had a higher 'Brix level than 'Hayward', 16.14' to 12.40'Brix respectively. However, pH level had no significant difference, but the NaOH volume spent in titration was significantly different between cultivars, higher on 'Hayward' than 'Jintao' (Table 19).

As on the previous situations, "Brix had significant differences between cultivars, however, "Brix, pH and the volume of NaOH spent in titration had no significant differences during shelf-life. At these 4 final sampling dates, shelf-life did not provide significant differences on the results, however between cultivars there were significant differences on "Brix and NaOH volume spent, and not on pH. 'Jintao' still obtained a higher "Brix value than 'Hayward' (Table 19).

**Table 19 -** Mean values of °Brix, pH and NaOH volume spent in titration (mL) between 'Hayward' and 'Jintao' kiwifruits from NA, after shelf-life.

	,	°Brix	pН	NaOH volume
Cultivar	'Hayward'	12.40b	3.34	21.58a
	'Jintao'	16.14a	3.36	17.69b
Shelf-life ('Hayward')	10/02/2016	12.53	3.38	21.04
,	24/02/2016	11.98	3.29	21.78
	09/03/2016	12.61	3.40	22.07
	23/03/2016	12.47	3.30	21.46
Shelf-life ('Jintao')	10/02/2016	15.69	3.37	16.99
( = === ,	24/02/2016	16.06	3.33	18.04
	09/03/2016	16.42	3.35	18.26
	23/03/2016	16.38	3.38	17.48
ANOVA (p-valu	ies)			
Shelf-life		0.1802	0.1143	0.1553
Cultivar		<0.0001*	0.4828	<0.0001*
Cultivar*Shelf-	life	0.3281	0.3322	0.9906

#### 5. Free-sugars

# 5.1. 'Hayward' kiwifruits from CA and NA storage

Four sugars were quantified, fructose, glucose, sucrose and galactose (Table 20). On the first sampling date, the predominant sugars were fructose, with an average value of 13.26mg/g FW, followed by glucose 10.74mg/g FW, and on smaller amounts, sucrose with an average value of 5.63mg/g FW and galactose with 1.85mg/g FW. Free-sugars concentrations increased through storage time with no significant differences due to storage method, and similar results were found by Barboni *et al.* (2010). The main sugars identified in *A. deliciosa* cultivars were fructose and glucose on similar amounts and sucrose on smaller amounts (Nishiyama *et al.*, 2008).

All analysed free-sugars increased through storage time (Table 20), and on the last sampling date, the contents of each soluble sugar were very similar between storage atmosphere and this behaviour corresponds to previous studies referring to eating-ripe 'Hayward' kiwifruits (Nishiyama *et al.*, 2008; Nunes-Damaceno *et al.*, 2013; D'Evoli *et al.*, 2015; Sivakumaran *et al.*, 2016). Also, glucose and fructose constant increase and evolution speed, through storage time was met by Barboni and Chiaramonti (2006).

**Table 20** - Mean values of fructose (mg/g FW), glucose (mg/g FW), sucrose (mg/g FW) and galactose (mg/g FW) of 'Hayward' kiwifruits after CA and NA on different sampling dates.

		Fructose	Glucose	Sucrose	Galactose
Atmosphere	CA	30.31	24.75	11.85	4.42
	NA	31.57	25.38	11.10	4.41
Sampling dates	11/11/15	13.26c	10.74c	5.63b	1.85d
	06/01/16	32.86b	25.67b	13.83a	4.30c
	03/02/16	35.69b	29.42b	12.73a	5.26b
	16/03/16	41.97a	34.43a	13.71a	6.26a
Sampling dates	11/11/15	13.20	10.94	4.93	1.82
(CA)	06/01/16	29.46	23.88	13.99	4.05
	03/02/16	36.22	29.91	14.56	5.23
	16/03/16	42.38	34.26	13.93	6.59
Sampling dates	11/11/15	13.33	10.55	6.32	1.87
(NA)	06/01/16	36.25	27.46	13.67	4.56
	03/02/16	35.15	28.92	10.90	5.30
	16/03/16	41.57	34.60	13.50	5.93
ANOVA (p-values	s)				
Atmosphere		0.2900	0.5763	0.3222	0.9818
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dates*A	tmosphere	0.0731	0.4868	0.1374	0.3134

Kiwifruits have an average starch content of 40% of dry weight, which is achieved a month prior to commercial harvest, and the starch hydrolysis has its peak on the first weeks of storage, after harvest, and all starch is converted by 10 weeks of storage (Richardson *et al.*, 1997). Initially, glucose is lower than fructose and remains until fruit is ripe, however when it becomes overripe, glucose concentration exceeds fructose by 40% (Jayashiva, 2012). Sucrose had a slight increase and then remained constant on the last sampling dates, but when it becomes overripe it decreases (Garcia *et al.*, 1998). Sucrose tends to remain constant as it synthetises from starch, and at the same time hydrolyses to glucose and fructose (Mack *et al.*, 2017). Galactose is associated to the kiwifruits cell wall and the fruit's softening. Cell wall associated galactose was found to be reduced by 70% after softening when compared to harvest, being released in a different form rather than free galactose or was directly metabolised (Mack *et al.*, 2017).

The content of each soluble sugar increases during postharvest ripening, and tend to stabilize when achieve an eating-ripe stage (Nishiyama *et al.*, 2008). During ripening, starch is converted mainly in sugars, specially glucose and in smaller amounts fructose and sucrose (Jayashiva, 2012). Kiwifruits with a higher content in soluble solids, and fructose being the sweetest sugar, followed by sucrose and glucose, tend to be more sweet, and consequently an elevated consumer acceptability (Nunes-Damaceno *et al.*, 2013).

All free-sugars concentrations were not influenced by storage atmosphere, only time provided highly significant differences with a general increase and the predominance of fructose.

## 5.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

Four free-sugars were quantified, fructose, glucose, sucrose and galactose for 'Hayward' and 'Jintao' kiwifruits (Table 21). All sugars were significantly different between cultivars. 'Jintao' had higher concentrations of sugars than 'Hayward'. Fructose was predominant on both cultivars followed by glucose, sucrose and galactose, and until 8 weeks of storage, all sugars had an increasing trend and then stagnated after 12 weeks and increased to the last sampling date, Barboni *et al.* (2010) had similar results and reported a stagnation of sugars concentrations after 15 weeks of storage.

Nishiyama *et al.* (2008) also reported higher concentrations of free-sugars on *A. chinensis* cultivars than 'Hayward' with similar results. The differences in concentrations of sugars as well as acids influence the final flavour of the fruit. Nunes-Damaceno *et al.* (2013) reported that the overall acceptance of consumers towards fruits are their overall higher content in 'Brix, greater soluble solids, as well as higher concentrations of fructose and glucose. On the present study, 'Jintao' overcomes 'Hayward' on the different sugars analysed.

Highly significant differences were obtained between cultivars, where 'Jintao' had superior concentrations on all free-sugars analysed. Sugars had a general increase trend with fructose being predominant on both cultivars.

**Table 21** - Mean values of fructose (mg/g FW), glucose (mg/g FW), sucrose (mg/g FW) and galactose (mg/g FW) of 'Hayward' and 'Jintao' kiwifruits on different sampling dates.

		Fructose	Glucose	Sucrose	Galactose
Cultivar	'Hayward'	30.15b	23.99b	10.37b	4.06b
	'Jintao'	41.53a	36.65a	18.39a	5.89a
Sampling dates	11/11/15	21.81c	21.60c	16.48a	3.08b
	06/01/16	39.00b	32.94ab	15.94ab	5.47a
	03/02/16	37.98b	31.00b	11.62c	5.55a
	16/03/16	44.59a	35.73a	13.46bc	5.81a
Sampling dates ('Hayward')	11/11/15	16.11	13.32d	8.93d	2.25
( may waru )	06/01/16	33.38	25.65c	12.28cd	4.11
	03/02/16	34.82	29.06bc	9.69d	5.13
	16/03/16	36.31	27.93bc	10.57d	4.75
Sampling dates ('Jintao')	11/11/15	27.51	29.87bc	24.02a	3.90
( Jiiitau )	06/01/16	44.61	40.24a	19.61ab	6.82
	03/02/16	41.13	32.94b	13.55cd	5.97
	16/03/16	52.88	43.54a	16.35bc	6.87
ANOVA (p-value	es)				
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dates		<0.0001*	<0.0001*	0.0003*	<0.0001*
Sampling dates*	Cultivar	0.0992	0.0012*	0.0001*	0.0510

## 5.3. Kiwifruits behaviour after one week shelf-life

No significant differences were obtained on shelf-life on the different concentrations of sugars on both storage atmospheres and cultivars (Table 22).

On the previous situations of sugars concentrations in CA and NA, as well as 'Hayward' and 'Jintao', sugars concentrations kept increasing. Barboni *et al.* (2010) reported a stagnation on sugars concentrations after 15 weeks of storage, and on the present study, kiwifruits had 18 weeks of storage on day 0, and with another week of shelf-life at day 7, where a stagnation on sugars concentrations was also obtained, except on sucrose that increased on shelf-life for 'Jintao'.

**Table 22 -** Mean values of fructose (mg/g FW), glucose (mg/g FW), sucrose (mg/g FW) and galactose (mg/g FW) of 'Hayward' kiwifruits from CA and NA, and 'Jintao' kiwifruits from NA on shelf-life, 16/03/2016 (Day 0) and 23/03/2016 (Day 7).

		Fructose	Glucose	Sucrose	Galactose
'Hayward'	CA	43.45	34.11	14.01	6.12
(Atmosphere)	NA	41.45	32.82	13.40	5.81
Shelf-life	Day 0	41.97	34.43	13.71	6.26
	Day 7	42.93	32.50	13.70	5.67
Atmosphere	CA*Day 0	42.38	34.26	13.93	6.59
(Shelf-life)	CA*Day 7	44.53	33.96	14.09	5.65
	NA*Day 0	41.57	34.60	13.50	5.93
	NA*Day 7	41.33	31.04	13.31	5.69
'Jintao'	Day 0	52.88	43.54	16.35b	6.87
(Shelf-life)	Day 7	56.11	45.19	17.56a	7.92
ANOVA (p-valu	ues)				
'Hayward'					
Atmosphere		0.2408	0.4724	0.5236	0.3750
Shelf-life		0.5700	0.2871	0.9888	0.0966
Atmosphere*Sh	nelf-life	0.4785	0.3673	0.8524	0.3197
'Jintao'					
Shelf-life		0.2175	0.2117	0.0497*	0.1000

The one-week shelf-life experiment did not provide significant differences on the freesugars concentrations as well as no differences were spotted between storage atmosphere and shelf-life for 'Hayward' and the comparison with 'Jintao'.

## 6. Organic acids

# 6.1. 'Hayward' kiwifruits from CA and NA storage

Table 23 represents the acids analysed where quinic and citric acids were predominant, followed by malic and ascorbic acids. Quinic acid was the only acid with a significant difference regarding its storage atmosphere, higher for NA, and was not influenced by storage time. However, all other analysed acids had no significant differences between storage atmosphere, but were significantly different regarding their storage time. Organic acids give fruit tartness and reduce bacterial spoilage and along with sugars, are responsible for fruit taste (Nishiyama *et al.*, 2008).

Quinic and citric acids were predominant on both storage atmospheres, with average values of 7.64mg/g FW and 7.58mg/g FW, respectively (Table 23). Malic acid comes next with an average value of 1.69mg/g FW, and at last ascorbic acid with an average value of 1.17mg/g FW.

Table 23 - Mean values of quinic (mg/g FW), malic (mg/g FW), citric (mg/g FW) and ascorbic (mg/g FW) acids

of 'Hayward' kiwifruits after CA and NA, on different sampling dates.

,		Quinic acid	Malic acid	Citric acid	Ascorbic acid
Atmosphere	CA	7.36b	1.70	7.45	1.15
	NA	7.91a	1.68	7.70	1.19
Sampling dates	11/11/15	7.20	1.40c	8.05a	1.17ab
	06/01/16	8.03	2.07a	7.94a	1.18ab
	03/02/16	7.56	1.74b	7.30a	1.06b
	16/03/16	7.77	1.55bc	7.02a	1.28a
Sampling dates	11/11/15	6.84	1.41	7.74	1.10
(CA)	06/01/16	7.57	1.93	7.68	1.16
	03/02/16	7.62	1.93	7.49	1.10
	16/03/16	7.42	1.53	6.89	1.25
Sampling dates	11/11/15	7.56	1.39	8.35	1.25
(NA)	06/01/16	8.50	2.21	8.21	1.20
	03/02/16	7.49	1.55	7.10	1.02
	16/03/16	8.12	1.58	7.15	1.31
ANOVA (p-values	s)				
Atmosphere		0.0372*	0.8370	0.3890	0.2386
Sampling dates		0.1470	<0.0001*	0.0454*	0.0006*
Sampling dates*A	tmosphere	0.4814	0.0336*	0.6080	0.1352

Levels not connected by the same letter are significantly different, at the same column.

Similar results were obtained by Nishiyama *et al.* (2008), Nunes-Damaceno *et al.* (2013), Yi *et al.* (2016) and Ma *et al.* (2017). The most important organic acids on kiwifruit are quinic, citric and malic (Nishiyama *et al.*, 2008). There was a slight increase of the acids content from the first sampling date to the second, with malic acid having a clear increase on this period and then stagnated on the third and fourth sampling date.

Quinic acid content was lower in CA when compared to NA, which means CA fruits had a lower perception of astringency than NA, and this acid is what gives 'Hayward' its distinctive acid flavour and is associated to health benefits (Nishiyama *et al.*, 2008). Ascorbic

acid remained stable at the first two sampling dates, then decreased on the third and then achieved its highest value on the last sampling date. However, Barboni *et al.* (2010) reported that ascorbic acid did not vary throughout storage period. Quinic acid had a higher perception on the acidity of the fruit than citric and malic acid at equivalent molar concentrations, resulting in a more astringent fruit. Quinic acid is also very important for the characteristic flavour of 'Hayward' kiwifruits (Nishiyama *et al.*, 2008). Although quinic acid does not have its physiological and nutritional importance established, it has attracted great interest, as it serves as a precursor for the biosynthesis of polyphenols, such as chlorogenic acids and flavonoids, in plants, and these dietary polyphenols act as a radical trapping antioxidant, helping to prevent several degenerative diseases such as cardiovascular diseases and cancers (Nishiyama *et al.*, 2008).

Organic acids are accumulated during fruit growth and then are used as respiratory substrates during fruit ripening, and citric acid is one of the main contributors to fruit titratable acidity, which declines gradually during fruit development (Moing *et al.*, 2001). Citric and malic acids play an important role on our health as they are important energy sources for the living cells via the tricarboxylic acid cycle (TCA), and *Actinidia* fruits are peculiar as often have similar content on quinic and citric acids (Nishiyama *et al.*, 2008).

Storage atmosphere resulted in a significant difference for quinic acid concentration, higher for NA, but the others were not influenced by atmosphere. Plants can use quinate as a carbon source for aromatic amino acids, or serve as storage or a transport form of carbon, and under energetically favourable conditions, carbon of shikimate of the shikimate pathway is also stored in the form of quinate and its derivatives as Herrmann (1995) explains, which can be an explanation for the accumulation of quinic acid in NA storage, as well as the metabolization of chlorogenic acid, an ester of caffeic acid and quinic acid (Olthof *et al.*, 2001). Malic and citric acids had significant differences through time, which can be due to the action of the TCA cycle where malate and citrate are accumulated, to form energy to be consumed by the living cells in their metabolic activity such as respiration (Berg *et al.*, 2002). Also, ascorbic acid is dominantly biosynthesised through the L-galactose pathway, where the main substrate is glucose made during photosynthesis (Bulley and Laing, 2016).

# 6.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

Of the analysed acids, quinic, citric and ascorbic acids were significantly different between 'Hayward' and 'Jintao', while malic acid had similar contents. Only malic and ascorbic acids were significantly different between sampling dates (Table 24).

**Table 24** - Mean values of quinic (mg/g FW), malic (mg/g FW), citric (mg/g FW) and ascorbic (mg/g FW) acids

of 'Hayward' and 'Jintao' kiwifruits after NA on different sampling dates.

		Quinic acid	Malic acid	Citric acid	Ascorbic acid
Cultivar	'Hayward'	7.85b	1.56	7.66a	1.17b
	'Jintao'	9.47a	1.58	5.67b	1.51a
Sampling dates	11/11/15	8.92	1.60ab	7.49	1.44a
	06/01/16	8.19	1.94a	6.74	1.17c
	03/02/16	8.69	1.37b	6.08	1.31b
	16/03/16	8.32	1.37b	6.34	1.43a
Sampling dates ('Hayward')	11/11/15	8.39	1.54	8.62	1.30
( Haywaru )	06/01/16	7.66	2.09	8.12	1.04
	03/02/16	7.72	1.45	7.08	1.10
	16/03/16	7.62	1.17	6.80	1.24
Sampling dates ('Jintao')	11/11/15	9.46	1.66	6.35	1.58
(Jintao)	06/01/16	8.71	1.79	5.36	1.30
	03/02/16	9.65	1.29	5.07	1.52
	16/03/16	10.05	1.57	5.89	1.63
ANOVA (p-values	s)				
Cultivar		0.0022*	0.8913	0.0003*	<0.0001*
Sampling dates		0.6603	0.0033*	0.1606	<0.0001*
Sampling dates*C	Cultivar	0.6356	0.1179	0.5146	0.1741

Levels not connected by the same letter are significantly different, at the same column.

On table 24, quinic and citric acids were predominant on both cultivars although having significant differences between them. 'Jintao' had a higher concentration of quinic and ascorbic acids, and a smaller concentration of citric acid, comparing to 'Hayward'. Quinic acid being present on higher concentrations on yellow-fleshed cultivars as 'Hort16A' and 'Jintao' was also obtained by Nishiyama *et al.* (2008) and Sivakumaran *et al.* (2016). Ma *et al.* (2017) obtained similar contents between 'Hayward' and yellow-fleshed cultivars. Malic acid having no significant difference between 'Hayward' and 'Jintao', and citric acid being higher on 'Hayward' than 'Hort16A' and 'Jintao' was also reported by Nishiyama *et al.* (2008),

Sivakumaran *et al.* (2016) and Ma *et al.* (2017). The organic acids concentrations obtained on 'Hayward' and 'Jintao' were similar to the ones obtained by Nishiyama *et al.* (2008), Sivakumaran *et al.* (2016) and Ma *et al.* (2017) that studied 'Hayward', 'Jintao' and other yellow-fleshed cultivars such as 'Hort16A'.

The higher ascorbic acid content on 'Jintao' or other yellow-fleshed cultivars such as 'Hort16A' than 'Hayward' was also obtained by Nishiyama *et al.* (2004) and Sivakumaran *et al.* (2016).

Organic acids along with sugars are known to influence fruit's taste, giving them tartness and reducing bacterial spoilage (Nishiyama *et al.*, 2008). Quinic acid is characterized by providing a higher perception of acidity to fruits as Nishiyama *et al.* (2008) reported, and on the present work, 'Jintao' is significantly different on this acid compared to 'Hayward', with a higher value which results in a kiwifruit with a higher perception of acidity. However, perception of flavour is not only due to the action of organic acids, but also with the combination of sugars, which are present on higher concentrations in 'Jintao' rather than 'Hayward' (Table 24).

Malic acid concentration did not vary between cultivar, quinic and ascorbic acids were present on higher concentrations in 'Jintao', while citric acid was higher for 'Hayward'. A difference in citric acid concentrations between cultivars stored in NA and as it is used as substrate in the TCA cycle for respiration can be due to an earlier harvest date for 'Jintao', and therefore undergoing through a longer metabolic activity than the other cultivar, however a general lower concentration in this acid is normal for 'Jintao' when compared to 'Hayward' (Nishiyama *et al.*, 2008). With this, also happens the accumulation of quinic acid as a product of the shikimate pathway due to a higher metabolic activity (Herrmann, 1995). As 'Jintao' has a higher availability of glucose than 'Hayward', this can be the reason for a higher concentration of ascorbic acid as glucose is the main substrate on the L-galactose pathway to biosynthesise ascorbic acid (Bulley and Laing, 2016). Only malic and ascorbic acids had significant differences through time.

#### 6.3. Kiwifruits behaviour after one week shelf-life

No significant differences were found on organic acids between CA and NA storage atmospheres on 'Hayward' and its effect on a 7-day shelf-life experiment. There was also no significant difference in organic acids on 7 days' self-life for 'Jintao' (Table 25).

There was no significant difference between storage atmosphere where the kiwifruits had been stored on this 7-day shelf-life experiment for 'Hayward', however, only ascorbic acid had a significant difference between the first day of shelf-life and the last (Table 25). The content of ascorbic acid tended to decrease along with fruit ripening as Tavarini *et al.* (2008) reported, together with weight loss which is particular important on this matter, a higher fresh weight loss or water loss, speeds up the degradation of ascorbic acid, where space that was once occupied by water, is now occupied by air, rich in O<sub>2</sub> that promotes oxidation.

**Table 25 -** Mean values of quinic (mg/g FW), malic (mg/g FW), citric (mg/g FW) and ascorbic (mg/g FW) acids of 'Hayward' kiwifruits from CA and NA, and 'Jintao' kiwifruits from NA on shelf-life, 16/03/2016 (Day 0) and 16/03/2016 + 7 days (Day 7).

· ·		Quinic acid	Malic acid	Citric acid	Ascorbic acid
'Hayward'	CA	7.52	1.48	7.07	1.21
(Atmosphere)	NA	7.84	1.54	6.79	1.20
Shelf-life	Day 0	7.77	1.55	7.02	1.28a
	Day 7	7.59	1.47	6.85	1.14b
Atmosphere	CA*Day 0	7.42	1.53	6.89	1.25
(Shelf-life)	CA*Day 7	7.63	1.44	7.26	1.18
	NA*Day 0	8.12	1.58	7.15	1.31
	NA*Day 7	7.56	1.50	6.44	1.10
'Jintao'	Day 0	10.05	1.57	5.89	1.63
(Shelf-life)	Day 7	9.99	1.44	6.17	1.49
ANOVA (p-val	ues)				
'Hayward'					
Atmosphere		0.3076	0.6586	0.4063	0.7798
Shelf-life		0.5730	0.4984	0.6059	0.0008*
Atmosphere*Sl	nelf-life	0.2196	0.9526	0.1170	0.0735
'Jintao'					
Shelf-life		0.9630	0.5661	0.7674	0.1797

Levels not connected by the same letter are significantly different, at the same column.

The shelf-life experiment had no influence on the organic acids concentrations, only a decrease with a significant difference was obtained in ascorbic acid, for 'Hayward' storage methods, which can be due to the environmental changes where the kiwifruits were put during the week. Ascorbic acid is prone to oxidation when left to open air, with the presence of oxygen, as happened on this experiment by leaving the kiwifruits on air conditions during one week

(Science Chef, 2016). Storage atmospheres had no influence in the outcome of the organic acids concentrations was well as no significant differences were reported on the shelf-life of 'Jintao'.

# 7. Antioxidant activity and vitamin C

## 7.1. 'Hayward' kiwifruits from CA and NA storage

In table 26 are exposed various antioxidant activity (AA) determination methods between 'Hayward' kiwifruits from CA and NA storage. There was a significant difference in AA between CA and NA for lipid peroxidation with a higher value for NA, and no significant differences for DPPH, reducing power and CUPRAC methods. For vitamin C content, the results shown a significant difference and higher value for NA compared to CA. Through sampling dates, there was a significant difference in AA on lipid peroxidation and CUPRAC methods.

A significant difference in lipid peroxidation from CA (34.75% inhibition) and NA (39.96% inhibition) was obtained regardless of sampling dates. An increase from the first two sampling dates to the third, and then a decrease on the last sampling date (Table 26) was observed for lipid peroxidation assay, the only with a significant difference between atmosphere. There was a significant difference through time on CUPRAC with clear decrease of the reduction potential of the antioxidants, from the first two sampling dates (3.18μM TE/g FW) to the last two (1.06μM TE/g FW), higher values were obtained by Gorinstein *et al.* (2009) and Park *et al.* (2011) with different sampling methods between authors and the present work.

This behaviour through time was also accompanied by the L-ascorbic acid, however with a significant difference through time and atmosphere with CA kiwifruits showing a higher value (1.43µM TE/g FW) on the last two sampling dates than NA (0.68µM TE/g FW). There was a significant difference on vitamin C content between CA (1.15mg/g FW) and NA (1.32mg/g FW) as well as through sampling dates with an increasing trend on CA, starting with a lower value (0.92mg/g FW) and increasing progressively to a maximum of 1.54mg/g FW. However, NA kiwifruits suffered a different variation but with an increasing trend also, starting with (0.95mg/g FW), achieving a maximum of 2.15mg/g FW on the second sampling date, then obtained the same value as the first sampling date (0.95mg/g FW) and finalized with 1.23mg/g FW (Table 26). A decrease in ascorbic acid was also observed by Yi *et al.* (2016) with an initial value of 1.03mg/g FW and with ripening decreased to 0.61mg/g FW, although on the present study higher concentrations were achieved.

Table 26 - Mean values of antioxidant activity determined by different methods, DPPH (%AA), FRAP (%AA), lipid peroxidation (%inhibition), CUPRAC ( $\mu$ M TE/g FW) and the contents of L-ascorbic acid ( $\mu$ M TE/g FW) and vitamin C (mg/g FW) of 'Hayward' kiwifruits after CA and NA on different sampling dates.

	ics.	DPPH	FRAP	Lipid peroxidation	CUPRAC	L-ascorbic acid	Vitamin C
Atmosphere	CA	43.55	92.39	34.75b	1.55	2.19	1.15b
	NA	43.34	92.52	39.96a	1.47	2.04	1.32a
Sampling dates	11/11/15	46.82	92.51	38.46ab	2.17a	3.31a	0.94b
	06/01/16	44.75	92.87	34.31b	2.04a	3.05a	1.54a
	03/02/16	41.13	92.36	41.67a	1.04b	1.29b	1.09b
	16/03/16	41.08	92.09	34.99b	0.80b	0.82b	1.39a
Sampling dates (CA)	11/11/15	49.06	92.48	35.45	1.99ab	3.00a	0.92c
	06/01/16	48.54	92.76	31.09	1.95ab	2.92a	0.93c
	03/02/16	40.21	91.74	39.43	1.36bc	1.83b	1.22bc
	16/03/16	36.39	92.57	33.02	0.92c	1.03bc	1.54b
Sampling dates (NA)	11/11/15	44.59	92.54	41.46	2.36a	3.63a	0.95c
	06/01/16	40.95	92.98	37.52	2.12ab	3.19a	2.15a
	03/02/16	42.05	92.97	43.91	0.73c	0.76c	0.95c
	16/03/16	45.77	91.61	36.97	0.68c	0.60c	1.23bc
ANOVA (p-v	alues)						
Atmosphere		0.9268	0.6806	0.0005*	0.5049	0.5071	0.0131*
Sampling da	tes	0.2217	0.4126	0.0017*	<0.0001*	<0.0001*	<0.0001*
Sampling da	tes*Atm.	0.0594	0.1551	0.9057	0.0319*	0.0443*	<0.0001*

Lipid peroxidation is one of the most used *in vivo* antioxidant assays, and is an autocatalytic process which is a common consequence of cell death, and malondialdehyde is one of the end products of the lipid peroxidation process, formed during oxidative degeneration. The lipid is a major component of cell membranes and its peroxidation is directly related to the peroxidative damage of cells *in vivo* (Alam *et al.*, 2013). Many factors affect antioxidant activity in fruits like genotypic variation and maturity, preharvest conditions to postharvest conditions. Also, a decrease in antioxidant activity is associated to a decrease in ascorbic acid content (Martin-Belloso and Fortuny, 2010).

A decrease in vitamin C through time was reported by Tavarini *et al.* (2008), with fresh fruits having higher contents than those cool-stored, and the loss of this vitamin was variable

between fruits and vegetables. Vitamin C consists of two forms, ascorbic acid and dehydroascorbic acid which are in a reversible equilibrium. It is easily oxidized and its degradation is enhanced when exposed to heat, light and heavy metal cations (Pisoschi and Negulescu, 2012). In prolonged cold-storage, vitamin C loss is registered due to auto-oxidation, degradation by condensation or enzyme oxidation, however many factors influence vitamin C concentrations, where time and temperature of storage also interfere (Oliveira *et al.*, 2011).

Different storage temperatures provoke different behaviours on antioxidant activity in fruits as Li *et al.* (2012) reported in their study that chilling storage of 1°C had higher loss of antioxidant activity than fruits and vegetables stored at 4°C.

Storage atmosphere only provided a significant difference on the antioxidant activity assay of lipid peroxidation and vitamin C content, both with a higher value for NA. Trough time, significant differences were obtained for lipid peroxidation, and a decrease trend with highly significant differences for CUPRAC as well as for L-ascorbic acid and vitamin C.

# 7.2. 'Hayward' vs. 'Jintao' kiwifruits from NA storage

There were highly significant differences on AA comparing 'Hayward' and 'Jintao' on the various methods done, with a higher AA for 'Jintao'. There were also significant differences through time, with oscillations between sampling dates but with a decreasing trend on both cultivars (Table 27).

'Hayward' had a lower general AA on all assays when comparing to 'Jintao'. On DPPH and FRAP, 'Hayward' had values of 42.32% AA and 92.13% AA, respectively, while 'Jintao' had 82.49% AA and 95.53% AA respectively, showing a significantly different and higher AA. At lipid peroxidation, 'Jintao' was also superior to 'Hayward', 48.63% to 41.11%, respectively. On CUPRAC assay, 'Jintao' also had a clear advantage over 'Hayward', 3.24 to 1.44μM TE/g FW and L-ascorbic with 5.13 to  $2.00\mu M$  TE/g FW. On vitamin C, 'Jintao' (1.60mg/g FW) also had a higher content than 'Hayward' (1.17mg/g FW).

There were significant differences through sampling dates on all assays (Table 27). 'Jintao' showed a decreasing trend through sampling dates, starting with a higher AA, then decreasing to the second sampling date or maintaining its AA until the last one. 'Hayward' showed a similar behaviour on these assays, starting with a higher value on the first sampling date, then decreasing to the second, and then stabilizing on the last two or showing an increase. CUPRAC assay and L-ascorbic acid content decreased until the third sampling date and then

stabilized to the last one, without significant differences between cultivars' behaviour through time.

**Table 27 -** Mean values of antioxidant activity determined by different methods, DPPH (%AA), FRAP (%AA), lipid peroxidation (%inhibition), CUPRAC (μM TE/g FW) and the contents of L-ascorbic acid (μM TE/g FW) and vitamin C (mg/g FW) of 'Hayward' and 'Jintao' kiwifruits on different sampling dates.

		DPPH	FRAP	Lipid peroxidation	CUPRAC	L-ascorbic acid	Vitamin C
Cultivar	'Hayward'	42.32b	92.13b	41.11b	1.44b	2.00b	1.17b
	'Jintao'	82.49a	95.53a	48.63a	3.24a	5.13a	1.60a
Sampling dates	s 11/11/15	68.64a	94.08ab	47.17a	3.70a	6.00a	1.22c
	06/01/16	51.09c	93.56ab	40.83b	2.74b	4.29b	1.82a
	03/02/16	63.29b	94.39a	49.57a	1.56c	2.19c	1.17c
	16/03/16	66.58ab	93.29b	41.93b	1.35c	1.77c	1.33b
Sampling dates	s 11/11/15	46.95d	92.42c	42.53bc	2.67	4.17	0.98d
('Hayward')	06/01/16	36.18e	92.57c	33.68d	1.78	2.61	1.68b
	03/02/16	42.60d	92.39c	51.37a	0.68	0.68	0.93d
	16/03/16	43.53d	91.15c	36.88cd	0.63	0.54	1.10d
Sampling dates	s 11/11/15	90.32a	95.74ab	51.80a	4.73	7.83	1.45c
('Jintao')	06/01/16	66.01c	94.55b	47.97ab	3.70	5.97	1.96a
	03/02/16	83.98b	96.38a	47.77ab	2.45	3.71	1.42c
	16/03/16	89.63ab	95.44ab	46.99ab	2.06	3.00	1.55bc
ANOVA (p-va	alues)						
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dat	es	<0.0001*	0.0192*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling dates*Cultiva	ır	<0.0001*	0.0151*	<0.0001*	0.4922	0.4447	0.0404*

Levels not connected by the same letter are significantly different, at the same column.

A higher antioxidant activity for *A. chinensis* kiwifruits rather than *A. deliciosa* was also reported by Du *et al.* (2008) and Skinner *et al.* (2011).

On vitamin C content, there was a significant difference between 'Jintao' (1.60mg/g FW) and 'Hayward' (1.17mg/g FW). Vitamin C behaviour on both cultivars was similar, with an increase from the first sampling date to the second, and then a decrease to third and a stabilization until the last sampling date (Table 27). Metabolic activity, oxidations and genotypic differences can explain variations on antioxidant activity and vitamin C contents on both cultivars through time of storage. Due to a higher concentration and availability of ascorbic acid in 'Jintao', which has a high antioxidant potential, it was registered a higher antioxidant

activity on the several methods used (Martin-Belloso and Fortuny, 2010). Other components such as carotenoids and phenolic compounds also influence antioxidant activity, and 'Jintao' kiwifruits have high carotenoid concentrations (Latocha *et al.*, 2015).

'Jintao' overcomes 'Hayward' with highly significant differences on all antioxidant activity methods as well as on L-ascorbic acid and vitamin C. Through sampling dates, oscillations on the results of the several methods are reported and a decrease is observed on the L-ascorbic content on both cultivars with a sharper decrease for 'Hayward' than 'Jintao'.

## 7.3. Kiwifruits behaviour after one week shelf-life

Significant differences were obtained on the antioxidant potential by CUPRAC and vitamin C content on 'Hayward' kiwifruits from CA and NA. However, a 7-day shelf-life period only showed a significant difference on vitamin C content on both cultivars and on the AA by DPPH on 'Jintao' (Table 28).

'Hayward' kiwifruits from CA  $(0.83\mu M\ TE/g\ FW)$  showed a higher antioxidant potential than those from NA  $(0.67\mu M\ TE/g\ FW)$ . The same behaviour was registered for vitamin C, also higher for CA  $(1.31mg/g\ FW)$  rather than NA  $(1.14mg/g\ FW)$ . However, 7-day shelf life period significantly affected and decreased the vitamin C content of 'Hayward' (on day 0 with  $1.39mg/g\ FW$  and on day 7 with  $1.06mg/g\ FW$ ) and 'Jintao' kiwifruits (on day 0 with  $1.55mg/g\ FW$  and on day 7 with  $1.40mg/g\ FW$ ). Even after 7 days, 'Jintao' still had a higher content of vitamin C than 'Hayward' (Table 28).

Shelf-life only interfered on vitamin C content with a general decrease for 'Hayward' on both atmospheres and 'Jintao'. Comparing CA and NA, CA had a sharper decrease on vitamin C than NA.

Vitamin C is easily oxidised and when fruits are exposed to a more oxygen rich environment, heat or light, and their metabolic activity is enhanced and therefore the oxidation of vitamin C happens. On this study, 7 days were enough to register a significant loss of vitamin C (Table 28), although L-ascorbic acid did not suffer from this period, which has great antioxidant properties, and works on a reversible equilibrium with dehydroascorbic acid, and both together end up as vitamin C (Pisoschi and Negulescu, 2012).

In antioxidant activity was registered a significant difference on shelf-life for 'Jintao' on DPPH method as one of the most used *in vitro* antioxidant activity assays. A loss of antioxidant activity was obtained and a lower capacity of free radical scavenging of the 'Jintao' sample was registered.

Table 28 - Mean values of antioxidant activity determined by different methods, DPPH (%AA), FRAP (%AA), lipid peroxidation (%inhibition), CUPRAC ( $\mu$ M TE/g FW), L-ascorbic acid ( $\mu$ M TE/g FW) and vitamin C (mg/g FW) of 'Hayward' kiwifruits from CA and NA, and 'Jintao' kiwifruits from NA on shelf-life, 16/03/2016 (Day 0) and 16/03/2016+7 days (Day 7).

	,	DPPH	FRAP	Lipid	CUPRAC	L-ascorbic	Vitamin C
				peroxidation		acid	
'Hayward' (Atmosphere)	CA	39.77	91.89	34.01	0.83a	0.87	1.31a
(Atmosphere)	NA	45.76	91.74	37.06	0.67b	0.59	1.14b
Shelf-life	Day 0	41.08	92.09	34.99	0.80	0.82	1.39a
	Day 7	44.46	91.54	36.08	0.70	0.64	1.06b
Atmospher e (Shelf-life)	CA*Day 0	36.39	92.57	33.02	0.92	1.03	1.54a
e (Shen-me)	CA*Day 7	43.16	91.21	35.01	0.74	0.71	1.08b
	NA*Day 0	45.77	91.61	36.97	0.68	0.60	1.23b
	NA*Day 7	45.75	91.87	37.15	0.66	0.58	1.05b
'Jintao' (Shelf-life)	Day 0	89.63a	95.44	46.99	2.06	3.00	1.55a
(Shen-me)	Day 7	80.32b	95.39	48.95	1.44	1.87	1.40b
ANOVA (p-va	alues)						
'Hayward'							
Atmosphere		0.0519	0.7144	0.0919	0.0483*	0.0516	0.0136*
Shelf-life		0.2574	0.1912	0.5349	0.2081	0.2104	<0.0001*
Atmosphere*5	Shelf-life	0.2545	0.0602	0.6074	0.3007	0.2858	0.0382*
'Jintao'							
Shelf-life		0.0234*	0.8286	0.2664	0.0695	0.0679	<0.0001*

#### 8. Carotenoids

## 8.1. 'Hayward' kiwifruits from CA and NA storage

From all the carotenoids analysed, lutein was the most abundant (Table 29), Montefiori  $et\ al.\ (2009)$  and D'Evoli  $et\ al.\ (2015)$  also reported that lutein was the most abundant carotenoid on 'Hayward' kiwifruits. All analysed carotenoids had a significant difference through storage time, except lutein, with a general decrease from the first sampling date to the second, and then stabilized. Montefiori  $et\ al.\ (2009)$  also reported this behaviour. No significant difference was found between CA and NA on carotenoids concentrations. Carotenoids are divided into carotenes that are characterized by the lack of oxygen, and xanthophylls that contain oxygen. The carotenes are  $\alpha$ -carotene,  $\beta$ -carotene and lycopene while xanthophylls are lutein, zeaxanthin, violaxanthin, capsanthin and neoxanthin (Mditshwa  $et\ al.\ 2017$ ).

Table 29 - Mean values of α-carotene ( $\mu g/g$  FW), β-carotene ( $\mu g/g$  FW), β-cryptoxanthin ( $\mu g/g$  FW), lycopene ( $\mu g/g$  FW), zeaxanthin ( $\mu g/g$  FW) and lutein ( $\mu g/g$  FW) of 'Hayward' kiwifruits regarding their storage atmosphere on different sampling dates.

	ruge umios		β-carotene	β-cryptoxanthin	Lycopene	Zeaxanthin	Lutein
Atmosphere	CA	2.38	1.92	2.02	1.19	2.00	8.08
	NA	2.47	1.99	2.10	1.26	2.08	7.78
Sampling dates	11/11/15	4.04a	3.34a	3.52a	2.31a	3.50a	7.51ab
	06/01/16	1.56c	1.22c	1.29c	0.70b	1.27c	6.80b
	03/02/16	1.94bc	1.54bc	1.62bc	0.89b	1.61bc	8.88a
	16/03/16	2.15b	1.71b	1.80b	1.00b	1.79b	8.54a
Sampling dates (CA)	11/11/15	3.59a	2.95b	3.11b	2.02b	3.09b	7.96
	06/01/16	1.65b	1.31c	1.37c	0.77c	1.36c	7.28
	03/02/16	2.00b	1.60c	1.68c	0.91c	1.67c	8.86
	16/03/16	2.26b	1.81c	1.91c	1.06c	1.89c	8.22
Sampling dates (NA)	11/11/15	4.50a	3.73a	3.94a	2.60a	3.91a	7.06
	06/01/16	1.47b	1.14c	1.20c	0.64c	1.19c	6.32
	03/02/16	1.88b	1.48c	1.56c	0.87c	1.55c	8.90
	16/03/16	2.03b	1.61c	1.70c	0.94c	1.69c	8.86
ANOVA (p-values)							
Atmosphere		0.5324	0.5240	0.5308	0.3809	0.5231	0.4295
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0009*
Sampling dates*Atmosphere		0.0245*	0.0137*	0.0143*	0.0082*	0.0140*	0.3588

Lutein, as the predominant carotenoid increased slightly through sampling dates, and its average concentration of 7.93 $\mu$ /g FW was almost the double than the obtained by Nishiyama *et al.* (2005) and D'Evoli *et al.* (2015), and  $\beta$ -carotene with an average concentration of 1.95 $\mu$ /g FW was also higher than the obtained by the same authors but similar to McGhie and Ainge (2002). Differences in results may be due to the use of different methodologies as Sivakumaran *et al.* (2016) reported on carotenoids assessment as other methods produced higher values due to interfering compounds. Sivakumaran *et al.* (2016) and Leontowicz *et al.* (2016) did not detected zeaxanthin on their studies for 'Hayward', although lutein was higher than  $\beta$ -carotene.

The increase of lutein concentrations through sampling dates results on the accumulation due to the conversion of chlorophyll-containing chloroplasts in carotenoid-containing chromoplasts during fruit ripening, although 'Hayward' never loses completely its

chlorophyll content, providing it with the green colour (McGhie and Ainge, 2002). Lutein originates from  $\alpha$ -carotene through hydroxylation accumulating at high levels in chloroplasts, while the hydroxylation of  $\beta$ -carotene leads to the production of  $\beta$ -cryptoxanthin and again through hydroxylation leads to the formation of zeaxanthin on which, under dark conditions is converted in violaxanthin (Ruiz-Sola and Rodríguez-Concepción, 2012; Acton, 2012). Lycopene is also used to synthesise lutein as Kim and DellaPenna (2006) observed, which can be explained by the statistical treatment with a decrease in lycopene through time and an increase in lutein.

Carotenoids such as  $\beta$ -carotene, lycopene, lutein and zeaxanthin are powerful antioxidants and free radical scavengers, preventing heart diseases and certain types of cancer. Provitamin A activity is also associated with  $\beta$ -carotene and lycopene (Nishiyama *et al.*, 2005). Although lutein and zeaxanthin do not have provitamin A activity, they are responsible for reducing age-related macular degeneration and cataract formation, and these carotenoids accumulate in the lens and macular region of the retina (Nishiyama *et al.*, 2005).

No significant differences were found between atmospheres on the carotenoids concentrations and only time provided highly significant differences, where all carotenoids had a decrease trend and stabilization, except lutein that had an increase trend. The degradation of carotenoids may occur by non-enzymatic (photo)oxidation or enzymatic oxidation, and these enzymes include peroxidases and lipoxygenases. In photosynthetic tissues, carotenoids can be destroyed by photochemical degradation, and in non-photosynthetic tissues this degradation happens by enzymatic activity. Yet, the mechanisms involved in the turnover and degradation of carotenoids are still little known (Ruiz-Sola and Rodríguez-Concepción, 2012).

## 8.2. 'Hayward' vs 'Jintao' kiwifruits from NA storage

Lutein was present in a higher quantity than all other carotenoids on both cultivars, and with a clear predominance on 'Hayward', while the least expressive was lycopene. There were significant differences between cultivars in all carotenoids, with 'Jintao' possessing higher concentrations than 'Hayward' except in lutein (Table 30).

All carotenoids had a similar behaviour through storage period and cultivar. All started with a higher amount of these components, then had a decreasing trend until 10 weeks of storage and after that time had a slight increase until the end of storage.

**Table 30** - Mean values of  $\alpha$ -carotene ( $\mu g/g$  FW),  $\beta$ -carotene ( $\mu g/g$  FW),  $\beta$ -cryptoxanthin ( $\mu g/g$  FW), lycopene ( $\mu g/g$  FW), zeaxanthin ( $\mu g/g$  FW) and lutein ( $\mu g/g$  FW) of 'Hayward' and 'Jintao' kiwifruits after NA on different sampling dates.

Oll C	different samp	0	•	0 1 11		7	
		α-carotene	β-carotene	<b>β-cryptoxanthin</b>	Lycopene	Zeaxanthin	Lutein
Cultivar	'Hayward'	2.15b	1.74b	1.83b	1.08b	1.81b	7.06a
	'Jintao'	2.89a	2.26a	2.38a	1.44a	2.36a	4.81b
Sampling dates	11/11/15	3.96a	3.26a	3.43a	2.23a	3.41a	5.41b
dates	06/01/16	1.70c	1.32c	1.39c	0.77c	1.37c	4.20c
	03/02/16	2.17b	1.69b	1.77b	1.02b	1.76b	7.09a
	16/03/16	2.24b	1.74b	1.83b	1.02b	1.81b	7.04a
Sampling	11/11/15	4.00a	3.34a	3.52a	2.31a	3.49a	6.91
dates ('Hayward')	06/01/16	1.24e	0.96d	1.01d	0.53d	1.00e	5.05
	03/02/16	1.69de	1.33cd	1.40cd	0.76cd	1.39de	8.21
	16/03/16	1.67de	1.32cd	1.39cd	0.74cd	1.38de	8.06
Sampling	11/11/15	3.93a	3.17a	3.34a	2.16a	3.32a	3.92
dates ('Jintao')	06/01/16	2.16cd	1.67bc	1.76bc	1.01bc	1.74cd	3.36
	03/02/16	2.66bc	2.04b	2.15b	1.28b	2.13bc	5.97
	16/03/16	2.81b	2.15b	2.27b	1.30b	2.25b	6.01
ANOVA (p-values)							
Cultivar		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Sampling date	Sampling dates*Cultivar		0.0004*	0.0004*	<0.0001*	0.0004*	0.0598
Sampling dates		<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*

Levels not connected by the same letter are significantly different, at the same column.

Highly significant differences were obtained between cultivars and sampling dates, with 'Jintao' overcoming 'Hayward' on all carotenoids except lutein, where the opposite was registered. Nishiyama *et al.* (2005) reported on their study that lutein concentration in *A. chinensis* cultivars was much lower than in 'Hayward' and other *A. deliciosa* cultivars, and reported higher concentrations of  $\beta$ -carotene in *A. chinensis* cultivars. With these values, the yellow colour of *A. chinensis* cultivars such as 'Jintao' is not mainly due to the abundance of these carotenoids but to the absence of chlorophyll which is degraded during ripening, and was below the detection limit (Nishiyama *et al.*, 2005). However the green flesh of 'Hayward' is due to the high accumulation of chlorophyll which is sufficient to hide the yellow colour of the carotenoids (McGhie and Ainge, 2002).

## 8.3. Kiwifruits behaviour after one week shelf-life

There were few significant differences on a 7-day shelf-life period on carotenoids, but still, CA for 'Hayward' kiwifruits managed to keep higher concentrations when compared to NA. However, 'Jintao' was superior in all carotenoids, except lutein when compared to 'Hayward' (Table 31). Shelf-life experiment only had significant differences on lutein concentration, with an increase from day 0 to day 7 on cultivars comparison. The remaining carotenoids did not have significant differences on shelf-life, nor the storage atmosphere had significant differences on shelf-life.

**Table 31 -** Mean values of α-carotene ( $\mu$ g/g FW), β-carotene ( $\mu$ g/g FW), β-cryptoxanthin ( $\mu$ g/g FW), lycopene ( $\mu$ g/g FW), zeaxanthin ( $\mu$ g/g FW) and lutein ( $\mu$ g/g FW) of 'Hayward' kiwifruits from CA and NA, and 'Jintao' kiwifruits from NA on shelf-life, 16/03/2016 (Day 0) and 16/03/2016 + 7 days (Day 7).

	<u> </u>			β-cryptoxanthin	•		Lutein
'Hayward'	CA	2.28a	1.81a	1.91a	1.09	1.89a	9.73
(Atmosphere)	NA	1.95b	1.54b	1.62b	0.92	1.60b	9.68
Shelf-life	Day 0	2.15	1.71	1.80	1.00	1.79	8.54b
	Day 7	2.08	1.64	1.72	1.01	1.71	10.87a
Atmosphere	CA*Day 0	2.26	1.81	1.91	1.06	1.89	8.22
(Shelf-life)	CA*Day 7	2.29	1.81	1.91	1.12	1.90	11.24
	NA*Day 0	2.03	1.61	1.70	0.94	1.69	8.86
	NA*Day 7	1.87	1.46	1.54	0.90	1.52	10.50
'Jintao' (Shelf-life)	Day 0	2.81	2.15	2.27	1.30	2.25	6.01b
	Day 7	2.65	2.01	2.11	1.27	2.10	7.30a
ANOVA (p-values)							
'Hayward'							
Atmosphere		0.0414*	0.0362*	0.0369*	0.0698	0.0363*	0.9143
Shelf-life		0.6549	0.5464	0.5581	0.9268	0.5428	<0.0001*
Atmosphere*S	Shelf-life	0.5483	0.5377	0.5497	0.6210	0.5345	0.1336
'Jintao'							
Shelf-life		0.1399	0.1042	0.1118	0.5398	0.1120	0.0452*

Levels not connected by the same letter are significantly different, at the same column.

The storage atmosphere of 'Hayward' kiwifruits registered significant differences on carotenoids concentrations with CA overcoming NA. However, shelf-life did not influence these concentrations, on both atmospheres as well as between cultivars. Lutein was an exception with an increase both in 'Hayward' and 'Jintao', as a product of continuous metabolic activity and accumulation due to the conversion of chlorophyll-containing chloroplasts in carotenoid-

containing chromoplasts during fruit ripening, on which  $\alpha$ -carotene is used as substrate to biosynthesise lutein (Ruiz-Sola and Rodríguez-Concepción, 2012).

## 9. Results synthesis

Of all the physicochemical changes occurred through sampling dates, storage atmosphere, different cultivar and shelf-life period, there are some that need to be highlighted and some tables were created to summarize it. Table 32 presents the main significant differences occurred between storage atmospheres for 'Hayward' kiwifruits.

**Table 32 -** Main differences between storing in CA or NA for 'Hayward' kiwifruits, using the average of the results from the sampling dates.

CA	NA
104.04a	90.56b
42.63a	35.10b
26.38a	25.44b
3.32b	3.37a
22.87a	22.20b
7.36b	7.91a
34.75b	39.96a
1.15b	1.32a
	104.04a 42.63a 26.38a 3.32b 22.87a 7.36b 34.75b

Levels not connected by the same letter are significantly different, at the same line.

On weight and size, CA was more efficient on preventing weight loss through sampling dates when compared to NA. On the overall skin or flesh firmness, CA was more efficient than NA (42.63N to 35.10N respectively) on keeping higher firmness which is crucial for marketability of kiwifruits, and preventing conservation problems from mechanical injuries which could induce ethylene production or the attraction of fungus as *Botrytis cinerea*. Other parameters studied that are not included in table 32 had no significant differences such as °Brix between kiwifruits stored in CA and NA. Variation in colour was also reported in C\* with a higher saturation for CA than NA. Also, pH proved to be higher in NA rather than CA. A higher NaOH volume spent in titration was obtained in CA kiwifruits meaning that more NaOH volume was needed to achieve a pH level of 8.2. The organic acids, particularly quinic acid had a significant difference between CA (7.36mg/g FW) and NA (7.91mg/g FW) resulting in a higher perception of acidity for NA kiwifruits, but concentrations did not vary through sampling dates. Malic, citric and ascorbic acids had some oscillations through sampling dates but had no

significant difference between CA and NA. Storage atmospheres had no influence on the freesugars concentrations, that only increased through time.

In antioxidant activity and vitamin C, NA kiwifruits obtained a superior value in both situations and several factors can influence one fruit's antioxidant activity and content in vitamin C such as pre- or postharvest conditions where time of storage or temperature play an important role (Martin-Belloso and Fortuny, 2010). There was a general trend for the antioxidant activity to decrease through time. Carotenoids demonstrated not to be influenced by storage atmosphere, but to have a general decrease through time, except lutein which increased.

In table 33 is reported the main differences occurred between cultivars on the several assays done on this study. Any absent parameters did not show significant differences.

'Hayward' had an average weight superior than 'Jintao', 90.56g to 85.70g respectively and reveals an overall skin and flesh firmness superior than 'Jintao' resulting in a fruit more resistant to storage and manipulation. When comparing 'Jintao' with 'Hayward' on 'Brix there was a clear superiority for 'Jintao' with an average of 15.04° to 11.03° respectively.

Through sampling dates there was a general loss of lightness, and 'Jintao' tended to be more colourful than 'Hayward' presenting a higher C\* value (26.65 to 25.44, respectively). 'Jintao' also showed higher concentrations on all free-sugars assessed which also increased through time. 'Jintao' had a higher concentration of quinic and ascorbic acids when compared to 'Hayward', but an inferior value of citric and a similar concentration of malic acid.

Comparing 'Jintao' with 'Hayward', the former was superior on all antioxidant activity determination methods as well as on L-ascorbic acid and vitamin C with highly significant differences. 'Jintao' also had higher concentrations on all carotenoids when compared to 'Hayward' except on lutein.

Table 33 - Main differences between 'Hayward' and 'Jintao' kiwifruits, using the average of the results from the

sampling dates.

sampling dates.	'Hayward'	'Jintao'
Weight (g)	90.56a	85.70b
Force on initial penetration (N)	26.87a	18.43b
C*	25.44b	26.65a
°Brix	11.03b	15.04a
pН	3.37b	3.46a
NaOH volume spent in titration (mL)	22.20a	17.27b
Fructose (mg/g FW)	30.15b	41.53a
Glucose (mg/g FW)	23.99b	36.65a
Sucrose (mg/g FW)	10.37b	18.39a
Galactose (mg/g FW)	4.06b	5.89a
Quinic acid (mg/g FW)	7.85b	9.47a
Citric acid (mg/g FW)	7.66a	5.67b
Ascorbic acid (mg/g FW)	1.17b	1.51a
DPPH (% antioxidant activity)	42.32b	82.49a
FRAP (% antioxidant activity)	92.13b	95.53a
Lipid peroxidation (% inhibition)	41.11b	48.63a
CUPRAC (µM TE/g FW)	1.44b	3.24a
L-ascorbic acid (µM TE/g FW)	2.00b	5.13a
Vitamin C (mg/g FW)	1.17b	1.60a
α-carotene (μg/g FW)	2.15b	2.89a
β-carotene (μg/g FW)	1.74b	2.26a
$\beta$ -cryptoxanthin ( $\mu g/g$ FW)	1.83b	2.38a
Lycopene (µg/g FW)	1.08b	1.44a
Zeaxanthin (µg/g FW)	1.81b	2.36a
Lutein (µg/g FW)	7.06a	4.81b

Levels not connected by the same letter are significantly different, at the same line.

In table 34 and table 35 are exposed the several differences occurred on shelf-life on 'Hayward' kiwifruits from CA and NA as well as the comparison between 'Hayward' and 'Jintao'.

**Table 34 -** Main differences between storing in CA or NA for 'Hayward' kiwifruits after shelf-life, using the average of the results from the sampling dates.

	CA		N.	A
	'Day 0'	'Day 7'	'Day 0'	'Day 7'
Force on initial penetration (N)	32.70	29.73	25.62	20.98
Vitamin C (mg/g FW)	1.54a	1.08b	1.23b	1.05b

Levels not connected by the same letter are significantly different, at the same line.

From the 7-day shelf-life period, it was also concluded that atmosphere had no interference and no significant differences on the initial and final skin or flesh firmness on kiwifruits in 'Day 0' and 'Day 7'. A decreasing trend from 'Day 0' to 'Day 7' was verified for vitamin C which is easily oxidised, on both atmospheres. The other parameters analysed showed little or no significant differences at all in the beginning of shelf-life and in the end of it (Table 34).

**Table 35 -** Main differences between 'Hayward' and 'Jintao' kiwifruits after shelf-life, using the average of the results from the sampling dates.

'Day 0'			
Day U	<b>'Day 7'</b>	'Day 0'	'Day 7'
28.88	24.85	19.48	17.38
22.58c	21.08d	28.44b	29.84a
13.71	13.70	16.35b	17.56a
1.28a	1.14b	1.63	1.49
41.08	44.46	89.63a	80.32b
1.39a	1.06b	1.55a	1.40b
8.54b	10.87a	6.01b	7.30a
	22.58c 13.71 1.28a 41.08 1.39a	22.58c 21.08d 13.71 13.70 1.28a 1.14b 41.08 44.46 1.39a 1.06b	22.58c       21.08d       28.44b         13.71       13.70       16.35b         1.28a       1.14b       1.63         41.08       44.46       89.63a         1.39a       1.06b       1.55a

Levels not connected by the same letter are significantly different, at the same line.

Table 35 presents the differences obtained between cultivars on shelf life, and on an overall firmness matter 'Hayward' had superior values than 'Jintao' however, the shelf-life period resulted in no significant differences on the initial and final 'Force on initial penetration'. An opposite behaviour was registered in the chromatic parameter C\* with a decrease in 'Hayward' from 'Day 0' to 'Day 7' and an increase for 'Jintao' and with this, 'Jintao' tended to raise its chroma and to continue superior to 'Hayward' in pulp colour saturation. The shelf-life period had no influence on free-sugars concentrations for 'Hayward' but induced an increase of sucrose in 'Jintao'. In organic acids, shelf-life only had influence on the ascorbic acid concentration which decreased from day 0 to day 7. Antioxidant activity determined by

DPPH suffered a decrease on 'Jintao', from 'Day 0' vitamin C. Lutein concentration increased from 'Day 0	

# **Chapter V - Conclusions**

One of the main results encountered was that storing in CA was overall more advantageous than NA for 'Hayward', which was more capable of preventing a higher weight loss and keeping firmness values. Kiwifruits on CA had their weight vary little from the initial sampling dates to the last, with few significant differences, while NA kiwifruits registered significant differences on weight on the same period, with a general decrease. Significant differences between atmospheres were obtained on firmness with CA kiwifruits registering overall higher values than NA, resulting in more resistant fruits to damage and with a prolonged marketability than NA fruits. Atmosphere did not provide significant differences on °Brix level of kiwifruits from both CA and NA, although significant differences were obtained on pH levels with a lower value for NA. The storage methods showed no significant differences on freesugars concentrations, but NA provided a higher quinic acid concentration which can result in kiwifruits with higher astringency. It is known that higher metabolic activity is associated with more intense physicochemical changes where quinic acid showed higher concentrations in NA kiwifruits, with the accumulation of quinate through the shikimate pathway as well as the metabolization of chlorogenic acid in quinic acid (Herrmann, 1995; Olthof et al., 2001). Antioxidant activity from kiwifruits in NA was higher on lipid peroxidation assay than kiwifruits from CA, as well as a higher content of vitamin C. So, for a better and prolonged marketability, CA storage was more efficient than NA on maintaining the fruit's weight and texture without influencing °Brix, although NA kiwifruits possessed higher content of vitamin C and antioxidant activity. By the end of the experimental period of this work, March 2016, kiwifruits had been on NA or CA storage for approximately 5 months and were in good conditions of marketability.

'Hayward' and 'Jintao' are from different *Actinidia* species and demonstrated different dimensions and average weight. These differences were evident on this work, with significant differences between cultivars with 'Hayward' surpassing 'Jintao' in weight and size, together with significant weight loss from the initial to the final sampling dates. 'Hayward' was overall more resistant to external forces than 'Jintao' with higher firmness values. Along with the genetic differences between cultivars, it was evidenced on 'Brix with 'Jintao' surpassing 'Hayward' with significant differences along with a higher pH value. 'Jintao' had a higher antioxidant activity on all assays used and a higher L-ascorbic acid concentration. This resulted in 'Jintao' being a more nutritionally attractive kiwifruit as well as being different from the

traditional kiwifruit by being yellow and sweeter when fully ripe, although more attention should be put on firmness maintenance especially on this cultivar.

The shelf-life period was also an important factor to evaluate as it determined how well 'Hayward' from CA or NA and 'Jintao' behaved. On this work, it was concluded that storage atmosphere had no influence on the shelf-life behaviour in a matter of weight loss, as this happened evenly for 'Hayward' kiwifruits from both atmospheres. As firmness is one of the most important factors of the shelf-life duration of kiwifruit, CA provided better results on keeping a firmer kiwifruit by the end of shelf-life. However, other nutritional traits can be harmed during shelf-life as vitamin C content on kiwifruits from both cultivars and storage atmospheres. Antioxidant activity assayed by DPPH registered a significant decrease on shelf-life for 'Jintao', while lutein registered an accumulation from day 0 to day 7 on both cultivars. Until the fruit reaches the consumer it is important to make sure that adequate handling of the fruits is done, so it does not suffer much from the environmental changes from the storage chambers to the shelfs and therefore, to the consumers.

On future studies, preharvest conditions or different agricultural practices could be an interesting object of comparison about their effects on postharvest behaviour of kiwifruits, as several studies indicate variances on the chemical compounds due to different cultural practices and production regions. On this subject, climate and soil conditions are pointed out by some authors as having an impact on carotenoids and phenolic compounds content and therefore influencing other characteristics as antioxidant activity, which is proven to be very important for our health as a free radical scavenger. 'Jintao' kiwifruits analysed on this study started with relatively high 'Brix for 3 weeks of storage, when compared to 'Hayward' kiwifruits however, several papers indicate difficulties in predicting an optimal harvest index for yellow-fleshed cultivars, where the yellow flesh is the main attribute of commercialization of these fruits, and therefore the harvest date must be connected to their flesh colour. Future research must emphasize the optimization of the harvest indexes and the correlations between different factors and variables. Also, it will be important to obtain scientific information to support the harvest time and the evolution of flesh colour in different cultivars. It will be of great importance the study of the behaviour of *Actinidia chinensis* under CA storage.

# **Chapter VI - References**

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