Universidade do Algarve

Innovative edible films to improve storage ability of fresh-cut apple cv. Bravo de Esmolfe

Adriano Emanuel Costa Martins

Dissertação para obtenção do Grau de Mestre em Hortofruticultura

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Trabalho realizado sob orientação de:
Prof.ª Maria Dulce Carlos Antunes

2015

Declaração de autoria de trabalho

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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Adriano Emanuel Costa Martins

Faro, 2015

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Sincerely,
Adriano E. C. Martins

Resumo

A maçã da cultivar Bravo de Esmolfe é produzida numa região delimitada no centro de Portugal, sendo um produto com denominação de origem protegida (DOP), bastante apreciado pelo consumidor. Formas alternativas de comercialização que valorizem o produto são geralmente bem acolhidas pelos produtores. A transformação em produtos minimamente processados é uma alternativa de escoamento dos produtos hortícolas que tem crescido nos últimos anos.

A procura por alternativas saudáveis e de fácil consumo tem aumentado em parte devido à consciencialização do consumidor quanto aos benefícios de uma alimentação saudável no seu dia-a-dia. Os produtos minimamente processados não são nenhuma novidade nos mercados, havendo uma gama abrangente, desde saladas, preparações para sopas, frutas e legumes pré-preparados, entre outros prontos a consumir ou a cozinhar.

Estes produtos por norma para manterem as suas qualidades recorrem a substâncias químicas ou processamento que altera as suas caraterísticas organoléticas. Por um lado, o consumidor exige que os produtos que consome tenham menor ou nenhuma quantidade de conservantes químicos, por outro lado exige uma frescura como tivessem sido preparados no momento.

Neste paradigma encontram-se os produtos minimamente processados da maçã que são bastante procurados pelo consumidor como alimento saudável e alternativo. A maça é um fruto que sofre acentuado escurecimento da sua polpa aquando do seu processamento, necessitando assim de alternativas saudáveis de conservação, baseadas em produtos geralmente reconhecidos como seguros (em inglês, GRAS – *Generally Recognised as Safe*).

Neste âmbito, as películas edíveis surgem como alternativa a tratamentos químicos e físicos que o consumidor tem procurado evitar. As películas edíveis são hoje em dia tidas como o futuro na preservação da qualidade e segurança alimentar de produtos minimamente processados. As películas edíveis podem conter um ou mais componentes, além de poderem também transportar antioxidantes, óleos essenciais, nutracêuticos, entre outros aditivos.

A constituição das películas varia conforme a especificidade do produto a ser tratado, sendo que na maçã 'Bravo de Esmolfe' estas deverão ter especial atenção na redução do escurecimento enzimático através da adição de agentes anti-escurecimento. Uma especial atenção também deve ser tida em relação à inativação de microrganismos que são outra fonte causadora de perdas e redução da vida útil do produto em armazenamento e nas prateleiras para ser comercializado.

O metabolismo das maçãs minimamente processadas também aumenta a sua atividade, sendo pelo aumento da respiração ou da produção de etileno, uma vez que o processamento inflige danos nos tecidos e rompimento das paredes celulares permitindo que substratos entrem em contacto, havendo assim uma necessidade de reduzir este impacto negativo.

Neste estudo, foram utilizadas maçãs Bravo de Esmolfe no seu estado ótimo de maturação, tendo sido adquiridas num supermercado local. O processamento consistiu numa lavagem inicial em água corrente, descartando-se frutos que apresentassem danos físicos ou desordens pós-colheita visíveis que pudessem causar interferência no estudo. O passo seguinte consistiu no corte das maçãs com um cortador especifico de forma a retirar o centro e pedúnculo não ficando sementes nas fatias daí resultantes, tendo estas sido colocadas imediatamente nas soluções de imersão com os diferentes tratamentos anti-escurecimento. No caso deste estudo, foram utilizados o ácido cítrico e o clorito de sódio.

De seguida, estas foram transferidas para as soluções de imersão com as substâncias hidrocoloídes formadoras de películas às quais tinham sido adicionados constituintes de óleos essenciais.

As combinações de películas utilizadas foram Alginato 2% + Eugenol 0,1%, Alginato 2% + Citral 0,15% + Eugenol 0,1%, Pectina 2% + Eugenol 0,2%, Pectina 2% + Citral 0,15%, películas estas que obtiveram resultados positivos em estudos anteriores.

Por último, uma imersão numa solução de cloreto de cálcio permitiu a formação de uma película homogénea à volta das fatias de maçã. As amostras foram armazenadas durante 8 dias em temperatura e humidade controlada, dentro de cuvetes cobertas com plástico PET de baixa densidade. Os estudos para aferir o desempenho dos tratamentos em armazenamento foram efetuados ao tempo 0 e ao fim de 2, 4, 6 e 8 dias em armazenamento a 4 ºC.

Foram analisadas as caraterísticas qualitativas da maçã (cor, firmeza, sólidos solúveis) bem como o peso, capacidade e atividade oxidante (TEAC, ORAC, fenóis totais e flavonoides), produção de CO_2, produção de etileno e atividade relativa da polifenol oxidase, bem como os conteúdos em ácidos orgânicos não voláteis e açúcares (Fructose, Glucose e Sacarose). Foram também feitos painéis de provadores bem como uma análise microbiológica.

As análises efetuadas demonstraram que é possível a conservação de maçã bravo de Esmolfe minimamente processada durante o período utilizado no estudo (8 dias) com manutenção satisfatória das suas características com as películas deste ensaio.

O estudo apontou diferenças entre os tratamentos anti-escurecimento, sendo melhor o clorito de sódio em relação ao ácido cítrico, principalmente ao nível da cor, firmeza e perda de peso.

Em relação à actividade antioxidante, em alguns casos de combinações com certas películas edíveis foram notadas algumas diferenças significantes, sendo que as combinações película/ agente anti-escurecimento que se destacaram das restantes positivamente foram as que tinham Clorito de Sódio na sua constituição.

A atividade da PPO foi reduzida em todos os tratamentos comparando com o controlo, mostrando assim uma capacidade inibidora da PPO por parte dos tratamentos reduzindo a incidência do escurecimento enzimático na maçã minimamente processada.

O painel de provadores mostrou que os tratamentos tornaram as fatias de maçã tratadas mais apetecíveis durante mais tempo. Alguns tratamentos com clorito de sódio receberam pontuações elevadas ao longo do ensaio, destacando-se de outros tratamentos com ácido cítrico como agente anti-escurecimento. Os tratamentos com películas edíveis tiveram melhor avaliação que os controlos e mantiveram-se ao longo do tempo acima da média.

Nas análises microbiológicas, verificou-se que não ocorreu uma grande atividade de microorganismos nas amostras, mas os valores no controlo foram superiores aos dos tratamentos com películas edíveis.

Em geral, não foram observadas diferenças significativas entre a pectina e alginato e, em alguns parâmetros de qualidade a combinação de citral + eugenol tiveram melhor desempenho, seguida por eugenol e citral.

Neste estudo, foi obtido uma noção geral da eficácia dos tratamentos anti-escurecimento utilizados, abrindo caminho a estudos futuros que promovem uma melhoria da simbiose entre película edível, óleos essenciais e agente anti-escurecimento para uma melhor conservação de maçã bravo de Esmolfe minimamente processada.

De futuro, estudos que aperfeiçoem a utilização de Clorito de sódio como agente anti – escurecimento em películas edíveis e sua combinação com outros tratamentos anti-oxidantes como o Ácido Ascórbico na mesma película é algo que deverá ser tido em consideração.

Abstract

In the past years, social awareness for a healthy lifestyle that benefit consumers, increased demand of minimally processed fruits that could be easily consumed. Minimally processed apples have been a product of choice in this category. The search for healthier preservatives to these products increased interest on edible coatings, as they can perform well in several parameters and also serve as carrier for other components.

'Bravo de Esmolfe' apple is an appreciated variety and minimally processing could be interesting to producers and consumers. However, this apple variety is known to become brown very easily after cut, therefore a capable edible coating completed with an efficient antibrowning agent is needed.

This study tested a group of edible coatings based on Alginate and Pectin with essential oils compounds (Eugenol and Citral) and with the addition of citric acid or sodium chlorite as antibrowning agents.

Experiments showed improved benefits of edible coatings in colour, firmness and weight loss during storage progression. Antioxidant activity assays (TEAC, ORAC, total phenols, Flavonoids) showed also benefits of edible coatings application as well as PPO activity. Concerning antibrowning, the edible coatings with sodium chlorite were the most efficient on preserving quality. All edible coatings were efficient in reducing microbial spoilage and did not affect negatively the sensorial properties of the fresh-cut apple. Generally, there were no differences between pectin and alginate and in some quality parameters the combination of citral+eugenol performed better followed by eugenol alone and citral.

KEYWORDS: sodium chlorite, citric acid, edible coatings, essential oils, antibrowning agents.

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1. Introduction

In the past years, social awareness for a healthy lifestyle that benefit consumers, such as the ingestion of higher amounts of fruits and vegetables, has increased. The change in consumer habits in a way that follow a healthy diet, as a more frenetic lifestyle, increased the demand for easy to eat healthy food.

Fresh-cut fruits and vegetables are products that are partially prepared so that no additional preparation is necessary for their use (Watada & Qi 1999). Among others, fresh-cut horticultural products stand out as convenient novel foods that fit the needs of a modern lifestyle as they combine technical content with an innovative food concept (Corbo & Speranza 2010).

The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruits or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience, and flavour while still maintaining its freshness (Corbo & Speranza 2010).They are prepared for restaurants, dining commons, fast food outlets and retail markets (Watada & Qi 1999).

Fresh-cut products are of increasing importance, since they are presented to the consumer in a state that allows for direct and immediate consumption (Antunes et al. 2012). However, those products are even more perishable than whole fruits, since cutting can induce a series of senescence associated responses to wounding, and are more susceptible to microbial spoilage (Antunes et al. 2012), so losses can be of great significance if postharvest correct management is not provided (Antunes et al. 2012).

Consumers expect fresh-cut product to be without defects, of optimum maturity and in fresh condition. Condition covers general appearance, sensory quality and nutritional quality (Watada & Qi 1999). By recognizing and controlling factors that have a deteriorative effect on quality, good quality fresh-cut product with sufficient shelf-life can be attained (Watada & Qi 1999). High quality can be attained by selecting produce at proper maturity and controlling deterioration with low temperatures and modified atmospheres (Watada & Qi 1999), and more recently edible coatings (Antunes et al. 2012; Bourtoom 2008).

Edible coatings are nowadays recognised as the future in quality preservation and food safety for minimally processed products, among those the apple. Most of the edible coatings are originated in natural products, from vegetable or animal origin, being able to incorporate other substances that increase microbial control and have anti-browning action in several cases.

Apple is one of the most common minimally processed product, being used for increasing its shelf life mainly anti-browning agents and modified atmosphere packaging. The apple cultivar ‘Bravo-de-Esmolfe’, is known to have a fast oxidation response to cut being the usual anti-browning agents not enough to give a shelf-life sufficient for marketing of this cultivar as minimally processed. However, its superior quality when compared with other apple varieties, presents itself as the ideal one to study the application of edible coatings.

The objective of this thesis was to study alternative edible coatings enriched with antimicrobial and antioxidant agents applied to fresh-cut ‘Bravo de Esmolfe’ apples to increase their shelf life.

2. Literature Review

2.1.‘Bravo de Esmolfe’ apples

‘Bravo de Esmolfe’ apple is a cultivar of *Malus Domestica* Borkh, produced in a delimited region, being a variety with a ‘Denominação de Origem Protegida’ (DOP) of region centre of Portugal, and express certain good quality determined by the edafo-climactic conditions present in the region.

When ripe, ‘Bravo de Esmolfe’ apples have specific organoleptic characteristics very appreciated by consumers:

- Colour: whitened epidermis, eventually with reddish spots, normally spotted and/ or rayed, russet in peduncle pit, expanding to cover in some cases 20% of epidermis;
- Aroma and flavour: intense aroma, enjoyable and *sui generis;* white pulp, juicy, sweet, with good taste quality;
- Shape: oblong-conical from medium to small size.

(Agricultura, n.d.)

2.2.Minimally Processed Products

Minimally processed or fresh-cut fruits and vegetables are growing in popularity, those are fruits or vegetables that have been physically modified maintaining their fresh appearance. This process includes selection, washing, sanitizing, pealing, cut, packaging, storage and marketing (Fontes et al. 2008).

Minimally processed fruits are characterized by a shorter shelf life than their whole counterparts, because of higher susceptibility to microbial spoilage, increased respiration rate and ethylene production, which is stimulated by wounding of the tissue. In addition, they may pose a food safety risk because they are consumed raw (Corbo & Speranza 2010).

Fruits and vegetables should not show any sign of damage or disease to be accepted as fit to minimally processing. Any rupture of the tissues inflicts a physiological reaction, bio-chemical reaction and/ or microbial spoilage, resulting in even shorter product shelf life.

The production and consumption of fresh-cut apples and other fresh-cut produce items is projected to continue growing as more consumers demand fresh, convenient and nutritious foods. The produce industry still faces two important challenges to the quality and

safety of fresh-cut product, i.e. control of enzymatic browning and control of microorganisms spoilage (Luo et al. 2011).

Minimally processed apples have a shorter shelf-life than their whole counterparts because of increased susceptibility to microbial spoilage, increased respiration rate, and ethylene production, which is stimulated by wounding of the tissue (Olivas et al. 2007). Researchers have given much attention and explored many approaches to solve the browning problem of fresh-cut apples (Martinez & Whitaker 1995). However, despite intensive research activities, concerns about off-flavors and odors, food safety, economic feasibility, and effectiveness of enzymatic inhibition have resulted in very few browning inhibitors demonstrating potential to be useful in the food industry (Luo et al. 2011; Soliva-Fortuny & Martín-Belloso 2003).

2.3.Processing, Handling and Mechanical damage

The physiology of minimally processed products is essentially that of wounded tissues. Wounding of fruit tissues induces a number of physiological processes that need to be minimised to get fresh-like quality products. In fresh-cut fruits, the greatest hurdle to commercial marketing is the limited shelf life, which is due to excessive tissue softening and cut surface browning (Soliva-Fortuny & Martín-Belloso 2003). It is well known that fruit processing promotes a faster physiological deterioration, biochemical changes and microbial degradation of the products which may result in degradation of its colour, texture and flavour, even when only slight processing operations are used (Corbo & Speranza 2010).

The ripening stage of the processed fruit has been shown to greatly influence the damage inflicted by mechanical operations on the cut fruit tissues. The existing studies on this matter show that the more advanced the ripening stage, the more susceptible the fruit is to wounding during processing (Soliva-Fortuny & Martín-Belloso 2003). The optimal stage of processing to minimize cutting damage also varies greatly depending on the species, cultivar and multiple crop harvest and post-harvest conditions (Soliva-Fortuny & Martín-Belloso 2003).

Each step during the production, packaging and storage, could potentially have an effect on nutrients and quality of the prepared produce (Corbo & Speranza 2010).

It is important to underline that cutting increases the area of injured tissues favouring elevated respiration, promoting further rapid deterioration and microbial proliferation (Corbo & Speranza 2010). Cell rupture from slicing of fruit is responsible for releasing intracellular products, such as enzymes, which can have a negative impact on the quality of cut apples, affecting the colour, flavour and texture (Ahvenainen 1996; Olivas et al. 2007).

The degradation changes that occur during senescence are induced or enhanced by the physical action of processing of fruits and vegetables. This response is noted particularly with cells or tissues adjacent to those that are damaged by the cutting action and when acids and hydrolysing enzymes of the vacuole are released. 'Wound ethylene' produced under these conditions can increase the permeability of membranes and perhaps reduce phospholipid biosynthesis, which can upset the dynamic processes of cellular structures and membrane integrity. This can contribute to the production of volatile long chain aldehydes, which are responsible for the 'wound respiration', and would rapidly utilize the reserve substrate (Watada et al. 1990). Also, extensive enzymatic degradation occurs in damaged membrane systems, causing loss of lipid components and loss of compartmentalization of enzymes and subtracts (Marangoni et al. 1996).

Watada & Qi (1999) states that fresh-cut products are vulnerable to discoloration because of damaged cells and tissues, and lack of protective skin. These exposed tissues have the potential of becoming dehydrated and/ or discoloured. For example, cutting and slicing carrots with a very sharp blade reduces the amount of damaged cells and dehydration when compared with those sliced with a regular culinary knife (Watada & Qi 1999).

The cut surfaces of any processed vegetable support better microbial growth. In fact, each step in the processing affects quality and micro flora of fresh-cut fruit and vegetables. For these reasons, the cutting and shredding must be performed with knives or blades as sharp as possible made from stainless steel (Allende et al. 2006).

The main steps throughout the processing chain of minimal processing fruits and vegetables are washing and disinfection. For this reason, guidelines for packing fresh or minimally processed fruits and vegetables generally specify a washing or sanitizing step to remove dirt, pesticide residues, and microorganisms responsibly for quality loss and decay (Allende et al. 2006). By careful washing, the shelf life of minimally processed produce can be prolonged by several days (Corbo & Speranza 2010). Special attention must be paid to some fruits such as apples or pears during cutting operations. Hence, the core and adjacent

tissues should be completely removed because susceptibility to browning is much higher than in other parts of the fruit (Soliva-Fortuny & Martín-Belloso 2003).

2.4.Fresh-Cut Metabolism

Minimally processed fruits and vegetables are very perishable due to their intrinsic characteristics. Wounds caused during processing stages, for example peeling and/ or cutting, increase the metabolic rates, enzyme reactions, and loss of water (Porte & Maia 2001).

Wounding of fruit tissues induces a number of physiological disorders that need to be minimised to get fresh-like quality products (Soliva-Fortuny & Martín-Belloso 2003).

Porte & Maia (2001), in their review explain that minimally processed fresh fruits or vegetables, suffer damage at a cellular level that enable the contact between enzymes and substrates, therefore originating biochemical changes like browning, smells and texture. Extrinsic factors like low temperature and modified atmosphere are essential to slow down physiological disorders, biochemical metabolism or microbiological evolution in minimally processed food that would affect organoleptic characteristics.

As reviewed by Soliva-Fortuny & Martín-Belloso (2003), the physiology of minimally processed products is essentially that of wounded tissues. Species and variety, O_2 and CO_2 concentrations, water vapour pressure, and the presence of inhibitors stand out as the most significant factors affecting it, paraphrasing Brecht (1995).The earliest physiological responses to wounding include a transient increase in ethylene production and an enhanced rate of respiration, which may be interlinked with the wound healing response of the tissue (Brecht 1995).Other consequences of wounding are chemical or physical in nature, such as oxidative browning reactions and lipid oxidation, or enhanced water loss. Appearance of new RNA and protein species in wounded tissues provides evidence for genomic control of the response (Brecht 1995).

Wounded tissues undergo accelerated deterioration and senescence. Minimizing the negative consequences of wounding in minimally processed fruits and vegetables will result in increased shelf life and greater maintenance of nutritional, appearance, and flavour quality in these products (Brecht 1995).

During the senescence of tissues induced or enhanced by the physical action of processing, the cellular structures and organelles suffer degradative changes that become superior to the biosynthetically changes in this stage. Changes like breaking lipid chains or

disorganisation of cellular membranes, that with the senescence process and following weakening of the cellular structure, make the ripe fruit tissue very susceptible to the process of senescence (Watada et al. 1990; Porte & Maia 2001).

2.5.Browning

Fresh-cut products are vulnerable to discoloration and dehydration because of damaged cells and tissues, and lack of protective skin (Watada & Qi 1999). Enzymatic browning is the primary physiological disorder that causes the decline of sensory quality and shelf life of fresh-cut apples (Luo et al. 2011). Colour was found to be a critical quality parameter on minimally processed apples, which can limit their shelf life (Rocha & Morais 2003). The colour of products, such as apple slices, is an important quality index. Brown apple slices are aesthetically unattractive.

Browning results from both enzymatic (PPO) and non-enzymatic oxidation of phenolic compounds. Browning usually impairs the sensory properties of products because of the associated changes in colour, flavour and softening (due probably to the action of pectic enzymes). Once cell walls and cellular membranes lose their integrity, enzymatic oxidation proceeds much more rapidly (Martinez & Whitaker 1995).

The initial products of oxidation are quinones, which rapidly condense to produce relatively insoluble brown polymers (melanins). Some non-enzymatic causes of browning in foods include the Maillard's reaction, auto-oxidation reactions involving phenolic compounds and the formation of iron-phenol complexes (Martinez & Whitaker 1995).

The most important factors that determine the rate of enzymatic browning of fruit and vegetables are the concentrations of active PPO and phenolic compounds present, the pH, the temperature and the oxygen availability of the tissue. Understanding the details of the enzymatic browning process is necessary in order to control it and to obtain a final product that is acceptable to consumers (Martinez & Whitaker 1995).

At least five causes of browning in processed and/or stored fruit and vegetables are known: enzymatic browning of the phenols, Maillard reaction, ascorbic acid oxidation, caramelization and formation of browned polymers by oxidized lipids. The oxidation of the o-diphenols to o-quinones by polyphenoloxidase (E.C. 1.14.18.1 :usually named PPO) is the most important cause of the change in col- or as the o-quinones quickly polymerize and

produce brown pigments (melanin) (Pizzocaro 1993). Enzymatic browning also causes a loss in the nutritional value through oxidation of ascorbic acid (Pizzocaro 1993).

Enzymatic browning does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the PPO that is present in the cytoplasm. Once tissues are damaged by slicing, cutting or pulping, however, the mixture of PPO and phenolic compounds consequently results in a rapid browning reaction. Enzymatic browning of fresh-cut products can lead to considerable economic losses, especially if browning occurs early in the product's projected shelf-life, but after the costs of processing, packaging and storage have been incurred (He & Luo 2007).

2.6. Respiration

The increase in respiration in wounded plant tissues is thought to be a consequence of elevated ethylene, which stimulates respiration. Starch breakdown is enhanced, and both the tricarboxylic acid cycle and electron transport chain are activated (Brecht 1995).

Reducing the respiration rate is an important factor when working to obtain viable minimally processed fruits and vegetables. Porte & Maia (2001) review, citing other authors, explain that plant respiration consists in oxidizing sugar and organic acids to obtain energy, producing as residuals Carbon dioxide (CO_2) and water.

The main physiological reactions caused by the rupture of tissues are the increase of respiration rate, and, in some cases, ethylene production. The respiration activity of minimally processed products increases 1.2–7.0-fold, or even higher than unprocessed, depending on the products, cutting grade, and temperature (Ahvenainen 1996; Lee et al. 2003). Because the rate of respiration indicates how quickly a product may deteriorate, increased respiration by tissue injury results in a greatly reduced shelf-life compared to whole fruits and vegetables (Lee et al. 2003). Such, reactions should be controlled to a minimum to achieve longer shelf-life of minimally processed produces.

Temperature plays a fundamental role in fruits and vegetables respiration, being that decreasing and stabilizing (without variations) also reduce respiratory rate and delays the senescence process (Porte & Maia 2001). Since fresh-cut products are held only for a short period and are highly perishable when compared with the whole product, a temperature which causes a slight amount of chilling injury is preferred over a temperature which causes

rapid natural deterioration (Watada & Qi 1999), therefore temperature is a very important factor to control in order to reduce respiration rates.

Also, lowering the O_2 level around fresh fruits and vegetables reduces their respiration rate in proportion to the O_2 concentration, but a minimum of about 1-3% O_2, depending on the commodity, is required to avoid a shift from aerobic to anaerobic respiration. Under such conditions, the glycolytic pathway replaces the Krebs cycle as the main source of the energy needed by the plant tissues. Pyruvic acid is no longer oxidized but is decarboxylated to form acetaldehyde, CO_2, and, ultimately, ethanol; this results in development of off-flavours and tissue breakdown (Kader 1986). Porte & Maia (2001) citing other author, refer that using controlled atmospheres could reduce oxygen consumption and CO_2 production. Kader (1986), identifies the reduction of O_2 and/ or elevation of CO_2 as the main reason for the benefit effects of modified/ controlled atmospheres.

2.7. Ethylene synthesis induction

Wounding plant tissues induces elevated ethylene production rates, sometimes within few minutes, but usually within 1h, with peak rates achieved usually within 6 to 12 hours, refers Brecht (1995) citing other authors. Wound ethylene may accelerate deterioration and senescence in vegetative tissues and promote ripening of climacteric fruits, such as apple (Brecht 1995). Wounding climacteric fruits may cause increased ethylene production, which can speed up the onset of the climacteric, resulting in a difference in physiological age between intact and sliced tissue (Watada et al. 1990; Brecht 1995).

Ethylene production is stimulated when plant tissues are injured and it can accumulate in packages of fresh-cut product, which can lead to undesirable effects (Watada & Qi 1999). Ethylene, a 2-carbon volatile, at concentrations as low as 0.1 pL/L, can induce a wide array of physiological responses, including altered geotropic growth, abscission, ripening, senescence, and physiological disorders. These responses can be beneficial or detrimental, depending upon the response and one's need (Watada 1986).

Porte & Maia (2001) citing Watada (1986) refers that ethylene induces synthesis of enzymes involved in fruit ripening with possible loss of firmness, probably due to enabling enzymes that hydrolyse the cellular wall. Kader (1985) reviewed that ethylene induces other physiological processes, resulting in fast membrane deterioration, loss of vitamin C and chlorophyll, abscission, firmness, and undesirable flavours in several fruits and vegetables.

2.8. Fresh-cut Microbiology

Wounding plant tissues makes them more susceptible to attack by plant pathogenic microorganisms and possibly more conducive to survival and growth of food poisoning microorganisms. Controlling fresh quality and growth of spoilage and pathogenic bacteria is a challenging problem for the fresh-cut fruit industry (Rojas-Graü et al. 2007).

During peeling, cutting and shredding, the surface of produce is exposed to air and to possible contamination with bacteria, yeasts and moulds. In the case of minimally processed vegetables, most of which fall into low-acid category (pH 5.8-6.0), the high humidity and the large number of cut surfaces can provide ideal conditions for the growth of microorganisms (Ahvenainen 1996).

The bacterial populations found on fruit and vegetables vary widely (Ahvenainen 1996). The high initial load of microorganisms makes it difficult to establish the cell-number threshold beyond which a product can be considered spoiled. Many studies show that a simple correlation does not exist between spoilage chemical markers, such as pH, lactic acid, acetic acid and carbon dioxide levels and sensory quality, and the total microbial cell load. In fact, different minimally processed fruit and vegetable products seem to have different spoilage patterns, which vary according to the characteristics of the raw materials (Ahvenainen 1996).

Fresh fruits and vegetables are among the more challenging food products to commercially produce and distribute. Fresh produce remains metabolically and developmentally active as it proceeds from the commercially appropriate time to harvest (horticultural maturity), to physiological maturity, to senescence and complete deterioration. During this period of development, several physiological and compositional changes occur (Barth et al. 2010).

Although infection and microbiological spoilage can proceed at any time during this developmental continuum, the period of greatest susceptibility to decay onset is during ripening and senescence (Barth et al. 2010). Losses due to postharvest spoilage or pathological decay are a result either of latent infections in the field that become active following harvest or of cross- contamination during harvest, processing, storage and distribution (Barth et al. 2010).

All this potential for infection is augmented in minimally processed fruits and vegetables, which because of their own processing characteristics, have wounds and large unprotected cut surfaces, making them more susceptible to microbial spoilage.

As processing and packaging technologies have improved during the last decade, microbiological spoilage or microbiological shelf life has become a major reason for sensory quality shelf life failure for most packaged fresh-cut fruits and vegetables. Contamination sources of fresh-cut fruits and vegetables include raw materials and contact with processing equipment (Barth et al. 2010). Microbiological spoilage defects of fresh-cut fruits and vegetables include microbial colony formation or visible microbial growth mainly due to microorganism proliferation, off-aroma and off-flavour formation mainly due to fermentation of sugar, soft-rot/water soak and sliminess due to enzymatic pectolyzation, and discoloration (Barth et al. 2010).

Growth of microorganisms subsequently forming visible colonies is a common cause of spoilage of fresh-cut fruits and vegetables (Barth et al. 2010). Abadias et al. (2008) in their study identified that fresh produce can be a vehicle for the transmission of bacterial, parasitic and viral pathogens capable of causing human illness and a number of reports refer to raw vegetables harbouring potential foodborne pathogens.

Raw fruit, whose internal tissues are normally sterile, is considered a potential target for a wide range of microorganisms, including human pathogens; in fact, the incidence of foodborne outbreaks caused by contaminated fresh fruit has recently increased. For example, numerous cases of Salmonella infection by consuming different fresh products are documented, as well as an Escherichia coli O157:H7 outbreak (Corbo & Speranza 2010).

The range of microorganisms recovered from raw fruit at harvest reflects very often the microflora present in the field and the contact with soil can add diverse human pathogenic microbes including *Enterobacter*, *Shigella*, *Salmonella*, *E. coli* O157:H7, *Bacillus cereus*, *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium botulinum*, as well as certain viruses (Hepatitis A Virus, Rotavirus and Norwalk disease virus) and parasites, such as *Giardia lamblia*, *Cyclospora cayetanensis* and *Cryptosporidium parvum*. These microbes remain outside on fruit surface as long as the skins are healthy and intact; any cuts or bruises that appear during the post-harvest processing operations allow their entry to the less protected internal soft tissue (Corbo & Speranza 2010). After harvesting, potable water is used to wash fruit and during washing some microorganisms will be removed from the product, nutrients will become available, and pathogens can be spread from contaminated parts to uncontaminated parts (Corbo & Speranza 2010).

The microflora present in unprocessed fruits and vegetables and processing facilities create a serious threat, when the objective is to achieve the longest shelf life possible for a minimally processed commodity.

2.9. Edible Coatings

The fresh-cut market trend has increased the demands to the food industry for seeking new strategies to increase storability and shelf life and to enhance microbial safety of fresh produce. The technology of edible coatings has been considered as one of the potential approaches for meeting this demand. Edible coatings from renewable sources, including lipids, polysaccharides and proteins, can function as barriers to water vapour, gases, and other solutes and also as carriers of many functional ingredients, such as antimicrobial and antioxidant agents, thus enhancing quality and extending shelf life of fresh and minimally processed fruits and vegetables (Lin & Zhao 2007). An edible coating is a method of extending shelf-life of fruits and vegetables that is growing in popularity and usage since it was recognized that packaging should be minimized for environmental reasons. Defined as a thin layer of edible material, which have been used as revetment of the food, it's function is to inhibit or reduce water loss, control respiration (O_2 and CO_2) and aromas, promoting semipermeable barriers (Fontes et al. 2008).

Low storage temperature and modified storage packaging (MAP) have been largely used to extend the shelf life of many whole and fresh-cut fruit and vegetable products, as they reduce the respiration rate, but most recently the use of edible coatings has been studied to extend shelf-life in fresh-cut produce (Antunes et al. 2012).

It is necessary to understand the mechanisms that cause fresh-cut produces spoilage, to understand the benefits of each edible coating and choose the most beneficial from a large quantity of options. For example, lipid-based edible coatings are excellent for preventing dehydration, and add brightness to the epidermis. Edible coating can also include some additives such as antimicrobials. These can effectively protect fresh-cut fruit against bacterial contamination by retaining preservatives on the surface of the cut fruit where they are needed, avoiding diffusion into the tissue (Antunes et al. 2012).

Edible coatings are gaining importance as an alternative to reduce the deterioration caused by minimal processing of fresh fruits. The semipermeable barrier provided by edible coatings extends shelf life by reducing moisture and solute migration, gas exchange,

respiration and oxidative reaction rates, as well as suppress physiological disorders of fresh-cut fruits. Edible coatings may also act as carriers of food additives such as anti-browning and antimicrobial agents, colorants, flavours, nutrients and spices (Rojas-Graü et al. 2009; Robles-Sánchez et al. 2013). Some polysaccharide-based coatings have been used to extend the shelf- life of fruits and vegetables, among them, alginate could be considered for edible film and coating because of their unique colloidal properties and their ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations like calcium (Ahvenainen 1996; Rojas-Graü et al. 2007; Robles-Sánchez et al. 2013).

The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products. These edible coatings can enhance the organoleptic properties of foods provided they contain various components (flavourings, colourings, sweeteners).

In food applications, edible coating solutions could be applied to food by several methods such as dipping, spraying, brushing and panning followed by drying. Components used for the preparation of edible films can be classified into three categories: hydrocolloids (such as proteins, polysaccharides, and alginate), lipids (such as fatty acids, acylglycerol, waxes) and composites (Bourtoom 2008).

Rojas-Graü et al. (2007) studied gellan-based edible coatings that showed ability to reduce respiration and gas exchange due to selective permeability to O_2 and CO_2. Since a certain degree of oxygen and carbon dioxide permeability is needed for respiration of living tissues, moderate barriers that allow a controlled respiratory exchange, avoiding anaerobic respiration, are considered more appropriate.

Edible coatings are applied on fresh-cut apples to produce a modified atmosphere, which reduces decay, delays ripening and colour changes, improves appearance, and functions as a carrier of antimicrobials, antibrowning agents, texture enhancers, nutraceuticals, flavours, and volatile precursors (Olivas et al. 2007).

Alginate films are potentially a good option for cut apples, since these films become stronger when cross-linked with Ca, and at the same time, adhere to the cut apple surface through this cross-linking (alginate-Ca-pectin). Alginate films are poor moisture-barriers, as they are hydrophilic films, however, the incorporation of calcium reduces their water vapour permeability, making alginate films water insoluble. The capacity of hydrocolloid-based films as water vapour barriers increases as their solubility in water decreases (Olivas et al. 2007).

2.10. Additional Components

Edible coatings have a high potential to carry active ingredients such as antibrowning agents, colorants, flavours, nutrients, spices and antimicrobial compounds that can extend product shelf- life and reduce the risk of pathogen growth on food surfaces (Rojas-Graü et al. 2009).

Edible coatings can also have food additives such as antioxidants, colorants, flavouring agents, and antimicrobial compounds. For example, organic acids (acetic, lactic, propionic, malic), bactericides (nisin, lacticin), enzymes (lysozyme, lactoperoxidase), peptides and natural antimicrobials (spices, essential oils, propolis) have been also incorporated into edible coatings (Antunes et al. 2012).

2.11. Essential Oils

For inhibiting the spoilage flora and to decrease the risk of pathogens in horticultural products some antimicrobial agents must be used in the formulation of edible films and coatings. GRAS antimicrobial compounds must be used not only to satisfy consumer demands but also for guarantee healthy foods (Antunes et al. 2012).

Essential oils and their compounds which have proved to have antioxidant and antimicrobial activities are promising compounds to be added to edible coatings increasing their benefits (Antunes et al. 2012).

Dipping of aqueous solutions containing antimicrobials is the most practical way to extend the microbial stability of fresh-cut fruits. However, application of antimicrobial agents directly on the food surface may have limited benefits because the active substances are rapidly neutralized or diffused from the surface into the food product, thus limiting the effect of the antimicrobial compound (Rojas-Graü et al. 2009). The utilization of antimicrobials in edible coatings has as advantage to maintain effective concentrations of the active compounds on the food surfaces in contrast to the direct application of the antimicrobials. In the latter, there is a rapid neutralization of the active compound or diffusion from the surface into the food product, decreasing the antimicrobial effect (Antunes et al. 2012**).** In this sense, antimicrobial edible films and coatings may provide increased inhibitory effects against spoilage and pathogenic bacteria by maintaining effective concentrations of the active compounds on the food surfaces (Rojas-Graü et al. 2009).

In the last years there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Essential oils and their constituents outstand as an alternative to chemical preservatives and their use in foods meets the demands of consumers for natural products (Rojas-Graü et al. 2009). Recent exploitation of natural products to control decay and extend storage life of perishables has received more and more attention. Essential oils are natural compounds isolated from aromatic plants. Some of these oils are generally recognized as safe (GRAS) for environment and human health. In this way, the interest in the use of such oils for a sustainable agriculture has increased and a lot of research has been done, proving for many cases, that plant essential oils and extracts may play the role as pharmaceuticals and food preservatives (Antunes et al. 2012).

Rojas-Graü et al. (2009) in review of the essential oils and their active constituents refers, based in other author's studies, that although their action against many microorganisms, including several pathogens is recognised, their mechanism of action has not been studied in great detail, but Burt (2004) reported that hydrophobicity is an important characteristic of EOs, which makes them able to pass through cell membranes and enter mitochondria, disturbing the internal structures and rendering the membranes more permeable. Due to the antimicrobial effect of essential oils and their components, their incorporation into edible coatings shows a great potential to increase shelf life and improve quality of fresh and fresh-cut fruits (Antunes et al. 2012).

Many factors must be considered in developing an anti- microbial edible coating, including the properties of the food, the coating and the effectiveness of the antimicrobial agents incorporated into the coating (Rojas-Graü et al. 2009).

Several studies have been made, combining the efficacy of alginate and gellan edible coatings with antimicrobial effect of essential oils to extend shelf life of minimally processed apples, but despite the positive results have been obtained, it became clear the major inconvenient it is the off-flavours compared with the original flavour of the produce (Rojas-Graü et al. 2009).

2.12. Anti-browning treatments

Fresh-cut fruits processing operations can induce undesirable changes in colour and appearance of these products during storage and marketing. The phenomenon is usually caused by the enzyme polyphenol oxidase (PPO), which in presence of oxygen, converts phenolic compounds into dark coloured pigments (Rojas-Graü et al. 2009). Enzymatic browning reactions in fruits are catalysed by PPO followed by non enzymatic formation of melanins (Lu et al. 2006).

Application of antioxidant treatments as dipping after peeling and/or cutting is the most common way to reduce browning of fresh-cut fruits (Rojas-Graü et al. 2009). Ascorbic acid is the most extensively used to avoid enzymatic browning of fruit due to the reduction of the o-quinones, generated by the action of the PPO enzymes, back to the phenolic substrates (Rojas-Graü et al. 2009). Rojas-Graü et al. (2009) citing others authors, also explain that ascorbic acid is oxidized to dehydroascorbic acid after a certain time, thus allowing the accumulation of o-quinones.

Treatment with calcium ascorbate is the major technology that has been widely employed by the fresh-cut apple industry to inhibit the enzymatic browning reaction and to maintain the quality and shelf life of fresh-cut apples (Luo et al. 2011). However, the reductive nature of calcium ascorbate (CaAs) makes proper sanitizing treatment for pathogen control a technical challenge due to the incompatibility of CaAs with the widely used oxidative sanitizing agents, i.e. chlorine, ozone and chlorine dioxide (He & Luo 2007).

The frequent reuse of browning control solutions for many batches of apples creates a high risk for food-borne illness outbreaks without proper sanitizing treatment to control the potential cross-contamination of apples by food-borne human pathogens. In fact, the contamination of sliced apples with human pathogenic bacterium, *Listeria monocytogenes*, has resulted in costly recalls in the US (Luo et al. 2011).

There is an urgent need for either a sanitizer that is compatible with the current widely used anti-browning solution, or preferably, a solution that can provide dual control of browning reaction and microbial growth to maintain the safety and quality of fresh-cut apples (Luo et al. 2011).

2.13. Statement of the problem and objectives

Fresh-cut demands a more challenging post-harvest treatment due to its increased perishability. The consumer are open to buy such produces as long it satisfies quality parameters (appearance, flavour, and texture), and also the product must be microbial safe and without signs of spoilage.

The convenience and attractiveness of fresh-cut produces can help the increase in consumption. Studies have been made to achieve extended shelf life, maintaining quality parameters, but the demand for non-chemical solutions requires the development of new approaches to this problem.

This study is designed to evaluate the effect of edible coatings enriched with essential oils and anti-browning agents developing a new approach to assure the quality and safety of minimally processed apple slices of ‘Bravo de Esmolfe’ apple cultivar.

3. Material and Methods

3.1.Plant material and sample preparation

In this study were used apples from 'Bravo de Esmolfe' cultivar, acquired in a local market in the beginning of January 2014. Prior to processing, the fruits have been individually visually checked for uniformity of size and freedom from defects, then have been washed with tap water to clean and sanitize the fruits. After washing, fruits were cut in 8 slices each, using a proper tool with sharp blades, which also removes the apple core which is not proper for consumption, therefore should not be present in the final product. Then, apple slices have been dipped in a primary solution with an anti-browning agent, before being dipped in a secondary solution with coatings and essential oils and, finally, in a calcium chloride solution (1%).

Primary solution of treatment consisted in a dilution of Citric Acid (2%) or Sodium Chlorite (0,5%). The secondary solution consisted in four different mixes of coatings with essential oils:

- Alginate (2%) + Eugenol (0.1%)
- Alginate (2%) + Citral (0.15%) + Eugenol (0.1%)
- Pectin (2%) + Eugenol (0.1%)
- Pectin (2%) + Citral (0.15%)

Fruit slices were dipped in the primary solution for 1 min, then in the second for 2 min.

After drip, 8 slices of apple have been put in each polyethylene terephtlate (PET) clamshell type container (8 cm x 10 cm x 4 cm), covered with low-density polyethylene film of 10 µc. Containers were stored in a cold chamber at a regular temperature (4ºC) and humidity (90-95%) during the entire study with the intention of simulating transport and retail conditions. Containers have been removed from the cold chamber after 2, 4, 6 and 8 days to be tested for some quality parameters and, part of samples, were stored at -80 for further analyses. Also at 3 and 6 days containers have been removed from cold chamber and used to access organoleptic characteristics with the help of a taste panel. For each treatment and time, three replications were used.

3.2. Quality Parameters

3.2.1. Colour

The colour was measured in each slice individually, with 3 readings per slice, by a Minolta Chroma Meter CR-300. This tool is used to analyse colour in fruits and vegetables, being the output a numeric scale of 3 parameters, colour, saturation and brightness. The instrument was calibrated in a standard white surface (Y=93.0; X= 0.3133; Z=93.85) as pre-established pattern in CIElab a*, b* e L* scale. The value of a* characterise readings from green (-a*) to red (+a*), the value of b* shows changes between blue (-b*) and yellow (+b*) colour. The value of L* shows the luminosity, going from white (L*=100) to black (L*=0) (McGuire 1992).

Also, Hue (hº) and Chroma (C*) values were obtained using the following formulas:

$$Hue\ (h*) = SE(a*> 0; \arctan\left(\frac{b*}{a*}\right) \times \frac{180}{3{,}1416}; \arctan(b*a*) \times \frac{180}{3{,}1416} + 180$$

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

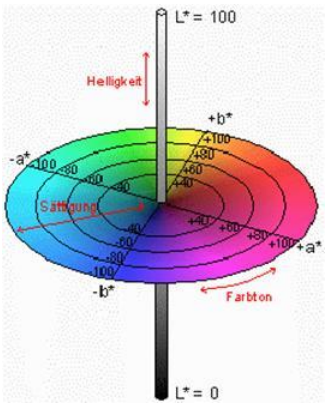

Figure 1 - CIElab colour system ("CIE L*a*b*")

3.2.2. Firmness

The firmness is a parameter that is based in the strength needed to penetrate the fruit pulp. Changes in apple slices firmness were measured with a fruit texture analyser Chatillon TCD200, with a cylindrical point of 11 mm diameter penetrating the sample to 7 mm. The firmness was measured in each apple slice pulp, being the results obtained in Newton (N).

3.2.3. Total Soluble Solids

The ºBrix measurement system is used to obtain the soluble solids content of an aqueous solution, as an indirect measurement of the sugars content. The measurement has been made in sample juices with a refractometer PAL-1 ATAGO, which gives a ºBrix value (%). The refractometer was calibrated with distilled water between each replication.

3.2.4. Weight Loss

Water loss is the main reason for weight loss in apple slices. Every replication box of each treatment was weighted at 0, 2, 4, 6 and 8 days, then the weight lost was calculated as percentage of initial weight as follow:

$$Weight\ loss = \frac{Weight_{initial} - Weight_{final}}{Weight_{initial}} \times 100$$

3.3. Respiration

The respiration was calculated as CO_2 production in every sample of treated apple slices during the entire study, following the methodology applied by Gago & Monteiro (2012).

CO_2 measurements were taken with a LI-COR LI-7000 CO_2 analyser, being each treatment sample measured at 0, 2, 4, 6 and 8 days. The respiration was calculated as CO_2 production rate as follow:

$$CO_2 = \frac{0{,}5 \times \Delta CO_2}{fruit\ sample\ weight}$$

$$\Delta CO_2 = CO_2S - CO_2R$$

Values are presented in μmol CO_2/ kg/ h.

3.4. Ethylene

Ethylene production was measured during this study (0, 2, 4, 6, and 8 days) following the method defined by Antunes et al, 2002.

Samples were encapsulated during 30 minutes in a closed container from which were withdrawn 1ml of gas-sample with a syringe and injected into a gas chromatograph.

Ethylene production was obtained comparing concentration in samples against a standard, which was also obtained injecting a 29 ppm ethylene control sample into a gas chromatograph.

The ethylene production was estimated as follow:

$$Ethylene\ (\mu L/Kg/h) = \left(\frac{29 \times\ sample\ area}{standard\ area}\right) \times \left(box\ volume - (fruit\ sample\ weight\ \times\ fruit\ density)\right) \times \left(\frac{1000}{fruit\ sample\ weight}\right) \times 2$$

The box volume used was 0.55 Litre.

Fruit density was calculated using a sample of 8 slices, using a simple method of water dislocation in a beaker and applying the formula:

$$\rho = \frac{m}{V}$$

3.5. Phenolic compounds

3.5.1. Extraction

To the following methods an extraction was made in order to obtain the appropriate solution to conduct the protocols.

From 10 grams of sample apple slices, juice was obtained with help of an instrument that uses pressure to extract the juice. Juice obtained in the process was put in Eppendorfs that were centrifuged at 5000 rpm to obtain a supernatant solution without floating solids. The eppendorfs were kept in a -80ºC freezer to maintain sample characteristics during the study till analyses.

3.5.2. Total phenols

Total phenol where quantified using a modified Folin – Ciocalteu method (Julkunen-titto, 1985; Wang et al, 1997). From the juice of each sample, a dilution of 1:4 (0.5 ml sample juice; 1.5 ml distilled water) has been made to adjust concentration to a Gallic acid standard curve.

A solution of Folin – Ciocalteu reagent was made in a 1:10 proportion (10 ml Folin – Ciocalteu; 90 ml distilled water). Also, a solution of Sodium Carbonate (Na_2CO_3) was prepared (7.5g Na_2CO_3; 100 ml distilled water).

In a spectrophotometer cuvette both reagents and a sample have been mixed (0.8 ml Sodium Carbonate solution; 0,2 ml sample; 1 ml Folin – Ciocalteu solution).

Before reading, the mixture was put in a vortex to homogenize and then left during 20 minutes at room temperature. Afterward, samples where centrifuged during 5 minutes at 5000 rpm. The absorbance reading was made in a spectrophotometer Ultrospect 1100 pro at 765 nm. The total phenolics content was estimated from a standard curve of Gallic acid and

the results expressed in mg of Gallic acid equivalent in 100 grams of fresh sample (mg GAE/ 100g F.W.).

Standard curve was made by reading absorbance in several Gallic acid concentrations (0,0325; 0,075; 0,125; 0,25 and 0,5 mg/ml). The method was the same that was used for the fruit samples.

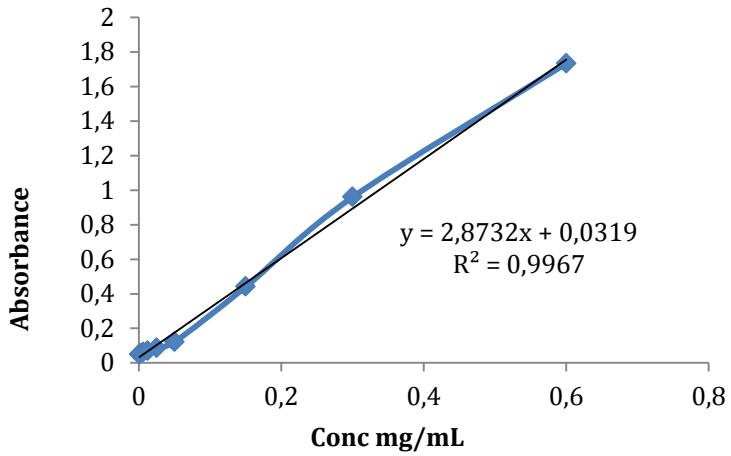

Figure 2 - Calibration curve for total phenols

3.6. Flavonoids

Flavonoids were obtained using a modified method from Miguel, M.G. et al, 2010. These compounds were quantified using an 2% Aluminium Chloride solution (0.4 mg $AlCl_3$: 200 ml Distilled water). For analysis, to 0,5 ml of sample (previously extracted) or standard, was added 0.5 ml of 2% $AlCl_3$ solution. After 1 hour at room temperature, the absorbance was measured at 420 nm in a spectrophotometer.

Previously, a calibration curve was obtained using Quercetin as a standard (mg Quercitin/ 100g F.W.).

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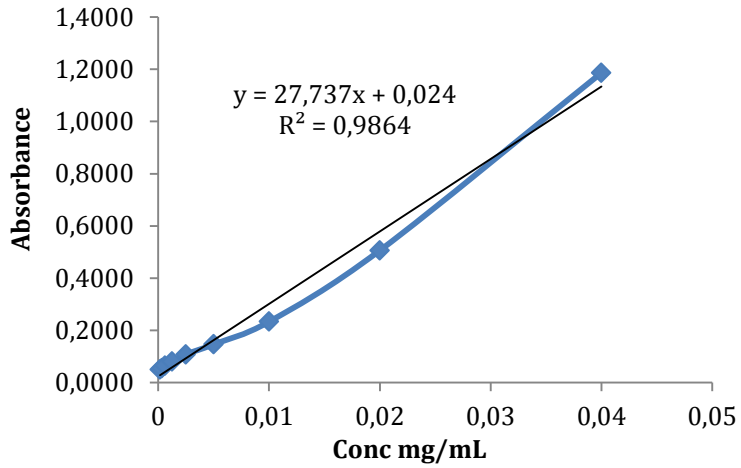

Figure 3 - Calibration curve for Flavonoids

3.7. ORAC (Oxygen Radical Absorbance Capacity)

The ORAC method objective is to determine the antioxidant capacity based in concentration reduction over time of an oxidative subtract (Fluorescein).

Dejian Huang et al (2002) method was adapted to determine ORAC in our samples. The analysis process was conducted in a Biotek Sinergy™ 4-Multidetector Microplate reader. A 96 well, black and sterile, plate (VMR) was used as support for samples, control probe and blanks.

In this assay, the control was Fluorescein (Sodium salt ,Sigma-Aldrich), which have been prepared from a stock solution (1mM), then a intermediate solution (0.4 ml stock solution; 10 ml phosphate tampon) and finally a daily solution, which was used to fill the well plate, using 0.4 ml intermediate solution in 10ml Phosphate tampon.

The assay also requires a blank which is ABAP (2,2- Azobis(methyl-propionamidine-dihydrocloride), Agros Organics 98%) that was prepared prior to being used solving 1gram in 10 ml Phosphate tampon solution 75 mM (pH 7.4).

Well plate was filled containing a column for the assay control (Fluorescein) and a column for the blank (ABAP), being the rest of wells samples.

Assay control wells had 150 µL Fluorescein solution and 50 µL Phosphate buffer. The Blank wells had 150 µL Fluorescein, 25 µL Phosphate buffer and 25 µL ABAP solution.

Sample wells were filled with 150 µL Fluorescein, 25 µL sample and 25 µLof ABAP solution. After plates being filled, readings took place during 1 hour and 30 minutes being the

gap between readings of each well 30 seconds, with a excitation wavelength of 485 nm and an emission wavelength of 528 nm.

Determination of the standard curve, was accomplished using a similar procedure to the one utilized to prepare the samples, in which the sample is changed to the different trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations (Fig. 4). The range of concentrations utilized to obtain the curve was 3.125; 6.25; 12.5; 25; 50 and 100 µM.

The results from these standards were used to calculate a calibration line that was used to obtain results in µM.TE/100g FW.

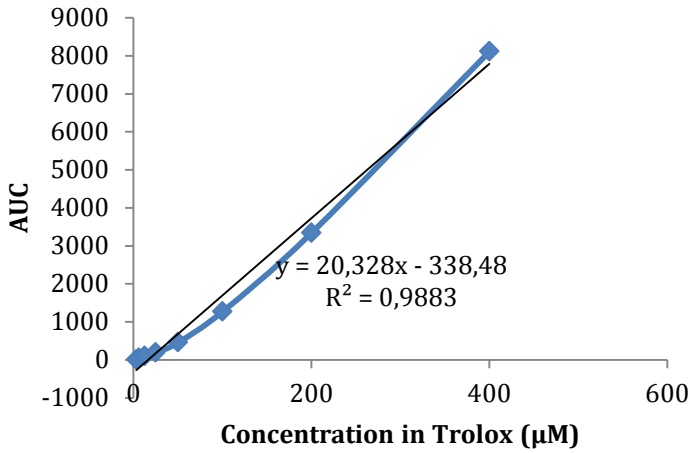

Figure 4 - Calibration curve for ORAC

3.8.TEAC Method (Trolox Equivalent Antioxidant Capacity)

In this assay, TEAC values were obtained applying an adapted method from Re, et al (1999). This assay requires an ABTS solution (2,2 – Azino – bis (3-ethylbenzothiazoline – 6 – suffonic acid) which was prepared dissolving ABTS into distilled water to a 7 mM concentration. After this, in order to produce ABTS radical cations (ABTS+), to the previous solution was added potassium persulfate (99%, Agros Organics) till a concentration of 2.45 mM were obtained. This solution was left in the dark at room temperature during 12-16 hours, as radicals tend to stabilize a while after and stay this way when correctly stored.

In the analysis, ABTS solution was dissolved in ethanol, to obtain an absorbance reading between 0.7 (+/- 0.02) at 735 nm (approximately 1 ml in 60 ml), being this a reading solution.

Absorbance reading procedure consisted in filling a cuvette with 990 µL of reading solution and registering the absorbance value, then adding 10 µL of sample or standard solution, perform an additional shaking and register the absorbance value after 6 minutes.

The standard curve is based on readings of a standard trolox solution (6 – Hidroxi – 2,5,7,8 – tetrametilcromo – 2 – carboxilic acid, Stock solution 2.5 mM), which have been stored at -20 ºC for posterior use in preparation of several concentrations of 500, 1000, 1500, 2000 and 2500 µM. At the end, a calibration line was obtained and a formula as well (Fig. 5).

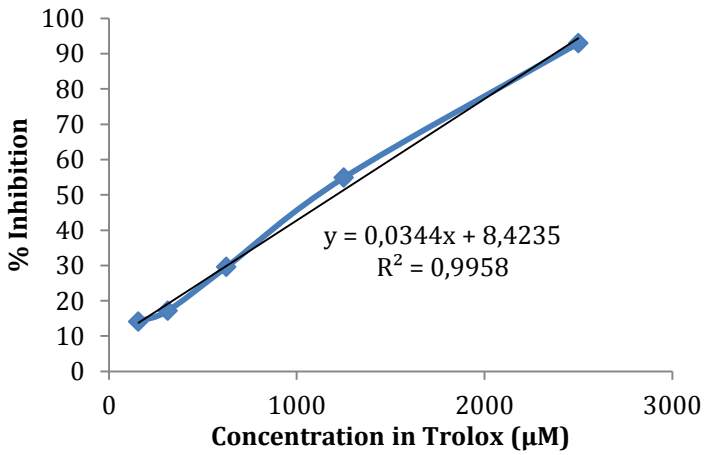

Figure 5 - Calibration curve for TEAC

With the previously obtained formula was possible to obtain the percentage (%) of absorbance inhibition, which later will be used to estimate the antioxidant capacity of samples, in µM.TE/ 100g sample.

$$Absorbance\ inhibition\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 – absorbance value of reading solution

A_1 – absorbance value of reading solution with sample or standard after 6 minutes

3.9. Polyphenol Oxidase (PPO) activity determination

3.9.1. Polyphenol oxidase extraction

This assay was adapted from the methodology explained in Soliva - Fortuny et al (2002). Polyphenol Oxidase (PPO) activity was obtained using a UV-vis spectrophotometer.

Initially a Mc Ilvane buffer was prepared by dissolving sodium phosphate dibasic heptahydrate (Panreac) ($Na_2HPO_4.7H_2O$) and citric acid (Sigma-Aldrich) in MilliQ water (Merck Millipore) (26,8g $Na_2HPO_4.7H_2O$; 5,25g Citric Acid; 1 liter MilliQ water). By using the McIlvane buffer solution, adding sodium chloride (Sigma-Aldrich) and Polyvinylpyrrolidone (PVPP) (Agros Organics), an extraction solution was obtained. Then, 3 grams of each sample were weighted into 50 ml Falcon tubes. Each Falcon tube was completed with 9 ml of extraction solution and homogenised. The homogenate was centrifuged during 30 minutes at 4ºC at 12000 rpm. Solids residues were discarded and the supernatant was filtered through a Whatman No. 1 paper. The entire process was conducted in cold temperatures, being the samples stored in a -80ºC freezer till the beginning of this assay.

3.9.2. Polyphenol oxidase activity measurement

To determine PPO activity 1 cm path cuvettes were filled with 3 ml of 0.05 M catechol and 75 µL of enzymatic extract. The mix was incubated during 5 minutes at 37ºC. Absorbance readings proceeded at 420 nm after incubation time. Inhibition was calculated using the following formula:

$$Relative\ activity\ \% = 100\frac{A}{A_0}$$

A – sample absorbance reading at 420 nm

A_0 – absorbance reading for 100% activity at 420 nm (average value used was 0,3607)

3.10. Extraction and Quantification of Non-volatile Organic Acids

This assay followed the methodology used in Terry et al. (2007).

In preparation for this assay, 50 ± 0.5 mg of freeze dried apple slices samples were dissolved into 3 ml of HPLC grade water to obtain extracts for organic acids determination. Sample extracts were later filtered through a 0.2 μm syringe filter before utilization in HPLC.

Main apple organic acids contents were determined using a HPLC system from Hitachi equipped with a diode array detector (DAD, L-2455, Elite LaChrom series, Hitachi,Japan) with multiple wavelength detector, degasser and cooled autosampler. Filtered samples were injected in a Purospher STAR RP18 column with 250x4.6 mm diameter, 5 μm particle size. (Merck Millipore, Germany) with an organic acid guard column (LiChroCART 4-4 Merck Millipore, Germany). The mobile phase used was an analytical grade KH_2PO_4 (25mM) dissolved in HPLC grade water. The pH adjustment to 2.5 was made using phosphoric acid. Flow rate of the mobile phase was 1.0 mL min^{-1} at isocratic conditions. System temperature was set at 35ºC in the thermostated column compartment (L-2300, Elite LaChrom series, Hitachi, Japan).

Output of the assay was compiled using EZ-Chrom Elite software which tools provided data on presence and quantity of each acid. Acid quantification was calculated by comparison of peak areas of samples to the standards.

3.11. Extraction and Quantification of Non-structural Sugars

Effect of treatments on sugar content of apple slices was analysed by the method described by Terry et al., (2007) and modified as described in Magwaza et al. (2012).

Sugar extraction was made with 150 +/- 0.5 mg of fruit powder obtained by freeze-drying, diluted in 3 ml of aqueous methanol 62.5% (v/v). Following extraction, concentrations of fructose, glucose and sucrose were determined using an Agilent 1200 series HPLC binary pump system (L-2130, Elite LaChrom series, Hitachi, Japan).

Diluted samples (1:10) were injected (20 μL) into a Purospher Star NH_2 (amino) column (4.6 mm diameter × 250 mm, 5 μm particle size; Merck Millipore, Germany) with an amino guard column (LiChroCART 4-4 Merck Millipore, Germany). System temperature was set at 30 ºC on the thermostated column compartment. The mobile phase used was HPLC grade

water: acetonitrile (25:75) at flow rate of 1.0 ml/min and the presence of carbohydrates was detected on a refractive index detector (RID, L-2490, Elite LaChrom series, Hitachi,Japan).

Sugars were quantified from a linear standard curve (0.05 – 1.25 mg/ml; average R^2= 0.99).

3.12. Microbial analysis

Samples were checked for microbial activity using the methodology described in Portuguese institute for Quality normative NP-2079.

At 0, 2, 4, 6 and 8 days sample were analysed to quantify aerobic mesophilic microorganisms, psychotropic microorganism, molds, and yeasts. From each sample, 10 grams of apple slices were placed inside a stomacher bag to which 90 ml of sterilized peptone water (Biokar Diagnostics) was added. Mixture was blended in a Stomacher during 30 seconds. Both PCA (Plante Count Agar, Biokar diagnostics) and Dicloran (Dichloran-rose bengal-chloramphenicol (DRBC) agar, Biokar Diagnostics) plates were inoculated with 100 μL of suspension previously prepared.

For the mesophilic microorganisms plates stood at 34ºC for 24 hours. In the molds and yeasts, Dichloran plates were incubated during 120 hours (5 days) at a temperature of 30ºC.

Results of microbial analysis will be shown in colony forming units per gram (CFU/g).

3.13. Sensory evaluation

In order to assess sensory quality of coated apple slices of this study, a group of semi-trained individuals, evaluated several parameters as, appearance, texture, aroma, acidity, sweetness and overall flavour, returning a classification for each parameter between 1 (dislike extremelly), 2 (dislike), 3 (slightly dislike), 4 (Neither like or Dislike), 5 (Like slightly), 6 (Like) and 7 (Excellent).

This assay was made at 0, 3 and 6 days. Results were expressed in averages calculated for each attribute.

4. Results and Discussion

4.1. Quality parameters

4.1.1. Colour

4.1.1.1. Luminosity (L*)

Figure 6 presents luminosity (L*) in the pulp of apple slices during this study.

L* values were expected to decrease being an indicator of greater browning in apple slices (Rojas‐Graü 2006). Results showed that L* value decreased as expected, meaning as storage time increased apple slices showed darkening (Fig. 6).

During the study, samples from each treatment had differences in browning intensity, but as showed in appendix 7.1, at the end of storage, Citric Acid treatments were the ones with lower L*, meaning that sodium chlorite was better for reducing browning.

Regarding the performance of both anti-browning agents that were tested, Sodium Chlorite perform better than Citric Acid, with the average L* values of apple slices with Sodium Chlorite treatments being superior to those obtained with Citric Acid (Appendix 7.23, ANOVA, $p<0,05$). This could be explained as either a result from oxidative browning reactions or from increasing pigment concentrations (Rojas‐Graü 2006; Rocha & Morais 2003) in result of cell wall damage due to processing.

Edible coatings containing Sodium Chlorite (SC) showed higher average L* values than control samples, what can be related to a slow consumption of substrates by polyphenol oxidase (PPO) (Rocha & Morais 2003).

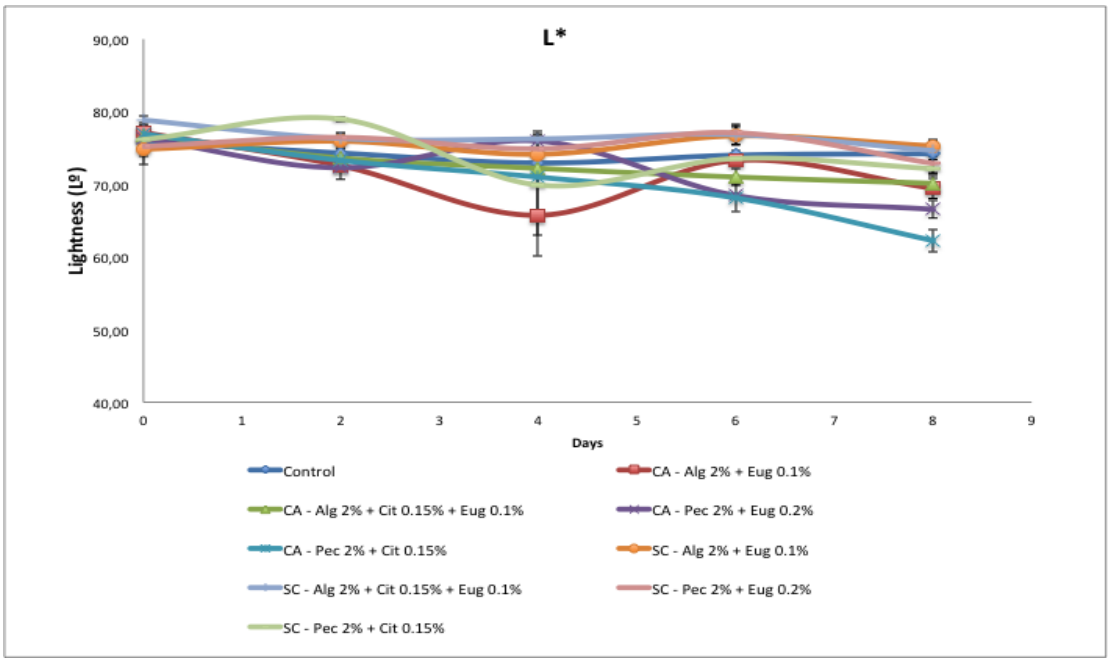

Figure 6 - Luminosity L* for treatments during storage progress

4.1.1.2. Colour parameter a*

The colour parameter a* characterises the readings from green (-a*) to red (+a*). As browning processes occur apple flesh colour tends to change from its normal pulp colour to more brown colours, which could be translated to an increase in a* values (Rocha & Morais 2003).

All treatments showed an increase in parameter a* average values through storage as expected (Fig. 7), with statistical evidence that some combinations withhold better colour in this parameter (appendix 7.2, Duncan test $p<0,05$). The objective was to maintain low values similar to those in day 0 during storage of apple slices. The Citric Acid treatments with citral or eugenol alone, were the ones that had higher changes in a* as verified also for luminosity (L*), as visible in figure 7 and appendix (7.2).

Sodium Chlorite average values were always below control, and when compared with Citric acid, the treatment performance was better as supported by the statistical significant results (appendix 7.2 and 7.23, $p<0,05$).

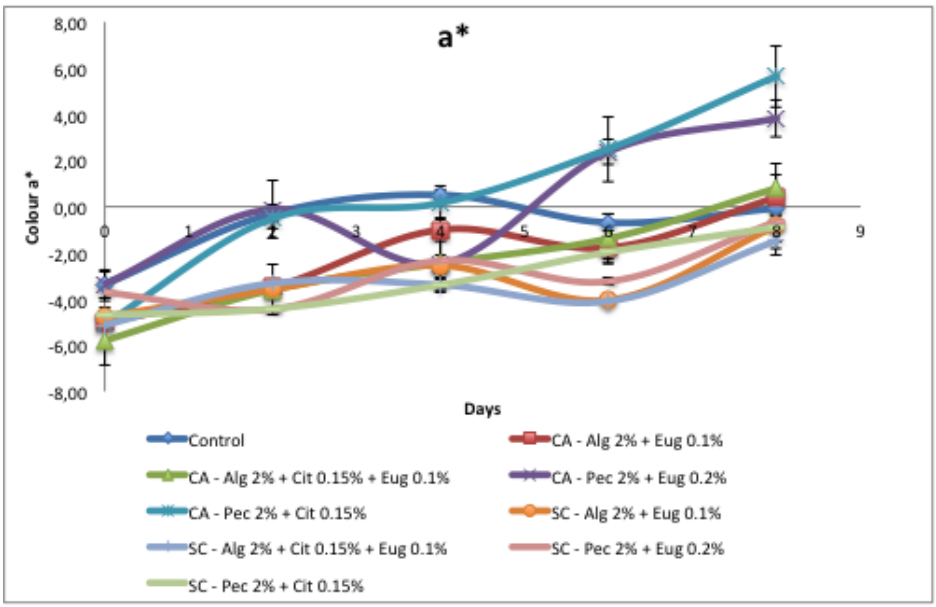

Figure 7 - Colour parameter a* for treatments during storage

4.1.1.3. Colour parameter b*

The colour value b* characterises colour changes from blue (-b*) to yellow (+b)*. 'Bravo de Esmolfe' apple flesh is quite white when mature, and when browning occurs it becomes yellowish before brown.

Statistical analysis shows a significant difference between treatments (Appendix 7.23, $p<0,05$), with Citric Acid with the pectin and citral treatments alone having higher changes than Sodium Chlorite treatments (Fig. 8). Despite the smaller importance of this parameter in this analysis, it follows the previous parameters (L* and a*), showing a better performance of Sodium Chlorite treatments in preserving colour.

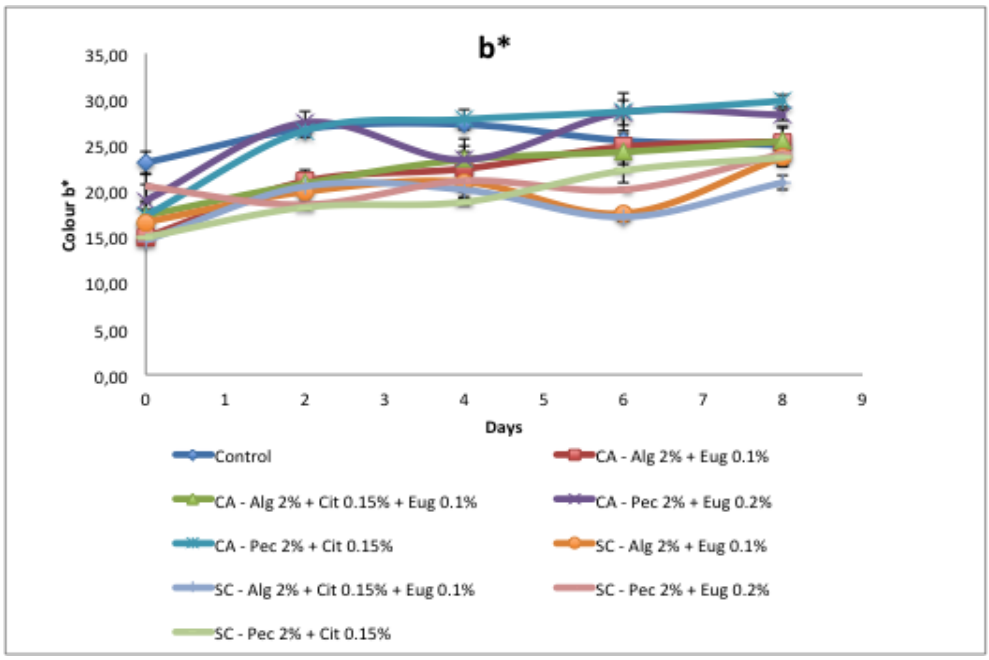

Figure 8 - Colour parameter b* for treatments during storage

4.1.1.4. Hue (hº)

The angle hue (hº) represents an angle in a colour wheel of 360º, with 0º, 90º, 180º, and 270º, representing the red, yellow, green, and blue, respectively (Rojas - Graü et al., 2006).

Like in the studies performed by Rojas – Graü et al., (2006), the ºhue values of the samples in this study decreased during storage time, which translates into an increase of surface browning (Fig. 9). Statistical analysis showed significant differences (Duncan test, $p<0,05$) between coatings, during storage, with the coating containing Alginate 2% + Citral 0,15% + Eugenol 0,1% + Sodium Chlorite maintaining stable values over the 8 days with no significant differences (Appendix 7.4).

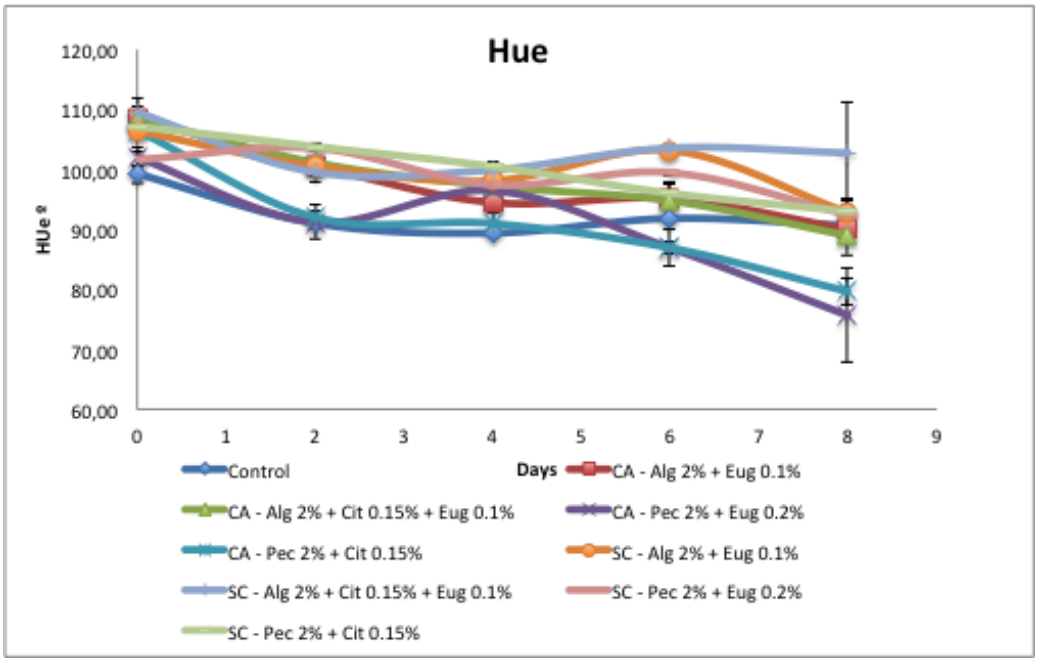

Figure 9 - Hue (hº) for treatments during storage

4.1.1.5. Chroma (C*)

Chroma (C*) values are used to show colour intensity or saturation, but it is not considered a parameter that relates directly to browning activity much, like parameter b*.

A slight increase in C* values is expected during storage time (Rocha & Morais 2003). In our study, Chroma showed a slight increase in all treatments, as expect as colour of our apple slices turned from white to brown (Fig. 10). Despite some differences during storage time, coatings behaved mostly in the same pattern. However, statistical analysis clarified some differences between Citric Acid and Sodium Chlorite (appendix 7.23, ANOVA, $p<0.05$), which provided a lower mean value to Sodium Chlorite. This difference can only be understood as support to browning parameters such L*, a* and ºhue which provided statistical significant results with less browning in Sodium Chlorite treated samples.

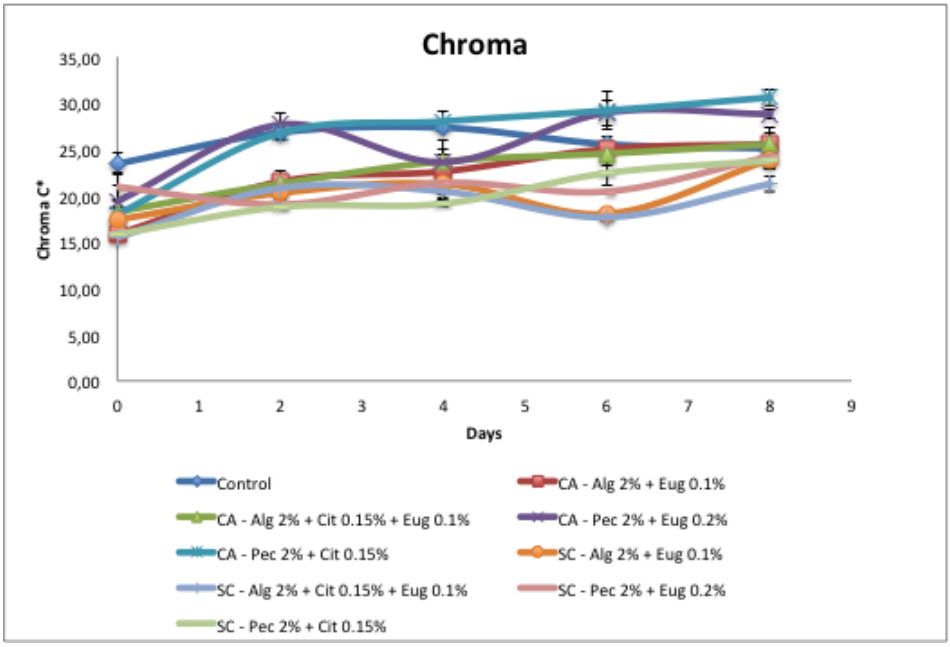

Figure 10 - Chroma (C*) for treatments during storage

4.1.2. Firmness

Usually in apple slices is expected to occur a decrease in firmness as storage progress due to action of endogenous enzymes on the cell wall degradation (Rocha & Morais 2003).

The control samples behaved as expected, showing a decrease during storage, but an increase in firmness occurred in some treatments (Fig. 11). This could be explained with the use of a calcium treatment as final dipping in our protocol. Firmness in apple slices tend to be affected by calcium treatments, in fact, the calcium cation is known to increase firmness and delay membrane lipid catabolism in apple fruits (Luo et al., 2011).

Treatments that contained sodium chlorite showed higher average values of firmness at the end of storage, and treatments with citric acid and eugenol alone or the combination with citral showed to maintain firmness values above control samples (Figure 11, appendix 7.6).

Overall, statistics showed a highly significant difference between both treatments (appendix 7.23, $p<0.05$), with Sodium Chlorite performing better, increasing firmness in the end of storage time in all combinations of coatings (appendix 7.6).

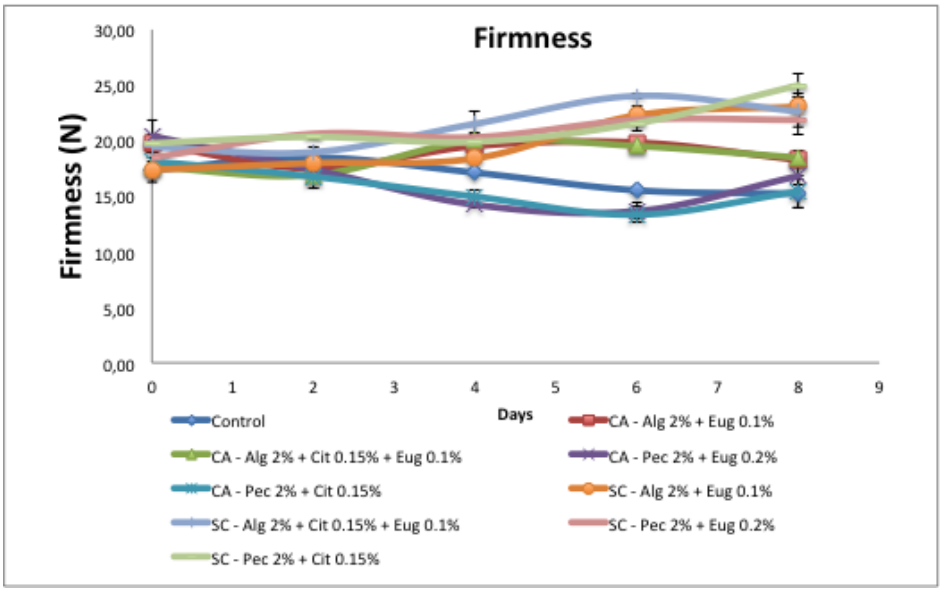

Figure 11- Firmness (N) of samples

4.1.3. Total Soluble Solids (TSS)

During ripening, fruits tend to reduce their content in acids and increase sugars (Olivas et al. 2007). The initial increase in sugars content might also be related to the breakdown of high molecular weight compounds such as starch and hemicellulose into low molecular weight compounds such as simple sugars (Rocha & Morais 2003).

TSS almost did not change through shelf life changing from 10 to 12 ºBrix (Figure 12). This is because fruit were ripe when used as fresh cut.

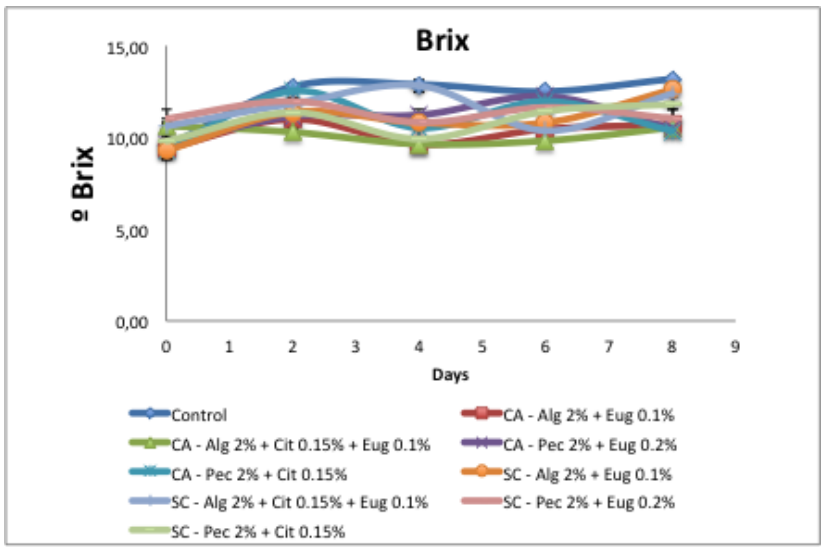

Figure 12 - Total Soluble Solids (º Brix)

4.1.4. Weight Loss

The percentage of weight lost is an indicator of water lost due to exposed tissue to dehydration. This study used minimally processed apples in slices, which created a large surface that, were exposed and due to processing at cellular level, membranes were damaged. This means that in all treated samples were expected weight loss similar or below the control samples as storage progressed, depending of the efficacy of each coating. However some coatings did not differ from control in weight loss (Fig. 13). Both the coatings (SC – Alg 2% + Eug 0,1% and SC – Alg 2% + Cit 0,15% + Eug 0,1%) were the ones that better reduced weight loss without significant difference between them.

Comparing Sodium Chlorite to Citric acid treatments, the latter was less effective (appendix 7.23, $p<0,05$). Sodium Chlorite showed as overall less weight loss with an average of 0,615% lower than the 0,842% from Citric Acid.

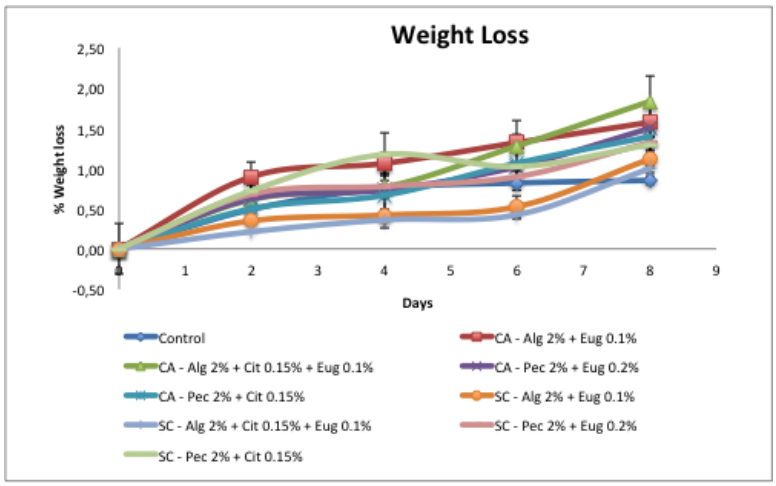

Figure 13 - Weight loss during storage

4.2. CO_2 production

The rate of respiration indicates how quickly a product may deteriorate. Increased respiration by tissue injury results in a greater reduced shelf-life compared to whole fruits and vegetables (Lee et al. 2003).

Figure 13 represents the percentages of CO_2 production measured during storage, showing a variation on respiration rates during that period. Respiration rate did not show a clear pattern of differences among treatments. However, considering the whole shelf life period, the values at the end were not significantly different than the ones at the beginning because fruit were ripe when prepared and wound respiration was declined.

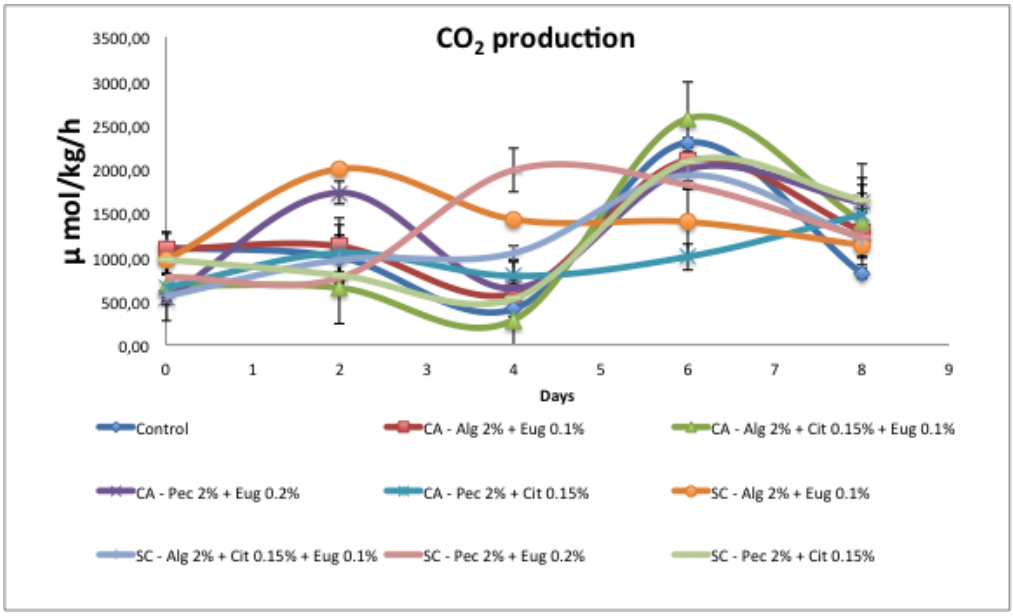

Figure 14 - CO2 production during storage

4.3. Ethylene

Wound ethylene may accelerate deterioration and senescence in vegetative tissues and promote ripening of climacteric fruits (Brecht 1995).

In our experiment, ethylene production was higher in the first days, decreasing to low values after day 2 and onwards (Fig. 15). The rapid decrease may be attributed to the fact that fruit were ripe, so overpass climacteric ethylene and also due to placement in low storage temperature (Antunes et al. 2000).

Also it is observed a higher decrease in ethylene production after 2 days storage in treated samples than control probably due to the reduction caused by coating. No significant difference where found between Citric Acid and Sodium Chlorite treatments when directly comparing average values (appendix 7.23, $p<0,05$).

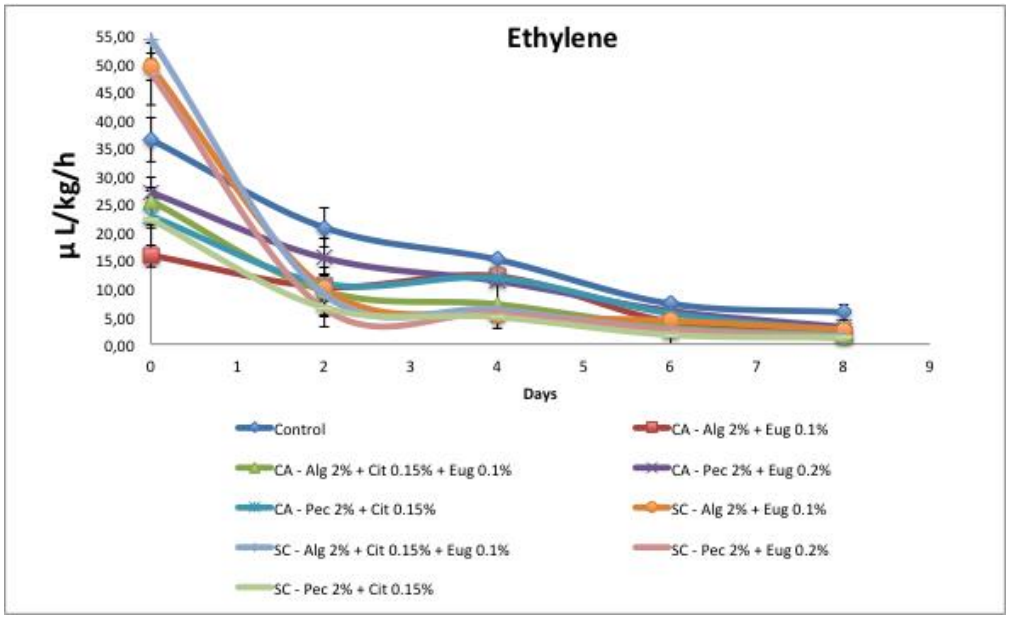

Figure 15 - Ethylene production during storage

4.4. Phenolic compounds

4.4.1. Total phenols

When cell structure is disrupted, phenolic compounds may be involved in both enzymatic and non-enzymatic browning reactions, with PPO catalysing the oxidation of phenolic substrates and the quinones formed can take part in secondary reactions, bringing the formation of dark secondary products (Rocha & Morais 2002).

This parameter showed significant differences between control and the rest of treatments (appendix 7.11, $p < 0,05$) during all storage period and no significant differences among them (Fig. 16). The decrease in total phenols means that edible coating treatments reduced the production of secondary metabolites.

Despite treatments positive effect, values obtained in this study did not show any difference between Citric acid and Sodium Chlorite solutions (appendix 7.23). Highest values for phenolic compounds were present in day 0 for treated samples, which decreased over time, contrary to control samples values.

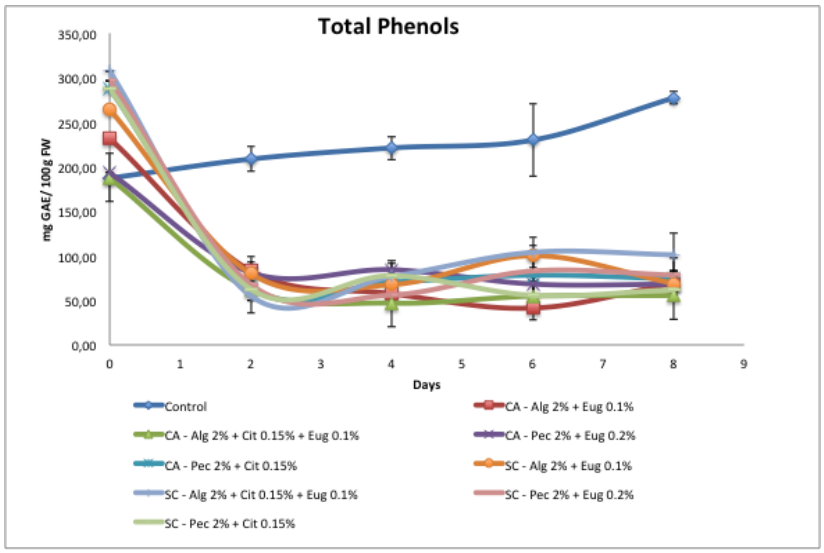

Figure 16 - Total phenols in samples during storage

4.4.2. Flavonoids

Flavonoids are a type of phenolic compound, being the most important polyphenol class (Miguel et al. 2010). As observed in total phenols, flavonoid values were significantly lower in treated samples as compared to control (Fig. 17, appendix 7.12, $p<0,05$).

Statistical analysis between Citric Acid and Sodium Chlorite average values showed significant difference in performance, being the latter more effective (Sodium Chlorite average = 1,239 mg GAE/100g FW) (appendix 7.23, $p<0,05$).

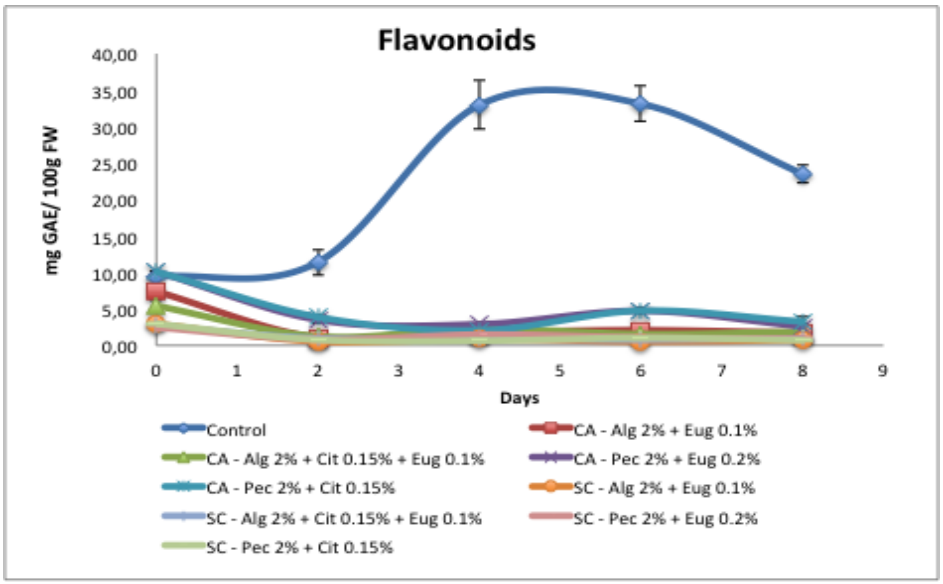

Figure 17 - Flavonoid content of samples in mg GAE/ 100g Fresh Weight

4.5. ORAC (Oxygen Radical Absorbance Capacity)

This assay is used to measure oxidative degradation of a substrate, in this case Fluorescein, the greater the antioxidant capacity of a fruit the less oxidative degradation will occur, as antioxidants should protect the fluorescent molecule. Results are presented in Trolox Equivalent based on the standard curve obtained previously.

Substances used in treatments, such as citric acid and sodium chlorite, could have a slight effect in reducing oxidative degradation of fluorescein, and therefore produce better values of antioxidant capacity for treated samples. This happened in fact for ORAC since edible coatings had higher values than control (Figure 18). Among treatments, there is no significant difference (appendix 7.23, $p>0,05$) that could help identify a treatment that performed better in this parameter.

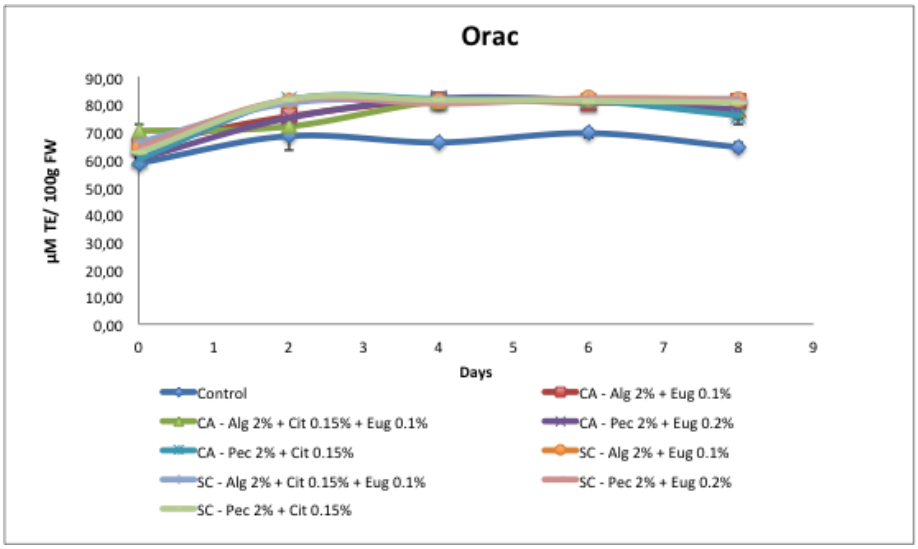

Figure 18 - ORAC values during storage of samples

4.6. TEAC Method (Trolox Equivalent Antioxidant Capacity)

The method is based on the ability of antioxidant molecules to quench the long-lived ABTS, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analog (Pellegrini et al. 2003).

Analysing values obtained, within the day 0 its possible to determine significant diffences (appendix 7.14, $p<0,05$) among treatments, with the treatment of SC+Pec+Cit coating presenting the biggest antioxidant capacity in the beggining.

Despite a good start for all treatments when compared with non-treated samples, in the entire storage duration of this study no significant differences in performance occured between Citric Acid and Sodium Chlorite treatments.

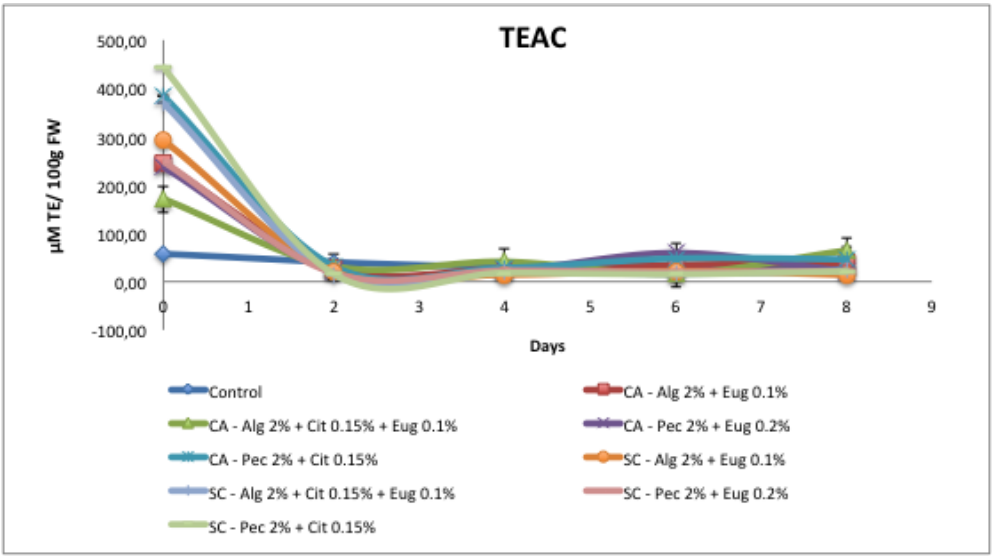

Figure 19 - TEAC values during storage of samples

4.7. Polyphenol Oxidase activity determination

Polyphenol oxidase (PPO) is seen as the principal path of enzymatic browning in apples, so it is expected that a reduction in its activity translate into less browning in apple flesh. PPO catalyses the transformation of polyphenolic subtracts in secondary metabolites with dark colour. Results are shown based on that the relative activity of PPO in control samples just after cut, presenting 100% relative activity in day 0 and the other measurements as percentage of this value.

The edible coatings decreased PPO relative activity (Figure 20). Inspite of a not clear pattern through storage, at the end of storage period the lower PPO values were in citric acid edible coatings.

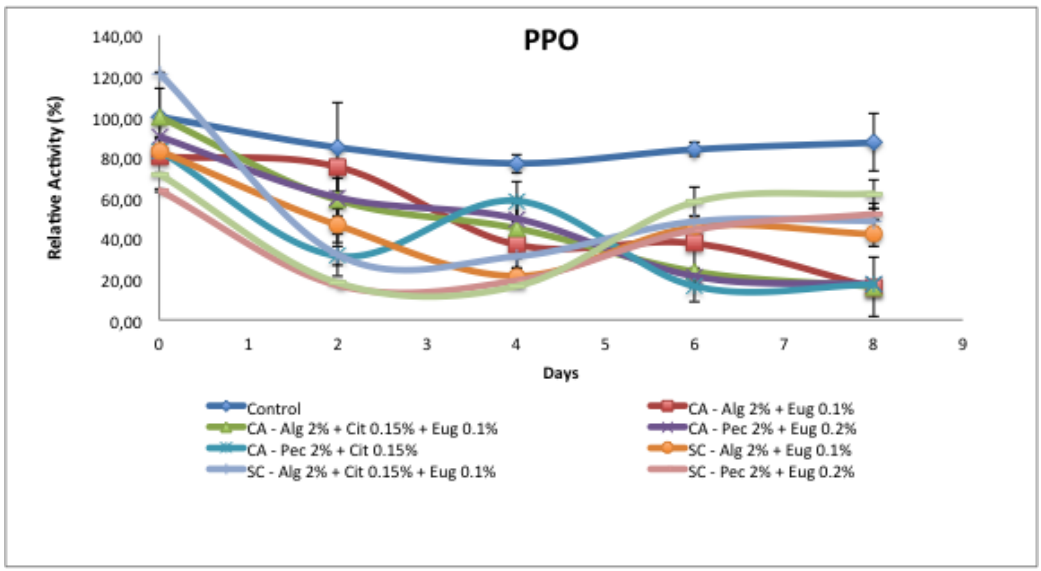

Figure 20 -PPO relative activity (%) during storage

4.8.Microbial analysis

4.8.1. Mesophilic microorganisms

Quantitative evaluation of mesophilic microorganisms was made in this study to assess the antimicrobial effect of treatments.

Control samples showed greater values than samples that were subject to treatments, as expected, showing that even a careful processing with a pre-wash with water and dipping in water do not remove all microorganisms (Fig. 21).

The group of samples that had citric acid and the group of samples with sodium chlorite, have some undefined behaviours within them. However at the end of the experiment all edible coatings had lower mesophilic microorganisms than control.

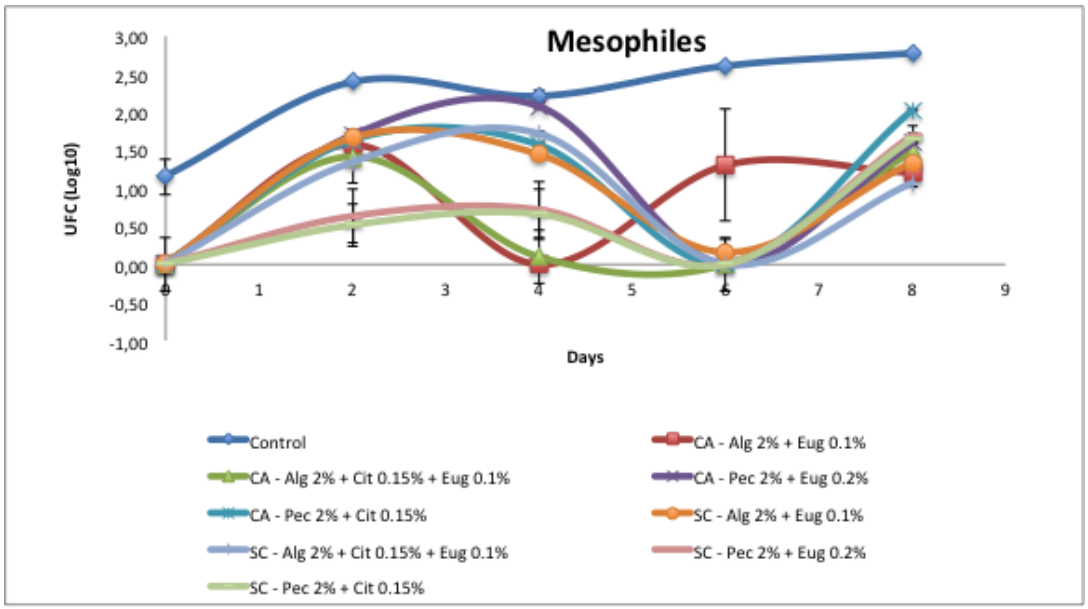

Figure 21 -UFC of mesophilic microorganisms in samples

4.8.2. Moulds and yeasts

Quantitative evaluation of moulds and yeasts in samples showed a small number of microorganisms. Results only show a higher amount of moulds and yeasts in control samples, higher than treated samples, after 4 days, with some treated samples showing higher amounts of moulds and yeasts before that (Fig. 22). This contamination does not comply with the results expected, in which a non-treated sample should present higher amounts of moulds and yeasts. External factors may have altered results.

After day 4, treated samples performed significantly better than control samples (appendix 7.16, $p<0,05$), showing that in long-term storage treatments improve quality of apples slices reducing spoilage due to moulds and yeasts damage.

No significant differences were obtained when comparing Citric Acid and Sodium Chlorite (appendix 7.23).

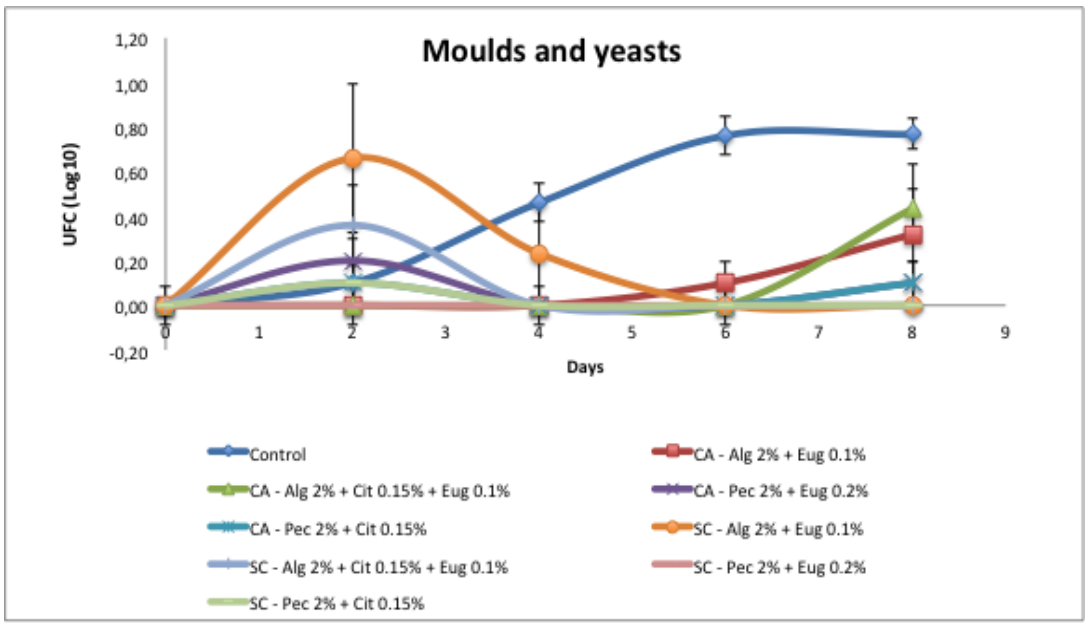

Figure 22 - UFC of moulds and yeasts in samples

4.9. Non-volatile Organic Acids

Ripening of fruits tends to reduce the content in acids and increase in sugars (Olivas et al. 2007), therefore acid consumption is expected during storage if senescence processes still active.

Our results were resumed to two acids, oxalic and malic, as other acids only appeared in some samples or/ in residual quantities, or our column didn't had the capacity to perform a correct reading.

Inspite of some changes in oxalic acid values were similar at the end of experiment to the ones after cut (Fig. 21). Average value of oxalic acid in Sodium Chlorite samples was higher than Citric acid average (1.4793 vs. 1.1583) being statistically significant such difference (appendix 8).

Malic acid followed similar pattern. However, for this acid average value for Citric acid treatments was higher than Sodium Chlorite average value (6.271 vs. 4.7345), mainly due to higher values at the beginning of storage period (Figure 22).

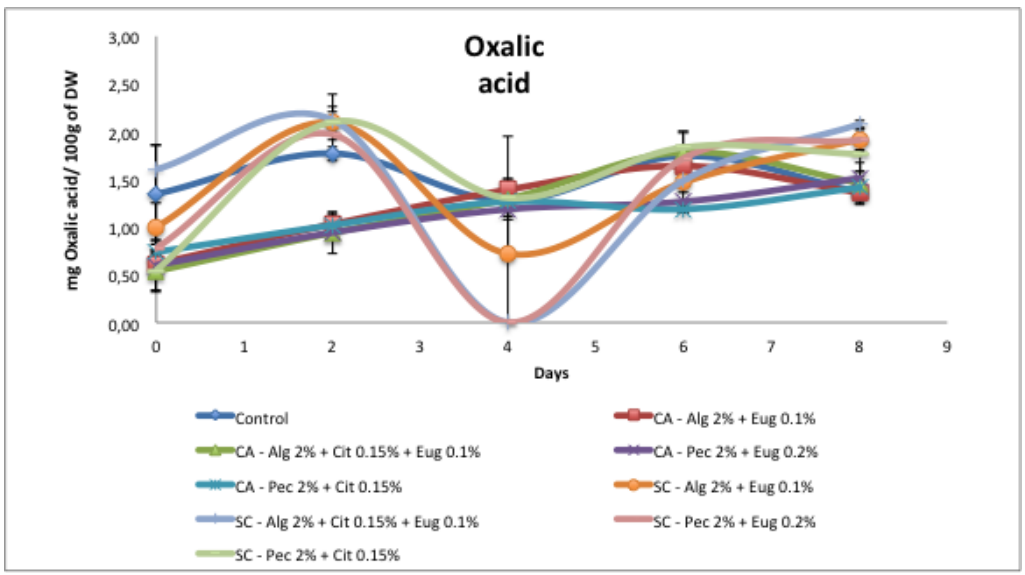

Figure 23- Oxalic acid content obtained in HPLC assay

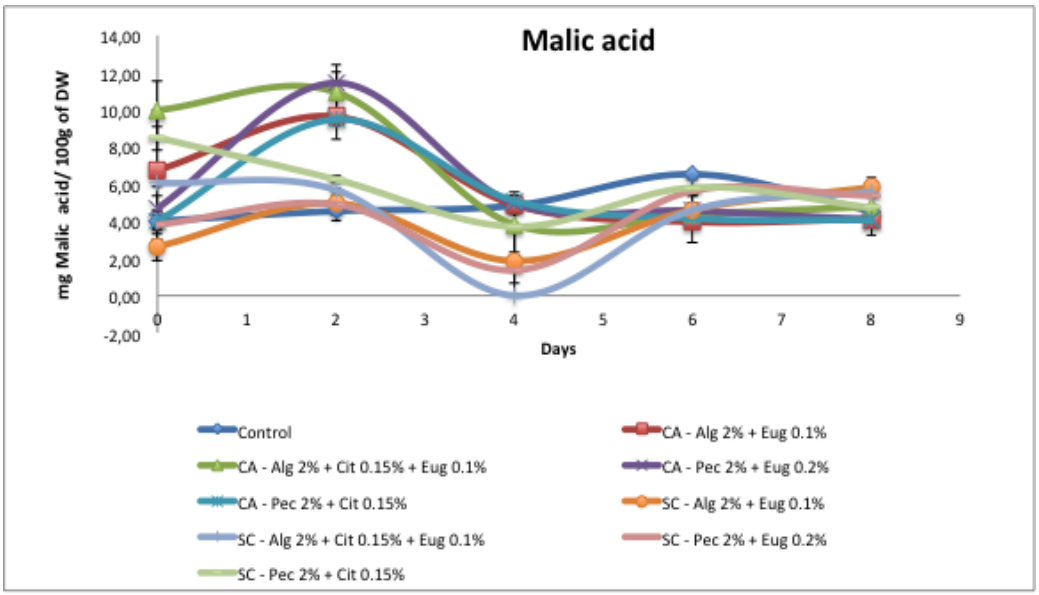

Figure 24 - Malic acid content obtained in HPLC assay

4.10. Non-structural sugars

Samples were analysed to determine variation in sugars during storage and comparison of treatment effect.

During storage, due to transformation of acids in sugars that occur in several senescence mechanisms an increase in sugar content is expect. Treatments should reduce this effect, maintaining the same level of sugars. Also initial increase in sugar contents might also be related to the breakdown of high molecular weight compounds such as starch and hemicellulose into low molecular weight compounds such as simple sugars (Rocha & Morais 2003).

This assay evaluated contents of Fructose, Glucose and Sucrose. All three sugars presented higher means in samples treated with Sodium Chlorite that samples with Citric acid treatment with statistical significance found in all sugars (appendix 7.23). Generally, sugars content did not change much through storage time. This may be because fruits were ripen when treated as fresh-cut.

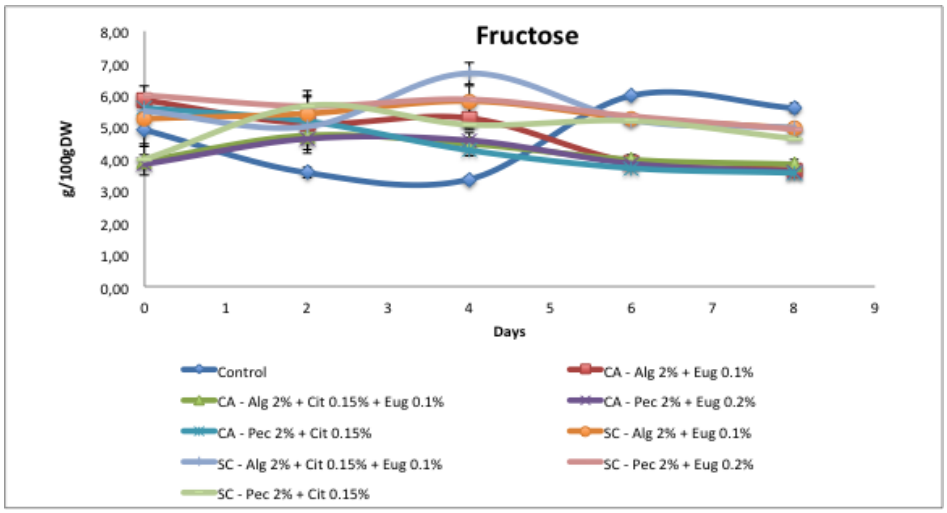

Figure 25 - Fructose in samples (g/ 100g Dry Weight)

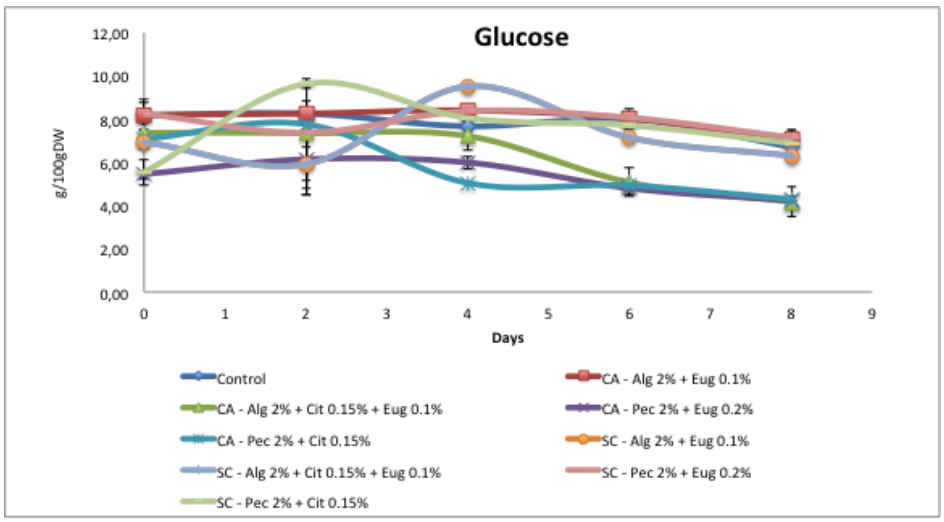

Figure 26 - Glucose in samples (g/ 100g Dry Weight)

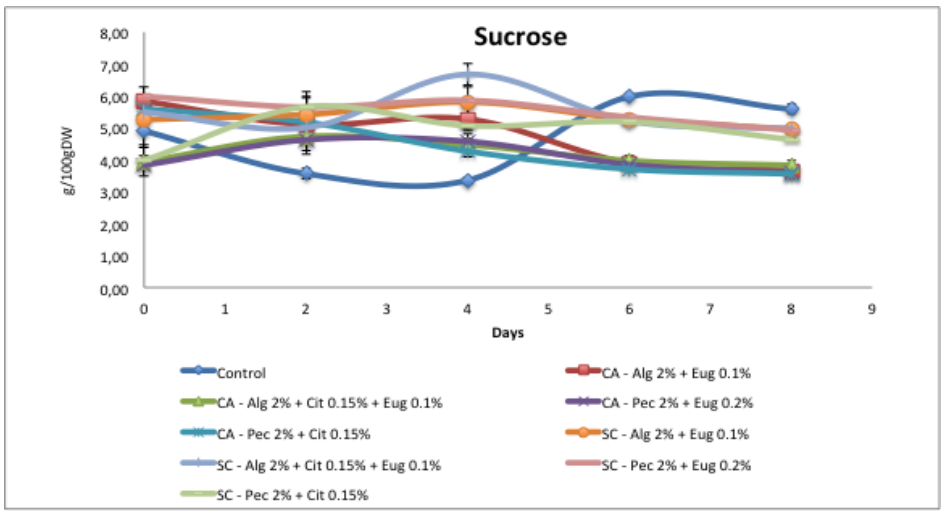

Figure 27 - Sucrose in samples (g/ 100 g Dry Weight)

4.11. Sensorial analysis

Taste panel classified samples in a scale from 1 (bad) to 7 (excelent), in two evalution periods (3 and 6 days of storage).

Samples treated with sodium chlorite appear to have slightly better acceptance than the ones treated with citric acid during the entire “shelf-life” (Table 1).

Sodium Chlorite treatment with Pectin and Eugenol achieve a higher average in first tasting, and was the second best in second tasting, being Sodium Chlorite with Alginate and Eugenol the best after 6 days.

Appearance is the most important parameter in fresh-cut produces and this study Sodium Chlorite treated samples perfomed better than Citric acid samples. Citric acid samples, showed signs of browning earlier than other samples resulting in lower values as presented in the following tables.

Treatments that resulted in poor appearence does not have any interest to our research.

Sodium Chlorite samples showed also higher flavour averages, which is also important to fresh cut produces with treatments. Sodium Chlorite with Alginate + Eugenol and Sodium Chlorite with Pectin + Eugenol presented the highest values for flavour.

Table 1 - Sensorial evaluation for parameters and overall acceptance

Parameters	Days		Citric Acid				Sodium Chlorite			
		Control	Alg 2% + Eug 0.1%	Alg 2% + Cit 0.15% + Eug 0.1%	Pec 2% + Eug 0.2%	Pec 2% + Cit 0.15%	Alg 2% + Eug 0.1%	Alg 2% + Cit 0.15% + Eug 0.1%	Pec 2% + Eug 0.2%	Pec 2% + Cit 0.15%
Appearance	3 days	2,5	2,7	3,0	6,2	3,2	4,7	4,1	4,9	5,1
	6 days	3,2	3,2	3,2	3,3	2,7	5,0	4,8	5,0	4,5
Aroma	3 days	4,5	3,9	3,8	3,8	3,9	5,3	4,2	5,0	5,4
	6 days	4,2	4,2	5,2	5,5	4,7	5,3	4,7	5,0	4,3
Texture	3 days	4,3	3,5	3,8	4,7	3,4	5,2	4,4	5,2	5,5
	6 days	3,5	4,7	5,0	3,5	4,0	5,5	5,0	5,8	5,3
Sweetness	3 days	5,0	4,1	4,1	4,9	3,6	5,5	3,9	5,6	5,4
	6 days	4,2	3,7	5,3	4,2	4,8	5,8	5,2	5,7	4,7
Acidity	3 days	4,9	3,8	3,7	5,3	4,0	5,7	4,2	5,5	5,6
	6 days	4,3	3,7	5,0	3,8	4,5	5,5	4,5	5,2	4,0
Flavour	3 days	4,5	3,5	3,5	4,6	2,9	4,8	3,4	5,3	5,1
	6 days	4,0	3,7	5,3	3,8	4,7	5,5	4,7	5,5	3,8
Overall acceptance	3 days	4,3	3,6	3,7	4,9	3,5	5,2	4,0	5,3	5,3
	6 days	3,9	3,8	4,8	4,0	4,2	5,4	4,8	5,4	4,5

Colour scale based on taste panel analysis:

- Green – Good or best samples
- Yellow – Average samples
- Red – Bad or worst samples

5. Conclusion and Future perspectives

In this study, alternative antibrowning substances were compared with positive outcome in a number of quality parameters in fresh-cut 'Bravo de Esmolfe' apples.

Edible coatings were efficient in maintaining most quality parameters through storage as compared to control.

Colour and firmness were better maintained in sodium chlorite treatments.

Regarding the total soluble solids, they were maintained stable during storage in almost all treated samples, since fruit were ripe when processed.

Respiration and ethylene production were reduced with the application of coatings which reduced exposition of tissues.

Total phenols were higher in controls indicating the oxidative process.

The most antioxidant activity measurements showed that all edible coatings were good for preserving antioxidant activity as compared to control.

Also the PPO measurements indicate that edible coatings reduce oxidative processes mainly for sodium chlorite treatments.

Microbial analysis revealed also a reduction in microbial spoilage when edible coatings were applied.

In this study, sensorial analysis of apple slice samples revealed a general acceptance of all edible coatings and for the subtle changes it brought to apple organoleptic characteristics.

Sodium chlorite was better to preserve quality than citric acid.

Generally, there were no differences between pectin and alginate and in some quality parameters the combination of citral+eugenol performed better followed by eugenol alone and citral.

Minimally processed apples have been commercialized for several years, but demand of healthier fresh food alternatives with less chemical compounds for conservation is growing, so the alternatives presented in this study can be applied to market demand.

Further study can also be developed regarding achieving a symbiosis of natural compounds (new essential oils, antibrowning agents) that can increase storage and shelf life for minimally processed apples.

The application of these concepts to cultivars like ‘Bravo de Esmolfe’, the one present in this study, could create renewed interests to diversify a traditional and well-appreciated product and provide a viable alternative to produce extra income to producers.

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7. Appendix

7.1. Luminosity (L*) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

L

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	75,897	aA	74,300	aA	72,943	abA	74,013	aA	74,210	aA	75,897	aA	74,300	cA	72,943	aA	74,013	abA	74,210	aA
Alg 2% + Eug 0.1%	77,100	aA	72,567	aAB	65,733	bB	73,277	aAB	69,453	bAB	74,760	aA	75,940	bcA	74,143	aA	76,663	abA	75,293	aA
Alg 2% + Cit 0.15% + Eug 0.1%	76,730	aA	73,620	aB	72,197	abBC	71,007	abC	70,130	bC	78,787	aA	76,313	bAB	76,253	aAB	76,843	abAB	74,660	aA
Pec 2% + Eug 0.2%	76,300	aA	72,287	aB	75,977	aAB	68,470	bC	66,547	bC	75,203	aAB	76,463	bA	74,903	aAB	77,097	aA	72,883	aB
Pec 2% + Cit 0.15%	76,963	aA	73,293	aAB	71,000	abBC	68,130	bC	62,253	cD	76,130	aA	78,983	aA	69,903	aA	73,547	bA	72,167	aA

7.2. Colour parameter a* for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

a

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	-3,370	aB	-0,303	aA	0,510	aA	-0,690	bA	-0,073	bA	-3,370	aB	-0,303	aA	0,510	aA	-0,690	aA	-0,073	aA
Alg 2% + Eug 0.1%	-5,013	bD	-3,503	bCD	-1,003	bAB	-1,777	bBC	0,397	bA	-4,783	bcC	-3,580	bBC	-2,550	bB	-4,043	cBC	-0,840	aA
Alg 2% + Cit 0.15% + Eug 0.1%	-5,797	bD	-3,597	bC	-2,447	cBC	-1,377	bB	0,800	bA	-5,127	cC	-3,317	bB	-3,387	bB	-4,093	cB	-1,480	aA
Pec 2% + Eug 0.2%	-3,350	aC	-0,117	aB	-2,447	cBC	2,383	aA	3,823	aA	-3,737	abB	-4,440	cB	-2,337	bAB	-3,237	cB	-0,647	aA
Pec 2% + Cit 0.15%	-5,090	bC	-0,513	aB	0,160	abB	2,507	aB	5,653	aA	-4,707	bcD	-4,423	cD	-3,390	bC	-1,970	bB	-0,887	aA

7.3. Colour parameter b* for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

b

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	23,043	aB	26,787	aA	27,243	aA	25,507	aAB	25,007	bAb	23,043	aB	26,787	aA	27,243	aA	25,507	aAB	25,007	aAB
Alg 2% + Eug 0.1%	14,972	cB	21,055	bA	22,434	aA	24,817	aA	25,394	bA	16,616	bD	19,923	bcBC	20,987	bAB	17,550	cCD	23,713	abA
Alg 2% + Cit 0.15% + Eug 0.1%	17,334	bcC	20,827	bB	23,422	aAB	24,253	aA	25,414	bA	14,625	bC	20,493	bA	20,130	bA	17,175	cB	20,915	bA
Pec 2% + Eug 0.2%	18,989	bC	27,436	aA	23,422	aB	28,553	aA	28,296	abA	20,434	aAB	18,520	cB	21,095	bAB	20,144	bAB	24,042	aA
Pec 2% + Cit 0.15%	17,241	bcB	26,590	aA	27,805	aA	28,640	aA	29,804	aA	14,984	bC	18,219	cB	18,801	bB	22,217	bA	23,640	abA

7.4. Hue (hº) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

hue

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	99,173	bA	91,003	bB	89,373	cB	91,803	abB	90,647	aB	99,173	bA	91,003	cB	89,373	bB	91,803	dB	90,647	aB
Alg 2% + Eug 0.1%	108,483	aA	100,665	aB	94,465	abCD	95,104	aC	90,017	aD	106,334	aA	100,522	bB	20,987	cD	17,550	eD	92,810	aC
Alg 2% + Cit 0.15% + Eug 0.1%	108,576	aA	101,136	aB	97,203	aC	94,765	aC	88,941	aD	109,383	aA	99,531	bA	99,908	aA	103,556	aA	102,737	aA
Pec 2% + Eug 0.2%	101,821	bA	91,222	bAB	97,203	aAB	86,874	bBC	75,640	bC	101,494	bA	103,530	aA	97,425	aAB	99,443	bA	92,464	aB
Pec 2% + Cit 0.15%	106,545	aA	92,025	bB	90,965	bcB	86,902	bB	79,634	abC	107,269	aA	103,805	aB	100,477	aC	96,008	cD	92,999	aE

7.5. Chroma (C*) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Chroma

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	23,437	aB	26,833	aA	27,340	aA	25,560	abAB	25,087	cAB	23,437	aB	26,833	aA	27,340	aA	25,560	aAB	25,087	aAB
Alg 2% + Eug 0.1%	15,792	bB	21,520	bA	22,619	aA	25,041	abA	25,557	bcA	17,347	bC	20,290	bcBC	21,248	bAB	18,039	cC	23,893	abA
Alg 2% + Cit 0.15% + Eug 0.1%	18,288	bC	21,300	bB	23,702	cAB	24,541	bA	25,543	bcA	15,505	bC	20,812	bA	20,450	bA	17,672	cB	21,270	bA
Pec 2% + Eug 0.2%	19,428	bC	27,665	aA	23,702	aB	28,952	abA	28,808	abA	20,923	aAB	19,054	cB	21,379	bAB	20,448	bAB	24,230	abA
Pec 2% + Cit 0.15%	17,982	bB	26,766	aA	28,026	aA	29,196	aA	30,604	aA	15,812	bC	18,756	cB	19,139	bB	22,436	bA	23,769	abA

7.6. Firmness (N) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Firmness

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Controlo	17,400	aAB	18,493	aA	17,117	bABC	15,527	bBC	15,227	aC	17,400	bAB	18,493	bcA	17,117	cABC	15,527	cBC	15,227	bC
Alg 2% + Eug 0.1%	19,723	aA	17,497	aB	19,520	aA	19,837	aA	18,263	aAB	17,377	bB	17,987	CB	18,410	bcB	22,287	bA	23,067	aA
Alg 2% + Cit 0.15% + Eug 0.1%	17,610	aBC	16,920	aC	20,020	aA	19,520	aAB	18,477	aABC	19,367	abB	18,970	bB	21,520	aAB	24,027	aA	22,627	aA
Pec 2% + Eug 0.2%	20,433	aA	17,410	aB	20,020	cCD	13,653	cD	16,817	aBC	18,423	abB	20,620	aAB	20,313	abAB	21,880	bA	21,870	aA
Pec 2% + Cit 0.15%	18,157	aA	16,760	aA	14,960	cAB	13,333	cB	15,420	aAB	19,820	aB	20,337	aB	19,897	abB	21,560	bB	24,987	aA

7.7. Total Soluble Solids (ºBrix) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

TSS

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	9,517	aB	12,767	aA	12,883	aA	12,500	aA	13,183	aA	9,51,67	aB	12,767	aA	12,883	aA	12,500	aA	13,183	aA
Alg 2% + Eug 0.1%	9,367	aC	10,983	bcA	9,617	cBC	10,450	bABC	10,683	bAB	9,267	aC	11,367	bB	10,800	bB	10,800	abB	12,600	abA
Alg 2% + Cit 0.15% + Eug 0.1%	10,650	aA	10,283	cAB	9,633	cB	9,817	bAB	10,533	bAB	10,633	aB	11,833	bAB	12,817	aA	10,367	cB	12,383	abA
Pec 2% + Eug 0.2%	9,567	aC	11,150	bcAB	9,633	bAB	12,233	aA	10,483	bBC	11,050	aA	11,967	abA	10,817	bA	11,633	bA	11,033	bA
Pec 2% + Cit 0.15%	9,267	aC	12,517	abA	10,517	bB	1,967	aA	10,350	bB	9,767	aA	11,350	bA	9,917	bA	11,417	bA	11,600	abA

7.8. Weight loss (%) for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Weight

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control			0,493	aB	0,750	abA	0,817	aA	0,850	bA			0,493	abB	0,750	abA	0,817	abA	0,850	bA
Alg 2% + Eug 0.1%			0,897	aB	1,063	aB	1,323	aAB	1,573	aA			0,350	bB	0,423	bB	0,527	bcB	1,117	abA
Alg 2% + Cit 0.15% + Eug 0.1%			0,610	aC	0,767	abBC	1,280	aB	1,833	aA			0,213	bC	0,357	bBC	0,420	cB	0,997	abA
Pec 2% + Eug 0.2%			0,620	aC	0,767	abC	1,020	aB	1,500	aA			0,680	aB	0,777	abB	0,897	aAB	1,323	aA
Pec 2% + Cit 0.15%			0,500	aBC	0,667	bB	1,067	aAB	1,393	abA			0,723	aB	1,170	aA	1,023	aAB	1,293	aA

7.9. Respiration for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test ($p < 0,05$). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Respiration (CO_2)

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	1106,703	aB	994,053	abB	167,817	aB	2686,223	abA	806,687	aB	1106,703	aB	994,053	bB	167,817	dB	2686,223	aA	806,687	bB
Alg 2% + Eug 0.1%	1088,450	aB	1118,667	abB	582,380	aB	3454,303	aA	1292,130	aB	963,837	aB	3214,567	aA	1425,500	abB	1396,377	aB	1134,090	abB
Alg 2% + Cit 0.15% + Eug 0.1%	677,510	abBC	647,277	bBC	284,303	aC	2571,327	abA	1405,343	aB	553,847	aC	966,057	bBC	1043,690	bcBC	1929,027	aA	1207,910	abB
Pec 2% + Eug 0.2%	527,837	bC	1729,610	aB	284,303	aC	3076,673	aA	1617,113	aB	779,293	aC	762,817	bC	1987,770	aA	1814,577	aAB	1223,603	abBC
Pec 2% + Cit 0.15%	654,457	abB	1042,723	abAB	783,380	aAB	1001,490	bAB	1482,247	aA	973,373	aB	789,803	bB	523,900	cdB	2084,723	aA	1644,427	aA

7.10. Ethylene production for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test ($p < 0,05$). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Ethylene

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Controlo	36,357	aA	20,710	aB	15,000	aB	7,167	aC	5,653	aC	36,357	bA	20,710	aB	15,000	aB	7,167	aC	5,653	aC
Alg 2% + Eug 0.1%	15,697	cA	10,180	bB	12,037	aB	3,923	bcC	1,817	bC	49,187	abA	9,993	bB	5,327	bBC	4,143	bC	2,610	bC
Alg 2% + Cit 0.15% + Eug 0.1%	35,397	bA	9,453	bB	7,057	bBC	2,997	cBC	1,597	bC	53,940	aA	8,867	bB	6,103	bBC	2,600	bcC	1,383	bC
Pec 2% + Eug 0.2%	26,907	bA	15,313	abB	7,057	abB	5,887	abC	3,010	bC	48,003	abA	6,333	bB	5,467	bB	2,453	bcB	1,140	bB
Pec 2% + Cit 0.15%	22,830	bcA	10,730	bB	11,723	aB	5,433	abC	2,410	bC	22,027	cA	6,473	bB	4,773	bB	1,627	cB	1,140	bB

7.11. Total Phenols for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Total Phenols

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	187,107	dB	208,603	aB	221,123	aAB	229,910	aAB	273,310	aA	187,107	eB	208,603	aB	221,123	aAB	229,910	aAB	277,310	aA
Alg 2% + Eug 0.1%	232,030	bA	83,337	bB	57,473	cBC	41,320	bC	68,910	bBC	264,040	dA	80,063	bB	67,557	bB	100,070	bB	68,943	bB
Alg 2% + Cit 0.15% + Eug 0.1%	187,730	dA	62,250	bB	46,927	cB	54,703	bB	55,527	bB	307,320	aA	55,150	bC	76,810	bBC	103,930	bB	101,177	bB
Pec 2% + Eug 0.2%	193,470	cA	81,153	bB	46,927	bB	68,347	bB	68,157	bB	296,520	bA	68,033	bB	56,047	bB	82,953	bB	78,400	bB
Pec 2% + Cit 0.15%	288,360	aA	64,737	bB	70,533	bcB	78,237	bB	74,193	bB	288,260	cA	62,683	bB	77,663	bB	55,663	bB	61,880	bB

7.12. Flavonoids for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Flavonoids

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	9,390	bC	11,413	aC	32,967	aA	33,117	aA	23,517	aB	9,390	aC	11,413	aC	32,967	aA	33,117	aA	23,517	aB
Alg 2% + Eug 0.1%	7,350	cA	1,087	cC	1,760	bB	2,090	bB	1,710	bB	2,900	bA	0,553	bB	0,880	bB	0,543	bB	0,743	bB
Alg 2% + Cit 0.15% + Eug 0.1%	5,380	dA	1,260	bcB	2,287	bB	1,570	bB	1,867	bB	2,670	cA	0,980	bB	0,950	bB	0,783	bB	0,977	bB
Pec 2% + Eug 0.2%	10,200	aA	3,520	bcC	2,287	bCD	4,743	bB	2,590	bD	2,420	dA	0,810	bB	1,500	bB	1,100	bB	0,903	bB
Pec 2% + Cit 0.15%	10,180	aA	3,900	bB	2,063	bC	4,757	bB	3,263	bBC	2,900	bA	0,773	bC	0,643	bC	1,037	bB	0,717	bc

7.13. ORAC for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

ORAC

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	58,550	dB	68,337	bA	66,030	bAB	69,407	bA	64,350	bAB	58,550	dB	68,337	bA	66,030	bAB	69,407	bA	64,350	bAB
Alg 2% + Eug 0.1%	65,130	bC	75,490	abB	81,410	aA	80,567	aAB	80,917	aAB	64,220	bB	81,237	aA	80,963	aA	81,930	aA	81,833	aA
Alg 2% + Cit 0.15% + Eug 0.1%	70,020	aB	71,837	abB	80,923	aA	81,513	aA	77,830	aA	66,100	aC	80,317	aB	81,283	aA	81,797	aA	81,857	aA
Pec 2% + Eug 0.2%	36,000	eC	74,917	abB	80,923	aA	81,283	aAB	77,970	aAB	64,480	bC	81,523	aA	80,150	aB	81,927	aA	81,540	aA
Pec 2% + Cit 0.15%	60,080	cC	81,513	aA	81,897	aA	82,177	aA	75,837	aB	62,200	cB	81,650	aA	81,430	aA	81,130	aA	80,636	aA

7.14. TEAC for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

TEAC

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	58,527	cA	40,120	aB	29,733	aC	23,803	abC	28,977	aC	58,527	eA	40,120	aB	29,733	aC	23,803	aC	28,977	aC
Alg 2% + Eug 0.1%	243,250	bA	28,113	abB	26,387	aB	34,623	abB	38,867	aB	292,830	cA	20,077	bBC	14,380	cC	21,700	aB	14,123	cC
Alg 2% + Cit 0.15% + Eug 0.1%	170,590	dA	31,393	abBC	41,037	aBC	16,303	bC	64,367	aB	370,190	bA	14,760	bC	20,240	bBC	15,917	aC	22,723	bB
Pec 2% + Eug 0.2%	238,140	cA	20,973	bC	41,037	aC	59,153	aB	21,393	aC	248,570	dA	17,253	bC	22,823	bB	19,647	aBC	21,407	bBC
Pec 2% + Cit 0.15%	383,810	aA	31,233	abB	28,313	aB	47,883	abB	46,227	aB	442,430	aA	15,400	bB	17,880	bcB	15,360	aB	21,033	bB

7.15. PPO relative activity for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

PPO activity

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	100,000	aA	84,783	aA	76,940	aA	83,787	aA	87,107	aA	100,000	bA	84,783	aA	76,940	aA	83,787	aA	87,107	aA
Alg 2% + Eug 0.1%	80,240	dA	75,160	aA	36,963	bB	37,483	bB	15,960	bC	83,040	cA	46,530	bB	21,503	bC	45,567	bB	42,103	bB
Alg 2% + Cit 0.15% + Eug 0.1%	99,440	aA	58,860	abB	44,987	bB	23,713	cC	16,250	bC	121,150	aA	31,993	bB	31,160	bB	48,323	bB	48,740	bB
Pec 2% + Eug 0.2%	89,720	bA	60,157	abB	44,987	abB	21,373	cC	17,270	bC	63,510	eA	16,930	bC	19,257	bC	44,243	bB	51,983	bB
Pec 2% + Cit 0.15%	82,810	cA	31,393	bC	58,523	abB	16,523	cC	17,457	bC	71,670	dA	18,990	bB	16,493	bB	57,900	bA	62,047	abA

7.16. Fungus activity for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Fungus

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	0,000	aC	0,100	aC	0,460	aB	0,760	aA	0,767	aA	0,000	aC	0,100	abC	0,460	aB	0,760	aA	0,767	aA
Alg 2% + Eug 0.1%	0,000	bA	0,000	aA	0,000	bA	0,000	bA	0,317	aA	0,000	aB	0,660	aA	0,233	abAB	0,000	bB	0,000	bB
Alg 2% + Cit 0.15% + Eug 0.1%	0,000	bA	0,000	aA	0,000	bA	0,000	bA	0,433	aA	0,000	aB	0,360	abA	0,000	bB	0,000	bB	0,000	bB
Pec 2% + Eug 0.2%	0,000	bA	0,200	aA	0,000	bA	0,000	bA	0,100	aA	0,000	aA	0,000	bA	0,000	bA	0,000	bA	0,000	bA
Pec 2% + Cit 0.15%	0,000	bA	0,100	aA	0,000	bA	0,000	bA	0,100	aA	0,000	aA	0,100	abA	0,000	bA	0,000	bA	0,000	bA

7.17. Mesophiles activity for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Mesophiles

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	1,153	aD	2,397	aBC	2,213	aC	2,607	aAB	2,777	aA	1,153	aD	2,397	aBC	2,113	aC	2,607	aAB	2,777	aA
Alg 2% + Eug 0.1%	0,000	bB	1,573	bcA	0,000	cB	1,303	bA	1,207	dA	0,000	bC	1,663	bA	1,443	bAB	0,160	0,000	1,340	####
Alg 2% + Cit 0.15% + Eug 0.1%	0,000	bB	1,417	cA	0,100	cB	0,000	cB	1,483	cA	0,000	bD	1,347	bB	1,717	abA	0,000	bD	1,073	dC
Pec 2% + Eug 0.2%	0,000	bD	1,700	bB	2,077	aA	0,000	cD	1,590	cC	0,000	bB	0,633	cB	0,717	cB	0,000	bB	1,697	bA
Pec 2% + Cit 0.15%	0,000	bD	1,633	bcB	1,570	bC	0,000	cD	2,027	bA	0,000	bC	0,517	cBC	0,660	cB	0,000	bC	1,647	bA

7.18. HPLC – Oxalic acid for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Oxalic Acid

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	1,337	aA	1,773	aA	1,273	aA	1,747	aA	1,367	aA	1,337	abA	1,773	aA	1,273	aA	1,747	aA	1,367	bA
Alg 2% + Eug 0.1%	0,610	aC	1,027	bB	1,393	aA	1,640	aA	1,363	aA	1,387	abAB	2,097	aA	0,723	aB	1,473	aAB	1,920	aAB
Alg 2% + Cit 0.15% + Eug 0.1%	0,530	aD	0,973	bC	1,247	aB	1,593	aA	1,470	aAB	2,087	aA	2,123	aA	0,000	aC	1,483	aB	2,080	aA
Pec 2% + Eug 0.2%	0,750	aB	0,937	bB	1,310	aA	1,263	bA	1,440	aA	0,837	bC	1,970	aA	0,000	aD	1,720	aB	1,913	aAB
Pec 2% + Cit 0.15%	0,733	aC	1,020	bBC	1,267	aAB	1,190	bA	1,410	aB	0,770	bB	2,100	aA	1,307	aAB	1,833	aAB	1,763	aAB

7.19. HPLC – Malic acid for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test ($p < 0,05$). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Malic Acid

	Citric Acid									Sodium Chlorite								
	0		2		4		6		8	0		2		4		6		8	
Control	4,013	bB	4,547	aB	4,843	aB	6,527	aA	4,553	aB	4,013	bB	4,547	bB	4,843	aB	6,527	aA	4,553	bB
Alg 2% + Eug 0.1%	13,177	aA	9,633	bA	4,883	aB	3,967	cB	4,130	aB	3,987	bAB	4,967	abAB	1,860	abB	4,617	bAB	5,830	aA
Alg 2% + Cit 0.15% + Eug 0.1%	8,037	abB	10,950	bA	3,817	bC	4,377	bcC	4,783	aC	5,637	bA	5,680	abA	0,000	bC	4,547	bB	5,577	aA
Pec 2% + Eug 0.2%	5,683	bB	11,443	bA	4,957	aBC	4,563	bBC	4,100	aC	4,397	bA	4,903	abA	1,357	abB	5,627	abA	5,370	aA
Pec 2% + Cit 0.15%	4,130	bB	9,480	bA	5,100	aB	4,153	bcB	4,057	aB	9,770	aA	6,250	aB	3,717	abB	5,807	abB	4,743	bB

7.20. Sugars – Fructose quantification for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test ($p < 0,05$). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Sugars -Fructose

	Citric Acid									Sodium Chlorite								
	0		2		4		6		8	0		2		4		6		8	
Control	9,773	bC	9,950	bC	10,827	cC	14,317	aA	12,873	aB	9,777	bC	9,950	cC	10,827	bC	14,317	aA	12,873	aB
Alg 2% + Eug 0.1%	14,897	aA	12,273	abB	12,667	aB	11,900	bBC	10,767	bC	9,773	bC	9,950	cC	10,827	bC	14,317	aA	12,873	aB
Alg 2% + Cit 0.15% + Eug 0.1%	10,020	bB	15,580	aA	11,910	abB	12,193	bB	11,373	bB	13,830	aB	15,223	aA	13,717	aB	12,707	cC	13,707	aB
Pec 2% + Eug 0.2%	10,327	bA	11,396	abA	12,333	abA	11,520	bA	10,530	bA	14,807	aA	13,153	bB	12,937	aB	13,560	bB	13,207	aB
Pec 2% + Cit 0.15%	14,810	aA	13,257	abB	11,650	bC	10,870	bC	11,373	bC	13,553	aA	14,110	abA	12,790	aA	12,960	bcA	12,610	aA

7.21. Sugars – Glucose quantification for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Sugars -Glucose

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	8,183	aAB	8,250	aA	7,660	bAB	8,000	aAB	6,710	aB	8,183	aAB	8,250	aA	7,660	cAB	8,000	aAB	6,710	aB
Alg 2% + Eug 0.1%	8,377	aA	8,253	aB	8,397	aB	8,020	aB	7,037	aA	7,250	aB	5,903	aB	9,510	aA	7,140	bB	6,300	aB
Alg 2% + Cit 0.15% + Eug 0.1%	7,647	aA	7,343	aAB	7,207	bAB	5,070	bBC	4,160	bC	7,250	aB	5,903	aB	9,510	aA	7,140	bB	6,300	aB
Pec 2% + Eug 0.2%	5,210	bA	6,133	aA	5,990	cA	4,793	bA	4,227	bA	7,540	aA	7,373	aA	8,370	bA	8,060	aA	7,147	aA
Pec 2% + Cit 0.15%	7,770	aA	7,757	aA	5,043	dC	4,923	bC	4,293	bC	6,757	aC	9,627	aA	8,043	bcB	7,697	aBC	6,890	aC

7.22. Sugars – Sucrose quantification for Citric Acid and Sodium Chlorite registered during storage. Letters indicate significant difference between means, as showed by Duncan test (p< 0,05). Lower case letters were used to show differences within coatings in each period. Upper case letters were used to show differences within periods in each coating.

Sugars -Sucrose

	Citric Acid										Sodium Chlorite									
	0		2		4		6		8		0		2		4		6		8	
Control	4,887	aB	3,563	aC	3,353	cC	5,977	aA	5,587	aAB	4,887	aC	3,563	bB	3,353	cC	5,977	aA	5,587	aAB
Alg 2% + Eug 0.1%	5,390	aA	5,103	aAB	5,277	aAB	3,900	bcBC	3,660	bcC	5,283	aB	5,417	aB	5,807	bA	5,273	bB	4,960	bC
Alg 2% + Cit 0.15% + Eug 0.1%	3,963	bA	4,730	aA	4,470	bA	3,973	bA	3,830	bA	5,810	aB	5,007	aB	6,677	aA	5,217	bB	4,977	bB
Pec 2% + Eug 0.2%	3,910	bAB	4,623	aA	4,580	bA	3,850	bcAB	3,587	bcB	6,003	aA	5,650	aA	5,860	abA	5,337	bA	4,930	bcA
Pec 2% + Cit 0.15%	5,370	aA	5,180	aA	4,270	bB	3,707	cC	3,560	cC	4,957	aAB	5,663	aA	5,070	bAB	5,187	bAB	4,633	cB

7.23. Significant values within Citric Acid and Sodium Chlorite for each assay (p< 0,05).

Parameters	Citric Acid Mean	Sodium Chlorite Mean	Sig.
L	71.6517	75.3468	0***
a	-1.0168	-3.1487	0***
b	23.8345	19.7111	0***
Hue	94.3709	92.4115	0,573 N.S.
Chroma	24.2478	20.1236	0***
Weight	0.8423	0.6145	0,02**
Firmness	17.4278	20.7672	0***
TSS	10.5642	11.1808	0,003**
CO2	1283.9477	1320.9583	0,811 N.S.
Ethylene	10.2713	12.0773	0,461N.S.
TEAC	79.734	82.4355	0,9 N.S.
Phenols	98.092	117.6582	0,182N.S.
Flavonoids	3.724	1.2392	0***
ORAC	74.915	77.9102	0,079 N.S.
PPO	46.7048	47.1167	0,935 N.S.
HPLC - Oxalic acid	1.1583	1.4793	0,004*
HPLC - Malic acid	6.271	4.7345	0,003**
Fungus	0.0675	0.0677	0,997 N.S.
Mesophiles	0.884	0.7307	0,289 N.S.
Sugars - Fructose	12.064	13.0305	0,002**
Sugars - Glucose	6.3825	7.4855	0***
Sugars - Sacarose	4.3467	5.3858	0***

*** - p< 0,001 = highly significant differences
** - p<0,01 = high significant differences
* - p<0,05 = significant differences
N.S. – p>0,05 = No significant difference within means.