UNIVERSIDADE DO ALGARVE

Innovative edible coatings to improve storage of small fruits and fresh-cut

Adriana Cavaco Guerreiro

Tese para obtenção do grau de Doutor em Ciências Agrárias

Especialidade de Ciência dos Alimentos

Trabalho efectuado sob a orientação de:

Professora Doutora Maria Dulce Carlos Antunes

Professora Doutora Maria da Graça Costa Miguel

Professora Doutora Maria Leonor Faleiro

2015

UNIVERSIDADE DO ALGARVE

Innovative edible coatings to improve storage of small fruits and fresh-cut

Adriana Cavaco Guerreiro

Tese para obtenção do grau de Doutor em Ciências Agrárias

Especialidade de Ciência dos Alimentos

Trabalho efectuado sob a orientação de:

Professora Doutora Maria Dulce Carlos Antunes

Professora Doutora Maria da Graça Costa Miguel

Professora Doutora Maria Leonor Faleiro

2015

Título tese: Innovative edible coatings to improve storage of small fruits and fresh-cut

Autora: Adriana Cavaco Guerreiro

Este trabalho foi realizado na condição de bolseira de doutoramento no projecto:

PTDC/AGR-ALI/121085/2010 - INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut fruits.

Declaração de autoria de trabalho

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

_____

(Adriana Cavaco Guerreiro)

Agradecimentos

Elaborar uma tese de Doutoramento é sempre um processo único de aprendizagem. O enriquecimento científico, profissional e pessoal foi uma constante nestes últimos anos. Foi necessários ultrapassar várias etapas, que sem a ajuda e conhecimentos daqueles que tornaram a realização deste trabalho possível, seria impossível de contornar, por isso, não poderia deixar de lhes expressar a minha profunda gratidão.

À professora Doutora Maria Dulce Carlos Antunes, orientadora desta tese, pelo grande rigor científico, pelo sentido prático, pela exigência, pelo empenho e interesse demonstrado desde o primeiro dia, pela amizade e atenção. Muito obrigada por esta oportunidade.

À professora Doutora Maria da Graça Costa Miguel, coorientadora, sempre disposta a ensinar, pela sua contribuição, apoio e estímulo, sem os quais não me teria sido possível desenvolver este trabalho.

À professora Doutora Maria Leonor Faleiro, coorientadora, por ter partilhado comigo a sua área de investigação, pelo rigor, incentivo, acompanhamento científico e pela amizade. Estarei sempre grata.

À Professora Olga Martín-Belloso da Universidade Lleida (Espanha) e ao Professor Leon Terry, Universidade de Cranfield (Ingraterra), por me terem recebido nas suas equipas de trabalho e terem partilhado comigo os seu conhecimentos, que me permitaram evoluir e contribuir para a elaboração deste trabalho, o meu profundo agradecimento.

Aos amigos que estiveram comigo ao longo desta etapa e que direta ou indiretamente contribuíram para que eu chegasse até aqui. Agradeço em especial à Doutora Custódia Gago que se revelou uma grande amiga e excelente companhia, que

foi o meu braço direito nesta jornada, sempre foi capaz de me animar e muito me motivou e incentivou.

A todas as pessoas que nas diferentes fases deram apoio laboratorial para a execução deste trabalho: Luísa Coelho, Liseta Viegas, João Duarte, Elsa Rodrigues, Ana Isabel Viera, Adriano Martins e Vasco Afonso.

Um agradecimento muito especial ao meu David por toda a paciência que teve comigo, pelo amor e carinho e pelo apoio que me deu mesmo nos momentos mais difíceis, sem ele teria sido muito mais difícil: obrigada por tudo!

E, finalmente, agradeço aos meus pais por todo o amor, carinho, apoio e pela paciência que tiveram que ter nos dias menos bons. Agradeço pelo incentivo para continuar a estudar e por nunca se terem arrependido do esforço e por sempre me terem dado as melhores condições e nunca terem deixado que nada me faltasse.

Muito Obrigada!

Resumo

O consumo de frutos frescos e saladas tem vindo a aumentar nos últimos anos devido à maior consciência dos consumidores para uma alimentação saudável. Devido ao estilo de vida do consumidor, o interesse pelos frutos frescos tem vindo a crescer, uma vez que são apresentados ao consumidor num estado que permite o seu consumo direto e imediato.

Os frutos frescos, particularmente os frutos minimamente processados, são altamente perecíveis e as perdas podem ser de grande dimensão se não se aplicarem as técnicas corretas de pós-colheita. A elevada perecibilidade destes produtos requer o estudo de técnicas, seguras para a saúde e o ambiente, que minimizem os processos que conduzem à senescência rápida, para que os mesmos possam permanecer de boa qualidade por um período maior, de modo a ser rentável para as empresas.

Nos frutos, temperaturas baixas combinadas com outros fatores como as coberturas edíveis e óleos essenciais têm por objetivo reduzir os processos metabólicos, que conduzem à senescência. Dado que os componentes de óleos essenciais eugenol e citral têm propriedades antioxidantes e antimicrobianas, estes foram testados em conjunção com coberturas edíveis (alginato e pectina) com diferentes concentrações e em vários frutos [medronho, framboesa, morangos e uma variedade de maçã portuguesa (*Malus domestica* Borkh.) cv. 'Bravo de Esmolfe' minimamente processada] para selecionar os mais efectivos na conservação destes frutos, sem alterar a sua aceitabilidade e isentos de citotoxicidade.

Nos últimos tempos tem-se explorado a utilização alternativa dos vários produtos agrícolas para além dos habitualmente usados. No caso do medronheiro (*Arbutus unedo* L.), o seu fruto, colhido de arbustivas que existem espontaneamente nas florestas, é atualmente usado em Portugal quase exclusivamente para fabrico de

aguardente. Nos últimos anos o interesse por esta cultura tem aumentado e vários pomares foram implantados.

A maçã é um dos frutos mais consumidos a nível mundial. A maçã 'Bravo de Esmolfe' é uma cultivar portuguesa com Denominação de Origem Protegida (DOP), bastante apreciada pelas suas características organoléticas únicas. No entanto, quando minimamente processada oxida fácilmente e deteriora-se com uma grande rapidez, sendo importante encontrar soluções que permitam contornar estes constrangimentos. As coberturas edíveis apresentam-se como uma solução viável.

As framboesas (*Rubus idaeus* L.) e os morangos (*Fragaria x ananassa* Duch*)* são de grande importância económica e amplamente consumidos em fresco, congelados ou em formas processadas. Para o consumo em fresco, as framboesas e os morangos são colhidos quando estão com uma cor vermelho brilhante e podem ser armazenadas a baixas temperaturas (0-2ºC) por apenas alguns dias.

O objetivo do presente trabalho foi testar o efeito dos componentes dos óleos essenciais, citral e eugenol, usados como aditivos de coberturas edíveis à base de alginato e de pectina na preservação da qualidade nutricional e sensorial e na segurança alimentar dos frutos medronho, framboesa, morango e maçã minimamente processada (*Malus domestica* Borkh.) cv. 'Bravo de Esmolfe'. Este trabalho foi iniciado com um breve estudo de diferentes hidrocoloides comerciais aplicados a um fruto minimamente processado (manga) durante a sua vida de prateleira a 4 ºC. Foram estudados alguns parâmetros qualitativos e quantitativos, que permitiram selecionar os dois melhores hidrocoloides (alginato e pectina) a utilizar nos trabalhos seguintes.

Inicialmente pretendeu-se encontrar a concentração mínima inibitória (CMI) dos componentes de óloes essencias a ser aplicados para ser usada nas películas. Os parâmetros gerais de qualidade [firmeza, cor, teor de sólidos solúveis, perda de peso], os

parâmetros de qualidade nutracêutica [açúcares e ácidos orgânicos, fenóis totais, atividade antioxidante [DPPH (*1,1-diphenyl-2-picrylhydrazyl*), ABTS *(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid*) e ORAC (oxygen radical absorbance capacity)], o crescimento microbiano, assim como as trocas gasosas (CO_2 e etileno) e as concentrações de etanol e acetaldeído foram avaliados ao longo do tempo de armazenamento. A apreciação por consumidores foi avaliada por um painel de provadores.

No medronho iniciámos o estudo com 11 combinações de coberturas edíveis (alginato 1 e 2% em combinação com citral e eugenol a valores CMI de 0.15 e 0.1%, respetivamente, e o dobro do valor CMI). Após 0, 14 e 28 dias foram retiradas amostras e realizadas análises físico-químicas [Cor (CIE L* hº C*), firmeza, teor de sólidos solúveis, perda de peso e atividade antioxidante pelo método TEAC (*Trolox equivalent antioxidant capacity*)], evolução da carga microbiana (microrganismos aeróbios mesófilos, psicrófilos, fungos e leveduras) e painel de provadores. No final deste estudo, selecionaram-se as duas melhores películas que foram novamente aplicadas e estudadas. Neste caso, para além das análises acima referidas foram analisados os seguintes parâmetros de qualidade nutracêutica [açúcares e ácidos orgânicos, fenóis totais, atividade antioxidante [TEAC e ORAC], produção de CO_2 e etileno, etanol e acetaldeído) aos 0, 14, 21 e 28 dias de armazenamento a 0.5 ºC. Do trabalho efetuado concluiu-se que das películas edíveis utilizadas, a que melhor preservou as características do fruto foi o alginato 1% + eugenol 0,1% + citral 0,15%.

Para a maçã minimamente processada, o estudo foi iniciado com 24 combinações de coberturas edíveis enriquecidas com componentes de óleos essenciais (alginato e pectina a 1 e 2% e eugenal e citral a CMI e dobro da CMI), e após 0, 7 e 14 dias foram retiradas amostras e realizadas análises físico-químicas [Cor (CIE L* hº C*), firmeza,

teor de sólidos solúveis, perda de peso, TEAC], evolução microbiana e avaliação por painel de provadores. Neste caso foram seleccionadas as 4 melhores formulações (duas de alginato e duas de pectina) que foram novamente aplicadas à maçã minimamente processada. Neste estudo, e tendo em atenção, o rápido escurecimento da maçã, optou-se por testar três agentes anti-escurecimento conhecidos pelo seu efeito antioxidante (ácido ascorbico, ácido citrico e clorito de sódio), sendo as amostras analisadas aos 0, 2, 4, 6 e 8 dias. Em cada uma das amostragens foram realizadas várias análises qualitativas e quantitativas, nomeadamente: cor (L*, hº e C*), firmeza, teor de sólidos solúveis, perda de peso, actividade antioxidante (TEAC, DPPH e ORAC), fenóis totais, flavonoides, etanol, acetaldeído, etileno, CO_2 e a determinação da carga microbiana (microrganismos aeróbios mesófilos, psicrófilos e fungos e leveduras) e avaliação por painel de provadores. As coberturas edíveis aplicadas foram eficientes na preservação da maçã 'Bravo de Esmolfe' minimamente processada durante oito dias a 4ºC, sendo alginato 2% + citral 0.15% + eugenol 0.1% a melhor seguida de alginato 2% + eugenol 0.1%.

Nos estudos realizados no morango e framboesa foram formuladas 24 combinações de coberturas edíveis enriquecidas com componentes de óleos essenciais (alginato e pectina a 1 e 2% e eugenol e citral a CMI e dobro da CMI), para cada fruto, e após 0, 14 e 21 dias foram retiradas amostras e realizadas análises físico-químicas [Cor (CIE L * h º C*), firmeza, teor de sólidos solúveis, perda de peso, TEAC], evolução microbiana e painel de provadores. Após análise dos resultados obtidos foram seleccionadas as 4 melhores formulações (duas de alginato e duas de pectina) que foram novamente aplicadas aos morangos e framboesas. Em cada uma das amostragens (0, 5, 10 e 15 dias) foram realizadas várias análises qualitativas e quantitativas, nomeadamente: cor (L*, hº e C*), firmeza, teor de sólidos solúveis, perda de peso,

, etanol, acetaldeído, etileno, CO_2 e a determinação da carga microbiana (microrganismos aeróbios mesófilos, psicrófilos e fungos e leveduras). Foram também realizados painéis de provadores em todos os ensaios. Após análise dos resultados obtidos concluiu-se que as duas melhores coberturas edíveis para o morangos foram pectina 2% + citral 0.15% e alginato 2% + citral 0.15% + eugenol 0.1%, enquanto que para a framboesa as melhores formulações foram pectina 1% + citral 0.15% + eugenol 0.1% e alginato 2% + citral 0.15%.

De modo a reduzir o número de formulações de películas edíveis a aplicar na pós-colheita de frutos, dos resultados deste estudo podemos recomendar para morangos alginato 2% + Citral 0.15%+ eugenol 0.1%, maçã 'Bravo de Esmolfe' minimamente processada alginato 2% + eugenol 0.1% com ácido ascórbico, framboesa petina 1% + citral 0.15% + eugenol 0.1% e para o medronho alginato 1% + citral 0.15%+ eugenol 0.1%.

Palavras-Chave: alginato; pectina; citral; eugenol; maçã; pequenos frutos

Abstract

Edible coatings enriched with essential oils or their constituents have been studied for their effect on increasing food storage life. The objective of the present study was to find the best edible coating formulations based on polysaccharides enriched with essential oils compounds to increase storage life of small fruit and fresh-cut. In the first year of this study, edible coating formulations based on alginate and pectin enriched with citral and eugenol were tested on Arbutus unedo berries, strawberries, raspberries and fresh-cut 'Bravo de Esmolfe'apples. General quality parameters [color CIE ($L^*h^oC^*$), firmness, soluble solids content (SSC), weight loss, trolox equivalent antioxidant capacity (TEAC), microbial growth and taste panels] were evaluated through cold storage. From them, two edible coatings which better preserved shelf-life for each polysaccharide (alginate and pectin) were chosen. The previous selected edible coatings were tested for cytotoxicity, then applied to the same fruit for studying their effect on nutritional and sensory parameters [color CIE ($L^*h^oC^*$), firmness, soluble solids content, weight loss, microbial growth, taste panels, phenol compounds (total phenols, flavonoids, anthocyanins), sugars, organic acids, antioxidant activity (TEAC, ORAC (oxygen radical absorbance capacity) and DPPH (*1,1-diphenyl-2-picrylhydrazyl*) and taste panels]. Ethylene and CO_2 production as well as ethanol and acetaldehyde were also measured. The edible coating which better preserved fruit quality while increasing storage life was selected for commercial recommendation. Those were for arbutus berries alginate 1% + eugenol 0.1% + citral 0.15%, for fresh-cut 'Bravo de Esmolfe' apple was alginate 2% + eugenol 0.1% plus dip in ascorbic acid, strawberries was alginate 2% + citral 0.15 % + eugenol 0.1% and for raspberrieswas pectin 1% + citral 0.15%+ eugenol 0.1%.

Key-Words: alginate; pectin; citral; eugenol; apple; small fruits

Aims and Chapters

The present work aim was find the best edible coating formulations for preserving quality and increase shelf-life of arbutus berries, raspberries, strawberries and a fresh-cut apple cv. 'Bravo de Esmolfe'. To achieve this goal was used the following approach. First were selected the polysacharydes which better served as base for the edible coatings as well as their best concentrations. Then citral and eugenol were selected due to their antimicrobial and antioxidant properties. The minimum inhibitory concentrations (MIC) of those essential oils compounds against main food borne pathogens were determined. After that, combination of the edible coatings based on the best polysacharydes, alginate and pectin, at two concentrations enriched with the essential oils compounds, citral and eugenol, were tested at MIC and double MIC for arbutus berries, strawberries, raspberries and fresh-cut 'Bravo de Esmolfe' apple. General quality parametes were measured [color CIE ($L^*h^oC^*$), firmness, soluble solids content, weight loss, trolox equivalent antioxidant capacity (TEAC), microbial growth and taste panels] Then the 2 best edible coatings either for alginate or pectin were selected for a second group of experiments. Those edible coatings were tested for cytotoxicity. In those experiments, the previously selected edible coatings were tested again for each fruit species by checking their effect on main general and nutritional quality parameters [color CIE ($L^*h^oC^*$), firmness, soluble solids content, weight loss, microbial growth, taste panels, phenol compounds (total phenols, flavonoids, anthocyanins), sugars, organic acids, TEAC, ORAC (oxygen radical absorbance capacity) and DPPH (*1,1-diphenyl-2-picrylhydrazyl*), , ethylene, CO_2 production and ethanol and acetaldehyde] preservation through storage, to come out with the best edible coatings for each fruit.

Ten chapters were developed.

Chapter I provides a general introduction, it presents an overview of the state-of-the-art. Quality concepts, postharvest of fruit, edible coatings, polysaccharides and essential oils are the main subjects in this study.

The objective of Chapter II was to compare the effectiveness of alginate, pectin, carboxymethyl celluloseand chitosan based coatings in preserving quality of fresh-cut mango. Effects of the coatings on antioxidant properties, β-carotene, firmness and colour were evaluated for 7 days at 4ºC, since it is known that the different origins and concentrations of edible coatings as well as food matrix have a relevant effect on fresh-cut fruits.

Chapter III has the objective to determine the effect of two essential oil constituents (citral and eugenol), when incorporated in polysaccharide edible coatings, on post-harvest quality, safety and shelf-life-extension of *Arbutus unedo* fruits (named either strawberry tree fruit or arbutus berries).

Chapter IV is the continuation of the previous chapter, here more information and analysis on the use of alginate as an edible coating to preserve *A. unedo* berries fruit quality, was the main objective of this paper to select the best for commercial recommendation.

The objective of Chapter V was to determine the effect of citral and eugenol, when incorporated in polysaccharide edible coatings based on alginate or pectin, on the quality, safety and shelf-life extension of fresh-cut 'Bravo de Esmolfe' apple. At Chapter VI the objective was to evaluate the best edible coatings from previous chapter and add anti-browning agents to evaluate their effect on increasing shelf-life of 'Bravo de Esmolfe' fresh-cut apple.

In Chapter VII and Chapter VIII was studied the effect of edible coatings enriched with essential oils components on strawberries storage life. First were studied

many edible coatings combinations effect on general quality parameters through storage and were chosen the best edible coatings formulations that better preserve this fruit. Then those best edible coatings formulations were studied for their effect in maintaining most sensorial and nutritional quality parameters through strawberries storage to select the best.

Raspberries were studied in chapters IX and X. At chapter IX we studied the combinations of edible coatings formulation based on alginate and pectin enriched with the essential oils components (citral and eugenol) effect on raspberries storage. The four edible coatings chosen at chapter IX were used in chapter X in a new experiment, studying more nutritional and sensorial parameters in order to select the best formulation who provides the uphold at storage of raspberries.

Finally, Chapter XI provides a synthesis of the results of the preceding chapters and futures taking into account the main contributions of this work.

Contents

List of Figures

List of Tables

Acronyms and Symbols

AA- Ascorbic Acid

AAPH- 2,2'-Azobis-2-methyl-propanimidamide, dihydrochloride

ABTS - 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

AL- Sodium Alginate

A_0 - Absorbance of the control

A_1 - Absorbance in the presence of the sample

C -Celsius

Ca^+ - Calcium

$CaCl_2$ – Calcium Chlorite

Cit - Citral

CFU - Colony forms unit

CH- Chitosan

CMI – Concentração minima inibitória

CMC- Carboxil Methyl Cellulose

CO_2 – Carbon dioxide

DPPH - 1,1-diphenyl-2-picrylhydrazyl

EC- Edible Coating

EO – Essential oils

EOC – Essential oils components

Eug - Eugenol

GRAS – Generally recognized as safe

HPLC - High-performance liquid chromatography

Kg- Kilogram

MAP –M odified atmosphere packaging

MCP - Methylcyclopropene

Min – Minute

mg - Miligram

mL – Mililiter

mmol- Milimol

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NaCl - Sodium chloride

NaOCl - Sodium hypochlorite

NaOH - Sodium hydroxide

nm- Nanometer

O_2 – Oxygen

ORAC- Oxygen Radical Absorbance Capacity

PBS –Phosphate Buffer Solution

PCA – Plate Count Agar

PE- Pectin

PHA - Polyhydroxyalkonoates

TE- Trolox Equivalent

TEAC - Trolox Equivalent Antioxidant Activity

Trolox- 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

s- Seconds

SSC – Solid Soluble Content

µg - Microgram

µL – Micro liter

µM- Micro molar

µmol –Micro mole

Chapter I

General Introduction

1. Fruit Quality and Postharvest

The quality of food product depends on its organoleptic, nutritional, and hygienic characteristics, but these can change during storage and commercialization. These alterations are mainly due to the interactions between food and the surrounding environment (Debeaufort et al., 1998).

Quality is a subjective concept, which varies with the type of consumers, depending, for example, on nationality, age and eating habits, what may be relevant for some, for others it may not be (Kader, 2008). However, there is a consensus that factors, such as visual appearance, texture, flavor, absence of defects, nutritional value, and more recently safety (chemical and microbiological contamination), are part of a set of attributes, which define the quality (Table 1.1).

Table 1.1. Major quality attributes of fresh fruits and vegetables (adapted from Lin and Zhao, 2007)

Quality factor	Primary concerns
Appearance (visual)	Size
	Shape and form
	Color, intensity, uniformity
	Gloss
	Defect
Texture (mouth-feel)	Firmness/softness
	Crispness
	Juiciness
	Toughness(fibrousness)
Flavor(taste, aroma)	Sweetness
	Acidity
	Astringency
	Bitterness
	Volatile compounds
Nutritional value	Vitamins
	Minerals
Safety	Toxic substances
	Chemical contaminants
	Microbial contamination

Fruits and vegetables undergo many physiological changes during postharvest storage, including tissue softening, increase in sugar level, and decrease in organic acid levels, degradation of chlorophyll accompanied by the synthesis of anthocyanins or carotenoids upon maturation, production and losses of volatile flavor compounds,

decrease in phenolic and amino acid contents, and breakdown of cell materials due to respiration (Lin and Zhao, 2007).

Eating quality of fresh-cut fruit products is not only influenced by the stage of ripeness at cutting but also is highly dependent on the postharvest history of fruit before processing (Oms-Oliu et al., 2010). Operations including peeling, coring, cutting and/or slicing are critical to delimit the shelf life of fresh-cut fruit commodities. Wounding stresses result in metabolic activation, becoming apparent with increased respiration rate and, in some cases, ethylene production. During mechanical operations, cut surfaces are damaged, releasing enzymes which spread through the tissue and come into contact with their substrates (Soliva-Fortuny and Martín-Belloso, 2003). The softening of fresh-cut fruit is mainly due to the enzymatic degradation of the cell wall, which is mainly composed of cellulose, hemicellulose and pectins. Enzymes, such as pectinmethylesterase (PME) and polygalacturonase (PG) generally play an important role in the fruit softening (Oms-Oliu et al., 2010). Wounding of fruit tissues induces a number of physiological disorders that need to be minimized to get fresh-like quality products. In fresh-cut fruits, the greatest hurdle to the commercial marketing is the limited shelf-life, which is due to excessive tissue softening and cut surface browning (Soliva-Fortuny and Martín-Belloso, 2003). Browning is also a major concern related to the extension of shelf-life of fresh-cut fruit, and strongly affects the consumer's purchase decision (Oms-Oliu et al., 2010).

Post-harvest diseases of fruits and vegetables are a major problem in produce storage and significantly affect the cost of food production and produce trade. Ready to sell fresh or processed food carries a higher value than the same crop in the field because of the cumulative cost of production, harvesting, storage, distribution, and sales (Maftoonazad et al., 2007).

Harvesting practices determine the extent of variability in maturity and physical injuries, once physical injuries lead to accelerated loss of water and vitamins and increased susceptibility to decay by fungi or pathogens during postharvest life (Sharma and Singh, 2000; Kader, 2002)

Temperature and relatively humidity directly affect postharvest respiration and transpiration of fruits and vegetables, with elevated temperature speeding up respiration, leading to increased ethylene production and high CO_2 level, and thus changes in flavor, taste, color, texture, appearance, and nutrients of the produce (Lin and Zhao, 2007). Appropriate postharvest handling operations should be applied, including controlling temperature (cooling) and relative humidity, atmosphere (O_2 and CO_2 levels), cleaning, waxing, and packaging applications (Sharma and Singh, 2000; Kader, 2002; Lin and Zhao, 2007).

Postharvest losses of fruit and vegetables depend on the species, harvest, transport and storage, and they may represent about 20-25% of total production in industrialized countries and more than 50% in developing countries, in which handling and storage techniques are not optimal (Manso, 2013).

Various postharvest physical, chemical and gaseous treatments may be applied to maintain fresh-like quality with high nutritional value and meet safety standards of fresh produce, these postharvest treatments are typically combined with appropriate management of storage temperatures (Table 1.2).

The food packaging material is an important factor in the food industry and it is dominated by petroleum-based polymers. Nevertheless, the research involving the production and characterization of edible biodegradable films and coatings has grown considerably, mainly due to the interest in minimizing the ecological impact caused by the use of synthetic non-biodegradable packaging materials (Marques, 2012).

Table 1.2. Overview of postharvest treatments of fresh produce (Adapted from Mahajan et al., 2014)

Postharvest Treatment	Benefits	Limitations	Commercial example application
Heat treatment	Reduction of chilling injury, delay of ripening, killing of critical insect contaminants and controls decay	High-energy costs and added labor	Potato, tomato, carrot, strawberry, asparagus, Broccoli, beans, kiwi, celery, lettuce, melon, Grape, plum, peach, spinach and rocket leaves.
Edible coating	Provides a partial barrier, minimizes moisture loss; establishes modified atmosphere; preserves colour and texture; retains natural aroma	Cost of scaling up, lack of edible materials with desired properties, regulatory challenges	Apples, pears, carrots, celery, strawberry andmushrooms.
Irradiation	Inhibits sprouting of tubers, bulbs and roots, meets quarantine requirements for export trade and recognized as a safe process	Capital intensive, lack of harmonization of regulations, slow consumer acceptance owing to perceived association with radioactivity	Potato, onion, strawberry and mango.
Antimicrobial and anti-browning agents	Retards browning, deterioration of texture and microbial growth	Inaccessible sites for treatments within fresh produce, such as calyx and wax area	Apple, strawberry, lettuce, melon, orange, prune, tomato, grapes and fresh-cut produce.
Nitric oxide	Inhibits ethylene biosynthesis, reduces respiration rate, water loss, browning, and lower incidence of postharvest diseases	Commercial application depends upon the development of a smart carrier/controlled release system for NO	Apple, banana, kiwifruit, mango, peach, pear, plum, strawberry, tomato, papaya, loquat, jujube fruit and bayberry.
Sulfur dioxide	Prevents postharvest decay	Higher concentration may induce injuries and sulfit e residues pose a health risk	Grapes, litchi, fig, banana, lemon, apple and blueberries.
Ozone	Easily incorporated into existing cold storage, washing system, better efficacy than chlorine	Does not penetrate natural openings, further research is needed to improve application	Apples, cherries, carrots, garlic, kiwi, onions, peaches, plums, potatoes and table grapes.
Ethylene	Triggers ripening process thereby improves fruit colour and quality	Need of optimum ethylene concentration, storage conditions for faster and more uniform ripening	Banana, avocado, persimmon, tomato, kiwifruit, mango and citrus fruits.
1-MCP	Maintains fruit cell wall integrity and peel colour, and develop aroma and flavour	It can increase susceptibility to CO2 injury and chilling disorders. Additional exposure time is required for fruit to recover its ability to ripen normally	Apple, avocado, banana, broccoli, cucumber, date, kiwifruit, mango, melon, nectarine, papaya, peach, pear, pepper, persimmon, pineapple, plantain, plum, squash and tomato.

Controlled atmosphere storage	Retards senescence, associated biochemical and physiological changes, reduction in decay severity	Capital intensive, fruit volumes must be high and extended storage periods are needed to make investment economical	Apple, pear, avocado, strawberry, cherry, cabbages, kiwifruit, avocados, persimmon, pomegranate, asparagus, banana, broccoli, cranberry, mango, melon, nectarine, peaches and plums.
MAP	Delay in respiration, senescence, and slows down rate of deterioration	Condensation inside the package resulting in microbial growth and decay of produce	Strawberry, banana, cherries, carrots, fresh-cut fruits, salad mix and leafy green vegetables.

2. Edible coatings

The use of edible coatings on fruits and minimally processed fruits consists on the application of a layer of any edible material on the surface of a cut-fruit with the purpose of providing it with a modified atmosphere, retarding gas transfer, reducing moisture and aroma loss, delaying color changes, and improving the general appearance of the product through storage (Olivas and Barbosa-Cánovas, 2005). 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 (Ahvenainen, 1996).

The utilization of edible films in fruits has been conducted for centuries with the purpose of increasing storage time(Pavlath and Orts, 2009). For example, Chinese people since the twelfth and thirteenth centuries used wax for coating oranges and lemons(Baldwin et al., 2011). However, the application of coatings was not only reserved to fruits, because since, at least in the sixteenth century it was reported that they also applied fat on meat cuts to prevent shrinkage (Debeaufort et al., 1998). More recently, in nineteenth century, other types of materials were suggested for preserving meat and other foodstuffs, such as gelatin films (Debeaufort et al., 1998; Olivas and

Barbosa-Cánovas, 2005). In the same century, sucrose was initially applied as an edible protective coating on nuts, almonds, and hazelnuts to prevent oxidation and rancidness during storage (Debeaufort et al., 1998). The more important application of edible films and coatings until now, and particularly since the 1930s, concerns the use of an emulsion made of waxes and oil in water that was spread on fruits to improve their appearance, such as their shininess, color, softening, onset of mealiness, carriage of fungicides, and to better control their ripening and to retard water loss (Debeaufort et al., 1998; Pavlath and Orts, 2009).

Edible films have been widely used since then on whole fruits like orange, grapefruit, lemon, apple, and pear, mainly with the purpose of reducing water loss, with waxes being the most commonly employed materials (Olivas and Barbosa-Cánovas, 2005; Rojas-Graü et al., 2009a; Dhall, 2013).

Edible coatings from renewable sources, including lipids, polysaccharides and proteins, can function as barriers to water vapor, 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 and Zhao, 2007). Defined as a thin layer of edible material, which have been used as revetment of the food, its function is to inhibit or reduce water loss, control respiration (O_2 and CO_2) and aromas, promoting semi-permeable barriers (Table 1.3) (Olivas and Barbosa-Cánovas, 2005; Marques, 2012).

Table 1.3. Potentials and requirements for edible coatings adapted from (adapted from Olivas and Barbosa-Cánovas, 2005).

Potential uses of edible coatings	Produce a modified atmosphere in the fruit Reduce decay Delay ripening of climacteric fruits Reduce water loss Delay color changes Improve appearance Reduce aroma loss Reduce exchange of humidity between fruit pieces Carriers of antioxidants and texture enhancers Carriers of volatile precursors Impart color and flavor Carriers of nutraceuticals
Requirements for edible coatings	Stability under high relative humidity GRAS (generally recognized as safe) components Good water vapor barrier Efficient oxygen and carbon dioxide barrier Good mechanical properties Adhesion to the fruit Colorless and tasteless1 Pleasant to taste Physico-chemical and microbial stability Reasonable cost

Low storage temperature and modified atmosfere 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)

The basic materials used to produce edible and biodegradable coatings/films in food packaging are the ones directly extracted from biomass. The most commonly available are extracted from marine and agricultural products and they are based in proteins, polysaccharides and lipids. A schematic diagram of the types of bio-based polymers or biopolymers is shown in Figure 1.1. Bio-based polymers may be divided into three main categories based on their origin and production (Weber, 2000):

•Category 1: Polymers directly extracted/removed from biomass; examples are polysaccharides, such as starch and cellulose and proteins like casein and gluten.

•Category 2: Polymers produced by classical chemical synthesis using renewable bio-based monomers; a good example is polylactic acid, a biopolyester polymerised from lactic acid monomers (the monomers themselves may be produced via fermentation of carbohydrate feedstock).

•Category 3: Polymers produced by microorganisms (either wild strains or genetically modified); to date, this group of bio-based polymers consists mainly of polyhydroxyalkonoates (PHA), but developments with bacterial cellulose are in progress.

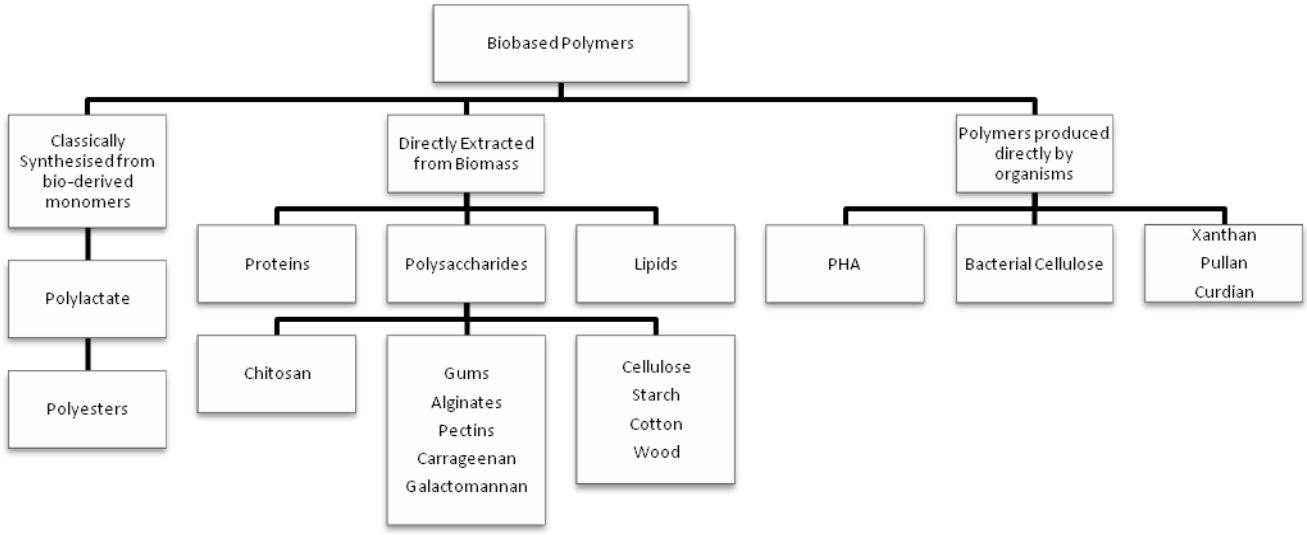


Figure 1.1 - Schematic representation of bio-based polymers based on their origin and method of production (Tuil et al., 2000).

Edible coatings do not intend to replace traditional packaging materials but they can additionally control moisture and gases and be supporters of additives (Campos et al., 2010). Edible coatings have been recognized for more innovative uses beyond their current uses and have a high potential to carry active ingredients, such as anti-browning agents, colorants, flavors, nutrients, spices and antimicrobial compounds that can extend product shelf-life and reduce the risk of pathogen growth on food surfaces (Zúñiga et al., 2012).

It is necessary to understand the mechanisms that cause fresh/fresh-cut produces spoilage, to understand the benefits of each edible coating and choose the most beneficial from a large quantity of options. 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 semi-permeable 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, flavors, nutrients and spices (Rojas-Graü et al., 2009b; Robles-Sánchez et al., 2013). The aim is to produce natural biopolymer-based coatings materials with specific properties, which may be consumed together with the food (Bravin et al., 2006). A great diversity of materials is used to produce edible coatings. They are extracted from marine and agricultural animals and plants e.g. lipids, polysaccharides and proteins. Edible coatings formulated with the desired properties can be utilized by the food industry to meet challenges associated with long term quality, market safety, nutritional value and economic production cost. With regard to the fresh products industry, the potential benefits of using edible coatings include:

1. Providing a moisture barrier on the surface of the product thus minimizing the problem of moisture loss. Moisture loss during postharvest storage of fresh products leads to weight loss and changes in texture, flavor and appearance;
2. Providing sufficient gas barriers for controlling gas exchange between the fresh product and its surrounding atmosphere, slowing down respiration and delaying deterioration.

3. The gas-barrier function could in turn retard the enzymatic oxidation and protect the fresh product from browning discoloration and texture softening during storage;
4. Restricting the exchange of volatile compounds between the fresh product and its surrounding environment, again by providing gas barriers, which prevents both the loss of natural volatile flavor compounds and color components from fresh product and the acquisition of different odors;
5. Protecting the product from physical damage caused by mechanical impact, pressure, vibrations and other mechanical factors;
6. Acting as carriers of functional ingredients, such as antimicrobial and antioxidant agents, nutraceuticals, and color and flavor ingredients for reduction of microbial loads, delaying oxidation and discoloration, and improving the overall quality (Rooney, 2005).

A schematic representation of the functional properties and potential benefits of an edible coating on fresh fruits and vegetables is presented in Figure 1.2.

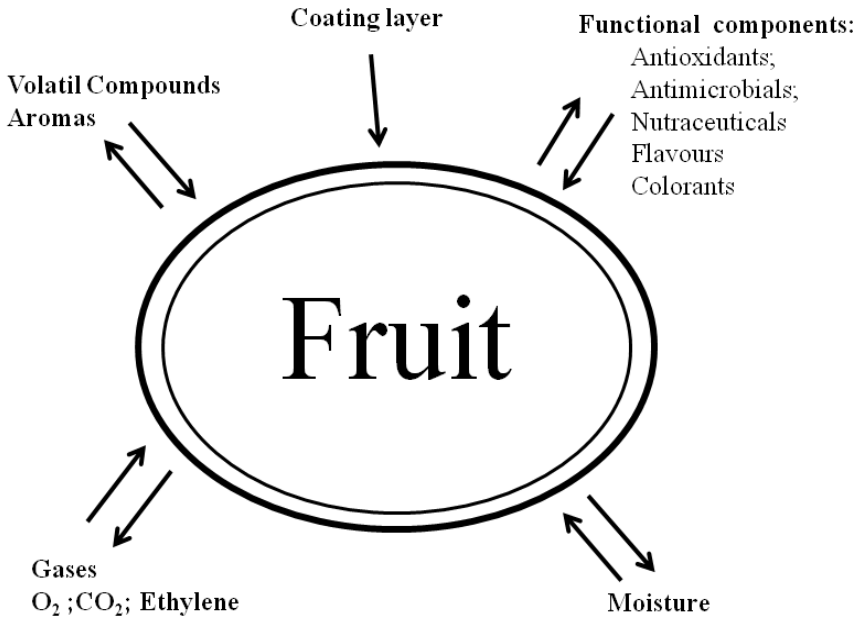


Figure 1.2. Functional properties of an edible coating on fresh fruits and vegetables (Lin and Zhao, 2007).

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).

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 coatings can be classified into three categories: hydrocolloids (such as proteins and polysaccharides), lipids (such as fatty acids, acylglycerol, waxes) and composites (Bourtoom, 2008).

Edible coating technology is a promising method to preserve the quality of fresh fruits and vegetables. Research and development efforts are leading to an improvement of the functional characteristics of the coatings, which depends on the properties of the fruit to be preserved or enhanced. The use of several edible coatings on different fruits and vegetables is listed in Table 1.4.

Table 1.4 Edible coating formulations using to improved quality of fruits and vegetables**.**

Coating Material	Composition	Fruit/vegetable	References
Aloe vera coating	5% aloe vera	Kiwifruit	(Benítez et al., 2015)
Brilloshine	Sucrose esters and wax (shine)	Apple, avocado, melons and citrus	(Baldwin, 1994)
Carboxymethyl cellulose(CMC)	1.5% CMC/1% Chitosan	Citrus, Guavas	(Arnon et al., 2014) (McGuire and Hallman, 1995)
Carnauba-shellac wax	carnauba-shellac wax and carnauba-shellac wax + tea tree oil and carnauba-shellac wax + lemongrass oil carnauba-shellac wax + rosewood oil	Apples Guavas	(Jo et al., 2014b) (McGuire and Hallman, 1995)
Chitosan	Chitosan and Tween 80 Chitosan (1%, w/v) Chitosan (2%, w/v) Chitosan (0.5–2.0%, w/v) Chitosan (0.02g/mL)	Strawberry, Apple, pear, plum, tomato, mango, papaya, Kiwifruit.	(Han et al., 2004) (Lee et al., 2003) (Gonzalez-Aguilar et al., 2008) (Dhall, 2013) (Benítez et al., 2015) (Li et al., 2015a)
FreshSeal™	Polyvinyl alcohol, starch and surfactant	Avocado, cantaloupe, mangoes and papaya	(Olivas and Barbosa-Cánovas, 2005)
Food Coat	composed of fatty acids derivatives and polysaccharides in alcohol solution)	Cactus Pear	(Palma et al., 2015)
Gellan	0.5% gellan	Apple Melon	(Rojas-Graü et al., 2008) (Oms-Oliu et al., 2008)
Gum arabic	Gum arabic 5%, 10%, 15% and 20% (w/v)	Tomato	(Ali et al., 2013)

Nature-seal™	Cellulose-based edible coating	Mango and tomato Pome fruit	(Nisperos-Carriedo et al., 1991) (Dhall, 2013)
Nu-coatFlo, Ban-seel	Sucrose esters of fatty acids and sodium salt of CMC	Apple, banana, guava melon, pear, and plum	(Baldwin, 1994)
Nutri-save	N,O carboxyl methyl chitosan	Apple, pear	(Olivas and Barbosa-Cánovas, 2005)
Pectin	2% pectin	Melon	(Oms-Oliu et al., 2008)
Pomfresh	Composed of a mixture of organic acids and antioxidant compounds	Cactus Pear	(Palma et al., 2015)
Prolong	Mixture of sucrose fatty acid esters, sodium CMC, and mono and diglycerides	Mango Pear	(Dhall, 2013) (Nisperos-Carriedo et al., 1991)
Semperfresh™	Sucrose esters with high proportion of short-chain unsaturated fatty acid esters, sodium salts of CMC, and mixed mono and diglycerides	Pears Apple Tomato Mango Blueberries Kiwifruit	(Olivas and Barbosa-Cánovas, 2005) (Nisperos-Carriedo et al., 1991) (Gonzalez-Aguilar et al., 2008) (Duan et al., 2011) (Fisk et al., 2008)
Sodium Alginate	Alginate/apple puree + (0.3–0.6% (w/v) vainillin or 1–1.5% (v/v) lemongrass or 0.1–0.5% (v/v) oregano oil) Alginate + (2.5% MA+ 0.7% lemongrass, or 0.3% cinnamon oil) Alginate + (2.5% MA+ 0.3% palmarosa oil) Alginate + lemongrass 0.1, 0.2 and 0.1%	Apples, melon, pineapple, kiwifruit, sweet cherry fruit, plum, strawberry, blueberries	(Rojas-Graü et al., 2007a) (Raybaudi-Massilia et al., 2008b) (Raybaudi-Massilia et al., 2008a) (Oms-Oliu et al., 2008) (Fan et al., 2009) (Díaz-Mula et al., 2012) (Valero et al., 2013) (Azarakhsh et al., 2014) (Benítez et al., 2015) (Montero-Calderón et al., 2008) (Duan et al., 2011)
Tal Prolong	Mixture of sucrose fatty acid esters, sodium CMC, and mono and diglycerides	Mango Pears	(Nisperos-Carriedo et al., 1991) (Olivas and Barbosa-Cánovas, 2005)
Zein	Corn zein protein	Tomato	(Zapata et al., 2008)

2.1. Polysaccharide-based coatings

Polysaccharides that have been evaluated or used for forming films and coatings include starch and starch derivatives, cellulose derivatives, alginates, carrageenan, various plant and microbial gums, chitosan, and pectinates; These coatings can be utilized to modify the internal atmosphere, thereby reducing respiration of fruits and vegetables (Nisperos-Carriedo et al., 1991; Bourtoom, 2008).

Due to the hydrophilic nature of polysaccharides, the advantages of using these materials are more apparent as a gas barrier rather than retarding water loss. However, certain polysaccharides, applied in the form of high-moisture gelatinous coatings, can

effectively retard moisture loss of food by functioning as sacrificing agents rather than moisture barriers (Bourtoom, 2008).

2.1.1. Sodium alginate based edible coating

Alginates, which are extracted from brown seaweeds of the *Phaephyceae* class, are salts of alginic acid, a natural anionic polysaccharide heteropolymers that consists of (1,4) linked β-D-mannuronate (M) and its C5-epimer, α-L-guluronate (G) residues, arranged in blocks of M-, G-,and alternating G/M domains (Gennadios et al., 1997; Bruchet and Melman, 2015). Calcium alginate is the most commonly ionically cross-linked hydrogel that is used in biomedical industry for wound treatment, cell encapsulation, and as a scaffold for tissue engineering (Kuo and Ma, 2001; Tan and Takeuchi, 2007; Boateng et al., 2008; Griffin and Kasko, 2012; Lee and Mooney, 2012; Bruchet and Melman, 2015). The dominant role in the cross-linking belongs to poly-guluronate domains that bind Ca^{2+}cations in egg-box fashion (Figure 1.3) (Grant et al., 1973; Li et al., 2007; Sikorski et al., 2007); (Bruchet and Melman, 2015).

Alginates have the ability to form uniform, transparent, water-insoluble and thermo-irreversible gels at room temperature, by cross-linking with di- or trivalent ions (Bruchet and Melman, 2015). Other properties of alginates are their high availability, biodegradability and low price compared to natural casings (Olivas et al., 2007; Comaposada et al., 2015).

Sensory evaluation data showed that alginate coatings fixed in calcium propionate solutions had better flavor than coatings fixed in $CaCl_2$ solutions, however, because calcium propionate has weaker ionizing properties than $CaCl_2$, immersion time in calcium propionate solution had to be longer to obtain coatings of similar strength to those fixed in a $CaCl_2$ solution (Bruchet and Melman, 2015).

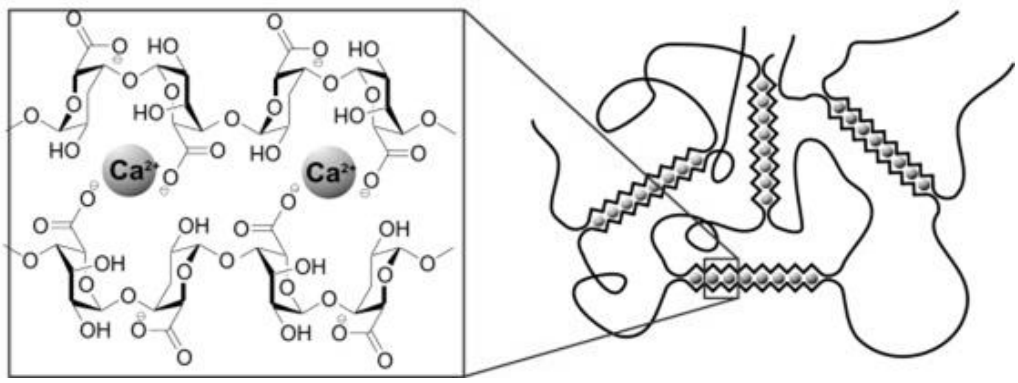


Figure 1.3. Structure of alginate and its binding of calcium cations in egg-box model (Grant et al., 1973; Bruchet and Melman, 2015).

2.1.2. Pectin based edible coating

Pectin is an anionic biopolymer soluble in water that shows low oxygen permeability (O_2P), it is one of the major structural polysaccharides of higher plant cells and is primarily composed of linear homogalacturonan (α-1,4-galacturonic acids) chains interspersed with branched rhamnogalacturonan (α-1,4-galacturonic acid to α-1,2-rhamnose) chains (the neutral sugar branches are attached through rhamnose residues) (Kang et al., 2007; Medeiros et al., 2012). Pectin is one of the main components in citrus by-products and it is considered that pectin is widely applicable in foodstuffs because of its various properties. The hydrocolloidal and polyelectrolytic properties of pectin determine its unique abilities, such as: strong water retention in colloidal systems together with their stabilization; easy plasticization with glycerol; due to its hydrophobic groups, ability to adsorb organic lipoid substances; an expressive cation exchange ability forming its restorative action (Baeva and Panchev, 2005).

Pectin is an important polysaccharide with applications in foods, pharmaceuticals, and a number of other industries. Its importance in the food sector lies on its ability to form a gel in the presence of Ca^{2+} ions or a solute at low pH. Depending on the pectin, coordinate bonding with Ca^{2+} ions or hydrogen bonding and hydrophobic

interactions are involved in the gel formation. In low-methoxyl pectin, gelation results from ionic linkage via calcium bridges between two carboxyl groups belonging to two different chains in close contact with each other. On the other hand, in high-methoxyl pectin, the cross-linking of pectin molecules involves a combination of hydrogen bonds and hydrophobic interactions between the molecules (Figure 1.4). A number of factors, namely pH, presence of other solutes, molecular size, degree of methoxylation, number and arrangement of side chains, and charge density on the molecule influence the gelation of pectin. In the food industry, pectin is used in jams, jellies, frozen foods, and more recently in low-calorie foods as a fat and/or sugar replacer. In the pharmaceutical industry, it is used to reduce blood cholesterol levels and gastrointestinal disorders. Although present in the cell walls of most plants apple pomace and orange peel are the two major sources of commercial pectin due to the poor gelling behavior of pectin from other sources (Thakur et al., 1997).

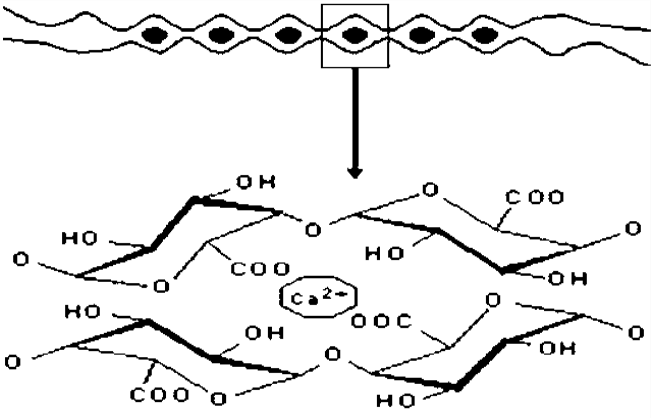


Figure 1.4. Structure of pectin and its binding of calcium cations in egg-box model (Shukla et al., 2011)**.**

2.2. Incorporation of active compounds in edible coatings

As mentioned above edible coatings have the capability to act as carriers for a wide range of food additives, including antioxidants, anti-browning agents, various antimicrobials, colorants, and flavors that can extend product shelf-life and reduce the

risk of pathogen growth on food surfaces and enhance the sensory quality of wrapped or coated food (Vargas et al., 2008).

The selection of incorporated active agents should be limited to edible, food grade compounds since they have to be consumed along with the coatings. In addition when some compounds are incorporated it is important to determine their impact on coatings functionality because there is a possibility of changing their basic functional properties, such as their vapor and gas barrier properties, or solute transport properties. The influence of an ingredient on coatings functionality depends on its concentration in the matrix, stability, chemical structure, degree of dispersion in the coating, and also on its interaction with the polymer (Suppakul et al., 2003).

The incorporation of essential oils on edible coatings has been investigated and the results show promising results on the maintenance of fruits proprieties and improvement of shelf-life.

2.2.1. Essential oils and essential oils components

Essential oils (EOs) are odorous, volatile products of an aromatic plant's secondary metabolism, normally formed in special cells or groups of cells. EOs are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) and can be obtained by expression, enfleurage or extraction but the method of steam distillation is the most commonly used for commercial production (Burt, 2004; Oussalah et al., 2007).

The main limitations to an industrial use of these substances as preservatives are their organoleptic impact and their variable composition (which can be reflected in their different proprieties), the action of single constituents of the essential oils has been studied to identify the most active molecules to balance the intrinsic variability of

essential oils (Patrignani et al., 2013). Along served as agents in food and beverages, and due to their resourceful content of antimicrobial compounds, they possess potential as natural agents for food preservation (Burt, 2004; Hyldgaard et al., 2012). Antimicrobial activity is assigned to a number of small terpenoids and phenolic compounds (e.g. citral and eugenol), which also in pure form demonstrate high antibacterial activity (Oussalah et al., 2007).

Although most of the EOs are classified as Generally Recognized as Safe (GRAS) their use as food preservatives is often limited due to flavoring considerations since effective antimicrobial doses may exceed organoleptic acceptable levels (Moreira et al., 2005). In order to achieve effective antimicrobial activity in direct food applications, high concentrations of EOs are generally needed, which might impact inappropriate flavors and odors in the product (Emiroğlu et al., 2010).

A new approach to overcome is the use of antimicrobial packaging techniques as a promising type of active packaging. In antimicrobial packaging, antimicrobial agents can be incorporated into the packaging material, coated on the surface of packaging film or a sachet containing antimicrobial compound can be added into the package (Emiroğlu et al., 2010). As part of an edible coating can reduce microbial growth and improve the quality of the fruit (Raybaudi-Massilia et al., 2008b; Jo et al., 2014a). EO coating in an emulsion form can enhance microbial safety of fresh fruits (Rojas-Graü et al., 2007a). Edible films can reduce the diffusion of antimicrobial compounds into the product since the EO forms is part of the chemical structure of the film and interacts with the polymer and the plasticizer. Antimicrobial compounds release from the edible films depends on many factors, including electrostatic interactions between the antimicrobial agent and the polymer chains, osmosis, structural changes induced by the presence of antimicrobial, and environmental conditions(Avila-Sosa et al., 2012). Compared with

direct application, smaller amounts of antimicrobial agents would be needed when edible films are used as carriers in order to achieve a specific food shelf life due to a gradual release on food surfaces (Ruiz-Navajas et al., 2013).

2.2.1.1. Citral

Citral($C_{10}H_{16}O$) is a mixture of two isomers, geranial and neral (Fig.1.5), which are acyclic α, β-unsaturated monoterpene aldehydes naturally occurring in many essential oils from citrus fruits or other herbs or spices (Patrignani et al., 2013). Citral or 3,7-dimethyl-2,6-octadienal is one of the most important flavoring compound used widely in beverages, foods, and fragrances for its characteristic flavor profile (Maswal and Dar, 2014). Citral, reportedly anti-fungal and anti-bacterial activity without acquisition of resistance to its own or to antibiotics (Apolónio et al., 2014; Siroli et al., 2014; Zheng et al., 2015).

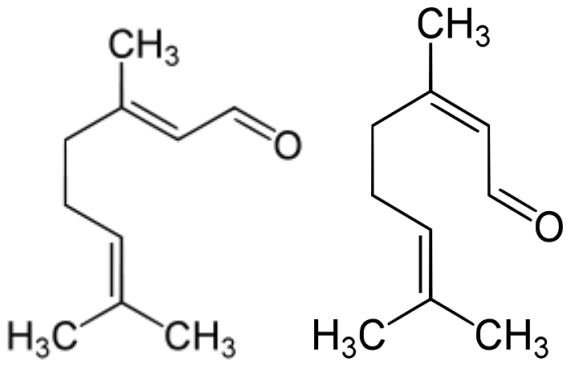


Figure 1.5. The chemical structures of geranial (*trans*-citral) and neral (*cis*-citral).

2.2.1.2. Eugenol

Eugenol ($C_{10}H_{12}O_2$), a natural phenolic compound, is a clear to pale yellow oily liquid extracted from buds and leaves of clove (*Eugenia caryophylata* Thumb) and from cinnamons (Fig 1.6) (Amiri et al., 2008; Xu et al., 2015). Eugenol constitutes the most significant active component of clove oil (85 to 95%) in addition to *iso*-eugenol and methyleugenol (Cowing et al., 2015; Yoshimura et al., 2015). Eugenol is a phenyl-

propanoid with a characteristic aroma and exhibits antifungal and antibacterial properties, and has been long known for its analgesic, local anesthetic and anti-inflammatory effects (Hemaiswarya and Doble, 2009; Devi et al., 2010; Apolónio et al., 2014; Li et al., 2015b).

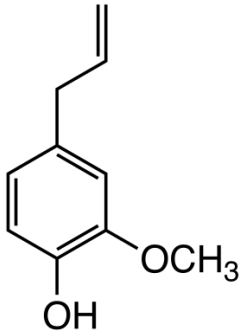


Figure 1.6. Chemical structure of eugenol.

3. References

Ahvenainen, R. New Approaches in Improving the Shelf Life of Minimally Processed Fruit and Vegetables. *Food Sci. Technol.* **1996**, *71*, 179–187.

Ali, A.; Maqbool, M.; Alderson, P. G.; Zahid, N. Effect of Gum Arabic as an Edible Coating on Antioxidant Capacity of Tomato (Solanum Lycopersicum L.) Fruit during Storage. *Postharvest Biol. Technol.* **2013**, *76*, 119–124.

Amiri, A.; Dugas, R.; Pichot, A. L.; Bompeix, G. In Vitro and in Vivo [corrected] Activity of Eugenol Oil (Eugenia Caryophylata) against Four Important Postharvest Apple Pathogens. *Int. J. Food Microbiol.* **2008**, *126*, 13–19.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Apolónio, J.; Faleiro, M. L.; Miguel, M. G.; Neto, L. No Induction of Antimicrobial Resistance in Staphylococcus Aureus and Listeria Monocytogenes during Continuous Exposure to Eugenol and Citral. *FEMS Microbiol. Lett.* **2014**, *354*, 92–101.

Arnon, H.; Zaitsev, Y.; Porat, R.; Poverenov, E. Effects of Carboxymethyl Cellulose and Chitosan Bilayer Edible Coating on Postharvest Quality of Citrus Fruit. *Postharvest Biol. Technol.* **2014**, *87*, 21–26.

Avila-Sosa, R.; Palou, E.; Jiménez Munguía, M. T.; Nevárez-Moorillón, G. V.; Navarro Cruz, A. R.; López-Malo, A. Antifungal Activity by Vapor Contact of Essential Oils Added to Amaranth, Chitosan, or Starch Edible Films. *Int. J. Food Microbiol.* **2012**, *153*, 66–72.

Azarakhsh, N.; Osman, A.; Ghazali, H. M.; Tan, C. P.; Mohd Adzahan, N. Lemongrass Essential Oil Incorporated into Alginate-Based Edible Coating for Shelf-Life Extension and Quality Retention of Fresh-Cut Pineapple. *Postharvest Biol. Technol.* **2014**, *88*, 1–7.

Baeva, M.; Panchev, I. Investigation of the Retaining Effect of a Pectin-Containing Edible Film upon the Crumb Ageing of Dietetic Sucrose-Free Sponge Cake. *Food Chem.* **2005**, *92*, 343–348.

Baldwin, E. A.; Hagenmaie, R.; Park, H. J. *Edible Coatings and Films to Improve Food Quality*; Second Edi.; CRC Press, 2011; Vol. 6.

Baldwin, E. *Edible Coatings for Fresh Fruits and Vegetables: Past, Present, and Future.*; Krochta, J.; Technomic Publishing Company, Lancaster, PA, 1994.

Benítez, S.; Achaerandio, I.; Pujolà, M.; Sepulcre, F. Aloe Vera as an Alternative to Traditional Edible Coatings Used in Fresh-Cut Fruits: A Case of Study with Kiwifruit Slices. *LWT - Food Sci. Technol.* **2015**, *61*, 184–193.

Boateng, J. S.; Matthews, K. H.; Stevens, H. N. E.; Eccleston, G. M. Wound Healing Dressings and Drug Delivery Systems: A Review. *J. Pharm. Sci.* **2008**, *97*, 2892–2923.

Bourtoom, T. Review Article Edible Films and Coatings : Characteristics and Properties. *Int. Food Res. J.* **2008**, *15*, 237–248.

Bravin, B.; Peressini, D.; Sensidoni, A. Development and Application of Polysaccharide-Lipid Edible Coating to Extend Shelf-Life of Dry Bakery Products. *J. Food Eng.* **2006**, *76*, 280–290.

Bruchet, M.; Melman, A. Fabrication of Patterned Calcium Cross-Linked Alginate Hydrogel Films and Coatings through Reductive Cation Exchange. *Carbohydr. Polym.* **2015**, *131*, 57–64.

Burt, S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods--a Review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253.

Campos, C. a.; Gerschenson, L. N.; Flores, S. K. Development of Edible Films and Coatings with Antimicrobial Activity. *Food Bioprocess Technol.* **2010**, *4*, 849–875.

Comaposada, J.; Gou, P.; Marcos, B.; Arnau, J. Physical Properties of Sodium Alginate Solutions and Edible Wet Calcium Alginate Coatings. *LWT - Food Sci. Technol.* **2015**, *64*, 212–219.

Cowing, D.; Powell, A.; Johnson, M. Evaluation of Different Concentration Doses of Eugenol on the Behaviour of Nephrops Norvegicus. *Aquaculture* **2015**, *442*, 78–85.

Debeaufort, F.; Quezada-Gallo, J. a; Voilley, a. Edible Films and Coatings: Tomorrow's Packagings: A Review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 299–313.

Devi, K. P.; Nisha, S. A.; Sakthivel, R.; Pandian, S. K. Eugenol (an Essential Oil of Clove) Acts as an Antibacterial Agent against Salmonella Typhi by Disrupting the Cellular Membrane. *J. Ethnopharmacol.* **2010**, *130*, 107–115.

Dhall, R. K. Advances in Edible Coatings for Fresh Fruits and Vegetables: A Review. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 435–450.

Díaz-Mula, H. M.; Serrano, M.; Valero, D. Alginate Coatings Preserve Fruit Quality and Bioactive Compounds during Storage of Sweet Cherry Fruit. *Food Bioprocess Technol.* **2012**, *5*, 2990–2997.

Duan, J.; Wu, R.; Strik, B. C.; Zhao, Y. Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions. *Postharvest Biol. Technol.* **2011**, *59*, 71–79.

Emiroğlu, Z. K.; Yemiş, G. P.; Coşkun, B. K.; Candoğan, K. Antimicrobial Activity of Soy Edible Films Incorporated with Thyme and Oregano Essential Oils on Fresh Ground Beef Patties. *Meat Sci.* **2010**, *86*, 283–288.

Fan, Y.; Xu, Y.; Wang, D.; Zhang, L.; Sun, J.; Sun, L.; Zhang, B. Effect of Alginate Coating Combined with Yeast Antagonist on Strawberry (Fragaria×ananassa) Preservation Quality. *Postharvest Biol. Technol.* **2009**, *53*, 84–90.

Fisk, C. L.; Silver, A. M.; Strik, B. C.; Zhao, Y. Postharvest Quality of Hardy Kiwifruit (Actinidia Arguta "Ananasnaya") Associated with Packaging and Storage Conditions. *Postharvest Biol. Technol.* **2008**, *47*, 338–345.

Gennadios, a; Hanna, M. a; Kurth, L. B. Application of Edible Coatings on Meats, Poultry and Seafoods: A Review. *Food Sci. Technol. Technol.* **1997**, *30*, 337–350.

Gonzalez-Aguilar, G.A.; Celis, J.; Sotelo-Mundo, R. R.; de la Rosa, L. A.; Rodrigo-Garcia, J.; Alvarez-Parrilla, E. Physiological and Biochemical Changes of Different Fresh-Cut Mango Cultivars Stored at 5 °C. *Int. J. Food Sci. Technol.* **2008**, *43*, 91–101.

Grant, G. T.; Morris, E. R.; Rees, D. A.; Smith, P. J. C.; Thom, D. Biological Interactions between Polysaccharides and Divalent Cations: The Egg-Box Model. *FEBS Lett.* **1973**, *32*, 195–198.

Griffin, D. R.; Kasko, A. M. Photodegradable Macromers and Hydrogels for Live Cell Encapsulation and Release. *J. Am. Chem. Soc.* **2012**, *134*, 13103–13107.

Han, C.; Zhao, Y.; Leonard, S. .; Traber, M. . Edible Coatings to Improve Storability and Enhance Nutritional Value of Fresh and Frozen Strawberries (Fragaria × Ananassa) and Raspberries (Rubus Ideaus). *Postharvest Biol. Technol.* **2004**, *33*, 67–78.

Hemaiswarya, S.; Doble, M. Synergistic Interaction of Eugenol with Antibiotics against Gram Negative Bacteria. *Phytomedicine* **2009**, *16*, 997–1005.

Hyldgaard, M.; Mygind, T.; Meyer, R. L. Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components. *Front. Microbiol.* **2012**, *3*, 12.

Jo, W. S.; Song, H. Y.; Song, N. B.; Lee, J. H.; Min, S. C.; Song, K. B. Quality and Microbial Safety of "Fuji" Apples Coated with Carnauba-Shellac Wax Containing Lemongrass Oil. *LWT - Food Sci. Technol.* **2014a**, *55*, 490–497.

Jo, W.-S.; Song, H.-Y.; Song, N.-B.; Lee, J.-H.; Min, S. C.; Song, K. Bin. Quality and Microbial Safety of "Fuji" Apples Coated with Carnauba-Shellac Wax Containing Lemongrass Oil. *LWT - Food Sci. Technol.* **2014b**, *55*, 490–497.

Kader, A. A. Perspective Flavor Quality of Fruits and Vegetables. *J. Sci. Food Agric.* **2008**, *88*, 1863–1868.

Kader, A. Quality Parameters of Fresh-Cut Fruit and Vegetable Products. In *Fresh-cut fruits and vegetables*; Lamikanra; O, Eds.; CRC Press LLC: Boca Raton, Fla, 2002; pp. 11–20.

Kang, H. J.; Jo, C.; Kwon, J. H.; Kim, J. H.; Chung, H. J.; Byun, M. W. Effect of a Pectin-Based Edible Coating Containing Green Tea Powder on the Quality of Irradiated Pork Patty. *Food Control* **2007**, *18*, 430–435.

Kuo, C. K.; Ma, P. X. Ionically Crosslinked Alginate Hydrogels as Scaffolds for Tissue Engineering: Part 1. Structure, Gelation Rate and Mechanical Properties. *Biomaterials* **2001**, *22*, 511–521.

Lee, J. Y.; Park, H. J.; Lee, C. Y.; Choi, W. Y. Extending Shelf-Life of Minimally Processed Apples with Edible Coatings and Antibrowning Agents. *LWT - Food Sci. Technol.* **2003**, *36*, 323–329.

Lee, K. Y.; Mooney, D. J. Alginate: Properties and Biomedical Applications. *Prog. Polym. Sci.* **2012**, *37*, 106–126.

Li, H.; Wang, Y.; Liu, F.; Yang, Y.; Wu, Z.; Cai, H.; Zhang, Q.; Wang, Y.; Li, P. Effects of Chitosan on Control of Postharvest Blue Mold Decay of Apple Fruit and the Possible Mechanisms Involved. *Sci. Hortic. (Amsterdam).* **2015a**, *186*, 77–83.

Li, L.; Fang, Y.; Vreeker, R.; Appelqvist, I.; Mendes, E. Reexamining the Egg-Box Model in Calcium-Alginate Gels with X-Ray Diffraction. *Biomacromolecules* **2007**, *8*, 464–468.

Li, W.; Chen, H.; He, Z.; Han, C.; Liu, S.; Li, Y. Influence of Surfactant and Oil Composition on the Stability and Antibacterial Activity of Eugenol Nanoemulsions. *LWT - Food Sci. Technol.* **2015b**, *62*, 39–47.

Lin, D.; Zhao, Y. Innovations in the Development and Application of Edible Coatings for Fresh and Minimally Processed Fruits and Vegetables. *Compr. Rev. Food Sci. Food Saf.* **2007**, *6*, 60–75.

Maftoonazad, N.; Ramaswamy, H. S.; Moalemiyan, M.; Kushalappa, A. C. Effect of Pectin-Based Edible Emulsion Coating on Changes in Quality of Avocado Exposed to Lasiodiplodia Theobromae Infection. *Carbohydr. Polym.* **2007**, *68*, 341–349.

Mahajan, P. V; Caleb, O. J.; Singh, Z.; Watkins, C. B.; Geyer, M. Postharvest Treatments of Fresh Produce. *Philos. Trans. A. Math. Phys. Eng. Sci.* **2014**, *372*, 20130309.

Manso, T. C. M. Isolamento E Produção de Agentes de Controlo Biológico Das Principais Doenças de Pós-Colheita, Em Citrinos E Pomóideas, PhD Thesis. Universidade do Algarve, 2013.

Marques, S. C. P. Edible Coatings Based on Chitosan - Beeswax Emulsions, Master Thesis. Universidade Nova de Lisboa, 2012.

Maswal, M.; Dar, A. A. Formulation Challenges in Encapsulation and Delivery of Citral for Improved Food Quality. *Food Hydrocoll.* **2014**, *37*, 182–195.

McGuire, R. G.; Hallman, G. J. Coating Guavas with Cellulose- or Carnauba-Based Emulsions Interferes with Postharvest Ripening. *HortScience* **1995**, *30*, 294–295.

Medeiros, B. G. de S.; Pinheiro, A. C.; Carneiro-da-Cunha, M. G.; Vicente, A. A. Development and Characterization of a Nanomultilayer Coating of Pectin and Chitosan – Evaluation of Its Gas Barrier Properties and Application on "Tommy Atkins" Mangoes. *J. Food Eng.* **2012**, *110*, 457–464.

Montero-Calderón, M.; Rojas-Graü, M. A.; Martín-Belloso, O. Effect of Packaging Conditions on Quality and Shelf-Life of Fresh-Cut Pineapple (Ananas Comosus). *Postharvest Biol. Technol.* **2008**, *50*, 182–189.

Moreira, M. R.; Ponce, A. .; del Valle, C. E.; Roura, S. I. Inhibitory Parameters of Essential Oils to Reduce a Foodborne Pathogen. *LWT - Food Sci. Technol.* **2005**, *38*, 565–570.

Nisperos-Carriedo, M.; Baldwin, E.; Shaw, P. Development of an Edible Coating for Extending Postharvest Life of Selected Fruits and Vegetables. *Proc. Fla. State Hort. Soc.* **1991**, *104*, 122–125.

Olivas, G. I.; Barbosa-Cánovas, G. V. Edible Coatings for Fresh-Cut Fruits. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 657–670.

Olivas, G. I.; Mattinson, D. S.; Barbosa-Cánovas, G. V. Alginate Coatings for Preservation of Minimally Processed "Gala" Apples. *Postharvest Biol. Technol.* **2007**, *45*, 89–96.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon. *LWT - Food Sci. Technol.* **2008**, *41*, 1862–1870.

Oms-Oliu, G.; Rojas-Graü, M. A.; González, L. A.; Varela, P.; Soliva-Fortuny, R.; Hernando, M. I. H.; Munuera, I. P.; Fiszman, S.; Martín-Belloso, O. Recent Approaches Using Chemical Treatments to Preserve Quality of Fresh-Cut Fruit: A Review. *Postharvest Biol. Technol.* **2010**, *57*, 139–148.

Oussalah, M.; Caillet, S.; Saucier, L.; Lacroix, M. Inhibitory Effects of Selected Plant Essential Oils on the Growth of Four Pathogenic Bacteria: E. Coli O157:H7, Salmonella Typhimurium, Staphylococcus Aureus and Listeria Monocytogenes. *Food Control* **2007**, *18*, 414–420.

Palma, A.; Schirra, M.; Aquino, S. D. Effect of Edible Coating on Ascorbic Acid , Betalains , Organic Acids , Sugar , Polyphenol Content and Antioxidant Activity in Minimally Processed Cactus Pear (Opuntia Ficus-Indica). *Acta Hortic.* **2015**, *1067*, 127–134.

Patrignani, F.; Tabanelli, G.; Siroli, L.; Gardini, F.; Lanciotti, R. Combined Effects of High Pressure Homogenization Treatment and Citral on Microbiological Quality of Apricot Juice. *Int. J. Food Microbiol.* **2013**, *160*, 273–281.

Pavlath, A. E.; Orts, W. Edible Films and Coatings: Why, What, and How? In *Edible Films and Coatings for Food Applications*; Springer Science + Busines Media, L., Ed.; Springer New York: New York, NY, 2009; pp. 1–23.

Raybaudi-Massilia, R. M.; Rojas-Graü, M. A; Mosqueda-Melgar, J.; Martín-Belloso, O. Comparative Study on Essential Oils Incorporated into an Alginate-Based Edible Coating to Assure the Safety and Quality of Fresh-Cut Fuji Apples. *J. Food Prot.* **2008a**, *71*, 1150–1161.

Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Martín-Belloso, O. Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon. *Int. J. Food Microbiol.* **2008b**, *121*, 313–327.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Raybaudi-Massilia, R. M.; Soliva-Fortuny, R. C.; Avena-Bustillos, R. J.; McHugh, T. H.; Martín-Belloso, O. Apple Puree-Alginate Edible Coating as Carrier of Antimicrobial Agents to Prolong Shelf-Life of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2007**, *45*, 254–264.

Rojas-Graü, M. A.; Tapia, M. S.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Maintain Quality of Fresh-Cut Fuji Apples. *LWT - Food Sci. Technol.* **2008**, *41*, 139–147.

Rojas-Graü, M. A.; Soliva-Fortuny, R.; Martín-Belloso, O. Edible Coatings to Incorporate Active Ingredients to Fresh-Cut Fruits: A Review. *Trends Food Sci. Technol.* **2009a**, *20*, 438–447.

Rojas-Graü, M. A.; Soliva-Fortuny, R.; Martín-Belloso, O. Edible Coatings to Incorporate Active Ingredients to Fresh-Cut Fruits: A Review. *Trends Food Sci. Technol.* **2009b**, *20*, 438–447.

Rooney, M. L. *Innovations in Food Packaging*; Elsevier, 2005.

Ruiz-Navajas, Y.; Viuda-Martos, M.; Sendra, E.; Perez-Alvarez, J. A.; Fernández-López, J. In Vitro Antibacterial and Antioxidant Properties of Chitosan Edible Films Incorporated with Thymus Moroderi or Thymus Piperella Essential Oils. *Food Control*, 2013, *30*, 386–392.

Sharma, R.; Singh, R. Harvesting, Postharvest Handling and Physiology of Fruits and Vegetables. In *Postharvest technology of fruits and vegetables Vol. 1. Handling, processing, fermentation and waste management.*; Verma LR, Joshi VK, E., Ed.; Indus Publishing Co.: Tagore Garden, New Delhi, 2000; pp. 94–147.

Shukla, S.; Verma, K.; Jain, D.; Verma, S. Pectin-Based Colon-Specific Drug Delivery. *Chronicles Young Sci.* **2011**, *2*, 83.

Sikorski, P.; Mo, F.; Skjåk-Braek, G.; Stokke, B. T. Evidence for Egg-Box-Compatible Interactions in Calcium-Alginate Gels from Fiber X-Ray Diffraction. *Biomacromolecules* **2007**, *8*, 2098–2103.

Siroli, L.; Patrignani, F.; Serrazanetti, D. I.; Tabanelli, G.; Montanari, C.; Tappi, S.; Rocculi, P.; Gardini, F.; Lanciotti, R. Efficacy of Natural Antimicrobials to Prolong the Shelf-Life of Minimally Processed Apples Packaged in Modified Atmosphere. *Food Control* **2014**, *46*, 403–411.

Soliva-Fortuny, R. C.; Martín-Belloso, O. New Advances in Extending the Shelf-Life of Fresh-Cut Fruits: A Review. *Trends Food Sci. Technol.* **2003**, *14*, 341–353.

Suppakul, P.; Miltz, J.; Sonneveld, K.; Bigger, S. W. Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and Its Applications. *J. Food Sci.* **2003**, *68*, 408–420.

Tan, W.-H.; Takeuchi, S. Monodisperse Alginate Hydrogel Microbeads for Cell Encapsulation. *Adv. Mater.* **2007**, *19*, 2696–2701.

Thakur, B. R.; Singh, R. K.; Handa, A. K. Chemistry and Uses of Pectin--a Review. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 47–73.

Tuil, R.; Fowler, P.; Lawther, M.; Weber, C. J. *Biobased Packaging Materials for the Food Industry*; Weber, C. J., Ed.; Frederiksberg C, Denmark, 2000.

Valero, D.; Díaz-Mula, H. M.; Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Serrano, M. Effects of Alginate Edible Coating on Preserving Fruit Quality in Four Plum Cultivars during Postharvest Storage. *Postharvest Biol. Technol.* **2013**, *77*, 1–6.

Vargas, M.; Pastor, C.; Chiralt, A.; McClements, D. J.; González-Martínez, C. Recent Advances in Edible Coatings for Fresh and Minimally Processed Fruits. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 496–511.

Xu, H.-X.; Zheng, X.-S.; Yang, Y.-J.; Tian, J.-C.; Lu, Y.-H.; Tan, K.-H.; Heong, K.-L.; Lu, Z.-X. Methyl Eugenol Bioactivities as a New Potential Botanical Insecticide against Major Insect Pests and Their Natural Enemies on Rice (Oriza Sativa). *Crop Prot.* **2015**, *72*, 144–149.

Yoshimura, T.; Shimasaki, T.; Teramoto, N.; Shibata, M. Bio-Based Polymer Networks by Thiol–ene Photopolymerizations of Allyl-Etherified Eugenol Derivatives. *Eur. Polym. J.* **2015**, *67*, 397–408.

Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D.; Serrano, M. Use of Alginate or Zein as Edible Coatings to Delay Postharvest Ripening Process and to Maintain Tomato (Solanum Lycopersicon Mill) Quality. *J. Food Agric.* **2008**, *88*, 1287–1293.

Zheng, S.; Jing, G.; Wang, X.; Ouyang, Q.; Jia, L.; Tao, N. Citral Exerts Its Antifungal Activity against Penicillium Digitatum by Affecting the Mitochondrial Morphology and Function. *Food Chem.* **2015**, *178*, 76–81.

Zúñiga, G. E.; Junqueira-Gonçalves, M. P.; Pizarro, M.; Contreras, R.; Tapia, A.; Silva, S. Effect of Ionizing Energy on Extracts of Quillaja Saponaria to Be Used as an Antimicrobial Agent on Irradiated Edible Coating for Fresh Strawberries. *Radiat. Phys. Chem.* **2012**, *81*, 64–69.

Chapter II

Fresh-cut mango quality changes through shelf-life as affected by four edible coatings: Alginate, Carboxymethilcellulose, Pectin and Chitosan

Adriana C. Guerreiro[1]

Supervisors: O. Martín-Belloso[2] & M.D.C. Antunes[1]

[1] *University of Algarve, Faculdade de Ciências e Tecnologia, IBB-CBV, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal*
[2] *University of Lleida, Department of Food Technology, Rovira Roure 191, 25198 Lleida, Spain*

Training Report

Preformed at Department of Food Technology, University of Lleida

Fresh-cut mango quality changes through shelf-life as affected by four edible coatings: Alginate, Carboximethilcellulose, Pectin and Chitosan

Abstract

Edible coatings based on polysaccharides were used to preserve quality of fresh-cut cold-stored mangos. Four edible coatings were evaluated at two different concentrations. The treatments were: NT (Non-treated fresh-cut mango), AL 1% (coating with Sodium Alginate), AL 2% (coating with Sodium Alginate), PE1 % (coating with Pectin), PE 2 % (coating with Pectin) CMC 0.5 % (coating with Carboxy Methyl Cellulose) CMC 1 % (coating with Carboxy Methyl Cellulose) CH 0.5 % (coating with Chitosan) and CH 1 % (coating with Chitosan). Mangoes were washed with water and sodium hipocholoride 0.1%, peeled and manually sliced into cubes of 2 cm^3. Then, 20 cubes were placed in polypropylene plastic trays and thermosealed. Storage was at 4 ºC ±1 ºC and on days 0, 2, 4 and 7 samples were taken to perform physicochemical and biochemical analysis (color CIEL*a*b*, firmness, β-caroten and antioxidant activity by DPPH method). Lightness (L*) was preserved by edible coatings through time except CH, ºHue had no changes among treatments and chroma was better in PE. Firmness was better in AL from the beginning of the experiment. The β-caroten and antioxidant activity (DPPH) were higher in CH. Edible coatings used, generally preserved well the quality parameters up to 7 days cold storage. The AL showed higher flesh firmness, but CH was better for preserving or increasing β-caroten and antioxidant activity.

Key words: Mango; Edible coatings; Antioxidant activity; β-carotene; Storage.

1. Introduction

Fresh and fresh-cut fruits are ready-to-eat products, which shall maintain freshness and nutritional quality through shelf-life. However, the peeling and cutting operations accelerate the metabolic activities of plant tissues, making the minimally processed products more perishable than fresh fruits and vegetables (Chiumarelli and Hubinger, 2012). Low storage temperature and modified atmosphere within the package (MAP) are used to extend the shelf-life of many fresh and fresh-cut fruit and vegetables, as they reduce respiration rate, surface damage and browning (Antunes et al., 2012). More recently, it has been reported that edible coatings can extend shelf-life in fresh-cut product (Fisk et al., 2008; Gonzalez-Aguilar et al., 2008). Edible coatings do not pretend to replace traditional packaging materials but they can additionally control moisture and gases and be supporters of additives (Campos et al., 2010).

Edible coatings have been recognized for more innovative uses beyond their current uses. They 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 (Zúñiga et al., 2012). For their formulation, there can be used polysaccharides, proteins and lipids and they must result neutral with respect to colour and flavour. Edible coatings made of polysaccharides, particularly starches, are good film-forming and have low oxygen permeability, implying in decrease of respiration rate of fresh-cut products (Campos et al., 2010). Alginate derived from marine brown algae (*Phaeophyceae*) and pectin extracted from apple waste or from the peel of citrus fruits are common polysaccharides used as gelling agents in food industry (Oms-Oliu et al., 2008). Cellulose is the structural material of plant cell walls and it is composed of linear chains of (1→4)-β-D-glucopyranosyl units. Chemical substitution of some hydroxyl groups

along the chain gives origin to cellulose esthers: nonionic and ionic as carboxymethylcellulose (CMC) (Campos et al., 2010). Chitosan is a linear polysaccharide composed of randomly distributed b-(1e4)-linked D-glucosamine and N-acetyl-D-glucosamine units. It is derived from natural sources by deacetylation of chitin which is harmless to humans, pets, wildlife, and the environment; and has been studied for efficacy in inhibiting decay and extending shelf life of fruits (Wang and Gao, 2012). Coatings constituted of polysaccharides have been tested in common fruits such as: apples, pears and strawberries (Cerqueira et al., 2009). Nevertheless there is less research focused in different edible coatings applied in tropical fruits. Mango (*Mangifera indica L.*) is the most preferred tropical fruit around the world due to its attractive colour, good flavour, taste, and its high content of bioactive compounds such as ascorbic acid, β-carotene and phenolic compounds. All these bioactive compounds are good antioxidants and their daily intake in the diet has been related to prevention of degenerative processes such as cardiovascular diseases and cancer (Liu et al., 2002). Thus, consumption of mango can provide significant amounts of bioactive compounds with antioxidant activity to the human diet (Robles-Sánchez et al., 2013). Since it is well known that the different origins and concentrations of edible coatings and as well as food matrix have a relevant effect on fresh-cut fruits, the objective of the present work was to compare the effectiveness of alginate, pectin, CMC and chitosan based coatings in preserving quality of fresh-cut mango. Effects of the coatings on antioxidant properties, β-carotene, firmness and colour were evaluated for 7 days at 4ºC.

2. Material and methods

2.1. Materials

Mature green mangoes (*Mangifera indica L. cv*. Tommy Atkins) were purchased from a local wholesale market in Lleida, Spain, and transported to the laboratory. The mangoes were selected to eliminate damaged, defective or unripe fruits.

Food grade Sodium alginate (Manucol LD, FMC-biopolymers, USA), low-methoxyl pectin (Across Organics, Fair Lawn, NJ), Carboxy Methyl Cellulose and Chitosan (Sigma-Aldrich Co. Steinhein, Germany) were the biopolymers used for coating formulations. Glacial acetic acid (Scharlau Chemie SA, Sentmenat, Barcelona) was added to dissolve chitosan and sodium hidroxyde (Scharlau Chemie SA, Sentmenat, Barcelona) to adjust the pH. Calcium chloride (Sigma-Aldrich Chemic, , Germany) was used to induce cross linking reaction. Ascorbic acid (AA) (Scharlau, Barcelona, Spain) was added as antibrowning agent. Tetrahidrofuran (THF) and Methanol (MeOH) were purchased in Scharlau Chemie SA (Barcelona, Spain) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka Sigma-Aldrich Chemic, , Germany).

2.2. Methods

2.2.1. Edible coatings

The coating forming solutions based on sodium alginate, pectin or carboxy methyl cellulose were formulated as described by Rojas-Graü et al (2007)(María A. Rojas-Graü, Raybaudi-Massilia, Robert C. Soliva-Fortuny, Avena-Bustillos, McHugh, Olga Martín-Belloso 2007). The chitosan coating was prepared by dissolving chitosan in distilled water with glacial acetic acid and adjusting to pH 5.0 (Jiang and Li, 2001). Ascorbic acid 1% was added as anti-browning agent in the edible coating solutions according to previous works(Robles-Sánchez et al., 2009) .

Four edible coatings were evaluated at two different concentrations. The treatments were named NT: Non-treated fresh-cut mango; AL 1%: fresh-cut mango coated with Sodium Alginate (10g/L); AL 2%: fresh-cut mango coated with Sodium Alginate (20g/L); PE 1%: fresh-cut mango coated with Pectin (10g/L); PE 2%: fresh-cut mango coated with Pectin (20g/L); CMC 0.5%: fresh-cut mango coated with Carboxy Methyl Cellulose (0.5g/L); CMC 1%: fresh-cut mango coated with Carboxy Methyl Cellulose (10g/L); CH 0.5%: fresh-cut mango coated with Chitosan (5g/L); CH 1%: fresh-cut mango coated with Chitosan (10g/L).

Four mangoes (3 kg) were used in each treatment which were washed with water and sodium hipocholoride 0.1%, peeled and sliced manually into cubes of 2 cm^3. Each treatment was performed in two steps: first mango cubes were dipped into the edible coating solution+AA for 2 min; the excess of coating material was allowed to drip off for 30s before the second dip in the calcium chloride solution for 2 min. Then, 20 cubes were placed in polypropylene plastic trays (18 x 12 x 2.5) and thermo-sealed using a packaging machine, then stored at 4 ºC ±1 ºC until analysis. On days 0, 2, 4 and 7 two trays were taken to perform the physicochemical and biochemical analysis. Two trays were taken at each sampling time to perform the analyses, and five mango pieces from each replicate were randomly withdrawn to carry out repetitions.

2.2.2. Physical measurements

Color of cut mango was measured with a color meter (Minolta Chroma Meter Model CR- 400, Minolta, Tokyo, Japan). The equipment was set up for illuminant D65 and 10º observer angle and calibrated using a standard white reflector plate. Color was measured using the CIE L*, a*, b* scale. The L* represents color lightness (0 = black and 100 = white). The a* scale indicates in the maximum the red (+a*) and in the

minimum the green color (-a*) while the b* axis ranged from yellow (+b*) to blue (−b*). Hue was calculated as hº = arctan (b*/a*) and color saturation (chroma) as C* = $(a^{*2} + b^{*2})^{0.5}$(McGuire, 1992). To determine darkening during storage, the color of each fruit was evaluated just after cutting and again after the different storage periods. Firmness was measured in order to obtain the maximum penetration force expressed in Newton (N). A Texture Analyser TA-XT2 (Stable Micro Systems Ltd., Surrey, England, UK) with 4 mm diameter rod was used. The downward distance was set at 20 mm at a rate of 5 mm s^{-1} and automatic return.

2.2.3. Biochemical measurements

β-carotene was determined according to Robles- Sánchez et al (2009). Five grams of mango cubes were added to 20 mL of tetrahydrifuran (THF) and homogenized with an Ultra-Turrax T 25 (IKA® WERKE, Germany). The content of β-carotene was detected by spectrophotometer (CECIL CE 2021 Cecil Instruments Ltd, Cambridge, UK) at 470nm. The concentration of β-carotene was calculated using an external standard and expressed as mg β-carotene/ 100g of fresh weight.

Antioxidant activity of coated fresh-cut mangoes was based on scavenging ability of antioxidants toward the stable radical DPPH (Sharma and Bhat, 2009). Tubes with 10g of fresh-cut mango were centrifuged at 5000 rpm, 4 ºC ± 0.5 during 20 minutes. A 10 µL of supernatant extract was mixed with 3.9 mL of a 0.0634 mM DPPH solution in methanol and shacked vigorously. Tubes were placed in a dark place for 30 min. A control reaction was prepared as above, without extract, and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 515 nm. Radical- scavenging activity was expressed as inhibition percentage and was calculated using the following equation:

$$\%radical - scavenging\ activity = \frac{Control\ Abs - Sample\ Abs}{Control\ Abs} \times 100$$

2.3. Statistical analysis

Statistical analyses were carried out with the SPSS 20.0 software (SPSS Inc.). Two-way ANOVA and Duncan's Multiple-Range Test ($P<0.05$) for comparisons among treatments over time was performed. Each treatment consisted of 3 replications.

3. Results and discussion

3.1. Color and Firmness

Color is one of the most important attributes for consumer acceptance of fresh-cut mangos. The color parameter lightness (L*) value decreased significantly over time only in Chitosan coating either at 0.5% or 1%, mainly in the first 2 days (Table 2.1). In 0.5% CH values remained constant thereafter, while a second significant decrease occurred from 2 to 4 days on CH at 1%. In alginate and pectin coating no significant changes in L* were observed through time. Also, there was no significant effect of different edible coatings tested at each sampling time, meaning that the decrease observed for chitosan coating was not so high. This decrease in L* value may be due to the fact that some compounds present in fruits, such as amino-acids and phenolics are related to the enzymatic browning and the reduction of L* value, so darkening (Oliveira et al., 2011a). Darkening is characteristic of fresh-cut fruit due to oxidation of tissues after cutting (Oms-Oliu et al., 2010).

In fresh- cut fruits also a* and b* parameters are related to the acceptance or rejection of the produce. In our study no significant changes were found through storage time for none treatment (Table 2.1). For the differences among treatments, only PE 2% can be considered has having slightly higher a* values than the other treatments.

The yellowness parameter (b*) only decreased significantly in first 2 and 4 days for CMC 0.5% and CH 1%, respectively (Table 2.1). After that the values remained constant. In general, b* values showed no significant differences among treatments at $P < 0.05$. Accordingly Hue showed no significant differences among treatments and over time (Table 2.1). Chroma, which represents color intensity, decreased significantly through time only in CMC and CH treatments mainly in the first 2 days of storage (Table 2.1). At the end of the experiment the chroma (light intensity) was higher in PE treatments.

Browning effect due to oxidation could limit shelf-life of fresh-cut mango cubes, and commonly promote colour changes(Chiumarelli and Hubinger, 2012). Also, Chien et al. (2005) found better color preservation in chitosan (0-2%) coating than control except for yellow colour (b*), and a decrease through time. However, as in our experiment, the darkening observed in chitosan was not so high and the better colour intensity in PE of our experiment was not significantly different from other treatments. Other studies observed in mango coated fruits with alginate a decrease in L* and Hue values through time, except when AA was added to edible coatings as in our studies (Robles-Sánchez et al., 2013). Different anti-browning agents have started to be tested in mango fruit in order to reduce the non desirable browning colour and to extend shelf-life (Chiumarelli and Hubinger, 2012). The addition of AA to all treatments may had a significant contribute to this behaviour(Gonzalez-Aguilar et al., 2008).

It was found that firmness decreased significantly mostly in the first 2 days in all treatments except PE and CMC 0.5% (Table 2.1). However, those treatments had significantly lower firmness at the beginning of the experiment which was obtained just after the coating treatment (day 0). PE, CMC and CH had an interaction with mango thus there was a loss of firmness in the initial moment of the coating formation. For NT

and AL showed higher firmness than other treatments just after the treatment. A significant decrease in firmness was observed in the first 2 days of storage for NT, AL and CMC 1% treatments, then remained constant (Table 2.1). CH showed a significant decrease after 4 days and Pe and CMC 0.5% showed no significant changes because they were already soft at the beginning of the experiment. At the end of the experiment firmness was better kept in NT and AL treatments.

A decrease in firmness in fresh-cut fruit is expected because of the release of water and other compounds as a consequence of the cutting process(Gonzalez-Aguilar et al., 2008). As in our experiment, it was found a higher significant decrease in firmness for some mango cultivars in the first 2-3 days of cold storage (Gonzalez Aguilar et al., 2008). This decrease was slightly reduced mostly with antioxidant treatment. Our results show difference between coatings hence the biopolymer forming the edible coating solution could have an influence to firmness along the storage. This effect in firmness has been also observed by Dang et al. (2008),and Qi et al. (2011)who studied the effect of dipping treatments using antibrowning and coatings in mango and apple respectively. Similar results were observed for fresh-cut kiwi fruit (Antunes et al., 2010). The different responses between coatings may be due to the polysaccharide chain and the alliance when mango is dipped in calcium chloride. In this sense Dang et al. (2008) concluded that the use of wax coatings could enhance the effect in firmness delaying the ripening by decreasing the activity of softening enzymes due to the modified internal atmosphere in the coated fruit. In the conditions of our experiment AL was the best for preserving firmness.

Table 1 Color and firmness of fresh-cut mango after 7 days stored at 4±1ºC. Values represent the mean ± standard deviation of three replicates.

Quality Parameters	Days	NT	AL 1%	AL 2%	PE 1%	PE 2%	CMC 0,5%	CMC 1%	CH 0.5%	CH 1%
Lightness (L*)	0	73.184 ± 0.53 ^{aA}	72.763 ± 0.905 ^{aA}	72.649 ± 5.45 ^{aA}	69.043 ± 2.811 ^{aA}	72.219 ± 2.848 ^{aA}	72.526 ± 0.78 ^{aA}	72.564 ± 0.532 ^{aA}	74.446 ± 1.695 ^{aA}	73.99 ± 0.184 ^{aA}
	2	71.862 ± 1.579 ^{aA}	70.081 ± 0.85 ^{aA}	72.484 ± 3.177 ^{aA}	67.251 ± 4.329 ^{aA}	67.686 ± 4.393 ^{aA}	67.444 ± 0.705 ^{aA}	68.138 ± 2.507 ^{aA}	67.821 ± 1.048 ^{bA}	68.321 ± 0.652 ^{bA}
	4	70.118 ± 0.205 ^{aA}	69.888 ± 3.935 ^{aA}	68.348 ± 2.051 ^{aA}	63.475 ± 8.149 ^{aA}	66.651 ± 4.591 ^{aA}	68.529 ± 4.584 ^{aA}	67.903 ± 3.14 ^{aA}	67.28 ± 1.298 ^{bA}	66.58 ± 0.173 ^{cA}
	7	68.712 ± 0.821 ^{aA}	70.68 ± 2.584 ^{aA}	68.984 ± 1.402 ^{aA}	66.888 ± 5.211 ^{aA}	67.548 ± 6.64 ^{aA}	67.344 ± 1.494 ^{aA}	66.49 ± 2.16 ^{aA}	66.228 ± 2.507 ^{bA}	66.064 ± 0.115 ^{cA}
a*	0	-7.669 ± 1.272 ^{aA}	-7.419 ± 1.137 ^{aAB}	-7.313 ± 0.739 ^{aABC}	-6.143 ± 1.008 ^{aABC}	-5.703 ± 0.537 ^{aC}	-5.866 ± 0.617 ^{aBC}	-6.811 ± 0.228 ^{aABC}	-6.868 ± 0.163 ^{aABC}	-6.438 ± 0.686 ^{aABC}
	2	-7.55 ± 1.087 ^{aA}	-7.41 ± 2.524 ^{aA}	-6.413 ± 1.092 ^{aA}	-5.196 ± 1.402 ^{aA}	-5.13 ± 0.651 ^{aA}	-6.129 ± 0.2 ^{aA}	-5.958 ± 0.863 ^{aA}	-5.491 ± 0.164 ^{aA}	-5.13 ± 0.788 ^{aA}
	4	-6.001 ± 1.126 ^{aA}	-7.361 ± 0.776 ^{aA}	-7.188 ± 1.117 ^{aA}	-4.758 ± 1.492 ^{aA}	-5.304 ± 1.105 ^{aA}	-6.195 ± 0.757 ^{aA}	-5.466 ± 0.91 ^{aA}	-5.151 ± 1.115 ^{bA}	-5.509 ± 0.15 ^{aA}
	7	-6.414 ± 0.112 ^{aABC}	-7.214 ± 0.985 ^{aA}	-7.103 ± 1.237 ^{aAB}	-4.664 ± 1.625 ^{aDE}	-4.285 ± 0.336 ^{aE}	-6.169 ± 0.794 ^{aBCD}	-5.315 ± 0.697 ^{aBCDE}	-5.49 ± 0.636 ^{bCDE}	-5.665 ± 0.071 ^{aBCDE}
b*	0	46.046 ± 1.554 ^{aA}	42.155 ± 6.512 ^{aAB}	39.699 ± 8.3 ^{aAB}	50.5 ± 0.636 ^{aAB}	52.264 ± 7.289 ^{aB}	47.991 ± 0.094 ^{aAB}	48.97 ± 2.44 ^{aAB}	46.375 ± 0.173 ^{aAB}	46.394 ± 0.949 ^{aAB}
	2	46.201 ± 0.87 ^{aA}	43.231 ± 9.152 ^{aA}	43.829 ± 11.429 ^{aA}	44.489 ± 1.989 ^{aA}	46.881 ± 8.54 ^{aA}	40.821 ± 1.108 ^{aA}	42.808 ± 4.646 ^{aA}	37.993 ± 7.11 ^{aA}	44.678 ± 2.022 ^{aA}
	4	45.076 ± 0.527 ^{aA}	44.51 ± 4.932 ^{aA}	42.074 ± 6.058 ^{aA}	40.586 ± 7.034 ^{aA}	43.861 ± 8.929 ^{aA}	42.105 ± 2.659 ^{bA}	43.336 ± 6.125 ^{aA}	42.675 ± 0.127 ^{aA}	40.504 ± 0.2 ^{bA}
	7	45.042 ± 4.061 ^{aA}	41.009 ± 8.049 ^{aA}	41.424 ± 8.925 ^{aA}	46.8 ± 2.737 ^{aA}	48.578 ± 7.184 ^{aA}	40.368 ± 1.16 ^{bA}	41.675 ± 4.667 ^{aA}	41.995 ± 0.499 ^{aA}	39.248 ± 0.711 ^{bA}
Hue (h^{o})	0	99.483 ± 1.854 ^{aA}	100.202 ± 3.04 ^{aA}	100.758 ± 3.228 ^{aA}	96.941 ± 1.213 ^{aA}	96.327 ± 1.457 ^{aA}	96.968 ± 0.712 ^{aA}	97.934 ± 0.652 ^{aA}	98.424 ± 0.228 ^{aABC}	97.909 ± 0.991 ^{aA}
	2	99.292 ± 1.485 ^{aA}	100.25 ± 5.365 ^{aA}	98.783 ± 3.651 ^{aA}	96.627 ± 1.487 ^{aA}	96.418 ± 1.945 ^{aA}	98.545 ± 0.503 ^{aA}	98.026 ± 1.992 ^{aA}	98.344 ± 1.297 ^{aA}	96.578 ± 1.293 ^{aA}
	4	97.574 ± 1.319 ^{aA}	99.496 ± 2.005 ^{aA}	99.889 ± 2.873 ^{aA}	96.603 ± 0.944 ^{aA}	97.178 ± 2.867 ^{aA}	98.354 ± 0.488 ^{aA}	97.34 ± 2.21 ^{aA}	96.883 ± 1.496 ^{bA}	97.746 ± 0.246 ^{aA}
	7	98.143 ± 0.864 ^{aA}	100.285 ± 3.309 ^{aA}	100.117 ± 3.795 ^{aA}	95.641 ± 1.642 ^{aA}	95.067 ± 0.352 ^{aA}	98.706 ± 1.347 ^{aA}	97.363 ± 1.757 ^{aA}	97.453 ± 0.941 ^{bCDE}	98.216 ± 0.248 ^{aA}
Chroma (C*)	0	46.693 ± 1.323 ^{aAB}	42.833 ± 6.213 ^{bB}	40.398 ± 8.022 ^{bB}	50.878 ± 0.51 ^{aA}	52.582 ± 7.186 ^{aA}	48.35 ± 0.168 ^{aA}	49.443 ± 2.385 ^{aA}	46.881 ± 0.148 ^{aAB}	46.842 ± 0.846 ^{aAB}
	2	46.822 ± 0.683 ^{aA}	43.956 ± 8.575 ^{bAB}	44.339 ± 11.139 ^{aAB}	44.799 ± 2.138 ^{abAB}	47.174 ± 8.416 ^{aA}	41.28 ± 1.066 ^{bB}	43.233 ± 4.481 ^{bAB}	38.392 ± 7.059 ^{bB}	44.977 ± 1.919 ^{aAB}
	4	45.48 ± 0.671 ^{aA}	45.128 ± 4.738 ^{aA}	42.71 ± 5.78 ^{abB}	40.867 ± 7.159 ^{bB}	44.208 ± 8.726 ^{bA}	42.559 ± 2.74 ^{bB}	43.696 ± 5.961 ^{bAB}	42.992 ± 0.007 ^{bB}	40.877 ± 0.178 ^{bB}
	7	45.499 ± 4.005 ^{aAB}	41.673 ± 7.75 ^{bB}	42.073 ± 8.579 ^{abB}	47.041 ± 2.884 ^{aA}	48.767 ± 7.186 ^{aA}	40.842 ± 1.026 ^{bB}	42.022 ± 4.54 ^{bB}	42.355 ± 0.412 ^{bB}	39.654 ± 0.693 ^{bC}
Firmness (N)	0	4.321 ± 0.818 ^{aABC}	5.728 ± 0.265 ^{aA}	4.586 ± 3.118 ^{aAB}	1.366 ± 0.137 ^{aD}	1.712 ± 0.177 ^{aCD}	1.872 ± 0.237 ^{aCD}	2.019 ± 0.063 ^{aBCD}	2.008 ± 0.478 ^{aBCD}	1.877 ± 0 ^{aCD}
	2	3.4 ± 0.962 ^{bA}	2.485 ± 0.542 ^{bABC}	2.687 ± 0.334 ^{bAB}	1.351 ± 0.169 ^{aC}	1.629 ± 0.01 ^{aBC}	1.708 ± 0.049 ^{aBC}	1.585 ± 0.046 ^{bBC}	1.903 ± 0.118 ^{aBC}	1.732 ± 0.11 ^{aBC}
	4	3.823 ± 0.203 ^{bA}	2.909 ± 0.844 ^{bB}	2.648 ± 0.845 ^{bB}	1.469 ± 0.003 ^{aC}	1.517 ± 0.002 ^{aC}	1.587 ± 0.137 ^{aC}	1.592 ± 0.002 ^{bC}	1.578 ± 0.017 ^{bC}	1.419 ± 0.001 ^{bC}
	7	3.775 ± 0.189 ^{bA}	3.189 ± 0.675 ^{bAB}	2.617 ± 0.662 ^{bB}	1.37 ± 0.049 ^{aC}	1.683 ± 0.045 ^{aC}	1.645 ± 0.144 ^{aC}	1.311 ± 0.061 ^{bC}	1.413 ± 0.12 ^{bC}	1.311 ± 0.033 ^{bC}

Values in the same column followed by the same lower case letter, and in the same row followed by the same upper case letter are not significantly different by Duncan's multiple range test ($P<0.05$).

3.2. β-Carotene and antioxidant activity

Generally the edible coatings applied to fresh-cut mangoes of our experiment maintained better bioactive compound β-carotene compared to non coated ones NT (Fig. 2.1). The best results were obtained for PE and CH and better at the lower concentrations for both coatings, CH 0.5% and PE 1%. The initial content of β-Carotene for control samples was 0.453 µg/100 g of fresh weight, being lower than that reported for other cultivars (Gonzalez-Aguilar et al., 2008). Robles- Sánchez R (2009)observed higher values of β-Carotene at the end of the storage in mango cubes treated with alginate. In our study β-caroten of fresh-cut mango treated with AL was maintained trough storage, only CH coating showed an increase with the higher β-caroten content at the end of the experiment (Fig. 2.1).

Tropical fruits, particularly mango, are rich in health promoting biocompounds such as β-Carotene and antioxidants. It is necessary to reduce the perishability to preserve and to promote their liberation from the tissues. It is important to point out that although minimally processing of fruit accelerates senescence of fresh-cut tissues, it can promote an increase in β-carotene content. Considering this hypothesis mango ripening produces an increase in β-Carotene content, which could be retarded in low-temperatures, and may be due to an increase in mevalonic acid and feraniol synthesis, which lead to higher levels of total carotenes (Litz, 1997). According to our results, it is suggested that the fresh-cut mango dipped in CH coating enhance the content of β-Carotene, maybe due to the better protection of this coating against the oxygen (Jiang and Li, 2001). These results corroborated the importance to find out clear mechanisms involved in the prevention of carotenes oxidation and biosynthesis in fresh-cut mango.

Antioxidant capacity is used to evaluate the antioxidant potential of the tissue. The antioxidant activity determined by the DPPH (2,2-diphenyl-2- picrylhydrazil) method allows evaluating the capacity of samples to scavenge free radicals such as DPPH. In our experiment, the antioxidant activity measured by the method DPPH showed an increase during the first 4 days storage in all treatments and decreased significantly thereafter ($P < 0.05$) (Fig. 2.2). The only exception was PE 1% which decreased in the first 2 days and remained almost constant thereafter. At the end of the experiment only CH treatment showed significantly higher antioxidant activity than AL.

Changes during storage and the use of different polysaccharide-based edible coatings may promote differences in the potential antioxidant capacity. As in other studies using other fruits and vegetables our results presented an increase in DPPH during the first 4 days (Oms-Oliu et al., 2008; Ali et al., 2013). Probably as in other studies the incorporation of antioxidant agents as AA are important to enhance the radical scavenging capacity (Antunes et al., 2010).

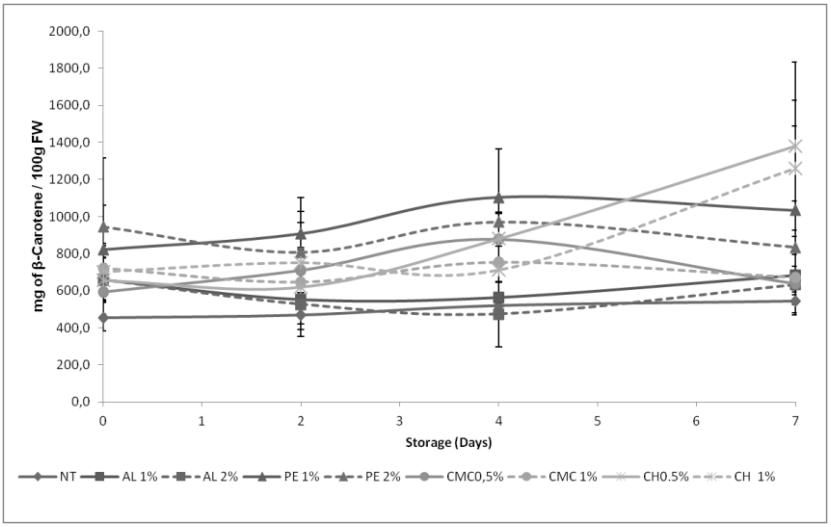


Figure. 2.1. β-Carotene recorded through time, in fresh-cut mango stored at 4±1ºC, Values represent the mean ± standard deviation of three replicates.

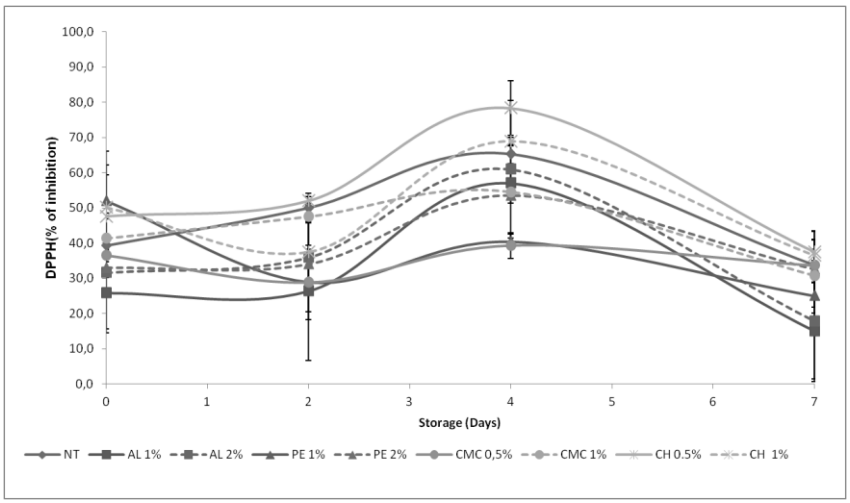


Figure 2 Antioxidant activity determined by DPPH recorded through time, in fresh-cut mango stored at 4±1ºC. Values represent the mean ± standard deviation of three replicates.

4. Conclusion

In this study we conclude that the fresh-cut mango can be stored for at least 7 days at 4±0.5 °C, maintaining an attractive appearance and flavor and edible coatings improved some of the quality attributes. The use of edible coating in these types of fruit could be considered as safe and effective treatment. The use of pectin- or alginate-based formulations may reduce wounding stress and best maintained most quality attributes of the commodity, although Chitosan showed the higher antioxidant activity and β-caroten content. We think that alginate could be recommended for commercial purposes because of its lower cost as compared to the high economic value of low methoxyl pectins for industrial applications.

5. References

Ali, A.; Maqbool, M.; Alderson, P. G.; Zahid, N. Effect of Gum Arabic as an Edible Coating on Antioxidant Capacity of Tomato (Solanum Lycopersicum L.) Fruit during Storage. *Postharvest Biol. Technol.* **2013**, *76*, 119–124.

Antunes, M. D. C.; Dandlen, S.; Cavaco, A. M.; Miguel, G. Effects of Postharvest Application of 1-MCP and Postcutting Dip Treatment on the Quality and Nutritional Properties of Fresh-Cut Kiwifruit. *J. Agric. Food Chem.* **2010**, *58*, 6173–6181.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Campos, C. a.; Gerschenson, L. N.; Flores, S. K. Development of Edible Films and Coatings with Antimicrobial Activity. *Food Bioprocess Technol.* **2010**, *4*, 849–875.

Cerqueira, M. a.; Lima, Á. M.; Teixeira, J. a.; Moreira, R. a.; Vicente, A. a. Suitability of Novel Galactomannans as Edible Coatings for Tropical Fruits. *J. Food Eng.* **2009**, *94*, 372–378.

Chiumarelli, M.; Hubinger, M. D. Stability, Solubility, Mechanical and Barrier Properties of Cassava Starch – Carnauba Wax Edible Coatings to Preserve Fresh-Cut Apples. *Food Hydrocoll.* **2012**, *28*, 59–67.

Dang, K. T. H.; Singh, Z.; Swinny, E. E. Edible Coatings Influence Fruit Ripening, Quality, and Aroma Biosynthesis in Mango Fruit. *J. Agric. Food Chem.* **2008**, *56(4)*, 1361–1370.

Fisk, C. L.; Silver, A. M.; Strik, B. C.; Zhao, Y. Postharvest Quality of Hardy Kiwifruit (Actinidia Arguta "Ananasnaya") Associated with Packaging and Storage Conditions. *Postharvest Biol. Technol.* **2008**, *47*, 338–345.

Gonzalez-Aguilar, G. a.; Celis, J.; Sotelo-Mundo, R. R.; de la Rosa, L. a.; Rodrigo-Garcia, J.; Alvarez-Parrilla, E. Physiological and Biochemical Changes of Different Fresh-Cut Mango Cultivars Stored at 5 °C. *Int. J. Food Sci. Technol.* **2008**, *43*, 91–101.

Jiang, Y.; Li, Y. Effects of Chitosan Coating on Postharvest Life and Quality of Longan Fruit. *Food Chem.* **2001**, *73*, 139–143.

Litz, R. E. *The Mango :botany, Production and Uses*; Oxon; New .; 1997.

Liu, M.; Li, X. Q.; Weber, C.; Lee, C. Y.; Brown, J.; Liu, R. H. Antioxidant and Antiproliferative Activities of Raspberries. *J. Agric. Food Chem.* **2002**, *50*, 2926–2930.

McGuire, R. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255.

Oliveira, I.; Baptista, P.; Malheiro, R.; Casal, S.; Bento, A.; Pereira, J. A. Influence of Strawberry Tree (Arbutus Unedo L.) Fruit Ripening Stage on Chemical Composition and Antioxidant Activity. *Food Res. Int.* **2011**, *44*, 1401–1407.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon. *LWT - Food Sci. Technol.* **2008**, *41*, 1862–1870.

Qi, H.; Hu, W.; Jiang, A.; Tian, M.; Li, Y. Extending Shelf-Life of Fresh-Cut "Fuji" Apples with Chitosan-Coatings. *Innov. Food Sci. Emerg. Technol.* **2011**, *12*, 62–66.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G. A.; Martín-Belloso, O. Effect of Minimal Processing on Bioactive Compounds and Antioxidant Activity of Fresh-Cut Kent Mango (Mangifera Indica L.). *Postharvest Biol. Technol.* **2009**, 384–390.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Raybaudi-Massilia, R. M.; Soliva-Fortuny, R. C.; Avena-Bustillos, R. J.; McHugh, T. H.; Martín-Belloso, O. Apple Puree-Alginate Edible Coating as Carrier of Antimicrobial Agents to Prolong Shelf-Life of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2007**, *45*, 254–264.

Sharma, O. P.; Bhat, T. K. DPPH Antioxidant Assay Revisited. *Food Chem.* **2009**, *113*, 1202–1205.

Wang, S. Y.; Gao, H. Effect of Chitosan-Based Edible Coating on Antioxidants, Antioxidant Enzyme System, and Postharvest Fruit Quality of Strawberries (Fragaria X Aranassa Duch.). *LWT - Food Sci. Technol.* **2012**, *52*, 1–9.

Zúñiga, G. E.; Junqueira-Gonçalves, M. P.; Pizarro, M.; Contreras, R.; Tapia, A.; Silva, S. Effect of Ionizing Energy on Extracts of Quillaja Saponaria to Be Used as an Antimicrobial Agent on Irradiated Edible Coating for Fresh Strawberries. *Radiat. Phys. Chem.* **2012**, *81*, 64–69.

Chapter III

The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage

A Guerreiro[1], C Gago[1], M Faleiro[2], M Miguel[1] & M Antunes[1]

[1]Faculdade de Ciências e Tecnologia, IBB-CBV, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal
[2]Faculdade de Ciências e Tecnologia, IBB-CBME, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Postharvest Biology and Technology 100 (2015) 226–233

Postharvest Biology and Technology 100 (2015) 226–233

Contents lists available at ScienceDirect

Postharvest Biology and Technology

The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage

Adriana C. Guerreiro[a], Custódia M.L. Gago[a], Maria L. Faleiro[b], Maria G.C. Miguel[a], Maria D.C. Antunes[a,*]

[a] Faculdade de Ciências e Tecnologia, IBB-CBV, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal
[b] Faculdade de Ciências e Tecnologia, IBB-CBME, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

article info

Article history:
Received 23 June 2014
Received in revised form 3 September 2014
Accepted 15 September 2014

Keywords:
Edible coating
Alginate Citral
Eugenol Arbutus
berries Storage

abstract

The effect of coating *Arbutus unedo* fresh fruit with alginate-based edible coatings enriched with the essential oils compounds (EOC) eugenol (Eug) and citral (Cit) was studied. The minimum inhibitory concentrations (MIC) against the main postharvest pathogens were determined for Eug and Cit giving values of 0.10 and 0.15 (w/v), respectively. Twelve formulations of edible coatings were used: sodium alginate (AL) was tested at 1 and 2% (w/v) with incorporation of Eug and Cit at MIC and double MIC or their combination at MIC. Arbutus berries were dipped in those solutions for 2 min, and then stored at 0.5 °C. Control consisted of uncoated fruit. On days 0, 14 and 28, samples were taken to perform physicochemical and biochemical analysis [color CIE (L^*, $h°$), firmness, soluble solids content (SSC), weight Loss, trolox equivalent antioxidant capacity (TEAC), microbial growth and taste panels]. Results showed that edible coatings of 1% AL were the best to maintain most quality attributes of the commodity through storage at 0.5 °C. The incorporation of Cit and Eug into the alginate edible coatings improved the coatings in most cases, AL 1% + Eug 0.20% and AL 1% + Cit 0.15% + Eug 0.10% being those that better preserved sensory and nutritional attributes and reduced microbial spoilage. Thus, these coatings may be useful for improving postharvest quality and storage life of fresh arbutus fruit.

1. Introduction

Until recently, flavor and appearance were the most impor- tant attributes of fruit and other fresh vegetables, but currently, consumers are more concerned about food safety and nutritional values. Fruit and vegetables are rich in natural antioxidants, nutrients, vitamins and fiber (Olaimat and Holley, 2012). The increasing demand for dietary products with antioxidant properties has focused interest on fresh fruit as natural sources of these compounds (Chiumarelli and Hubinger, 2012; Guerreiro et al., 2013).

However, fresh fruit and vegetables are highly perishable, and losses can be of great importance if correct postharvest measures are not provided. The high perishability of small fruit requires a search for the best (safe for health and environment) techniques to counteract the metabolic processes leading to rapid senescence, so that they can be of high quality for longer times, in order to be profitable for the enterprises dealing with them.

Edible coatings of different composition have been tested and used to prolong storage life of fresh and fresh-cut fruit, reducing metabolic processes and retarding microbial growth, and can also create a protective barrier to reduce respiration and transpiration rates, retarding senescence while preserving quality (Fisk et al., 2008; Gonzalez-Aguilar et al., 2008; Vargas et al., 2008; Antuneset al., 2012; Dhall, 2013; Valencia-Chamorro et al., 2013).

Polysaccharides, proteins and lipids can be used for the formulation of edible coatings and they must be neutral with respect to color and flavor. Edible coatings made of polysaccharides, particularly starches, contain good film-forming compounds and have low oxygen permeability, implying a decrease of respiration rates of fresh products (Campos et al., 2010). Alginate derived from marine brown algae (*Phaeophyceae*) and pectin extracted from apple waste or from the peel of citrus fruit are common polysaccharides used as gelling agents in the food industry (Oms-Oliu et al., 2008).

Edible coatings have also been recognized for more innova- tive uses beyond their current ones. They have a high potential to carry active ingredients such as anti-browning agents, colorants, flavors, nutrients, spices and antimicrobial compounds that can extend product shelf-life and reduce the risk of pathogen growth on food surfaces (Gonzalez-Aguilar et al., 2008; Oms-Oliu et al., 2010; Antunes et al., 2012; Zúñiga et al., 2012).

Some essential oils and their constituents have been shown to have a role as pharmaceuticals and food preservatives due to their,

* Corresponding author. Tel.: +351 289800900; fax: +351 289818419.
E-mail address: mantunes@ualg.pt (M.D.C. Antunes).

http://dx.doi.org/10.1016/j.postharvbio.2014.09.002
0925-5214/

among others, antioxidant and antimicrobial capacity, and are generally recognized as safe (GRAS) (Hammer et al., 1999; Antunes and Cavaco, 2010; Miguel, 2010). Since the chemical composition of plant-derived products, such as essential oils, is highly variable and may involve many compounds, the utilization of single compound instead of essential oils is a better approach to obtain an edible coating with constant characteristics as requested by the market (Miguel, 2010).

The strawberry tree (*Arbutus unedo* L.) belongs to the family *Ericacea* and is native to the Mediterranean region (Barros et al., 2010). Usually its fruit are used in the production of an alcoholic distillate, a very aromatic and appreciated drink (containing 40–60% alcohol/volume). Recently, its interest as a fresh fruit for consumption has increased due to its high nutritional value (Pallauf et al., 2008; Fortalezas et al., 2010; Guerreiro et al., 2013).

To our knowledge only a report from the present authors refers to storage of *Arbutus unedo* L. fruit and none exist on the use of edible coatings (Guerreiro et al., 2013). The objective of this study was to determine the effect of the essential oils constituents (EOC) citral (Cit) and eugenol (Eug) when incorporated into alginate-based edible coatings, on postharvest quality, safety and shelf-life extension of *Arbutus unedo* L. fruit (also named the strawberry tree fruit or arbutus berries).

2. Materials and methods

2.1. Fruit source

The fruit of the strawberry tree (*Arbutus unedo* L.) were harvested in the mountain "Caldeirão", in Algarve Region, Portugal, in mid-November, when they were ripe (with red color, firmness of 5.22 ± 0.42 N and soluble solids content (SSC) 22.6 ± 0.4 (°Brix), and immediately transported to the postharvest laboratory at the University of Algarve, where they were selected for uniformity of size and freedom from defects, for the experiments.

2.2. Chemicals

Food grade sodium alginate (AL) (Sigma–Aldrich Chemic, Steinhein, Germany) was the biopolymer used for coating formulations. Calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce cross linking reaction and ascorbic acid (Scharlau, Barcelona, Spain) was added as an anti-browning agent. Citral and eugenol were purchased from Sigma–Aldrich Chemic, Steinhein, Germany.

2.3. Edible coatings

The coating-forming solutions based on sodium alginate were formulated as described by Rojas-Graü et al. (2007a). Ascorbic acid 1% was added as an anti-browning agent in the edible coating solutions according to previous work (Robles-Sánchez et al., 2009).

Twelve different formulations of edible coatings were prepared. The treatments were: Control (fruit not coated or dipped in any treatment); AL 1% (strawberry tree fruit coated with sodium alginate (10 g/L)); AL 1% + Citral 0.15% (citral 1.50 g/L); AL 1% + Citral 0.3% (citral 3.00 g/L); AL 1% + Eugenol 0.1% (eugenol 1.00 g/L); AL 1% + Eugenol 0.2% (eugenol 2.00 g/L); AL 1% + Citral 0.15% + Eugenol 0.1%; AL 2% (strawberry tree fruit coated with sodium alginate (20 g/L)); AL 2% + Citral 0.15%; AL 2% + Citral 0.3%; AL 2% + Eugenol 0.1%; AL 2% + Eugenol 0.2% and AL 2% + Citral 0.15% + Eugenol 0.1%.

Each treatment was performed in two steps: first, arbutus berries were dipped into the edible coating solution + ascorbic acid for 2 min; the excess of coating material was allowed to drip off for 30 s before the second dip in the calcium chloride solution for 1 min. Then, 8 fruit were placed in polypropylene plastic trays (8 cm × 10 cm × 4 cm), perforated in the cover, and stored at 0.5 °C until analysis. On days 0, 14 and 28, three trays per treatment were taken to perform the analyses. Experiments were repeated twice.

Table 1
Table of microorganisms used for determination of the minimum inhibitory concentration (MIC) of citral and eugenol.

Microorganisms	Origin	Source
Escherichia coli DMS 1077	Feces from diphtheria convalescent patient	German Collection of Microorganisms and Cell Cultures
Salmonella enterica subspecie *enterica* serovar Typhimurium ATCC 14028	Animal tissue (chicken heart and liver. 4 weeks old)	American Type Culture Collection
Listeria monocytogenes EGD	Clinical Inflammation. University ofLeicester. UK	Dept Inf. Immun. and
Botrytis cinerea DSM 877	Isolated by American Cyanamid Co. No. N51	German Collection of Microorganisms and Cell Cultures
Penicillium digitatum DSM 2748	Citrus fruit	German Collection of Microorganisms and Cell Cultures
Penicillium expansum DSM 1282	Mold fermented sausage	German Collection of Microorganisms and Cell Cultures

2.4. General quality parameters

The color of the arbutus berries was measured using a Minolta Chroma meter CR-300 (EC Minolta, Japan) using the CIELab scale (L^*, a^* and b^*). The L^* represents color lightness (0 = black and 100 = white). Hue was calculated as °Hue = arctan (b^*/a^*) (McGuire, 1992). The firmness of the fruit was determined by puncture with a Chatillon TCD200 and a Digital Force Gauge DFIS 50 (Jonh Chatillon & Sons, Inc., USA) fitted with a 4 mm diameter probe at a depth of 7 mm. For the determination of the total soluble solids content (SSC) the °Brix was measured by a digital refractometer PR1 ATAGO CoLTD (Japan), in the fruit juice. Weight loss was expressed as percentage of the initial weight.

2.5. Trolox equivalent antioxidant activity (TEAC)

The preformed radical monocation of 2,2ʳ-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was generated according to the modified method of Re et al. (1999), as described in Antunes et al. (2010). For the assay, 10 μL of the juice was added to 990 μL of ABTS radical cation solution. The absorbance was monitored spectrophotometrically at 750 nm for 6 min (Shimadzu spectrophotometer 160-UV, Tokyo, Japan). The antioxidant activity of each sample was calculated using the following equation: scavenging effect (SE%) = (1 − As/Ao) × 100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 min. The values were compared with the curve for Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations of 156.25, 312.5, 625.0, 1250 and 2500 μM and the values given as μM Trolox equivalent antioxidant capacity.

2.6. Determination of minimum inhibitory concentration (MIC)

The antimicrobial activities of Eug and Cit against the microorganisms depicted in Table 1 were tested by the agar diffusion assay. The tested concentrations for Cit and Eug were 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.15, 0.20, 0.25 and 0.30% (w/v) diluted in ethanol 96%.

Original cultures were kept at -80 °C. The bacterial strains were recovered in tryptic soy agar (TSA). The bacterial cultures were inoculated in fresh TSA plates and fungi and yeasts in potato dextrose agar (PDA). Bacteria growth was for 24 h at 37 °C, and for fungi and yeasts the incubation was at 25 °C during 48-72 h. From each plate a loop was used to prepare a suspension with a turbidity value of 0.5 of the McFarland scale (10^8 CFU/mL). Decimal dilutions of the bacterial suspension were plated in TSA agar plates with the appropriate component concentration. The antibiotic chloramphenicol and ethanol were used as positive control (30 μg/mL). As negative control each microbial culture was grown in the absence of EOC. The lowest concentration that inhibited the visible bacte- rial growth was considered the MIC value. The MIC value for fungi was determined as described by Camele et al. (2012). Molds strains were maintained in PDA. The MIC value was determined by drop- ping, under axenic conditions, 10 μL aliquots of single suspensions containing 1×10^4 conidia/mL of the single species onto the surface center of Petri dishes containing PDA and prepared with the same percentage of Tween-20 and the component concentration. Three replicates for each tested compound dose were provided in PDA plugs from *Penicillium digitatum*, *P. expansum* and *Botritys cinerea* and transferred as above into Petri dishes containing only PDA, PDA plus 0.2% Tween 20 or PDA with Cit or Eug. The inhibitory effects of the EOC against each tested microorganism was determined after a 3–10 days incubation period at 25 °C, *e.g.*, when control colonies margins reached plate edges.

The checkerboard determination of Cit and Eug were done as described by Orhan et al. (2005). All combinations of the concentrations used for MIC determinations (0.01–0.30%) were tested. A total of 50 μL of medium was distributed into each well of the microplates. The first component (Cit or Eug) of the combination was serially diluted along the ordinate. An inocu- lum with a density similar to 0.5 McFarland turbidity standard was prepared for each bacteria. Each microtiter well was inoc- ulated with 100 μL of the bacterial inoculum (5×10^5 CFU/mL), and the plates were incubated at 30 °C during 48 h under aerobic conditions.

The fractional inhibitory concentration (FIC) index (XFIC) was calculated on the basis of the following: XFIC = FIC A + FIC B, where FIC A is the MIC of component A in the combination/MIC of component A alone, and FIC B is the MIC of component B in the combination/MIC of component B alone. A FIC index of <0.5 indicates synergism, >0.5–1 indicates additive effects, >1 to <2 indifference, and ≥2 is considered to be antagonism.

Three biological replicates and three technical were done ($n = 9$).

2.7. Microbial counts

Microbial counts were determined for each treatment. The microbiological parameters that were determined included counts of aerobic mesophilic and psychrophilic bacteria and molds and yeasts. The counts of aerobic mesophilic and psychrophilic were done according to the standard Portuguese NP-4405 (2002) using the Plate Count Agar medium (Biokar, Paris, France). The counts of molds and yeasts were performed according to ISO 21527-2 (2008) using Dicloran Rose-Bengal Cloranfenicol Agar (Biokar, Paris, France). Ten gram of each sample was trans- ferred to 90 mL of peptone water (Oxoid) and homogenized. Decimal dilutions were prepared using the same diluent. The

incubation temperature for yeasts and molds was 25±1 °C during 48-72 h, for aerobic mesophilic bacteria was 30 ± 1 °C during 24–72 h and was 6.5 ± 1 °C during 5 to 10 days for psychrophilic bacteria. Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram fresh weight.

2.8. Sensory evaluation

A taste panel was performed with 15 semi-trained panelists on the base of a 7-point hedonic scale (1 = dislike extremely, 4 = neither like nor dislike, 7 = like very much) for the sensory parameters: appearance, aroma, texture, taste and overall acceptance according to Gol et al. (2013) with some modifications. Panelists consisted of Faculty students and staff members who were trained at the beginning of the experiment to become familiar with the characteristics of the fruit. All parameters were evaluated at harvest and after 14 days, while after 28 days only appearance was evaluated due to reduced sample material. Sensory tests were carried out in a sensory laboratory equipped with individual sensory compartments.

2.9. Statistical analysis

Statistical analysis was carried out with the SPSS 20.0 software (IBM, Inc.). The experimental design was a complete randomized block design.

Two-way analysis of variance (ANOVA) was performed using treatments and storage time as factors. Duncan's multiple-range test ($P < 0.05$) for comparisons of means was performed.

Hierarchical cluster analysis (HCA) was utilized to investigate the similarities and dissimilarities among the formulations with respect to analyzes. For classification, the Ward's Minimum Vari- ance Method was utilized. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. The grouping derived from HCA was used to interpret the results of the dendo- gram and Principal Component Analysis (PCA) was performed by Chemoface 1.5 software.

3. Results and discussion

3.1. Antibacterial and antifungal activity

All the tested EOC were active against the used microorganisms. The MIC value of Eug and Cit was 0.10 and 0.15%, respectively for all the bacteria tested. Our findings are in accordance with the reported by Apolónio et al. (2014). However, Hemaiswarya and Doble (2009) report a MIC of 20 mM (0.33%) for Eug against Gram negative bacteria. This difference may be associated with several factors, including different strains tested and differences in the methodology used (Faleiro, 2011; Hyldgaard et al., 2012).

Both Eug and Cit were active against all the tested molds show- ing a similar MIC value of 0.08%. Since EOC in combination can interact and can display different effects, such as synergism and antagonism, these effects were evaluated between Eug and Cit. All the paired combinations showed indifferent or antagonist effect (FIC index >1) against all tested microorganisms.

According to these results the several formulations were prepared using (1) each compound at the MIC and the double concentration of MIC, and (2) in combination the MIC value of each EOC was used.

Since experiments included 13 treatments, there was too much data to show. For this reason, data presented is for treatments with Al 1% (Tables 2–4), since the best edible coatings were selected from them. Tables with results for all treatments are available electron- ically as Supplementary Tables 2A, 3A and 4A.

Supplementary Tables 2A, 3A and 4A related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2014.09.002.

3.2. Quality parameters

Although with a slight decrease through storage time in all treatments except AL 2% + Cit 0.3%, the color L^* value did not show

Table 2
Color parameters (L^* and $h°$), total soluble solids content (SSC), firmness, weight loss and antioxidant activity of *Arbutus unedo* L. fresh fruits covered with different edible coating formulations, through 28 days storage at 0.5 °C.

Treatments	Days	Control	AL 1%	AL 1% + Cit 0.15%	AL 1% + Cit 0.3%	AL 1% + Eug 0.1%	AL 1% + Eug 0.2%	AL 1% + Cit 0.15% + Eug 0.1%
Lightness (L^*)	0	39.75 aA	39.75 aA	39.75 aA	39.75 aA	39.75 aA	39.75 aA	39.75 aA
	14	35.31 abB	35.89 aAB	36.59 aAB	38.38 aB	36.13 aB	36.49 aAB	37.87 aB
	28	32.44 aC	33.82 aC	35.34 aABC	36.32 aABC	37.31 aABC	37.04 aABC	37.39 aBC
Hue angle ($h°$)	0	47.08 aA	47.08 aA	47.08 aA	47.08 aA	47.08 aA	47.08 aA	47.08 aA
	14	41.33 bABC	39.25 bABC	39.61 bABC	43.97 abA	37.98 bC	38.09 bC	41.06 abABC
	28	38.90 bAB	35.32 bAB	35.96 bAB	39.72 bAB	38.88 bAB	36.16 bAB	38.39 bAB
SSC (°Brix)	0	22.60 aA	22.60 aA	22.60 abA	22.60 aA	22.60 aA	22.60 aA	22.60 aA
	14	19.07 aB	21.87 aAB	20.67 aAB	23.47 aAB	21.00 aAB	19.97 aAB	23.93 aAB
	28	22.27 aB	24.29 aAB	23.20 aAB	24.67 aA	23.47 aAB	23.60 aAB	24.40 aA
Firmness (N)	0	5.21 aA	5.21 aA	5.21 aA	5.21 aA	5.21 aA	5.21 aA	5.21 aA
	14	1.93 bB	2.32 bAB	3.07 bAB	3.19 bAB	2.16 bB	3.50 bA	2.08 bB
	28	1.60 bCD	2.22 bBC	2.63 bB	1.69 cBCD	2.59 bBC	2.61 bB	1.81 bBC
Weight loss (%)	0	0.00 cA	0.00 cA	0.00 cA	0.00 cA	0.00 cA	0.00 cA	0.00 cA
	14	2.02 bB	2.80 bA	2.93 bA	2.74 bA	3.17 bA	3.02 bA	2.78 bA
	28	3.75 aB	4.88 aAB	4.81 aAB	5.27 aAB	5.87 aA	5.21 aAB	5.20 aAB
Antioxidant activity (μM TE/100 g)	0	4215.68 aA	4215.68 aA	4215.68 aA	4215.68 aA	4215.68 aA	4215.68 aA	4215.68 aA
	14	1020.92 bBC	865.61 bC	1558.61 bBC	1345.08 bBC	1321.53 bBC	1709.02 bB	2349.09 bB
	28	546.15 cC	758.02 bBC	659.65 cC	581.96 cC	655.39 cC	782.45 cBC	970.00 cBC

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 3
Yeasts and molds and aerobic mesophillic microorganisms of *Arbutus unedo* L. fresh fruits covered with different edible coating formulations, through 28 days storage at 0.5 °C.

Treatments	Days	Control	AL 1%	AL 1% + Cit 0.15%	AL 1% + Cit 0.3%	AL 1% + Eug 0.1%	AL 1% + Eug 0.2%	AL 1% + Cit 0.15% + Eug 0.1%
Yeast and molds (Log CFU/g)	0	3.53 aA	3.53 aA	3.53 aA	3.53 aA	3.53 aA	3.53 aA	3.53 aA
	14	3.20 aA	3.19 aA	3.27 aA	1.57 aBC	1.13 bCD	0.77 abCD	1.76 aB
	28	3.26 bB	1.53 bD	0.00 bE	0.00 bE	0.00 bE	0.00 bE	1.23 aD
Aerobic mesophilic microrganisms (Log CFU/g)	0	2.92 aA	2.92 aA	2.92 aA	2.92 aA	2.92 aA	2.92 aA	2.92 aA
	14	3.45 aA	3.26 aA	3.26 aA	3.11 aAB	2.65 aC	2.59 aC	2.52 aC
	28	3.52 aAB	3.67 aA	2.79 aBC	2.56 aC	0.53 bF	0.33 bF	1.20 aDE

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

statistically significant differences (Table 2). However, after 14 days storage, AL 2% + Cit 0.15% showed significantly higher L^* value than control, AL 1% + Cit 0.3%, AL 1% + Eug 0.1% and AL 1% + Cit 0.15% + Eug 0.1%. In fact treatments with 2% AL had the highest L^* values, most close to those at harvest time, leading to the suggestion that AL concentration is more efficient than EOC to preserve natural lightness (L^*) of eating ripe arbutus berries up to 14 days storage. However, after 28 days storage, control together with AL 1% had the significantly lower L^* values which means darker fruit according to McGuire (1992). This may be attributed to a higher rate of

Table 4
Sensory evaluation of *Arbutus unedo* L. fresh fruit covered with different edible coating formulations through 28 days storage at 0.5 °C. Appearance, texture, aroma, taste and overall liking were evaluated at harvest and after 14 days storage, while after 28 days only appearance was evaluated.

Treatments	Days	Control	AL 1%	AL 1% + Cit 0.15%	AL 1% + Cit 0.3%	AL1% + Eug 0.1%	AL1% + Eug 0.2%	AL 1% + Cit 0.15% + Eug 0.1%
Appearance	0	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA
	14	3.20 bB	4.20 aAB	4.60 aAB	4.80 aAB	4.40 aAB	3.80 aAB	4.40 aAB
	28	3.00 bB	4.33 aAB	4.33 aA	4.33 aA	5.00 aA	5.00 aAB	5.00 aA
Texture	0	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA
	14	3.40 bBC	4.20 aAB	3.80 aB	3.60 bBC	5.00 aA	5.20 aA	3.80 aBC
Aroma	0	4.40 aA	4.40 aA	4.40 aA	4.40 aA	4.40 aA	4.40 aA	4.40 aA
	14	4.20 aA	4.60 aA	4.20 aA	4.40 aA	4.40 aA	4.40 aA	4.40 aA
Taste	0	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA	5.00 aA
	14	3.80 bB	5.00 aA	3.00 bBC	1.80 bC	4.40 aAB	5.00 aA	4.40 aAB
Overall Liking	0	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA	4.80 aA
	14	3.65 bB	4.50 aA	3.90 bAB	3.65 bB	4.55 aA	4.60 aA	4.25 aA

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test, or ANOVA when comparing only 0–14 days storage ($P < 0.05$).

deterioration in fruit of these treatments. As for 14 days, AL 2% gave the highest L^* values, closest to harvest, but in this case when combined with Cit 0.3%, followed by the synergetic AL 2% + Cit 0.15%+Eug 0.1%.

From the above it appears that AL 2% combined with Cit was the most efficient in maintaining lightness, 0.15% being enough for 14 days and 0.3% for 28 days.

The °hue represents the angle at the color wheel (McGuire, 1992). At harvest, the °hue was 47.08, locating the ripe arbutus berries in the orange color as other authors have shown (Guerreiro et al., 2013 and references therein).

The °hue decreased significantly through 28 days storage time, due to the orange color turning to red in accordance with results of Guerreiro et al. (2013), except in the treatment AL 2% + Cit 0.15% + Eug 0.1% where it was maintained (Table 2). In fact, all treatments showed a significant decrease from 0 to 14 days, and then the decrease was not significant up to 28 days, with the exception of all treatments with Cit plus 2% AL and 1% AL+0.3% Cit. Again, as for L^*, treatments with 2% AL was more efficient in keeping color of the berries fruit, and in this case Cit was clearly favorable.

Color L^* and °hue values for all treatments are within the range found by other authors for ripe arbutus berries (Guerreiro et al., 2013 and references therein).

According to Valero et al. (2013) and Azarakhsh et al. (2014) the coating of plum fruit and fresh-cut pineapple with alginate was significantly effective in maintaining color parameters as compared to uncoated samples, as in our case probably due to the reduced advance in senescence caused by the protective effect of alginate edible coating. As in our study, Rojas-Graü et al. (2007b), when using oregano, lemongrass and vanillin essential oils into alginate edible coatings for fresh-cut apples, found lower changes in color in vanillin (0.3 and 0.6%) edible coatings than in the other treatments, although all edible coatings were better than in the control. Also, when lemongrass essential oil was added to alginate edi- ble coatings, in a percentage up to 0.5%, and applied to fresh-cut pineapple, the coating did preserve better color than the control but without significant differences among treatments (Azarakhsh et al., 2014). In our case, edible coatings enriched with essential oils constituents also did preserve better color, with Cit giving slightly better results.

The total soluble solids content (SSC) was 22.6 °Brix at harvest, corresponding to a eating ripe value (Guerreiro et al., 2013). Through storage, there was not a significant change in SSC for any treatment (Table 2). However, among treatments there were differences. After 14 days, the control showed significantly lower SSC values than AL 2% + Cit 0.3%. After 28 days, the control and AL 2% at both Eug concentrations showed lower SSC than AL plus Cit 0.3%. During postharvest storage, metabolic processes continue as a result of fruit ripening and senescence, which continues by converting starch and organic acids to sugars to be used in metabolic processes (Duan et al., 2011). Due to the non-significant differences in SSC for each treatment through storage, it means that fruit were ripe at harvest and no great changes occurred through storage as it is common for ripe fruit. Duan et al. (2011) found no effect of applying edible coatings on SSC in blueberries through storage. However, Velickova et al. (2013) and Gol et al. (2013) reported slower sugar metabolism through storage in coated strawberries. In our study, although some statistically significant differences among treatments occurred, they are meaningless since no signifi- cant differences were observed through storage for each treatment (Table 2).

Fruit softening, one of the most important quality deterioration parameters during postharvest storage is generally caused by the hydrolysis of starch to sugar and mainly the degradation of pectin in the fruit cell wall associated with fruit ripening. The firmness of arbutus berries at harvest was ~5.2 N (Table 2). Similar values were reported by other authors for ripe fruit (Guerreiro et al., 2013 and references therein). Generally, the firmness was significantly better maintained ($P < 0.05$) with the edible coatings than for the control. Through storage time, firmness decreased in all treatments mostly in the first 14 days, then remained almost constant.

This behavior is in accordance with the results of Guerreiro et al. (2013) and for other fruit not treated with edible coatings (Cordenunsi et al., 2003; Shin et al., 2008). The maintenance of the firmness is an important factor for increasing storage life of fresh products. Duan et al. (2011) found, for blueberries, no differences in firmness between sodium alginate and chitosan coatings. Valero et al. (2013) found that alginate coating in plums slowed down softening. Azarakhsh et al. (2014) found for fresh-cut pineapple a decrease in softening in alginate edible coatings enriched with essential oil from lemongrass up to 0.3%, being for 0.5% the firmness similar to control. Also Rojas-Graü et al. (2007b) report a decrease in firmness with lemongrass at 1.5% attributed to the low pH of this edible coating. As expected, in our case, the values of firmness decreased trough storage, but coatings helped to maintain firmness as compared to control.

The benefit of all edible coatings on firmness retention as compared to untreated fruit (control) may be mainly attributed to the inclusion of the dip in the calcium chloride solution, which was used for crosslinking the edible coatings polymers (Olivas and Barbosa-Cánovas, 2005; Rojas-Grau et al., 2008).

The effect of calcium for firmness preservation during storage of fresh and fresh-cut fruit has been extensively studied (Martin-Diana et al., 2007; Antunes et al., 2010).

However, Rojas-Graü et al. (2007b) report an improvement in firmness of alginate edible coatings when essential oils of vanillin and oregano were incorporated, which were not clear in the present research.

After 14 days storage, all edible coatings treatments lost more weight than control (Table 2). The weight loss was higher in treatments with Eug and AL 2% + 0.15% Cit than control after 28 days storage and the other treatments did not differ among them.

It is expected from some edible coatings composition, such as chitosan, that the coatings will create a semi-permeable bar- rier which reduces respiration, water loss and oxidation (Maqbool et al., 2011; Gol et al., 2013). Azarakhsh et al. (2014) found reduced weight loss in fresh-cut pineapple coated with alginate based ediblecoatings. However, Rojas-Grau et al. (2008) report no differences in gas exchange in alginate edible coatings and controls what may also happen to water permeability.

In fact, polysaccharide edible coatings such as alginate-based ones (Rojas-Grau et al., 2008 and references therein) may not have the ability to reduce water loss *per se*, needing additional lipid incorporation for water loss reduction improvement. Duan et al. (2011) confirm our results by reporting higher water loss in polysaccharide-based edible coatings than controls. They report the higher permeability to hydrophilic properties of those edible coatings. Also, in our case the first weight measurements were just after edible coating application, which means that through storage, loss of water could be also from the edible coating by itself and not only from the fruit, which was the case of the control, since the control did not have any kind of washing or treatment.

Antioxidant activity is an important quality factor attributed to fruit and vegetables for improving human health. The antioxidant activity as measured by TEAC method showed high values similar to those of other authors (Guerreiro et al., 2013). TEAC values decreased with storage time except for AL 2% and Al 2% + Cit 0.3% in which the decrease was significant only after 14 days storage (Table 2). After 28 days storage, AL 2% and AL 2% + Cit 0.15% + Eug 0.1% gave the higher antioxidant values and control the lowest.

The decrease in antioxidant activity through time may be attributed to the breakdown of cell structure as the fruit senesce

(Macheix et al., 1990). Edible coatings may provide a barrier to reduce oxygen intake and so reducing oxidative processes (Bonilla et al., 2012). The antioxidant effect of some essential oils constituents may also have contributed to reduce the decrease in antioxidant activity probably due to its antioxidant effect (Antunes and Cavaco, 2010 and references therein). Also, Gol et al. (2013) found better preservation of phenols and ascorbic acid with chitosan edible coatings than control.

Some edible coatings did not show significant differences from the control, and this may be attributed to the ineffectiveness of some alginate edible coatings in reducing gas exchange as reported by Rojas-Grau et al. (2008).

According to Robles-Sánchez et al. (2013) antioxidant activ- ity in fresh-cut mangoes covered with the edible coating Alginate 2% + Ascorbic Acid 1%, expressed as TEAC, was significantly higher than in alginate alone and control fruit. This may be attributed to the ascorbic acid added, which is in accordance with our work.

3.3. Microbial analyses

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration. Is has been referred that some essential oils and their constituents have an antimicrobial effect (Antunes and Cavaco, 2010 and references therein). Additionally, edible coatings, by creating a cover to fruit surface, also protect from pathogen spoilage (Rojas-Grau et al., 2008) and the inclusion of EO or EOC improves this behavior (Raybaudi-Massilia et al., 2008).

Our experiment showed higher yeasts and molds and aerobic microorganisms in control fruit through storage (Table 3). However they do not differ significantly from AL 1% and AL 2% after 14 days and after 28 days from Al 2% for yeasts and molds and 1% for aerobic microorganisms. It is clear that Eug treatments followed by the combination of Cit 0.15% + Eug 0.1% were the most efficient in controlling yeasts and molds through storage. Although after 14 days, counts were higher in some treatments for 2% AL than for 1% AL, most treatments did not differ significantly and, after 28 days, treatments with 1% AL were more efficient in reducing yeast and molds than 2% AL. This may be due to the higher carbohydrate content of AL 2% which may be substrate for microbial growth as also reported by Bierhals et al. (2011).

Aerobic microorganisms followed a similar pattern as for yeast and molds (Table 3). Rojas-Graü et al. (2007b) and Azarakhsh et al. (2014) also found that edible coatings based on alginate did not reduce microbial spoilage by themselves but they did when some essential oils were incorporated, as in our case. It is noteworthy that in all cases, either yeasts and molds or aerobic microorganisms did not reach after 28 days storage the limits reported by the Institute of Food Science and Technology as the limit of acceptance of fruit products for consumption which is of 10^6 CFU/g (Bierhals et al., 2011) and Stannard (1997). No growth of psycrophilic aerobic bacteria was observed through storage time in any treatment.

3.4. Sensory analyses

The acceptance by consumers of fruit treated with edible coatings is a major issue, since they can change sensory properties of the fruit. The taste panel is of great importance because treat- ments and storage time can change the edible quality of fruit and edible coatings are usually consumed with the food product (Rojas- Graü et al., 2009). However most work has not included taste panels when testing new edible coatings.

A complete sensory evaluation was done after 14 days storage, while after 28 days only appearance was evaluated. Interestingly all edible coatings gave a good appearance which did not significantly change through 28 days storage time (Table 4). Only the control had a significant decrease after 14 days storage then remained constant until 28 days. In the case of our experiments, after 14 days storage, the appearance of the control was 3.0–3.2, which is under the average acceptable value (4 in a scale of 7). For texture there was a statistically significant decrease ($P < 0.05$) from 0 to 14 days storage in control, AL 2% and AL 1% + Cit 0.3%, showing no statistically significant changes with the other treatments (Table 4). In this manner, after 14 days storage treatments with Eug and AL 2% + Cit 0.15% + Eug 0.1% had the highest texture preference values. For aroma no statistically significant changes ($P < 0.05$) were detected from 0 to 14 days storage (Table 4). Also, although a stronger smell was due to Cit application, mostly at double MIC concentrations, no statistically significant changes in aroma were found because panelists were recording it as beneficial to fruit aroma. Taste was the most affected by Cit inclusion into edible coatings, mostly at higher concentrations (Table 4).

Taking into account the overall liking of arbutus berries treated with edible coatings, the sensory panel gave a good overall acceptance after 14 days, not significantly different from that at harvest, for all treatments except for the control and treatments with Cit 0.3%, with lower values. This was attributed mainly to lower values of taste due to the intense change caused by this Cit concentration to the natural taste of the fruit, and control due to a faster advance in senescence. Also for Cit at double MIC, texture was influenced. This is in agreement with results for firmness which report better firmness retention in Eug edible coatings (Table 2). Overall liking was significantly lower at 14 days storage in arbutus berries treated with AL 1% + Cit 0.3% and control than in the other treatments which did not show significant differences among them and were scored as good (>4 in a scale of 7).

There is no doubt that alginate edible coatings improved stor- age, as evaluated by panelists, of arbutus berries as compared to non-coated fruit (control). Similar results were found for alginate edible coatings used in fresh-cut pineapple (Azarakhsh et al., 2014). The incorporation of EOC into these edible coatings did improve storage life for longer as compared to control, and did not change sensory properties except for Cit mainly at double MIC which had a negative influence mainly on taste. Some authors found also no significant changes in sensory attributes by adding some EO to alginate edible coatings at low concentrations in other fruit (Rojas-Graü et al., 2007b; Azarakhsh et al., 2014). At high concentrations (0.5 and 1%), lemongrass reduced sensory properties of fresh-cut apples and pineapple, respectively as oregano at 0.1% for apple. Some EO or their EOC, although with a good effect on reducing spoilage growth and even appearance, may have a negative effect by changing natural taste of the coated fruit which was the case of Cit at double MIC, in our case.

Raybaudi-Massilia et al. (2008) as in our work found no changes in sensory properties in alginate edible coatings applied to fresh- cut melon, but did find when EO or EOC were applied depending on the EO's and their concentrations.

3.5. Formulation selection

The Hierarchical cluster and PCA analyses gave a main group, rounded by a circle, with closer characteristics as shown in Fig. 1. Control (1), AL 2% (8) and AL 2% + Citral 0.3% (10) are separated among them and from the main group.

Taking into account for each quality parameter measured the mean closest value to the one at harvest for color, higher value for firmness, °Brix and antioxidant activity, and lower value for weight loss and microbial spoilage and from the above discussion,it is clear that the main group showed the better performance in preserving quality characteristics through storage time. From this group we selected the 2 edible coatings which were better accepted by consumers in general. There were selected the edible coatings AL 1% + Eug 0.2% and AL 1% + Cit 0.15% + Eug 0.1% because they preserve

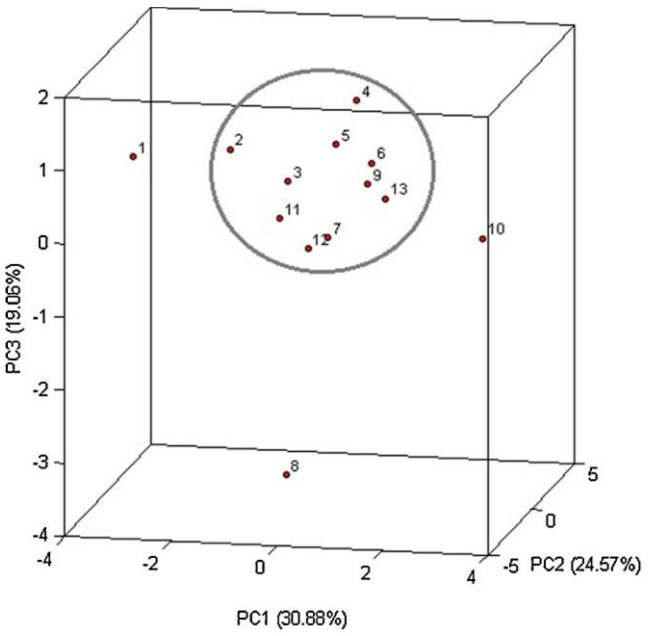


Fig. 1. Loading plot for principal component analysis (PCA) of the 13 treatments measured and showed in the above tables. 1 – Control; 2 – AL 1%; 3 – AL 1% + Cit 0.15%; 4 – AL 1% + Cit 0.3; 5 – AL 1% + Eug 0.1%; 6 – AL 1% + Eug 0.2%; 7 – AL 1% + Cit 0.15% + Eug 0.1%; 8 – AL 2%; 9 – AL 2% + Cit 0.15%; 10 – AL 2% + Cit 0.3%; 11 – AL 2% + Eug 0.1%; 12 – AL 2% + Eug 0.2%; 13 – AL 2% + Cit 0.15% + Eug 0.1%.

most quality characteristics well and reduce microbial growth and had good scores in sensory evaluation.

4. Conclusions

In this study, we conclude that arbutus berries can be stored for at least 28 days at 0.5 °C, with the edible coatings of this experiment with a good attractive appearance. The use of alginate-based formulations is appropriate to maintaining most quality attributes of the commodity. Alginate could be recommended for commercial purposes due its lower cost in comparison to other polysaccharides used in industrial applications of high economic value. For *Arbutus unedo* L. berries, 1% AL is enough, higher concentrations not being necessary.

The results of the present research showed that edible coatings based on AL 1% when combined with Eug at double MIC (0.2%) or combined with the synergetic effect of Eug and Cit at MIC (0.1 and 0.15, respectively) where the most efficient in preserving quality parameters, reduce microbial spoilage and preserving sensory properties of *Arbutus unedo* L. fresh fruit, thus improving storage life.

Acknowledgements

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010 – INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

References

Antunes, M.D.C., Cavaco, A.M., 2010. The use of essential oils for postharvest decay control. A review. Flavour Fragr. J. 25, 351–366.

Antunes, M.D.C., Dandlen, S., Cavaco, A.M., Miguel, G., 2010. Effects of postharvest application of 1-MCP and postcutting dip treatment on the quality and nutritional properties of fresh-cut kiwifruit. J. Agric. Food Chem. 58, 6173–6181.

Antunes, M., Gago, C., Cavaco, A., Miguel, M.G., 2012. Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. Recent Pat. Food. Nutr. Agric. 4, 114–122.

Apolónio, J., Faleiro, M.L., Miguel, M.G., Neto, L., 2014. No induction of antimicrobial resistance in *Staphylococcus aureus* and *Listeria monocytogenes* during continu- ous exposure to eugenol and citral. FEMS Microbiol. Lett. 354, 92–101.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., Mohd Adzahan, N., 2014. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. Postharvest Biol. Tech- nol. 88, 1–7.

Barros, L., Carvalho, A.M., Morais, J.S., Ferreira, I.C.F.R., 2010. Strawberry-tree, blackthorn and rose fruit: detailed characterisation in nutrients and phytochemicals with antioxidant properties. Food Chem. 120, 247–254.

Bierhals, V.S., Chiumarelli, M., Hubinger, M.D., 2011. Effect of casava starch coating on quality and shelf life of fresh-cut pineapple (*Ananas comosus* L. Merril cv. 'Pérola'). J. Food Sci. 76, 62–72.

Bonilla, J., Atarés, L., Vargas, M., Chiralt, A., 2012. Edible films and coatings to prevent the detrimental effect of oxygen on food quality: possibilities and limitations. J. Food Eng. 110, 208–213.

Camele, I., Altieri, L., De Martino, L., De Feo, V., Mancini, E., Rana, G.L., 2012. In vitro control of post-harvest fruit rot fungi by some plant essential oil components. Int. J. Mol. Sci. 13, 2290–2300.

Campos, C.A., Gerschenson, L.N., Flores, S.K., 2010. Development of edible films and coatings with antimicrobial activity. Food Bioprocess Technol. 4, 849–875.

Chiumarelli, M., Hubinger, M.D., 2012. Stability, solubility, mechanical and barrier properties of cassava starch – Carnauba wax edible coatings to preserve fresh- cut apples. Food Hydrocolloid. 28, 59–67.

Cordenunsi, B.R., Nascimento, J.R.O., Lajolo, F.M., 2003. Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. Food Chem. 83, 167–173.

Dhall, R.K., 2013. Advances in edible coatings for fresh fruits and vegetables: a review. Crit. Rev. Food Sci. 53, 435–450.

Duan, J., Wu, R., Strik, B.C., Zhao, Y., 2011. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. Postharvest Biol. Technol. 59, 71–79.

Faleiro, M.L., 2011. The mode of antibacterial action of essential oils. In: Méndez-Vilas, A. (Ed.), Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. Formatex, Badajoz, Spain, pp. 1143–1156. Fisk, C.L., Silver, A.M., Strik, B.C., Zhao, Y., 2008. Postharvest quality of hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') associated with packaging and storage conditions. Postharvest Biol. Technol. 47, 338–345.

Fortalezas, S., Tavares, L., Pimpão, R., Tyagi, M., Pontes, V., Alves, P.M., McDougall, G., Stewart, D., Ferreira, R.B., Santos, C.N., 2010. Antioxidant properties and neuroprotective capacity of strawberry tree fruit (*Arbutus unedo*). Nutrients 2, 214–229.

Gol, N.B., Patel, P.R., Rao, T.V.R., 2013. Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biol. Technol. 85, 185–195.

Gonzalez-Aguilar, G.A., Celis, J., Sotelo-Mundo, R.R., De La Rosa, L.A., Rodrigo-Garcia, J., Alvarez-Parrilla, E., 2008. Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5 °C. Int. J. Food Sci. Technol. 43, 91–101.

Guerreiro, A.C., Gago, C.M.L., Miguel, M.G.C., Antunes, M.D.C., 2013. The effect of temperature and film covers on the storage ability of *Arbutus unedo* L. fresh fruit. Sci. Hortic. 159, 96–102.

Hammer, K.A., Carson, C.F., Riley, T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86, 985–990.

Hemaiswarya, S., Doble, M., 2009. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. Phytomedicine 16, 997–1005.

Hyldgaard, M., Mygind, T., Meyer, R.L., 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3 (12), 1–24.

ISO 21527-2, 2008. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Yeasts and Moulds – Part 2: Colony Count Technique in Products with Water Activity Less Than or Equal to 0.95. International Standards Organization, Switzerland.

Macheix, J.J., Fleuriet, A., Bilot, J., 1990. Fruit Phenolics. CRC Press, Inc., Florida, USA.

Maqbool, M., Ali, A., Alderson, P.G., Zahid, N., Siddiqui, Y., 2011. Effect of a novel edible composite coating based on gum arabic and chitosan on biochemical and physiological responses of banana fruit during cold storage. J. Agric. Food Chem. 59, 5474–5482.

Martin-Diana, A.B., Ricoa, D., Friasa, J.M., Barat, J.M., Henehana, G.T.M., Barry-Ryan, C., 2007. Calcium for extending the shelf life of fresh whole and minimally processed fruit and vegetables: a review. Trends Food Sci. Technol. 18, 210–218.

McGuire, R., 1992. Reporting of objective color measurements. HortScience 27, 1254–1255.

Miguel, M., 2010. Antioxidant activity of medicinal and aromatic plants. A review. Flavour Fragr. J. 25, 291–312.

NP-4405, 2002. Food Microbiology – General Rules for Microorganism Counts. Colonies Count at 30 °C. Instituto Português da Qualidade, Lisboa, Portugal (in Portuguese).

Olaimat, A.N., Holley, R.A., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol. 32, 1–19.

Olivas, G.I., Barbosa-Cánovas, G.V., 2005. Edible coating for fresh-cut fruits. Crit. Rev. Food Sci. Nutr. 45, 657–670.

Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2008. Using polysaccharide-based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. LWT Food Sci. Technol. 41, 1862–1870.

Oms-Oliu, G., Rojas-Graü, M.A., González, L.A., Varela, P., Soliva-Fortuny, R., Hernando, M.I.H., Munuera, I.P., Fiszman, S., Martín-Belloso, O., 2010. Recent

approaches using chemical treatments to preserve quality of fresh-cut fruit: a review. Postharvest Biol. Technol. 57, 139–148.

Orhan, G., Bayram, A., Zer, Y., Balci, I., 2005. Synergy tests by E test and checker-board methods of antimicrobial combinations against *Brucella melitensis*. J. Clin. Microbiol. 43, 140–143.

Pallauf, K., Rivas-Gonzalo, J.C., del Castillo, M.D., Cano, M.P., de Pascual-Teresa, S., 2008. Characterization of the antioxidant composition of strawberry tree (*Arbu- tus unedo* L.) fruit. J. Food Compos. Anal. 21, 273–281.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J., Martín-Belloso, O., 2008. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. Int. J. Food Microbiol. 121, 313–327.

Re, R., Pellegrini, N., Proteggente, A., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. 26, 1231–1237.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G.A., Martín-Belloso, O., 2009. Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut Kent mango (*Mangifera indica* L.). Postharvest Biol. Technol. 51, 384–390.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G., Martin-Belloso, O., 2013. Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh- cut Kent mangoes. LWT Food Sci. Technol. 50, 240–246.

Rojas-Graü, M.A., Avena-Bustillos, R.J., Olsen, C., Friedman, M., Henika, P.R., Martín-Belloso, O., Pan, Z., McHugh, T.H., 2007a. Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate–apple puree edible films. J. Food Eng. 81, 634–641.

Rojas-Graü, M.A., Raybaudi-Massilia, R.M., Soliva-Fortuny, R.C., Avena-Bustillos, R.J., McHugh, T.H., Martín-Belloso, O., 2007b. Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. Posthar- vest Biol. Technol. 45, 254–264.

Rojas-Grau, M.A., Tapia, M.S., Martín-Belloso, O., 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fugi apples. LWT Food Sci. Tech- nol. 41, 139–147.

Rojas-Graü, M.A., Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2009. The use of packaging techniques to maintain freshness in fresh-cut fruit and vegetables: a review. Int. J. Food Sci. Technol. 44, 875–889.

Shin, Y., Ryu, J.A., Liu, R.H., Nock, J.F., Watkins, C.B., 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. Postharvest Biol. Technol. 49, 201–209.

Stannard, C., 1997. Development and use of microbiological criteria for foods. Food Sci. Technol. Today 11, 137–176.

Valencia-Chamorro, S.A., Palou, L., Del Rio, M.A., Pérez-Gago, M.B., 2013. Antimi-crobial edible films and coatings for fresh and minimally processed fruits and vegetables: a review. Crit. Rev. Food Sci. 51, 872–900.

Valero, D., Díaz-Mula, H.M., Zapata, P.J., Guillén, F., Martínez-Romero, D., Castillo, S., Serrano, M., 2013. Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. Postharvest Biol. Technol. 77, 1–6.

Vargas, M., Pastor, C., Chiralt, A., McClements, D.J., González-Martínez, C., 2008. Recent advances in edible coatings for fresh and minimally processed fruit. Crit. Rev. Food Sci. Nutr. 48, 496–511.

Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V.D., Moldão-Martins, M., 2013. Impact of chitosan-beeswax edible coatings on the quality of fresh straw-berries (*Fragaria ananassa* cv Camarosa) under commercial storage conditions. LWT Food Sci. Technol. 52, 80–92.

Zúñ iga, G.E., Junqueira-Gonc, alves, M.P., Pizarro, M., Contreras, R., Tapia, a., Silva, S., 2012. Effect of ionizing energy on extracts of *Quillaja saponaria* to be used as an antimicrobial agent on irradiated edible coating for fresh strawberries. Radiat. Phys. Chem. 81, 64–69.

Chapter IV

Nutritional quality of Arbutus unedo fresh fruit as affected by edible coatings

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria L Faleiro[2]
Maria G C Miguel[1], Maria D C Antunes[1]

[1]Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Submitted

Nutritional quality of Arbutus unedo fresh fruit as affected by edible coatings

Abstract

Arbutus berries (*Arbutus unedo* L.) have been shown to be of excellent sensory and nutritional quality for fresh consumption. The effect of two alginate edible coatings which have been proved to increase storage life of arbutus fresh berries was investigated to confirm and guarantee their effectiveness.

Arbutus berries were dipped in the edible coatings sodium alginate (AL) 1%+ eugenol (Eug) 0.2% and AL1% + Eug0.1% + citral (Cit) 0.15% for 2 minutes, and then stored at 0.5 ºC. Control fruit did not have any dip treatment. On days 0, 14, 21 and 28, samples were taken to perform physicochemical and biochemical analysis [color CIE ($L^*h^oC^*$), firmness, soluble solids content, weight loss, microbial growth, taste panels, phenol compounds, sugars, organic acids, antioxidant activity, ethanol, acetaldehyde, CO_2 and ethylene]. Citotoxicity of the edible coatings was also evaluated. Both edible coatings did not show cytotoxicity and were effective on reducing microbial spoilage and ethylene production. Control fruit showed a climacteric pattern. Edible coatings did not significantly affect phenols and antioxidant activity and performed better than control in the taste panel. AL 1% + Eug 0.1% + Cit 0.15% was slightly better than AL 1% + Eug 0.2% in initial reduction of CO_2 and keeping firmness and color. Based on these quality characteristics, we confirmed that arbutus berries are well preserved in terms of general quality parameters and nutritional value in AL 1% + Eug 0.1% + Cit 0.15% followed by AL1% + Eug 0.2%, for at least 28 d at 0.5 ºC.

Key-Words: Alginate, Citral , Eugenol, Arbutus berries, Storage, fruit quality.

1. Introduction

The utilization of active edible coatings in food products offers many advantages because of their edibility, aesthetic appearance, selective permeability to gases (CO_2 and O_2), good mechanical properties, non-toxicity, non-polluting properties and low cost (Azevedo et al., 2014). Moreover, these edible coatings, by themselves or acting as carriers of food additives (e.g. antimicrobials, antioxidants) play an important role in food preservation extending the shelf life (Elsabee and Abdou, 2013)

Alginate is a salt of alginic acid, a polymer of D-mannuronic acid (M) and L-guluronic acid (G), has been used as a base for edible coatings. These coatings are poor moisture-barriers, as they are hydrophilic films, however, the incorporation of calcium reduces their water vapour permeability, making alginate films water insoluble (Olivas et al., 2007).

The incorporation of antimicrobial agents, as essential oils, into edible coatings can enhance the functionality of coatings in protecting food from microbial spoilage and thus extending their shelf life. Some of these essential oils have the advantage in being generally recognized as safe (GRAS) (Hammer et al., 1999; Antunes and Cavaco, 2010; Miguel, 2010; Azevedo et al., 2014; Jo et al., 2014a). Citral and eugenol, which are essential oil constituents, have been used successfully when incorporated into edible coatings for *Arbutus unedo* fresh berries storage (Guerreiro et al., 2015a).

Arbutus unedo L. (*Ericaceae* family), commonly known as strawberry tree, is an evergreen shrub endemic to the Mediterranean region, but also encountered in other regions with hot summers and mild rainy winters (Celikel et al., 2008). Its fruits (berries) are spherical, about 2–3 cm in diameter, dark red, and tasty only when fully ripe, in the autumn (Oliveira et al., 2011a). A. *unedo* berries have considerable importance in local agricultural communities where they are used for the production of

alcoholic beverages, jams, jellies, and marmalades (Pallauf et al., 2008; Oliveira et al., 2011a; Guerreiro et al., 2013).

A. unedo berries, in general, are very much appreciated by consumers, the degree of acceptance depending on organoleptic properties, such as colour, texture and flavour. In addition, *A. unedo* berries consumption has reported beneficial health effects due to their antioxidant compounds such as vitamin C, carotenoids, polyphenols and anthocyanins (Alarcão-E-Silva et al., 2001; Pallauf et al., 2008; Oliveira et al., 2011b; Guerreiro et al., 2013, 2015a).

The perishable nature of A. *unedo* berries makes the use of cold storage necessary to delay changes related to ripening, such as ethylene production, respiration rate, softening, pigment changes and weight loss. However, cold storage is not sufficient to preserve A. *unedo* berries quality at optimum levels during transportation and marketing, therefore, appropriate postharvest technologies combined with cold storage are needed, such as edible coatings enriched with essential oils components.

Appropriate cold temperatures as well as alginate-based edible coatings have been effective in maintaining postharvest quality of A. *unedo* berries (Guerreiro et al., 2015a). These authors tested a group of 13 edible coatings combination and, based on the principal quality characteristics (colour, firmness, SSC, weight loss, antioxidant activity and microbial spoilage) over storage, two corresponding were selected AL1%+Eug0.2% and AL1%+Cit0.15%+Eug0.1%. However, more information on the detailed effect of these aforementioned edible coatings on most nutritional quality parameters through storage is required.

2. Material and Methods

2.1. Plant Material

The fruits were harvested from the mountain "Caldeirão", in Algarve Region, Portugal, in mid-November, when they were ripe (with red colour, with 3-4 N firmness and ºBrix of 22%), and immediately transported to the postharvest laboratory at the University of Algarve, where they were selected for uniformity of size and freedom from defects, for the experiments.

2.2. Edible Coatings preparation

The coating forming solutions based on food grade sodium alginate (AL) (Sigma-Aldrich Chemic, Steinhein, Germany), citral(Cit) (Sigma-Aldrich Chemic, , Germany) and eugenol(Eug) (Sigma-Aldrich Chemic, , Germany), were formulated as described by Rojas-Graü et al.(2007a) and Guerreiro et al. (2015b). Ascorbic acid (Sigma-Aldrich Chemic, , Germany) 1% was added to all edible coatings as anti-browning agent and $CaCl_2$ (Sigma-Aldrich Chemic, , Germany) at 1g /100mL was used as final dip for cross-link (Robles-Sánchez et al., 2013; Guerreiro et al., 2015a).

The treatments were: Control, AL 1g/100 mL (AL1%) + Eug 0.2g/100 mL (Eug 0.2%), and AL1% + Cit 0.15g/100 mL (Cit 0.15%) + Eug 0.1%.

The fruits were dipped into the edible coating solution for 2 min, allowed to drip for 30s, and dipped again in the calcium chloride solution for 1 min, then drip-dried again (Guerreiro et al., 2015a). Afterwards, 8 randomly arbutus berries were placed in polypropylene plastic trays (8cm x 10cm x4 cm), covered with a perforated polypropylene film and stored at 0.5 ºC until analyses. On days 0, 14, 21 and 28, three trays per treatment (replications) were taken for quality evaluation. Controls did not have any kind of treatment or dip.

2.3. General quality parameters

Colour of fruits was measured using a Minolta Chroma meter CR-300 (ECMinolta, Japan) using the CIELab scale (L*, hº, C*) (McGuire, 1992). The firmness of the pulp was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS50 (Jonh Chatillon&Sons, Inc. USA) using a piston cylinder of 8 mm diameter at a depth of 7 mm. The soluble solids content (SSC) in expressed juice was measured as ºBrix, using a digital refractometer PR1ATAGO CoLTD (Japan). Weight loss was expressed as percentage of initial weight.

2.4.Microbial counts and sensory evaluation

The microbiological parameters that were determined included counts of aerobic mesophilic and psycrophilic bacteria and moulds and yeasts as described in Guerreiro et al. (2015a).

A taste panel was performed with 20 semi-trained panellists on the base of a 7-point hedonic scale (1-bad; 7-excellent) for the sensory parameters: Appearance, aroma, texture, sweetness, acidity, flavour and overall acceptance. All parameters were evaluated at harvest and after 14 and 21 days.

2.5. Total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) modified for microplates. Gallic acid was used as standard for calibration curve. The sample (80 µL) and 20 µL of sodium carbonate (75 $g.L^{-1}$) were added to 100 µL of 10% (w/v) Folin–Ciocalteu reagent. After

30 min of reaction at room temperature, the absorbance was measured at 765 nm (Tecan Infinite M200, Swiss).

2.6. Flavone and flavonol content

The content of these group of compounds was quantified as described by Miguel et al. (2010) and modified for microplate reading. Briefly, to 100 µL of sample or standard, 100 µL of 2% $AlCl_3$ ethanol solution were added. After 1h at room temperature, the absorbance was measured at 420 nm on a microplate reader Tecan Infinite M200, Swiss. Quercetin was used as a standard for the construction of the calibration curve.

2.7. Anthocyanins

The total anthocyanins content was measured using a modified pH differential method (Lee et al., 2005). Absorbance of anthocyanins at 520 nm and 700 nm in different pH buffers (pH 1.0 and 4.5) were measured. Absorbance readings were converted to total mg of cyanidin 3-glucoside per 100 g fresh weight of sample (Guerreiro et al., 2013).

2.8. Antioxidant Activity

2.8.1. Extraction for antioxidant activity measurement

For antioxidant activity *A. unedo* juice was obtained after squeezing *A. unedo* flesh with an UltraTurrax T18 (IKA, Germany) for 2 min then centrifuge 5 min at 5000 rpm.

2.8.2. Trolox Equivalent Antioxidant Activity (TEAC)

The trolox equivalent antioxidant activity was measured according to Re et al., (1999) and modify for microplates. For the assay, 3 μL of *A. unedo* juice was added to 197 μL of of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation solution). The absorbance was monitored at 750 nm for 6 min (Tecan Infinite M200, Swiss). The antioxidant activity of each sample was calculated by the equation: scavenging effect (SE %)=(1-As/Ao)x100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 min. The values were compared with the curve for several Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations and the values given as mmol Trolox.Kg^{-1} fresh weight.

2.8.3. Oxygen Radical Absorbance Capacity (ORAC)

The antioxidant activity by the method ORAC measures the ability of samples for scavenging peroxyl radicals.

The ORAC method used, with fluorescein (FL) as the fluorescent probe, was that described by (Ou et al., 2001) and modify by (Guerreiro et al., 2013). The ORAC values are calculated according to a previous work (Prior and Cao, 1999) and are expressed as mmol Trolox.Kg^{-1} fresh weight.

2.9.Ethanol and Acetaldehyde

Fruit ethanol content was quantified using the kit K-ETOH 02/11 Megazyme (Ireland), specific for the determination of ethanol, according to manufacturer instructions. Absorbance was measured by spectrophotometry using a Tecan Infinite M200 microplate reader at 340 nm.

The quantification of acetaldehyde was determined using the kit K-ACHYD Megazyme (Ireland), specific for the determination of acetaldehyde, according to manufacturer instructions, the absorbance was measured at 340nm (Tecan Infinite M200, Switzerland).

2.10. Extraction and quantification of sugars

Extraction and quantification of sugars (fructose, glucose and sucrose) was based on a method described by Terry et al. (2007) and modified as described in Magwaza et al., 2012. Briefly, a 150 ± 0.5 mg of fruit powder was extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentrations of fructose, glucose and sucrose were determined in an HPLC binary pump system (1200 series, Agilent Technologies, UK). Twenty micro litres (20 µL) of a diluted sample solution (1:10) was injected into a Rezex RCM monosaccharide Ca^{+}(8%) column of 7.8mm×300mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} guard column of 3mm×4mm (Phenomenex). The thermostated column compartment (G1316A, Agilent) temperature was set at 80ºC. The mobile phase used was HPLC-grade water at a flow rate of 0.6 $mL.min^{-1}$ and the presence of carbohydrates was detected on a refractive index detector (RID, G1362A, Agilent Technologies). Sugars were quantified from a linear standard curve (0.05–1.25 $mg.mL^{-1}$).

2.11. Extraction and quantification of non-volatile organic acids

Non-volatile organic acids (citric, ascorbic, malic, tartaric and oxalic acid) were extracted and determined using the method described by Crespo et al. (2010) with slight modifications by Magwaza et al. (2013). Briefly, 50±0.5mg of freeze dried samples were cold extracted for 5min in 3mL of HPLC water. The flocculate was filtered

through a 0.2 µm syringe filter before HPLC analysis. Citric, ascorbic, malic, tartaric and oxalic acid concentrations were determined on a HPLC binary pump system equipped with a diode array detector (DAD) with multiple wavelength detector, degasser and cooled autosampler. The filtered sample extract was injected into a Prevail organic acid column (4.6 mm diameter × 250 mm, 5 m particle size; Alltech, UK) with an organic acid guard column. Temperature of the column was set to 35ºC using a thermostated column compartment (G1316A, Agilent). The mobile phase used was 0.2% HPLC-grade aqueous metaphosphoric acid at a flow rate of 1.0 mL/min. Non-volatile organic acids were detected at 210 nm except for ascorbic acid which was detected at 245 nm and quantified using linear standard curves (0.01–1.25 $mg.mL^{-1}$).

2.12. Ethylene and CO_2 gas analysis

Ethylene measurements were performed by withdrawing a 0.5 ml headspace gas sample from the jars with a syringe, and injecting it into a Trace 1300 (Thermo Scientific) gas chromatograph, equipped with a TG-Bond Alunina (Na_2SO_4) 30 m x 0.53 mm x 10µm (Thermo Scientific) at 60 °C and a flame ionisation detector at 120 °C. The carrier gas was N_2 at a flow rate of 35 $mL.min^{-1}$. Respiration was calculated by CO_2 production in the gas phase of the jars, measured in Li-6400 portable (Li-Cor) using a flow rate of 0.5 $\mu mol.s^{-1}$, and read for 5 min.

2.13. Cells Culture and Cytotoxicity

THP-1 leukemia and human intestinal Caco-2 cell lines were kept in 10 mL dishes at 5% CO_2 in Dulbecco's Modified Eagle Medium (1000 $mg.mL^{-1}$ glucose, 110 mg. mL^{-1} pyruvate, and 580 mg. mL^{-1} glutamine) supplemented with 10% fetal bovine

serum, 1% non-essential amino acids, 100 µg. mL^{-1} penicillin, and 100 µg.mL^{-1} streptomycin.

MTT is a standard colorimetric assay for measuring the activity of enzymes that reduce yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan in living cells (Klewicka et al., 2012). Cytotoxicity was determined using the method described by Girón-Calle et al. (2010) with slight modifications. In our study citotoxicity was estimated in the two treatments, and tested at 1 day, 4 days and 6 days. Tissue cells in 96 well microplates were exposed to MTT by addition of fresh medium containing the reagent so that the final concentration was 0.5mg.mL^{-1}, and were incubated for 1h in a CO_2 incubator. Reduced MTT was solubilized by addition of the same volume of 0.1 M HCl in isopropanol. Absorbance at 570 nm with a background reference wavelength of 630 nm was measured using a plate reader (Tecan Infinite M200, Switzerland) and calculated according the follows equations:

$$\%cell\ viability = \frac{Sample\ O.D}{Control\ O.D} \times 100$$

$$\%cytotoxicity = 100 - \%cell\ viability$$

2.1.Statistical Analysis

Statistical analysis was carried out using the SPSS 20.0 software (SPSS Inc.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among treatments was performed.

3. Results

3.1. Quality parameters

For the colour parameters, the parameter lightness (L*), which indicates darkening of the fruits, the values decreased significantly in the first 14 days, probably due faster advance in ripening and senescence, in all treatments, then remained constant (Table 1). However, control fruit did show significantly higher darkening from 14 to 28 d as compared to edible coating treatments which did not show significant differences between them. This indicates that edible coatings retarded senescence.

In the ºhue parameter the values decreased significantly from 0 to 14 days and remained constant thereafter (Table 1). Again the higher decrease was observed in control and AL 1% + Eug 0.2%, being the treatment AL 1% + Cit 0.15% + Eug 0.1% the one with fewer changes in ºhue. Chroma (C*) had a similar behavior, except that the edible coating AL 1% + Cit 0.15% + Eug 0.1% did not show changes in C* values through storage time.

From the results of these experiments, it seems that the edible coating AL 1% + Cit 0.15%+Eug 0.1% is the most efficient in reducing the postharvest ripening or senescence processes related to colour changes.

The firmness decreased significantly from 0 to 14 d storage and remained more or less constant thereafter in all treatments (Table 1). A more pronounced decrease in firmness during the initial storage process has been already reported in Guerreiro et al. (2015a) and for other fruits not treated with edible coatings (Cordenunsi et al., 2003; Shin et al., 2008; Guerreiro et al., 2013). In our experiment, the edible coating AL 1% + Cit 0.15% + Eug 0.1% showed the least decrease in firmness up to 14 days (Table 1). Although, thereafter the differences were not significant, 14 d is considered an

important period for storage of high perishable fruit, showing that this edible coating is of significant importance for better preserving strawberry tree fruit.

The SSC did not significantly change through storage time and no significant changes among treatments were found, except at 14d storage where AL1%+Cit0.15%+Eug0.1% showed significantly higher values, proving its beneficial effect mentioned for firmness.

The weight loss is indicative of the rate of water loss and fruit shrivelling over storage time, decreasing their freshness. Weight loss observed in our study showed a significant increase through storage in all treatments without significant differences among them (Table 1). This confirms the water permeability of polysaccharide edible coatings.

3.2. Microbiological Evaluation

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration, mainly the development of yeasts and moulds.

In control fruit, the development of yeasts and moulds and aerobic mesophilics through storage time was observed (Table 1). It was evident that the edible coatings suppressed yeast and mould development up to 28 days (Table 1).In the case of aerobic mesophilic organisms, also a great reduction was observed from 0 to 14 d and the complete growth inhibition was observed thereafter (Table 1).

No growth of psycrotrophic bacteria was observed during the storage period.

Table 1 Color parameters (L*, Hue^{o} and Chroma), firmness (N), SSC (^{o}Brix), weight loss (%), moulds and yeasts and aerobic mesophillic microorganisms of arbutus berries covered with different alginate (AL) based edible coating (EC) formulations during storage at 0.5^{o}C. Values represent the mean of six replicates taken at 0, 14, 21 and 28 d.

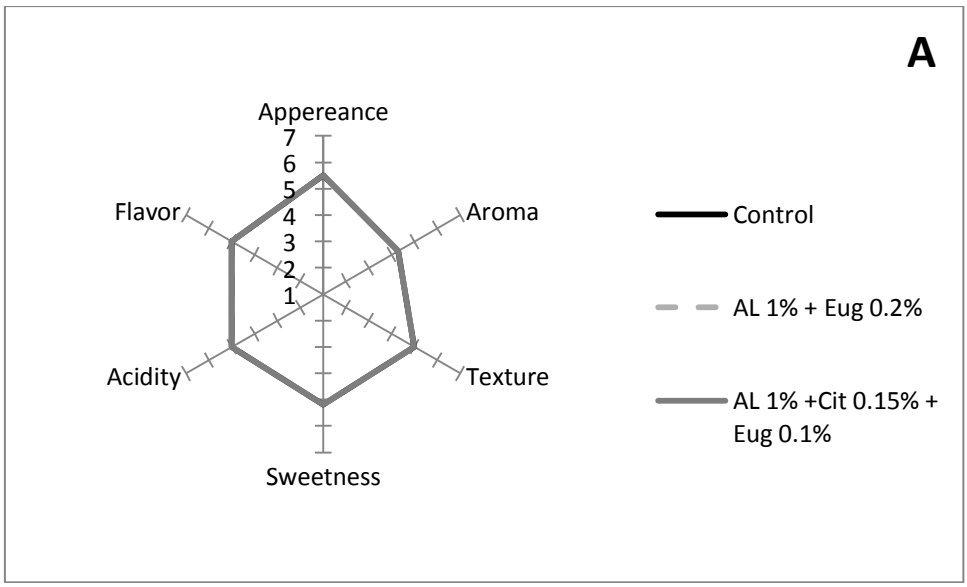
Quality parameters	Days	Control			AL 1% + Eug 0.2%			AL 1% +Cit 0.15% + Eug 0.1%		
		Mean	SE		Mean	SD		Mean	SD	
Lightness(L*)	0	45.77 ± 2.48		aA	43.07 ± 1.02		aA	41.05 ± 3.37		aA
	14	27.75 ± 2.59		bB	33.83 ± 1.35		bA	35.02 ± 0.38		bA
	21	27.67 ± 1.20		bB	32.55 ± 0.86		bA	34.43 ± 1.10		bA
	28	28.98 ± 0.53		bB	32.24 ± 0.65		bA	31.13 ± 0.63		bA
Hue(h^{o})	0	56.40 ± 2.72		aA	52.00 ± 1.63		aAB	46.20 ± 3.42		aB
	14	31.94 ± 1.83		bB	38.83 ± 1.27		bA	37.31 ± 0.54		bA
	21	35.39 ± 0.60		bC	37.82 ± 0.66		bB	39.72 ± 0.29		bA
	28	35.69 ± 0.26		bB	36.94 ± 0.45		bA	35.44 ± 0.26		bB
Chroma(C*)	0	50.56 ± 2.11		aA	51.15 ± 2.78		aA	48.04 ± 4.31		aA
	14	36.33 ± 2.46		bA	41.64 ± 0.66		bA	40.99 ± 1.11		aA
	21	36.82 ± 0.98		bB	40.60 ± 0.92		bAB	41.42 ± 1.62		aA
	28	38.08 ± 1.27		bA	40.21 ± 0.76		bB	39.88 ± 0.26		aA
Firmness(N)	0	3.56 ± 0.44		aB	5.67 ± 0.60		aAB	6.91 ± 1.10		aA
	14	1.00 ± 0.24		bB	0.84 ± 0.05		bB	1.55 ± 0.08		bA
	21	1.15 ± 0.30		bA	1.06 ± 0.25		bA	0.89 ± 0.06		bA
	28	0.97 ± 0.10		bA	0.71 ± 0.01		bB	1.00 ± 0.04		bA
SSC (^{o}Brix)	0	23.10 ± 1.37		aA	22.40 ± 0.85		aA	22.70 ± 0.44		bA
	14	20.67 ± 0.84		aB	19.97 ± 1.28		aB	25.10 ± 0.50		aA
	21	22.90 ± 1.30		aA	21.40 ± 0.26		aA	22.20 ± 0.52		bA
	28	23.93 ± 0.57		aA	22.80 ± 1.29		aA	21.33 ± 0.67		bA
WeightLoss(%)	0	0.00 ± 0.00		cA	0.00 ± 0.00		cA	0.00 ± 0.00		cA
	14	1.95 ± 0.26		bA	1.24 ± 0.50		bA	2.67 ± 0.44		bA
	21	2.63 ± 0.32		bAB	2.39 ± 0.37		bB	3.97 ± 0.49		bA
	28	4.66 ± 0.41		aA	3.84 ± 0.40		aA	5.15 ± 0.60		aA
Yeast and moulds (Log CFU.g^{-1})	0	1.49 ± 0.12		aA	1.49 ± 0.12		aA	1.49 ± 0.12		aA
	14	1.73 ± 0.09		aA	0.00 ± 0.00		bB	0.00 ± 0.00		bB
	21	1.77 ± 0.04		aA	0.00 ± 0.00		bB	0.00 ± 0.00		bB
	28	1.78 ± 0.15		aA	0.00 ± 0.00		bB	0.00 ± 0.00		bB
Aerobic mesophilics (Log CFU.g^{-1})	0	3.51 ± 0.03		aA	3.51 ± 0.03		aA	3.51 ± 0.03		aA
	14	3.08 ± 0.04		cA	0.33 ± 0.33		bC	1.70 ± 0.32		bB
	21	3.33 ± 0.02		bA	0.00 ± 0.00		bB	0.00 ± 0.00		cB
	28	3.49 ± 0.01		aA	0.00 ± 0.00		bB	0.00 ± 0.00		cB

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.3. Sensory evaluation

Subjective sensory evaluation is of great importance because treatments and storage time can change the edible quality of fruits, and edible coatings are usually consumed with the food product (Rojas-Graü et al., 2009c).

In our work, just after harvest, non-treated fruit had a good sensory evaluation (>4 in a scale of 1-dislike very much to 7-like very much) (Fig. 1A). After 14 d of storage at 0.5ºC the taste panel showed that both treatments with alginate had a good sensory evaluation for all parameters (Fig. 1B). Both edible coatings scored better than control for appearance, flavour and texture, yet had similar values for sweetness, aroma and acidity. At 21d storage, the results showed that both edible coatings had higher overall ranking in the taste panel than control, since scored higher in all parameters except sweetness, in spite of acidity being better classified for AL 1%+Eug 0.2% and sweetness equal for all treatments (Fig. 1C).



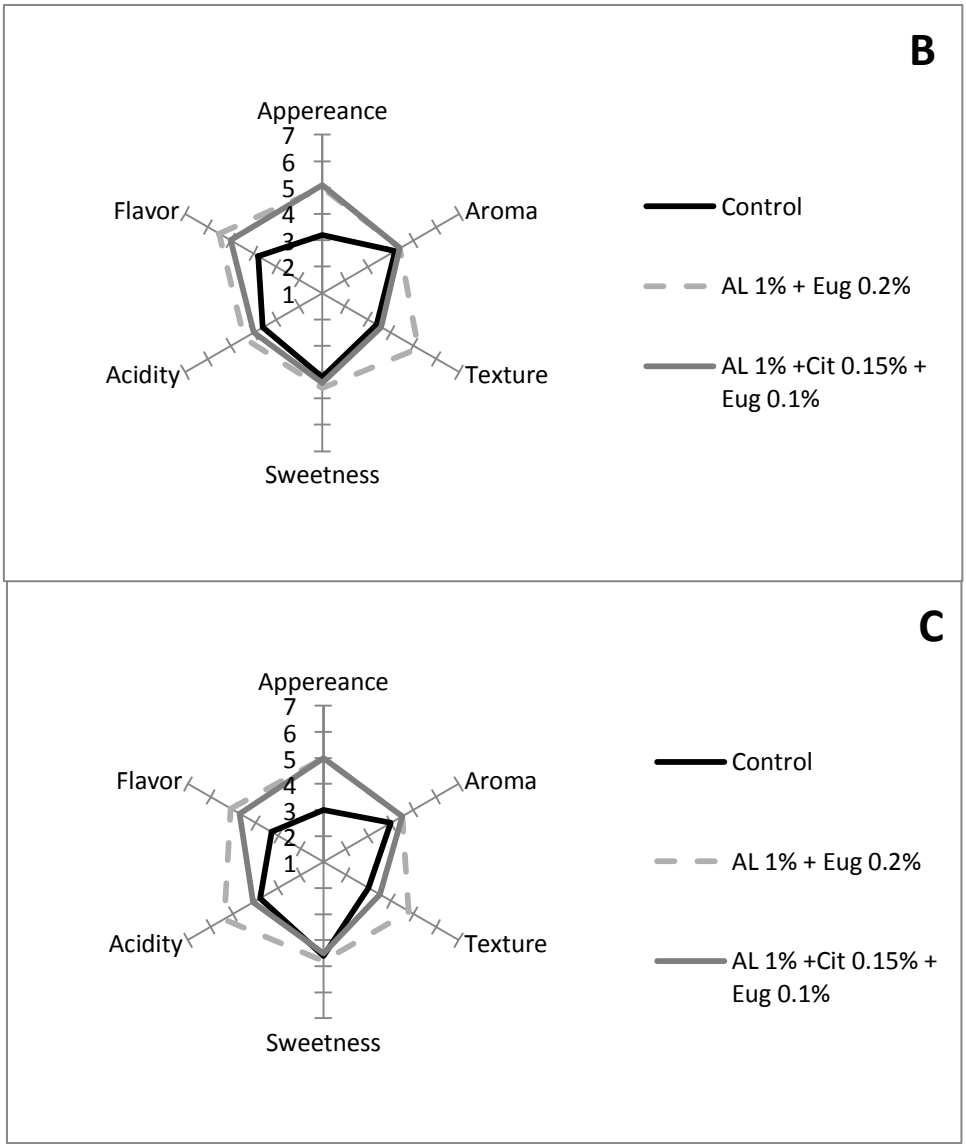


Figure 1 Taste panel of arbutus berries covered with different alginate based edible coating formulations during storage at 0.5 ºC. Values represent the mean of twenty replicates taken at 0(A), 14(B) and 21(C) days.

3.4. Total phenols

The phenolic compounds are extensively distributed in fruits and vegetables and their beneficial effects on health have been widely studied (Chun et al., 2005). Total phenols content almost did not change through storage, being, after 28 d at 0.5 ºC, with similar values to the ones just after treatments (0 d) in all treatments, in spite of some changes through storage (Table 2). No significant differences were observed among treatments.

3.5. Flavone and flavonol content

The *A. unedo* fruits showed, just after treatments (0 d), higher flavonoids content in edible coating treatments than control (Table 2). However, after 14 and 28 d storage, differences were not significant among treatments. With the exception of Al 1%+Eug 0.1%, which did not change through storage time, the other two treatments decreased significantly from 0 to 14 d, being constant thereafter.

3.6. Anthocyanins

The anthocyanins content increased significantly from 0 to 14 d in control and 21 to 28 d in AL 1%+Eug 0.2%, while in AL 1%+Cit 0.15%+Eug 0.1% was higher from the beginning and was constant through storage (Table 2). However, after 21 and 28 d storage there was no significant differences among treatments.

3.7. Antioxidant Activity (TEAC and ORAC)

Antioxidant capacity has been used to evaluate the antioxidant potential status of tissue, which is a function of the type and amount of bioactive compounds present. There are many methods used to determine the total antioxidant capacity, and it is important to point out that all of them have some limitations. It has been observed that some antioxidant assays give different trends in the same sample. For that reason multiple methods to generate an 'antioxidant profile' might be needed (Robles-Sánchez et al., 2013). That was the procedure, which was followed in the present research.

The antioxidant activity measured by TEAC and ORAC did not show significant changes through storage for each treatment, neither significant difference among treatments (Table 2).

Table 2. Total phenols, flavonoids, anthocyanins, TEAC and ORAC content of arbutus berries covered with different alginate based edible coating formulations during storage at 0.5ºC. Values represent the mean of six replicates taken at 0, 14, 21 and 28 d.

Quality Parameters	Days	Control				AL 1% + Eug 0.2%				AL 1% +Cit 0.15% + Eug 0.1%			
		Mean		SE		Mean		SE		Mean		SE	
Total Phenols (mg of Gallic acid.100g^{-1} FW)	0	784.5	±	13.9	aA	790.4	±	15.5	aA	829.7	±	20.1	aA
	14	771.7	±	8.8	aA	766.3	±	29.6	aA	705.2	±	30.4	cA
	21	694.1	±	18.7	bB	784.7	±	7.1	aA	734.6	±	10.9	bcB
	28	819.2	±	19.5	aA	812.8	±	13.6	aA	804.9	±	21.1	abA
Flavonoids (mg of Quercitin .100g^{-1} FW)	0	3.50	±	0.35	aA	1.59	±	0.02	aB	1.81	±	0.13	aB
	14	1.93	±	0.15	bA	1.45	±	0.32	aA	1.34	±	0.09	bA
	21	1.47	±	0.09	bA	1.23	±	0.06	aB	1.04	±	0.02	bB
	28	1.64	±	0.27	bA	1.60	±	0.14	aA	1.24	±	0.15	bA
Anthocyanins (mg of Cyanidin 3-O-glucoside .100g^{-1} FW)	0	15.73	±	1.20	cB	16.40	±	1.32	bAB	21.99	±	2.28	aA
	14	22.06	±	0.81	bA	15.14	±	2.02	bB	24.67	±	4.87	aA
	21	21.25	±	1.18	bA	18.70	±	3.49	bA	17.01	±	2.62	aA
	28	31.55	±	0.70	aA	27.58	±	2.17	aA	28.44	±	5.82	aA
TEAC (mM TE. Kg^{-1}FW)	0	295.60	±	26.94	aA	310.73	±	27.77	aA	319.41	±	10.88	aA
	14	348.72	±	32.46	aA	340.34	±	9.46	aA	339.89	±	11.89	aA
	21	335.16	±	3.23	aA	341.47	±	7.94	aA	328.11	±	15.34	aA
	28	320.06	±	26.77	aA	333.38	±	8.53	aA	319.13	±	4.95	aA
ORAC (mM TE.Kg^{-1}FW)	0	47.02	±	0.18	aA	46.86	±	0.28	aA	46.40	±	0.45	aA
	14	45.51	±	0.85	aA	47.41	±	0.39	aA	47.06	±	0.38	aA
	21	47.14	±	0.20	aA	46.96	±	0.30	aA	46.97	±	0.19	aA
	28	47.03	±	0.38	aA	47.35	±	0.05	aA	47.45	±	0.30	aA

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.8. Gas exchange

The effects of alginate-based edible coatings on respiratory activity and ethylene production were studied. Ethylene production of control fruit was higher in control fruit just after treatments and followed a climacteric pattern, as shown in Figure 1A. Interestingly, both edible coatings initially reduced and then inhibited ethylene production in strawberry tree berries.

Figure 2B shows the production of CO_2 through storage. Just after treatments, CO_2 production was higher in controls followed by AL 1%+Eug 0.2% and AL 1%+Cit 0.15%+Eug 0.2% (Fig. 2B). However, after 3 d storage there was a significant decrease in all treatments being CO_2 production values similar and then remained more or less constant thereafter.

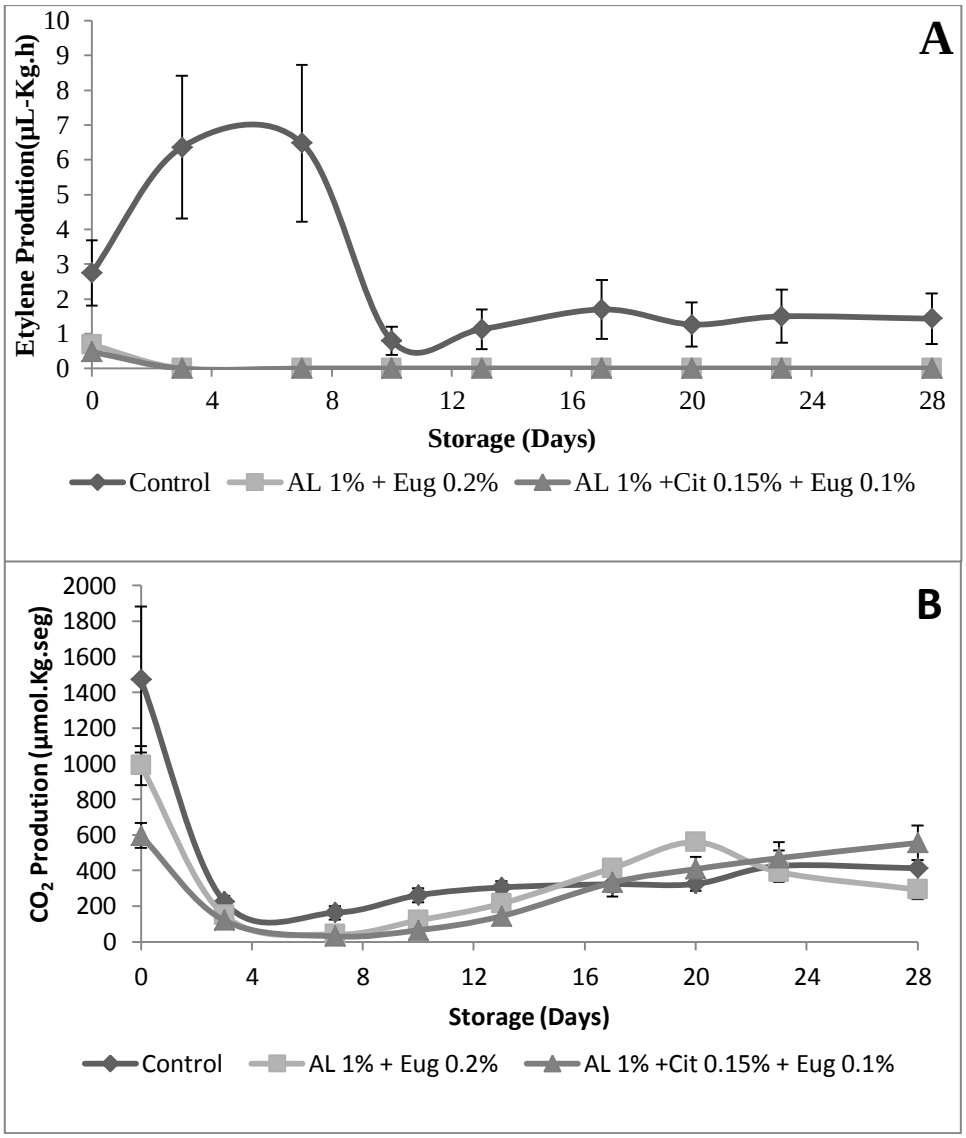


Figure 2 Ethylene production and CO_2 production of arbutus berries covered with different alginate (AL) based edible coating (EC) formulations during storage at 0.5ºC. Values represent the mean of six replicates taken at 0, 14, 21 and 28 days.

3.9. Ethanol and Acetaldehyde

Ethanol and acetaldehyde content were low and did not show significant differences among treatments through storage (Table 3). Values ranged between 0.149-1.250 mg.100 g^{-1} fresh weight for ethanol and 0.167-1.013 mg.100 g^{-1} fresh weight for acetaldehyde.

Table 3 Ethanol and acetaldehyde of arbutus berries covered with different alginate (AL) based edible coating (EC) formulations during storage at 0.5ºC. Values represent the mean of six replicates taken at 0, 14, 21 and 28 days.

Quality Parameters	Days	Control				AL 1% + Eug 0.2%				AL 1% +Cit 0.15% + Eug 0.1%			
		Mean		SE		Mean		SE		Mean		SE	
Ethanol (mg.100g^{1} FW)	0	0.816	±	0.537	aA	0.149	±	0.149	aA	0.000	±	0.000	aA
	14	0.776	±	0.281	aA	0.182	±	0.182	aAB	0.000	±	0.000	aB
	21	0.436	±	0.436	aA	0.464	±	0.464	aA	0.841	±	0.638	aA
	28	1.053	±	0.072	aA	1.254	±	0.523	aA	0.888	±	0.347	aA
Acetaldehyde (mg.100g^{1} FW)	0	0.167	±	0.105	aA	1.030	±	0.548	aA	0.000	±	0.000	aA
	14	0.776	±	0.281	aA	0.844	±	0.218	aA	0.475	±	0.294	aA
	21	0.847	±	0.163	aA	0.533	±	0.342	aA	1.013	±	0.243	aA
	28	0.658	±	0.251	aA	0.737	±	0.369	aA	0.894	±	0.596	aA

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.10. Sugars

Fructose was in higher quantity in *A. unedo* fresh fruit than glucose (Table 4). Fructose was lower in control than coated fruit just after edible coatings application. After 14 d of storage there were no significant differences among treatments in fructose thereafter. In control, fructose increased from 0 to 14 d remaining constant thereafter. Fruit coated with AL 1% + Eug0.2% did not change fructose values during storage. Although there was a decrease in AL 1% + Cit 0.15% + Eug 0.1 % treated fruit from 0 to 14 d storage, fructose increased thereafter being at the end of the experiments with values similar to those at the beginning of the experiment.

Glucose was higher in AL 1% + Eug 0.2% arbutus berries just after coating followed by AL1%+Cit0.15%+Eug0.1% and control (Table 4). Glucose increased through storage in control and AL1% + Cit0.15% + Eug0.1%, while increase was not significant in AL1% + Eug0.2% coated arbutus berries. At 14 and 21 d storage, AL 1% + Cit 0.15% + Eug 0.1 % had lower values of glucose than the other treatments, but at the end of storage, differences were not significant.

3.11. Non-volatile organic acids

Non-volatile organic acids measured in our study were oxalic, malic and ascorbic acid. Malic acid was the major organic acid found, followed by ascorbic and oxalic acids (Table 4). Ascorbic and oxalic acids were lower in control than edible coating treatments just after coating application (day 0), while malic acid did not show significant differences among treatments. After 14 d storage ascorbic and malic acids did not show significant differences among treatments. Ascorbic acid remained almost constant through 28 d storage in all treatments. Oxalic acid increased from 0 to 14 d in control and remained stable thereafter and in edible coatings treatments.

Malic acid showed significantly lower values at the end of storage time than just after edible coatings application for all treatments (Table 4). Among treatments, the AL 1% +Cit 0.15% + Eug 0.1 showed lower values than the other treatments after 14 d and lower than AL 1% + Eug 0.2% after 21 d. However, at the beginning and end of the experiment there were no significant differences among treatments.

Table 4 Organic acids and sugars of arbutus berries covered with different alginate based edible coating formulations during storage at 0.5ºC. Values represent the mean of six replicates taken at 0, 14, 21 and 28 d.

Quality Parameters	Days	Control				AL 1% + Eug 0.2%				AL 1% +Cit 0.15% + Eug 0.1%			
		Mean		SE		Mean		SE		Mean		SE	
Fructose (mg.g^{1} DM)	0	59.17	±	2.35	bB	219.11	±	25.52	aA	186.79	±	5.11	aA
	14	184.89	±	22.15	aA	177.02	±	20.61	aA	107.80	±	1.13	bB
	21	155.08	±	18.52	aA	178.98	±	25.44	aA	172.44	±	19.19	aA
	28	149.67	±	9.59	aA	201.05	±	12.86	aA	214.40	±	7.60	aA
Glucose (mg.g^{1} DM)	0	42.55	±	0.18	cC	80.46	±	1.99	aA	66.86	±	7.25	bB
	14	59.41	±	9.30	bA	69.40	±	7.51	aA	31.59	±	2.23	cB
	21	93.87	±	1.96	aA	78.85	±	10.91	aAB	56.13	±	6.79	bB
	28	74.82	±	2.64	abA	89.54	±	6.26	aA	83.21	±	6.97	aA
Oxalic Acid (mg.100g^{1} DM)	0	59.34	±	3.06	bA	134.54	±	8.85	aA	151.21	±	5.54	aA
	14	178.90	±	12.18	aA	159.06	±	9.78	aA	154.92	±	6.15	aA
	21	148.96	±	13.87	aA	154.95	±	7.14	aA	135.98	±	3.57	aA
	28	158.63	±	15.19	aA	142.11	±	10.03	aA	169.25	±	6.51	aA
Malic Acid (mg.100g^{1} DM)	0	1416.42	±	80.12	aA	1354.41	±	28.69	aA	1539.81	±	53.44	aA
	14	1421.24	±	16.77	aA	1411.72	±	12.91	aA	1293.60	±	44.12	aA
	21	1507.49	±	5.50	aA	1768.23	±	127.04	aA	1354.56	±	31.81	aA
	28	1222.25	±	62.43	aA	1353.10	±	125.99	aA	1253.07	±	72.59	aA
Ascorbic Acid (mg.100g^{-1} DM)	0	628.02	±	20.69	aA	701.55	±	58.39	aA	716.91	±	13.13	aA
	14	559.10	±	50.19	aA	616.97	±	55.18	aA	660.14	±	25.77	aA
	21	637.91	±	39.27	aA	684.47	±	56.62	aA	596.23	±	38.67	aA
	28	528.40	±	78.45	aA	652.02	±	59.11	aA	742.31	±	85.90	aA

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.12. Cytotoxicity

The cytotoxic properties of the formulations used for coating the fruits were evaluated on the THP-1 and differentiated Caco-2 cells (confluent monolayer). Either control or samples with edible coating did not show cytotoxicity for THP-1 or Caco-2 cells, cell viability of 98-100% and 80-100%, respectively.

4. Discussion

The decrease in the color parameters (L*, hue and chroma) during storage can be attributed to the increase in weight loss and anthocyanins synthesis making the fruit

redder and darker as observed for other red fruit (Esti et al., 2002; Vicente et al., 2002; Han et al., 2004; Vargas et al., 2006; Gol et al., 2013). Coating the arbutus berries with the edible coatings reduced this tendency as compared to control, with the AL 1%+Cit 0.15%+Eug 0.1% better retarding the ripening/senescence process. This behaviour has been observed for other coatings based on chitosan or alginate in other fruit (Han et al., 2004; Valero et al., 2013; Azarakhsh et al., 2014). Moreover, better results were obtained when essential oils were incorporated into those edible coatings (Azarakhsh et al., 2014; Rojas-Graü et al., 2007). Such results may be attributed to the stabilization of anthocyanins by the edible coatings compounds as reported by Han et al. (2004).

The early decline in postharvest firmness is a common process in most fruit which is attributed to faster tissue ripening/senescence and cell wall breakdown (Antunes and Sfakiotakis, 2002; Vargas et al., 2006). In our experiment, considering the whole storage period (28 d), there is no significant effect of the edible coatings in preserving firmness of the arbutus fruits. Similar behaviour was observed for raspberries coated with chitosan (Han et al., 2004; Tezotto-Uliana et al., 2014). This may be because coated arbutus berries were difficult to dry completely and the wet surface lead to an increase in softening and pectin solubilisation as compared to control fruit (Han et al., 2004; Tezotto-Uliana et al., 2014).

However the AL 1%+Cit 0.15%+Eug 0.1% edible coating showed higher firmness from just after coating application to 14 d storage. This may be of significant importance for these high perishable fruit. Guerreiro et al. (2015a) found a better firmness maintenance when using some edible coatings enriched with essential oils as compared to control in fresh arbutus berries. Also Valero et al. (2013) found that alginate coated plums slowed down softening.

Fruits were ripe at harvest and our values are in accordance to Guerreiro et al. (2015a) who found ±22 °Brix in arbutus berries at harvest and the values were maintained through storage. This indicates that arbutus berries were ripe when harvested and no significant senescence was observed during the storage period. This is supported by Duan et al. (2011) who did not find changes in coated or uncoated blueberries during storage. However, Velickova et al. (2013) and Gol et al. (2013) observed a decrease in the SSC content in strawberries at the end of storage and attributed this to respiration.

Weight loss during storage at low temperature has been observed for arbutus berries, strawberries and red raspberries (Vicente et al., 2002; Shin et al., 2008; Krüger et al., 2011; Guerreiro et al., 2013). In agreement with Guerreiro et al. (2015a) weight loss was not reduced by the edible coatings in the current study as compared to control. In contrast, Tezotto-Uliana et al. (2014) and Han et al. (2004) found that weight loss was reduced when applying chitosan coatings to strawberry and raspberry fruits, due to a reduction on water permeability created by coating. In fact, it is expected that coatings serve as semi-permeable barrier against oxygen, carbon dioxide and moisture, thus reducing respiration, water loss and oxidation reactions (Gol et al., 2013; Valero et al., 2013; Guerreiro et al., 2015a). As in our case, edible coatings did not reduce weight loss as compared to control in blueberries (Duan et al., 2011). However, some coating material like alginate, which is a polysaccharide, may not reduce water loss probably due to their hydrophilic properties (Duan et al., 2011; Guerreiro et al., 2015a).

One of the main reasons for incorporating essential oils into edible coatings is their antimicrobial effect (Raybaudi-Massilia et al., 2008b; Antunes et al., 2012). In fact, both edible coatings were able to inhibit microbial development in arbutus berries. Reduction of microbial development due to the incorporation of essential oils into edible coatings was also reported for other fruit (Azarakhsh et al., 2014; Guerreiro et al.,

2015a). This is a very important effect since fruit spoilage by food borne pathogens is one of the main causes of postharvest losses.

Sensory change due to edible coatings application is an important issue since this can decrease consumer acceptance. So, taste panels are of significant importance when testing new edible coatings. The taste panel gave higher sensory scores in both edible coatings than control after 14 and 21 d, meaning that the concentrations of the essential oils and alginate were good enough to reduce senescence/spoilage as compared to control without changing the sensorial properties of the fruit. In many cases, when higher concentrations of the edible coatings are used, they may reduce ripening/senescence evolution but sensorial properties are also affected (Vargas et al., 2006; Azarakhsh et al., 2014). However, at lower concentrations they can be also effective without reducing sensory attributes as in our case (Gol et al., 2013; Guerreiro et al., 2015a; Rojas-Graü et al., 2007). In the present experiment, the better sensory evaluation of coated fruit can be attributed to reduced senescence/spoilage.

Ethylene production in *A. unedo* fruit has not been reported up to now. According to our reports, arbutus berries seem to be climacteric fruit since fruits harvested at the yellow stage become red and ripe after harvest (Data not shown). The results of our experiment (control) show a climacteric pattern of ethylene production (Antunes and Sfakiotakis, 2000; Alexander and Grierson, 2002; Pech et al., 2008). Since ethylene production of the control was already about $3\mu.kg^{-1}.hr^{-1}$ at harvest, and CO_2 production was high at the same time, it seems that arbutus berries had initiated the ethylene onset and the respiration burst was before the one of ethylene, as happens for a group of climacteric fruit (Antunes and Sfakiotakis, 2000). Interestingly, both edible coatings reduced ethylene production and respiration rate immediately after application; this being more pronounced for AL1%+Cit0.15%+Eug0.1%. However, respiration

becomes lower and similar in all treatments after 3 days storage, probably because climacteric CO_2 production was finished. The ethylene burst, in our experiment, finished after 9d, but ethylene production continued higher in control fruit than in coated ones.

Rojas-Grau et al. (2008) found also reduced ethylene production when applying alginate and gellan edible coatings to fresh-cut apples than controls, while CO_2 production did not show changes among treatments. Similarly, Valero et al. (2013) found reduced ethylene production in whole plum fruits when applying alginate edible coatings.

Acetaldehyde and ethanol are indicators of the degree of anaerobic fermentation that is taking place and its accumulation occurs when internal atmosphere is affected and gas exchange is restricted (Beaulieu et al., 1997; Raybaudi-Massilia et al., 2008b). The appearance of these fermentative metabolites (ethanol and acetaldehyde) as a result of anaerobic respiration is often associated with off- flavours and its presence might be detrimental to quality (Rojas-Graü et al., 2007). In our case the levels of ethanol and acetaldehyde were very low. This indicates that neither the edible coatings nor 28 d storage at 0.5 ºC induced an advance in senescence due to reduced internal oxygen to a level that causes anaerobic respiration. This is in agreement with CO_2 production which similar in all treatments. With these results it seems also, that the reduction in ethylene is not only due to a decrease in edible coatings permeability to gases but to internal atmosphere changes, which needs further investigation.

Fructose and glucose were higher in coated fruit than in control just after edible coats application probably due to the coating composition. The fact that differences were decreasing through storage and did not exist at the end of storage period seems to be due to the decreased effect of edible coating through time. Small changes in those

sugars through storage time have been reported for other fruit including arbutus berries (Antunes et al., 2010, 2012; Guerreiro et al., 2013).

Non-volatile organic acids are natural components of many fruits and vegetables. The main organic acid in arbutus berries was malic followed by ascorbic and oxalic as reported by Ruiz-Rodríguez et al. (2011). Ayaz et al. (2000) reported fumaric and malic acids as the major ones in Turkish *A. unedo* fruits, and Alarcão-E-Silva et al. (2001) reported quinic and malic acids as the main acids in Portuguese arbutus berries. Malic acid is known to be the main organic acid in many fruits. The low quantity of oxalic acid is important since is considered toxic in high quantities reducing calcium absorption (Guil et al., 1996; Ruiz-Rodríguez et al., 2011).

The content of ascorbic acid was expected to be higher in edible coating treatments than in control, since ascorbic acid 1% was added to edible coatings. Such differences occurred just after treatments, but after 14 d differences between coated fruit and control disappeared due to a slight decrease in the ascorbic acid of the edible coated fruit. Similar behaviour was found when fresh-cut kiwifruit were treated with ascorbic acid 2% (Antunes et al., 2010). This is probably due to ascorbic acid degradation in the coatings through storage, while internal ascorbic acid was not degraded in none fruit.

The parental cell line of the human intestinal Caco-2 cell line, originally obtained from a human colon adenocarcinoma, undergoes in culture a process of spontaneous differentiation that leads to the formation of a monolayer of cells. In this phase, the cells express several morphological and functional characteristics of the mature enterocyte (Sambuy et al., 2005). For this reason they may be used for evaluating the cytotoxicity of compounds which may be in contact or be absorbed by intestinal mucosa, such as the edible coatings.

Our edible coatings were not cytotoxic as reported by Zou et al. (2012) when using cocoa procyanidins–gelatin–chitosan nanoparticles with TPH-1 cells, and Hermans et al. (2012) when using chitosan-coated nanoparticules in contact to keratinocyt epithelial cells.

5. Conclusions

Arbutus unedo fruits showed a climacteric pattern, with both edible coating reducing ethylene production. Both edible coatings reduced microbial spoilage, and preserved well most quality parameters without significantly reducing nutritional value and did not show cytotoxicity. The use of edible coating in arbutus berries can be considered as safe and effective treatment. The use of alginate-based formulations may reduce wounding stress, maintained most quality attributes of the commodity and could be recommended for commercial purposes due its lower cost in comparison to other polysaccharides used in industrial applications of high economic value.

6. Acknowledgements

This research was supported by Fundação para a Ciência e Tecnologia, Portugal, Project: PTDC/AGR-ALI/121085/2010 - INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

7. References

Alarcão-E-Silva, M. L. C. M. M.; Leitão, a. E. B.; Azinheira, H. G.; Leitão, M. C. a. The Arbutus Berry: Studies on Its Color and Chemical Characteristics at Two Mature Stages. *J. Food Compos. Anal.* **2001**, *14*, 27–35.

Alexander, L.; Grierson, D. Ethylene Biosynthesis and Action in Tomato: A Model for Climacteric Fruit Ripening. *J. Exp. Bot.* **2002**, *53*, 2039–2055.

Antunes, M. D. C.; Sfakiotakis, E. M. Effect of High Temperature Stress on Ethylene Biosynthesis , Respiration and Ripening of " Hayward " Kiwifruit. *Postharvest Biol. Technol.* **2000**, *20*, 251–259.

Antunes, M. D. C.; Sfakiotakis, E. . Ethylene Biosynthesis and Ripening Behaviour of "Hayward" Kiwifruit Subjected to Some Controlled Atmospheres. *Postharvest Biol. Technol.* **2002**, *26*, 167–179.

Antunes, M. D. C.; Dandlen, S.; Cavaco, A. M.; Miguel, G. Effects of Postharvest Application of 1-MCP and Postcutting Dip Treatment on the Quality and Nutritional Properties of Fresh-Cut Kiwifruit. *J. Agric. Food Chem.* **2010**, *58*, 6173–6181.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Antunes, M. M. D. C.; Cavaco, A. M. A. The Use of Essential Oils for Postharvest Decay Control. A Review. *Flavour Fragr. J.* **2010**, *25*, 351–366.

Ayaz, F. a.; Kucukislamoglu, M.; Reunanen, M. Sugar, Non-Volatile and Phenolic Acids Composition of Strawberry Tree (Arbutus Unedo L. Var.ellipsoidea) Fruits. *J. Food Compos. Anal.* **2000**, *13*, 171–177.

Azarakhsh, N.; Osman, A.; Ghazali, H. M.; Tan, C. P.; Mohd Adzahan, N. Lemongrass Essential Oil Incorporated into Alginate-Based Edible Coating for Shelf-Life Extension and Quality Retention of Fresh-Cut Pineapple. *Postharvest Biol. Technol.* **2014**, *88*, 1–7.

Azevedo, A. N.; Buarque, P. R.; Cruz, E. M. O.; Blank, A. F.; Alves, P. B.; Nunes, M. L.; Santana, L. C. L. D. A. Response Surface Methodology for Optimisation of

Edible Chitosan Coating Formulations Incorporating Essential Oil against Several Foodborne Pathogenic Bacteria. *Food Control* **2014**, *43*, 1–9.

Beaulieu, J. C.; Peiser, G.; Saltveit, M. E. Acetaldehyde Is a Causal Agent Responsible for Ethanol-Induced Ripening Inhibition in Tomato Fruit. *Plant Physiol.* **1997**, *113*, 431–439.

Celikel, G.; Demirsoy, L.; Demirsoy, H. The Strawberry Tree (Arbutus Unedo L.) Selection in Turkey. *Sci. Hortic. (Amsterdam).* **2008**, *118*, 115–119.

Chun, O. K.; Kim, D. O.; Smith, N.; Schroeder, D.; Han, J. T.; Chang, Y. L. Daily Consumption of Phenolics and Total Antioxidant Capacity from Fruit and Vegetables in the American Diet. *J. Sci. Food Agric.* **2005**, *85*, 1715–1724.

Cordenunsi, B. R.; Nascimento, J. R. O.; Lajolo, F. M. Physico-Chemical Changes Related to Quality of Five Strawberry Fruit Cultivars during Cool-Storage. *Food Chem.* **2003**, *83*, 167–173.

Crespo, P.; Bordonaba, J. G.; Terry, L. A.; Carlen, C. Characterisation of Major Taste and Health-Related Compounds of Four Strawberry Genotypes Grown at Different Swiss Production Sites. *Food Chem.* **2010**, *122*, 16–24.

Duan, J.; Wu, R.; Strik, B. C.; Zhao, Y. Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions. *Postharvest Biol. Technol.* **2011**, *59*, 71–79.

Esti, M.; Cinquanta, L.; Sinesio, F.; Moneta, E.; Matteo, M. Di. Physicochemical and Sensory Fruit Characteristics of Two Sweet Cherry Cultivars after Cool Storage. *Food Chem.* **2002**, *76*, 399–405.

Girón-Calle, J.; Alaiz, M.; Vioque, J. Effect of Chickpea Protein Hydrolysates on Cell Proliferation and in Vitro Bioavailability. *Food Res. Int.* **2010**, *43*, 1365–1370.

Gol, N. B.; Patel, P. R.; Rao, T. V. R. Improvement of Quality and Shelf-Life of Strawberries with Edible Coatings Enriched with Chitosan. *Postharvest Biol. Technol.* **2013**, *85*, 185–195.

Guerreiro, A. C.; Gago, C.; Miguel, M.; Antunes, M. The Effect of Temperature and Film Covers on the Storage Ability of Arbutus Unedo L. Fresh Fruit. *Sci. Hortic. (Amsterdam).* **2013**, *159*, 96–102.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Effect of Alginate-Based Edible Coatings Enriched with Essential Oils Constituents on Arbutus Unedo L. Fresh Fruit Storage. *Postharvest Biol. Technol.* **2015a**, *100*, 226–233.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Use of Polysaccharide-Based Edible Coatings Enriched with Essential Oils to Improve Shelf-Life of Strawberries. *Postharvest Biol. Technol.* **2015b**, *110*, 51–60.

Guil, J. L.; Torija, M. E.; Giménez, J. J.; Rodríguez-García, I.; Giménez, A. Oxalic Acid and Calcium Determination in Wild Edible Plants. *J. Agric. Food Chem.* **1996**, *44*, 1821–1823.

Hammer, K. a; Carson, C. F.; Riley, T. V. Antimicrobial Activity of Essential Oils and Other Plant Extracts. *J. Appl. Microbiol.* **1999**, *86*, 985–990.

Han, C.; Zhao, Y.; Leonard, S. .; Traber, M. . Edible Coatings to Improve Storability and Enhance Nutritional Value of Fresh and Frozen Strawberries (Fragaria × Ananassa) and Raspberries (Rubus Ideaus). *Postharvest Biol. Technol.* **2004**, *33*, 67–78.

Hermans, K.; Van Den Plas, D.; Everaert, A.; Weyenberg, W.; Ludwig, A. Full Factorial Design, Physicochemical Characterisation and Biological Assessment of Cyclosporine A Loaded Cationic Nanoparticles. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 27–35.

Jo, W. S.; Song, H. Y.; Song, N. B.; Lee, J. H.; Min, S. C.; Song, K. B. Quality and Microbial Safety of "Fuji" Apples Coated with Carnauba-Shellac Wax Containing Lemongrass Oil. *LWT - Food Sci. Technol.* **2014**, *55*, 490–497.

Klewicka, E.; Nowak, A.; Zduńczyk, Z.; Juśkiewicz, J.; Cukrowska, B. Protective Effect of Lactofermented Red Beetroot Juice against Aberrant Crypt Foci Formation, Genotoxicity of Fecal Water and Oxidative Stress Induced by 2-Amino-1-Methyl-6-phenylimidazo[4,5-B] Pyridine in Rats Model. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 895–904.

Krüger, E.; Dietrich, H.; Schöpplein, E.; Rasim, S.; Kürbel, P. Cultivar, Storage Conditions and Ripening Effects on Physical and Chemical Qualities of Red Raspberry Fruit. *Postharvest Biol. Technol.* **2011**, *60*, 31–37.

Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.* **2005**, *88*, 1269–1278.

Magwaza, L. S.; Opara, U. L.; Terry, L. a.; Landahl, S.; Cronje, P. J.; Nieuwoudt, H.; Mouazen, A. M.; Saeys, W.; Nicolaï, B. M. Prediction of "Nules Clementine" Mandarin Susceptibility to Rind Breakdown Disorder Using Vis/NIR Spectroscopy. *Postharvest Biol. Technol.* **2012**, *74*, 1–10.

Magwaza, L. S.; Opara, U. L.; Terry, L. A.; Landahl, S.; Cronje, P. J. R.; Nieuwoudt, H. H.; Hanssens, A.; Saeys, W.; Nicolaï, B. M. Evaluation of Fourier Transform-NIR Spectroscopy for Integrated External and Internal Quality Assessment of Valencia Oranges. *J. Food Compos. Anal.* **2013**, *31*, 144–154.

McGuire, R. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255.

Miguel, M. G.; Nunes, S.; Dandlen, S. A.; Cavaco, A. M.; Antunes, M. D. Phenols and Antioxidant Activity of Hydro-Alcoholic Extracts of Propolis from Algarve, South of Portugal. *Food Chem. Toxicol.* **2010**, *48*, 3418–3423.

Miguel, M. Antioxidant Activity of Medicinal and Aromatic Plants. A Review. *Flavour Fragr. J.* **2010**, *2009*, 291–312.

Olivas, G. I.; Mattinson, D. S.; Barbosa-Cánovas, G. V. Alginate Coatings for Preservation of Minimally Processed "Gala" Apples. *Postharvest Biol. Technol.* **2007**, *45*, 89–96.

Oliveira, I.; Baptista, P.; Malheiro, R.; Casal, S.; Bento, A.; Pereira, J. A. Influence of Strawberry Tree (Arbutus Unedo L.) Fruit Ripening Stage on Chemical Composition and Antioxidant Activity. *Food Res. Int.* **2011a**, *44*, 1401–1407.

Oliveira, I.; Guedes de Pinho, P.; Malheiro, R.; Baptista, P.; Pereira, J. A. Volatile Profile of Arbutus Unedo L. Fruits through Ripening Stage. *Food Chem.* **2011b**, *128*, 667–673.

Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.

Pallauf, K.; Rivas-Gonzalo, J. C.; del Castillo, M. D.; Cano, M. P.; de Pascual-Teresa, S. Characterization of the Antioxidant Composition of Strawberry Tree (Arbutus Unedo L.) Fruits. *J. Food Compos. Anal.* **2008**, *21*, 273–281.

Pech, J. C.; Bouzayen, M.; Latché, a. Climacteric Fruit Ripening: Ethylene-Dependent and Independent Regulation of Ripening Pathways in Melon Fruit. *Plant Sci.* **2008**, *175*, 114–120.

Prior, R. L.; Cao, G. In Vivo Total Antioxidant Capacity: Comparison of Different Analytical Methods. *Free Radic. Biol. Med.* **1999**, *27*, 1173–1181.

Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Martín-Belloso, O. Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon. *Int. J. Food Microbiol.* **2008**, *121*, 313–327.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Raybaudi-Massilia, R. M.; Soliva-Fortuny, R. C.; Avena-Bustillos, R. J.; McHugh, T. H.; Martín-Belloso, O. Apple Puree-Alginate Edible Coating as Carrier of Antimicrobial Agents to Prolong Shelf-Life of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2007a**, *45*, 254–264.

Rojas-Graü, M. a.; Avena-Bustillos, R. J.; Olsen, C.; Friedman, M.; Henika, P. R.; Martín-Belloso, O.; Pan, Z.; McHugh, T. H. Effects of Plant Essential Oils and Oil Compounds on Mechanical, Barrier and Antimicrobial Properties of Alginate-Apple Puree Edible Films. *J. Food Eng.* **2007b**, *81*, 634–641.

Rojas-Graü, M. A.; Tapia, M. S.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Maintain Quality of Fresh-Cut Fuji Apples. *LWT - Food Sci. Technol.* **2008**, *41*, 139–147.

Rojas-Graü, M. A.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. The Use of Packaging Techniques to Maintain Freshness in Fresh-Cut Fruits and Vegetables: A Review. *Int. J. Food Sci. Technol.* **2009**, *44*, 875–889.

Ruiz-Rodríguez, B.-M.; Morales, P.; Fernández-Ruiz, V.; Sánchez-Mata, M.-C.; Cámara, M.; Díez-Marqués, C.; Pardo-de-Santayana, M.; Molina, M.; Tardío, J. Valorization of Wild Strawberry-Tree Fruits (Arbutus Unedo L.) through Nutritional Assessment and Natural Production Data. *Food Res. Int.* **2011**, *44*, 1244–1253.

Sambuy, Y.; De Angelis, I.; Ranaldi, G.; Scarino, M. L.; Stammati, a.; Zucco, F. The Caco-2 Cell Line as a Model of the Intestinal Barrier: Influence of Cell and Culture-Related Factors on Caco-2 Cell Functional Characteristics. *Cell Biol. Toxicol.* **2005**, *21*, 1–26.

Shin, Y.; Ryu, J.-A.; Liu, R. H.; Nock, J. F.; Watkins, C. B. Harvest Maturity, Storage Temperature and Relative Humidity Affect Fruit Quality, Antioxidant Contents and Activity, and Inhibition of Cell Proliferation of Strawberry Fruit. *Postharvest Biol. Technol.* **2008**, *49*, 201–209.

Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of Water Deficit Irrigation and Inoculation with Botrytis Cinerea on Strawberry (Fragaria X Ananassa) Fruit Quality. *J. Agric. Food Chem.* **2007**, *55*, 10812–10819.

Tezotto-Uliana, J. V.; Fargoni, G. P.; Geerdink, G. M.; Kluge, R. A. Chitosan Applications Pre- or Postharvest Prolong Raspberry Shelf-Life Quality. *Postharvest Biol. Technol.* **2014**, *91*, 72–77.

Valero, D.; Díaz-Mula, H. M.; Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Serrano, M. Effects of Alginate Edible Coating on Preserving Fruit Quality in Four Plum Cultivars during Postharvest Storage. *Postharvest Biol. Technol.* **2013**, *77*, 1–6.

Vargas, M.; Albors, A.; Chiralt, A.; González-Martínez, C. Quality of Cold-Stored Strawberries as Affected by Chitosan-Oleic Acid Edible Coatings. *Postharvest Biol. Technol.* **2006**, *41*, 164–171.

Velickova, E.; Winkelhausen, E.; Kuzmanova, S.; Alves, V. D.; Moldão-Martins, M. Impact of Chitosan-Beeswax Edible Coatings on the Quality of Fresh Strawberries (Fragaria Ananassa Cv Camarosa) under Commercial Storage Conditions. *LWT - Food Sci. Technol.* **2013**, *52*, 80–92.

Vicente, A.; Martínez, G.; Civello, P.; Chaves, A. Quality of Heat-Treated Strawberry Fruit during Refrigerated Storage. *Postharvest Biol. Technol.* **2002**, *25*, 59–71.

Zou, T.; Percival, S. S.; Cheng, Q.; Li, Z.; Rowe, C. a.; Gu, L. Preparation, Characterization, and Induction of Cell Apoptosis of Cocoa Procyanidins-Gelatin-Chitosan Nanoparticles. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 36–42.

Chapter V

Edible coatings enriched with essential oils for extending the shelf-life of 'Bravo de Esmolfe' fresh-cut apples

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria L Faleiro[2]
Maria G C Miguel[1], Maria D C Antunes[1]

[1]*Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal*

[2]*Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal*

In Press

International Journal of Food Science and Technology

Edible coatings enriched with essential oils for extending the shelf-life of 'Bravo de Esmolfe' fresh-cut apples

Summary

Edible coatings based on sodium alginate (AL) and pectin(PE) at 1% and 2%(w/v) enriched with eugenol(Eug) and citral(Cit) at MIC (0.10 and 0.15%) and double MIC were used to preserve the quality of fresh-cut apples 'Bravo de Esmolfe'. Samples were taken, through 14d at 4ºC, for analysis of color CIE(L*hºC*), firmness, ºBrix, weight loss, antioxidant activity(TEAC), microbial growth and taste panels. With those quality characteristics, 3 groups were formed by the Principal component and hierarchical cluster analysis, for each coating base (AL or PE). Based on, for each quality parameter measured, the mean closest value to the one at harvest for color, higher value for firmness, ºBrix and TEAC, and lower value for weight loss and microbial spoilage, the best group was selected for AL and PE. From each group, two edible coating with the highest scores in overall acceptability were selected for fresh-cut apples: AL2%+Eug0.1%, AL2%+Cit0.15%+Eug0.1%, PE2%+Cit0.15% and PE2%+Eug0.2%.

Key-Words: Edible coating, Alginate, Pectin, Fresh-cut, Apples

Introduction

Fresh-cut fruit are ready to eat products, whose interest is growing in food market. However, peeling and cutting operations accelerate the metabolic activities of plant tissues, making fresh-cut fruit more perishable than fresh fruit (Chiumarelli & Hubinger, 2012). The marketing of fresh-cut fruit, such as apples, is strongly influenced by colour changes due to enzymatic reactions of phenolic

compounds (Perez-Gago, *et al.*, 2006). Therefore, fresh-cut fruit requires the application of preservation technologies, such as, low storage temperature and modified atmosphere within the package, which can be complemented with antimicrobial and antioxidant agents to extend their shelf-life by reducing respiration rate, surface contamination and browning (Antunes *et. al.*, 2010). Also, edible coatings can be another strategy to extend shelf-life by maintaining fresh quality and nutritive value (Fisk *et al.*, 2008; Gonzalez-Aguilar *et al.*, 2008).

Edible coatings can be ingredient carriers and act as barrier of water vapour losses, thus extending product shelf-life by reducing the risk of pathogen growth on food surfaces (Antunes *et al.*, 2012; Oms-Oliu *et al.*, 2010; Zúñiga *et al.*, 2012). Alginate derived from sea brown algae (*Phaeophyceae*), is a linear unbranched polymer containing mannuronic and guluronic acids, with an instantaneous gelation with calcium or bivalent ions (Rojas-Graü *et al.*, 2008). Pectin extracted from apple waste or citrus fruits peel is a homopolymeric linear chain of galacturonic acid units, and form strong films when gelation conditions are meet (Oms-Oliu *et al.*, 2008). Both are natural common polysaccharides used as gelling agents in the food industry and recognized as safe (GRAS).

Essential oils have been investigated for their antimicrobial and antioxidant effects in food preservation and are also recognized as GRAS (Antunes & Cavaco, 2010; Jo *et al.*, 2014; Miguel, 2010). Citral and eugenol, which are essential oil compounds, have been successfully used when incorporated into edible coatings for *Arbutus unedo* fresh berries storage (Guerreiro *et al.*, 2015).

Malus domestica Borkh. cv. Bravo de Esmolfe is a Portuguese apple cultivar produced in northern Portugal which has high acceptability by consumers. The phytochemical and nutritional characterization and cardiovascular protective properties of 'Bravo de Esmolfe' apples have been reported (Reis *et al.*, 2009; Serra

et al., 2010, 2012; Silva *et al.*, 2014). 'Bravo de Esmolfe' apple demand is increasing due to its particular flavour, aroma and sweetness; nevertheless, such organoleptic attributes rapidly deteriorate over storage (Moldão-Martins *et al.*, 2003). When compared with some common apple cultivars, 'Bravo de Esmolfe' presented higher contents of fibre, protein, sugars, ß-carotene, vitamin E and B, K, Mg, phenolics and antioxidant activity and were preferred by consumers (Feliciano et al, 2010; Serra et al., 2010). For this reason, some efforts have been made for preserving their fruit freshness closer to the market quality standards. Modified atmosphere packaging and edible coatings are techniques already assayed to increase the storage life of 'Bravo de Esmolfe' fresh apples, but not fresh-cut (Moldão-Martins *et al.*, 2003; Rocha *et al.*, 2004).

Besides their faster postharvest deterioration as compared to other apple cultivars, 'Bravo de Esmolfe' apples show a faster browning after slicing due to a rapid oxidation. Such darkening hampers, up to now, the possibility of its use as fresh-cut fruit. Edible coatings with some components of essential oils with proven antioxidant activity may be one solution to overcome that problem.

To our knowledge there are no reports on fresh-cut 'Bravo de Esmolfe' apple. The objective of this study was to determine the effect of citral and eugenol, when incorporated into polysaccharide layer edible coatings based on alginate or pectin, on the quality, safety and shelf-life extension of fresh-cut 'Bravo de Esmolfe' apple.

Material and Methods

Material

'Bravo de Esmolfe' apples were purchased from the market when they were at eating- ripe stage (16.2-16.6 ºBrix and 38.6-40.4 N firmness). At the postharvest

laboratory in the University of Algarve, fruits were selected for uniformity of size and freedom from defects for use in the experiments.

Food grade sodium alginate (AL) and pectin (PE) (Sigma-Aldrich Chemic, , Germany) were the biopolymers used for coating formulations. Calcium chloride, citral (Cit) and eugenol (Eug) were from Sigma-Aldrich Chemic, , Germany. Ascorbic acid was from Scharlau, Barcelona, Spain.

Edible Coatings preparation

The coating forming solutions based on AL, as well as on PE, were formulated as described by Rojas-Graü et al. (2007) and Guerreiro et al. (2015). Ascorbic acid 1% was added to all edible coatings as anti-browning agent and CaCl2 at 1 g /100 mL was used as final dip for cross-link (Guerreiro et al., 2015; Robles-Sánchez et al., 2013).

The minimum inhibitory concentrations (MIC) for Eug and Cit were determined in a previous work (Guerreiro *et al.,* 2015). Concentrations of MIC and double MIC were used for Eug (0.1 and 0.2 g /100 mL) and Cit (0.15 and 0.3 g /100 mL).

The treatments were: Control, AL 1 g/100 mL (AL1%), AL1%+Cit 0.15 g/100 mL (Cit 0.15%), AL1%+Cit 0.3 g/100 mL (Cit 0.3%), AL1%+Eug 0.1 g/100 mL (Eug 0.1%), AL1%+Eug 0.2% g/100 mL (Eug 0.2%), AL1%+Cit 0.15%+Eug 0.1%, AL 2 g/100 mL (AL2%), AL 2%+Cit 0.15%; AL2%+Cit 0.3%; AL2%+Eug 0.1%; AL2%+Eug 0.2% and AL2%+Cit 0.15%+Eug 0.1%. PE treatments were formulated in the same manner except that PE was used instead of AL.

Fruits were washed in tap water and manually cut into 8 pieces with an appropriate apple-cutting device, with sharp blades. Then, apple slices were dipped

into the edible coating solution for 2 min, allowed to drip for 30 sec, dipped in the calcium chloride solution for 1 min, then dripped again (Guerreiro et al., 2015). Afterwards, 8 randomly apple slices were placed in polypropylene plastic trays (8 cm x 10 cm x 4 cm), covered with a linear low density polyethylene film 10 µm thick (permeability characteristics: O_2-6,000 $cm^3.m^{-2}$. 24 hr.bar; CO_2 45,000 $cm^3.m^{-2}$. 24 hr.bar; water vapor-157 $g.m^{-2}$.24hr.bar), and stored at 4 ºC until analyses. On days 0, 7 and 14, three trays per treatment (replications) were taken for quality evaluation. Controls did not have any kind of treatment, except initial tap washing and slices cut.

Quality parameters

Colour was measured by a Minolta chroma meter CR-300 (ECMinolta, Japan) using the CIELab scale (L*,hº*,C*) (McGuire, 1992). The firmness of the pulp was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS50 (Jonh Chatillon&Sons, Inc. USA) using a piston cylinder of 11 mm diameter at a depth of 7 mm. The soluble solids content (ºBrix) was measured by a digital refractometer PR1ATAGO CoLTD (Japan), in apple fruit's juice. Weight loss was expressed as percentage of initial weight.

Trolox Equivalent Antioxidant Activity (TEAC)

The antioxidant activity was measured according to the modified method of Re *et al.* (1999). Apple juice was obtained after squeezing apple flesh with an UltraTurrax T 18 (IKA, Germany) for 2 min then centrifuge 5 minutes at 5000 rpm. For the assay, 10 µL of apple juice was added to 990 µL of ABTS radical cation solution. The absorbance was monitored at 750 nm for 6 min (Shimadzu spectrophotometer 160-UV, Tokyo, Japan). The antioxidant activity of each sample

was calculated by the equation: scavenging effect (SE %)=(1-As/Ao)x100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 minutes. The values were compared with the curve for several Trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations and the values given as mM Trolox equivalent antioxidant capacity.

Microbial evaluation

The microbiological parameters that were determined included counts of aerobic mesophilic, psychrophilic bacteria, and molds and yeasts. Aerobic mesophilic and psychrophilic counts were done according to the Portuguese NP-3788 (2002) using Plate Count Agar medium (Biokar, Paris, France). The counts of molds and yeasts were performed according to the standard ISO 21527-2:2008 by using Dichloran Rose- Bengal Chloranfenicol Agar (Biokar, Paris, France). Ten grams of each sample were homogenized with 90 mL of peptone water (Oxoid). Decimal dilutions were prepared using the same diluent. The incubation temperature for yeasts and molds was 25±1 ºC during 48-72 h and for aerobic mesophilic bacteria was 30±1 ºC during 24-72 h and 6.5±1 ºC during 5 to 10 d for psychrophilic bacteria. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram of fresh weight.

Sensory Evaluation

A taste panel was performed with 15 panellists on the base of a 7-point hedonic scale: 1-dislike definitely; 2-dislike; 3-dislike mildly; 4-neither like nor dislike; 5-like mildly; 6-like; 7-like definitely. Overall liking was calculated as a mean of the sensory parameters evaluated. Panellists consisted of faculty students and staff to

whom a selection and training was performed before experiments (Guerreiro *et al.*, 2015).

Statistical Analysis

Statistical analysis was done with SPSS 20.0 software (IBM, Corp.) Two-way ANOVA and Duncan's multiple-range test ($P<0.05$) for comparisons among treatments was performed. To explore the similarities and dissimilarities among the formulations with respect to analyses was used the hierarchical cluster analysis (HCA) and for classification the Ward's Minimum Variance Method. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. The grouping derived from HCA was used to interpret the results of the dendogram and Principal Component Analysis (PCA) which was performed by Chemoface 1.5 software (Nunes *et al.*, 2012).

Results and Discussion

Quality parameters

The analysis of the results considered the sampling times 0 and 7 d shelf-life for all quality parameters measured. The 14^{th} day measurements were not take into account since fruits were considered unacceptable by the taste panel.

Colour parameters namely, lightness (L*) value, chroma and ^{o}hue of coated and uncoated fresh-cut apples during 7 days of storage at 4 ^{o}C are shown in Table 1. Generally, edible coatings were not significantly effective in maintaining L* values as compared to uncoated samples (control). The exceptions were AL1% and PE 1% + Eug 0.1%, where higher L* values (closer to the ones just after cutting)

were observed. The use of Cit and Eug revealed to be less effective when AL was used than PE, because at least 3 formulations (AL1% + Cit 0.3%, AL1% + Eug 0.2% and AL1% + Cit 0.15% + Eug 0.1%) had lower L* values than the control, so were darker. Such was not observed when PE was used, independent on the combination assayed (Table 1). Nevertheless, only PE+ Eug 0.1% was significantly lighter (higher L* values) than control. Such results indicate that AL at lower concentration is able to ameliorate the lightness, but the presence of Cit or Eug exert a negative effect on the surface of fresh-cut apples. In contrast, the presence of Eug in the formulation with PE, at lower concentration, presents a positive effect on the lightness of samples. Also, alginate at lower concentration may act as an oxygen barrier limiting the action of polyphenol oxidases, but the presence of Cit or Eug exert a negative effect. Similarly, the addition of Eug at MIC to the PE 1% has the same effect.

For ^{o}hue and chroma values, the presence of Cit or Eug in PE formulations did not produce any effect when compared to the control or to those samples only coated with PE, as well as for AL chroma measurements (Table 1). For the AL formulations, only the treatment AL 1%+Cit 0.15%+Eug 0.1% had significantly lower ^{o}hue values than control (Table 1).

Although using other type of edible coatings, Pérez-Gago *et al.* (2005) showed that apple pieces coated with whey protein-based coatings had higher L*, and lower b*, a*, and browning index values than hydroxypropyl methylcellulose-based coated and uncoated apple pieces. In the present work, the effect of Cit or Eug in the formulations was also dependent on the type of edible coating used. The presence of ascorbic acid, an anti-browning agent did not greatly prevent the darkening as reported by other authors (Rojas-Graü *et al.*, 2008; Azarakhsh *et al.*, 2014).

It is clear, for both AL and PE edible coatings, that all treated fresh-cut apples have higher firmness than controls (Table 2). This can be partially explained by the use of $CaCl_2$ dip, since is widely known that calcium preserve firmness (Antunes & Cavaco, 2010; Antunes *et al.*, 2013; Guerreiro *et al.*, 2015; Olivas & Barbosa-Cánovas, 2005; Rojas-Graü *et al.*, 2008). When looking at the PE edible coatings with Cit it is visible that AL or PE 1% + Cit 0.3% and PE 2% + Cit 0.15 or Cit 0.3% have the lower firmness values, although they are only significantly lower than AL 2% +Cit 0.15% + Eug 0.1% and AL 1%, for AL. For the PE edible coatings differences were significant only from PE 2% + Eug 0.2%. It seems that Cit is less effective in preserving firmness

of apple fresh-cut than Eug, probably because Cit, mainly at higher concentrations, can damage cell membranes making them softer, as reported by Raybaudy-Massilia *et al.* (2008). However, when higher AL concentrations are used, 2% in this case, this effect was counteracted as observed for AL2%+Cit 0.3%, probably because of diluted effect of Cit. In fact, generally firmness was significantly higher in AL than in PE treatments ($P=0.000$).

Rojas-Graü *et al.* (2007) found that alginate edible coatings applied to fresh-cut apples were effective in controlling moisture loss, thus loss of firmness. Olivas *et al.*, (2007) reported that the effect of calcium in keeping the texture of apple slices is probably higher than the effect of alginate coatings in preventing water loss, since softening of apples can be attributed more to cell wall degradation than to a reduction in turgor pressure. In our case, the incorporation of $CaCl_2$, for polymerization of the coatings, was probably the main cause of preserving firmness (Rojas-Graü et al., 2008). However, the differences among coatings also show that there is additional effect of the coating.

It is observed in Table 2 that the lower values of soluble solids content

(ºBrix) were in the edible coatings constituted by Cit 0.15%+Eug 0.1% for both AL and PE. However, although with some statistically significant differences, the ºBrix values ranged between 15.5-17.0% for all treatments, and were not significantly different from just after fruit-cutting values, what was expected since fruit were already ripe.

According to Olivas *et al.* (2007) for fresh-cut apples the ºBrix was not significantly affected by coating. These results disagree with those reported by Gol *et al.*, (2013) who showed a decrease in the total soluble solids content in strawberries during storage and attributed it to respiration. In our case, although without significant differences from most edible coatings, the ºBrix values of AL and PE at 1%+Cit 0.15%+Eug 0.1% were the lowest and significantly different from control. This may be due to lower respiration rate, which slightly increased for the same additives when edible coatings base increased to double.

Weight loss is also an indicator of freshness of fruits, which increases during shelf-life of fresh-cut fruit mainly due to water loss (Antunes *et al.*, 2010). Edible coatings are expected to reduce weight loss of fresh and fresh-cut fruit by reducing water loss (Olivas *et al.*, 2007; Perez-Gago *et al.*, 2006).

The most visible finding is that weight loss was higher in control AL fruit than in control PE (Table 2). Such differences should be lower. The possible explanation is because AL and PE treatments occurred one week apart, since AL and PE have similar water vapor sorption isotherms (Galus & Lenart, 2012). Nevertheless, all AL based edible coats reduced significantly the 'Bravo de Esmolfe' fresh-cut weight loss as compared to control, being the best AL2%+Cit 0.15%+Eug 0.1% followed by AL2%+Eug 0.1% (Table 2). In PE based edible coats, although lower, PE1% plus Eug at both concentrations or with the synergistic effect with 0.15% Cit, did not significantly differ in weight loss from control. The treatments that better

reduced weight loss were PE at both 1 and 2% or when combined with Cit 0.15% for PE1% and in both lower Cit and Eug concentrations (0.15% and 0.1%, respectively) for PE2% (Table 2).

Our results were consistent with previous studies showing a reduction in weight loss due to the effects of coatings composition, which served as semi-permeable barrier against moisture loss (Gol *et al.,* 2013; Valero *et al.,* 2013). Edible coatings act as an extra layer which also coats the stomata leading to a decrease in transpiration and in turn, to a reduction in weight loss. Moreover, differences in the ability to reduce weight loss are attributed to the different water vapor permeability of the polysaccharides used in the formulation of the edible coating (Vargas *et al.*, 2008). However, when applied to small entire *Arbutus unedo* fruits, some edible coatings were not so efficient as in the fresh-cut apples of the present experiment (Guerreiro *et al.*, 2015). This may be because fresh-cut fruit has much higher predisposition to lose water than entire fruit.

Antioxidant Activity (TEAC)

Antioxidant activity as measured by TEAC was significantly higher, almost double, in PE than in AL treatments (Table 3). This can be partially explained by the higher antioxidant activity of the fruit of PE experiment from the beginning, as controls have also higher TEAC values in PE than in AL. Nevertheless, several samples had higher antioxidant activity than slices just after cut, particularly when pectin 2% was used and independent on the concentrations of Cit or Eug assayed (Table 3). However, when pectin 2% was used alone did not present such activity. TEAC showed the lowest values for AL in treatment AL2%+Cit 0.15% and for PE in PE1%+Cit 0.15%. The highest values were for AL in AL2%+Cit 0.15%+Eug 0.1% or with both Eug concentrations, and for PE in PE2%+Eug 0.2% without

significant differences from the other PE 2% with essential oils (Table 3).

According to Robles-Sánchez *et al.* (2013), antioxidant activity in fresh-cut mangoes covered with an alginate edible coating enriched with ascorbic acid, expressed as TEAC or DPPH, was significantly higher than in alginate alone and control fruits. They attribute it to the ascorbic acid added to the coating. Also, Antunes *et al.* (2010; 2013) report an increase in fresh-cut kiwifruit and tomato antioxidant activity due to dips in ascorbic acid solutions. In our study, all coatings had ascorbic acid at 1 g/100 mL nevertheless not all had higher antioxidant activity than control (Table 3). Some authors (Mateos-Aparicio *et al.*, 2010; Urias-Orono *et al.*, 2010) have demonstrated the antioxidant capacity of pectin obtained from diverse sources. Urias-Orona *et al.* (2010) attributed such property of pectin to its richness in galacturonic acid. As generally, it seems that the higher concentrations of PE are better to preserve antioxidant activity when combined with the synergistic effect of Cit and/or Eug, whereas for AL, the possible synergistic effect with those aromatic additives only occur when higher concentration of AL is used and in the presence of Eug (both concentrations) and Eug+Cit. Also, the higher significant values of antioxidant activity in PE than in AL are attributed mainly to the addition of Cit and Eug at both concentrations and their combination in PE 2%. The antioxidant capacity of Eug is known (Gülçin, 2010). Although the anti-inflammatory activity of Cit (Miguel, 2010), its antioxidant capacity has not been found (Guimarães *et al.*, 2011), nevertheless the association with pectin and/or eugenol may exert a synergistic effect.

Microbial Quality

Food spoilage microorganisms are one of the main causes of fresh fruit

deterioration. No growth of psychotropic bacteria was observed during the storage period (data not shown). The counts of moulds and yeasts were higher in control and AL1% plus Cit and for PE in control and PE without additives (Table 3). Eug or Cit or their combination was equally efficient in reducing moulds and yeasts growth except PE1%+Cit 0.3% with higher values. For AL edible coatings, the most effective in reducing moulds and yeasts were the combination of Cit 0.15%+Eug 0.1% at both AL concentrations. Interestingly, PE was significantly better in reducing moulds and yeasts than AL, only when essential oils were added.

For the aerobic mesophilic microorganisms, the lower values were when both AL and PE coatings were at 2% with incorporation of Cit and Eug at double MIC (0.3% and 2%, respectively) (Table 3). Higher counts of aerobic mesophilic microorganisms were found in AL1% alone and in all PE1% treatments except PE1%+Cit 0.3%. Overall, the total counts for aerobic mesophilic microorganisms and moulds and yeasts were low, complying with the permissible standard limits (Stannard, 1997).

The main objective of introducing essential oils and/or their constituents into edible coatings as antimicrobials and antioxidant agents (Antunes *et al.,* 2012). According to Azarakhsh *et al.* (2014) alginate-based coating formulation with lemongrass oil significantly reduced the total microorganisms in coated fresh-cut pineapple during storage whereas uncoated and other coats failed to reduce the microbial population. Raybaudi-Massilia *et al.,* (2008) and Rojas-Graü *et al.* (2007) found no effect of alginate on microbial reduction, but observed it when some essential oils or their active compounds were added. Our work showed that the combination of Cit 0.15%+Eug 0.1% had significant effect mainly on reducing yeasts and molds in both edible coatings. Moreover, in PE2% also Cit and Eug showed significantly lower yeasts and molds counts than PE alone or in control fruits.

Sensory evaluation

The taste panel is of great importance because treatments and storage time change the edible quality of fruits. However, most research do not include taste panels when testing new edible coatings. In our work, after 7 d of storage at 4 ºC the taste panel showed that all treatments had a good sensory appreciation (>3.5 in a scale of 1-dislike definitely to 7-like definitely) (Table 4).

According to taste panels the treatments which scored higher as overall liking were AL or PE with Eug 0.1% or 2% (Table 4). The combination of Cit 0.15%+ Eug 0.1% gave also good score. Treatments with Cit scored lower mainly because they changed the natural flavour of the fruit. The 14 d storage was too long showing the appearance of fruit values from 1.8 to 3.9 for both edible coatings (Table 4). The concentrations of AL or PE used (1 or 2%) did not affect taste panel scores.

According to Azarakhsh *et al.* (2014), the incorporation of low concentrations up to 0.3 g/100 mL of lemongrass into alginate-based coating formulation did not have effect on sensory attributes of coated samples. However, incorporation of 0.5 g/100 mL lemongrass affected the sensory attributes of coated samples. In our work it seems that only Cit alone at higher concentration gives a significant change in the organoleptic characteristics of the fruit, so is not suitable for the market.

Edible coatings selection

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were utilized to investigate the similarities and dissimilarities among the formulations for each parameter, AL or PE, with respect to the analysed quality parameters. HCA and PCA gave similar grouping for both edible coatings. For

AL, control was separated alone, and edible coatings made 2 groups (Fig. 1). Taking into account that the best edible coatings are the ones which show the mean closest value to the one just after apple cut for colour; higher value for firmness, ºBrix and antioxidant activity; and lower value for weight loss and microbial spoilage, the group rounded by a circle is the one with the best characteristics. To select from them the two best formulations, we looked from this group the ones which scored higher in the taste panel and come out with AL2%+Eug 0.1% and AL2%+Cit 0.15+Eug 0.1%.

For PE edible coatings, a similar procedure was done and from the edible coatings rounded by the circle, were selected PE2%+Cit 0.15% and PE2%+Eug 0.2% (Fig. 2).

Conclusions

In this study we conclude that fresh-cut 'Bravo de Esmolfe' apple can be stored for 7 days at 4 °C with a good attractive appearance with edible coatings based on AL or PE. Fresh-cut 'Bravo de Esmolfe' apples were better preserved in terms of sensory and nutritional quality for alginate edible coatings with AL2%+Eug 0.1% and AL2%+Cit 0.15%+Eug 0.1% and for pectin with PE2%+Cit 0.15% and PE2%+Eug 0.2%.

Acknowledgements

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010- INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

References

Antunes, M. & Cavaco, A. (2010). The use of essential oils for postharvest decay control. A review. *Flavour and Fragrance Journal*, **25,** 351-366.

Antunes, M.D.C., Dandlen, S., Cavaco, A. M. & Miguel, G. (2010). Effects of postharvest application of 1-MCP and postcutting dip treatment on the quality and nutritional properties of fresh-cut kiwifruit. *Journal of Agricultural and Food Chemistry*, ***58*****(10),** 6173–81.

Antunes, M.D.C., Rodrigues, D., Pantazis, V., Cavaco, A.M., Siomos, A.S. & Miguel, G. (2013). Nutritional quality changes of fresh-cut tomato during shelf life. *Food Science and Biotechnology*, **22(5),** 1–8.

Antunes, M.D.C., Gago, C.M., Cavaco, A. & Miguel, M.G. (2012). Edible Coatings Enriched with Essential Oils and their Compounds for Fresh and Fresh-cut Fruit. *Recent Patents on Food, Nutrition & Agriculture*, **4(11),** 114–122.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P. & Mohd Adzahan, N. (2014). Lemongrass essential oil incorporated into alginate-based edible coating for shelf- life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology*, **88,** 1–7.

Chiumarelli, M. & Hubinger, M.D. (2012). Stability, solubility, mechanical and barrier properties of cassava starch - Carnauba wax edible coatings to preserve fresh-cut apples. *Food Hydrocolloids*, **28(1),** 59–67.

Feliciano, R.P., Antunes, C., Ramos, A., Serra, A.T., Figueira, M.E., Duarte, C.M.M., Carvalho, A. & Bronze, M.R. (2010). Characterization of traditional and exotic apple varieties from Portugal. Part 1 – Nutritional, phytochemical and sensory evaluation. *Journal of Functional Foods*, **2,** 35-45.

Fisk, C.L., Silver, A.M., Strik, B.C. & Zhao, Y. (2008). Postharvest quality of hardy kiwifruit (Actinidia arguta "Ananasnaya") associated with packaging and storage conditions. *Postharvest Biology and Technology*, **47(3),** 338–345.

Galus, S. & Lenart, A. (2012). Development and characterization of composite edible films based on sodium alginate and pectin. *Journal of Food Engineering*, **115(4),** 459–465.

Gol, N.B., Patel, P.R. & Rao, T.V.R. (2013). Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. *Postharvest Biology and Technology*, **85,** 185–195.

Gonzalez-Aguilar, G., Celis, J., Sotelo-Mundo, R.R., d Rosa, L., Rodrigo-Garcia, J. & Alvarez-Parrilla, E. (2008). Physiological and biochemical changes of different fresh-cut mango cultivars stored at 5°C. *International Journal of Food Science & Technology*, **43(1),** 91–101.

Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., & Antunes, M.D.C. (2015). The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage. *Postharvest Biology and Technology*, **100,** 226–233.

Guimarães, L.G.L., Cardoso, M.G., Sousa, P.E., Andrade, J. & Vieira, S.S. (2011). Antioxidant and fungitoxic activities of the lemongrass essential oil and citral. *Revista Ciência Agronómica* **42 (2),** 464-472. ISSN 1806-6690

Gülçin, I. (2010). Antioxidant activity of food constituents: an overview. *Archives of Toxicology*, **86,** 345–391.

Jo, W.S., Song, H.Y., Song, N.B., Lee, J.H., Min, S.C. & Song, K. B. (2014). Quality and microbial safety of "Fuji" apples coated with carnauba-shellac wax containing lemongrass oil. *LWT - Food Science and Technology*, **55(2),** 490–497.

Mateos-Aparicio, I., Mateos-Peinado, C., Jimenez-Escrig, A. & Ruperez, P. (2010). Multifunctional antioxidant activity of polysaccharide fractions from the soybean byproduct okara. *Carbohydrate Polymers*, **82,** 245–250.

McGuire, R. (1992). Reporting of objective color measurements. *HortScience*, **27(12),** 1254–1255.

Miguel, M. (2010). Antioxidant activity of medicinal and aromatic plants. A review. *Flavour and Fragrance Journal*, **25,** 291–312.

Moldão-Martins, M., Beirão da Costa, S.M. & Beirão da Costa, M.L. (2003). The effects of edible coatings on postharvest quality of the "Bravo de Esmolfe" apple. *European Food Research and Technology*, **217(4),** 325–328.

NP-3788:2002. (n.d.). Microbiologia Alimentar. *Regras Gerais Para a Contagem de Microrganismos a 30 ^{o}C.*, (IPQ, Portugal.).

Nunes, C., Freitas, M.P., Pinheiro, A.C.M. & Bastos, S.C. (2012). Chemoface: A novel free user-friendly interface for chemometrics. *Journal of the Brazilian Chemical Society*, **23(11),** 2003–2010.

Olivas, G.I. & Barbosa-Cánovas, G.V. (2005). Edible coatings for fresh-cut fruits. *Critical Reviews in Food Science and Nutrition*, **45(7-8),** 657–670.

Olivas, G.I., Mattinson, D.S. & Barbosa-Cánovas, G.V. (2007). Alginate coatings for preservation of minimally processed "Gala" apples. *Postharvest Biology and Technology*, **45(1),** 89–96.

Oms-Oliu, G., Rojas-Graü, M.A., González, L.A., Varela, P., Soliva-Fortuny, R., Hernando, M.I.H., & Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, **57(3),** 139–148.

Oms-Oliu, G., Soliva-Fortuny, R. & Martín-Belloso, O. (2008). Using polysaccharide- based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. *LWT - Food Science and Technology*, **41(10),** 1862–1870.

Pérez-Gago, M.B., Serra, M. & Río, M. (2006). Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biology and Technology*, **39(1),** 84–92.

Pérez-Gago, M.B., Serra, M., Alonso, M., Mateos, M. & Río, M. (2005) effect of whey protein- and hydroxypropyl methylcellulose-based edible composite coatings on color change of fresh-cut apples. *Postharvest Biology and Technology*,**36**: 77-85.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J. & Martín-Belloso, O. (2008). Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *International Journal of Food Microbiology*, **121(3),** 313–27.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, ***26*(98)**, 1231–1237.

Reis, S.F.A.R., Rocha, S.M., Barros, A. S., Delgadillo, I. & Coimbra, M.A. (2009). Establishment of the volatile profile of "Bravo de Esmolfe" apple variety and identification of varietal markers. *Food Chemistry*, **113(2),** 513–521.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G. & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh- cut Kent mangoes. *LWT - Food Science and Technology*, **50(1),** 240–246.

Rocha, A.M.C.N., Barreiro, M.G. & Morais, A.M.M.B. (2004). Modified atmosphere package for apple "Bravo de Esmolfe." *Food Control*, **15(1),** 61–64.

Rojas-Graü, M.A., Raybaudi-Massilia, R.M., Soliva-Fortuny, R.C., Avena-Bustillos, R. J., McHugh, T.H. & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology*, **45(2),** 254–264.

Rojas-Graü, M.A., Tapia, M.S. & Martín-Belloso, O. (2008). Using polysaccharide- based edible coatings to maintain quality of fresh-cut Fuji apples. *LWT - Food Science and Technology*, **41(1),** 139–147.

Serra, A.T., Matias, A.A., Frade, R.F.M., Duarte, R.O., Feliciano, R.P., Bronze, M.R. & Duarte, C.M.M. (2010). Characterization of traditional and exotic apple varieties from Portugal. Part 2 – Antioxidant and antiproliferative activities. *Journal of Functional Foods*, **2(1),** 46–53.

Serra, A. T., Rocha, J., Sepodes, B., Matias, A. a, Feliciano, R. P., de Carvalho, A., Figueira, M. E. (2012). Evaluation of cardiovascular protective effect of different apple varieties - Correlation of response with composition. *Food Chemistry*, ***135*(4)**, 2378–86.

Silva, A.S., Nunes, C., Coimbra, M.A. & Guido, L.F. (2014). Composition of pectic polysaccharides in a Portuguese apple (*Malus domestica* Borkh. cv Bravo de Esmolfe), *Scientia Agrícola*, **71(4),** 331–336.

Stannard, C. (1997). Development and use of microbiological criteria for foods. *Food Science and Technology* Today, **11,** 137-176.

Urias-Orona, V., Huerta-Oros, J., Carvajal-Millán, E., Lizardi-Mendoza, J., Rascón- Chu, A. & Gardea A.A. (2010). Component analysis and free radicals scavenging activity of *Cicer arietinum* L. husk pectin. *Molecules,* **15(10),** 6948-6955.

Valero, D., Díaz-Mula, H.M., Zapata, P.J., Guillén, F., Martínez-Romero, D., Castillo, S. & Serrano, M. (2013). Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. Postharvest Biology and Technology, **77**, 1–6.

Vargas, M., Pastor, C., Chiralt, A., McClements, D. J., & González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, *48*(6), 496–511.

Zúñiga, G. E., Junqueira-Gonçalves, M. P., Pizarro, M., Contreras, R., Tapia, A., & Silva, S. (2012). Effect of ionizing energy on extracts of Quillaja saponaria to be used as an antimicrobial agent on irradiated edible coating for fresh strawberries. Radiation Physics and Chemistry, 81(1), 64–69.

Table 1 Color parameters (L*, Hueº and Chroma) of fresh-cut apples, just after cut, and covered with different alginate (AL) and pectin (PE) based edible coating (EC) formulations after 7 d storage at 4ºC.

Values in the same column followed by a different letter are significantly different by Duncan's multiple range test (P<0.05).

Treatments	L		Hue		Chroma	
	AL	PE	AL	PE	AL	PE
Just after cut	78.895 a	78.244 a	99.446 a	99.812 a	24.293 b	24.910 b
Control	70.104 cd	69.838 cd	89.224 bcde	87.868 b	31.559 a	32.248 a
EC 1%	73.343 b	70.707 bcd	90.225 b	88.963 b	31.880 a	31.388 a
EC 1% +Cit 0.15%	71.135 bcd	71.030 bcd	88.453 bcdef	88.638 b	32.885 a	32.546 a
EC 1% +Cit 0.3%	68.702 e	71.199 bcd	88.867 bcdef	88.469 b	32.560 a	32.965 a
EC 1% + Eug 0.1%	70.029 cde	73.071 b	87.147 ef	88.921 b	31.826 a	30.600 a
EC 1% + Eug 0.2%	68.002 e	70.631 bcd	88.295 bcdef	88.215 b	32.639 a	33.044 a
EC 1% +Cit 0.15% + Eug 0.1%	68.596 e	72.329 bc	86.801 f	90.225 b	32.552 a	32.445 a
EC 2%	71.734 bcd	71.672 bcd	89.539 bcd	89.636 b	32.785 a	32.590 a
EC 2% +Cit 0.15%	69.510 de	70.981 bcd	88.236 bcdef	89.589 b	32.241 a	31.960 a
EC 2% +Cit 0.3%	70.204 cde	71.577 bcd	88.421 bcdef	89.267 b	32.403 a	33.308 a
EC 2% + Eug 0.1%	70.104 cde	71.967 bcd	87.744 cdef	88.599 b	31.357 a	31.164 a
EC 2% + Eug 0.2%	71.918 bc	72.325 bc	90.595 b	89.389 b	31.840 a	31.757 a
EC 2% +Cit 0.15% + Eug 0.1%	72.095 bc	72.387 bc	90.053 bc	89.489 b	31.468 a	32.356 a
Significance level for AL*PE (ANOVA)	0.001		0.319		0.0	

Table 2 Firmness, ºBrix and weight loss of fresh-cut apples, just after cut, and covered with different alginate (AL) and pectin (PE) based edible coating (EC) formulations after 7 d storage at 4ºC.

Treatments	Firmness(N)		ºBrix (%)		Weight loss (%)	
	AL	PE	AL	PE	AL	PE
Just after cut	38.57 ef	40.427 abcd	16.58 ab	16.17 a	0.00 h	0.00 g
Control	35.86 f	33.65 e	17.00 ab	16.33 a	1.22 a	0.64 a
EC 1%	45.56 a	40.94 abcd	16.39 bc	15.88 ab	0.89 bcd	0.28 ef
EC 1% +Cit 0.15%	43.12 abc	38.88 bcd	16.25 bcd	15.80 ab	0.89 bcd	0.35 cdef
EC 1% +Cit 0.3%	40.94 cde	38.83 cd	15.71 ef	15.78 ab	0.88 bcd	0.40 cde
EC 1% + Eug 0.1%	41.92 bcde	39.97 abcd	16.01 cdef	16.04 ab	0.70 def	0.59 ab
EC 1% + Eug 0.2%	42.25 abcd	40.92 abcd	16.39 bc	15.88 ab	0.82 bcde	0.65 a
EC 1% +Cit 0.15% + Eug 0.1%	43.12 abc	39.92 abcd	15.63 f	15.52 b	0.83 bcde	0.58 ab
EC 2%	42.57 abc	40.71 abcd	16.58 ab	15.79 ab	0.74 cdef	0.30 def
EC 2% +Cit 0.15%	43.41 abc	37.93 d	16.22 bcd	16.32 a	0.75 cdef	0.32 cdef
EC 2% +Cit 0.3%	43.50 abc	38.83 cd	16.16 bcd	16.36 a	0.87 bcd	0.39 cde
EC 2% + Eug 0.1%	42.41 abc	39.62 abcd	16.27 bcd	16.00 ab	0.58 f	0.34 cdef
EC 2% + Eug 0.2%	42.94 abc	42.33 ab	16.02 cdef	16.12 a	0.79 bcdef	0.38 cde
EC 2% +Cit 0.15% + Eug 0.1%	45.18 ab	41.43 abc	15.88 def	16.02 ab	0.36 g	0.46 bc
Significance level AL*PE (ANOVA)	0.000		0.061		0.00 0	

Values in the same column followed by a different letter, for each parameter, are significantly different by Duncan's multiple range test (P<0.05).

Table 3 Antioxidant activity (TEAC), moulds and yeast and aerobic mesophilic microorganisms of fresh-cut apples, just after cut, and covered with different alginate (AL) and pectin (PE) based edible coating (EC) formulations after 7 d storage at4ºC.

Treatments	Antioxidant Activity (µM.TE/10				Moulds and yeast (Log $_{10}$CFU/g)				Aerobic mesophilic			
	AL		PE		AL		PE		AL		PE	
Just after cut	87.1	a	96.4	cde	2.08	b	1.97	c	3.30	d	3.50	c
Control	46.4	bcdef	77.7	fgh	2.90	a	2.88	a	3.81	a	3.41	cde
EC 1%	50.1	bcde	115.0	abc	2.62	cd	2.74	a	3.79	a	3.79	ab
EC 1% +Cit 0.15%	49.8	bcde	64.3	h	3.00	a	0.99	d	3.74	a	3.79	ab
EC 1% +Cit 0.3%	48.6	bcdef	94.0	def	2.95	a	2.51	b	3.74	a	3.67	b
EC 1% + Eug 0.1%	44.7	bcdef	104.7	abcd	2.3	fg	0.99	d	3.71	ab	3.74	ab
EC 1% + Eug 0.2%	41.8	def	99.9	bcde	1.04	i	0.99	d	3.25	d	3.46	cd
EC 1% +Cit 0.15% + Eug 0.1%	40.5	ef	74.2	gh	1.04	i	0.99	d	3.61	b	3.86	a
EC 2%	46.9	bcdef	73.5	gh	2.35	ef	2.73	a	3.78	a	3.27	efg
EC 2% +Cit 0.15%	39.3	f	103.1	abcd	2.13	gh	0.99	d	3.42	c	3.27	efg
EC 2% +Cit 0.3%	43.1	cdef	114.3	abc	2.07	h	0.99	d	3.25	d	3.15	g
EC 2% + Eug 0.1%	51.5	bcd	107.2	abcd	1.04	i	0.99	d	3.61	b	3.21	fg
EC 2% + Eug 0.2%	51.9	bc	121.4	a	2.69	bc	0.99	d	1.94	e	2.94	h
EC 2% +Cit 0.15% + Eug 0.1%	53.2	b	107.0	abcd	1.04	i	0.99	d	3.61	b	3.34	ef
Significance level AL*PE (ANOVA)	0.000				0.00				0.535			

Values in the same column followed by different lower case letter are significantly different by Duncan's multiple range test ($P<0.05$).

Table 4 Sensory evaluation of fresh-cut apples, just after cut, and covered with different alginate based edible coating formulations through storage at 4ºC. Appearance, aroma, texture, sweetness, acidity, flavor and overall acceptance were evaluated at harvest and after 7 d storage, while after 14 days only appearance was evaluated. Values represent the mean of 15 replicates.

Treatments	Appearance		Aroma		Texture		Sweetness		Acidity		Flavour		Overall Liking		Appearance At 14 Days	
	AL	PE	AL	PE	AL	PE	AL	PE	AL	PE	AL	PE	AL	PE	AL	PE
Just after cut	5.1	5.4	5.7	5.7	6.1	5.6	6.2	5.9	6.0	5.8	6.2	5.9	5.8	5.9	-	-
Control	3.7	3.6	5.0	4.3	3.3	4.1	4.4	4.3	5.1	4.7	4.1	4.1	4.2	4.4	2.0	1.8
EC 1%	4.1	3.8	4.7	4.5	5.6	4.8	5.9	4.5	5.8	4.0	6.1	4.3	4.9	4.9	3.9	3.5
EC 1% +Cit 0.15%	4.2	4.5	4.4	3.8	4.9	5.0	4.6	4.5	4.8	5.3	4.3	4.0	4.6	4.6	3.0	3.0
EC 1% +Cit 0.3%	4.0	3.8	4.4	4.0	5.3	4.5	4.7	4.5	5.3	4.0	4.2	3.5	4.4	4.3	1.5	3.0
EC 1% + Eug 0.1%	4.7	4.8	5.1	4.5	5.4	5.5	5.6	5.5	5.9	5.5	5.8	5.3	5.3	5.5	1.5	3.3
EC 1% + Eug 0.2%	4.9	4.0	4.9	5.0	5.3	5.8	4.8	5.8	5.4	5.8	5.6	5.8	5.2	5.6	1.0	2.8
EC 1% +Cit 0.15% + Eug 0.1%	4.0	4.8	4.9	4.5	5.6	4.8	5.2	5.3	5.1	5.3	5.1	5.3	4.9	5.2	2.5	3.0
EC 2%	4.2	4.0	4.7	4.5	5.6	4.5	5.7	4.8	5.6	5.0	5.6	4.8	4.9	5.1	3.0	3.3
EC 2% +Cit 0.15%	4.3	4.8	4.7	4.3	4.7	5.0	4.6	4.8	4.8	5.0	4.6	4.5	4.7	4.7	2.0	3.3
EC 2% +Cit 0.3%	4.2	3.5	5.0	5.0	5.2	4.5	5.4	4.0	4.8	3.5	3.9	4.0	4.5	4.1	2.0	2.8
EC 2% + Eug 0.1%	4.7	4.5	5.1	4.5	5.4	5.0	5.6	5.5	5.9	5.0	5.8	5.0	5.2	5.4	1.5	3.0
EC 2% + Eug 0.2%	4.6	3.0	5.4	4.5	5.6	5.0	5.3	5.0	5.3	5.0	4.9	4.5	4.9	4.9	1.8	3.3
EC 2% +Cit 0.15% + Eug 0.1%	4.3	4.0	5.3	4.3	5.2	5.0	5.2	5.0	5.3	5.3	5.2	5.3	4.9	5.2	1.8	3.3

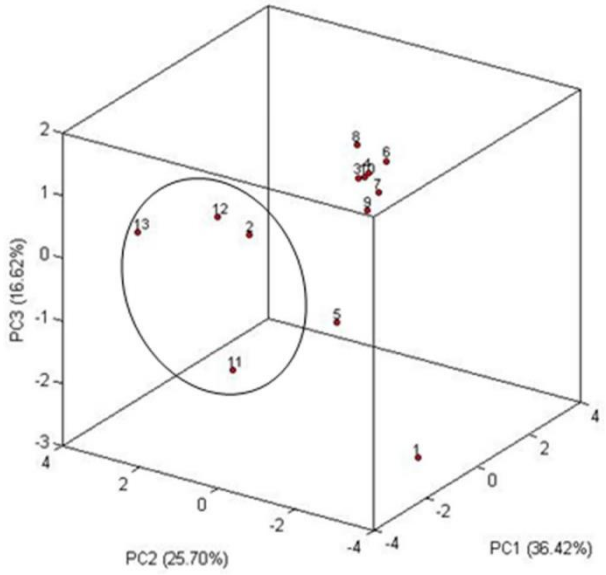


Figure 1 Loading plot for principal component analysis (PCA) of the 13 Alginate (AL) treatments shown in the above tables. 1- Control; 2- AL 1%; 3- AL 1% + Citral 0.15%; 4- AL 1% + Citral 0.3; 5- AL 1% + Eugenol 0.1%; 6- AL 1% + Eugenol 0.2%; 7- AL 1% + Citral 0.15% + Eugenol 0.1%; 8- AL 2%; 9- AL 2% + Citral 0.15%;10- AL 2% + Citral 0.3%;11- AL 2% + Eugenol 0.1%; 12- AL 2% + Eugenol 0.2%;13 - AL 2% + Citral 0.15% + Eugenol 0.1%.

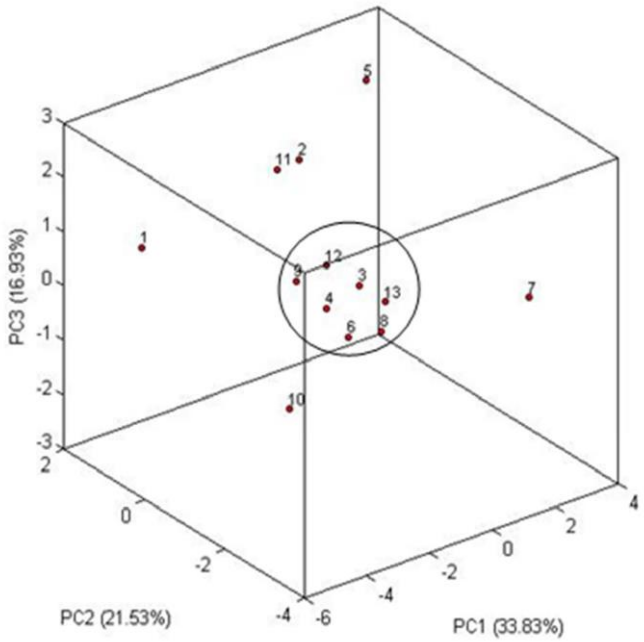


Figure 2 Loading plot for principal component analysis (PCA) of the 13 Pectin (PE) treatments shown in the above tables. 1- Control; 2- PE 1%; 3- PE 1% + Citral 0.15%; 4- PE 1% + Citral 0.3; 5- PE 1% + Eugenol 0.1%; 6- PE 1% + Eugenol 0.2%; 7- PE 1% + Citral 0.15% + Eugenol 0.1%; 8- PE 2%; 9- PE 2% + Citral 0.15%;10- PE 2% + Citral 0.3%;11- PE 2% + Eugenol 0.1%; 12- PE 2% + Eugenol 0.2%;13 - PE 2% + Citral 0.15% + Eugenol 0.1

Chapter VI

The effect of edible coatings on the nutritional quality of 'Bravo de Esmolfe' fresh-cut apple through shelf-life

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria L Faleiro[2]
Maria G C Miguel[1], Maria D C Antunes[1]

[1]*Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal*

[2]*Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal*

Submitted

The effect of edible coatings on the nutritional quality of 'Bravo de Esmolfe' fresh-cut apple through shelf-life

Abstract

The effect of edible coatings in combination with anti-browning agents on fresh-cut 'Bravo de Esmolfe' apple was studied. Four formulations of edible coatings were used: 1) sodium alginate at 2% with eugenol 0.1%; 2) sodium alginate at 2% with citral 0.15% and eugenol 0.1%; 3)Pectin 2% with eugenol 0.2%; 4) Pectin 2% with citral 0.15%. Three anti-browning agents were used, ascorbic and citric acids were used at 1% and sodium chlorite was used at 0.05%. Fresh-cut apples were immersed into those solutions for 2 minutes, and then stored at 4 ºC. On days 0, 2, 4, 6 and 8, samples were taken to perform physicochemical and biochemical analysis [color, browning index, firmness, soluble solids content (SSC), weight loss, microbial growth, taste panels, phenol compounds (total phenols, flavonoids), sugars content, antioxidant activity and ethylene production]. Based on those best quality characteristics, we conclude that fresh-cut apples were better preserved in terms of general quality parameters and nutritional value with AL2% + Eug 0.1% plus dip in ascorbic acid.

Key-Words: Alginate, pectin, citral, eugenol, 'Bravo de Esmolfe', fruit quality.

1. Introduction

Malus domestica Borkh. Cv. Bravo de Esmolfe is an emblematic Portuguese apple cultivar classified as Protected Designation and Origin (PDO), which corresponds to a traditional product produced under strict conditions and labeled with a specific law of protected designation (Moldão-Martins, Beirão da Costa, & Beirão da Costa, 2003).

The demand for fresh-cut fruit has increased considerably due to their content of vitamins, phenols and other antioxidants related to the prevention of various cancers and degenerative diseases. However, fresh-cut processing causes quality deterioration associated with tissue breakdown that results in metabolic, physicochemical and textural changes (Zambrano-Zaragoza et al., 2014). One of the main challenges for the fresh-cut fruit industry is the browning effect that develops as a result of the polyphenol oxidase activity that occurs after peeling and cutting. Browning effect must be treated with a browning inhibitor that impedes the development of brown discoloration (Gonzalez-Aguilar et al., 2008). Browning-inhibitor formulations generally contain reducing agents, such as organic acid, cysteine, honey, $CaCl_2$ and polyphosphates among others (Zambrano-Zaragoza et al., 2014).

Edible coatings are one option for reducing the deterioration caused by minimal processing of fresh-cut fruits, and for extending shelf life, because they form a semi-permeable barrier consisting of O_2, CO_2, moisture and solute movement that serves to reduce respiration index, weight loss, and oxidation reactions rates (Perez-Gago, Serra, Alonso, Mateos, & Del Río, 2005).

Different structural materials have been used in edible film elaboration, such as proteins, lipids and polysaccharides. Polysaccharides include alginate, cellulose, chitosan, starch and pectin. Pectin is one of the main components of the plant cell wall, contributing to tissue integrity and rigidity and it is considered one of the most complex macromolecules in nature (Espitia et al. 2014). Alginate is a salt of alginic acid, a polymer of D-mannuronic acid and L-guluronic acid, and is isolated from brown algae, is used as an edible coating because of its unique colloidal properties and its ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations, such as calcium (Jiang, 2013). Citral and eugenol, which are essential oil constituents,

have been used successfully when incorporated into edible coatings, against food spoilage microorganisms (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015b)

In a previous work, two edible coatings based on pectin and two based on alginate were selected, among 13 coatings, as the best for preserving fresh-cut 'Bravo de Esmolfe' apples main quality characteristics (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015a). However, other sensorial and nutritional parameters remain to be studied as well as their combination with anti-browning agents. So, the objective of this study was to evaluate the effect of different, previously selected, edible coatings and anti-browning agents on the sensorial and nutritional quality attributes on 'Bravo de Esmolfe' fresh-cut apple through shelf-life.

2. Material and Methods

2.1. Material

'Bravo de Esmolfe' apples were purchased from the market when they were eating-ripe (16.2-16.6 ºBrix and 38.6-40.4 N firmness), ready to prepare as fresh-cut. At the postharvest laboratory in the University of Algarve, fruits were selected for uniformity of size and freedom from defects and used in the experiments.

Food grade sodium alginate (AL) and pectin (PE) (Sigma-Aldrich Chemic, , Germany) were the biopolymers used for coating formulations. Calcium chloride (Sigma-Aldrich Chemic, , Germany) was used to induce cross linking reaction and ascorbic acid (Scharlau, Barcelona, Spain), citric acid (Sigma-Aldrich Chemic, , Germany) and calcium chlorite (Sigma-Aldrich Chemic, , Germany) were added to coatings as anti-browning agent. Citral (Cit) and eugenol (Eug) were from Sigma-Aldrich Chemic, , Germany.

2.2. Edible Coatings preparation

The coating forming solutions were formulated as described by Rojas-Graü et al. (2007) and Guerreiro et al. (2015a). The three anti-browning agent solutions consisted of ascorbic or citric acid at 10 g/L (1%) and sodium chlorite was used at 0.5g/L (0.05%). $CaCl_2$ at 10 g/L (1%) was used as final dip for cross-link (Guerreiro et al., 2015b; Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martin-Belloso, 2013).

The edible coating treatments were: Control, AL 2 g/100 mL (AL 2%)+ Eug 0.1 g/100 mL (Eug 0.1%), AL2%+Cit 0.15 g/100 mL (Cit 0.15%)+Eug 0.1, PE 2 g/100 mL (PE 2%)+ Eug 0.2% and PE 2% + Cit 0.15%.

Fruits were manually cut into 8 pieces with an appropriate apple-cutting device, with sharp blades. Then, apple slices were dipped for 1 min in an anti-browning solution (ascorbic acid, citric acid or sodium chlorite), after that into the edible coating solution for 2 min, allowed to drip off excess coating for 30 sec, and dipped again in the calcium chloride solution for 1 min, then drip again. Afterwards, 8 randomly apple slices were placed in polypropylene plastic trays (8 cm x 10 cm x 4 cm), covered with a linear low density polyethylene film 10 µm thick (permeability characteristics:O_2- 6,000 cm^3/m^2. 24 hr.bar; CO_2 45,000 cm^3/m^2. 24 hr.bar; water vapor- 157 g/m^2. 24hr.bar), and stored at 4 ºC until analyses. On days 0, 2, 4, 6 and 8, three trays per treatment were taken for quality evaluation.

2.3. Quality parameters

Color of fruits was measured by a Minolta Chroma meter CR-300 (EC Minolta, Japan) using the CIELab scale (L*,a* and b*). (McGuire, 1992). The browning index

(BI) was calculated and used as an indicator of intensity of brown color and was calculated as follows (Olivas, Mattinson, & Barbosa-Cánovas, 2007):

$$\mathrm{BI} = \frac{[100(x - 0.31)]}{0.172}$$

Where:

$$x = \frac{(a* + 1.75L*)}{(5.646L* + a* - 3.012b*)}$$

The firmness of the pulp was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS50 (Jonh Chatillon & Sons, Inc. USA) using a piston cylinder of 11 mm diameter at a depth of 7 mm. The soluble solids content (ºBrix) were measured using a digital refractometer PR1ATAGO CoLTD (Japan), in apple juice. Weight loss was expressed as percentage of initial weight.

2.4.Microbial counts

Microbial counts were determined for each treatment. The microbiological parameters that were determined included counts of aerobic mesophilic, psychrophilic microorganisms, and molds and yeasts. The counts of aerobic mesophilic, psychrophilic and molds and yeasts were done as described in a previous work (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015c). Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram fresh weight.

2.5.Sensory Evaluation

A taste panel was performed with 15 semi-trained panelists on the base of a 7-point hedonic scale (1-bad; 7-excellent) for the sensory parameters: Appearance, aroma, texture, sweetness, acidity, flavor and overall acceptance. All parameters were evaluated at harvest and after 3 and 6 days.

2.6. Total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965) modified for microplates. The sample (80 µL) and 20 µL of sodium carbonate (75 g/L) were added to 100 µL of 10% (w/v) Folin–Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm (Tecan Infinite M200, Swiss). Gallic acid was used as standard for calibration curve.

2.7. Flavonoids content

The content of these groups of compounds were quantified as described by (Miguel, Nunes, Dandlen, Cavaco, & Antunes, 2010) and modified for using microplates. Quercetin was used as a standard for the construction of the calibration curve. Sample or standard (100 µL) was added to 100 µL of 2% $AlCl_3$ ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm (Tecan Infinite M200, Swiss).

2.8. Antioxidant Activity

2.8.1. Trolox Equivalent Antioxidant Activity (TEAC)

The antioxidant activity was measured according to Re et al., (1999) modified for microplates adaptation. Apple juice was obtained after squeezing apple slices flesh with an UltraTurrax T 18 (IKA, Germany) for 2 min, then centrifuge for 5 minutes at 5000 rpm. For the assay, 3 µL of apple juice was added to 197 µL of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation solution). The absorbance was monitored at 750 nm for 6 min (Tecan Infinite M200, Swiss). The antioxidant activity of each sample was calculated by the equation: scavenging effect (SE)%= (1-

A_S/A_0)x100, where A_0 stands for the absorbance of the control at time 0 and A_S for the absorbance in the presence of the sample after 6 minutes. The values were compared with the curve for several Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations and the values given as mM Trolox equivalent antioxidant capacity.

2.8.2. Oxygen Radical Absorbance Capacity (ORAC)

ORAC is a method for measuring antioxidant activity, which measures the ability of samples for scavenging peroxyl radicals.

The ORAC method used had fluorescein (FL) as the fluorescent probe (Ou, Hampsch-Woodill, & Prior, 2001). As the ORAC assay is extremely sensitive, the samples must be diluted appropriately before analysis to avoid interference. In each well, 150 μL of fluorescein working solution and 25 μL apple juice, blank (75 mM phosphate buffer), or standard (Trolox) were placed. The plate was covered with a lid and incubated in the preheated (37 °C) microplate reader for 10 min with a previous shaking of 3 min (Tecan Infinite M200, Swiss). The *2,2'-Azobis-2-methyl-propanimidamide, dihydrochloride* (AAPH) was added to each well of the plate, except for the control and blank. The final volume of the assay was 200 μL. The fluorescence was read every minute during 90 min at excitation of 485 nm and emission of 527 nm. The ORAC values are calculated according to a previous work (Prior & Cao, 1999). Briefly, the net area under the curve (AUC) of the standards and samples was calculated. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values are calculated using the regression equation between Trolox concentration and the net AUC and are expressed as mmol Trolox/100 fresh weight.

2.9.Extraction and quantification of sugars and sweetness index

Extraction and quantification of sugars (fructose, glucose and sucrose) was based on a method described by Terry et al., (2007) and modified as described in Magwaza et al., 2012. Briefly, a 150 ± 0.5 mg of fruit lyophilized powder was extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentrations of fructose, glucose and sucrose were determined in an HPLC binary pump system (L-2130, Elite LaChrom series, Hitachi, Japan). Ten micro litres (10 µL) of a diluted sample solution (1:10) was injected 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). The thermostated column compartment temperature was set at 35ºC. The mobile phase used was HPLC-grade water at a 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 R2= 0.99).

Sugars have different sweetness impact. Since sucrose is 1.35 times sweeter than glucose and fructose is 2.3 times sweeter than glucose, a sweetness index concept was used to estimate the total sweetness perception. Glucose was assigned a sweetness value of one, sucrose 1.35 and fructose 2.3 (Keutgen & Pawelzik, 2007; Qian, 2005). Total sweetness index = 1 glucose + 1.35 sucrose + 2.3 fructose.

2.10. Ethylene production

Ethylene measurements were performed by withdrawing a 0.5 ml headspace gas sample from the jars with a syringe, and injecting it into a Trace 1300 (Thermo Scientific) gas chromatograph, equipped with a TG-Bond Alunina (Na_2SO_4)

30mx0.53mmx10µm (Thermo Scientific) at 60 °C and a flameionisation detector at 120°C. The carrier gas was N_2 at a flow rate of 35 ml/min.

2.11. Statistical Analysis

Statistical analysis was carried out with the SPSS 20.0 software (SPSS Inc.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among edible coatings treatments and for anti-browning treatment within each edible coating were performed.

3. Results and Discussion

3.1. General quality parameters

The browning index (BI), which integrates the color parameters of CIELab, is an indicator of the intensity of brown color (Olivas et al., 2007). The browning of pulp is a main issue in fresh-cut apples especially in 'Bravo de Esmolfe' cultivar.

The results of this experiment show that control had the higher values of BI as expected (Table 1). All the edible coatings reduced the BI being the best the AL ones. Taking into account the additional application of anti-browning agents, the most efficient was, for all edible coatings, the ascorbic acid, followed by citric acid and sodium chlorite.

Browning effect and the loss of color are common characteristics of fresh-cut fruit due to the tissues' damage provoked by peeling and slicing, which can induce enzymatic and non-enzymatic browning reactions promoting loss of natural color (Chiumarelli & Hubinger, 2012; Oms-Oliu et al., 2010). The differences due to the effect of the additional anti-brownings was higher than the one of the edible coatings (Salvia-trujillo, Rojas-graü, Soliva-fortuny, & Martín-belloso, 2015). Fresh-cut 'Fugi'

apples coated with nanoemulsions edible coatings with incorporation of essential oils, might induce the browning of cut apple surface by two mechanisms: (i) phenolic compounds from essential oils might be substrate themselves for PPO (polyphenol oxidase) activity; and (ii) an increase in the permeability of plant cell membrane due to volatile compounds might cause a higher leakage of PPO and polyphenols from the cell cytoplasm. Such was not the case of ours edible coatings, despite PE 2% + Cit 0.15% being the less effective showed better results in reducing BI in comparison to the control. Zambrano-Zaragoza et al. (2014) and Jo et al. (2014), using xanthan gum and carnauba-shellac wax based nanoemulsion containing lemongrass oil, observed similar results as ours. Also the use of alginate was reported to reduce the browning of fresh-cut pineapple (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan,2014).

Fruit ripening and softening are natural complex processes in fruit, softening seems to be a consequence of progressive cell wall modification and disassembly by enzyme action, leading to the solubilisation and depolymerisation of pectins and hemicelluloses (Cavaco, Pinto, Antunes, Silva, & Guerra, 2009). The maintenance of the firmness is an important factor for increasing shelf -life of fresh-cut products.

Firmness decreased through storage in control, while slightly increase in edible coated fruit probably due to the continued drying effect of the edible coatings (Table 1). The exception is when citric acid was used as anti-browning in which firmness was maintained for AL and decreased for PE edible coatings. Generally all edible coatings were efficient in maintaining firmness. When using additional anti-browning agents, the ascorbic acid and sodium chlorite showed similar firmness values, while citric acid the lower (Table 1). Rojas-Graü et al. (2007) reported similar results. Also, firmness of fresh-cut melon was better maintained with the use of the alginate edible coatings (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). Nevertheless, the immersion in

$CaCl_2$ for cross link reactions done in all edible coating treatments can be the reason for the higher firmness as reported previously (Olivas et al., 2007). Those authors reported that the effect of calcium in keeping the texture of apple slices is probably higher than the effect of alginate coatings. On the contrary, the softening of fresh-cut apples after applying edible coatings with essential oils was reported by Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso (2008), and attributed to the low pH of film forming solutions, which might cause the acid hydrolysis of pectic acid in fruit cell walls. Also, it has been suggested that such degradation of the texture might be caused by the penetration of the essential oils on the cell tissue of the fruit, producing structural changes (Salvia-trujillo et al., 2015). In our case this was not significant probably because essential oils concentrations were low. The combination of citric acid with the coatings was the one that caused lower firmness probably due to lower pH.

The objective of post-harvest and/or post-cut fruit treatments is to retard the metabolic processes, including the conversion of starch and organic acids to sugars to be used in the metabolic processes, which occur on postharvest as a result of ripening and senescence (Duan, Wu, Strik, & Zhao, 2011).

The SSC almost did not change through storage in coated fruit while slightly increased in control. This supports the fact that edible coatings retard the physiological ripening process, despite fruit were at the good ripening stage for fresh-cut preparation (Table 1).

There are reports regarding an increase in the SSC/titrable acidity during the storage of apples which could be attributed to the fact that insoluble polysaccharides are hydrolyzed to mono-and di-saccharides and malic acid is consumed for metabolism during storage (Hussain, Meena, Dar, & Wani, 2012; Jha, Rai, & Shrama, 2012; Jo et al., 2014). However, Olivas et al. (2007) reported no significant differences in the SSC

of fresh-cut apples treated with different alginate based edible coatings and control. Similar behavior to our experiments was reported for fresh-cut 'Red Delicious' apples with nano-coatings with α-tocopherol and xanthan gum (Zambrano-Zaragoza et al., 2014).

This can be explained due to the climacteric behavior of apples. When they are prepared at the physiological ripening SSC can be maintained or decreased due to senescence, when prepared before the complete physiological ripening they may increase SSC through shelf-life.

Weight loss is also an indicator of freshness of fruits and increases during shelf-life of fresh-cut fruit mainly due to water loss (Antunes, Dandlen, Cavaco, & Miguel, 2010).

In this study, weight loss increased through storage in all treatments without significant differences among edible coatings and control (Table 1). Nevertheless, when using additional anti-browning, PE coatings did not show significant differences among anti-browning agents, while in AL the sodium chlorite performed better than citric or ascorbic acids.

It is expected weight loss to occur in the shelf-life of fresh-cut fruit (Soliva-Fortuny & Martín-Belloso, 2003). The migration of water from fruit to the environment is considered the main cause of weight loss of fruit during storage (Duan et al., 2011). Edible coatings are considered to reduce weight loss due to their effects as semi-permeable barrier against moisture loss (Gol, Patel, & Rao, 2013; Valero et al., 2013), as it has been demonstrated in a wide range of fruit including apricot, pepper, peach, sweet cherry, and litchi (Ayranci & Tunc, 2003; Díaz-Mula, Serrano, & Valero, 2012; Hassimotto, Pinto, & Lajolo, 2008). Moreover, differences in the ability to reduce weight loss are attributed to the different water vapor permeability of the

polysaccharides used in the formulation of the edible coating (Vargas, Pastor, Chiralt, McClements, & González-Martínez, 2008). According to some authors, the addition of glycerol as plasticizer to the coating gave good results in terms of reducing weight loss in tomato, apple and strawberry (Abreu & Beirao-da-Costa, 2003; Garcia-Viguera et al., 1998; Serrano et al., 2008). However, when applied to small entire *Arbutus unedo* fruits, some edible coatings were not so efficient as in the fresh-cut of the present experiment (Guerreiro et al., 2015b). This may be because fresh-cut fruit has much higher predisposition to lose water than entire fruit and the polysaccharides of this experiment are more permeable to water than coatings containing lipids.

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration. No growth of psychotropic microorganisms was observed during the storage period (data not shown). Results of molds and yeast counts showed that the edible coatings treatments of this experiment showed lower values than control samples (Table 1). However, at the end of the experiment, AL coatings with the lower concentration of Eug were slightly less efficient than PE with Cit only in the last day of shelf-life.

Also mesophilic microorganisms' counts were lower in treated samples than in control, being AL edible coatings more efficient than PE in the last days of shelf-life (Table 1).

When looking at the additional anti-brownings there are not significant differences among them in none edible coatings for yeasts and molds control, while for mesophilic microorganisms, citric acid is generally better for reducing them in AL and in PE edible coatings the sodium chlorite performed better (Table 1). Overall, the total counts for aerobic mesophilic microorganisms and molds and yeasts were low, complying with the permissible standard limits (Stannard, 1997).

Literature has been referring the main objective of introducing essential oils and/or their constituents into edible coatings as antimicrobials and antioxidant agents (Antunes et al., 2012). According to Azarakhsh et al. (2014) alginate-based coating formulation with incorporation of lemongrass oil significantly reduced the total microorganisms and yeast and mold counts in coated fresh-cut pineapple samples as compared to controls and the same behavior was reported for 'Fuji' apples coated with carnauba- shellac wax containing lemongrass oil (Jo et al., 2014). Raybaudi-Massilia et al (2008) found no effect of alginate on microbial reduction, but observed it when some essential oils or their active compounds were added as in our case. Rojas-Graü et al. (2007) indicated that an alginate coating by itself did not reduce the psychrophilic aerobic bacteria or yeast and mold counts on fresh-cut 'Fuji' apples.

3.2. Total phenols and flavonoids

Phenolic compounds make up a class of phytochemicals that play an important role in the nutritional and sensory properties of various fruits and vegetables (Sandhu & Gu, 2010).

The results of the edible coatings showed that edible coatings did not affect the total phenols since no significant differences were observed among treatments and control (Table 2). However, the results in total phenols showed differences among anti-browning agents within the edible coatings, being total phenols significantly higher in ascorbic dips than in the other treatments for all edible coatings (Table 2). Phenols increased in control and ascorbic acid treatments for all edible coatings mainly in the first 2 shelf-life days, while they decreased for the other anti-browning dips. This behavior of ascorbic acid dips may be due to the antioxidant capacity of ascorbic acid as reported for other fruit (Antunes et al., 2010; Antunes et al., 2013) .

Table 1 Browning Index, firmness (N), soluble solids content (SSC), weight loss, molds and yeasts and aerobic mesophilic microorganisms of fresh-cut 'Bravo de Esmolfe' apples covered with different alginate and pectin based edible coating formulations during storage at 4ºC. Values represent the mean of three replicates taken at 0, 2, 4, 6 and 8 days.

Quality Parameters	Days	Control	Alginate 2% + Eugenol 0.1%			Alginate 2% + Citral 0.15% + Eugenol .0.1%			Pectin 2% + Eugenol 0.2%		
		No Treated	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite
Browning Index (BI)	0	31.28±0.57 ^{d} **A**	14.53±1.74 ^{bB}	15.85±1.89 ^{eA}	19.30±1.72 ^{dB} **C**	18.03±2.28 ^{dA}	18.85±0.89 ^{eA}	14.84±1.18 ^{dB} **C**	15.99±2.51 ^{cB}	24.19±3.25 ^{dA}	26.67±1.25 ^{bA} **B**
	2	42.26±0.65 ^{b} **A**	22.83±1.59 ^{aB}	29.21±1.23 ^{dA}	25.65±1.23 ^{bA} **B**	29.85±1.37 ^{bA}	28.20±0.98 ^{dA}	26.74±1.86 ^{bA} **B**	26.99±1.28 ^{bC}	45.60±0.60 ^{bA}	22.24±1.05 ^{cB} **B**
	4	45.21±0.46 ^{a} **A**	25.59±1.36 ^{aC}	38.87±0.79 ^{cA}	29.36±0.72 ^{bB} **B**	23.79±0.81 ^{cC}	35.03±1.63 ^{cA}	26.08±0.90 ^{bB} **B**	20.66±0.32 ^{cC}	32.90±0.64 ^{cA}	29.42±0.57 ^{aA} **B**
	6	40.45±0.71 ^{bc} **A**	23.57±1.28 ^{aB}	37.81±0.59 ^{bA}	21.00±0.24 ^{cB} **AB**	37.14±0.13 ^{aA}	38.61±1.22 ^{bA}	20.29±2.36 ^{cB} **AB**	29.20±0.65 ^{aB}	54.29±1.00 ^{aA}	25.92±0.60 ^{bB} **AB**
	8	39.60±0.61 ^{c} **A**	23.00±1.10 ^{aC}	42.74±0.62 ^{aA}	44.07±1.01 ^{aB} **A**	25.33±0.55 ^{cC}	43.56±0.57 ^{aA}	35.86±2.65 ^{aB} **A**	25.23±1.62 ^{bC}	47.03±0.76 ^{bA}	30.98±1.18 ^{aB} **A**
Firmness(N)	0	17.40±0.19 ^{ab} **B**	17.20±0.66 ^{cA}	19.72±0.80 ^{bcA}	17.38±1.10 ^{bA} **AB**	19.09±0.96 ^{bA}	17.61±0.90 ^{bcA}	19.37±0.17 ^{bA} **AB**	18.53±0.47 ^{aA}	20.43±1.47 ^{aA}	18.42±0.59 ^{bA} **A**
	2	19.79±0.24 ^{a} **A**	17.93±0.45 ^{cA}	17.49±0.53 ^{cB}	17.98±0.39 ^{bA} **A**	19.98±0.70 ^{abA}	16.92±0.48 ^{cB}	18.97±0.39 ^{bA} **A**	19.58±2.21 ^{aA}	17.41±0.84 ^{bA}	20.62±0.07 ^{abA} **A**
	4	16.48±0.02 ^{abc} **B**	18.95±1.01 ^{cdA}	19.52±0.27 ^{aA}	18.41±0.17 ^{abA} **A**	18.97±0.40 ^{bA}	20.02±0.21 ^{aA}	21.52±1.19 ^{abA} **A**	20.88±0.82 ^{aA}	14.23±0.25 ^{cdB}	20.31±0.44 ^{abA} **AB**
	6	15.53±0.25 ^{bc} **C**	21.03±1.05 ^{abA}	19.83±0.28 ^{abB}	22.28±0.83 ^{aA} **A**	21.68±0.91 ^{aAB}	19.52±0.72 ^{abB}	24.03±0.20 ^{aA} **A**	21.90±0.48 ^{aA}	13.65±0.71 ^{dB}	21.88±0.13 ^{aA} **AB**
	8	15.23±1.28 ^{c} **B**	22.01±0.92 ^{aA}	18.26±0.59 ^{abcB}	23.07±1.27 ^{aA} **A**	20.21±0.10 ^{bAB}	18.48±0.31 ^{abcB}	22.63±1.38 ^{aA} **A**	21.98±0.71 ^{aA}	16.81±0.82 ^{bcB}	21.87±1.34 ^{aA} **A**
SSC (ºBrix)	0	9.52±0.74 ^{b} **A**	11.18±0.12 ^{aA}	9.37±0.09 ^{aA}	9.27±0.42 ^{bA} **A**	10.12±0.66 ^{abA}	10.65±0.26 ^{aA}	10.63±0.42 ^{bA} **A**	10.30±0.18 ^{aAB}	9.57±0.43 ^{dB}	11.05±0.45 ^{aA} **A**
	2	12.77±0.20 ^{a} **A**	10.28±0.02 ^{abA}	10.98±0.56 ^{abB}	11.37±0.02 ^{abA} **C**	11.12±0.33 ^{aAB}	10.28±0.49 ^{abB}	11.83±0.27 ^{abA} **C**	11.38±0.16 ^{abA}	11.15±0.62 ^{abA}	11.97±0.43 ^{aA} **BC**
	4	12.88±0.11 ^{a} **A**	10.62±0.40 ^{abAB}	9.62±0.26 ^{bB}	10.80±0.25 ^{aA} **B**	9.80±0.14 ^{bB}	9.63±0.32 ^{bB}	12.82±0.36 ^{aA} **B**	10.02±0.55 ^{bA}	11.22±0.26 ^{abA}	10.82±0.43 ^{aA} **B**
	6	12.50±0.03 ^{a} **A**	10.28±0.50 ^{abA}	10.45±0.38 ^{abB}	10.80±0.36 ^{bAB} **C**	10.68±0.14 ^{abAB}	9.82±0.13 ^{abB}	10.37±0.23 ^{bAB} **C**	11.08±0.39 ^{abB}	12.23±0.21 ^{aA}	11.63±0.13 ^{aAB} **B**
	8	13.18±0.08 ^{a} **A**	9.60±0.44 ^{bB}	10.68±0.31 ^{abB}	12.60±0.37 ^{aA} **B**	9.78±0.22 ^{bAB}	10.53±0.09 ^{abB}	12.38±0.87 ^{aA} **B**	10.53±0.24 ^{abA}	10.48±0.33 ^{cdA}	11.03±0.54 ^{aA} **B**
WeightLoss (%)	0	0.00±0.00 ^{d} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{dA}	0.00±0.00 ^{dA} **A**	0.00±0.00 ^{dA}	0.00±0.00 ^{dA}	0.00±0.00 ^{dA} **A**	0.00±0.00 ^{dA}	0.00±0.00 ^{dA}	0.00±0.00 ^{cA} **A**
	2	0.49±0.06 ^{c} **A**	0.95±0.08 ^{aA}	0.90±0.19 ^{cB}	0.35±0.04 ^{cC} **A**	1.01±0.07 ^{cA}	0.61±0.10 ^{cB}	0.21±0.01 ^{cC} **A**	0.74±0.09 ^{cA}	0.62±0.05 ^{cA}	0.68±0.19 ^{bA} **A**
	4	0.75±0.04 ^{b} **A**	1.08±0.16 ^{aA}	1.06±0.12 ^{bcB}	0.42±0.07 ^{bcC} **A**	1.21±0.04 ^{bA}	0.77±0.08 ^{bcB}	0.36±0.10 ^{bcC} **A**	0.80±0.06 ^{cA}	0.73±0.05 ^{cA}	0.78±0.15 ^{bA} **A**
	6	0.82±0.10 ^{a} **A**	1.20±0.14 ^{aA}	1.32±0.10 ^{bB}	0.53±0.13 ^{bB} **A**	1.34±0.04 ^{abA}	1.28±0.25 ^{bB}	0.42±0.05 ^{bB} **A**	1.01±0.03 ^{bA}	1.02±0.02 ^{bA}	0.90±0.17 ^{aA} **A**
	8	0.82±0.17 ^{a} **A**	1.29±0.14 ^{aA}	1.57±0.17 ^{aB}	1.12±0.12 ^{aB} **A**	1.40±0.05 ^{aAB}	1.83±0.25 ^{aB}	1.00±0.05 ^{aB} **A**	1.30±0.06 ^{aA}	1.50±0.11 ^{aA}	1.32±0.16 ^{aA} **A**
Molds and Yeast (Log 10 CFU/g)	0	0.00±0.00 ^{c} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.66±0.66 ^{aA} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.36±0.36 ^{aA} **A**	0.00±0.00 ^{bA}	0.20±0.20 ^{aA}	0.00±0.00 ^{aA} **A**
	2	0.20±0.10 ^{c} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.23±0.23 ^{bA} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.00±0.00 ^{bA} **A**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.00±0.00 ^{aA} **A**
	4	0.46±0.09 ^{b} **A**	0.00±0.00 ^{bA}	0.10±0.10 ^{aA}	0.00±0.00 ^{bA} **B**	0.19±0.10 ^{aA}	0.00±0.00 ^{aA}	0.00±0.00 ^{bA} **B**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.00±0.00 ^{aA} **B**
	6	0.76±0.09 ^{a} **A**	0.07±0.07 ^{bA}	0.32±0.32 ^{aA}	0.00±0.00 ^{bA} **B**	0.00±0.00 ^{bA}	0.43±0.30 ^{aA}	0.00±0.00 ^{bA} **B**	0.20±0.10 ^{aA}	0.10±0.10 ^{aA}	0.00±0.00 ^{aA} **B**
	8	0.77±0.07 ^{a} **A**	0.67±0.11 ^{aA}	0.00±0.00 ^{aA}	0.00±0.00 ^{bA} **C**	0.00±0.00 ^{bA}	0.00±0.00 ^{aA}	0.00±0.00 ^{bA} **C**	0.13±0.07 ^{abA}	0.00±0.00 ^{aA}	0.00±0.00 ^{aA} **BC**
Mesophilic Microorganisms	0	1.15±0.23 ^{d} **A**	0.00±0.00 ^{dA}	0.00±0.00 ^{bA}	0.00±0.00 ^{dA} **B**	0.00±0.00 ^{dA}	0.00±0.00 ^{bA}	0.00±0.00 ^{dA} **B**	0.00±0.00 ^{dA}	0.00±0.00 ^{dA}	0.00±0.00 ^{bA} **B**
	2	2.40±0.05 ^{bc} **A**	2.27±0.01 ^{bA}	1.57±0.12 ^{aB}	1.67±0.03 ^{bB} **B**	2.01±0.01 ^{aA}	1.42±0.10 ^{aB}	1.34±0.04 ^{bB} **B**	2.16±0.02 ^{bA}	1.70±0.03 ^{bA}	0.63±0.35 ^{bB} **B**
	4	2.21±0.07 ^{c} **A**	0.00±0.00 ^{dB}	0.00±0.00 ^{bC}	1.45±0.02 ^{aA} **BC**	0.57±0.13 ^{cB}	0.10±0.10 ^{bC}	1.72±0.03 ^{aA} **BC**	0.78±0.18 ^{cB}	2.08±0.04 ^{aA}	0.72±0.37 ^{bB} **B**
	6	2.61±0.00 ^{ab} **A**	2.40±0.04 ^{aA}	1.30±0.74 ^{bB}	0.16±0.16 ^{dB} **B**	2.03±0.03 ^{aA}	0.00±0.00 ^{bB}	0.00±0.00 ^{dB} **B**	2.49±0.03 ^{aA}	0.00±0.00 ^{dB}	0.00±0.00 ^{bB} **B**
	8	2.78±0.00 ^{a} **A**	1.44±0.03 ^{cA}	1.21±0.10 ^{aA}	1.34±0.03 ^{cB} **C**	1.51±0.02 ^{bA}	1.48±0.03 ^{aA}	1.07±0.05 ^{cB} **C**	1.92±0.02 ^{bA}	1.59±0.02 ^{cC}	1.70±0.02 ^{aB} **B**

Values in the same column followed by different lower case, in the same row followed by different upper case for each anti-browning within coats and bold upper case for each edible coating, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Robles-Sánchez et al.(2013) found in fresh-cut mangos uncoated and coated with alginate plus ascorbic acid a constant phenolic content through shelf-life. According to Oms-Oliu et al. (2008) in fresh-cut 'Piel del Sapo' melon uncoated and coated with alginate, gellan and pectin, the initial phenolic content was maintained or slightly decreased during the first week of storage, but then it increased up at 12-day storage. In our case the increase was in the first days of storage and maintenance occurred thereafter in ascorbic acid and uncoated samples, on the other hand citric acid and sodium chlorite showed opposite behavior in the first 5 d.

In general, as phenols, the values of flavonoids increased through shelf-life and were higher in ascorbic acid dips than in other treatments (Table 2). However, all edible coatings had lower flavonoids content than control. It was found for fresh-cut mangos coated with alginate minimal influence of the treatment applied on fresh-cut samples, being storage time, which promoted changes on this parameter (Robles-Sánchez et al., 2013). Similar effect was found for hydroxypropyl methylcellulose–lipid edible coatings in 'Oronules' mandarins (Contreras-Oliva, Rojas-Argudo, & Pérez-Gago, 2012).

Wang & Gao (2012) showed similar behaviour to our experiment in strawberries coated with chitosan but stored at higher temperature 10ºC.

3.3. Antioxidant activity

The free-radical scavenging capacities of samples are shown in Table 2. The antioxidant activity measured by ORAC method, increased in all treatments through storage, while TEAC method showed a decrease in control, decrease in all coats with citric acid and sodium chlorite and maintenance of its values in ascorbic acid treatments.

No significant influence of the edible coatings was observed through storage, despite some statistically significant differences on TEAC values (Table 2). When using the ORAC method, antioxidant activity was higher in edible coatings than control. This is in agreement to what was observed for phenolic content, since increase in total phenolic content is related with the enhancement of antioxidant capacity (Ali, Maqbool, Alderson, & Zahid, 2013). Higher ORAC values in chitosan coated strawberries was also reported for strawberries but mostly at the end of storage (Wang & Gao, 2012). Oms-Oliu et al (2008) using gellan, alginate and pectin found also an increase in phenolics compounds related to the enhancement of antioxidant capacity in fresh-cut melon. According to Robles-Sánchez et al. (2013) antioxidant activity in fresh-cut mangoes covered with the edible coating alginate 2% + ascorbic Acid 1%, expressed as TEAC, was significantly higher than in alginate alone and control fruits. This was attributed to the ascorbic acid added at higher concentration (2%), which is in accordance to our work.

3.4. Ethylene production

Besides being climacteric, fresh-cut apple may have wounding induced ethylene production due to cut. The ethylene production was higher at the beginning of the experiment decreasing significantly from 2 days of storage (Table 2). This high ethylene production was due to the wounding effect of the fruit, since climacteric ethylene production may have finished since fruit were used ripe (Antunes & Sfakiotakis, 2000; Soliva-Fortuny & Martín-Belloso, 2003).

Edible coatings reduced ethylene production as compared to control (Table 2). There is no influence of the anti-browning agent in AL based edible coatings, but in PE citric acid had higher values. Reduction of ethylene production due to edible coatings

application was already reported for fresh-cut apples coated with alginate containing lemongrass essential oil nano-emulsion (Salvia-trujillo et al., 2015), fresh-cut 'Fuji' apples coated with gellan and alginate-based edible coatings (Rojas-Graü et al., 2007) and plums coated with alginate (Valero et al., 2013). On the other hand, (Oms-Oliu et al. (2008) report no effect of gellan, alginate and pectin edible coatings on fresh-cut melon ethylene production.

3.5. Sugars content and sweetness index

The analysis of sugar composition in fresh-cut 'Bravo de esmolfe' apple indicated fructose in higher concentrations followed by glucose and sucrose (Table 3). Despite some statistically significant differences, sugars did not change much through shelf-life. All sugars had lower values in edible coatings than in control at the last period of storage (6 and 8 d), indicating that edible coatings were in a slighter lower stage of ripening which is in accordance with the SSC values. Fruit dipped in sodium chlorite had generally higher sugars content than the other dips. The sweet index showed similar behavior but at day 8 differences were not significant (Table 3). Sugar content of coated tomato was lower than control being control at more advanced ripeness through storage as in our case(Zapata et al., 2008).

On the other hand minimally processed cactus pear coated with "Food coat"(composed of fatty acids derivatives and polysaccharides in alcohol solution) and "Pomfresh" (composed of a mixture of organic acids and antioxidant compounds) showed no influence on sugars content (Palma, Schirra, & Aquino, 2015).

Table 2 Phenols totals, flavonoids, TEAC, ORAC and ethylene of fresh-cut 'Bravo de Esmolfe' apples covered with different alginate and pectin based edible coating formulations during storage at 4ºC. Values represent the mean of three replicates taken at 0, 2, 4, 6 and 8 days.

Quality Parameters	Days	Control	Alginate 2% + Eugenol 0.1%			Alginate 2% + Citral 0.15% + Eugenol .0.1%			Pectin 2% + Eugenol 0.2%			Pectin 2% + Citral 0.15%		
		No Treated	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite
Total Phenols (mg of Gallic acid.100g^{-1} FW)	0	187.1±3.3 ^{b} **A**	135.2±22.0 ^{bB}	232.0±0.3 ^{aB}	264.0±0.3 ^{aA} **A**	119.5±21.0 ^{bC}	187.7±0.3 ^{aB}	307.3±0.3 ^{aA} **A**	132.6±21.^{bA}	193.5±0.3 ^{aA}	296.5±0.3 ^{aA} **A**	105.9±12.0 ^{bA}	288.4±0.3 ^{aA}	288.3±0.3 ^{aA} **A**
	2	208.6±14.2 ^{b} **A**	352.4±25.9 ^{aA}	83.3±16.2 ^{bB}	80.1±13.5 ^{cB} **A**	252.9±20.8 ^{aA}	62.2±12.5 ^{bB}	55.1±5.0 ^{cB} **A**	353.3±30.^{aA}	81.2±4.3 ^{bB}	68.0±1.8 ^{bB} **A**	303.9±7.3 ^{aA}	64.7±6.7 ^{bB}	62.7±12.9 ^{bB} **A**
	4	221.1±13.0 ^{ab} **A**	318.3±16.8 ^{abA}	57.5±6.7 ^{bB}	67.6±17.5 ^{bcB} **A**	251.9±125.3 ^{aA}	46.9±5.5 ^{bB}	76.8±17.4 ^{bcB} **A**	348.7±22.^{aA}	84.4±6.3 ^{bB}	56.0±14.6 ^{bB} **A**	397.0±3.0 ^{aA}	70.5±1.7 ^{bB}	77.7±8.2 ^{bB} **A**
	6	229.9±40.4 ^{ab} **A**	389.5±6.1 ^{aA}	41.3±5.9 ^{bC}	100.1±21.3 ^{bB} **A**	353.0±6.9 ^{aA}	54.7±10.1 ^{bC}	103.9±7.1 ^{bB} **A**	341.2±25.^{abA}	68.3±15.5 ^{bB}	83.0±15.0 ^{bB} **A**	367.1±18.8 ^{aA}	78.2±8.4 ^{bB}	55.7±15.2 ^{bB} **A**
	8	277.3±6.8 ^{a} **A**	375.2±6.3 ^{aA}	68.9±4.0 ^{bC}	68.9±12.7 ^{bB} **A**	334.2±25.3 ^{aA}	55.5±5.0 ^{bC}	101.2±23.7 ^{bB} **A**	325.7±23.^{abA}	68.2±5.8 ^{bB}	78.4±19.3 ^{bB} **A**	312.3±6.7 ^{aA}	74.2±9.1 ^{bB}	61.9±12.4 ^{bB} **A**
Flavonoids (mg of Quercitin .100g^{-1} FW)	0	3.13±3.13 ^{c} **A**	1.23±1.23 ^{bB}	7.35±0.06 ^{aA}	2.90±0.06 ^{aB} **B**	0.86±0.86 ^{cC}	5.38±0.06 ^{aA}	2.67±0.06 ^{aB} **B**	2.55±2.55^{bC}	10.20±0.06 ^{aA}	2.42±0.06 ^{aB} **B**	2.61±2.61 ^{bB}	10.18±0.06 ^{aA}	2.90±0.06 ^{aB} **B**
	2	11.42±1.79 ^{c} **A**	9.29±2.89 ^{aA}	1.09±0.08 ^{bB}	0.55±0.11 ^{bB} **B**	4.88±1.46 ^{bcA}	1.26±0.00 ^{bB}	0.98±0.15 ^{bB} **B**	10.46±2.2^{aA}	3.52±0.46 ^{cB}	0.81±0.01 ^{bA} **B**	8.32±4.17 ^{abA}	3.90±0.19 ^{bA}	0.78±0.07 ^{bcA} **B**
	4	32.97±3.31 ^{a} **A**	6.77±0.29 ^{aA}	1.76±0.15 ^{bB}	0.88±0.18 ^{bB} **B**	6.93±2.08 ^{abA}	2.29±0.87 ^{bB}	0.95±0.08 ^{bB} **B**	7.00±0.69^{abA}	2.90±0.04 ^{cdB}	1.50±0.36 ^{bB} **B**	8.89±0.74 ^{abA}	2.06±0.69 ^{cB}	0.64±0.03 ^{cB} **B**
	6	33.12±2.36 ^{a} **A**	9.49±0.41 ^{aA}	2.09±0.14 ^{bB}	0.54±0.06 ^{bB} **B**	10.73±2.48 ^{aA}	1.57±0.17 ^{bB}	0.78±0.23 ^{bB} **B**	7.92±1.33^{abA}	4.74±0.19 ^{bB}	1.10±0.27 ^{bC} **B**	11.96±1.93 ^{aA}	4.76±0.44 ^{bB}	1.03±0.11 ^{bB} **B**
	8	23.52±1.22 ^{b} **A**	5.48±0.82 ^{abA}	1.71±0.25 ^{bB}	0.74±0.09 ^{bB} **B**	5.92±1.05 ^{abcA}	1.87±0.36 ^{bB}	0.98±0.26 ^{bB} **B**	8.61±0.63^{aA}	2.59±0.29 ^{dB}	0.90±0.26 ^{bC} **B**	9.52±1.22 ^{abA}	3.26±0.76 ^{bcB}	0.72±0.13 ^{cB} **B**
ORAC (µM TE.100g-^{1} FW)	0	19.52±19.52 ^{b} **A**	19.99±19.99 ^{bB}	65.13±0.40 ^{bA}	64.22±0.40 ^{cB} **A**	22.10±22.10 ^{bC}	70.02±0.40 ^{bA}	66.10±0.40 ^{cB} **A**	18.82±18.^{aB}	36.00±0.40 ^{cAB}	64.48±0.40 ^{cA} **A**	21.68±21.68 ^{bA}	60.08±0.40 ^{cA}	62.20±0.40 ^{bA} **A**
	2	68.34±5.12 ^{a} **A**	59.71±0.47 ^{aB}	75.49±3.65 ^{bB}	81.24±0.06 ^{bA} **A**	63.17±2.53 ^{aC}	71.83±0.03 ^{bB}	80.32±0.44 ^{bA} **A**	64.38±1.0^{bB}	74.92±3.00 ^{bAB}	81.52±0.25 ^{aA} **A**	65.42±0.86 ^{aB}	81.52±0.39 ^{aA}	81.65±0.49 ^{aA} **A**
	4	66.03±0.90 ^{ab} **B**	61.96±1.20 ^{aB}	81.41±0.37 ^{aA}	80.96±0.90 ^{abA} **A**	65.03±2.27 ^{aB}	80.92±0.38 ^{aA}	81.28±0.32 ^{abA} **A**	66.45±3.3^{bB}	81.99±0.48 ^{aA}	80.15±0.60 ^{bA} **A**	61.90±0.23 ^{aB}	81.90±0.36 ^{aA}	81.43±0.06 ^{aA} **A**
	6	69.41±1.53 ^{a} **A**	63.38±2.41 ^{aB}	80.57±0.69 ^{aA}	81.93±0.29 ^{aA} **A**	61.96±2.72 ^{aB}	81.51±0.81 ^{aA}	81.80±0.24 ^{aA} **A**	61.47±1.3^{bB}	81.28±0.61 ^{abA}	81.93±0.20 ^{aA} **A**	62.32±1.63 ^{aB}	81.17±1.25 ^{aA}	81.13±0.74 ^{aA} **A**
	8	64.35±1.57 ^{ab} **B**	64.45±1.59 ^{aB}	80.92±0.63 ^{aA}	81.83±0.65 ^{aA} **A**	61.74±1.08 ^{aB}	77.83±3.10 ^{aA}	81.86±0.17 ^{aA} **A**	63.04±1.8^{bB}	77.97±3.34 ^{abA}	81.54±0.37 ^{aA} **A**	59.25±0.17 ^{aB}	75.84±3.29 ^{bA}	80.64±1.09 ^{aA} **AB**
TEAC (µM TE.100g^{-1} FW)	0	35.42±17.98 ^{a} **B**	43.32±21.85 ^{aB}	243.25±0.03 ^{aB}	292.83±0.03 ^{aA} **A**	40.72±20.58 ^{aC}	170.59±0.03 ^{aB}	370.19±0.03 ^{aA} **A**	37.79±18.^{aC}	238.14±0.03 ^{aB}	248.57±0.03 ^{aA} **AB**	35.79±19.06 ^{aC}	383.81±0.03 ^{aB}	442.40±0.02 ^{aA} **A**
	2	40.12±4.99 ^{b} **A**	43.08±2.52 ^{aA}	28.11±4.18 ^{bcB}	20.08±4.00 ^{cC} **B**	40.38±2.65 ^{aA}	31.39±3.13 ^{bcB}	14.76±0.53 ^{cC} **B**	43.55±2.7^{aB}	20.97±1.24 ^{cA}	17.25±1.55 ^{cA} **B**	38.19±4.17 ^{aA}	31.23±4.59 ^{bA}	15.40±2.82 ^{bB} **B**
	4	29.73±1.88 ^{c} **A**	18.58±6.81 ^{aB}	26.38±1.77 ^{bcA}	14.38±1.63 ^{bcB} **A**	30.60±12.97 ^{aA}	41.03±14.47 ^{bcA}	20.24±0.79 ^{bcB} **A**	42.64±0.9^{aA}	22.62±4.80 ^{cB}	22.82±2.13 ^{bB} **A**	43.58±1.88 ^{aA}	28.31±3.99 ^{bB}	17.88±1.48 ^{bC} **A**
	6	23.80±0.27 ^{c} **B**	36.77±1.58 ^{aA}	34.62±11.86 ^{cB}	21.70±2.06 ^{cB} **B**	39.91±4.03 ^{aA}	16.31±0.54 ^{cB}	15.92±3.38 ^{cB} **B**	44.66±1.1^{aA}	59.15±20.54 ^{bA}	19.65±1.42 ^{bcB} **A**	47.06±3.74 ^{aA}	47.89±11.47 ^{bA}	15.36±3.61 ^{bB} **AB**
	8	28.98±0.82 ^{c} **AB**	48.33±1.27 ^{aA}	38.86±20.00 ^{bA}	14.12±0.85 ^{bB} **A**	50.97±3.48 ^{aA}	64.37±23.36 ^{bA}	22.72±1.39 ^{bB} **A**	10.18±12.^{bA}	21.40±2.07 ^{cA}	21.41±0.57 ^{bcA} **B**	43.49±2.06 ^{aA}	46.22±24.06 ^{bA}	21.04±2.09 ^{bA} **AB**
Ethylene (µL.kg.h^{-1})	0	36.36±3.97 ^{a} **A**	23.56±2.46 ^{aB}	15.69±1.98 ^{aB}	49.15±2.47 ^{aA} **A**	23.72±1.38 ^{aB}	25.38±0.58 ^{aB}	53.91±3.39 ^{aA} **A**	23.50±3.1^{aB}	26.89±0.96 ^{aB}	47.97±5.55 ^{aA} **AB**	21.38±2.29 ^{aA}	22.81±2.19 ^{aA}	22.01±5.20 ^{aA} **B**
	2	20.71±3.46 ^{b} **A**	9.51±2.17 ^{bA}	10.17±0.72 ^{bA}	8.06±3.80 ^{bA} **B**	5.35±0.93 ^{bB}	9.44±3.89 ^{bA}	8.86±1.00 ^{bA} **B**	5.13±0.79^{bB}	15.30±3.32 ^{bA}	6.33±3.27 ^{bB} **B**	7.17±2.17 ^{bA}	10.72±1.57 ^{bA}	6.47±1.71 ^{bA} **B**
	4	7.45±1.86 ^{b} **A**	4.01±0.66 ^{cB}	12.03±1.21 ^{bcA}	5.33±0.19 ^{bcA} **B**	2.49±0.45 ^{cB}	7.05±2.45 ^{bcA}	6.10±0.80 ^{bcA} **B**	3.29±0.89^{bB}	11.17±0.43 ^{bA}	5.46±1.41 ^{bB} **B**	2.77±0.69 ^{bcB}	11.71±1.06 ^{bA}	4.77±0.81 ^{bB} **B**
	6	7.17±0.62 ^{c} **A**	2.06±0.53 ^{cA}	3.92±0.30 ^{bcA}	4.14±1.29 ^{cA} **B**	2.14±0.43 ^{cA}	3.00±0.31 ^{bcA}	2.60±0.26 ^{cA} **B**	1.65±0.19^{bB}	5.88±0.61 ^{cA}	2.45±0.37 ^{bB} **B**	1.85±0.36 ^{cB}	5.43±1.00 ^{cA}	1.63±0.32 ^{bB} **B**
	8	5.66±1.35 ^{c} **A**	0.77±0.18 ^{cA}	1.82±0.11 ^{cA}	2.64±0.91 ^{cA} **B**	1.49±0.31 ^{cA}	1.60±0.41 ^{cA}	1.40±0.18 ^{cA} **B**	0.88±0.12^{bB}	3.01±0.33 ^{cA}	1.15±0.29 ^{bB} **B**	1.24±0.33 ^{cB}	2.41±0.34 ^{cA}	1.15±0.30 ^{bB} **B**

Values in the same column followed by different lower case, in the same row followed by different upper case for each anti-browning within coats and bold upper case for each edible coating, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Table 3 Sugars of fresh-cut 'Bravo de Esmolfe' apples covered with different alginate and pectin based edible coating formulations during storage at 4ºC. Values represent the mean of three replicates taken at 0, 2, 4, 6 and 8 days.

Quality Parameters	Days	Control			Alginate 2% + Eugenol 0.1%						Alginate 2% + Citral 0.15% + Eugenol .0.1%						Pectin 2% + Eugenol 0.2%					
		No Treated			Ascorbic Acid		Citric Acid		Sodium Chlorite		Ascorbic Acid		Citric Acid		Sodium Chlorite		Ascorbic Acid		Citric Acid		Sodium Chlorite	
Fructose (mg.g^{-1} DW)	0	9.8±0.3	c	**B**	8.3±0.5	bC	14.5±0.2	bB	9.8±0.3	bA **AB**	10.4±0.8	bB	10.2±0.1	bB	13.8±0.0	bA **AB**	9.3±0.4	cB	10.7±0.3	aB	15.8±0.6	aA
	2	9.9±0.6	c	**C**	10.6±0.0	aAB	12.3±0.9	aA	9.9±0.6	aA **A**	10.3±0.1	bB	15.6±2.3	aA	15.2±0.2	aA **A**	9.4±0.6	cB	11.4±1.5	aB	13.2±0.6	bA
	4	10.8±0.3	c	**B**	10.6±0.3	aB	12.7±0.2	bB	10.8±0.3	bA **A**	13.0±0.4	aA	11.9±0.2	bB	13.7±0.4	bA **A**	13.4±0.2	aA	12.3±0.2	aA	12.9±0.1	bA
	6	14.3±0.3	a	**A**	9.6±0.2	aB	11.9±0.2	bA	14.3±0.3	cA **B**	11.0±0.6	abA	12.2±0.4	bA	12.7±0.1	cA **B**	9.6±0.8	cB	11.5±0.6	aB	13.6±0.2	bA
	8	12.9±0.5	b	**A**	10.5±0.5	aB	10.8±0.1	bA	12.9±0.5	bA **AB**	12.1±1.2	abA	11.4±0.2	bA	13.7±0.3	bA **AB**	11.1±0.2	bcB	10.5±0.2	aB	13.2±0.1	bA
Glucose (mg.g^{-1} DW)	0	8.19±0.71	ab	**A**	7.21±0.49	bA	8.21±0.12	aA	6.95±0.35	bA **A**	9.07±0.59	aA	7.38±0.13	aA	6.95±0.35	bA **A**	7.59±0.34	aA	5.43±0.21	aB	8.26±0.48	aA
	2	8.25±0.56	a	**A**	8.98±0.10	aA	8.25±0.23	aA	5.91±1.44	bA **A**	8.36±0.01	aA	7.35±1.63	aA	5.91±1.44	bA **A**	7.61±0.41	aA	6.14±1.37	aB	7.37±2.22	aA
	4	7.66±0.22	ab	**AB**	7.46±0.22	bC	8.40±0.13	abB	9.51±0.07	aA **AB**	7.76±0.35	abB	7.21±0.21	abB	9.51±0.07	aA **AB**	8.08±0.05	aA	5.99±0.30	aB	8.37±0.10	aA
	6	8.00±0.19	ab	**A**	4.81±0.13	cC	8.02±0.41	bcB	7.14±0.08	bA **B**	5.84±0.47	cB	5.07±0.04	bcB	7.14±0.08	bA **B**	5.13±0.45	bB	4.79±0.32	aB	8.06±0.19	aA
	8	6.71±0.32	b	**A**	5.61±0.34	cC	7.04±0.41	bB	6.30±0.26	bA **A**	6.37±0.80	bcA	4.16±0.01	bB	6.30±0.26	bA **A**	5.61±0.08	bB	4.23±0.00	aB	7.15±0.34	aA
Sucrose (mg.g^{-1} DW)	0	4.89±0.50	b	**A**	4.19±0.23	aB	5.83±0.22	aA	5.27±0.01	bA **A**	4.65±0.28	aA	3.95±0.04	aA	5.50±0.31	bA **A**	4.44±0.07	bB	3.81±0.09	abB	6.01±0.28	aA
	2	3.57±0.16	c	**B**	3.82±0.13	aB	5.11±0.82	aA	5.42±0.12	bA **AB**	3.93±0.02	bA	4.73±1.21	aA	5.01±0.39	bA **AB**	3.29±0.23	cB	4.63±0.47	aA	5.65±0.47	aA
	4	3.36±0.10	c	**B**	3.15±0.08	bB	5.27±0.38	aB	5.81±0.13	aA **A**	4.93±0.16	aB	4.47±0.02	aB	6.68±0.34	aA **A**	5.68±0.09	aA	4.58±0.24	aB	5.86±0.44	aA
	6	5.98±0.03	a	**A**	3.84±0.03	aB	3.90±0.12	aB	5.27±0.03	bA **B**	4.57±0.06	aB	3.97±0.03	aB	5.22±0.01	bA **B**	4.49±0.23	bB	3.85±0.08	abC	5.34±0.06	aA
	8	5.59±0.15	ab	**A**	4.19±0.25	aA	3.66±0.05	aB	4.96±0.00	bA **B**	4.63±0.15	aA	3.83±0.06	aB	4.98±0.09	bA **B**	4.65±0.07	bA	3.59±0.03	bB	4.93±0.12	aA
Sweet Index	0	37.27±2.06	b	**A**	31.96±1.85	bC	49.44±0.86	cC	36.67±1.04	bA **A**	39.20±2.76	abB	36.25±0.45	cC	46.17±0.78	bA **A**	35.03±1.43	bB	35.17±0.96	abB	52.76±2.19	aA
	2	35.95±2.25	b	**B**	38.49±0.34	aB	43.37±3.42	aA	36.10±3.08	bA **A**	37.42±0.38	bB	49.57±8.57	aA	47.68±2.31	bA **A**	33.62±2.02	bB	38.59±5.49	aAB	45.24±4.24	bA
	4	37.09±1.10	b	**B**	36.13±1.13	abB	44.65±1.19	bB	42.25±0.99	aA **A**	44.38±1.51	aAB	40.63±0.74	bB	50.07±1.34	aA **A**	46.58±0.56	aA	40.53±1.19	aB	46.04±0.81	bA
	6	49.00±0.85	a	**A**	32.02±0.62	bC	40.66±1.01	bcB	47.20±0.75	cA **B**	37.33±1.90	bB	38.48±1.05	bcB	43.41±0.42	cA **B**	33.26±2.52	bC	36.50±1.78	abB	46.45±0.76	bA
	8	43.86±1.73	ab	**A**	35.41±1.83	abB	36.74±0.67	cB	42.60±1.47	cA **A**	40.35±3.75	abAB	35.48±0.58	cB	44.54±1.10	cA **A**	37.44±0.74	abC	33.29±0.47	bB	44.18±0.65	bA

Values in the same column followed by different lower case, in the same row followed by different upper case for each anti-browning within coats and bold upper case for each edible coating, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.6. Sensory evaluation

Results of the sensory evaluation of fresh-cut apples non-coated and coated are plotted in Table 4. In our work, after 6 days of storage the taste panel showed that the edible coating treatments had a good sensory appreciation (>4 in a scale of 1-bad to 7-excellent), while control was not suitable for consumption according to panel scores (Table 4). It is worth to notice that when using ascorbic acid as anti-browning agent, appearance was lower than when using ascorbic acid or sodium chlorite. The formulation that scored better as overall was Al 2% + Eug 0.2% with ascorbic acid.

According to Azarakhsh et al. (2014), the incorporation of concentrations up to 0.3% (w/v) of lemongrass into alginate-based coating formulation did not have effect on sensory attributes of coated fresh-cut pineapple. However, incorporation of 0.5% (w/v) lemongrass affected the sensory attributes of coated samples. Rojas-Graü et al., (2007) reported that vanillin incorporated into alginate edible coatings up to 0.3% were the most effective in terms of sensory quality after 2 weeks fresh-cut apples storage as compared to lemongrass and oregano oils additives, being this last the one with lower scores. Perdones, Sánchez-gonzález, Chiralt, & Vargas (2012) using chitosan–lemon essential oil coatings in strawberries report the overall differences considering the non-coated samples were not significant. In our study the coatings or essential oils were at low concentrations, being beneficial for general sensorial quality parameters.

Table 4 Sensory evaluation of fresh-cut 'Bravo de Esmolfe' apples covered with different alginate and pectin based edible coating formulations during storage at 4ºC. Values represent the mean of 15 replicates taken at 0, 3 and 6 days.

Quality Parameters	Days	Control	Alginate 2% + Eugenol .0.1%			Alginate 2% + Citral 0.15% + Eugenol 0.1%			Pectin 2% + Eugenol 0.2%			Pectin 2% + Citral 0.15%		
		No Treated	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite	Ascorbic Acid	Citric Acid	Sodium Chlorite
Appereance	0	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
	6	2.0	5.6	3.2	5.0	4.8	3.2	4.8	4.4	3.3	5.0	4.3	3.3	4.8
Aroma	0	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7
	6	3.8	5.1	4.2	5.3	5.3	5.2	4.7	4.0	3.8	5.0	3.9	4.3	4.3
Texture	0	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
	6	2.1	4.5	4.7	5.5	3.3	5.0	5.0	3.9	3.5	5.7	4.9	5.0	5.3
Sweetness	0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
	6	4.0	5.3	3.7	5.8	3.2	5.3	5.2	3.8	4.2	5.7	4.2	5.3	4.7
Acidity	0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
	6	4.6	5.3	3.8	5.5	4.0	5.0	4.5	4.0	3.8	5.2	3.3	5.2	4.0
Flavor	0	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
	6	2.3	5.2	3.7	5.5	4.1	5.3	4.7	4.9	3.8	5.5	3.4	5.3	3.8
Overall Aceptance	0	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
	6	3.1	5.2	3.9	5.4	4.1	4.8	4.8	4.1	3.8	5.3	4.0	4.8	4.5

4. Conclusions

Outcome from this study indicate the possibility of using edible coatings to develop ready-to-eat fresh-cut 'Bravo de Esmolfe' apples market. The solution for success is using an appropriate coating material and anti-browning agent. The use of the edible coatings of this experiment can be considered as safe and effective treatment. The edible coating that better performed on reducing wounding stress and best maintained most quality attributes of the commodity was Al 2% + Eug 0.1% using ascorbic acid as anti-browning agent.

5. Acknowledgements

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010 - INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

6. References

Abreu, M.; Beirao-da-Costa, S. Use of Mild Heat Pre-Treatments for Quality Retention of Fresh-Cut "Rocha"pear. *Postharvest Biol. Technol.* **2003**, *30*, 153–160.

Ali, A.; Maqbool, M.; Alderson, P. G.; Zahid, N. Effect of Gum Arabic as an Edible Coating on Antioxidant Capacity of Tomato (Solanum Lycopersicum L.) Fruit during Storage. *Postharvest Biol. Technol.* **2013**, *76*, 119–124.

Antunes, M. D. C.; Sfakiotakis, E. M. Effect of High Temperature Stress on Ethylene Biosynthesis , Respiration and Ripening of " Hayward " Kiwifruit. *Postharvest Biol. Technol.* **2000**, *20*, 251–259.

Antunes, M. D. C.; Dandlen, S.; Cavaco, A. M.; Miguel, G. Effects of Postharvest Application of 1-MCP and Postcutting Dip Treatment on the Quality and Nutritional Properties of Fresh-Cut Kiwifruit. *J. Agric. Food Chem.* **2010**, *58*, 6173–6181.

Antunes, M. D. C.; Rodrigues, D.; Pantazis, V.; Cavaco, A. M.; Siomos, A. S.; Miguel, G. Nutritional Quality Changes of Fresh-Cut Tomato during Shelf Life. *Food Sci. Biotechnol.* **2013**, *22*, 1–8.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Ayranci, E.; Tunc, S. A Method for the Measurement of the Oxygen Permeability and the Development of Edible Films to Reduce the Rate of Oxidative Reactions in Fresh Foods. *Food Chem.* **2003**, *80*, 423–431.

Azarakhsh, N.; Osman, A.; Ghazali, H. M.; Tan, C. P.; Mohd Adzahan, N. Lemongrass Essential Oil Incorporated into Alginate-Based Edible Coating for Shelf-Life Extension and Quality Retention of Fresh-Cut Pineapple. *Postharvest Biol. Technol.* **2014**, *88*, 1–7.

Cavaco, A. M.; Pinto, P.; Antunes, M. D.; Silva, J. M. Da; Guerra, R. "Rocha" Pear Firmness Predicted by a Vis/NIR Segmented Model. *Postharvest Biol. Technol.* **2009**, *51*, 311–319.

Chiumarelli, M.; Hubinger, M. D. Stability, Solubility, Mechanical and Barrier Properties of Cassava Starch – Carnauba Wax Edible Coatings to Preserve Fresh-Cut Apples. *Food Hydrocoll.* **2012**, *28*, 59–67.

Contreras-Oliva, A.; Rojas-Argudo, C.; Pérez-Gago, M. B. Effect of Solid Content and Composition of Hydroxypropyl Methylcellulose-Lipid Edible Coatings on Physico-

Chemical and Nutritional Quality of "Oronules" Mandarins. *J. Sci. Food Agric.* **2012**, *92*, 794–802.

Díaz-Mula, H. M.; Serrano, M.; Valero, D. Alginate Coatings Preserve Fruit Quality and Bioactive Compounds during Storage of Sweet Cherry Fruit. *Food Bioprocess Technol.* **2012**, *5*, 2990–2997.

Duan, J.; Wu, R.; Strik, B. C.; Zhao, Y. Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions. *Postharvest Biol. Technol.* **2011**, *59*, 71–79.

Espitia, P. J. P.; Du, W.-X.; Avena-Bustillos, R. D. J.; Soares, N. D. F. F.; McHugh, T. H. Edible Films from Pectin: Physical-Mechanical and Antimicrobial Properties - A Review. *Food Hydrocoll.* **2014**, *35*, 287–296.

Garcia-Viguera, C.; Zafrilla, P.; Artes, F.; Romero, F.; Abellan, P.; Tomas-Barberan, F. A. Colour and Anthocyanin Stability of Red Raspberry Jam. *J. Sci. Food Agric. 784565573 1998 Dec* **1998**, *78*, 565–573.

Gol, N. B.; Patel, P. R.; Rao, T. V. R. Improvement of Quality and Shelf-Life of Strawberries with Edible Coatings Enriched with Chitosan. *Postharvest Biol. Technol.* **2013**, *85*, 185–195.

Gonzalez-Aguilar, G. a.; Celis, J.; Sotelo-Mundo, R. R.; de la Rosa, L. a.; Rodrigo-Garcia, J.; Alvarez-Parrilla, E. Physiological and Biochemical Changes of Different Fresh-Cut Mango Cultivars Stored at 5 °C. *Int. J. Food Sci. Technol.* **2008**, *43*, 91–101.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. Edible Coatings Enriched with Essential Oils for Extending the Shelf-Life of 'Bravo de Esmolfe. *Int. J. Food Sci. Technol.* **2015a**, *in press*.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Effect of Alginate-Based Edible Coatings Enriched with Essential Oils Constituents on Arbutus Unedo L. Fresh Fruit Storage. *Postharvest Biol. Technol.* **2015b**, *100*, 226–233.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Use of Polysaccharide-Based Edible Coatings Enriched with Essential Oils to Improve Shelf-Life of Strawberries. *Postharvest Biol. Technol.* **2015c**, *110*, 51–60.

Hassimotto, N. M. A.; Pinto, M. D. S.; Lajolo, F. M. Antioxidant Status in Humans after Consumption of Blackberry (Rubus Fruticosus L.) Juices with and without Defatted Milk. *J. Agric. Food Chem.* **2008**, *56*, 11727–11733.

Hussain, P. R.; Meena, R. S.; Dar, M. a.; Wani, a. M. Effect of Post-Harvest Calcium Chloride Dip Treatment and Gamma Irradiation on Storage Quality and Shelf-Life Extension of Red Delicious Apple. *J. Food Sci. Technol.* **2012**, *49*, 415–426.

Jha, S. N.; Rai, D. R.; Shrama, R. Physico-Chemical Quality Parameters and Overall Quality Index of Apple during Storage. *J. Food Sci. Technol.* **2012**, *49*, 594–600.

Jiang, T. Effect of Alginate Coating on Physicochemical and Sensory Qualities of Button Mushrooms (Agaricus Bisporus) under a High Oxygen Modified Atmosphere. *Postharvest Biol. Technol.* **2013**, *76*, 91–97.

Jo, W. S.; Song, H. Y.; Song, N. B.; Lee, J. H.; Min, S. C.; Song, K. B. Quality and Microbial Safety of "Fuji" Apples Coated with Carnauba-Shellac Wax Containing Lemongrass Oil. *LWT - Food Sci. Technol.* **2014**, *55*, 490–497.

Keutgen, A.; Pawelzik, E. Modifications of Taste-Relevant Compounds in Strawberry Fruit under NaCl Salinity. *Food Chem.* **2007**, *105*, 1487–1494.

Magwaza, L. S.; Opara, U. L.; Terry, L. a.; Landahl, S.; Cronje, P. J.; Nieuwoudt, H.; Mouazen, A. M.; Saeys, W.; Nicolaï, B. M. Prediction of "Nules Clementine" Mandarin Susceptibility to Rind Breakdown Disorder Using Vis/NIR Spectroscopy. *Postharvest Biol. Technol.* **2012**, *74*, 1–10.

McGuire, R. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255.

Miguel, M. G.; Nunes, S.; Dandlen, S. A.; Cavaco, A. M.; Antunes, M. D. Phenols and Antioxidant Activity of Hydro-Alcoholic Extracts of Propolis from Algarve, South of Portugal. *Food Chem. Toxicol.* **2010**, *48*, 3418–3423.

Moldão-Martins, M.; Beirão da Costa, S. M.; Beirão da Costa, M. L. The Effects of Edible Coatings on Postharvest Quality of the "Bravo de Esmolfe" Apple. *Eur. Food Res. Technol.* **2003**, *217*, 325–328.

Olivas, G. I.; Mattinson, D. S.; Barbosa-Cánovas, G. V. Alginate Coatings for Preservation of Minimally Processed "Gala" Apples. *Postharvest Biol. Technol.* **2007**, *45*, 89–96.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon. *LWT - Food Sci. Technol.* **2008**, *41*, 1862–1870.

Oms-Oliu, G.; Rojas-Graü, M. A.; González, L. A.; Varela, P.; Soliva-Fortuny, R.; Hernando, M. I. H.; Munuera, I. P.; Fiszman, S.; Martín-Belloso, O. Recent Approaches Using Chemical Treatments to Preserve Quality of Fresh-Cut Fruit: A Review. *Postharvest Biol. Technol.* **2010**, *57*, 139–148.

Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.

Palma, A.; Schirra, M.; Aquino, S. D. Effect of Edible Coating on Ascorbic Acid , Betalains , Organic Acids , Sugar , Polyphenol Content and Antioxidant Activity in Minimally Processed Cactus Pear (Opuntia Ficus-Indica). *Acta Hortic.* **2015**, *1067*, 127–134.

Perdones, A.; Sánchez-gonzález, L.; Chiralt, A.; Vargas, M. Effect of Chitosan – Lemon Essential Oil Coatings on Storage-Keeping Quality of Strawberry. *Postharvest Biol. Technol.* **2012**, *70*, 32–41.

Perez-Gago, M. B.; Serra, M.; Alonso, M.; Mateos, M.; Del Río, M. A. Effect of Whey Protein- and Hydroxypropyl Methylcellulose-Based Edible Composite Coatings on Color Change of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2005**, *36*, 77–85.

Prior, R. L.; Cao, G. In Vivo Total Antioxidant Capacity: Comparison of Different Analytical Methods. *Free Radic. Biol. Med.* **1999**, *27*, 1173–1181.

Qian, P. I. M. Objective Flavor Comparison of Oregon Strawberries and Those from Other Climatic Condition. **2005**.

Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Martín-Belloso, O. Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon. *Int. J. Food Microbiol.* **2008**, *121*, 313–327.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Raybaudi-Massilia, R. M.; Soliva-Fortuny, R. C.; Avena-Bustillos, R. J.; McHugh, T. H.; Martín-Belloso, O. Apple Puree-Alginate Edible Coating as Carrier of Antimicrobial Agents to Prolong Shelf-Life of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2007a**, *45*, 254–264.

Rojas-Graü, M. a.; Avena-Bustillos, R. J.; Olsen, C.; Friedman, M.; Henika, P. R.; Martín-Belloso, O.; Pan, Z.; McHugh, T. H. Effects of Plant Essential Oils and Oil Compounds on Mechanical, Barrier and Antimicrobial Properties of Alginate-Apple Puree Edible Films. *J. Food Eng.* **2007b**, *81*, 634–641.

Salvia-trujillo, L.; Rojas-graü, M. A.; Soliva-fortuny, R.; Martín-belloso, O. Use of Antimicrobial Nanoemulsions as Edible Coatings : Impact on Safety and Quality Attributes of Fresh-Cut Fuji Apples. *Postharvest Biol. Technol.* **2015**, *105*, 8–16.

Sandhu, A. K.; Gu, L. Antioxidant Capacity, Phenolic Content, and Profiling of Phenolic Compounds in the Seeds, Skin, and Pulp of Vitis Rotundifolia (Muscadine Grapes) As Determined by HPLC-DAD-ESI-MS(n). *J. Agric. Food Chem.* **2010**, *58*, 4681–4692.

Serrano, M.; Martínez-Romero, D.; Guillén, F.; Valverde, J. M.; Zapata, P. J.; Castillo, S.; Valero, D. The Addition of Essential Oils to MAP as a Tool to Maintain the Overall Quality of Fruits. *Trends Food Sci. Technol.* **2008**, *19*, 464–471.

Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

Soliva-Fortuny, R. C.; Martín-Belloso, O. New Advances in Extending the Shelf-Life of Fresh-Cut Fruits: A Review. *Trends Food Sci. Technol.* **2003**, *14*, 341–353.

Stannard, C. Development and Use of Microbiological Criteria. *Food Sci. Technol.* **1997**, *11*, 137–177.

Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of Water Deficit Irrigation and Inoculation with Botrytis Cinerea on Strawberry (Fragaria X Ananassa) Fruit Quality. *J. Agric. Food Chem.* **2007**, *55*, 10812–10819.

Valero, D.; Díaz-Mula, H. M.; Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Serrano, M. Effects of Alginate Edible Coating on Preserving Fruit Quality in Four Plum Cultivars during Postharvest Storage. *Postharvest Biol. Technol.* **2013**, *77*, 1–6.

Vargas, M.; Pastor, C.; Chiralt, A.; McClements, D. J.; González-Martínez, C. Recent Advances in Edible Coatings for Fresh and Minimally Processed Fruits. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 496–511.

Wang, S. Y.; Gao, H. Effect of Chitosan-Based Edible Coating on Antioxidants, Antioxidant Enzyme System, and Postharvest Fruit Quality of Strawberries (Fragaria X Aranassa Duch.). *LWT - Food Sci. Technol.* **2012**, *52*, 1–9.

Zambrano-Zaragoza, M. L.; Mercado-Silva, E.; Del Real L., A.; Gutiérrez-Cortez, E.; Cornejo-Villegas, M. A.; Quintanar-Guerrero, D. The Effect of Nano-Coatings with A-Tocopherol and Xanthan Gum on Shelf-Life and Browning Index of Fresh-Cut "Red Delicious" Apples. *Innov. Food Sci. Emerg. Technol.* **2014**, *22*, 188–196.

Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D.; Serrano, M. Use of Alginate or Zein as Edible Coatings to Delay Postharvest Ripening Process and to Maintain Tomato (Solanum Lycopersicon Mill) Quality. *J. Food Agric.* **2008**, *88*, 1287–1293.

Chapter VII

The use of polysaccharides-based edible coatings enriched with essential oils to improved shelf-life of strawberries

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria L Faleiro[2] Maria G C Miguel[1], Maria D C Antunes[1]

[1]Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Postharvest Biology and Technology 110 (2015) 51-60

Postharvest Biology and Technology 110 (2015) 51–60

Contents lists available at ScienceDirect

Postharvest Biology and Technology

The use of polysaccharide-based edible coatings enriched with essential oils to improve shelf-life of strawberries

Adriana C. Guerreiro[a], Custódia M.L. Gago[a], Maria L. Faleiro[b], Maria G.C. Miguel[a], Maria D.C. Antunes[a,*]

[a] Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal
[b] Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 17 June 2015
Accepted 27 June 2015
Available online xxx

Keywords:
Alginate
Pectin
Citral
Eugenol
Quality

ABSTRACT

Edible coating formulations have been developed to increase shelf-life of some horticultural products. The objective of this research was to study the effect of edible coatings based on sodium alginate (AL) and pectin (PE) enriched with essential oils constituents (citral and eugenol) on the shelf-life extension of strawberries. AL and PE were tested at 1 and 2% (w/v) and were enriched with eugenol (Eug) at 0.1 and 0.2% and citral (Cit) at 0.15 and 0.3%. Strawberries were dipped in those solutions for 2 min, then stored at 0.5 °C. Measurements of color CIE (L*, a*, b*, h°, C*), firmness, soluble solids content (SSC), weight loss, trolox equivalent antioxidant capacity (TEAC), microbial growth and taste panels were accomplished at 0, 7 and 14 d storage. With those quality characteristics, hierarchical cluster analysis formed 3 groups either for AL or PE based edible coatings. Taking into account the mean closest values to the one at harvest for color, higher value for firmness, SSC and antioxidant activity, and lower value for weight loss and microbial spoilage, the best group was selected. From the selected groups, the 2 edible coating formulations which had higher score on taste panels were considered the best for preserving quality through shelf-life of strawberries. Those edible coatings were for AL the AL 2% + Eug 0.1%; AL 2% + Cit 0.15% + Eug 0.1% and for PE the PE 2% + Eug 0.1%; PE 2% + Cit 0.15%.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.), a very appreciated fruit worldwide, is highly perishable with a short postharvest life mainly due to their high metabolism and microbial decay (Gol et al., 2013). The shelf-life of fresh strawberries at temperatures from 0 to 4 °C is usually around 5 d (Vargas et al., 2006). Many preservation techniques including refrigeration, modified or controlled atmosphere and heat treatments have been applied to strawberries to increase their shelf-life (Harker et al., 2000; Velickova et al., 2013).

The use of edible coatings enriched with antimicrobial or antioxidants has proved to be efficient in preserving the quality during storage of many fruit (Antunes et al., 2012; Campos et al., 2011; Guerreiro et al., 2015; Oms-Oliu et al., 2010; Zúñiga et al., 2012). Polysaccharide-based edible coatings, such as alginate (AL) and pectin (PE), are often used due to their capacity to form rigid and stable gels (Campos et al., 2011; Salmieri and Lacroix, 2006).

The essential oils, which are bioactive compounds have been used as food preservatives (Jo et al., 2014) and canbe added toedible coatings to increase their effect in preserving fruit quality and reducing microbial spoilage, thus increasing their storage life (Guerreiro et al., 2015; Salmieri and Lacroix, 2006; Vu et al., 2011). Eugenol and citral are examples of plant-derived essential oils which have been reported as good antimicrobial agents in edible films for extending the shelf-life of fresh-cut fruits (Hyldgaard et al., 2012; Raybaudi-Massilia et al., 2008a,b; Rojas-Graü et al., 2007a,b). Some edible coatings based on chitosan have been studied to improve strawberry fruit shelf-life (Vargas et al., 2006; Velickova et al., 2013; Vu et al., 2011). Vu et al. (2011) report the addition of essential oils to chitosan edible coatings and their effect on strawberry fruit decay. However, essential oils can change sensory or nutritional properties, thus reducing consumer's acceptability. Also, essential oils composition can change from year to year due to plant cultural practices, being the use of sole compounds a better approach to obtain an efficient edible coating (Guerreiro et al., 2015; Miguel, 2010).

The objective of this study was to determine the effect of citral (Cit) and eugenol (Eug), when incorporated in polysaccharide edible coatings based on AL and PE, on the shelf-life extension of strawberries.

* Corresponding author. Fax: +351 289818419.
E-mail address: mantunes@ualg.pt (M.D.C. Antunes).

http://dx.doi.org/10.1016/j.postharvbio.2015.06.019
0925-5214/

2. Material and methods

2.1. Material

Strawberries were purchased from a local market (Algarve, Portugal) at the harvest day, then immediately transported to the Postharvest laboratory at the University of Algarve. Then fruit were selected for uniformity of size and freedom of defects and treatments were applied within six hours after harvest in the laboratory environment set at 18 °C.

Food grade sodium alginate (AL), pectin (PE), calcium chloride, citral (Cit), eugenol (Eug) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid and 2,2⁰-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) were purchased from Sigma–Aldrich Chemic, Steinhein, Germany. Ascorbic acid was from Scharlau, Barcelona, Spain.

Plate count agar medium and dicloran rose-bengal cloranfeni- col agar were purchased from Biokar, Paris, France.

2.2. Methods

2.2.1. Edible Coatings

The edible coating formulations were done as described in Guerreiro et al. (2015). As for the previous authors, ascorbic acid 1% was added as anti-browning agent and calcium chloride 1% was used to induce cross linking reaction (Robles-Sánchez et al., 2009).

The concentrations of Cit and Eug were based on a previous determination of the minimum inhibitory concentration (MIC) for main food borne pathogens (Guerreiro et al., 2015).

The treatments were control (no addition of coating), AL or PE 1%, AL or PE 1% + Cit 0.15%, AL or PE 1% + Cit 0.3%, AL or PE 1% + Eug 0.1%, AL or PE 1% + Eug 0.2%, AL or PE 1% + Cit 0.15% + Eug 0.1%, AL or PE 2%, AL or PE 2% + Cit 0.15%, AL or PE 2% + Cit 0.3%, AL or PE 2% + Eug 0.1%, AL or PE 2% + Eug 0.2% and AL or PE 2% + Cit 0.15% + Eug 0.1%.

Strawberries were dipped into the edible coating solution for 2 min and allowed to drip off for 30 s. Then, they were dipped in a calcium chloride 1% (w/v) solution plus ascorbic acid 1% (w/v) for 1 min and dripped again. After that, 8 fruits per replication/treatment were placed in polypropylene plastic trays (8 cm × 10 cm × 4 cm), which were perforated in the cover, and stored at 0.5 °C until analyses. Sample analysis were performed just before treatments (day 0), and after 7 and 14 d storage. Three trays per treatment (replications) were used for each sampling time. Experiments were repeated twice.

2.2.2. General Quality Parameters analysis

A Minolta Chroma meter CR-300 (EC Minolta, Japan) was used to measure the color of the strawberries using the CIELab scale (L^*, a^* and b^*). The L^* represents color lightness (0 = black and 100 = white). Hue was calculated as h° = arctan (b^*/a^*) and color saturation (chroma) as $C^* = (a^{*2} + b^{*2})^{0.5}$ (McGuire, 1992). The firmness of strawberries was measured by puncture with a Chatillon TCD200 and a Digital Force Gauge DFIS 50 (Jonh Chatillon & Sons, Inc., USA) using a piston cylinder of 4 mm diameter at a depth of 7 mm. For the determination of the soluble solids content (%) was used a digital refractometer PR1 ATAGO CoLTD (Japan), in the fruit's juice. Fruit weight was measured at every sampling time in the same fruits and weight loss was expressed as the percentage of the initial weight.

2.2.3. Trolox Equivalent Antioxidant Activity (TEAC)

The preformed radical monocation of 2,2⁰-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was produced according to Re et al. (1999) with modifications Guerreiro et al., (2015). 10 mL of the juice was added to 990 mL of ABTS radical cation solution. The absorbance was spectrophotometrically monitored at 750 nm for 6 min in a Shimadzu spectrophotometer 160-UV, Tokyo, Japan. The antioxidant activity was considered using the following equation: scavenging effect% (SE%) = (1 — As/Ao) × 100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 min. The values were compared with the curve for several Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations in mM Trolox equivalent antioxidant capacity.

2.2.4. Microbial analysis

Microbial analysis included the counts of aerobic mesophilic and psicrophilic bacteria and molds and yeasts. The counts of aerobic mesophilic and psicrophilic were done according to the standard Portuguese NP-3788 (2002) using the Plate Count Agar medium (Biokar, Paris, France). The count of molds and yeasts was performed according to ISO 21527-2:2008 using Dicloran Rose-Bengal Cloranfenicol Agar (Biokar, Paris, France). The incubation temperature for yeasts and molds was 25 ⊤ 1 °C during 48–72 h, for aerobic mesophilic bacteria was 30 ⊤ 1 °C during 24–72 h and 6.5 ⊤ 1 °C during 5 to 10 d for psychrophilic bacteria. Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram fresh weight.

2.2.5. Sensory analysis

The sensory analysis included a taste panel constituted by 15 semi-trained panelists on the base of a 7-point hedonic scale: 1-dislike definitely; 2-dislike; 3-dislike mildly; 4-neither like nor dislike; 5-like mildly; 6-like; 7-like definitely. Sensory parameters evaluated were appearance, texture, aroma, taste and overall liking. Overall liking was calculated as a mean of the sensory parameters evaluated.

Panelists were recruited from Faculty students and staff to who was ministered a training at the beginning of the experiments to become familiar with the fruits.

2.2.6. Statistical analysis

Statistical analysis was carried out with the SPSS 20.0 software (IBM, Corp.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among treatments was performed. Hierarchical cluster analysis (HCA) was utilized to investigate the similarities and dissimilarities among the formulations. For classification, Ward's minimum variance method was utilized, with the squared Euclidean distance as dissimilarity measure. The grouping derived from HCA was used to interpret the results of the dendogram.

3. Results and discussion

3.1. Quality parameters

The L^* value of color (variation between 0 = black and 100 = white) of strawberries showed small changes along 14 d shelf life at 0.5 °C, changing from 38.97 to 45.65 for AL and from 39.70 to 46.2 for PE (Tables 1 and 2). Although with significant differences in some treatments, maximum changes for each treatment did not exceed the value 4.5 for L^*, which did not alter significantly the color parameter. There were no significant differences between AL and PE ($P = 0.088$). Similar behavior was observed for hue color value (Tables 1 and 2).

Strawberry fruit of 7 cultivars stored at 0 °C for 7 d become darker but h° value did not change (Sacks and Shaw, 1993). In our case, we found no significant changes either in L^* or h° through 14 d storage in non-coated fruit. Although there were statistically significant changes in some edible coating treatments, they were not of significance in terms of quality change.

Table 1
Color parameters (L^* and h°), firmness, weight loss, antioxidant activity and microbial analysis of strawberries covered with different alginate (AL) based edible coating formulations during storage at 0.5 °C. Parameters were evaluated at harvest (before treatments) and after 7 and 14 d storage. Values represent the mean ± standard error of three replicates.

	Days	Control	AL 1%	AL 1% + Cit 0.15%	AL 1% + Cit 0.3%	AL 1% + Eug 0.1%	AL 1% + Eug 0.2%	AL 1% + Cit 0.15% + Eug 0.1%	AL 2%	AL 2% + Cit 0.15%	AL 2% + Cit 0.3%	AL 2% + Eug 0.1%	AL 2% + Eug 0.2%	AL 2% + Cit 0.15% + Eug 0.1%
Lightness (L^*)	0	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 aA	43.4 ± 0.4 abA	43.4 ± 0.4 aA
	7	42.2 ± 0.6 aBCDE	42.3 ± 0.1 bBCDE	39.6 ± 0.9 bEF	39.9 ± 0.8 bEF	41.0 ± 1.0 bCDEF	43.0 ± 0.6 aABCD	45.1 ± 1.2 aA	44.0 ± 1.0 aAB	40.3 ± 0.9 bDEF	39.0 ± 0.7 bF	39.8 ± 0.8 bEF	45.6 ± 1.2 aA	43.7 ± 0.7 aABC
	14	42.3 ± 0.8 aABC	43.0 ± 0.4 abAB	43.1 ± 0.5 aAB	40.9 ± 0.8 bCD	40.2 ± 0.4 bD	42.4 ± 0.7 aABC	44.1 ± 0.8 aA	44.2 ± 0.4 aA	41.9 ± 0.6 abBCD	44.0 ± 0.7 aA	43.0 ± 0.3 aABC	41.1 ± 0.3 bBCD	41.2 ± 0.6 bBCD
Hue angle (h°)	0	34.3 ± 0.8 aA	34.3 ± 0.8 bA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA	34.3 ± 0.8 aA
	7	36.5 ± 0.8 aAB	36.9 ± 0.7 aA	34.0 ± 1.2 aBCD	31.3 ± 1.0 abDEF	29.1 ± 0.6 bF	31.3 ± 0.2 aDEF	31.7 ± 1.0 abDEF	30.8 ± 0.5 bEF	33.5 ± 0.5 aCDE	34.9 ± 1.0 aABC	34.4 ± 1.5 aABC	31.1 ± 0.6 bEF	30.8 ± 0.9 bEF
	14	36.3 ± 0.5 aA	36.3 ± 0.5 abA	31.1 ± 1.4 aBCD	29.0 ± 1.1 bDE	33.1 ± 0.7 aBC	30.9 ± 1.9 aBCD	30.5 ± 0.6 bCD	34.0 ± 0.3 aAB	31.7 ± 1.5 aBCD	25.9 ± 0.2 bE	29.0 ± 0.9 bDE	30.3 ± 0.6 bCD	30.5 ± 1.1 bCD
Firmness (N)	0	6.0 ± 0.4 bA	6.0 ± 0.4 bA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 bA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 bA	6.0 ± 0.4 bA	6.0 ± 0.4 aA
	7	7.7 ± 0.6 aA	7.8 ± 0.5 aA	5.8 ± 0.3 aBCD	5.4 ± 0.3 aCD	6.9 ± 0.7 aABCD	5.3 ± 0.8 aD	6.7 ± 0.6 aABCD	8.0 ± 0.2 aA	7.1 ± 0.1 aABC	5.9 ± 0.2 aBCD	7.2 ± 0.4 abAB	6.0 ± 0.4 bBCD	6.0 ± 0.8 aBCD
	14	6.3 ± 0.2 abAB	4.3 ± 0.3 cCDE	3.6 ± 0.1 bE	3.4 ± 0.4 bE	5.3 ± 0.5 aBCD	5.6 ± 0.5 aBC	5.2 ± 0.7 aBCD	6.2 ± 0.8 abAB	4.0 ± 0.6 bDE	3.6 ± 0.0 bE	7.6 ± 0.4 aA	7.5 ± 0.4 aA	4.6 ± 0.7 aCDE
Weight loss (%)	0	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA
	7	2.1 ± 0.0 bB	2.4 ± 0.2 bAB	3.0 ± 0.2 bA	3.1 ± 0.0 bA	2.4 ± 0.1 bAB	2.4 ± 0.2 bAB	2.7 ± 0.2 bAB	2.1 ± 0.3 bB	2.8 ± 0.2 bAB	2.7 ± 0.2 bAB	2.7 ± 0.6 bAB	2.7 ± 0.1 bAB	2.9 ± 0.1 bA
	14	3.3 ± 0.2 aD	4.0 ± 0.2 aCD	5.5 ± 0.1 aA	4.9 ± 0.1 aABC	4.0 ± 0.3 aCD	4.5 ± 0.5 aABC	5.1 ± 0.4 aAB	4.1 ± 0.5 aBCD	4.4 ± 0.2 aBC	4.5 ± 0.3 aABC	4.7 ± 0.7 aABC	4.4 ± 0.2 aBC	4.9 ± 0.1 aABC
Antioxidant activity ($mMkg^{-1}$)	0	451 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA	4515 ± 43 aA
	7	4084 ± 80 aAB	5199 ± 2 aA	4669 ± 53 aAB	3916 ± 86 aB	5249 ± 9 aA	5015 ± 14 aAB	5157 ± 4 aA	4986 ± 22 aAB	5245 ± 3 aAB	5164 ± 5 aA	5106 ± 11 aAB	5196 ± 13 aA	5143 ± 6 aA
	14	2676 ± 3 aA	2667 ± 5 bA	2626 ± 3 bA	2521 ± 17 aA	2703 ± 8 bA	2653 ± 4 bA	2544 ± 8 bA	2642 ± 4 bA	2658 ± 2 bA	2455 ± 14 bAB	1939 ± 38 bC	2458 ± 10 bAB	2033 ± 26 bBC
Yeast and moulds (Log_{10} CFU g^{-1})	0	2.5 ± 0.0 cA	2.5 ± 00 aA	2.5 ± 0.0 aA	2.5 ± 0.0 bA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 bA	2.5 ± 0.0 bA	2.5 ± 0.0 bA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 aA
	7	3.3 ± 0.1 bAB	2.6 ± 0.3 aABC	2.6 ± 0.0 aABC	4.1 ± 0.2 aA	0.8 ± 0.8 aD	0.7 ± 0.7 bD	3.1 ± 0.1 aABC	3.7 ± 0.0 aA	0.7 ± 0.7 cD	3.1 ± 0.4 aABC	1.7 ± 0.8 abBCD	1.3 ± 0.7 aCD	0.8 ± 0.8 bD
	14	4.0 ± 0.0 aA	2.4 ± 0.6 aB	0.8 ± 0.4 bDE	1.3 ± 0.2 bCD	1.6 ± 0.3 aBCD	1.9 ± 0.1 abBC	1.9 ± 0.3 cBC	3.9 ± 0.4 aA	4.3 ± 0.0 aA	1.2 ± 0.1 bCD	0.0 ± 0.0 bE	0.9 ± 0.5 aCDE	1.5 ± 0.2 abBCD
Aerobic mesophilic microorganisms (Log_{10} CFU g^{-1})	0	2.9 ± 0.0 cA	2.9 ± 0.0 bA	2.9 ± 0.0 bA	2.9 ± 0.0 aA	2.9 ± 0.0 a	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA
	7	3.6 ± 0.0 bA	3.1 ± 0.2 abAB	2.5 ± 0.1 bBC	2.1 ± 0.3 bCD	0.0 ± 0.0 bF	1.2 ± 0.2 bDE	0.0 ± 0.0 bF	1.4 ± 0.4 bDE	0.8 ± 0.4 bEF	1.5 ± 0.8 aDE	0.7 ± 0.3 bEF	0.0 ± 0.0 cF	0.0 ± 0.0 bF
	14	4.5 ± 0.0 aA	4.4 ± 0.0 aA	4.7 ± 0.0 aA	3.0 ± 0.0 aB	2.7 ± 0.0 aB	2.6 ± 0.0 aB	0.0 ± 0.0 bE	1.2 ± 0.1 bD	0.8 ± 0.4 bD	2.1 ± 0.1 aC	0.9 ± 0.5 bD	0.7 ± 0.3 bD	0.0 ± 0.0 bE

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 2
Color parameters (L^* and h°), firmness, weight loss, antioxidant activity and microbial analysis of strawberries covered with different pectin (PE) based edible coating formulations during storage at 0.5 °C. Parameters were evaluated at harvest (before treatments) and after 7 and 14 d storage. Values represent the mean ± standard error of three replicates.

	Days	Control	PE 1%	PE 1% + Cit 0.15%	PE 1% + Cit 0.3%	PE1% + Eug 0.1%	PE1% + Eug 0.2%	PE 1% + Cit 0.15% + Eug 0.1%	PE 2%	PE 2% + Cit 0.15%	PE 2% + Cit 0.3%	PE2% + Eug 0.1%	PE2% + Eug 0.2%	PE 2% + Cit 0.15% + Eug 0.1%
Lightness (L^*)	0	41.6 ± 0.6 aA	41.6 ± 0.6 aA	41.6 ± 0.6 aA	41.6 ± 0.6 bA	41.6 ± 0.6 bA	41.6 ± 0.6 bA	41.6 ± 0.6 aA	41.6 ± 0.6 aA	41.6 ± 0.6 bA	41.6 ± 0.6 bA	41.6 ± 0.6 aA	41.6 ± 0.6 bA	41.6 ± 0.6 bA
	7	39.7 ± 1.6 aE	39.5 ± 0.9 aDE	43.8 ± 0.8 ABC	42.4 ± 0.4 abCDE	43.9 ± 0.9 bABC	45.8 ± 0.6 aAB	44.4 ± 1.6 aABC	44.1 ± 1.1 aABC	42.7 ± 0.7 bBCD	41.6 ± 0.6 bCDE	43.3 ± 1.5 aABC	46.2 ± 0.5 aA	46.1 ± 0.6 aA
	14	41.5 ± 0.1 aE	41.6 ± 0.4 aE	42.2 ± 0.7 aD	44.1 ± 0.9 aBCD	44.9 ± 0.3 aABCD	44.9 ± 0.7 aABCD	45.1 ± 0.7 aABC	44.5 ± 0.6 aABCD	45.4 ± 0.9 aABC	43.2 ± 0.1 aCD	44.6 ± 0.6 aABCD	45.6 ± 0.3 aAB	45.9 ± 0.4 aA
Hue angle (h°)	0	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA	38.9 ± 0.5 aA
	7	35.4 ± 1.1 abA	35.3 ± 0.3 bA	31.6 ± 0.3 cCD	32.4 ± 1.0 bBCD	31.9 ± 0.4 bBCD	31.7 ± 0.1 bCD	31.9 ± 1.0 bBCD	33.1 ± 0.7 bABC	33.5 ± 0.4 bAB	32.4 ± 0.4 bBCD	32.6 ± 0.7 bBCD	31.4 ± 0.7 bCD	30.0 ± 0.8 bD
	14	33.6 ± 1.5 bA	31.5 ± 0.7 cBCD	33.0 ± 0.3 bAB	31.4 ± 0.3 bBCD	29.9 ± 1.0 bCD	30.6 ± 0.6 bCD	30.0 ± 0.5 bCD	30.4 ± 1.4 bCD	31.6 ± 0.9 bBCD	31.3 ± 1.1 bBCD	32.5 ± 0.2 bABC	29.3 ± 0.7 bD	29.2 ± 0.8 bD
Firmness (N)	0	6.2 ± 0.1 aA	6.2 ± 0.1 bA	6.2 ± 0.1 aA	6.2 ± 0.1 bA	6.2 ± 0.1 bA	6.2 ± 0.1 aA	6.2 ± 0.1 aA	6.2 ± 0.1 bA	6.2 ± 0.1 aA	6.2 ± 0.1 aA	6.2 ± 0.1 abA	6.2 ± 0.1 bA	6.2 ± 0.1 aA
	7	7.0 ± 0.4 aAB	8.2 ± 0.5 aAB	6.4 ± 0.4 aB	8.0 ± 0.6 aAB	7.5 ± 0.5 aAB	6.5 ± 0.4 aB	7.4 ± 0.7 aAB	8.6 ± 0.7 aA	7.6 ± 0.8 aAB	7.6 ± 0.5 bAB	7.5 ± 0.4 aAB	7.7 ± 0.3 aAB	7.4 ± 0.8 aAB
	14	5.8 ± 0.8 aAB	3.8 ± 0.5 cCDE	3.5 ± 0.6 bDE	4.2 ± 0.5 cBCDE	5.0 ± 0.2 cABCD	5.4 ± 0.7 aABC	6.0 ± 0.5 aA	2.8 ± 0.3 cE	3.5 ± 0.5 bDE	2.7 ± 0.2 cE	5.5 ± 0.7 bABC	5.6 ± 0.4 cAB	5.6 ± 0.3 aAB
Weight Loss (%)	0	0.0 ± 4.8 bA	0.0 ± 4.8 cA	0.0 ± 4.8 cA	0.0 ± 4.8 cA	0.0 ± 4.8 cA	0.0 ± 4.8 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA	0.0 ± 0.0 cA
	7	2.2 ± 1.2 bDE	3.1 ± 5.6 bABC	2.0 ± 6.4 bE	2.7 ± 2.0 bBCD	2.5 ± 1.4 bCDE	2.7 ± 1.6 bBCD	3.5 ± 1.2 bA	2.5 ± 1.9 bCDE	1.9 ± 1.3 bE	2.4 ± 1.4 bDE	2.2 ± 0.9 bDE	2.8 ± 2.1 bBCD	3.2 ± 3.9 bAB
	14	4.6 ± 1.6 aDE	5.8 ± 4.5 aBCD	4.8 ± 6.0 aCDE	6.2 ± 3.6 aBC	5.1 ± 17.2 aBCDE	4.6 ± 3.1 aDE	6.4 ± 0.4 aB	9.9 ± 4.2 aA	4.0 ± 2.0 aE	5.6 ± 3.3 aBCD	4.2 ± 2.8 aE	6.2 ± 8.5 aBC	5.9 ± 6.2 aBCD
Antioxidant activity ($mMkg^{-1}$)	0	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA	5351 ± 48 aA
	7	5313 ± 58 aA	5337 ± 31 aA	5334 ± 11 aA	5361 ± 16 aA	5359 ± 32 aA	5386 ± 51 aA	5352 ± 78 aA	5300 ± 88 aA	5199 ± 96 aA	5280 ± 153 aA	5292 ± 123 aA	5381 ± 23 aA	5335 ± 119 aA
	14	2651 ± 15 bAB	2447 ± 136 bAB	2601 ± 38 bAB	2531 ± 83 bAB	2519 ± 172 bAB	2539 ± 91 bAB	2496 ± 147 bAB	2214 ± 207 bB	2399 ± 193 bAB	2468 ± 135 bAB	2602 ± 66 bAB	2711 ± 17 bA	2452 ± 76 bAB
Yeast and moulds (Log_{10} CFU g^{-1})	0	2.5 ± 0.0 cA	2.5 ± 0.0 bA	2.5 ± 0.0 aA	2.5 ± 0.0 bA	2.5 ± 0.0 bA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 bA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 aA	2.5 ± 0.0 aA
	7	4.3 ± 0.0 aA	4.0 ± 0.0 aA	2.9 ± 0.2 aB	3.1 ± 0.1 aB	3.0 ± 0.0 aB	0.0 ± 0.0 bD	2.1 ± 0.1 aBC	0.0 ± 0.0 cD	1.3 ± 0.7 ab	0.0 ± 0.0 cD	1.4 ± 0.7 aD	1.3 ± 0.7 bD	0.0 ± 0.0 cD
	14	4.0 ± 0.0 bA	3.4 ± 0.3 aAB	1.3 ± 0.3 bDEF	1.6 ± 0.4 cDEF	1.0 ± 0.0 cFG	0.0 ± 0.0 bH	1.9 ± 0.5 aCDE	3.2 ± 0.0 aB	0.0 ± 0.0 bH	2.2 ± 0.0 bC	2.0 ± 0.0 aCDE	0.9 ± 0.4 cF	1.3 ± 0.0 bEFG
Aerobic mesophilic microrganisms (Log_{10} CFU g^{-1})	0	2.9 ± 0.0 cA	2.9 ± 0.0 cA	2.9 ± 0.0 cA	2.9 ± 0.0 cA	2.9 ± 0.0 bA	2.9 ± 0.0 bA	2.9 ± 0.0 bA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA	2.9 ± 0.0 aA
	7	3.2 ± 0.1 bA	3.3 ± 0.0 bA	3.4 ± 0.0 bA	3.5 ± 0.0 bA	2.4 ± 0.1 cA	0.8 ± 0.4 cC	0.3 ± 0.3 cCD	2.2 ± 0.0 bB	0.0 ± 0.0 bD	0.7 ± 0.3 bC	0.7 ± 0.3 bC	0.0 ± 0.0 cD	0.0 ± 0.0 cD
	14	5.6 ± 0.0 aA	5.6 ± 0.0 aA	5.0 ± 0.0 aA	5.0 ± 0.0 aA	5.0 ± 0.0 aA	5.0 ± 0.0 aA	4.0 ± 0.0 aB	2.8 ± 0.0 aC	0.0 ± 0.0 bE	0.8 ± 0.4 bD	0.3 ± 0.3 bDE	0.8 ± 0.4 bD	0.7 ± 0.3 bD

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

As in our case, there was no additional benefit of applying chitosan edible coatings on strawberry h° values either when enriched with oleic acid or beeswax or alginate combined with an yeast antagonist (Vargas et al., 2006; Velickova et al., 2013). However, those authors found lower darkening in coated strawberries than in uncoated controls. In our case we found no additional benefit of the tested edible coatings since control fruit color did not change significantly during 14 d storage. Ribeiro et al. (2007) also found no effect of starch, chitosan or carrageenan edible coatings on strawberry color.

The benefit of coating on preserving fruit color is mainly on reducing darkening, and has been attributed for fresh-cut fruit due to their higher susceptibility to oxidation as reported for fresh-cut apples, melon and pineapple (Perez-Gago et al., 2005; Raybaudi-Massilia et al., 2008a,b; Olivas et al., 2007; Rojas-Graü et al., 2007b; Rojas-Graü et al., 2008; Bierhals et al., 2011; Azarakhsh et al., 2014). The chroma and soluble solids content (SSC) were not significantly affected by the edible coatings (data not shown).

According to Duan et al. (2011) for blueberries, SSC (%) were not significantly affected by cold storage or coating (sodium alginate and chitosan) treatments. These results differ from those reported by Gol et al. (2013) and Velickova et al. (2013) who showed a decrease in the total soluble solids content in strawberries, at the end of storage, and attributed it to respiration, when using other edible coatings.

Firmness is an important quality parameter for fresh fruit, which decreases during storage as a result of cell wall degradation and loss of turgor. The application of some edible coatings improved the firmness as compared to control and just harvested fruit, either for AL or PE (Tables 1 and 2). In fact, after 7 d storage, AL 1% and 2% alone and AL 2% + Eug 0.1% showed higher firmness values than just harvested fruit without treatment (Table 1). After 14 d storage, the highest firmness values were observed in AL 2% or AL 2% combined with both concentrations of Eug.

In PE treated fruit, the improvement in firmness after 7 d storage did not follow a clear pattern, but was mainly in PE 2% treatments (Table 2). However, after 14 d storage it is clear that the PE treated fruit with Eug at both concentrations or the combination of Eug plus Cit at MIC concentrations gave the highest firmness values without significant differences among them either for PE 1 or 2%.

From the above it seems that both AL and PE edible coatings preserve well the firmness, mainly at 2%, which is improved by Eug at both concentrations or in combination with Cit in both AL or PE treatments. The action of Cit addition seems detrimental for firmness, mainly at higher concentrations (0.3%) and later on storage time (14 d) since a negative effect on firmness with values even lower than controls were observed (Tables 1 and 2). Generally, there was no significant difference between AL and PE as base for edible coatings ($P = 0.447$).

The beneficial effect of coating applications on the strawberry firmness has been reported for coatings prepared from cactus mucilage, chitosan-oleic acid coatings, chitosan coatings in combination with calcium dips and chitosan–beeswax (Del-Valle et al., 2005; Vargas et al., 2006; Hernández-Muñoz et al., 2008; Velickova et al., 2013).

Rojas-Graü et al. (2007b) found that alginate edible coatings enriched with vanillin (up to 6%) and oregano (1%) applied to fresh-cut apples were effective in improving firmness. However, lemongrass containing coatings and oregano at 5% induced severe texture softening. They attributed it to the lower pH of those edible coating solutions. Also, lemongrass has as main compound citral, confirming our results. Guerreiro et al. (2015) also reported a better effect of Eug than Cit on *Arbutus unedo* fresh fruit storage when using alginate based edible coatings. In our case this happened also in pectin based edible coatings.

Some authors (Ribeiro et al., 2007; Hernández-Muñoz et al., 2008) attributed the beneficial effect of their coatings to the use of calcium on them. The beneficial effect of calcium on firmness retention is widely known (Antunes et al., 2010, 2012). In our case, the differences in the coatings cannot be attributed only to calcium since all coatings had the same calcium dip. Moreover, as for Rojas-Graü et al. (2007b) despite of calcium dips, some essential oils concentrations decreased firmness mainly at higher concentrations as observed when we used Cit (0.3%), while others improved firmness as Eug or the mixture of Eug with Cit at MIC concentrations.

Weight loss is also an indicator of freshness of fruits. Weight loss increased during storage in all samples as expected. The edible coatings of this experiment did not decrease weight loss as compared to uncoated samples (control) (Tables 1 and 2). Moreover, for AL the edible coatings with Cit at both concentrations and for PE the PE 1% + Cit 0.3%, PE 1% + Cit 0.15% + Eug 0.1% and PE 0.2% gave significantly higher weigh loss than control. The weight loss in the other treatments did not significantly differ from control. Generally, there were no significant differences between the use of AL or PE ($P = 0.204$).

It is known that migration of water from fruit to the environment is the major cause of weight loss of fruit during storage (Duan et al., 2011). Edible coatings act as an extra layer which also coats the stomata leading to a decrease in transpiration and in turn, to a reduction in weight loss, as it has been demonstrated in a wide range of fruit including apricot, pepper, peach, sweet cherry and litchi (Ayranci and Tunc, 2003; Hassimotto et al., 2008; Díaz-Mula et al., 2012). Moreover, differences in the ability to reduce weight loss are attributed to the different water vapor permeability of the polysaccharides used in the formulation of the edible coatings (Vargas et al., 2008).

Weight loss during storage at low temperature was also observed for arbutus berries, strawberries and red raspberries (Guerreiro et al., 2013; Krüger et al., 2011; Shin et al., 2008; Vicente et al., 2002). Previous studies showed a reduction in weight loss for strawberries due to the effects of coatings composition, which served as semi-permeable barrier against moisture loss (Gol et al., 2013; Valero et al., 2013). However, these authors used different coatings based on chitosan. Also, they used fruit immersed in distilled water as control while our controls were just harvested fruits without any treatment.

In fact, Duan et al. (2011), when using control blueberries without any treatment, found lower weight loss than most polysaccharide based edible coatings used, while fruit washed in sanitized water were the ones that lost more weight. Guerreiro et al. (2015) found similar results as ours in arbutus berries coated with alginate based edible coatings, but here the higher weight loss was for the combination of alginate with eugenol.

It is known that transpiration is usually reduced by epidermal cell layer and cuticle (Valero et al., 2013). Some of our edible coatings, especially the ones with higher Cit concentration, gave higher weigh loss than control. The firmness loss was also verified when higher concentrations of Cit were used, as reported above. Such results are related and may be attributed to pectic acid undergoing acid hydrolysis, according to Ponting et al. (1971). However, this may be dependent on the concentration and combination with other components, since lower concentrations of Cit or its combination with Eug did not produce the same effect when Cit 0.3% was used. They also reported that Cit combined with 2-(*E*)-hexenal promoted a better retention of the initial character- istics of fresh-cut apples, which means lower loss of firmness than the control. Nevertheless, the authors did not report a possible mechanism of action of those molecules on enzymatic reactions and/or cellular modifications responsible for the results obtained.

3.2. Trolox equivalent antioxidant activity (TEAC)

Antioxidant activity is an important quality factor promoting health in food and is used to evaluate the antioxidant potential of the fruit tissue. The antioxidant activity determined by the TEAC method allows the evaluation of the capacity of samples to scavenge free radicals such as ABTS.

The TEAC values for both AL and PE edible coatings showed a reduction for most treatments only from 7 to 14 d (Tables 1 and 2). After 14 d storage only AL 2% + Eug 0.1% gave significantly lower antioxidant activity than the other treatments except AL 2% + Cit 0.15% + Eug 0.1% (Table 1). In the case of PE, PE 2% + Eug 0.2% gave the highest TEAC and AL2% the lowest with significant differences ($P < 0.05$) between them but not from the other treatments (Table 2). Generally, strawberries coated with PE based edible coatings showed higher antioxidant activity than the AL ones ($P = 0.014$).

There are few works reporting the antioxidant activity changes through cold storage of fruit. Piljac-Žegarac and Šamec (2011) reported a more or less constant antioxidant activity, as measured by TEAC method, through cold storage for strawberries, cherries, raspberries, red currants, cherry and sour cherry. These authors stored strawberries for 16 d and generally found lower values than the ones of our experiment.

Guerreiro et al. (2015) found that AL 2% and AL 2% + Cit 0.15% + Eug 0.1% gave higher TEAC values than control in *Arbutus unedo* fresh fruit, after 28 d cold storage. Also, Robles-Sánchez et al. (2013) found higher antioxidant activity in fresh-cut mangoes covered with Alginate 2% + Ascorbic Acid 1% edible coating than in alginate alone and control fruits. They attributed it to the ascorbic acid added. In our case, all the treatments, except control, were dipped in 1% ascorbic acid but no additional increase in antioxidant activity was found in strawberries, this was also observed for some edible coatings used in *Arbutus unedo* fruits (Guerreiro et al., 2015). It seems that there is no response in strawberry fruit to edible coatings probably because these fruit have the inherent capacity to preserve antioxidant activity in the cold even without any treatment as control did.

3.3. Microbial quality

The yeast and molds counts increased through storage time in both AL and PE treatments (Tables 1 and 2). In control, this increase was constant up to 14 d storage. However, when edible coatings were applied, mainly the ones with essential oils and with AL or PE at higher concentrations (2%), the yeast and molds development on fruits decreased. Up to 14 d shelf-life, the counts of yeast and molds was under the limits for those microorganisms in food (5 Log_{10} CFU/g) even in control (Stannard, 1997).

Counts of aerobic mesophilic microrganisms increased during storage up to 14 d in control and all AL 1% treatments (Table 1). Similar results were obtained for PE 1% (Table 2). Interestingly, for both PE and AL, the 2% concentration with inclusion of essential oils constituents reduced the counts of aerobic mesophilic microrganisms (Tables 1 and 2).

After 14 days shelf-life, the treatment with AL 2% + 0.15% Cit + 0.1% Eug were the most eficient in reducing aerobic mesophilic microorganisms followed by all AL 2% treatments which had higher effect than the AL 1% ones (Table 1). Control had the higher values as well as AL 1% and AL1% + Cit 0.15%. Also here the maximum permited limits for food borne microorganisms development in food (5 Log_{10} CFU/g) were not reached (Stannard, 1997). In the case of PE edible coatings the same pattern was obtained, but here there were not significant differences among PE 1% treatments which were not also different from control (Table 2). Moreover, control and AL 1% pass the permissible limits for aerobic mesophilic microrganisms in food by reaching 5.61 and 5.58 Log_{10} CFU/g, respectively, making those treatments not recommendable for more than 7 d storage.

Generally there were significant differences in edible coatings based on AL and PE for aerobic mesophilic microrganisms ($P = 0.034$) with AL giving the best results, but not for yeast and molds ($P = 0.219$).

No growth of psychotropic bacteria was observed during the storage period.

In the present work it is clear that the use of Eug and Cit was beneficial for reducing microbial spoilage as reported by other authors (Antunes et al., 2012).

However, in other studies (Fan et al., 2009; Gol et al., 2013; Han et al., 2004) the decay incidence in strawberries coated with just chitosan and alginate was significantly reduced in comparison to uncoated control fruit. In contrast, in our edible coatings AL or PE, by themselves alone, were not efficient especially when at lower concentrations (1%). Similar results were obtained for AL based edible coatings by Rojas-Graü et al. (2007b) for fresh-cut apple and Azarakhsh et al. (2014) for fresh-cut pineapple.

The mechanisms involved in the microbial growth inhibition by these compounds were not studied in the present work. The mechanisms of growth inhibition, cell injury and inactivation induced by Cit are not yet fully understood; nevertheless, both for Cit or other terpenoids it is common to observe the disruption of the cell membrane (Somolinos et al., 2009). Cit may increase the membrane permeability of fungi by decreasing total lipid and ergosterol contents of the cells, with the consequent release of cell constituents, and leakage of potassium ions (Tao et al., 2014).

The antimicrobial activity of Eug has been reported, and has being linked to its ability to disturb the permeability of the cell membrane, the inhibition of enzymes, such as ATPase, histidine decarboxylase, amylase and the release of the cellular content (Hyldgaard et al., 2012).

Other interesting result was that, in every edible coating formulation based on AL or PE at each concentration (1 or 2%), the coatings with higher Cit (0.3%) were the less effective, contrarily to that in arbutus berries in which citral, at the same concentration, was effective in reducing microbial spoilage, mainly yeasts and molds (Guerreiro et al., 2015). This may be because of the damage caused by this high Cit concentration on strawberry epidermis cells, as observed for firmness and weight loss, making those fruit more susceptible to spoilage.

3.4. Sensory evaluation

Literature has been referring the main objective of introducing essential oils and/or their constituents into edible coatings as antimicrobials and antioxidant agents, nevertheless taking into account their acceptability by consumers (Antunes et al., 2012). However most works do not include taste panels when testing new edible coatings. Our study aims to fill this gap.

In Tables 3 and 4 it is visible that after 14 d storage at 0.5 °C, the strawberries had a maximum visual appearance evaluation of3 corresponding to “dislike mildly” in the sensory panel scale, sono treatment was susceptible for consumption. After 7 d ofstorage at 0.5 °C the taste panel showed for most treatments an evaluation over 4 (neither like nor dislike), which is the minimum acceptable for marketing, and none scored below 3.5. As overall liking, the treatments that scored higher were AL 1 or 2% + Eug 0.1% and AL 2% + Cit 0.15%, nevertheless without significant differences from AL at both concentrations and control (Table 3).

For PE the overall rating was obtained for PE 1 or 2% + Citral 0.15% with no significant differences with PE at 2% and control (Table 4).

Table 3
Sensory evaluation of strawberries covered with different alginate (AL) based edible coating formulations during storage at 0.5 °C. Appearance, texture, aroma, taste and overall liking were evaluated at harvest (before treatments) and after 7 d storage, while after 14 d only appearance was evaluated. Values represent the mean ± standard error of 15 replicates.

	Days	Control	AL 1%	AL 1% + Cit 0.15%	AL 1% + Cit 0.3%	AL1% + Eug 0.1%	AL1% + Eug 0.2%	AL 1% + Cit 0.15% + Eug 0.1%	AL 2%	AL 2% + Cit 0.15%	AL 2% + Cit 0.3%	AL2% + Eug 0.1%	AL2% + Eug 0.2%	AL 2% + Cit 0.15% + Eug 0.1%
Appearance	0	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA	6.1 ± 0.3 aA
	7	5.8 ± 0.4 aA	5.5 ± 0.4 aA	4.6 ± 0.5 bAB	4.4 ± 0.5 bAB	4.6 ± 0.6 bAB	3.9 ± 0.5 bB	5.1 ± 0.4 aAB	5.3 ± 0.3 aAB	5.8 ± 0.3 aA	5.3 ± 0.4 aAB	5.3 ± 0.4 aAB	4.4 ± 0.5 bAB	4.9 ± 0.4 bAB
	14	4.0 ± 0.4 bA	2.0 ± 0.4 bBCD	1.0 ± 0.0 cD	1.0 ± 0.5 cBC	2.3 ± 0.3 cCD	1.5 ± 0.3 cCD	1.3 ± 0.3 bCD	1.3 ± 0.3 bCD	1.3 ± 0.3 bCD	1.3 ± 0.3 bBC	2.3 ± 0.5 bAB	1.8 ± 0.4 cCD	1.3 ± 0.3 cCD
Texture	0	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA	5.9 ± 0.4 aA
	7	5.4 ± 0.3 aAB	5.3 ± 0.3 aAB	4.6 ± 0.3 bABCD	3.8 ± 0.4 bCD	5.6 ± 0.3 aA	4.3 ± 0.4 bBCD	5.1 ± 0.1 aAB	4.9 ± 0.5 bABC	5.6 ± 0.2 aA	3.9 ± 0.4 bCD	5.6 ± 0.3 aA	4.3 ± 0.5 bBCD	3.5 ± 0.5 bD
Aroma	0	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA	6.0 ± 0.4 aA
	7	4.5 ± 0.3 bABC	4.5 ± 0.3 bABC	3.9 ± 0.3 bBCD	3.8 ± 0.4 bCD	4.5 ± 0.2 bABC	3.4 ± 0.3 bD	4.9 ± 0.4 bA	4.8 ± 0.3 bAB	5.0 ± 0.3 bA	3.9 ± 0.3 bBCD	4.6 ± 0.3 ABC	3.9 ± 0.2 bBCD	4.1 ± 0.3 bABCD
Taste	0	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA
	7	4.5 ± 0.5 bAB	4.8 ± 0.5 bAB	4.3 ± 0.3 bABC	3.4 ± 0.6 bBCD	5.1 ± 0.4 bA	3.6 ± 0.5 bBCD	4.1 ± 0.4 bABC	4.5 ± 0.6 bAB	4.5 ± 0.4 bAB	3.0 ± 0.4 bCD	5.1 ± 0.4 bA	2.8 ± 0.3 bD	3.5 ± 0.3 bBCD
Overall liking	0	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA	5.9 ± 0.3 aA
	7	4.8 ± 0.4 abAB	4.8 ± 0.4 bAB	4.3 ± 0.3 bBC	3.7 ± 0.5 bCD	5.0 ± 0.3 bA	3.9 ± 0.4 bCD	4.5 ± 0.3 bBC	4.8 ± 0.4 bAB	5.0 ± 0.3 bA	3.8 ± 0.4 bCD	5.1 ± 0.3 aA	3.5 ± 0.4 bD	4.1 ± 0.4 bC
	14	4.0 ± 0.3 bA	2.0 ± 0.3 cBCD	1.0 ± 0.2 cD	1.0 ± 0.3 cBC	2.3 ± 0.2 cCD	1.5 ± 0.2 cCD	1.3 ± 0.2 cCD	1.3 ± 0.2 cCD	1.3 ± 0.2 cCD	1.3 ± 0.2 cBC	2.3 ± 0.3 bAB	1.8 ± 0.3 cCD	1.3 ± 0.2 cCD

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 4
Sensory evaluation of strawberries covered with different pectin (PE) based edible coating formulations during storage at 0.5 °C. Appearance, texture, aroma, taste and overall liking were evaluated at harvest (before treatments) and after 7 d storage, while after 14 d only appearance was evaluated. Values represent the mean ± standard error of 15 replicates.

	Days	Control	PE 1%	PE 1% + Cit 0.15%	PE 1% + Cit 0.3%	PE 1% + Eug 0.1%	PE 1% + Eug 0.2%	PE 1% + Cit 0.15% + Eug 0.1%	PE 2%	PE 2% + Cit 0.15%	PE 2% + Cit 0.3%	PE 2% + Eug 0.1%	PE 2% + Eug 0.2%	PE 2% + Cit 0.15% + Eug 0.1%
Appearance	0	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 aA	6.0 ± 0.0 A
	7	5.8 ± 0.2 aA	5.0 ± 0.4 aABC	5.3 ± 0.3 aAB	5.0 ± 0.4 aABC	5.3 ± 0.3 aAB	3.6 ± 0.5 bD	3.9 ± 0.5 bCD	4.8 ± 0.4 bABCD	5.5 ± 0.2 aA	4.6 ± 0.3 bABCD	5.4 ± 0.4 aA	4.1 ± 0.5 bBCD	4.0 ± 0.5 bCD
	14	2.5 ± 0.3 bAB	1.8 ± 0.3 bBCD	2.3 ± 0.3 bABC	1.5 ± 0.3 bCD	2.3 ± 0.5 bABC	1.5 ± 0.3 cCD	1.8 ± 0.3 cBCD	1.0 ± 0.3 cD	1.3 ± 0.0 bD	1.0 ± 0.3 cD	2.8 ± 0.3 bA	2.3 ± 0.3 cABC	1.8 ± 0.3 cBCD
Texture	0	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA
	7	5.3 ± 0.5 aAB	5.5 ± 0.4 aA	5.0 ± 0.5 aAB	5.3 ± 0.3 aAB	5.5 ± 0.5 aA	4.6 ± 0.5 bAB	4.6 ± 0.3 bAB	5.3 ± 0.3 aAB	5.8 ± 0.3 aA	4.9 ± 0.2 bAB	5.4 ± 0.3 aA	4.6 ± 0.3 bA	4.1 ± 0.4 bB
Aroma	0	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA	6.3 ± 0.2 aA
	7	4.6 ± 0.3 bBCD	4.5 ± 0.2 bBCD	5.6 ± 0.2 aA	4.5 ± 0.2 bBCD	4.8 ± 0.3 bBC	4.1 ± 0.1 bBCD	3.8 ± 0.2 bD	4.9 ± 0.3 bAB	4.8 ± 0.3 bBC	4.1 ± 0.4 bBCD	4.3 ± 0.3 bBCD	4.1 ± 0.4 bBCD	3.9 ± 0.4 bBCD
Taste	0	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA	6.3 ± 0.3 aA
	7	5.0 ± 0.4 aAB	5.0 ± 0.3 aAB	5.0 ± 0.3 aAB	3.9 ± 0.5 bBC	4.9 ± 0.7 bAB	3.3 ± 0.4 bC	3.5 ± 0.4 bC	5.4 ± 0.5 aA	5.4 ± 0.4 aA	3.3 ± 0.5 bC	4.9 ± 0.4 bAB	3.8 ± 0.5 bBC	3.0 ± 0.4 bC
Overall Liking	0	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA	6.1 ± 0.2 aA
	7	5.0 ± 0.3 aAB	4.8 ± 0.3 bBC	5.1 ± 0.3 aA	4.4 ± 0.3 bBCD	5.0 ± 0.4 aAB	3.9 ± 0.4 bCD	3.8 ± 0.3 bCD	5.1 ± 0.4 bAB	5.2 ± 0.3 bA	4.0 ± 0.4 bBCD	5.0 ± 0.3 bBC	4.1 ± 0.4 bBCD	3.7 ± 0.4 bB
	14	2.5 ± 0.2 bAB	1.8 ± 0.2 cBCD	2.3 ± 0.2 bABC	1.5 ± 0.2 cCD	2.3 ± 0.3 bABC	1.5 ± 0.2 cCD	1.8 ± 0.2 cBCD	1.0 ± 0.2 cD	1.3 ± 0.1 cD	1.0 ± 0.2 cD	2.8 ± 0.2 cA	2.3 ± 0.2 cABC	1.8 ± 0.2 cBCD

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

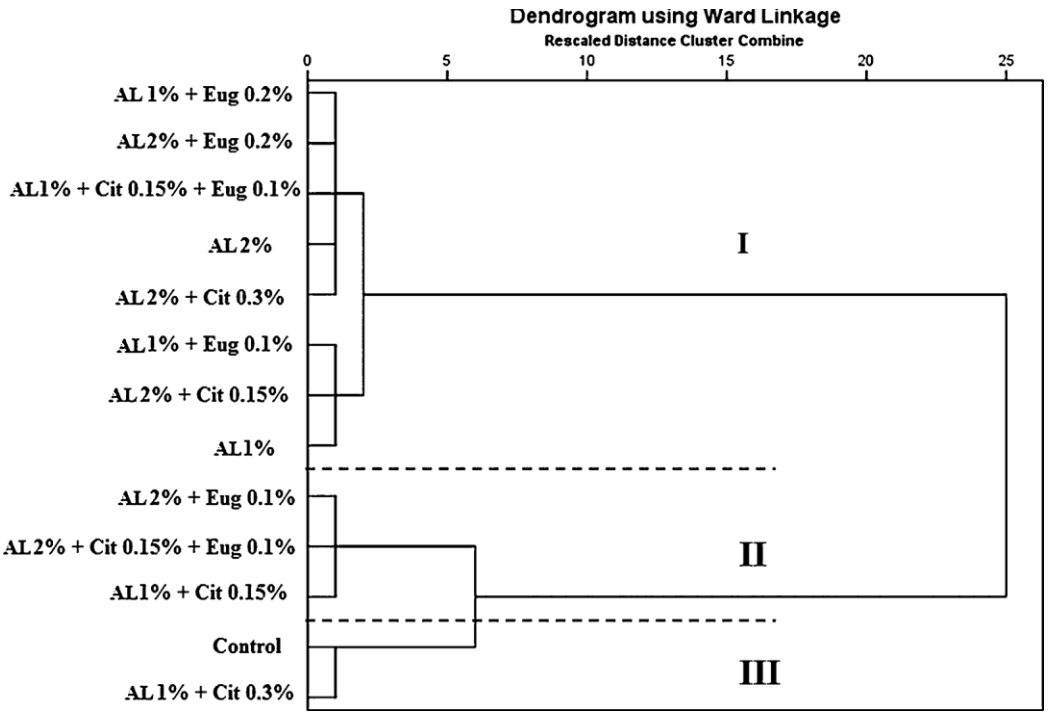


Fig. 1. Dendogram of 13 treatments with alginate (AL) edible coatings containing different essential oils based on the measured physicochemical variables.

Gol et al. (2013) observed from the results of the sensory analysis that the coatings with carboxilmethyl cellulose, hydropropyl-methyl cellulose at 1% and their combinations with chitosan had significantly improved the shelf-life of strawberries as compared to control, while maintaining the overall acceptability at high scores. Our results are in agreement with those reported by Rojas- Graü et al. (2007b) and Azarakhsh et al. (2014) who refer changes in sensory panel of fresh-cut apple or pineapple, respectively,by the adition of essential oils, making fruit unaceptable by consumers, depending on the essential oil and/or its concentration. In our study, the use of essential oil constituents at lower concentrations achieved better score than when double MIC was used, despite their effect on microbial reduction (Tables 3 and 4).

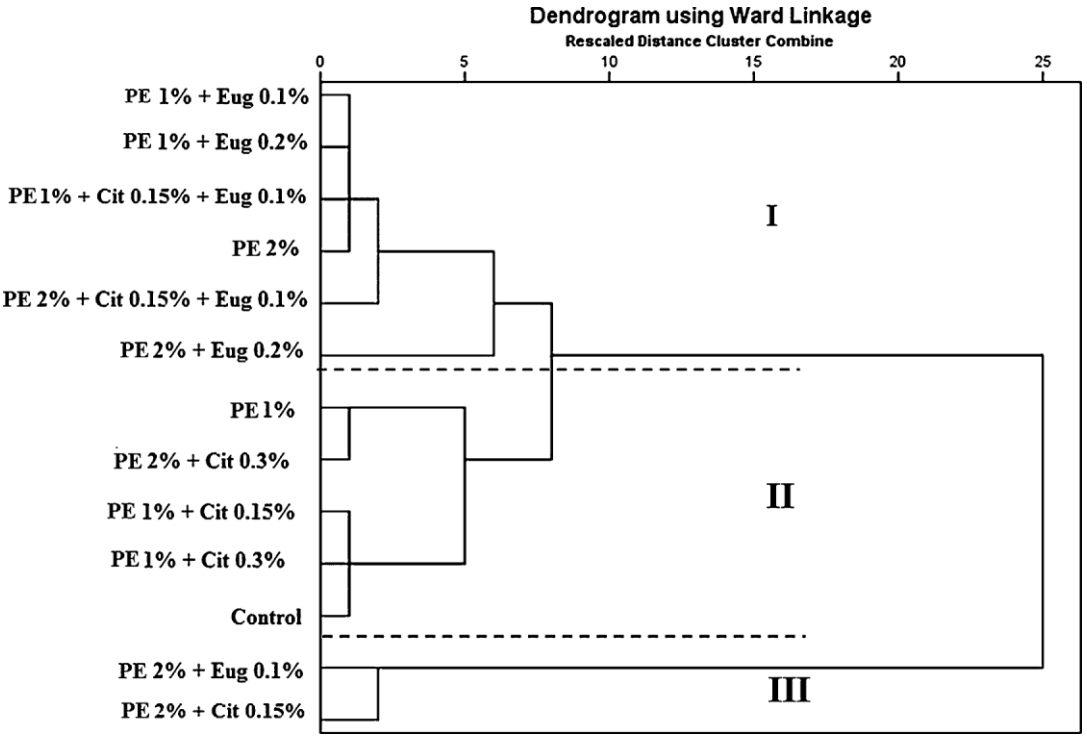


Fig. 2. Dendogram of 13 treatments with pectin (PE) edible coatings containing different essential oils based on the measured physicochemical variables.

3.5. Formulations selection

Among the multiple edible coatings tested we decided to select 2 for each AL or PE based edible coatings taking into account the higher benefit of each quality parameter evaluated through storage.

First, a hierarchical cluster analysis (HCA) was carried out to identify similar edible coatings groups according to all quality parameters measured, except taste panel. The original variables used for HCA analysis were the mean values for the quality parameters (L^*, a^*, b^*, h°, chroma, SSC, firmness, TEAC, weight loss, counts of yeast and molds and aerobic mesophilic microorganisms). The dendogram of Fig. 1 depict the group separation for alginate based coatings. Three main groups can be distinguished, according to the grouping derived from HCA. Taking into account mean group values for all evaluated quality parameters, considering that the best group is the one with better quality properties (values closer to the ones at harvest for color; higher firmness, SSC and antioxidant activity; and lower weight loss and microbial counts), group II was the best followed by group I and III. From the group with the best edible coatings for preserving physicochemical strawberry fruit quality (group II), we wanted to select two with higher scores in the taste panel. The edible coating with the highest score in overall liking was AL 2% + Eug 0.1% (Table 4). The AL 2% + Cit 0.15% + Eug 0.1% did not significantly differ from AL 1% + Cit 0.15%, so the first was chosen because had the same concentration of AL as the edible coating first selected.

A similar approach was done for pectin based edible coatings. The dendogram in Fig. 2 shows also three main groups. The edible coatings group with better properties to preserve strawberry quality was group III, followed by group I and II. Group III evolved only two edible coatings, which had high taste panel scores. Those coatings were PE 2% + Eug 0.1% and PE 2% + Cit 0.15%.

4. Conclusions

We conclude that, in spite of no significant differences were observed for the measured general quality parameters between the majority of edible coatings and control over storage, coatings were important in reducing microbial spoilage of strawberry fresh fruits. Taste panels showed that strawberry fruit could be stored with good sensory properties up to 7 d, but after 14 d the appreciation by panelists indicate that they are not marketable any more. Generally, there was no significant difference in using AL or PE as polysaccharide base for the edible coatings. Concerning all quality parameters evaluated (color, firmness, soluble solids content, weight loss and antioxidant activity), as well as, microbial spoilage and sensory evaluation the edible coatings that better performed during storage were for AL the AL 2% + Eug 0.1% and AL 2 % + Cit 0.15% + Eug 0.10% and for PE the PE 2% + Eug 0.1% and PE 2% + Cit 0.15%.

The selected edible coatings will be used in future experiments where more general and nutritional quality parameters will be evaluated.

Acknowledgement

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010—INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

References

Antunes, M.D.C., Dandlen, S., Cavaco, A.M., Miguel, G., 2010. Effects of postharvest application of 1-MCP and postcutting dip treatment on the quality and nutritional properties of fresh-cut kiwifruit. J. Agric. Food Chem. 58, 6173–6181.

Antunes, M., Gago, C., Cavaco, A., Miguel, M.G., 2012. Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. Recent Patents Food Nutr. Agric. 4, 114–122.

Ayranci, E., Tunc, S., 2003. A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. Food Chem. 80, 423–431.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., Mohd Adzahan, N., 2014. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. Postharvest Biol. Technol. 88, 1–7.

Bierhals, V.S., Chiumarelli, M., Hubinger, M.D., 2011. Effect of cassava starch coating on quality and shelf life of fresh-cut pineapple (*Ananas Comosus* L. Merril cv Pérola). J. Food Sci. 76, 62–72.

Campos, C.A., Gerschenson, L.N., Flores, S.K., 2011. Development of edible films and coatings with antimicrobial activity. Food Bioprocess Technol. 4, 849–875.

Díaz-Mula, H., Serrano, M., Valero, D., 2012. Alginate coatings preserve fruit quality and bioactive compounds during storage of sweet cherry fruit. Food Bioprocess Technol..

Del-Valle, V., Hernández-Muñoz, P., Guarda, A., Galotto, M.J., 2005. Development of a cactus-mucilage edible coating (*Opuntia* ficus *indica*) and its application to extend strawberry (*Fragaria ananassa*) shelf-life. Food Chem. 91, 751–756.

Duan, J., Wu, R., Strik, B.C., Zhao, Y., 2011. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. Postharvest Biol. Technol. 59, 71–79.

Fan, Y., Xu, Y., Wang, D., Zhang, L., Sun, J., Sun, L., Zhang, B., 2009. Effect of alginate coating combined with yeast antagonist on strawberry (*Fragaria* × *ananassa*) preservation quality. Postharvest Biol. Technol. 53, 84–90.

Gol, N.B., Patel, P.R., Rao, T.V.R., 2013. Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biol. Technol. 85, 185–195.

Guerreiro, A., Gago, C., Miguel, M., Antunes, M., 2013. The effect of temperature and film covers on the storage ability of *Arbutus unedo* L. fresh fruit. Sci. Hortic. (Amsterdam) 159, 96–102.

Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., Antunes, M.D.C., 2015. The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. Postharvest Biol. Technol. 100, 226–233.

Han, C., Zhao, Y., Leonard, S., Traber, M., 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus ideaus*). Postharvest Biol. Technol. 33, 67–78.

Harker, F.R., Elgar, H.J., Watkins, C.B., Jackson, P.J., Hallett, I.C., 2000. Physical and mechanical changes in strawberry fruit after high carbon dioxide treatments. Postharvest Biol. Technol. 19, 139–146.

Hassimotto, N., Da Silva Pinto, M., Lajolo, F.M., 2008. Antioxidant status in humans after consumption of blackberry (*Rubus fruticosus* L.) juices with and without defatted milk. J. Agric. Food Chem. 11727–11733.

Hernández-Muñoz, P., Almenar, E., Valle, V., Del Velez, D., Gavara, R., 2008. Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria* × *ananassa*) quality during refrigerated storage. Food Chem. 110, 428– 435.

Hyldgaard, M., Mygind, T., Meyer, R.L., 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3, 1–24.

Jo, W.-S., Song, H.-Y., Song, N.-B., Lee, J.-H., Min, S.C., Song, K.B., 2014. Quality and microbial safety of Fuji apples coated with carnauba-shellac wax containing lemongrass oil. LWT—Food Sci. Technol. 55, 490–497.

Krüger, E., Dietrich, H., Schöpplein, E., Rasim, S., Kürbel, P., 2011. Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit. Postharvest Biol. Technol. 60, 31–37.

McGuire, R., 1992. Reporting of objective color measurements. HortScience 27, 1254–1255.

Miguel, M., 2010. Antioxidant activity of medicinal and aromatic plants. A review. Flavour Fragance J. 2009, 291–312.

NP-3788, 2002. Microbiologia Alimentar. *Regras Gerais Para a Contagem de Microrganismos a* 30 °C. IPQ, Portugal.

Olivas, G.I., Mattinson, D.S., Barbosa-Cánovas, G.V., 2007. Alginate coatings for preservation of minimally processed Gala apples. Postharvest Biol. Technol. 45, 89–96.

Oms-Oliu, G., Rojas-Graü, M.A., González, L.A., Varela, P., Soliva-Fortuny, R., Hernando, M.I.H., Munuera, I.P., Fiszman, S., Martín-Belloso, O., 2010. Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: a review. Postharvest Biol. Technol. 57, 139–148.

Perez-Gago, M.B., Serra, M., Alonso, M., Mateos, M., del Río, M.A., 2005. Effect of whey protein—and hydroxypropyl methylcellulose-based edible composite coatings on color change of fresh-cut apples. Postharvest Biol. Technol. 36, 77– 85.

Piljac-Žegarac, J., Šamec, D., 2011. Antioxidant stability of small fruits in postharvest storage at room and refrigerator temperatures. Food Res. Int. 44, 345–350.

Ponting, J.D., Jackson, R., Watters, G., 1971. Refrigerated apple slices: effects of pH, sulphites and calcium on texture. J. Food Sci. 36, 349–350.

Raybaudi-Massilia, R.M., Mosqueda-Melgar, J., Martín-Belloso, O., 2008a. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. Int. J. Food Microbiol. 121, 313–327.

Raybaudi-Massilia, R.M., Rojas-Graü, M.A., Mosqueda-Melgar, J., Martín-Belloso, O., 2008b. Comparative study on essential oils incorporated into na alginate-based

edible coating to assure the safety and quality of fresh-cut Fuji apples. J. Food Prot. 71,1150–1161.
Re, R., Pellegrini, N., Proteggente, A., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Ribeiro, C., Vicente, A.A., Teixeira, J.A., Miranda, C., 2007. Optimization of edible coating composition to retard strawberry fruit senescence. Postharvest Biol. Technol. 44, 63–70.
Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G. A., Martín-Belloso, O., 2009. Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut Kent mango (*Mangifera indica* L.). Postharvest Biol. Technol. 384–390.
Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G., Martin-Belloso, O., 2013. Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh- cut Kent mangoes. LWT—Food Sci. Technol. 50, 240–246.
Rojas-Graü, M., Avena-Bustillos, R.J., Olsen, C., Friedman, M., Henika, P.R., Martín-Belloso, O., Pan, Z., McHugh, T.H., 2007a. Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate- apple puree edible films. J. Food Eng. 81, 634–641.
Rojas-Graü, M.A., Raybaudi-Massilia, R.M., Soliva-Fortuny, R.C., Avena-Bustillos, R.J., McHugh, T.H., Martín-Belloso, O., 2007b. Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples.
Postharvest Biol. Technol. 45, 254–264.
Rojas-Graü, M.A., Tapia, M.S., Martín-Belloso, O., 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. LWT—Food Sci.
Technol. 41,139–147.
Sacks, E.J., Shaw, D.V., 1993. Color change in fresh strawberry fruit of seven genotypes stored at 0 ºC. HortScience 28, 209–210.
Salmieri, S., Lacroix, M., 2006. Physicochemical properties of alginate/ polycaprolactone-based films containing essential oils. J. Agric. Food Chem. 54, 10205–10214.
Shin, Y., Ryu, J.-A., Liu, R.H., Nock, J.F., Watkins, C.B., 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. Postharvest Biol. Technol. 49,201–209.
Somolinos, M., García, D., Condón, S., Mackey, B., Pagán, R., 2009. Inactivation of Eschericihai coli by citral. J. Appl. Microbiol. 108,1928–1939.
Stannard, C., 1997. Development and use of microbiological criteria for foods. Food Sci. Technol. Today 11,137–177.
Tao, N., Yang, Q.O., Jia, L., 2014. Citral inhibits growth of *Penicillium italicum* by a membrane damage mechanism. Food Control 41, 116–121.
Valero, D., Díaz-Mula, H.M., Zapata, P.J., Guillén, F., Martínez-Romero, D., Castillo, S., Serrano, M., 2013. Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. Postharvest Biol. Technol. 77,1– 6.
Vargas, M., Albors, A., Chiralt, A., González-Martínez, C., 2006. Quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings. Postharvest Biol. Technol. 41,164–171.
Vargas, M., Pastor, C., Chiralt, A., McClements, D.J., González-Martínez, C., 2008. Recent advances in edible coatings for fresh and minimally processed fruits. Crit. Rev. Food Sci. Nutr. 48, 496–511.
Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V.D., Moldão-Martins, M., 2013. Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv Camarosa) under commercial storage conditions. LWT—Food Sci. Technol 52, 80–92.
Vicente, A., Martı'nez, G., Civello, P., Chaves, A., 2002. Quality of heat-treated strawberry fruit during refrigerated storage. Postharvest Biol. Technol. 25, 59– 71.
Vu, K.D., Hollingsworth, R.G., Leroux, E., Salmieri, S., Lacroix, M., 2011. Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries. Food Res. Int. 44, 198–203.
Zúñiga, G.E., Junqueira-Gonçalves, M.P., Pizarro, M., Contreras, R., Tapia, A., Silva, S., 2012. Effect of ionizing energy on extracts of *Quillaja saponaria* to be used as an antimicrobial agent on irradiated edible coating for fresh strawberries. Radiat. Phys. Chem. 81, 64–69.

Chapter VIII

Improving the shelf-life of strawberry fruit with edible coatings enriched with essential oils

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria L Faleiro[2] Maria G C Miguel[1], Maria D C Antunes[1]

[1]Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Submitted

Improving the shelf-life of strawberry fruit with edible coatings enriched with essential oils

Abstract

The increased consumer demand for higher quality food in combination with the environmental need to reduce disposable packaging waste, led to an increased interest in research on edible coatings. In this study, four formulations of edible coatings were used: sodium alginate was tested at 2% with eugenol 0.1% and with citral 0.15% plus eugenol 0.1%; and pectin at 2% with eugenol 0.1%, and Pectin 2% with citral 0.15%. At 0, 5, 10 and 15 d, samples were taken to perform physicochemical and biochemical analysis [colour CIE (L^*,h^o,C^*), firmness, soluble solids content (SSC), weight loss , microbial growth, taste panels, phenol compounds (total phenols, flavonoids, anthocyanins), sugars, organic acids, antioxidant activity, CO_2, ethanol, acetaldehyde and coat cytotoxicity].

Edible coatings were efficient in preserving most sensorial and general quality attributes of fruits while not affecting their antioxidant activity, sugars or organic acids. Edible coatings were not cytotoxic and were efficient in reducing microbial spoilage mainly the alginate ones. Taste panel showed that fruit of control were not suitable for consumption after 14 d storage, while the edible coated could keep that long with good sensorial and nutritional quality. The edible coating which better preserved the overall quality parameters through storage was Alginate 2% + Citral 0.15 % + Eugenol 0.1%.

Key-Words: Strawberries, alginate, pectin, citral, eugenol, storage.

1. Introduction

Post-harvest diseases of fruits and vegetables are a major problem in fresh horticultural produce storage and significantly affect the cost of food production and produce trade (Maftoonazad et al., 2007). Attempts to reduce food losses and maintain the quality of fresh food over a longer period of time has been a priority for the food industry and the use of edible coatings for food applications has attracted great interest as sustainable alternative packaging materials and as a mean of improving food safety and quality (Velickova et al., 2013; Leceta et al., 2015).

Strawberry (*Fragaria* × *ananassa Duch*.) is a non-climacteric fruit and is a relevant source of bioactive compounds due to high levels of vitamin C, vitamin E, β-carotene and phenolic compounds such as anthocyanins, substances related to health benefits (Gol et al., 2013). Strawberry fruit have a very short shelf-life and senescent period due to their susceptibility to mechanical injury, excessive texture softening, physiological disorders and infection caused by several pathogens that can rapidly reduce the quality of fruit, and that make marketing a challenge (Vu et al., 2011).

Pectin and alginate, both belonging to the group of polyuronates, are two characteristic examples of natural ionic polysaccharides undergoing chain–chain association and forming hydrogels upon addition of divalent cations (e.g. Ca^{2+}), both polysaccharides form synergistic mixed gels in the presence of Ca^{2+} (Galus and Lenart, 2012).

The effectiveness against several pathogens of different antimicrobial substances such as lysozyme, nisin, organics acids, essential oils and their derivatives incorporated into the edible films have shown to be satisfactory (Raybaudi-Massilia et al., 2008). Citral and eugenol, which are essential oil constituents, have been used successfully when incorporated into edible coatings (Guerreiro et al., 2015a).

Strawberries are a soft fruit with high respiration and softening rates, making the availability of high quality strawberries challenging. Due to its high metabolism, strawberries must be kept at 4–5ºC, which can extend their high quality for up to 6 or 7 days (Tanada-Palmu and Grosso, 2005). The use of edible coating in strawberries can also be an alternative to improve their shelf life. In a previous work were chosen, among 13, the 4 edible coatings formulation which better preserved through shelf life the main quality parameters in strawberries (2 for pectin and 2 for alginate based coats) (Guerreiro et al., 2015b). The main objective of this research was to evaluate the effect of these 4 edible coatings in maintaining most sensorial and nutritional quality parameters through strawberries shelf-life.

2. Materials and Methods

2.1. Materials

Strawberries were obtained from a local producer (Algarve, Portugal), at the harvest day, then immediately transported to the Postharvest laboratory at the University of Algarve where fruit were selected for uniformity of size and freedom from defects for use in the experiments. Food grade sodium alginate (AL), pectin (PE), citral (Cit), eugenol (Eug) and calcium chloride were purchased from Sigma-Aldrich Chemic, , Germany, and ascorbic acid (AA) from Scharlau, Barcelona, Spain.

2.2. Methods

2.2.1. Edible coatings preparation

The coating forming solutions based on AL and PE, were formulated as described by Rojas-Graü et al. (2007a) and Guerreiro et al. (2015a). The treatments were: Control (non-treated fruit), AL 2% + Eug 0.1%, AL2% + Cit 0.15% + Eug 0.1%,

PE 2% + Eug 0.1% and PE 2% + Cit 0.15 %. Glycerol 1% (w/v) was added to the edible coatings to increase their flexibility, hence avoiding splitting on the coated fruit, and ascorbic acid 1% (w/v) was added as antioxidant agent. $CaCl_2$ 1% (w/v) was used as final dip for cross-linking (Robles-Sánchez et al., 2013; Guerreiro et al., 2015a). The fruits were immersed into the edible coating solution for 2 min, allowed to drip for 30 sec, and immersed again in the calcium chloride solution for 1 min, then dripped again (Guerreiro et al., 2015a, 2015b). Afterwards, 6 randomly strawberries were placed in polypropylene plastic trays (8cm x 10cm x 4 cm), clamshell type, and stored at 0.5 ºC until analyses. On days 0, 5, 10 and 15, three trays per treatment (replications) were taken for quality evaluation.

2.2.2. Colour, firmness, soluble solids content and weight loss

Colour measurements were based on CIE (Commission International de I'Eclairage) L*a*b* scale by using a Minolta Chroma meter CR-300 (ECMinolta, Japan). The L* represents colour lightness (0 = black and 100 = white). Hue was calculated as h^o = arctan (b*/a*) and colour saturation (chroma) as $C^* = (a^2+b^2)^{0.5}$ (McGuire, 1992). The firmness of the pulp was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS50 (Jonh Chatillon&Sons, Inc. USA) using a piston cylinder of 4 mm diameter at a depth of 7 mm. The soluble solids content (%) was measured using a digital refractometer PR1ATAGO CoLTD (Japan), in the strawberry juice. Weight loss was expressed as percentage of initial weight.

2.2.3. Microbial counts

Microbial counts were determined for each treatment. The microbial parameters determined included counts of aerobic mesophilic microorganisms and molds and

yeasts. The counts of aerobic mesophilic microorganisms were done according to the standard Portuguese NP-3788 (2002) using the Plate Count Agar medium (Biokar, Paris, France). The counts of molds and yeasts were performed according to ISO 21527-2:2008 using Dicloran Rose-Bengal Cloranfenicol Agar (Biokar, Paris, France). Sample preparation was done as reported before (Guerreiro et al., 2015b). Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram fresh weight.

2.2.4. Sensory Evaluation

A taste panel was performed with 15 panellists on the base of a 7-point hedonic scale (1-bad; 7-excellent) for the sensory parameters: Appearance, aroma, texture, sweetness, acidity, flavour and overall acceptance. All parameters were evaluated at harvest and after 7 and 14 d storage.

2.2.5. Total phenols content

Total phenols content was determined according to the Folin–Ciocalteu colourimetric method Singleton and Rossi (1965) modified for microplates. The sample (80 μL) and 20 μL of sodium carbonate (75 g/L) were added to 100 μL of 10% (w/v) Folin–Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm (Tecan Infinite M200, Swiss). Gallic acid was used as standard for the calibration curve.

2.2.6. Flavonoids content

The content of these groups of compounds was quantified as described by Miguel et al. (2010) and modified for using microplates of 96 wells (Miguel et al.,

2010). Sample or standard (100 µL) was added to 100 µL of 2% $AlCl_3$ ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm (Tecan Infinite M200, Swiss). Quercetin was used as a standard for the construction of the calibration curve.

2.2.7. Anthocyanins

The total anthocyanin content was measured by using a modified pH differential method (Lee et al., 2005). Absorbance at 520 nm and 700 nm in different pH buffers (pH 1.0 and 4.5) were measured, respectively. Absorbance readings were converted to total mg of cyanidin 3-glucoside per 100 g fresh weight of sample using the molar extinction coefficient of 26,900 and absorbance of A. Anthocyanin pigment concentration was, therefore, expressed as Cyanidin-3-glucoside equivalents, as follows:

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

In which:

$A=(A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm}-A_{700nm})_{pH4.5;}$ MW=449.2 g/mol=value of the molecular weight of Cyanidin-3-glucoside; DF=dilution factor; E=26,900 molar extinction coefficient for Cyanidin-3-glucoside, in L x mol^{-1} x cm^{-1};10^3=factor for conversion from g to mg;1=path length in cm.

2.2.8. Trolox Equivalent Antioxidant Activity (TEAC)

The antioxidant activity was measured according to Re et al. (1999), modified for microplates. Strawberry juice was obtained after squeezing apple slices flesh with an UltraTurrax T 18 (IKA, Germany) for 2 min, then centrifuge for 5 min at 5000 rpm. For the assay, 3 µL of strawberry juice was added to 197 µL of *2,2'-azinobis-(3-*

ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation solution). The absorbance was monitored at 750 nm for 6 min (Tecan Infinite M200, Swiss). The antioxidant activity of each sample was calculated by the equation: scavenging effect (SE %) = (1-As/Ao) x 100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 min. The values were then compared with the curve for several Trolox (*6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid*) concentrations and the values given as mM Trolox equivalent antioxidant capacity per 100g fresh weight.

2.2.9. Oxygen Radical Absorbance Capacity (ORAC)

The antioxidant activity by the method ORAC, gives the ability of samples for scavenging peroxyl radicals. The ORAC method used, with fluorescein (FL) as the fluorescent probe, was that described by Ou et al. (2001). As the ORAC assay is extremely sensitive, the samples must be diluted appropriately before analysis to avoid interference. In each well, 150 μL of fluorescein working solution and 25 μL strawberry juice, blank (75 mM phosphate buffer), or standard (Trolox) were placed. The plate was covered with a lid and incubated at 37 °C for 10 min with a previous shaking of 3 min (Tecan Infinite M200, Swiss). The *2,2'-Azobis-2-methyl-propanimidamide, dihydrochloride* (AAPH) was added to each well of the plate, except for the control and blank. The final volume of the assay was 200 μL. The fluorescence was read every minute for 90 min at excitation of 485 nm and emission of 527 nm. The ORAC values were calculated according to a previous work Prior and Cao (1999). Briefly, the net area under the curve (AUC) of the standards and samples was calculated. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using

the regression equation between Trolox concentration and the net AUC and were expressed as mmol Trolox/100 fresh weight.

2.2.10. DPPH Assay

For the antioxidant activity measurements by the DPPH method, fifteen microliters of strawberry fruit juice were added to 185 µL of 60 µM methanolic solution of DPPH (Brand-Williams et al., 1995). Absorbance measurements were read at 517 nm, after 30 min of incubation time at room temperature (A1). Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control (A0). The percentage inhibition [(A0–A1/A0)*100] was compared with the curve for several Trolox concentrations and the values given as mM Trolox equivalent antioxidant capacity.

2.2.11. Extraction, quantification of sugars and sweetness index

Extraction and quantification of sugars (fructose, glucose and sucrose) was based on a method described by Terry et al. (2007) and modified as described in Magwaza et al. (2012). Briefly, 150 ± 0.5 mg of fruit powder were extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentrations of fructose, glucose and sucrose were determined in an HPLC binary pump system (L-2130, Elite LaChrom series, Hitachi, Japan). Ten microliters of a diluted sample solution (1:10) were injected 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). The column compartment temperature was set at 35ºC. The mobile phase used was HPLC-grade water at a 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). Sugars have different sweetness impact. Since sucrose is 1.35 times sweeter than glucose and fructose is 2.3 times sweeter than glucose, a sweetness index concept was used to estimate the total sweetness perception. Glucose was assigned a sweetness value of one, sucrose 1.35 and fructose 2.3 (Qian, 2005; Keutgen and Pawelzik, 2007). Total sweetness index = 1 glucose + 1.35 sucrose + 2.3 fructose.

2.2.12. Extraction and quantification of non-volatile organic acids

Non-volatile organic acids (citric, ascorbic, malic, quinic, oxalic and shikimic acid) were extracted and determined using a method described by Crespo et al.(2010) with modifications (Magwaza et al., (2013). Briefly, 50 ± 0.5 mg of freeze dried samples were cold extracted for 5 min in 3 mL of HPLC water. The flocculate was filtered through a 0.2 µm syringe filter before HPLC analysis. Citric, ascorbic, malic, tartaric and oxalic acid concentrations were determined in a HPLC binary pump system equipped with a diode array detector (DAD, L-2455, Elite LaChrom series, Hitachi,Japan) with multiple wavelength detector, degasser and cooled auto-sampler. The filtered sample extract (20 µL) was injected into a Purospher Star RP-18 column (4.6 mm diameter × 250 mm, 5 µm particle size; Merck Millipore, Germany) with an organic acid guard column (LiChroCART 4-4 Merck Millipore, Germany). Temperature of the column was set at 35 ºC in a column compartment (L-2300, Elite LaChrom series, Hitachi,Japan). The mobile phase used was 0.2% HPLC-grade aqueous metaphosphoric acid at a flow rate of 1.0 mL/min. Non-volatile organic acids were detected at 210 nm except for ascorbic acid which was detected at 240 nm and quantified by using linear standard curves (0.01–2.5 mg/mL).

2.2.13. Ethanol and Acetaldehyde quantification

For the quantification of the ethanol existing in samples was used the kit K-ETOH 02/11 Megazyme (Ireland), specific for the determination of ethanol, according to manufacturer instructions. Absorbance was measured by spectrophotometry using a microplate reader (Tecan Infinite M200, Swiss) at 340nm. The quantification of acetaldehyde was determined by the kit K-ACHYD Megazyme (Ireland), specific for the determination of acetaldehyde, according to manufacturer instructions. The absorbance was measured by spectrophotometry at 340nm (Tecan Infinite M200, Swiss).

2.2.14. Respiration rate

Respiration rate was calculated as CO_2 production (µmol/g/s) in the gas phase of a jar, connected to a Li-6400 portable (Li-Cor) IRGA, where was used a flow rate of 0.5µmol/s, and reads for 5 min.

2.2.15. Cells Culture and Cytotoxicity

THP-1 and Caco-2 cells were kept in 10 mL dishes at 5% CO_2 in Dulbecco's Modified Eagle Medium (1000 mg/ml glucose, 110 mg/ml pyruvate, and 580 mg/ml glutamine) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 100 µ/ml penicillin, and 100 µg/ml streptomycin.

MTT is a standard colorimetric assay for measuring the activity of enzymes that reduce yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan in living cells (Klewicka et al., 2012). Cytotoxicity was determined using the method described by Girón-Calle et al. (2010) with slight modifications. In

our study we analyse the cytotoxicity only in the coatings formulations, without fruits, at 1, 4 and 6 days (Girón-Calle et al., 2010). Cells in 96 well microplates were exposed to MTT by addition of fresh medium containing the reagent, so that the final concentration was 0.5 mg/ml, and were incubated for 1 h in the CO_2 incubator. Reduced MTT was solubilized by addition of the same volume of 0.1 N HCl in isopropanol. Absorbance at 570 nm with a background reference wavelength of 630 nm was measured using a plate reader and calculated according the follows equations:

$$\%cell\ viability = \frac{Sample\ O.D}{Control\ O.D} \times 100$$

$$\%cytotoxicity = 100 - \%cell\ viability$$

2.2.16. Statistical Analysis

Statistical analysis was carried out with the SPSS 20.0 software (SPSS Inc.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among treatments through storage dates was performed.

3. Results

3.1. Quality parameters

Strawberry colour is a very important attribute for consumer product acceptance. Lightness (L*) increased through storage at 0.5 ºC (Table 1), meaning that peel fruit colour become whiter. However, in coated fruit the increase was statistically significant from 0 to 5 d, while in control was from 0 to 5 and from 5 to 10 d. Nevertheless, with the exception of 5 d where PE 2% + Cit 0.15% was significantly lower than AL 2% + Cit 0.15% + Eug 0.1%, no significant differences were found among treatments. Hue value did not show significant changes through storage in control, AL 2% + Eug 0.1% and PE 2% + Cit 0.15%, while it decreased from 0 to 5 d in AL 2% + Cit 0.15% + Eug

0.1% and from 0 to 10 d in PE2% + Eug 0.1% (Table 1). When comparing treatments in each sampling time, Hue values behaved similarly to L*. Chroma did not significantly change in control, while decreased through storage in all edible coatings treatments (Table 1). Nevertheless, as for the other colour parameters, differences among treatments did not exist, with the exception of the 10th d. From the results of this experiment we can conclude that the edible coatings of this experiment did not influence colour changes for up to 15 d storage.

The firmness, one of the main factors affecting storage ability of fruit, did not show significant changes through storage in control fruit, but increased significantly from 0 to 10 d in Eug edible coatings and from 0 to 5 d in the other edible coating treatments (Table 1). This increase may be related to the properties of the edible coating material to coating drying. There were no significant differences among treatments during the experiment, except after 5 d in which AL 2% + Eug 0.1% had significantly higher firmness than control.

Changes in the SSC of strawberries over storage time are shown in Table 1. The SSC values were maintained stable until day 10 and then significantly decreased up to 15 d in all treatments. However, values for control did not significantly differ from the ones at harvest. This may be due to the starting process of senescence. Nevertheless no significant changes in SSC were found among treatments although at the end of storage control had SSC values similar to the ones at harvest.

Strawberries showed a progressive loss of weight during storage (Table 1). There was no significant differences among treatments up to 5 d, while at 10 and 15 d weight loss was significantly higher in AL + Eug 0.1% than in the other treatments.

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration. There was no microbial growth in none fruit treatment at the beginning of

the experiment (Table 1). In control fruit, either yeasts and molds or mesophilic microorganisms increased from 0 to 5 d storage then remained constant. For yeasts and molds there was a significant increase from 0 to 5 d in all edible coatings except for PE 2% + Cit 0.15%, in which the increase was significant from 5 to 10 d. Nevertheless, edible coatings with AL significantly reduced yeasts and molds counts and at the end of the experiment their growth was not observed. Generally, edible coatings did reduce yeasts and molds counts in comparison to control, being the best AL 2% + Eug 0.1% and AL 2% + Cit 0.15% + Eug 0.1%. All edible coatings from 0 to 5 d, except PE 2% + Cit 0.15% showed increasing counts of mesophilic microorganisms. PE 2% + Cit 0.15% edible coating totally inhibited the microbial growth during all the experiment (Table 1). Edible coatings were efficient in controlling mesophilic microorganisms through storage in comparison to control being PE 2% + Cit 0.15% the best followed by AL 2% + Cit 0.15% + Eug 0.1%.

3.2. Phenolics contents (Total phenols, flavonoids and anthocyanins)

Total phenols almost did not change trough storage in any treatment (Table 2). However, significant differences among treatments were observed, being PE 2% + Cit 0.15% significantly higher than AL 2% + Cit 0.15% + Eug 0.1% after 5 d storage.

Flavonoid content determined as quercetin based flavonoids, almost did not change up to 10 d storage in all treatments, but significantly decreased from 10 to 15 d (Table 2). Generally, values were higher in PE 2% + Cit 0.15% and lower in AL edible coatings.

Table 1 Color parameters (L*, hº, C*),firmness (N), soluble solids content (SSC), weight loss, molds and yeasts and aerobic mesophilic microorganisms in strawberries covered with different alginate and pectin based edible coating formulations during storage at 0.5ºC. Values represent the mean of three replicates ± standard error taken at 0, 5, 10 and 15 days.

Quality Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15% + Eug 0.1%		PE 2% + Eug 0.1%		PE 2% + Cit 0.15%	
Lightness (L*)	0	31.0±1.2	cA	33.3±2.7	bA	33.1±2.7	bA	34.7±2.0	bA	33.0±1.5	bA
	5	36.5±0.3	bAB	38.1±0.5	aAB	39.0±0.5	aA	37.9±1.4	abAB	35.5±1.0	abB
	10	40.9±1.5	aA	40.5±0.7	aA	40.2±0.7	aA	40.9±0.6	aA	38.2±1.0	aA
	15	37.1±0.4	bA	39.6±0.2	aA	39.1±0.2	aA	39.6±0.5	aA	38.4±1.3	aA
Hue (hº)	0	29.6±0.7	aA	30.5±1.2	aA	33.4±1.2	aA	32.1±2.4	aA	31.1±1.8	aA
	5	32.6±1.9	aA	28.2±1.8	abAB	27.6±0.5	bAB	28.1±2.2	abAB	26.4±1.8	aB
	10	26.9±2.1	aA	25.3±0.6	bA	23.8±1.2	bA	23.4±0.5	bA	25.9±1.2	aA
	15	27.1±1.9	aA	26.6±1.3	abA	25.9±1.8	bA	22.4±0.9	bA	27.7±1.7	aA
Chroma (C*)	0	32.2±2.9	aB	39.1±0.7	aA	40.1±2.0	aA	39.8±2.7	aA	41.6±1.6	aA
	5	36.3±2.4	aA	33.0±1.0	bA	33.8±0.5	bA	33.7±1.6	bA	33.1±1.2	bA
	10	30.1±0.5	aAB	29.4±0.3	cAB	30.4±0.9	bcAB	27.2±1.9	abB	32.5±0.5	bA
	15	29.9±1.6	aA	28.8±1.2	cA	29.0±0.9	cA	31.1±0.6	cA	29.9±2.2	bA
Firmness (N)	0	11.2±1.9	aA	12.3±1.9	bA	11.4±0.8	bA	10.1±1.0	bA	9.8±1.0	bA
	5	10.7±0.9	aB	14.3±1.0	abA	13.6±0.6	aAB	13.2±1.5	abAB	11.5±1.0	bAB
	10	13.5±1.0	aB	16.6±0.5	aA	14.2±0.6	aAB	15.6±0.6	aAB	14.0±0.2	aAB
	15	12.9±1.9	abA	13.6±1.2	abA	12.8±0.5	abA	13.0±1.7	abA	14.9±0.3	aA
SSC (ºBrix)	0	3.6±0.4	abA	3.8±0.4	aA	3.6±0.0	aA	4.0±0.1	aA	3.9±0.1	aA
	5	3.9±0.2	aA	4.0±0.1	aA	3.9±0.2	aA	4.2±0.2	aA	4.0±0.1	aA
	10	4.2±0.2	aA	4.0±0.1	aA	3.8±0.1	aA	3.8±0.1	abA	3.8±0.1	aA
	15	3.1±0.1	bA	3.0±0.3	bA	3.1±0.2	bA	3.4±0.1	bA	3.2±0.2	bA
Weight Loss (%)	0	0.0±0.0	dA	0.0±0.0	bA	0.0±0.0	cA	0.0±0.0	bA	0.0±0.0	dA
	5	2.4±0.3	cA	3.5±0.6	aA	3.3±0.7	bA	5.0±1.5	aA	3.4±0.1	cA
	10	3.7±0.1	bB	6.4±1.1	aA	4.5±0.8	abAB	6.5±1.5	aA	5.3±0.2	bA
	15	5.2±0.1	aB	8.0±2.0	aA	5.7±0.8	aB	7.7±1.5	aA	7.2±0.1	aAB
Moulds and Yeasts (Log $_{10}$ CUF/g)	0	0.0±0.0	bA	0.0±0.0	bA	0.0±0.0	bA	0.0±0.0	bA	0.0±0.0	bA
	5	1.3±0.3	aAB	0.7±0.2	aBC	0.5±0.1	aC	1.7±0.0	aA	0.3±0.3	bC
	10	0.8±0.1	aA	0.3±0.1	bB	0.2±0.2	bB	1.2±0.1	aA	1.2±0.4	aA
	15	0.9±0.1	aA	0.0±0.0	bB	0.0±0.0	bB	1.0±0.0	aA	0.6±0.3	abAB
Mesophilic Microorganisms (Log $_{10}$ CUF/g)	0	0.0±0.0	bA	0.0±0.0	cA	0.0±0.0	bA	0.0±0.0	bA	0.0±0.0	aA
	5	1.7±0.0	aA	0.9±0.0	aB	0.5±0.2	aC	0.9±0.1	aB	0.0±0.0	aD
	10	1.7±0.0	aA	0.4±0.2	bC	0.0±0.0	bD	0.9±0.0	aB	0.0±0.0	aD
	15	1.8±0.0	aA	0.7±0.0	abB	0.4±0.1	aC	0.7±0.0	aB	0.0±0.0	aD

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Anthocyanin content behaved similarly to flavonoids through storage for each treatment (Table 2). When comparing treatments, just after treatment application, control and AL 2% + Eug 0.1% were significantly lower than AL 2 % + Cit 0.15% + Eug 0.1% and PE 2% + Cit 0.1%. After 10 d all treatments were similar and after 15 d PE treatments were significantly lower than control and AL 2 % + Cit 0.15% + Eug 0.1%.

3.3. Antioxidant activity

Antioxidant capacity has been used to evaluate the antioxidant potential status of tissue, which is a function of the type and amount of bioactive compounds present. When measuring antioxidant capacity of strawberries by the methods ORAC and TEAC, no significant changes were observed through storage time or among treatments (Table 2). However, when using the DPPH method, edible coatings showed significantly higher values than control being, at the end of storage, AL 2 % + Cit 0.15% + Eug 0.1% the best treatment.

3.4. Respiration rate, ethanol and acetaldehyde

Ethylene production and acetaldehyde of the strawberries of this experiment were not detected in none treatment. The respiration rate calculated by the CO_2 production, showed no significant changes among treatments up to 5 d storage (Table 2). After 10 d control showed significantly lower CO_2 production and at the end of storage was PE 2% + Cit 0.15% that had the lowest values.

The values of ethanol were similar at the beginning of the experiment and increased significantly from 0 to 5 d storage in all treatments (Table 2). Then they decrease from 5 to 10 d and increased again in day 15 for control and PE coatings, while decreased and remained constant in AL coatings. However, at the end of storage there were not significant differences among treatments.

Table 2 Total phenols, flavonoids, anthocyanins, antioxidant activity, ethanol and CO_2 production of strawberries covered with different alginate and pectin based edible coating formulations during storage at 0.5ºC. Values represent the mean of three replicates ± standard error, taken at 0, 5, 10 and 15 days.

Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15% + Eug 0.1%		PE 2% + Eug 0.1%		PE 2% + Cit 0.15%	
Total Phenols (mg of Gallic acid/100g^{-1} FW)	0	157.22±12.18	cA	157.52±10.37	aAB	152.15±6.54	aB	181.28±5.05	aA	173.21±5.04	bcAB
	5	187.76±4.80	bAB	176.68±7.79	aAB	156.96±13.23	aB	182.05±4.81	aAB	187.90±6.60	abA
	10	143.42±7.11	aA	172.75±6.79	aAB	169.09±5.31	aB	191.69±4.91	aA	192.18±5.27	abA
	15	136.41±5.97	bA	132.41±22.29	aAB	115.63±13.10	bB	121.99±7.76	bAB	160.21±4.83	bA
Flavonoids (mg of Quercitin .100g^{-1} FW)	0	11.55±2.05	aA	7.94±1.01	aB	8.86±0.76	aB	12.78±0.50	bA	13.74±0.82	aA
	5	15.16±0.16	aA	11.00±0.50	aB	7.41±0.88	aC	16.01±0.70	aA	12.97±0.79	aB
	10	14.03±1.33	aA	9.04±0.87	aB	9.29±0.53	aB	14.24±0.74	abA	12.11±1.07	aA
	15	7.95±0.39	aA	6.53±2.59	aAB	4.67±0.77	bAB	3.51±0.31	cB	7.36±0.14	aA
Anthocyanins (mg of Cyanidin 3-O-glucoside .100g^{-1} FW)	0	234.92±35.01	aB	243.86±27.56	abB	340.88±30.69	aA	282.92±31.78	bAB	343.00±16.18	aA
	5	295.53±32.83	aA	292.60±14.61	aB	293.41±32.85	aB	410.68±20.58	aA	281.42±27.74	abB
	10	287.18±22.08	aAB	244.57±31.30	abA	293.13±23.31	aA	278.31±31.93	bA	221.62±21.97	bA
	15	180.89±35.14	aA	150.83±39.90	bAB	158.19±31.93	bA	113.03±11.96	cB	103.96±7.90	cB
ORAC (mm TE.100g^{-1} FW)	0	4.27±0.13	aA	4.42±0.01	aA	4.41±0.01	aA	4.41±0.05	aA	4.42±0.02	aA
	5	4.54±0.10	aB	4.40±0.01	aAB	4.40±0.02	aAB	4.34±0.05	aB	4.38±0.01	aAB
	10	4.35±0.03	aB	4.44±0.03	aA	4.41±0.04	aA	4.43±0.05	aA	4.37±0.03	aA
	15	4.38±0.02	abA	4.41±0.02	aA	4.39±0.01	aA	4.42±0.00	aA	4.40±0.02	aA
TEAC (mm TE.100g^{-1} FW)	0	37.91±0.97	abA	34.12±3.79	aB	34.72±2.44	aB	37.84±1.99	aAB	47.80±6.62	aA
	5	32.68±0.67	aA	39.43±2.79	aA	34.72±1.91	aA	45.61±11.31	aA	39.70±5.13	aA
	10	36.84±1.94	aA	32.02±2.26	aA	34.51±3.81	aA	36.01±4.41	aA	33.24±0.78	aA
	15	33.37±3.75	bA	37.05±3.70	aA	38.30±5.85	aA	33.04±2.11	aA	32.28±3.80	aA
DPPH (mm TE.100g^{-1} FW)	0	161.80±7.54	dA	322.15±41.09	aA	280.81±28.15	aA	294.58±21.17	aA	240.17±13.35	abAB
	5	145.78±13.22	cA	262.05±44.48	abA	197.73±43.67	abAB	107.52±2.90	bB	294.41±33.82	aA
	10	170.99±24.02	bB	165.11±42.60	bAB	127.06±17.59	bAB	94.51±12.44	bB	94.49±3.09	cB
	15	96.08±12.58	aB	182.71±10.79	bB	307.71±42.29	aA	211.33±46.09	aAB	154.86±43.29	bcB
Ethanol (mg.100g^{-1} FW)	0	12.79±4.73	bA	10.00±3.38	bA	10.38±1.27	bA	11.47±4.74	bA	13.68±3.20	bA
	5	45.75±8.46	aAB	25.57±5.07	aB	22.12±3.05	aB	31.16±9.36	aAB	42.64±9.29	aA
	10	22.39±1.10	aA	21.03±5.72	aA	22.84±5.04	aA	18.37±5.82	bA	19.67±3.67	bA
	15	27.67±2.53	aA	23.21±6.91	aA	25.21±3.80	abA	31.57±7.88	aA	37.63±9.59	aA
CO_2 (µmol.g.seg^{-1})	0	21.10±0.64	bA	22.50±2.27	bA	23.00±0.12	cA	21.20±0.91	cA	22.77±0.81	cA
	5	23.33±0.47	aA	25.63±2.21	abA	29.07±2.45	bA	25.37±0.47	bA	26.73±0.96	bA
	10	22.80±0.31	aA	29.53±1.70	aB	36.27±0.26	aA	34.23±0.88	aA	33.93±1.31	aA
	15	21.13±0.45	aA	21.57±0.57	bAB	23.73±0.12	cA	22.77±0.38	cAB	18.67±0.83	dB

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.5. Non- volatile organic acids and non-structural carbohydrates

Non-volatile organic acids are natural components of many fruit and vegetables. Non-volatile organic acids found in our study were oxalic, malic, ascorbic, citric, quinic and shikimic acids (Table 3). Citric and quinic acids were the major non-volatile organic acids. Citric acid increased through storage in all treatments except AL 2% + Eug 0.1% which remained constant (Table 3). Malic acid behaved similarly but the exception was PE2% + Cit 0.15% (Table 3). Shikimic acid did not show differences through storage in edible coated fruit but increased in control (Table 3). In spite of some changes through storage, at the end of the experiment there were not significant differences among treatments in those organic acids. Oxalic acid increased through storage in all treatments except PE 2% + Cit 0.15% which remained constant (Table 3). At the end of storage there were no significant differences among treatments. Ascorbic acid increased through storage in all treatments and was higher in AL 2% + Cit 0.15% + Eug 0.1% and PE edible coatings than control and AL 2% + Eug 0.1%.

Nonstructural carbohydrates were equally affected by treatments. Fructose did not change trough storage except in Al 2% + Cit 0.15% + Eug 0.1% which increased from 0 to 5 d storage (Table 3). With the exception of day 5, there were no significant differences among treatments. Glucose behaved similarly to fructose but the increase was in control and Al 2% + Cit 0.15% + Eug 0.1% (Table 3). No significant differences among treatments were observed at the end of the experiment. Sucrose did not show significant differences over storage in AL 2% + Eug 0.1%, but decreased in control from 5 to 10 d and from 10 to 15 d in the other treatments (Table 3). The sweet index decreased in control from 5 to 10 d, increased in AL 2% + Cit 0.15% + Eug 0.1% from 0 to 5 d and was constant through time in the other treatments (Table 3). Nevertheless,

no significant differences among treatments were observed after 10 days until the end of the experiment.

Table 3 Organic acids and sugars of strawberries covered with different alginate and pectin based edible coating formulations during storage at 0.5ºC. Values represent the mean of three replicates ± standard error taken at 0, 5, 10 and 15 days.

Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15% + Eug 0.1%		PE 2% + Eug 0.1%		PE 2% + Cit 0.15%	
Oxalic Acid (mg.100g^{1} DW)	0	1105±124	cA	754±48	bB	854±47	cB	1332±77	aA	1330±201	aA
	5	711±134	bAB	1471±112	aA	1284±160	bA	1177±150	aA	1364±91	aA
	10	949±176	aA	1233±104	aAB	1255±42	bAB	788±123	bC	1630±173	aA
	15	1348±89	bA	1177±109	aA	1472±117	aA	1365±88	aA	1194±91	aA
Malic Acid (mg.100g^{1} DW)	0	209±13	aA	213±12	bC	561±46	aA	395±37	aB	296±18	aC
	5	279±47	aA	267±6	abA	320±38	bA	356±65	abA	345±46	aA
	10	383±70	aA	314±73	abAB	327±54	bAB	213±26	bB	429±53	aA
	15	420±55	aA	391±34	aA	283±25	bA	332±56	abA	353±53	aA
Ascorbic Acid (mg.100g DW)	0	168±6	aB	184±4	bB	195±12	bB	237±12	bA	190±15	bB
	5	199±10	aA	211±13	bBC	284±8	aAB	285±33	bAB	325±38	aA
	10	274±41	aAB	210±45	bB	303±33	aAB	258±10	bAB	355±30	aA
	15	262±30	aA	277±14	aB	355±38	aAB	414±37	aA	390±27	aA
Citric Acid (mg.100g^{1} DW)	0	2066±171	aA	2601±114	aAB	2844±203	bA	1915±198	bC	1921±158	bC
	5	1933±95	aB	2617±62	aB	2650±209	bB	3244±247	aA	2992±193	aAB
	10	3329±188	aB	2489±305	aBC	3085±225	abAB	1788±144	bC	3471±297	aA
	15	3639±215	abA	3048±293	aA	3655±173	aA	3221±361	aA	3311±261	aA
Quinic Acid (mg/100g^{1} DW)	0	1512±67	abA	2825±488	aB	3297±139	aA	3498±164	aA	3400±49	aA
	5	2594±112	aA	3071±88	aAB	3171±397	aAB	3335±37	aAB	3456±281	aA
	10	3026±651	aA	3349±399	aA	3232±235	aA	2454±104	bA	3466±249	aA
	15	3179±171	bA	3103±317	aA	3699±130	aA	3612±357	aA	3322±126	aA
Shikimic Acid (mg.100g^{1} DW)	0	54.8±1.6	dA	52.4±1.1	aAB	48.6±1.3	aB	51.3±1.2	aAB	48.3±2.4	aB
	5	47.1±0.3	cA	47.2±0.6	aA	46.9±2.1	aA	48.7±2.4	aA	53.1±2.8	aA
	10	49.7±5.4	bB	50.5±4.9	aA	48.8±2.9	aA	42.2±1.2	aA	52.1±4.5	aA
	15	50.4±3.3	aB	48.7±4.0	aA	47.9±0.7	aA	50.7±5.9	aA	49.5±2.8	aA
Fructose (mg.g^{1} DW)	0	3.5±0.1	bA	3.3±0.3	aA	3.1±0.1	bA	3.3±0.1	aA	3.3±0.1	bA
	5	3.7±0.0	aAB	3.8±0.1	aA	3.7±0.0	aAB	3.6±0.0	aB	3.7±0.0	aAB
	10	3.5±0.1	aA	3.5±0.0	aA	3.6±0.0	aA	5.0±1.5	aA	3.5±0.1	abA
	15	3.6±0.1	aA	3.7±0.1	aA	3.7±0.1	aA	3.8±0.2	aA	3.6±0.1	abA
Glucose (mg/g^{1} DW)	0	4.5±0.1	bA	4.3±0.3	aA	4.1±0.2	bA	4.2±0.2	aA	4.2±0.1	aA
	5	4.9±0.0	aA	5.0±0.1	aA	4.8±0.1	aA	4.5±0.1	aB	4.2±0.0	aC
	10	5.0±0.2	aA	4.8±0.2	aAB	5.1±0.1	aA	4.7±0.1	aAB	4.4±0.1	aB
	15	4.4±0.0	aA	4.5±0.0	aA	4.4±0.1	bA	4.4±0.2	aA	4.2±0.1	aA
Sucrose (mg.g^{-1} DW)	0	5.3±0.1	aA	4.2±0.2	aB	4.1±0.3	aB	4.6±0.2	aB	4.4±0.1	aB
	5	4.6±0.2	aA	3.8±0.2	aB	4.5±0.2	aA	4.5±0.1	aA	4.8±0.0	aA
	10	3.3±0.6	aA	4.0±0.5	aAB	3.8±0.3	aAB	4.7±0.2	aA	4.2±0.2	aAB
	15	3.2±0.1	aA	3.3±0.1	aA	2.7±0.3	bA	3.3±0.4	bA	3.3±0.3	bA
Sweet Index (SI)	0	19.8±0.4	aA	17.6±1.4	aAB	16.8±0.9	bB	18.0±0.8	aAB	17.7±0.2	bAB
	5	19.6±0.2	aA	18.9±0.1	aBC	19.3±0.2	aAB	18.7±0.2	aC	19.2±0.1	aABC
	10	17.6±1.0	aA	18.2±0.8	aA	18.6±0.5	aA	21.1±2.0	aA	18.0±0.4	abA
	15	17.0±0.3	aA	17.5±0.2	aA	16.4±0.2	bA	17.6±1.1	aA	17.0±0.7	bA

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

3.6. Sensory evaluation

Results of the sensory evaluation of strawberries are shown in Table 4. Control fruit were not suitable for consumption after 14 d storage since panelists gave scores under 4, the minimum acceptable for marketing. All edible coatings had acceptable quality up to 14 d storage. Better scores as overall liking were in AL 2% + Eug 0.1%, followed by PE 2% + Cit 0.15%, AL 2% + Cit 0.15% + Eug 0.1% and PE 2% + Eug 0.1%.

Table 4 Taste panel of strawberries covered with different alginate and pectin based edible coating formulations during storage at 0.5ºC. Values represent the mean of 15 replicates ± standard error, taken at 0, 7 and 14 days.

Treatment	Days	Appearance	Aroma	Texture	Sweetness	Acidity	Flavour	Overall Liking
Control	0	6.4	5.3	6.3	6.3	6.0	6.3	6.1
	7	4.4	4.2	4.6	4.3	4.4	4.1	4.3
	14	1.8	2.6	3.4	3.4	3.0	2.0	2.7
AL 2% + Eug 0.1%	0	6.4	5.3	6.3	6.3	6.0	6.3	6.1
	7	5.8	4.7	5.5	5.2	4.8	5.7	5.3
	14	4.0	4.6	5.6	4.8	4.4	4.4	4.6
AL 2% + Cit 0.15% + Eug 0.1%	0	6.4	5.3	6.3	6.3	6.0	6.3	6.1
	7	6.0	4.5	4.0	4.5	4.2	5.7	4.8
	14	4.2	4.0	3.8	4.0	3.6	4.0	4.1
PE 2% + Eug 0.1%	0	6.4	5.3	6.3	6.3	6.0	6.3	6.1
	7	4.8	4.0	3.7	3.8	3.9	4.3	4.1
	14	3.4	3.8	4	4.2	3.6	3.2	3.7
PE 2% + Cit 0.15%	0	6.4	5.3	6.3	6.3	6.0	6.3	6.1
	7	5.8	4.6	4.4	4.6	4.8	5.6	5.0
	14	4	4.4	3.6	4.6	4.8	4.2	4.3

3.7. Cytotoxicity

The cytotoxic properties of the formulations used for coating the fruits were evaluated on the THP-1 and CaCo-2 cancer cells. No coating showed cytotoxicity, being values of cell viability around 100% in THP-1 and 80-100% in CaCo-2.

4. Discussion

Generally the edible coatings of this experiment did not significantly influenced colour. According to our work, Del-Valle et al. (2005) coated strawberries with cactus-mucilage edible coating, and found that coating neither modify the original colour of strawberries, nor delayed browning in storage at 5 ºC. On the other hand, Hernández-Muñoz et al. (2008) using chitosan but higher temperature storage (10 ºC), found that uncoated fruit were significantly darker than coated fruit throughout storage and chitosan concentration of the coating solution gave rise to significant differences in fruit colour. The same authors report that colour changes in strawberry fruit are greatly influenced by storage temperature and it is expected that colour differences between control and coated strawberries to be more accentuated in fruit stored at higher temperatures. Ribeiro et al. (2007) found no effect of starch, chitosan or carrageenan edible coatings on color of strawberries stored at 0 to 5 ºC and Velickova et al. (2013) found lower darkening in coated strawberries than in uncoated controls stored at 20 ºC. According to our results and the referred above, it seems that the low temperature effect overcome the effect of the edible coatings for color development.

The loss of firmness is one of the main factors limiting quality and the postharvest shelf-life of strawberries, which soften rapidly through ripening, mainly through degradation of the middle lamella of the cell wall of cortical parenchyma cells (Fan et al., 2009). The edible coatings of the present experiment did increase firmness while control did not show significant differences through storage probably due to the characteristics of the edible coatings by themselves. In fact, edible coated fruit were immersed in $CaCl_2$ solution for crosslink edible coatings. The beneficial effect of calcium on firmness retention is widely known (Antunes et al. (2010, 2012), Hernández-Muñoz et al. (2008) and Ribeiro et al. (2007) attributed the beneficial effect

of their coatings to the use of calcium on them. In our case, the increase in firmness in edible coated fruit could be attributed to calcium since control did not have any dip.

The beneficial effect of the coating applications on the strawberry firmness were reported for other authors using coatings prepared from cactus mucilage, chitosan-oleic acid coatings, chitosan and chitosan-beeswax (Del-Valle et al., 2005; Vargas et al., 2006; Hernández-Muñoz et al., 2008; Velickova et al., 2013). Nevertheless, in our case, when comparing treatments in each sampling time, although higher values were found in edible coatings tan control, differences were not significant, whit the exception of AL 2% + Eug 0.1% which was significantly higher than control up to 10 d storage and without significant differences at the end of storage. This suggests the good effect of edible coatings on firmness, which is decreasing in late storage and was better for strawberries with the coating combination AL 2% + Eug 0.1%.

It is not expected a significant change in SSC through shelf-life in non-climacteric fruit, as strawberry, since it is harvested at the eating ripe stage. A small decrease can be expected in late storage due to the senescence process. This is what happened in our experiment. Nevertheless, the fact that control showed values at the end of storage similar to the ones at harvest shows the beneficial effect of the edible coatings on retarding senescence. Similar behavior was found in strawberries coated with other formulations which were attributed to respiration (Hernández-Muñoz et al., 2008, Velickova et al., 2013; Gol et al., (2013). Contrarily, Duan et al. (2011) found blueberries SSC not significantly affected by cold storage or coating (sodium alginate and chitosan) treatments.

Loss of weight in fresh fruit and vegetables is mainly due to the loss of water caused by transpiration and respiration processes (Fan et al., 2009). Prior studies using chitosan coating showed a reduction in weight loss for strawberries, which served as

semi-permeable barrier against moisture loss (Gol et al., 2013; Valero et al., 2013). In our case, edible coatings had similar weight loss as control, except AL 2% + Eug 0.1% which showed even higher values. These differences in the ability to reduce weight loss are attributed to the different water vapor permeability of the polysaccharides used in the formulation of the edible coatings (Vargas et al., 2008). Duan et al. (2011) and Guerreiro et al. (2015a) confirm our results by reporting higher water loss in some polysaccharide based edible coatings than in controls.

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration. Generally, edible coatings did reduce yeasts and molds and mesophilic microorganisms, as comparing to control. The same behavior was observed for other authors who reported a decrease in decay incidence in strawberries coated with chitosan and alginate as compared to uncoated-control fruit (Han et al., 2004; Fan et al., 2009; Perdones et al., 2012; Gol et al., 2013). Nevertheless, none treatment pass the upper safety limits of microbial growth (10^6 CFU/g) established for fresh fruit products (IFPA, 2003).

Some authors reported a progressive decrease in phenolic content over the entire storage period in chitosan coated strawberries (Wang and Gao, 2012; Gol et al., 2013). Ghasemnezhad (2010) and Ali et al. (2013) found the same behavior, but those authors reported that, in apricot and tomato coated with chitosan and gum arabic, respectively, maintained (tomato) or increased (apricot) antioxidant activity and phenol content as compared to control. In our case, there was no clear additional benefit of the edible coatings on phenols, flavonoids and anthocyanins as compared to control.

The report of hydroxypropyl methylcellulose–lipid edible coatings in ‘Oronules’ mandarins showed no important effect of coating application on the level of the different flavonoids (Contreras-Oliva et al., 2012). Also, Robles-Sánchez et al. (2013)

study on fresh-cut mangos coated with alginate, showed that flavonoids content was minimally influenced by the treatment applied on fresh-cut samples, being storage time which promoted changes on this parameter. On the other hand, some authors report that anthocyanin content increased in control strawberries, during the whole storage period, while alginate or chitosan edible coatings reduce anthocyanins development (Fan et al., 2009; Gol et al., 2013). Wang and Gao (2012) showed similar behaviour to our experiment in strawberries coated with chitosan but stored at higher temperature 10ºC. It is likely that our fruit were completely ripe at harvest so no significant changes occurred during normal storage.

Antioxidant capacity has been used to evaluate the antioxidant potential status of tissue, which is a function of the type and amount of bioactive compounds present. There are many methods used to determine the total antioxidant capacity, it is important to point out that all of them have some limitations, and it has been observed that some antioxidant assays give different trends in the same sample, for that reason multiple methods to generate an 'antioxidant profile' might be needed (Ou et al., 2001; Robles-Sánchez et al., 2013). In our case, no effect of edible coatings as compared to control was found for ORAC and TEAC methods, while DPPH was higher in edible coatings than control. Wang and Gao (2012) found for strawberries, that the decline in antioxidant activity in untreated fruit at the end of storage might be due to senescence and decay, this indicating that chitosan treatment not only can extend shelf life, but also can retain higher antioxidant activity in strawberries after prolonged storage.

According to Oms-Oliu et al. (2008) using gellan, alginate and pectin the increase in phenolic compounds was related to the enhancement of antioxidant capacity in fresh-cut melon. In our case since no significant changes were found in phenols, they were found in antioxidants. Nevertheless, the higher values of DPPH in edible coatings

confirm that there is some benefit of edible coatings in preserving antioxidant activity. Robles-Sánchez et al. (2013) attributed the higher antioxidant activity in fresh-cut mangoes covered with Alginate 2% + Ascorbic Acid 1%, expressed as TEAC and DPPH to the ascorbic acid in the coatings, which is in accordance to our work.

Edible coatings can act as a gas barrier reducing the respiration as observed by the use of chitosan coatings in fresh-cut fruit and in cold-stored strawberries (Vargas et al., 2006; Hernández-Muñoz et al., 2008; Perdones et al., 2012). Bierhals et al. (2011) also report that cassava starch coatings were efficient in reducing respiration rate in fresh-cut pineapple. Nevertheless, Oms-Oliu et al. (2008) using gellan, alginate and pectin, found that the production of CO_2 in fresh-cut melon was similar in coated and uncoated fruits, as in our case, showing the permeability of alginate and pectin to CO_2 gas exchange.

Similarly, and due to the permeability of the coatings, the ethanol content was low and similar in coated samples and control. Rojas-Graü et al., (2007b) report the presence of ethanol and acetaldehyde to be influenced by the type of essential oil and its concentration when using alginate as base. In our case, the essential oils constituents used were in much lower concentration.

Zapata et al. (2008) reported for tomato coated with alginate and zein, the composition in sugar and organic acids were lower in control than coated fruit and attributed it to a more advanced ripening stage in control. On the other hand, Palma et al. (2015) using "Food coat" (composed of fatty acids derivatives and polysaccharides in alcohol solution) and "Pomfresh" (composed of a mixture of organic acids and antioxidant compounds) in minimally processed cactus pear, reported that organic acids and sugars content remained almost constant during the storage period and were not influenced by treatments. Similar results were found in our experiment. The exception

was ascorbic acid which was lower in control than in coated samples, mainly in PE. This may be attributed to the ascorbic acid added to the coating which may be better preserved in pectin based coats.

The taste panel showed us that control fruit could not be preserved in commercial conditions until 14 d since showed in all attributes values lower than the minimum acceptable (4). All edible coatings scored over 4 so were acceptable for consumption, despite PE 2% + Eug 0.1% being in the limits (3.7) as overall liking. Perdones et al. (2012) using chitosan-lemon essential oil coatings in strawberries report that the overall differences among coated and non-coated samples were not significant. In the cases of Hernández-Muñoz et al. (2008) and Gol et al. (2013) the taste panel showed a preference for coated fruit due to the glossy appearance imparted by the coating, as in our coatings.

The formulations used in our experiments were not cytotoxic, so no health problems caused by them shall be expected at those concentrations. Similar results were obtained for other edible coatings, such as cocoa procyanidins–gelatin–chitosan nanoparticles and chitosan-coated nanoparticules (Hermans et al., 2012; Zhou et al., 2014).

5. Conclusions

Based on our results, alginate and pectin edible coatings can be used as natural postharvest treatments for increasing strawberries storage life, by delaying microbial spoilage and improve fruit organoleptic attributes.

Taking into account the coating effect on all general, sensorial and nutritional quality preservation through storage, the best edible coating was AL 2% + Cit 0.15% + Eug 0.1%.

Funding This work was supported by Fundação para a Ciência e Tecnologia, Portugal, Project: PTDC/AGR-ALI/121085/2010 - INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

6. References

Ali, A.; Maqbool, M.; Alderson, P. G.; Zahid, N. Effect of Gum Arabic as an Edible Coating on Antioxidant Capacity of Tomato (Solanum Lycopersicum L.) Fruit during Storage. *Postharvest Biol. Technol.* **2013**, *76*, 119–124.

Antunes, M. D. C.; Dandlen, S.; Cavaco, A. M.; Miguel, G. Effects of Postharvest Application of 1-MCP and Postcutting Dip Treatment on the Quality and Nutritional Properties of Fresh-Cut Kiwifruit. *J. Agric. Food Chem.* **2010**, *58*, 6173–6181.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Bierhals, V. S.; Chiumarelli, M.; Hubinger, M. D. Effect of Cassava Starch Coating on Quality and Shelf Life of Fresh-Cut Pineapple (Ananas Comosus L. Merril Cv "Pérola"). *J. Food Sci.* **2011**, *76*, 62–72.

Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT - Food Sci. Technol.* **1995**, *28*, 25–30.

Contreras-Oliva, A.; Rojas-Argudo, C.; Pérez-Gago, M. B. Effect of Solid Content and Composition of Hydroxypropyl Methylcellulose-Lipid Edible Coatings on Physico-Chemical and Nutritional Quality of "Oronules" Mandarins. *J. Sci. Food Agric.* **2012**, *92*, 794–802.

Crespo, P.; Bordonaba, J. G.; Terry, L. A.; Carlen, C. Characterisation of Major Taste and Health-Related Compounds of Four Strawberry Genotypes Grown at Different Swiss Production Sites. *Food Chem.* **2010**, *122*, 16–24.

Del-Valle, V.; Hernández-Muñoz, P.; Guarda, A.; Galotto, M. J. Development of a Cactus-Mucilage Edible Coating (Opuntia Ficus Indica) and Its Application to Extend Strawberry (Fragaria Ananassa) Shelf-Life. *Food Chem.* **2005**, *91*, 751–756.

Duan, J.; Wu, R.; Strik, B. C.; Zhao, Y. Effect of Edible Coatings on the Quality of Fresh Blueberries (Duke and Elliott) under Commercial Storage Conditions. *Postharvest Biol. Technol.* **2011**, *59*, 71–79.

Fan, Y.; Xu, Y.; Wang, D.; Zhang, L.; Sun, J.; Sun, L.; Zhang, B. Effect of Alginate Coating Combined with Yeast Antagonist on Strawberry (Fragaria×ananassa) Preservation Quality. *Postharvest Biol. Technol.* **2009**, *53*, 84–90.

Galus, S.; Lenart, A. Development and Characterization of Composite Edible Films Based on Sodium Alginate and Pectin. *J. Food Eng.* **2012**, *115*, 459–465.

Ghasemnezhad, M. Effect of Chitosan Coatings on Some Quality Indices of Apricot (Prunus Armeniaca L.) During Cold Storage. **2010**, *8*, 25–33.

Girón-Calle, J.; Alaiz, M.; Vioque, J. Effect of Chickpea Protein Hydrolysates on Cell Proliferation and in Vitro Bioavailability. *Food Res. Int.* **2010**, *43*, 1365–1370.

Gol, N. B.; Patel, P. R.; Rao, T. V. R. Improvement of Quality and Shelf-Life of Strawberries with Edible Coatings Enriched with Chitosan. *Postharvest Biol. Technol.* **2013**, *85*, 185–195.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Effect of Alginate-Based Edible Coatings Enriched with Essential Oils Constituents on Arbutus Unedo L. Fresh Fruit Storage. *Postharvest Biol. Technol.* **2015a**, *100*, 226–233.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Use of Polysaccharide-Based Edible Coatings Enriched with Essential Oils to Improve Shelf-Life of Strawberries. *Postharvest Biol. Technol.* **2015b**, *110*, 51–60.

Han, C.; Zhao, Y.; Leonard, S. .; Traber, M. . Edible Coatings to Improve Storability and Enhance Nutritional Value of Fresh and Frozen Strawberries (Fragaria × Ananassa) and Raspberries (Rubus Ideaus). *Postharvest Biol. Technol.* **2004**, *33*, 67–78.

Hermans, K.; Van Den Plas, D.; Everaert, A.; Weyenberg, W.; Ludwig, A. Full Factorial Design, Physicochemical Characterisation and Biological Assessment of Cyclosporine A Loaded Cationic Nanoparticles. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 27–35.

Hernández-Muñoz, P.; Almenar, E.; Valle, V. Del; Velez, D.; Gavara, R. Effect of Chitosan Coating Combined with Postharvest Calcium Treatment on Strawberry (Fragaria × Ananassa) Quality during Refrigerated Storage. *Food Chem.* **2008**, *110*, 428–435.

IFPA. *Guía de Seguridad Alimentaria Para La Industria de Productos Vegetales Frescos Cortados*; Gil MI, G. J., Ed.; Alexandria, Virginia, 2003.

Keutgen, A.; Pawelzik, E. Modifications of Taste-Relevant Compounds in Strawberry Fruit under NaCl Salinity. *Food Chem.* **2007**, *105*, 1487–1494.

Klewicka, E.; Nowak, A.; Zduńczyk, Z.; Juśkiewicz, J.; Cukrowska, B. Protective Effect of Lactofermented Red Beetroot Juice against Aberrant Crypt Foci Formation, Genotoxicity of Fecal Water and Oxidative Stress Induced by 2-Amino-1-Methyl-6-phenylimidazo[4,5-B] Pyridine in Rats Model. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 895–904.

Leceta, I.; Molinaro, S.; Guerrero, P.; Kerry, J. P.; Caba, K. De. Postharvest Biology and Technology Quality Attributes of Map Packaged Ready-to-Eat Baby Carrots by Using Chitosan-Based Coatings. *Postharvest Biol. Technol.* **2015**, *100*, 142–150.

Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.* **2005**, *88*, 1269–1278.

Maftoonazad, N.; Ramaswamy, H. S.; Moalemiyan, M.; Kushalappa, a. C. Effect of Pectin-Based Edible Emulsion Coating on Changes in Quality of Avocado Exposed to Lasiodiplodia Theobromae Infection. *Carbohydr. Polym.* **2007**, *68*, 341–349.

Magwaza, L. S.; Opara, U. L.; Terry, L. a.; Landahl, S.; Cronje, P. J.; Nieuwoudt, H.; Mouazen, A. M.; Saeys, W.; Nicolaï, B. M. Prediction of "Nules Clementine" Mandarin Susceptibility to Rind Breakdown Disorder Using Vis/NIR Spectroscopy. *Postharvest Biol. Technol.* **2012**, *74*, 1–10.

Magwaza, L. S.; Opara, U. L.; Terry, L. A.; Landahl, S.; Cronje, P. J. R.; Nieuwoudt, H. H.; Hanssens, A.; Saeys, W.; Nicolaï, B. M. Evaluation of Fourier Transform-NIR Spectroscopy for Integrated External and Internal Quality Assessment of Valencia Oranges. *J. Food Compos. Anal.* **2013**, *31*, 144–154.

McGuire, R. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255.

Miguel, M. G.; Nunes, S.; Dandlen, S. A.; Cavaco, A. M.; Antunes, M. D. Phenols and Antioxidant Activity of Hydro-Alcoholic Extracts of Propolis from Algarve, South of Portugal. *Food Chem. Toxicol.* **2010**, *48*, 3418–3423.

NP-3788:2002. Microbiologia Alimentar. *Regras gerais para a contagem microrganismos a 30 ^{o}C.*

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon. *LWT - Food Sci. Technol.* **2008**, *41*, 1862–1870.

Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe. *J. Agric. Food Chem.* **2001**, *49*, 4619–4626.

Palma, A.; Schirra, M.; Aquino, S. D. Effect of Edible Coating on Ascorbic Acid , Betalains , Organic Acids , Sugar , Polyphenol Content and Antioxidant Activity in Minimally Processed Cactus Pear (Opuntia Ficus-Indica). *Acta Hortic.* **2015**, *1067*, 127–134.

Perdones, A.; Sánchez-gonzález, L.; Chiralt, A.; Vargas, M. Effect of Chitosan – Lemon Essential Oil Coatings on Storage-Keeping Quality of Strawberry. *Postharvest Biol. Technol.* **2012**, *70*, 32–41.

Prior, R. L.; Cao, G. In Vivo Total Antioxidant Capacity: Comparison of Different Analytical Methods. *Free Radic. Biol. Med.* **1999**, *27*, 1173–1181.

Qian, P. I. M. Objective Flavor Comparison of Oregon Strawberries and Those from Other Climatic Condition. **2005**.

Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Martín-Belloso, O. Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon. *Int. J. Food Microbiol.* **2008**, *121*, 313–327.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.

Ribeiro, C.; Vicente, A. A.; Teixeira, J. A.; Miranda, C. Optimization of Edible Coating Composition to Retard Strawberry Fruit Senescence. *Postharvest Biol. Technol.* **2007**, *44*, 63–70.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier

of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Raybaudi-Massilia, R. M.; Soliva-Fortuny, R. C.; Avena-Bustillos, R. J.; McHugh, T. H.; Martín-Belloso, O. Apple Puree-Alginate Edible Coating as Carrier of Antimicrobial Agents to Prolong Shelf-Life of Fresh-Cut Apples. *Postharvest Biol. Technol.* **2007a**, *45*, 254–264.

Rojas-Graü, M. a.; Avena-Bustillos, R. J.; Olsen, C.; Friedman, M.; Henika, P. R.; Martín-Belloso, O.; Pan, Z.; McHugh, T. H. Effects of Plant Essential Oils and Oil Compounds on Mechanical, Barrier and Antimicrobial Properties of Alginate-Apple Puree Edible Films. *J. Food Eng.* **2007b**, *81*, 634–641.

Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

Tanada-Palmu, P. S.; Grosso, C. R. F. Effect of Edible Wheat Gluten-Based Films and Coatings on Refrigerated Strawberry (Fragaria Ananassa) Quality. *Postharvest Biol. Technol.* **2005**, *36*, 199–208.

Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of Water Deficit Irrigation and Inoculation with Botrytis Cinerea on Strawberry (Fragaria X Ananassa) Fruit Quality. *J. Agric. Food Chem.* **2007**, *55*, 10812–10819.

Valero, D.; Díaz-Mula, H. M.; Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Serrano, M. Effects of Alginate Edible Coating on Preserving Fruit Quality in Four Plum Cultivars during Postharvest Storage. *Postharvest Biol. Technol.* **2013**, *77*, 1–6.

Vargas, M.; Albors, A.; Chiralt, A.; González-Martínez, C. Quality of Cold-Stored Strawberries as Affected by Chitosan-Oleic Acid Edible Coatings. *Postharvest Biol. Technol.* **2006**, *41*, 164–171.

Vargas, M.; Pastor, C.; Chiralt, A.; McClements, D. J.; González-Martínez, C. Recent Advances in Edible Coatings for Fresh and Minimally Processed Fruits. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 496–511.

Velickova, E.; Winkelhausen, E.; Kuzmanova, S.; Alves, V. D.; Moldão-Martins, M. Impact of Chitosan-Beeswax Edible Coatings on the Quality of Fresh Strawberries (Fragaria Ananassa Cv Camarosa) under Commercial Storage Conditions. *LWT - Food Sci. Technol.* **2013**, *52*, 80–92.

Vu, K. D.; Hollingsworth, R. G.; Leroux, E.; Salmieri, S.; Lacroix, M. Development of Edible Bioactive Coating Based on Modified Chitosan for Increasing the Shelf Life of Strawberries. *Food Res. Int.* **2011**, *44*, 198–203.

Wang, S. Y.; Gao, H. Effect of Chitosan-Based Edible Coating on Antioxidants, Antioxidant Enzyme System, and Postharvest Fruit Quality of Strawberries (Fragaria X Aranassa Duch.). *LWT - Food Sci. Technol.* **2012**, *52*, 1–9.

Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D.; Serrano, M. Use of Alginate or Zein as Edible Coatings to Delay Postharvest Ripening Process and to Maintain Tomato (Solanum Lycopersicon Mill) Quality. *J. Food Agric.* **2008**, *88*, 1287–1293.

Zhou, W.; Liu, W.; Zou, L.; Liu, W.; Liu, C.; Liang, R.; Chen, J. Storage Stability and Skin Permeation of Vitamin C Liposomes Improved by Pectin Coating. *Colloids Surf. B. Biointerfaces* **2014**, *117C*, 330–337.

Chapter IX

Raspberry fresh fruit quality as affected by pectin- and alginate-based edible coatings enriched with essential oils

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria G C Miguel[1], Maria L Faleiro[2], Maria D C Antunes[1]

[1]Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Scientia Horticulturae 194 (2015) 138–146

Scientia Horticulturae 194 (2015) 138–146

Contents lists available at ScienceDirect

Scientia Horticulturae

Raspberry fresh fruit quality as affected by pectin- and alginate-based edible coatings enriched with essential oils

Adriana C. Guerreiro[a], Custódia M.L. Gago[a], Maria L. Faleiro[b], Maria G.C. Miguel[a], Maria D.C. Antunes[a,*]

[a] Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[b] Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

article info

Article history:
Received 27 May 2015
Received in revised form 25 July 2015
Accepted 1 August 2015

Keywords:
Eugenol
Citral
Storage
Coatings
Quality
Shelf-life

abstract

The effects of optimized edible coatings based on sodium alginate (AL) or pectin (PE) with the essential oil constituent additives, citral (Cit) and eugenol (Eug), were studied on fresh raspberries quality during storage at 0.5 °C. Several formulations of edible coatings were used as treatments: AL and PE were tested at 1% and 2% (w/v) with Cit and Eug at minimum inhibitory concentrations (MIC) (0.15 and 0.1%, respectively), at double the MIC concentration and their combination at MIC. Raspberries were immersed in each solution (treatment) for 2 min, and then cooled at 0.5 °C. On days 0, 7, and 14th, samples were removed and used for the following physicochemical and biochemical analysis: color CIE (L^*, °hue), firmness, soluble solids concentration (SSC), weight loss, trolox equivalent antioxidant capacity (TEAC), microbial growth, and taste panels. Color parameter L^* and TEAC were not significantly affected by the coatings. Cit at higher concentrations reduced °hue and firmness and increased weight loss. The SSC decreased mainly in controls. Edible coatings enriched with Cit and Eug were effective at reducing microbial spoilage. Taste panels showed lower scores in Cit 0.3% treatments and raspberries were considered not acceptable after 14 days storage. No significant differences were observed between PE and AL. Raspberries immersed in water (control + water) performed worse than fruit stored without any immersion or wash (control). With the results of the physicochemical and biochemical parameters measured, 3 similar treatment groups were formed by the principal component analysis (PCA) and hierarchical cluster analysis (HCA), either for AL or PE coatings. The group which had better performance was selected for AL- and PE-based edible coatings. From each group the two best edible coating formulations with the higher sensory evaluation were selected. Those confirmed that raspberries were better preserved in terms of general sensory attributes with coatings of AL 2% + Cit 0.15% and AL 2% + Eug 0.1%, and with PE 1% + Eug 0.1% followed by PE 1% + Cit 0.15% + Eug 0.1%.

1. Introduction

Red raspberries (*Rubus idaeus* L.) are of high economic importance and widely consumed in fresh, frozen, or in processed forms, such as jams and juices. In addition to their attractive color and flavor, raspberries contain a unique phytochemical profile. Specifically they are rich in ellagitannins and anthocyanins, which distinguishes them from other berries and fruits (Rao and Snyder, 2010).

The postharvest life of berries is generally determined by their susceptibility to water loss, softening, mechanical injuries, and especially to the presence of postharvest pathogens (Tezotto-Uliana et al., 2014). Various studies have proposed strategies to control postharvest pathogens, while preserving the quality of this fruit, such as modified atmospheres, forced-air cooling or other cooling processes, heat shock, osmotic treatments, irradiation, and edible coatings (Velickova et al., 2013).

Edible coatings have been of increasing interest because of their capacity to reduce respiration and transpiration rates, while increase storage periods and the retention of berry firmness (Azevedo et al., 2014; Tezotto-Uliana et al., 2014; Velickova et al., 2013; Vu et al., 2011). Edible coatings also provide good mechanical properties, are non-toxic and non-polluting, and can be applied at low cost.

The incorporation of antimicrobial agents, such as essential oils or their compounds, into edible coatings can enhance the functionality of coatings in protecting food from microbial spoilage and thus extending their postharvest life and quality (Antunes et al.,

* Corresponding author.
E-mail address: mantunes@ualg.pt (M.D.C. Antunes).

http://dx.doi.org/10.1016/j.scienta.2015.08.004
0304-4238/

2012). Because the chemical composition of plant-derived products, such as essential oils, are highly variable with season and cultural practices, the utilization of sole compounds instead of naturally produced essential oil mixtures is a better approach to obtain an edible coating with constant characteristics (Azevedo et al., 2014; Miguel, 2010).

Eugenol, the main constituent of the essential oil isolated from clove flower buds [*Syzygium aromaticum* (L.) (Merrill & Perry)] has antioxidant, antimicrobial, antinociceptive, and antiviral activities (Cortés-Rojas et al., 2014). Citral is a mixture of two stereoiso- meric monoterpenes aldehydes: the *E*-isomer specifically referred as geranial or citral A (40–62%), and the Z-isomer (25–38%) known as neral or citral B. This isomer mixture can be isolated from the essential oil of *Cymbopogon citratus* (lemongrass) and *Litsea cubeba.* Citral is used in traditional medicine as antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic, and sedative. Citral also possesses antimicrobial activity and insecticidal property (Maswal and Dar, 2014).

Polysaccharide edible coatings have good coating-forming and low oxygen permeability properties, as well as, the capacity to decrease the respiration rate of fresh-cut products (Campos et al., 2011). Sodium alginate (AL) is a polysaccharide derived from brown sea algae (Phaeophyceae) and is a linear unbranched polymer containing mannuronic and guluronic acids (Rojas-Graü et al., 2008). Pectin (PE) based edible coatings are extracted from apple waste or citrus peel and are homopolymeric linear chain of galacturonic acid units (Oms-Oliu et al., 2008).

The objective of this research was to study the effects of edible coatings based on AL or PE with Cit and/or Eug incorporated, on the storage ability and fruit quality of fresh red raspberries. Knowledge of how essential oils influence storage ability could prolong the postharvest lifespan of fresh raspberry, which could increase the market window and have positive economic implications.

1. Materials and methods

1.1. Edible coatings formulations

Pectin (PE) and sodium alginate (AL) (Sigma–Aldrich Chemic, Steinhein, Germany) were the biopolymers used for coating formulations and calcium chloride (Sigma–Aldrich Chemic, Steinhein, Germany) was used to induce cross linking reaction (Olivas et al., 2007). The essential oils components, citral (Cit) and eugenol (Eug), were purchased from Sigma–Aldrich Chemic, Steinhein, Germany. The coating forming solutions based on AL or PE were for- mulated as described by Rojas-Graü et al. (2007). Ascorbic acid (Scharlau, Barcelona, Spain) at 1% (w/v) was added as anti- browning agent in the edible coating solutions according to previous work (Robles-Sánchez et al., 2009).

The treatments were control (non-treated fruit), control + water (fruit immersed in distilled water for the same time as edible coatings) and edible coatings formulations as described in Table 1.

Concentrations of Cit and Eug were the minimum inhibitory concentrations (MIC) and double MIC determined in a previous work (Guerreiro et al., 2015).

The raspberries (*Rubus idaeus* L.) were from the group of Driscoll's cultivars and were purchased from the local market within 4 h after harvest and immediately transported to the postharvest laboratory at the University of Algarve, where they were selected for the experiments. The experiments were performed within 6 h postharvest at room temperature of 18 °C.

In each treatment, raspberries were first hand-immersed into the edible coating solution for 2 min. Excess of coating material was allowed to drip off for 30 s before the berries were immersed for a second time in the calcium chloride solution for 1 min. After dripping dry for 30 s again, 8 fruits per replication were placed in polypropylene plastic, clamshell type, containers (8 × 10 × 4 cm), perforated in the cover, and stored at 0.5 °C until analysis. On days 0, 7, and 14th, three containers per treatment were taken to perform the analyses. Experiments were repeated twice.

Table 1

Table of coating formulations, using alginate and pectin.

Alginate (AL)	Pectin (PE)
AL 1% (10 g L^{-1})	PE 1% (10 g L^{-1})
AL 1% + Cit 0.15% (Cit 1.5 g L^{-1})	PE 1% + Cit 0.15% (Cit 1.5 g L^{-1})
AL 1% + Cit 0.3% (Cit 3.0 g L^{-1})	PE 1% + Cit 0.3% (Cit 3.0 g L^{-1})
AL 1% + Eug 0.1% (Eug 1.0 g L^{-1})	PE 1% + Eug 0.1% (Eug 1.0 g L^{-1})
AL 1% + Eug 0.2% (Eug 2.0 g L^{-1})	PE 1% + Eug 0.2% (Eug 2.0 g L^{-1})
AL 1% + Cit 0.15% + Eug 0.1%	PE 1% + Cit 0.15% + Eug 0.1%
AL 2% (20 g L^{-1})	PE 2% (20 g L^{-1})
AL 2% + Cit 0.15%	PE 2% + Cit 0.15%
AL 2% + Cit 0.3%	PE 2% + Cit 0.3%
AL 2% + Eug 0.1%	PE 2% + Eug 0.1%
AL 2% + Eug 0.2%	PE 2% + Eug 0.2%
AL 2% + Cit 0.15% + Eug 0.1%	PE 2% + Cit 0.15% + Eug 0.1%

1.2. Determination of qualitative parameters

The color of the raspberries was measured using a Minolta Chroma meter CR-300 (EC Minolta, Japan) using the CIE (L^*, a^*, b^*) scale. Before measuring, the colorimeter was calibrated with a white standard calibration plate. Hue was calculated as h° = arctan (b^*/a^*) (McGuire, 1992). The firmness of the fruits was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS 50 (Jonh Chatillon & Sons, Inc., USA) using a piston cylinder of 4 mm diameter at a depth of 7 mm. A digital refractometer (PR1 ATAGO CoLTD., Japan), was used for the determination of °Brix/soluble solids concentration (SSC) through analysis of the raspberry juice. Weight loss was expressed as the percentage of the initial weight.

1.3. Trolox equivalent antioxidant activity (TEAC)

TEAC was measured as the preformed radical monocation of 2,2^{r}-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by the modified method of Re et al. (1999). For the assay, 10 μL of the juice was added to 990 μL of ABTS radical cation solution. The absorbance was monitored spectrophotometrically at 734 nm till stabilization, after 6 min (Shimadzu spectrophotometer 160-UV, Tokyo, Japan). The antioxidant activity of each sample was calculated using the following equation: scavenging effect % (SE %) = $(1 - A_S/A_0) \times 100$, where A_0 stands for the absorbance of the control at time 0 and A_S for the absorbance in the presence of the sample after 6 min. The values were compared with the curve for several Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations and expressed as mm Trolox equivalent antioxidant capacity.

1.4. Microbial counts

The microbiological parameters that were determined included counts of aerobic mesophilic and psychrophilic bacteria and molds and yeasts. The counts of aerobic mesophilic and psychrophilic microorganisms were done according to the Portuguese NP-3788 standard (2002) using the Plate Count Agar medium (Biokar, Paris, France). The count of molds and yeasts was performed according to ISO 21527-2 (2008) using Dicloran Rose-Bengal Cloranfenicol Agar (Biokar, Paris, France). Ten gram of each sample were transferred to 90 mL of peptone water (Oxoid) and homogenized at their designated sampling times. The incubation conditions for yeasts and molds was 25 ± 1 °C during 48–72 h, 30 ± 1 °C for 24–72 h for aerobic mesophilic bacteria, and 6.5 ± 1 °C during 5–10 days for

psychrophilic bacteria. Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram of fresh weight.

1.5. Taste panel

Taste panels were performed with 15 panelists on the base of a 7-point hedonic scale: 1—dislike definitely; 2—dislike; 3—dislike mildly; 4—neither like nor dislike; 5—like mildly; 6—like; 7—like definitely. Overall liking was calculated as a mean of the sensory parameters evaluated. Panelists consisted of faculty staff and students who were trained to be familiar with the fruit taste panel before experiments.

1.6. Statistical analysis

Statistical analysis was carried out with the SPSS 20.0 software (IBM, Corp.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among treatments were performed.

The similarities and dissimilarities among the formulations with respect to measured parameters were utilized to investigate hierarchical cluster analysis (HCA) by using the SPSS 20.0 software (IBM, Corp.). For classification, the Ward's Minimum Variance Method was utilized and the squared Euclidean distance was used as the dissimilarity measure for Ward's method. The grouping derived from HCA was used to interpret the results. Principal component analysis (PCA), to bring out grouping of edible coatings with similar effects on the quality parameters studied, was performed using the statistical software *Chemoface* version 1.5 (Nunes et al., 2012).

2. Results and discussion

2.1. General quality parameters

Fruit color is an important indicator of fruit ripeness and is used by consumers to make conclusions on the ripeness and freshness of the raspberry fruit (Mikulic-petkovsek et al., 2015; Krüger et al., 2011). The lightness, color L^* parameter, which indicates darkening of the fruits, generally did not change until 14 days storage in any AL treatment (Table 2). °Hue color parameter decreased mainly from 0 to 7 days in all treatments indicating an increase in redness and advanced ripening (McGuire, 1992). After 14 days, treatments with the lowest °hue were AL 1% with both Cit concentrations (Table 2).

The PE edible coatings decreased L^* mainly from 0 to 7 days, except for the control and PE 1% + Eug 0.1% and PE 1% + Cit 0.15% + Eug 0.1%, which did not change significantly (Table 3). The lowest values of L^* (darker fruit) were in PE 2% based edible coatings. The °hue decreased significantly from 0 to 7 days in all treatments, suggesting increased red color due to the synthesis of anthocyanins (Han et al., 2004). The exception were edible coatings enriched with Eug, where the significant decrease was only from 7 to 14 days, meaning that these treatments reduced the ripening process (Table 3). The lower °hue values were in PE 2% + Cit 0.15% after 7 days and PE 2% + Cit 0.3% after 14 days, suggesting more advanced ripening in these treatments. Krüger et al. (2011) found for the raspberry color values a decrease in L^* and °hue values with storage due to advanced ripening.

In raspberries, Han et al. (2004) found a decrease of the °hue during storage when chitosan-based edible coatings were used either in the presence or absence of some additives (vitamin E or calcium), although the changes were more pronounced when additives associations were used. In this experiment, for both AL and PE, the addition of Cit acted negatively by increasing color changes in comparison to other coating treatments. Eug or its combination with Cit at MIC were the best to preserve color through storage.

Firmness which is one of the most important physical attributes in maintaining the postharvest quality of raspberries, decreased mainly from 7 to 14 days in all treatments except control (Tables 2 and 3). Fruit immersed only in water (control + water) induced faster softening than if the fruits had no treatment at all (control) (Tables 2 and 3). This indicates that the immersion by itself induce softening independently from the ingredients included into the edible coats. After 14 days, both edible coatings showed lower firmness when Cit was added and this was more visible in PE (Tables 2 and 3). It seems that Eug is better than Cit in reducing firmness loss, probably because Cit cause damage in cell walls (Somolinos et al., 2009). However, coating did not significantly reduce softening in raspberries as reported previously for other coating compositions (Tezotto-Uliana et al., 2014; Han et al., 2004). Those authors attributed this to the fact that immersion leaves the fruit wet, leading to faster softening. This is in accordance to our results which show slightly higher firmness in non-treated (control) raspberries.

The inclusion of $CaCl_2$ in coatings has been suggested to increase the retention of fruit firmness in the postharvest life of fresh-cut fruit (Olivas et al., 2007). In our case, although $CaCl_2$ was used in our coating treatments, such an effect was not observed. This may be due to the high sensitivity of raspberries to softening, which the effects of $CaCl_2$ could not counteract the effects of wetting.

Raspberries are a non-climacteric fruit and, as such, only slight changes of SSC are expected. In particular, only slight increases and decreases occur during ripening and senescence, respectively, as starch and organic acids in the fruit are converted to sugars for metabolic processes (Duan et al., 2011). In our experiment, SSC increased only in treatment AL 2% + Eug 0.1%, while decreased in the control and control + water from 7 to 14 days (Table 2). PE treatments seemed better to preserve SSC, while controls did not show significant differences trough time and Cit increased SSC mainly from 7 to 14 days (Table 3). However, as for AL coatings, SSC had the lowest values in control and control + water at the end of the experiment showing that sugar metabolism (respiration) advanced faster than in coated fruit (Gol et al., 2013; Velickova et al., 2013).

Weight loss is also an indicator of fruit freshness. Weight loss increased significantly through storage across all treatments. Interestingly the control fruit without any treatment were observed to have less weight loss than treatments (Tables 2 and 3). When immersions were performed, the weight loss was higher in control + water than in edible coatings treatments (Tables 2 and 3). This may be attributed to the fact that immersion can damage cells of very sensitive fruit, leading them to lose more water through storage. Weight loss during storage at low temperature is expected and was also observed for arbutus berries, strawberries and red raspberries (Guerreiro et al., 2013; Krüger et al., 2011; Shin et al., 2008; Vicente et al., 2002). Weight loss up to 4–5% does not significantly affect fruit freshness and consequently consumer acceptance (Nunes, 2015). In the case of our experiment, weight loss was within this range for up to 7 days, except for AL and PE 1% + Cit 0.3%, PE 2% + Cit 0.3% and PE 2% + Cit 0.15% + Eug 0.1% (Tables 2 and 3). Our data suggests that higher concentrations of Cit can damage raspberry cells, as it does for firmness. Han et al. (2004) found weigh loss of cold-stored raspberries increased up to 11% in 14 days when stored at 2 °C, which is consistent with our results. Also, chitosan coatings slightly reduced weight loss as compared to controls (Han et al., 2004). However, controls for those authors were fruit immersed in water (control + water). Moreover, differences in weight loss have been attributed to the different water vapor permeability of the polysaccharides or additives and their concen-

Table 2

Quality parameters of raspberries coated with alginate edible coatings (AL) enriched with citral (Cit) and eugenol (Eug) during storage at 0.5 °C.

	Days	Control		Control + water		AL 1%		AL 1% + Cit 0.15%		AL 1% + Cit 0.3%		AL 1% + Eug 0.1%		AL 1% + Eug 0.2%		AL 1% + Cit 0.15% + Eug 0.1%		AL 2%		AL 2% + Cit 0.15%		AL 2% + Cit 0.3%		AL 2% + Eug 0.1%		AL 2% + Eug 0.2%		AL 2% + Cit 0.15% + Eug 0.1%	
Lightness (L^*)	0	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA	33.7	aA
	7	32.6	aAB	30.2	aABC	30.2	aAB	29.9	bABC	30.2	bBC	29.7	bAB	32.4	aAB	31.1	aABC	33.0	aA	29.7	bBC	31.2	aAB	33.1	aA	28.0	bC	32.8	aA
	14	33.2	aA	33.8	aA	35.1	aA	34.1	aA	34.0	aA	33.4	aA	33.7	aA	34.2	aA	33.5	aA	34.0	aA	33.2	aA	33.4	aA	33.5	aA	30.4	aB
Hue angle (h°)	0	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA	20.9	aA
	7	13.5	bA	8.3	cABC	8.5	cABC	13.7	bA	13.7	bA	13.4	bA	5.8	cD	7.6	cBCD	8.5	cBCD	12.7	bA	11.4	cAB	8.7	cBCD	8.8	cBCD	10.4	cBCD
	14	14.6	bAB	15.3	bABCD	13.8	bABC	9.1	cE	9.4	cDE	10.1	bABC	14.3	bABCD	13.2	bA	13.7	bABCD	11.0	bBCDE	15.0	bAB	13.4	bABCD	10.6	bCDE	13.8	bABCD
Firmness (N)	0	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA	2.8	aA
	7	2.1	aABC	2.2	abA	2.3	aABCD	1.1	bE	1.0	bDE	1.3	bABC	2.0	bABC	2.0	abAB	2.2	abAB	1.1	bE	1.4	abCDE	1.9	abABCD	2.1	bABC	1.6	bBCDE
	14	2.0	aA	1.8	bAB	1.9	bAB	1.2	bCDE	0.8	bE	1.0	bDE	1.9	bAB	1.4	bBCDE	1.7	bB	1.0	bCDE	0.9	bDE	1.6	bBC	1.9	bAB	1.4	bBCD
SSC (° Brix)	0	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	aA	7.8	bA	7.8	aA	7.8	aA
	7	7.9	aA	7.5	aABC	7.3	abABC	8.3	aAB	7.8	aAB	7.8	aAB	6.9	bABC	7.2	aABC	7.3	aABC	6.7	bC	7.8	aAB	7.3	bABC	7.9	aAB	7.4	bABC
	14	7.1	bDEF	6.5	bF	6.9	bEF	8.2	aB	8.2	aB	7.3	bCDEF	7.6	aBCDE	7.5	aBCDE	7.9	aBC	7.9	aBC	7.9	aBC	9.1	aA	8.1	aBC	7.9	aBC
Weight loss (%)	0	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA
	7	1.2	bE	3.8	bDE	2.4	bDE	4.5	bABC	5.9	bA	3.7	bDE	4.4	bABC	4.6	bBC	4.9	bABC	4.4	bABC	4.3	bBC	3.7	bCD	4.3	bBC	4.4	bDC
	14	3.4	aE	5.5	aDE	4.1	aABC	6.1	aA	7.6	aCD	5.1	aABC	6.2	aABC	6.2	aBCD	6.2	aABC	5.5	aBCD	7.0	aAB	4.9	aCDE	5.5	aBCD	5.7	aBCD
Antioxidant activity (μM TE 100 g^{-1})	0	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA	2709	aA
	7	2677	aAB	3094	aA	2404	aB	3164	aAB	2801	aAB	2850	aA	3149	aA	3009	aA	2991	aAB	3147	aA	3099	aA	3064	aA	3117	aA	3086	aA
	14	3211	aA	3103	aA	3170	aA	3225	aA	3152	aAB	3025	aA	3244	aA	3184	aAB	3162	aAB	3091	aABC	2775	aC	2407	aABC	2494	aBC	2440	aBC
Yeast and molds (Log$_{10}$ CFU g^{-1})	0	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA
	7	1.4	bCD	1.5	bABC	0.0	cE	0.3	bD	0.0	bE	0.0	bE	0.0	bE	1.1	bBCD	1.3	bABC	0.0	bE	2.1	aA	0.0	cE	0.0	cE	1.7	abAB
	14	1.7	abA	1.5	bAB	1.0	bBC	0.0	cD	1.4	abAB	0.6	bCD	0.0	bD	0.0	cD	0.7	cCD	0.0	bD	0.0	bD	0.7	bCD	0.9	bBC	0.0	bD
Aerobic mesophilics (Log$_{10}$ CFU g^{-1})	0	1.8	aA	1.8	aA	1.8	aA	1.8	bA	1.8	abA	1.8	aA	1.8	aA	1.8	aA	1.8	aA	1.8	abA	1.8	aA	1.8	aA	1.8	aA	1.8	aA
	7	2.5	aA	2.2	aABCD	0.4	bG	1.3	bEFG	2.4	aAB	1.9	aBCDE	0.8	bFG	1.6	aBCDE	1.8	aBCDE	1.4	aDEF	2.4	aA	1.1	abEFG	1.4	abDEF	1.6	abCDE
	14	1.8	aA	1.8	aA	0.0	bD	1.6	abAB	1.2	bABC	1.6	aAB	0.8	bC	0.8	bC	0.7	bC	0.7	bC	0.0	bD	0.0	bD	0.7	bC	1.2	bABC

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

A.C. Guerreiro et al. / Scientia Horticulturae 194 (2015) 138–146

Table 3

Quality parameters of raspberries coated with pectin edible coatings (PE) enriched with citral (Cit) and eugenol (Eug) during storage at 0.5 °C.

	Days	Control		Control + water		PE 1%		PE 1% + Cit 0.15%		PE 1% + Cit 0.3%		PE 1% + Eug 0.1%		PE 1% + Eug 0.2%		PE 1% + Cit 0.15% + Eug 0.1%		PE 2%		PE 2% + Cit 0.15%		PE 2%+Cit 0.3%		PE 2% + Eug 0.1%		PE 2% + Eug 0.2%		PE 2% + Cit 0.15% + Eug 0.1%	
Lightness (L^*)	0	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA	33.2	aA
	7	31.7	aABCD	30.6	bABCDE	29.6	bCDEF	29.7	bCDEF	29.6	bCDEF	33.1	aAB	30.6	aABCDE	33.5	aA	29.7	bCDEF	30.2	bBCDEF	29.1	bCDEF	27.7	bEF	29.1	bCDEF	27.2	bF
	14	33.3	aA	29.8	bBCDE	30.6	bABC	29.4	bBCDE	29.7	bBCDE	30.2	aABCD	30.4	aABCD	32.3	aAB	30.5	bBC	30.5	bBCD	30.1	bBCDE	27.7	bCDE	27.2	cDE	26.9	bE
Hue angle (h°)	0	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA	23.7	aA
	7	18.7	bCDEF	20.7	bABCDE	17.5	bDEFG	15.1	bFG	14.2	bG	23.4	aABC	21.1	aBCDE	15.5	bFG	20.8	bABCDE	13.8	cG	20.2	bABCDE	23.8	aAB	25.3	aA	20.9	aABCDE
	14	17.7	bAB	16.4	cABC	17.9	bA	15.3	bABC	14.8	bBC	16.3	bABC	15.9	bABC	15.2	bABC	17.5	cAB	16.8	bAB	13.3	cC	16.8	bAB	15.6	bABC	16.0	bABC
Firmness (N)	0	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA
	7	2.0	aA	2.1	aA	1.7	abBC	1.5	abBC	1.5	abBC	1.7	abBC	1.7	abBC	1.6	bBC	1.9	aB	1.8	abBC	1.3	bC	1.7	abBC	1.2	cC	1.4	bBC
	14	1.9	aA	1.7	bAB	1.1	bDEF	1.2	bDEF	1.3	bCDEF	1.5	bBCD	1.4	bBCDE	1.3	cCDEF	1.1	bEF	1.0	bF	1.0	cF	1.4	bBCDE	1.4	bBCDE	1.1	cEF
SSC (° Brix)	0	6.9	aA	6.9	aA	6.9	aA	6.9	bA	6.9	bA	6.9	aA	6.9	bA	6.9	aA	6.9	aA	6.9	aA	6.9	bA	6.9	aA	6.9	aA	6.9	aA
	7	7.2	aBC	7.3	aABC	7.2	aBC	7.5	abABC	7.3	abABC	7.5	abABC	7.6	bABC	7.5	aABC	7.1	aC	7.9	aAB	7.9	bA	7.5	aABC	7.3	aABC	7.3	aABC
	14	6.9	aD	7.3	aCD	7.3	aBCD	8.1	aAB	8.3	aA	7.8	BABC	8.1	aABC	7.8	aABC	7.7	aABC	7.9	aABC	8.1	aAB	7.8	aABC	7.7	aABC	7.9	aABC
Weight loss (%)	0	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA	0.0	cA
	7	1.6	bE	3.5	bD	3.6	bD	4.9	bBC	5.5	bAB	1.9	bE	4.2	bCD	3.4	bD	3.9	bCD	4.3	bCD	5.3	bAB	4.7	bBC	4.1	bCD	6.1	bA
	14	4.3	aE	6.9	aCDE	7.5	aCDE	8.6	aBCD	10.3	aABC	6.2	aDE	5.8	aDE	7.5	aCDE	7.9	aCDE	9.1	aBCD	9.1	aBCD	9.0	aBCD	9.0	aBCD	10.8	aABC
Antioxidant activity (μM TE 100 g^{-1})	0	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA	2935	aA
	7	2859	aAB	2293	aAB	2977	aAB	2673	aB	2744	aAB	3005	aA	2871	aAB	2953	aAB	2854	aAB	3003	aAB	2957	aAB	2923	aAB	2975	aAB	2945	aC
	14	3064	aAB	3025	aAB	3053	aAB	3007	aAB	2886	aAB	3095	aA	3038	aAB	2913	aAB	2920	aAB	2811	aBC	2904	aAB	2904	aAB	2992	aC	2615	aC
Yeast and moulds (Log_{10} CFU g^{-1})	0	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA	2.1	aA
	7	1.4	bC	2.5	aA	2.0	aB	0.0	cE	0.0	bE	1.4	bC	0.8	bD	0.0	bE	0.0	cE	0.0	bE	0.0	cE	1.9	aB	0.0	cE	0.0	bE
	14	1.7	abA	1.4	bAB	1.0	bABC	1.4	bAB	0.0	bC	0.7	cBC	0.0	cC	0.0	bC	1.2	bABC	0.0	bC	0.7	bBC	0.8	bABC	0.8	bABC	0.0	ABC
Aerobic mesophilics (Log_{10} CFU g^{-1})	0	1.9	bA	1.9	bA	1.9	bA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	aA	1.9	bA	1.9	aA	1.9	aA	1.9	aA
	7	2.5	aB	2.3	aAB	3.4	aA	1.9	aC	1.1	bEF	1.8	aCD	2.1	aBC	0.7	bG	1.0	bFG	1.3	bEF	2.4	aAB	1.1	bEF	1.4	bEF	1.5	abDE
	14	2.4	aA	1.9	bA	1.8	bA	1.9	aA	0.7	cC	0.0	bD	0.7	bC	0.0	cD	0.0	cD	0.7	cC	0.0	cD	0.0	cD	0.7	cC	1.3	cB

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

trations used in the formulation of the edible coating (Vargas et al., 2008).

2.2. Trolox equivalent antioxidant activity (TEAC)

The antioxidant activity determined by the TEAC method allows for the evaluation of the capacity of samples to scavenge free radicals such as ABTS. The antioxidant activity was high and did not significantly change throughout the experiment (Tables 2 and 3). Of note is that, when comparing treatments after 14 days storage, some treatment with the higher concentrations of AL or PE showed slightly lower values of antioxidant activity (AL 2% + Cit 0.15%, PE 2% + Eug 0.2% and PE 2% + Eug 0.1% + Cit 0.15%).

According to Robles-Sánchez, et al. (2013) antioxidant activity (expressed as TEAC) in fresh-cut mangoes covered with the edible coating, Alginate 2% + ascorbic acid 1%, was significantly higher than fruit covered with only Alginate 2% or control fruits. Similar results were obtained for ascorbic acid immersion in fresh-cut kiwifruit (Antunes et al., 2010). The authors attributed these differences to ascorbic acid which is an antioxidant. In our experiment, all edible coating treatments had similar ascorbic acid content. However, controls with no ascorbic acid treatments obtained similar TEAC values as edible coatings. This is probably because raspberry has greater antioxidant activity than kiwifruit and mango (Giovanelli et al., 2014; Li et al., 2014; Park et al., 2014) and the entire fruit was used in the experiment, while the other experiments utilized fresh-cut fruit. Combined, it is possible the ascorbic acid acid did not significantly affect the high antioxidant capacity inherent in the raspberry fruit. The values of antioxidant activity found for raspberries are close of those reported by Giovanelli et al. (2014).

2.3. Microbial evaluation

Yeast and mold counts were significantly higher in control and control + water for both AL- and PE-based edible coatings (Tables 2 and 3). After 14 days shelf-life, only the AL 1% + Cit 0.3% showed similar counts of yeast and mold to control and control + water (Table 2). When the PE edible coating was tested, the same results were observed for PE 1% + Cit 0.15% (Table 3). Other than these two treatments, the addition of essential oils constituents reduced microbial counts for both AL- and PE-based edible coatings. For aerobic mesophilic microorganisms, the edible coatings AL 1% + Cit 0.3%, AL 2% + Cit 0.3% and PE 1% + Cit 0.3% showed limited action on the reduction of this microbial parameter (Tables 2 and 3). As for yeast and mold counts also the addition of essential oils resulted in reduction of aerobic mesophilic microorganisms. It is noteworthy that all treatments complied with the permissible standard limits for microbial loads through 14 days storage (Stannard, 1997).

Essential oils or their constituents have been shown to reduce microbial spoilage in fresh-cut pineapple, fresh-cut apple and arbutus berries in alginate based edible coatings (Guerreiro et al., 2015; Azarakhsh et al., 2014; Rojas-Graü et al., 2007). Moreover, Rojas-Graü et al. (2007) indicated that an alginate coating by itself alone did not reduce the psychrophilic aerobic bacteria or yeast and mold counts on fresh-cut 'Fuji' apples, while Tezotto-Uliana et al. (2014) found reduced raspberry decay rates with increased chitosan coating up to 2%. Our experiment was consistent with these previous findings and found that coating with either AL or PE reduced microbial spoilage when essential oil constituents were added.

2.4. Sensory evaluation

When treatments are applied to food products, taste panels are of great significance since treatments and storage time can change the edible quality of fruits, mainly when additives with strong flavor as essential oils are added. However, limited use of taste panels on such studies is observed.

In our study, after 7 days of storage at 0.5 °C, the taste panel showed that all treatments had good sensory attributes (>3.5 on a scale of 1-dislike definitely to 7-like definitely), (Tables 4 and 5). According to the taste panel, control and treatments with MICs of Eug and Cit scored higher than double MIC and control + water treatments for AL based edible coatings. For the PE edible coatings, the treatments with Eug at both MIC and double MIC and control, achieved better ratings than PE with Cit or the control + water (Table 5).

After 14 days, the raspberries were scoring under 4 for all AL treatments, denoting the fruit were not acceptable (Table 4). This may be attributed to the high weight loss observed after 14 days (Table 2), since weight loss reduces fruit freshness, influencing appearance and decreasing consumer's acceptance. Similar behavior was observed for PE-based edible coatings, except PE 1% with both Eug concentrations or when combined with Cit 0.15%, which were the treatments with less weight loss (Table 3). Rojas-Graü et al. (2007) report that vanillin incorporated into alginate edible coatings up to 0.3% were the most effective in terms of sensory quality after 2 weeks in fresh-cut apples as compared to lemongrass and oregano oils additives.

According to Azarakhsh et al. (2014), the incorporation of concentrations up to 0.3% (w/v) of lemongrass into alginate-based coating formulation did not have effect on the sensory attributes of fresh-cut pineapple. Lemongrass odor and taste were not detected by panelists at these concentrations (Azarakhsh et al., 2014). However, incorporation of 0.5% lemongrass negatively affected the sensory attributes of coated samples. In our case, Eug at double MIC concentration (0.2%) did not decrease sensory evaluation scores throughout the storage time, while Cit at 0.3% (double MIC) had reduced sensory evaluation scores and the microbial load was not as reduced.

2.5. Formulations selection

Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were used to explore the similarities and dissimilarities among the formulations with respect to the different analyzed quality parameters. HCA and PCA gave 3 main similar groups for both AL- and PE-based edible coatings (Figs. 1 and 2). The means were analyzed taking into account that the most suitable edible coatings had means with high harvest color, firmness, SSC, and antioxidant potential, while having lower values for percentage weight loss and microbial spoilage. The group enclosed by a circle represents in both AL and PE the edible coatings which were considered to have the best qualities on preserving fruit quality during cold storage (Figs. 1 and 2).

For the determination of the best AL and PE formulations, we examined within the chosen group and considered ones that scored highest in the taste panel. The results of this approach revealed for alginate the AL 2% + Cit 0.15% and AL 2% + Eug 0.1% as the best. For pectin-based edible coatings were the PE 1% + Eug 0.1% followed by PE 1% + Cit 0.15% + Eug 0.1%.

3. Conclusions

Fresh raspberry can be stored for at least 7 days at 0.5 °C, when edible coatings of AL and PE are applied at 1–2% concentrations and enriched with Eug and Cit at MIC concentrations (0.1 and 0.15%, respectively). Fruit quality declined by 14 days of storage and led to reduced consumer acceptance. Raspberries immersed in water (control + water) performed worse than fruit stored without any immersion or wash (control), so if hygienic conditions can be

Table 4

Taste panel of raspberries coated with alginate edible coatings (AL) enriched with citral (Cit) and eugenol (Eug) during storage at 0.5 °C.

	Days	Control		Control + water		AL 1%		AL 1% + Cit 0.15%		AL 1% + Cit 0.3%		AL 1% + Eug 0.1%		AL 1% + Eug 0.2%		AL 1% + Cit 0.15% + Eug 0.1%		AL 2%		AL 2% + Cit 0.15%		AL 2% + Cit 0.3%		AL 2% + Eug 0.1%		AL 2% + Eug 0.2%		AL 2% + Cit 0.15% + Eug 0.1%	
Appearance	0	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA	6.1	aA
	7	5.6	aA	4.8	bAB	5.5	aA	4.6	bAB	4.4	bAB	4.6	bAB	3.9	bB	5.1	aAB	5.3	aAB	5.8	aA	5.3	aAB	5.3	aAB	4.4	bAB	4.9	bAB
	14	3.3	bA	1.5	cAB	2.0	bAB	1.0	cB	2.3	cAB	1.5	cAB	1.3	cAB	1.3	bAB	1.0	bB	1.3	bAB	2.3	bAB	3.0	bAB	1.8	cAB	1.3	cAB
Texture	0	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA	5.9	aA
	7	5.3	aAB	4.1	bCD	5.3	aA	4.6	bABCD	3.8	bCD	5.6	aA	4.3	bBCD	5.1	aAB	4.9	bABC	5.6	aA	3.9	bCD	5.6	aA	4.3	bBCD	3.5	bD
Aroma	0	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA
	7	4.4	bABC	4.3	bABC	4.5	bABC	3.9	bBCD	3.8	bCD	4.5	bABC	3.4	bD	4.9	bA	4.8	bAB	5.0	aA	3.9	bBCD	4.6	bABC	3.9	bBCD	4.1	bABCD
Taste	0	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA
	7	4.3	bAB	4.1	bABCD	4.8	bA	4.3	bABC	3.4	bBCD	5.1	aA	3.6	bBCD	4.1	bABC	4.5	bAB	4.5	bAB	3.0	bCD	5.1	aA	2.8	bD	3.5	bBCD
Overall liking	0	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA	6.0	aA
	7	4.9	bAB	4.3	bBC	5.0	bAB	4.3	bBC	3.8	bC	5.0	aA	3.8	bC	4.8	bB	4.8	bAB	5.2	aA	4.0	bC	5.2	aA	3.8	bC	4.0	bBC

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 5

Taste panel of raspberries coated with pectin edible coatings (PE) enriched with citral (Cit) and eugenol (Eug) during storage at 0.5 °C.

	Days	Control		Control + water		PE 1%		PE 1% + Cit 0.15%		PE 1% + Cit 0.3%		PE 1% + Eug 0.1%		PE 1% + Eug 0.2%		PE 1% + Cit 0.15% + Eug 0.1%		PE 2%		PE 2% + Cit 0.15%		PE 2% + Cit 0.3%		PE 2% + Eug 0.1%		PE 2% + Eug 0.2%		PE 2% + Cit 0.15% + Eug 0.1%	
Appearance	0	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA	6.4	aA
	7	5	bABC	4.8	bABC	5.1	bABC	4.7	bBC	4.9	bABC	5.9	abA	5.6	aAB	5.2	bABC	5.2	bABC	4.7	bBC	4.7	bBC	4.4	bC	5.6	aAB	4.4	bC
	14	3.7	cABC	3	cBC	3.3	cABC	3	cBC	2.7	cC	5	bAB	5.3	aA	4.7	bABC	3	cBC	3.3	bABC	3.3	cABC	2.7	cC	3.7	bABC	3.3	bABC
Texture	0	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA	5.4	aA
	7	4.8	aAB	4	aBCDE	4.7	aABC	4.3	aABCD	4.1	aBCD	5.2	aA	4.6	aABCD	4.1	aBCD	4.6	aABCD	3.7	bCDE	2.9	bE	3.3	bDE	3.9	bBCDE	4.3	aABCD
Aroma	0	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA	4.9	aA
	7	4.1	aAB	4.1	aAB	4.6	aAB	4.1	aAB	4.9	aA	4.7	aAB	4.8	aAB	4.7	aAB	4.4	aAB	3.9	B	4.4	aAB	4.1	aAB	4.4	aAB	4.1	aAB
Taste	0	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA	5.8	aA
	7	4.7	bAB	4.1	bABC	4.9	aA	4.3	bABC	4.1	bABC	5	aA	4.9	aA	4.1	bABC	4.8	bA	3.3	bBC	3	bC	3.7	bABC	4.6	bAB	3.9	bABC
Overall liking	0	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA	5.5	aA
	7	4.6	bAB	4.3	bBC	4.8	bA	4.4	bBC	4.5	bB	5.2	aA	4.9	aA	4.5	bBC	4.8	bA	3.9	bC	3.8	bC	3.9	bC	4.6	bAB	4.2	bBC

Values in the same column followed by different lower case letter and in the same row followed by different upper case letter, for each parameter, are significantly different by Duncan's multiple range test ($P < 0.05$).

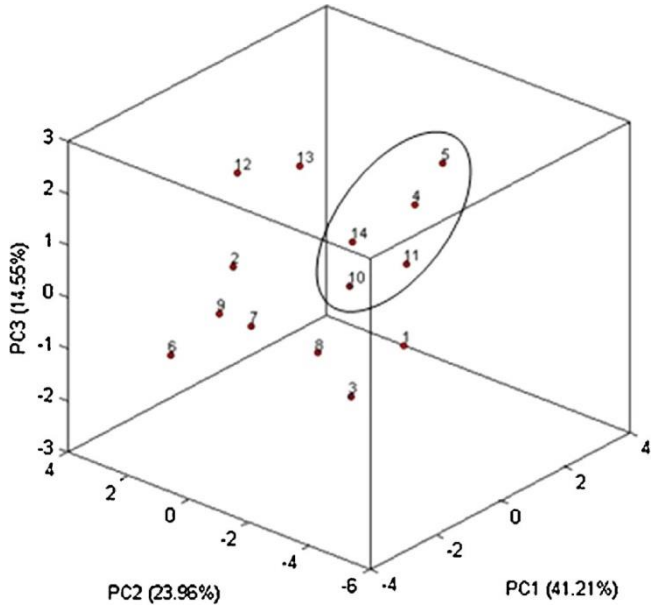


Fig. 1. Principal component analysis (PCA) plot of the 14 alginate (AL) treatments measured and showed in the above tables. 1—Control; 2—Control + water; 3—AL 1%; 4—AL 1% + Cit 0.15%; 5—AL 1% + Cit 0.3; 6—AL 1% + Eug 0.1%; 7—AL 1% + Eug 0.2%; 8—AL 1% + Cit 0.15% + Eug 0.1%; 9—AL 2%; 10—AL 2% + Cit 0.15%; 11—AL 2% + Cit 0.3%; 12—AL 2% + Eug 0.1%; 13—AL 2% + Eug 0.2%; 14—AL 2% + Cit 0.15% + Eug 0.1%.

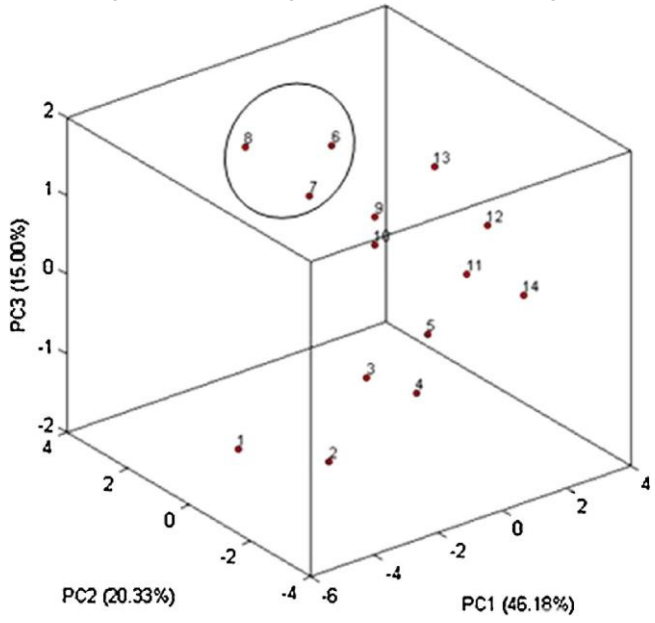


Fig. 2. Principal component analysis (PCA) plot of the of the 14 pectin (PE) treatments measured and showed in the above tables. 1—Control; 2—Control + water; 3—PE 1%; 4—PE 1% + Cit 0.15%; 5—PE 1% + Cit 0.3%; 6—PE 1% + Eug 0.1%; 7—PE 1% + Eug 0.2%; 8—PE 1% + Cit 0.15% + Eug 0.1%; 9—PE 2%; 10—PE 2% + Cit 0.15%; 11—PE 2% + Cit 0.3%; 12—PE 2% + Eug 0.1%; 13—PE 2% + Eug 0.2%; 14—PE 2% + Cit 0.15% + Eug 0.1%.

provided pre-, during and post-harvest, coating is not necessary. However, for fruit needing cleansing, raspberry fruits were better preserved in terms of general sensory attributes with coatings of AL 2% + Cit 0.15% and AL 2% + Eug 0.1%, and for PE with PE 1% + Eug 0.1% followed by PE 1% + Cit 0.15% + Eug 0.1%. The use of edible coating in raspberries is considered a safe and effective treatment. Future research will focus on the effect of those four edible coatings on preserving raspberries nutritional quality though storage, to select the best for commercial purposes.

Acknowledgment

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010—INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

References

Antunes, M.D.C., Dandlen, S., Cavaco, A.M., Miguel, G., 2010. Effects of postharvest application of 1-MCP and postcutting dip treatment on the quality and nutritional properties of fresh-cut kiwifruit. J. Agric. Food Chem. 58, 6173–6181.

Antunes, M.D.C., Gago, C.M.C., Cavaco, A., Miguel, M.G., 2012. Edible coatings enriched with essential oils and their compounds for fresh and fresh-cut fruit. Recent Pat. Food Nutr. Agric. 4, 114–122.

Azarakhsh, N., Osman, A., Ghazali, H.M., Tan, C.P., Mohd Adzahan, N., 2014. Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. Postharvest Biol. Technol. 88, 1–7.

Azevedo, A.N., Buarque, P.R., Cruz, E.M.O., Blank, A.F., Alves, P.B., Nunes, M.L., Santana, L.C.L.A., 2014. Response surface methodology for optimisation of edible chitosan coating formulations incorporating essential oil against several foodborne pathogenic bacteria. Food Control 43, 1–9.

Campos, C.A., Gerschenson, L.N., Flores, S.K., 2011. Development of edible films and coatings with antimicrobial activity. Food Bioprocess. Technol. 4, 849–875.

Cortés-Rojas, D.F., Souza, C.R.F., Oliveira, W.P., 2014. Clove (*Syzygium aromaticum*): a precious spice. Asian Pac. J. Trop. Biomed. 4, 90–96.

Duan, J., Wu, R., Strik, B.C., Zhao, Y., 2011. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. Postharvest Biol. Technol. 59, 71–79.

Giovanelli, G., Limbo, S., Buratti, S., 2014. Effects of new packaging solutions on physico-chemical, nutritional and aromatic characteristics of red raspberries (*Rubus idaeus* L.) in postharvest storage. Postharvest Biol. Technol. 98, 72–81.

Gol, N.B., Patel, P.R., Rao, T.V.R., 2013. Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. Postharvest Biol. Technol. 85, 185–195.

Guerreiro, A., Gago, C., Miguel, M., Antunes, M., 2013. The effect of temperature and film covers on the storage ability of *Arbutus unedo* L. fresh fruit. Sci. Hortic. 159, 96–102.

Guerreiro, A.C., Gago, C.M.L., Faleiro, M.L., Miguel, M.G.C., Antunes, M.D.C., 2015. The effect of alginate-based edible coatings enriched with essential oils constituents on *Arbutus unedo* L. fresh fruit storage. Postharvest Biol. Technol. 100, 226–233.

Han, C., Zhao, Y., Leonard, S., Traber, M., 2004. Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus ideaus*). Postharvest Biol. Technol. 33, 67–78.

ISO 21527-2, 2008. Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 2: Colony Count Technique in Products with Water Activity Less Than or Equal to 0.95. International Standards Organization, Switzerland.

Krüger, E., Dietrich, H., Schöpplein, E., Rasim, S., Kürbel, P., 2011. Cultivar, storage conditions and ripening effects on physical and chemical qualities of red raspberry fruit. Postharvest Biol. Technol. 60, 31–37.

Li, L., Wang, S., Chen, J., Xie, J., Wu, H., Zhan, R., Li, W., 2014. Major antioxidants and in vitro antioxidant capacity of eleven mango (*Mangifera indica* L.) cultivars. Int. J. Food Prop. 17, 1872–1887.

Maswal, M., Dar, A.A., 2014. Formulation challenges in encapsulation and delivery of citral for improved food quality. Food Hydrocolloids 37, 182–195.

McGuire, R., 1992. Reporting of objective color measurements. HortScience 27, 1254–1255.

Miguel, M., 2010. Antioxidant activity of medicinal and aromatic plants. A review. Flavour Fragr. J. 2009, 291–312.

Mikulic-petkovsek, M., Rescic, J., Schmitzer, V., Stampar, F., Slatnar, A., Koron, D., Veberic, R., 2015. Changes in fruit quality parameters of four Ribes species during ripening. Food Chem. 173, 363–374.

NP-4405, 2002. Food Microbiology—General Rules for Microorganism Counts. Colonies Count at 30 °C. Instituto Português da Qualidade, Lisboa, Portugal (In Portuguese).

Nunes, M.C.N., 2015. Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. Postharvest Biol. Technol. 107, 43–54.

Nunes, C.A., Freitas, M.P., Pinheiro, A.C.M., Bastos, S.C., 2012. Chemoface: a novel free user-friendly interface for chemometrics. J. Braz. Chem. Soc. 23, 2003–2010.

Olivas, G.I., Mattinson, D.S., Barbosa-Cánovas, G.V., 2007. Alginate coatings for preservation of minimally processed Gala apples. Postharvest Biol. Technol. 45, 89–96.

Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2008. Using polysaccharide-based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. LWT Food Sci. Technol. 41, 1862–1870.

Park, Y.-S., Namiesmik, J., Vearasilp, K., Leontowicz, H., Leontowicz, M., Barasch, D., Nemirovski, A., Trakhtenberg, S., Gorinstein, S., 2014. Bioactive compounds and the antioxidant capacity in new kiwi fruit cultivars. Food Chem. 165, 354–361.

Rao, A.V., Snyder, D.M., 2010. Raspberries and human health: a review. J. Agric. Food Chem. 58, 3871–3883.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G., Martin-Belloso, O., 2013. Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. LWT Food Sci. Technol. 50, 240–246.

Robles-Sánchez, R.M., Rojas-Graü, M.A., Odriozola-Serrano, I., González-Aguilar, G.A., Martín-Belloso, O., 2009. Effect of minimal processing on bioactive compounds and antioxidant activity of fresh-cut Kent mango (*Mangifera indica* L.). Postharvest Biol. Technol., 384–390.

Rojas-Graü, M.A., Raybaudi-Massilia, R.M., Soliva-Fortuny, R.C., Avena-Bustillos, R.J., McHugh, T.H., Martín-Belloso, O., 2007. Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. Postharvest Biol. Technol. 45, 254–264.

Rojas-Graü, M.A., Tapia, M.S., Martín-Belloso, O., 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. LWT Food Sci. Technol. 41, 139–147.

Shin, Y., Ryu, J.-A., Liu, R.H., Nock, J.F., Watkins, C.B., 2008. Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. Postharvest Biol. Technol. 49, 201–209.

Somolinos, M., García, D., Condón, S., Mackey, B., Pagán, R., 2009. Inactivation of *Eschericihai coli* by citral. J. Appl. Microbiol. 108, 1928–1939.

Stannard, C., 1997. Development and use of microbiological criteria for foods. Food Sci. Technol. Today, 11.

Tezotto-Uliana, J.V., Fargoni, G.P., Geerdink, G.M., Kluge, R.A., 2014. Chitosan applications pre- or post-harvest prolong raspberry shelf-life quality. Postharvest Biol. Technol. 91, 72–77.

Vargas, M., Pastor, C., Chiralt, A., McClements, D.J., González-Martínez, C., 2008. Recent advances in edible coatings for fresh and minimally processed fruits. Crit. Rev. Food Sci. Nutr. 48, 496–511.

Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V.D., Moldão-Martins, M., 2013. Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv. Camarosa) under commercial storage conditions. LWT Food Sci. Technol. 52, 80–92.

Vicente, A., Martıı´nez, G., Civello, P., Chaves, A., 2002. Quality of heat-treated strawberry fruit during refrigerated storage. Postharvest Biol. Technol. 25, 59–71.

Vu, K.D., Hollingsworth, R.G., Leroux, E., Salmieri, S., Lacroix, M., 2011. Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries. Food Res. Int. 44, 198–203.

Chapter X

Raspberry storage ability as affected by edible coatings enriched with essential oils

Adriana C Guerreiro[1], Custódia M L Gago[1], Maria G C Miguel[1], Maria L Faleiro[2], Maria D C Antunes[1]

[1]Faculdade de Ciências e Tecnologia, MeditBio, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

[2]Faculdade de Ciências e Tecnologia, CBMR, Edf. 8, Campus de Gambelas, 8005-139 Faro, Portugal

Submitted

Raspberry storage ability as affected by edible coatings enriched with essential oils

Abstract

This work aimed to study the effect of alginate and pectin based edible coatings enriched with essential oils components in raspberries storage ability. Four formulations of edible coatings selected in a previous work were used: sodium alginate(AL) 2%+eugenol 0.1%, AL 2%+citral (Cit) 0.15%, Pectin(PE) 1%+Eug 0.1% and PE 1%+Cit 0.15%+Eug 0.1%. At 0, 5, 10 and 15 d, samples were taken to perform analysis of colour, firmness, soluble solids content, weight loss, microbial growth, taste panels, phenolic compounds, sugars, organic acids, antioxidant activity, acetaldehyde, ethylene, CO_2 production and taste panel. Results showed that edible coatings did not have significant effect on raspberries general quality. They were efficient in controlling microbial food spoilage and were accepted by consumers up to 14 d while control fruit were unacceptable. Based on the major effect of edible coatings on reducing microbial spoilage and the taste panel, the best coating was PE 1%+Cit 0.15%+Eug 0.1%.

Key-Words: Alginate, pectin, eugenol, citral, raspberries, fruit quality.

1. Introduction

Raspberry (*Rubus idaeus* L.) is a member of the *Rosaceae* family and is grown primarily for its edible berries which typical flavor makes these fruit easily recognized and appreciated by consumers (Morales et al., 2014).

Consumption of fresh fruit and vegetables is beneficial to human health. However, they are highly perishable and during postharvest handling and storage, losses of vitamins and other phytonutrients are expected, although losses vary by nutrient,

genotype, physical damage, temperature and storage environment (Olivas and Barbosa-Cánovas, 2005; Wang, 2007). Because of raspberry high perishability, a rapid decrease in temperature is a critical point for reducing respiration and slowing deterioration (Morales et al., 2014). Besides that, other postharvest technologies can be applied to increase storage ability.

One of them are edible coatings which are traditionally used to improve food appearance and conservation due to their environmentally friendly nature, they act as barriers to moisture and oxygen during processing, handling, and storage, and not only retard food deterioration, but also improve safety, due to their natural biocide activity or to the incorporation of antimicrobial compounds (Hassanpour, 2015). Alginate is a natural polysaccharide extracted from brown sea algae (*Phaeophyceae*), it is composed of two uronic acids: β-D-mannuronic acid and α-L-guluronic acid and it is located in the intracellular matrix as a gel containing sodium, calcium, magnesium, strontium and barium ions and is known as a hydrophilic biopolymer that has a coating function because of its well-studied unique colloidal properties, which include its use for thickening, suspension forming, gel forming and emulsion stabilizing (Acevedo et al., 2012; Gol et al., 2015). Pectin is a complex anionic polysaccharide composed of β-1,4-linked D-galacturonic acid residues, wherein carboxyl groups of uronic acid are either fully (HMP, high methoxyl pectin, DE, degree of esterification > 50%) or partially (LMP, low methoxyl pectin, DE < 50%) methyl esterified (Galus and Lenart, 2012).

The aim of this study was to evaluate the effect of different edible coatings on the quality attributes of raspberries.

2. Materials and Methods

2.1.Material

Strawberries were obtained from a local producer at the harvest day (Algarve, Portugal), then immediately transported to the Postharvest laboratory at the University of Algarve where fruits were selected for uniformity of size and freedom from defects for use in the experiments.

Food grade sodium alginate (AL) and pectin (PE) (Sigma-Aldrich Chemic, Steinhein, Germany) were the biopolymers used for coating formulations. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce cross linking reaction and ascorbic acid (AA) (Scharlau, Barcelona, Spain).

.

2.2.Edible Coatings preparation

The coating forming solutions based on AL and PE, were formulated as described by Guerreiro et al. (2015b). Ascorbic acid 1%(w/v) was added to all edible coatings as anti-browning agent and $CaCl_2$ at 1 g. 100^{-1} mL was used as final dip for cross-link.

The treatments were: Control, AL 2 g.100^{-1} mL (AL 2%)+ Eug 0.1 g.100^{-1} mL (Eug 0.1%), AL2%+Cit 0.15 g.100^{-1} mL (Cit 0.15%), PE 1 g.100^{-1} mL (PE1%)+ Eug 0.1% and PE 1% + Cit 0.15% + Eug 0.1%.

The fruit were dipped into the edible coating solution for 2 min, allowed to drip for 30 sec, and dipped in the calcium chloride solution for 1 min, then drip again. Afterwards, 8 randomly raspberries were placed in polypropylene plastic trays (8 cmx10 cmx4 cm), clamshell type, and stored at 0.5 ºC until analyses. On days 0, 5, 10 and 15, three trays per treatment (replications) were taken for quality evaluation. Controls did not have any kind of treatment.

2.3.Quality parameters

Color of fruits was measured by a Minolta Chroma meter CR-300 (ECMinolta, Japan) using the CIELab scale (L*,hº*,C*) (McGuire, 1992; Guerreiro et al., 2015a).

The firmness of the pulp was determined by puncture with a Chatillon TCD200 and Digital Force Gauge DFIS50 (Jonh Chatillon&Sons, Inc. USA) using a piston cylinder of 4 mm diameter at a depth of 7 mm. The soluble solids content (ºBrix) was measured using a digital refractometer PR1ATAGO CoLTD (Japan), in raspberry juice. Weight loss was expressed as percentage of initial weight.

2.4.Microbial counts

Microbial counts were determined for each treatment. The microbiological parameters that were determined included counts of aerobic mesophilic microorganisms and molds and yeasts were preformed according to (Guerreiro et al., 2015b). Experiments were done in triplicate. Results were expressed as Log_{10} CFU (Colony Forming Unit) per gram fresh weight.

2.5.Sensory Evaluation

A taste panel was performed with 15 semi-trained panelists on the base of a 7-point hedonic scale (1-bad; 7-excellent) for the sensory parameters: Appearance, aroma, texture, sweetness, acidity, flavor and overall acceptance. All parameters were evaluated at harvest and after 7 and 14 days storage.

2.6.Total phenolic content

Total phenolic content was determined according to the Folin–Ciocalteu colorimetric method Singleton & Rossi (1965) modify for microplates. The sample (80

µL) and 20 µL of sodium carbonate (75 $g.L^{-1}$) were added to 100 µL of 10% (w/v) Folin–Ciocalteu reagent. After 30 min of reaction at room temperature, the absorbance was measured at 765 nm (Tecan Infinite M200, Swiss). Gallic acid was used as standard for calibration curve.

2.7.Flavonol content

The content of these groups of compounds were quantified as described by Miguel et al. (2010) and modify for using microplates of 96 wells. Sample or standard (100 µL) was added to 100 µL of 2% $AlCl_3$ ethanol solution. After 1h at room temperature, the absorbance was measured at 420 nm (Tecan Infinite M200, Swiss). Quercetin was used as a standard for the construction of calibration curve.

2.8.Anthocyanins

The total anthocyanin content was measured using a modified pH differential method (Lee et al., 2005; Guerreiro et al., 2013). Absorbance of anthocyanin at 520 nm and 700 nm in different pH buffers (pH 1.0 and 4.5) were measured, respectively. Absorbance readings were converted to total mg of cyanidin 3-glucoside per 100 g fresh weight of sample.

2.9.Antioxidant Activity

2.9.1. Trolox Equivalent Antioxidant Activity (TEAC)

The antioxidant activity was measured according to Re at al. (1999) and modified for microplates. Raspberry juice was obtained after squeezing apple slices flesh with an UltraTurrax T 18 (IKA, Germany) for 2 min then centrifuge 5 minutes at 5000 rpm. For the assay, 3 µL of raspberry juice was added to 197 µL of of 2,2'-

azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS radical cation solution). The absorbance was monitored at 750 nm for 6 min (Tecan Infinite M200, Swiss). The antioxidant activity of each sample was calculated by the equation: scavenging effect (SE %)=(1-As/Ao)x100, where Ao stands for the absorbance of the control at time 0 and As for the absorbance in the presence of the sample after 6 minutes. The values were compared with the curve for several Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations and the values given as mm Trolox equivalent antioxidant capacity.

2.9.2. Oxygen Radical Absorbance Capacity (ORAC)

The antioxidant activity by the method ORAC measures the ability of samples for scavenging peroxyl radicals. The ORAC method used, with fluorescein (FL) as the fluorescent probe, was that described by Guerreiro et al. (2013). Final ORAC values are calculated using the regression equation between Trolox concentration and the net AUC and are expressed as mmol Trolox.100^{-1} fresh weight.

2.10. Extraction, quantification of sugars and sweetness index

Extraction and quantification of sugars (fructose, glucose and sucrose) was based on a method described by Terry et al. (2007). Briefly, a 150 ± 0.5 mg of fruit powder was extracted in 3 mL 62.5% (v/v) aqueous methanol. Following extraction, the concentrations of fructose, glucose and sucrose were determined in an HPLC binary pump system (L-2130, Elite LaChrom series, Hitachi, Japan). Ten micro litres (10 µL) of a diluted sample solution (1:10) was injected 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). The

thermostated column compartment temperature was set at 35ºC. The mobile phase used was HPLC-grade water at a flow rate of 1.0 mL.min^{-1} 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.

Sugars have different sweetness impact. Since sucrose is 1.35 times sweeter than glucose and fructose is 2.3 times sweeter than glucose, a sweetness index concept was used to estimate the total sweetness perception. Glucose was assigned a sweetness value of one, sucrose 1.35 and fructose 2.3(Qian, 2005; Keutgen and Pawelzik, 2007). Total sweetness index = 1 glucose+ 1.35 sucrose + 2.3 fructose.

2.11. Extraction and quantification of non-volatile organic acids

Non-volatile organic acids (citric, ascorbic, malic, tartaric and oxalic acid) were extracted and determined using a method described by Crespo et al. (2010). Briefly, 50 ± 0.5 mg of freeze dried samples were cold extracted for 5 min in 3 mL of HPLC water. The flocculate was filtered through a 0.2 µm syringe filter before HPLC analysis. Organic acids concentrations were determined on a HPLC binary pump system equipped with a diode array detector (DAD, L-2455, Elite LaChrom series, Hitachi,Japan) with multiple wavelength detector, degasser and cooled autosampler. The filtered sample extract was injected into a Purospher Star RP-18 column (4.6 mm diameter × 250 mm, 5 µm particle size; Merck Millipore, Germany) with an organic acid guard column (LiChroCART 4-4 Merck Millipore, Germany). Temperature of the column was set to 35ºC using a thermostated column compartment (L-2300, Elite LaChrom series, Hitachi,Japan). The mobile phase used was 0.2% HPLC-grade aqueous metaphosphoric acid at a flow rate of 1.0 mL.min^{-1}. Non-volatile organic acids was

detected at 210 nm except for ascorbic acid which was detected at 245 nm and quantified using linear standard curves.

2.12. Acetaldehyde quantification

For the quantification of the acetaldehyde existing in samples were used the kit K-ETOH 02/11 Megazyme (Ireland) and K-ACHYD Megazyme (Ireland), respectively. The determination of ethanol and acetaldehyde were preformed according to manufacturer instructions. Absorbance was measured by spectrophotometry using a microplate reader (Tecan Infinite M200, Swiss) at 340nm.

2.13. CO_2 production

Respiration was calculated by CO_2 production in the gas phase of the jars, measured in Li-6400 portable (Li-Cor) using a flow rate of 0.5µmol/s^{-1}, and read for 5 min.

2.14. Cells Culture and Cytotoxicity

Cells were kept in 10 mL dishes at 5% CO_2 in Dulbecco's Modified Eagle Medium (1000 mg.ml^{-1} glucose, 110 mg/ml pyruvate, and 580 mg.ml^{-1} glutamine) supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 100 µg.ml^{-1} penicillin, and 100 µg.ml^{-1} streptomycin.

MTT is a standard colorimetric assay for measuring the activity of enzymes that reduce yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan in living cells (Klewicka et al., 2012). Cytotoxicity were determined using a method described by Girón-Calle, Alaiz, & Vioque (2010) with slight modifications. In our study we analyze the citotoxicity only in the coatings formulations

without fruits, and tested at 24h, 4days and 6 days. Cells in 96 well microplates were exposed to MTT by addition of fresh medium containing the reagent so that the final concentration was 0.5mg.ml^{-1}, and were incubated for 1h in the CO_2 incubator. Reduced MTT was solubilized by addition of the same volume of 0.1 N HCl in isopropanol. Absorbance at 570 nm with a background reference wavelength of 630 nm was measured using a plate reader and calculated according the follows equations:

$$\%cell\ viability = \frac{Sample\ O.D}{Control\ O.D} \times 100$$

$$\%cytotoxicity = 100 - \%cell\ viability$$

2.15. Statistical Analysis

Statistical analysis was carried out with the SPSS 20.0 software (SPSS Inc.). Two-way ANOVA and Duncan's multiple-range test ($P < 0.05$) for comparisons among treatments was performed.

3. Results

3.1.The effect of edible coatings on quality parameters and microbial ability

Change in color is one of the factors that determine the quality of fresh raspberries. The surface color L* value showed a significant increase through storage in all treatments (Table 1). At the beginning of the experiment, just after fruit coating, L* value was higher (whiter) in control than in coated fruit, maybe due to coating formulation. At the end of the experiment control and PE 1% + Eug 0.1% had significantly lower values than AL 2% + Eug 0.1%. The ºhue values decreased through storage time in strawberries of all treatments (Table 1). At the beginning of the experiment, the ºhue was higher in control than coated fruit, but at the end of storage period there were no significant differences among treatments. Chroma decreased through storage in all treatments (Table 1). Despite no significant differences among

treatments were observed, fruit of AL treatments showed significantly lower Chroma values than the other treatments.

Firmness decreased through storage in all treatments (Table 1). With the exception of day 5, there were no significant differences in firmness among treatments.

Despite of some statistically significant differences, raspberries SSC changes from 4.8 to 6.1 % (Table 1), from where we can conclude that edible coatings did not affect SSC.

The weight loss increased through storage time in all treatments (Table 1). However, increase was higher in edible coatings than in control. From the edible coatings the one that showed lower weight loss was PE 1% + Cit 0.15% + Eug 0.1%.

Molds and yeasts and aerobic mesophilic microorganisms were present in all treatments at the beginning of the experiment (Table 1). The PE coatings were efficient in controlling yeasts and molds development, while all edible coatings inhibited mesophilic microbial growth. Control showed the higher microbial development.

Total phenols almost did no change through storage in none treatment (Table 2). Nevertheless, control had higher values at harvest than coated fruit, while at the end of the experiment, AL 2% + Cit 0.15% and PE 1% + Eug 0.1% were significantly lower than control and PE 1% + Cit 0.15% + Eug 0.1%.

Flavonoids were higher, just after coating application, in control followed by PE treatments and AL (Table 2). They did increase in PE 1% + Cit 0.15% + Eug 0.1% and were maintained in the other treatments. At the end of storage, control and PE 1% + Cit 0.15% + Eug 0.1% had significantly higher flavonoids than the other treatments.

Table 1 Color parameters (L*, hº, C*), firmness (N), soluble solids content (SSC), weight loss, moulds and yeasts and aerobic mesophilic microorganisms of raspberries covered with different alginate and pectin based edible coating formulations during storage at 0.5 ºC. Values represent the mean of three replicates ± standard error taken at 0, 5, 10 and 15 days.

Quality Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15%		PE 1% + Eug 0.1%		PE 1% + Cit 0.15% + Eug 0.1%	
Lightness (L*)	0	36.8±1.3	cA	30.3±0.4	cB	30.5±0.4	bB	33.2±0.7	cB	31.0±0.3	cB
	5	34.9±0.7	bAB	37.1±0.6	bA	37.4±0.6	aA	36.2±0.2	bAB	37.0±0.6	bA
	10	35.2±0.3	aA	37.5±0.8	bA	37.8±0.8	aA	36.3±0.7	bAB	37.9±0.3	abA
	15	38.2±0.3	bA	40.1±0.4	aA	39.3±0.4	aAB	38.2±0.3	aB	39.0±0.2	aAB
Hue (hº)	0	31.3±2.3	aA	25.5±0.5	cB	25.7±2.1	aB	28.3±0.7	aAB	25.9±0.9	aB
	5	24.7±0.5	aA	20.3±0.5	cB	20.0±0.6	bB	20.0±0.6	bB	19.4±0.7	bB
	10	21.1±0.2	aA	17.5±0.4	bC	20.4±0.5	bBC	19.4±0.8	bB	19.1±0.6	bBC
	15	17.7±0.3	aA	17.1±0.3	aA	16.6±0.8	bA	16.7±0.4	cA	17.1±0.7	bA
Chroma (C*)	0	27.3±2.4	aB	28.2±1.1	cA	28.4±1.6	aA	28.3±0.8	aA	27.4±0.9	aA
	5	23.0±0.3	aA	17.9±0.5	cB	18.4±0.5	bB	19.1±0.5	bB	19.0±1.1	bB
	10	20.3±0.3	aAB	15.3±0.1	bC	16.7±0.9	bBC	17.7±0.8	bcB	17.4±0.7	bBC
	15	18.0±0.3	aA	14.8±0.1	aC	15.1±0.7	bC	16.2±0.6	cBC	16.9±0.7	bAB
Firmness (N)	0	2.7±0.4	aA	2.5±0.1	aA	2.6±0.2	aA	2.4±0.1	abA	2.3±0.1	aA
	5	3.2±0.1	aB	2.6±0.1	aB	2.4±0.2	aB	2.8±0.1	aAB	2.5±0.2	aB
	10	2.4±0.2	aB	1.8±0.1	bA	2.1±0.1	aA	2.2±0.2	bcA	2.3±0.2	aA
	15	1.9±0.2	abA	1.7±0.1	bA	2.0±0.2	aA	1.8±0.1	cA	2.0±0.1	aA
SSC (ºBrix)	0	5.2±0.2	abA	4.8±0.0	bB	4.9±0.2	aAB	5.2±0.1	aAB	5.3±0.1	bA
	5	5.4±0.0	aA	4.9±0.1	bA	5.2±0.3	aA	5.1±0.1	aA	5.3±0.1	bA
	10	5.5±0.0	aA	5.4±0.0	aB	5.2±0.1	aB	5.4±0.1	aAB	5.8±0.2	aA
	15	6.1±0.2	bA	5.5±0.0	aB	5.0±0.1	aC	5.4±0.1	aBC	5.7±0.1	abAB
Weight Loss (%)	0	0.0±0.0	dA	0.0±0.0	dA	0.0±0.0	dA	0.0±0.0	dA	0.0±0.0	dA
	5	1.5±0.2	cA	4.2±0.1	cA	3.6±0.4	cAB	3.1±0.3	cB	3.7±0.4	cAB
	10	2.7±0.2	bB	6.5±0.1	bA	5.9±0.5	bAB	5.2±0.3	bB	5.6±0.4	bAB
	15	3.9±0.2	aB	8.6±0.3	aA	7.9±0.6	aA	7.3±0.5	aA	7.5±0.4	aA
Moulds and Yeasts	0	1.6±0.0	bA	1.5±0.0	cB	1.7±0.0	aA	0.6±0.1	aC	0.6±0.0	abC
	5	2.1±0.1	aAB	1.9±0.0	aA	1.2±0.2	bB	0.0±0.0	bC	0.6±0.3	aB
	10	2.1±0.1	aA	1.8±0.0	aB	1.4±0.0	abC	0.0±0.0	bD	0.0±0.0	bD
	15	2.2±0.0	aA	1.7±0.0	bB	1.3±0.0	abC	0.0±0.0	bD	0.0±0.0	bD
Mesophilic Microorganisms	0	1.3±0.3	bA	0.7±0.0	aBC	1.6±0.0	aA	0.3±0.3	aC	0.4±0.1	aC
	5	1.1±0.0	aA	0.0±0.0	bB	0.0±0.0	bB	0.0±0.0	aB	0.0±0.0	bB
	10	1.2±0.1	aA	0.0±0.0	bB	0.0±0.0	bB	0.0±0.0	aB	0.0±0.0	bB
	15	1.6±0.0	aA	0.0±0.0	bB	0.0±0.0	bB	0.0±0.0	aB	0.0±0.0	bB

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Anthocyanins were higher in control and AL 2% + Cit 0.15% than in the other treatments at the beginning of the experiment (Table 2). Control did not change through storage but the other treatments had some changes. Nevertheless, after 10 d control, AL 2% + Eug 0.1% and PE 1% + Eug 0.1% had significantly higher anthocyanins, while at the end of storage control was the one with higher values.

The antioxidant activity as measured by ORAC method did not show significant changes among treatments or over storage (Table 2).

When measuring antioxidant activity by the TEAC method, PE treatments had higher values than the other treatments at the beginning of the experiment (Table 2). Values of ORAC increased in all treatments, except PE 1% + Cit 0.15% + Eug 0.1% treatment, and at the end of storage there were no significant differences among treatments.

Treatments did not significantly affect acetaldehyde or CO_2 production (Table 2).

Oxalic acid increased though storage in control and AL treatments while in PE coated raspberries was maintained constant (Table 3). There were no significant differences among treatments at the end of the storage period.

Ascorbic acid increased at the beginning of storage and then decreased in all treatments (Table 3). However, at the end of storage AL treatments had significantly higher ascorbic acid than the other treatments.

Citric acid decreased mainly at the end of the storage in all treatments (Table 3). At the end of storage PE 1% + Cit 0.15% + Eug 0.1% had significantly lower values than the other treatments.

Quinic acid decreased mostly from 5 to 10 d storage in all treatments except PE 1% + Eug 0.1%, which did not significantly change through storage (Table 3). At the

end of the experiment this edible coating showed significantly higher quinic acid than the other treatments.

Shikimic acid also decreased through storage in all treatments, despite a small increase from 0 to 5 d in edible coated raspberries (Table 3). At the end of storage, shikimic acid showed higher values in AL 2% + Eug 0.1% than in the other treatments, except control.

Fructose increased from 0 to 5 d in all treatments, then remained constant, except PE 1% + Cit 0.15% + Eug 0.1% in which it increased, then decreased again from 5 to 10 d (Table 3). Although control showed the higher values at 10 d storage, at the end of the experiment fructose values did not differ among treatments.

Sucrose increased from 0 to 5 d in control and PE treatments, while it remained constant in the AL ones (Table 3). Nevertheless, no significant differences among treatments were found at 10 and 15 d storage.

The sweet index did not show significant differences among treatments at the beginning of the experiment (Table 3). There was an increase in the sweet index from 0 to 5 days in all treatments but at the end of storage there were no significant differences among treatments.

The taste panel sowed that control fruit were not suitable for consumption at 14 d after storage since overall liking scored under the minimum acceptable value, which is 4 (Table 4). All edible coatings preserve raspberries in good commercial quality for up to 14 d, being the PE 1% + Cit 0.15% + Eug 0.1% the best, followed by AL 2% + Eug 0.1%.

Table 2 Total phenols totals, flavonoids, anthocyanins, TEAC, ORAC, acetaldehyde and CO_2 production of raspberries covered with different alginate and pectin based edible coating formulations during storage at 0.5 ºC. Values represent the mean ± standard error of three replicates taken at 0, 5, 10 and 15 days.

Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15%		PE 1% + Eug 0.1%		PE 1% + Cit 0.15% + Eug 0.1%	
Total Phenols (mg of Gallic acid.100g^{-1} FW)	0	121.95±4.56	cA	85.72±1.53	aB	83.06±2.83	abB	94.01±3.65	aB	91.65±5.86	aB
	5	120.95±5.11	bAB	102.23±5.54	aA	110.11±14.50	aA	108.12±2.18	aA	114.01±11.71	aA
	10	92.10±10.01	aA	87.51±7.92	aA	89.97±6.18	abA	93.33±11.94	aA	101.75±3.18	aA
	15	108.96±5.20	bA	97.87±6.94	aAB	73.51±4.98	bC	85.61±4.68	aBC	104.07±2.77	aA
Flavonoids (mg of Quercitin .100g^{-1} FW)	0	8.03±0.31	aA	2.40±0.15	cC	2.53±0.14	bC	6.13±0.31	aB	5.78±0.28	bB
	5	8.60±0.36	aA	4.41±0.26	aC	5.41±1.31	aABC	5.08±1.29	aBC	7.85±1.14	aAB
	10	5.67±0.76	aA	3.19±0.07	bB	3.16±0.27	abB	4.83±0.66	aA	5.44±0.07	bA
	15	7.65±0.38	aA	3.67±0.21	bC	2.79±0.43	bC	5.00±0.37	aB	6.64±0.20	abA
Anthocyanins (mg of Cyanidin 3-O-glucoside .100g^{-1} FW)	0	54.14±5.92	aB	34.24±5.58	aB	51.12±6.14	aA	27.59±2.46	bB	33.13±4.13	bB
	5	59.73±7.48	aA	47.71±1.55	aA	56.58±9.39	aA	54.22±7.74	aA	57.11±3.20	aA
	10	68.29±11.46	aAB	41.96±1.42	aAB	32.03±9.74	aB	64.15±10.42	aA	32.62±3.35	bB
	15	70.26±7.41	aA	42.35±8.76	aB	40.32±3.31	aB	42.57±2.31	aB	39.08±2.05	bB
ORAC (µM TE.100g^{-1} FW)	0	45.14±2.31	aA	48.44±0.85	aA	49.38±2.11	aA	48.25±1.82	aA	46.45±0.37	aA
	5	47.26±0.62	aB	46.48±0.61	aA	49.37±0.99	aA	46.88±0.76	aA	49.40±1.80	aA
	10	49.59±0.09	aB	48.89±0.74	aA	49.70±0.93	aA	48.18±1.25	aA	49.27±0.38	aA
	15	48.59±0.87	abA	48.67±1.00	aA	48.94±0.36	aA	47.59±0.31	aA	47.49±0.74	aA
TEAC (µM TE.100g^{-1} FW)	0	43.46±5.43	abA	40.10±2.61	aB	45.28±6.95	bB	49.12±7.49	bAB	66.07±3.46	bA
	5	97.90±9.48	aA	63.78±12.72	aB	53.30±6.19	abB	65.05±3.73	aB	52.71±7.81	abB
	10	61.95±3.98	aA	58.63±15.84	aA	64.88±0.19	aA	46.81±2.34	bA	57.88±7.17	abA
	15	50.03±1.66	bA	55.65±9.77	aA	61.54±1.93	aA	46.39±2.42	bA	44.18±4.21	aA
Acetaldehyde (mg.100g^{-1} FW)	0	1.68±0.55	dA	0.96±0.51	aA	2.10±0.27	abA	2.63±1.42	aA	1.53±0.63	aA
	5	2.96±1.03	cA	1.94±0.72	aA	2.75±1.56	abA	0.46±0.46	aA	8.24±7.34	aA
	10	1.78±0.50	bB	2.05±1.11	aA	3.64±0.64	aA	1.77±0.90	aA	1.91±1.01	aA
	15	2.16±1.14	aB	1.65±0.59	aA	0.25±0.25	bA	0.37±0.31	aA	2.47±1.24	aA
CO_2 Production (µmol.Kg.seg^{-1})	0	10.37±0.90	bA	10.27±0.32	aA	12.30±0.31	aA	11.07±1.32	aA	11.90±1.05	aA
	5	10.00±1.15	aAB	6.57±0.15	aB	8.00±1.08	aAB	10.33±0.27	abA	10.43±0.78	aA
	10	6.83±0.09	aA	6.93±2.94	aA	7.47±2.17	aA	7.40±0.78	bA	7.53±0.75	aA
	15	10.00±0.06	aA	11.33±0.57	aA	8.60±1.60	aA	9.20±1.06	abA	9.30±0.72	abA

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Table 3 Organic acids and sugars of raspberries covered with different alginate and pectin based edible coating formulations during storage at 0.5 ºC. Values represent the mean of three replicates ± standard error taken at 0, 5, 10 and 15 days.

Parameters	Days	Control No Treated		AL 2% + Eug 0.1%		AL 2% + Cit 0.15%		PE 1% + Eug 0.1%		PE 1% + Cit 0.15% + Eug 0.1%	
Oxalic Acid (mg.100g^{-1} DW)	0	1775.4±140.8	cA	1241.5±149.4	bA	1799.2±115.5	bA	1568.5±471.7	aA	1680.1±338.8	aA
	5	1866.8±49.3	bAB	2169.5±105.9	aAB	2412.7±151.6	aA	1913.7±133.4	aB	2125.5±71.4	aAB
	10	2062.7±67.9	aA	2018.6±85.5	aA	1833.6±142.8	bAB	1090.1±545.2	aB	1397.1±50.7	abAB
	15	1075.1±121.1	bA	1430.8±92.1	bA	1432.8±93.2	bA	893.6±450.2	aA	484.3±484.3	bA
Ascorbic Acid (mg.100g^{-1} DW)	0	266.2±17.0	aA	378.5± 14.6	bBC	543.7±33.7	bA	375.8±55.7	abBC	415.2±46.6	bB
	5	307.6±8.1	aA	691.8± 63.9	aA	737.6±35.0	aA	497.4±45.5	aB	543.9±14.2	aB
	10	389.8±43.3	aA	501.8± 61.0	bAB	549.1±22.0	bA	459.2±41.5	aAB	360.7±57.4	bB
	15	240.8±21.6	aA	445.5± 22.2	bA	438.3±25.7	cA	276.0±53.2	bB	308.8±3.9	bB
Citric Acid (mg.100g^{-1} DW)	0	12160.5±1500.2	aB	8556.3±1004.8	bcB	12896.7±861.3	aA	10150.2±1164.4	aBC	11443.0±1181.7	aAB
	5	11138.4±327.1	aA	13199.0±998.8	aAB	14008.6±981.2	aA	11253.0±797.4	aB	11370.7±155.3	aB
	10	10722.4±355.9	aAB	10258.5±750.5	bA	9671.8±799.2	bA	5861.3±2932.2	bA	8366.9±584.0	bA
	15	5398.7±715.9	aA	6841.4±424.4	cAB	7464.8±258.8	bA	5542.9±863.7	bAB	5101.4±899.8	bB
Quinic Acid (mg.100g^{-1} DW)	0	1913.1±123.1	aA	2174.5±104.5	abA	2141.6±182.4	bA	1839.9±341.8	aA	1958.6±247.2	aA
	5	2072.1±39.3	aB	2543.1±22.4	aA	2829.7±138.0	aA	1545.7±86.0	aC	1716.9±123.6	aC
	10	1292.1±83.5	aB	1890.6±203.4	bcA	1756.0±108.0	bA	1529.4±306.0	aAB	1057.4±166.0	bB
	15	827.4±54.8	abA	1626.1±124.0	cB	1117.0±78.4	cC	2296.5±224.2	aA	589.2±205.4	bD
Shikimic Acid (mg.100g^{-1} DW)	0	55.5±7.4	abA	42.2±3.7	bA	45.5±1.5	bA	47.2±2.7	aA	44.8±1.4	bA
	5	43.9±4.5	aA	51.3±0.9	aB	51.9±0.8	aB	49.2±1.1	aB	47.9±0.3	aB
	10	45.5±0.7	aA	44.0±0.6	bAB	43.0±0.8	bABC	39.9±2.3	bBC	39.0±1.0	cC
	15	36.6±0.4	bA	38.7±0.1	bA	37.6±1.2	cAB	36.3±0.6	bB	36.1±0.6	cB
Fructose (mg.g^{-1} DW)	0	2.0±0.2	dA	1.7±0.2	bA	1.8±0.2	bA	1.7±0.0	bA	1.5±0.0	cA
	5	2.6±0.1	cA	2.2±0.1	abC	2.4±0.1	aBC	2.5±0.1	aAB	2.7±0.1	aA
	10	2.7±0.1	bB	2.4±0.1	aB	2.3±0.1	aB	2.3±0.1	aB	2.3±0.1	bB
	15	2.4±0.1	aB	2.3±0.2	aA	2.3±0.0	aA	2.5±0.1	aA	2.3±0.1	bA
Glucose (mg.g^{-1} DW)	0	3.2±0.1	bA	2.9±0.2	bA	2.9±0.3	bA	2.9±0.0	bA	2.8±0.0	cA
	5	4.1±0.1	aAB	3.5±0.1	abC	3.8±0.1	aBC	4.4±0.3	aB	5.0±0.3	aAB
	10	3.5±0.1	aA	3.4±0.2	abC	3.9±0.1	aAB	4.0±0.1	aA	4.2±0.1	bA
	15	4.4±0.1	aA	4.1±0.3	aA	4.0±0.0	aA	4.4±0.1	aA	3.9±0.1	bA
Sucrose (mg.g^{-1} DW)	0	4.8±0.4	bA	4.4±0.4	aA	4.6±0.5	aA	4.7±0.1	bA	4.0±0.1	bA
	5	6.2±0.3	aA	5.2±0.2	aB	5.4±0.1	aB	5.2±0.2	aB	5.2±0.1	aB
	10	5.6±0.1	aA	4.7±0.4	aA	5.0±0.2	aA	5.4±0.1	aA	5.5±0.3	aA
	15	5.4±0.1	aA	5.1±0.4	aA	5.2±0.2	aA	5.6±0.1	aA	5.1±0.1	aA
Sweetness Index (SI)	0	13.9±0.6	aA	12.8±1.3	aA	13.3±1.4	bA	13.2±0.3	bA	11.7±0.2	cA
	5	18.5±0.7	aA	15.6±0.7	aB	16.6±0.3	aAB	17.2±0.5	aAB	18.4±0.6	aA
	10	17.3±0.4	aA	15.1±0.7	aB	16.0±0.7	abAB	16.5±0.4	aAB	16.9±0.6	abAB
	15	17.2±0.4	aA	16.2±1.3	aA	16.3±0.3	aA	17.7±0.5	aA	16.0±0.4	bA

Values in the same column followed by different lower case and in the same row followed by different upper case, for each parameter, are significantly different by Duncan's multiple range test ($P<0.05$).

Table 4 Sensory evaluation of raspberries covered with different alginate and pectin based edible coating formulations during storage at 0.5 ºC. Values represent the mean of 15 replicates taken at 0, 5 and 10 days.

Treatment	Days	Appareance	Aroma	Texture	Sweetness	Acidity	Flavor	Ovarall Liking
Control	0	6.1	5.1	6.0	6.2	6.1	6.4	6.0
	7	5.4	4.7	5.5	5.2	4.8	5.1	5.1
	14	3.1	2.8	4.0	3.8	3.4	2.4	3.3
AL 2% + Eug 0.1%	0	6.1	5.1	6.0	6.2	6.1	6.4	6.0
	7	5.1	4.3	3.8	4.4	4.1	3.8	4.3
	14	3.9	4.6	5.6	4.8	4.4	4.2	4.6
AL 2% + Cit 0.15%	0	6.1	5.1	6.0	6.2	6.1	6.4	6.0
	7	4.6	4.2	4.7	4.5	4.4	4.2	4.4
	14	3.7	4.0	3.5	4.0	3.5	4.0	3.8
PE 1% + Eug 0.1%	0	6.1	5.1	6.0	6.2	6.1	6.4	6.0
	7	4.7	3.9	3.6	3.6	3.6	3.6	3.9
	14	3.8	3.8	4.2	4.4	4.0	3.8	4.0
PE 1% + Cit 0.15% + Eug 0.1%	0	6.1	5.1	6.0	6.2	6.1	6.4	6.0
	7	5.6	4.6	4.1	4.5	4.7	4.7	4.7
	14	5.0	4.4	3.8	4.8	5.0	5.0	4.7

4. Discussion

Changes in color due to coating fruits have been observed for blueberries and fresh-cut apples (Jo et al., 2014; Zambrano-Zaragoza et al., 2014; Abugoch et al., 2015). Mantilla et al. (2013) reported that changes in color are expected in high concentrations of sodium alginate coatings due to the whitish appearance of it. In our case, the color did not change much due to alginate due to its low concentration, despite L to have slightly higher values at the end of storage. Generally, edible coatings did not affect significantly the color of the raspberries.

One of the main factors used to determine fruit quality and postharvest shelf life is the rate and extent or firmness loss during the storage of soft fruit, which is attributed to the degradation of cell wall components, mainly pectins, due to the action of specific

enzymes such as polygalacturonase (Tanada-Palmu and Grosso, 2005). In our study, a decrease in firmness was observed through storage but without significant changes among treatments. Tezotto-Uliana (2014) obtained similar results to those observed in the present study.

While SSC is an indirect measurement of fruit sugars, higher ºBrix represents more ripe fruit. In this case, in general, values were similar with a slight increase, in particular in control samples. It seems that a slight ripening occurred in storage, despite raspberry to be considered a climacteric fruit. In such a way the edible coatings slightly retarded the increase in SSC. As in our case, others found higher maintenance of SSC in raspberries coated with other edible coatings (Gol et al., 2013; Velickova et al., 2013; Hassanpour, 2015). However those authors found a decrease in SSC content in strawberries at the end of storage and attributed it to respiration. In our case it seems that the ripening was not completed in terms of SSC when fruit were harvested.

Weight loss is an indicator of freshness of fruits. Han et al. (2004) found reduced weight loss in raspberries coated with chitosan as compared to control. In our case, weight loss was higher in edible coatings than in control. This may be due to the coating, which does not dry immediately and still continue to lose water through storage, as well as the non-impermeability of our coating to water loss.

Food spoilage microorganisms are one of the main causes of fresh fruit deterioration. Some authors refer that the main objective of introducing essential oils and/or their constituents into edible coatings is their effect as antimicrobial agents (Antunes, Gago, Cavaco, & Miguel, 2012). According to Azarakhsh et al. (2014) alginate-based coating formulation with lemongrass oil significantly reduced the total microorganisms counts and yeast and mold counts in coated fresh-cut pineapple during shelf-life whereas uncoated and other used coats failed to reduce the microbial

population. Similar results were reported by (Brasil et al., 2012) in fresh-cut papaya with a multilayered edible coating made of chitosan and pectin. In fact, the main benefits of the edible coatings of the present experiment were the elimination of the microbial mesophilic population, and although both showed good results, the PE showed to be more efficient than AL for controlling yeasts and molds.

Phenolic compounds are secondary metabolites contributing for the color and sensory characteristics of fruits and vegetables (Balasundram et al., 2006). Ghasemnezhad (2010) and Ali et al. (2013) reported a decrease in phenolic content of apricot and tomato coated with chitosan and gum arabic, respectively, due to senescence. (Serrano et al., 2006) in grapes coated with *Aloe vera* gel found maintenance of total phenolics during the first 14 days at 1°C, and a slight decrease from this time until the end of the cold storage. In our work, no significant changes occurred through storage or due to coating application. However, flavonoids had a slight decrease through storage and were higher at the end of storage and significantly lower than control except PE 1% + Cit 0.15% + Eug 0.1%.

Contreras-Oliva et al. (2012) using hydroxypropyl methylcellulose–lipid edible coatings in 'Oronules' mandarins showed, in general, coating application had not an important effect on the level of the different flavonoids although some significant differences. This was the case of our work for the PE edible coatings if we look at all storage period. Also, Robles-Sánchez et al. (2013) study in fresh-cut mangos using alginate coats, found minimally changes in flavonoids by the coatings, being storage time which promoted changes on this parameter.

Anthocyanins are a group of phenolic compounds responsible for the red-blue color of many fruit and vegetables. The increase in the total amount of anthocyanin during storage time may be due to the continued biosynthesis of phenolic compounds after

harvest, related to the ripening processes (García-Alonso, 2004; Hassanpour, 2015). Hassanpour (2015) report that the total anthocyanin content was significantly higher in *Aloe vera* gel coated raspberries and increased as storage time advanced. In our case, anthocyanins increased through storage but at the end of the experiment their values were lower in edible coatings than control, being the coatings with Eug the more similar to control.

Fruits and vegetables contain many different antioxidant components; these include carotenoids, vitamins, phenols, flavonoids, dietary glutathionine, and endogenous metabolites (Wang & Lin, 2000). Wang & Gao (2012) found for strawberries that the decline in antioxidant activity in untreated fruit at the end of storage might be due to senescence and decay, this indicated that chitosan treatment not only can extend shelf life, but also can retain higher antioxidant activity in strawberries after prolonged storage.

According to Robles-Sánchez et al. (2013) antioxidant activity in fresh-cut mangoes covered with the edible coating alginate 2% + ascorbic acid 1%, expressed as TEAC, was significantly higher than in alginate alone and control fruits. In our experiment edible coatings did not affect significantly the antioxidant activity. Higher levels of both acetaldehyde and ethanol increase in ripening fruit. Acetaldehyde is an aroma constituent in most plant tissues and ethanol is an indicator of the degree of anaerobic fermentation, as a result of anaerobic respiration is often associated with off-flavors and its presence might be detrimental to quality (Beaulieu et al., 1997; Raybaudi-Massilia et al., 2008; Rojas-Graü et al., 2008).

The values of acetaldehyde were low and were not affected by the edible coatings. Contreras-Oliva et al. (2012) in ‘Oronules’ mandarins using hydroxypropyl methylcellulose–lipid edible coatings found increased concentrations of ethanol and

acetaldehyde as compared to uncoated ones, which confirms the creation of a modified atmosphere into the fruit. The edible coatings of our experiment did not create such a permeable barrier. This is confirmed by the values of CO_2 production which were also not affected by the edible coatings as compared to controls.

Similarly Oms-Oliu et al. (2008) using gellan, alginate and pectin, found that the production of CO_2 in fresh-cut melon was similar in fruits coated and fruits uncoated. However, other authors found with other edible coatings and mostly for fresh-cut fruit, reduced respiration rate (Bierhals et al., 2011; Contreras-Oliva et al., 2012).

No significant influence of coating on organic acids was found except that ascorbic acid was higher in coated fruit than in control. This may be attributed to the application of ascorbic acid to coats as reported by other authors (Antunes, Dandlen, Cavaco, & Miguel, 2010; Antunes et al., 2013).

Generally there was not a significant effect of edible coatings in the sugar content. Zapata et al. (2008) in tomato using alginate and zein as edible coatings report the composition in sugar and organic acids demonstrated that control fruits were at a more advanced ripening stage than coated tomatoes, with lower sugar and organic acid concentration. On the other hand Palma, Schirra, & Aquino (2015) using "Food coat" (composed of fatty acids derivatives and polysaccharides in alcohol solution) and "Pomfresh" (composed of a mixture of organic acids and antioxidant compounds) in minimally processed cactus pear, report that organic acids and sugar content remained almost constant during the storage period and was not influenced by treatments, as in our case.

Control raspberries of the present experiment were not suitable for consumption after 14 d of storage since scored below 4 in a scale of 1-bad to 7-excellent. Edible coated fruit had overall flavor in a rage of 4 or over after 14 d storage, being the best the

PE 1% + Cit 0.15% + Eug 0.1%. As in our work, other authors who incorporated essential oils at low concentration into edible coatings, did not found a negative effect of the essential oil on sensory properties (Perdones et al., 2012; Azarakhsh et al., 2014). Also, the non-cytotoxicity of the edible coatings used in this experiment make them good for use in raspberries. Other authors found similar results in other edible coatings based on chitosan (Hermans et al., 2012; Zou et al., 2012).

5. Conclusions

The alginate and pectin edible coatings of this experiment did not affect significantly the general physicochemical and nutritional properties of raspberries. Their effect was mostly on reducing food spoilage microorganisms and consequently shelf-life in good consumer acceptance quality. In that way we can state that these coatings can be used as natural postharvest treatments in raspberries with the aim to delay the postharvest ripening process and to maintain fruit quality up to 14 d at 0.5 ºC, while uncoated fruits could not reach that time in good consumer acceptance conditions. Based on the major effect of the edible coatings on reducing microbial spoilage and the scores of the taste panel we select as the best coating for increasing raspberry storage the PE 1% + Cit 0.15% + Eug 0.1%.

6. Acknowledgements

This research was supported by FCT, Project: PTDC/AGR-ALI/121085/2010 - INNCOAT-Innovative edible coatings to improve storage of small fruit and fresh-cut.

7. References

Abugoch, L.; Tapia, C.; Plasencia, D.; Pastor, A.; Castro-Mandujano, O.; López, L.; Escalona, V. Shelf Life of Fresh Blueberries Coated with Quinoa Protein/chitosan/sunflower Oil Edible Film. *J. Sci. Food Agric.* **2015**.

Acevedo, C. A.; López, D. a.; Tapia, M. J.; Enrione, J.; Skurtys, O.; Pedreschi, F.; Brown, D. I.; Creixell, W.; Osorio, F. Using RGB Image Processing for Designing an Alginate Edible Film. *Food Bioprocess Technol.* **2012**, *5*, 1511–1520.

Ali, A.; Maqbool, M.; Alderson, P. G.; Zahid, N. Effect of Gum Arabic as an Edible Coating on Antioxidant Capacity of Tomato (Solanum Lycopersicum L.) Fruit during Storage. *Postharvest Biol. Technol.* **2013**, *76*, 119–124.

Antunes, M. D. C.; Dandlen, S.; Cavaco, A. M.; Miguel, G. Effects of Postharvest Application of 1-MCP and Postcutting Dip Treatment on the Quality and Nutritional Properties of Fresh-Cut Kiwifruit. *J. Agric. Food Chem.* **2010**, *58*, 6173–6181.

Antunes, M. D. C.; Rodrigues, D.; Pantazis, V.; Cavaco, A. M.; Siomos, A. S.; Miguel, G. Nutritional Quality Changes of Fresh-Cut Tomato during Shelf Life. *Food Sci. Biotechnol.* **2013**, *22*, 1–8.

Antunes, M. D.; Gago, C.; Cavaco, A. M.; Miguel, M. G. Edible Coatings Enriched with Essential Oils and Their Compounds for Fresh and Fresh-Cut Fruit. *Recent Patents Food, Nutr. Agric.* **2012**, *4*, 114–122.

Azarakhsh, N.; Osman, A.; Ghazali, H. M.; Tan, C. P.; Mohd Adzahan, N. Lemongrass Essential Oil Incorporated into Alginate-Based Edible Coating for Shelf-Life Extension and Quality Retention of Fresh-Cut Pineapple. *Postharvest Biol. Technol.* **2014**, *88*, 1–7.

Balasundram, N.; Sundram, K.; Samman, S. Phenolic Compounds in Plants and Agri-Industrial by-Products: Antioxidant Activity, Occurrence, and Potential Uses. *Food Chem.* **2006**, *99*, 191–203.

Beaulieu, J. C.; Peiser, G.; Saltveit, M. E. Acetaldehyde Is a Causal Agent Responsible for Ethanol-Induced Ripening Inhibition in Tomato Fruit. *Plant Physiol.* **1997**, *113*, 431–439.

Bierhals, V. S.; Chiumarelli, M.; Hubinger, M. D. Effect of Cassava Starch Coating on Quality and Shelf Life of Fresh-Cut Pineapple (Ananas Comosus L. Merril Cv "Pérola"). *J. Food Sci.* **2011**, *76*, 62–72.

Brasil, I. M.; Gomes, C.; Puerta-Gomez, a.; Castell-Perez, M. E.; Moreira, R. G. Polysaccharide-Based Multilayered Antimicrobial Edible Coating Enhances Quality of Fresh-Cut Papaya. *LWT - Food Sci. Technol.* **2012**, *47*, 39–45.

Contreras-Oliva, A.; Rojas-Argudo, C.; Pérez-Gago, M. B. Effect of Solid Content and Composition of Hydroxypropyl Methylcellulose-Lipid Edible Coatings on Physico-Chemical and Nutritional Quality of "Oronules" Mandarins. *J. Sci. Food Agric.* **2012**, *92*, 794–802.

Crespo, P.; Bordonaba, J. G.; Terry, L. A.; Carlen, C. Characterisation of Major Taste and Health-Related Compounds of Four Strawberry Genotypes Grown at Different Swiss Production Sites. *Food Chem.* **2010**, *122*, 16–24.

Galus, S.; Lenart, A. Development and Characterization of Composite Edible Films Based on Sodium Alginate and Pectin. *J. Food Eng.* **2012**, *115*, 459–465.

García-Alonso, M. Evaluation of the Antioxidant Properties of Fruits. *Food Chem.* **2004**, *84*, 13–18.

Ghasemnezhad, M. Effect of Chitosan Coatings on Some Quality Indices of Apricot (Prunus Armeniaca L.) During Cold Storage. **2010**, *8*, 25–33.

Girón-Calle, J.; Alaiz, M.; Vioque, J. Effect of Chickpea Protein Hydrolysates on Cell Proliferation and in Vitro Bioavailability. *Food Res. Int.* **2010**, *43*, 1365–1370.

Gol, N. B.; Patel, P. R.; Rao, T. V. R. Improvement of Quality and Shelf-Life of Strawberries with Edible Coatings Enriched with Chitosan. *Postharvest Biol. Technol.* **2013**, *85*, 185–195.

Gol, N. B.; Chaudhari, M. L.; Rao, T. V. R. Effect of Edible Coatings on Quality and Shelf Life of Carambola (Averrhoa Carambola L.) Fruit during Storage. *J. Food Sci. Technol.* **2015**, *52*, 78–91.

Guerreiro, A. C.; Gago, C.; Miguel, M.; Antunes, M. The Effect of Temperature and Film Covers on the Storage Ability of Arbutus Unedo L. Fresh Fruit. *Sci. Hortic. (Amsterdam).* **2013**, *159*, 96–102.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Effect of Alginate-Based Edible Coatings Enriched with Essential Oils Constituents on Arbutus Unedo L. Fresh Fruit Storage. *Postharvest Biol. Technol.* **2015a**, *100*, 226–233.

Guerreiro, A. C.; Gago, C. M. L.; Faleiro, M. L.; Miguel, M. G. C.; Antunes, M. D. C. The Use of Polysaccharide-Based Edible Coatings Enriched with Essential Oils to Improve Shelf-Life of Strawberries. *Postharvest Biol. Technol.* **2015b**, *110*, 51–60.

Han, C.; Zhao, Y.; Leonard, S. .; Traber, M. . Edible Coatings to Improve Storability and Enhance Nutritional Value of Fresh and Frozen Strawberries (Fragaria × Ananassa) and Raspberries (Rubus Ideaus). *Postharvest Biol. Technol.* **2004**, *33*, 67–78.

Hassanpour, H. Effect of Aloe Vera Gel Coating on Antioxidant Capacity, Antioxidant Enzyme Activities and Decay in Raspberry Fruit. *LWT - Food Sci. Technol.* **2015**, *60*, 495–501.

Hermans, K.; Van Den Plas, D.; Everaert, A.; Weyenberg, W.; Ludwig, A. Full Factorial Design, Physicochemical Characterisation and Biological Assessment of Cyclosporine A Loaded Cationic Nanoparticles. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 27–35.

Jo, W. S.; Song, H. Y.; Song, N. B.; Lee, J. H.; Min, S. C.; Song, K. B. Quality and Microbial Safety of "Fuji" Apples Coated with Carnauba-Shellac Wax Containing Lemongrass Oil. *LWT - Food Sci. Technol.* **2014**, *55*, 490–497.

Keutgen, A.; Pawelzik, E. Modifications of Taste-Relevant Compounds in Strawberry Fruit under NaCl Salinity. *Food Chem.* **2007**, *105*, 1487–1494.

Klewicka, E.; Nowak, A.; Zduńczyk, Z.; Juśkiewicz, J.; Cukrowska, B. Protective Effect of Lactofermented Red Beetroot Juice against Aberrant Crypt Foci Formation, Genotoxicity of Fecal Water and Oxidative Stress Induced by 2-Amino-1-Methyl-6-phenylimidazo[4,5-B] Pyridine in Rats Model. *Environ. Toxicol. Pharmacol.* **2012**, *34*, 895–904.

Lee, J.; Durst, R. W.; Wrolstad, R. E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int.* **2005**, *88*, 1269–1278.

Mantilla, N.; Castell-Perez, M. E.; Gomes, C.; Moreira, R. G. Multilayered Antimicrobial Edible Coating and Its Effect on Quality and Shelf-Life of Fresh-Cut Pineapple (Ananas Comosus). *LWT - Food Sci. Technol.* **2013**, *51*, 37–43.

McGuire, R. Reporting of Objective Color Measurements. *HortScience* **1992**, *27*, 1254–1255.

Miguel, M. G.; Nunes, S.; Dandlen, S. A.; Cavaco, A. M.; Antunes, M. D. Phenols and Antioxidant Activity of Hydro-Alcoholic Extracts of Propolis from Algarve, South of Portugal. *Food Chem. Toxicol.* **2010**, *48*, 3418–3423.

Morales, M. L.; Callejón, R. M.; Ubeda, C.; Guerreiro, a.; Gago, C.; Miguel, M. G.; Antunes, M. D. Effect of Storage Time at Low Temperature on the Volatile Compound Composition of Sevillana and Maravilla Raspberries. *Postharvest Biol. Technol.* **2014**, *96*, 128–134.

Olivas, G. I.; Barbosa-Cánovas, G. V. Edible Coatings for Fresh-Cut Fruits. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 657–670.

Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Enhance Quality and Antioxidant Properties of Fresh-Cut Melon. *LWT - Food Sci. Technol.* **2008**, *41*, 1862–1870.

Palma, A.; Schirra, M.; Aquino, S. D. Effect of Edible Coating on Ascorbic Acid , Betalains , Organic Acids , Sugar , Polyphenol Content and Antioxidant Activity in Minimally Processed Cactus Pear (Opuntia Ficus-Indica). *Acta Hortic.* **2015**, *1067*, 127–134.

Perdones, A.; Sánchez-gonzález, L.; Chiralt, A.; Vargas, M. Effect of Chitosan – Lemon Essential Oil Coatings on Storage-Keeping Quality of Strawberry. *Postharvest Biol. Technol.* **2012**, *70*, 32–41.

Qian, P. I. M. Objective Flavor Comparison of Oregon Strawberries and Those from Other Climatic Condition. **2005**.

Raybaudi-Massilia, R. M.; Mosqueda-Melgar, J.; Martín-Belloso, O. Edible Alginate-Based Coating as Carrier of Antimicrobials to Improve Shelf-Life and Safety of Fresh-Cut Melon. *Int. J. Food Microbiol.* **2008**, *121*, 313–327.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237.

Robles-Sánchez, R. M.; Rojas-Graü, M. A.; Odriozola-Serrano, I.; González-Aguilar, G.; Martin-Belloso, O. Influence of Alginate-Based Edible Coating as Carrier of Antibrowning Agents on Bioactive Compounds and Antioxidant Activity in Fresh-Cut Kent Mangoes. *LWT - Food Sci. Technol.* **2013**, *50*, 240–246.

Rojas-Graü, M. A.; Tapia, M. S.; Martín-Belloso, O. Using Polysaccharide-Based Edible Coatings to Maintain Quality of Fresh-Cut Fuji Apples. *LWT - Food Sci. Technol.* **2008**, *41*, 139–147.

Serrano, M.; Valverde, J. M.; Guillén, F.; Castillo, S.; Martínez-Romero, D.; Valero, D. Use of Aloe Vera Gel Coating Preserves the Functional Properties of Table Grapes. *J. Agric. Food Chem.* **2006**, *54*, 3882–3886.

Singleton, V. L.; Rossi, J. A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

Tanada-Palmu, P. S.; Grosso, C. R. F. Effect of Edible Wheat Gluten-Based Films and Coatings on Refrigerated Strawberry (Fragaria Ananassa) Quality. *Postharvest Biol. Technol.* **2005**, *36*, 199–208.

Terry, L. A.; Chope, G. A.; Bordonaba, J. G. Effect of Water Deficit Irrigation and Inoculation with Botrytis Cinerea on Strawberry (Fragaria X Ananassa) Fruit Quality. *J. Agric. Food Chem.* **2007**, *55*, 10812–10819.

Tezotto-Uliana, J. V.; Fargoni, G. P.; Geerdink, G. M.; Kluge, R. A. Chitosan Applications Pre- or Postharvest Prolong Raspberry Shelf-Life Quality. *Postharvest Biol. Technol.* **2014**, *91*, 72–77.

Velickova, E.; Winkelhausen, E.; Kuzmanova, S.; Alves, V. D.; Moldão-Martins, M. Impact of Chitosan-Beeswax Edible Coatings on the Quality of Fresh Strawberries (Fragaria Ananassa Cv Camarosa) under Commercial Storage Conditions. *LWT - Food Sci. Technol.* **2013**, *52*, 80–92.

Wang, S. Y.; Lin, H. S. Antioxidant Activity in Fruits and Leaves of Blackberry, Raspberry, and Strawberry Varies with Cultivar and Developmental Stage. *J. Agric. Food Chem.* **2000**, *48*, 140–146.

Wang, S. Y.; Gao, H. Effect of Chitosan-Based Edible Coating on Antioxidants, Antioxidant Enzyme System, and Postharvest Fruit Quality of Strawberries (Fragaria X Aranassa Duch.). *LWT - Food Sci. Technol.* **2012**, *52*, 1–9.

Wang. Fruits with High Functional Foods. In *Functional Food Ingredients and Nutraceuticals*; Taylor & Francis Group, L., Ed.; 2007; pp. 371–413.

Zambrano-Zaragoza, M. L.; Mercado-Silva, E.; Del Real L., A.; Gutiérrez-Cortez, E.; Cornejo-Villegas, M. A.; Quintanar-Guerrero, D. The Effect of Nano-Coatings with A-Tocopherol and Xanthan Gum on Shelf-Life and Browning Index of Fresh-Cut "Red Delicious" Apples. *Innov. Food Sci. Emerg. Technol.* **2014**, *22*, 188–196.

Zapata, P. J.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Valero, D.; Serrano, M. Use of Alginate or Zein as Edible Coatings to Delay Postharvest Ripening Process and to Maintain Tomato (Solanum Lycopersicon Mill) Quality. *J. Food Agric.* **2008**, *88*, 1287–1293.

Zou, T.; Percival, S. S.; Cheng, Q.; Li, Z.; Rowe, C. a.; Gu, L. Preparation, Characterization, and Induction of Cell Apoptosis of Cocoa Procyanidins-Gelatin-Chitosan Nanoparticles. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 36–42.

Chapter XI

General Conclusions and Future Perspectives

1. General Conclusions

The study presented in this thesis is the result of a plan that aimed to find the best edible coating formulations for better preserving quality and increase shelf-life of *Arbutus unedo* berries, raspberries, strawberries and fresh-cut apple (*Malus domestica* Borkh.) cv. 'Bravo de Esmolfe'. Among the results obtained, the following conclusions must be highlighted:

- The edible coatings based on alginate or pectin enriched with the essential oils compounds citral and eugenol, in appropriate concentrations (MIC or double MIC), are good for preserving small fruit and fresh-cut. The efficiency of coating formulations depended on the fruit species studied.
- The use of edible coatings in arbutus berries can be considered as safe and effective treatment (reducing microbial spoilage and without showing cytotoxicity) allowing fruits to be stored for at least 28 days at 0.5°C. The use of alginate-based formulations were good to maintain most quality attributes of the commodity and can be recommended for commercial purposes due its lower cost in comparison to other polysaccharides used in industrial applications of high economic value. Although both edible coatings were good for preserving general sensory and nutritional quality properties, AL 1% + Cit 0.15% + Eug 0.1% was slightly better since in some periods of storage was better for color and firmness preservation and CO_2 initial reduction than AL 1% + Eug 0.2%.
- Alginate and pectin edible coatings can be used to maintain the quality in fresh-cut 'Bravo de Esmolfe' apple. The coating extended the shelf life up to 8 d at 4 ºC while uncoated fruits were unacceptable at that time. Our results suggest that the edible coatings of this study may be a useful non-chemical method of

maintaining fresh-cut apples quality and extend their shelf life, being the best treatment Al 2% + Eug 0.1% with the anti-browning agent ascorbic acid.

- Based on our results, alginate and pectin edible coatings can be used as natural postharvest treatments in strawberries storage by delaying microbial spoilage of strawberries and improve fruit quality. Taking into account the coating effect on all general, sensorial and nutritional quality preservation through storage, the best edible coatings were PE 2% + Cit 0.15% and AL 2% + Cit 0.15% + Eug 0.1%.
- In our study we concluded that alginate and pectin treatments could be used in raspberries with the aim to delay the postharvest senescence process and to maintain fruit quality. The edible coatings extended the shelf life up to 15 d at 0.5 ºC while uncoated fruits were unacceptable at that time. Our results suggest that the edible coatings of our study may be a useful non-chemical method of maintaining raspberry fruit quality and extending their postharvest life. Nevertheless, raspberries were better preserved in terms of sensory and nutritional quality with PE 1% + Cit 0.15% + Eug 0.1% followed by Al 2% + Cit 0.15%.

In order to have less different formulations for different fruit species, although all of them being efficient, from the results of this research we can recommend the use of alginate as the base, for strawberries AL2%+Cit 0.15%+Eug 0.1%; for fresh-cut 'Bravo de Esmolfe' apple we recommend AL2% + Eug 0.1% plus dip in ascorbic acid; for Arbutus unedo berries the AL 1% + Eugenol 0.1% + Citral 0.15%. In case of raspberries we recommend the used of PE 1%+Cit 0.15%+Eug 0.1%.

2. Future Perspectives

This thesis opens a series of possibilities concerning the development of new edible coating formulations and better understand their effect when applied to different fresh and fresh-cut fruits. However there are still some aspects that shall be studied in further research:

- The application of edible coatings in different fresh fruit and vegetables, other than the ones of this study in order to study their effect on extended shelf life preserving quality;
- New formulations of edible coatings with different polysaccharides and/or essential oils components;
- Characterization the chosen edible coatings in what concerns their optical, superficial, structural, mechanical, hygroscopic and barrier properties;
- Gas permeability studies with gas mixtures;
- Testing the effectiveness of best edible coatings when expose to a microbial contamination;
- Testing different anti-browning agents, in order to further extend the storage and shelf-life;
- Study the formulations of edible coatings for their application at a commercial scale.