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XXXV CICLO

Biopolymer active coating to extend the shelf life of minimally processed fruits and vegetables

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Summary and aim of the PhD project

Fruit and vegetables are key elements of a healthy and balanced diet providing humans with essential nutrients and bioactive compounds, including vitamins, organic acid, carotenoids, minerals, fibre, and polyphenols (Prasanna et al., 2007). The increased consumers' attention to "healthy" food attributes (such as "freshness", "naturalness" and "nutritional value") and overall sustainability of production and processing methods has contributed to a growing demand for regional and locally produced/supplied and less processed food. A wide variety of minimally processed fruits and vegetables products has been offered on the market, resulting in increased consumption and consequently intake of fresh fruits and vegetables, benefitting the agrifood economy and human well-being. Although minimal processing methods, such as washing, cutting and modified atmosphere packaging, have been demonstrated to preserve the freshness of the products (Raso & Barbosa-Cánovas, 2003), the shelf-life of fruits and vegetables is still limited.

Edible biopolymer coating can be used as preservation technology to extend the shelf life of minimally processed fruits and vegetables, slowing down their quality decay through the reduction of mass transfer of gas, moisture or aromas. In addition, coatings can be used as carriers of active additives that can contribute to the shelf-life extension and/or improve food nutritional quality of minimally processed fruits and vegetables (Yousuf et al., 2018, Valentino et al., 2020, Khan et al., 2021).

The main factors contributing to fruit and vegetables' physiological deterioration are respiration, transpiration and ethylene production. However, also chemical-physical and nutritional properties are for the freshness and nutritional value of the product.

Respiration and ripening of fruits and vegetables involve gas exchange with the environment; carbon dioxide, oxygen, water and other metabolic by-products, such as ethylene and other volatile compounds, are the main substances exchanged during storage. The coatings applied on the product can form a thin layer of film that can modify the gas exchange rate between the product and the environment, and thus can control transpiration, respiration and other metabolic processes that lead to loss of quality. However, the low oxygen concentration in the product could lead to anaerobic respiration, which can result in product deterioration due to the production of off-flavours and accelerated senescence (Kays and Paull 2004). Thus, the impact of coating should be evaluated by monitoring the product's main physiological, chemical-physical and nutritional quality indices during storage. Moreover, to properly evaluate the impact of the coating on the shelf-life of the product it is important to study the kinetics of the product alteration and describe it by mathematical modelling.

Starting from these hypotheses, these specific goals have been addressed along with the PhD project:

- developing active coatings based on proteins, and/or polysaccharides and/or lipids enriched with antioxidant compounds at different concentrations to obtain stable solutions;
- implementing coatings with plasma activated water as the dispersing solution of solid compounds;
- understanding the effect of the coatings on different food products by measuring the respiration rate;
- exploring the effect of the coatings on the alteration kinetics (microbiological, nutritional, and physical-chemical quality) of minimally processed fruits and vegetables;
- quantifying degradation kinetics of the critical quality indicators with kinetics curve, obtained with a mathematical model to estimate/predict product shelf life.

The work was organized in three different study cases:

1) Minimally processed fennel
2) Minimally processed pear
3) Minimally processed apples

Sintesi e obiettivi del progetto di dottorato

Frutta e verdura sono elementi chiave di una dieta sana ed equilibrata, fornendo nutrienti essenziali e composti bioattivi, tra cui vitamine, acidi organici, carotenoidi, minerali, fibre e polifenoli (Prasanna et al., 2007). Negli ultimi anni si è assistito a un crescente interesse dei consumatori verso prodotti salutari, caratterizzati da attributi come "freschezza", "naturalità" e "valore nutrizionale". Inoltre, la sostenibilità complessiva dei metodi di produzione e lavorazione ha contribuito a far crescere la domanda di alimenti minimamente processati. Il marcato ha offerto un'ampia varietà di prodotti ortofrutticoli minimamente lavorati, con conseguente aumento del consumo e quindi dell'assunzione di frutta e verdura fresca, a beneficio dell'economia agroalimentare e del benessere umano. Sebbene sia stato dimostrato che i metodi di lavorazione minima, come il lavaggio, il taglio e il confezionamento in atmosfera modificata, preservino la freschezza dei prodotti (Raso & Barbosa-Cánovas, 2003), la durata di conservazione di frutta e verdura minimamente processata è ancora limitata. L'applicazione di un coating biopolimerico potrebbe essere un'alternativa per conservare frutta e verdura minimamente lavorata. Questa tecnologia può migliorare la conservazione dei prodotti alimentari, grazie al rallentamento del decadimento qualitativo attraverso la riduzione del trasferimento di massa di gas, umidità o aromi. Inoltre, i rivestimenti edibili possono essere utilizzati come vettori di composti attivi, i quali possono contribuire all'estensione della shelf-life e/o migliorare la qualità nutrizionale di frutta e verdura minimamente lavorate (Yousuf et al., 2018, Valentino et al., 2020, Khan et al., 2021). Durante lo sviluppo del coating, è necessario evitare che esso possa influenzare negativamente la concentrazione di ossigeno all'interno del prodotto. Infatti,

una bassa concentrazione di ossigeno all'interno del prodotto potrebbe portare a una respirazione anaerobica, con conseguente deterioramento del prodotto dovuto alla produzione di off-flavours e all'accelerazione della senescenza (Kays e Paull 2004). La respirazione cellulare e la maturazione di frutta e verdura comportano uno scambio di gas con l'ambiente. L'anidride carbonica, l'ossigeno, l'acqua e altri prodotti secondari del metabolismo, come l'etilene, sono le principali sostanze scambiate durante la conservazione. I rivestimenti superficiali modificano il tasso di scambio di gas tra l'ambiente e il prodotto fresco, controllando così la traspirazione, la respirazione e altri processi metabolici che portano alla perdita di qualità. I principali fattori che contribuiscono al deterioramento fisiologico di frutta e verdura sono la respirazione, la traspirazione e la produzione di etilene. Tuttavia, anche le proprietà chimico-fisiche e nutrizionali sono importanti per controllare come cambiare gli indici di qualità di frutta e verdura. Attraverso l'applicazione di coating, la shelf-life di frutta e verdura minimamente lavorata sarà incrementata e le proprietà chimico-fisiche e nutrizionali migliorate. Partendo da questa ipotesi, con il progetto di dottorato sono stati affrontati questi obiettivi specifici:

- sviluppare rivestimenti attivi a base di proteine e/o polisaccaridi e/o lipidi arricchiti con composti antiossidanti a diverse concentrazioni per ottenere soluzioni stabili;
- realizzare rivestimenti utilizzando acqua attivata al plasma come soluzione disperdente dei composti solidi;
- comprendere l'effetto del rivestimento su diversi prodotti alimentari misurando il tasso di respirazione;
- studiare l'effetto del rivestimento sulle cinetiche di alterazione (qualità microbiologica, nutrizionale e fisico-chimica) dei prodotti minimamente processi conservati in specifiche condizione di conservazione;
- quantificare la cinetica di degradazione degli indicatori critici di qualità con una curva cinetica, ottenuta con un modello matematico per stimare/prevedere la shelf-life del prodotto.

Overview of PhD research activities

Publications in journals

M. Valentino, S. Volpe, F. A. Di Giuseppe, S. Cavella, E. Torrieri (2020). Active Biopolymer Coating Based on Sodium Caseinate: Physical Characterization and Antioxidant Activity. Coatings, 10 (8) 706, 1-12.

M. Valentino, A. Trotta, S. Volpe, E. Torrieri (2020). Coating edibili antiossidanti a base di biopolimeri per la conservazione di frutta e verdura minimamente processata. Rivista COM.PACK, 14-17.

M. Valentino (2021). Coating edibili per la conservazione di frutta e verdura fresca (4/2022). Rivista online Food hub. https://www.food-hub.it/media/2022/04/29/coating-edibili/.

M. R. Khan, S. Volpe, **M. Valentino**, N. A. Miele, S. Cavella, E. Torrieri (2021). Active Casein Coatings and Films for Perishable Foods: Structural Properties and Shelf-Life Extension. Review, Coatings, 11, 899.

M. Valentino, S. Volpe, E. Torrieri (2022). Impiego di coating attivi a base di biopolimeri per preservare la qualità di pere minimamente processate. Rivista COM.PACK, 26-28.

Conference proceedings

M. Valentino (2021). Biopolymer active coatings to extend shelf life of minimally processed fruits and vegetables, In Proceedings of First Virtual (XXV) Workshop on the "Developments in the Italian phD Research on Food Science Technology and Biotechnology", Palermo, Italy.

M. Valentino. Biopolymer active coatings to extend shelf life of minimally processed fruits and vegetables, In Proceedings of First Virtual (XXVI) Workshop on the "Developments in the Italian phD Research on Food Science Technology and Biotechnology", Asti, Italy, 2022.

Conference

<u>Poster sessions</u>

M. Valentino, F. Colonna, S. Volpe, S. Cavella, R. Di Monaco. E. Torrieri (2022). "Impact of caseinate based edible coating on the sensory quality of minimally processed pears" Eurosense, Turku, Finlandia.

M. Valentino, G. Rossi, O. Schlüter, E. Torrieri (2022). "Insect-based biopolymers for developing functional coating: effect on the quality of fresh-cut apples" INSECTA conference, Germany.

M. Valentino, O. Schlüter, E. Torrieri (2022). "Plasma activated water to develop functional edible coating: effect on the quality of fresh-cut apples" EFFoST conference, Dublino, Irlanda.

<u>Oral presentation</u>

M. Valentino. "Biopolymer active coating to extend the shelf-life of minimally processed fruits and vegetables" Workshop phD student in Food Science 2022, Asti, Italia.

M. Valentino, S. Volpe. S. Cavella, P. Masi, E. Torrieri. (2022). "Effect of biopolymer active coating on alteration kinetics of minimally processed fennels stored at different temperatures "SLIM conference, Bogotà, Colombia.

Awards

Winner of the **STARPLUS 2020**, in 2021. Project title: PLASMA treatments to optimize the functional properties of BIOpolymer Coating; Acronym: PLASMACOBIO.

Other publications

S. Puleo, **M. Valentino**, P. Masi, R. Di Monaco (2021). Hardness sensitivity: Are old, young, female and male subjects all equally sensitive? Food Quality and Preference, 90 104118.

S. Volpe, **M. Valentino**, R. Muhammad, E. Torrieri (2022). Application of Releasing Systems in Active Packaging for Dairy Products. Releasing Systems in Active Food Packaging, pp: 353-372.

Chapter 1

1. Introduction

Fruit and vegetables are key elements of a healthy and balanced diet providing humans with essential nutrients and bioactive compounds, including vitamins, organic acid, carotenoids, minerals, fibre, and polyphenols (Prasanna et al., 2007). Although the consumption was below the minimum recommendation of 400 grams by the WHO, the trend towards natural, sustainable and locally produced fruits and vegetables is positive. The increased consumers' attention to "healthy" food attributes (such as "freshness", "naturalness" and "nutritional value") and overall sustainability of production and processing methods has contributed to a growing demand for regional and locally produced/supplied and less processed food. A wide variety of minimally processed fruits and vegetables products has been offered on the market, resulting in increased consumption and consequently intake of fresh fruits and vegetables, benefitting the agrifood economy and human well-being. Although minimal processing methods, such as washing, cutting and modified atmosphere packaging (MAP), have been demonstrated to preserve the freshness of the products (Raso & Barbosa-Cánovas, 2003), the shelf life of fruits and vegetables is still limited. Moreover, currently available chemical sanitisers, besides being perceived negatively by consumers, are not considered as a solution since they are not efficient for microbial reduction and are harmful to human health and the environment(Raso & Barbosa-Cánovas, 2003). The relatively high costs to procure minimal processing technologies require high innovation and investment capacity. The need for more effective and sustainable post-harvesting and processing techniques to improve safety and retain nutritional and sensory properties of fruits and vegetables with a prolonged shelf-life is urgent. The main advantages are maintaining freshness and quality of produce, slowing down deteriorative processes and extending the shelf-life. In recent years there has been a large diffusion among the consumers of fresh-cut fruits and vegetables; these products undergo mini processing that includes trimming, peeling, washing and cutting; finally, they are packed to consumers' nutrition, convenience maintaining freshness (Torrieri et al., 2008). The influence of minimal processing unit operations causes disruptions of cells, and since the product remains biologically and physiologically active, there is a shifting of cellular processes and interactions in response to the damage, which induces an increase in respiration rate, transpiration and enzymatic activities after harvest (Dea et al., 2011). An optimal combination of non-thermal sanitization, preservation and stabilization methods can improve safety (inactivation of pathogens and spoilage microorganisms) while preserving the nutritional and organoleptic quality and prolonging the shelf-life of fresh and minimally processed fruit and vegetable products. Combining and modulating non-thermal technologies with minimally processing operations can be obtained freshly, healthy, convenient, sustainable and locally produced and

additive-free food which is safe and nutritious. Among non-thermal technology high pressure processing, pulsed electric fields, plasma-activated water and electrolysed water can be used to preserve the quality decay of fruits and vegetables.

1.1 Non-thermal technology to preserve fruits and vegetables

1.1.1 High-pressure processing

High-pressure processing is a good alternative to heat treatments to destroy food-borne pathogens and inactivate enzymes. High pressures affect molecular volumes, which destabilize the molecular non-covalent interactions in macromolecules, like enzymes and membranes of bacteria, resulting in disruption of the three-dimensional structure and ultimately leading to denaturation and inactivation. Contrarily, low-molecular-weight compounds are only slightly affected by high-pressure processing, therefore, the primary structure of aroma compounds, pigments, vitamins, and other compounds are rarely affected. Hence, high pressure has a wide potential for producing unique fruits and vegetable products due to the targeted impact on proteins/enzymes. For these reasons, high-pressure processing is widely used in the food industry, but mainly for the production of low-acid or high-salt products e.g. juices and ready-to-eat meat products. These commercial uses, together with scientific studies (Barba et al., 2017) prove that high-pressure processing can produce microbial-safe and stable products with improved quality characteristics like taste and appearance. Yet, no overall high-pressure processing solution exists for treating fruits and vegetables raw materials and most product development relies on experimental trials and empirical knowledge. Since high-pressure processing involves multifactor set-up such as pressure level, holding time, process temperature, and sample preparation, investigations are still required for developing effective formulations and processing conditions. Enzymatic reactions are other important factors that have a major impact on the quality, especially colour, texture and flavour, of fruits and vegetable products (Netsanet et al., 2014; Oey et al., 2008). Generally, the effect of high-pressure processing on enzyme activity depends on their origin as well as their environment. In addition, the effect of high-pressure processing on sensory quality is still limited due to the fact that sensory property is highly product dependent, stressing the importance of target investigations. Pressure-induced matrix disruption can also be used to increase the bioaccessibility and bioavailability of nutrients, phytonutrients and minerals in fruits and vegetables-based drinks and products (Barba et al., 2015). However, conflicting results exist, for example, studies showed that the bioaccessibility of β-carotene from HP-treated carrots was increased, while the bioaccessibility of lycopene from HP-treated tomato juice was not improved. This underlines the importance of more studies into the interaction of the applied high-pressure processing process and food matrix and structure on consequences on the bioaccessibility and bioavailability of micronutrients and bioactive compounds. This would allow the rational application

of high-pressure processing for intelligently manipulating fruits and vegetables matrix structures to improve the bioaccessibility and bioavailability of specific bioactive and micronutrients. The advantages of high-pressure processing over traditional heat techniques make the pressure technology an intelligent processing method: independent of food shape or size; uniformity of treatment throughout food; the primary structure of molecules remains intact; reduced treatment times; positive consumer appeal; approved by a regulatory agency.

1.1.2 Pulsed electric fields

Pulsed electric fields are a good pre-treatment technology able to enhance the extraction of bioactive compounds. Pulsed electric field technology consists of electrical treatment of a short time (from several nanoseconds to several milliseconds) with pulse electric field strength from 100 to 300 V/cm to 20–80 kV/cm. Pulsed electric fields are a non-thermal technique that increases mass transfer thanks to the electroporation under the effect of electric pulses. Under the effect of pulsed electric fields, the biological membrane is electrically pierced and losses its semi-permeability temporarily or permanently, which can allow the selective recovery of high-added value compounds from different matrices. Briefly, this process accelerates the release of intracellular compounds and increases the extraction rates and yields of different components from vegetal matrices with low energy consumption and low environmental impact (Barbosa-Pereira et al., 2018). Electroporation allows solvent permeability and extractability favouring the use of an eco-friendly solvent such as water at ambient temperatures instead large amounts of organic solvents (Redondo et al., 2018). Since the concentration of bioactive compounds in fruits and vegetable coproducts & by-products is relatively low, their content in the extracts needs to be concentrated to obtain a feasible process at an industrial scale. The elimination of water and/or another solvent by evaporation may denature the heat-sensitive compounds. Thus, membrane separation technology offers several advantages low energy consumption and mild operating conditions for the separation of thermolabile compounds. Moreover, they show a selective recovery, easy adaptation for industrial scale-up, clean environmentally benign, cost-effective and generation of lesser waste (Castro-Muñoz et al., 2016).

1.1.3 Plasma-activated water

Plasma is a gas containing free electrons, ions, and neutral particles. Complex plasma chemistry is driven by electrons. They perform ionization, which is necessary to sustain the plasma, and they are responsible for atomic/molecular excitation, dissociation, and production of radicals and metastable molecular states. The result is an active gaseous medium that can be safely used without thermal damage to the surrounding materials. It has been used for surface modification, water disinfection, and biomedical applications. The industrial application of this technology as an alternative to conventional disinfectant methods of water treatment at the industrial level is still limited. Plasma

treatment is also regarded as a potential alternative to another chemical (e.g. chlorine treatment) or physical methods (e.g. high-pressure, pulsed electric fields, ionizing irradiation). Advantages of plasma processes are: (i) high efficiency at low temperatures (generally < 70 °C); (ii) precise generation of plasmas suitable for the intended use; just-in-time production of the acting agent; (iii) low impact on the internal product matrix; application free of water or solvents; no residues; (iv) in package treatments; resource-efficient (Schlüter et al., 2013). One important application that takes advantage of long-living species is plasma-activated water (PAW). Plasma-activated water is created by exposing water to a suitable plasma source, leading to the formation of the required long-living species as a result of a cascade of reactions occurring after plasma impact. Plasma-activated water can then be used as an antimicrobial liquid for the decontamination of food surfaces, which could have several advantages. Up to now, a handful of studies have been published on this topic. Whereas it is Zhang et al. (2006) and Ikawa et al. (2010) observed the formation of different reactive species in liquids and evaluated their direct effect on microbial inactivation, other groups focused on their long-term action (Zhang et al., 2006; Ikawa et al., 2010). It studied the microbial disinfection efficacy of plasma-activated water produced with gliding electric discharges using planktonic as well as adherent cells of *S. epidermidis, L. mesenteroides, H. alvei* and *S. cerevisiae* (Kamgang-Youbi et al., 2009). It was founded that planktonic cells were more susceptible to the treatment than adherent ones and that bacteria were more susceptible than yeasts. The authors suggest that the antimicrobial effect of the plasma-activated water used in this study can be explained by a combined effect of hydrogen peroxide, nitrites, nitrates and acidification. Particularly for the planktonic cells of *H. alvei*, they found that approximately 80 % of the decontamination can be explained by the action of a mixture of these effects. Similarly treated water showed antimicrobial activity over a period of several days, and also highlighted the complexity of plasma-activated water solutions since multiple chemical components exert varying biological effects on differing time scales (Traylor et al., 2011). Water exposed to plasma for 3 hours was shown to sustain its antimicrobial efficacy for at least 7 days. Except for the samples treated for longer, the authors demonstrated that both the hydrogen peroxide, as well as nitrite levels, correlated well with the log reductions. It was concluded that further species must have been present, on which further studies should focus. Different approaches have been used to investigate the inactivation mechanisms of plasma-activated water, such as optical emission spectroscopy, x-ray photoelectron spectroscopy, atomic absorption spectroscopy and transmission electron microscopy (Zhang et al., 2013). For the case of *S. aureus*, it is proposed that ROS affect and damages the redox state of antioxidants first, followed by penetrating the bacterial membrane and damaging the cell structure, resulting in the death of the cells. Other results have shown that the idea of plasma-activated water could also be transferred to other applications. Plasma-treated apple juice

showed similar characteristics in terms of long-term antimicrobial activity (Surowsky et al., 2014). Applied plasma-activated water on fruits and vegetables showed a high antimicrobial potential (Ma et al., 2016) on strawberries (Fernández et al., 2013; Misra et al., 2014), cherry tomatoes (Ziuzina et al., 2014), mushrooms (Agun et al., 2019) tomatoes (Hou et al., 2021) and cabbage (Bacchetti et al., 2014). However, plasma-activated water have a good effect on the fruits and vegetables and showed a higher firmness, colour index and total soluble solid in plasma-activated water-treated berries compared to control samples after 8 days of storage (Ma et al., 2016). Another potential effects of plasma-activated water could be the decrease of the browning enzyme activity reaction in fresh-cut fruits (Xu et al., 2016).

1.1.4 Electrolysed Water

Electrolysed water has strong bactericidal effects on most pathogenic bacteria that are important to food safety. Electrolysed water is produced by passing a diluted salt solution through an electrolytic cell, within which the anode and cathode are separated by a membrane. By subjecting the electrodes to direct current voltages, negatively charged ions such as chloride and hydroxide in the diluted salt solution move to the anode to give up electrons and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid and hydrochloric acid, while positively charged ions such as hydrogen and sodium move to the cathode to take up electrons and become hydrogen gas and sodium hydroxide (Hsu, 2005). This process rises to 3 types of electrolysed water depending on the pH: acid, basic and neutral. This electrolysed water is less oxidative and corrosive, but at the same time, maintains its antimicrobial capacity. The main reason for its popularity is the simplicity of production and application which exhibits antimicrobial activity against a variety of microorganisms and eliminates most common types of viruses, bacteria, fungi, and spores in a relatively short amount of time (usually within 5 to 20 s) in food products, food processing surfaces, and non-food surfaces. Recent studies (Li et al., 2018; Kim et al., 2018, Ma et al., 2017; Tango et al., 2018) demonstrate the efficacy of electrolysed water on broccoli, against several pathogens and minimally processed fruits and vegetables respectively, at lab scale. It has been demonstrated that electrolyzed water can be used as an alternative to chlorine-based products for the disinfection of minimally processed fruits and vegetables. Electrolysed water can control spoilage microorganisms in washing water, reduce cross-contamination phenomena and delay fruit and vegetable decay. Some combined applications of electrolysed water proved to be very effective in both shelf-life extension and microbial inhibition of minimally processed fruits and vegetables. Future research needs to consider varieties of further combined applications of physical, chemical and bio-preservation technologies, which may allow better maintenance of the fresh-like characteristics of the raw produce.

1.2 Biomaterial for food packaging application

Another non-thermal possibility to preserve minimally processed fruits and vegetables is through biopolymer coatings. They are a promising preservation technology that allows extending the shelf-life of food products, slowing down their quality decay through the reduction of mass transfer of gas, moisture or aromas. In addition, coatings can be used as carriers of active additives that can contribute to the shelf-life extension and/or improve food nutritional quality of MPF&V (Yousuf et al., 2018, Valentino et al., 2020, Khan et al., 2021). Polymers belong to the category of bio-based materials, in which organic carbon is derived exclusively from renewable biological resources. The class of biopolymers encompasses all the polymeric compounds produced by living organisms and are mainly represented by polysaccharides, proteins and nucleic acids. Generally, proteins and polysaccharides have played a major role in the food industry, presenting a lot of applications. Protein in the food system can be denatured by heat, and pH, whereas polysaccharides are branched or linear polymers of sugars; moreover polysaccharides may also be synthesized by microorganisms such as exopolysaccharides (Telis, 2012). Among the many kinds of polysaccharides, cellulose and chitin are the most important biomass resources, being the most abundant organic compounds on Earth. Biomaterials are not necessarily edible, and not completely biodegradable according to official definitions, but their main advantage consists in the ease with which they can be eliminated (compostable) and in the low environmental impact. Biobased polymers can be classified in several ways, based on their chemical composition, origin, synthesis method, application etc. The traditional way of classifying biobased packaging materials has been to divide them into three categories based on their origin and production. Various natural sources from which biopolymers can be extracted are shown in Figure 1.

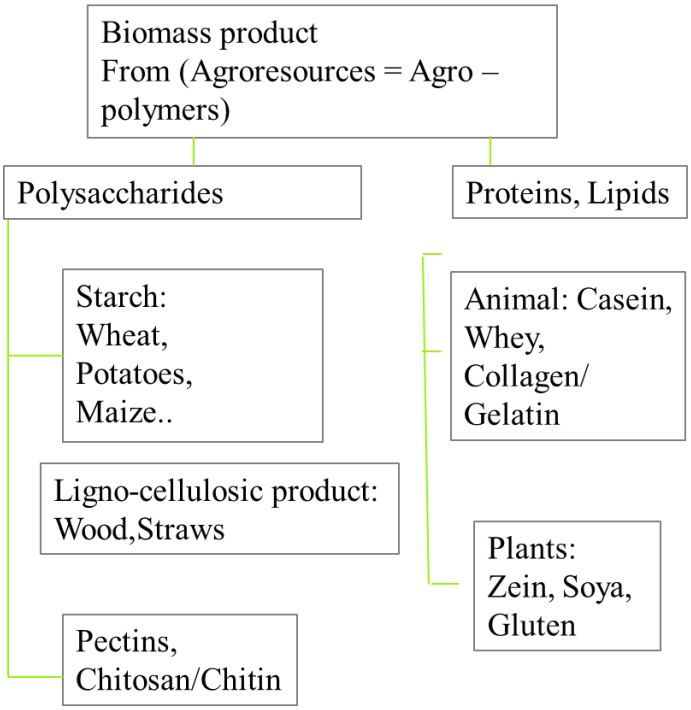

Figure 1. Different categories of bio-based material for edible and biodegradable films

The polymers, most commonly available, are extracted from marine and agricultural animals and plants. Examples are polysaccharides such as cellulose, starch, and chitin and proteins such as casein, whey, collagen and soy, protein of house cricket. All these polymers are, by nature, hydrophilic and somewhat crystalline factors causing processing and performance problems, especially concerning the packaging of moist products. On the other hand, these polymers make materials with excellent gas barriers. Edible coatings are produced from edible biopolymers and food-grade additives: hydrocolloids (such as proteins or polysaccharides), lipids (such as fatty acids, acylglycerols or waxes) and composites of them. The production of edible and biodegradable films by combining various polysaccharides, proteins and lipids is considered with the aim of taking advantage of the properties of each compound and the synergy between them (Falguera et al., 2011). Edible coatings and film are usually classified according to their structural material. They are a mixture of a high molecular weight polymer, solvent and a plasticizer but there are several ingredients such as antimicrobial compounds, antioxidants, flavours, and colouring that can be incorporated (Krochta et al., 1994).

1.2.1 Protein

Functional properties of proteins include the ability to form films and coatings. Proteins are polymers with specific amino acid sequences and molecular structures. Depending on the sequential order of the amino acids, the protein can be assuming different structures along the polymer chain which can determine the secondary, tertiary, and quaternary structures. The secondary, tertiary, and quaternary

structures of proteins can be easily modified to optimize the protein configuration, protein interactions, and resulting film properties (Perez-Gago, 2012). Coatings based on proteins are edible or biodegradable, depending on the formulation, formation method, and modification treatments (Krochta, 2002). The structure of proteins can be modified by a physical and chemical agent, including heat, mechanical treatment, pressure, irradiation, lipid interface, acids and alkalis, and metal ions. This modification can optimize protein configuration, protein interactions, and resulting film properties. Proteins have multiple sites for chemical interaction as a function of their diverse aminoacidic functional groups, which can allow for property improvement and tailoring. Chemical changes can improve the stability of coatings (Dargaran et al., 2009). As long as food-grade proteins and additives are used and only protein changes due to heating, pH modification, salt addition, enzymatic modification, and water removal occur, the resulting film or coating is edible (Krochta and DeMulder-Johnston, 1997). Due to its hydrophilic nature, the protein-based coating does not have good barrier proprieties against water vapour, but they have good barrier properties against gas, such as oxygen and carbon dioxide. The majority of the protein-based film has good mechanical and organoleptic properties (Krochta, 2002; Dargaran et al., 2009). Generally, the protein used for fresh-cut fruits and vegetables is whey protein (Yong Cho et al., 2002a; Anker et al., 2002; Shaw et al., 2002), casein extracted from milk (Belyamani et al., 2014; Barreto et al., 2003), soy protein (Pol et al., 2002; Yong Cho et al., 2002b; Yong Cho et al., 2007; Mariniello et al., 2003) fish protein (Bourtoom et a., 2006), egg protein (Gennadios et al., 1996; Wongsaulak et al., 2006; Di Pierro et al., 2006).

1.2.1.1 Casein

Casein is the major protein in milk. It is a unique protein because it is only synthesized in the mammary gland and is found nowhere else in nature. Casein exists in the form of micelles containing all four casein species complexed with colloidal calcium phosphate. Each micelle consists of an average of 104 peptide chains with molecular weights of about 105 kDa. The casein micelles are stable to most common milk processes such as heating, compacting, and homogenization. Micellar integrity is preserved by extensive electrostatic and hydrogen bonding, and hydrophobic interactions. Four principal components of αs1-, αs2-, β-, and κ-caseins are identified. Their molecular weights range from 19 to 25 kDa. (Perez-Gago, 2012). The primary structure of the four casein fractions contains many hydrophobic amino acid residues with non-polar sidechains (35 to 45% total residues). The uneven distribution of these amino acids results in hydrophobic ends and patches. The caseins are amphipathic proteins having hydrophobic and hydrophilic ends and, thus, are especially used as emulsifiers. This feature helps the formation of stable composite protein-lipid emulsions for coating wet surfaces. However, caseins are generally considered hydrophilic because their hydrophobicity

values are lower than that of valine (7.05 kJ/residue). Among the casein fractions, β-casein is the most hydrophobic, and αs2-casein is the most hydrophilic. Isoelectric casein is water-insoluble. Application to the food field requires sodium or potassium caseinate with high water solubility. This can be also achieved by dispersing casein in water and adjusting with alkaline solution pH to between 6.5 and 7.0. The most common casein product is soluble water caseinate. It is normally manufactured by dissolving fresh acid casein curd in sodium hydroxide followed by spray drying. Other soluble caseinates prepared similarly include potassium, calcium, magnesium, and ammonium caseinates. Casein can easily form a coating due to its open secondary structure. The chemical and physical forces that may change the balances of the intermolecular interactions can perceivably modify the coating properties. Adjusting the pH, changing the drying rate, and adding functional additives such as plasticizers, hydrophobic ingredients, and crosslinking ions, are examples of approaches used by investigators.

1.2.1.2 Insect flour

Entomophagy, i.e., the eating of insects, is exercised traditionally in 113 countries all over the world (MacEvilly, 2000) and more than 2000 insect species that are considered edible have been counted to date (Jongema, 2012). The class of insects belongs just like the crustaceans (e.g. shrimp, crab, lobster, krill) to the arthropods and counts more than a million species. Insects are further divided into orders such as coleoptera (true beetles), diptera (flies), hemiptera (bugs), homoptera (treehoppers), hymenoptera (wasps, bees, ants), isoptera (termites), lepidoptera (butterflies and moths), orthoptera (grasshopers, locusts, crickets) (Capinera, 2008). In comparison to conventional livestock in general, insects have a higher feed conversion efficiency (Nakagaki & Defoliart, 1991) i.e. need less amount of feed for the production of 1 kg biomass, have a higher fecundity (e.g. the common house cricket lays up to 1,500 eggs over a period of about a month (Nakagaki & Defoliart, 1991), are mostly omnivorous and can be raised on organic waste, equally nutritious and take up less space in the rearing process. It has even been indicated that insects might contribute fewer greenhouse gases than pigs and cattle (Oonincx et al., 2010). For industrial mass production of insects, automation technologies as well as safety procedures have to be developed to ensure an economic production process of safe food and feed products derived from insects. In addition, consumer acceptance has to be improved. Insects have an attractive nutritional profile with a protein content varying between 35 and 61% and a balanced aminoacidic profile, meeting the requirements of the World Health Organization (WHO) for amino acids (Rumpold & Schlüter, 2013).

Regarding the protein quality, it was observed that the protein of the house cricket *Acheta domesticus* was superior to soy protein (Finke et al., 1989) and that the removal of the chitin further improves the quality of the insect protein since some of the proteins are linked to the chitin. A chitin removal via

alkaline extraction resulted in increased digestibility of bee protein from 71.5 to 94.3% (Ozimek et al., 1985). An overview of the amino acid spectra of a selection of commonly eaten insects. Regarding the amino acid requirements for human nutrition, the selected insects show a high-quality amino acid profile with high contents of phenylalanine and tyrosine and generally meet the requirements except for the amino acid methionine. The cricket *Acheta domesticus* contains high-quality proteins (Finke, Defoliart, & Benevenga, 1989).

The protein and lipid contents of the house cricket (20-25 and 4-7 g/100 g fresh weight, respectively) are comparable to those of conventional animal sources such as beef or chicken (Kulma et al., 2019) House crickets have also been studied as a resource for protein extraction and fractionation (Laroche et al., 2019a; Ndiritu et al., 2017; Udomsil et al., 2019; Yi et al., 2013), with a reported 20–40% protein yield in a liquid fraction and an approximately 60–75% purity (Laroche et al., 2019a; Ndiritu et al., 2017). Furthermore, they have been used as a starting material for fat extraction and isolation with several methods (Laroche et al., 2019a) and solvents (Ramos-Bueno et al., 2016). The fat yield of house crickets has been reported to reach 25% (Laroche et al., 2019). Frozen, dried and powdered cricket (*Acheta domesticus*) meal thanks to Regulation 169/2022 then Regulation 188/2022 was included as a novel food. The International Platform of Insects as Food and Feed (IPIFF), has published a set of useful guidelines for labelling insect foods based on Regulations 1169/2011 and 1924/2006 (NHCR, Nutrition and Health Claims Regulation).

1.2.2 Polysaccharides

Polysaccharides are widely used as edible coatings and films; they have considerable molecular weight and are water-soluble. They dissolve and form intensive hydrogen bonds with water. Because of the size and configuration of their molecules, polysaccharides can thicken and/or gel aqueous solutions as a result of both hydrogen bonding between polymer chains and intermolecular friction when subjected to shear. In solution, polymer molecules may arrange themselves into an ordered structure, called a micelle that is stabilized or fortified by intermolecular hydrogen bonds. The micelle traps and immobilizes water and, depending on the extent of the intermolecular association, the water is either thickened, as measured by a parameter called viscosity, or converted into a gel that possesses both liquid and solid-like characteristics or viscoelasticity.

Polysaccharides can exhibit either a neutral charge (e.g., acetate esters, methyl ethers, other neutral sugars), negative charge (e.g., carboxylate, sulphate groups), or positive charge (e.g., amino groups) due to the presence various chemical groups attached to individual monosaccharide units. All of these structural features of polysaccharides contribute to their differences in solubility, synergy or incompatibility with each other or with other ingredients (e.g., proteins, minerals, acids and lipids), thickening, gelling, and emulsifying properties and, more importantly, their film-forming properties

(Neito et al., 2009). Polysaccharides edible coating are generally poor moisture barriers due to their hydrophilic nature and soluble in water, but in contrast they have moderately low oxygen permeability and, at the same time, selective permeability to O_2 and CO_2. Typically, polysaccharide-based coatings have been applied very often to fruits and vegetables, either fresh or minimally processed, to reduce their respiration by creating modified atmosphere conditions inside the product, provide a partial barrier to moisture, improve mechanical handling properties, carry additives, as well as contribute to the retention and even the production of volatile compounds (Dea et al., 2012; Gill & Gill, 2005; Oms-Oliu et al., 2008; Yingyua et al., 2006).

The polysaccharides that can be used for the production of films and coatings include starch and its derivatives (Garcia et al., 2000; Phan The et al., 2009a; Petersson et al., 2005; Bertuzzi et al., 2007; Garcia et al., 2006), cellulose and its derivate (Brindle et al., 2008), chitosan (Garcia et al., 2004; Rivero et al., 2009; Chillo et al., 2008, Volpe at al., 2017), pectin (Maftoonazad et al., 2007; Liu et al., 2006; Giancone et al.,2008), agarose (Phan The et al., 2009a; Phan The et al., 2009b), arabinose (Phan The et al., 2009b) and alginate (Olivas et al., 2008).

1.2.2.1. Chitosan

Chitosan is a naturally occurring polysaccharide whose commercial forms are essentially produced from N-deacetylation of chitin. Chitin is chemically composed of repeating units of 1,4-linked 2-deoxy-2-acetamido-α-D-glucose, and chitosan refers to a family of partially N-acetylated 2-deoxy-2-amino-α-glucan polymers derived from chitin. The difference between chitin and chitosan is essentially related to the possibility to solubilize the polymer in dilute acidic media. Thus, when their structure can be dissolved in this kind of solvent, it corresponds to chitosan, in the reverse case, to chitin. Therefore the degree of acetylation (DA), which is related to the balance between the two kinds of residues, is essential to define these two terms. When DA is below 60%, the polymer is soluble in dilute acidic solutions. This behaviour is related to the fact that the protonation of the amino groups of glucosamine residues contributes to the disruption of hydrogen bonding, the solvation of the cationic sites, and then to the solubilisation when the balance between solvent/polymer and polymer/polymer interactions becomes favourable. (Domard & Domard, 2002). When DA becomes over 60%, we enter the range of chitin and the chains become completely insoluble in water. This insolubility has to be related to the numerous hydrogen bonds occurring between the alcohol, amide, and ether functions distributed on the repeating units all along the polymer chains. They also correspond to hydrophobic interactions due to the presence of the methyl groups of the acetamide functions and to the -CH and -CH_2 of the glucosides rings. Another important parameter to take into account is the molecular weight; for thermodynamic reasons, the solubility of neutral polymers is known to decrease with an increase in their molecular weight. The former behaviour has been

identified in the case of high molecular-weight chitosan, whose aggregation capacity increases with molecular weight. Chitosan has been extensively studied for coating applications because of its film-forming properties; by the attraction of oppositely charged molecules, chitosan, owing to its cationic polyelectrolyte nature, spontaneously forms water-insoluble complexes with anionic polyelectrolytes such as alginate, carrageenan, xanthan, various polyphosphates, and organic sulphates. This method is frequently used for enzyme immobilization into gel beads, which is achieved by adding an anionic polyelectrolyte solution containing the enzyme into an acidic chitosan solution (Soliva Fortuny et al., 2012). Polysaccharide coatings may be used to preserve the quality of several food commodities. The oxygen and moisture barrier properties of these coatings can protect fresh fruit and vegetables from dehydration and, in some cases, even retard their respiration rate. Polysaccharide coatings can also be used to prevent the oxidation of lipid ingredients or to reduce the loss of food colours and flavours. Some of the most common applications of edible coatings are for improving the quality and extending the shelf life of foods.

1.2.3 Lipids and waxes

Lipids are usually added to food coatings to impart hydrophobicity and thereby reduce moisture loss (Baldwin et al., 1997). An example of a coating lipid is white mineral oil, which consists of a mixture of liquid paraffinic and naphthenic hydrocarbons, allowing for use as a food release agent and as a protective coating agent for fruits and vegetables in an amount not to exceed good manufacturing practices. Waxes are the most common lipid used in coatings, so much so that the word wax is commonly used to denote any coating, whether it includes a lipid substance such as beeswax, carnauba wax, polyethene wax, or candelilla wax. Examples of such usage are storage wax, shipping wax, solvent wax, and water wax. Beeswax, also known as white wax, is secreted by honeybees for comb building. The wax is harvested by centrifuging the honey from the wax combs and then melting it with hot water, steam, or solar heating. The wax is subsequently refined with diatomaceous earth and activated carbon and is finally bleached with permanganates or bichromates. Beeswax consists mostly of monofunctional alcohols C_{24}–C_{33}, hydrocarbons C_{25}–C_{33}, and long-chain acids C_{24}–C_{34} (Tulloch, 1970). This wax is very plastic at room temperature but becomes brittle at colder temperatures. It is soluble in most other waxes and oils. Beeswax is considered a GRAS substance and is allowed for direct use in food. Maximum allowed levels are 0.065% in chewing gum, 0.005% in confections and frostings, 0.04% in hard candy, 0.1% in soft candy, and 0.002% in all other categories. Waxes such as carnauba, beeswax, paraffin, rice bran, and candelilla have been used in combination with other lipids, resins, or polysaccharides to coat fresh fruits and vegetables such as citrus, apples and strawberry (Ahmad et al., 1979; Claypool, 1939; Dalal et al., 1971; Durand et al., 1984; Hitz and Haut, 1942, Miele et al., 2022).

1.2.3. Proteins polysaccharides interaction

Proteins and polysaccharides are present together in many kinds of food systems, and both types of food macromolecules contribute to the structure, texture and food stability through their thickening or gelling behaviour and surface properties. Most structural elements present in foods at the supramolecular (or microstructural) level are thermodynamically metastable and at non-equilibrium (e.g. amorphous phase), where the nature and kinetics of interactions between them are largely unknown and uncontrolled. Knowledge of the thermodynamics of simple mixtures provides a reference point to assess the potential behaviour of the extremely complex multi-component system that is real food and the effect on its variables such as temperature, pH, ionic strength, concentration, and so on (Tolstoguzov, 1997). An understanding of polymer science principles is essential for following the evolution of food materials science. The basic premise of this science is that since most foods are formed by polymers, they must comply with the principles and theories that apply to synthetic polymers. It tries to interpret physical and chemical phenomena in the food system through concepts such as the thermodynamic incompatibility of polymer solutions, the glass transition, state diagrams, polymer rheology, etc. Material science is a well-developed discipline that, building on chemistry and physics, covers such subjects as internal properties of materials, phase transitions and phase equilibrium, strength and fracture of materials, and surface and transport properties (Aguilera et al., 1999).

1.3 Edible Coating

Biopolymer coatings are a promising preservation technology that allows extending the shelf-life of minimally processed vegetables, slowing down their quality decay through the reduction of gas transfer, moisture or aromas (Figure 2) (Baldwin et al., 1995, Ahvenainen 1996; Yousuf et al., 2018; Volpe et al., 2019; Khan et al., 2021). Renewable and biodegradable polymers as well as natural additives are currently considered sustainable alternatives for food packaging applications, enhancing resource efficiency and reducing environmental negative issues associated with packaging wastes after their useful life. In addition, coatings can be used as carriers of active additives that can contribute to the shelf-life extension and/or improve food nutritional quality of minimally processed fruits and vegetables (Baldwin et al., 1995; Hua Han et al., 2005; Valentino et al., 2020). Among active compounds, propyl gallate has shown the most promising action for its antimicrobial and antioxidant properties. In foods, propyl gallate has been employed as an additive (E310) since 1948 to protect fats, oils, and fat-containing food from peroxide-induced rancidity (Lillian, 2007). Successful active packaging systems are achieved via an adequate understanding of produce physiological characteristics and properties of the packaging material, coupled with optimum equilibrium atmospheric conditions for the specific product (Torrieri et al., 2008).

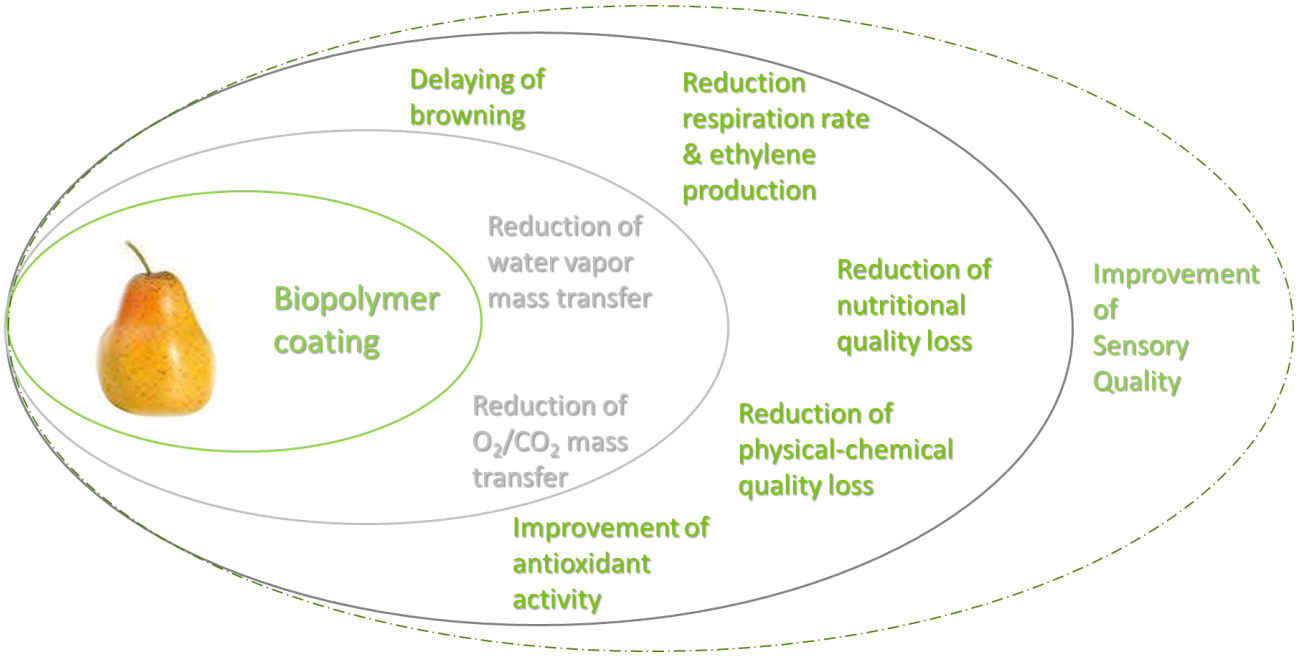

Figure 2. Effect of the coating on the product

1.3.1 Edible coating formation

Coating application consists of applying a liquid or a powder ingredient as a base product and can be obtained in different ways. The simplest way to apply a film is direct to form the solution; depending on the concentration of the coating solution, the product will absorb an appropriate amount of material necessary to form the desired layer, which when dried, forms a protective layer on the food surface (Pavlath and Orts, 2009). Generally, the application of coatings requires a four-step process:

1. Deposition of coating material (solution, suspension, emulsion, or powder) on the surface of the product to be coated through spraying, brushing, spreading, or casting.
2. Adhesion of coating material (solution, suspension, emulsion or powder) to the food surface.
3. Coalescence (film-forming step) of the coating on the food surface.
4. Stabilization of the continuous coating layer on its support or food product through coacervation by drying, cooling, heating, or coagulation.

1.3.2 Different technique to apply coatings

The dipping method lends itself to food products that require several applications of coating materials or require uniform coating on an irregular surface. After dipping, an excess coating material is allowed to drain from the product and it is then dried or allowed to solidify. Final formed coatings may be less uniform than coatings applied by other methods, and multiple dipping (with draining and drying steps between dipping operations) sometimes may be necessary to ensure full coverage (Krochta et al. 1994). The spraying method allows for obtaining thinner, more uniform coating over a food surface than those applied by dipping. However, spray-coating requires that the bottom surface

of the product be coated in a separate operation after the application of the initial coating and drying. In this framework, the product must then be turned to expose the bottom for subsequent coating application. Spray-coating is preferred for items possessing a large surface area (Dangaran et al., 2009). The spray-coating technique can be used alone or in combination with a pan, drum, screw and fluidized-bed coaters. Spraying makes it possible to deposit either thin or thick layers of aqueous solution or suspensions and molten lipids. The spraying nozzle plays a critical role in the coating process. Spraying efficiency depends on the pressure, fluid viscosity, temperature and surface tension of the coating liquid, as well as nozzle shape or design. This in turn affects the flow rate, the size of the droplets, spraying distance and angle, and overlap rate (Debeaufort and Volley, 2009). Spraying or dipping techniques are reported in Figure 3.

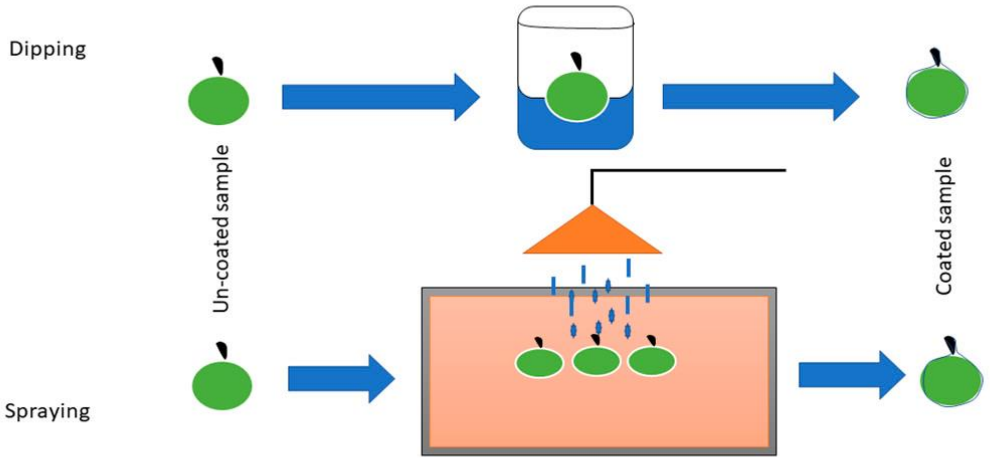

Figure 3. Dipping and spraying technique to apply coating on the product (Khan et al., 2021)

1.4 Quality indices of fruits and vegetables

The edible coating improves the quality and extends the shelf life of minimally processed fruit and vegetables. Edible films or coatings can reduce respiration and hence increase the shelf life of a commodity. In the selection of a coating, several considerations should be addressed to avoid extremely low oxygen concentrations inside the commodity. Low oxygen concentration in the product could lead to anaerobic respiration, which can result in the deterioration of the product due to the production of off-flavours and accelerated senescence (Kays and Paull 2004). Respiration and ripening of fruits and vegetables involve gas exchange with the environment. Carbon dioxide, oxygen, water and other metabolic by-products, such as ethylene and other volatile compounds, are the main substances exchanged during storage. Surface coatings modify the gas exchange rate between the environment and fresh produce, and thus control transpiration, respiration and other metabolic processes that lead to loss of quality. Moreover, edible coatings decrease water vapour transmission rate by forming a barrier on the fruit or vegetable surface; in the case of minimally-processed fruits, there is usually very high water activity present at the surface, making it difficult to develop a coating that delays water loss, since the capacity of films to work as barriers to water vapour

decreases as water activity increases in the commodity (or relative humidity of the environment) (Hagenmaier and Shaw 1990).

Moreover, the production and development of fresh-cut fruits and vegetables must fulfil the following two major aims. The first aim is to extend the shelf life of fresh-cut products by keeping them fresh while preserving their nutritional quality, sensory attributes, and food safety aspects. The second aim is to ensure a long enough shelf life of such products that are adequate to make its regional distribution possible (Siddiq et al., 2020, Laurila & Ahvenainen, 2002). During storage and distribution, the packaging of fresh-cut produce plays a critical role in quality preservation and shelf-life extension. Since fruits and vegetables consist of living tissue, subsequent physiological and biochemical changes which cause detrimental changes in the quality and shelf life of products are common after harvesting. The main factors contributing to fruit and vegetables' physiological deterioration are respiration, transpiration and ethylene production. However, also chemical physical and nutritional properties are important to control how to change the quality indices of fruits and vegetables.

1.4.1 Physiological changes of fruit and vegetables

1.4.1.1 Respiration

Any type of food undergoes continuous biochemical, biological and physiological processes; enzymatic reactions occur in fruits and vegetables during respiration where oxygen is consumed, carbon dioxide is produced, and heat and energy are released. Respiration is required to keep produce alive and to support any developmental changes. When oxygen composition is lowered, respiration decreases and hence, senescence does as well. However, when oxygen concentration falls below the threshold level anaerobic respiration occurs, which in turn causes ethanol production, off-flavour formation and loss of harvested produce (Kays & Paull 2004). The respiration activity of a product is influenced by storage temperature, type of processing, oxygen-to-carbon dioxide ratio, and the absolute value of oxygen concentration itself (Pavlath & Ortis, 2009). Moreover, some unit operations such as cutting and wounding can cause an increase in respiration, thereby increasing the production of carbon dioxide and consumption of oxygen, further causing a decrease in stored reserves.

1.4.1.2 Transpiration

One important factor, which is necessary to preserve the quality of fruits and vegetables, is the avoidance of water loss. Most of the fresh produce contains from 65% to 85% of water when harvested. After harvesting, the water supply is broken but the loss of water continues; if weight loss exceeds 5% of weight, produce will appear too shrunken to be saleable (Bai & Plotto, 2009). Cutting, slicing, and peeling, etc., of fruits and vegetables, will increase the water transpiration rate due to exposure of tissue following the removal of the natural epidermal layer, and will also increase the surface area of exposure (Toivonen & DeEll 2002). Water loss through transpiration cannot be

replaced and is problematic since the loss of small amounts of water will severely impact production quality. When water is lost, the turgor of production decreases, as does firmness; moreover, water stress causes metabolic alterations and changes in enzyme activation causing accelerated senescence, reduced flavour and aroma, a decline in nutritional value, and increased susceptibility to chilling injury and pathogen invasion (Olivas & Barbosa-Canova, 2009). Respiration rate is affected by the difference between the water vapour pressure inside and outside the tissue, air movement, packaging or coating and surface area; the RH in the product is almost 100% versus 90% to 95% in a well-maintained storage room and 30% to 80% on the market shelves; thus, to prevent the water loss is important to keep the product in a moist atmosphere as much as possible. Temperature plays an important role because higher temperatures raise the water activity of molecules, increasing water loss. Moreover, the faster the surrounding air moves, the quicker water is lost. Thus, the ideal storage condition should be as low a temperature as possible (above chilling injury temperature) and relative humidity of 90%or above (Bai & Plotto, 2009). The water loss rate varies with the type of produce: generally, leafy green vegetables such as spinach and leaf lettuce, lose water quickly because they have a high surface-area to volume-ratio and a thin waxy cuticle with many pores; other multi-layered products, such as onion or iceberg lettuce, are carrying an inherent protection because water transport between leaves occurs via the dwarf stem.

1.4.1.3 Ethylene production

Ethylene is a hormone produced when a vegetable or fruit undergoes stress. Ethylene triggers ripening and senescence and is partially responsible for changes in the flavour, colour and texture of fruits and vegetables (Kays & Paull 2004). On the other hand, the removal of ethylene will slow ripening and senescence. Controlled atmosphere (CA) or modified atmosphere (MA) storage of produce can reduce ethylene production/action, preserving the quality of fruits and vegetables for longer periods.

1.4.2 Chemical-physical and nutritional properties

Among several chemical-physical parameters, firmness is a key quality attribute because besides being able to be pleasant, it conditions the possibility that other compounds contained in the cell (sugars, acids, volatile substances) can be extracted from the cell with mastication and so can be perceived by the consumers (Zerbini 2002; Maringgal et al., 2020). Polyphenol concentrations and antioxidant capacity in foods vary according to numerous genetic, environmental, and technological factors, some of which may be controlled to optimize the polyphenol content of foods (Manach et al., 2004). The postharvest life of fruits and vegetables has been traditionally defined in terms of visual appearance (freshness, colour, and absence of decay or physiological disorders) and texture (firmness, juiciness, and crispness). Although this concept involves aesthetic appeal and mechanical properties associated with quality, it disregards flavour and nutritional quality (Ayala-Zavala et al., 2004).

Flavour plays an important role in consumer satisfaction and influences further consumption of fruits and foods in general (Pelayo et al., 2003). Fruits and vegetables form an important part of our diet mainly as a source of energy, vitamins, minerals, and antioxidants. Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and are enhanced by physical damage, extended storage duration, high temperatures, low relative humidity, and chilling injury of chilling-sensitive commodities (Navarro et al., 2006; Lee et al., 2000). Ascorbic acid is the best-known chemical agent for reducing the browning reaction (Sapers et al. 2001). It is a labile vitamin that loses activity due to several factors, including pH, moisture content, oxygen, temperature and metal ion catalysis (Uddin et al., 2001). Ascorbic acid prevents the oxidation of phenols by itself being a substrate for oxidation and converting to dehydro-ascorbic acid thereby inhibiting or delaying browning (Arora et al., 2018).

1.5 Kinetics models

1.5.1 Reaction Order

Chemical reactions occur in foods during processing and storage. Some reactions result in a quality loss and must be minimized, whereas others result in the formation of a desired flavour or colour and must be optimized to obtain the best product quality. Kinetics is a science that involves the study of chemical reaction rates and mechanisms. An understanding of reaction mechanisms coupled with the quantification of rate constants will facilitate the selection of the best conditions of a process or storage so that the desired characteristics will be present in the product.

Reaction order is the sum of the exponents of reactant concentration in terms of the rate equation.

Zero or first-order kinetics, traditionally used to describe degradation reactions in foods, may be generally written as (Giannakourou & Taoukis, 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005):

$$\frac{dQ(t)}{dt} = -kQ^n \qquad \textit{Eq.(1)}$$

The equation may be integrated easily obtaining the well-known decay functions. In particular, first kinetic order (n=1) the equation is:

$$Q = Q_i - kt \qquad \textit{Eq.(2)}$$

whereas

$$Q = Q_i e^{-kt} \qquad \textit{Eq.(3)}$$

Where:

Q(t) is the concentration of the quality index at the time *t*, *k* is the rate constant, and n is the kinetic order of the equation.

The second-order unimolecular reaction is characterized by a hyperbolic relationship between the concentration of the reactant or product and time. A linear plot will be obtained if *1/A* is plotted against time. Second-order bimolecular reactions may also follow the following rate equation:

$$Ln\frac{Q}{Q0} = -kt \qquad Eq.(4)$$

Where:

k0 is a pseudo-first-order rate constant: *k0 =- kB (B=reactor)*.

A second-order bimolecular reaction will yield a similar plot of the concentration of the reactant against time as a first-order unimolecular reaction, but the reaction rate constant will vary with different concentrations of the second reactant. An example of a second-order bimolecular reaction is the aerobic degradation of ascorbic acid. Oxygen is a reactant, and a family of pseudo-first-order plots will be obtained when ascorbic acid degradation is studied at different levels of oxygen availability. Different kinetics are reported in Figure 4.

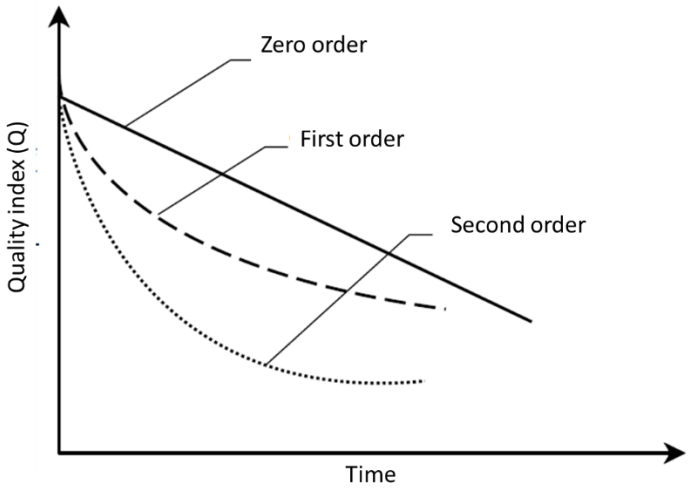

Figure 4. Variation of a quality index as a function of time, according to the kinetics of order zero, first and second.

This type of reaction is usually followed not in terms of the concentration of the reactant but by some manifestation of the completion of the reaction in terms of a physical property change. Examples are protein gelation measured as an increase in the strength of the gel, nonenzymatic browning reaction in solid foods, textural changes during cooking, sensory flavour scores during storage, and so forth. The magnitude of the attribute measured usually levels off not because of depletion of the reactants, but because the measuring technique could no longer detect any further increase in intensity. Reactions of this type could be fitted to the following:

$$ln\left(1 - \frac{C}{C*}\right) = \pm kt \qquad Eq.\ (5)$$

where *C** is the value of the measured attribute when it remained constant at long reaction times, and *C* is the value at any time during the transient stage of the process (Toledo et al., 2018).

1.5.2 Temperature Dependence of Reaction Rates

1.5.2.1 The Arrhenius Equation

The activated complex theory for chemical reaction rates is the basis for the Arrhenius equation which relates reaction rate constants to the absolute temperature.

The effect of temperature on critical quality indices degradation is expressed in terms of activation energy (Ea) as described by the Arrhenius equation:

$$k = Aexp\left(-\frac{E_a}{RT}\right) \qquad Eq.\ (6)$$

Where:

k=specific constant kinetic; *A* is pre-exponential, temperature-independent constant; E_a is activation energy (J mol^{-1}), independent of temperature; *R* is gas constant (8.31 J mol^{-1} K^{-1}); *T* is absolute temperature (°Kelvin).

Let T0=the reference temperature at which k= k_0:

$$k = Qexp^{-Ea/RT0};\ k = Qexp^{-Ea/RT} \qquad Eq.(7)$$

Taking the ratio of the two equations:

$$\frac{k}{k0} = exp^{-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T0}\right)} \qquad Eq.\ (8)$$

The negative sign is placed on the exponent of the Arrhenius equation so that a positive activation energy will indicate an increasing reaction rate constant with increasing temperature. Using Eq. (8), the rate constant at any temperature can be determined from the activation energy and the rate constant *k0* at a reference temperature, *T0* (Toledo et al., 2018).

1.5.3 Determination of reaction kinetic parameters

The reaction rate constant is usually determined at constant temperature by measuring changes in the concentration of reactant or concentration of reaction product with time. Because the reaction rate can be affected by the presence of interfering compounds, pH, and water activity, kinetic parameters are determined with the reacting compound contained in a specific substrate. Model systems may be used to ensure that the substrate composition is constant during the determination of the kinetic parameters. However, literature data indicate variations in the value of the kinetic parameters for the same reaction in different food products. Indeed, different studies found that using zero, first, or second-order kinetics is possible to determine the quality degradation reactions (Zanoni et al., 2005, Nisha et al., 2005, Giannakourou & Taoukis, 2003; Rodrigo et al., 2007). These models fitted well with several food degradation reactions, such as microbial growth (Corradini & Peleg, 2007), antioxidant changes (Muley et al., 2022; Oms-Oliu et al., 2009), browning of orange juice (Manso et al., 2001) vitamin C degradation during thermal treatments of fresh fruits and vegetables (Corradini

& Peleg, 2007, Maftoonazad & Ramaswamy, 2019; Muley et al., 2022) and total polyphenols content during storage time (Esua et al., 2019). The temperature dependence of the reaction rate constant is determined by conducting the kinetic studies at several constant temperatures and determining the z value or activation energy for the reaction. A typical technique for the determination of the kinetic parameters is to derive a linear form of the reaction rate equation applying regression analysis to the transformed data. This approach however has been shown to have limitations because of the smoothing out effect of the transformation used to linearize the data. One method that can be used is nonlinear curve fitting. Another approach is the use of the Solver feature in Microsoft Excel or XSTAT. Although Solver only allows the manipulation of one variable to minimize the least square error, an iteration method may be employed to fit two-parameter equations to the data (Toledo et al., 2018).

1.6 Project outline

The PhD thesis focuses on the development of active coatings based on proteins, and/or polysaccharides and/or lipids enriched with antioxidant compounds and quantifying degradation kinetics of the critical quality indicators, obtained with a mathematical model to estimate/predict product shelf life. Firstly, the active coating was developed to select the best sodium caseinate concentration by evaluating the capacity of the coating to reduce the product respiration rate at 10 °C of minimally processed fennels. Quality indices, including microbial load, weight loss, firmness, TA, TSS, pH, total colour change, total antioxidant capacity, total polyphenols and vitamin C content were measured at set intervals during storage (**chapter 2A**). Secondly, critical quality indices of fennels were modelled with kinetic models. Pseudo zero, first order and Arrhenius-type models well describe the firmness, ΔE, total antioxidant capacity, total polyphenols and vitamin C content of the products over time at different temperatures (**chapter 2B**). Therefore, the best concentration of sodium caseinate was used to develop a blend, adding guar gum, beeswax and propyl gallate and it was applied on minimally processed pears. A preliminary test was conducted to study the respiration rate. Then, the effect of the blend on the chemical-physical and nutritional parameters of minimally processed pears was studied at different temperatures and relative humidity (**chapter 3A**). Firmness, vitamin C, antioxidant capacity and total polyphenol content were selected as critical quality indices for shelf-life pears. Pseudo first order well described the quality changes of the product over time in the range of temperatures tested (**chapter 3B**). Finally, a coating based on chitosan and house cricket protein obtained with and without plasma-activated water was developed and applied on fresh-cut apples to study the effect on the quality of the product (**chapter 4)**. Respiration and transpiration rates at different relative humidity were measured at 5 °C. Secondary, chemical-physical (weight loss, colour, hardness, pH and total soluble solids) and nutritional properties (antioxidant capacity, total polyphenols content and vitamin C) were studied during storage at set integrate. The first-order kinetic model was found to be the best fit for the browning index, antioxidant activity, total polyphenols content and vitamin C, whereas the zero-order kinetic model was used to fit hardness values. Finally, **chapter 5**, provides final remarks and reflects on potentials and limits of the project. Main conclusions and a brief consideration on future perspective are also given.

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Chapter 2A

Effects of active sodium caseinate coating on minimally processed fennels quality during storage at different temperatures

Abstract

The aim of this work was to study the effect of an active coating based on sodium caseinate (SC) and propyl gallate (PG) on the quality of minimally processed fennel. To this end, a preliminary activity has been performed to select the best SC concentration to develop the active coating by evaluating the capacity of the coating to reduce the product respiration rate to 10 °C. Quality indices, including microbial load, weight loss, firmness, TA, TSS, pH, total colour change, total antioxidant capacity, total polyphenols and vitamin C content were measured at set intervals during storage. Results showed that the product respiration rate was reduced to a maximum of 20% by using the SC coating at 8%. The pH, TA and TSS did not change significantly during storage. The active coating did not reduce microbial growth, colour changes and weight loss, whereas it showed a protective effect on firmness changes and nutritional loss.

Keywords: Edible coating, propyl gallate, antioxidant coating, chemical physical properties, nutritional properties.

1. Introduction

Fennel (*Foeniculum vulgare* Mill. Subsp. *Vulgare* var. *azoricum*) belongs to the *Apiaceae* family and it is native to the Mediterranean region (Azeez *et al.*, 2002). It is mainly cultivated in Italy, Spain, and France, nevertheless, Italy is one of the largest fennel producers. The fennel edible part is the swollen basal part called "grumolo", normally known as "bulb" and can be eaten as a fresh product in salads or cooked. Due to its fleshy, crispy texture and aromatic flavour is particularly appreciated by consumers of South Europe. Moreover, thanks to its low energy content of only 9 kcal 100 g^{-1}, the high amount of fibre, and mineral salt content, in particular potassium (394 mg 100 g^{-1} fresh product), and vitamin C (12 mg 100 g^{-1} fresh product), fennels are an ideal food for a healthy diet To increase its consumption and diffusion, minimally processed operation and modified atmosphere packaging can be used to increase the product convenience and shelf life (Artés et al., 2002a; Capotorto et al., 2018; Escalona et al., 2005). However, the shelf life of minimally processed (MP) fennels is still limited.

Biopolymer coatings are a promising preservation technology that allows extending of the shelf-life of minimally processed vegetables (MPV), slowing down their quality decay through the reduction of gas, moisture or aromas transfer from or to the environment (Ahvenainen 1996; Baldwin et al., 1995; Yousuf et al., 2018; Volpe et al., 2019; Khan et al., 2021). In addition, coatings can be used as carriers of active additives that can contribute to improving the food nutritional quality of MPV and extend its (Baldwin et al., 1995; Hua Han et al., 2005; Valentino et al., 2020).

Casein-based coatings have shown promising results in extending the shelf life of fresh-cut fruits and vegetables related to their excellent gas barrier characteristics and good mechanical properties (Khan et al., 2021). However, no previous works are reported on the effect of edible coatings on the quality preservation of MP fennel. Caseinate coatings at different concentrations have been applied on MP fennel to estimate the coating thickness as a function of the solid concentration. Results showed that the dry coating thickness ranged between 0,7 to 6,4 μm for SC samples between 4% and 14%, respectively (Valentino et al., 2020). Moreover, results showed that sodium caseinate was a good substrate for the dispersion of antioxidant compounds as gallic acid showed good antioxidant capacity against DPPH or ABTS analysis (Valentino et al., 2020). However, gallic acid is not a safe antioxidant for food products; therefore, propyl gallate ($C_{10}H_{12}O_5$), a generally recognized safe antioxidant (GRAS), could be used as an alternative. Thus, the objective of this work was to study the effect of the active coating based on sodium caseinate and propyl gallate (SC+PG) on the quality preservation of minimally processed fennel. A second side objective was to select the physico-chemical quality indices critical for the product shelf life. To this end, a preliminary activity has been performed to select the best sodium caseinate concentration to develop the active coating by evaluating the capacity

of the coating to reduce the product respiration rate at 10 °C. Then, the effect of sodium caseinate active coating on the quality of minimally processed fennel during storage at 4 °C, 10 °C and 15 °C for 15, 12 and 8 days has been evaluated. Quality was monitored by measuring physical-chemical (weight loss %, colour, pH, °Brix, TA, firmness) and nutrition (antioxidant capacity, total polyphenol and vitamin C content) indices, defining microbial loads and microbiota dynamics.

2. Materials and Methods

2.1 Materials

Sodium caseinate from bovine milk, glycerol, propyl gallate, sodium hydroxide, riboflavin, sodium bicarbonate, potassium buffer, glacial acetic acid, methanol, Folin-Ciucalteu, and DPPH reagent were purchased from Sigma-Aldrich (Milan, Italy). Fennels were supplied by Commerciale Export Company (Pagani, Italy).

2.2 Methods

2.2.1 Coating solution preparation

Sodium caseinate (SC) solutions with protein concentrations at 8%, 10%, and 12% (g mL^{-1}) were obtained by dispersing SC powder in deionized water and stirring continuously for 4 h at room temperature as reported by Valentino et al. (2020). Glycerol (Gly) was added as a plasticizer to obtain a Gly/SC weight ratio of 0.1. To obtain active coating, propyl gallate at 0.13 mg mL^{-1} was also added after the solubilisation of sodium caseinate at room temperature.

2.2.2 Minimally processed fennel preparation

Fennels (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv *Augusto* and *Tiziano*) were cultivated in Puglia and Calabria. Samples (about 400-500 g each) were preliminary processed by Commerciale Export cutting the external part of the product with a sharp knife. Then, the fennels were transported to the laboratory of the Agricultural Science Department of the University of Napoli Federico II and they were stored for 24 h at 4°C before processing. Fennel bulbs were cut by using a sharp knife to eliminate the bottom and top parts, washed under tap water and dried with a tissue. The fennel samples were divided into three batches. Two batches were further processed to obtain the coated fennels with and without the PG: each fennel bulb was dipped by hand into the SC solutions (at different concentrations or with PG) (500 mL) for 2 min and then quickly withdrawn and drained on metallic grids over the glass vessel containing the solution (Valentino et al., 2020). The third batch of uncoated fennels was used as control. Fennels were dried at 30°C and 50% relative humidity (RH) for one hour in a circulating air system chamber (MMM Medcenter Einrichtungen GmbH, Munich, Germany) before characterization or packaging.

2.2.3 Respiration rate measurement

The O_2 consumption (R_{O2}) and CO_2 production (R_{CO2}) rates of minimally processed fennels uncoated

(control) and coated with sodium caseinate solutions at different concentration (8%, 10%, 12%) was measured at 10°C using a modified closed system (Torrieri et al., 2009). The products (about 0.5 kg) were placed in steel jars (4000 mL) and conditioned at test temperature for two hours under a constant air flux (1.6 $mL \cdot s^{-1}$) previously humidified. The temperature and relative humidity inside the jar were monitored using a data logger (MINI TH Giorgio Bormac S.r.l., Modena, Italy). After equilibrium, the inlet and outlet valves were closed and the gas composition was monitored over time with an O_2/CO_2 gas analyzer (accuracy of 0.5%), equipped with a needle (Check Mate 9900 O_2/CO_2; Ringsted, Denmark). The experimental time was 48h. At constant time intervals (Δt), 3 mL of the gas mixture was drawn from the jar headspace and analyzed using the gas analyzer.

The free volume (Vf) inside the jar was calculated by using Eq. (1):

$$Vf = V - \frac{W}{\rho} \qquad \textit{Eq. (1)}$$

where V is the volume of the jar (mL), W is the weight of the fennel (kg), and ρ the apparent density of the fennel (760 $kg \cdot m^{-3}$).

R_{O2} and R_{CO2} and respiration quotient, RQ, was calculated as reported by Torrieri et al. (2010) and expressed as mol kg^{-1} s^{-1}.

2.2.4 Packaging and storage conditions

Fennel samples (about 1 kg, 2 for each package) coated with SC at 8% (SC) or with SC + PG, and without the SC coating (control) were packed in polypropylene trays (26x16.5x10 cm) and wrapped with LDPE film. All samples were stored at 4°C, 10°C and 15°C for a maximum of 15 days. At different storage times, microbiota analyses (culture-dependent and -independent approaches), physical-chemical (weight loss, colour, pH, titrable acidity, total soluble solids and firmness) and nutrition (antioxidant capacity, total polyphenol and vitamin C content) properties were evaluated as following described.

2.2.5 Microbial counts

At each sampling point (0, 7 and 15 days), a portion of fennels stored at 4 °C (about 50 g) was diluted 1:10 in quarter-strength Ringer's solution (Oxoid, Basingstoke, UK) and homogenized in a Stomacher (2 min at 250 rpm). Serial decimal dilutions were plated on different media: PCA (Plate Count Agar, Oxoid) to determine the total psychrotrophic aerobic counts, incubated at 20°C for 48 h; Lactic Acid Bacteria counted on MRS agar (Oxoid) incubated for 48 h at 30°C; *Enterobacteriaceae* counted on VRBGA (Violet Red Bile Glucose Agar, Oxoid) incubated at 30°C for 24-48 h*; Escherichia coli* counted on TBX (Tryptone Bile X-GLUC, HiMedia, Mumbai, India) incubated at 42 °C for 48 h; *Pseudomonas* spp., counted on PSA (*Pseudomonas* Agar Base added with 1 vial/500ml of *Pseudomonas* cetrimide-fucidin-cephalosporin selective supplement, both from

Oxoid), incubated at 20°C for 48 h; yeasts and moulds on DRBC agar (Dichloran Rose Bengal Chloramphenicol, Oxoid) supplemented with 100 mg/L of chloramphenicol, incubated at 28 °C for 5 days. Results were expressed as Colony Forming Units (CFU)/g.

2.2.6 Microbiota dynamics by culture-independent, high-throughput sequencing

At the same sampling point, fennels were washed with Ringer's buffer and microorganisms were detached from the surface by shaking. The solution containing the microorganisms was then collected and centrifuged at 13,000 g for 2 min. Cell pellets were collected and used for DNA extraction, carried out by using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The V3-V4 region of the 16S rRNA gene (about 460 bp) was amplified using primer S-D-Bact-0341F/S-D-Bact-0785R and conditions previously reported (Berni Canani et al., 2017). Amplicons were independently barcoded and then pooled in an equimolar pool using the Microlab STARlet workstation (Hamilton) according to the Illumina metagenomic sequencing library preparation protocol. Equimolar pool of amplicons was sequenced on a MiSeq platform, yielding to 250 bp, paired-end reads.

Reads were imported into QIIME 2 (q2cli version 2020.11.1; Bolyen et al., 2019). Primers were trimmed and sequences were quality checked, denoised and merged through the plugin 'dada2', using parameters "--p-chimera-method pooled", "--p-pooling-method pseudo", "--p-min-fold-parent-over-abundance 10" and "--p-max-ee 2". Representative sequences were mapped against the Greengenes 13_8 database (McDonald et al., 2012). Taxonomic assignment was carried out with the 'feature-classify' plugin ('classify-consensus-vsearch' method). Amplicon Sequence Variant (ASV) table was collapsed at the genus level. Chloroplast contamination was removed from the ASV tables, and the relative abundance of other taxa was recalculated. Statistical analyses and plotting were carried out in R environment (http://www.r-project.org).

2.2.7 Physical-chemical properties

The weight loss of fennels during storage was determined by using a gravimetric method. Fennel samples were weighed before and after different storage times by using a balance (accurate to 0.01 g) (Mark Ben 3000, Monza, Italia). The weight loss was calculated as:

$$\left(\frac{wi-wf}{wi}\right)*100 \qquad \textit{Eq. (9)}$$

where *wi* was the initial weight of the fennel and *wf* was the fennel weight after storage and expressed as a percentage.

The colour of the fennels was determined with an electronic eye (visual analyser VA400 IRIS, Alpha MOS, France) equipped with a CCD camera (resolution 2592×1944 pixels and 24 bits). The camera was equipped with a 25 mm f1:2.2 Basler lens by Fujion and it was mounted in a light box equipped

with top and bottom lightning (each position using 4 x 4 White LED) which was stabilized for 15 min before use (Tretola et al., 2017). Raw images were processed in RGB scale and subsequently converted in Cie L* a* b* scale using Alphasoft software (version 16.0). For each image, the white background was automatically removed and the L*, a* and b* measured in the different parts of the fennel sample (bottom, top and side) were averaged (Cevoli et al., 2023). Total colour change (ΔE) was also calculated (ASTM E1910), using the chromatic coordinates of the fresh samples as reference:

$$\Delta E = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b^2)]} \qquad \textit{Eq. (10)}$$

Firmness (N) was measured at four opposite points per bulb in the equatorial zone using a compression test with a TMS-Pro texture analyzer (Food Technology Corporation) by using a 6.3 mm diameter plate and with load-bearing cells (500 N). Firmness has been calculated as the maximum force required to compress the sample up to a depth of 8mm. Tests were run with a crosshead speed of 25 mm/min (Artés et al., 2002a). Data were acquired through Texture Lab Pro Software.

The titratable acidity (TA), pH, and total soluble solids (TSS) were measured by using the fennel juice; it was extracted using 200 g of fennel tissue in the centrifuge for domestic use (Kenwood, AT 320 A). 10 mL juice was used to measure the TA by titration with 0.01 N NaOH to an endpoint at pH 8.1 (AOAC 1984) and expressed as g of oxalic acid·L^{-1} (Escalona et al., 2005). For pH, 10 mL of fennel juice was used and this parameter was monitored through a pH meter (Eutech Instruments Pte Ltd., Singapore). A few drops of the fennel juice obtained were used to measure TSS content (°Brix) with a digital refractometer (Atago PR32- Palette, Tokyo, Japan). Six measurements were carried out on each sample.

2.2.8 Nutritional quality

The total antioxidant capacity (TAC) was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Volpe et al. (2019), with slight modifications. 0.5 g of freeze-dried sample was added in 10 mL of methanol/water (80:20) solution, mixed with constant shaking at a temperature room for 60 minutes, and put on an ultrasound bath for 30 minutes. The sample was centrifuged (Hermle Z 326 K, Germany, European Union) at 10,000 rpm for 15 minutes (Pérez-Jiménez et al., 2008). The pellet was discarded and the supernatant was retained and mixed (100 μL) with 4.9 mL of DPPH solution (methanol+DPPH 0.1 Mm) to initiate the reaction. The absorbance was read using a spectrophotometer UV-VIS (UV-550 Jasco, Japan) at 515 nm after 30 minutes of incubation at room temperature in the dark. TAC was expressed as mg of Trolox equivalents g^{-1} of dry matter (mg TE g_{dm}^{-1}) using a Trolox standard curve (0–625 mg mL^{-1}). The TAC value was normalized concerning the TAC value of the fresh sample.

To measure the total phenolic content (TPC), 0.5g of freeze-dried sample was crushed with mortar and pestle with 10 mL of sodium bicarbonate (6%); the solution was filtered through a paper filter and 0.5 mL of the filtrate was added with 2.5mL of Folin-Ciocalteau reagent and 2 mL of sodium bicarbonate. The samples were incubated for 1h at 35°C and then for 1h at 6 °C. After 2 h of incubation in the dark, the absorbance was read at 760 nm against a blank (2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium bicarbonate), using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). The TPC was calculated based on the calibration curves of gallic acid (0-8 mg mL^{-1}) and expressed as mg of gallic acid equivalents g^{-1} of dry matter (mg GAE g_{dm}^{-1}). TPC values were normalized respect to the TPC value of the fresh sample.

Vitamin C of fennels was extracted by homogenizing 1 g of product tissue with 10 mL of glacial acetic acid solution in water (8%) for 1 min by using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA). The homogenate was centrifugated at 7000 rpm for 7 minutes. The sample was filtered through a paper filter, and the supernatant was collected. Then, 5 mL of glacial acetic acid solution was added to the pellet and centrifugated at 7000 rpm for 7 minutes. This procedure was replicated four times for vitamin C extraction. Then, the method reported by Jung et al. (1994), with minor modifications, was used to determine vitamin C content in MP fennels. 1 mL of sample filtered was added in 4 mL of riboflavin stock solution, and 0.06g riboflavin in 100 mL 0.01 M potassium buffer (pH=7.5). Absorbances of samples before and after light storage (5500 Lux) were measured at 265 nm using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). Differences in absorbance of samples before and after 15 min-illumination were used for the calculation of ascorbic acid. The vitamin C content was calculated based on the calibration curves of ascorbic acid in the buffer of riboflavin (0-10.5 μg mL^{-1}) and expressed as mg of vitamin C g^{-1} of dry matter (mg of Vit C g_{dm}^{-1}). Vit C values were normalized respect to the Vit C value of the fresh sample.

2.3 Statistical analysis

The results are reported as the average of replications of each sample ± standard deviation.

The effect of SC concentration on respiration rate was studied by one-way ANOVA analysis. Duncan's test was carried out to find the source of the significant differences within the samples examined.

Multivariate ANOVA analysis has been carried out to evaluate the effect of the independent parameters storage time (t), temperature (T) and coating treatment (C) and its interaction on physical, chemical and nutritional quality indices of MP fennel with a full factorial experimental. Three levels of treatments (control, SC, SC + PG), 5 levels of times (0, 5, 8, 12 and 15 days) at 4°C, 4 levels of time (0, 5, 8 and 12 days) at 10°C and 4 levels of time (0, 2, 5 and 7 days) at 15°C were studied. Duncan's test was carried out to find the source of the significant differences within the samples

examined. Significant differences were defined at $p \leq 0.05$. Data were analyzed using SPSS software (SPSS Inc. 28.0, Chicago, IL, USA, 2022).

Data Availability Statement

The raw sequence reads generated in this study have been deposited in the Sequence Read Archive (SRA) of the NCBI under accession number PRJNA835434.

3. Results and Discussions

3.1 Respiration rate measurement

The edible coating can modify the internal atmosphere of coated fruits or vegetables due to its semipermeable oxygen and carbon dioxide barrier properties (Miele et al., 2022) hence reducing respiration and delaying ripening and senescence processes (Tahir et al., 2019). The RR_{O2} of MP fennel ranges between $10.5 \pm 0.3 \times 10^{-6}$ mol kg^{-1} s^{-1} and $8.1 \pm 0.1 \times 10^{-6}$ mol kg^{-1} s^{-1} as a function of coating composition, whereas the RR_{CO2} ranges between $6.6 \pm 0.9 \times 10^{-6}$ mol kg^{-1} s^{-1} and $8.6 \pm 0.3 \times 10^{-6}$ mol kg^{-1} s^{-1}. RQ ranges between 0.7 and 0.9 for the samples with and without coatings (Table 1). In general normal RQ values in the literature are reported as ranging from 0.7 to 1.3 (Kader, 1987). ANOVA analysis showed that SC coatings affected significantly RR_{O2} and RR_{CO2} of MP fennels. Indeed, 8% SC was able to reduce a maximum of 20% of the respiration concerning control. On the other hand, the sample with 12% SC showed higher values of RR_{O2} ($10.5 \pm 0.3 \times 10^{-6}$ mol kg^{-1} s^{-1}) and RR_{CO2} ($8.5 \pm 0.3 \times 10^{-6}$ mol kg^{-1} s^{-1}) than other samples ($p \leq 0.05$). Similar results were reported by Lerdthanangkul & Krochta (1996) who showed that edible coating based on 8% of sodium caseinate was able to reduce the RR of bell peppers by about 10% than the control samples.

Table 1. Averages and standard deviations of respiration rate expressed as a rate of oxygen consumption (RR_{O2}) and carbon dioxide production (RR_{CO2}) in the air at 10°C.

	RR_{O2} mol*kg^{-1}s^{-1}	**RR_{CO2} mol*kg^{-1}s^{-1}**	**RQ**
Samples	**10^{-6}**		
Control	10.1±0.1c	6.6±0.9a	0.7
8% SC	8.1±0.1a	6.8±0.1a	0.8
10%SC	8.7±0.5b	7.4±0.3b	0.8
12%SC	10.5±0.3d	8.6±0.3c	0.9

Lowercase letters represent statistically different differences ($p \leq 0.05$) during storage time.

With these results on RR, the SC at 8% was chosen as the optimal SC concentration for producing the active coating with PG.

3.2 Packaging and storage conditions

3.2.1 Microbiota analysis by culture-dependent and -independent approaches

Microbial populations of fennels coated in a sodium caseinate coating with (SC+PG) or without (SC) the addition of propyl-gallate were evaluated by culture-dependent and –independent methods after 7 and 15 days of refrigerated storage (4°C). the use of coating or activated coating did not reduce

microbial growth. On the contrary, SC always showed counts similar to C for all the populations counted both at 7 and 15 days of storage, except for yeasts and moulds at 7 days, which were higher in SC. Moreover, the addition of PG to the coating (SC+PG) always led to higher counts compared with controls for all the microbial populations (Table 2).

Microbiota dynamics were evaluated by 16S rRNA sequencing. Pseudomonas relative abundance increased during storage in all the samples, but it represented > 70% of the microbial community in fennels coated in active coating (Figure 1). In control and CS samples, several *Enterobacteriaceae* taxa were found, including *Pantoea, Rahnella, Serratia* and other unidentified species. The results suggest an impact of propyl gallate in modulating the microbiota composition, inhibiting some Enterobacteria species, thus boosting the increase in *Pseudomonas* spp., in agreement with culture-based results.

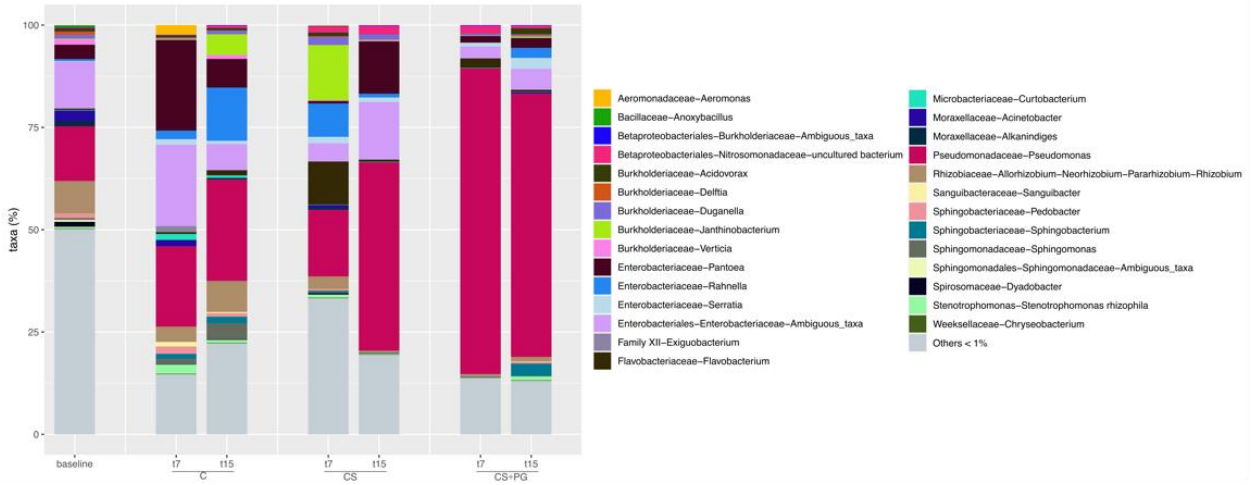

Figure 1. Stacked bar plot showing the mean relative abundance of bacterial taxa in the fennel samples analysed. Only taxa with abundance > 1% in at least one group are shown.

Table 2. Microbial counts were monitored during the storage of control fennels and fennels covered with SC or SC + PG.

Storage time (at 4 °C)	**0 d**	**7 d**			**15 d**		
		C	**SC**	**SC+PG**	**C**	**SC**	**SC+PG**
TMC (20 °C)	4.52	6.20^{a}	6.40^{a}	7.39^{b}	7.27^{a}	7.17^{a}	8.47^{b}
Enterobacteriaceae	1.33	3.00^{a}	2.94^{a}	5.40^{b}	5.04^{a}	4.58^{a}	5.63^{a}
LAB	3.00	<1^{a}	<1^{a}	<1^{a}	<1^{a}	<1^{a}	1.30^{b}
Pseudomonas	4.30	6.22^{a}	6.55^{a}	7.76^{b}	7.08^{a}	7.36^{a}	8.44^{b}
Escherichia coli	<1	<1	<1	<1	<1	<1	<1
Yeasts and moulds	3.21	3.62^{a}	4.54^{b}	5.89^{c}	4.62^{a}	4.35^{a}	5.96^{b}

TMC, Total Microbial Counts at 20 °C; LAB, Lactic Acid Bacteria. The data are mean of 3 replicate samples and are reported as Log CFU/g. Statistical Significance: different letters in the same row indicate significant differences among the three treatments at each sampling time (C, SC, SC + PG; paired t-tests, $p \leq 0.05$).

3.2.2 Physical-chemical properties

Different storage conditions can influence the senescence of MP product, indeed small quantities of organic acids in plants (e.g., in the tricarboxylic acid or Krebs cycle, glyoxylate cycle, or shikimic acid pathway) change and they can monitor using TA and pH as control parameters.

The results of ANOVA analyses showed that the independent factors of time, temperatures and treatments did not significantly affect the chemical indices pH, TA, and TSS, thus in Table 3 the average values are reported.

These results are in agreement with different studies conducted on minimally processed fennels in which is reported that pH, TA and TSS did not change as a function of process parameters (Capotorto et al., 2018) or storage condition (Artés et al., 2002a; Escalona et al., 2005).

Weight loss of MP fennels increased during storage time up to a maximum of 2% after 15 days at 4 °C (Table 2). The effect of the coating on the weight loss was not statistically significant ($p \leq 0.05$), whereas the storage time and temperature significantly affected the weight loss ($p \leq 0.05$). Thus, in Table 3 data are reported as the average of treatments for each time and each temperature. From a commercial point of view, a weight loss between 5% to 10% is considered critical for the acceptability of the product (Volpe et al., 2018; Ben-Yeoshua &Rodov 2003). Thus, weight loss is not a critical quality parameter for the shelf life of MP fennel packed in a specific condition.

For the colour, L, a*, b* and ΔE of the bottom, top and side were used to study the differences between treatments, time and temperature (Table 2). L values of MP fennels decreased by about 10% over time with a significant difference ($p \leq 0.05$) for all samples. The a* values remained always negative for all the samples, however, a gradual increment of the parameter was observed after 2 days of storage (Table 2). In particular, the parameter a* of the bottom part of MP fennels significantly increased ($p \leq 0.05$)by about 50%, 80% and 65% after 15, 12 and 8 days of storage at 4, 10 and 15 °C, respectively. In contrast, ANOVA analysis showed that storage time did not significantly affect the parameter a* of the top and side parts of MP fennels. The b* parameter increased for all the samples during storage time by about 15%, as reported in Table 3. ANOVA showed that the treatment did not affect the b* parameter ($p \leq 0.05$).

Table 3. Weight loss, pH, TA, °Brix and colour (bottom, side and top part) (average ±SD) of MP fennel stored for 15 days at different temperatures.

		T (°C)		Time (days)						df	F	Sign. (p≤0.05)
	Variables			0	2	5	8	12	15			
Treatments*time*temperature	weight loss	4		0±0a		0.9±0.1b	1.40±0.2c	1.77±0.3d	2±0.4e	9	2.3	0.02
		10		0±0a		0.9±0.1b	1.39±0.2c	1.81±0.3d				
		15		0±0a	0.5±0.2b		1.5±0.2c					
	pH			6.2±0.1						9	1.9	0.07
	TA			0.45±0.1						9	0.4	0.91
	TSS			5.0±0.4						9	0.3	0.95
	L	4	bottom	81±2c		77±2bc	72±3ab	71±4ab	68±3a	9	4.3	<0.001
		10		81±2b		74±5ab	72±5b	72±5b				
		15		81±2b	73±3ab	75±3a	74±4a					
	a*	4		-4±1a		-2±1b	-2±1b	-2±1b	-2±1b	9	1.8	0.3
		10		-4±1a		-1±1b	-1±1b	-1±1b				
		15		-4±1a	-4±1a	-3±1a	-1±1b					
	b*	4		29±2a		30±2ab	31±2abc	34±1c	33±2bc	9	5	<0.001
		10		29±2a		31±1ab	30±1ab	33±3b				
		15		29±2a	31±1ab	32±1b	31±2ab					
	ΔE	4		0a		7±2b	12±3bc	14±4bc	16±5c	9	4.3	<0.001
		10		0a		11±4b	12±3b	13±3b				
		15		0a	6±3b	6±3b	8±2b					
	L	4	top	67±2a		70±3a	64±3a	62±5a	60±5a	9	1.1	0.34
		10		67±2a		62±3a	62±4a	61±5a				
		15		67±2a	64±5a	63±5a	59±6a					
	a*	4		-5±1a		-3±1a	-3±0a	-2±3a	-2±3a	9	3.3	0.002
		10		-5±1a		-1±3b	-1±1ab	-1±2ab				
		15		-5±1a	-4±1a	-3±3ab	-1±1b					
	b*	4		29±2a		32±1a	29±0a	30±4a	30±4a	9	3.1	0.003
		10		29±2a		27±4a	30±2a	31±4a				
		15		29±2a	31±3a	29±3a	30±1a					
	ΔE	4		0a		6±1ab	10±4ab	12±4b	14±4b	9	2.1	0.04
		10		0a		8±3b	9±3b	11±3b				
		15		0a	8±3b	8±2b	10±3b					
	L	4	side	77±2bc		78±3c	71±3abc	71±2ab	67±5a	9	3.1	0.003
		10		77±2b		72±2a	72±3a	70±3a				
		15		77±2a	72±5a	72±4a	71±6a					
	a*	4		-5±1a		-4±0a	-4±0a	-5±0.2a	-4±2a	9	2.6	0.01
		10		-5±1a		-2±0c	-4±1b	-3±1bc				
		15		-5±1a	-5±2a	-5±1a	-3±1b					
	b*	4		29±2a		30±1ab	29±2b	32±2b	31±2ab	9	1.2	0.31
		10		29±2a		29±1a	30±2a	31±3a				
		15		29±2a	30±1a	30±1a	30±1a					
	ΔE	4		0a		4±1ab	10±5ab	11±5b	14±5b	9	1.8	0.07

10	0a		7±2b	7±2b	9±3b
15	0a	3±1b	4±0b	5±1c	

Lowercase letters represent statistically different differences ($p \leq 0.05$) during storage time.

ΔE parameter of the bottom, top and side parts of the MP fennels increased for all the samples. In the specific, the ΔE of the bottom part increased during storage time up to 16% after 15 days at 4 °C, 14% after 12 days at 10 °C and 8% after 8 days at 15 °C. However, the ΔE parameter of samples stored for two days of storage was statistically different to the value of the fresh samples ($p \leq 0.05$). Also for the top and side parts, ΔE increased during storage time. In particular, ΔE of the side part increased by 15%, 9% and 5% at 4, 10 and 15 °C respectively after 15, 12 and 8 days, whereas average values of ΔE top increased by about 13% for all the samples. ΔE parameter has been used as a global indicator of colour changes and its variation was correlated to consumer acceptability: if $0 < \Delta E < 1$ the observer does not notice the difference, and $1 < \Delta E < 2$ only the experienced observer can notice the difference, $2 < \Delta E < 3.5$ even the inexperienced observer notices the difference, $3.5 < \Delta E < 5$ a clear colour difference is noticed, $5 < \Delta E$ the observer notices two different colours (Mokrzycki & Tatol 2012).

The colour changes observed on MP fennel are mainly caused by the oxidation of phenolics and quinones, catalyzed by oxidative enzymes, including polyphenol oxidase (PPO) and peroxidases (POD). Quinones then polymerize to form dark pigments, leading to a browning appearance (Garcia & Barret 2002, Volpe et al., 2019). Browning may be prevented by inhibiting the activity of PPO by removing one of its necessary reaction components, O_2, enzyme, Cu^{2+} contained on its active site, or substrate (Richardson & Hyslop, 1985; Lambrecht, 1995), or by mechanical or chemical methods (Garcia & Barret, 2002). Usually, the coating can act as a barrier of oxygen, reducing the oxidation process. However, our results showed that the SC coating was not able to control the alteration process and reduce oxidation.

Figure 2 reported the values of firmness of the MP fennels stored at 4 °C (A), 10°C (B) and 15 °C (C). Firmness can decrease due to several chemical and physical cell structure changes such as loss in turgor pressure caused by cell membrane disruption or changes in the pectin substances (Du et al., 2022). This physical parameter decreased by about 25% during storage time for coated and control samples stored at different temperatures. ANOVA analysis showed that storage time, temperature, treatments and their interactions have a significant effect on firmness ($p \leq 0.05$). In the specific, coated samples showed a higher value of firmness with respect to control samples after 12, 8 and 2 days of storage at 4, 10 and 15 °C, respectively. Also, Lerdthanangkul & Krochta (1996) reported that 8% SC applied on bell peppers preserved the firmness loss with respect to uncoated samples during the

time.

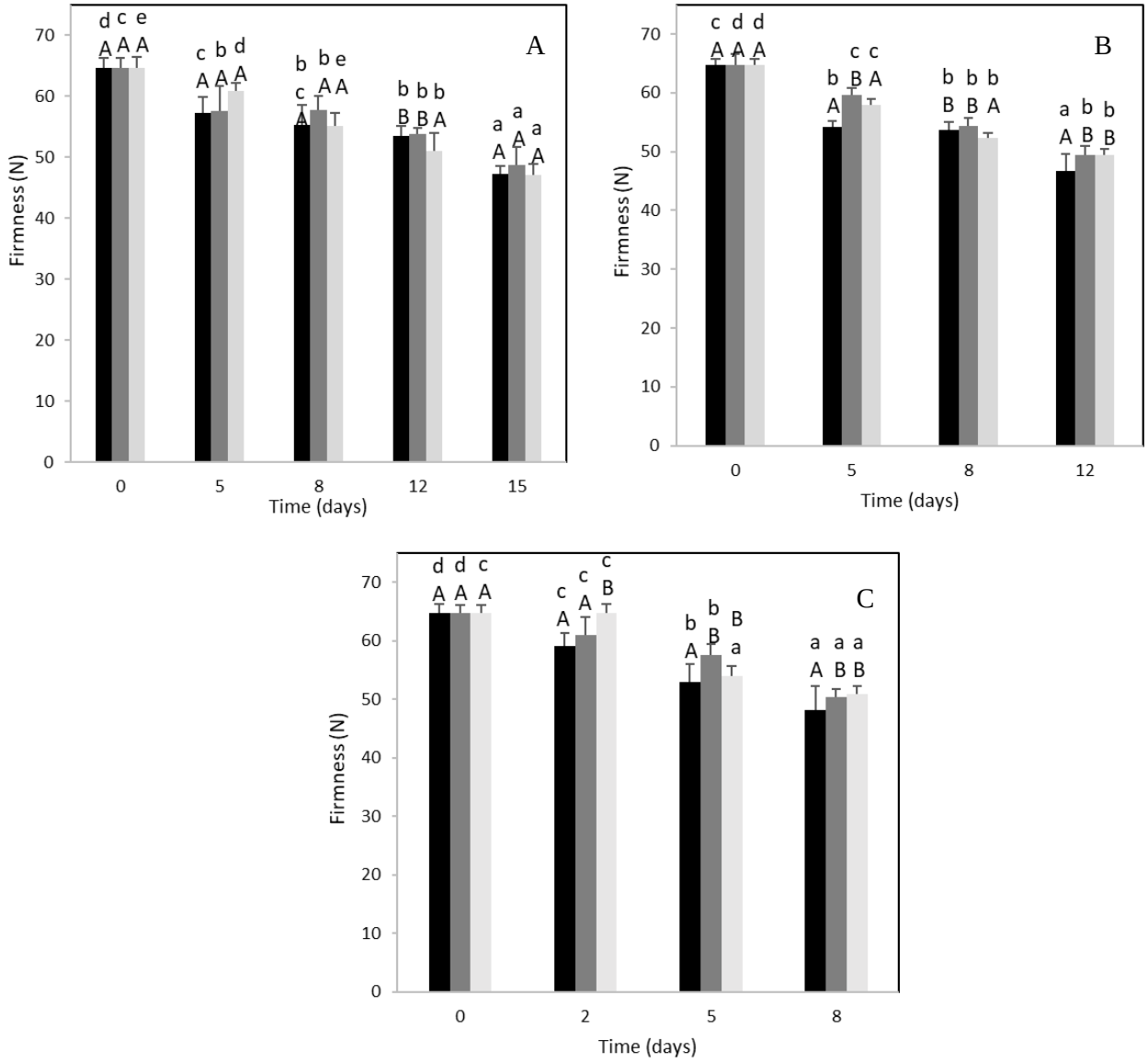

Figure 2. Firmness of MP fennels stored at 4°C for 15 days (A) at 10°C for 12 days (B) and at 15°C after 8 days (C) of control (■) 8%CAS (■) and 8%CAS+PG (■). Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

3.2.2 Nutrition properties of fresh fennel

Figures 3, 4 and 5 show the results of the TAC, TPC and vitamin C content, respectively.

Storage time, coating and temperature had a significant effect on TAC with significant interaction between factors ($p \leq 0.01$). During storage time it decreased for all treatments and temperatures. In Figure 3 are reported values of the total antioxidant capacity of MP fennels stored at 4°C (A), 10 °C (B) and 15 °C (C). For control samples, TAC decreased from 50 to 80 % during storage as a function

of temperature. SC coating had a negative impact on the TAC decrease of MP fennel during storage at 4°C. Whereas, at 10°C and 15°C, SC samples showed a behaviour similar to SC+PG samples. Nevertheless, the presence of the SC+PG coating preserved the slowing of TAC of MP fennel of a maximum of 30% after 15 days at 4 °C. With the exception of the samples stored at 10°C, the quantity of TAC for MP-coated samples is always 50% higher than for control samples. For samples stored at 10 °C, SC+PG samples showed always a higher content of TAC with respect to control samples, with a maximum difference of 30% after 5 days of storage.

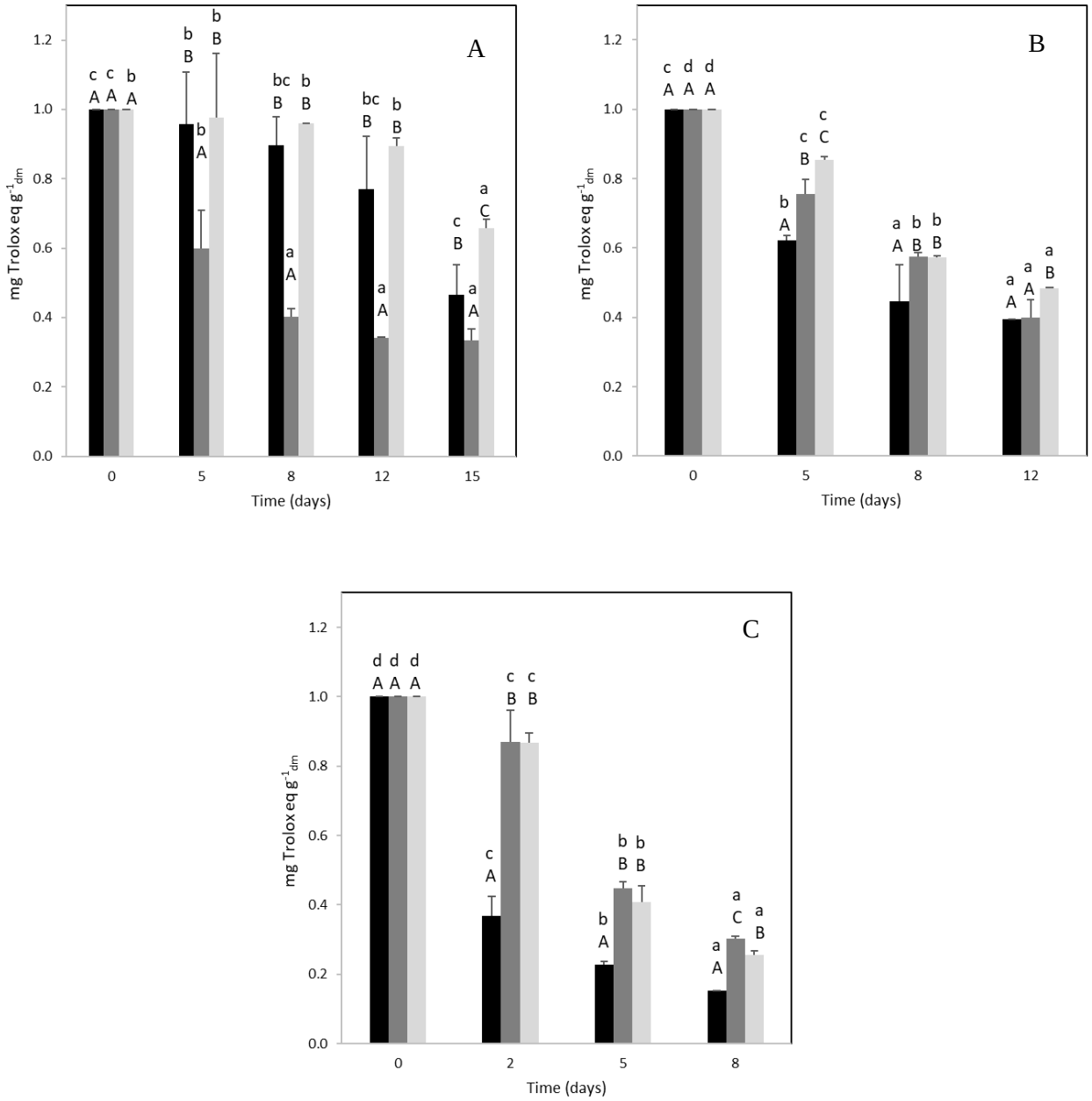

Figure 3. Antioxidant capacity of MP fennels stored at 4°C for 15 days (A) 10°C for 12 days (B) and 15°C for 8 days (C) of control (■) 8% SC (■) and 8% SC+PG (■). Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

Several studies have highlighted that the presence of antioxidant compounds dissolved in the edible coating, namely polyphenols associated with dietary fibres derived from oranges and apples, preserved the TAC of the product (Figuerola et al., 2005, Marín et al., 2007; de Moraes Crizel et al., 2013). The results of this work confirm the results previously obtained by Valentino et al., (2020). In particular, SC+PG coating acts as a good antioxidant and reduces the loss of nutritional properties of MP fennel. Moreover, similar results were obtained when a coating based on SC and chitosan has been applied to MP apples (Volpe et al., 2019).

In Figure 4 are reported values of total polyphenols content in MP fennels stored at 4°C (A), 10 °C (B) and 15 °C (C). ANOVA analysis showed that the storage time ($p \leq 0.05$), temperature ($p \leq 0.05$) and treatment ($p \leq 0.05$) had a significant effect on the TPC of MP fennel. Total polyphenols content increased during storage time, due to sample cutting and, thus, by the activation of polyphenol oxidase. Moreover, SC+PG coating preserved the increase in total polyphenol content of 10% after 15 days at 4 °C, 17% after 12 and 8 days at 10 °C and 15 °C respectively, with statistically significant differences ($p \leq 0.05$) with respect to control samples. Also, coating with only SC 8 % preserved the TPC of MP fennel about 8% after 15 days with respect to control for all temperatures, with statistically significant differences after the second days of storage ($p \leq 0.05$).

Physiological maturity plays a key factor in influencing the level of phytochemicals. There are little informations about phenolic content evolution during growth in vegetables and results are controversial (Tiwari & Cummins, 2013). Quinic acid could influence phenolic content in vegetables because it is an efficient precursor of aromatic amino acids: in plants, it can be converted into shikimic acid which can be further metabolized to aromatic biosynthesis (Minamikawa, 1976). Indeed, shikimic acid is an intermediate compound in the pathway for the biosynthesis of L-phenylalanine that is utilized in the phenylpropanoid metabolism to synthesize phenolic compounds (Cisneros-Zevallos et al., 2014). These results are in agreement with Capotorto et al., (2018), that they had studied the effect of an anti-browning solution applied on freshly cut fennel stored at 4 °C for 6 days. However, TPC increased during the time also for other vegetables, for example, in “Cool Guard” lettuce harvested at three stages of maturity phenolics were higher in the immature and mature stages (0.13 and 0.14 mg g^{-1} respectively) than in the over-mature stage (0.11 mg g^{-1}) (Couture et al., 1993; Chutichudet et al. 2011). Moreover, Pandjaitan et al., (2005) found the highest level of total phenolics as well as total flavonoids in the middle leaves of spinach plants, suggesting that these compounds were synthesized in leaves at early stages of maturity, decreasing during the final maturity. The increase in TPC is due to the interaction of phenolic components with polyphenol oxidase (PPO) and peroxidase (POD) enzymes during fennel cutting, also causing of an increase in ΔE, as reported in the previous paragraph.

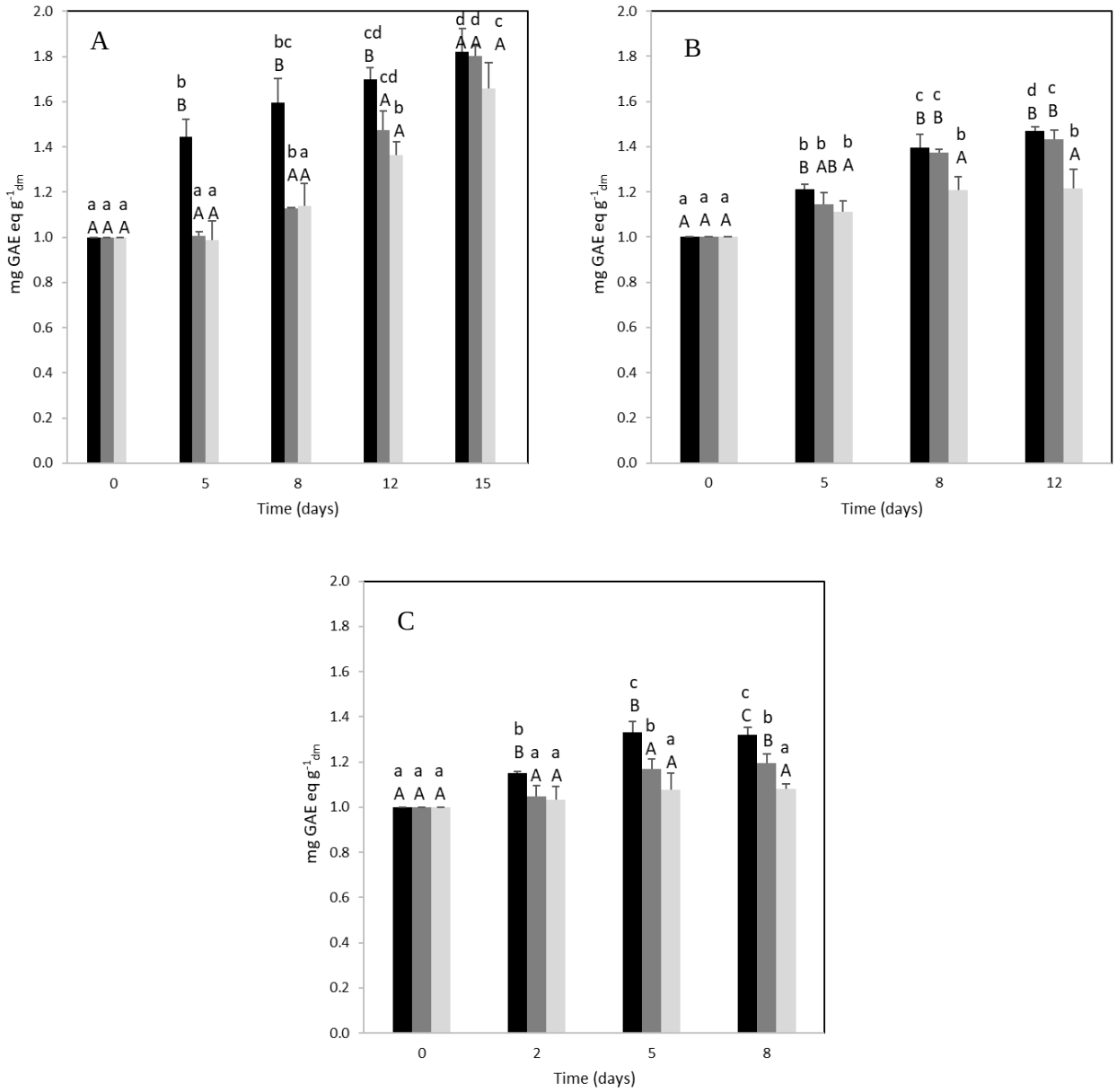

Figure 4. Total polyphenol content of MP fennels stored at 4°C for 15 days (A) 10°C for 12 days (B) and 15°C for 8 days (C) of control (■) 8% SC (■) and 8% SC + PG (■). Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

As reported for the TAC, also Vitamin C content of the samples stored at different temperatures with and without coating decreased during storage time, as reported in Figure 5. For control samples, vitamin C decreased by about 60% after 8, 12 and 15 days at 4, 10 and 15 °C with statistically significant differences ($p \leq 0.05$). Nevertheless, coating with the antioxidant compound preserved the slowing of vitamin C of MP fennel of 33% after 15 days at 4 °C, 40% after 12 days at 10 °C and 35% after 8 days at 15 °C. ANOVA analysis showed that statistically significant differences ($p \leq 0.05$) between coated and uncoated samples were only at time 8 days for samples at 4 and 10 °C and time

2 days ($p \leq 0.05$) for the samples stored at 15 °C.

The decrease in ascorbic acid (AA) depends on its oxidation in DHAA by the enzyme ascorbate oxidase (AAO) which has been proposed to be the major enzyme responsible for the enzymatic degradation of AA (Mehlhorn, 1990; Saari' et al., 1996). Ascorbate oxidase is associated with rapidly growing regions in the plant (Lee & Kader, 2000); therefore this enzyme, in general, is largely present in fresh fennel when the younger sheathes represented the higher portion of the total fennel sheathes, catalyzing the degradation of AA in DHAA. However, DHAA can be further degraded to diketogluconic acid (Parviainen and Nyyssonen, 1992).

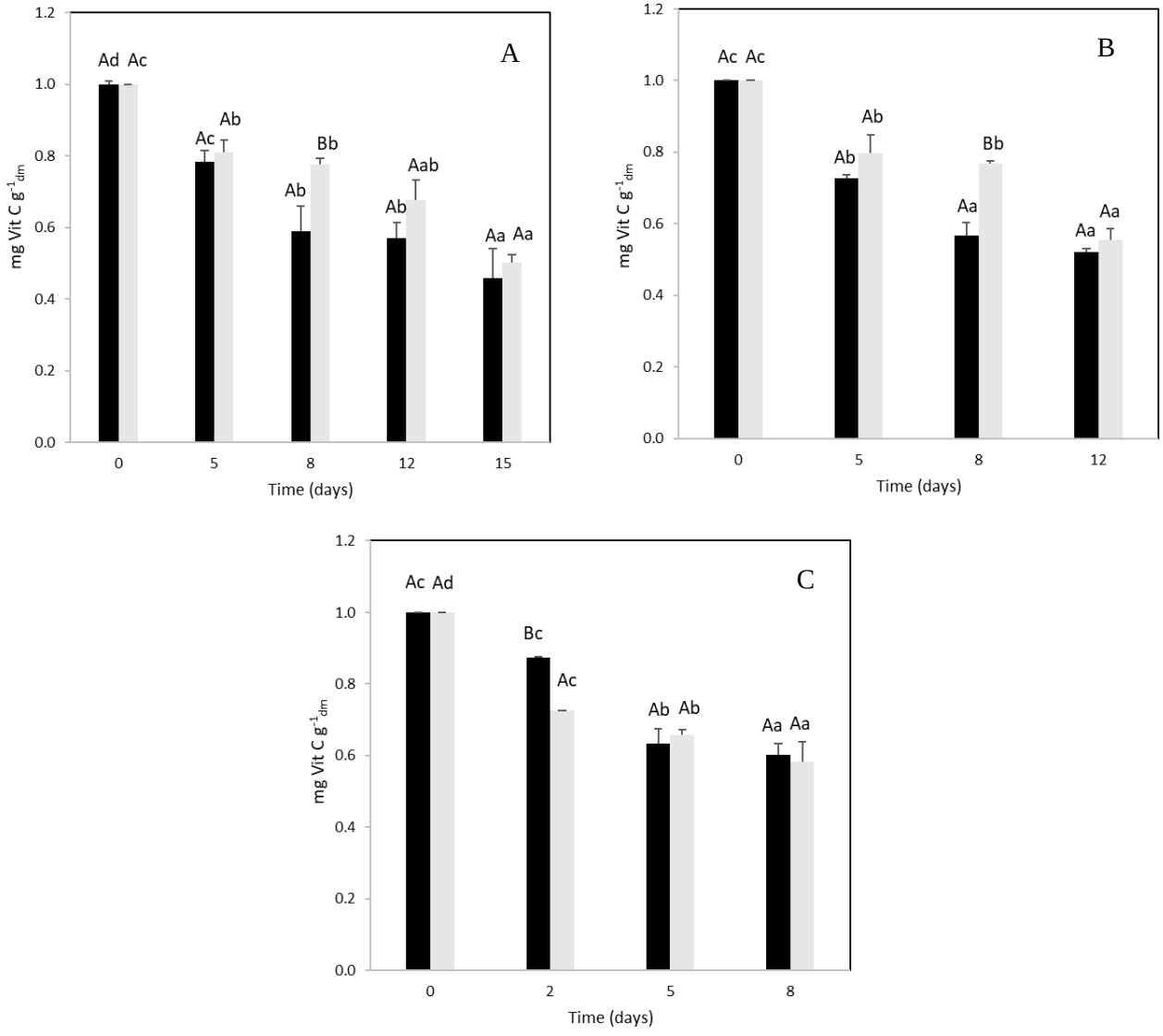

Figure 5. Vitamin C content of MP fennels stored at 4°C for 15 days (A) 10°C for 12 days (B) and 15°C for 8 days (C) of control (■) 8% and 8% SC+PG (■). Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

In accordance with the present data, Weston, (1997) reported in snap beans and other green vegetables that ascorbic acid tends to increase with maturation and decrease with advanced maturation. Similar behaviour of vitamin C was observed also by Capotorto et al., (2018) on fresh-cut fennels stored at 5 °C for 6 days, where ascorbic acid decreased for samples with and without an anti-browning solution.

4 Conclusions

Our study showed that SC at 8% was the best concentration to prepare active coatings based on SC and PG. The active coating (SC+PG) was not able to preserve the colour of the MP fennel and did not affect the weight loss and firmness loss of the product during storage. However, active coating preserved the nutritional quality of MP fennel, by reducing the decrement of antioxidant capacity by about 30%, of vitamin C by about 35%, and of TPC by about 10% compared with control samples. Moreover, the storage temperature affected the efficacy of the coating which showed better efficacy at 4°C and 10°C.

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Chapter 2 B

Mathematical model of critical quality indices of minimally processed fennel with and without active coating

Abstract

The objectives of this work were: (i) to describe the critical quality changes of MP fennel by kinetic modelling and (ii) to evaluate the effect of storage temperature and active coating on the alteration kinetics. The quality-change relationship between samples over time and temperature is mainly studied by firmness, total antioxidant capacity and vitamin C content. Pseudo zero and first order and Arrhenius type models well described the quality changes of the product over time in the range of temperatures tested. The active coating was able to preserve the nutritional quality of the products by reducing the antioxidant capacity decrease by 40 % and vitamin C reduction by 20 %. Samples with the active coating are less sensitive to temperature for ΔE (Ea 42 kJ/mol), antioxidant capacity (Ea 113 kJ/mol) and firmness (21 kJ/mol) than the control sample (Ea $_{\Delta E}$ 53, Ea $_{TAC}$ 130 and Ea $_{firmness}$ 46 kJ/mol). Different results were obtained for vitamin C, the control sample was less sensitive than the active sample, with a value of 10 and 24 kJ/mol respectively. In conclusion, the kinetic model is a useful tool for shelf-life prediction. Biopolymer coating impacts the alteration kinetics by preserving nutritional quality.

Keywords: Firmness, nutritional properties, pseudo zero order, pseudo first order, Arrhenius model

1. Introduction

To properly predict the quality change of a product during storage or the effect of an innovative process on food shelf life, is useful to follow a kinetic modelling approach based on the knowledge of the rate of alteration process and the relationships between kinetics parameters and factors activating or slowing down these processes. Understanding the fundamental processes is critical for quality modelling and quality control (Datta & Sablani, 2007).

Alteration reactions are mostly (bio) chemical and physical in nature. These changes occur at a certain rate and with specific kinetics. Kinetic modelling allows us to quantify these changes and their rates. In the literature, the theoretical aspects of bacterial growth and compound degradation are well-established and there is a large amount of information regarding the mathematical modelling (Rao et al., 1981; Burton 1954; Wilkinson et al., 1981; Lund 1977). For shelf-life modelling, empirical models based on kinetic models (zero-, first-, or second pseudo-order) are commonly used (Labuza, 1982; Zanoni et al., 2005, Nisha et al., 2005, Giannakourou & Taoukis, 2003; Nisha et al., 2005, Rodrigo et al., 2007). Several food degradation reactions, such as antioxidant changes (Oms-Oliu et al., 2009), microbial growth (Corradini & Peleg, 2007); vitamin C degradation (Corradini & Peleg, 2007) and browning (Manso et al., 2001) have been described by kinetic models.

Well-known are the mathematical models that describe the impact of environmental factors on the main alteration mechanisms. Storage temperature is one of the environmental factors that mainly affect the rate of the alteration reaction and Arrhenius type-equation is successfully used to predict the quality changes at different storage temperatures. On the other hand, the relationships between new preservation technologies (e.g. edible coatings) and decay kinetics are not well explained by the mathematical model. Although the benefits of these technologies are well known, the results are not always easily transferable.

The alteration mechanisms of minimally processed fennels during storage are mainly related to the senescence processes, microbial growth, enzymatic browning and oxidation processes which in turn affect the safety of the product and the sensory and nutritional properties (chapter 2). The critical quality indices related to sensory and nutritional properties were the colour, the firmness, the total antioxidant capacity, the total phenol content and the vitamin C content. Previous results (chapter 2) showed that an edible antioxidant coating based on sodium caseinate and propyl gallate was able to preserve the nutritional quality of minimally processed fennel at specific storage conditions. Thus, the aims of this work were i) to describe the critical quality indices of minimally processed fennel by kinetic modelling and (ii) to evaluate the effect of storage temperatures and active coating on alteration kinetics.

2. Materials and Methods

Fennel samples were processed as described in the previous section (chapter 2 A) and stored for 21 days at 4°C, 18 days at 10°C and 15 days at 15°C. Physical-chemical (colour and firmness) and nutrition (antioxidant capacity, total polyphenol and vitamin C content) properties were evaluated as described in chapter 2A. To properly describe the changes in critical quality indices as a function of time, additional storage time was analyzed. In particular, for products stored at 4°C, analysis after 2 days and 18 days were added to the previous data (0, 5, 8, 12, 15 days) for a total of seven times; at 10°C sample 18 days was also analyzed for a total of five times (0, 5, 8, 12, 18 days); at 15°C samples stored for 1, 12 and 15 days were also analyzed for a total of 6 times (0, 1, 2, 5, 7, 12, 15 days).

2.1 Mathematical modelling: zero and first-order kinetic models

Zero and first-order kinetics, traditionally used to describe degradation reactions in foods, may be generally written as (Giannakourou & Taoukis, 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005):

$$\frac{dQ(t)}{dt} = -kQ^n \qquad Eq.(1)$$

The equation may be integrated easily obtaining the well-known decay functions. In particular, for pseudo-zero order kinetic model, the following equation can be used to predict the quality of a product as a function of storage time:

$$Q = Q_i - kt \qquad Eq.(2)$$

whereas for pseudo-first kinetic order (n=1), the equation will be:

$$Q = Q_i e^{-kt} \qquad Eq.(3)$$

Or

$$Q = Qeq + (Q0 - Qeq)e^{-kt} \qquad Eq.(4)$$

where

Q_i is the concentration of the quality index at time zero, *Q(t)* is the concentration of the quality index at the time *t*, *Q(eq)* is the concentration of the quality index at the time at equilibrium, *k* is the rate constant, and n is the kinetic order of the equation. The negative sign is generally referred to as a decrease in quality. However, if the quality indices related to the alteration process increase during storage, the function will have a positive sign.

An Arrhenius-type equation has been used to describe the changes in the kinetic constant as a function of the storage temperature:

$$k = Aexp\left(-\frac{E_a}{RT}\right) \qquad Eq.\ (5)$$

Where:

k= kinetic constant at temperature (T) ; *A* is pre-exponential, temperature-independent constant; E_a is activation energy (J mol^{-1}), independent of temperature; *R* is gas constant (8.31 J mol^{-1} K^{-1}); *T* is absolute temperature (°Kelvin).

2.2 Statistical analysis

Linear and non-linear regression were used to estimate the kinetic constants by using XLSTAT 16.0 (Addinsoft, France, 2023). The regression coefficients (R^2) and the root mean square error (RMSE) were calculated to evaluate the goodness of the model to describe data. The highest the values of R^2 and the lowest the values of MSE and RMSE, the better the fitting of the models to experimental data. The adequacy of the fitted model was also assessed using an analysis of residuals which permits confirmation the of validity of the assumptions regarding the independence and normal distribution of the errors. Paired t-test analysis was carried out to find the source of the significant differences within the samples examined statistically significant difference was defined at $p \leq 0.05$.

3. Results and Discussions

In Figure 1 the ΔE values of control and SC+PG samples stored at 4°C (A 1 and 2 respectively), 10 °C (B1 and 2 respectively) and 15 °C (C1 and C2 respectively) are reported. A pseudo-first-order model, as reported in Eq. 4, well described the increment of ΔE over time (R^2 ranging from 0.79 to 0.92, MSE ranging from 3.7 and 12.9, and RMSE ranging from 1.9 to 4.2 (Table 1). In Table 2 the average values of the kinetic constant for the ΔE changes are reported. Except for samples stored at 10°C, the active coating caused a significant increment of the kinetic constant of about 30% than the control sample ($p \leq 0.05$). Thus, the active coating had a negative impact on the browning of the minimally processed fennel. However, if the acceptability value of ΔE is fixed at 5, which represents the value at which consumers will recognize the colour difference, we can conclude that the time to reach that value is very short for both control (4 days) and active coated samples (2 days). Thus, browning is a limited alteration mechanism for minimally processed fennel and a different technological approach should be followed to reduce the rate of browning.

Table 1. Goodness of fitting of the pseudo-first-order model used to estimate ΔE of MP fennels with and without coating at different temperatures over time.

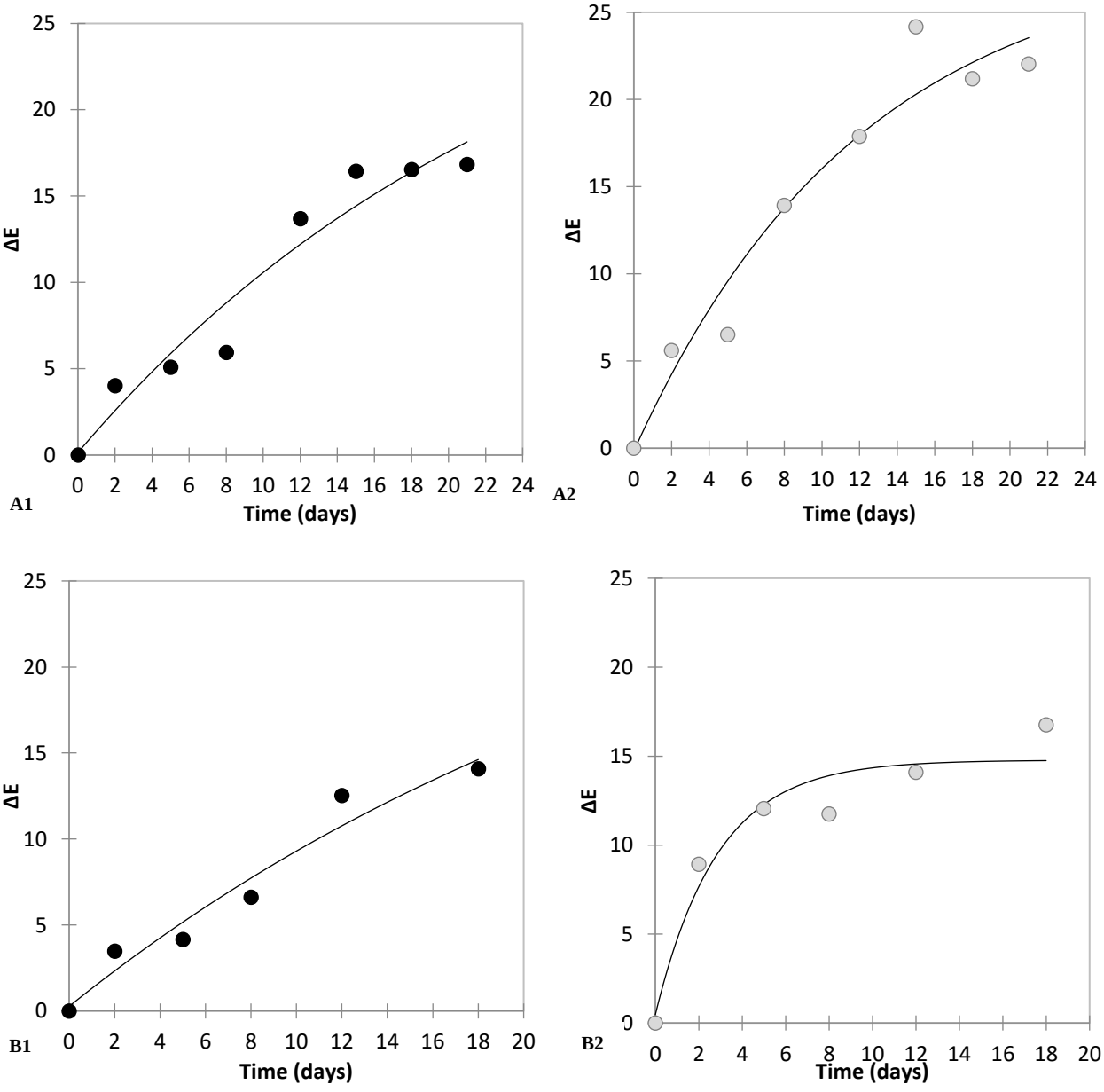
		ΔE			
Treatments	T (°C)	Kinetic	MSE	RMSE	R^2
Control	4	First order	12.9	3.6	0.79
	10		3.7	1.9	0.78
	15		17.8	4.2	0.80
SC+PG	4		6.0	2.4	0.92

	10	4.6	2.1	0.75
	15	4.4	2.1	0.92

Table 2. Kinetic constants of ΔE of control and SC+PG samples stored at 4, 10 and 15°C.

	4°C		10°C		15°C	
Samples	**k(day^{-1})**	**Q_{eq}**	**k(day^{-1})**	**Q_{eq}**	**k(day^{-1})**	**Q_{eq}**
Control	0.038^{a}±0.019	31±2	0.040^{a}±0.003	31±2	0.028^{a}±0.012	31±2
SC+PG	0.082^{b}±0.001	31±3	0.34^{b}±0.009	31±1	0.036^{b}±0.021	31±2

Letters indicate significant differences between the treatments at each temperature ($p \leq 0.05$)



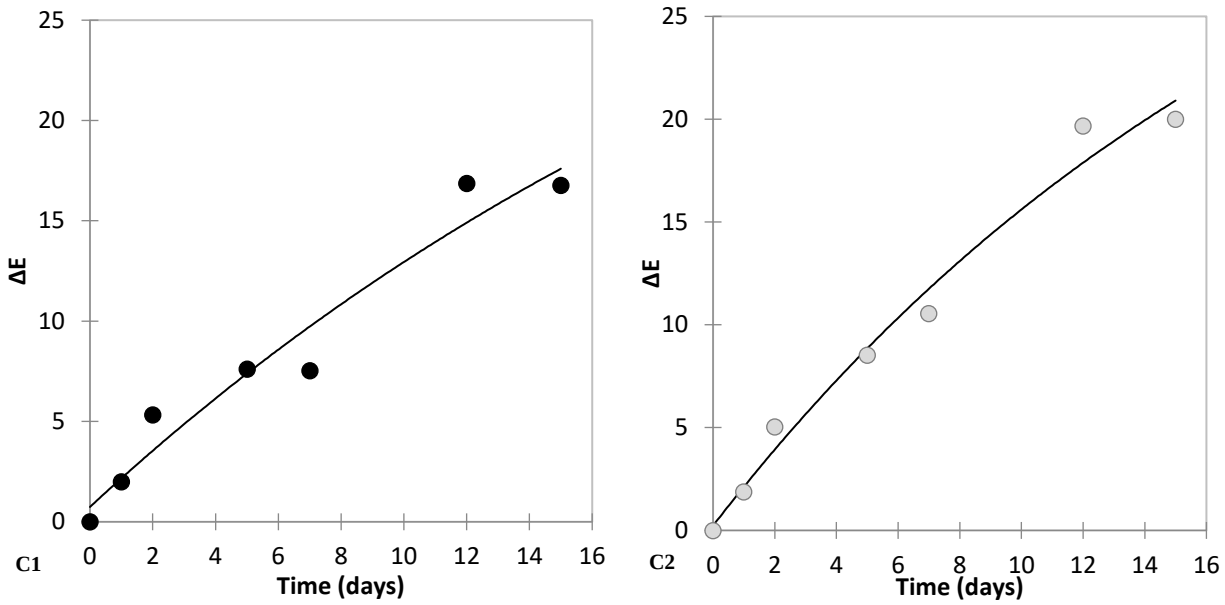


Figure 1. Values of ΔE of control (●) and of SC+PG (●) samples stored at 4°C for 21 days (A1 and A2 respectively), 10°C for 18 days (B1 and B2 respectively), and 15°C for 15 days (C1 and C2 respectively). ── is the data predicted by the model.

Maftonazad and colleagues (2019) found that ΔE parameter of lime fruit was fitted well with pseudo zero order in the presence or absence of an edible pectin coating. The effect of coatings on browning control greatly depends on intrinsic factors such as the biopolymer, the antioxidant compound and the fresh-cut commodity (Sanchís et al., 2016). For example, pectin-based coatings added as antioxidants significantly reduced the browning of fresh-cut pears (Oms-Oliu et al., 2008).

Figure 2 shows the firmness changes of control and SC+PG samples stored at 4°C (A1 and 2 respectively), 10 °C (B1 and B2 respectively) and 15 °C (C1 and C2 respectively). The firmness loss was well described by a pseudo-zero order kinetic model (R^2 ranging from 0.72 to 0.97, MSE ranging from 0.001-0.004 and RMSE of 0.03 and 0.04) (Table 3). The kinetic constants of firmness loss (Table 4) obtained for the samples with and without coatings were 0.016, 0.022 and 0.028 $days^{-1}$ for samples stored at 4, 10 and 15°C respectively. Furthermore, the active coating was able to reduce firmness degradation by about 20% more than control samples only for samples stored at 15°C. A similar effect of pectin coating on avocados (Maftoonazad & Ramaswamy, 2005) and lime fruits (Maftoonazad & Ramaswamy, 2019) was observed.

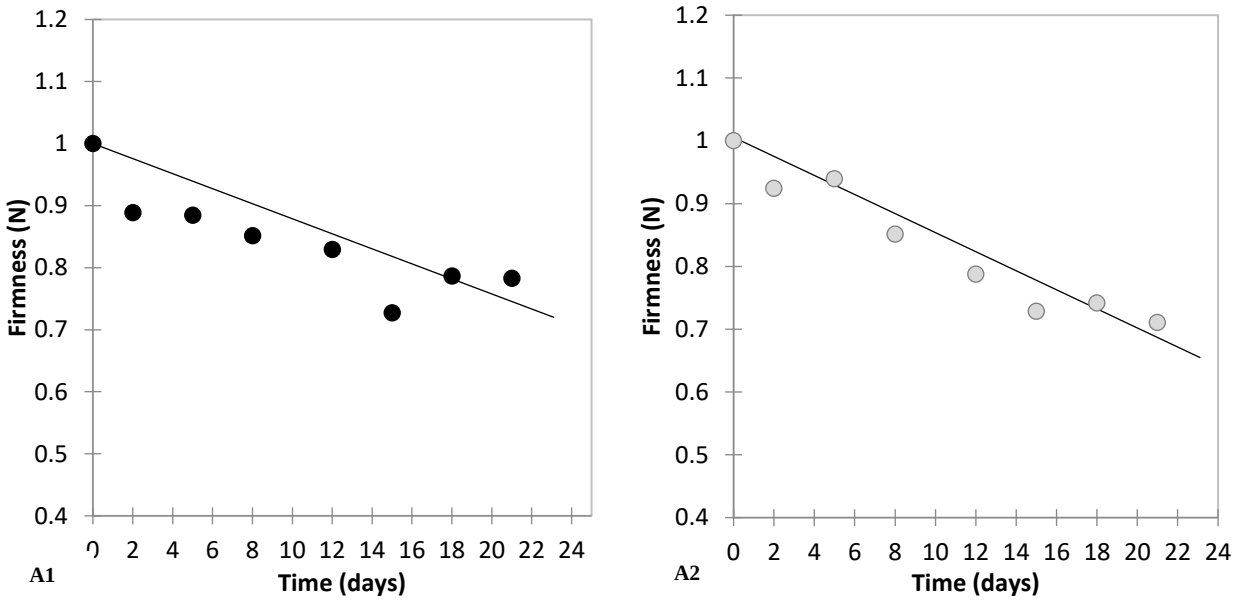
Table 3. Goodness of fitting of pseudo-zero order model used to estimate firmness of MP fennels with and without coating at different temperatures over time.

Firmness					
Treatments	T (°C)	Kinetic	MSE	RMSE	R^2
Control	4	Zero-order	0.002	0.04	0.72
	10		0.002	0.04	0.84
	15		0.001	0.03	0.97
SC+PG	4		0.001	0.03	0.93
	10		0.002	0.04	0.84
	15		0.001	0.04	0.92

Table 4. Constant kinetics of firmness of control and SC+PG samples stored at 4, 10 and 15°C.

Samples	$\mathbf{k_{4°C}}$	$\mathbf{k_{10°C}}$	$\mathbf{k_{15°C}}$
Control	0.014^{a}±0.002	0.028^{a}±0.010	0.031^{a}±0.001
SC+PG	0.016^{a}±0.010	0.018^{a}±0.001	0.023^{b}±0.001

Letters indicate significant differences between the treatments in each temperature ($p \leq 0.05$)



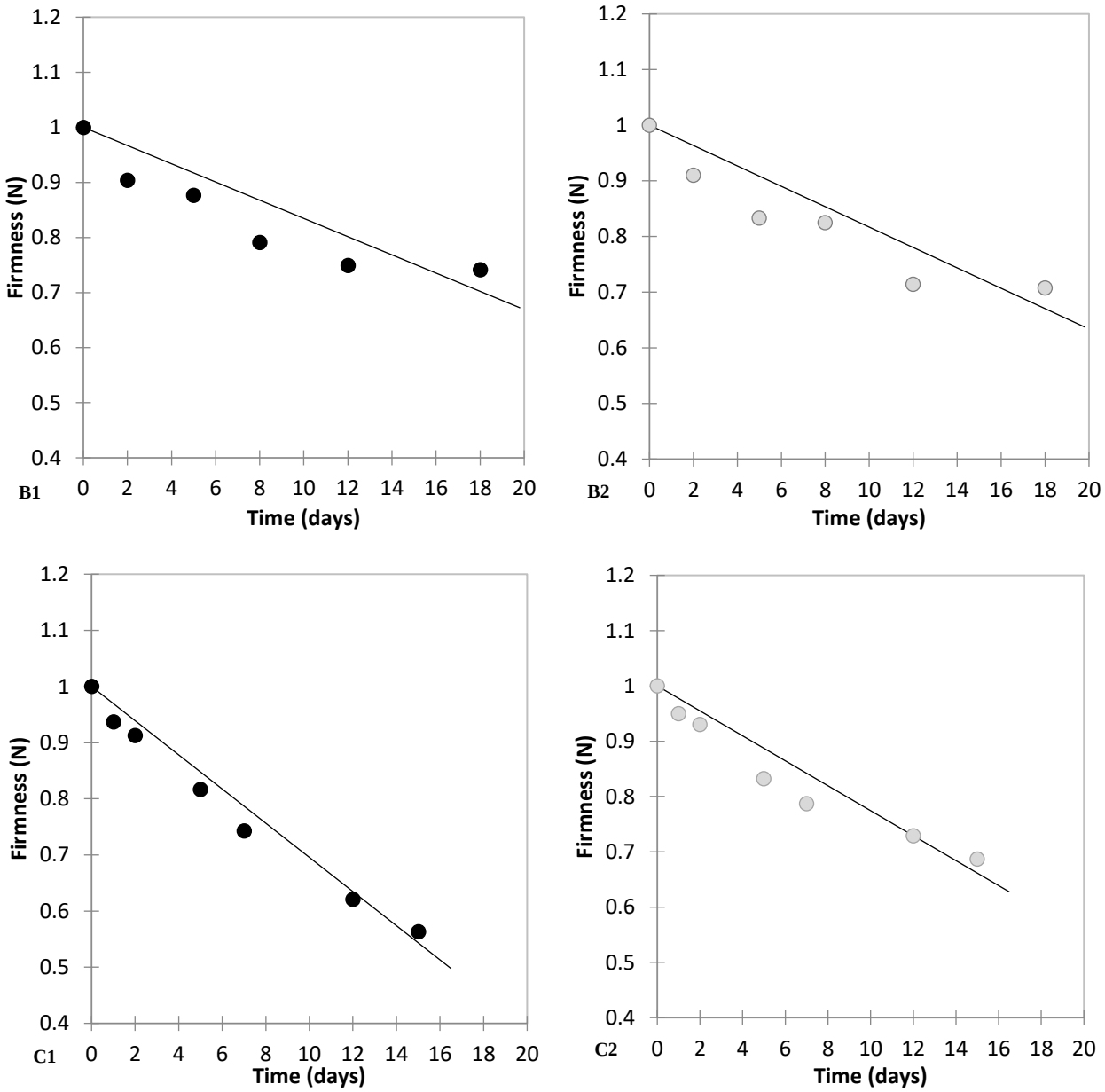


Figure 2. Values of firmness of control (●) and of SC+PG (●) samples stored at 4°C for 21 days (A1 and A2 respectively), 10°C for 18 days (B1 and B2 respectively), and 15°C for 15 days (C1 and C2 respectively).— is the data predicted by the model.

Also, other authors reported that the pseudo-zero order well describes the firmness loss during the stored time. In the specific, Amodio et al. (2013) studied the shelf life of fresh-cut melons and reported the hat zero-order kinetic model was found to be the best model to predict firmness loss showing a correlation coefficient of 0.99, with MSE of about 0.1 and an RMSE of 0.84. The same results were shown for cabbage firmness which decrement was explained by using a zero-order kinetic model with R^2 ranging from 0.89 to 0.97 (Jaiswal & Abu-Ghannam, 2013), and for sichuan (pseudo zero order; R^2= 0.92) (Du et al., 2022). However, the firmness loss of processed products has been also predicted

by using a pseudo-first-order kinetic model (R^2 = 0.93) (Maftoonazad & Ramaswamy, (2019).
In Figure 3 the TAC of control and SC+PG samples at 4°C (A 1 and A2 respectively), 10 °C (B1 and B2 respectively) and 15 °C (C1 and C2 respectively) are reported. High values of R^2 ranging from 0.79 to 0.97 and RMSE of 0.07 and 0.12 was obtained by using a pseudo-first-order kinetic model. A good correlation between experimental and predicted data was observed, as reported in Table 6. The Kinetic constants of TAC are reported in Table 7. The active coating was able to preserve the loss of TAC of about 30% for samples stored at 4 °C and 10 °C and of about 50% for samples stored at 15°C, concerning the control sample ($p \leq 0.05$). In the literature, there are no studies on the mathematical modelling of the antioxidant capacity of fennel, whether fresh or minimally processed. However, the kinetic constant of the reduction of TAC of cabbage was about 0.110 day-1 at 15°C in presence of a coating (Jaiswal & Abu-Ghannam, 2013). The kinetics constant of TAC depends on temperature, as reported by Oms-Oliu and colleagues (2009). Fresh-cut watermelon samples stored at temperatures up to 15°C had greater antioxidant capacity than those stored at 20 °C. The samples stored at 20°C underwent a substantial depletion of TAC during the first two days, retaining 30 % of the initial TAC after 14 days of storage. On the other hand, the antioxidant capacity retention of fresh-cut watermelon stored at temperatures lower than 20 °C varied from 55 % to 65 % after 2 weeks.

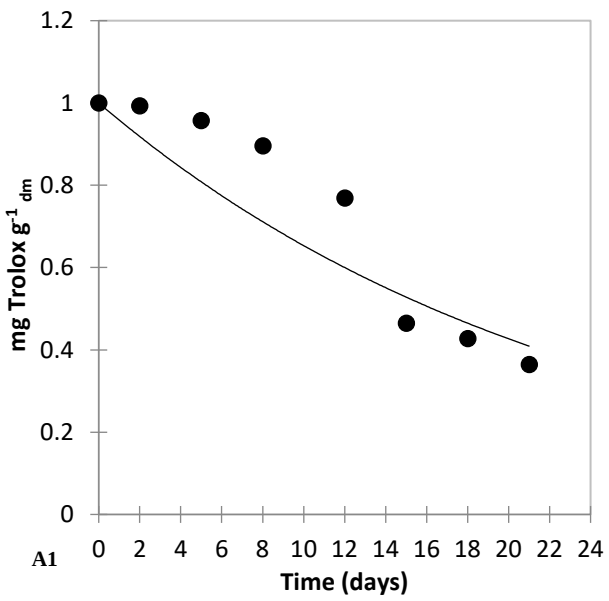
Table 6. Goodness of fitting of pseudo-first-order model used to estimate the total antioxidant capacity of MP fennels with and without coating at different temperatures over time.

Total antioxidant capacity					
Treatments	T (°C)	Kinetic	MSE	RMSE	R^2
Control	4	First order	0.02	0.12	0.79
	10		0.01	0.09	0.85
	15		0.01	0.12	0.86
SC+PG	4		0.01	0.10	0.76
	10		0.006	0.08	0.90
	15		0.005	0.07	0.97

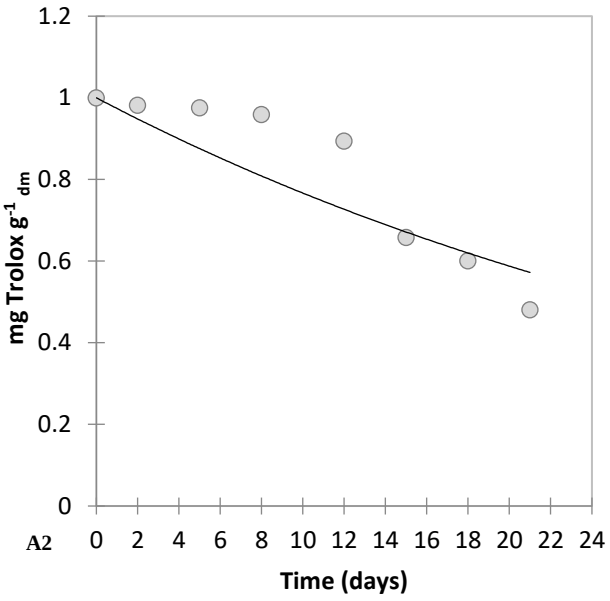
Table 7. Constant kinetics of total antioxidant capacity of control and SC+PG samples stored at 4, 10 and 15°C.

Samples	$\mathbf{k_{4°C}}$	$\mathbf{k_{10°C}}$	$\mathbf{k_{15°C}}$
Control	0.035^{b}±0.007	0.088^{b}±0.008	0.304^{b}±0.029
SC+PG	0.024^{a}±0.003	0.061^{a}±0.002	0.147^{a}±0.005

Letters indicate significant differences between the treatments in each temperature ($p \leq 0.05$)


1.2
1
0.8
0.6
0.4
0.2
0
mg Trolox g-1 dm
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (days)

A1


1.2
1
0.8
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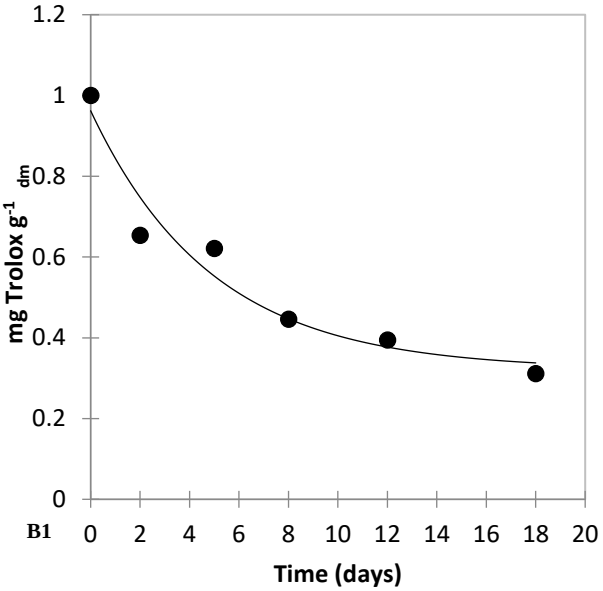
0
mg Trolox g-1 dm
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (days)

A2


1.2
1
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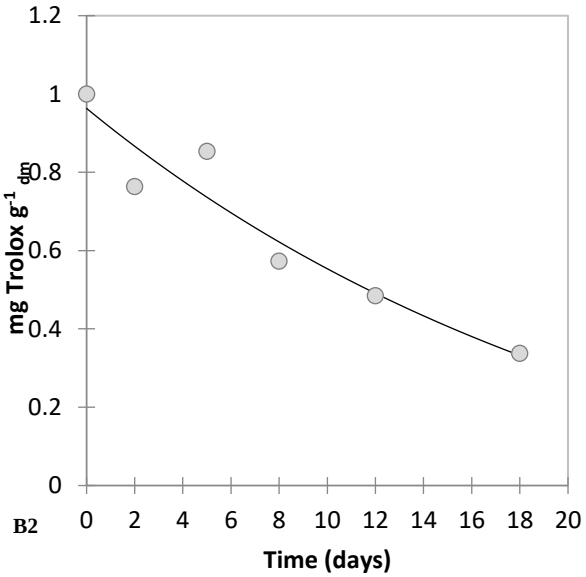
0
mg Trolox g-1 dm
0 2 4 6 8 10 12 14 16 18 20
Time (days)

B1


1.2
1
0.8
0.6
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0.2
0
mg Trolox g-1 dm
0 2 4 6 8 10 12 14 16 18 20
Time (days)

B2

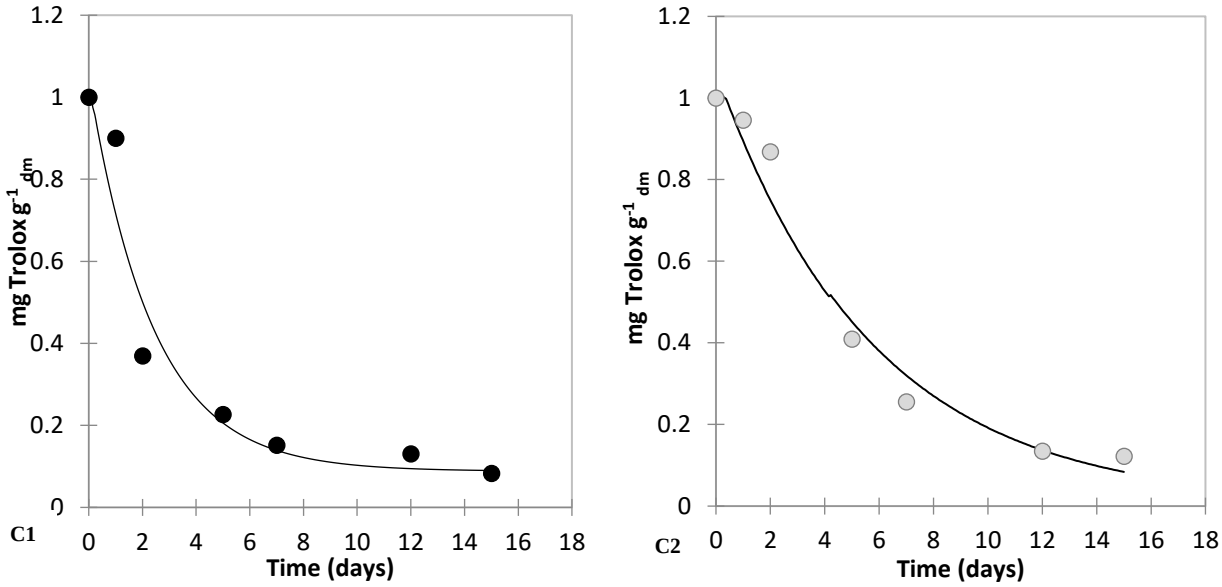


Figure 3. Values of total antioxidant capacity of control (●) and of SC+PG (○) samples stored at 4°C for 21 days (A1 and A2 respectively), 10°C for 18 days (B1 and B2 respectively), and 15°C for 15 days (C1 and C2 respectively).— is the data predicted by the model.

However, by comparing the results obtained of MP fennel with cabbage, the first-order kinetic model showed a high degree of fit, being the most suitable with the highest R^2 value, ranging from 0.95 to 0.97 and mean square errors (MSE) 0.003 for DPPH free radical scavenging capacity (Jaiswal & Abu-Ghannam, 2013). A different model was applied on fresh-cut watermelon stored at 5°C, as reported Oms-Oliu et al., (2009) which applied the Weibull model and it showed a high value of R^2 (0.98) for AC.

In Figure 4 are reported values of TPC of control and SC+PG samples at 4°C (A 1 and A2 respectively), 10 °C (B1 and B2 respectively) and 15 °C (C1 and C2 respectively).

Total polyphenol content was modelled using the pseudo-zero kinetic model. A good correlation between the experimental and model predicted was demonstrated, indeed there were obtained high values of R^2 ranging from 0.82 to 0.92, MSE of 0.008 and 0.7 and RMSE of 0.03 and 0.16 (Table 7). However, Table 8 are reported the average values of the kinetic constants of TPC, and it is possible to see that active coating preserved the increment of TPC about 32 %, 60 % and 20 % than the control samples stored at 4, 10 and 15 °C respectively with statistically significant difference ($p \leq 0.05$). Contrary to other attributes, k values of TPC did not depend on temperature. Values of kinetic constant were lower than values of other authors (Esua et al., 2019; Li et al., 2022). However, it should be pointed out that these results are compared with mushrooms and tomatoes.

Table 7. Goodness of fitting of pseudo-zero order model used to estimate total polyphenols content of MP fennels with and without coating at different temperatures over time.

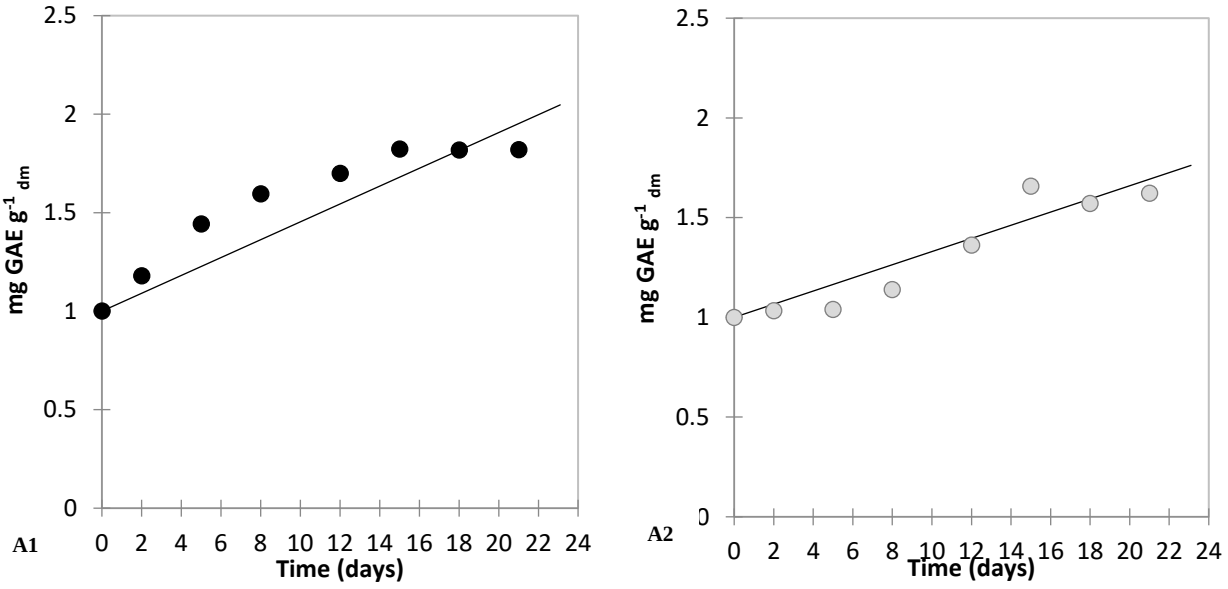
Total polyphenols content					
Treatments	T (°C)	Kinetic	MSE	RMSE	R^2
Control	4	Zero-order	0.01	0.14	0.82
	10		0.008	0.08	0.85
	15		0.03	0.16	0.93
SC+PG	4		0.01	0.11	0.85
	10		0.005	0.07	0.65
	15		0.07	0.27	0.83

Table 8. Constant kinetics of total polyphenols content of control and SC+PG samples stored at 4, 10 and 15°C.

Samples	$\mathbf{k_{4°C}}$	$\mathbf{k_{10°C}}$	$\mathbf{k_{15°C}}$
Control	$0.041^{b}\pm0.003$	$0.032^{b}\pm0.002$	$0.09^{a}\pm0.005$
SC+PG	$0.036^{a}\pm0.001$	$0.013^{a}\pm0.004$	$0.103^{b}\pm0.005$

Letters indicate significant differences between the treatments in each temperature ($p \leq 0.05$)

Several reports documented that the increase in phenolic content was associated with the up-regulation of browning levels (Campos-Vargas and Saltveit, 2002; Li et al., 2022).



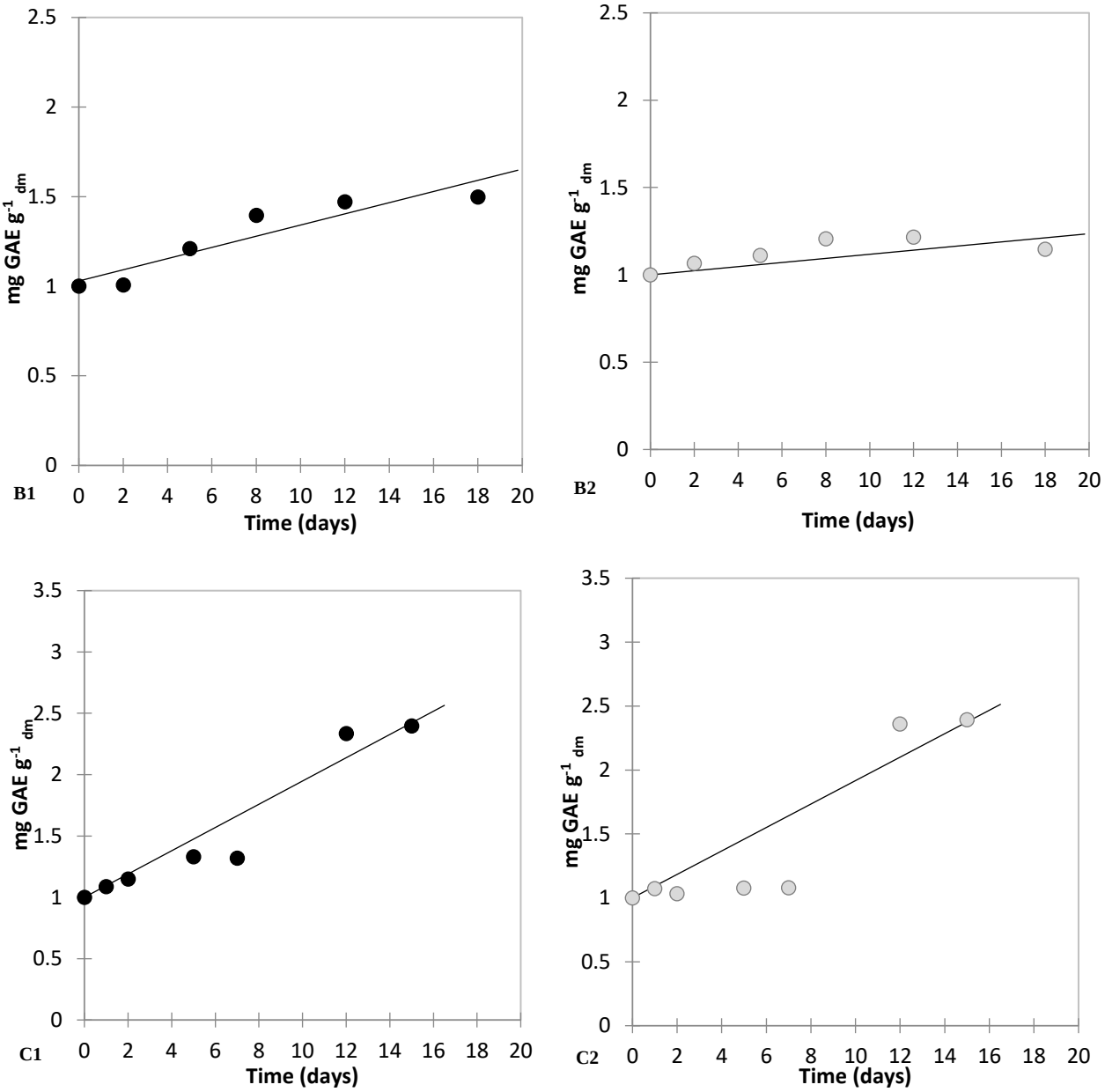


Figure 4. Values of total polyphenols content of control (●) and of SC+PG (○) samples stored at 4°C for 21 days (A1 and A2 respectively), 10°C for 18 days (B1 and B2 respectively), and 15°C for 15 days (C1 and C2 respectively).
—is the data predicted by the model.

Total phenolic content fitted better with zero-order kinetic models, according to Li et al., (2022) on fresh shiitake mushrooms stored at 5, 10, or 15°C for 15 days. The same results were obtained on tomatoes treated with a combination of ultrasound and ultraviolet-C irradiation (Esua et al., 2019). Therefore, the active coating applied on MP fennel shows a slowing down of TPC increase compared to control samples, demonstrating a protective effect.

Also, vitamin C content was modelled using the pseudo-first-order model. In Figure 5 are reported values of vitamin C content of control and SC+PG samples at 4°C (A 1 and A2 respectively), 10 °C (B1 and B2 respectively) and 15 °C (C1 and C2 respectively). In Table 9 are reported R^2, ranging

from 0.84 to 0.92, MSE values between 0.002 and 0.1, and RMSE values between 0.05 and 0.11. Vitamin C content decreased during storage time, but it was faster for uncoated than coated samples for all temperatures. The kinetic constants of vitamin C decrease for control and active samples were reported in Table 10. The decrement of vitamin C was 20 % than control samples stored at 4 and 10° C during storage time, whereas active coating preserved 10 % of samples compared with control samples stored at 15 °C, with statistically significant difference ($p \leq 0.05$) between the treatments. However, active coating applied on the samples with PG preserved with statistically significant difference ($p \leq 0.05$) than uncoated samples. Vitamin C content loss was more pronounced under higher temperatures than under refrigerated storage conditions, in agreement with Qiu & Wang (2015). They reported that vitamin C in "Satsuma" mandarins stored at 4 °C was much higher compared to those stored at 20 °C. Other studies demonstrated that 10°C can be the best temperature to store fruits, for example, sweet oranges or in lime fruit (Rab et al., 2012; Maftoonazad & Ramaswamy, 2019). Fruit stored at 10 °C lost vitamin C at a rate of 0.0067 and 0.0074 per day for coated and uncoated limes, respectively, whilst those stored at 15 and 25 °C had a rate of 0.0091 and 0.028 per day for uncoated limes and 0.0073 and 0.0180 per day for coated samples (Maftoonazad & Ramaswamy, 2019). Moreover, since the inverse of the scale factor of the Weibull has rate units, it was possible to calculate a rate constant of 0.0581 d^{-1} for the degradation of vitamin C in fresh-cut melons (Amodio et al., 2013). Similar results were obtained from Oms-Oliu et al. (2009) who modelled the vitamin C degradation with the Weibull model and reported a rate constant of 0.019 d^{-1} for watermelon samples stored at 5°C.

Table 9. Goodness of fitting of pseudo-first-order model used to estimate Vitamin C of MP fennels with and without coating at different temperatures over time.

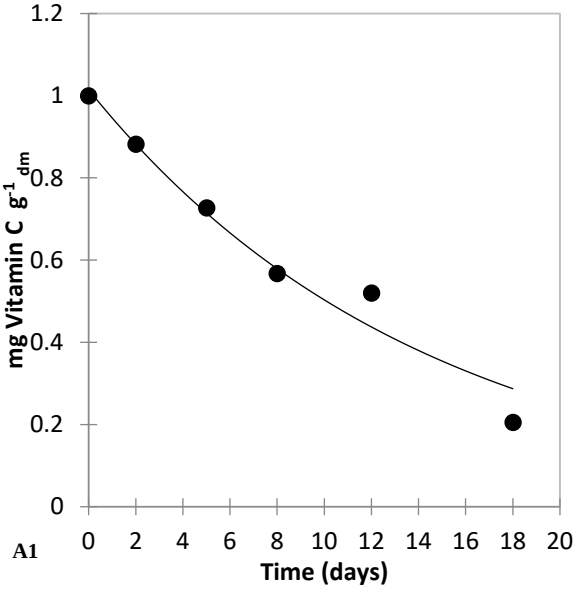
Vitamin C content					
Treatments	T (°C)	Kinetic	MSE	RMSE	R^2
Control	4	First order	0.01	0.11	0.84
	10		0.003	0.05	0.90
	15		0.002	0.05	0.91
SC+PG	4		0.01	0.11	0.85
	10		0.006	0.08	0.91
	15		0.005	0.07	0.92

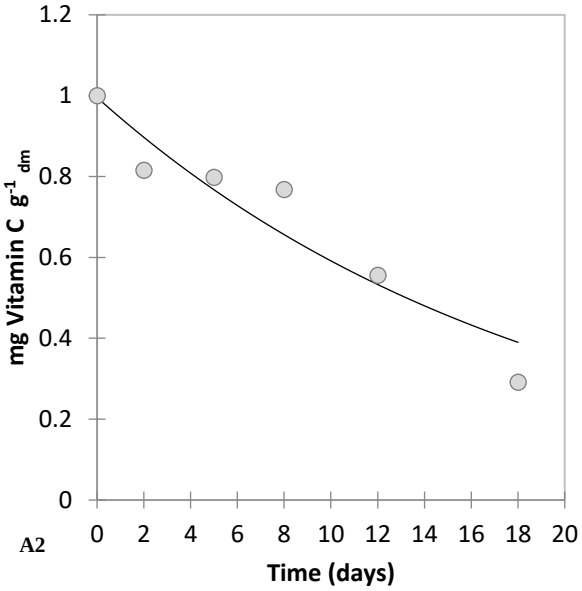
Table 10. Constant kinetics of vitamin C of control and SC+PG samples stored at 4, 10 and 15°C.

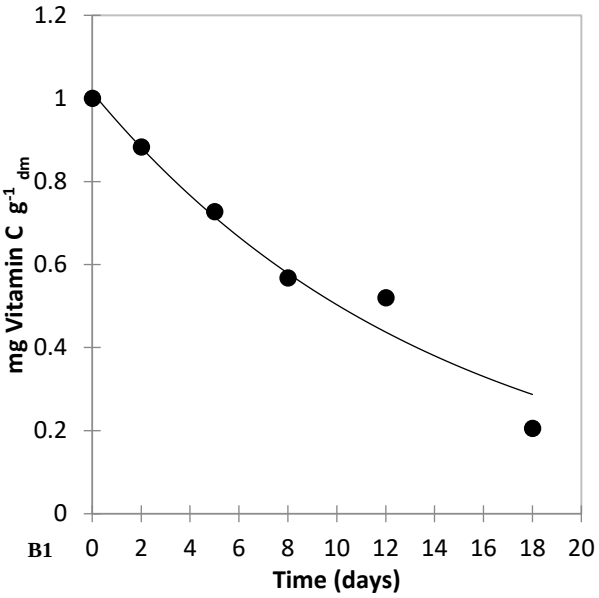
Samples	$\mathbf{k_{4°C}}$	$\mathbf{k_{10°C}}$	$\mathbf{k_{15°C}}$
Control	0.062^{b}±0.011	0.064^{b}±0.003	0.073^{b}±0.003

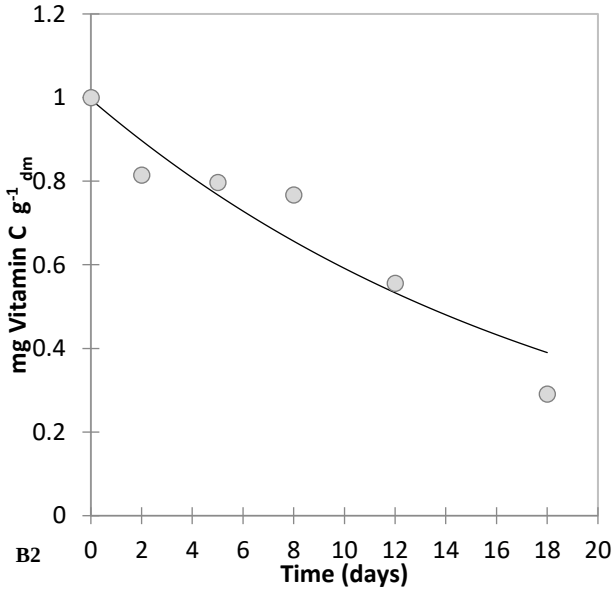
SC+PG	0.046[a]±0.003	0.050[a]±0.003	0.069[a]±0.001

Letters indicate significant differences between the treatments in each temperature ($p \leq 0.05$)









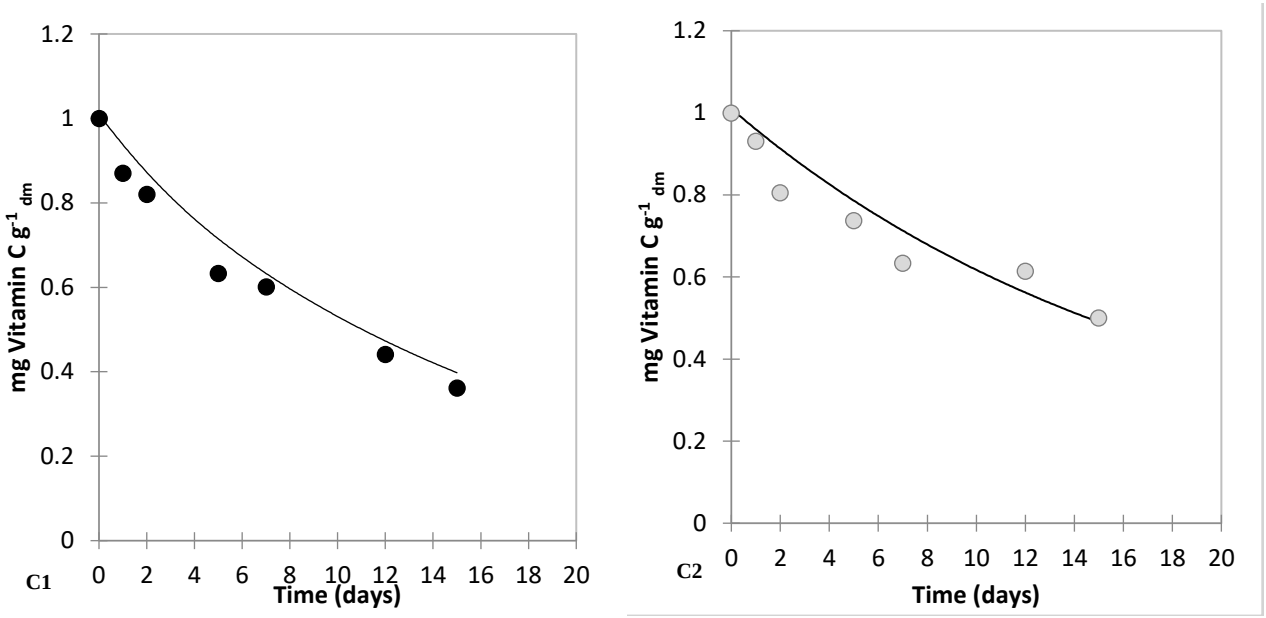


Figure 5. Values of vitamin C content of control (●) and of SC+PG (●) samples stored at 4°C for 21 days (A1 and A2 respectively), 10°C for 18 days (B1 and B2 respectively), and 15°C for 15 days (C1 and C2 respectively). ⟶is the data predicted by the model.

Pseudo first order was used to describe the kinetic of lime fruit stored at 10 and 25 °C (Maftoonazad & Ramaswamy, 2019). However, as demonstrated by Gil et al., (2006) and Amodio et al., (2013), vitamin C can also degrade by following Weibull model.

3.6 Activation energy

Figure 6 shows the logarithmic of the reaction rate constant of ΔE (A), firmness (B), antioxidant capacity (C) and vitamin C (D) of MP fennels with and without coating as function of the inverse of the absolute temperature. The dependence of the k value by temperature was adequately described by the Arrhenius relationship, with a high R^2 (0.98). The values of Ea for samples stored with and without coating were reported in Table 11. Therefore, TAC reduction seems to be more susceptible to temperature rise than other parameters studied of MP fennels. In general, samples with the active coating are less sensitive to temperature for ΔE, antioxidant capacity (Ea 113 kJ/mol) and firmness (21 kJ/mol) than the control sample (Ea_{AC} 130 and $Ea_{firmness}$ 46 kJ/mol). Different results were obtained for vitamin C, the control sample was less sensitive than the active sample, with a value of 10 and 24 kJ/mol, respectively. There were no reported values of Ea of TPC because this parameter not depends on temperature, as reported in the preceding paragraph. In the literature is reported that TPC activation energy observed in fresh-cut watermelon was the lowest compared with the values obtained for the other antioxidant properties. Thus, TPC accumulation seems to be less affected by temperature increases compared to AC and vitamin C content (Giannakourou & Taoukis, 2003; Oms-

Oliu et al., 2009).

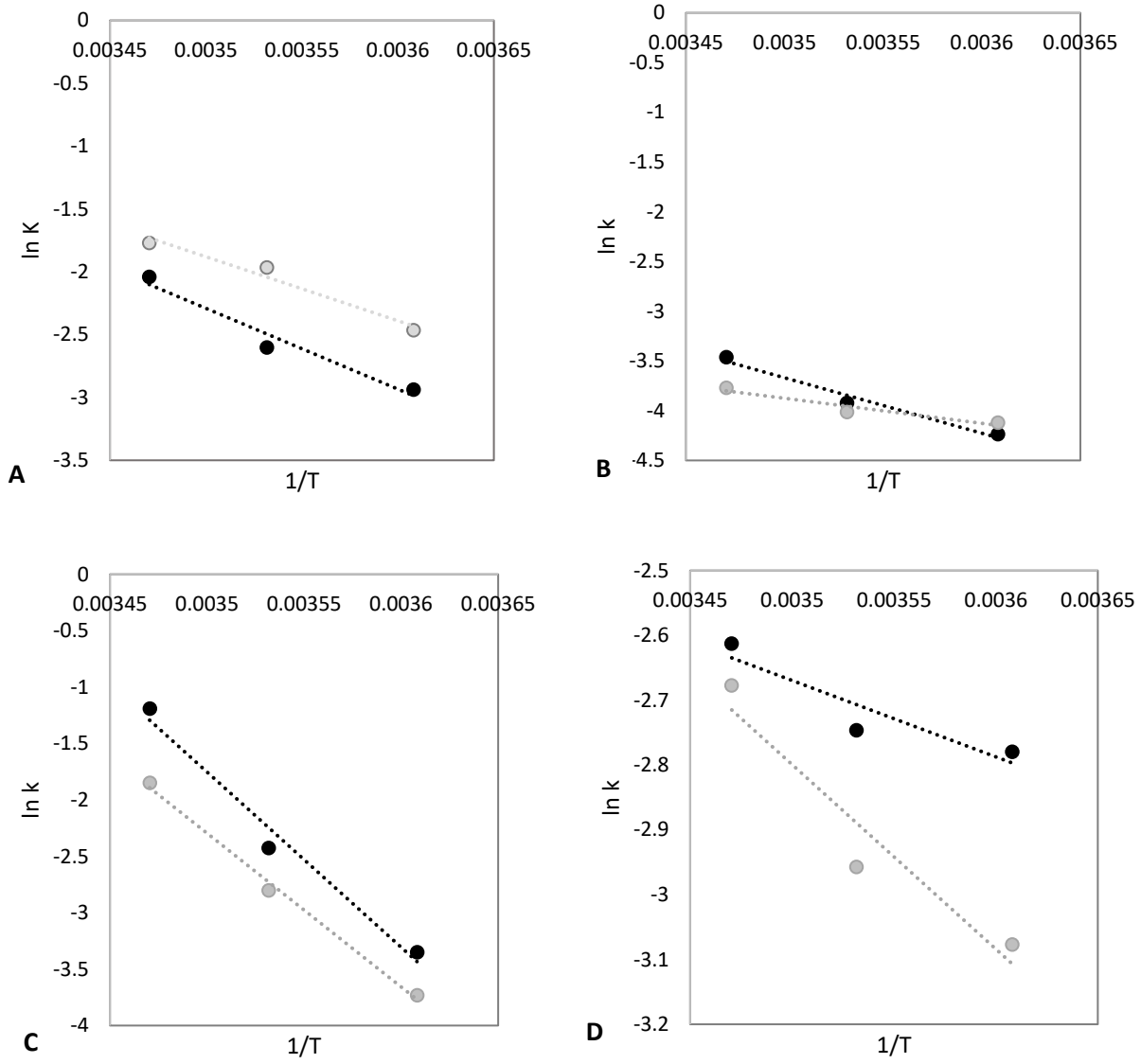


Figure 6. Reaction rate constant of ΔE, (A), firmness (B), antioxidant capacity (C), vitamin C (D) of MP fennels without coating (●) and SC+PG (●) as affected by storage temperature on a log-lin scale.

Therefore, vitamin C losses seem to be more susceptible to temperature rise than the decrease in antioxidant capacity of MP fennels. Vitamin C degradation in fresh-cut produce seems to be less susceptible to temperature increases than in frozen green vegetables, whose activation energies for vitamin C degradation were about 98–112 kJ mol^{-1} (Giannakourou & Taoukis, 2003).

Table 11. Values of Ea of ΔE, firmness, TAC and vitamin C content of MP fennels control and SC+PG

	Quality indices			
Samples	ΔE	Firmness	TAC	Vitamin C
			Ea (kJ/mol)	
Control	53	46	130	10
SC+PG	42	21	113	24

4. Conclusions

A good correlation was observed between experimental data e data predicted by the model. Pseudo-zero and first-order models well described the firmness and nutritional properties loss, as antioxidant capacity, total polyphenols content and vitamin C of MP fennels during storage time at 4, 10 and 15 °C. In the specific, pseudo zero order modelled better firmness and total polyphenols content, whereas pseudo first order modelled better antioxidant capacity and vitamin C content. Firmness decrease showed the lowest rate of decrement, whereas colour changes and nutritional loss showed the highest rate of decrement. However, the coating enriched with propyl gallate showed to preserve 20 % firmness and vitamin C content, 40 % antioxidant capacity and 50 % total polyphenol content of MP fennels stored at an average temperature of 10 °C over time. TAC reduction seems to be more susceptible to temperature rise than other parameters studied of MP fennels. Moreover, samples with the active coating are more sensitive to temperature for ΔE (Ea 42 kJ/mol), antioxidant capacity, (Ea 113 kJ/mol) and firmness (21 kJ/mol) than the control sample (Ea_{AC} 130 and $Ea_{firmness}$ 46 kJ/mol). Different results were obtained for vitamin C, the control sample was less sensitive than the active sample, with a value of 10 and 24 kJ/mol respectively.

Thus, the active coating can be a technological solution to preserve the nutritional quality of the product and to reduce the dependence on quality changes from temperature fluctuation during storage. Validation should be performed to confirm the values estimated. However, the kinetic model can be a useful tool to predict the quality changes of the product during distribution to optimize the food chain management and quality assurance management.

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Chapter 3 A

Study of the effect of biopolymer active coating on minimally processed pears stored at different temperatures and relative humidity

Abstract

The aims of this work were (i) to study the effect of coating developed with sodium caseinate, guar gum, beeswax and propyl gallate with respiration rate of minimally processed (MP) pears and (ii) to evaluate the effect of the active coating on pears quality at different storage times, relative humidity and temperatures. The samples were washed and dried; the active samples were coated with solution; the control samples were without coating. Minimally processed pears were stored at 10 and 20 °C for 30 and 15 days, respectively at 70 and 95 % RH. Quality indices, including weight loss, firmness, soluble solids content, titratable acidity, pH, total colour change, antioxidant capacity, total polyphenol content and vitamin C content were measured at set intervals during storage. Coating reduced by 50% O_2 consumption and CO_2 production than control samples at 4, 10 and 20°C. However, active coating was able to preserve firmness of the product. Regardless of the storage temperature and humidity, coating showed to preserve firmness of MP pears by about 50 % than uncoated samples. Moreover, storage temperature and relative humidity played a key role. In fact, 70 % RH was able to preserve about 30 % of the antioxidant capacity and total polyphenols compared to samples stored at 20°C. In addition, relative humidity also affected the effect of the coating; in fact, the coating was unable to preserve the vitamin C content at 20°C and 95%RH, unlike the samples stored at 70 % RH where the active coating was able to preserve 25 % of the vitamin C content compared to the uncoated sample. In conclusion, coating applied to pears significantly preserved firmness, and nutritional properties, but best conditions to highlight the effect of the coating on pears are 10°C at 70 % RH. In future other authors could be studied the effect of coating using kinetic modelling on firmness, antioxidant capacity, total polyphenols content and vitamin C.

Keywords: MP product, respiration rate, nutritional properties, firmness.

1. Introduction

Pear *(Pyrus spp. Pyrus communis* L. cv. Conference) is an important fruit due to its high nutritional antioxidant properties (Singh et al., 2019). Most important indications for the deterioration of quality, as well as storage life, are reduction in firmness, cell turgor pressure and cell wall degradation. One parameter that influences the rapid maturation of pears is the temperature associated with relative humidity (Dai et al., 2020). However, pears are climacteric fruit ripened in association with a rise in ethylene production and respiration rate during storage (Singh et al., 2019). Some studies have been reported to prolong the shelf life of pears using edible coatings that can improve the chemical-physical properties, reporting the ability of technology to retard changes in moisture, oxygen, aromas and solute transport (de Moraes et al., 2012). However, the edible coating can be used as a carrier transfer of antioxidants, antimicrobials, colorants, flavours, fortifying nutrients, and spices in film formulation (Pranoto et al., 2005). One of the most sought-after technologies in recent years has been the incorporation of antioxidant agents into packaging materials because oxidation is a major problem affecting food quality (Güçbilmez et al., 2007). Edible coatings can be obtained from different types of materials, but the most used ones are made of polysaccharides (de Moraes et al., 2012); however, certain limitations or disadvantages have been found due to their hygroscopic nature. Recent studies, as reported by Khan et al., (2021) and Miele et al., (2022), have shown that sodium caseinate can be a good protein for the development of biopolymer coatings. Furthermore, it can be used with support, enriched with beeswax, guar gum and emulsifiers, allowing a complete coating to preserve fresh fruit. Moreover, different authors found the high efficiency of candelilla wax edible coatings to improve the shelf life of fresh-cut fruits (Saucedo-Pompa et al., 2007), avocado (Saucedo-Pompa et al., 2009), and bell peppers (Ochoa-Reyes et al., 2013).

The objective of this work was to study the effect of biopolymer coating developed with sodium caseinate (SC), guar gum (GG), beeswax (BB), emulsions and propyl gallate (PG), as an antioxidant compound, on minimally processed pears stored at different temperatures and relative humidity. Then, the effect of the active coating on pears quality has been evaluated at different storage times and temperatures and critical quality indices were selected.

2. Materials and Methods

2.1 Materials

Sodium caseinate from bovine milk (SC), glycerol (GLY), propyl gallate (PG), tween 80 (T), span 80 (S), and guar gum (GG), sodium hydroxide, riboflavin, sodium bicarbonate, potassium buffer, glacial acetic acid, methanol, Folin-Ciucalteu, and DPPH reagent were purchased from Sigma-Aldrich (Milan, Italy). Beeswax was purchased from Agraria Ughetto Apicoltura (Giaveno, Torino, Italia). *Conference* pears fruit were purchased from a local supermarket (Sole 365, Portici, Italy).

2.2 Methods

2.2.1 Coating solution preparation

Coating solution was prepared as reporter by Miele et al., (2022). In the specific, SC 8% (weight (w)/volume (v)) was obtained by dispersing SC powder in deionized water and stirring continuously for 1.3 h at 90 °C; GLY was added (weight ratio of 0.1). Then GG (0.2 % (w/v)) was added under stirring for another 30 min at 90 °C. Next, BW (2 % (w/v)) and surfactants (T/S ratio 1:1) were added, and the mixture was stirred in a double-walled reactor, for 10 min a 90 °C, to allow the wax to melt. Surfactants were obtained by mixing tween 80 (HLB 15) and span 80 (HLB 4.2). The ratio BW:S was constant and equal to 4:1. Emulsification of the hot sample was achieved using an Ultra-Turrax T25 system (15,000 rpm for 5 min) (IKA-Werke, Staufen im Breisgau, Germany). The emulsions were stirred at a constant rate of 230 rpm until room temperature was reached so that the wax droplets could solidify (Miele et al., 2022). Finally, PG (0.13 mg mL^{-1}) was added to the solution, as antioxidant compound.

2.2.2 Coating application and thickness estimation

To estimate the thickness of the coating on the product, the approach reported by Valentino et al., (2020) has been followed. Considering a truncated cone geometry for pears, as reported by Miele et al., 2022 studying the strawberry, and taking into account that the surface tension force is surpassed by the viscous and gravity forces, the average liquid thickness (havg) has been estimated as follows:

$$havg = \frac{q}{A} = \frac{2}{3} k \left(\frac{\eta Z}{\rho g t}\right)^{1/2} \qquad Eq.(11)$$

where q was the coating volume (cm^3), A is the surface area of the pear (cm^2), η is the viscosity of the coating solution, Z is the height of the pears (cm), ρ is the solution density, g is the gravitational acceleration, t is the draining time and K is the dimensionless flow factor that can be experimentally determined by linear regression of Eq. (1) (Cisneros-Zevallos & Krochta, 2003).

The dry film thickness (Havg, μm) on pears at a given draining time has been estimated as function of dry coating load (Dc, g cm^{-2}) and calculated as:

$$Havg = \frac{Dc}{\rho f} * 10 \qquad Eq.\ (2)$$

$$Dc = \rho c havg 1000 \qquad Eq.\ (3)$$

where ρ is the coating solution density (g cm^{-3}), c is the concentration of solids in solution (g*g^{-1}), ρf is the dry film density (g cm^{-3}).

2.2.3 Scanning electron microscopy analysis

Interactions between coatings and fruit surfaces were also investigated by evaluating the adhesion of

coating suspensions into fruit surfaces using scanning electron microscopy (LEO EVO 40, Zeiss, Oberkochen, Germany). The products were mounted on bronze stubs using double-sided tape and then placed on specimen stubs with the cross-section oriented upward and were coated with a thin layer of gold using a DC sputter coater (AGAR B7340, Agar Scientific Ltd, Stansted, UK). Digital images of the film cross-sections were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

2.2.4 Processing

Before processing, *Conference* pears fruit harvested at the commercial maturity stage were stored at 4°C before the test; samples were qualitatively selected based on colour, size, and absence of defects to obtain a homogeneous batch. Pears were washed under tap water for 2 minutes and dried with a tissue. Thus, each pear (200 g) was dipped by hand into the SC solutions (400 mL) for 2 min and then quickly withdrawn and drained on metallic grids over the glass vessel containing the solution. Coated and uncoated pears were dried at 30°C and 50% relative humidity (RH), for one hour in a circulating air system chamber (MMM Medcenter Einrichtungen GmbH, Munich, Germany) (Valentino et al., 2020).

2.2.5 Respiration rate measurement

The O_2 consumption (R_{O2}) and CO_2 production (R_{CO2}) rates of minimally processed pears uncoated (control) and active coated as measured at 4, 10 and 20°C using a modified closed system. The products (about 0.200 kg) were placed in steel jars (4000 mL) and conditioned at test temperature for two hours under a constant air flux (1.6 $mL \cdot s^{-1}$) previously humidified (Torrieri et al., 2009). The temperature and relative humidity inside the jars were monitored using a data logger (MINI TH Giorgio Bormac S.r.l., Modena, Italy). After equilibrium, the inlet and outlet valves were closed and the gas composition was monitored over time with an O_2/CO_2 gas analyzer (accuracy of 0.5%), equipped with a needle (Check Mate 9900 O_2/CO_2; Ringsted, Denmark). The experimental time was 48h. At constant time intervals (Δt), 3 mL of gas mixture was drawn from the jar headspace and analysed using the gas analyser.

The free volume (Vf) inside the jar was calculated by using Eq. (4):

$$Vf = V - \frac{W}{\rho} \qquad Eq.\ (4)$$

where V is the volume of the jar (mL), W is the weight of the pear (kg), and ρ the apparent density of the pear (1500 $kg \cdot m^{-3}$).

R_{O2} and R_{CO2} expressed as mol kg^{-1} $s^{-1}$and respiration quotient, RQ were calculated as reported by Torrieri et al. 2009.

The effect of temperature on RR is expressed in terms of activation energy (Ea) as described by the

Arrhenius equation:

$$k = Aexp\left(-\frac{E_a}{RT}\right) \qquad Eq.\ (5)$$

Where:

k=specific constant; *A* is pre-exponential, temperature-independent constant; E_a is activation energy (J mol^{-1}), independent of temperature; *R* is gas constant (8.31 J mol^{-1} K^{-1}); *T* is absolute temperature (°Kelvin).

2.2.6 Packaging and storage condition

Minimally processed pears with and without coating were stored at 10 and 20°C at 70 % and 95 % RH for a maximum of 30 days. At different storage times, physical-chemical (weight loss, colour, pH, °Brix, TA, firmness) and nutrition (total antioxidant capacity, total polyphenol and vitamin C content) were evaluated as following described.

2.2.7 Physical-chemical properties

The weight loss of pears during storage was determined by using a gravimetric method. Pear samples were weighed before and after different storage times by using a balance (accurate to 0.01 g) (Mark Ben 3000, Monza, Italia). The weight loss was calculated as:

$$\left(\frac{wi-wf}{wi}\right)*100 \qquad Eq.(6)$$

where *wi* was the initial weight of pear and *wf* was the fennel weight after storage and expressed as a percentage.

The colour of the pears was determined with an electronic eye (visual analyser VA400 IRIS, Alpha MOS, France) equipped with a CCD camera (resolution 2592×1944 pixels and 24 bits). The camera was equipped with a 25 mm f1:2.2 Basler lens by Fujion and it was mounted in a light box equipped with top and bottom lightning (each position using 4 x 4 White LED) which was stabilized for 15 min before use (Tretola et al., 2017). Raw images were processed in RGB scale and subsequently converted in Cie L* a* b* scale using Alphasoft software (version 16.0). For each image, the white background was automatically removed and the L*, a* and b* measured pears (Cevoli et al., 2023). Total colour change (ΔE) was also analysed (ASTM E1910), where the sample time 0 was used to reference sample:

$$\Delta E = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b^2)]} \qquad Eq.\ (7)$$

Firmness (N) was measured at 4 opposite points per pear in the equatorial zone using a compression test with a TMS-Pro texture analyser (Food Technology Corporation) by using a 9 mm diameter plate and with load-bearing cells (500 N). Firmness has been calculated as the maximum force required to compress the sample up to a depth of 8mm. Tests were run with a crosshead speed of 9

mm/min(Rosenbloom et al., 2020). Data were acquired through Texture Lab Pro Software.

Juice of pears was extracted using 100 g of pears tissue in the blender (Fisher, Warings laboratory science LB20ES). TA was analysed by mixing one part of juice with 10 parts of distilled water and the mixture was titrated with 0.1 N NaOH to an endpoint pH of 8.0. TA (as percent malic acid) (Siddiq et al., 2020).

$$\%\, malic\ acid = \frac{ml_{NaOH\ used} * N_{NaOH} * PE_{NaOH}}{Ve_{Camp}} * 100 \qquad \textit{Eq. (8)}$$

For pH analysis, a pH meter (Eutech Instruments Pte Ltd., Singapore) was used in which 5 mL of pear juice was used. Few drops of the pears juice obtained were used to measure the total soluble solids (TSS) content with a digital refractometer (Atago PR32- Palette, Tokyo, Japan) (Kowalczyk et al., 2017). Three measurements were carried out on each sample.

2.2.8 Microbiological analysis

For microbial counts, whole pear samples were immersed in 100 ml of Ringer's solution and rubbed to allow microbial cells attached to the peel to disperse in the liquid. From the mother dilution of the pear samples and the coating solutions, the serial decimal dilutions were obtained and seeded in plates by pour plate technique using PCA (Plate Count Agar) medium for determination of mesophilic bacterial counts, and by spread plate technique on DRBC (Dichloran Rose Bengal Chloramphenicol) agar for yeast and mould counts. PCA and DRBC plates were incubated at 30°C and 28°C for 48-72 hours, respectively. The results were expressed in CFU/cm^2 (Colony Forming Units/cm^2) considering the average area of a pear equal to 200 cm^2 and in CFU/ml for liquid samples. Microbiological analysis was performed on: freshly prepared solution (SLZ0); solution after immersion of 13 pears (SLZ13); solution after immersion of 26 pears (SLZ26); control pears stored at 10 °C for 6 days (PC6); coated pears stored at 10 °C for 6 days(PA6); control pears stored at 10 °C for 15 days (PC15); coated pears stored at 10 °C for 15 days (PA15); control pears stored at 10 °C for 21 days (PC21); coated pears stored at 10 °C for 21 days (PA21).

2.2.9 Nutritional quality

The total antioxidant capacity (TAC) was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Volpe et al. (2019), with slight modifications. 0.5 g of freeze-dried sample was added in 10 mL of methanol/water (80:20) solution, mixed with constant shaking at temperature room for 60 minutes, and put on an ultrasound bath for 30 minutes. The sample was centrifuged (Hermle Z 326 K, Germany, European Union) at 10,000 rpm for 15 minutes (Pérez-Jiménez et al., 2008). The pellet was discarded and the supernatant was retained and mixed (100 µL) with 4.9 mL of DPPH solution (methanol+DPPH 0.1 Mm) to initiate the reaction. The absorbance was read using a spectrophotometer UV-VIS (UV-550 Jasco, Japan) at

515 nm after 30 minutes of incubation at room temperature in the dark. TAC was expressed as mg of Trolox equivalents g^{-1} of dry matter (mg TE g_{dm}^{-1}) using a Trolox standard curve (0–625 mg mL^{-1}).

To measure the total phenolic content (TPC), 0.5g of freeze-dried sample was crushed with mortar and pestle with 10 mL of sodium bicarbonate (6%); the solution was filtered through a paper filter and 0.5 mL of the filtrate was added with 2.5mL of Folin-Ciocalteau reagent and 2 mL of sodium bicarbonate. The samples were incubated for 1h at 35°C and then for 1h at 6 °C. After 2 h of incubation in the dark, the absorbance was read at 760 nm against a blank (2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium bicarbonate), using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). The TPC was calculated based on the calibration curves of gallic acid (0-8 mg mL^{-1}) and expressed as mg of gallic acid equivalents g^{-1} of dry matter (mg GAE g_{dm}^{-1}) (Dai et al., 2020).

Vitamin C of pears was extracted by homogenizing 1 g of product tissue with 10 mL of glacial acetic acid solution in water (8%) for 1 min by using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA). The homogenate was centrifugated at 7000 rpm for 7 minutes. The sample was filtered through paper filter, and the supernatant was collected. Then, 5 mL of glacial acetic acid solution was added to the pellet and centrifugated at 7000 rpm for 7 minutes. This procedure was replicated four times for vitamin C extraction. Then, the method reported by Jung et al. (1994), with minor modifications, was used to determine vitamin C content in MP fennels. 1 mL of sample filtered was added in 4 mL of riboflavin stock solution, and 0.06g riboflavin in 100 mL of 0.01 M potassium buffer (pH=7.5). Absorbances of samples before and after light storage (5500 Lux) were measured at 265 nm using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). Differences in absorbance of samples before and after 15 min-illumination were used for the calculation of ascorbic acid. The vitamin C content was calculated based on the calibration curves of ascorbic acid in the buffer of riboflavin (0-10.5 μg mL^{-1}) and expressed as mg of vitamin C g^{-1} of dry matter (mg of Vit C g_{dm}^{-1}).

2.3 Statistical analysis

The results are reported as the average of replications of each sample ± standard deviation.

The effect of coating on respiration rate was studied by one-way ANOVA analysis. Duncan's test was carried out to find the source of the significant differences within the samples examined.

Multivariate ANOVA analysis has been carried out to evaluate the effect of the independent parameters storage time (t), temperature (T), treatment coating (C) and relative humidity (RH) and their interaction on physical, chemical and nutritional quality indices of MP pears with a full factorial experimental. Two levels of treatments (control and active coating), 4 levels of times (0, 6, 9 and 13 days) at 10°C, 4 levels of time (0, 6, 7 and 9 days) at 20°C at 95% RH, whereas 8 levels of time (0, 3, 6, 9, 15, 20, 24 and 30 days) at 10°C and 8 levels of time (0, 1, 3, 6, 9, 13 and 15 days) at 20 °C at 70% RH were studied. Paired t-test was carried out to find the source of the significant differences

within the samples examined; whereas Dancan's test was used to find differences during storage time. Significance of difference was defined at $p \leq 0.05$. Data were analyzed using SPSS software (SPSS Inc. 28.0, Chicago, IL, USA, 2022).

3 Results and Discussions

3.1 Coating thickness

The average liquid thickness (h_{avg}) and the dry coating thickness estimated on pears (H_{avg}) were found to be 30 ± 3 and 3 ± 0.1μm. Valentino and colleagues (2020) reported that for SC at 8 %, the average dry coating thickness on fennel was 2 μm. Thus, blending sodium caseinate with other polymers, allowed increasing in the viscosity of the solution and the thickness of the coating on the product, as reported by Miele et al., (2022). They showed that the coating developed with SC 8%, guar gum and beeswax is able to increase coating thickness on strawberries with an average h_{avg} and H_{avg} 63 ± 8 and 4.5 ± 0.6 μm.

3.2 Scanning electron microscopy analysis

Figure 1 shows both cross-section and surface samples of uncoated (A and C) and coated (B and D) pear peels. Coating applied on MP pears was evenly distributed and showed few voids. Coating also showed spherical and small globules, perhaps emulsion droplets, with "humps" and crater-like holes, suggesting an improved gas and moisture barrier (Bosquez-Molina et al., 2003; Deng et al., 2018). Hence, coating with a rigid and dense matrix and good dispersion of emulsion droplets over the fruit surface was evaluated on pears during time and high RH cold storage, with uncoated and coated.

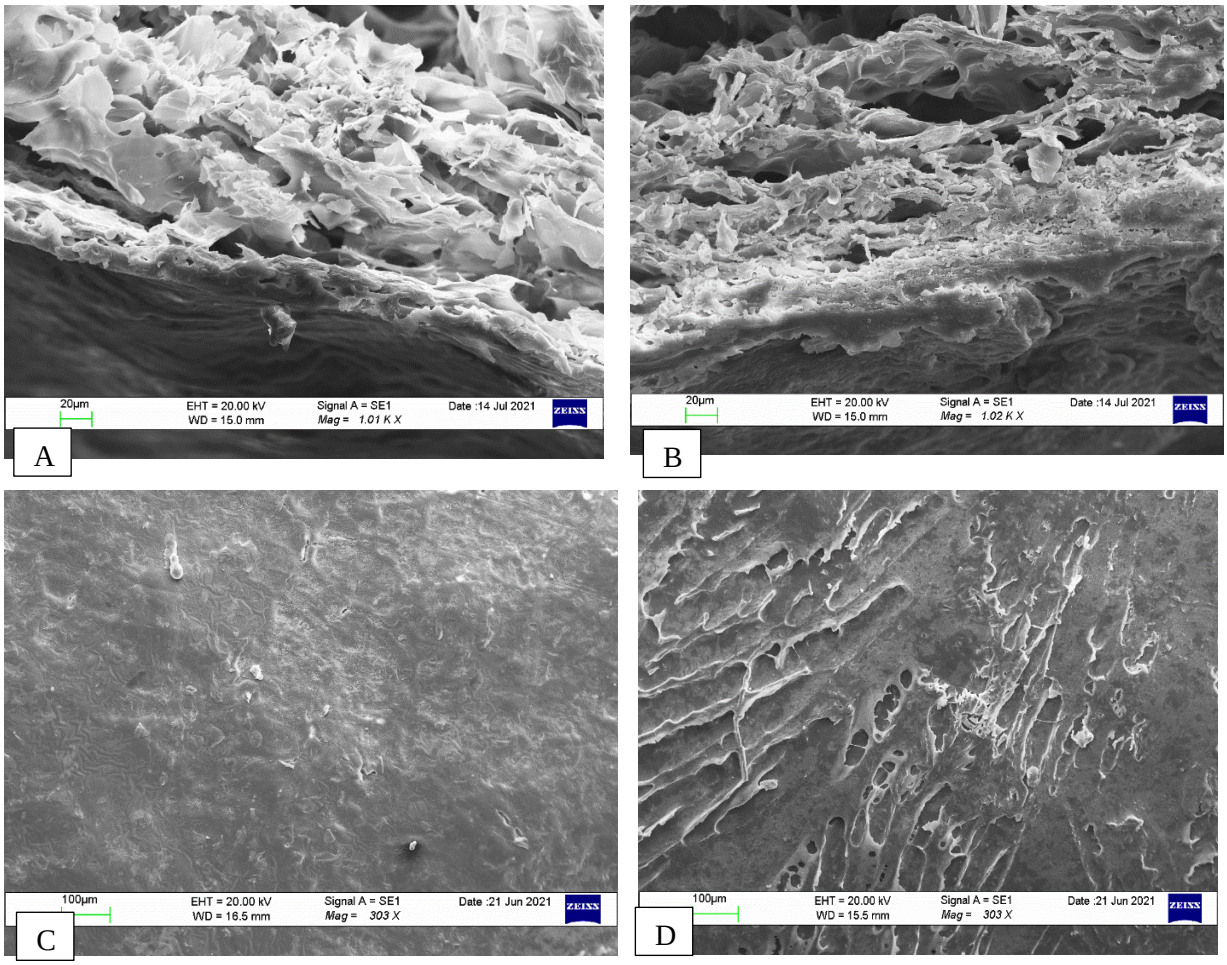


Figure 1. Scanning electron microscopy (SEM) micrographs of cross-sections and surfaces of uncoated (A and C) and coated (B and D) pear peels; Digital images were collected at an accelerating voltage of 20kv.

3.3 Respiration rate

In Figure 2 A and B are reported the values of RR_{O2} and RR_{CO2}. Results showed that coating had affected significantly on RR_{O2} and RR_{CO2} of MP pears. It can be seen that for all temperatures considered (4, 10 and 20 °C) the coating is able to reduce the rate of oxygen consumption and rate of carbon dioxide production by about 50 % than control with statistically significant differences ($p \leq 0.05$). The uncoated and coated samples showed values of RR_{O2} of 4 ± 1 and 2 ± 0.5 mol kg^{-1} s^{-1} at 4 °C, 7 ± 1 and 4 ± 1 mol kg^{-1} s^{-1} at 10 °C, and 15 ± 2 and 6±1 mol kg^{-1} s^{-1}at 20 °C, respectively with statistically significant difference ($p \leq 0.05$). Same results were obtained on RR_{CO2}, with statistically significant differences ($p \leq 0.05$) between samples, but coated pears showed lower values of production of CO_2 than uncoated samples, as reported in Figure 2 B.

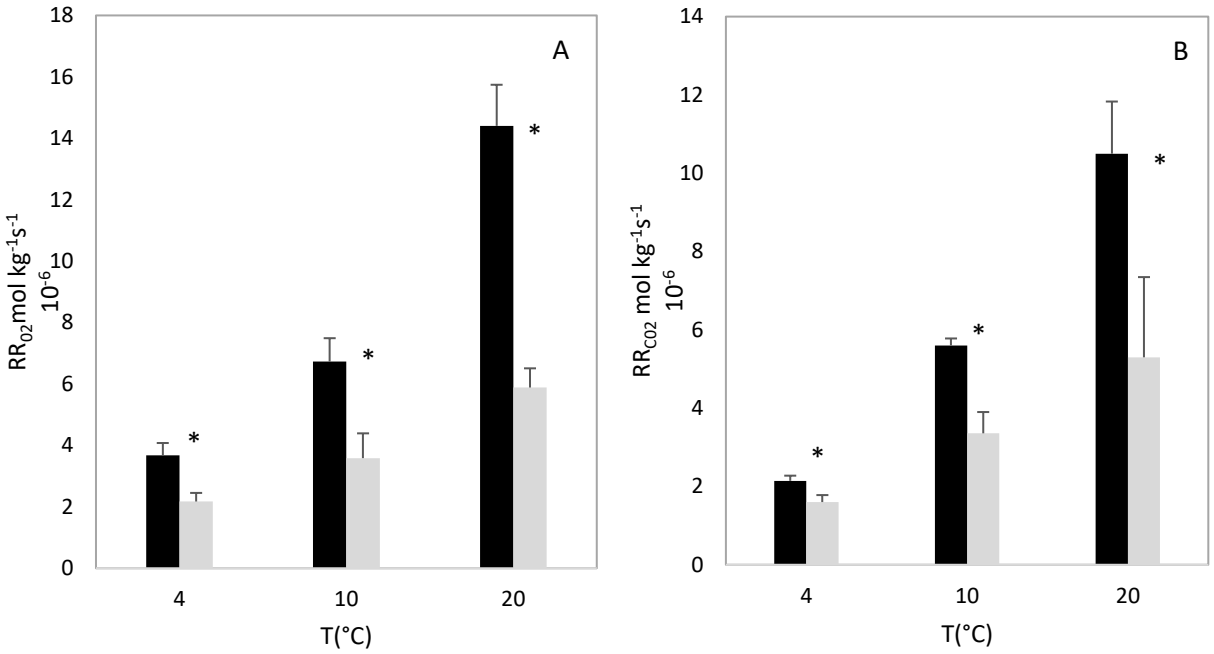


Figure 2. Averages of respiration rate expressed as the rate of oxygen consumption (A) and carbon dioxide production (B) in the air at 4,10 and 15°C of control (●) and active (●) MP pears. Statistically significant differences between the treatments are reported with asterisks (*) ($p \leq 0.05$).

These results, therefore, suggest that the coating preserved respiration rate of pears, as observed by (Lin et al., 2008); in the specific coating developed with chitosan significantly ($p < 0.01$) reduced about 70 % respiration rate compared than control pears. In addition, increasing the storage temperature shows an increase in oxygen consumption and an increase in carbon dioxide production. Thus, the Arrhenius equation expressing the dependence of the reaction rate on temperature and activation energy (Ea) can be measured (Figure 3). Values of Ea were 58.37 kJ mol^{-1} for O_2 and 48.08 kJ mol^{-1} for CO_2 for the control sample. In the case of the edible coated sample, these values are 60 and 67 kJ mol^{-1} respectively. Fitting of the Ln of RR_{O2} and RR_{CO2} depending on 1/T describes the phenomenon well, with a high R^2 value (0.98-0.99).

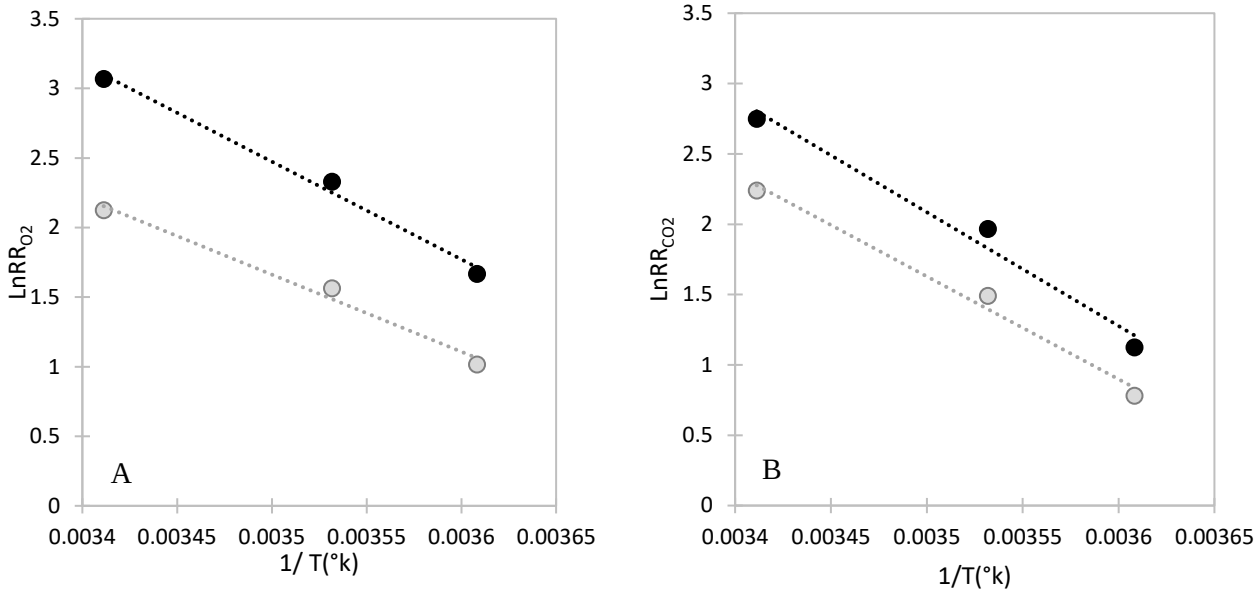


Figure 3. Reaction rate constant of temperature of RR_{O2} (A), RR_{CO2} (B) of control (●), and active(●) MP pear as affected by storage temperature on a log-lin scale

Table 1 are reported the values of RQ of samples stored at 4, 10 and 20°C with and without coating. Ranges of RQ are between 0.6 and 0.9 for the samples with and without coating. Theoretically, the respiration quotient at the cellular level has a value close to 1 when no fermentation occurs in the cell (Lammertyn et al., 2003). However, when the gas exchange of a pear can be determined in a closed system, the respiration quotient, RQ pear, typically has a value between 0.7 and 0.8 (de Wild & Peppelenbos, 2001), suggesting that more O_2 has been consumed than CO_2 has been produced. Lammertyn et al., (2001) found an RQ pear of 0.7-0.9 for respiration measurements on intact pears.

Table1. RQ of MP pears with and without coating stored at 4, 10 and 20°C.

	RQ	
T(°C)	Control	Active
4	0.6	0.7
10	0.8	0.9
20	0.7	0.9

3.4 Effect of coating on storage condition

The results showed that the interaction between independent factors, such as time, temperature, relative humidity and treatments did not affect significantly the weight loss, colour parameters, TSS, antioxidant capacity, total polyphenols content and vitamin C content, whereas there were statistically significant ($p \leq 0.05$) interaction for ΔE, pH, TA and firmness variables chemical-physical and nutritional properties (Table 2).

Table 2. Interaction between dependent and independent parameters of MP pears

	Variables	F	Sign. (p ≤ 0.05)
RH* Treatment* Temperature* time	weight loss	0.02	0.87
	L*	0.33	0.58
	a*	0.75	0.39
	b*	0.24	0.63
	ΔE	2.7	0.11
	pH	0.88	0.35
	TSS	0.00	0.99
	TA	2.5	0.13
	Firmness	0.51	0.48
	Antioxidant capacity	0.06	0.81
	Total polyphenols content	0.12	0.72
	Vitamin C	0.10	0.6

3.4.1 Chemical physical properties

Figure 4 shows the values of weight loss over time for the control and coated sample at the two temperatures and relative humidity considered. After 9 days of storage at 20 °C and 95% RH the mean values were 0.2 ± 0.04% and 0.31 ± 0.1 of the control (Figure 4 A) and active (Figure 4 B) samples respectively, with statistically significant differences during storage time ($p \leq 0.05$). Similar results were obtained for samples stored at 10 °C at 95 %RH (Figure 4 B). Statistically significant difference ($p \leq 0.05$) was reported after 5 and 9 days of storage for the control and active sample. Figure 4 C shows the weight loss of pears over time at 20 °C at 70 % RH. In particular, value increase of 4.9 ± 0.3% for the control sample and 4.3 ± 0.3% for the active sample after 15 days of storage. Similar data were also obtained at 10 °C at 70 % RH (Figure 4 B). Values increased with significant differences over time ($p \leq 0.05$). Indeed, after 30 days of storage, weight loss was 4.3 ± 0.3 % for the control sample and 4.5 ± 0.4 % for the active sample.

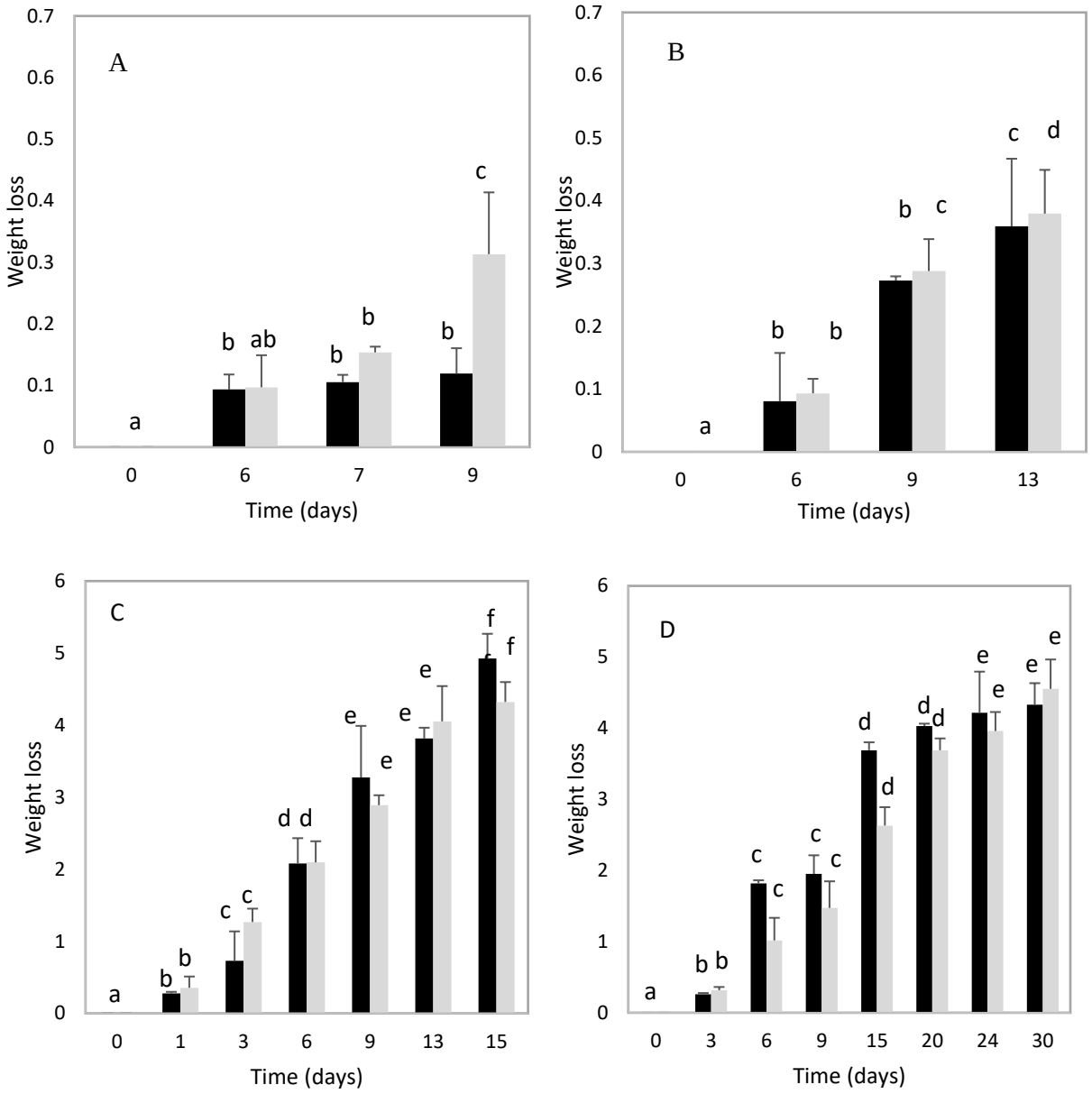


Figure 4. Weight loss of control (■) and active (■) of MP pears stored at 20°C 95 % RH for 9 days (A), at 10°C 95 % RH for 13 days(B), 20°C 70%RH for 15 days (C) and 10°C 70%RH for 30 days(D). Statistically, significant differences are reported with uppercase letters ($p \leq 0.05$).

Comparing the values of the samples stored at the two different relative humidity, it can be seen that as the RH increases there is a decrease in weight loss. Paired samples T-test did not show statistically significant differences between treatments, so the edible coating applied does not affect this quality index. Other authors reported the negative effect of coating on the MP pears, as demonstrated by Kowalczyk et al., (2017); coated fruits showed a significantly higher weight loss than uncoated ones. However, coating can be preserving weight loss of MP pears, as reported by de Moraes et al., (2012). They found that samples coated with 0.5 % carrageenan and 2 % alginate had a lower weight loss than the control. However, this could be due to external storage conditions and not because the coating

does not affect this index.

The values of L*, a* and b* of MP pears with and without coating are reported in Table 3. Control sample stored at 20 °C (70 and 95% RH), compared with the coated samples, showed an increase of the parameters L and b* while the parameter a * (green) was constant during storage. The change in peel colour in ripening pears is due to chlorophyll degradation and carotene accumulation, as reported by Chitarra et al (2005). In the coated sample, on the other hand, an increase in the L parameter and the b* parameter was observed while the a* parameter appears to be variable during storage. However, samples stored at 10 °C (70 and 95 % RH) the parameter L for the active sample decreases more slowly than the control sample, but there were no statistically significant differences ($p \leq 0.05$) between the two samples. The values of a* for the control sample increased over time; whereas parameter b* for the coated samples showed an increment after 5 days of storage, however this parameter for control sample showed non-constant trend, but there were no statistically significant differences ($p \leq 0.05$) between uncoated and coated sample. It can be considered that Conference pears do not have a homogeneity surface, and, for this reason, it is difficult to have colour homogeneity.

Table 3. Average value and standard deviation of colour (L*, a* and b*) of MP pears with and without coating stored at different temperatures, RH and times.

				Time (days)										
T (°C)	**%RH**		**Parameters colour**	**0**	**1**	**3**	**6**	**7**	**9**	**13**	**15**	**20**	**24**	**30**
10	**70**	**C**	**L***	74±4 ^{abA}		65±1 ^{aA}	68±2 ^{aA}		71±5 ^{aA}		63±5 ^{aA}	63±2 ^{aA}	66±2 ^{aA}	74±3 ^{bA}
			a*	-4±1 ^{aA}		-1±1 ^{bA}	-2 ± 1 ^{bA}		4 ± 1 ^{cA}		5±2 ^{cB}	5±1 ^{cB}	1±1 ^{bA}	5±2 ^{cA}
			b*	46±3 ^{bA}		48±1 ^{aB}	49±2 ^{bB}		51,22±4,05 ^{bA}		48±3 ^{bA}	47±3 ^{bA}	49±2 ^{bA}	55±1 ^{cB}
		A	**L***	74±3 ^{aA}		69±5 ^{aB}	72±5 ^{aB}		77±2 ^{aB}		73±4 ^{aB}	72±5 ^{aB}	73±4 ^{aB}	72±2 ^{aB}
			a*	-4±1 ^{aA}		-2±1 ^{abA}	-2±1 ^{abA}		1±1 ^{cdeA}		2±2 ^{bcA}	2±1 ^{deA}	1±1 ^{bcdA}	5±2 ^{eB}
			b*	46ì±3 ^{aA}		46±3 ^{aA}	47±5 ^{aA}		52±2 ^{aA}		47±3 ^{aA}	48±4 ^{aA}	47±4 ^{aA}	51±3 ^{aA}
20		**C**	**L***	74±3 ^{bA}	72±2 ^{abA}	71±3 ^{bA}	71±3 ^{abA}		72±2 ^{abA}	72±3 ^{abA}	70±1 ^{bA}			
			a*	-4±1 ^{aA}	-4±1 ^{aA}	-1±1 ^{bA}	1±1 ^{bA}		1±1 ^{bcB}	2±1 ^{cdB}	3±1 ^{aB}			
			b*	46±3 ^{aA}	47±1 ^{aA}	47±2 ^{aA}	51±1 ^{cB}		51±2 ^{bB}	55±2 ^{bc}	54±1			
		A	**L***	74±4 ^{cA}	72±1 ^{bcA}	71±2 ^{aA}	72±2 ^{cB}		69±2 ^{abA}	72±3 ^{cA}	70±2 ^{aA}			
			a*	-4±1 ^{aA}	-4±2 ^{aA}	-1±1 ^{aA}	-2±1 ^{aA}		-3±2 ^{aA}	-3±2 ^{aA}	-2±3 ^{aA}			

			b*	46±3 aA	47±1 bA	48± 2 bA	44±1 abA		48±2 abA	55±2 abA	50±3 abA
10		**C**	**L***	66 ± 2 abA			67±3 bA		67±2 aA	69±2 bA	
			a*	- 3±2 a A			- 1±3 ab A		-3±3 bA	1±4 ab A	
			b*	38±2 aA			48±2 cB		44±7 bA	49±2 cA	
		A	**L***	66±3 aA			67±3 aA		68±3 aA	67±4 aA	
			a*	- 3±2 a A			- 1±1 b B		-5±3 abA	- 2±2 ab A	
			b*	38±2 aA			47±3 bA		47±4 bA	48±4 bA	
20	**95**	**C**	**L***	66±3 aA		69± 2 aB		68±3 abA	72±3 cB		
			a*	- 3±2 a A		- 2±1 a A		- 1±1 a B	-3±3 aA		
			b*	38±2 aA		45± 1 bB		45±5 bA	53±3 cB		
		A	**L***	66±2 aA		57± 2 bA		72±2 bcB	69±2 cA		
			a*	- 3±2 a A		1±1 a bB		- 5±1 b A	-2±1 cA		
			b*	38±2 aA		39± 2 bA		48±3 bA	45±1 abA		

Statistically significant differences over time are reported with uppercase letters ($p \leq 0.05$), whereas statistically significant differences for each temperature and time are reported with lowercase letters ($p \leq 0.05$).

ΔE of the pear changed gradually during storage, due to an increase in respiration rate and enzymatic processes, including browning or other reactions (Dai et al., 2020). In the specific, in Figure 5 A values of samples stored at 20 °C at 95% RH are shown. ΔE increased over time by 17% and 7% after 9 days of storage for the control and active sample respectively. Only at time 9 days the active and control samples were significantly statistically different ($p \leq 0.05$). Similar results were obtained for samples stored at 10 °C at 95% RH. However, no statistically significant differences emerged between the samples with and without coating after 12 days. By increasing the storage time and decreasing the temperature, the coating showed a protective effect on the change in ΔE. In fact, samples stored for 15 days at 20°C at 70% RH (Figure 5 C) showed an increment of ΔE of 12 % and 8%. After 6 days of storage, there were statistically significant differences ($p \leq 0.05$) between the control and active pears, except for day 13 where there were no significant differences between treatments. By storing the samples at 10 °C at 70% RH for up to 30 days, the ΔE value increased by 14 and 12% for control and active samples respectively. There were statistically significant

differences ($p \leq 0.05$) between treatments at times 3, 15 and 20 days. ΔE values increased with statistically significant differences ($p \leq 0.05$) over time for all samples.

The results obtained for colour are in agreement with other studies. Specifically, Dai and colleagues (2020) observed ΔE increased for the control and active samples, while L decreased and the b* value remained constant. de Moraes et al., (2012) observed that control samples showed greater variation in the parameters a* and b* than coated samples; the control sample showed more yellow colour than the active sample at the end of the storage period. They also observed that for the sample with 2 % alginate, the values of L* decreased significantly ($p \leq 0.05$) during storage, and the values of a * and b * varied significantly ($p \leq 0.05$), but this variation was less than that observed for the control.

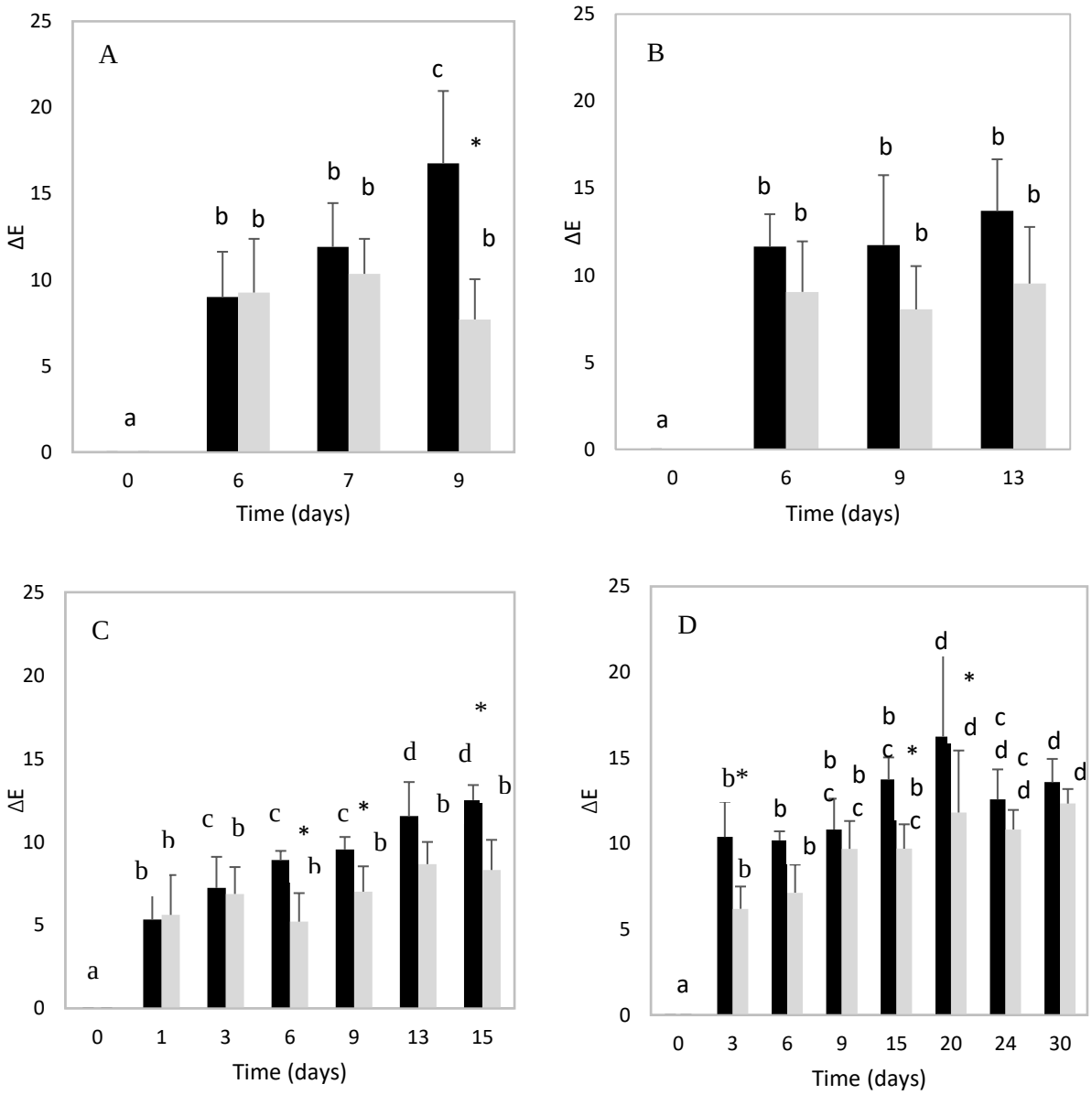
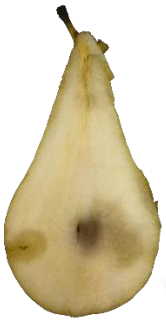


Figure 5. ΔE of control (■) and active (▒) MP pears stored at 20°C 95%RH (A), at 10°C 95%RH (B), 20°C 70%RH (C) and 10°C 70%RH (D). Statistically significant differences over time are reported with uppercase letters ($p \leq 0.05$) whereas

statistically significant differences between the treatments, for each temperature and time are reported with * ($p \leq 0.05$).

As shown in the images (Table 4) it is possible to evidence of the capacity of the coating to preserve the green colour of the pears, due to the high amount of chlorophyll. There is a visible difference between the control pears, which were yellow in colour, with yellowed flesh and numerous lesions compared to the pears with the coating.

Table 4. Images of pears stored at 10 and 20 °C at 70 %RH over time.

	Control		**Active**	
Time(days)	**10°C**			
0				
15				
30				
	20°C			

0				
6				
15				

Figure 6 reported the values of firmness of MP pears. In the specific, Figure 6 A reported the values of the samples stored at 20 °C at 95% RH; after 9 days firmness decreased by 20 and 5% for control and active samples respectively, showing a slowdown of this parameter decrease of 42 % of samples with coating compared to the sample control. Moreover, paired samples t-test analysis showed significant statistical differences ($p \leq 0.05$) after 7 days of storage. The same results were obtained for samples stored at 10 °C at 95% RH after 12 days (Figure 6 B). Good results were obtained also for the samples stored at 70% RH. In fact, firmness decreased by 85 and 60% for the samples stored at 20 °C (Figure 6 C) after 15 days for uncoated and coated pears showing statistically significant differences ($p \leq 0.05$) between the treatments as early as time 1. However, active coating preserved the firmness of samples by about 60 % comparing control pear. Same results were obtained for samples stored at 10 °C for 30 days (Figure 6 D). The paired t-test showed statistically significant differences ($p \leq 0.05$) between the control and active sample.

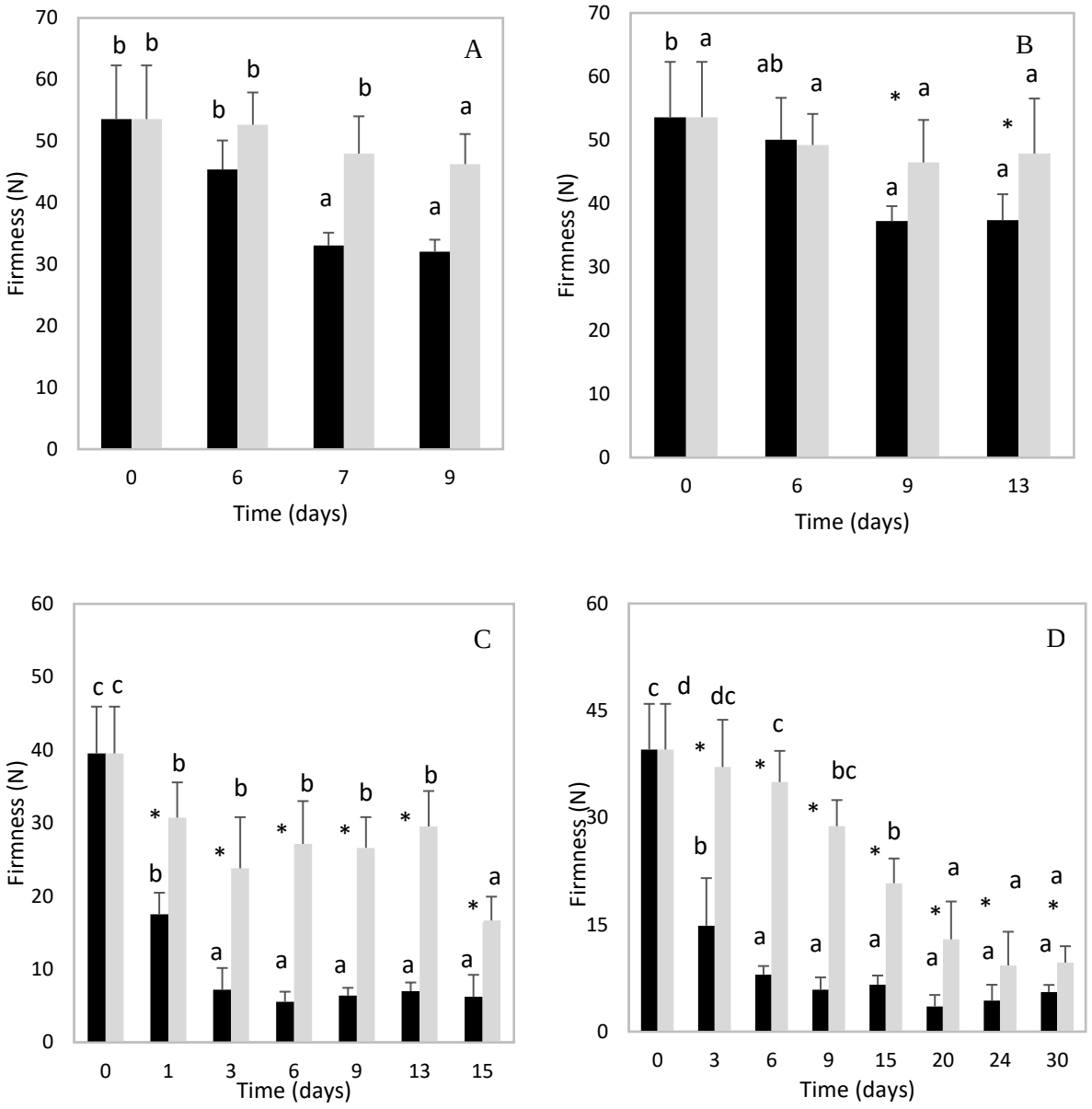


Figure 6. Firmness (N) of control (■) and active (■) MP pears stored at 20°C 95%RH (A), at 10°C 95%RH (B), 20°C 70%RH (C) and 10°C 70%RH (D). Statistically significant differences over time are reported with uppercase letters ($p \leq 0.05$) whereas statistically significant differences between the treatments, for each temperature and time are reported with * ($p \leq 0.05$).

With the obtained results, it is possible to say that the coating, both at 10 and 20 °C (70 and 95 % RH) slowed down the decrease in firmness by about 50% than the control sample, preserving firmness for MP pears. Furthermore, the results are in agreement with Dai et al., (2020); in which they showed the preservation effect in the firmness of pears coated with a coating produced with starch nanocomposite and reinforced with starch nanocrystals than to control samples.

Table 6 reported the average values of pH, TA and TSS over time for control and coated samples. pH and TA decreased during storage time, whereas TSS increased for coated and uncoated samples. MP

pears stored at 10 and 20 °C at 95% RH showed no statistically significant differences between the control and active sample for the pH; but, the coating preserved the pH of samples stored at 70% RH. In fact, the active coating slows down the increase of pH after 3 days at 20 °C and after 9 days at 10 °C at 70% RH with statistically significant differences ($p \leq 0.05$) between treatments. TSS increased for coated and uncoated samples, however for samples stored at 95% RH the differences were not statistically significant; while for the samples stored at 70% RH, t-test analysis showed statistically significant differences ($p \leq 0.05$) between the control and active sample after long storage time. In contrast to TSS, TA decreased during the storage time of MP pears with and without coating (Table 6). The control sample showed a decrement of TA of 20 %, whereas coated sample showed a decrease of 27 %. The control and active samples were not statistically different, in fact after 12 days of TA was about 0.22 %. Also, for samples stored at 10 and 20 °C at 70% RH, TA decreased during storage. After 6 days of storage at 20 °C, the coating preserved the decrease of TA by about 15 % with statistically significant differences ($p \leq 0.05$) compared to the control sample. TA decreased by about 35% after 30 days of storage for the two treatments at 10 °C. However, the differences between the control and active sample were statistically significantly different ($p \leq 0.05$) at times 3, 9 and 15 days. Coating could have an effect on microbial growth that limits the acidification and fermentation of the samples (de Moraes et al., 2012). The biopolymer coating, generally, applied on MP pears slows down the pH change (Cruz et al., 2015). These authors reported that coating developed with 3% of candelilla wax, 4% gum Arabic, 0.15 jojoba oil and 0.015% pomegranate polyphenols preserved pH than to the control sample.

Table 6. Averages and SD of pH, °Brix and TA of MP pear with and without coating stored at 10 and 20°C at 70 and 95%RH.

Parameters	Treatments	T(°C)	RH (%)	Time (days)										
				0	1	3	6	7	9	13	15	20	24	30
pH	C	20	70	5.3± 0.1dA	5.3± 0.1dA	4.7± 0.3dA	4.6±0. 1aA		4.4±0. 1aA	4.3±0. 1aA	4.3±0. 1aA			
	A			5.3±0. 1dA	5.3±0. 1dA	5.1±0. 1bA	5.2±0. 1cB		5.3±0. 1aB	5.1±0. 1aB	5.1±0. 1aB			
	C		95	5.2±0. 1b			5.2±0. 1ab	5.1±1 a	5.1±0. 1a					
	A			5.2±0. 1b			5.2±0a	5.2±0 a	5.1±0 a					
	C	10	70	5.3±0. 1eA		5.1±0. 1dA	4.9±0. 1cA		4.5±0. 1aA		4.7±0. 1bA	4.9±0. 1cA	4.9±0. 1cA	5.1±0. 1dA
	A			5.3±0. 1cA		4.9±0. 2aA	4.9±0. 1abA		4.9±0. 1abA		5±0.1a bA	4.9±0. 1abA	5±0.1a bA	5.1±0. 1abA

	C		9	5.2±0.1b			5.1±0b			5.1±0.1ab	5±0.1a				
	A		5	5.2±0.1b			5.1±0.1ab			5.1±0.1a	5±0.1a				
°Brix	C		7	12±1aA	14±1bcA	14±1dB	13±1cdA			13±1cdA	13±0bA	13±1bA			
	A	20	0	12±1aA	14±1bcA	13±1bcA	13±1dbA			14±1cA	14±1bcA	14±1bcA			
	C		9	14±1aA			14±1aA	14±1aA		14±1aA					
	A		5	14±1aA			14±1abA	14±1abA		14±1bB					
	C		7	12±1aA		12±1aA	12±1aA			14±1bA		13±1bA	14±1cA	15±1bA	15±1bA
	A	10	0	12±1aA		14±1bB	14±1bB			13±1bA		14±1cB	15±1dB	15±1cdB	15±1dB
	C		9	14±1aA			14±1aA			14±1aA	14±1bB				
	A		5	14±1aA			14±1aA			14±1aA	15±1aA				
TA (% malic acid)	C		7	0.16±0.01eA	0.14±0.00dA	0.13±0.02cA	0.12±0.00aB			0.12±0.01bB	0.11±0.00aB	0.12±0.01bB			
	A	20	0	0.16±0.01eA	0.13±0.01dA	0.11±0.01cA	0.08±0.03aA			0.10±0.01bA	0.08±0.01aA	0.10±0.01bcA			
	C		9	0.29±0.02bA			0.28±0.02bA	0.24±0.01aB		0.23±0.1aB					
	A		5	0.29±0.02bA			0.28±0.04bA	0.21±0.01aA		0.20±0.00aA					
	C		7	0.16±0.01eA		0.11±0.02dA	0.09±0.02abA			0.08±0.00aA		0.08±0.00aA	0.10±0.02bcA	0.10±0.01bcA	0.10±0.01cdA
	A	10	0	0.16±0.01cA		0.14±0.00cA	0.10±0.01bA			0.09±0.00aB		0.10±0.01aB	0.09±0.00aA	0.12±0.02bA	0.10±0.01cA
	C		9	0.29±0.02cA			0.28±0.02cA			0.25±0.01bA	0.22±0.01aA				
	A		5	0.29±0.02cA			0.21±0.01bA			0.19±0.02bA	0.19±0.02aA				

Statistically significant differences over time are reported with uppercase letters ($p \leq 0.05$), whereas statistically significant differences for each temperature and time are reported with lowercase letters ($p \leq 0.05$).

These results are in agreement with de Moraes et al., (2012) indeed, during storage they observed a significant ($p \leq 0.05$) increment of pH of coated and uncoated samples, but samples with coating, developed with alginate and carrageenan, differed significantly ($p \leq 0.05$) from the control sample for the evaluated period. Moreover, the increment of TSS is due to the ripening of the fruit during storage (Kowalczyk et al., 2017). On the other hand, the coating slowed the decrease of malic acid, and these results are in agreement with Kowalczyk and colleagues (2017) and Dai et al., (2020). Also,

for these authors, the coating developed with polysaccharide and wax was able to slow the decrease of TA.

3.4.2 Nutritional properties

Table 7 reported the values of nutritional properties of minimally processed pears.

The antioxidant capacity of samples stored at 20 °C at 95% RH (Table 7) showed a decreasing trend of about 55% over time for all treatments. However, antioxidant capacity of samples stored at 10 °C at 95 % RH, decreased after 12 days by 70 % and 40 % for control and active samples respectively, showing a protective effect of coating with statistically significant differences ($p \leq 0.05$) since time 6 days respect to control samples. The coating applied to the pears showed to preserve samples at both 10 and 20 °C at 70% RH, as reported Table 7. Antioxidant capacity decreased by about 75 and 50% for uncoated and coated samples at 10 and 20 °C. However, paired t-test showed that there were statistically significant differences between the treatments. Active coating applied on MP pears was able to slow down about a 50% decrease of antioxidant capacity concerning control samples. However, ANOVA analysis reported statistically significant differences ($p \leq 0.05$) during storage time for all samples. Also, in other studies coating was able to preserve the antioxidant capacity of minimally processed pears, as reported by Kou et al., (2014) and Lin et al., (2008). They found that coating developed with chitosan, maintained the quality of pears, which was associated with the antioxidant capability of the fruit.

Table 7 reported values of total polyphenols content of minimally processed pears at 10 and 20°C at 70 and 95 % RH. During storage time total polyphenols content decreased 64% after 9 and 13 days at 20 and 10°C respectively at 95 % RH, showing no statistically significant differences between coated and uncoated samples. On the other hand, coating applied on pears stored at 20 and 10 °C at 70% RH showed to preserve decrease in polyphenols compared with the control sample. Coating was able to slow down decrement of total polyphenols content by about 15 and 50% for samples stored at 20 and 10°C, with statistically significant differences ($p \leq 0.05$) after 3 days of storage. However, ANOVA analysis showed that the samples differed statistically ($p \leq 0.05$) for each temperature, RH and treatment during storage time.

Table 7. Average value and standard deviation of TAC, TPC and vitamin C of MP pears with and without coating stored at different temperatures, RH and times.

Parameters	Treatments	T(°C)	RH (%)	Time (days)											
				0	1	3	5	6	7	9	13	15	20	24	30
TAC	C	20	70	1.9±0.2cA	0.81±0.0bA	0.81±0.19bA		0.75±0.14bA		0.69±0.2bA	0.62±0.02bA	0.55±.17aA			
	A			1.9±0.2dB	1.9±0.14dB	1.9±0.16dB		1.5±0.3cB		1.09±0.4bA	0.89±0.01aA	0.82±0.01aA			
	C		95	1.8±0.1cA				1.4±0.2bA	1.3±0.3bA	0.8±0.5aA					
	A			1.8±0.1cA				1.4±0.3bA	1.3±0.2bA	1.05±0.2aA					
	C	10	70	1.9±0.2cAdA		0.9±0.09cA		0.85±0.25bA		0.65±0.15bA		0.64±0.02bA	0.46±0.04aA	0.42±0.05aA	0.40±0.05aA
	A			1.9±0.2eA		1.9±0.15dB		1.78±0.02cB		1.59±0.07bB		0.93±0.23aB	0.88±0.03aB	0.83±0.04aB	0.83±0.05aB
	C		95	1.8±0.1cA			1.5±0.1bA			0.53±0.1aA	0.55±0.02aA				
	A			1.8±0.1cA			1.3±0.1bB			1.07±0.07aB	1.01±0.12aB				
TPC	C	20	70	61±6eA	52±3dA	32±1cA		27±7bA		11±5aA	15±4aA	14±3aA			
	A			61±6cA	57±3cB	40±4bB		29±4aB		25±6aB	26±5aB	24±1aB			
	C		95	54±5cA				30±3bA	17±3aA	17±1aA					
	A			54±5cA				28±2bA	19±3aA	19±1aA					
	C	10	70	61±6dA		38±4cA		28±1bA		26±1aA		26±3aA	23±1aA	23±1aA	26±3aA
	A			61±6cA		54±3bB		39±1aB		36±2aB		37±1aB	38±3aB	37±1aB	37±1aB
	C		95	54±5cA			29±3bA			26±4bA	20±2aA				
	A			54±5cA			25±4bA			23±1bA	16±1aA				
VITC	C	20	70	0.13±0.01dA	0.05±0.01cA	0.04±0.00bA		0.02±0.00aA		0.02±0.01aA	0.02±0.01aA	0.02±0.01aA			
	A			0.13±0.01eA	0.07±0.00dB	0.05±0.00cB		0.04±0.00bA		0.03±0.00aA	0.03±0.00aA	0.03±0.00aA			
	C		95	0.13±0.01bA				0.02±0.00aA	0.02±0.01aA	0.01±0.00aA					

A			0.13± 0.01d A			0.04± 0.00c B	0.02± 0.00b A	0.01± 0.00a A						
C	10	70	0.13± 0.01g A	0.08± 0.00f A		0.05± 0.01e A		0.04± 0.00d A		0.03± 0.01c A	0.02± 0.00b A	0.01± 0.00a A	0.01± 0.00a A	
A			0.13± 0.01g A	0.11± 0.01f B		0.08± 0.01e B		0.06± 0.01d B		0.05± 0.00c B	0.04± 0.00b B	0.03± 0.00a B	0.03± 0.00a B	
C		95	0.13± 0.01c A		0.02± 0.01b A			0.01± 0.01a A	0.01± 0.02a A					
A			0.13± 0.01d A		0.04± 0.01c B			0.03± 0.00b B	0.01± 0.01a A					

Statistically significant differences over time are reported with uppercase letters ($p \leq 0.05$), whereas statistically significant differences for each temperature and time are reported with lowercase letters ($p \leq 0.05$).

It is demonstrated in the literature that applied coating, e.g., developed with chitosan, has preserved about 50% the decrease total polyphenols compared with the control sample, as reported by Kou et al., (2014). However, content polyphenolics have previously been shown to be present at higher levels in the peel than in the flesh, the ratio depending on the cultivar (Wolfe et al., 2003).

Table 7 are reported results of vitamin C content of pears. Samples stored at 20 °C and 95 % RH showed that vitamin C decreased by 85 % with statistically significant differences ($p \leq 0.05$) only at time 5 days between coated and uncoated. Instead, vitamin C content decreased by about 90 and 75% for control and active samples after 13 days, reporting statistically significant differences ($p \leq 0.05$) at times 7 and 9 (Table 7). Furthermore, coating applied on pears stored at 20 and 10 °C at 70% RH showed to preserve vitamin C content by about 25% compared with the control sample, with statistically significant differences ($p \leq 0.05$) after 1 day of storage. The vitamin C content of the control pears was reduced by 50% after 3 days, while the sample with coating after 9 days at 10 °C and after 6 days at 20 °C, showed the capacity of active coating to preserve MP pears. ANOVA analysis showed that the samples differed statistically ($p \leq 0.05$) for vitamin C content from each temperature, RH and treatment during storage time.

Good effect of coating, developed with chitosan and ascorbic acid, was reported by Lin et al., (2008) on MP pears. Indeed, the active coating was able to preserve the fruit, by about 30%, then control samples. However, the vitamin C content depends on the cultivar, picking, growing location and ripeness of the fruit.(Galvis Sánchez et al., 2003; Veltman et al., 2000). Previous studies have shown lower vitamin C content than those found in the present work (7.2 mg 100 g^{-1} for Conference pears and 6.1–8.2 mg 100 g^{-1} for Rocha pears (Veltman et al., 2000).

3.4.3 Microbiological analysis

The results for total mesophilic bacterial counts and yeasts and moulds are shown in Table 8. In general, for both microbial groups analysed, no significant differences are evident between treated pears and control samples during storage.

Table 8. Total mesophilic and yeast and mould bacterial counts on pear samples

Samples	Sample Yeast and mould	Total bacterial count at 30 °C
	Log UFC/ml (SLZ) and Log UFC/cm^2 (pears)±SD	
SLZ0	**< 1**	**4.54±0.08**
SLZ13	**2.16±0.22**	**2.78±0.11**
SLZ26	**2.18±0.14**	**3.38±0.11**
PC6	**1.45±0.25**	**0.7±0.35**
PA6	**1.85±0.11**	**0.7±0.44**
PC15	**1.20±0.21**	**2.41±0.22**
PA15	**2.86±0.15**	**3.92±1.2**
PC21	**2.11±0.19**	**1.36±0.65**
PA21	**1.52±0.02**	**2.48±0.20**

4 Conclusion

The coating applied on MP pears showed good properties of ripening and senescence. Indeed, coating reduced 50 % O_2 consumption and CO_2 production to control samples at 4, 10 and 20 °C. Moreover, active coating was able to preserve the colour and firmness of the product. Regardless of the storage temperature and humidity, coating showed to preserve firmness of MP pears by about 50 % than uncoated samples. The coating, due to the effect of propyl gallate, was able to slow down the decrease in the selected nutritional parameters; it preserved the antioxidant capacity by 50 % and the total polyphenol content by 40 %. The coating developed with protein, lipid, and polysaccharides was able to preserve the nutritional properties of minimally processed pears. In addition, storage temperature and relative humidity played a key role. In fact, stored at 10°C and 70 % RH were able to preserve about 30 % of the antioxidant capacity and total polyphenols compared to samples stored at 20°C. In addition, relative humidity also affected the effect of the coating; in fact, the coating was unable to preserve the vitamin C content at 20°C, unlike the samples stored at 70 % RH where the active coating was able to preserve 25 % of the vitamin C content compared to the uncoated sample. In conclusion, coating applied to pears significantly preserved firmness, AC, TPC and vitamin C content., but best conditions to highlight the effect of the coating on pears are 10°C at 70 % RH. In future other authors could be studied the effect of coating using kinetic modelling on firmness, antioxidant capacity, total polyphenols content and vitamin C.

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Chapter 3B

Effect of biopolymer active coating on alteration kinetics of minimally processed pears stored at different temperatures and relative humidity

Abstract

The objectives of this work were: (i) to describe the critical quality changes of MP pears at different storage conditions by kinetic modelling and (ii) to evaluate the effect of storage active coating on alteration kinetics. The samples were treated as reported in the first case study. Quality indices, including physical and nutritional, were measured at set intervals during storage. Experimental data were fitted to the models by least-squares procedure. The results showed that firmness, vitamin C, antioxidant capacity and total polyphenol content were selected as critical quality indices for the product shelf life. Pseudo-first order well described the quality changes of the product over time in the range of temperatures tested. The active coating was able to preserve the firmness of about 64 % and 92% of pears stored at 95% and 70% RH respectively. It preserved the nutritional quality of the products by reducing the antioxidant capacity, total polyphenols content and vitamin C decrease of more than 50% than control samples. Moreover, active coating preserved MP pears, but the preservation effect of active coating was depend on for the RH, indeed pears stored at 70% RH showed better effect than pears stored at 95% RH at both temperatures. In conclusion, kinetic model used is a useful tool for shelf life prediction.

Keywords: blend, nutritional properties, firmness, pseudo-first-order

1. Introduction

Pears with good eating quality have juicy, buttery and melting texture with a good pear flavour (Zerbini, 2002). However, there are two different types of consumers: consumers who like pears which are soft, juicy and sweet (Italian people), and consumers that prefer pears with a crispy and juicy texture to a buttery and melting one (Hoehn et al. 2003). *Conference* pears picked at 4 dates, stored in air or controlled atmosphere (CA) for 5-6 months and ripened at 20 °C were firm immediately ex-store; then they became softer but crisp and juicy, then buttery and melting; finally, the pears became mealy and exhibited an overripe note (Hoehn et al. 2003). Generally, the quality of MPF&V is primarily evaluated with safety, chemical-physical, nutritional and sensorial properties (Ma et al., 2017). Moreover, the production and development of fresh-cut fruits and vegetables must fulfil the following two major aims. First aim is to extend the shelf life of fresh-cut products by keeping them fresh while preserving their nutritional quality, sensory attributes, and food safety aspects. Second aim is to ensure a long enough shelf life of such products that is adequate to make its regional distribution possible (Siddiq et al., 2020, Laurila & Ahvenainen, 2002). Among several chemical-physical parameters, firmness is a key quality attribute because besides being able to be pleasant, it conditions the possibility that other compounds contained in the cell (sugars, acids, volatile substances) can be extracted from the cell with mastication and so can be perceived by the consumers (Zerbini, 2002, Maringgal et al., 2020) To determinate the quality degradation of fruits and vegetables it possible use the kinetics models. Different studies found that using zero, first, or second-order kinetics is possible to determine the quality degradation reactions (Zanoni et al., 2005, Nisha et al., 2005, Giannakourou & Taoukis, 2003; Nisha et al., 2005, Rodrigo et al., 2007). These models fitted well with several food degradation reactions, such as microbial growth (Corradini & Peleg, 2007), antioxidant changes (Muley et al., 2022; Oms-Oliu et al., 2009), browning of orange juice (Manso et al., 2001) vitamin C degradation during thermal treatments of fresh fruits and vegetables (Corradini & Peleg, 2007, Maftoonazad & Ramaswamy, 2019; Muley et al., 2022) and total polyphenols content during storage time (Esua et al., 2019). Moreover, studies are not reported in literature of the use of the kinetics model to study degradation reactions of MP pears with and without active coating. The aims of the four part of my thesis were: (i) to describe the critical quality changes of minimally processed pear at different storage conditions by kinetic modelling and (ii) to evaluate the effect of active coating on alteration kinetics.

2. Materials and Methods

Pear samples were processed as described in the previous section (chapter 3A). Physical-chemical (firmness) and nutrition (antioxidant capacity, total polyphenol and vitamin C content) properties

were evaluated as critical quality indices of minimally processed pears (described in chapter 3A). Nutritional properties (total antioxidant capacity, total polyphenol and vitamin C content) were normalized to the initial values of the fresh sample.

2.1 Mathematical modelling: zero and first-order kinetic models

First-order kinetics, traditionally used to describe degradation reactions in foods, may be generally written as (Giannakourou & Taoukis, 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005):

$$\frac{dQ(t)}{dt} = -kQ^n \qquad Eq.(1)$$

The equation may be integrated easily obtaining the well-known decay functions. In particular, first kinetic order (n=1) the equation is:

$$Q = Q_i e^{-kt} \qquad Eq.(2)$$

Or

$$Q = Qeq + (Q0 - Qeq)e^{-kt} \qquad Eq.(3)$$

where

Q_i is the concentration of the quality index at time zero, *Q(t)* is the concentration of the quality index at the time *t*, *Q(eq)* is the concentration of the quality index at the time at equilibrium, *k* is the rate constant, and n is the kinetic order of the equation. The negative sign is generally referred to as a decrease of quality. However, if the quality indices related to the alteration process increase during storage, the function will have a positive sign.

2.2 Statistical analysis

Linear and non-linear regression were used to estimate the kinetic constants using XLSTAT 16.0 (Addinsoft, France, 2023). The regression coefficients (R^2) and the root mean square error (RMSE) were calculated to evaluate the goodness of the model to describe data. The highest the values of R^2 and the lowest the values of MSE and RMSE, the better the fitting of the models to experimental data. The adequacy of the fitted model was also assessed using an analysis of residuals which permits confirmation the of validity of the assumptions regarding the independence and normal distribution of the errors. Paired t-test analysis was carried out to find the source of the significant differences within the samples examined statistically significant difference was defined at $p \leq 0.05$.

3. Results and Discussions

By using a first kinetic model, the quality attributes studied have shown significant changes over time. For the evaluation parameter, firmness, antioxidant capacity, total polyphenol content and Vitamin C, first order showed good correlation coefficients. In particular, R^2 and RMSE values ranged between 0.74 to 0.99 and 0.001 and 0.16, respectively, indicating the high ability of the model

chosen to fit experimental data. In Figure 1 are reported values of firmness of control and active samples stored at 20 °C and 10 °C at 95 % and 70 % RH. A good correlation was observed between experimental and predicted models, indeed high values of R^2 ranging from 0.75 to 0.98 and RMSE of 0.001 and 0.012 (Table 1) were obtained using a pseudo-first-order kinetic model. Comparing the results obtained with data in literature, pseudo-first-order is better to describe how decreased firmness during storage time. Maringgal and colleagues (2020) studied papayas with and without edible coating for 12 days at 12 °C and they reported that first-order kinetic model was found to be the best to estimate experimental firmness data, showing a correlation coefficient of 0.84. Similar results were found for tomatoes during a storage period of up to 20 days at various temperatures (van Dijk et al., 2006). Moreover, Maftoonazad & Ramaswamy, (2005) during storage of avocados at 5, 10 and 20 °C with and without coating, developed with pectin-based emulsion, for various times textural softening followed a first-order kinetic model (R^2 0.94). Furthermore, the firmness of lime fruits stored at 10 and 25 °C decreased with pseudo-first-order during storage time with an R^2 of 0.93 (Maftoonazad & Ramaswamy 2019).

Table 1. Goodness of fitting of pseudo-first-order model used to estimate firmness of MP pears with and without coating at different temperatures and RH over time.

			Firmness		
Samples	%RH	T (°C)	MSE	RMSE	R^2
Control	95	10	0.007	0.081	0.925
		20	0.003	0.057	0.938
Active		10	0.001	0.027	0.963
		20	0.001	0.038	0.985
Control	70	10	0.012	0.108	0.805
		20	0.019	0.139	0.808
Active		10	0.004	0.063	0.984
		20	0.010	0.099	0.749

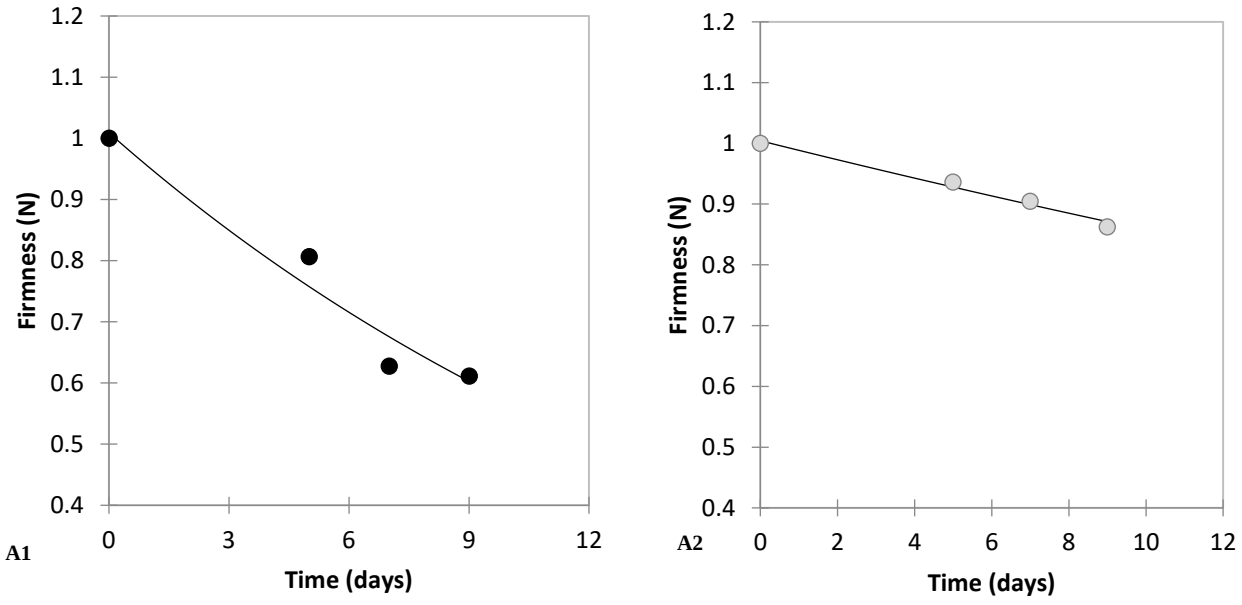
In Table 2 are reported kinetic constant of firmness. The values were -0.06±0.01 and -0.02±0.01 day^{-1} for samples stored at 20 °C at 95% RH uncoated and coated respectively, whereas values of constant kinetics of samples stored at 10 °C were -0.04±0.01 and -0.02±0.00 day^{-1} uncoated and coated respectively. However, by decreasing the relative humidity at 70% RH the values of constant kinetics increased. As reported in Table 2, samples stored at 20 °C without and with coating showed a value of k of -0.63 ± 0.05 and-0.03± 0.01 day^{-1} respectively, while the values of samples stored at 10 °C were -0.29 ± 0.03 and -0.04 ± 0.00 control and active samples, respectively. Active coating applied on MP pears was able to preserve firmness than control products of about 57%, 71%, 84% and 95% stored at 10 and 20 °C at 95% RH and 10 and 20 °C at 70% RH respectively. However, t-test analysis

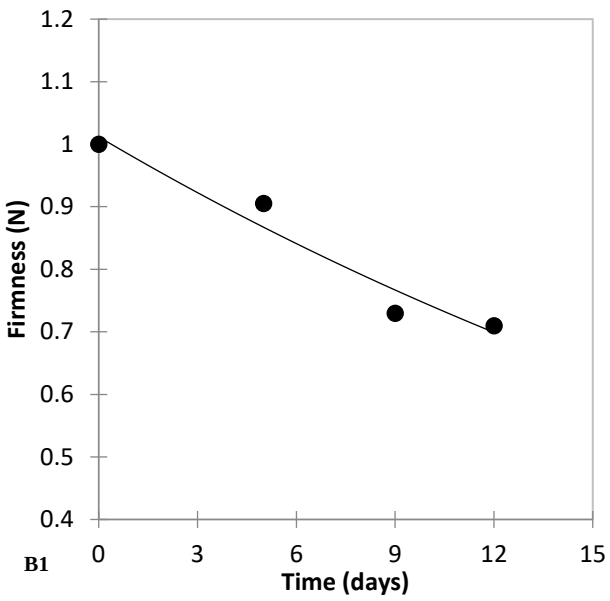
showed statistically significant differences ($p \leq 0.05$) for the firmness of MP pears between the kinetics constant for different treatments for each temperature and each relative humidity. Moreover, 70% RH is better than 95% RH to store the MP pears with active coating because relative humidity showed to preserve the firmness of the fruit at about 88% than samples stored at 95 % RH, with an average value of 64 %.Previous studies on tomatoes (van Dijk et al., 2006), pectin-coated avocados (Maftoonazad & Ramaswamy, 2005) and lime fruits (Maftoonazad & Ramaswamy, 2019) observed similar results.

Table 2. Constant kinetics of firmness, antioxidant capacity, total polyphenol content and vitamin C content of control and active MP pears stored at 10 and 20°C at 70 and 90% RH.

			Firmness	**TAC**		**TPC**		**Vitamin C**	
%RH	T (°C)	Samples	k (day $^{-1}$)	k (day $^{-1}$)	Q_{eq}	k (day $^{-1}$)	Q_{eq}	k (day $^{-1}$)	Q_{eq}
95	10	Control	-0.04±0.01^{a}	-0.10±0.02^{a}	0.25	-0.28±0.03^{a}	0.38	-0.39±0.01^{a}	0.10
		Active	-0.02±0.00^{b}	-0.05±0.01^{b}	0.25	-0.24±0.01^{a}	0.38	-0.31±0.01^{b}	0.10
	20	Control	-0.06±0.01^{a}	-0.07±0.01^{a}	0.25	-0.10±0.01^{a}	0.38	-0.37±0.02^{a}	0.10
		Active	-0.02±0.01^{b}	-0.06±0.01^{a}	0.25	-0.20±0.03^{a}	0.38	-0.21±0.01^{b}	0.10
70	10	Control	-0.29±0.03^{a}	-0.4±0.02^{a}	0.25	-0.36±0.0^{a}	0.38	-0.16±0.01^{a}	0.10
		Active	-0.04±0.00^{b}	-0.05±0.03^{b}	0.25	-0.25±0.00^{b}	0.38	-0.10±0.00^{b}	0.10
	20	Control	-0.63±0.05^{a}	-2.37±0.01^{a}	0.25	-0.25±0.00^{a}	0.38	-1.43±0.06^{a}	0.10
		Active	-0.03±0.01^{b}	-0.01±0.01^{b}	0.25	-0.27±0.01^{b}	0.38	-0.75±0.00^{b}	0.10

Letters indicate significant differences between the treatments in each temperature and relative humidity ($p \leq 0.05$)

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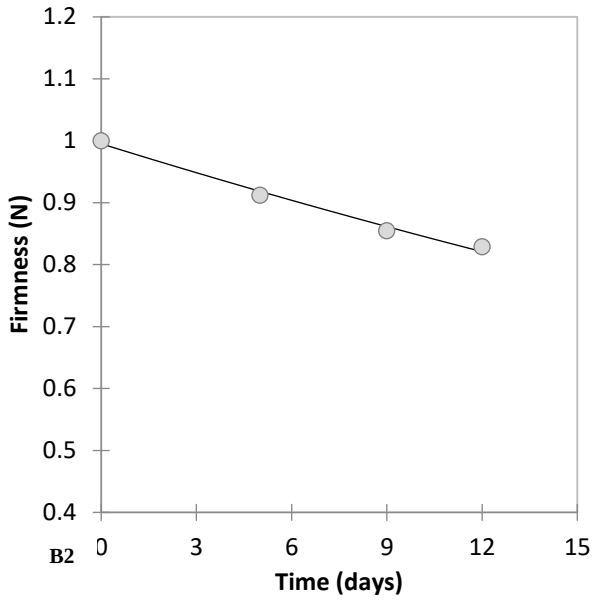
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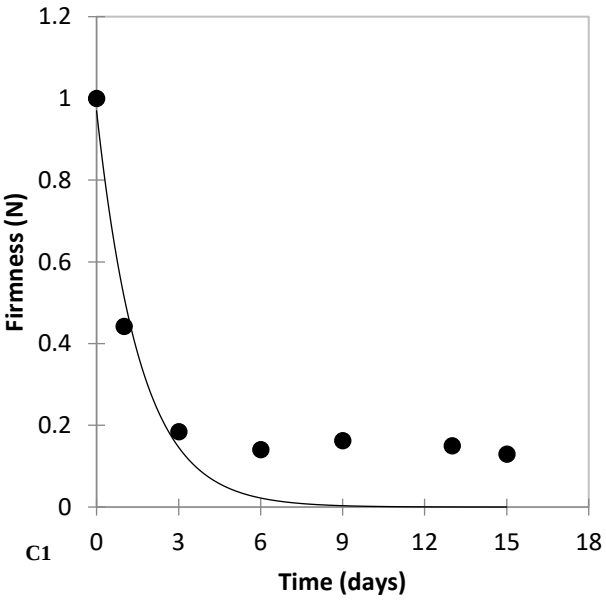
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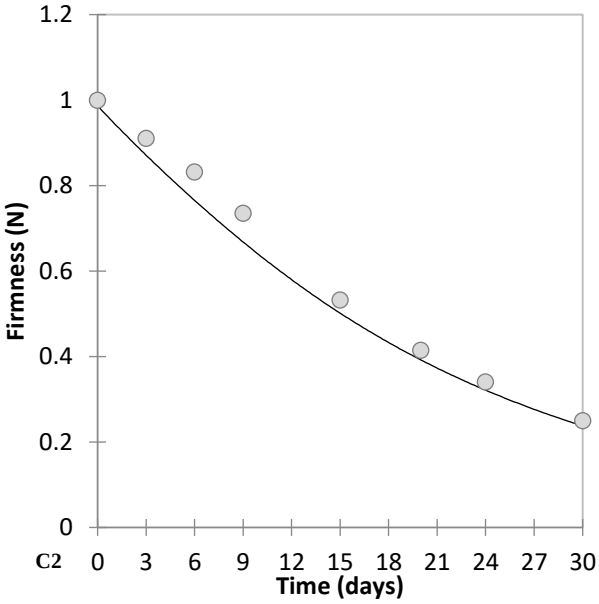
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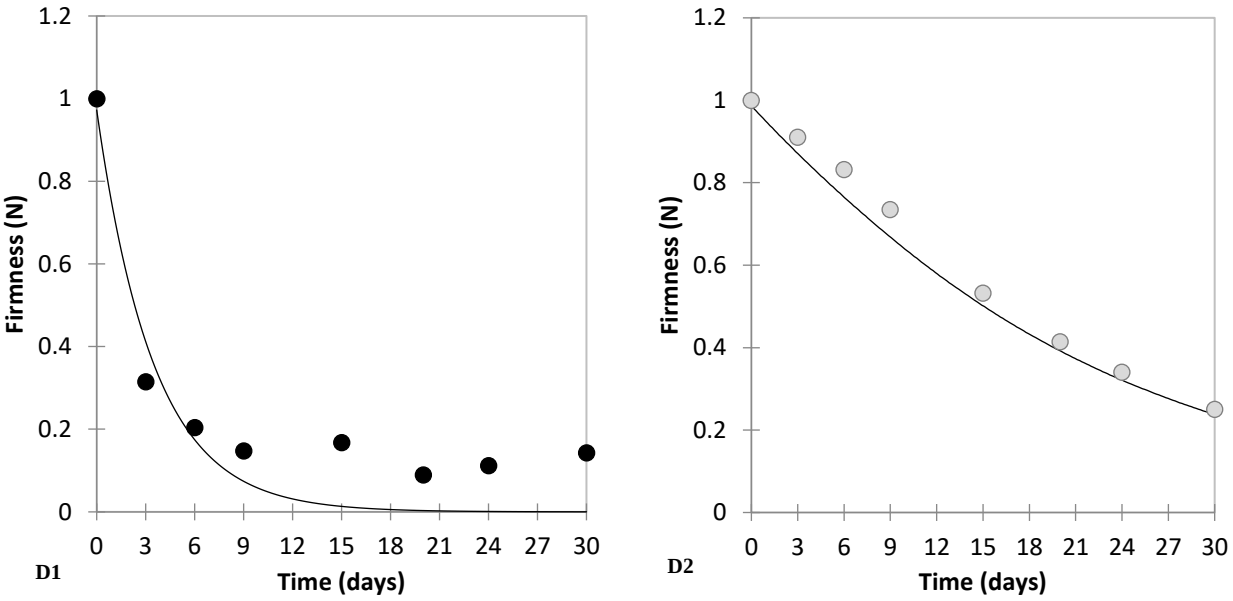


Figure 1. Values of firmness of control (●) and of active (●) samples stored at 20°C at 95% RH for 9 days (A1 and A2 respectively), 10°C at 95% RH for 12 days (B1 and B2 respectively), 20°C at 70 % RH for 15 days (C1 and C2 respectively), and 10°C at 70% RH for 30 days (D1 and D2 respectively). ── is the data predicted by the model.

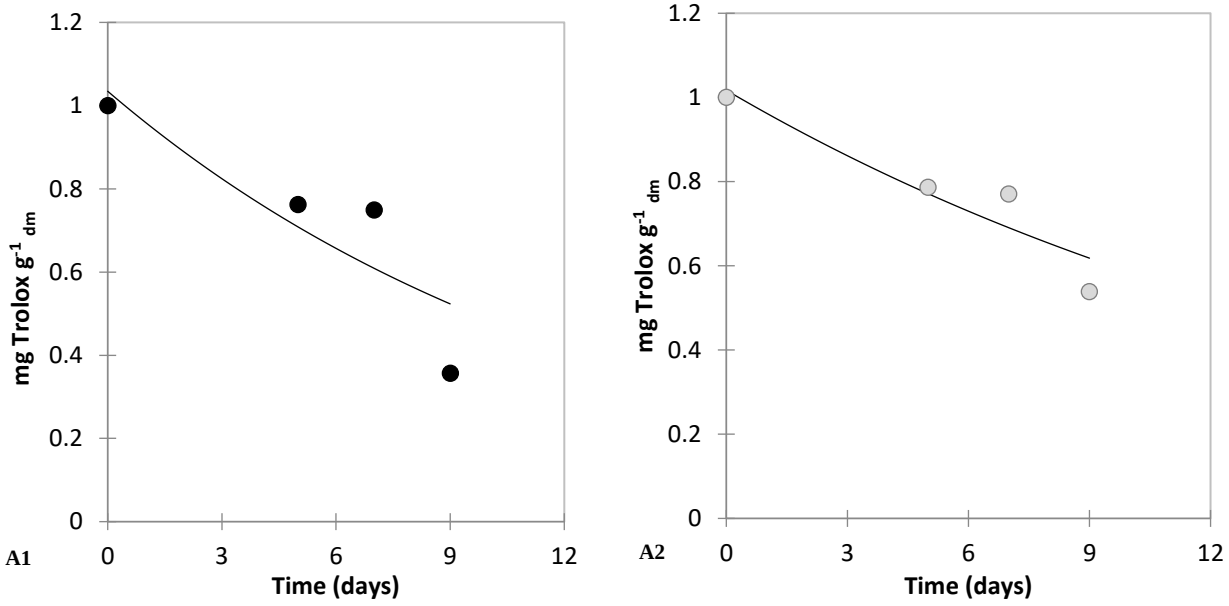
A pseudo-first-order model, as reported in Eq. 3, well described the decrement of TAC over time R^2 value, ranging from 0.74 to 0.99; MSE ranging between 0.011 and 0.033 and RMES between 0.07 and 0.18. Figure 2 reported experimental and predicted values of TAC of control and active samples stored at 20 °C and 10 °C at 95 % and 70 % RH. The degradation of antioxidant capacity was faster for the control samples than for the samples with active coating, confirming the impact of coatings applied on MP pears stored at different temperatures ad relativity humidity for different days. By comparing the results obtained, pseudo first order gave good accuracy (higher R^2, lower RMSE, lower AIC and 95% Confidence Interval) as compared to second order for antioxidant capacity of strawberries stored at 5, 10, 20, 26 and 32 °C (Muley et al., 2022). However, Oms-Oliu et al., (2009) showed that Weibull model is able to describe the antioxidant capacity of fresh-cut watermelon stored at 5 °C, as reported with high value of R^2 0.98.

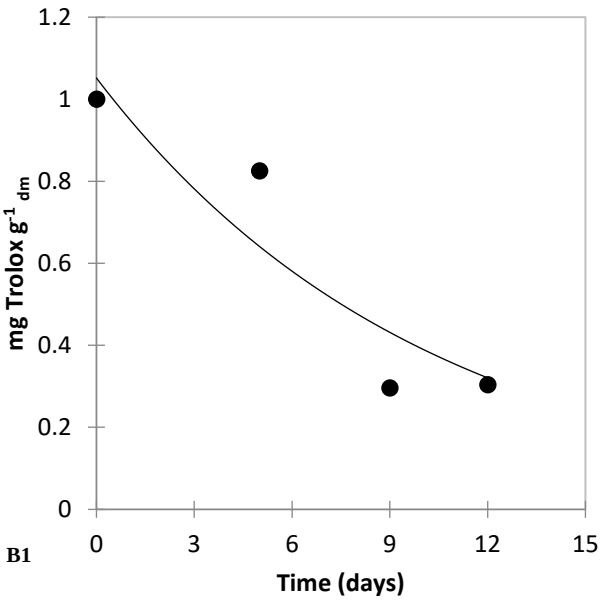
Table 3. Goodness of fitting of pseudo-first-order model used to estimate firmness of MP pears with and without coating at different temperatures and RH over time.

Total antioxidant capacity					
Samples	%RH	T(°C)	MSE	RMSE	R^2
Control	95	10	0.021	0.144	0.87
		20	0.023	0.162	0.83
Active		10	0.005	0.073	0.89
		20	0.017	0.131	0.85
Control	70	10	0.021	0.144	0.75

	20	0.033	0.180	0.74
Active	10	0.011	0.103	0.98
	20	0.013	0.113	0.99

Kinetic constants of TAC are reported in Table 2. Values of kinetic constant of control and active samples were negative because TAC decreased for all samples during storage time; this nutritional parameter was shown to reach values at the equilibrium of 0.25. Furthermore, active coating showed lower values of kinetic constant than control samples. In particular, active coating preserved antioxidant capacity about 24% of samples stored at 20 °C at 95 and 70% RH compared with control samples; whereas preserved 50% and 80% samples stored at 10 °C at 95 and 70% RH respectively with statistically significant differences ($p \leq 0.05$) respect control sample. Thus, coating applied on pears with antioxidant compound preserved with statistically significant difference ($p \leq 0.05$) the decrement of antioxidant capacity during storage time. Similar results were obtained by Oms-Oliu (2009) and colleagues on fresh-cut watermelon. Samples stored at temperatures up to 15 °C had greater antioxidant capacity than those preserved at 20 °C. Fresh-cut watermelon stored at 20 °C underwent a substantial depletion during the first two days, retaining 30% of the initial AC after 14 storage days. On the other hand, antioxidant capacity retention of fresh-cut watermelon stored at temperatures lower than 20 °C varied from 55% to 65% after 2 weeks.

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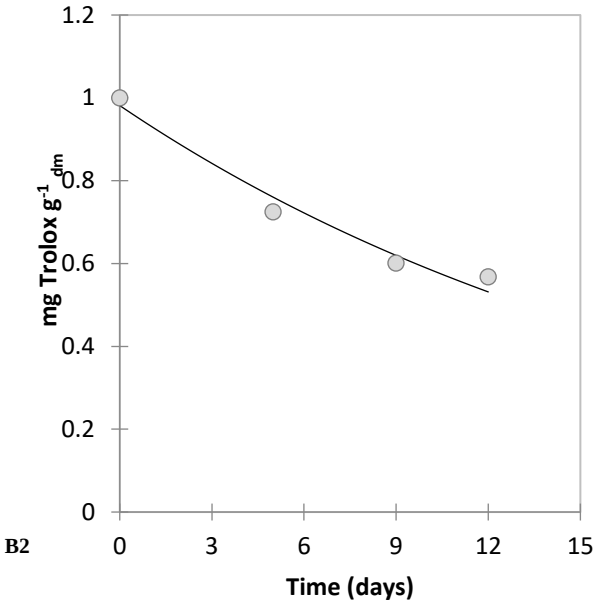
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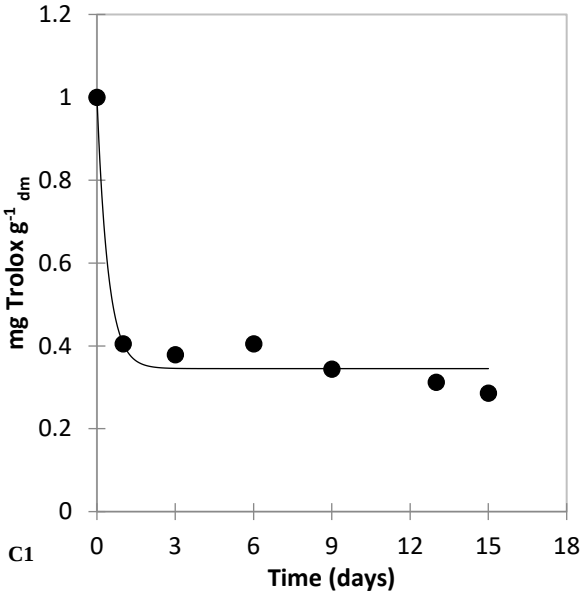
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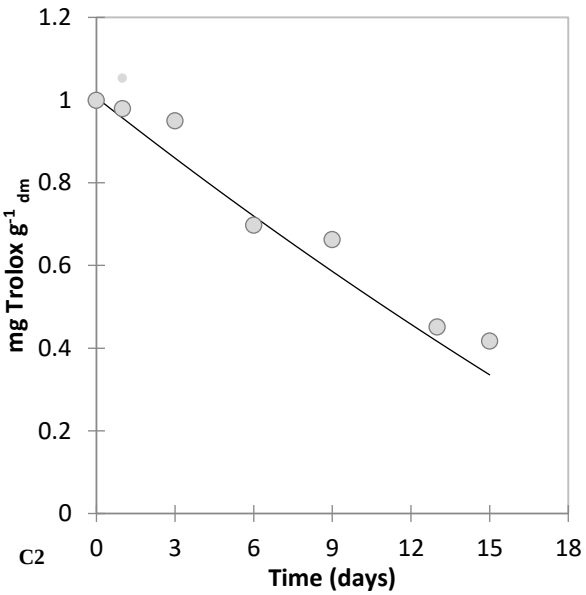
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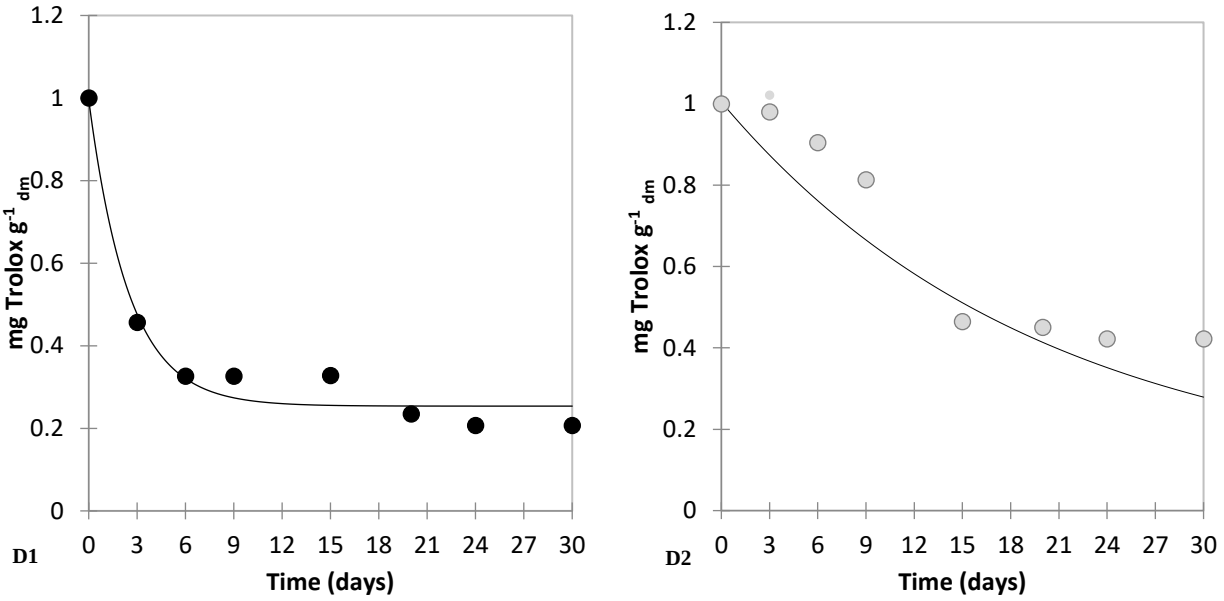


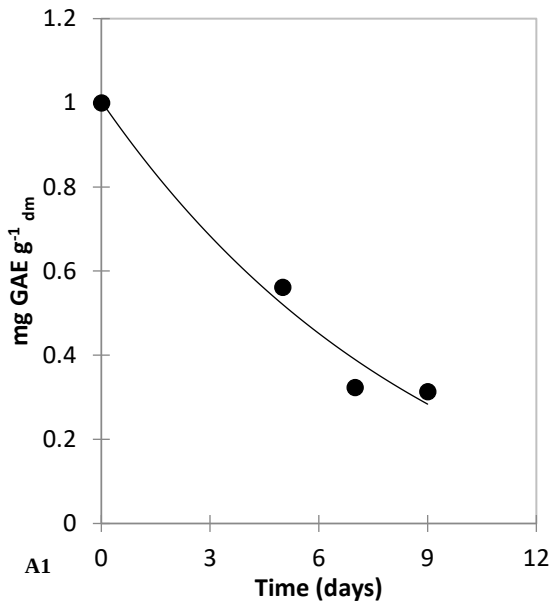
Figure 2. Values of total antioxidant capacity of control (●) and of active (●) samples stored at 20°C at 95% RH for 9 days (A1 and A2 respectively), 10°C at 95% RH for 12 days (B1 and B2 respectively), 20°C at 70 % RH for 15 days(C1 and C2 respectively), and 10°C at 70% RH for 30 days (D1 and D2 respectively). —— is the data predicted by the model.

A pseudo-first-order model, as reported in Eq. 3, well described the decrement of TPC over time R^2 value, ranging from 0.79 to 0.91; MSE ranging between 0.005 and 0.033 and RMES between 0.07 and 0.12 (Table 4). In Figure 3 are reported total polyphenols content values of control and active samples stored at 20 °C and 10 °C at 95 % and 70 % RH. Also, Muley et al., (2022) reported that pseudo first order gave good accuracy (higher R^2, lower RMSE, lower AIC and 95% Confidence Interval) as compared to second order for TPC of strawberries stored at 5, 10, 20, 26 and 32 °C. However, results of kinetic modelling show that TPC retention during the combination of ultrasound and ultraviolet-C irradiation of tomatoes followed first-order model. The R^2 adj and sum of squared error were in the range of 0.896–0.984 and 0.353–1.045, respectively (Esua et al., 2019a). TPC decreases for all samples reaching a value at equilibrium of 0.35, as shown in Table 2.

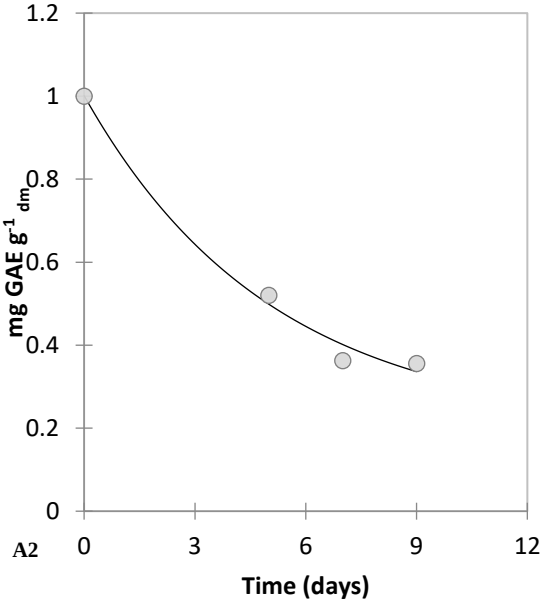
Good effect of active edible coating was obtained on total polyphenols content, as reported in Table 2. During storage time active coating was able to slow the decrease of TPC about 73% of samples stored at 20 °C at 95% RH and 52% of pears stored at 70% RH. Paired samples t-test did not report statistically significant differences between control and active samples, but there were statistically significant differences ($p \leq 0.05$) between the control and active samples stored at 70% RH at 10 and 20 °C; indeed active coating showed to preserve about 55% MP pears with statistically significant differences ($p \leq 0.05$) than control pears. Values of kinetic constant were similar to values reported on other products: tomatoes and mushrooms (Esua et al., 2019; Li et al., 2022).

Table 4. Goodness of fitting of first kinetic used to estimate total polyphenol content of MP pears with and without coating at different temperatures and RH over time.

Total polyphenol content					
Samples	%RH	T(°C)	MSE	RMSE	R^2
Control	95	10	0.013	0.113	0.90
		20	0.005	0.074	0.91
Active		10	0.007	0.084	0.89
		20	0.006	0.075	0.90
Control	70	10	0.012	0.107	0.79
		20	0.008	0.088	0.89
Active		10	0.014	0.108	0.85
		20	0.015	0.123	0.88



A1



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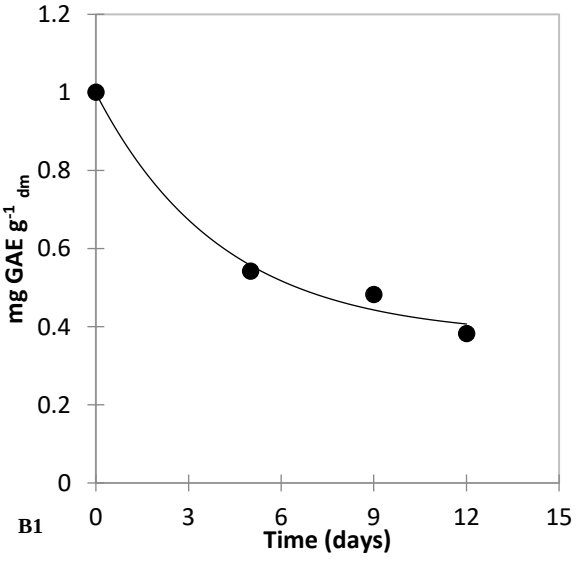

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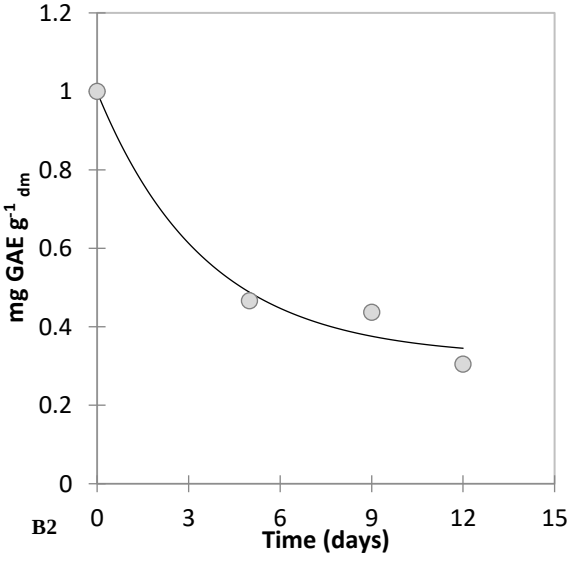

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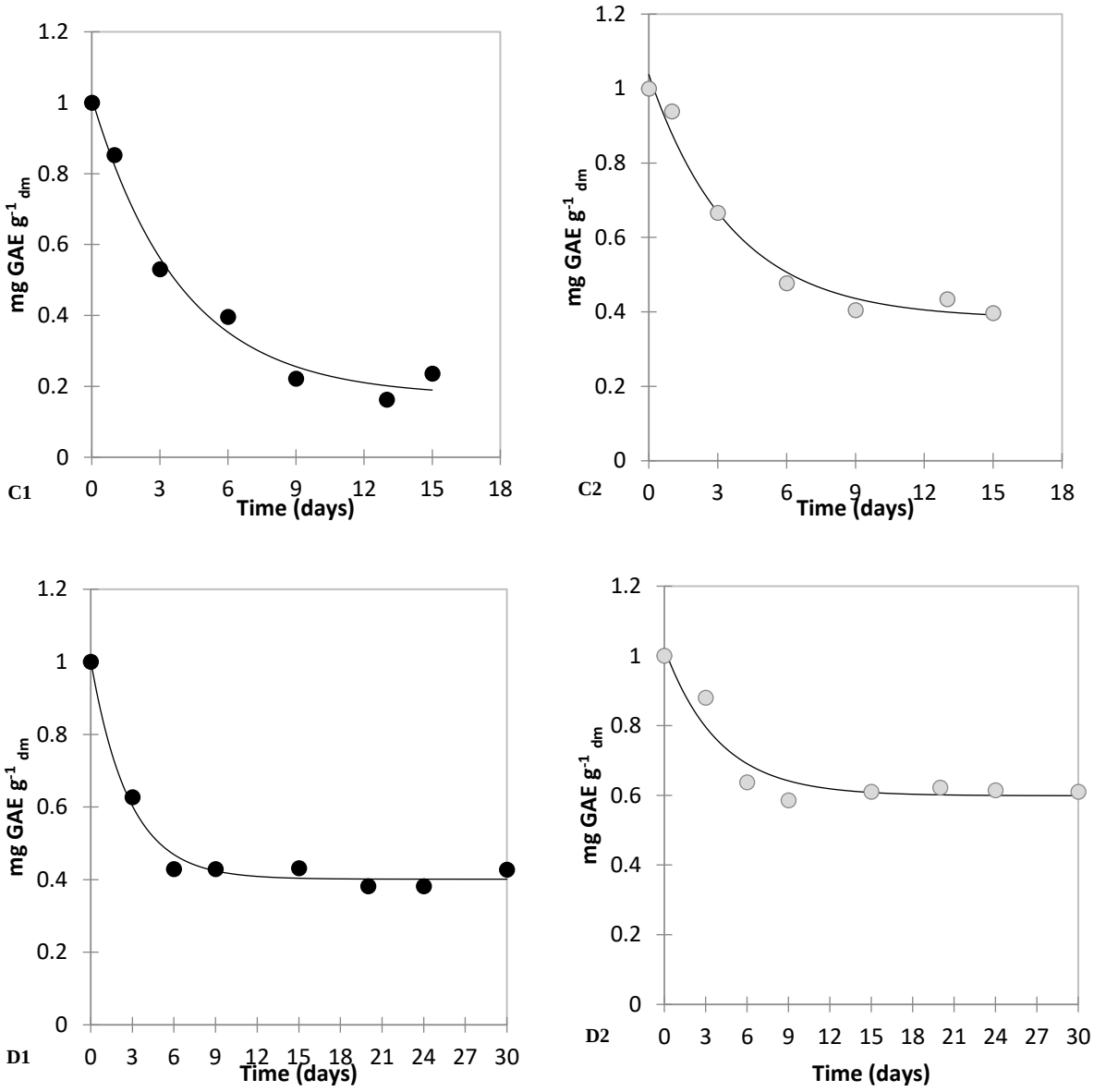


Figure 3. Values of total polyphenols capacity of control (●) and of active (●) samples stored at 20°C at 95% RH for 9 days (A1 and A2 respectively), 10°C at 95% RH for 12 days (B1 and B2 respectively), 20°C at 70 % RH for 15 days(C1 and C2 respectively), and 10°C at 70% RH for 30 days (D1 and D2 respectively).── is the data predicted by the model.

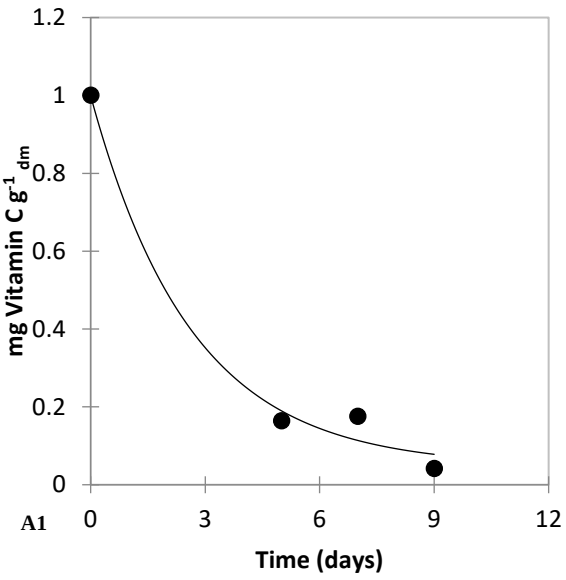
In Figure 4 are reported vitamin C content values of control and active samples stored at 20 °C and 10 °C at 95 % and 70 % RH. Also for vitamin C content pseudo-first-order model, as reported in Eq. 3, well described the decrement of this nutritional property (R^2 value ranging from 0.78 to 0.98; MSE ranging between 0.00 and 0.02 and RMES between 0.01 and 0.1) (Table 5).

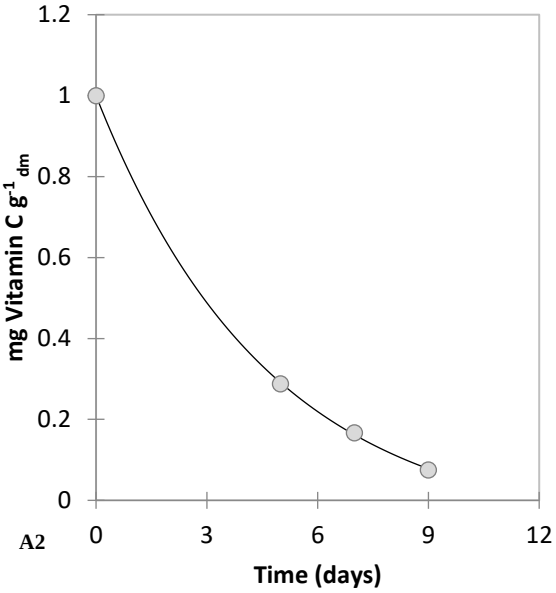
Vitamin C content decreased during storage time, but it was faster for uncoated than coated samples for all temperatures and RH, confirming the impact of coating applied on MP pears. Nevertheless, by comparing the results obtained with other products, pseudo zero order kinetic was used to describe the degradation of vitamin C content of strawberries (stored at 5, 10, 20, 26 and 32 °C) and lime fruit

(stored at 10 and 25 °C) with high R^2 and low RMSE values (Muley et al., 2022; Maftoonazad & Ramaswamy, 2019). However, as demonstrated by Gil et al., (2006) and Amodio et al., (2013), vitamin C can also degrade by following Weibull model of fresh-cut fruits. Vitamin C content loss was more pronounced under higher temperatures than under refrigerated storage conditions for fruits, in agreement with Qiu & Wang (2015), Maftoonazad & Ramaswamy, (2019)and Muley et al., (2022). During storage time coating was able to preserve vitamin C 45% concerning control samples stored at 10 °C, whereas it preserved 23% and 77% vitamin C of samples stored at 95 and 70% RH at 20 °C for 9 and 12 days respectively comparing with control, with statistically significant difference ($p \leq 0.05$) between the treatments. Active coating was applied on the samples with PG preserved with statistically significant difference ($p \leq 0.05$) the decrement of vitamin C for MP pears stored at two different temperatures and RH (Table 2). Other authors reported lower values of kinetics constant found on lime fruit, fresh-cut melon and watermelon (Maftoonazad & Ramaswamy, 2019; Amodio et al., 2013; Oms-Oliu et al. 2009). Fruit stored at 10 °C lost vitamin C at a rate of 0.0067 and 0.0074 per day for coated and uncoated limes, respectively, while those stored at 15 and 25 °C had a rate of 0.0091 and 0.028 per day for uncoated limes and 0.0073 and 0.0180 per day for coated samples (Maftoonazad & Ramaswamy, 2019). Oms-Oliu et al. (2009) obtained similar results, modelled the vitamin C degradation with the Weibull model and reported a rate constant of 0.019 d^{-1} for watermelon samples stored at 5 °C.

Table 5. Goodness of fitting of first kinetic used to estimate vitamin C of MP pears with and without coating at different temperatures and RH over time.

Vitamin C content					
Samples	RH	T (°C)	MSE	RMSE	R^2
Control	95	10	0.000	0.018	0.98
		20	0.002	0.045	0.94
Active		10	0.006	0.081	0.88
		20	0.001	0.024	0.97
Control	70	10	0.003	0.057	0.89
		20	0.024	0.125	0.79
Active		10	0.005	0.071	0.92
		20	0.017	0.102	0.78


1.2
1
0.8
0.6
0.4
0.2
0
mg Vitamin C g-1 dm
A1
0
3
6
9
12
Time (days)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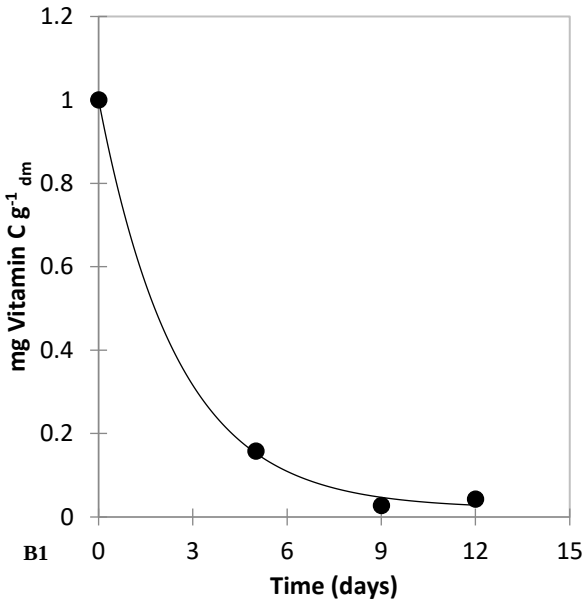
1.2
1
0.8
0.6
0.4
0.2
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mg Vitamin C g-1 dm
B1
0
3
6
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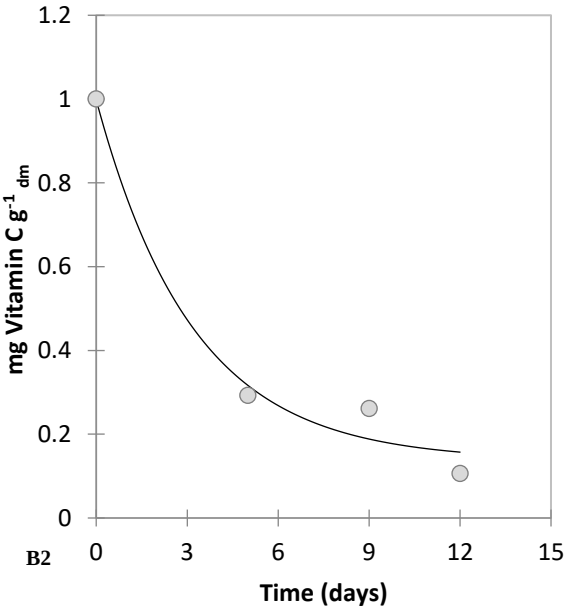
12
15
Time (days)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mg Vitamin C g-1 dm
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Time (days)

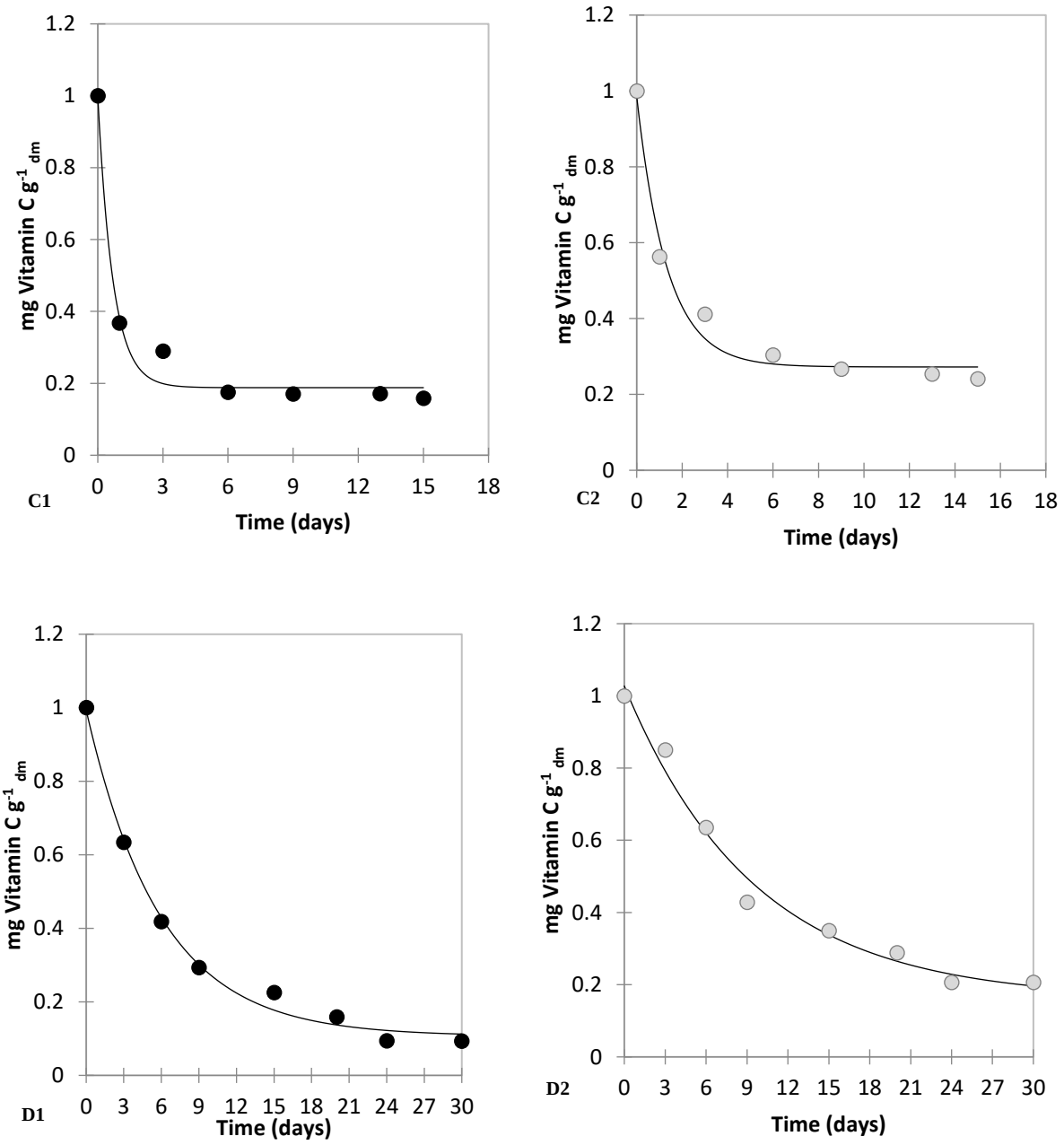

Figure 4. Values of vitamin C of control (●) and of active (●) samples stored at 20°C at 95% RH for 9 days (A1 and A2 respectively), 10°C at 95% RH for 12 days (B1 and B2 respectively), 20°C at 70 % RH for 15 days(C1 and C2 respectively), and 10°C at 70% RH for 30 days (D1 and D2 respectively).— is the data predicted by the model.

4. Conclusions

Good correlation was observed between experimental and model predicted using the pseudo-first-order model to fit physical and nutritional properties of MP pears during storage time at 10 and 20 °C at 70 and 95% RH. Physical and nutritional properties decreased during storage time for all samples. However, the coating enriched with PG was able to preserve firmness about 64 % and 92 % of pears stored at 95% and 70% RH respectively. Additionally, coating slows down antioxidant capacity about 24% of samples stored at 20 °C; whereas preserved 70% samples stored at 10 °C. Furthermore,

degradation of vitamin C was slowed down by active coating that showed to preserve samples about 45% concerning control samples stored at 10 °C, whereas 23% and 77% of samples stored at 95 and 70% RH respectively at 20 °C. During storage time, active coating was able to slow the decrease of total polyphenols content about 73% of samples stored at 20° C at 95% RH and 52% of pears stored at 70% RH. In conclusion, the active coating preserves MP pears, but the preservation effect of active coating is dependent for the RH, indeed pears stored at 70% RH showed better effect than pears stored at 95% RH at both temperatures.

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Chapter 4

Plasma-activated water and proteins extracted by house cricket to develop functional edible coating: effect on the quality of fresh-cut apples

Abstract

Edible coatings are a promising sustainable preservation technology able to extend the shelf-life of food products. Plasma-activated water (PAW) is an important application of the cold atmospheric pressure plasma that can be used as an antimicrobial liquid for decontamination of food surfaces. The objective of this work was to evaluate the possibility to use PAW to develop edible coating. Due to the low pH of the PAW, a biopolymer soluble at low pH must be used. In this work, two natural biopolymers were tested: low molecular weight chitosan (LMW) and proteins extracted by house cricket (PE). PE and LMW were used at different concentrations to optimize the solution stability in PAW. Then, the optimal coating obtained with and without PAW was applied to fresh-cut apples to study the effect on the quality of the product. Firstly, respiration rate (RR_{CO2}) and transpiration rate (TR) at 60, 76, 86 and 96% of RH were measured at 5°C. Secondary, chemical-physical (weight loss, colour, hardness, pH and total soluble solids) and nutritional properties (antioxidant capacity, total polyphenols content and vitamin C) were studied during storage at 5°C for 13 days. Results showed that PAW was a good solvent for cricket protein in 1 and 2%, whereas chitosan was not soluble in PAW at any concentration. The optimal coating composition was a blend of 2 % protein dissolved in PAW and 2% chitosan dissolved in 1% acetic solution (ratio 1:1). The first-order kinetic model was found to be the best fit for browning index, antioxidant activity, total polyphenols content and vitamin C, whereas zero-order kinetic model was used to fit hardness values. Coatings were not able to preserve the browning index during storage, but they slowed down firmness degradation by about 10%, antioxidant capacity and vitamin C content by about 20%, and total polyphenol content (30%) of the fresh-cut apples compared to the control samples. However, no differences were observed between coating prepared with or without PAW.

Keywords: blend solution, kinetic constants, respiration rate, nutritional properties.

1. Introduction

Developments in packaging technology and edible coatings for foods have shown promising results in extending the shelf-life of fresh-cut fruits and vegetables (Khan et al., 2021; Valentino et al., 2020; Volpe et al., 2019). Furthermore, coatings can be used as a carrier of active compounds such as antimicrobial or antioxidant; an alternative to the addition of antimicrobial substances could be the application of plasma technology. Plasma-activated water (PAW) is an important application, which can be used as a means of sanitization in the food sector, it has a high antimicrobial potential (R. Ma et al., 2016), indeed directly applied the cold atmospheric gas plasma for the microbial inactivation of strawberries (Fernández et al., 2013; Misra et al., 2014), cherry tomatoes (Ziuzina et al., 2014), mushrooms (Agun et al., 2019) tomatoes (Hou et al., 2021) and cabbage (Bacchetti et al., 2014).

However, PAW has a good effect on the F&V and showed a higher firmness, colour index and total soluble solids in PAW-treated berries compared to control samples after 8 days of storage (R. Ma et al., 2016). Other potential effects of PAW could be the decrease of the browning enzyme activity reaction in fresh-cut fruits (Xu et al., 2016). PAW could be used to dissolve protein insect flour extract, because these products dissolved with good results in an acidic solution, as reported by Rumpold & Schlüter (2013) Insects have an attractive nutritional profile with a protein content varying between 35 and 61% and a balanced aminoacidic profile, meeting the requirements of the World Health Organization (WHO) for amino acids (Rumpold & Schlüter, 2013) The protein and lipid contents of the house cricket (20-25 and 4-7 g/100 g fresh weight, respectively) are comparable to those of conventional animal sources such as beef or chicken (Kulma et al., 2019) House crickets have also been studied as a resource for protein extraction and fractionation (Laroche et al., 2019a; Ndiritu et al., 2017; Udomsil et al., 2019; Yi et al., 2013), with a reported 20–40% protein yield in a liquid fraction and an approximately 60–75% purity (Laroche et al., 2019a; Ndiritu et al., 2017). Furthermore, they have been used as a starting material for fat extraction and isolation with several methods (Laroche et al., 2019a) and solvents (Ramos-Bueno et al., 2016). The fat yield of the house crickets has been reported to reach 25% (Laroche et al., 2019a) The objective of this work was divided into two steps. The first was to develop coatings with different % of chitosan with low molecular weight (LMW) and protein extract to house cricket (PE) dissolved in acetylated water and PAW. Thus, the second step was to apply the best blend solution on fresh-cut apples and finally shelf-life test was conducted on fresh-cut apples for 13 days at 5°C studying the physiological (respiration and transpiration rate), chemical-physical (weight loss, colour, mechanical properties, pH, and °Brix) and nutritional properties (antioxidant capacity, total polyphenols content and vitamin C content).

2. Materials and Methods

2.1 Materials

Chitosan (low molecular weight (LMW), deacetylation degree, 75–85%), acetic acid (99–100% glacial) and glycerol were purchased from Sigma-Aldrich (Steinheim am Albuch, Germany). Living house crickets (*A. domesticus*) were purchased at an adult age from Tropic-Shop (Nordhorn, Germany). Apples were purchased from a local supermarket (Rewe, Potsdsm, Germany).

2.2 Methods

2.2.1 PAW

For the production of plasma-activated water (PAW), plasma-processed air needs to generate, which is then mixed with water to form PAW. Therefore, a microwave-driven discharge set-up was used. The microwaves had a frequency of 2.45 GHz, and the supply power was in the range of 1.1 kW. Accordingly, the gas temperature was about 4000 K at a gas flux of 18 standard litres per minute (slm) of air. For higher volume rates of plasma processed air (PPA), a second stage of a microwave setup was developed and combined with the plasma source PLexc®. This innovative double-stage plasma source, called PLexc2®, worked at atmospheric pressure and produced 90 slm (standard litres per minute) of PPA continuously. PLexc2® and all support units for cooling water, microwave power, pressurized air and process control were combined in an auxiliary decontamination unit. The generation of PPA and PPW (Figure x) was realized by using compressed air as working gas for the microwave-driven discharge PLexc®. By ignition of the plasma, the compressed air is processed into reactive nitrogen species (RNS, ca. 3%) (Andrasch et al., 2017). These meta-stable and stable compounds can react by oxidation with organic matter, e.g., microbial contamination. The PPA was used to produce plasma processed water (PPW) inside an international bulk container (IBC). During the experiments, 1.5 m^3 in a total of PPW was produced. Over a time period of 3 h, the PPW was finally prepared before its use for the washing process.

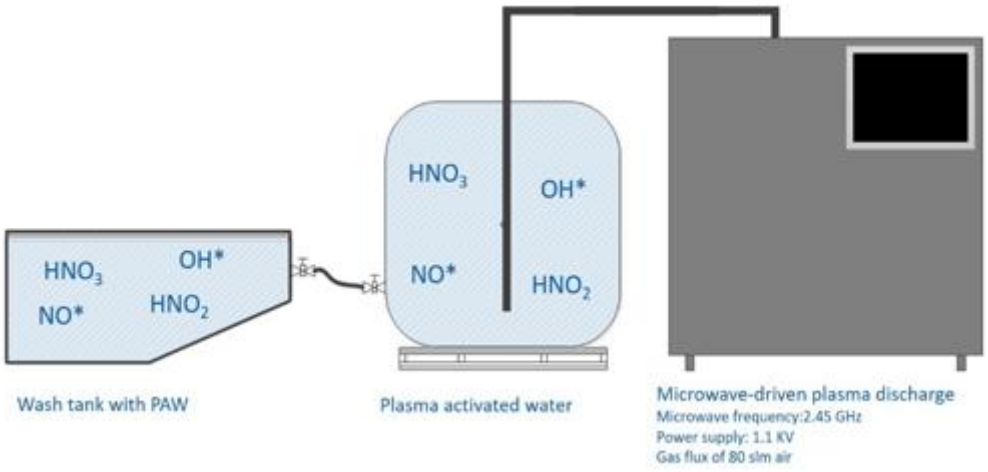


Figure 1 Schematic drawing of the PAW producing system

2.2.2 Protein extraction

Living house crickets (*A. domesticus*) were stored for 2 h inside a cold room at 4 °C to reduce movement activity. Afterwards, crickets were freeze inactivated at -20 °C packed in plastic pouches. Before any treatment, they were washed with cold water to remove impurities and then ground fresh for 10 seconds with a Retsch Mill (Retsch Grindomix, Retsch GmbH, Germany). Two different steps were used to extract proteins. The first step was defatted powder with n-hexane (s/l ratio = 1:40, temperature room 1 h). Then, the mixture was centrifuged (temperature room, 10 min, 3200 x g) and the supernatant was removed; the pellet was left to dry inside a fume hood. Thus, the powder was mixed with NaOH (0.5 M) at a solid/liquid ratio of 1:15 and stirred for 60 min at room temperature and centrifuged at 10000 x g, 10 min, 20 °C (Psarianos et al., 2022). Supernatants from the first and second alkaline solubilization were pooled and the pH was adjusted to 4.3–4.5 to induce protein precipitation with 2 M HCl before centrifugation (2500 g, 15 min, 4 °C). After protein precipitation, the supernatant was removed, and the residue containing precipitated proteins was washed twice with distilled water at pH 4.5 and centrifuged (2500 g, 10 min, 4 °C). The residue was freeze-dried (Laroche et al., 2019b).

2.2.3 Coating solution preparation

After extraction, 1 and 2 % of the proteins extracted (PE) were dissolved in PAW (pH < 2) for 4 hours and in 1% of acetic solution (AW) 0.1 M and the solutions were centrifuged. Different concentrations of LMW (1, 1.5, 2, and 3 % w /v) were used to study the dissolution minimum and maximum of polysaccharides chosen in AW and PAW. Solutions were prepared by adding LMW in AW and in PAW under stirring for 4 hours at room temperature and glycerol was added as a percentage of the solids (10% weight/solid). The effect of LMW concentrations on solution stability was studied and centrifugated 10 mL at 3900 rpm for 10, 20 and 30 minutes. Then, four different blend solutions were prepared to study the different ratios of LMW and PE as reported in Table 1. The blend solutions were prepared by adding dropwise the solution of PE to LMW with a 1:1 ratio; glycerol was added as a percentage of the solids (weight ratio of 0.1). The solution was stirred for 4 hours at room temperature. Four different solutions were prepared to study the different ratios of LMW and PE at, as reported in Table 1.

Table 1. Different ratios of LMW and PE used to develop blend solutions

Biopolymers	Solvents	Code
LMW 1%	AW	COATING A
PE 1%	PAW	

LMW 1%	AW	COATING B
PE 1%	AW	
LMW 2%	AW	COATING 1
PE 2%	AW	
LMW 2%	AW	COATING 2
PE 2%	PAW	

2.2.4 Coating application

2.2.4.1 Samples preparation

Apples selected for uniform size and appearance, without damages, were washed with tap water for 3 min; then, apples were cut into cylinders. Samples were immersed in an anti-browning solution (1% citric acid, 1% ascorbic acid, 1% calcium chloride) for two minutes (Volpe et al., 2019). Then, samples were immersed in the biopolymer solutions for 1 min. Coated and uncoated apple slices were put on a grid and allowed to dry in the climatic chamber at 30° C, 50% RH for 30 min (Valentino *et al*., 2020). The samples without coating were used as control, whereas fresh-cut apples with coatings 1 and 2 as used as active samples.

2.2.4.2 Transpiration rate

Weight loss technique was used to evaluate the transpiration rate (TR). Three pieces of apple with and without coatings were stored in three containers, located in walk-in cooling room, and maintained at 5°C in air. Relative humidity within the test containers should be controlled by using saturated salt solutions of sodium chloride, potassium chloride, potassium nitrate and distilled water giving 60, 76, 86, and 96 % RH, respectively (Volpe et al., 2018).

2.2.4.3 Measurement of CO_2 release

To evaluate CO_2 release of the apples was used slices (n = 12) from each replication were used to analyse the respiration rates (RR) by measuring the CO_2 release (mg $kg^{-1}h^{-1}$) at ambient air within a custom-made gas exchange system (Rux et al., 2017) at 4 °C (storage temperature) for 3 h. Briefly, apple slices were rapid, within minutes, transferred to a clean plastic tray and placed in one of nine acrylic glass cuvettes (volume 8.2 L). In each chamber, changes in CO_2 concentrations were measured with GMP222 CO_2 sensors (Vasalia, Helsinki, Finland) and data was recorded by a NetDAQ 2645A data logger (Fluke Deutschland GmbH, Glottertal, Germany). From changes in CO_2 concentrations over time, the fresh mass-based CO_2 release rates (RR, equivalent to respiration rates) were calculated as mg $kg^{-1}1\ h^{-1}$(Caleb et al., 2016):

$$RR = \frac{\Delta CO2 * V}{FM * \Delta t}$$

where ΔCO_2 reflects the increase in the CO_2 concentrations (mg L^{-1}) during measurement, V the free

cuvette air volume (L), FM the total fresh mass of measured apple slices (kg) and Δt the duration of measurement (h).

2.2.5 Packaging and storage Condition of fresh-cut apples

Apple samples (42 g) were packed by using polyethene pouch (0.15 m×0.10 m) and stored at 5° C for 13 days (Volpe et al., 2019) in air. After 0, 1, 3, 6, 8, 10 and 13 days, the headspace gas composition (O_2 % - CO_2 %), the colour, the pH, mechanical properties, °Brix, vitamin C content, total polyphenol content, and antioxidant capacity were monitored.

2.2.5.1 Headspace Gas Analysis

O_2 and CO_2 concentration (% v/v) in the package headspace is monitored by means of a portable PBI Dansensor A/S analyzer (accuracy±0.1%), by sampling with a needle 2–3 mL of gas from the package headspace.

2.2.5.2 Chemical-physical properties

Weight loss of apples during storage was determined by using the gravimetric method. Samples were weighted by using a balance (accurate to 0.01 g). The weight loss was calculated as follows:

$$\left(\frac{wi-wf}{wi}\right)*100 \qquad \textit{Eq. (1)}$$

Where *wi* was the initial weight and *wf* weight after storage and expressed as a percentage.

Tissue colour parameters (CIE L*, a*, and b*) were measured with a Minolta (Konica Minolta Sensing Inc., Tokyo, Japan) CM-2600d spectrophotometer (illuminant: D65; illumination area: 6 mm; specular component excluded) The instrument was calibrated using a white standard plate. Total colour change (ΔE) was also analysed (ASTM E1910):

$$\Delta E = \sqrt{[(\Delta L)^2 + (\Delta a)^2 + (\Delta b^2)]} \qquad \textit{Eq. (2)}$$

Whereas the browning Index (BI) was measured (Maskan, 2001):

$$BI = \frac{\lfloor 100(x-0.31)\rfloor}{0.17} \; where \; x = \frac{(a+1.75L)}{(5.645L+a-3.012b)} \qquad \textit{Eq. (3)}$$

Seven measurements were performed on each sample.

A compression test was performed with a SMS XT Plus texture analyser (Stable Micro Systems, Godalming, UK, fitted with an SMS-P/4 cylindrical probe of 4 mm diameter), tissue strength of apples was measured as the maximum compression force (N) at 8 mm indentation (test speed 4 $mm \cdot min^{-1}$ Rojas-Graü et al., 2008).

Afterwards, fresh-cut apples were juiced with a garlic press for analysing the soluble solid content (TSS) and pH. TSS (°Brix) was measured with a DR301-95 electronic refractometer (A.KRÜSS

Optronic GmbH, Hamburg, Germany) (Rux et al., 2021) and pH was measured at room temperature using a Cyber Scan pHmeter (Volpe et al., 2019).

2.2.5.3Nutritional quality

The total antioxidant capacity (TAC) was studied by evaluation of the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Volpe et al. (2019), with slight modifications. 0.5 g of freeze-dried sample was added in 10 mL of methanol/water (80:20) solution, mixed with constant shaking at a temperature room for 60 minutes, and put on an ultrasound bath for 30 minutes. The sample was centrifuged (Hermle Z 326 K, Germany, European Union) at 10,000 rpm for 15 minutes (Pérez-Jiménez et al., 2008). The pellet was discarded and the supernatant was retained and mixed (100 µL) with 4.9 mL of DPPH solution (methanol+DPPH 0.1 Mm) to initiate the reaction. The absorbance was read using a spectrophotometer UV-VIS (UV-550 Jasco, Japan) at 515 nm after 30 minutes of incubation at room temperature in the dark. TAC was expressed as mg of Trolox equivalents g^{-1} of dry matter (mg TE g_{dm}^{-1}) using a Trolox standard curve (0–625 mg mL^{-1}).

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method described by Rocha & Morais, (2002) with slight modifications. 0.5g of lyophilized sample was crushed by mortar and pestle with 10 mL of 6% sodium bicarbonate and they were filtered through a paper filter and 0.5 mL of the filtrate solution was added with 2.5 mL of Folin-Ciocalteau reagent and 2 mL of sodium bicarbonate. The samples were incubated for 1 h at 35 °C and then for 1 h at 6 °C. After 2 h of incubation in the dark, the absorbance was read at 760 nm against a blank (2.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium bicarbonate), using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). The total phenolic content was calculated based on the calibration curves of gallic acid (0-8 mg mL^{-1}) and expressed as mg of gallic acid equivalents g^{-1} of dry matter.

Vitamin C of pears was extracted by homogenizing 1 g of product tissue with 10 mL of glacial acetic acid solution in water (8%) for 1 min by using an Ultraturrax (IKA, T18 Basic; Wilmington, NC, USA). The homogenate was centrifugated at 7000 rpm for 7 minutes. The sample was filtered through a paper filter, and the supernatant was collected. Then, 5 mL of glacial acetic acid solution was added to the pellet and centrifugated at 7000 rpm for 7 minutes. This procedure was replicated four times for vitamin C extraction. Then, the method reported by Jung et al. (1994), with minor modifications, was used to determine vitamin C content in MP fennels. 1 mL of sample filtered was added in 4 mL of riboflavin stock solution, and 0.06g riboflavin in 100 mL of 0.01 M potassium buffer (pH=7.5). Absorbances of samples before and after light storage (5500 Lux) were measured at 265 nm using a spectrophotometer UV-VIS (UV-550 Jasco, Japan). Differences in absorbance of samples before and after 15 min-illumination were used for the calculation of ascorbic acid. The vitamin C content was calculated based on the calibration curves of ascorbic acid in the buffer of riboflavin (0-10.5 µg mL^{-}

^{1}) and expressed as mg of vitamin C g^{-1} of dry matter (mg of Vit C g_{dm}^{-1}).

2.3 Mathematical modelling: zero and first-order kinetic models

Zero and first-order kinetics, traditionally used to describe degradation reactions in foods, may be generally written as (Giannakourou & Taoukis, 2003; Polydera et al., 2005; Zanoni et al., 2005; Nisha et al., 2005):

$$\frac{dQ(t)}{dt} = -kQ^n \qquad Eq.(4)$$

The equation may be integrated easily obtaining the well-known decay functions. In particular, for the pseudo-zero order kinetic model, the following equation can be used to predict the quality of a product as a function of storage time:

$$Q = Q_i - kt \qquad Eq.(5)$$

whereas for pseudo-first kinetic order (n=1), the equation will be:

$$Q = Q_i e^{-kt} \qquad Eq.(6)$$

Or

$$Q = Qeq + (Q0 - Qeq)e^{-kt} \qquad Eq.(7)$$

where

Q_i is the concentration of the quality index at time zero, *Q(t)* is the concentration of the quality index at the time *t*, *Q(eq)* is the concentration of the quality index at the time at equilibrium, *k* is the rate constant, and n is the kinetic order of the equation. The negative sign is generally referred to as a decrease of quality. However, if the quality indices related to the alteration process increase during storage, the function will have a positive sign.

2.4 Statistical analysis

The results are reported as the average of replications of each sample ± standard deviation.

The effect of blends on respiration and transpiration rate were studied by one-way ANOVA analysis. Duncan's test was carried out to find the source of the significant differences within the samples examined.

Multivariate ANOVA analysis has been carried out to evaluate the effect of the independent parameters storage time (t), and coating treatment (C) and its interaction on physical, chemical and nutritional quality indices of fresh cut apples with a full factorial experimental. Three levels of treatments (control, coating1 and coating 2) and 7 levels of times (0, 1, 3, 6, 8, 10 and 13) at 5°C were studied. Duncan's test was carried out to find the source of the significant differences within the samples examined. Significant differences were defined at $p \leq 0.05$. Data were analyzed using SPSS software (SPSS Inc. 28.0, Chicago, IL, USA, 2022).

Linear or non-linear regression were used to estimate the kinetic constants using XLSTAT 16.0 (Addinsoft, France, 2023). The regression coefficients (R^2) and the root mean square error (RMSE) and MSE were calculated to evaluate the goodness of the model to describe data. The highest the values of R^2 and the lowest the values of RMSE, the better the fitting of the models to experimental data. The adequacy of the fitted model was also assessed by means of an analysis of residuals which permits to confirm of the validity of the assumptions regarding the independence and normal distribution of the errors. ANOVA analysis was performed for each constant kinetics of quality attributes in order to evaluate the significant differences ($p \leq 0.05$) between the treatments. Data were submitted for analysis of variance by means of SPSS (v28 Chicago, IL, USA, 2022). ANOVA analysis was carried out to find the source of the significant differences within the samples examined; the significance of difference was defined at $p \leq 0.05$.

3. Results and discussions

3.1 Coating solution preparation

LMW was not dissolved in PAW because pH was too low, about < 2; for this reason, was discarded from subsequent tests. Indeed chitosan is insoluble in organic solvents, in acids at high concentrations and in alkali; it is also insoluble in aqueous solution at pH ≥ 6, except for low molecular weight samples. On the other hand, it is soluble in aqueous acidic media, following protonation of amino groups in the repeating unit; this polycationic structure is unique, other polysaccharides being usually neutral or anionic (Terbojevich & A Muzzarelli, 2000). Different results were obtained for PE. Indeed they dissolved in both PAW and AW and were stable after centrifugation. Protein extracted from insect flour had a higher solubility both in alkaline (pH = 12) and in acidic solutions (pH = 2) (Bußler et al., 2016). Based on these previous results, to develop a blend only coating 1 and 2 were used. These two different blend solutions were applied to fresh-cut apples and physiological, chemical physical and nutritional properties were studied.

3.2 Respiration and transpiration rate

Figure 2 shows the TR and RR results of samples coated with coating 1 and 2 and without coating. The respiration rate (Figure A) of samples without coating is 17.1 ± 0.4 mg * kg^{-1} h^{-1}, while the average value of samples with coating 1 and 2 was 11±1 mg*kg^{-1} h^{-1} and 9.9 ±1 mg*kg^{-1} h^{-1} respectively. There were no statistically significant differences ($p \leq 0.05$) between the samples with coatings, but statistically significant differences ($p \leq 0.05$) with respect to the control sample. Due to the coatings, a reduction of respiration rate of 40% was observed compared to control samples, as reported by Volpe et al., (2019). For their properties, biopolymer coating can create a reduction of carbon dioxide. When using edible films and coatings on minimally processed fruit and vegetables, a modified atmosphere can be created around the product reducing the respiration rate (Rojas-Graü

et al., 2008). Indeed, biopolymer coating based on protein and chitosan due to their hydrophilic nature can act as barrier to non polar substances such as oxygen or carbon dioxide. Thus, by using the coating technology, developed with chitosan and PE in PAW, in combination with refrigeration temperature the utilization of the modified atmosphere packaging technology can be avoided. Similar results were reported by Solís-Contreras et al., (2021) who showed that coating developed with chitosan was able to reduce the respiration rate of minimally processed apples stored at 25 °C of almost 40% due to oxygen barrier properties of coating. Coatings developed with chitosan is able to reduce respiration rate of fresh cut fruits, hence senescence, which leading to less damage to biological membranes, and lower cell wall hydrolytic enzyme activity, is reduced (Carvalho et al., 2016).

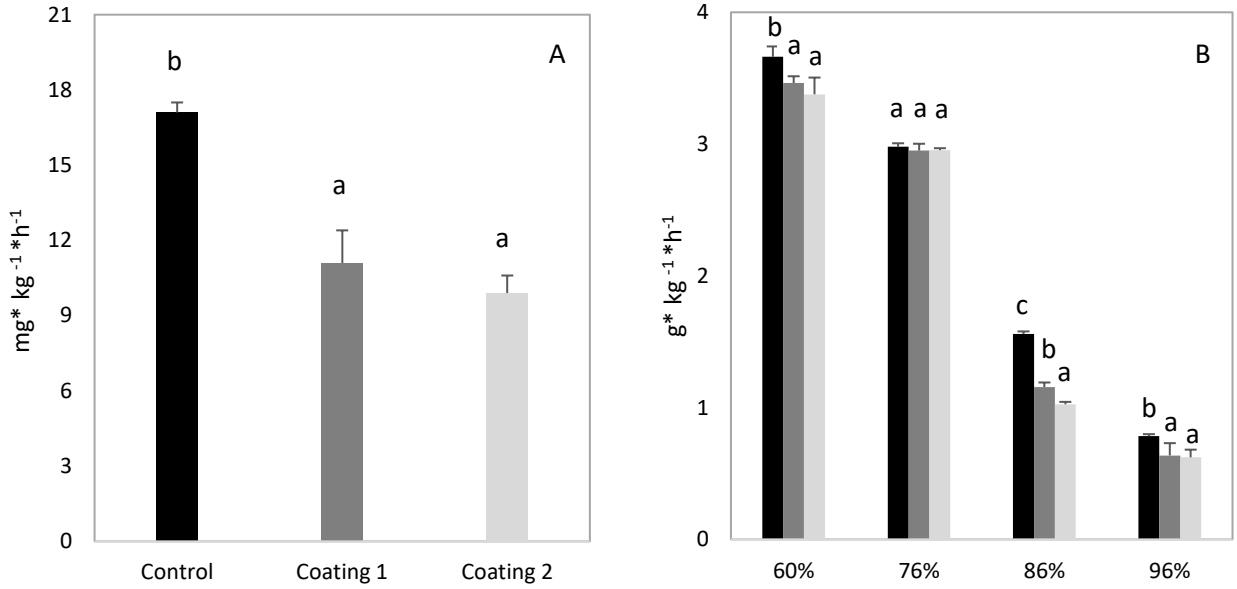


Figure 2. Average values and standard deviation of physiological properties of control (●), coating 1 (●) and 2 (●) samples stored at 5°C. Different letters correspond to significantly different samples for the sample effect ($p \leq 0.05$).

TR results of the samples stored at 5 °C and 60%, 76%, 86% and 96% of RH are shown in Figure 2 B. As the relative humidity increases, there is a decrease in the rate of transpiration (Volpe et al., 2018), moreover, the samples with coatings show lower values than the sample without coatings at 60, 86 and 96% RH. The presence of the coatings reduced TR of 6%, 30%, and 20% of samples stored at 60%, 86%, and 96%, respectively. Statistically significant differences ($p \leq 0.05$) emerged between the samples with and without coatings, with the exception of the sample stored at 76% RH. In this case, no statistically significant differences were observed among the samples, whose TR showed an average value of 2.97 ± 0.04 g $kg^{-1}h^{-1}$.

The results are in agreement with those obtained on mushroom, strawberry and pomegranate arils (Caleb et al., 2013; Mahajan et al., 2008; Sousa-Gallagher et al., 2013). TR of fresh-cut apples is of the same order of magnitude as the TR mushrooms (range from 0.29 to 5.2 g kg^{-1} h^{-1}) (Mahajan et

al., 2008), but higher than TR of strawberries (range 0.24-1.16 g kg^{-1} h^{-1}) (Sousa-Gallagher et al., 2013) at the similar range of temperature and relative humidity. The higher TR of fresh-cut apples than other fruit and vegetables can be justified considering that the water loss rate varies with the type of product and the effect of the coating applied on apples. With these results, coatings 1 and 2 preserved the physiological properties of fresh-cut apples stored at 5 °C.

3.3 Storage conditions of fresh-cut apples

In Table 2 reported the interaction between treatment and time. There were affect significantly on the variables chemical-physical and nutritional properties, only for total polyphenols content there were no statistically significant interactions between time and treatments.

Table 2. Interaction between dependent and independent parameters of fresh-cut apples

	Variables	F	Sign. ($p \leq 0.05$)
Treatment*time	$\%O_2$	11.4	< 0.001
	$\%CO_2$	17.1	< 0.001
	Weight loss	1.19	0.319
	L	12.85	< 0.001
	a*	14.33	< 0.001
	b*	5.15	< 0.001
	ΔE	10.2	< 0.001
	BI	5.6	< 0.001
	pH	1.97	0.052
	°Brix	3.43	0.001
	Hardness	4.27	< 0.001
	TAC	5.55	< 0.001
	TPC	1.72	0.097
	Vitamin C	12.5	< 0.001

3.3.1 Headspace Gas Analysis

Figure 3 shows the headspace gas composition of minimally processed apples stored at 5 °C for 13 days. As expected, the % of oxygen decreased during storage time whereas the carbon dioxide increased due to the respiration rate of the product and the permeability constant of the film. The oxygen changes from 20 % to 16 ± 0.2 % for control samples, whereas for coated samples the equilibrium gas composition was reached after 6 days and the value of oxygen was 19 ± 0.1 % (Figure 3 A). Similar results were obtained for carbon dioxide, as reported in Figure 3 B. % CO_2 were 1.7 ± 0.3% and 0.3 ± 0.1% for control and coated samples respectively after 13 days. The equilibrium values were reached after 4 days of storage for coated samples and after 10 days of storage for control samples. These results can be justified by the reduction of respiration rate due to the presence of the coating, in agreement with the results reported in the previous paragraph. Indeed, ANOVA showed different statistically significant between control and samples with coatings ($p \leq 0.05$) for % O_2 and

CO_2. Same results were reported by Volpe et al., 2019) that fresh-cut apples with and without coating developed with chitosan and protein. Authors reported lower values are a similar range of temperature and storage time. In the specific, O_2 changes from 21% to 6.6 ± 0.2% for control samples, whereas for coated samples the equilibrium gas composition was reached after 4 days and the value of oxygen was 11.6 ± 0.1. However, carbon dioxide changed during stored time, for control samples reached a value of 10.4 ± 0.4% whereas for coated samples the value was 7.2 ± 0.5%.

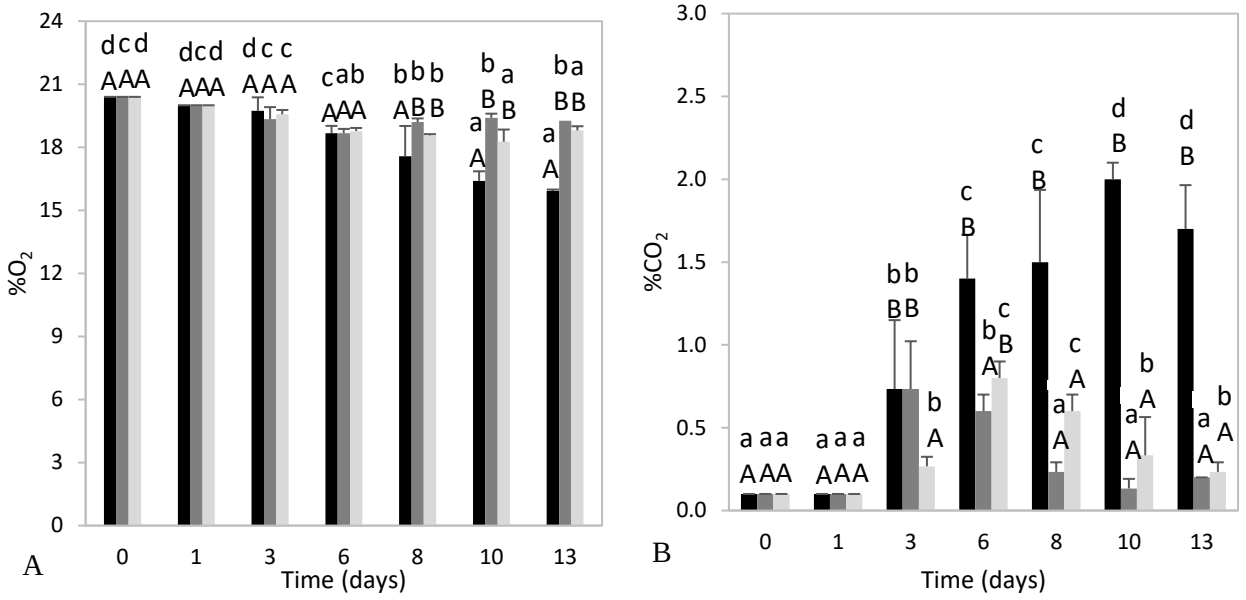


Figure 3. Headspace gas composition (O_2%, (A); CO_2%, (B)) of minimally processed apples stored at 5 °C for 13 days for control sample (●) coating 1 (●) and coating 2 (●). Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

3.3.2 Chemical-physical properties

Table 3 reported weight loss of fresh-cut apples stored at 5 °C for 13 days. During storage time weight loss decreased to 2% for all the samples. ANOVA showed weight loss increased with statistically significant differences ($p \leq 0.05$) during storage time for coated and uncoated samples, however only after 8 days there were different statistically significant ($p \leq 0.05$) between control and samples with coatings. Similar results of weight loss during storage time were reported by Cofelice et al., (2019) with alginate / essential oil nanoformulations applied on fresh-cut apples during 14 days of storage. Moreover, Qi et al., (2011) compared uncoated apple slices with chitosan-ascorbic acid-$CaCl_2$-coated slices and chitosan-citric acid-$CaCl_2$-coated slices, and after 2 days of storage. Uncoated apple slices lost around 19% of their weight, while coated apple slices lost 15% of their weight, and the weight loss gradually continued until the end of the 8 days. Also, Solís-Contreras et al., (2021) reported that weight loss decreased during storage time for apple slices for 25 days at 4 °C with and without coating (chitosan, guar gum and cinnamon essential oil). Active coating showed to reduce the rate of weight

loss with difference with control samples.

Parameters of colour, L*, a*, b* and ΔE are reported in Table 3. L* decreased during storage time by 10 and 2 % for control and coatings samples respectively. Instead, a* and b* increased during storage time for all samples ($p \leq 0.05$), as reported in Table 3. In the specific, a* from -2.2 ± 0.1 until increased from 0 ± 0.8 for control samples, whereas until 6.5 ± 1.0 for samples with coatings. The same results were obtained for b* from a value of 23.8 ± 0.4 increased to 30 ± 4 for all samples. Active coatings showed an increment of colour than control samples, indeed ANOVA reported that coating 2 showed a higher decrement of L* and increment of a* and b* than control and coating 1 ($p \leq 0.05$) samples. The parameter ΔE increased during time with significant variation since the first storage time ($p \leq 0.05$), reaching after 13 days a value of 11 and 23 for uncoated and coated samples respectively, with statistically significant differences ($p \leq 0.05$) between the samples (Table 3). Anyway, coating developed with chitosan and sodium caseinate was able to preserve the fresh-cut apples, as reported by Volpe et al., (2019). Indeed authors showed that coating preserved parameters L* and ΔE respect control samples. ΔE assured a slowly increasing of this parameter during storage time with significant effect after 1, 4 and 7 days of storage. The coating did not have any effect on the variation of the parameter a*, while it had a negative effect on the b*.

Table 3 reported illustrated BI of fresh-cut apples stored for 13 days at 5 °C. BI increased gradually during storage time ($p \leq 0.05$) by 50% and 63% for control and coatings samples respectively. Coated apples presented higher browning index values during the storage period than the uncoated ones from day 1 of storage with statistically significant differences ($p \leq 0.05$). Browning of fruit tissues is strongly associated with not only the activity of polyphenol oxidase and the concentration of phenolic compounds but also oxygen content and temperature of the tissues (Madinez & Whitaker, 1995). Immerging fresh-cut fruits in adequate liquids largely reduces the oxygen concentration in tissues and, thus, helps preventing browning (Rux et al., 2017). Despite that, it is important the colour of the solution used. In our case, the solutions were brown colour due to insect protein. However, processing of the insect powder further affected visual appearance of the flour fractions produced. Experiments showed that colour formation was most likely due to enzymatic browning reactions and also depended on the protein concentration of the respective extract (Bußler et al., 2016). To confirm this Perez-Gago et al., (2006) showed that using white edible coatings (prepared with whey protein isolate, whey protein concentrate or hydroxypropyl methylcellulose all three mixed with beeswax or carnauba wax) significantly reduced BI compared to the uncoated apple slices during a 7 days trail and at 5 °C.

Another important quality parameter evaluated in this study was hardness, as reported in Table 3. The loss of hardness can be attributed to the maturation, ripening and/or early degradation of tissues. Tissue strength is determined by cell size, biochemical and biophysical cell wall properties, cell-to-

cell adhesion and tissue turgor (Toivonen & Brummell, 2008). However, the action of hydrolytic and pectolytic enzymes on the pectic substances had a negative effect on the cell wall, with decreased crystallinity of cellulose, and thinning of the cell walls (Qi et al., 2011). During storage time, the hardness of the fresh-cut apples decreased from an initial value of 12 ± 1 N to a final value of 2.5 ± 0.5 N after 13 days of storage at 5 °C. ANOVA analysis showed a significant effect of the storage time and coating on the hardness parameter. Moreover, coatings helped to retard tissue softening during storage, indeed the effect of the coating was significant at 1, 3 and 6 days time ($p \leq 0.05$). The results obtained are in agreement with Qi et al., (2011); Solís-Contreras et al., (2021). Authors reported that coating developed with chitosan and proteins preserved the hardness of the fresh-cut apples compared with uncoated samples, despite hardness decreasing during the storage time for all samples.

In Table 3 are reported values of TSS and pH. During storage time pH decreased from an initial value of 3.9 ± 0.0 to a value of 3.6 ± 0.0 for coated and uncoated samples (Table 3). The coating has a significant effect on the pH ($p \leq 0.05$) which decreased slightly during storage time, with a significant effect after 1, 3, 10 and 13 days of storage Also Volpe et al., (2019) found that pH increased also for fresh-cut apples stored with chitosan and sodium caseinate until 5 respect control samples with an average value of 5.06.

Table 3. % Weight loss, colour (L*, a*, b*, ΔE, BI), Ph,°Brix and hardness of control and samples with coatings at different storage time.

		Chemical-physical properties								
Samples	**Time (days)**	**Weight loss**	**L***	**a***	**b***	**ΔE**	**BI**	**PH**	**°Brix**	**Hardness**
Control	0	0.0±0.0^{Aa}	78±1 Ad	-2.2±0.1Aa	24±1Aa	0±0Aa	33±1aA	3.9±0.0Ad	12.1±0.1Ac	12±1fA
	1	0.5±0.1^{Aa}	74±1Bdc	-2.8±0.7Aa	27±2Aa	6±1Ab	40±5abA	3.2±0.0Aa	11.8±0.1Bc	8.3±1.1eA
	3	0.6±0.3Aa	77±2Bc	-3.2±0.8Aa	27±3Aa	4±3Abc	38±6bA	3.3±0.0Aa	11.5±0.3Cbc	7.4±0.5dA
	6	0.9±0.1Aab	72±3Ccb	-0.4±1.6Abc	30±2Ab	10±3Acd	53±8cA	3.6±0.8Ac	11.6±0.2Bbc	7.2±0.6dA
	8	0.9±0.0Aab	73±3Bb	-0.6±1.6Ac	30±2Ab	9±3Ad	51±9cdA	3.4±0.0Aab	11.0±0.6Aab	6.6±0.8cA
	10	1.4±0.9Aab	73±3Cb	0.2±0.9Ac	33±3Ab	11±4Adc	59±9deA	3.4±0.0Aab	10.7±0.2Ba	5.3±0.8bA
	13	2.1±1.8Ab	68±3Ca	0.0±0.8Ac	32±4Ab	14±.2Ae	63±11eA	3.4±0.0Aab	11.1±0.3Bab	2.5±0.3aA
Coating 1	0	0.0±0.0Aa	79±1Aa	-2.2±0.1Aa	24±1Aa	0±0Aa	33±1aA	3.9±0.0Ad	12.1±0.1Ad	12±1fA
	1	0.6±0.1Aab	66±2Ab	2.3±0.8Cb	31±2Bcd	15±2Bb	64±6bC	3.5±0.0Ba	10.2±0.1Ab	11±0.4eB
	3	0.6±0.2Aab	65±2Ab	4.1±1.5Bc	32±1Bd	17±3Bb	69±5bB	3.5±0.0Bab	11.0±0.2Bc	9.8±1.2dcB
	6	1.0±0.1Ab	58±3Ac	6.8±0.7Bd	28±2Ab	22±3Bc	73±9cB	3.6±0.0Ac	10.8±0.2Ac	6.7±1.1cB
	8	1.6±0.3Cc	58±2Ac	6.5±0.6Bd	29±1Abc	23±2Bc	76±5cdB	3.5±0.1Abc	10.8±0.5Ab	5.9±1.2bA
	10	1.7±0.9Adc	58±3Ac	6.7±0.9Cd	30±2Ab	23±4Cc	79±10eC	3.5±0.0Bbc	9.2±0.1Aa	5.3±0.3bA
	13	2.1±0.7Ad	58±2Ac	6.5±1.0Bd	29±1Abc	23±3Bc	77±8deB	3.5±0.0Cbc	9.9±0.3Ab	2.5±0.7aA
Coating 2	0	0.0±0.0Aa	78±1Ae	-2.2±0.1Aa	24±0Aa	0±0Aa	33±1aA	3.9±0.0Ad	12.1±0.1Ad	12±1eA
	1	0.3±0.0Ab	73±2Bd	-1.4±0.9Bb	28±3Ab	8±3Ab	47±8bB	3.6±0.0Cbc	10.9±0.6Ac	9.9±1.9dB

3	0.4±0.1Ab	67±2Ac	2.8±0.8Bc	32±1Bc	15±2Bb	66±6cB	3.6±0.0Bab	10.3±0.1Cab	10.1±1.3dB
6	0.9±0.2Ab	64±2Bab	5.2±0.9Bd	31±3Ac	18±3Cc	71±10dB	3.7±0.0Ac	10.6±0.2Abc	7.5±0.8cB
8	1.3±0.5Bc	61±4Ab	6.3±0.8Bd	31±2Ac	21±3Bc	77±7dB	3.5±0.1Aa	10.5±0.2Abc	7.1±0.6cA
10	2.1±0.3Ac	63±3Bab	5.5±0.4Be	30±2Ab	18±2Bd	69±5deB	3.6±0.1Bbc	9.9±0.5Aa	5.5±1.7bA
13	2.1±0.3Ac	61±2Ba	6.4±0.6Be	31±2Ac	21±2Bd	77±6eB	3.6±0.0Cbc	9.7±0.2Aa	2.6±1.1aA

Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

All treatments showed a gradual decrease of °Brix during storage at 5 °C (Table 3). These parameters were higher for control ($p \leq 0.05$) than coated samples that showed a value of 9 ± 0.5 from the initial °Brix (12 %). However, significant effects between the treatments were observed after 1, 3, 6 and 10 days of storage ($p \leq 0.05$). The ripening in postharvest storage causes variations in °Brix as a result of the hydrolytic changes in polysaccharides (Sapper & Chiralt, 2018). Main substrates of respiratory metabolism, sugars and acids are consumed, causing corresponding changes in °Brix of fruits during storage (Chen et al., 2013). Thus, high contents of °Brix in fresh-cut apples with coating 2 after the 13 days of storage may be due to the inhibition of PAW treatment on the respiratory rate of fresh-cut apples, which consequently decreased the consumption of sugars and acids during storage. Our results are in agreement with Ma et al., (2017) that studied Chinese bayberries treated with PAW. The authors reported that after the storage of 8 days, the TSS value of the control decreased to 10 %, while that of the PAW-treated fruits decreased to 11 %. Different results were obtained by Solís-Contreras et al., (2021), Thakur et al., (2019), and Zhelyazkov et al., (2014) because °Brix increased during storage time.

3.3.3 Nutritional properties

Table 4 reported the TAC of fresh-cut apples during storage at 5 °C. Treatments ($p \leq 0.05$) and storage time ($p \leq 0.05$) had a significant effect on antioxidant capacity with significant interaction between factors ($p \leq 0.05$). The antioxidant capacity decreased about 70% for control and coating 1 during storage time, whereas coating 2 decreased by 56% after 13 days, showing that coating 2 is able to reduce about 20% the decrement of antioxidant capacity with respect to other 2 treatments with statistically significant differences ($p \leq 0.05$) during storage time. Coatings developed with PAW preserved antioxidant capacity because have a protective effect again oxygen which is an activation factor for the oxidation reactions. However, coating developed with chitosan and proteins is able to protect fresh cut apples, as reported by Volpe et al., (2019). Also in this study antioxidant capacity decrease was slower for coted samples respect to control samples, with significant different during storage time, except for the samples stored for 14 days of storage.

Table 4. Total antioxidant capacity, total polyphenols content and vitamin C of control and samples with coatings at different storage times.

		Nutritional properties		
Samples	**Time (days)**	**TAC** $mg_{TROLOX}*g^{-1}$ $_{dm}$	**TPC** $mg_{GAE}*g^{-1}$ $_{dm}$	**VIT C** mg $_{vit\ C}*g^{-1}$ $_{dm}$
Control	0	2.34±0.01	141±8	0.6±0.1
	1	1.4±0.1	128±7	0.43±0.01
	3	1.06±0.08	113±6	0.35±0.01
	6	0.79±0.2	97±5	0.27±0.01
	8	0.69±0.05	92±5	0.24±0.01
	10	0.81±0.08	88±3	0.17±0.06
	13	0.78±0.07	78±2	0.08±0.01
Coating 1	0	2.34±0.01	141±8	0.6±0.1
	1	1.33±0.09	112±6	0.43±0.01
	3	1.17±0.04	110±7	0.41±0.03
	6	0.96±0.11	107±3	0.32±0.02
	8	0.86±0.09	107±2	0.26±0.01
	10	0.77±0.09	86±5	0.19±0.01
	13	0.71±0.03	82±6	0.09±0.01
Coating 2	0	2.34±0.01	141±8	0.6±0.1
	1	1.41±0.11	136±8	0.47±0.00
	3	1.3±0.04	122±3	0.39±0.01
	6	1.18±0.09	112±3	0.34±0.02
	8	1.21±0.11	111±6	0.25±0.00
	10	0.95±0.14	85±2	0.23±0.01
	13	0.95±0.07	85±5	0.12±0.01

Lowercase letters represent statistically significant differences ($p \leq 0.05$) during storage time, whereas uppercase letters represent statistically significant differences ($p \leq 0.05$) among treatments.

Apples are a food rich in phenolic components with biological activities. Specifically, chlorogenic acid, caffeic acid, catechin, phlorizin, quercetin, rutin, and epicatechin are present in high amounts (Pająk et al., 2017). However, during the processing of cutting and peeling phenylalanine ammonia lyases are activated and that stimulates the synthesis of phenols contrarily a decrease in levels of phenolic components is attributed to PPO (Cofelice et al., 2019). Table 4 reported the values of the total polyphenols content of fresh-cut apples stored at 5 °C. During storage TPC decreased about 44%, 42% and 39% for control, coating 1 and coating 2 respectively. ANOVA analysis evidenced that there were statistically significant differences since time 1 days, whereas between the treatments there were during time 1, 3 and 6 days. Coating 1 and 2 maintained a similar behaviour during the storage period related to the total phenolic content. Thus, applying coatings developed with chitosan and proteins considerably reduces the loss of total phenolic content. However, coating developed with

1% of CH can increase the total polyphenols content of fresh-cut apples, as reported by Karagöz & Demirdöven, (2019). Vitamin C content in fresh-cut apples during storage at 5 °C as reported in Table 4. Vitamin C content decreased about 50% for control samples after 6 days, whereas after 8 days for samples with coatings. ANOVA showed that during storage time vitamin C content decreased with statistically significant differences ($p \leq 0.05$). Furthermore, samples coated with edible coating showed statistically significant differences ($p < 0.05$) and higher levels of such variables being maintained, throughout storage. Thus, at the end of the storage period vitamin C content of coated fresh-cut apples was 42% higher than for uncoated control fruit, respectively. These results are in agreement with Carvalho and colleagues., (2016), which studied the effect of coating with 2% CH of fresh-cut melon stored at 4 °C for 20 days and found that coating was able to preserve vitamin C content about 33% than uncoated samples. The coating developed with CH is able, also, to preserve vitamin C in other fruits, such as bananas and strawberries. Indeed, chitosan coating has been associated with reducing vitamin C loss to 11% in Cavendish banana (Suseno et al., 2014) compared to 30% found for control, and to 30% compared to 42% for control, in "Earliglow" strawberry (Wang & Gao, 2013).

3.4 Kinetic constants and mathematical modelling

Among the quality parameters studied, browning index, hardness, antioxidant capacity, total polyphenol content and Vitamin C were selected to describe the kinetic variation over time. Table 5 reported the values of the kinetics constant of fresh-cut apples stored at 5 °C for 13 days. The browning index showed an average value of 0.04 day^{-1} for all samples, without statistically significant differences between the treatments. Our values are lower than those reported for fresh sliced mushrooms stored at refrigerated temperature ($k = 97\ day^{-1}$) (Oliveira et al., 2012). It is important to evidence that different values of kinetic constants of BI depend on different treatments and cultivars of the product (Arora et al., 2018).

Kinetic constants of the hardness of samples with coatings showed lower values than control samples, with an average value of 0.058 day^{-1}, reserving about 10% hardness of the product with statistically significant differences ($p \leq 0.05$) than the control sample (Table 5). The value of kinetic constants of hardness is of the same order of magnitude as the hardness of lime fruits (range 0.047-0.057 $days^{-1}$) at 10 °C with and without coating (developed with pectin) (Maftoonazad & Ramaswamy, 2019). In this study the authors found that coating preserved hardness of lime fruits about 22%. Edible coatings applied on fruits directly affect fruit hardness by delaying the ripening process and decreasing the activity of cell wall degrading enzymes. In addition, coatings were able to maintain the hardness by limiting respiration and transpiration rates, which are the primary physiological activities involved in depleting storage reserves (Ergun & Satici, 2012; Menezes & Athmaselvi, 2016).

Table 5. Constant kinetics of browning index, hardness, antioxidant capacity, total polyphenol content and vitamin C content of control coating 1 and 2 of fresh-cut apples stored at 5°C.

Treatments	**BI**		**Hardness**	**TAC**		**TPC**	**Vitamin C**
	k ($days^{-1}$)	Q_{eq}	k ($days^{-1}$)	k ($days^{-1}$)	Q_{eq}	k ($days^{-1}$)	k ($days^{-1}$)
Control	-0.045±0.009a	2.8	-0.065±0.002a	-0.072±0.014b	0.39	-0.043±0.003b	-0.125±0.005b
Coating 1	-0.034±0.002a	2.8	-0.059±0.002b	-0.064±0.001a	0.39	-0.027±0.003a	-0.102±0.003a
Coating 2	-0.043±0.002a	2.8	-0.058±0.009b	-0.065±0.003a	0.39	-0.028±0.003a	-0.098±0.002a

Different letters correspond to significantly different samples for treatments for each quality index ($p \leq 0.05$).

Kinetics constant of the antioxidant capacity of coatings was 0.65 day^{-1} (Table 5), showing a few effects of coating (about 10%) to preserve of fresh cut apples stored at 5 °C. Furthermore, kinetic constants obtained on fresh-cut apples are higher than different cultivars of apples (Starkrimson, Royal gala and Scarlet spur) with an average value of 1.5 min^{-1} (Arora et al., 2018) at room temperature stored at 80 minutes and of fresh-cut watermelon (about 0.037 day^{-1}) at 5 °C during 14 days (Oms-Oliu et al., 2009). On the other hand, the kinetic constant of the antioxidant capacity of fruits depends a variety of factors including thermal processing (Dewanto et al., 2002), and unit operations such as slicing, peeling and storage regime (Piga et al., 2003).

The same results were obtained for total polyphenols content, indeed sample control showed higher values than coatings samples (0.028 $days^{-1}$), reporting the preservation of total polyphenols content about 34% (Table 5). ANOVA analysis showed that there were statistically significant differences ($p \leq 0.05$) between coatings and control samples, and the combination of PAW with LMW and PE showed good effect con fresh-cut apples. On the other hand, values of the kinetic constants of apples were higher than other authors (Arora et al., 2018) that reported an average value of 1.2 min^{-1} during storage at room temperature for 80 minutes.

Finally, values of kinetic constants of vitamin C are reported in Table 5. Also for this nutritional index, coatings showed to preserve products about 20% than uncoated samples. ANOVA test showed that there were statistically significant differences between samples with and without coating ($p \leq 0.05$). Our results were lower than different cultivars of apples and lime (Arora et al., 2018; Maftoonazad & Ramaswamy, 2019). Indeed average values of apples stored at temperature room were 1.5 min^{-1} (Arora et al., 2018), whereas limes stored at 10 °C showed values of 0.0067 and 0.0074 per day for coated and uncoated (Maftoonazad & Ramaswamy, 2019). Figure 8 showed the experimental data and the predicted model of BI, hardness, TAC, TPC and vitamin C of the control

sample, coating 1 and 2 after 13 days.

First kinetic model showed good correlation coefficients for the evaluation of the browning index, antioxidant capacity, total polyphenol content and Vitamin C. In particular, R^2 values ranged between 0.65 to 0.98, RMSE values between 0.01 to 0.36 and MSE values between 0.00 and 0.13, indicating the high ability of the model chosen to fit experimental data. Instead, for the evaluation of hardness, zero-order fitted well the data, with R^2 and RMSE values ranging between 0.84 and 0.91 and 0.06 to 0.10, respectively. These results confirm that zero or first-order kinetics is able to describe how physical and nutritional properties of fresh cut apples change with and without coatings. Table 6 reported values of MSE, RMSE and R^2.

Table 6. Goodness of fitting of pseudo-zero of first order model used to estimate browning index, hardness, antioxidant capacity, total polyphenols content and vitamin C of fresh cut apples with and without coatings at 5°C.

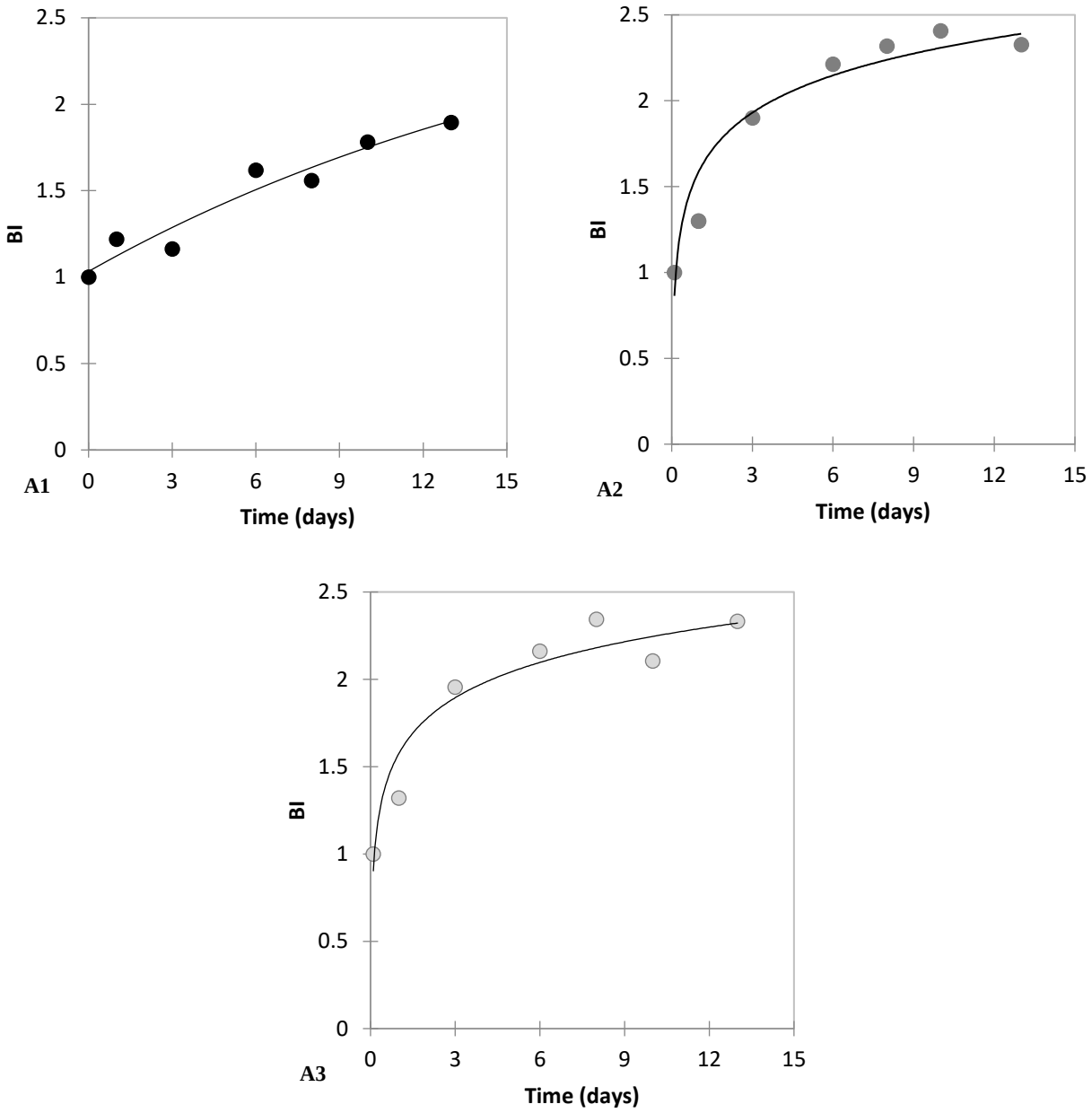
		Control	Coating 1	Coating 2
Browning Index	MSE	0.03	0.13	0.09
	RMSE	0.17	0.36	0.31
	R^2	0.79	0.65	0.69
Hardness	MSE	0.01	0.00	0.01
	RMSE	0.10	0.06	0.08
	R^2	0.84	0.97	0.91
TAC	MSE	0.02	0.02	0.02
	RMSE	0.13	0.13	0.70
	R^2	0.71	0.7	0.69
TPC	MSE	0.01	0.01	0.01
	RMSE	0.07	0.10	0.08
	R^2	0.81	0.72	0.8
Vitamin C	MSE	0.00	0.00	0.00
	RMSE	0.06	0.07	0.04
	R^2	0.95	0.93	0.98

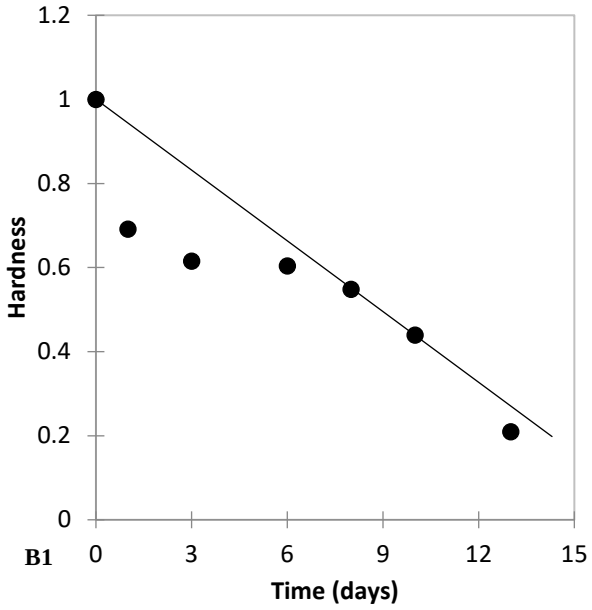
Browning index decreased following pseudo-first-order kinetics, as reported in Eq. 7, as reported in Figure 4 values of BI control (A1), coating 1 (A2) and coating 2 (A3) using first-order. However, BI of processed products does not always follow pseudo-first-order kinetics, as reported by Oliveira et al., (2012); the specific BI of fresh sliced mushrooms stored at refrigerated temperature following Weibull model increased with an R^2 of 0.93.

Pseudo-zero-order is better to describe how decreased hardness during storage time (Figure 4 B1, B2 and B3 for control, coating 1 and 2 respectively). Confirming this, different authors reported that zero order first order kinetic model was found to be the best to estimate experimental hardness data, showing a correlation coefficient of about 0.90 on fresh-cut melons (Amodio et al., 2013), on cabbage (Jaiswal & Abu-Ghannam, 2013), and sichuan sauerkraut (Dai et al., 2020). Furthermore, the

hardness of processed products does not always follow pseudo zero order kinetics, as reported by different authors (Maringgal et al., 2020; Maftoonazad & Ramaswamy, 2019; Maftoonazad & Ramaswamy, 2005).

By comparing the results obtained of nutritional properties degradation followed first-order-kinetics also for other authors, as reported by Arora et al., (2018) that studied kinetics of fresh cut apples of three different cultivars. However, also for other fruits and vegetables pseudo first order was able to describe well the kinetics of nutritional properties, showing high correlation coefficient on strawberries (Muley et al., 2022) and lime fruit (Maftoonazad & Ramaswamy, 2019).

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Time (days)

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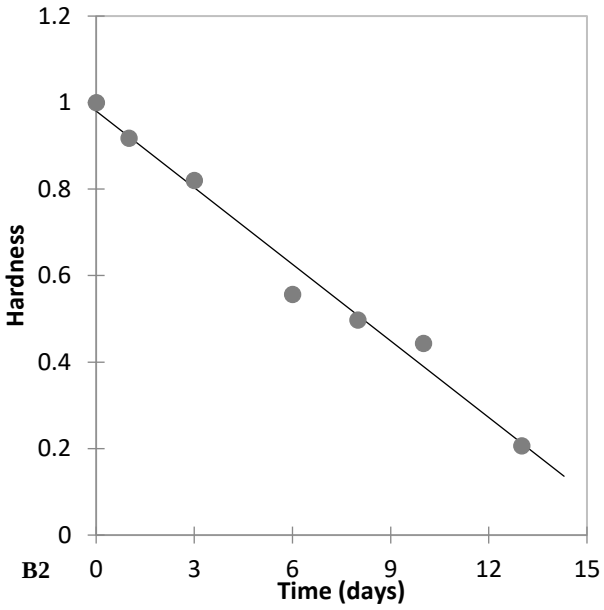
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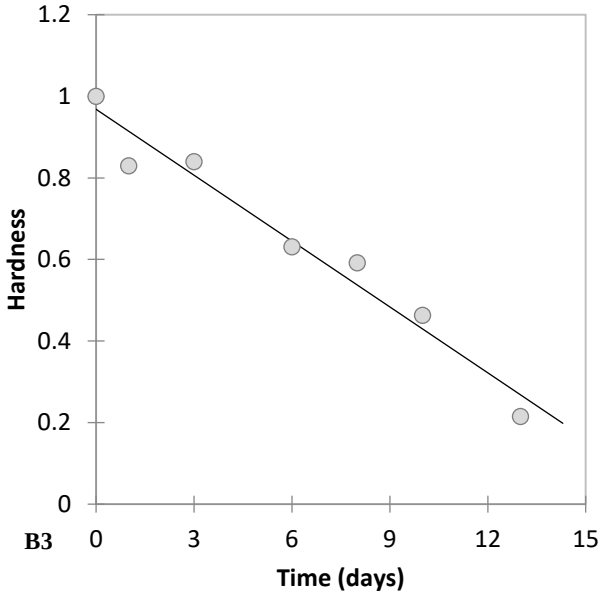
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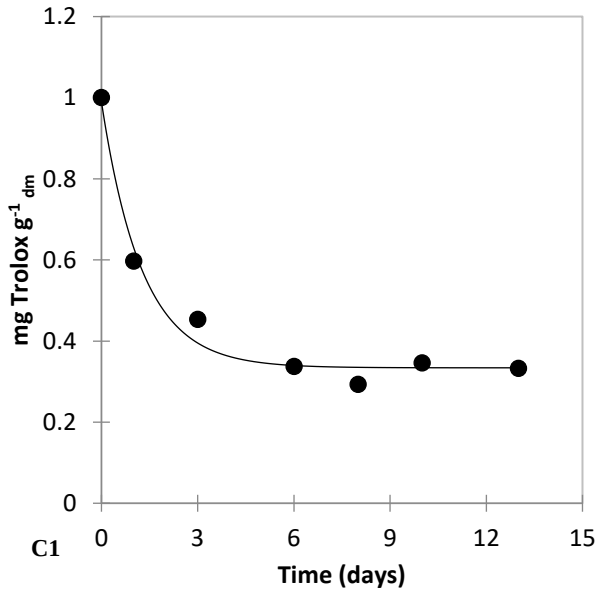
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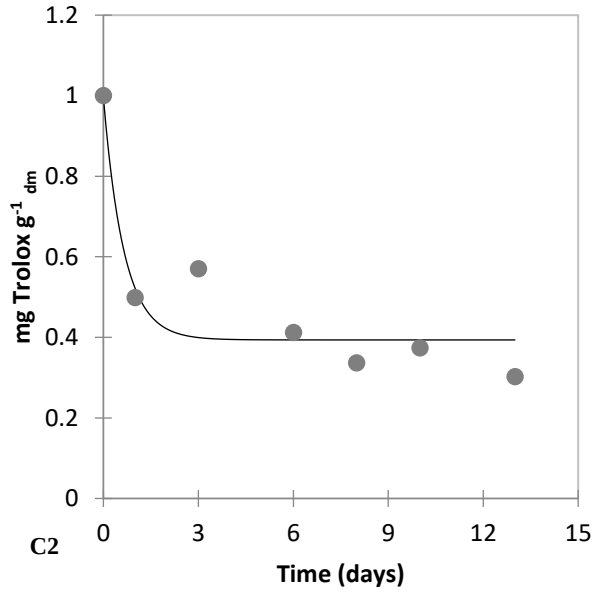
C1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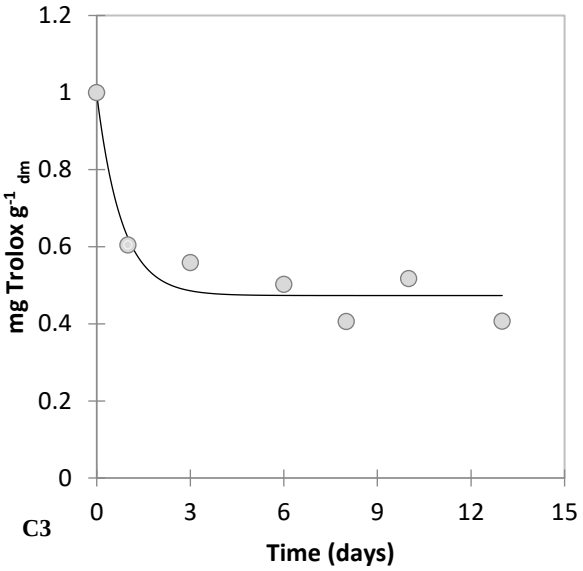
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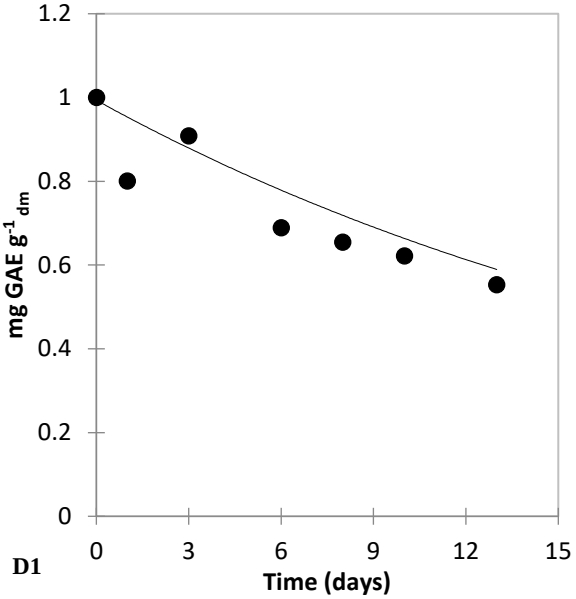
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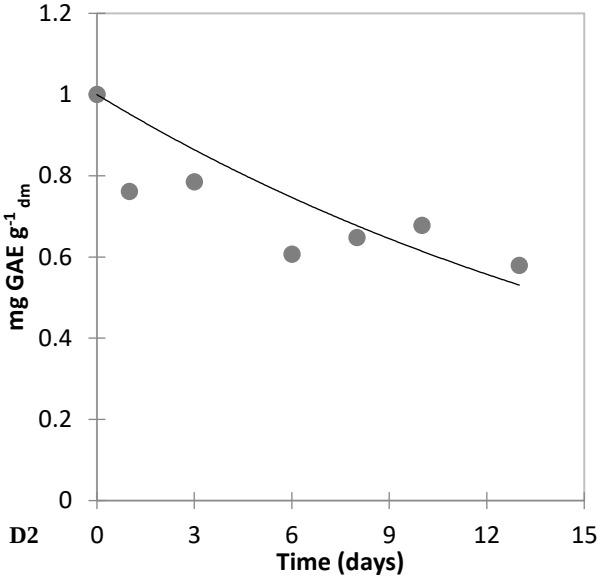
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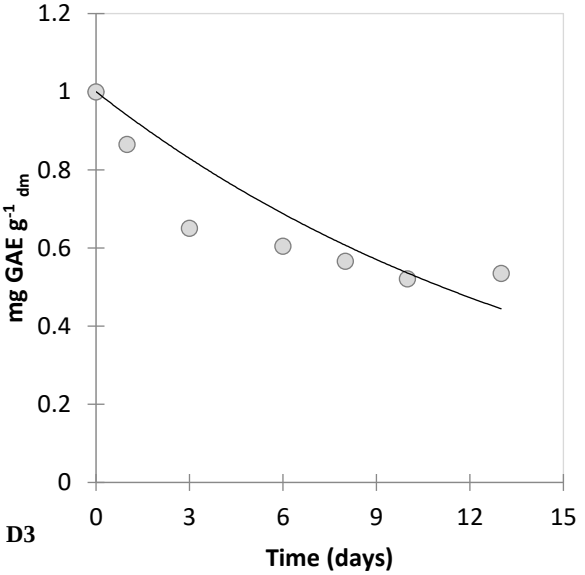
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D3

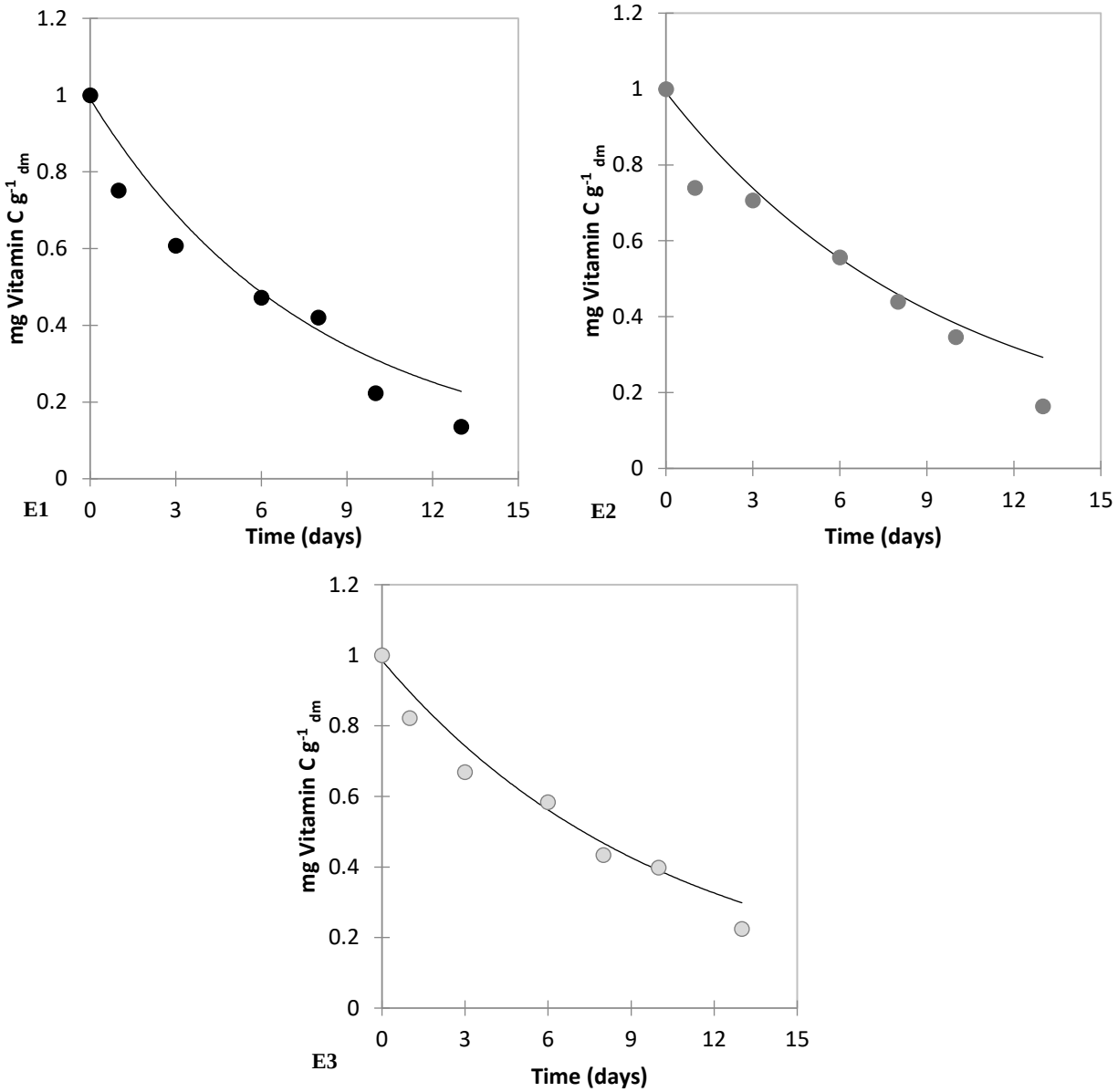

Figure 4. Values of BI (A1,A2 and A3 respectively), hardness (B1,B2 and B3 respectively),TAC (C1,C2 and C3 respectively), TPC (D1,D2 and D3 respectively) and vitamin C (E1,E2 and E3 respectively) of control (●) coating 1 (●) and coating 2 (●) of samples stored at 5 °C for 13 days. — is the data predicted by the model.

4. Conclusions

Plasma-activated water was a good solvent for cricket protein in 1 and 2 %, whereas chitosan was not soluble in PAW at any concentration. The optimal coating composition was a blend of 2 % of protein dissolved in PAW and 2% chitosan dissolved in 1% acetic solution (ratio 1:1). Coatings preserved the physiological properties of the samples about 40% and the variation of some quality parameters during storage time. The first order kinetic model was found to be the best fit for browning index, antioxidant activity, total polyphenols content and vitamin C, whereas zero order kinetic model was used to fit hardness values. Coatings were not able preserve browning during storage, but they slowed

down firmness degradation about 10%, antioxidant capacity and vitamin C content about 20%, and total polyphenol content (30%). In the conclusion, coatings developed with chitosan and proteins of cricket was able to preserve fresh cut apples stored at 5 °C for 13 days.

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CHAPTER 6

6.1 Final remarks and future perspective

In the present PhD thesis, biopolymer active coatings applied on minimally processed fruits and vegetables were investigated and interesting findings were highlighted.

Sodium caseinate is a good solid compound to develop a coating, indeed 8% of SC was the best concentration to prepare active coatings based on SC and PG.

By applying active coating on MP fennels, using the dipping method, the firmness and nutritional quality of MP fennel were preserved. Moreover, the storage temperature affected the efficacy of the coating which showed better efficacy at 4 °C and 10 °C. In addition, the critical parameters of minimally processed fennels were physical (firmness and colour) and nutritional (total antioxidant capacity, total polyphenols content and vitamin C). Pseudo-zero and first-order and Arrhenius type models well described the quality changes of the product over time in the range of temperatures tested. The active coating was able to preserve the nutritional quality of the products by reducing the antioxidant capacity decrease by 40% and vitamin C reduction by 20%. Samples with the active coating are less sensitive to temperature for ΔE (Ea 42 kJ/mol), antioxidant capacity (Ea 113 kJ/mol) and firmness (21 kJ/mol) than the control sample (Ea $_{\Delta E}$ 53, Ea $_{TAC}$ 130 and Ea $_{firmness}$ 46 kJ/mol). Different results were obtained for vitamin C, the control sample was less sensitive than the active sample, with a value of 10 and 24 kJ/mol respectively.

Subsequently, the biopolymer solution was enriched with guar gum, as a polysaccharide compound, and beeswax, as a lipid compound. The developed blend was applied on minimally processed pears and showed good properties of ripening and senescence. Indeed, coating reduced 50% O_2 consumption and CO_2 production than control samples at 4, 10 and 20 °C. Moreover, the active coating was able to preserve firmness and nutritional properties. These parameters were also critical for pears and the pseudo-first-order model well describes the decrement of physical and nutritional properties. Coating was able to preserve firmness about 75% than control samples. Additionally, coating slows down the antioxidant capacity about 40%, 20% of vitamin C and total polyphenols content by 30% than control samples.

Plasma-activated water (PAW) is an important application of the cold atmospheric pressure plasma that can be used as an antimicrobial liquid for the decontamination of food surfaces. The coating was developed using PAW; due to the low pH of the PAW, a biopolymer soluble at low pH must be used. PAW was a good solvent for cricket protein in 1 and 2%, whereas chitosan was not soluble in PAW at any concentration. The optimal coating

composition was a blend of 2 % protein dissolved in PAW and 2% chitosan dissolved in 1% acetic solution (ratio 1:1). The first-order kinetic model was found to be the best fit for browning index, antioxidant activity, total polyphenols content and vitamin C, whereas zero-order kinetic model was used to fit hardness values. Coatings were not able to preserve the browning index during storage, but they slowed down firmness degradation by about 10%, antioxidant capacity and vitamin C content by about 20%, and total polyphenol content (30%) of the fresh-cut apples compared to the control samples. However, no differences were observed between coating prepared with or without PAW.

In conclusion, coatings developed in this study were able to preserve the properties of minimally processed fennels, pears and fresh-cut apples. Selecting the critical quality indices are important to study kinetic models. The kinetic model is a useful tool for shelf-life prediction.

Further investigations should be conducted on the application of the studied biopolymer coatings on other types of products, determining the critical parameters and kinetic constants of the specific product.