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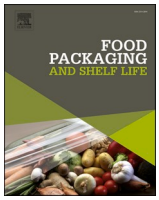

Improving the post-harvest quality of fruits during storage through edible packaging based on guar gum and hydroxypropyl methylcellulose

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ABSTRACT

Fruits are particularly susceptible to post-harvest decay. In this context, bio-based coatings could be a useful and sustainable approach to overcome this problem. The EU Commission is very restrictive about food additives and materials in contact with food, regulated respectively by Reg. UE (CE) 1333/2008 and Reg. UE (CE) 10/2011. In this research, different percentages of hydroxypropyl methylcellulose (H)-guar gum (G) (1–2% w/v) and potassium sorbate (PS) (0–2% w/v) were used to produce coatings to be applied on tomatoes (*Solanum lycopersicum*) and oranges (*Citrus sinensis*). The efficacy of the coatings was firstly evaluated through an accelerated test (28 °C 90% RH, 20 days) on artificially wounded fruits, and then under storage conditions (8 °C 95% RH, 6 weeks). The physical and chemical parameters of the fruits were periodically analyzed and used to evaluate the effect of the coatings on the fruit quality through a multivariate approach (PCA). All coatings were effective in preserving fruits under refrigeration against *Penicillium spp.* and *Alternaria spp.*, but PS led to significant fruit weight loss despite its strong fungistatic activity. Biopolymers showed potential for preserving fruit freshness, but alternative antifungal agents without adverse weight effects should be explored using a similar statistical approach.

1. Introduction

Fruits are an important source of minerals, vitamins, sugars, antioxidant, anti-inflammatory, anti-cancer, and anti-tumor compounds, in a daily healthy diet (Kharchoufi et al., 2018; Nishino, Murakoshi, Tokuda, & Satomi, 2009; Okwu, 2008). Due to the high content of nutrients and their intrinsic high concentration of water (~75–95%), fruits, are particularly susceptible to post-harvest.

The natural metabolic processes that lead fruits to maturity, such as respiration, transpiration, and ethylene production, can significantly influence their quality. Additionally, fruits are susceptible to biological contamination by bacteria, molds, and insects, which can further impact their freshness and shelf life. Understanding and managing these processes is essential for ensuring optimal fruit preservation and reducing spoilage (Jafarzadeh, Mohammadi Nafchi, Salehabadi, Oladzad-abbasabadi, & Jafari, 2021). The maturation and ripening of fruits are mainly related to the respiration process and ethylene biosynthesis (Kou, Wu, & Guo, 2018). Generally, after the beginning of this physiological process, fruits initiate an autocatalytic system, gradually softening over time due to pectin cell wall degradation and a decrease in fruit weight caused by the conversion of starch into available sugars. Moreover, they develop flavor and aroma, and change color due to pigment accumulation and chlorophyll degradation (Iqbal et al. 2017; Kou et al., 2021). The differences in the expression of endogenous hormones and biochemical processes that involve various fruits allow to categorize them into two groups: climacteric and non-climacteric fruits. Two examples that can illustrate the difference between the two groups are oranges (i.e. *Citrus sinensis*), and tomatoes (i.e. *Solanum lycopersicum*). Oranges and tomatoes account for approximately ~75 and ~189 million tons of global production worldwide, respectively (FAOSTAT, 2018). Tomatoes are climacteric fruits that undergo a process of ripening after detachment from the parent plant. As reported by De Vries et al. (1995), ethylene is the main hormone that causes fruit maturation, with 85–90% of this hormone being released from the stem scar region. Ethylene is an endogenous hormone produced by the fruit that may induce a peak in the respiration process, leading to the consumption of O_2 and release of CO_2, increased sensitivity to external ethylene, enhanced autocatalytic processes, and production of VOCs (Volatile

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Organic Compounds). These processes result in a decrease in fruit firmness, degradation of chlorophyll, and accumulation of anthocyanins, carotenoids, and sugars (Chen, Qin, & Tian, 2020; Iqbal et al., 2017). The condition of hypoxia created inside tomatoes, due to the respiration process, triggers the activation of enzymes that lead to the production of ethanol and acetaldehyde (Paul, Pandey, & Srivastava, 2012). In contrast, oranges are non-climacteric fruits, characterized by an inversely proportional relationship between the ripening process and ethylene. As the ripening process continues, the biosynthesis of this hormone declines (Iqbal et al., 2017). Normally, non-climacteric fruits ripen very slowly or do not ripen at all after harvest, and they do not respond to treatment with ethylene hormone (Paul et al., 2012). Citrus fruits are not canonical non-climacteric fruits; they respond to endogenous and exogenous ethylene through the degradation of chlorophyll and the production and accumulation of carotenoids, causing a color change in the flavedo (Goldschmidt, Huberman, & Goren, 1993). However, this hormone has only a slight effect on the maturation and ripening of the fruits themselves (Goldschmidt, 1997). The maturation process leads to the availability of carbon sources that microorganisms and insects could use for their growth and proliferation. For example, it is reported that every year 45% of the fruits are globally wasted due to contamination related to the bad management of the harvesting process from the moment it is detached from the plant to the retail (Guimarães et al., 2019; Manhongo, Chimphango, Thornley, & Röder, 2022). Contamination of the fruits not only causes food waste but also poses a risk to human health. Certain bacteria and molds can produce dangerous metabolites and mycotoxins that are resistant to heat, physical, or chemical treatments. If consumers mistakenly ingest such contaminated products, it can lead to serious health issues (Saleh & Al-Thani, 2019). For instance, *Alternaria alternata* is a widely distributed fungus known to induce post-harvest decay in many different fruits and vegetables, including tomatoes (Troncoso-Rojas & Tiznado-Hernández, 2014). The mycotoxins produced by this pathogenic fungus can lead to food poisoning. Studies have indicated that these toxins can cause keratitis in humans (Xu et al., 2013) and damage DNA in carcinoma cells (Fehr et al., 2009). *Penicillium spp.* is the primary genre of fungi causing post-harvest decay in *Citrus spp.* (Palou, 2014). This genre can also produce mycotoxins that may adversely affect human health. Among them, patulin is a particularly concerning mycotoxin, known for its immunotoxicity, cytotoxicity, and genotoxicity in humans (Rovetto *et al.* 2023). Insects exacerbate the problem of post-harvest decay and contribute to a worldwide fruit waste of 10–20%, annually. In developing countries, this percentage can escalate up to 50% of the total fruit waste (Adedeji et al., 2020). The post-harvest decay process is not the only factor responsible for food waste along the food supply chain. The fruits can be subject to accidental damage. As well, they can have inherent defects which may conflict with quality standards, leading to their disposal as waste. This risk is related to all the levels of the supply chain, including transportation, packaging, processing, retailing, storing, etc. (Bernstad, Cánovas, & Valle, 2017). To avoid waste generation, especially during storage and retail, fruits can be kept in refrigerated conditions, or coated, waxed, packed, and treated with chemical compounds to prolong their shelf-life. For example, oranges are typically waxed or treated with shellac (insect-derived resin) to limit weight loss and to increase the gloss (Khorram, Ramezanian, & Hosseini, 2017; Rojas-Argudo, del Río, & Pérez-Gago, 2009). These treatments provide an excellent barrier to water and gases but might induce, in some cases, the production of off-flavor due to the alteration of the internal atmosphere (Saberi et al., 2018). In addition, the intensive use of chemical fungicides for fruit preservation has led to a general concern related to human health risks and to environmental pollution linked to the residues that these compounds can generate (Fagundes, Palou, Monteiro, & Pérez-Gago, 2015; Guimarães et al., 2019). These concerns have led the authorities to limit and, in some cases, ban conventional synthetic fungicides (Palou, Smilanick, & Droby, 2008). In view of a gradual transition toward a green approach in the sector of fruit production, prospective alternatives to conventional coatings and treatments could be investigated among the GRAS molecules (Generally Recognized As Safe), a list of compounds promulgated by the American "Food and Drug Administration" (FDA), the authority that is responsible for examining and approving whether or not food compounds are eligible for human safety. Besides, the use of natural and GRAS substances for food processing and packaging is widely preferred by consumers, and thus could give to the products a plus-value from a commercial standpoint (Dhall, 2013; Khorram et al., 2017).

Edible films produced from GRAS biopolymers are regarded as an effective alternative to conventional treatments to preserve fruits (Ali, Maqbool, Ramachandran, & Alderson, 2010; Fagundes et al., 2015; Ghosh, Dey, Bhowmick, Medda, & Dutta, 2014; Khorram et al., 2017; Palou, Pérez-Gago, & Valencia-Chamorro, 2014; Ruelas-Chacon et al., 2017; Valencia-Chamorro, Pérez-Gago, del Río, & Palou, 2009). Some examples of polysaccharides, that belong to this category, are guar gum and hydroxypropyl methylcellulose. Guar gum is a non-ionic polysaccharide derived from the endosperm of the seeds of *Cyamopsis tetragonoloba*. The chemical structure of guar gum is characterized by a linear chain of D-mannopyranose branched together with galactose residues (Heyman, De Vos, Depypere, Van der Meeren, & Dewettinck, 2014; Ruelas-Chacon et al., 2017). This polymer has been widely used due to its ability as a thickener, its low price compared to other gums, its high availability, and its biodegradability (Ruelas-Chacon et al., 2017). In the study of Saberi et al. (2018), guar gum and pea starch were blended with oleic acid and used to create an edible coating for oranges, providing a good barrier against the loss of moisture, reducing the respiration rate, the disease index, and the production of ethylene. The same result was obtained by Ruelas-Chacon et al. (2017), that used guar gum to coat tomatoes, delaying the ripening, respiration rate, and the overall loss of quality parameters of the fruits during storage. Hydroxypropyl methylcellulose is a cellulose derivate with promising applications in the food packaging sector, since it is transparent, odorless, and provides a good barrier against oil (Bigi *et al.* 2023). This polymer was blended with lipids (beeswax and shellac) to improve the shelf-life of oranges and tomatoes, without the production of off-flavor, and without affecting ethanol and acetaldehyde content (Fagundes, Pérez-Gago, Monteiro, & Palou, 2013; Fagundes et al., 2015; Guimarães et al., 2019). In the study of Fagundes, Palou, Monteiro, and Pérez-Gago (2014), a coating based on HPMC-Beeswax, with the addition of PS, was applied to cherry tomato fruits. According to sensory evaluations, the coating only slightly affected the flavor until the 42nd day of testing, and no statistical differences were found between treated and untreated samples. Similarly, Cloete *et al.* (2022) reported a minimal impact on the overall acceptability of a coating based on xanthan gum and PS (0.5% w/v), which was applied on fresh-cut tomatoes. In the case of citrus fruits, off-flavour generated during storage are typically related to the production of ethanol and acetaldehyde, particularly under anaerobic conditions caused by the presence of a homogeneous layer of coating covering the fruits. Martínez-Blay, Pérez-Gago, de la Fuente, Carbó, and Palou (2020), Guimarães et al. (2019), and Valencia-Chamorro et al. (2009) demonstrated that the production of these compounds in citrus fruits is cultivar-dependent. Nevertheless, the addition of 2% PS to an HPMC-Beeswax coating did not statistically alter the flavor difference between coated and uncoated samples. This was evident from the lower level of ethanol detected compared to other studies on the same fruits. GRAS salts have been extensively studied on fruits in numerous scientific studies as antimicrobial and antifungal compounds. In particular, potassium sorbate is one of the most effective salts used as a food additive. The activity of this salt is strictly dependent on pH. At low values, the alteration of the transport balance through the fungal cell membranes increases, unlike other organic and inorganic salts, whose average antifungal activity increases as pH increases (Guimarães et al., 2019; Hervieux, Yaganza, Arul, & Tweddell, 2002; Martínez-Blay *et al.* 2020; Palou *et al.* 2014). However, the efficacy of every GRAS salt is difficult to predict due to the countless variables involved in an in vivo

test, like the cultivar of the fruits, the specific pathogens that could contaminate the products, the conditions of temperatures and humidity, etc. (Martínez-Blay et al., 2020). Potassium sorbate (PS) is one of the most used GRAS salts to hinder contamination, growth of molds, and increase the shelf-life of foods (Stopforth, & Kudron, 2020). As reported by different authors (Fagundes et al., 2013; Palou et al., 2014; Valencia-Chamorro et al., 2009; Valencia-Chamorro, Palou, del Río, & Pérez-Gago, 2011), PS is an effective food preservative, particularly active in aqueous solutions or in a polymeric matrix (that permit the release of this compound) at the concentration of 2% against *Penicillium spp.* and *Alternaria spp*. Potassium sorbate has been included in different coating formulations applied to different fruits to test the antifungal activity of the salt against *P. digitatum*, *P. italicum* (Valencia-Chamorro *et al.* 2009; Valencia-Chamorro *et al.* 2011), *C. gloeosporioides* (Martínez-Blay et al., 2020), *C. musae* (Al Zaemey, Magan, & Thompson, 1993), *L. theobromae* (Guimarães et al., 2019; Zhang et al.,2014), *B. cinerea*, *A. alternata* (Fagundes et al., 2013), and *M. fructicola* (Karaca, Pérez-Gago, Taberner, & Palou, 2014), showing an excellent antifungal activity when compared to other GRAS organic and inorganic acids.

The impact of the time and types of coatings is generally investigated one variable at a time. This method does not consider the interaction between the variables along the storage shelf-life test and the influence of the samples over the analyzed parameters (Bigi, Haghighi, De Leo, Ulrici, & Pulvirenti, 2021; Chaichi, Badii, Mohammadi, & Hashemi, 2019). In the present study, different formulations of hydroxypropyl methylcellulose (H) and guar gum (G), with or without potassium sorbate (PS), were applied to two types of fruits characterized by different ripening processes (*Citrus sinensis* and *Solanum lycopersicum*). An accelerated and storage test was conducted, and the physical and chemical parameters of the fruits were analyzed at each sampling time. A principal component analysis (PCA) was used to monitor the progress of the parameters throughout the entire storage test and evaluate the effectiveness of the formulated coatings with antifungal activity. The study aimed to establish a cost-effective technique for monitoring the ripening process and demonstrate the efficacy of the coatings under refrigerated storage conditions for preserving fruits.

2. Materials and method

2.1. Materials and Reagents

The materials for the preparation of film-forming solutions were provided by Flinn Scientific (Batavia, USA), ACEF SPA (Piacenza, Italy), and CARLO ERBA Reagents S.r.l. (Milano, Italy); respectively for guar gum (25 kDa), hydroxypropyl methylcellulose (hydroxypropyl 5–8%, methoxy 28–30%), and pure granulated potassium sorbate. Potato dextrose agar (PDA) was purchased from Biolife (Milano, Italy). Sodium hypochlorite (liquid solution 6–14%) and sodium hydroxide (pellets ≥98%) were purchased from Merck (Darmstadt, Germany).

2.2. In vitro antifungal activity

The in vitro tests were performed through a disk diffusion assay, according to the protocol of Haghighi et al. (2019) with slight modification. *Penicillium spp.* and *Alternaria spp.* strains were isolated from decayed oranges and tomatoes and grown on PDA medium for 7 days at 25 °C. The strains were standardized to a concentration of 10^5 spores/mL using a Bürker chamber for the antifungal test, as described by Fagundes et al. (2015). Sterile PDA Petri dishes were inoculated with standardized strains of *Penicillium* spp. and *Alternaria* spp. molds. Filter paper disks were then aseptically transferred to the center of each plate and soaked with 100 μl of a sterile 2% (w/v) PS solution. The plates were incubated at 25 °C and monitored for radial mycelial growth (RMG) for 3, 5, and 7 days. The inhibition halo diameters were measured with a caliper, and the mean and standard deviation were calculated.

Table 1
Scheme of the produced film-forming solutions.

Formulations	Guar gum	Hydroxypropyl methylcellulose	Potassium sorbate
G	2%	/	/
G_PS	2%	/	2%
GH_PS	1%	1%	1%
H	/	2%	/
H_PS	/	2%	2%

2.3. Preparations of film-forming solutions

Guar gum (G) and hydroxypropyl methylcellulose (H) powders were used to prepare two film-forming solutions by dissolving 2% (w/v) of each polymer in 100 mL of distilled water. To prepare the guar gum solution, the mixture was stirred at 800 rpm and heated to 60 °C for 30 min (Ruelas-Chacon et al., 2017) using a magnetic stirrer/hot plate (Argo Lab, Carpi, Italy). The hydroxypropyl methylcellulose solution was prepared according to the method described by Fagundes et al. (2015), using a magnetic stirrer/hot plate (Argo Lab, Carpi, Italy) set at 90 °C with a stirring speed of 800 rpm until complete solubilization. The opaque solution was stirred at 1200 rpm until clear and was then cooled down to room temperature (~25 °C) with low stirring. Potassium sorbate (PS) was added to both the film-forming solutions (2% w/v) at ambient temperature for 1 h at 800 rpm, as described by Fagundes et al. (2013). After solubilization, any excessive bubbles were removed by centrifugation (Remi Elektrotechnik Ltd., Vasai, India) to ensure the purity of the solution. Another film-forming solution was prepared by blending two solutions of G (2%) and H (2%), added respectively with a concentration of PS of 1% (w/v). The two film-forming solutions were then blended in a 1:1 (w/w) ratio using a magnetic stirrer (Argo Lab, Carpi, Italy), and stirred (800 rpm) until complete homogenization. The final mixture was degassed by centrifugation (Remi Elektrotechnik Ltd., Vasai, India) to remove air bubbles. All the formulations produced in this study are summarised in Table 1. All the thickness of the produced stand-alone films were measured with a micrometer (Model IP65, SAMA Tools, Viareggio, 146 Italia) at five random positions on stripes of 10 × 1.5 cm, and enlisted in Table 1S.

2.4. Accelerated test

An accelerated shelf-life test was conducted to determine whether there is a difference in the effectiveness of the antifungal compound against the tested pathogens under ideal conditions (in vitro) and actual conditions (in vivo) due to the numerous variables related to typical storage conditions. A total of 144 oranges (*Citrus sinensis*) and tomatoes (*Solanum lycopersicum*) were purchased from a local supermarket and selected for size, color, texture, and absence of any type of injuries. In the case of oranges, the selection was based on the uniformity of the maturity index according to Regulation (EU) N° 543/2011, with an orange peel color of at least 4/5 of the total surface of the fruits (Lado, Rodrigo, & Zacarías, 2014). For tomatoes, the fruits were chosen at the light red stage based on the color classification chart provided by USDA (Ruelas-Chacon et al., 2017). Following the protocol of Kharchoufi et al. (2018), the fruits were immersed in a sanitizing solution of 11.5 g/L NaOCl for 3 min and washed repeatedly with sterile distilled water to eliminate all the traces of sanitizer. The fruits were air-dried, artificially wounded (2 injuries in the apical and 2 in the basal parts of every fruit) with a sterile needle and inoculated with 20 μl of 10^5 spores/mL standardized physiological solution of *Penicillium spp.* for oranges, and *Alternaria spp.* for tomatoes. The fruits were placed under a microbiological hood (Angelantoni Life Science, Milano, Italy) to let the inoculum dry and attach to the wounds, and then each fruit was coated with a specific film-forming solution (according to the experimental design) by spraying method. The equipment used to apply the coating on the fruit

surface included an air compressor (EN3–9.5, ELGI Italia S.p.a., Torino, Italy), equipped with an air drying and purification system to avoid any external contamination, and a spray gun suitable for food treatment. A negative control group (C) of uncoated fruits was also included in the study. The fruits were placed in a climacteric chamber for 20 days (T5 = 5th day, T10 = 10th day, T15 = 15th day, T20 = 20th day) at 26 °C and 90% RH for an accelerated test of shelf life. Samples were randomized into 4 blocks of 36 fruits, with six replicates of each fruit for each sampling time. Disease incidence (DI) and severity (DS) were evaluated every 5 days following the protocols described by Scuderi *et al.* (2009) and Zeng, Cao, and Jiang (2006). DI parameter was calculated as the number of fruits contaminated by fungal pathogens out of the 6 samples, for every time of sampling. DS was measured using an empirical scale ranging from 1 to 4, where 1 indicated the absence of molds (0% incidence), 2 indicated the presence of some traces of molds (35% incidence), 3 indicated the presence of mycelium (65% incidence), and 4 indicated the presence of sporulation (90% incidence). Before the analysis of variance, the data were normalized using the acrsine squareroot of the values related to DI and DS parameters. Average DS was calculated following the formula (1) provided by Parafati, Vitale, Restuccia, and Cirvilleri (2015):

$$DS = \frac{\sum (Ci)}{N} * 100 \quad (1)$$

where: *DS* stands for the average severity index, *C* represents the number of fruits in each class, *i* (1-to-4) are the class values, and *N* represents the total number of evaluated fruits.

2.5. Storage shelf-life test

A storage shelf-life test was disposed to ensure the consistency of the experiment and simulate the real storage conditions. Fruits of the same cultivar were chosen from a single supermarket. In total, 180 fruits were selected and sanitized using 11.5 g/L NaOCl for 3 min, followed by repeated washing with sterile distilled water and air-drying. The fruits were then coated using the spraying technique with formulations that were identified as significantly effective in the accelerated test compared to the control. The coated fruits were randomized based on the coating formulations and placed in different trays inside a climacteric chamber set at 10 °C and 90% RH for 42 days (T1 = 1st week, T2 = 2nd week, T3 = 3rd week, T4 = 4th week, T5 = 5th week, T6 = 6th week) to simulate storage conditions (Jafarzadeh et al., 2021). Every week, 5 samples of each formulation were evaluated based on specific fruit quality parameters. A negative control group (C) of uncoated fruits was included in the study. For the oranges, a series of commercially waxed fruits as a positive control (T) were included in the experiment at each sampling time for comparison with our formulation. However, for tomatoes, no positive control was included because there were no commercial treatments available for comparison. Disease incidence (DI) and severity (DS) were evaluated every 7 days to monitor the fruits for any signs of decay.

Table 2
Radial mycelial growth (RMG) of the different solutions prepared, with (0.2% and 2%) and without potassium sorbate (C), against *Alternaria spp.* and *Penicillium spp.*

RMG (cm)	*Alternaria spp.*	*Penicillium spp.*
C	N.D.^{Ac}	N.D.^{Ac}
0.2%	0.55 ± 0.03^{Bb}	1.39 ± 0.13^{Ab}
2%	1.13 ± 0.14^{Ba}	5.17 ± 0.3^{Ac}

Values are given as mean ± SD (n = 5). N.D. means as not detected.

2.6. Weight loss

The weight loss rate of the fruits was recorded at day 0 and at each sampling time. The values were calculated as a percentage of weight loss from the beginning of the experiment, following the standard protocol (AOAC, 2012).

2.7. Peel color analysis

The color of the peel was measured at the beginning of the experiment and at each sampling time to monitor any color changes. Three equidistant measurements were made along the longitudinal axis of the sample for every fruit tested (Fagundes et al., 2015; Saberi et al., 2018). A Spectrophotometer CM-600d (Konica Minolta Inc., Osaka, Japan) was used to measure the color of the fruits using the CIELAB color space, with the following parameters: lightness (L*), red to green (a*), and blue to yellow (b*). These parameters were also used to calculate the ΔE, which represents the overall color difference (2) between the reference value at T0 and the values measured at each time of sampling (Bigi et al., 2023).

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (2)$$

2.8. Soluble Solid Content (SSC)

The soluble solids content (SSC) of freshly squeezed orange and tomato juice was measured in triplicate following the official protocol (AOAC, 2012) using a refractometer ABBE ARW-ORT (ARW Misure, Vicenza, Italy). The measurements were taken at ambient temperatures and expressed in Brix°.

2.9. Titratable acidity (TA)

10 mL of freshly squeezed orange and tomato juice were diluted with 30 mL of distilled water. The titration of the solution was carried out in triplicate using NaOH 0.1 N until pH 8.1 was reached. The values were then calculated as the amount of citric acid in grams per 100 g of fruits (Fagundes et al., 2015).

2.10. pH

A pH meter (XS PH 8 + DHS, Modena, Italy) was used for the determination of pH from ten milliliters of freshly squeezed orange and tomato juice. Three juice samples for every formulation were measured and recorded for statistical analysis (Fagundes et al., 2015).

2.11. Firmness

At each time of sampling, the texture of the fruits was determined using a texture analyzer (Zwick/Roell DO-FB0.5 TS model 2002, Genova, Italy). This device is equipped with a cylindrical stainless probe (8 mm in diameter) that penetrates the surface of the fruit until 1 cm deep. The fruit firmness was evaluated at three different points: apical, median, and basal regions. The force applied by the piston was measured at the end of the deformation and expressed in N/cm^2 (Islam, Dash, & Das, 2023; Olmo, Nadas, & Garcia, 2000).

2.12. Statistical Analysis

The results of the shelf-life tests were analyzed for every parameter through two different one-way ANOVA tests, one between the samples without the influence of time, and one within every time of sampling. A Duncan's multiple comparison test post hoc was also carried out with a significance level of 0.05. A bar plot was created for every parameter, where each bar represents the average ± standard deviation for each

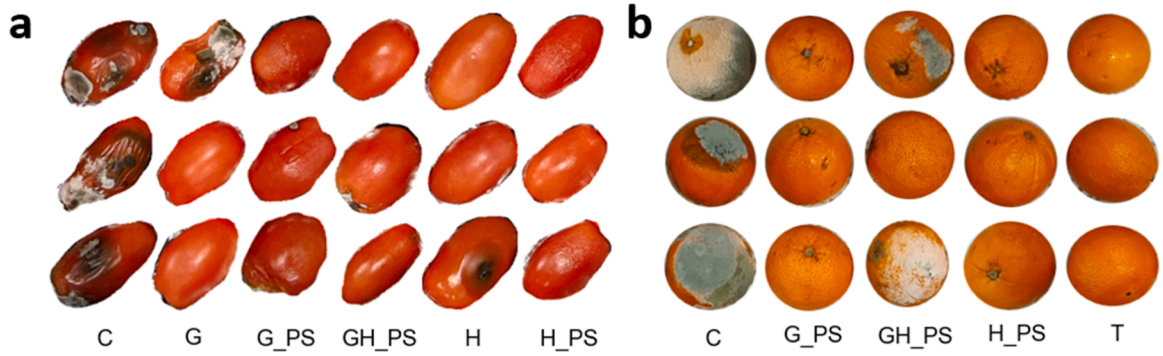

Fig. 1. Visual effect at T4 of the different formulations on a) tomatoes and b) oranges.

sample for every time of sampling. A Principal Component Analysis (PCA) was performed to describe the relationship between the coatings and the results of the different analyses. All statistical analyses were performed using RStudio software, version 2022.12.0 + 353.

3. Results and discussion

3.1. In vitro test

The disk diffusion assay performed in this study revealed that even 0.2% (w/v) of PS was enough to restrain the growth of the two strains of molds, as shown in Table 2. However, this effect is clear against *Penicillium spp.* (1.39 ± 0.13) but not enough for *Alternaria spp.* (0.55 ± 0.03), whose inhibition radius tends to decrease after the seventh day of incubation. This phenomenon is probably related to the fact that the performance as an antifungal compound of PS tends to decrease over time (Martínez-Blay et al., 2020). The fungicidal or fungistatic activity of this compound is strictly related to pH, temperature, the richness of available nutrients of the substrate (for molds growth) where the PS is applied, the concentration of sorbate, exposure time, and sensitivity of the mold strain to the organic acid salt (Liewen & Marth, 1985; Schroeder & Bullerman, 1985). Marth, Capp, Hasenzahl, Jackson, and Hussong (1966) and Fadda et al. (2015) observed a fungistatic rather than a fungicidal activity of PS. In the first case, *Penicillium roqueforti* was able to deplete completely PS at the concentration of 3700 mg L^{-1} after 6 days of incubation. In the second case, *Penicillium expansum* decreased PS from 1000 mg L^{-1} to 45 mg L^{-1} within 48 h, resulting in rapid mold growth after 72 h of the experiment. The degradation of the chemical compound is probably related to the capacity of *Penicillium spp.* to decarboxylate sorbic acid, generating inactive substances and neutralizing the effect of the PS (Marth et al., 1966). Another example was given by Montaño, Sánchez, Casado, Beato, and De Castro (2013), who found that various Lactobacillus species obtained from green olives could be grown on MRS broth in the presence of PS. However, at the concentration of 2% used in this experiment, the halos of inhibition were abundant and persistent over time. Cause of the wide literature about the efficacy of this salt, the radial mycelial growth registered in this study, and the countless variables differentiating in vivo from in vitro tests; the concentration of 2% of PS was chosen as the best to potentially preserve the fruits in the following in vivo tests.

Different capital letters in the same row indicate significant differences ($p < 0.05$).

Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

3.2. Accelerated test

The stress conditions to which the fruits (either coated with different film-forming solutions or not coated) were exposed during the accelerated shelf-life test resulted in significant differences between the samples. The suboptimal storage conditions exacerbated the differences between the samples, leading to the rapid deterioration of the fruits. In the case of oranges, the disease incidence (Fig. S1) was higher in the absence of PS (with mean values over time of 74.30 ± 28% for C, 55.76 ± 21.08% for G, and 55.29 ± 22.89% for H, against values of 27.84 ± 17.56 for G_PS, 34.88 ± 21.69 for GH_PS, and 23.62 ± 17.70 for H_PS), leading to the proliferation of mold due to the high humidity and temperature. The disease severity results confirmed these observations, indicating that the formulations with PS were more effective in controlling mold growth, as shown in Fig. S2. Therefore, we decided to include only the oranges coated with film-forming solutions containing PS in the final shelf-life storage test, to assess the efficacy of this antifungal molecule over an extended period at cold temperatures. For tomatoes, the differences between the control and treated samples were less pronounced at T15 and T20, likely due to the inherent perishability of the fruit. Notably, the only significant differences were observed between the coated and uncoated samples for both the DI and DS parameters, as shown in Fig. S3-S4. As a result, we decided to include all the samples in the final shelf-life storage test.

3.3. Storage shelf-life test

3.3.1. DS and DI

During the sampling period, the DI and DS were recorded, and the values increased due to the contamination by molds and the ripening of fruits. In the case of oranges, the analysis of DI (Fig. S5) showed that, from T2 to T6, the uncoated samples (i.e., control, C) were significantly more susceptible to disease by fungal pathogen than the other samples (average values over time of 50.53 ± 38.87 for DI, and 42.48 ± 36.38 for DS), as confirmed by the DS parameter (Fig. S6). This may be based on the fact that the efficacy of the PS molecule tends to decrease over time, allowing molds to take over the fruits (Martínez-Blay et al., 2020). A possible solution to this problem could be the encapsulation of the antifungal organic acid salt in a thicker film-forming solution, which would provide a slower release of the substance over time. Kowalczyk et al. (2020) encapsulated PS at different concentrations (0.5–2% w/v) in pullulan, gelatin, and the blend of these polymers. Gelatin showed better performance in terms of the release of the antifungal compound due to its superior gelling properties compared to pullulan. However, it is important to note that tomatoes are climacteric fruits, which makes them more perishable than oranges. This difference in perishability may lead to more evident and statistically significant variations between samples. Differences between samples were already observed in the first week of the shelf-life test. Uncoated samples had a higher rate of contamination compared to the other samples, as shown in Fig. S7. On the other hand, G_PS and H_PS formulations showed good results from the beginning to the end of the experiment, with an average DI value lower than ~50% over time. The DS results were similar to those observed for the DI parameter but with slighter differences between the coated samples (Fig. S8). These analyses confirmed that the efficacy of

antifungal GRAS salt on the mold (Fig. 1) is strictly related to the considered variables, such as properties of the fruits, the specifical activity of the salts against the pathogens considered, and the pathosystem (Martínez-Blay et al., 2020). Moreover, the results obtained from in vitro tests can differ significantly from those obtained in vivo due to the numerous variables involved in real marketing conditions (Martínez--Blay et al., 2020).

3.3.2. Weight loss

The weight of the fruits physiologically decreases over time due to the gradient of water vapor pressure and the diffusion of vapor through the layer that separates the fruits from the outer environment, typically the peel (Yaman & Bayoındırlı, 2002). The thickness of the layer, its permeability, temperature, and relative humidity are crucial factors that affect weight loss. Furthermore, the maturation and ripening of the fruits contribute to weight loss due to the respiration process that occurs over time, leading to withering, the release of CO_2, and an increase in sugar concentration in the fruits. Polysaccharide-based biopolymers can establish a semi-permeable barrier that can delay the respiration process, reduce the exchange of water vapor and gas from the surface of the fruits, and reduce weight loss (Khorram et al., 2017). Dong & Wang (2018) found that guar gum decreased weight loss over time in sweet cherries, creating an efficient semi-permeable barrier to gas and moisture. Hydroxypropyl methylcellulose-based coatings also provided a sufficient water barrier to reduce the weight loss of mangoes, decreasing the value to 5% and 10% at 10 ° and 30 °C, respectively, during 18 days of storage (Klangmuang & Sothornvit, 2018). Saberi et al. (2018) found that the presence of free hydroxyl groups in the polymer matrix can create a bond with the cutin of the fruit peels, forming a barrier that hinders the exchange with the environment. In the current study, the 2% concentration of the polymers was not enough to significantly reduce the weight loss of the fruits, probably due to the low concentration of the biopolymers used in this experiment (Ali *et al.* 2010). However, the presence of potassium sorbate in the coating formulations increased the weight loss of the fruits, as reported by Martínez-Blay et al. (2020) and Valencia-Chamorro et al. (2009). As a result, the coating G_PS, GH_PS, and H_PS formulations performed worse than C and T in terms of weight loss (Fig. S9-S10).

3.3.3. Color

The color of fruits is an important parameter to control because it is an essential factor in the consumer's choice of fruit (Singh, & Reddy, 2006).

At the beginning of the experiment, the color of the oranges already satisfies the minimum requirements for maturity index, without the presence of green spots over the surface of the fruits (Reg. EU 543/2011; OECD, 2010). The tomatoes were light red at T0 (USDA, 1997). The color differences were calculated as ΔE between T0 and each time of sampling. Generally, the peel color by ΔE increased over time as expected from the literature (Gao, Liu, Kan, Chen, & Chen, 2019). Ripening of the fruits causes a degradation of chlorophyll in favor of carotenoids, with a decrease in values of L* (lightness) and b* (blue--yellow) in both oranges and tomatoes. In oranges, ΔE drastically increased between T3 and T4 for every sample, but from T4 to T6 there was no significant change (Fig. S11). Only GH_PS and T samples were statistically different from each other during the entire test, with GH_PS having the worst value of ΔE and T having the best value. For tomatoes (Fig. S12), the values increased over time, particularly for GH_PS. C and G samples started with high values, but C increased more than G. G maintained more or less the same value from T1 to T6 (6.58 ± 2.66).

3.3.4. Solid soluble content (SSC)

In the present research, the solid soluble content (SSC) of the samples increases over time, and this effect is related to the ripening of the fruits. During this process, enzymes are produced to convert complex polysaccharides into simple sugars, which are sources of the respiration process that ultimately leads to fruit senescence. For example, in oranges, galactosidase, and glucosidase cause the degradation of pectin (Khorram et al., 2017), resulting in the release of soluble polysaccharides that can affect the SSC (Iglesias & Echeverría, 2009). In tomatoes, phosphorolytic and hydrolytic enzymes contribute to raising the SSC levels by shifting the balance from starch synthesis to degradation (Klangmuang & Sothornvit, 2018; Luengwilai & Beckles, 2009). In this study, the samples coated with potassium sorbate gave the worst results for this parameter, probably due to the previously discussed effect of this molecule on the respiration rate (Valencia-Chamorro et al., 2009). Until T3 for tomatoes and T4 for oranges, the differences between samples were not statistically significant, except for the control samples, which were consistently the worst. After these times, the differences became more pronounced week by week. In the case of oranges, the waxed ones (T) had lower values of Brix° after six weeks of storage (13.03 ± 0.15), although not significantly different from the coated ones (Fig. S13). This effect is probably due to the thicker peel of oranges, which mediates the influence of PS, resulting in better retention of humidity and gas. In the case of tomatoes, formulations G and H had lower values of Brix° (Ali et al., 2010) in comparison with those containing PS (Fig. S14).

3.3.5. Titratable acidity (TA)

The TA of the samples decreased over time, which is a natural process during fruit maturation and ripening. Organic acids, such as citric acid and malic acid, are the main sources of respiration in highly respiring fruits (Ghosh et al., 2014). Citric acid tends to decrease during maturation, while malic acid remains relatively stable after its initial accumulation in unripe fruits (Agius, von Tucher, Poppenberger, & Rozhon, 2018; Lo Piero, Lo Cicero, & Puglisi, 2014). Therefore, it is expected that TA gradually declines over time during the storage period (El anany, Hassan, & Ali, 2009). Naeem, Abbas, Ali, and Hasnain (2018) found that guar gum polymer efficiently decreases the loss of TA, while essential oils in the formulations tested on mangoes by these authors significantly worsened the loss of TA. From the T0, all the oranges treated and untreated met the requirement ratio of sugar/acid to be classified as minimally mature (OECD, 2010). The different coatings tested in this study did not seem to have a significant effect on TA. The only difference observed between the fruits was the initial TA values at T1, which is a naturally unpredictable trait connected to the inherent natural variation of one fruit compared to another, challenging to estimate only visually. However, over time, this effect tended to equalize for every sample, as shown by Fig. S15-S16. Due to the absence of a clear pattern of decrease over time, it is difficult to conclude that the coatings influenced TA.

3.3.6. pH

The acidity of all the samples increased during the time of storage depending on the type of coating (Baldwin et al., 1999). The pH parameter is generally indirectly correlated to the TA values of the fruit juice during post-harvest storage (Pulela, Maboko, Soundy, & Amoo, 2022). In fact, due to the metabolism of the fruits, the concentration of the polysaccharides rises during maturation due to acid hydrolysis, while the organic acids decrease with maturation due to the respiration process (Singh, & Sharma, 2017). The juice obtained from conventionally waxed orange samples (i.e., T samples) had the lowest value (4.48 ± 0.14), while G_PS had the highest (4.51 ± 0.08) at the end of the shelf-life test. The GH_PS and H_PS had nearly the same results as the C sample, with constant growth throughout the entire test (Fig. S17). For tomato juice, the H and G samples were better than the others, with lower values at the end of the experiment; in particular, H was stable from T2 to T4 (4.7 ± 0.03) and from T5 to T6 (4.79 ± 0.00). The C sample was the worst, with the highest pH value, as shown in Fig. S18.

3.3.7. Firmness

The texture of fruits tends to decrease over time, like the weight

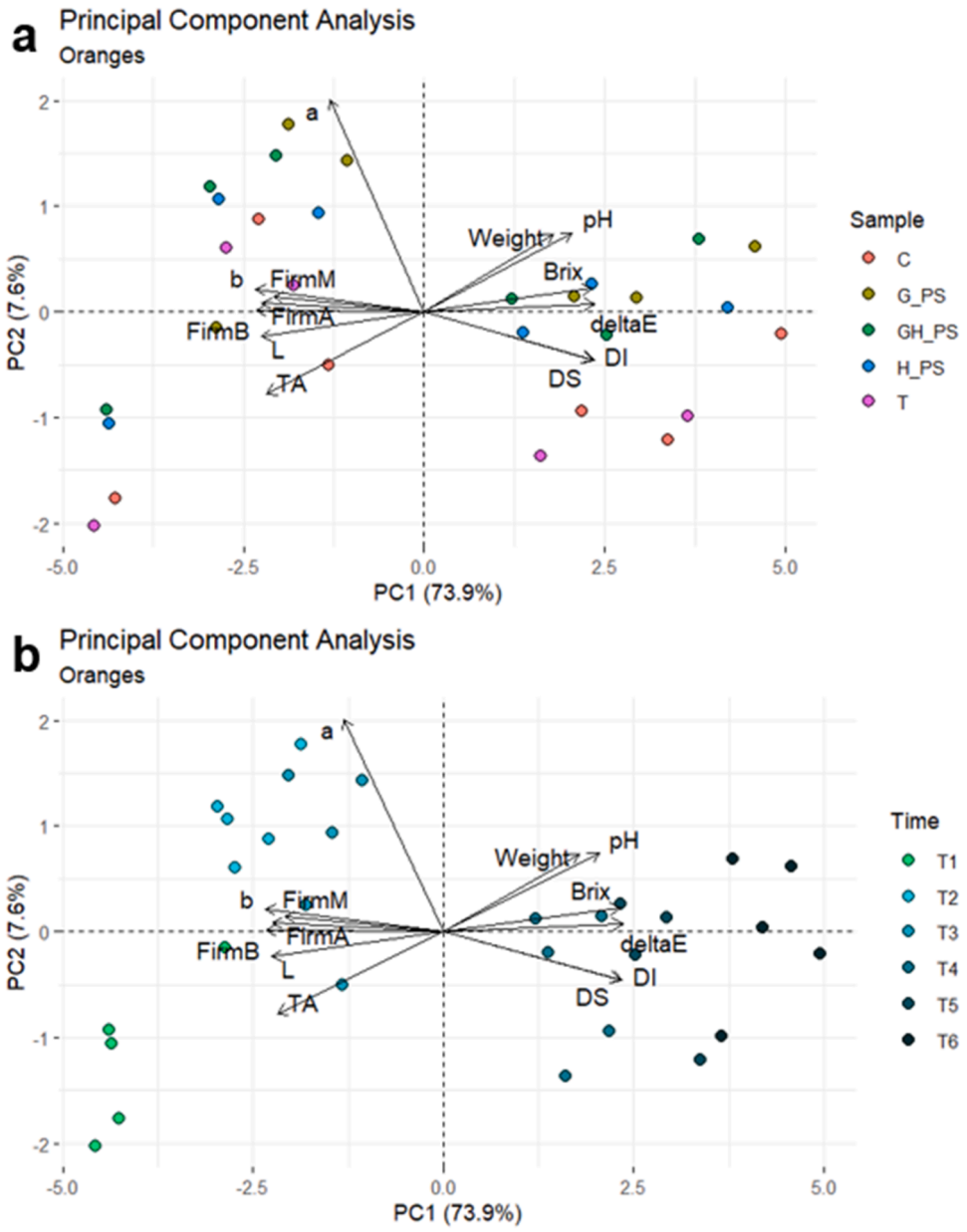

Fig. 2. Biplot of a) the means of the samples at b) the different times of oranges stored at 10 °C and 90% RH for 42 days.

parameter. Fruit firmness is an important quality factor for consumers (Singh, & Reddy, 2006), and its degradation during storage is related to moisture loss, enzymatic degradation of pectin and starch, and their soluble components. Some researchers suggest a possible correlation between firmness and weight loss, but the opinions are contrasting (Hagenmaier, 2000; Navarro-Tarazaga, Del Río, Krochta, & Pérez-Gago, 2008; Perez-Gago, Rojas, & Del Rio, 2002; Valencia-Chamorro et al., 2009). Pectin is primarily degraded by polygalacturonase and pectin-esterase enzymes, which increase during fruit maturation and ripening. The presence of a barrier that mediates gas exchange with the outer environment can limit the respiration process by reducing oxygen intake and retaining carbon dioxide (Yaman & Bayoindirli, 2002). Based on this assumption, it is possible to state that there is a strict correlation between the respiration rate, weight loss, and the firmness of the fruit itself (Ali et al., 2010; Klangmuang & Sothornvit, 2018). In the present case, the high degree of moisture loss negatively influenced the firmness of the fruits (Valencia-Chamorro et al., 2009), due to the disaggregating behavior of PS. This behavior causes a higher water vapor permeability of coatings, which is directly proportional to the concentration of this antifungal compound (Guimarães et al., 2019; Martínez-Blay et al., 2020; Valencia-Chamorro et al., 2009; Zactiti & Kieckbusch, 2006). No significant effect of different coatings was detected Martínez-Blay et al., 2020 probably because of the low concentrations of polymers that could not provide a good barrier to moisture, unequivocally resulting in the excessive softening of the fruits regardless of the type of coatings (Fig. S19-S20-S21-S22-S23-S24).

3.3.8. *Marketing acceptability*

Overall, over time, the coated oranges remained in the "Extra" category, as described by Regulation (EU) N° 543/2011, until the period between the T1 and T2. After that time, the samples coated with G_PS and GH_PS started to manifest physical defects and contamination, leading them to be unequivocally classified in class II or lower. However, H_PS and T samples were the best in terms of marketing quality until T3, with a classification in class I. The C samples performed the worst, belonging to class II prematurely at T2. The average shelf-life of oranges

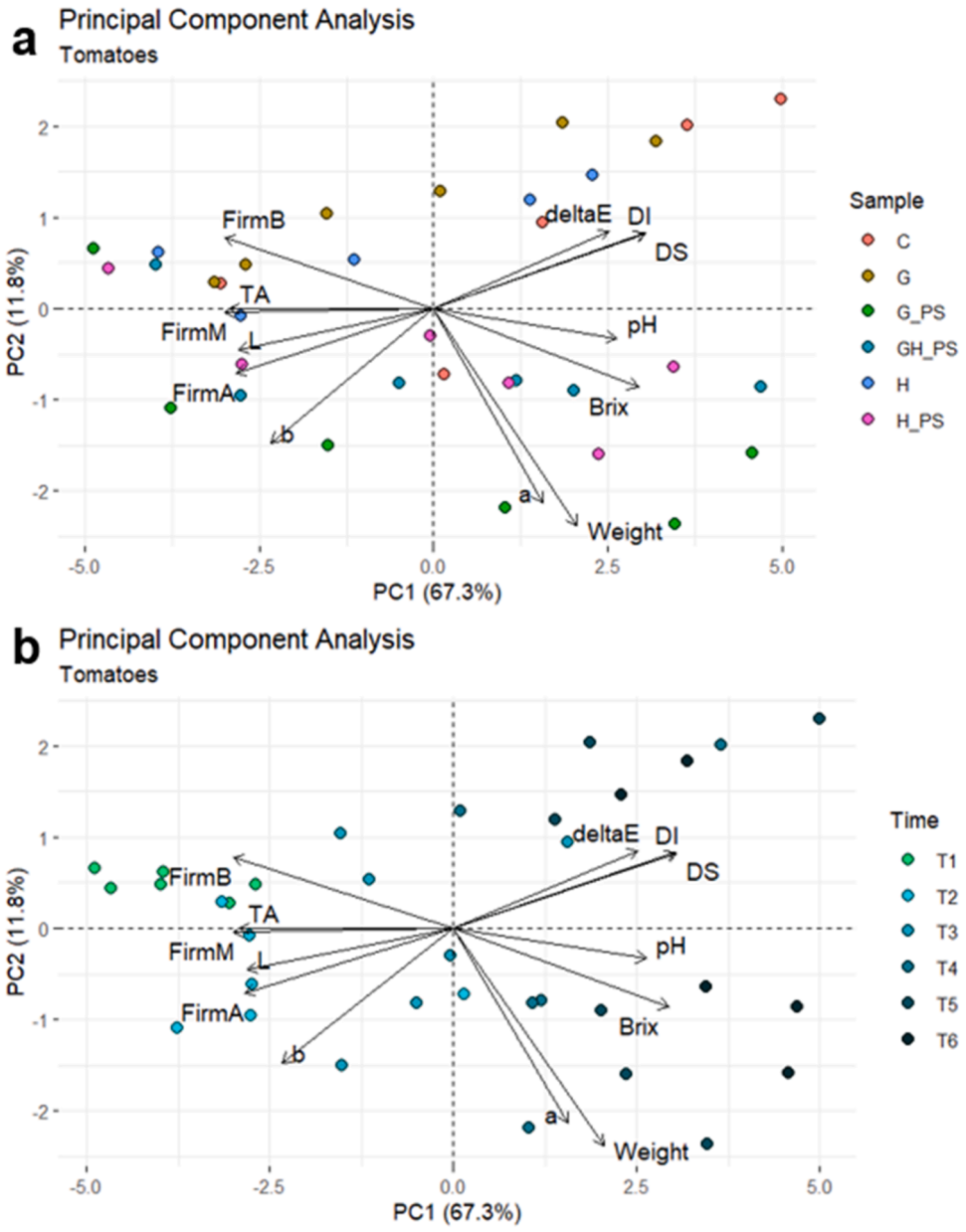

Fig. 3. Biplot of a) the means of the samples at b) the different times of tomatoes stored at 10 °C and 90% RH for 42 days.

is estimated to be 18 days under storage conditions of 10 °C (Khathir, Yuliana, Agustina, & Putra, 2019). In this case, the shelf life of the samples coated with H_PS formulation was prolonged to more than 3 weeks from T0. At T0, the tomatoes were unripe, turgid, clean, fresh in appearance, free from pests, and odorless. In this case, the samples underwent sudden changes (due to the characteristic climacteric behavior) that led to the ripening of the fruits within two weeks of storage conditions. Using the same classification used for oranges (Reg. EU 543/2011; OECD, 2019), the untreated samples (C) were classified as class II in the period from T1 to T2, and decaying prematurely thereafter. The other samples reached a class II at T2, except for G_PS, which was still classified as "Extra" at this time. The latter samples reached class II at T3, beginning a generalized process of decay. Normally, the shelf-life of tomatoes under refrigerated conditions is 12 days (Jany, Sarker, Mazumder, & Shidker, 1970), at ambient temperature, it ranges between 7 and 10 days (Duguma, 2022; Nasrin, Molla, Hossaen, Alam, & Yasmin, 1970). In the current work, the G_PS formulation enhanced the shelf life of tomato fruits in refrigerated conditions for up to 3 weeks, which is an interesting result considering the quick ripening process of this fruit.

3.3.9. *Principal component analysis (PCA)*

The principal component analysis (PCA) is a multivariate statistical method that was chosen to assess the presence of correlations between variables related to the physiological conditions of the samples and time of storage (Bigi et al., 2021; Petriccione *et al.* 2015). The first two principal components (PCs) accounted for 81.5% and 79.1% of the variability of the models for oranges and tomatoes, respectively. The correlation plots revealed that the first two PCs were enough to explain most of the variance of the models, and PC2 (in both cases) accounted for the variable "time.".

For oranges, PC1 was positively correlated with pH, weight loss, DI, DS, Brix, and ΔE; these variables were also correlated with each other, particularly DI and DS, ΔE and Brix, weight loss, and pH. In contrast, PC2 was positively correlated with firmness, a* , and b* , and negatively correlated with L* and TA. The biplot (Fig. 2) clearly shows how the

maturation and ripening of the fruits influence these parameters. The weight loss, pH, Brix, and ΔE increased for all samples during the time of sampling, but to a greater extent for the samples with PS. C and T had lower levels for these parameters but a higher percentage of DI and DS. This result reflects the well-known characteristic of PS to permit a higher degree of loss of humidity of the products (if used for coating) and, at the same time, to enhance the antimicrobial property. The PCA of tomatoes revealed a different result with less correlation between variables but more significance of samples within a single parameter. This effect is probably related to the higher degree of perishability of this fruit compared to oranges. PC1 was positively correlated with weight loss, a* , Brix, pH, ΔE, DI, and DS, with a higher correlation between ΔE, DI, and DS, a* and weight loss, and pH and Brix. At the same time, PC1 was negatively correlated with firmness, L* , b* , and TA, as shown in Fig. 3. It is interesting to observe the distribution of the samples as a function of time. In fact, after the T2 it was possible to observe different patterns of distribution for C, G, H, and G_PS, GH_PS, H_PS; this effect is related to the behavior of the samples as a function of the different variables. The samples without PS had a higher degree of DI% and DS%, likely due to the absence of an antifungal compound, and high values of ΔE, leading to faster maturation. In contrast, the samples with PS showed higher levels of weight loss, likely due to the effect of PS. The losses of humidity also influenced the Brix° values, as sugars became more concentrated. The values of firmness were not clear in this case, and TA was indiscriminately lower for all samples at the beginning of the experiment, increasing over time.

The methodology used in this experiment was effective for monitoring and controlling the process of ripening and contamination of fruits during the test period. This approach made it possible to use affordable physical and chemical techniques to create specific profiles of the different coatings used, verifying their efficacy in prolonging the freshness and integrity of the fruits. This method could be further enhanced by applying Near-Infrared (NIR) spectroscopy, an instrument that can instantly measure fruit parameters and simultaneously check their quality over time without the need for invasive and expensive techniques (Bureau et al., 2009; Ventura, De Jager, De Putter, & Roelofs, 1998). The technology for applying such tests and monitoring fruits during storage conditions already exists. Now, it is only a matter of disseminating the method and implementing it at an industrial level. The major constraint of this study, and other works aimed at developing new edible coatings or films based on biopolymers, is the difficulty in finding compounds that are safe for food contact, suitable for human consumption, and regulated by international organizations for the country in which they are to be marketed (Fagundes et al., 2013). Additionally, these molecules often have limited antimicrobial and antifungal activity, greatly restricting their application and preventing widespread use in the food industry (Fagundes et al., 2015).

4. Conclusion

In this work, PS was selected as an antifungal compound for its efficacy against fungal strains belonging to the genus *Alternaria spp*. and *Penicillium spp*. *In vitro* tests confirmed the antifungal activity of PS against the target strains, demonstrating inhibitory activity sufficient for both genres at a concentration of 2%. PS was included in all formulations for an accelerated test, which showed significant results preserving the coated fruits compared to uncoated ones. Furthermore, the final storage test showed good results in slowing down mold contamination and prolonging storage times when compared to uncoated samples. However, the presence of PS led to a significant increase in weight loss during the test period. The performed multivariate test demonstrated to be a suitable approach to explain the evolution of quality parameters of the coated fruits with the used formulations. Future research should focus on improving coating formulations by exploring higher polymer concentrations or using another efficient molecule that can enhance water retention during fruit maturation.

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CRediT authorship contribution statement

Enrico Maurizzi: Conceptualization, Data curation, Writing-Original draft, Software, Formal analysis; **Francesco Bigi:** Methodology, Investigation, Writing – Review & Editing, Validation, Visualization; **Luisa Antonella Volpelli:** Writing – Review & Editing, Supervision, Resources; **Andrea Pulvirenti:** Supervision, Resources, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2023.101178.

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