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Antifungal Polyvinyl Alcohol Coatings Incorporating Carvacrol for the Postharvest Preservation of *Golden Delicious* Apple

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Abstract: Different polyvinyl alcohol (PVA) coating formulations incorporating starch (S) and carvacrol (C) as the active agent were applied to *Golden Delicious* apples to evaluate their effectiveness at controlling weight loss, respiration rate, fruit firmness, and fungal decay against *B. cinerea* and *P. expansum* throughout storage time. Moreover, the impact of these coatings on the sensory attributes of the fruit was also analyzed. The application of the coatings did not notably affect the weight loss, firmness changes, or respiration pathway of apples, probably due to the low solid surface density of the coatings. Nevertheless, they exhibited a highly efficient disease control against both black and green mold growths, as a function of the carvacrol content and distribution in the films. The sensory analysis revealed the great persistence of the carvacrol aroma and flavor in the coated samples, which negatively impact the acceptability of the coated products.

Keywords: PVA; starch; weight loss; firmness; respiration rate; *P. expansum*; *B. cinerea*

1. Introduction

Postharvest blue mold, caused by *Penicillium expansum*, and gray mold, caused by *Botrytis cinerea*, are two of the most common fungal diseases in apples, pears, and a number of other pectin-rich fruits [1–3]. Initial infection most often occurs at sites of fruit injury, such as bruises, natural openings, or puncture wounds. Although infections may start in the field, infected spots often become evident post-harvest, and expand while fruits are in storage due to the combination of intrinsic factors, such as the high sugar content, water activity, and ideal pH; together with favorable environmental conditions, such as low temperatures and high humidity, which permit the postharvest deterioration of the harvested fruit, this causes considerable economic losses [2,4,5]. In pome fruits, disease symptoms include soft, light brown watery lesions. Sometimes, infection can develop from placing rotted fruit next to healthy fruit, spoiling entire lots [6].

Traditionally, the postharvest management of fresh fruit and vegetable decay involves the use of synthetic chemical fungicides. However, growing public concern over the health and environmental hazards associated with the increased levels of chemical fungicides and the lack of approval for the renewal of some of the most effective active molecules has led to the development of safe, alternative, and natural methods of post-harvest disease control. The application of active coatings using antimicrobial compounds of natural origin for fruit preservation purposes could solve some of the challenges associated with stable quality, nutritional value, health safety, and economic production costs [7]. These coatings can modify the internal gas composition and reduce the water loss through

the regulation of O_2, CO_2, and water vapor exchange between the fruit and the external atmosphere. These modifications could affect the physiological behavior of the coated products, associated with the shelf life of produce, and, in some cases, even modify their characteristics prior to consumption [8].

The incorporation of active ingredients, such as antimicrobial agents (essential oils or their components) into the coating matrix represents an additional advantage, since it permits the reduction of the doses of the active compounds while maintaining their effectiveness [9]. Moreover, the application of this technology could minimize one of the major drawbacks of using essential oil-based compounds, such as its potential phytotoxicity; this shows up as spotting on fruit skin, leading to a loss of marketability and to its strong aroma/flavor, which could affect the organoleptic properties of the product, leading to sensory incompatibilities of the selected active compound with the target fruit.

Of the natural antimicrobial compounds, thymol, eugenol, carvacrol and other terpenoids, and phenolic acids from plant essential oils have been widely reported to effectively inhibit mycelial growth and spore germination through fungistatic and/or fungicidal actions against several microorganisms in both in vitro and in vivo studies [10–13]. Of them, the monoterpenoid phenol carvacrol (one of the major constituents of oregano and thyme essential oils) is considered as a safe food additive in Europe and the USA due to the "generally recognized as safe" status [14]. Carvacrol exhibited antibacterial and antioxidant activity and several studies have demonstrated its effectivity against several food-related spoilage fungi such as *Fusarium* spp., several *Aspergillus* and *Penicillium* strains, *Cladosporium* spp., *Botrytis cinerea*, *Fusarium oxysporum* and *Rhizopus oryzae*, etc. [15–20].

Starch, widely used as a food coating/packaging polymer, is available from diverse plant sources, low cost, and biodegradable. Due to their hydrophilic nature, starch films are highly water sensitive and exhibit poor water vapor barrier properties [21]. To overcome these problems, starch is often blended with other biopolymers in order to obtain coatings/films with enhanced properties. To this end, different studies into starch-polyvinyl alcohol (PVA) blend films have been carried out [22–25]. PVA is a hydrophilic, nontoxic synthetic polymer, biocompatible and biodegradable, resulting in an eco-friendly material [26]. PVA has also received FDA approval for close contact with food products, making it widely used as a cold and hot water-soluble film for diverse packaging applications, including food products, detergents, pharmaceuticals, and agricultural chemicals. Recently, PVA has been submitted to FDA approval for use as a component of a water-soluble edible film containing dry food ingredients (GRAS Notice no. 676, 2018). PVA films have good oxygen and aroma barrier properties, good transparency and high tensile strength, and flexibility. Some authors reported that blend films based on starch-PVA presented several advantages over pure starch films, due to the formation of interpenetrated polymer networks with positive effects on the mechanical and water barrier properties of the composite films [22].

In this study, PVA-starch coatings incorporating carvacrol were applied to apples to evaluate: (1) The postharvest behavior of coated fruit in terms of weight loss, respiration rates, and fruit firmness, (2) the antifungal efficacy of these coatings applied as a curative treatment against *B. cinerea* and *P. expansum*, (3) the sensory acceptance of the coated product.

2. Materials and Methods

2.1. Materials

Polyvinyl alcohol (PVA) (M_w: 13,000–23,000, 87%–89% hydrolyzed) was purchased from Sigma-Aldrich Química S.L. (Madrid, Spain), native potato starch was supplied by Roquette Laisa España S.A. (Benifaió, Valencia, Spain), and carvacrol (C) was provided by Sigma-Aldrich (Steinheim, Germany). Glycerol, used as a starch plasticizer and methanol were supplied by Panreac Química S.A. (Castellar de Vallès, Barcelona, Spain).

Apples (*Malus domestica Borkh cv. Golden Delicious*) were purchased from a local packinghouse (Valencia, Spain). The fruit was chosen according to its uniform shape, size, color, and the absence of surface defects.

2.2. Preparation of the Coatings Forming Dispersions and Films

The coating forming dispersions (CFD) were prepared on the basis of previous studies [22]. Thus, starch (2.5% *w*/*w*) was dispersed in distilled water and kept at 95 °C for 30 min to induce complete starch gelatinization. Meanwhile, 2.5% (*w*/*w*) PVA aqueous dispersion was obtained under stirring at 90 °C for 30 min. Both solutions were cooled down to reach room temperature and afterwards, glycerol was added to the starch dispersion (0.25 *g*/*g* of polymer). Carvacrol, used as an antifungal agent, was incorporated into the PVA dispersion (40% or 80% with respect to the PVA) and homogenized for 4 min at 12,500 rpm using an Ultra Turrax rotor-stator homogenizer (DI 25 Basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany). The starch and PVA dispersions were mixed in the adequate proportion to obtain the different CFD. Table 1 shows the different CFD formulations and their respective solid composition.

Table 1. Mass fraction (X) (*g*/100) and viscosity of the different components in each coating forming dispersion (CFD) and carvacrol retention percentage in the dry films. Mean values (and standard deviation). (S: Starch; PVA: Polyvinyl alcohol; Gly: Glycerol; and C: Carvacrol).

Formulation	CFD					Film
	X_{PVA}	X_S	X_{GLY}	X_C	μ (mPa·s)	Carvacrol Retention (%)
100PVA:0S	2.5	-	-	-	2.92 (0.12) [a]	-
100PVA:0S-C40	2.5	-	-	1	2.89 (0.09) [a]	59 (2) [a]
75PVA:25S-C40	1.875	0.625	0.156	0.75	3.51 (0.02) [b]	44.3 (0.4) [c]
50PVA:50S-C40	1.25	1.25	0.312	0.5	5.33 (0.05) [c]	47 (1) [c]
50PVA:50S-C80	1.25	1.25	0.312	1	6.04 (0.04) [d]	54 (2) [b]

[a–d]: Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

Standalone films were also obtained in order to evaluate the final carvacrol content expected in the coatings after their drying. To this end, a mass of the formulations containing 1.5 g of total solids was spread evenly onto Teflon casting plates (150 mm in diameter) to provide a density of solid of 84 g/m^2. The films were dried under natural convection for approximately 48 h at 25 °C and 45% relative humidity (RH). After drying, the films were peeled off the casting surface and conditioned at 0% RH (using P_2O_5) and at 25 °C.

2.3. Characterization of Coating Forming Dispersions and Carvacrol Retention in the Films

2.3.1. Rheological Behavior of the Dispersions

The rheological behavior of the different formulations was analyzed in triplicate at 25 °C by means of a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) by using a sensor system of coaxial cylinders, type Z34DIN Ti. Measurements were taken between 0–150 s^{-1} where Newtonian behavior could be assumed and the viscosity values (μ) of the dispersions were determined.

2.3.2. Carvacrol Retention in the Films

To quantify the retention of the active compound during film formation, a known mass of dried film was placed in triplicate in amber vials containing 15 mL of an aqueous solution of methanol 50% (*v*/*v*), hermetically sealed and kept under stirring at 300 rpm for 24 h at 25 °C to promote carvacrol extraction. Subsequently, aliquots of the sample extract were measured as to the absorbance (A) at 275 nm, using a spectrophotometer (Evolution 201 UV-Vis, Thermo Fisher Scientific Inc., Shanghai, China), as previously described by [27]. The carvacrol concentration (*C*) in the films was determined by means of a calibration curve obtained with the carvacrol solutions in the same solvent containing

between 1118 and 71.28 µg/mL ($C = 0.014A + 0.0048$; $r^2 = 0.9995$). As blank samples, the extract of the corresponding film without carvacrol was considered (2.3.2).

The carvacrol distribution in the films was analyzed through the microstructure of the film's cross-sections. To this end, the film samples were previously conditioned in desiccators containing P_2O_5 in order to eliminate the water content; then, they were immersed in liquid nitrogen to obtain cryo-fractured cross-sections. All of the samples were mounted on copper stubs and platinum coated. The images were obtained by Field Emission Scanning Electron Microscopy (FESEM) (ZEISS®, model ULTRA 55, Oberkochen, Germany), using an accelerating voltage of 2 kV.

2.4. Quality of Coated Fruit

The apples were cleaned and disinfected by immersion in a 1% sodium hypochlorite solution, thoroughly rinsed with tap water, and air-dried at room temperature before the coating application. The samples were dipped in the different CFD for 3 s, while the non-coated samples (controls) were immersed in a water bath. The CFD were allowed to drip off and afterwards, the applied coatings were dried by natural convection for about 24 h at room temperature and stored at 25 °C and 65% RH for 14 days. Five pieces of fruit were considered for each formulation.

2.4.1. Surface Density of Solids (SDS)

The SDS of each coating was evaluated by weighing each fruit with a precision balance (Kern PFB 120-3, Balinguen, Germany) before and after the coating application to obtain the CFD adhered mass, as it has been reported by other authors [21]. To calculate the total adhered solids, the mass fraction of each CFD was considered and the SDS ($g \cdot m^{-2}$) was calculated by applying Equation (1), according to [28]:

$$SDS = \frac{(m_C - m_0) \cdot X_{SCFS}}{m_0} \cdot \rho \cdot \frac{1}{S_e} \quad (1)$$

where m_C is the mass of the coated apple, m_0 is the mass of the uncoated apple, X_{SCFS} is the mass fraction of the solids of the CFD (g solids/g solution), and ρ is the apple density (0.9 $g \cdot cm^{-3}$). To obtain the specific surface ($Se = 6/d$, m^2 particles m^{-3} fruit), the average diameter (d) was calculated considering a spherical geometry for the fruit.

2.4.2. Weight Loss Rate

Fruit weight loss during storage was determined using an analytical balance (ME235P, Sartorius, Wertheim, Germany) before and after 3, 7, and 14 days of the storage period. The mass loss was referred to the initial mass of each fruit, and the results were expressed as a relative mass loss rate (day^{-1}), which was obtained from the slope of the fitted straight line to the relative weight loss versus time. Five repetitions were considered for each formulation (coated and non-coated).

2.4.3. Respiration Rates

A closed system was used to measure the respiration rate, according to the method proposed by [29], with some modifications. Thus, two apples were placed into 0.940 L hermetic glass jars with a septum in the lid for sampling gas in the headspace at different times. Gas sampling was carried out every 30 min for 4 h by means of a needle connected to a gas analyzer (CheckMate 9900 PBI Dansensor, Ringsted, Denmark). Two replicates per treatment were performed after 3, 7, and 14 days of the storage period. The respiration rate (R_i) of the samples in terms of CO_2 generation and O_2 consumption was determined from the slope of the fitted linear equation, according to Equation (2). The respiration quotient (RQ) was determined as the ratio between CO_2 production and the O_2 consumption.

$$y_{it} = y_{i0} \pm 100 \cdot R_i \cdot \frac{M}{V} \cdot t \quad (2)$$

where y_{it} is the gas concentration (%O_2, %CO_2) at time t, y_{i0} is the initial gas concentration, R_i is the respiration rate (mL·kg^{-1}·h^{-1}), M is the mass of the samples, V is the volume (mL) of headspace, and t is time.

2.4.4. Fruit Firmness

Fruit firmness was measured through a puncture test using a texture analyzer (Stable Micro Systems, TA.XT plus, Haslemere, England) with a 50 N load cell equipped with an 11-mm diameter cylindrical probe, applying a modification of the method proposed by [30]. A small skin area was removed from four opposite sides of each fruit in the equatorial zone where the puncture test was carried out. The probe penetrated the flesh at 10 mm·min^{-1} and the force and distance at the break point of the flesh (Fmax, N, dmax, mm) were determined. Four measurements were taken around the equatorial plane of the apple in five different samples for each treatment at 0 and after 14 days of storage.

2.5. In Vivo Antifungal Assays

Stock cultures of *B. cinerea* (CECT-20973) and *P. expansum* (CECT-20906) were supplied by the Spanish Type Culture Collection (Burjassot, Valencia, Spain). These fungal strains were inoculated on to potato dextrose agar (PDA; Scharlab, Barcelona, Catalonia, Spain) in the dark and incubated at 25 °C until sporulation. Conidia were scraped from the cultures using a sterile loop and subsequently filtered and transferred to test tubes with sterile distilled water and 0.01% Tween 85. The suspensions were adjusted by means of an haemocytometer at 3×10^4 conidia·mL^{-1} for *B. cinerea* and 1×10^5 conidia·mL^{-1} for *P. expansum*, according to other studies [31,32].

Each fruit was wounded (approximately 1.6 mm in diameter and 2 mm deep) at one point of the fruit equator using the tip of a stainless-steel rod and inoculated with a micropipette with 100 µL of the correspondent spore suspension of *B. cinerea* and 20 µL in the case of *P. expansum*.

For the assessment of the coatings' curative activity, the fruit was first inoculated with the different fungal strain and after 24 h, samples were coated as previously described. Once dried, the pieces of fruit were placed on to perforated plastic trays avoiding any direct contact between them and incubated at 20 °C and 85% ± 5% RH. Twelve pieces of fruit were used per treatment. The control fruit was also inoculated using the same procedure, and afterwards immersed in water as previously described.

Disease incidence (% of infected fruit) and severity (lesion diameter) were assessed after 2, 5, 7, 9, and 12 days of incubation at 20 °C and 55% RH. The lesion diameter (mm) was evaluated by using the ImageJ 1.52a software (National Institutes of Health, Bethesda, Maryland, USA).

2.6. Sensory Analysis

A 40-member non-trained panel carried out the sensory evaluation of the samples 2 days after the coating application was performed. The sensory analysis was performed using whole fresh apples and, as control, uncoated fresh samples were used. All the samples, which were previously cut into wedges (1/8 of the whole apple), were presented to the judges at the same time. The judges were asked to evaluate the samples in terms of appearance and aroma, flavor, and overall preference using a 9-point hedonic scale (1 = "dislike extremely", 9 = "like extremely").

This sensory evaluation poses no hazard to human health taking into account the low level of carvacrol ingested and that carvacrol has been recognized as a food additive and as a flavoring substance by the Food and Agriculture Organization of the United Nations (FAO) and European Food Safety Authority (EFSA), respectively [33,34]. The samples were randomly presented with a three-digit code. All of the evaluations were conducted in an EU homologated sensory room.

The panelists were supplied with a rating sheet containing information on the evaluation procedure, in addition to the general oral instructions and individual clarifications as required. The panelists were also required to cleanse their palate with mineral water between the testing of different samples. The panelist's average responses were considered for each attribute.

2.7. Statistical Analysis

The statistical analyses of the results were performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVI.II (StatPoint Technologies Inc., Warrenton, VA, USA). Fisher's least significant difference (LSD) test was used at the 95% confidence level to determine significant differences between means.

3. Results

3.1. Rheological Behavior of the Coating Forming Dispersions

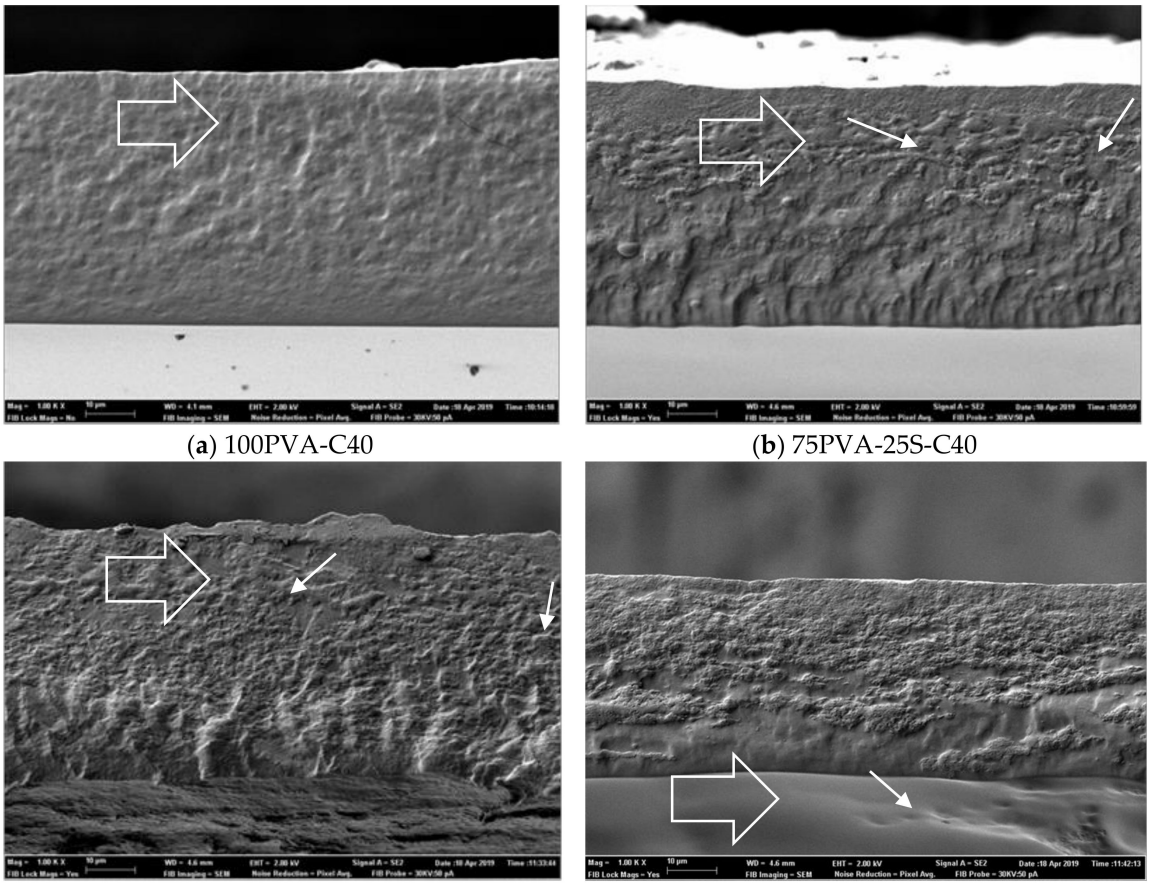
The gravitational drainage of CFD after application occurs before the coating dries, depending on the liquid viscosity, greatly affecting the thickness of the applied coating, which, in turn, determines its barrier capacity. All the formulations exhibited Newtonian behavior in the low shear rate range considered (0–150 s^{-1}) at the solid concentrations used. The gravitational drainage occurs at low shear rates, in the order of 1–10 s^{-1} [35] and the obtained viscosity values correspond to this range. The viscosities of the CFD are shown in Table 1. The incorporation of carvacrol was observed to have no significant effect ($p > 0.05$) on the viscosity value of the pure PVA solution. This could be attributed to the changes in the PVA concentration in the continuous phase due to its interfacial adsorption at the oil-water interphase, as observed by other authors for some polymer solutions containing essential oils [36]. On the other hand, the viscosity significantly rose ($p < 0.05$) when the starch ratio or carvacrol content increased in the polymer blends (Table 1), in agreement with the thickening power of starch or the rise in the dispersed phase concentration (carvacrol). The different values in the viscosity of CFD will affect the coating thickness or surface density of the solids in the coatings.

3.2. Carvacrol Retention in the Films

The carvacrol retention in the films for each formulation is also shown in Table 1. Remarkable losses of carvacrol were expected during the film drying step due to the emulsion destabilization (droplet flocculation and creaming) that occurs in line with the water evaporation and the steam drag effect at the film surface of the creamed droplets, as reported by other authors for cast films containing carvacrol or similar volatile compounds [27,37,38]. Nevertheless, the CA-loaded films exhibited moderate retention values, of about 44%–59% depending on the formulation. The maximum retention capacity was found for the 100PVA:0S-C40 coating (Table 1) with similar values to those found by other authors for PVA films with carvacrol [39]. The retention of carvacrol was promoted by the presence of residual acetyl groups in the PVA chains. As reported by [40], the acetyl groups undergo ionization, generating negative charges in the polymer chain that can interact with the acidic phenolic group of CA. This mechanism promotes the binding of CA to the polymer chains, thus contributing to an increase in its effective retention in the polymer matrix, since the bonded carvacrol is not emulsified and so, insensitive to the emulsion destabilization and evaporation by the steam drag effect [39,41]. Nevertheless, the low viscosity of the polymer aqueous phase was a limitation for the carvacrol retention in emulsified systems during the coating drying step. In emulsified polymer systems, high values of viscosity contribute to reducing the volatile losses since high viscosity limits the creaming and surface evaporation. The relatively low viscosity of the used CFD led to moderate retention values, in comparison with other studies that considered 15% PVA and 2% CA in the aqueous dispersion [41].

The addition of starch to the CFD significantly ($p < 0.05$) decreased the carvacrol retention despite the increase in viscosity. This can be attributed to the lower degree of affinity of the starch and carvacrol compared with that of PVA-C, and the subsequent increase in the emulsified carvacrol content sensitive to destabilization processes and evaporation by means of the steam drag effect. For the same polymer composition in the formulation, the retention efficiency increased as the carvacrol content rose; this can be explained by the increase in the viscosity and the reduction in the creaming rate when the concentration of dispersed phase rose.

The FESEM micrographs of the films obtained with carvacrol (Figure 1) confirmed the abovementioned effects. In the film with only PVA, very small carvacrol droplets were observed, which indicates the PVA's stabilizing effects in carvacrol (bonded or emulsified) that limit the coalescence and creaming phenomena, increasing the carvacrol retention in the film. In PVA-starch blend films, coalescence of carvacrol can be observed forming big liquid clusters entrapped into the polymer matrix during the film forming process (small arrows in Figure 1). In the starch-PVA blend with the highest ratio of carvacrol, the top part of the film during the film-forming step and water evaporation appeared completely flooded by carvacrol, thus revealing the coalescence and creaming of the carvacrol droplets during this step. Differences in the film microstructure and carvacrol distribution could affect the carvacrol release, thus affecting its antifungal action.



(**a**) 100PVA-C40 (**b**) 75PVA-25S-C40

(**c**) 50PVA-50S-C40 (**d**) 50PVA-50S-C80

Figure 1. FESEM micrographs of the cross section of PVA composite films containing carvacrol. Big arrows indicate the upper part of the film in contact with the air during the drying process (top) and small arrows, the presence of carvacrol liquid clusters. (**a**) 100PVA-C40; (**b**) 75PVA-25S-C40; (**c**) 50PVA-50S-C40; (**d**) 50PVA-50S-C80.

3.3. Effect of Coatings on the Postharvest Quality of Apples

Table 2 shows the values of the surface density of solids (SDS), the rate of the relative weight loss, the fruit firmness parameters, and the respiration rates of the apple samples after two different storage times. These parameters allow for the evaluation of the relevance of the coatings on the quality changes in apple during storage.

Table 2. Surface solids density (SSD), weight loss rate, force and distance at the failure point (Fmax, dmax), O_2 consumption rate and CO_2 production rate, and respiration quotient (RQ) of coated and uncoated (control) apples.

Property	Control	100PVA:0S	100PVA:0S-C40	75PVA:25S-C40	50PVA:50S-C40	50PVA:50S-C80
SSD (g/m^2)	-	0.352 (0.112) ^{a}	0.30 (0.07) ^{a}	0.22 (0.08) ^{a}	0.49 (0.13) ^{b}	0.53 (0.09) ^{b}
Weight loss rate (day^{-1})	−0.34 (0.08) ^{a}	−0.32 (0.12) ^{a}	−0.295 (0.012) ^{a}	−0.33 (0.07) ^{a}	−0.34 (0.05) ^{a}	−0.35 (0.05) ^{a}
F_{max} (N)	31 (3) ^{a}	33 (7) ^{a}	32 (4) ^{a}	34 (4) ^{a}	32 (4) ^{a}	29 (3) ^{a}
d_{max} (mm)	3.9 (0.2) ^{bc}	4.0 (1.3) ^{bc}	4.4 (0.2) ^{c}	3.9 (0.6) ^{bc}	3.7 (0.5) ^{bc}	3.5 (0.4) ^{ab}
R_{O2} t = 3 *	11 (5) ^{ab}	6 (5) ^{a}	4.8 (1.5) ^{a}	11.9 (0.6) ^{ab}	9 (4) ^{ab}	15 (2) ^{b}
R_{CO2} t = 3 *	16 (4) ^{b}	6.6 (0.9) ^{a}	11.9 (1.4) ^{b}	13 (3) ^{ab}	12 (6) ^{ab}	14.5 (1.9) ^{b}
RQ t = 3	1.4 (0.5) ^{a}	0.8 (0.5) ^{a}	1.8 (0.3) ^{a}	1.358 (0.003) ^{a}	1.3 (0.6) ^{a}	1.26 (0.03) ^{a}
R_{O2} t = 14 *	11 (3) ^{bc}	6 (0.5) ^{a}	6.7 (1.2) ^{ab}	13 (3) ^{c}	12 (5) ^{bc}	10.5 (0.8) ^{bc}
R_{CO2} t = 14 *	16 (10) ^{bc}	3 (4) ^{a}	9.7 (1.4) ^{b}	16.2 (0.8) ^{bc}	10 (3) ^{b}	19 (2) ^{c}
RQ t = 14	1.46 (0.13) ^{bc}	1.11 (0.07) ^{a}	1.708 (0.107) ^{c}	1.308 (0.097) ^{ab}	1.435 (0.102) ^{b}	1.38 (0.07) ^{b}

*: ($mL \cdot kg^{-1} \cdot h^{-1}$). $^{a–c}$: Different superscript letters within the same row indicate significant differences ($p < 0.05$).

The coating thickness will affect the coating efficiency and its value would be related with the obtained SDS value, although the homogeneity of the solid distribution will also have a significant impact. SDS depends on the amount of coating that adheres to the surface of the fruit and the total solid content of the formulation, and it is greatly affected by the wettability, extensibility, and viscosity of the CFD [42]. The obtained SDS values ranged between 0.3–0.5 g/m^2, depending on the formulation. The amount of adhered solids was relatively low in comparison with similar studies, where thicker dispersions were used [42]. Likewise, the 50PVA:50S-C40 and 50PVA: 50S-C80 coatings presented significantly higher SDS values ($p < 0.05$) compared to the rest of the dispersions, which can be attributed to their higher viscosity values (Table 2). A high degree of viscosity limits the gravitational drainage of the applied dispersion before the drying process is applied to the coating, and so promotes the retention of a greater surface density of the CFD. Therefore, a greater coating thickness could be expected for the thickest formulations of 1:1 starch-PVA blends with carvacrol. This would also imply a greater amount of active compound on the fruit surface.

The firmness of the fruit was evaluated through the maximum force and the penetrated distance values at the failure point, since it represents the deformability of the flesh associated with differences in the cell turgor [42]. At initial time (t = 0), the uncoated control fruit exhibited a force value of 29 ± 3 N and a deformation distance of 2.9 ± 0.4 mm. After two storage weeks, the distance at failure point increased in all (coated and uncoated) of the apple samples with respect to the initial value, thus indicating the loss of cellular turgor throughout time due to the progressive dehydration of the samples at the surface level, which favors the sample deformation without break. However, no significant changes occurred in the maximum force value, which presents similar values for every sample. The samples exhibiting the greatest SDS values have the lowest deformability values, while those coated with pure PVA with carvacrol (with a low SDS value) were the most deformable. However, non-significant differences were found regarding the weight loss rate of the different samples (uncoated and coated) during the 14 storage days. This reflects a mild barrier effect of the coatings to water exchanges, which may be due to the highly hydrophilic nature of the polymers that enhanced the water vapor permeability and to the low amount of adhered solids, which reflected the very limited thickness of the coatings. An increase in the polymer concentration in the CFD, and so in viscosity, could enhance the coating thickness and boost the barrier effect to water exchanges. However, the industrial application of the CFD points to the need for low viscosity in order to facilitate their manipulation.

The effect of the coatings on the respiration rate of apples (uncoated and coated samples) was evaluated through O_2 consumption and CO_2 generation and the respiration quotient (RQ) after 3 and 14 days of storage (Table 2). In uncoated apples (control), these values were 11 ± 5 and 16 ± 4 $mL \cdot kg^{-1} \cdot h^{-1}$ for O_2 consumption and CO_2 generation, respectively, coinciding with other studies [42]. Coatings can serve as gas barriers, which could reduce respiration rates [43], due to a blockage of the surface pores. A lower respiration rate is associated with a lower exchange of

gases and, therefore, a more limited availability of oxygen to allow respiration. However, changes in the internal atmosphere of the fruit depend on the type of material used, the homogeneity and thickness of the coating, and potential interactions with the natural wax of the fruits, etc. [42,44]. In general, a decrease in the O_2 consumption and CO_2 production was observed in the coated samples, although the great variability found made this decrease non-significant in most cases, despite the fact that these hydrophilic coatings represent an excellent barrier to oxygen [45]. Samples coated with pure PVA, containing or not carvacrol, exhibited the lowest respiration rates in line with the lower oxygen permeability of PVA [22]. The variability in the values can be attributed to the natural variability in fruits and to the small amount of coating material deposited on the surface that may lead to the incomplete coverage of the fruit once the coating dries [46]. In general, no significant changes in the respiration patterns were observed throughout the storage period.

The respiration quotient (RQ) ranges from 0.7–1.3 in aerobic respiration depending on the metabolic substrate [47]. In general, this respiratory quotient did not change over time, which shows that there were no changes in the metabolic pathways during the time of analysis. Nor were any significant differences found in the RQ of the different treatments, except for the formulation with pure PVA with carvacrol, where the RQ was higher after both 3 and 14 days of storage, in line with the lowest oxygen consumption rates with normal CO_2 production rates. Carvacrol incorporation in pure PVA did not reduce the great barrier capacity of PVA to oxygen, but increased the CO_2 production, in comparison with pure PVA.

These analyses led to the conclusion that applied coatings were not effective at controlling the water vapor exchanges of the fruits which produced a slight increase in the fruit deformability during storage, although pure PVA based coatings limited the oxygen consumption to a greater extent than those of PVA-starch blends with carvacrol. This compound did not induce negative changes in the metabolic pathway of apple, as deduced from the values of the respiration quotient. Likewise, it is remarkable that neither the respiration pattern nor the symptoms observed on the fruit surface pointed to any phytotoxicity of the carvacrol in the coated fruits.

3.4. Fungal Decay of Apples

Numerous studies have previously shown that the application of coatings with the incorporation of essential oils, or some of their pure components, contributed to the control of various diseases caused by pathogens in the postharvest storage of fruit (including *B. cinerea* and *P. expansum*) [48–50]. Nevertheless, a wide variability in the efficiency of the disease control can be found due to numerous factors that influence the antifungal properties of the coatings. The nature of the coating matrix, the type and concentration of antifungal compounds used, the species and strains of the target postharvest pathogens, the cultivar and the physical and physiological conditions of the host fruit and the postharvest environmental conditions are among the most important.

The carvacrol concentrations in the coatings, taking into account its mass fraction in the CFD and assuming the same retention percentage as in the films, were: 0.17, 0.10, 0.07, and 0.14 g C/g dry coating for the 100PVA:0S-C40, 75PVA:250S-C40, 50PVA:50S-C40, and 50PVA:50S-C80 formulations, respectively. This implies samples with a higher or lower content of the active compound, whose release rate will also influence the effectiveness at controlling fungal growth when the minimal inhibitory concentration is reached at the infection point. In terms of the carvacrol release, different behaviors could be expected as a function of the carvacrol load in the coating and its distribution in the polymer matrix as a consequence of the different PVA-starch-carvacrol interactions and the final film microstructure (Figure 1). The greater chemical affinity of PVA with carvacrol could limit its release from the matrix when it is richer in PVA. Likewise, the presence of starch in coating formulations could partially inhibit the action of the antimicrobial agent due to its nutritional effect that can favor the growth of fungi, such as *Botrytis* [42]. All of these aspects can contribute to the different growth inhibition behavior observed for the coatings in each fungus.

Figure 2 shows the incidence level of both fungi as a function of storage time both for the control samples and those coated with the different formulations. The incidence percentage reached a practically constant value from 5–8 storage days onwards, depending on the treatment, which was affected by the type of coating and fungus. A 100% incidence of *Botrytis* was observed for both the control samples and those coated with pure PVA without carvacrol, whereas lower percentages (67%–83%) were reached in samples coated with formulations containing carvacrol. In the case of *Penicillium*, the asymptotic incidence level was lower than in *Botrytis*, being more sensitive to the different treatments. Specifically, the greatest incidence level of *Penicillium* was observed for the treatment with pure PVA without carvacrol, which could be related with the ability of *Penicillium* to use PVA as a carbon source for growth purposes [51,52]. On the other hand, the lowest incidence level was detected for the treatments with starch-PVA blends with carvacrol and the intermediate values for both the control samples and those coated with pure PVA with carvacrol. These results indicate the different sensitivity of each fungus to the coating action and carvacrol effect. Whereas every coating with carvacrol reduced the incidence level of *Botrytis*, the incidence of *Penicillium* was only notably affected by the coatings formed by PVA-starch blends, where a faster release of the active compound could be expected due to its weaker bonding to the polymer matrix.

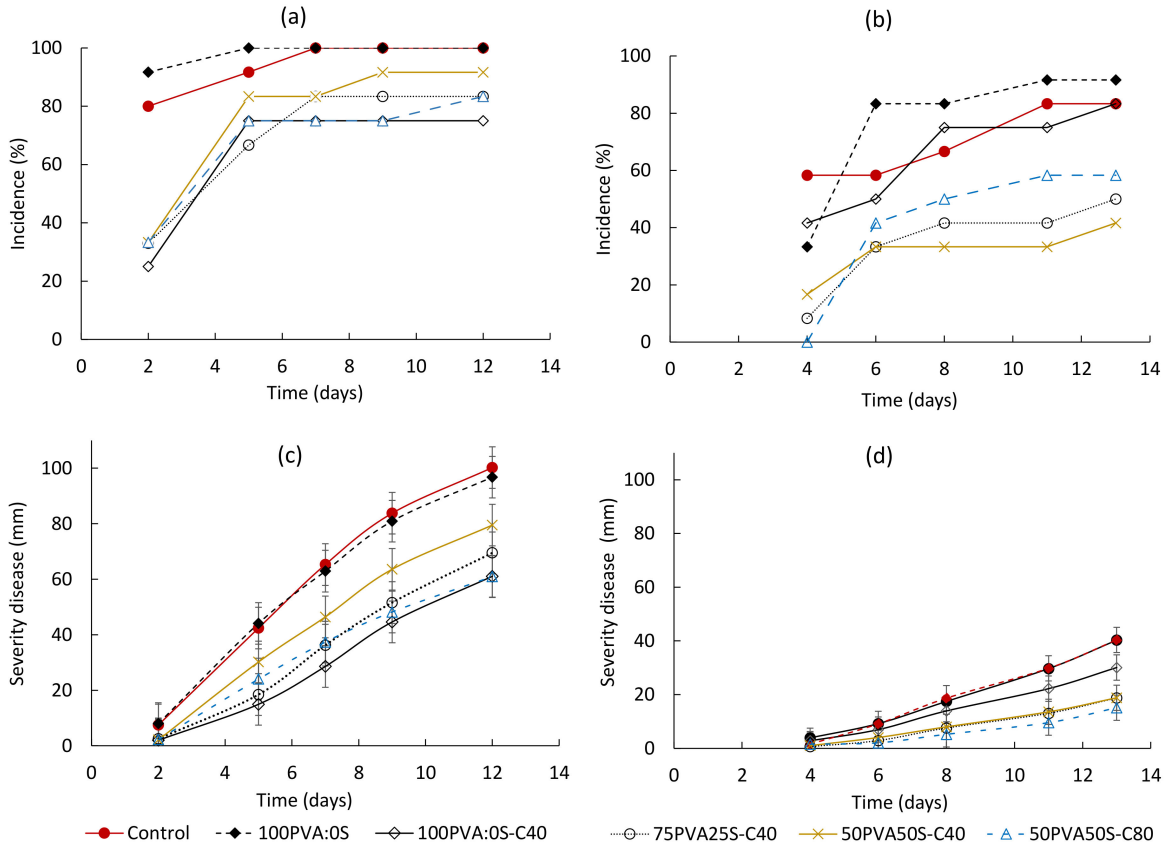


Figure 2. Incidence (%) and severity disease (mm) in apple inoculated with (**a**,**c**) *Botrytis cinerea* and (**b**,**d**) *Penicillium expansum* throughout the incubation time.

Figure 2 also shows the values of the diameter of the lesions (mm), which represent the severity of the disease caused by both fungi (*B. cinerea* and *P. expansum*) in the fruit throughout the storage period. The MANOVA analysis showed that both factors, coating treatment and storage time, significantly affected ($p < 0.05$) the disease's severity, without there being any significant interactions. The disease's severity increased throughout storage time in every case, more slowly in the samples coated with films containing carvacrol, without any significant differences between the control samples and those coated with PVA without carvacrol. The values of disease severity observed for *Penicillium* were a

great deal lower than for *Botrytis*, with different trends concerning the effect of the carvacrol content. In *Penicillium*, the coatings with pure PVA and carvacrol reduced the growth of the lesion diameters to a lower extent than the blend coatings of starch and PVA with carvacrol. In contrast, the reduction in the growth of the fungal lesion for *Botrytis* was less significant with the 50PVA50S-CA40 coating, which contained the lowest final concentration of carvacrol. Therefore, both the concentration of the active compound and its release rate from the polymer matrix affected the fungal growth, depending on the fungal physiology and its sensitivity to the active compound in the different growth stages.

The antifungal activity of carvacrol has been related to the severe damage to the fungal membranes and cell walls, which led to the morphological deformations, collapse and deterioration of the conidia, and/or hyphae [19]. Antifungal effects were also observed by other authors for thymol essential oil (EO) in *Red Fuji* apple [53]. These authors showed that, additionally to the known antifungal activity of thymol essential oil (with thymol and carvacrol as major components), the efficacy of thyme essential oil was also related to the induction of host resistance, since the state of alertness is activated in the fruit after the oil application. Nevertheless, these authors also pointed out that the direct application of the EO provoked the appearance of a certain degree of phytotoxicity in the samples, which was not observed in the samples submitted to the studied treatments. However, the progressive volatilization of carvacrol may reduce its antifungal action throughout the storage time.

To summarize, the application of carvacrol-loaded coatings exerted a positive antifungal effect on apples, as significant reductions in the severity and incidence level of both *P. expansum* and *B. cinnerea* were observed. Thus, after 12 storage days, the severity of the damage was reduced by around 30%, and even by up to 33%, with respect to the control samples when using carvacrol-loaded coatings for *Botrytis* and *Penicillium*, respectively. Likewise, in the same period of time, the incidence level decreased by up to 27% and by around 40% for *Botrytis* and *Penicillium*, respectively.

3.5. Sensory Evaluation

The results of the sensory analysis carried out with the formulations applied to whole apples are summarized in Figure 3. As can be observed, the appearance of the apple was not significantly affected by the coating applications. On the contrary, the judges found significant differences ($p < 0.05$) regarding the aroma and flavor attributes between uncoated samples or ones coated only with pure PVA and those samples treated with the active coatings due to the negative impact of the odor and taste of carvacrol. Similar results have been previously observed by other authors for other carvacrol-loaded coatings [54].

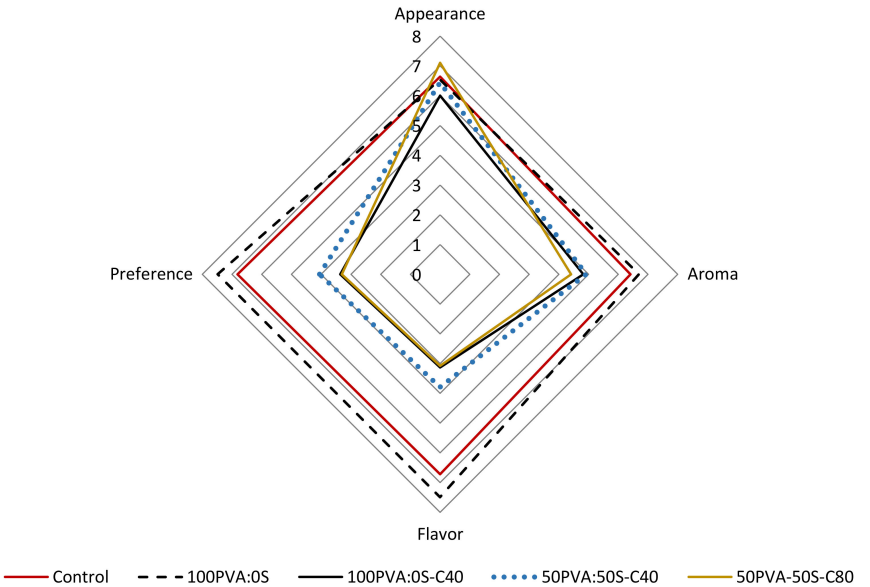


Figure 3. Sensory profile of the uncoated (control) and the different coating formulations applied onto whole fresh apples in terms of appearance, aroma, flavor, and preference.

4. Conclusions

None of the coating formulations reduced the weight loss or promoted significant changes in the respiration rates in apples, probably due to the low surface solid density of these coatings. Despite that, the application of carvacrol-loaded coatings was effective at reducing the incidence and severity of the black and blue molds caused by *B. cinnerea* and *P. expansum*. An analysis of the impact of the carvacrol-loaded coating on the apple sensory attributes revealed that the threshold of unpleasantness of aroma and flavor perception was reached when the coatings were applied and the aroma and flavor of the coated apples were negatively affected. Therefore, the application of these kinds of coatings to apples is recommended only as a pre-harvest treatment because of the high antifungal efficiency and low degree of fitotoxicity. Further studies are needed to evaluate if the organoleptic properties of the product change when those coatings are applied as a pre-harvest treatment.

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