coatings

MDPI

Article

The Antifungal and Antiviral Activity of Coatings Containing Zinc Oxide Nanoparticles and *Verbascum* L. or *Formitopsis betulina* Extracts and Their Influence on the Quality of Strawberries after Storage

Małgorzata Mizielińska [1,*], Magdalena Ordon [1], Weronika Burdajewicz [1,2], Paweł Nawrotek [3], Józef Sternal [2] and Marcin Okręglicki [2]

[1] Center of Bioimmobilisation and Innovative Packaging Materials, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology in Szczecin, Janickiego 35, 71-270 Szczecin, Poland; magdalena.ordon@zut.edu.pl (M.O.); w.burdajewicz@onet.pl (W.B.)
[2] MarDruk Opakowania sp. z o.o., sp.k. ul. Krakowska 83c, 34-120 Andrychów, Poland; technolog@mardrukopakowania.pl (J.S.); marcin@mardrukopakowania.pl (M.O.)
[3] Center for Nanotechnology Research and Education, Department of Microbiology and Biotechnology, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Piastów Avenue 45, 70-311 Szczecin, Poland; pawel.nawrotek@zut.edu.pl
* Correspondence: malgorzata.mizielinska@zut.edu.pl

Abstract: The goal of this study was to analyze the antifungal and antiviral activity of coatings based on *Formitopsis betulina*, *Verbascum* L. and *Uncaria tomentosa* extracts with ZnO nanoparticles as active compounds. The other purpose was to investigate the impact of polypropylene bags coated with the obtained antiviral/antifungal coatings on the microbial quality/purity of strawberries. The results of this study showed that the analyzed coatings inhibited *Candida albicans* growth completely. They did not inhibit the growth of *Fusarium oxysporum*, but they decreased its number. Additionally, all layers demonstrated a high activity against the Φ 6 bacteriophage particles. Analyzing the microbial purity of the strawberries after storage, it was noticed that the modified bags with *Verbascum* L. (ZnVL) and *F. betulina* (ZnFb) extracts and the addition of the nano ZnO had a significant effect on the decrease of the total count and on the number of yeast and mold. After 144 h of storage of the strawberries, the ZnVL coating was found to be more effective than the ZnFb layer. However, after 216 h of storage, ZnVL was more active against yeast and mold, but the packaging covered with the ZnFb coating was more effective against bacteria.

Keywords: active packaging; strawberries; *Verbascum* L. extract; *Formitopsis betulina* extract; antifungal properties; antiviral properties

Citation: Mizielińska, M.; Ordon, M.; Burdajewicz, W.; Nawrotek, P.; Sternal, J.; Okręglicki, M. The Antifungal and Antiviral Activity of Coatings Containing Zinc Oxide Nanoparticles and *Verbascum* L. or *Formitopsis betulina* Extracts and Their Influence on the Quality of Strawberries after Storage. *Coatings* **2024**, *14*, 260. https://doi.org/10.3390/coatings14030260

Academic Editors: Frederic Debeaufort, Manuela Pintado and Fátima Poças

Received: 17 January 2024
Revised: 15 February 2024
Accepted: 16 February 2024
Published: 22 February 2024

1. Introduction

According to its nutrient profile, the strawberry represents a healthy food choice; it contains dietary fiber (contributing to a controlled calorie intake through its satiating effect) and fructose (contributing to the regulation of blood sugar levels by slowing digestion). It is worth mentioning that strawberries contain extremely high amounts of vitamin C and folate. A major class of phenolic compounds from strawberries is represented by flavonoids flavanols, followed by hydrolysable tannins and phenolic acids [1,2]. Unfortunately, strawberries have a short shelf-life due to their high perishability and the fact that they are susceptible to mechanical injury, water loss, physiological deterioration and decay. The post-harvest preservation of fresh strawberries is highly complex owing to their high metabolic activity. The short shelf-life of these fruits has limited the marketability of this product, and losses can reach up to 40% during storage [3]. Microbial contamination of

strawberries also has an impact on their quality and shelf-life. Fungal pathogens are considered to be one of the main reasons for post-harvest losses of strawberries. Unfortunately, the physiological characteristics of strawberries, e.g., low pH, high sugar concentration, soft texture, as well as an optimum high-water activity, provide an ideal environment for mold/fungal strain growth. The most widespread strawberry pathogens are *Fusarium oxysporum*, *Phytophthora fragariae*, *Colletotrichum acutatum*, *Verticillium dahliae*, *Botrytis cinerea*, *Macrophomina phaseolina*, *Xanthomonas fragariae*, *Rhizopus stolonifer* and *Penicillium expansum, Cladosporium* sp. [4–6].

Appropriate preservation methods and the selection of the proper packaging materials can minimize losses and extend their shelf-life [3]. To reduce the number of microorganisms responsible for strawberry spoilage, bioactive compounds can be coated on their surface (edible coatings) [7,8]. Edible coatings, when applied as a thin layer on the surface of food products, act as a barrier against gases, vapors and solutes and may maintain food quality at a high level. They may potentially extend the shelf-life through the reduction of microorganisms responsible for fruit decay [6–8]. The active agents can also be incorporated into the packaging material [6]. Active packaging for fresh fruits can play an important role in their quality preservation and their safety [6]. Polypropylene films coated with antimicrobial layers release active agents into the product, reducing or inhibiting microorganism growth, which may prolong the shelf life of fruits [6,9–11].

Verbascum (family *Scrophulariaceae*) consists of a large group of plants with many applications in traditional medicine. *Verbascum* L. contains bioactive agents, mainly polyphenols and iridoids that have several biological activities, such as antimicrobial, antidiabetic, antioxidant, anticancer, cardiovascular and neuroprotective activities. It is important that *Verbascum* L. has also been found to have antiviral properties. Its aqueous and alcoholic extracts have been used in therapy, improving the prognosis of persons with COVID-19 [12,13]. Birch polypore (*Fomitopsis betulina*) is an edible, medicinal mushroom (when young). *F. betulina* consists (beyond others chemicals) mostly of triterpenoids, especially lanostane derivatives, which are responsible for the effectiveness of this fungus [9,14]. *Uncaria tomentosa* is a non-toxic, medicinal plant that was confirmed to have antimicrobial, antioxidant, anti-inflammatory, antineoplastic, anti-conceptive and immunostimulant properties [15,16]. The findings of the previous experiments [9] demonstrated that *F. betulina*, *Verbasculum* L. and *Uncaria tomentosa* extracts had antibacterial properties which greatly depended on their concentrations. Ag or ZnO nanoparticles were confirmed to have high antimicrobial activity [6,10,17]. Additionally, according to Poças, F. and Franz, R. [18], ZnO nanoparticles were confirmed to be safe. An earlier experiment performed by the authors [10] showed that an active coating containing geraniol with the addition of ZnO nanoparticles demonstrated higher activity than coatings which contained nanoparticles or geraniol alone as active compounds. Zinc oxide nanoparticle properties were confirmed to be biocompatible and safe. They offer very good stability, as well as semi-conducting behavior, high transparency and high UV absorption capabilities (shielding properties). This is why they are widely used as antimicrobial compounds for active coatings/packaging applications [11,19–25]. The authors' [10] findings led to the theory that the introduction of the ZnO nanoparticles (separately) into the *F. betulina* or into the *Verbasculum* L. or the *U. tomentosa* extracts may cause a synergistic effect between two active agents. Other experiments carried out by the authors [26] also confirmed these assumptions. The coatings including *F. betulina* or *U. tomentosa* extracts with zinc oxide nanoparticles as active agents, which were found to be highly active against *Staphylococcus aureus*, *Escherichia coli* and *B. atrophaeus* cells. The active coatings were used by the authors for cooked ham storage tests (initially sliced by a shop assistant). The results of the experiments showed that both of the analyzed packaging materials were active. However, the film covered with the coating based on the *U. tomentosa* extract (with the addition of nano ZnO) had a greater effect on the microbial quality of the slices of cooked ham than the coating containing *F. betulina* extract. To summarize, it was suggested that due to their antibacterial properties, the packaging

coated with these active layers tested and described by the authors could be applicable to preserve food products and even extend their shelf life.

As an assumption [9,10,26], it might be predicted that the introduction of ZnO nanoparticles into the *F. betulina*, *Verbascum* L. or *U. tomentosa* extracts (separately) might lead to antifungal and antiviral activity of the layers on the packaging material surfaces. The coatings containing zinc oxide nanoparticles as an additive will be UV resistant due to their UV-shielding properties, and they will maintain the plant extracts' active compound effectiveness [12,16–20]. As a conclusion [4–6], to analyze the antifungal properties of the coatings' selected strains, *Candida albicans* PCM 2566 and *Fusarium oxysporum* CCM F-545 were used. To analyze the antiviral properties, a SARS-CoV-2 surrogate, phage phi 6 DSM-21518, was used.

The first aim of this study was to analyze the antifungal and antiviral properties of the coatings based on *F. betulina*, *Verbascum* L. and *U. tomentosa* extracts with ZnO nanoparticles as active compounds. Another focus of this work was to investigate the impact of coated/active PP films on the microbial quality of strawberries.

2. Materials and Methods

2.1. Materials

The Leibniz Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) as well as the Polish Collection of Microorganisms and the Czech Collection of Microorganisms (Brno, Czech Republic) delivered the microbes which were used to analyze the antifungal and antiviral properties of the coatings in the experiments. These included a *Candida albicans* PCM 2566, a *Fusarium oxysporum* CCM F-545 and a *Pseudomonas syringae* van Hall 1902 DSM 21482. The Φ 6 phage (DSM-21518) was used as a SARS-CoV-2 surrogate.

Polypropylene films (A4, 20 µm) were delivered by a MarDruk company (An-drychów, Poland). *Verbascum* L. (flowers), *Uncaria tomentosa* (bark) and *Fomitopsis betulina* (fungus) (Planteon, Borków Stary, Poland), as well as a powder of ZnO nanoparticles AA 44899 (particles size: 70 nm) (Thermo Fisher GmbH, Kandel, Germany), were used as antifungal and antiviral agents. The 70 GU279686 varnish (a solvent dispersion, Hubergroup, Warsaw, Poland) was applied as the coating system. This dispersion contained propan-1-ol, ethyl acetate, resin acids and rosin acids, fumarated. Ethanol (purity 99.8%, EUROCHEM BGD Sp. z o.o. Tarnów, Poland) was used as a solvent to obtain the plant extracts. The Saburaud broth (containing 2% dextrose) and Saburaud agar (chemical composition: peptone 10.0 g/L, glucose 40 g/L, agar 15.0 g/L, chloramfenikol 0.05 g/L) were used to investigate the antifungal properties of all obtained coatings. Luria-Bertani (LB chemical composition: NaCl 5 g/L, Tryptone 10 g/L, Yeast Extract 5 g/L) broth and Agar-Agar (Merck, Darmstadt, Germany) were applied to investigate the antiviral activity of these coatings. To determine the microbial quality analysis of strawberries (before and after strawberry storage), the following experiments were carried out. To verify the total bacterial count of the strawberries, PPS (PPS: 0.1% *m*/*v* peptone, 0.85% *m*/*v* NaCl) and PCA (with yeast extract, glucose and casein peptone, BTL, Łódź, Poland) mediums were used. All of the mentioned mediums were prepared according to the Merck manufacturer's instructions. To verify yeast molds and counts, Saburaud agar was used (Merck, Darmstadt, Germany). All mediums were then weighed and later dissolved in 1 L of distilled water and sterilized at 121 °C for 15 min.

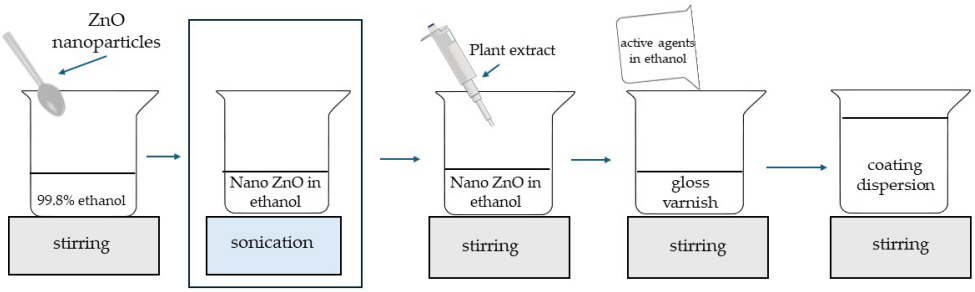
2.2. Extract Preparation

The dry plants *Uncaria tomentosa* (Ut), *Verbascum* L. (VL) and *Fomitopsis betulina* (Fb) were poured/put (separately) into a TM6 Thermomix (VORWERK, Wrocław, Poland). The dry powder was obtained from plants with the grounding parameters 7600 rpm, 20s. Then, 100 g of each/selected powder was separately poured/put into 100 mL of the 99.8% ethanol. As a next step, these solutions were incubated in a microwave (Amica, Wronki, Poland) for 5 min at 70 °C. The solutions were then extracted/shaken for 1 h at 70 °C (speed rate 150 rpm, Ika, Staufen im Breisgau, Germany). The extraction was performed according

to the methods which were used in previous experiments [9,27,28]. After extraction, the powders were filtered from the extracts with a Büchner funnel. Further, they were filtered using a 0.2 µm filter and dried (dry mass: 13.74%, 13.5%, 21.72% for *F. betulina*, *Verbascum* L. and *U. tomentosa*, respectively).

2.3. ZnUt, ZnVL and ZnFb Coating Preparation

Coating dispersions were prepared according to Scheme 1. In brief, the ZnO nanoparticles (0.03 g) were weighed and introduced into 45 mL of 99.88% ethyl alcohol. As a first step of the experiments, the nanoparticles were stirred for 60 min (500 rpm). Then, dispersion was sonicated for 30 min (procedure: cycle: 0.5; amplitude: 20%), while at the same time, preparations of the 2nd and the 3rd nano ZnO dispersions were carried out as described above. Then, 5 mL of the *U. tomentosa* extract (Ut) was added into the first system. The same amount of *Verbascum* L. extract was added into the 2nd system and 5 mL of *F. betulina* (Fb) extract was introduced into the 3rd system. Next, the dispersions were stirred (15 min, 500 rpm). The dispersion of zinc oxide nanoparticles (50 mL) containing the studied extracts Ut, VL or Fb was added into the 50 mL of the gloss varnish (coating carrier) and stirred for 10 min (500 rpm).



Scheme 1. Coating dispersions preparation. Gloss varnish = the solvent dispersion 70 GU279686.

Polypropylene films (thickness: 20 µm) were then coated with the active layers (grammage: 2.2 g/m^2) with an Unicoater 409 (Erichsen, Hemer, Germany) at room temperature with a 40 µm diameter roller. The covered PP packaging films were dried at a temperature of 50 °C (10 min). PP film samples that were not covered were control samples (PP). The film squares (3 × 3 cm) were analyzed/tested for their antifungal and antiviral effectiveness.

2.4. Antifungal Analysis

The antifungal effectiveness of the coatings including the Ut, VL or Fb extracts with the addition of ZnO nanoparticles (coatings: ZnUt, ZnVL, ZnFb) against *Candida albicans* and *Fusarium oxysporum* was evaluated compared to the non-covered PP film samples according to the ASTM E 2180-01 standard [29].

2.5. Antiviral Activity Analysis

Initially, the purification of the phage 6 particles took place according to Bhetwal et al. [30]. Next, a pure Φ 6 lysate was obtained according to Bonilla et al. [31]. The antiviral effectiveness of the active layers containing plant extracts and nanoparticles was compared to the non-coated films and tested according to an ISO 22196-2011 standard with a slight modification [32]. Then, bacteriophage amplification was performed using the Skaradzińska et al. method [33].

The analysis of the growth rate of the *Pseudomonas syringae* in real-time was performed according to the previous work [10] with some modifications. The bacteriophage lysate was cultivated/incubated with the non-coated PP film samples (control samples) and with the film-coated films according to the ISO 22196-2011 standard [32]. Next, the LB broth was introduced into 5 BioSan bioreactors (BS-010160-A04, BioSan, Riga, Latvia). Then, the overnight culture of *P. syringae* was added to 10 mL of Luria–Bertani broth and cultivated/incubated until OD = 0.2 (optical density) at 28°. Four Φ 6 lysates were

amplified in the respective host bacterial strain (one lysate after cultivation/incubation with the non-coated film (control sample), three lysates after their cultivation/incubation (separately) with the squares coated with layers containing ZnUt, ZnVL or ZnFb). Then, 10 µL of Φ 6 lysate (MOI = 1) was pipetted to a host culture (OD = 0.2) and incubated at 28° (until OD fall). It was possible to perform four tests (simultaneously), meaning that the antiviral activity of the 3 active coatings was analyzed during one experiment.

2.6. Bag Preparation

Initially, the polypropylene film was perforated (the holes' diameter was 5 mm) and then coated with the ZnVL or ZnFb layers (containing plant extracts and zinc oxide nanoparticles (separately) as active agents). A control sample (C) was prepared from the perforated PP film which was not covered with the active coatings. The non-coated and covered films were then cut into rectangles to obtain bags. As a next step, both the covered and non-covered films were sealed together (air conditions: HSE-3, RDM Test Equipment, Hertfordshire, UK) for the preparation of the bags. The seal parameters were set as follows: time—4 s, temperature—117 °C and pressure—4 kN.

2.7. Packaging and Storage

The fresh strawberries (Rumba) were bought from a local supplier (Szczecin, Poland) and immediately transported to the laboratory (CBIMO—Center of Bioimmobilisation and Innovative Packaging Materials). The fruits were then put into bags aseptically, including the following (Figure 1a–c):

a. Nine PP packages/bags (C) (control samples);
b. Nine PP packages/bags covered with ZnVL layer;
c. Nine PP packages/bags covered with ZnFb layer.

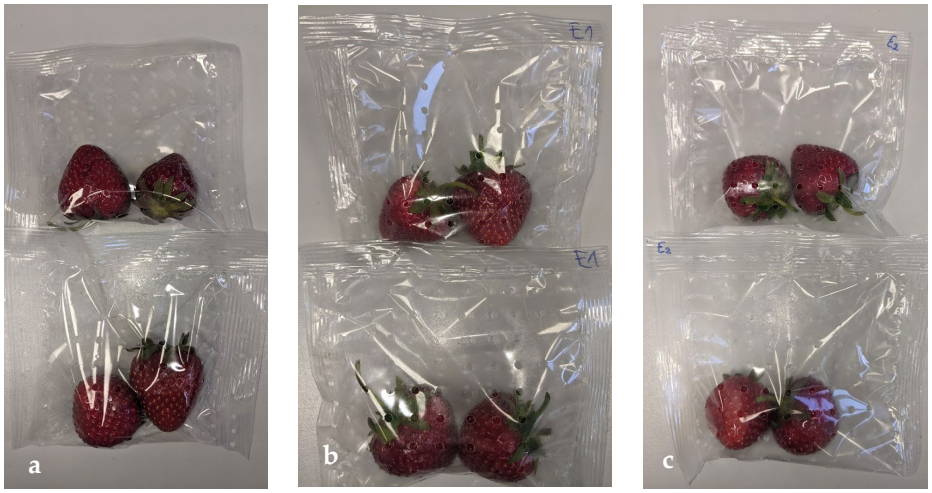


Figure 1. The strawberries: (**a**) in uncoated bag; (**b**) in bag coated with the ZnFb coating; (**c**) in bag covered with the ZnVL coating.

The non-active and the active packaging were welded (HSE-3, RDM Test Equipment, Elsenham, UK) under air conditions. Sealing parameters were set as described above.

The 27 bags (9 of each bag) containing strawberries were then kept at 5 °C. The 9 strawberry samples were analyzed after 72 h (three bags of each kind of bag) and after 144 h of storage. The last 9 samples were analyzed after 216 h of storage.

2.8. Microbial Quality Investigation

Microbiological quality examination was carried out on the strawberries before and after their storage (in non-antifungal and in antifungal bags). For each test, 10 ± 0.1 g of fruit sample was aseptically transferred into a sterile stomacher bag in a physiological

PPS solution (saline peptone: 0.1% *m*/*v* peptone; 0.85% *m*/*v* NaCl) and into a sterile stomacher bag in a Saburaud broth. The strawberries were then homogenized in a Bag Mixer (Interscience, Saint-Nom-la-Brèteche, France) for 120 s. As a next step, appropriate decimal dilutions were created in PPS or Saburaud broth. The total count was performed according to a standard PN-EN ISO 4833-2:2013-12 [34]. A yeast and mold count analysis was determined according to PN-ISO 21527-2:2009 [35].

2.9. Dry Mass Tests

The dry mass of the strawberries before storage and of the fruit samples after 72, 144 h and 216 h of storage (in packages/bags) was determined using a weight dryer (Radwag, Warsaw, Poland). The strawberry samples from each package were investigated in two repetitions.

*2.10. CIE L*a*b* Colour Properties*

The average of 9 trials/repetitions from strawberry spots were randomly selected to measure the color of the fruits. The test was performed using a colorimeter (NR 20 XE, EnviSense, Lublin, Poland) with other related data software. The color determination was performed through an aperture (8 mm diameter) using a CIE L*a*b* color space that was equipped with a standard 10 observer and Illuminant D65. The ΔE_{lab} (total color aberration) and ΔL (darkness and lightness difference) were the analyzed parameters. The EnviSense protocol was used to calculate the parameters.

2.11. SEM Tests of the Strawberries

The microstructure of the strawberries was examined before their introduction into the packages and after 72 h, 144 h and 216 h of storage using a scanning electron microscope. The SEM analysis was performed to visualize the fruits' micro-texture differences. The strawberries were set at 4 °C (solution of 2% glutaraldehyde in a 0.1 M sodium cacodylate, pH 7.4) for 18 h. As a next step, the strawberries were washed with 0.1 M sodium cacodylate and then dehydrated/made moistureless in serial dilutions (20%, 40%, 60%, 80% and 100%) of ice-cold (−20 °C) methyl alcohol at 120 min intervals. The samples of the strawberries were placed in a Petri dish for 5 min and then they were put on pin stubs. Then gold (thin layer) was deposited on the samples using a sputter coater at ambient conditions (Quorum Technologies Q150R S, Laughton, East Sussex, UK). The strawberry surface examination took place using SEM (scanning microscope: Vega 3 LMU microscope, Tescan, Brno-Kohoutovice, Czech Republic). The microscopic analysis of the samples was carried out through the use of a Vega 3 LMU microscope (Tescan, Brno-Kohoutovice, Czech Republic). A test was carried out with tungsten filament at 25°, and a voltage of 20 kV was used to capture pictures/images for the studied samples. All specimens were observed with magnifications of 100×, 500×, 1000× and 2000×.

2.12. Statistical Analysis

The statistical significance analysis (an analysis of variance followed by a one-way ANOVA test) was carried out to compare the results of the microbiological purity analysis. Where $p < 0.05$, the values were seen as significantly different. All analyses were carried out through the use of GraphPad Prism 8 (GraphPad Software, Version 9, San Diego, CA, USA).

3. Results

3.1. Antifungal Analysis

The results of this study showed that all of the analyzed coatings containing ZnO nanoparticles and plant extracts inhibited *C. albicans* growth completely, because the yeast colonies on the Saburaud agar were not noticed (in comparison to uncoated PP films, for which it was confirmed that the number of viable *C. albicans* cells was $1.1 \times 10^4 \pm 2.94 \times 10^3$ CFU/g). Additionally, the coatings ZnFb, ZnVL and ZnUt decreased the number of

F. oxysporum cells (Figure 2). It was noted that the ZnFb and ZnVL coatings were more effective against mold cells than the ZnUt layer. Statistical analysis proved/confirmed that the changes between the numbers of yeast and mold cells were noted to be significant (Figure 2).

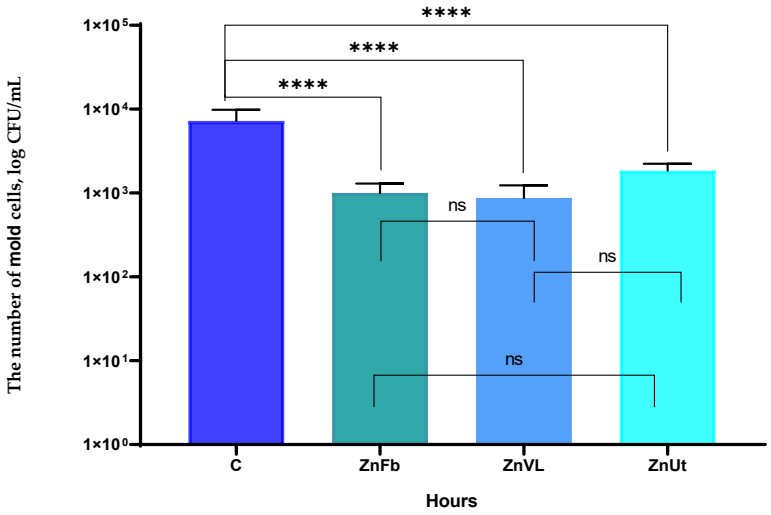


Figure 2. The effect of active layers on *F. oxysporum* growth. C—PP film; ZnFb—PP film coated with the layer with nano ZnO and Fb extract; ZnVL—PP film coated with the layer with nano ZnO and VL extract; ZnUt—PP film coated with the layer with nano ZnO and Ut extract. Error bars—standard deviation. One-way ANOVA; **** $p < 0.0001$; ns—$p > 0.5$.

3.2. Antiviral Analysis

The performed investigations led to the results showing that in the case of the coatings containing ZnO nanoparticles and *F. betulina*, *Verbascum* L. or *U. tomentosa* extracts, a complete reduction in the bacteriophage titer compared to the control sample (non-coated PP film) was noted (Table 1). As emphasized in Figure 3, an OD fall was noticed/noted after 11 h of bacteriophage particle cultivation with the host for uncoated PP film, meaning that the phage particles were active. Contradictory results were noted for the active coatings. As determined in Figure 3, an OD fall was not observed even after 23 h of phage cultivation with the host, meaning that the phi 6 phage particles were not active. It could be concluded that the ZnFb, ZnVL and ZnUt layers demonstrated a high antiviral effectiveness, resulting in the complete inactivation/elimination of the Φ 6 particles.

Table 1. The influence of coatings on phi 6 phage activity.

The Sample	The Number of Phi6 Cells [PFU/g] Phi 6 Phage
PP	$2.4 \times 10^7 \pm 2.38 \times 10^6$
ZnFb	0 ****
ZnVL	0 ****
ZnUt	0 ****

One-way ANOVA test: ****—$p < 0.0001$.

3.3. Microbial Purity Examination

It was indicated that the number of the total count isolated from the strawberries kept in the non-covered packages/bags (C—control sample) increased significantly after 72 h ($p < 0.0001$), 144 h ($p < 0.0001$) and 216 h ($p < 0.0001$) of storage under air conditions at 5 °C (as when compared to prestored sample "0"). As emphasized in Figure 4, the ZnFb coating with five percent of the *F. betulina* extract and ZnO nanoparticles had an influence on the growth increase of the mesophilic bacteria. It was noted that the number of living microorganisms decreased after 72 h of storage in comparison to samples that were stored

in packages/bags without coatings. However, a lower than one log increase in the number of mesophilic microorganisms was seen (when compared to sample "0"—pre-storage) for the strawberries stored for 72 h in bags covered with the Fb coating. Similar results were observed for the ZnVL active coating. Moreover, as seen in Figure 4, both of the active layers (ZnFb and ZnVL) significantly decreased the number of mesophilic bacteria compared to the samples stored in bags without any coatings after 144 h ($p < 0.0001$) and 216 h of storage ($p < 0.0001$), respectively. However, a slight increase ($p > 0.5$) in the amount of bacterial cells was noted when compared to the "0" sample (pre-storage). Additionally, it could be suggested that the coating with Fb extract was more active against mesophilic microbes/bacteria than the layer containing VL extract after 216 h of storage, and that the ZnVL active layer was more effective than the ZnFb layer after a shorter time (144 h) in storage.

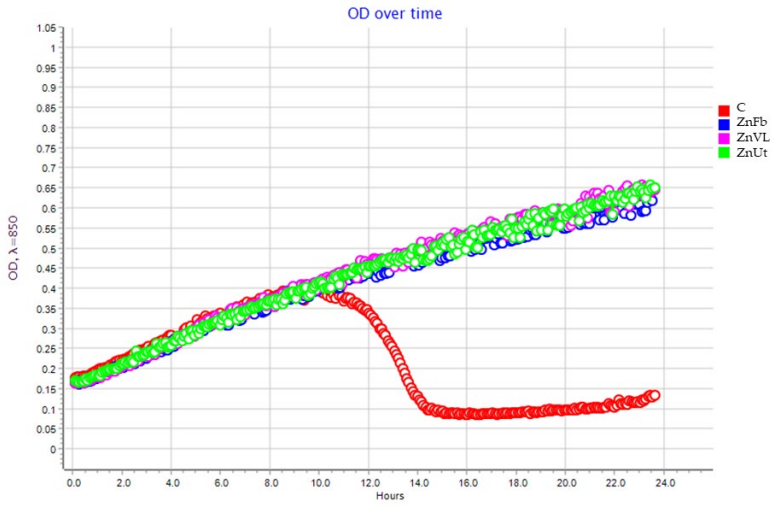


Figure 3. OD over time, tested for the host after 11 h of cultivation/incubation. Cultivation with Φ 6 particles after their cultivation/incubation with the PP films and covered with the coatings: ZnFb, ZnVL and ZnUt coatings; introduction of Φ 6 particles when OD − 0.2; amount of Φ 6 particles MOI − 1.

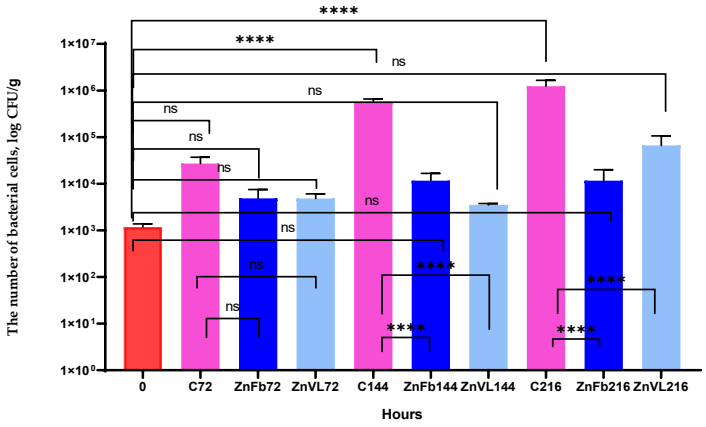


Figure 4. The mesophilic bacteria total count. Microorganisms were detected from the strawberries stored for 72 h, 144 h and 216 h. 0—sample "0"; C—strawberries, stored in PP packages/bags; ZnFb—strawberries, stored in PP packages/bags covered with the ZnFb layer; ZnVL—strawberries, stored in PP packages/bags covered with the ZnVL layer. Error bars—standard deviation. One-way ANOVA **** $p < 0.0001$; ns—$p > 0.05$.

As was emphasized in Figure 5, the polypropylene films covered with ZnVL and ZnFb layers had a significant effect on the total number/count of yeast and mold compared to the uncovered PP packages/bags after 72 h, 144 h and 216 h of storage. It was observed that the

amount of yeast and mold isolated from the samples stored for 72 h in the bags/packages without active coatings increased slightly. However, the amount of yeast and mold isolated from the strawberries taken from the active packages/bags was almost the same when compared to the "0" sample—pre-storage. A statistical analysis clearly demonstrated that any variances between the amounts of yeast and mold stored for 72 h were considered to be not significant ($p > 0.5$). An analysis of the microbial purity of the strawberries stored in PP films indicated that the number of microorganisms increased significantly ($p < 0.0001$) after 144 h and 216 f of storage. Alternatively, the total count of yeast and mold detected from the fruits stored in bags coated with the ZnVL layer did not change significantly ($p > 0.5$), even after 144 h of storage. In the case of strawberries in the ZnVL-coated bags, the number of microbes only increased after 216 h of storage. However, the growth in the amount of yeast and mold was lower than one log. This means that the packaging covered with the ZnVL layer was highly effective. The findings of this research show that the amount of yeast and mold cells detected from the fruit samples stored in bags covered in the ZnFb layer was lower than the number of these microorganisms isolated from the samples stored in uncoated bags after 144 h and 216 h, confirming that the ZnFb coating was also effective.

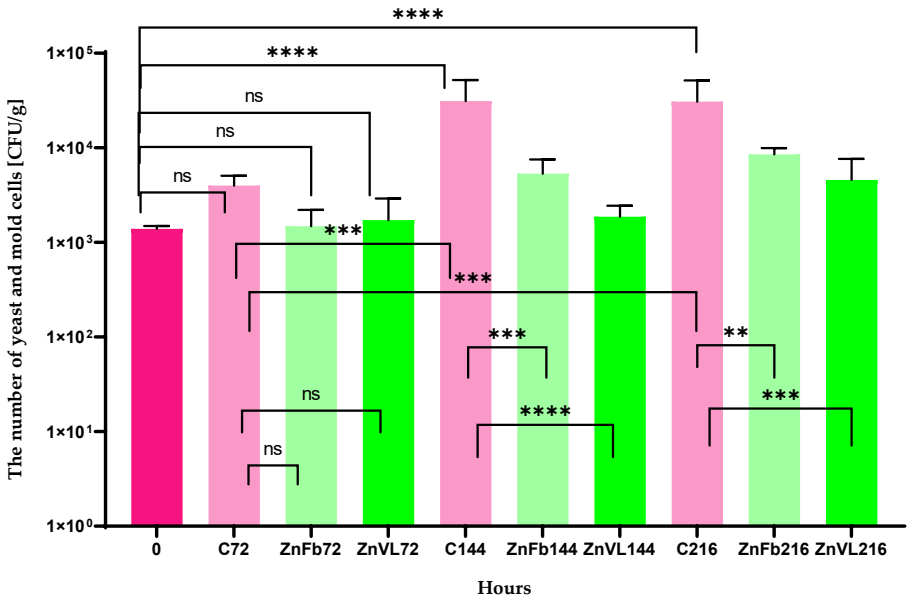


Figure 5. The total yeast and mold count. Microorganisms detected from the strawberries stored for 72 h, 144 h and 216 h. 0—sample "0"; C—strawberries, stored in PP packages/bags; ZnFb—strawberries, stored in PP packages/bags covered with the ZnFb layer; ZnVL—strawberries, stored in PP packages/bags covered with the ZnVL layer. Error bars—standard deviation. One-way ANOVA ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns—$p > 0.05$.

3.4. Dry Mass Analysis

The results determined that the strawberries' dry mass was 14.63%. Storing fruits in polypropylene packages/bags caused an increase of the dry mass of the strawberries to 15.06% after 72 h, to 17.04% after 144 h of storage and to 17.89% after 216 h, respectively. It was determined that for the strawberries' dry mass, that of the fruits which were kept in packages/bags covered with ZnFb and ZnVL layers was lower than the dry mass of the strawberries that were stored in packages/bags that remained uncoated after 72 h, 144 h and 216 h of storage, respectively. Additionally, after 72 h of storage time, a reduction in the dry mass value of strawberries taken from the active packages was observed compared to sample "0" (before storage). It is worth mentioning that the slight increase in the dry mass of the fruits from the active bags was noted after 144 h. After 144 h and 216 h, the dry mass of the samples was found to decrease. The ZnFb coating led to the observation of a slightly higher dry mass value than in the case of the ZnVL layer. Different results were

found in samples kept for 72 h (Table 2). The results that were obtained confirmed that the active coatings led to a lower weight loss of the fruits than in uncoated bags, contributing to lower water loss.

Table 2. The dry mass of strawberries after 72 h, 144 h and 216 h of storage.

Time [h]	Dry Mass [%]		
	C	ZnFb	ZnVL
0		14.63 ± 0.01	
72	15.06 ± 0.03 ****	12.72 ± 0.02 ****	13.59 ± 0.01 ****
144	17.04 ± 0.01 ****	15.38 ± 0.22 ****	15.06 ± 0.59 ****
216	17.89 ± 0.26 ****	14.57 ± 0.11 ****	14.41 ± 0.08 ****

One-way ANOVA test: ****—$p < 0.0001$. C—strawberries, stored in PP packages/bags; ZnFb—strawberries, stored in PP packages/bags covered with the ZnFb layer; ZnVL—strawberries, stored in PP packages/bags covered with the ZnVL layer.

*3.5. L*a*b* Analysis*

The results of this study show that the material used for packaging had an impact on ΔE_{lab} (Table 3). The ΔE_{lab} of strawberries that were added into the uncoated bags for 72 h was lower than the ΔE_{lab} of the fruit samples that were introduced to bags coated with ZnFb and ZnVL layers. Similar findings were noticed after 144 h of storage, and they were contrary to the results obtained after 216 h of storage. The highest ΔE_{lab} was observed in the case of strawberries kept in bags covered with ZnVL after 72 h. Similar results were noted for samples stored in bags with an ZnFb active layer, while the lowest ΔE_{lab} was observed for active packaging after 216 h of storage. To summarize, it was clearly confirmed that ΔE_{lab} was dependent on the film/material used for packaging.

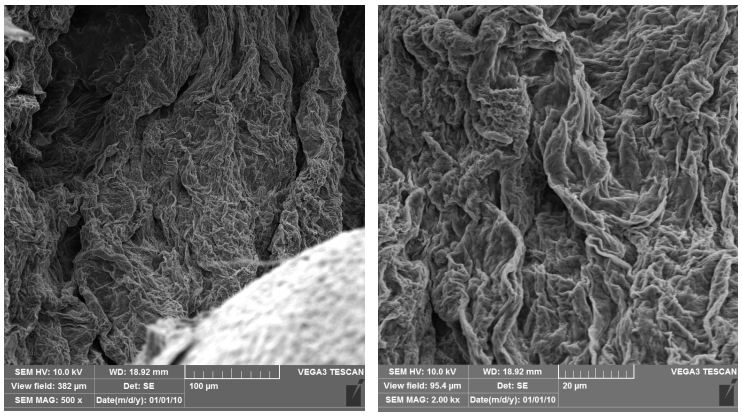
Table 3. The ΔE_{lab} of strawberries after 72 h, 144 h and 216 h of storage.

Time [h]		C	ZnFb	ZnVL
ΔE_{lab}	0	13.67 ± 1.14	13.67 ± 1.14	13.67 ± 1.14
ΔL		−10.75 ± 0.22	−10.75 ± 0.22	−10.75 ± 0.22
ΔE_{lab}	72	48.15 ± 5.63 ****	56.01 ± 1.63 ****	56.32 ± 2.95 ****
ΔL		−4.28 ± 1.49 **	1.50 ± 0.51 ****	−0.19 ± 0.23 ****
ΔE_{lab}	144	35.81 ± 1.67 ****	38.95 ± 2.69 ****	40.68 ± 1.68 ****
ΔL		14.12 ± 1.14 ****	19.55 ± 1.01 ****	16.89 ± 1.83 ****
ΔE_{lab}	216	40.46 ± 7.22 ****	30.80 ± 1.87 ****	31.48 ± 0.66 ****
ΔL		15.08 ± 1.64 ****	17.81 ± 1.35 ****	16.88 ± 1.13 ****

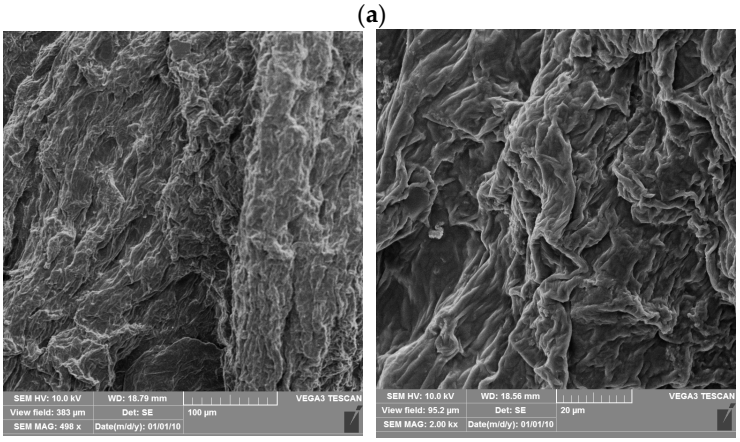
One-way ANOVA test: **—$p < 0.01$; ****—$p < 0.0001$. C—strawberries, stored in PP packages/bags; ZnFb—strawberries, stored in PP packages/bags covered with the ZnFb layer; ZnVL—strawberries, stored in PP packages/bags covered with the ZnVL layer.

3.6. SEM Analysis of the Strawberries

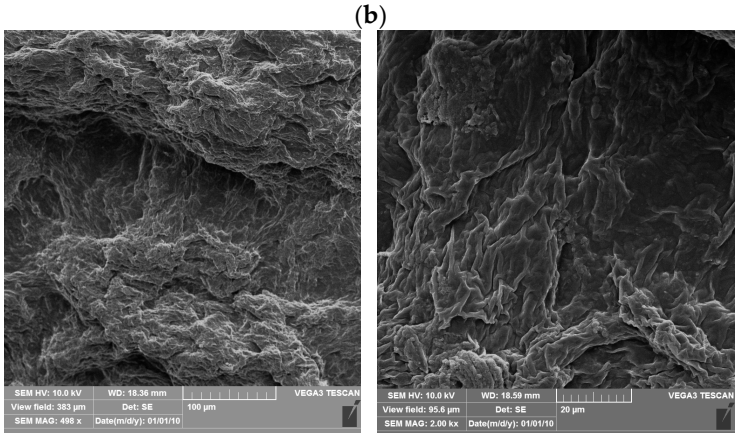
The microstructure of the strawberries before and after being stored was examined using SEM. Figure 6a shows that the fruit samples were clear in appearance, showing the rough and uneven surface (not homogenous looking) common in natural strawberries. A similar microstructure was observed in samples stored for 72 h in uncovered PP packages/bags (Figure 6b). Upon analyzing the effect of the storage on the microstructure of strawberries stored in uncoated packaging, it was clearly noted that the samples were more compact, more dense and less pleated compared to the fruits before storage (Figure 6c,d). After 72 h, 144 h and 216 h of storage in active packaging, the strawberries were observed to have a less compact and more pleated appearance with clearly visible irregular holes (Figure 6e–j). It was noted that the surface of the strawberries kept in active bags (active ZnVL and ZnFb) for 144 h and 216 h (Figure 6f,g,i,j) were less compact and dense than the microstructures of the fruits stored in PP packages/bags (Figure 6c,d), which corresponded to the higher water loss seen in strawberries kept in non-active packaging.



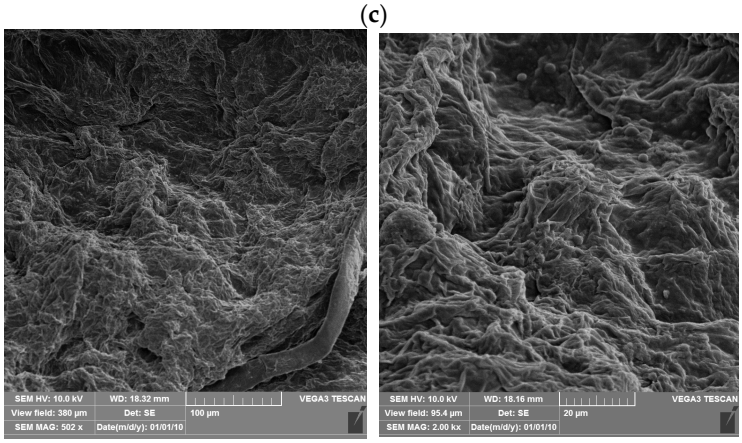
(**a**)



(**b**)

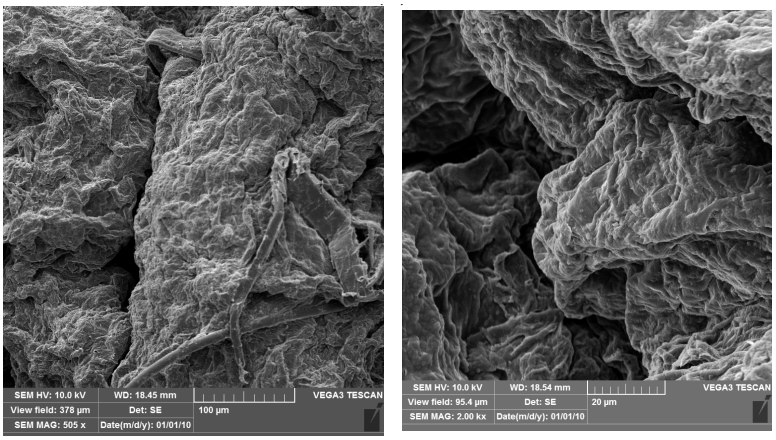


(**c**)

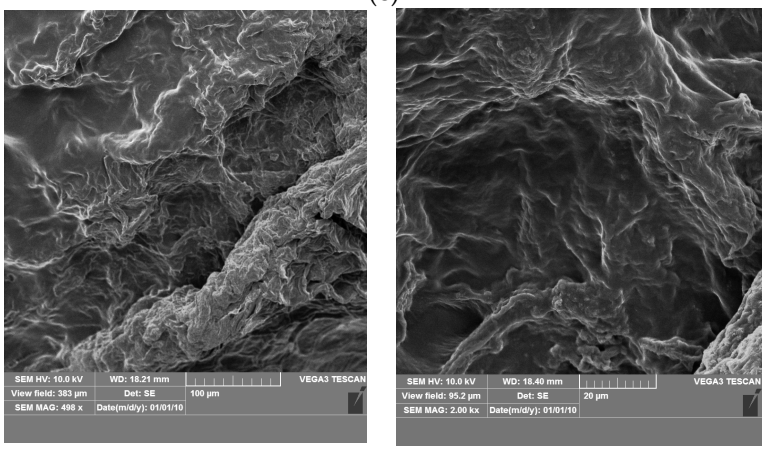


(**d**)

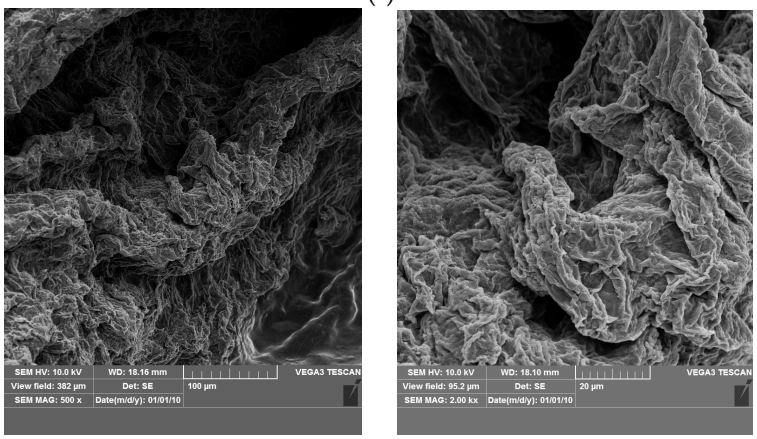
Figure 6. *Cont.*



(**e**)



(**f**)



(**g**)

Figure 6. *Cont*.

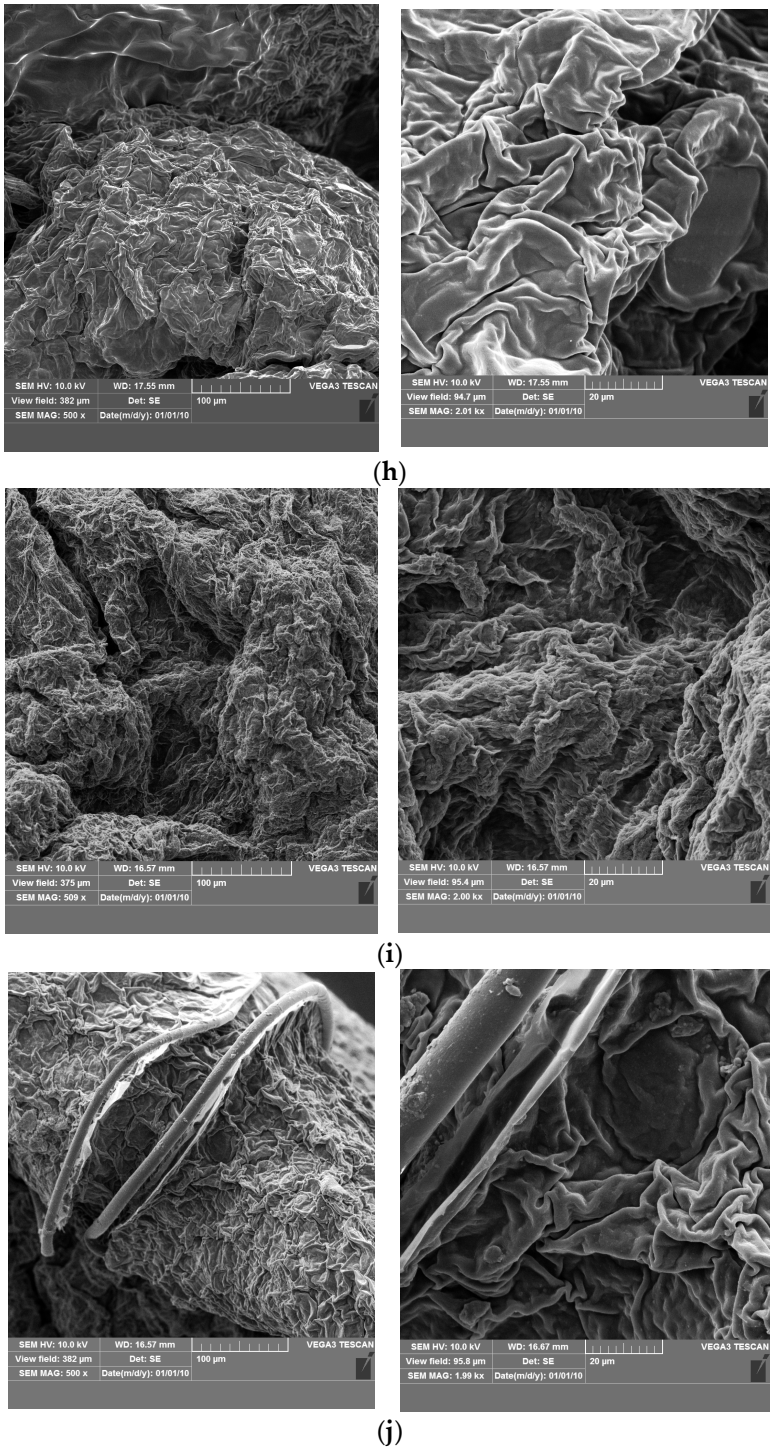


Figure 6. (**a**) Strawberries before storage (sample "0"). (**b**) Strawberries stored for 72 h in uncoated PP packages/bags. (**c**) Strawberries stored for 144 h in uncoated PP packages/bags. (**d**) Strawberries stored for 216 h in uncoated PP packages/bags. (**e**) Strawberries stored for 72 h in PP packages/bags covered with the ZnFb coating. (**f**) Strawberries stored for 144 h in PP packages/bags covered with the ZnFb coating. (**g**) Strawberries stored for 216 h in PP packages/bags covered with the ZnFb coating. (**h**) Strawberries stored for 72 h in PP packages/bags covered with the ZnVL coating. (**i**) Strawberries stored for 144 h in PP packages/bags covered with the ZnVL coating. (**j**) Strawberries stored for 216 h in PP packages/bags covered with the ZnVL coating.

4. Discussion

The results of this work showed that the active coatings which contained ZnO nanoparticles and *F. betulina*, *Verbascum* L. or *U. tomentosa* extracts in the coating carrier completely inhibited *C. albicans* growth. They did not inhibit the growth of *F. oxysporum*, but they decreased its number. However, the packaging covered with the ZnFb and ZnVL coatings was slightly more active against *F. oxysporum* than the ZnUt layer. The findings of the previous study confirmed that these coatings were also active against bacterial strains such as *S. aureus*, *E. coli* and *B. atrophaeus*. It was also concluded in a previous work that a Ut active coating was able to display a greater effect on the growth of bacteria than an Fb coating, though for a shorter time [26]. Due to their antibacterial effectiveness, they were also confirmed as retaining the quality and freshness of slices of cooked ham during secondary shelf-life (48 h) [26]. This is why the ZnVL and ZnFb active layers (more effective than ZnUt layer), which exhibit antibacterial and antifungal properties, were selected to preserve the strawberries during these storage tests. It is worth mentioning that the aforementioned active layers determined high antiviral effectiveness, proceeding from a complete inactivation/elimination of the active Φ 6 particles (SARS-CoV-2 surrogate). Similar results were obtained in previous research by the authors [9,35]. As confirmed earlier, active coatings containing a mixture of *Glycyrrhiza* L. and *S. baicalensis* extracts [9] and layers containing rosemary, pomegranate and raspberry seed extracts [36] were highly effective against the phi6 phage. However, the antiviral effectiveness of the layers described was higher than the antiviral effectiveness of the coatings, including zinc oxides nanoparticles and geraniol or carvacrol (added separately), which demonstrated moderate, antiviral activity as a result of a reduction in the phage titer (in the initial phase of cultivation/incubation), without a complete elimination of active phage particles [10]. It should be stated that the phi 6 phage is very similar to SARS-CoV-2, with a similar size (~80–100 nm) and with enveloped (by a lipid membrane) spike proteins [37]. Due to these similarities, it could be underlined that packaging materials covered with layers that are effective against Φ 6 will also be effective against SARS-CoV-2. As a conclusion, there is a suggestion that ZnFb and ZnVL coatings may be used as an external packaging layer to limit the spread of coronavirus particles through contact with human hands. The coronavirus particles were confirmed to spread via consumers' hands, packaging and surfaces [38].

Nevertheless, EU regulations on microbial criteria for foodstuffs (EC 2073/2005 and subsequent modifications) do not include the maximum levels of total mesophilic bacteria in fresh and pre-cut fruits [39], and the preservation of strawberries against microbial spoilage seems to be important. This is why, due to their antibacterial and antifungal properties, ZnFb and ZnVL coatings were used to preserve strawberries against decay. Analyzing the microbial purity of strawberries after storage at 5 °C, it was noted that the active packages/bags with the layers with *Verbascum* L. (ZnVL) and *F. betulina* (ZnFb) extracts and with the nano ZnO had a significant effect on the decrease of the total count (mesophilic bacteria) and on the number of the yeast and mold. It was noted that the amount of mesophilic microbes/bacteria detected in the strawberries from the sample "0" was 1.18×10^3 [log CFU/g] and the amount of yeast and mold was 1.39×10^3 [log CFU/g]. The total count of mesophilic bacteria and the number of yeast and mold increased significantly after 72 h, 144 h and 216 h of storage for the samples which were kept in the non-active bags. Similar results were also obtained by Sogvar et al. [7]. It was clear that the amount of living mesophilic microorganisms, yeast and mold isolated from the fruits stored in active packaging decreased after 72 h compared to samples that were stored in packages/bags without coatings. However, this decrease was very small. Moreover, as observed, both of the active layers (ZnFb and ZnVL) significantly reduced the total count and yeast and mold compared to the samples stored in packages/bags without any coverings after longer storage periods (144 h and 216). Similarly, Sogvar et al. [7] noted that the total count of mesophilic microbes/bacteria, yeast and mold detected from non-preserved strawberries was higher after storage (3, 6 and 9 days) then when compared to the fruits preserved by active coatings. However, the authors did not use active packaging

materials. They covered the fruits with a coating carrier containing ZnO nanoparticles and saw a clear improvement in its effectiveness. Antimicrobial packaging films produced from pullulan and Solid Lipid Nanoparticles (SLN) containing 1% *w*/*w* cinnamaldehyde were used by Trinetta et al. [5] to preserve strawberries during storage. The authors confirmed that strawberries kept in active packaging displayed lower yeast and mold counts than those fruits stored in control packaging. It should be emphasized that pullulan packaging films loaded with cinnamaldehyde SLN were not as effective in inhibiting total aerobic bacteria as in inhibiting the number of yeast and mold. Summarizing the obtained results, it should be noted that the active coatings preserved fruits against fungal contamination. Moreover, the active coatings created by the authors led to a lower weight loss of the fruits (corresponding to water loss) than the loss in weight that was observed in the case of the uncoated strawberries. It was observed that fruits stored in uncoated packaging demonstrated a greater increase in dry weight (greater water loss) than strawberries kept in active packaging, and that the water loss of the strawberries which were stored for 216 h in active packaging was not noted. While 3.92% of the water loss was observed for the samples stored for 216 h in uncoated PP films, more importantly, the water loss percentage was below 10% and therefore within the acceptable commercial weight loss range for fruit [40]. An SEM analysis performed by Álvarez-Barreto et al. [41] clearly shows that the microstructure of the uncoated strawberries had compact, pleated, rough and uneven surfaces, meaning that the appearance of the fruits had much more in common with natural strawberries. Corresponding results were obtained in this study.

Van et al. [42] showed that active, edible coatings preserved fruits against microbiological spoilage due to their antifungal properties. The results obtained by the authors also confirmed the findings of this work, which proved that active compounds in coatings may decrease the number of microorganisms responsible for fruit spoilage. However, the authors observed that after storage in the case of all strawberry samples, no significant differences were observed in the ΔE and ΔL values except in the case of the control sample, in which the color of the sample exhibited a significant change. Strawberries coated with the active coating demonstrated higher L* values than the uncoated strawberries, and the color of the covered fruits was lighter than that of the uncovered samples. Meanwhile, the ΔE values for all strawberries showed no significant differences during the storage period. Overall, the color of strawberries after storage was light red for coated strawberries and dark red for the uncoated. This suggests that the active, edible coatings slowed the color change of strawberries during storage. Contradictory results were observed in this work. The ΔE and ΔL values changed significantly for all analyzed samples, meaning that active coatings had no influence on the change of color. It should be underlined that the active coatings which slowed the change of the color were distributed on the surface of fruits, whereas the active coatings that protected the strawberries against microbial spoilage in this work were distributed on the surface of the PP films. This comparison might lead to the assumption that active packaging materials may preserve strawberries against microorganisms and water loss; however, they did not slow any color change.

5. Conclusions

The results of the current study show that all of the analyzed, active coatings containing ZnO nanoparticles and plant extracts inhibited *Candida albicans* growth completely. They did not inhibit the growth of *Fusarium oxysporum*, but they decreased their number. Additionally, all three active layers demonstrated high antiviral effectiveness, attributed to a complete inactivation of the Φ 6 phage particles (surrogate of the SARS-CoV-2). Upon analyzing the microbial purity of the strawberries after storage, it was noted that the active packaging based on *Verbascum* L. (ZnVL) and *F. betulina* (ZnFb) extracts with the addition of the nano ZnO had a significant effect on the decrease of the total count (mesophilic bacteria) and on the amount of the yeast and mold. After 144 h of storage of the strawberries, the ZnVL coating was found to be more effective than the ZnFb layer. However, after 216 h of storage, the ZnVL coating was found to be more active against yeast and mold cells,

but the packaging covered with the ZnFb coating was more effective against mesophilic bacteria (according to the total count results). To summarize, the active packaging materials may preserve strawberries against microorganisms and water loss; however, they may not be able to slow color change. It can also be assumed that if the packaging is coated with external layers which proved to be effective against the Φ6 phage, they would also be active towards SARS-CoV-2 particles.

Author Contributions: M.M., M.O. (Marcin Okręglicki) and J.S. conceived and designed the experiments. M.M. wrote the paper. M.O. (Magdalena Ordon) and W.B. performed the antifungal activity analysis; M.M. analyzed the data. P.N. performed antiviral properties tests; M.M. analyzed the data; M.M. performed lab tests; M.M. performed microscopic analysis including SEM analysis and analyzed the data; M.M. performed dry mass tests and analyzed the data; M.M., W.B., M.O. (Marcin Okręglicki) and J.S. contributed and prepared reagents/materials; M.O. (Marcin Okręglicki) and J.S. contributed analysis tools; M.M. performed statistical analysis and analyzed the data. All authors have read and agreed to the published version of the manuscript.

Funding: This research work was funded by the project POIR.01.01.01-00-0740/21: "Innovative, ecological, active packaging made of plastics, biodegradable and compostable films and paper intended for packaging food products with special, protective and functional properties"; it was co-financed by the European Union and the National Center for Research and Development.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: Authors Weronika Burdajewicz, Józef Sternal and Marcin Okręglicki were employed by the company MarDruk Opakowania sp. z o.o. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Frankowska, A.; Jeswani, H.K.; Azapagic, A. Life cycle environmental impacts of fruits consumption in the UK. *J. Environ. Manag.* **2019**, *248*, 109111. [CrossRef]
2. Giampieri, F.; Tulipani, S.; Alvarez-Suarez, J.M.; Quiles, J.L.; Mezzetti, B.; Battino, M. The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition* **2012**, *28*, 9. [CrossRef] [PubMed]
3. Caner, C.; Aday, M.S.; Demir, M. Extending the Quality of Fresh Strawberries by Equilibrium Modified Atmosphere Packaging. Eur. *Food Res. Technol.* **2008**, *227*, 1575. [CrossRef]
4. Mirmajlessi, S.M.; Destefanis, M.; Gottsberger, R.A.; Mänd, M.; Loit, E. PCR-based specific techniques used for detecting the most important pathogens on strawberry: A systematic review. *Syst. Rev.* **2019**, *4*, 9. [CrossRef]
5. Trinetta, V.; McDaniel, A.G.; Batziakas, K.; Yucel, U.; Nwadike, L.; Pliakoni, E. Antifungal Packaging Film to Maintain Quality and Control Postharvest Diseases in Strawberries. *Antibiotics* **2020**, *9*, 618. [CrossRef] [PubMed]
6. Mizielińska, M.; Bartkowiak, A. Innovations in food packaging materials. In *Food Preservation and Packaging—Recent Process and Technological Advancements*; Tumuluru, Y.S., Ed.; IntechOpen: London, UK, 2022. [CrossRef]
7. Sogvar, O.B.; Koushesh Saba, M.; Emamifar, A.; Hallaj, R. Influence of nano-ZnO on microbial growth, bioactive content and postharvest quality of strawberries during storage. *Innovative Food Sci. Emerg. Technol.* **2016**, *35*, 168–176. [CrossRef]
8. Abu Salha, B.; Gedanken, A. Extending the Shelf Life of Strawberries by the Sonochemical Coating of their Surface with Nanoparticles of an Edible Anti-Bacterial Compound. *Appl. Nano* **2021**, *2*, 14. [CrossRef]
9. Ordon, M.; Nawrotek, P.; Stachurska, X.; Schmidt, A.; Mizielińska, M. Mixtures of *Scutellaria baicalensis* and *Glycyrrhiza* L. Extracts as Antibacterial and Antiviral Agents in Active Coatings. *Coatings* **2021**, *11*, 1438. [CrossRef]
10. Mizielińska, M.; Nawrotek, P.; Stachurska, X.; Ordon, M.; Bartkowiak, A. Packaging Covered with Antiviral and Antibacterial Coatings Based on ZnO Nanoparticles Supplemented with Geraniol and Carvacrol. *Int. J. Mol. Sci.* **2021**, *22*, 1717. [CrossRef]
11. Mizielińska, M.; Łopusiewicz, Ł.; Mężyńska, M.; Bartkowiak, A. The Influence of Accelerated UV-A and Q-SUN Irradiation on the Antimicrobial Properties of Coatings Containing ZnO Nanoparticles. *Molecules* **2017**, *22*, 1556. [CrossRef]
12. Donn, P.; Barciela, P.; Perez-Vazquez, A.; Cassani, L.; Simal-Gandara, J.; Prieto, M.A. Bioactive Compounds of *Verbascum sinuatum* L.: Health Benefits and Potential as New Ingredients for Industrial Applications. *Biomolecules* **2023**, *13*, 427. [CrossRef] [PubMed]
13. Okasha, Y.M.; Fathy, F.I.; Fathy Soliman, F.; Fayek, N.M. The untargeted phytochemical profile of *Verbascum thapsus* L. with potent antiviral, antibacterial and anticancer activities. *S. Afr. J. Bot.* **2023**, *156*, 334. [CrossRef]

14. Sofrenić, I.; Anđelković, B.; Todorović, N.; Stanojković, T.; Vujisić, L.; Novaković, M.; Milosavljević, S.; Tešević, V. Cytotoxic triterpenoids and triterpene sugar esters from the medicinal mushroom *Fomitopsis betulina*. *Phytochemistry* **2021**, *181*, 112580. [CrossRef] [PubMed]
15. Herrera, D.R.; Durand-Ramirez, J.E.; Falcão, A.; Nogueira da Silva, E.J.L.; dos Santos, E.B.; Figueiredo de Almeida Gomes, B.P. Antimicrobial activity and substantivity of *Uncaria tomentosa* in infected root, canal dentin. *Braz. Oral Res.* **2016**, *30*, 61. [CrossRef]
16. Cahuana-Vasquez, R.A.; Soléo Ferreira dos Santos, S.; Koga-Ito, C.Y.; Cardoso Jorge, A.O. Antimicrobial activity of *Uncaria tomentosa* against oral human pathogens. *Braz. Oral. Res.* **2007**, *46*, 46. [CrossRef]
17. Wang, R.; Song, X.; Xiang, T.; Liu, Q.; Su, B.; Zhao, W.; Changsheng Zhao, C. Mussel-inspired chitosan-polyurethane coatings for improving the antifouling and antibacterial properties of polyethersulfone membranes. *Carbohydr. Polym.* **2017**, *168*, 310. [CrossRef] [PubMed]
18. Poças, F.; Franz, R. Overview on European Regulatory Issues, Legislation, and EFSA Evaluations of Nanomaterials. In *Nanotechnology for Food Packaging: Materials, Processing Technologies, and Safety Issues*; Cerqueira, M., Maria Lagaron, J., Pastrana Castro, L., Vicente., A., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 2018. [CrossRef]
19. Eskandari, F.; Mofidi, H.; Asheghi, B.; Mohammadi, F.; Gholami, A. Bringing resistance modulation to methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) strains using a quaternary ammonium compound coupled with zinc oxide nanoparticles. *World J. Microbiol. Biotechnol.* **2023**, *39*, 193. [CrossRef]
20. Karimi, M.; Sadeghi, E.; Khosravi Bigdeli, S.; Zahedifar, M. Optical properties, singlet oxygen, and free radical production ability with different UV irradiations and antimicrobial inhibitors against various bacterial species of ZnO: Eu nanoparticles. *Radiat. Phys. Chem.* **2023**, *212*, 111132. [CrossRef]
21. Wu, H.; Meng, Y.; Yu, M.; Yang, H. Modulating the antibacterial activity of ZnO/talc by balancing the monodispersity of ZnO nanoparticles. *Appl. Clay Sci.* **2023**, *242*, 107024. [CrossRef]
22. Sougandhi, P.R.; Jena, S.K.; Brahmanand, P.S.; Tej, M.B.; Kadiyala, N.K.; Rani, T.S.; Rao, M.V.B. Multifunctional Bio-Nanocomposite Films of Carboxymethyl Cellulose and Pectin with Incorporated Zinc Oxide Nanoparticles. *Lett. Appl. NanoBioSci.* **2023**, *12*, 85.
23. Nguyena, T.V.; Dao, P.H.; Khanh Linh Duong, K.L.; Duong, Q.H.; Vu, Q.T.; Nguyena, A.H.; Mac, V.P.; Lea, T.L. Effect of R-TiO2 and ZnO nanoparticles on the UV-shielding efficiency of water-borne acrylic coating. *Prog. Org. Coat.* **2017**, *110*, 114–121. [CrossRef]
24. El-Feky, O.M.; Hassan, E.A.; Fadel, S.M.; Hassan, M.L. Use of ZnO nanoparticles for protecting oil paintings on paper support against dirt, fungal attack, and UV aging. *J. Cult. Herit.* **2014**, *15*, 165–172. [CrossRef]
25. Kairyte, K.; Kadys, A.; Luksiene, Z. Antibacterial and antifungal activity of photoactivated ZnO nanoparticles in suspension. *J. Photochem. Photobiol. Biol.* **2013**, *128*, 78–84. [CrossRef]
26. Ordon, M.; Burdajewicz, W.; Sternal, J.; Okręglicki, M.; Mizielińska, M. The Antibacterial Effect of the Films Coated with the Layers Based on *Uncaria tomentosa* and *Formitopsis betulina* Extracts and ZnO Nanoparticles and Their Influence on the Secondary Shelf-Life of Sliced Cooked Ham. *Appl. Sci.* **2023**, *13*, 8853. [CrossRef]
27. Kubra, I.R.; Kumar, D.; Rao, L.J.M. Effect of microwave-assisted extraction on the release of polyphenols from ginger (*Zingiber officinale*). *Int. J. Food Sci. Tech.* **2013**, *48*, 1828–1833. [CrossRef]
28. Mandal, V.; Mohan, Y.; Hemalatha, S. Microwave Assisted Extraction—An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacogn. Rev.* **2007**, *1*, 7–18.
29. *ASTM E 2180-01*; Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials. ASTM International: West Conshohocken, PA, USA, 2001.
30. Bhetwal, A.; Maharjan, A.; Shakya, S.; Satyal, D.; Ghimire, S.; Khanal, P.R.; Parajuli, N.P. Isolation of Potential Phages against Multidrug-Resistant Bacterial Isolates: Promising Agents in the Rivers of Kathmandu Nepal. *BioMed. Res. Int.* **2017**, *2017*, 3723254. [CrossRef]
31. Bonilla, N.; Rojas, M.J.; Cruz, G.N.F.; Hung, S.H.; Rohwer, F.; Barr, J.J. Phage on tap–A quick and efficient protocol for the preparation of bacteriophage laboratory stocks. *Peer J.* **2016**, *4*, 2261. [CrossRef]
32. *ISO 22196-2011*; Measurement of Antibacterial Activity on Plastics and Other Non-Porous Surfaces. International Organization for Standardization: Geneva, Switzerland, 2011.
33. Skaradzińska, A.; Ochocka, M.; Śliwka, P.; Kuźmińska-Bajora, M.; Skaradziński, G.; Friese, A.; Roschanski, N.; Murugaiyan, J.; Roesler, U. Bacteriophage amplification–A comparison of selected methods. *J. Virol. Methods* **2020**, *282*, 113856. [CrossRef]
34. *PN-EN ISO 4833-2:2013-12;* Angielska, W. ISO: Geneva, Switzerland, 2013. Available online: https://sklep.pkn.pl/pn-en-iso-4833-2-2013-12e.html (accessed on 20 June 2023).
35. *PN-ISO 21527-2:2009*; Polska, W. ISO: Geneva, Switzerland, 2009. Available online: https://sklep.pkn.pl/pn-iso-21527-2-2009p.html (accessed on 20 June 2023).
36. Ordon, M.; Nawrotek, P.; Stachurska, X.; Mizielińska, M. Polyethylene films coated with antibacterial and antiviral layers based on CO2 extracts of raspberry seeds, of pomegranate seeds and of rosemary. *Coatings* **2021**, *11*, 1179. [CrossRef]
37. Fedorenko, A.; Grinberg, M.; Orevi, T.; Kashtan, N. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Sci. Rep.* **2020**, *10*, 22419. [CrossRef] [PubMed]
38. Arienzo, A.; Gallo, V.; Tomassetti, F.; Pitaro, N.; Pitaro, M.; Antonini, G. A narrative review of alternative transmission routes of COVID-19: What we know so far. *Pathog. Global Health* **2023**, *117*, 681695. [CrossRef] [PubMed]

39. Ortiz-Solà, J.; Viñas, I.; Colás-Medà, P.; Anguera, M.; Abadias, M. Occurrence of selected viral and bacterial pathogens and microbiological quality of fresh and frozen strawberries sold in Spain. *Int. J. Food Microbiol.* **2020**, *314*, 108392. [CrossRef]
40. Popescu, P.-A.; Palade, L.M.; Nicolae, I.-C.; Popa, E.E.; Miteluț, A.C.; Drăghici, M.C.; Matei, F.; Popa, M.E. Chitosan-Based Edible Coatings Containing Essential Oils to Preserve the Shelf Life and Postharvest Quality Parameters of Organic Strawberries and Apples during Cold Storage. *Foods* **2022**, *11*, 3317. [CrossRef]
41. Álvarez-Barreto, J.F.; Cevallos-Ureña, A.; Zurita, J.; Pérez, J.; León, M.; Ramírez-Cárdenas, L. Edible Coatings of Aloe Vera Gel and Carnauba Wax Microparticles to Increase Strawberry (*Fragaria ananassa*) Shelf Life In. *J. Fruit Sci.* **2023**, *23*, 181. [CrossRef]
42. Van, T.T.; Phuong, N.T.H.; Sakamoto, K.; Wigati, L.P.; Tanaka, F.; Tanaka, F. Effect of edible coating incorporating sodium carboxymethyl cellulose/cellulose nanofibers and self-produced mandarin oil on strawberries. *Food Packag. Shelf Life* **2023**, *40*, 101197. [CrossRef]