polysaccharides

Article

Optimised Extraction of Bioactives from Strawberry Lignocellulosic Byproducts for Edible Active Coatings in Fresh Fruits Preservation

Christian Cravotto [1], Marco Santin [2], Sunny Uchechukwu [1], Abdouramane Dosso [1], Patrizia Falabella [3,4], Maria-Beatrice Coltelli [5,*], Antonella Castagna [2] and Morad Chadni [1,*]

[1] URD Agro-Biotechnologies Industrielles (ABI), CEBB, AgroParisTech, 51110 Pomacle, France; christian.cravotto@gmail.com (C.C.); suaveyoucy@gmail.com (S.U.); abdouramane.dosso@agroparistech.fr (A.D.)
[2] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy; marco.santin@unipi.it (M.S.); antonella.castagna@unipi.it (A.C.)
[3] Department of Basic and Applied Sciences, University of Basilicata, 85100 Potenza, Italy; patrizia.falabella@unibas.it
[4] Spinoff XFlies s.r.l., University of Basilicata, 85100 Potenza, Italy
[5] Department of Civil and Industrial Engineering, University of Pisa, 56122 Pisa, Italy
* Correspondence: maria.beatrice.coltelli@unipi.it (M.-B.C.); morad.chadni@agroparistech.fr (M.C.)

Abstract

This study proposes a sustainable strategy to valorise strawberry lignocellulosic agro-industrial byproducts through the recovery of antioxidant and antimicrobial compounds (AOM) for use in active edible coatings. Subcritical water extraction (SWE), optimised using response surface methodology, was applied to maximise phenolic content and antioxidant capacity while minimising sugars' co-extraction. Optimal SWE conditions (120 °C, 5 min, and S/L ratio 40) yielded a total phenolic content (TPC) of 146.9 mg GAE/g DM and an antioxidant activity of 24.8 mg TE/g DM, comparable to ethanolic reflux extraction (138.4 mg GAE/g DM and 23.4 mg TE/g DM). Scale-up in a Parr pressurised reactor achieved 91.2% polyphenol recovery relative to accelerated solvent extraction (ASE). Purification using Amberlite® XAD 7 resin enhanced TPC purity and antioxidant activity more than 2.5-fold, producing a desorbed fraction with a polyphenol purity of 93.9% (*w*/*w*, dry basis) and no detectable sugars. The purified AOM was incorporated (1% *w*/*v*) into a 1.5% (w) chitosan solution obtained from *Hermetia illucens* pupal exuviae to produce a biopolymeric active coating. Application to strawberries was associated with a reduction in fungal infection severity (−72%) and incidence (−66.7%) under natural infection conditions. Although fruit firmness declined during storage, coated samples showed significantly better firmness retention. These results demonstrate the effectiveness of combining chitosan with phenolic extracts obtained by SWE to enhance microbial stability and maintain fruit quality.

Keywords: subcritical water extraction; strawberry byproducts; *Hermetia illucens* chitosan; active edible coating; fresh fruits preservation

Academic Editors: Klementina Pušnik Črešnar and Lidija Fras Zemljič

Received: 22 December 2025
Revised: 25 January 2026
Accepted: 10 February 2026
Published: 24 February 2026

1. Introduction

Strawberry (*Fragaria* × *ananassa*) is one of the most widely cultivated berries worldwide, with an annual global production of nearly 8 million tonnes [1]. In 2022, China (3.4 Mt) and the United States (1.3 Mt) were the largest producers, while Mediterranean countries such as Turkey (728 kt), Egypt (638 kt), and Spain (326 kt) also ranked among the

leading producers [2]. Due to their high moisture content and delicate tissues, strawberries are highly perishable, which has driven substantial industrial processing into juices, jams, purées, and other products [3].

The processing of fruits and vegetables generates considerable amounts of byproducts, including peels, seeds, stems, leaves, and non-edible fruits. These residues, often considered unavoidable waste, represent 7–20% of strawberry production [4]. Their heterogeneous composition has stimulated extensive research into valorisation strategies, including the recovery of functional ingredients, fermentation and bioprocessing, enzymatic conversion, and thermal or mechanical treatments [5].

Strawberry byproducts (SBPs) are particularly rich in phenolic compounds. Extracts from inedible fractions, such as the crown, stolon leaves, and flowers, have been reported to exhibit high phenolic content and strong antioxidant, antimicrobial, anti-obesity, anti-allergy, and other bioactive properties [6]. Key compounds identified include ellagitannins (e.g., agrimoniin), ellagic acid, and flavonoids, such as quercetin and kaempferol glycosides [1].

A promising application of SBPs' polyphenols is their incorporation into biodegradable active packaging. Active packaging systems not only act as physical barriers but also enhance food preservation through the controlled release of antioxidants or antimicrobial compounds [7]. Chitosan, a deacetylated derivative of chitin, has attracted wide interest as a film-forming biopolymer. At the industrial scale, chitosan is mainly obtained from the exoskeletons of crustaceans [8]. However, the growing market demand for this biopolymer has resulted in the need to identify alternative sustainable sources. In this context, insects—particularly *Hermetia illucens*—have emerged as promising candidates due to their efficiency as bioconverters [9,10]. Waste biomasses generated during *H. illucens* rearing, including pupal exuviae, offer an added-value substrate for chitin extraction and chitosan production, fitting well within a zero-waste circular economy framework [11]. Thanks to its biodegradability, antimicrobial activity, and oxygen barrier properties, chitosan is well suited for use as an active coating material in food preservation applications [10,12–16].

The enrichment of chitosan films with polyphenol-rich extracts has been shown to enhance their antioxidant and antimicrobial properties, offering a natural alternative to synthetic preservatives [17]. Recent studies have confirmed these benefits for strawberry preservation: Yu et al. reported that chitosan films plasticised with tomato byproduct extracts improved antioxidant properties and extended strawberry shelf life [18], while Zidan et al. showed that chitosan/polyethylene glycol films incorporating date palm fruit waste extract exhibited strong antimicrobial activity and effectively maintained strawberry quality during storage [19]. In both cases, bioactive-enriched coatings significantly delayed microbial spoilage and quality deterioration in fresh strawberries, highlighting their potential for direct application on fresh fruits.

Efficient recovery of polyphenols is, therefore, a critical step in this valorisation pathway [20]. Conventional solid–liquid extraction methods are typically characterised by long processing times, low extraction yields, and the use of organic solvents, which limit their sustainability [21]. Emerging green technologies, such as deep eutectic solvents (DESs) [22,23], microwave-assisted extraction [24,25], supercritical CO_2 extraction [26], and ultrasound-assisted extraction [3], have been tested for the recovery of phenolic compounds from SBPs, offering high extraction efficiency. Among emerging green technologies, subcritical water extraction (SWE) is recognised as a highly effective method for polyphenol recovery from plant matrices; however, its application to SBPs has been only scarcely investigated.

SWE has gained attention as a green and scalable method. It employs water maintained at 100–374 °C under sufficient pressure to remain in the liquid state [27]. Under these conditions, the dielectric constant of water decreases, enabling selective solubilisation of a

wide range of compounds, from polar phenolics to moderately non-polar molecules [28]. SWE is cost-effective, uses a non-toxic solvent, and avoids the environmental impact of organic solvent disposal. Furthermore, extraction selectivity can be tuned by temperature adjustment, processing times are reduced, and successful scale-up has been demonstrated, as reported for the multikilogram-scale recovery of phenolics from hazelnut skins [29]. Nevertheless, SWE is often poorly selective for polyphenols, as sugars and organic acids may be co-extracted.

To address this limitation, post-extraction purification is commonly required. In this study, macroporous resin adsorption was applied, as resins provide selective retention of phenolic compounds via hydrogen bonding and hydrophobic interactions, while allowing non-phenolic solutes to be washed out with water. Additional advantages include resin reusability, operational simplicity, and cost-effectiveness, making this approach suitable for both laboratory and industrial applications [30].

This study explores the valorisation of SBPs through the recovery of phenolic compounds using SWE followed by resin-based purification. The purified extracts are subsequently evaluated for their incorporation into chitosan-based edible active coatings. This integrated approach aims to enhance the preservation of fresh strawberries while providing a sustainable and circular strategy for the utilisation of agro-industrial residues.

2. Materials and Methods

2.1. Raw Material Preparation

Strawberry plants at the end of their productive cycle, collectively referred to as SBPs, were supplied by the University of Pisa (Pisa, Italy). The material was manually cleaned to remove adhering soil particles, weeds, and other extraneous vegetation. The byproducts consisted exclusively of above-ground biomass, mainly represented by leaves (approximately 75%), followed by stems (about 20%), while residual non-marketable fruits accounted for a minor fraction (around 5%), and flowers were almost absent. The SBP was dried in a ventilated oven at 45 °C until constant weight. The dried material was milled and sieved (<850 μm) to obtain a uniform particle size distribution. The processed SBP was stored in sealed containers at room temperature, in the dark, until extraction. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Hermetia illucens Chitosan Production

Bleached chitosan from *H. illucens* pupal exuviae was produced following Triunfo et al.'s 2022 protocol [31], with starting biomasses supplied by Xflies s.r.l. (Potenza, Italy). Specifically, insect chitosan had a deacetylation degree of 90% and viscosity-average molecular weight (Mv) of 35 kDa [31].

2.3. Accelerated Solvent Extraction (ASE)

Polyphenols were extracted from SBPs using an accelerated solvent extraction system (Dionex™ ASE™ 350, Thermo Fisher Scientific Inc., Sunnyvale, CA, USA). Extractions were carried out in 34 mL stainless-steel cells fitted with two cellulose filters. Each cell was loaded with 1–3 g of SM, depending on the solid-to-liquid (S/L) ratio required by the experimental design. Extraction parameters included a pressure of 10.34 MPa, a 50% flush volume, and a nitrogen purge time of 30 s. The obtained extracts were collected in 250 mL glass vials sealed with Teflon-lined caps, transferred to 50 mL volumetric flasks, and brought to volume with water.

2.4. Conventional Ethanolic Extraction

As a control, a conventional solvent extraction was performed using 70% aqueous EtOH. Briefly, SBP was extracted in a round-bottom flask under reflux conditions, with the solvent heated to its boiling point. An L/S ratio of 30 was applied, and the mixture was maintained for 1 h under continuous agitation. The residual plant material was subsequently subjected to a second extraction under identical conditions. The extracts were combined, brought to a defined volume, and analysed for global extraction yield, TPC, DPPH, and sugars.

2.5. Optimisation of ASE Conditions

A Box–Behnken design was used to optimise the extraction temperature, extraction time, and S/L ratio. The design consisted of 16 runs, including 3 replicates at the central point. The variable levels are shown in Table 1. Response surface methodology (RSM) was applied to identify the conditions maximising TPC, DPPH, and minimising the sugar content. Statistical analyses were performed using Design-Expert version 13 (Design-Ease Inc., Minneapolis, MN, USA). The selected parameter ranges were defined based on the prior experience of the research group in SWE of lignocellulosic agro-industrial residues and were consistent with previously published RSM-based SWE studies [32–34]. Specifically, the temperature domain deliberately included a conventional hot water condition at 70 °C and two subcritical regimes at 110 and 150 °C to assess the transition from non-pressurised to subcritical extraction.

Table 1. Ranges and variables used for the experimental designs.

Independent Variables	Unit	Symbol	Levels		
			Low (−1)	Middle (0)	High (1)
Time	min	A	5	17.5	30
Temperature	°C	B	70	110	150
L/S ratio	mL/g	C	10	30	50

2.6. Parr High-Pressure Reactor

The optimised SWE conditions established with the ASE system were scaled up using a Parr series 4540 high-pressure reactor (Parr Instrument Company, Moline, IL, USA) to obtain larger extract volumes. Approximately 15 g of SBP was extracted in 600 mL of water. The mixture was heated with an external heating jacket, with a heating rate of approximately 8–10 °C min^{-1}, and maintained at 120 °C for 5 min, under constant mechanical agitation. After extraction, the system was cooled, and the extracts were filtered under vacuum through a 1.6 µm paper filter before analysis.

2.7. Resin Purification

Amberlite® XAD-7 resin (Thermo Fisher Scientific, Waltham, MA, USA) was preconditioned by sequential rinsing with distilled water, EtOH 96%, and distilled water (1 h each, 25 °C, 140 rpm), followed by Büchner filtration and partial drying. The resin was resuspended in water and packed into a fixed-bed column (40 cm × 6 cm, ~300 mL of BV) connected to a peristaltic pump (KrosFlo® Research II TFF System, Spectrum Laboratories, Rancho Dominguez, CA, USA). About 3 L of Parr extract (10 bed volume, BV) was loaded at 5 BV/h in downward flow. The column was then washed with 10 BV of Milli-Q water to remove sugars, and phenolic compounds were eluted upward with 70% EtOH. Eluates were pooled, concentrated under reduced pressure at 45 °C, and freeze-dried. Purification efficiency was determined from TPC recovery and purity. Purification efficiency was assessed in terms of TPC recovery and TPC purity in the eluted fraction.

2.8. Analytical Methods

2.8.1. Total Phenolic Content (TPC)

TPC was measured using the Folin–Ciocalteu method. Briefly, 2 mL of distilled water, 25 µL of sample, 125 µL of 10% (*v*/*v*) Folin reagent, and 1 mL of 700 mM Na_2CO_3 were combined in a 12-well plate, making a final volume of 3.15 mL. Samples were incubated at 40 °C for 30 min, and absorbance was measured at 765 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Wilmington, DE, USA). Calibration curves were prepared using gallic acid (0–1000 µg/mL), and results were expressed as mg gallic acid equivalent (GAE) per gram of dry matter (DM) or extract (ex). All TPC measurements were performed on liquid samples. Crude aqueous extracts were analysed directly after appropriate dilution, while resin-purified fractions were analysed from the EtOH 70% solution after dilution with distilled water, applying suitable dilution factors to ensure absorbance values within the linear range of the calibration curve (0.1–1.0).

2.8.2. Antioxidant Activity (DPPH Assay)

Antioxidant capacity was determined using the DPPH radical scavenging assay. In a 96-well plate, 220 µL of DPPH solution (46.7 mg/L in EtOH) was mixed with 80 µL of sample (0.0125–0.25 mg/mL in EtOH) and incubated for 60 min at 25 °C in the dark. Absorbance was recorded at 515 nm. Results were expressed as mg Trolox equivalent (TE) per g DM.

2.8.3. Polyphenol Profiling (LC–MS QToF)

Polyphenols were identified by LC–MS QToF on an Agilent 1290 system with a PDA UV detector, coupled to a 6545 Q-ToF mass spectrometer (Agilent Technologies, Wilmington, DE, USA) equipped with a JetStream ESI source. Data were acquired in both positive and negative ion modes over an m/z range of 50–1000. The ESI source parameters were as follows: gas temperature 325 °C, gas flow 10 L/min, nebuliser 35 psi, sheath gas temperature 350 °C, sheath gas flow 11 L/min, capillary voltage 3500 V, nozzle voltage 2000 V, fragmentor 175 V, skimmer 65 V, and octopole RF 750 V. Accurate mass calibration was maintained using reference ions (m/z 121.0509 and 922.0098 in positive mode; m/z 112.9856 and 1033.9881 in negative mode). Chromatographic separations were performed on a Zorbax Eclipse Plus C18 column (1.8 µm, 50 × 2.1 mm) at 40 °C, with 0.1% formic acid in water (A) and acetonitrile (B) at 0.4 mL/min, using the following gradient: 0–7.5 min, 1–20% B; 7.5–17.5 min, 20–40% B; 17.5–21 min, 40–65% B; 21–25 min, 65–99% B; 25–30 min, 99% B; 30–31 min, 99–5% B. The injection volume was 1 µL, and the autosampler was set at 10 °C. UV detection was performed at 285, 320, and 360 nm. Compound identification was based on accurate mass measurements, isotopic pattern, and MS/MS fragmentation data, processed with MassHunter B.08.000 software and compared with spectral databases.

2.8.4. Simple Sugar Quantification (HPLC-RI)

Sugars were quantified using an Aminex HPX-87H column (300 × 7.8 mm; BioRad, Hercules, CA, USA) at 50 °C, with 4 mM H_2SO_4 as the mobile phase at 0.5 mL/min. Detection was performed using a Shodex RI-101 refractive index detector at 35 °C. Glucose (Gl) and fructose (Fr) were quantified using calibration curves (0.015–1 mg/mL), and results were expressed as g (Gl + Fr)/100 g DM.

2.9. Coating Solutions

Chitosan-based coating solutions were prepared according to Hassan et al. (2020) with slight modifications [35]. Briefly, the required amount of HIC was dissolved in 1% (*v*/*v*) lactic acid under continuous stirring using a magnetic stirrer (M2-A, ARGO LAB, Carpi,

Italy) for 16 h at room temperature to ensure complete dissolution. To improve wettability and film-forming properties, 0.2% (*v*/*v*) Tween 80 was added as a surfactant, and 2% (*v*/*v*) glycerol was incorporated as a plasticiser. Four different solutions were prepared: distilled water as a control, 1% (*v*/*v*) lactic acid as an acid control, a 1.5% (*w*/*v*) HIC coating solution, and 1.5% (*w*/*v*) HIC combined with 1% (*v*/*v*) antioxidant/antimicrobial resin-purified extract (AOM) from strawberry plant residues (as shown in Figure S1). The concentration of 1% (*v*/*v*) AOM extract was selected based on literature reports demonstrating efficacy of bioactive-enriched chitosan coatings at similar levels (typically 0.5–2% *v*/*v*) [36–38]. Besides, higher concentrations resulted in excessive darkening and opacity of the coating due to the deep brown colour of the purified extract and, therefore, were not considered.

2.10. Coating Application and Fruit Storage

Fresh strawberries (*F.* × *ananassa* cv. Sabrosa) were purchased from a local supermarket. Fruits were carefully selected for uniformity in size, colour, and ripeness stage, excluding any fruits showing physical damage, bruising, or visible signs of microbial infection. The coating solutions were applied to strawberry fruits using a cordless airbrush (PQ-30-1, Autolock, Shenzhen, China). During application, strawberries were placed on stainless-steel grids to allow excess solution to drain off, as shown in Figure S2. Each fruit was uniformly sprayed from approximately 7–8 cm to ensure complete surface coverage while avoiding excessive solution accumulation. The coating application was performed twice, with a two-hour interval between applications, to enhance coating uniformity and adhesion to the fruit surface. After the second application, the coated strawberries were allowed to air-dry at room temperature and were then stored in darkness at a constant temperature of 16 °C. This temperature was selected to simulate suboptimal but realistic post-retail conditions, such as fruit display in supermarkets or domestic storage, where temperature control is generally less stringent than in cold-chain distribution (0–4 °C). At 16 °C, quality deterioration and fungal development occur at measurable rates within a practical observation period, enabling meaningful comparison among treatments.

2.11. Assessment of Fungal Infection

Visual assessment of fungal infection was conducted at 1, 4, and 5 days post-treatment, examining and photographing the strawberries using standardised lighting conditions. The disease incidence, representing the incidence of visible fungal growth (presence/absence), was recorded for each fruit and expressed as a percentage. The disease severity was quantified using image analysis software (ImageJ version 1.54 g, National Institutes of Health, Bethesda, MD, USA) to determine the percentage of fruit surface area affected by fungal colonisation when present.

2.12. Determination of Fruit Quality Parameters

Fruit quality parameters were assessed on five strawberries (replicates) for each treatment at one, four, and five days post-treatment, following the methods by Parichanon et al. (2026) [39].

All strawberries were weighed individually at each sampling time (1, 4, and 5 days post-treatment) using an analytical balance (AS 220.R2 PLUS, RADWAG, Radom, Poland). To minimise variability due to differences in the initial fruit mass, the weight of each strawberry was normalised to its individual value at day 0 (T0 = 100%). Results were expressed as percentage of weight loss relative to the initial weight according to the following equation (Equation (1)):

$$Weight\ loss\ (\%) = \frac{(W_1 - W_t)}{W_1} \times 100, \quad (1)$$

where W_1 is the weight of the fruit (g) at 1 day post-treatment, and W_t is the weight at time t (g).

Firmness was measured on two opposing portions of the equatorial side of each strawberry fruit using a digital fruit penetrometer (53200, TURONI, Forlì, Italy) equipped with a 5 mm-diameter probe. The probe was inserted perpendicularly to the fruit surface at a constant speed until reaching a depth of approximately 5 mm, and fruit firmness values were expressed as newtons (N).

For chemical analyses, each fruit was homogenised and filtered through a 1 mm mesh sieve to remove solid residues to obtain a clear juice. Total soluble solid content (TSSC) was determined using a digital refractometer (HI96801, HANNA Instruments, Woonsocket, RI, USA) according to the standard method EN ISO 2173:2003 [40]. A few drops of filtered strawberry juice were placed on the refractometer prism at room temperature after calibration with distilled water, and results were expressed as degrees Brix (°Brix).

Titratable acidity (TA) was determined by titrating 5 g of filtered strawberry juice, diluted with 25 mL of distilled water, with 0.1 N NaOH to an endpoint of pH 8.2 using a digital burette (Titrette 4760161, BRAND, Wertheim, Germany). Titratable acidity was calculated as grams of citric acid equivalents per 100 g of juice (% *w*/*w*) using Equation (2):

$$TA\ (\%) = \frac{(\mathrm{V} \times N \times E)}{\mathrm{m}} \times 100, \quad (2)$$

where V is the volume of NaOH solution used (mL), N is the normality of NaOH solution (0.1 N), E is the equivalent weight of citric acid (64.04 g mol^{-1}), and m is the mass of the juice sample (g).

The pH of strawberry juice was measured at room temperature using a pH meter (pH 50+ DHS, XS Instruments, Carpi, Italy). The sugar–acid ratio (TSS/TA) was calculated as the ratio between total soluble solid content and titratable acidity, providing an indication of the balance between sweetness and acidity in the fruit. Finally, the ripening index (RPI) was determined as indicated by Santin et al. (2024) [41], using Equation (3):

$$RPI = \ln\left(100 \times \frac{FF}{\frac{TSSC}{TA}}\right), \quad (3)$$

where FR is firmness (N), TSSC is total soluble solids (°Brix), and TA is titratable acidity (g kg^{-1}).

2.13. Statistical Analysis

For firmness, total soluble solid content, titratable acidity, pH, and TSS/TA ratio determinations, five fruits per treatment and sampling time were analysed, while twelve fruits per treatment and sampling time were evaluated for fungal infection assessment. Data are presented as the mean ± standard error. Differences among treatments and storage times (days after treatment) were analysed by two-way analysis of variance (ANOVA; $p < 0.05$), followed by the Tukey–Kramer post hoc test. All statistical analyses were performed using JMP Pro version 18.0.2 (JMP Statistical Discovery LLC, Cary, NC, USA).

3. Results

3.1. Optimisation of Extraction Conditions

During the extraction of bioactive compounds using SWE, several factors—such as temperature, time, and solvent-to-solid (S/L) ratio—are known to significantly influence the recovery of bioactive compounds and antioxidant activity from plant materials [28]. A Box–Behnken experimental design was used to evaluate the effects of these parameters on four response variables: total phenolic content (TPC), TPC selectivity, DPPH radical scavenging

activity, and sugar co-extraction (glucose + fructose). The experimental matrix and ANOVA results are summarised in Table 2 and Table S1, respectively, and representative response surface plots are shown in Figure 1A–D.

Table 2. Box–Behnken design matrix and experimental responses for SBP extraction by SWE.

Run	Independent Variables			Responses			
	Time (min)	Temperature (°C)	L/S Ratio (mL/g)	TPC Yield (mg GAE/g DM)	TPC Selectivity (g GAE/100 g_{ex})	DPPH (mg TE/g DM)	Sugars (g G+F/100 g DM)
1	5	70	30	105.3	31.4	20.6	4.42
2	30	70	30	106.0	33.2	19.5	3.58
3	5	150	30	159.0	34.4	28.8	5.15
4	30	150	30	146.2	28.3	33.2	5.91
5	5	110	10	135.0	36.2	23.2	5.02
6	30	110	10	137.4	35.0	24.7	5.02
7	5	110	50	137.3	35.6	23.9	4.87
8	30	110	50	139.6	37.0	23.8	5.31
9	17.5	70	10	95.7	30.2	19.9	3.52
10	17.5	150	10	130.9	27.7	30.5	5.32
11	17.5	70	50	114.0	34.0	20.5	3.53
12	17.5	150	50	140.7	27.7	32.4	5.77
13	17.5	110	30	134.7	34.3	24.6	4.70
14	17.5	110	30	130.1	34.6	23.6	5.16
15	17.5	110	30	137.1	35.9	25.4	5.54
16	17.5	110	30	140.2	34.9	24.6	4.68

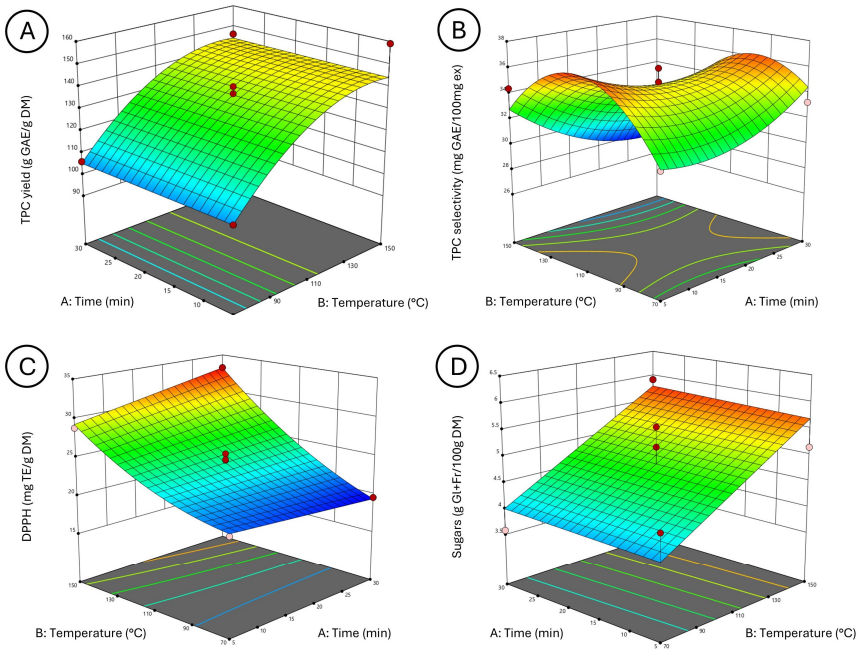

Figure 1. Response surface plots showing the effect of extraction time and temperature on (**A**) TPC yield, (**B**) TPC selectivity, (**C**) DPPH antioxidant activity, and (**D**) sugar co-extraction during SWE.

TPC yield followed a quadratic model (R^2 = 0.846; *p*-value < 0.0001) and was primarily driven by temperature (SS = 3035.8; *p*-value < 0.0001), whereas extraction time and S/L ratio were not significant (*p*-value = 0.807 and 0.289, respectively). TPC yields ranged from 95.7 to 159.0 mg GAE/g DM, with the maximum at 150 °C and 5 min (Run 3; Figure 1A). This strong temperature dependence is consistent with the enhanced diffusivity and reduced

dielectric constant of subcritical water, which promote the desorption and solubilisation of moderately polar phenolic compounds [42,43]. With increasing temperature, water viscosity decreases while surface tension and diffusivity increase, enhancing solute mass transfer from the plant matrix [44]. Moreover, elevated temperatures disrupt hydrogen bonding, van der Waals forces, and other weak interactions between solutes and the plant matrix, facilitating the release of phenolics [34,45].

In our study, working at L/S ratio between 1:10 and 1:50 (mL/g), no significant differences in TPC yield were observed, suggesting operation in the non-linear region where solvent volume no longer enhances extraction. Similar behaviour was observed in SWE studies where TPC yield increased only marginally between 1:15 and 1:30 (*w*:*v*), but decreased at lower ratios, such as 1:5 (*w*:*v*) [32]. Extraction time was not significant, as extending contact from 5 to 30 min did not increase TPC yield. At 150 °C, however, a reduction of ~8% was noted between 5 and 30 min (Runs 3–4), likely reflecting thermal degradation of polyphenols, as reported in previous SWE studies [34].

TPC selectivity reflects the relative preference of the extraction process for polyphenols over other co-extracted substances, such as sugars, organic acids, and proteins [46]. Thus, TPC selectivity provides additional insight into extract quality, complementing yield as a performance criterion. TPC selectivity fitted a quadratic model (R^2 = 0.858; *p*-value = 0.0006), with significant contributions from temperature (*p* = 0.0237), the AB interaction (*p* = 0.0191), and B^2 (*p*-value < 0.0001). Values ranged from 27.7 to 37.0 g GAE/100 g extract (Table 3). Selectivity peaked at intermediate temperatures (110 °C) and shorter extraction times, reflecting a compromise where phenolic recovery is maximised while co-extraction of non-phenolic solutes remains limited (Figure 1B). Similar patterns have been reported in SWE optimisation studies, where phenolic recovery is frequently accompanied by sugar solubilisation [47]. Consequently, the ratio of phenolics to sugars may represent a more relevant quality indicator than TPC yield alone [47,48].

Table 3. Regression models, equations, and statistical parameters describing the responses of SWE optimisation.

Responses	Model	Equation	R^2	R^2 Adjusted	Signal/Noise Ratio
TPC yield	Quadratic	$Y = 136.43 + 19.48B - 11.69B^2$	0.8462	0.8225	12.71
TPC selectivity	Quadratic	$Y = 34.67 - 0.4966A - 1.34B - 1.99AB + 1.46A^2 - 4.53B^2$	0.8582	0.7873	10.15
DPPH	Quadratic	$Y = 24.22 + 0.6001A + 5.54B + 1.37AB + 1.45B^2$	0.9789	0.9713	34.37
Sugars	Linear	$Y = 4.84 + 0.8887B$	0.7422	0.7238	12.70

DPPH followed a quadratic model (R^2 = 0.979; *p*(model) < 0.0001), with temperature as the dominant factor (*p*-value < 0.0001). Significant effects also arose from time (*p*-value = 0.0378), AB interaction (*p*-value = 0.0029), and B^2 (*p* = 0.0019). Values increased from 19.5 to 33.2 mg TE/g DM across the design space, with the maximum at 150 °C and 30 min (Run 4; Figure 1C). The increase with temperature is consistent with enhanced release of bound phenolics and improved solubilisation of polyphenols. Previous studies have shown that SWE reaches its maximum antioxidant potential under moderate conditions (130–150 °C), beyond which degradation reduces activity. For instance, the highest activity in distillery stillage was reported at 140 °C [32], while walnut bark extracts peaked at 130–140 °C [33]. In contrast, no clear decline was observed in the present study at the upper temperature tested (150 °C), suggesting a pronounced thermal stability of the phenolic compounds in this extract.

Sugar extraction was described by a linear model (R^2 = 0.742; *p*(model) = 0.0006), only influenced by temperature (*p*-value < 0.0001), while time and S/L ratio were not significant. Concentrations ranged from 3.52 to 5.91 g G+F/100 g DM, with the highest values again observed at 150 °C and 30 min (Run 4; Figure 1D). This behaviour aligns with the known increase in ionic product and hydrolytic capacity of subcritical water, which favours carbohydrate solubilisation [49]. High sugar release during SWE is usually regarded as undesirable because of the co-extraction of interfering compounds that usually require downstream purification. For instance, some studies focused on the valorisation of lignocellulosic matter have exploited this effect at elevated temperatures (>160 °C), leading to extensive hemicellulose solubilisation and consequently increasing the cellulose content in the insoluble fraction [50,51].

3.2. Extraction Optimisation

SWE was optimised to maximise phenolic recovery (TPC yield and selectivity and DPPH activity) while minimising sugar co-extraction. Extraction time and temperature were identified as significant factors (*p*-value < 0.05), whereas the S/L ratio was not statistically relevant within the tested range. However, for practical reasons related to matrix swelling during scale-up in the Parr reactor—where efficient stirring and filtration were required—an S/L ratio of 40 mL/g was selected. Optimisation was, therefore, performed by varying the extraction time and temperature, and the optimal conditions were determined using a desirability function.

The optimal SWE parameters were identified as 120 °C, 5 min, and a S/L ratio of 40 mL/g, with a desirability score of 0.55. Experimental results closely matched predicted values, with low errors of 4.29% for TPC yield, 2.10% for TPC selectivity, 0.12% for DPPH, and 9.18% for sugars (Table 4). These outcomes validate the robustness of the RSM model, consistent with other SWE optimisation studies where experimental deviations typically remain below 10% [34].

Table 4. Predicted versus experimental values of responses under optimal SWE conditions.

Responses	Target	Predicted	Experimental	% Error	Desirability	Optimal Values
TPC yield	Maximisation	140.62	146.92	+4.29	0.55	Time = 5 min Temp. = 120 °C L/S ratio = 40 mL/g
TPC selectivity	Maximisation	36.50	37.29	+2.10		
DPPH	Maximisation	24.78	24.81	+0.12		
Sugars	Minimisation	5.07	5.58	+9.18		

The optimised ASE process (SWE-ASE) was scaled up in a Parr high-pressure reactor (SWE-Parr) and compared with conventional extraction using 70% EtOH at boiling point (two successive cycles). As shown in Figure 2, TPC yields were not significantly different (*p*-value > 0.05) among SWE-ASE (146.9 mg GAE/g DM), SWE-Parr (134.0 mg GAE/g DM), and EtOH (138.4 mg GAE/g DM). EtOH extraction, however, achieved the highest TPC selectivity (42.3 g GAE/100 g extract), significantly greater than SWE values (*p*-value < 0.05), which was also reflected in its lower sugar co-extraction.

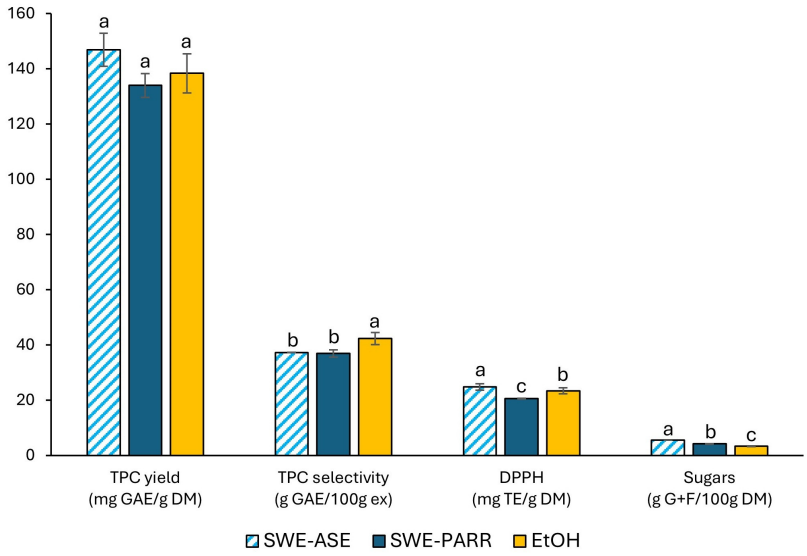

Figure 2. Comparison of TPC yield, TPC selectivity, DPPH, and sugar co-extraction in SWE-ASE, SWE-Parr, and EtOH extraction. Different letters (a–c) above bars indicate statistically significant differences ($p < 0.05$, Tukey's test).

Antioxidant activity followed the order SWE-ASE > EtOH > SWE-Parr, with SWE-ASE extracts reaching 24.8 mg TE/g DM. Although the differences were modest, these results indicate that SWE can yield antioxidant-rich extracts at least equivalent to conventional EtOH extraction. Comparable findings have been reported for spent coffee grounds, hazelnut skins, and grape pomace, where SWE matched or outperformed conventional S/L extraction with MeOH or EtOH in terms of TPC yield and antioxidant activity, while eliminating solvent residues and reducing processing costs [29,52,53]. Importantly, good scale-up reproducibility was confirmed using a Parr reactor: SWE-Parr recovered 91.2% of TPC and 82.9% of antioxidant activity compared to SWE-ASE. The slightly lower efficiency observed in the Parr system may be attributed to differences in heating dynamics and sample handling. While the ASE operates under constant pressure with immediate hot filtration, the Parr reactor requires a cooling phase prior to opening. During this step, the decrease in temperature and pressure increases water polarity and reduces the solubility of phenolic compounds. This temperature drop likely induces changes in solvent properties that promote partial precipitation or re-adsorption of solubilised compounds onto the solid matrix, ultimately reducing recovery.

Overall, the optimisation, scale-up reproducibility, and comparative performance demonstrate that SWE is a robust method for valorising SBP into bioactive-rich extracts suitable for food applications.

3.3. Resin Purification

Phenolic extracts obtained by SWE generally contain considerable amounts of co-extracted sugars, organic acids, and other hydrophilic solutes. These compounds dilute the phenolic fraction and may interfere with downstream applications. In particular, residual sugars in activated coatings are undesirable because they can serve as substrates for microbial growth, compromise film integrity, and hinder the activity of phenolic compounds [54]. To overcome this limitation, a purification step based on resin adsorption was applied.

Amberlite® XAD-7, a moderately polar acrylic ester resin, was selected due to its well-documented ability to adsorb phenolic compounds primarily through hydrogen bonding between the phenolic hydroxyl groups and the ester functionalities of the resin backbone [55], while allowing highly polar molecules such as sugars and organic acids

to be removed by simple washing. Compared to other macroporous resins, XAD-7 has demonstrated particularly high efficiency in polyphenol recovery, offering high recovery yields, reproducibility, and ease of regeneration, which makes it suitable for both laboratory- and industrial-scale processes [56].

As summarised in Table 5, resin purification markedly improved extract composition. The TPC increased from 36.9 g GAE/100 g_{ex} in the crude fraction to 93.9 g GAE/100 g_{ex} in the purified fraction, corresponding to a 2.5-fold enrichment.

Table 5. Composition and recovery parameters of strawberry matrix extracts before and after Amberlite® XAD-7 resin purification.

Parameters	SWE-Parr	SWE-Parr Purified
TPC (g GAE/100 g_{ex})	36.9 ± 0.42	93.9 ± 6.6
DPPH (g TE/100 g_{ex})	6.3 ± 0.10	17.6 ± 2.0
Glucose (g/100 g_{ex})	6.5 ± 0.1	ND
Fructose (g/100 g_{ex})	6.5 ± 0.1	ND
TPC recovery (%)	-	87.4 ± 4.3
Dry extract recovery (%)	-	34.4 ± 1.7

A similar enhancement was observed for antioxidant capacity, which rose from 6.3 to 17.6 g TE/100 g extract. These findings are consistent with previous reports showing significant enrichment of polyphenols and corresponding increases in antioxidant potential after resin adsorption [57,58]. At the same time, both glucose and fructose, present at 6.5 ± 0.1 g/100 g_{ex} in the crude fraction, were completely removed in the purified extract (not detected).

The process achieved a TPC recovery of 87.4% with a dry extract recovery of 34.4%, indicating that phenolic compounds were selectively retained by the resin and efficiently recovered in the desorbed fraction. The overall process yield (extraction plus purification) was 11.1%, corresponding to approximately 11 g of highly pure polyphenol-rich extract obtained from 100 g of dried SBP. Overall, purification with Amberlite® XAD-7 produced a sugar-free, phenolic-rich fraction with significantly enhanced antioxidant potential. Although SWE requires operation at elevated temperature and pressure, its sustainability should be evaluated at the process level, considering not only solvent consumption but also the energy demand associated with downstream operations. In this context, integrating SWE with resin-based purification enables direct concentration of polyphenols from aqueous extracts, significantly reducing the energy requirements of solvent-intensive removal steps, such as evaporation, spray drying, or freeze drying, which typically dominate the energy footprint of conventional aqueous extraction processes. In the present study, approximately 90% of the total phenolic content was recovered in the purified fraction using 70% EtOH, while reducing the solvent volume to about one-quarter of the initial extraction volume.

This purified extract was subsequently incorporated into bioactive chitosan coatings to evaluate its performance in postharvest strawberry preservation.

3.4. Polyphenol Profiling

Phenolic compounds in the extracts were characterised by LC–MS QToF using accurate mass measurement, isotopic distribution, and MS/MS fragmentation. A qualitative comparison of the LC–MS chromatograms suggests that the main phenolic features obtained by SWE–ASE were largely retained after scaling up in the Parr reactor, as the dominant peaks appeared at comparable retention times, as shown in Figure S3. Fourteen characteristic ions were identified across the EtOH, SWE-Parr, and SWE-Parr purified extracts, corresponding mainly to ellagitannins, ellagic acid derivatives, and flavonoid glycosides (Table 6). The

major constituents consistently detected included catechin (*m*/*z* 289.07), quercetin 3-*O*-xylosyl-glucuronide (*m*/*z* 609.11), ellagic acid (*m*/*z* 301.00), and querciturone (*m*/*z* 477.07), which have been previously reported in strawberry leaves, sepals, and stems [1,24,26].

Table 6. Observed and theoretical *m*/*z* values of polyphenols identified in EtOH, SWE-Parr, and SWE-Parr purified extracts (ionisation mode: $[M-H]^-$).

Peak	Proposed Compounds	Molecular Formula	RT (min)	Observed (*m*/*z*)	Theoretical (*m*/*z*)	Mass Error	EtOH	SWE-Parr	SWE-Parr purified
1	Pedunculagin ** or isomers	$C_{34}H_{24}O_{22}$	1.206	783.0686	783.0693	0.9 ppm	✔	Traces	Traces
2	Uralenneoside ** or Quinol glucuronide **	$C_{12}H_{14}O_8$	2.133	285.0622	285.0616	−2.1 ppm	✔	Traces	X
3	Salidroside ** or 4-methoxybenzyl glucoside *	$C_{14}H_{20}O_7$	2.407	345.1202	345.1191	−3.2 ppm	✔	✔	Traces
4	Procyanidin B1 * or B2 *	$C_{30}H_{26}O_{12}$	2.978	577.1367	577.1351	−2.8 ppm	✔	✔	✔
5	Catechin * or (-)-Epicatechin *	$C_{15}H_{14}O_6$	3.235	289.0723	289.0718	−1.7 ppm	✔	✔	✔
6	Quercetin 3-O-xylosyl-glucuronide **	$C_{26}H_{26}O_{17}$	6.457	609.1101	609.1097	−0.7 ppm	✔	✔	✔
7	Ellagic acid arabinoside** or Ellagic acid 4-O-xylopyranoside **	$C_{19}H_{14}O_{12}$	6.747	433.0423	433.0412	−2.5 ppm	✔	Traces	Traces
8	Ellagic acid 2-rhamnoside ** or Eschweilenol C **	$C_{20}H_{16}O_{12}$	6.904	447.0574	447.0569	−1.1 ppm	✔	Traces	✔
9	Ellagic acid **	$C_{14}H_6O_8$	7.103	300.9990	300.9990	0 ppm	✔	✔	✔
10	Querciturone ** or Quercetin-3′-glucuronide **	$C_{21}H_{18}O_{13}$	7.50	477.0688	477.0675	−2.7 ppm	✔	✔	✔
11	Luteolin-7-O-glucuronide * or Scuterallin **	$C_{21}H_{18}O_{12}$	9.033	461.0735	461.0725	−2.2 ppm	✔	Traces	Traces
12	Syringalide A **	$C_{23}H_{26}O_{10}$	9.77	461.1467	461.1453	−3.0 ppm	✔	Traces	✔
13	Eutigoside A ** or Osmanthuside A **	$C_{23}H_{26}O_9$	10.888	445.1515	445.1504	−2.5 ppm	✔	Traces	✔
14	Momordicoside E ** or Glucosyl passiflorate **	$C_{37}H_{60}O_{12}$	14.167	695.4019	695.4012	−1.0 ppm	✔	Traces	✔

* From internal database and ** from PubChem; ✔: present; X: absent

Ethanolic extraction yielded a broad spectrum of phenolic compounds, with pronounced signals corresponding to ellagitannins and flavonoid glycosides. The SWE-Parr extract generated a comparable profile, although peak intensities were generally lower, reflecting the slightly reduced selectivity of SWE observed in global assays (Figure 2). In comparison with EtOH extraction, several phenolic compounds, such as ellagic acid arabinoside (*m*/*z* 433.04) and luteolin-7-*O*-glucuronide (*m*/*z* 461.07), were detected only at trace levels. Agrimoniin, a high-molecular-weight ellagitannin characteristic of strawberry matrices, was detected in the EtOH extract but was not identified in the SWE and SWE

purified extracts under the applied analytical conditions. This behaviour may be attributed to the limited suitability of SWE for extracting intact ellagitannins and to possible thermal hydrolysis or structural transformation into lower-molecular-weight ellagic acid derivatives during SWE.

Amberlite® XAD-7 purification markedly improved extract composition by reducing early-eluting signals associated with sugars and other polar interferences, while enriching ellagitannins and flavonoid glycosides (Figure S3). This step led to a notable increase in the relative intensity of major peaks, such as catechin, querciturone, and ellagic acid. Notably, compounds originally present at trace levels the in crude extracts became readily detectable after purification (same concentration), indicating that resin adsorption efficiently concentrated both major and minor phenolic constituents. These findings are consistent with the known selective adsorption behaviour of Amberlite® XAD-7 towards polyphenols [56].

Overall, the combination of SWE and resin purification generated a phenolic profile closely resembling that of the EtOH extract, but with higher purity and complete removal of interfering solutes. The predominance of ellagitannins and flavonoid glycosides across all extracts supports their established role as the primary contributors to the antioxidant and antimicrobial activities of SBP [6].

3.5. Active Coating

3.5.1. Fungal Infection Incidence and Severity

The evaluation of the coating formulations and their impact on fungal infection and fruit quality is presented below. A visual assessment of the fruit surface over the storage period is presented in Figure 3.

Figure 3. Visual comparison of strawberries after 1 (**A**), 4 (**B**), and (**C**) 5 days of storage under different coating treatments. In each panel: top left, control with distilled water; top right, control with 1% lactic acid; bottom left, 1.5% HIC; bottom right, 1.5% HIC + 1% AOM.

Disease incidence showed no significant results when the interaction between storage time (days after treatment) and treatment factors was considered (Figure 4A). However, regardless of the treatment, the disease incidence increased significantly from day 4 to day 5 of storage by 70% (Table 7). Coating treatments did not result in statistically significant differences, although HIC + AOM showed lower values. Disease severity (% of necrotic area) also revealed strong effects of storage time and treatment alone, although their interaction gave no significant results (Figure 4B). Considering only the storage time, disease severity increased by 732% from day 4 to day 5. In relation to the coating treatment, regardless of the time, the HIC + AOM group achieved the greatest, statistically significant reduction in severity (72% relative to water), while HIC alone showed an intermediate reduction (38%). Treatment with lactic acid gave comparable results to those of the water control (Figure 4). It should be noted that fungal incidence and severity were evaluated

under natural infection conditions, without artificial inoculation of a specific pathogen. Therefore, the observed reduction in disease severity reflects the overall protective effect of the coatings during storage but does not allow attribution of the effect to direct antimicrobial activity against a specific fungal species.

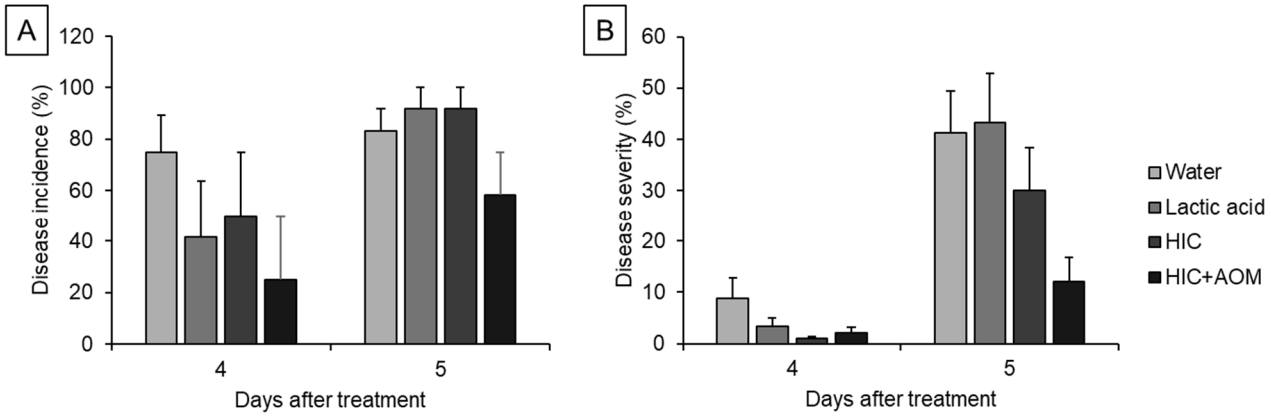

Figure 4. Disease incidence (**A**) and severity (**B**) of strawberries 4 and 5 days after treatment with water, 1% lactic acid, 1.5% HIC, or 1.5% HIC + 1% AOM. Bars represent means ± standard error.

Table 7. Mean effects of storage time (days after treatment) and treatment on disease incidence and disease severity (%) in coated strawberries.

Days After Treatment	Treatment	Disease Incidence (%)	Disease Severity (%)
4		47.9 ± 10.9 b	3.8 ± 1.2 b
5		81.3 ± 6.3 a	31.6 ± 4.2 a
	Water	79.2 ± 7.7	25.0 ± 5.6 a
	Lactic acid	66.7 ± 15.4	23.3 ± 6.4 a
	HIC	70.8 ± 15.0	15.5 ± 5.1 ab
	HIC + AOM	41.7 ± 15.4	7.0 ± 2.6 b
ANOVA			
Days after treatment (A)		**0.0156**	**<0.0001**
Treatment (B)		0.2042	**0.0104**
A × B		0.6660	0.0759

Upper section shows means across treatments for each time point (4 and 5 days). Middle section shows means across time points for each treatment. Lower section shows *p*-values from two-way ANOVA ($p < 0.05$). Values represent means ± standard error. Statistically significant effects ($p < 0.05$) are shown in bold. Different letters indicate statistically significant differences according to the Tukey–Kramer post hoc test ($p < 0.05$). Absence of letters indicates no significant differences.

These results are consistent with findings of Romanazzi et al. (2018) [59], who reported that chitosan inhibits fungal spore germination and slows the development of decay in infected fruits, due to both its antimicrobial action and its role as a semipermeable barrier. By lowering respiration and transpiration rates, chitosan coatings delay senescence. Chitosan's antimicrobial activity is mainly attributed to electrostatic interactions between its protonated amino groups and the negatively charged microbial cell walls, leading to increased membrane permeability, leakage of intracellular materials, metal–ion chelation, and interference with enzymatic systems essential for pathogens' survival [60,61]. Beyond these direct antimicrobial effects, chitosan films act as semipermeable barriers that reduce water loss and modulate gas exchange, thereby creating a microenvironment less favourable for pathogen growth [59,62,63]. More recently, chitosan coatings have also been recognised for their role in delaying senescence and reducing oxidative stress by stabilising the host tissue microenvironment [64,65].

The superior efficacy observed for HIC + AOM compared to HIC alone highlights the antimicrobial potential of SWE-Parr purified extract from strawberry plant residues. The wide spectrum of phenolic compounds detected in the plant extract, especially ellagitannins and flavonoid glycosides, can inhibit fungal spore germination, disrupt enzymatic activity, scavenge reactive oxygen species, and reduce oxidative damage to host tissues, thereby contributing to chitosan's antifungal effects [66].

Few studies indicate that chitosan-based coatings enriched with bioactive extracts (e.g., turmeric, green tea, and propolis) exhibit superior efficacy in mitigating decay in strawberry fruits, extending the shelf life and maintaining fruit quality [67]. Although direct studies on the application of strawberry-derived extracts to strawberry fruit are still scarce, ellagitannins and tannin-rich extracts from small fruits have demonstrated antifungal activity both in vitro and on fruit tissues [68], supporting our findings of enhanced protection when these extracts were incorporated into chitosan coatings.

The use of chitosan derived from *H. illucens* (HIC) is justified by studies showing comparable or better performance than crustacean chitosan in fruit preservation [10,16]. Guarnieri et al. (2024) reported that insect-derived chitosan extended the shelf life in grapes more than conventional chitosan [16]. Moreover, Triunfo et al. (2023) demonstrated that *H. illucens* chitosan effectively reduced fungal decay and enhanced the shelf life of strawberries [10]. Pupal exuviae chitosan coating was influenced by its physicochemical properties [31]. Specifically, the deacetylation degree influences chitosan antimicrobial properties, increasing its biological characteristics with higher deacetylation degrees [69,70]. Also, the molecular weight can be a determining factor. Indeed, the activity of chitosan used as a coating on strawberries infected with *Botrytis cinerea* has already been investigated in [71]. This study demonstrated that chitosan coating was effective in containing the proliferation of the fungus, but with specific differences related to chemical and physical properties of the biopolymer, specifically its molecular weight. Indeed, the study was carried out by testing bleached and unbleached chitosan from *H. illucens* pupal exuviae, and it was shown that bleached chitosan was less effective than unbleached chitosan, which is full of melanin pigments and other natural components. The reduced antifungal activity observed in bleached chitosan can be attributed to the loss of bioactive components and possible structural alterations induced by the bleaching process. In contrast, unbleached and bleached chitosan functionalised with natural extracts employed in a previous study [71] showed greater antifungal efficacy, suggesting that the presence of melanin or phenolic compounds, both native and added, contributes significantly to the overall biological activity. However, despite the relevant outcome of the aforementioned study, the experimental conditions under which antifungal efficacy was assessed in the present work must be carefully considered. Indeed, although the HIC + AOM coating showed a significantly greater reduction in disease severity compared to HIC alone, these results were obtained exclusively under natural infection conditions and, therefore, do not allow a definitive attribution of the observed effects to a direct antifungal mechanism. While previous studies demonstrated antifungal activity of pure *Hermetia illucens* chitosan against *Botrytis cinerea* under controlled challenge conditions [71], this evidence cannot be directly extended to the HIC + AOM formulation, in which interactions between chitosan and added phenolic compounds may alter both the efficacy and mode of action. Consequently, the reduced disease severity observed here may arise from a combination of indirect effects, such as barrier properties, delayed senescence, and enhanced tissue stability, in addition to potential antimicrobial activity. In this context, controlled pathogen challenge tests, particularly using *Botrytis cinerea*, represent a critical next step to confirm the antimicrobial function of the HIC + AOM formulation and to elucidate its underlying mechanism of action.

Mean effects of storage time (days after treatment) and coating treatments are reported in Table 7.

3.5.2. Commercial Quality Parameters and Shelf Life

Results related to the physicochemical parameters contributing to the commercial quality of strawberries are reported in Table 8. The intermolecular interactions created by the structure of chitosan allow the formation of electrostatic and hydrogen bonds, which are responsible for the formulation of chitosan films [72,73]. The film-forming ability of *H. illucens* chitosan has validated the use of these samples for the desired application, demonstrating their ability to form coatings linked to the characteristics of chitosan itself. Viscosity and molecular weight are two of the main factors influencing coating solution properties.

As already reported by Tafi et al. (2023) [15], low molecular weight and low viscosity can reduce the moisture retention effect of the coating, limiting the formation of the barrier on the surface of the treated fruit. For this reason, the functionalisation of the biopolymer with other substances, as reported in this work, can improve the film-forming activity, ensuring greater adhesion and, therefore, the formation of a thicker and more effective film.

Weight loss did not show any statistically significant results when the interaction between time (days after treatment) and treatment factors was considered. However, weight loss increased significantly over storage time regardless of the treatment (37.9% increase from day 4 to day 5). Among treatments, independently from the time, HIC + AOM caused a 26.9% lower weight loss compared to water, whereas lactic acid and HIC treatments resulted in non-significant differences.

Firmness decreased significantly over the storage time regardless of treatment, with an overall loss of 35% from day 1 to days 4–5. When treatments were compared across all time points, HIC resulted in a significantly greater reduction in firmness relative to the water control (by 27.4%), whereas HIC + AOM induced a smaller and statistically non-significant loss (12.9%).

These results reflect typical postharvest physiology of strawberry, marked by high respiration and enzymatic softening [74]. Chitosan coatings are widely reported to act as semipermeable barriers that reduce moisture loss and moderate gas exchange [62,63,66]. In our study, the barrier effect is inferred from weight loss and firmness data, although direct measurements of coating thickness, water vapour transmission rate, and oxygen permeability were not performed. However, the coating efficacy depends strongly on the film's physicochemical characteristics, such as polymer concentration, deacetylation degree, thickness, uniformity, and gas/water permeability [75]. In our study, the greater firmness loss observed with HIC alone compared to the water control is counterintuitive and may reflect inadequate barrier properties of the coating. Pure chitosan films can exhibit high water vapour permeability due to the polymer's hygroscopic nature, and suboptimal film formation during airbrush application may have created microscopic defects that accelerated moisture loss. The superior performance of HIC + AOM suggests that phenolic compounds enhanced film cohesion through hydrogen bonding and hydrophobic interactions with chitosan, improving barrier properties and reducing water vapour permeability, as previously reported in similar systems [76].

In line with the reduced weight loss observed in strawberries coated with the HIC + AOM formulation, superior performance of chitosan coatings enriched with bioactive extracts or essential oils in reducing fruit water loss compared with pure chitosan has been reported [67,77]. Weight loss in fruits mainly results from evapotranspiration and from the release of carbon and water during respiration [78]. The phenolic compounds may have reinforced the chitosan film network through molecular interactions, potentially reducing water vapour permeability. However, since no direct measurements of coating

thickness, water vapour transmission rate, or gas permeability have been performed, the barrier effect can only be inferred indirectly from the observed physiological responses. These effects likely stem from the ability of phenolics to interact with chitosan through hydrogen bonding, electrostatic, and hydrophobic interactions [79,80].

Table 8. Weight loss, firmness, total soluble solids (TSSs), titratable acidity (TA), TSS/TA ratio, and pH of strawberries treated with different coatings (water, lactic acid, HIC, and 1.5% HIC + AOM) and evaluated at 1, 4, and 5 days after treatment.

Days After Treatment	Treatment	Weight Loss (%)	Firmness (Kg)	TSS (°Brix)	TA (% Citric Acid)	TSS/TA Ratio	pH
1	Water	-	0.65 ± 0.08	6.96 ± 0.77	1.40 ± 0.15	5.09 ± 0.58	3.50 ± 0.03
	Lactic acid	-	0.80 ± 0.11	7.54 ± 0.22	1.46 ± 0.14	5.32 ± 0.45	3.53 ± 0.04
	HIC	-	0.68 ± 0.03	8.34 ± 0.19	1.50 ± 0.16	5.82 ± 0.59	3.59 ± 0.04
	HIC + AOM	-	0.76 ± 0.06	7.86 ± 0.36	1.11 ± 0.05	7.09 ± 0.09	3.53 ± 0.03
4	Water	16.1 ± 1.9	0.62 ± 0.05	10.20 ± 0.91	1.21 ± 0.10	8.64 ± 0.01	3.56 ± 0.05
	Lactic acid	18.5 ± 3.0	0.53 ± 0.05	9.32 ± 0.54	1.31 ± 0.18	7.53 ± 1.02	3.51 ± 0.04
	HIC	17.9 ± 1.8	0.34 ± 0.05	9.72 ± 0.54	1.22 ± 0.04	7.93 ± 0.35	3.58 ± 0.05
	HIC + AOM	11.9 ± 1.2	0.40 ± 0.01	8.80 ± 0.72	1.10 ± 0.05	8.12 ± 0.90	3.58 ± 0.04
5	Water	22.9 ± 3.0	0.59 ± 0.08	7.72 ± 0.11	1.21 ± 0.03	6.39 ± 0.01	3.53 ± 0.03
	Lactic acid	25.2 ± 3.9	0.49 ± 0.14	8.38 ± 0.45	1.19 ± 0.04	7.03 ± 0.22	3.48 ± 0.03
	HIC	24.3 ± 2.5	0.33 ± 0.03	8.88 ± 0.68	1.36 ± 0.11	6.63 ± 0.57	3.45 ± 0.03
	HIC + AOM	16.5 ± 1.9	0.45 ± 0.02	7.93 ± 0.41	1.34 ± 0.04	5.94 ± 0.35	3.41 ± 0.02
	Mean effect						
1		-	0.72 ± 0.04 a	7.68 ± 0.24 b	1.37 ± 0.07	5.83 ± 0.28 b	3.54 ± 0.02 a
4		16.1 ± 1.1 b	0.47 ± 0.04 b	9.51 ± 0.37 a	1.21 ± 0.05	8.06 ± 0.40 a	3.56 ± 0.02 a
5		22.2 ± 1.6 a	0.47 ± 0.04 b	8.23 ± 0.25 b	1.27 ± 0.03	6.50 ± 0.19 b	3.47 ± 0.02 b
	Water	13.0 ± 2.8 a	0.62 ± 0.04 a	8.29 ± 0.54	1.27 ± 0.06	6.71 ± 0.52	3.53 ± 0.02
	Lactic acid	14.6 ± 3.2 a	0.60 ± 0.07 ab	8.41 ± 0.32	1.32 ± 0.08	6.63 ± 0.43	3.50 ± 0.02
	HIC	14.1 ± 2.9 a	0.45 ± 0.05 b	8.98 ± 0.33	1.36 ± 0.07	6.80 ± 0.36	3.54 ± 0.03
	HIC + AOM	9.5 ± 2.0 b	0.54 ± 0.06 ab	8.20 ± 0.32	1.18 ± 0.04	7.05 ± 0.38	3.51 ± 0.03
	ANOVA						
Days after treatment (A)		**<0.0001**	**<0.0001**	**0.0002**	0.1154	**<0.0001**	**0.0056**
Treatment (B)		**0.0180**	**0.0330**	0.3690	0.1956	0.8374	0.6401
A × B		0.4476	0.3222	0.5394	0.2793	0.1771	0.2986

Weight loss is expressed relative to the individual initial weight. Data for day 1 are not shown because all values equal 0% by definition. Initial absolute weights (g) are provided in Table S2. The upper section shows individual treatment means at each time point. The middle section shows mean effects across time points for each treatment and across treatments for each time point. The lower section shows *p*-values from two-way ANOVA. Statistically significant effects ($p < 0.05$) are shown in bold. Different letters indicate statistically significant differences according to the Tukey–Kramer post hoc test ($p < 0.05$). Absence of letters indicates no significant differences.

TSS significantly increased by day 4 (+23.8%) and declined by day 5 (−13.5%), while no effects ascribable to the treatments were observed (Table 8). Titratable acidity (TA) was not affected by either storage time, treatments, or their interaction, while the TSS/TA ratio was influenced only by the time, with a significant 38.3% increase at day 4 followed by a 19.4% reduction by day 5. Similarly, also the pH was only influenced by the storage time, showing a statistically significant decrease (2.5%) by day 5.

The limited impact of the coatings on TSS, TA, and pH suggests that the sugar–acid balance, and consequently the overall taste, were well preserved. Therefore, lack of significant changes in these parameters is a positive outcome, indicating that fruit quality and consumer perception remain unaffected by the treatment.

4. Conclusions

This study proposed an integrated process for the valorisation of strawberry agro-industrial byproducts through the recovery and application of phenolic-rich extracts for food packaging. Subcritical water extraction, optimised by response surface methodology, provided high phenolic yields and antioxidant activity comparable to conventional ethanolic extraction while eliminating the use of organic solvents. Resin adsorption with Amberlite® XAD-7 effectively increased extract purity and antioxidant capacity, producing a sugar-free, polyphenol-enriched fraction suitable for food packaging applications. Critical parameters to be better investigated for future scale-up studies include heat and mass transfer efficiency, temperature and pressure control, solid–liquid ratio, and residence time during subcritical water extraction, as well as resin bed hydrodynamics, linear flow velocity, adsorption capacity, and regeneration performance during Amberlite® XAD-7 purification.

Incorporation of the purified extract into *Hermetia illucens* chitosan-based coatings resulted in a significant reduction of fungal decay and weight loss, while contributing to improved firmness retention in fresh strawberries without affecting other quality attributes. These results highlight the synergy between the antimicrobial and barrier properties of chitosan and the bioactivity of phenolic compounds recovered from strawberry plant byproducts.

Some limitations of the present study should be acknowledged. Physical characterisation of the coatings (thickness, water vapour transmission rate, and oxygen permeability) and phenolic release kinetics were not assessed. In addition, storage trials were conducted at 16 °C, which restricts direct extrapolation to cold-chain conditions (0–4 °C), and the extraction and coating production were performed at laboratory scale rather than under food-grade manufacturing conditions. Besides, a validation under controlled pathogen challenge conditions will be essential to fully substantiate the antimicrobial function of the proposed coating.

Overall, this work demonstrates that the combination of green extraction technologies and bio-based polymers offers a promising and sustainable approach to transform agro-industrial residues into functional materials for active food packaging. While the results are encouraging, further investigation is required to fully assess the performance and applicability of these systems. Future research should, therefore, focus on comprehensive coating characterisation, controlled release and barrier property studies, pathogen challenge tests, validation of release kinetics under cold-chain storage conditions, as well as food safety, sensory evaluation, and process scalability under food-grade conditions. In this context, ongoing research within the PLAMINPACK project aims to advance detailed physico-chemical characterisation, controlled pathogen challenge assays, and phenolic compound release kinetics, thereby providing deeper insight into the mechanisms responsible for the preservation effects observed in this study under real storage and distribution scenarios.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/polysaccharides7010025/s1, Figure S1: Panel A: Stock solution of AOM (antioxidants/antimicrobials) at 5% in 1% lactic acid before addition to the HIC solution. The strawberry waste extract shows a characteristic dark colour. Panel B: Comparison of final treatment solutions: (1) 1% lactic acid (control), (2) commercial chitosan (1.5%) in 1% lactic acid, (3) *Hermetia illucens* chitosan (HIC) (1.5%) in 1% lactic acid, and (4) HIC (1.5%) plus AOM (1% v/v) in 1% lactic acid. A progressive darkening is observed, with the HIC plus AOM solution showing the most pronounced brown and opaque appearance. Figure S2: Strawberry coating process. (A) Experimental setup showing strawberries arranged on grid trays for the four treatment groups (from left to right: water control, 1% lactic acid, 1.5% HIC, and 1.5% HIC + 1% AOM). (B) Close-up of the airbrush application technique showing the HIC + AOM solution being sprayed. (C) Treated strawberries after coating application. The grid arrangement facilitates air circulation and prevents contact between fruits during storage and monitoring. Table S1: Analysis of variance (ANOVA) for the effects of extraction parameters (time, temperature, and solid-to-liquid ratio) on TPC yield, TPC selectivity, antioxidant capacity (DPPH), and sugar co-extraction during SWE-ASE of SBPs. Figure S3: LC-MS/MS chromatograms of SBP extracts showing polyphenol profiles in negative ionisation modes. Table S2: Initial fruit weights (mean ± SD, g) for each treatment at day 0 (T0). These values correspond to the starting weights used to calculate percentage of weight loss.

Author Contributions: Conceptualisation, C.C., M.C., M.S., and A.C.; methodology, C.C., M.S., M.C., S.U., and A.D.; software, M.S.; validation, M.C., M.-B.C., P.F., and A.C.; formal analysis, C.C. and S.U.; investigation, S.U., M.S., A.D., and A.C.; resources, M.C., A.C., and M.-B.C.; data curation, C.C. and M.S.; writing—original draft preparation, C.C. and M.S.; writing—review and editing, M.C., A.C., P.F., A.D., and M.-B.C.; visualisation, C.C.; supervision, M.C. and M.-B.C.; project administration, M.C., A.C., and M.-B.C.; funding acquisition, M.C. and M.-B.C. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support has been provided by Partnership for Research and Innovation in the Mediterranean Area (PRIMA), to the "PLAnt-based antiMIcrobial aNd circular PACKaging for plant products" (PLAMINPACK) Project. Each partner is funded by its National Funding Agency (NFA): Ministero dell'Universitàe della Ricerca (MUR), Italy (project PRIMA23_00059, Directorial Decree No. 3313 of 13 March 2025) is funding Italian entities, and the French National Research Agency (ANR-24-P013-0004) and the communauté urbaine du Grand Reims, Département de la Marne et la Région Grand Est are funding the URD ABI.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: Author Patrizia Falabella was employed by the company Spinoff XFlies s.r.l. 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.

Abbreviations

The following abbreviations are used in this manuscript:

AOM	Antioxidant and antimicrobial compounds
ASE	Accelerated solvent extraction
ANOVA	Analysis of variance
BV	Bed volume
BV/h	Bed volume per hour
CO_2	Carbon dioxide
DES	Deep eutectic solvent
DM	Dry matter
DPPH	2,2 diphenyl 1 picrylhydrazyl

EtOH	Ethanol
GAE	Gallic acid equivalent
HIC	*Hermetia illucens* chitosan
HPLC RI	High-performance liquid chromatography refractive index
LC MS/MS	Liquid chromatography tandem mass spectrometry
LC MS QTOF	Liquid chromatography quadrupole time of flight
m/z	Mass-to-charge ratio
ND	Not detected
RPI	Ripening index
RSM	Response surface methodology
SBP	Strawberry byproduct
S/L ratio	Solid-to-liquid ratio
SD	Standard deviation
SWE	Subcritical water extraction
TA	Titratable acidity
TE	Trolox equivalent
TPC	Total phenolic content
TSS	Total soluble solid
TSSC	Total soluble solid content

References

1. Villamil-Galindo, E.; Van de Velde, F.; Piagentini, A.M. Strawberry Agro-Industrial by-Products as a Source of Bioactive Compounds: Effect of Cultivar on the Phenolic Profile and the Antioxidant Capacity. *Bioresour. Bioprocess.* **2021**, *8*, 61. [CrossRef]
2. Strawberry Production by Country. 2025. Available online: https://worldpopulationreview.com/country-rankings/strawberry-production-by-country (accessed on 25 August 2025).
3. Villamil-Galindo, E.; Gastélum-Estrada, A.; Chuck-Hernandez, C.; Antunes-Ricardo, M.; Reza-Zaldivar, E.E.; Piagentini, A.; Jacobo-Velázquez, D.A. Kinetic Ultrasound-Assisted Extraction as a Sustainable Approach for the Recovery of Phenolics Accumulated through UVA Treatment in Strawberry By-Products. *Foods* **2023**, *12*, 2989. [CrossRef] [PubMed]
4. Villamil-Galindo, E.; Van De Velde, F.; Piagentini, A.M. Extracts from Strawberry By-Products Rich in Phenolic Compounds Reduce the Activity of Apple Polyphenol Oxidase. *LWT* **2020**, *133*, 110097. [CrossRef]
5. Areti, H.A.; Muleta, M.D.; Abo, L.D.; Hamda, A.S.; Adugna, A.A.; Edae, I.T.; Daba, B.J.; Gudeta, R.L. Innovative Uses of Agricultural By-Products in the Food and Beverage Sector: A Review. *Food Chem. Adv.* **2024**, *5*, 100838. [CrossRef]
6. Zhu, Q.; Nakagawa, T.; Kishikawa, A.; Ohnuki, K.; Shimizu, K. In Vitro Bioactivities and Phytochemical Profile of Various Parts of the Strawberry (*Fragaria* × *Ananassa* Var. *Amaou*). *J. Funct. Foods* **2015**, *13*, 38–49. [CrossRef]
7. Cazón, P.; Mateus, A.R.; Silva, A.S. Advances in Active Packaging Using Natural Biopolymers and Fruit By-Products for Enhanced Food Preservation. *Food Res. Int.* **2025**, *213*, 116439. [CrossRef]
8. Hahn, T.; Tafi, E.; Paul, A.; Salvia, R.; Falabella, P.; Zibek, S. Current State of Chitin Purification and Chitosan Production from Insects. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 2775–2795. [CrossRef]
9. Scieuzo, C.; Franco, A.; Salvia, R.; Triunfo, M.; Addeo, N.F.; Vozzo, S.; Piccolo, G.; Bovera, F.; Ritieni, A.; Francia, A.D.; et al. Enhancement of Fruit Byproducts through Bioconversion by *Hermetia illucens* (*Diptera*: *Stratiomyidae*). *Insect Sci.* **2023**, *30*, 991–1010. [CrossRef]
10. Triunfo, M.; Guarnieri, A.; Ianniciello, D.; Coviello, L.; Vitti, A.; Nuzzaci, M.; Salvia, R.; Scieuzo, C.; Falabella, P. *Hermetia illucens*, an Innovative and Sustainable Source of Chitosan-Based Coating for Postharvest Preservation of Strawberries. *iScience* **2023**, *26*, 108576. [CrossRef]
11. Triunfo, M.; Guarnieri, A.; Ianniciello, D.; Coltelli, M.B.; Salvia, R.; Scieuzo, C.; De Bonis, A.; Falabella, P. A Comprehensive Characterization of *Hermetia illucens* Derived Chitosan Produced through Homogeneous Deacetylation. *Int. J. Biol. Macromol.* **2024**, *271*, 132669. [CrossRef]
12. Charles, A.P.R.; Rajasekaran, B.; Awasti, N.; Choudhary, P.; Khanashyam, A.C.; Majumder, K.; Wu, Y.; Pandiselvam, R.; Jin, T.Z. Emerging Chitosan Systems Incorporated with Polyphenols: Their Applications in Intelligent Packaging, Active Packaging, and Nutraceutical Systems—A Comprehensive Review. *Int. J. Biol. Macromol.* **2025**, *308*, 142714. [CrossRef]
13. Priyadarshi, R.; Rhim, J.-W. Chitosan-Based Biodegradable Functional Films for Food Packaging Applications. *Innov. Food Sci. Emerg. Technol.* **2020**, *62*, 102346. [CrossRef]

14. Triunfo, M.; Tafi, E.; Guarnieri, A.; Ianniciello, D.; Scieuzo, C.; Salvia, R.; Hahn, T.; Zibek, S.; Falabella, P. Usage of Chitosan from *Hermetia illucens* as a Preservative for Fresh Prunus Species Fruits: A Preliminary Analysis. *Chem. Biol. Technol. Agric.* **2023**, *10*, 101. [CrossRef]
15. Tafi, E.; Triunfo, M.; Guarnieri, A.; Ianniciello, D.; Salvia, R.; Scieuzo, C.; Ranieri, A.; Castagna, A.; Lepuri, S.; Hahn, T.; et al. Preliminary Investigation on the Effect of Insect-Based Chitosan on Preservation of Coated Fresh *Cherry tomatoes*. *Sci. Rep.* **2023**, *13*, 7030. [CrossRef] [PubMed]
16. Guarnieri, A.; Triunfo, M.; Ianniciello, D.; Tedesco, F.; Salvia, R.; Scieuzo, C.; Schmitt, E.; Capece, A.; Falabella, P. Insect-Derived Chitosan, a Biopolymer for the Increased Shelf Life of White and Red Grapes. *Int. J. Biol. Macromol.* **2024**, *275*, 133149. [CrossRef]
17. Zhu, F. Polysaccharide Based Films and Coatings for Food Packaging: Effect of Added Polyphenols. *Food Chem.* **2021**, *359*, 129871. [CrossRef]
18. Yu, J.; Xu, S.; Chen, R.; Shao, P. A Promising Bioactive Chitosan Film in Strawberry Fresh-Keeping: Plasticized with Tomato Processing by-Product Extract of Deep Eutectic Solvent. *Food Hydrocoll.* **2024**, *151*, 109859. [CrossRef]
19. Zidan, N.S.; Aziz Albalawi, M.; Alalawy, A.I.; Al-Duais, M.A.; Alzahrani, S.; Kasem, M. Modification of Edible Chitosan/Polyethylene Glycol Films Fortified with Date Palm Fruit Waste Extract as Promising Antimicrobial Food Packaging Materials for Fresh Strawberry Conservation. *Eur. Polym. J.* **2023**, *194*, 112171. [CrossRef]
20. Osorio-Tobón, J.F. Recent Advances and Comparisons of Conventional and Alternative Extraction Techniques of Phenolic Compounds. *J. Food Sci. Technol.* **2020**, *57*, 4299–4315. [CrossRef]
21. Aït-Kaddour, A.; Hassoun, A.; Tarchi, I.; Loudiyi, M.; Boukria, O.; Cahyana, Y.; Ozogul, F.; Khwaldia, K. Transforming Plant-Based Waste and by-Products into Valuable Products Using Various "Food Industry 4.0" Enabling Technologies: A Literature Review. *Sci. Total Environ.* **2024**, *955*, 176872. [CrossRef]
22. Vázquez-González, M.; Fernández-Prior, Á.; Bermúdez Oria, A.; Rodríguez-Juan, E.M.; Pérez-Rubio, A.G.; Fernández-Bolaños, J.; Rodríguez-Gutiérrez, G. Utilization of Strawberry and Raspberry Waste for the Extraction of Bioactive Compounds by Deep Eutectic Solvents. *LWT* **2020**, *130*, 109645. [CrossRef]
23. Oliva, E.; Mir-Cerdà, A.; Sergi, M.; Granados, M.; Sentellas, S.; Saurina, J. Green Extraction of Phenolic Compounds from Strawberry Waste Based on Natural Deep Eutectic Solvents. *Int. J. Food Sci. Technol.* **2024**, *59*, 3967–3977. [CrossRef]
24. Lin, D.; Ma, Q.; Zhang, Y.; Peng, Z. Phenolic Compounds with Antioxidant Activity from Strawberry Leaves: A Study on Microwave-Assisted Extraction Optimization. *Prep. Biochem. Biotechnol.* **2020**, *50*, 874–882. [CrossRef] [PubMed]
25. Terpinc, P.; Dobroslavić, E.; Garofulić, I.E.; Repajić, M.; Cegledi, E.; Dobrinčić, A.; Pedisić, S.; Levaj, B. Maximizing the Recovery of Phenolic Antioxidants from Wild Strawberry (*Fragaria vesca*) Leaves Using Microwave-Assisted Extraction and Accelerated Solvent Extraction. *Processes* **2023**, *11*, 3378. [CrossRef]
26. Sato, T.; Ikeya, Y.; Adachi, S.; Yagasaki, K.; Nihei, K.; Itoh, N. Extraction of Strawberry Leaves with Supercritical Carbon Dioxide and Entrainers: Antioxidant Capacity, Total Phenolic Content, and Inhibitory Effect on Uric Acid Production of the Extract. *Food Bioprod. Process.* **2019**, *117*, 160–169. [CrossRef]
27. Cheng, Y.; Xue, F.; Yu, S.; Du, S.; Yang, Y. Subcritical Water Extraction of Natural Products. *Molecules* **2021**, *26*, 4004. [CrossRef]
28. Zhang, J.; Wen, C.; Zhang, H.; Duan, Y.; Ma, H. Recent Advances in the Extraction of Bioactive Compounds with Subcritical Water: A Review. *Trends Food Sci. Technol.* **2020**, *95*, 183–195. [CrossRef]
29. Capaldi, G.; Voss, M.; Tabasso, S.; Stefanetti, V.; Branciari, R.; Chaji, S.; Grillo, G.; Cravotto, C.; Tagliazucchi, D.; Fiego, D.P.L.; et al. Upgrading Hazelnut Skins: Green Extraction of Polyphenols from Lab to Semi-Industrial Scale. *Food Chem.* **2025**, *463*, 140999. [CrossRef]
30. Aljawarneh, R.Y.A.; Che Zain, M.S.; Zakaria, F. Macroporous Polymeric Resin for the Purification of Flavonoids from Medicinal Plants: A Review. *J. Sep. Sci.* **2024**, *47*, 2400372. [CrossRef]
31. Triunfo, M.; Tafi, E.; Guarnieri, A.; Salvia, R.; Scieuzo, C.; Hahn, T.; Zibek, S.; Gagliardini, A.; Panariello, L.; Coltelli, M.B.; et al. Characterization of Chitin and Chitosan Derived from *Hermetia illucens*, a Further Step in a Circular Economy Process. *Sci. Rep.* **2022**, *12*, 6613. [CrossRef]
32. Mikucka, W.; Zielinska, M.; Bulkowska, K.; Witonska, I. Subcritical Water Extraction of Bioactive Phenolic Compounds from Distillery Stillage. *J. Environ. Manag.* **2022**, *318*, 115548. [CrossRef] [PubMed]
33. Kamiński, P.; Tyśkiewicz, K.; Fekner, Z.; Gruba, M.; Kobus, Z. The Influence of Subcritical Water Extraction Parameters on the Chemical Composition and Antioxidant Activity of Walnut (*Juglans regia* L.) Bark Extracts. *Appl. Sci.* **2022**, *12*, 12490. [CrossRef]
34. Khurun Hizar, S.A.; Putra, N.R.; Kobun, R.; Mohd Amin, S.F.; Roslan, J.; Ronie, M.E.; Ahmad Zaini, M.A.; Mamat, H.; Abdul Aziz, A.H. Subcritical Water Extraction on Phenolic, Flavonoid and Antioxidant Activity from *Orthosiphon stamineus* Leaves: Experimental and Optimization. *J. Eng. Res.* **2025**, *13*, 2801–2808. [CrossRef]
35. Hassan, J.; Anwar, R.; Khan, A.S.; Ahmad, S.; Malik, A.U.; Nafees, M.; Hussain, Z.; Inam-ur-Raheem, M. Chitosan-Based Edible Coating Delays Fungal Decay and Maintains Quality of Strawberries during Storage. *Int. J. Agric. Biol.* **2020**, *24*, 486–492. [CrossRef]

36. Zam, W. Effect of Alginate and Chitosan Edible Coating Enriched with Olive Leaves Extract on the Shelf Life of Sweet Cherries (*Prunus avium* L.). *J. Food Qual.* **2019**, *2019*, 8192964. [CrossRef]
37. Nair, M.S.; Saxena, A.; Kaur, C. Effect of Chitosan and Alginate Based Coatings Enriched with Pomegranate Peel Extract to Extend the Postharvest Quality of Guava (*Psidium guajava* L.). *Food Chem.* **2018**, *240*, 245–252. [CrossRef]
38. Yuan, G.; Chen, X.; Li, D. Chitosan Films and Coatings Containing Essential Oils: The Antioxidant and Antimicrobial Activity, and Application in Food Systems. *Food Res. Int.* **2016**, *89*, 117–128. [CrossRef]
39. Parichanon, P.; Santin, M.; Khemakhem, M.; Falher, T.; Petrucci, A.; Mencarini, D.; Sarrocco, S.; Conti, B.; Castagna, A. Mandarin Essential Oil-Flavoured Bio-Based Active Packaging for Quality Preservation and Fungal Control in Fresh Produce. *Postharvest Biol. Technol.* **2026**, *231*, 113906. [CrossRef]
40. *ISO 2173:2003*; Fruit and Vegetable Products—Determination of Soluble Solids—Refractometric Method. ISO: Geneva, Switzerland, 2003. Available online: https://www.iso.org/standard/35851.html (accessed on 6 November 2025).
41. Santin, M.; Parichanon, P.; Sciampagna, M.C.; Ranieri, A.; Castagna, A. Enhancing Tomato Productivity and Quality in Moderately Saline Soils through Salicornia-Assisted Cultivation Methods: A Comparative Study. *Horticulturae* **2024**, *10*, 655. [CrossRef]
42. Ko, M.-J.; Nam, H.-H.; Chung, M.-S. Subcritical Water Extraction of Bioactive Compounds from *Orostachys japonicus* A. Berger (*Crassulaceae*). *Sci. Rep.* **2020**, *10*, 10890. [CrossRef]
43. Mustafa, A.; Turner, C. Pressurized Liquid Extraction as a Green Approach in Food and Herbal Plants Extraction: A Review. *Anal. Chim. Acta* **2011**, *703*, 8–18. [CrossRef] [PubMed]
44. Nastić, N.; Švarc-Gajić, J.; Delerue-Matos, C.; Morais, S.; Barroso, M.F.; Moreira, M.M. Subcritical Water Extraction of Antioxidants from Mountain Germander (*Teucrium montanum* L.). *J. Supercrit. Fluids* **2018**, *138*, 200–206. [CrossRef]
45. Somat, H.A.; Thani, N.M.; Mustapha, W.A.W.; Lim, S.J.; Seng, N.S.S.; Rahman, H.A.; Razali, N.S.M.; Ali, M.M.; Kamal, S.M.M. Subcritical Water Extraction of Bioactive Compounds from Plant Materials: Recent Advances. *J. Future Foods* **2025**, *6*, 753–764. [CrossRef]
46. Herrero, M.; Cifuentes, A.; Ibanez, E. Sub- and Supercritical Fluid Extraction of Functional Ingredients from Different Natural Sources: Plants, Food-by-Products, Algae and microalgae: A Review. *Food Chem.* **2006**, *98*, 136–148. [CrossRef]
47. Palos-Hernández, A.; González-Paramás, A.M.; Santos-Buelga, C. Latest Advances in Green Extraction of Polyphenols from Plants, Foods and Food By-Products. *Molecules* **2025**, *30*, 55. [CrossRef]
48. Giombelli, C.; Iwassa, I.J.; Da Silva, C.; Bolanho Barros, B.C. Valorization of Peach Palm By-Product through Subcritical Water Extraction of Soluble Sugars and Phenolic Compounds. *J. Supercrit. Fluids* **2020**, *165*, 104985. [CrossRef]
49. Yuan, Y.; Shimizu, N.; Li, F.; Magaña, J.; Li, X. Buckwheat Waste Depolymerization Using a Subcritical Ethanol Solution for Extraction of Bioactive Components: From the Laboratory to Pilot Scale. *J. Environ. Chem. Eng.* **2023**, *11*, 109807. [CrossRef]
50. Freitas, P.A.V.; Martín-Pérez, L.; Gil-Guillén, I.; González-Martínez, C.; Chiralt, A. Subcritical Water Extraction for Valorisation of Almond Skin from Almond Industrial Processing. *Foods* **2023**, *12*, 3759. [CrossRef]
51. Maté, I.; Vargas, M.; Atarés, L.; Chiralt, A. Fractionation of Winemaking Grape Stalks by Subcritical Water Extraction to Obtain Added-Value Products. *Foods* **2024**, *13*, 3566. [CrossRef]
52. Fernandes, F.; Delerue-Matos, C.; Grosso, C. Valorization of Spent Coffee Grounds: Comparing Phenolic Content and Antioxidant Activity in Solid-Liquid vs. Subcritical Water Extraction Methods. *Biol. Life Sci. Forum* **2025**, *40*, 23. [CrossRef]
53. Aliakbarian, B.; Fathi, A.; Perego, P.; Dehghani, F. Extraction of Antioxidants from Winery Wastes Using Subcritical Water. *J. Supercrit. Fluids* **2012**, *65*, 18–24. [CrossRef]
54. Liyanapathiranage, A.; Dassanayake, R.S.; Gamage, A.; Karri, R.R.; Manamperi, A.; Evon, P.; Jayakodi, Y.; Madhujith, T.; Merah, O. Recent Developments in Edible Films and Coatings for Fruits and Vegetables. *Coatings* **2023**, *13*, 1177. [CrossRef]
55. Le, T.T.; Aymes, A.; Framboisier, X.; Ioannou, I.; Kapel, R. Adsorption of Phenolic Compounds from an Aqueous By-Product of Sunflower Protein Extraction/Purification by Macroporous Resins. *J. Chromatogr. Sep. Tech.* **2020**, *11*, 435. [CrossRef]
56. Toma, R.; Lemaire, J.; Flourat, A.L.; Marant, B.; Duval, M.; Allais, F.; Chadni, M. Optimizing Stilbene Recovery from Cell Cultures Media: A Comprehensive Study of the Adsorption Process. *Sep. Purif. Technol.* **2025**, *354*, 128983. [CrossRef]
57. Silva, E.; Pompeu, D.; Larondelle, Y.; Rogez, H. Optimisation of the Adsorption of Polyphenols from Inga Edulis Leaves on Macroporous Resins Using an Experimental Design Methodology. *Sep. Purif. Technol.* **2007**, *53*, 274–280. [CrossRef]
58. Seif Zadeh, N.; Zeppa, G. Recovery and Concentration of Polyphenols from Roasted Hazelnut Skin Extract Using Macroporous Resins. *Foods* **2022**, *11*, 1969. [CrossRef]
59. Romanazzi, G.; Feliziani, E.; Sivakumar, D. Chitosan, a Biopolymer with Triple Action on Postharvest Decay of Fruit and Vegetables: Eliciting, Antimicrobial and Film-Forming Properties. *Front. Microbiol.* **2018**, *9*, 2745. [CrossRef]
60. Rabea, E.I.; Badawy, M.E.-T.; Stevens, C.V.; Smagghe, G.; Steurbaut, W. Chitosan as Antimicrobial Agent: Applications and Mode of Action. *Biomacromolecules* **2003**, *4*, 1457–1465. [CrossRef]
61. Ke, C.-L.; Deng, F.-S.; Chuang, C.-Y.; Lin, C.-H. Antimicrobial Actions and Applications of Chitosan. *Polymers* **2021**, *13*, 904. [CrossRef]

62. Hernández-Muñoz, P.; Almenar, E.; Valle, V.D.; Velez, D.; Gavara, R. Effect of Chitosan Coating Combined with Postharvest Calcium Treatment on Strawberry (*Fragaria* × *Ananassa*) Quality during Refrigerated Storage. *Food Chem.* **2008**, *110*, 428–435. [CrossRef]
63. Vu, K.D.; Hollingsworth, R.G.; Leroux, E.; Salmieri, S.; Lacroix, M. Development of Edible Bioactive Coating Based on Modified Chitosan for Increasing the Shelf Life of Strawberries. *Food Res. Int.* **2011**, *44*, 198–203. [CrossRef]
64. Wang, S.-Y.; Herrera-Balandrano, D.D.; Jiang, Y.-H.; Shi, X.-C.; Chen, X.; Liu, F.-Q.; Laborda, P. Application of Chitosan Nanoparticles in Quality and Preservation of Postharvest Fruits and Vegetables: A Review. *Compr. Rev. Food Sci. Food Saf.* **2023**, *22*, 1722–1762. [CrossRef]
65. Dai, L.; Wang, X.; Zhang, J.; Li, C. Application of Chitosan and Its Derivatives in Postharvest Coating Preservation of Fruits. *Foods* **2025**, *14*, 1318. [CrossRef]
66. Muñoz-Tebar, N.; Pérez-Álvarez, J.A.; Fernández-López, J.; Viuda-Martos, M. Chitosan Edible Films and Coatings with Added Bioactive Compounds: Antibacterial and Antioxidant Properties and Their Application to Food Products: A Review. *Polymers* **2023**, *15*, 396. [CrossRef]
67. Martínez-González, M.d.C.; Bautista-Baños, S.; Correa-Pacheco, Z.N.; Corona-Rangel, M.L.; Ventura-Aguilar, R.I.; Del Río-García, J.C.; Ramos-García, M.d.L. Effect of Nanostructured Chitosan/Propolis Coatings on the Quality and Antioxidant Capacity of Strawberries During Storage. *Coatings* **2020**, *10*, 90. [CrossRef]
68. Klewicka, E.; Sójka, M.; Ścieszka, S.; Klewicki, R.; Milczarek, A.; Lipińska, L.; Kołodziejczyk, K. The Antimycotic Effect of Ellagitannins from Raspberry (*Rubus idaeus* L.) on Alternaria Alternata ŁOCK 0409. *Eur. Food Res. Technol.* **2020**, *246*, 1341–1349. [CrossRef]
69. Takahashi, T.; Imai, M.; Suzuki, I.; Sawai, J. Growth Inhibitory Effect on Bacteria of Chitosan Membranes Regulated with Deacetylation Degree. *Biochem. Eng. J.* **2008**, *40*, 485–491. [CrossRef]
70. Kong, M.; Chen, X.; Xue, Y.; Liu, C.; Yu, L.; Ji, Q.; Cha, D.S.; Park, H.J. Preparation and Antibacterial Activity of Chitosan Microshperes in a Solid Dispersing System. *Front. Mater. Sci. China* **2008**, *2*, 214–220. [CrossRef]
71. Vitti, A.; Coviello, L.; Triunfo, M.; Guarnieri, A.; Scieuzo, C.; Salvia, R.; Falabella, P.; Nuzzaci, M. In Vitro Antifungal Activity and in Vivo Edible Coating Efficacy of Insect-Derived Chitosan against *Botrytis cinerea* in Strawberry. *Int. J. Biol. Macromol.* **2024**, *279*, 135158. [CrossRef]
72. Muxika, A.; Etxabide, A.; Uranga, J.; Guerrero, P.; de la Caba, K. Chitosan as a Bioactive Polymer: Processing, Properties and Applications. *Int. J. Biol. Macromol.* **2017**, *105*, 1358–1368. [CrossRef]
73. Becerra, J.; Sudre, G.; Royaud, I.; Montserret, R.; Verrier, B.; Rochas, C.; Delair, T.; David, L. Tuning the Hydrophilic/Hydrophobic Balance to Control the Structure of Chitosan Films and Their Protein Release Behavior. *AAPS PharmSciTech* **2017**, *18*, 1070–1083. [CrossRef] [PubMed]
74. Petriccione, M.; Mastrobuoni, F.; Pasquariello, M.S.; Zampella, L.; Nobis, E.; Capriolo, G.; Scortichini, M. Effect of Chitosan Coating on the Postharvest Quality and Antioxidant Enzyme System Response of Strawberry Fruit during Cold Storage. *Foods* **2015**, *4*, 501–523. [CrossRef] [PubMed]
75. 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] [PubMed]
76. Feng, X.; Li, S.; Sun, Z.; Yuan, H.; Li, R.; Yu, N.; Zhang, Y.; Chen, X. The Preservation Effect of Chitosan-Hawthorn Leaf Extract Coating on Strawberries. *J. Food Prot.* **2024**, *87*, 100244. [CrossRef]
77. Araújo, J.M.S.; de Siqueira, A.C.P.; Blank, A.F.; Narain, N.; de Aquino Santana, L.C.L. A Cassava Starch–Chitosan Edible Coating Enriched with *Lippia sidoides* Cham. Essential Oil and Pomegranate Peel Extract for Preservation of Italian Tomatoes (*Lycopersicon esculentum* Mill.) Stored at Room Temperature. *Food Bioprocess Technol.* **2018**, *11*, 1750–1760. [CrossRef]
78. Das, D.K.; Dutta, H.; Mahanta, C.L. Development of a Rice Starch-Based Coating with Antioxidant and Microbe-Barrier Properties and Study of Its Effect on Tomatoes Stored at Room Temperature. *LWT—Food Sci. Technol.* **2013**, *50*, 272–278. [CrossRef]
79. Silva, N.C.; Chevigny, C.; Domenek, S.; Almeida, G.; Assis, O.B.G.; Martelli-Tosi, M. Nanoencapsulation of Active Compounds in Chitosan by Ionic Gelation: Physicochemical, Active Properties and Application in Packaging. *Food Chem.* **2025**, *463*, 141129. [CrossRef]
80. Fierri, I.; Chignola, R.; Stranieri, C.; Di Leo, E.G.; Bellumori, M.; Roncoletta, S.; Romeo, A.; Benetti, F.; Fratta Pasini, A.M.; Zoccatelli, G. Formulation, Characterization, and Antioxidant Properties of Chitosan Nanoparticles Containing Phenolic Compounds from Olive Pomace. *Antioxidants* **2024**, *13*, 1522. [CrossRef]