

Article

Active Pectin Films Enriched with Phenolic Acids: A Novel Strategy for Maintaining Postharvest Quality of Pears

Magdalena Mikus¹, Jolanta Małajowicz^{2,*}  and Sabina Galus^{1,*} ¹ Department of Food Engineering, Institute of Food Sciences, Warsaw University of Life Sciences, 02-776 Warsaw, Poland; magdalena_mikus@sggw.edu.pl² Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences, 02-776 Warsaw, Poland

* Correspondence: jolanta_malajowicz@sggw.edu.pl (J.M.); sabina_galus@sggw.edu.pl (S.G.)

Abstract

This study aimed to analyze the effect of various phenolic acids introduced into pectin films on their ability to inhibit microorganisms. The antimicrobial activity of six phenolic acids—gallic, protocatechuic, caffeic, sinapic, coumaric, and ferulic acids—was verified against *Bacillus subtilis* bacteria. No inhibitory effect was observed when the acids were introduced into the substrates with the films, as the polysaccharide films served as a breeding ground for microorganisms. Bacterial growth was inhibited when pure acid was introduced to the substrate. Gallic and caffeic acid, at concentrations of 50 and 75 mM/dm³, respectively, completely inhibited bacterial growth. However, studies on pears have shown that such concentrations of phenolic acids are unsuitable for fruit coatings, as they lead to cloudiness and impaired visual appeal. Consequently, the lowest effective concentration was applied to fruit, reducing the total bacterial count from 2.59 ± 0.04 to 1.88 ± 0.22 log CFU/mL and mold and yeast counts from 2.11 ± 0.09 to 1.63 ± 0.10 log CFU/mL. The coating produced with the lowest tested concentration of gallic acid reduced the pears' respiration rate. The amount of CO₂ released by coated fruit was approximately 4 mg/kg·h lower than that of uncoated pears, and the level of ethylene released was approximately 6 ppm lower. The addition of gallic acid at a concentration of 15 mM/dm³ to the coating reduced the growth of bacteria, yeasts, and molds. After 12 days of pear storage, the number of microorganisms in coated fruit was approximately 0.71 log CFU/mL lower for bacterial cells and 0.48 log CFU/mL lower for yeasts and molds.

Keywords: edible films; edible coatings; pectin; phenolic acids; antioxidant activity; *Bacillus subtilis*; microorganism inhibition; pears



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1. Introduction

Increasing consumer awareness of health, food quality, and environmental sustainability has intensified interest in minimally processed foods with extended shelf life and reduced use of synthetic preservatives [1]. In this context, edible coatings have emerged as an effective and sustainable strategy for preserving the quality of fresh fruits and vegetables [2,3]. These thin layers of biodegradable film-forming materials are applied directly to food surfaces, where they form a protective barrier after drying [4]. They help reduce moisture loss, regulate gas exchange, delay ripening processes, and maintain sensory and visual quality during storage [5]. Edible coatings and films are classified according to their structural components, which include proteins, lipids, polysaccharides, and composite materials [6,7].

Among these, polysaccharides such as starch, chitosan, carrageenan, cellulose, alginates, and pectin are widely used due to their biodegradability, edibility, and film-forming capacity [8]. In particular, pectin is considered a promising coating material because of its transparency, safety, and compatibility with bioactive compounds [9]. However, the hydrophilic nature of polysaccharide-based coatings limits their resistance to water vapor, which remains a significant drawback in food preservation applications [10]. Therefore, improving their functional performance through formulation strategies is an important research direction [11].

To overcome these limitations, polysaccharide-based coatings are often enriched with additional components, including proteins, lipids, or other polysaccharides, to improve barrier properties and extend their functionality in food systems [12]. More recently, increasing attention has been given to the incorporation of natural bioactive compounds, which can provide both antimicrobial and antioxidant activity while maintaining consumer demand for clean-label ingredients [13]. These compounds are frequently derived from plant sources and include essential oils, organic acids, and phenolic substances, which can enhance food stability and reduce microbial contamination [4,14]. Synergistic combinations of such compounds are often used to improve overall efficacy, particularly in active packaging systems.

Phenolic compounds represent one of the most important groups of plant-derived bioactives due to their broad functional properties. Phenolic acids, including hydroxycinnamic and hydroxybenzoic derivatives, are widely distributed in plant tissues and are characterized by significant antioxidant and antimicrobial activity [15]. Their activity is associated with their ability to neutralize free radicals, donate hydrogen or electrons, and chelate metal ions [16]. Structure–activity relationships indicate that the number and position of hydroxyl groups strongly influence their effectiveness, with hydroxycinnamic acids generally exhibiting higher antioxidant activity than hydroxybenzoic acids due to the presence of a conjugated side chain [17]. Owing to these properties, phenolic acids are increasingly considered promising functional ingredients in active food packaging systems aimed at extending shelf life and preserving quality [18].

Although a wide range of studies has focused on edible coatings containing essential oils and complex plant extracts, relatively less attention has been given to pectin-based systems enriched with individually selected phenolic acids. In particular, there is still limited understanding of how specific phenolic acids perform when incorporated into a polysaccharide matrix and applied directly to fresh produce. This represents an important research gap, especially in relation to systematic screening of antimicrobial effectiveness and subsequent validation under real storage conditions.

The genus *Bacillus* is considered one of the most ancient and taxonomically diverse groups within the family *Bacillaceae*. *Bacillus subtilis* is a Gram-positive bacterium that is widely distributed in soil, water, plant surfaces, and food environments [19]. It is known for its high environmental resistance and ability to form endospores, which allows survival under adverse conditions such as heat, desiccation, and UV radiation [20]. It is therefore frequently used as a model organism in studies on microbial contamination and food spoilage, as well as in evaluating the antimicrobial activity of bioactive materials.

This study aimed to develop pectin-based edible films enriched with selected phenolic acids, designed to inhibit microbial growth while preserving their functional properties. The effect of the coatings on pear quality during storage was evaluated. Antimicrobial activity was assessed against the Gram-positive bacterium *B. subtilis*, selected due to its widespread occurrence in soil, freshwater, and marine environments, as well as the gastrointestinal tract of animals and perishable food products. The experimental design consisted of two stages. In the first stage, edible coating formulations containing various

concentrations of phenolic acids were developed to identify the compound exhibiting the strongest inhibitory potential. In the second stage, pears were coated with the formulation containing the selected phenolic acid, and quality parameters were analyzed after 10 days of storage.

Although numerous studies have investigated edible coatings based on polysaccharides such as pectin, as well as coatings enriched with phenolic compounds for improving food quality and shelf life, these approaches have generally focused on single-component systems. In contrast, relatively few studies have explored the incorporation of phenolic acids directly into pectin-based matrices to develop multifunctional edible coatings. The combination of pectin with phenolic acids represents a promising strategy to simultaneously enhance the structural properties of the coating and introduce additional antioxidant functionality. This integrated approach may lead to synergistic effects between the biopolymer matrix and bioactive compounds; however, the stability, interactions, and functional performance of such systems remain insufficiently understood. Therefore, there is a clear research gap regarding the development and characterization of pectin–phenolic acid composite coatings for food preservation applications.

2. Materials and Methods

2.1. Materials

The research material consisted of edible films made of apple pectin (ZPOW “Pektowin” S.A., Jasto, Poland) incorporated with selected phenolic acids: ferulic acid, gallic acid, caffeic acid, coumaric acid, protocatechuic acid (Sigma-Aldrich, Saint Louis, MO, USA), and sinapic acid (Acros Organics, Poznań, Poland). Glycerol (Avantor Performance Materials, Gliwice, Poland) was used as a plasticizer. Mueller–Hinton Agar, Mueller–Hinton Broth (Graso Biotech, Starogard, Poland), and Sabouraud Dextrose Agar (Pol-Aura, Morąg, Poland) were used to grow the microorganisms. Physiological saline NaCl (BTL, Łódź, Poland) was used to prepare dilutions of the microbial cultures, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Sigma-Aldrich, Saint Louis, MO, USA) was used to determine the antioxidant activity of the resulting coatings.

Additionally, this study used *B. subtilis* bacterial cells from the collection of industrial microbial cultures at the Prof. W. Dąbrowski Institute of Agricultural and Food Biotechnology in Warsaw. The microbial cells were stored at 4 °C on a solid growth medium.

2.2. Film Preparation

Pectin-based films were prepared based on the method described by Mikus and Galus [21]. Aqueous film-forming solutions were from 5% apple pectin (Herbstreith & Fox GmbH Niemcy, Neuenbürg, Germany; 5 g/100 g of solution) with the addition of selected phenolic acids (protocatechuic acid, ferulic acid, coumaric acid, gallic acid, caffeic acid, and sinapic acid) at concentrations of 15, 25, 50, 75, and 100 mmol/dm³. The ingredients were heated to 50 °C and held for 20 min, which allowed the pectin to dissolve and improve its gelling properties. During heating, the solution was stirred at 500 rpm using a RCT Basic IKAMAG magnetic stirrer (IKA Sp. z o.o., Warsaw, Poland.) to obtain a clear solution. After the ingredients were evenly distributed, glycerol was added to the pectin solution at 30% relative to the pectin. This acted as a plasticizer, improving the film’s elasticity and mechanical properties. To obtain a constant film thickness of 100 ± 10 µm, 50 cm³ of each solution was poured into 10 cm diameter Petri dishes. The solutions were dried in a SLW 115 SMART PRO laboratory dryer (POL-EKO APARATURA sp. j., Wodzisław Śląski, Poland) at approximately 40 °C until the film was completely dry (approximately 24 h). After drying, the films were removed from the dishes and conditioned in a KBF 240 climatic chamber (Binder, Tuttlingen, Germany) at 25 °C and

50% relative humidity. At least three series of films were prepared from each mixture type. In model experiments involving microorganisms, the film was cut into approximately 1 cm × 1 cm pieces before use.

2.3. Determination of Antimicrobial Activity of Films in Solid Media

The antimicrobial activity of edible films against *B. subtilis* was assessed on a solid Mueller–Hinton medium. Surface inoculation was performed with a standardized bacterial inoculum at 0.5 on the McFarland scale, corresponding to approximately 1.5×10^8 CFU/mL. Films containing phenolic acids were cut into 1 cm × 1 cm squares and applied to the inoculated Petri dish surfaces. Antimicrobial activity was assessed by observing the bacterial growth inhibition zones.

2.4. Assessing the Antimicrobial Activity of Films in Liquid Media

Antimicrobial activity was also assessed in submerged cultures of *B. subtilis* bacteria in Mueller–Hinton broth. Flat-bottomed flasks were loaded with 100 cm³ of Mueller–Hinton medium and 1 g of foil containing the selected phenolic acid. The sterilized media were inoculated with 1 cm³ of inoculum at a cell concentration of 0.5 McFarland. The flasks were incubated at 36 °C for 48 h, shaking at 140 rpm. Control cultures were run in parallel, without the addition of films. Each culture was performed in triplicate.

2.5. Enumeration of Viable *B. Subtilis* Cells

The viable *B. subtilis* cell count was determined by plate counting. Furthermore, 9 cm³ of 0.85% saline solution was added to the test tubes, followed by cascading 1 cm³ of bacterial cell suspension and diluting them in the range of 10^{-6} and 10^{-10} . From the appropriate dilutions, 10 µL of the solution was removed, and the surface was inoculated onto solid Mueller–Hinton medium. After approximately 48 h of incubation, colonies of grown bacteria were counted, and the result was reported as log CFU/mL.

2.6. Determination of the Optical Density of the Media

To determine the optical density of the media (OD₆₀₀), 1 cm³ of cell suspension was collected at specified intervals into Eppendorf tubes. Samples were centrifuged in a MiniSpin plus centrifuge for 3 min at 12,000 rpm. The supernatant was decanted from the sediment, which was then washed with 1 cm³ of distilled water and then centrifuged again. After washing, the bacterial cells were resuspended in 1 cm³ of distilled water, and the absorbance was measured at 600 nm (Rayleigh UV-1601 Spectrophotometer, Donserv, Warsaw, Poland) after appropriate dilution. Each determination was performed in triplicate.

2.7. Analysis of the Antioxidant Activity of Medium with Films—DPPH Method

To prepare the DPPH radical solution, 100 mg of DPPH was dissolved in methanol to obtain a solution with a concentration of approximately 0.1 mM/dm³. The solution was stored in the dark throughout the analysis.

The antioxidant activity was determined for the culture medium (supernatant) obtained after incubation of the films in the medium. The culture medium was centrifuged to obtain a clear supernatant. Subsequently, 50 µL of the supernatant was mixed with 2.95 mL of DPPH solution in a cuvette. Each sample was thoroughly vortexed and incubated at room temperature in the dark for 20 min. After incubation, absorbance was measured at 517 nm against a blank sample (2.95 mL of methanol and 50 µL of water). The control sample consisted of 2.95 mL of DPPH radical solution and 50 µL of water. Absorbance was measured three times for each solution. Antioxidant activity was expressed as a percentage of DPPH reduction, calculated using the following formula:

$$\% \text{ DPPH reduction} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100\%$$

where A_{control} is the absorbance value of the control solution, and A_{sample} is the absorbance value of the test solution.

2.8. Coating Fruit with Edible Coating

“Konferencja” pears (*Pyrus communis* L.), sourced from Poland and harvested at commercial maturity (ready-for-consumption stage), were purchased in a local market and subsequently coated with a pectin formulation containing 15 mmol/dm³ of gallic acid by immersion for 30 s (Coated).

Excess coating was removed by natural drainage, and the pears were dried at room temperature for 24 h. Pears coated with the edible formulation without phenolic acid served as the control, while those immersed in distilled water for 30 s served as the reference (Uncoated). Coated pears were analyzed for weight loss, pH, total soluble solids, and respiration rate over 10 days at room temperature. Microbiological quality was assessed on day 12 of storage.

2.9. Determining Weight Loss

To determine the weight loss, the pears were weighed on a Radwag 60/C/1 analytical balance. The percentage of weight loss was calculated from the difference in weight between the pears directly coated with the edible coating and the fruit after a specified storage time. The analysis was performed in triplicate.

2.10. Determination of pH and Total Soluble Solids

The pH and total soluble solids were measured during 10 days of fruit storage. The pH was measured using a calibrated PH-100ATC pH meter. Dissolved solids were determined using a digital refractometer (Palette PR-32, Atago Co., Tokyo, Japan), and the results were expressed as °Brix. The determination was performed in triplicate.

2.11. Determination of Fruit Respiration

The respiration rate of coated fruit was assessed using an F-950 analyzer (Felix Instruments Inc., Camas, WA, USA). The release of two gases was verified: ethylene (C₂H₄) and carbon dioxide (CO₂). For analysis, the pears were placed in a chamber connected to a gas analyzer. The fruit was tightly closed, and the initial data were recorded. Samples were left in the chamber for 1 h.

2.12. Microbiological Analysis of Coated Fruit

After 12 days of storage, pears coated with a protective coating were subjected to microbiological testing to determine the total microbial counts and yeast and mold counts. All tests were performed in triplicate. To determine the microbial count, the pear (both the flesh and the surface) was ground and homogenized in sterile BagLight bags. The total microbial count was determined by plate culture: 1 cm³ of the sample was deep-plated after appropriate dilution onto an agar medium. After 48–72 h of cultivation at 28 °C, colonies were counted, and the result was reported as log CFU/mL. Fungal counts (yeasts and molds) were determined on Sabouraud Dextrose Agar. Samples were incubated at 28–30 °C for 48–96 h.

2.13. Statistical Analysis

Means and standard deviations were calculated in Microsoft Excel 2019. Statistical analysis was performed using Statistica 13 (StatSoft Sp. z o.o., Warsaw, Poland) using

one-way ANOVA with Tukey's test. Significant differences between samples were determined at $p \leq 0.05$.

3. Results and Discussion

3.1. Evaluation of the Effect of Selected Phenolic Acids Constituting the Films on *Bacillus Subtilis* Cell Growth

To verify the effect of phenolic acid addition to the films on their inhibitory activity, six different acids were tested—derivatives of hydroxybenzoic acid (gallic and protocatechuic acid) and hydroxycinnamic acid (caffeic, sinapic, coumaric, and ferulic acid). The concentration of phenolic acids in the films was approximately 15 mM/dm^3 . A total of 1 g of the film was added to microbiological media in which *Bacillus* cells were grown. *B. subtilis* was selected as a model microorganism to evaluate the inhibitory effects of edible coatings enriched with phenolic acids due to its well-characterized physiology and ubiquitous distribution in diverse environments. This bacterium is also relevant in food microbiology, as it can contribute to the spoilage of plant-based products. Therefore, it serves as a suitable indicator organism for assessing the antimicrobial efficacy of the tested coatings and their potential to prolong the shelf life of pears. The effect of phenolic acids was assessed by measuring the medium optical density (OD_{600}) and the number of viable cells grown from inoculations on solid media. The results of this experiment are presented in Table 1 and Figure 1.

Table 1. Optical density (OD_{600}) of media after 48 h cultivation of *B. subtilis* bacteria on Mueller–Hinton medium with the addition of films enriched with selected phenolic acids.

Type of Material	OD_{600}
Control	2.56 ± 0.29^a
Film without acid	4.92 ± 0.41^b
Caffeic acid	4.72 ± 0.36^b
Coumaric acid	3.43 ± 0.35^b
Ferulic acid	3.84 ± 0.34^b
Gallic acid	4.17 ± 0.39^b
Protocatechuic acid	4.28 ± 0.10^b
Sinapic acid	4.52 ± 0.42^b

Mean values $\pm$ standard deviations. Different superscript letters (^{a–b}) within the same column indicate significant differences between media with the addition of coatings enriched with different acids ($p < 0.05$).

The results showed that none of the phenolic acids used in films at the tested concentration of 15 mM/dm^3 had an inhibitory effect on *Bacillus* cells. In contrast, the addition of 1 g of pectin coating enriched with the selected phenolic acid increased the number of viable bacterial cells. This is evident both in the optical density results of the media and in the number of viable cells, which, after 24 h of culture, were nearly three-fold (medium with films containing sinapic or protocatechuic acid) to eight-fold (the medium with films containing ferulic or gallic acid) higher in media with added acids than in media without the added acid. Furthermore, in all media with added films (containing phenolic acids or not), microorganisms multiplied more intensively—this is confirmed by the results from both graphs. In the control sample (the medium without the coating), the optical density of the medium after 48 h of cultivation was approximately 2.56 ± 0.29 , while in each of the other samples, in which 1 g of coating was added, the OD_{600} ranged from 3.43 ± 0.35 for the medium with coumaric acid and 4.72 ± 0.36 for the medium with caffeic acid (Figure 1). This is also confirmed by the viable cell count results. In the control medium (without the coating), the number of *Bacillus* cells after 48 h of cultivation reached $0.33 \cdot 10^8 \text{ CFU/mL}$,

while in the media with the coating, it ranged from $2.33 \cdot 10^8$ CFU/mL (in the medium with gallic acid) to $15.67 \cdot 10^8$ CFU/mL (in the medium with the coating without phenolic acid).

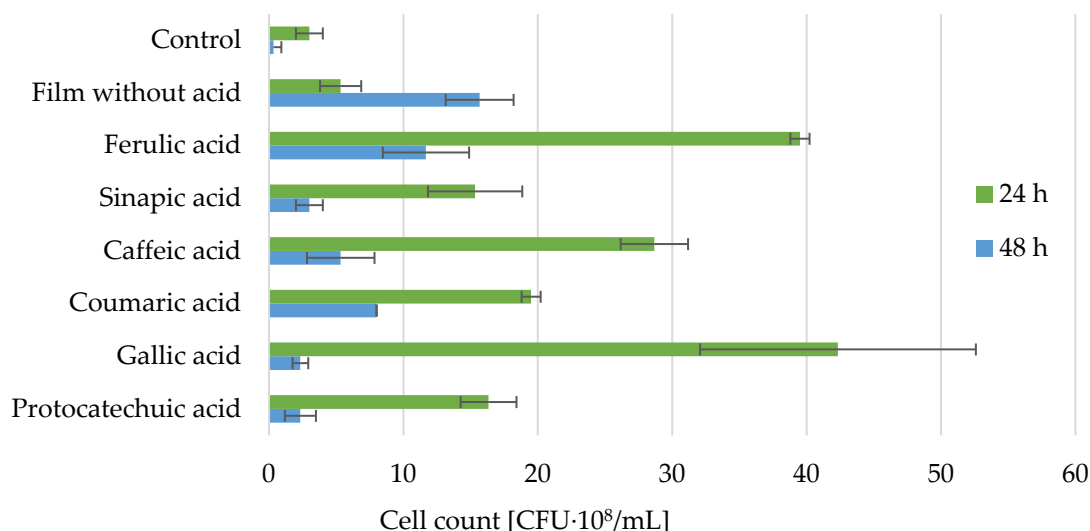


Figure 1. Number of viable *B. subtilis* cells grown for 48 h on Mueller–Hinton medium with the addition of films enriched with selected phenolic acids.

The above results demonstrate that pectin films introduced into the substrate can act as a breeding ground for microorganisms. Pectin is a polysaccharide, a high-molecular-weight heteropolymer with a high content of galacturonic acid—an oxidized form of *D*-galactose, which constitutes the main monomeric unit (approximately 65%) of the pectin molecule [22].

This polysaccharide can be degraded by enzymatic hydrolysis involving enzymes from the pectinase group. Three broader groups of pectinase can be identified: protopectinases (EC 3.2.1.99), pectinesterases (or pectin methyl esterase, EC 3.1.1.11), and pectin depolymerases (or endo-polygalacturonase, EC 3.2.1.15) [23]. Polygalacturonases are thought to play a special role in pectin hydrolysis [24].

The enhanced growth of *B. subtilis* cells observed in media supplemented with pectin, compared to the control medium, may be attributed to the ability of these bacteria to effectively utilize pectic substances as an additional carbon source. *Bacillus* spp. are known to produce extracellular pectinolytic enzymes, including polygalacturonases and pectate lyases, which hydrolyze pectin into assimilable oligogalacturonides and galacturonic acid. These degradation products may subsequently enter central metabolic pathways, thereby supporting bacterial growth and cellular metabolism [25].

Additionally, the presence of pectin in the culture medium may stimulate the secretion of pectinolytic enzymes and activate adaptive metabolic pathways associated with polysaccharide degradation and utilization. Previous studies demonstrated that pectin-rich substrates enhance the production of polygalacturonases by *Bacillus* strains, confirming their metabolic adaptation to pectic compounds [26].

Consequently, the availability of pectin in the medium likely increased nutrient accessibility and metabolic activity, resulting in more intensive bacterial proliferation compared to the control medium lacking this polysaccharide substrate.

3.2. The Effect of Phenolic Acid Concentration on the Growth of *Bacillus* Bacteria and the Antioxidant Activity of the Medium

The results obtained in the first stage of the experiment showed that testing the addition of phenolic acid to the medium, by incorporating it into a pectin coating, is unreliable for assessing *Bacillus* inhibition due to polysaccharide degradation and increased

microbial cell growth. Therefore, in the next stage of the study, it was decided to add phenolic acids directly to the medium. Additionally, at this stage of the study, various concentrations of phenolic acids were tested, ranging from 15 to 75 mM/dm³.

Table 2 presents the results for the number of viable *B. subtilis* cells grown for 48 h on Mueller–Hinton medium supplemented with selected phenolic acids at concentrations ranging from 15 to 75 mM/dm³.

Table 2. The number of live *B. subtilis* bacterial cells grown for 48 h on Mueller–Hinton medium with the addition of selected phenolic acids at concentrations ranging from 15 to 75 mM/dm³.

Phenolic Acid Concentration [mM/dm ³]	Number of Bacterial Cells [CFU/mL]					
	Protocatechuic Acid	Ferulic Acid	Coumaric Acid	Gallic Acid	Caffeic Acid	Control Substrate
15	>10 ⁹	>10 ⁹	10.5 ± 0.3 × 10 ¹⁰	1.2 ± 0.2 × 10 ⁴	4.9 ± 0.6 × 10 ⁶	-
25	>10 ⁹	>10 ⁹	11.1 ± 0.8 × 10 ¹⁰	0.4 ± 0.1 × 10 ⁴	4.1 ± 0.1 × 10 ⁶	-
50	>10 ⁹	>10 ⁹	10.2 ± 0.4 × 10 ¹⁰	0	0.8 ± 0.2 × 10 ⁶	-
75	>10 ⁹	>10 ⁹	<10 ⁹	0	0	-
-	-	-	-	-	-	3.5 ± 0.3 × 10 ⁹

The results in Table 2 indicate that the type of phenolic acid added to the medium influences its effect on *Bacillus* cells. Of the five phenolic acids tested, protocatechuic, ferulic, and coumaric acids did not exhibit any inhibitory effect on microbial growth. The number of multiplied cells (above 10⁹) was comparable to the control culture. Gallic and caffeic acids, however, inhibited bacterial growth at all tested concentrations. The number of viable cells, both with gallic acid and caffeic acid added, was approximately 3 (for caffeic acid) and 5 (for gallic acid) log units lower than the control culture. The data indicate a significantly stronger inhibitory effect of gallic acid. Even the lowest concentration of this compound used, at the level of 15 mM/dm³, significantly reduced the number of living cells (from 3.5 ± 0.3 × 10⁹ CFU/mL in the control culture to 1.2 ± 0.2 × 10⁴ CFU/mL in the phenolic acid culture (analysis after 48 h of multiplication)). Higher tested concentrations, above 50 mmol/dm³, completely inhibited the growth of microorganisms.

The literature describes many examples of the inhibitory effects of phenolic acids on the growth and metabolism of microorganisms. In the yeast *Saccharomyces cerevisiae*, cinnamic acid has been reported to negatively affect membrane integrity, leading to increased cell permeability [27]. This is supported by the research of Lobiuc et al. [28], which found that the key mechanism of action of phenolic acids involves excessive acidification, which alters the cell membrane potential. Dissociation of phenolic acids increases membrane permeability, thereby affecting the function of the sodium-potassium pump. Gram-positive bacteria are more susceptible to this inhibitory mechanism because they lack an outer membrane. It was found that high concentrations of phenolic acids (at the level of 1 mg/mL) have antibacterial activity against lactic acid bacteria such as *L. paraplantarum* LCH7, *L. plantarum* LCH17, *L. fermentum* LPH1, *L. fermentum* CECT 5716, *L. brevis* LCH23, and *L. coryniformis* CECT 5711 [29].

Phenolic acids, including gallic acid, can alter the charge, hydrophobicity, and permeability of membranes. In Gram-negative bacteria, disruption may occur through the chelation of divalent cations [30]. Cueva et al. [29] report that antimicrobial activity depends on both the species and strain of microorganisms, as well as on the number and position of substituents on the benzene ring. They report that phenolic acids, among others, dissociate at the cell membrane of Gram-positive pathogens and inhibit the growth of

bacteria such as *S. aureus* EP167 and *S. aureus* ATCC 29213, with IC values of 1000 µg/mL and 667 µg/mL, respectively.

The antibacterial activity of plant polyphenols may also result from interactions between the acids, proteins, and bacterial cell walls, altering the function of cytoplasmic membranes, inhibiting energy metabolism and DNA repair, or inhibiting cellular nucleic acid synthesis. At the molecular level, the planarity of the DNA backbone and the hydrophobic core allow polyphenols to penetrate the DNA helix during replication, recombination, repair, and transcription.

Borges et al. [31] evaluated the mechanism of action of gallic acid against selected strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Gallic acid showed antimicrobial activity against the bacteria tested, with MICs of approximately 500 µg/mL. Gallic acid induced irreversible changes in membrane properties (charge, intra- and extracellular permeability, and physicochemical properties) through alterations in hydrophobicity, a reduction in negative surface charge, and local rupture or pore formation in cell membranes, leading to the leakage of essential intracellular components.

The lack of an inhibitory effect of protocatechuic, ferulic, or coumaric acids on *Bacillus subtilis* cells can be explained by studies by Konzock et al. [32], among others. Scientists have reported that differences in the metabolism of microbial cells, in terms of the carbon sources they absorb, may influence tolerance to compounds commonly considered inhibitors. The literature data indicate that many compounds introduced into the medium, in addition to components ensuring optimal microbial growth, can constitute a stress factor for them. In many cases, this factor contributes to changes in gene expression, leading to the synthesis of proteins that are not produced under optimal growth conditions [33]. Microorganisms exposed to stress factors develop numerous adaptations that determine the microorganism's tolerance or resistance to a wide range of harmful compounds [34].

To assess the activity of the most effective inhibitory phenolic acids in the microbiological medium, we also analyzed their antioxidant properties using the DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. This is one of the most commonly used spectrophotometric methods, which utilizes a stable radical with an unpaired electron in the valence shell of one of the nitrogen atoms. The assay was performed on substrates containing gallic or caffeic acid. The results of this experiment are presented in Figure 2.

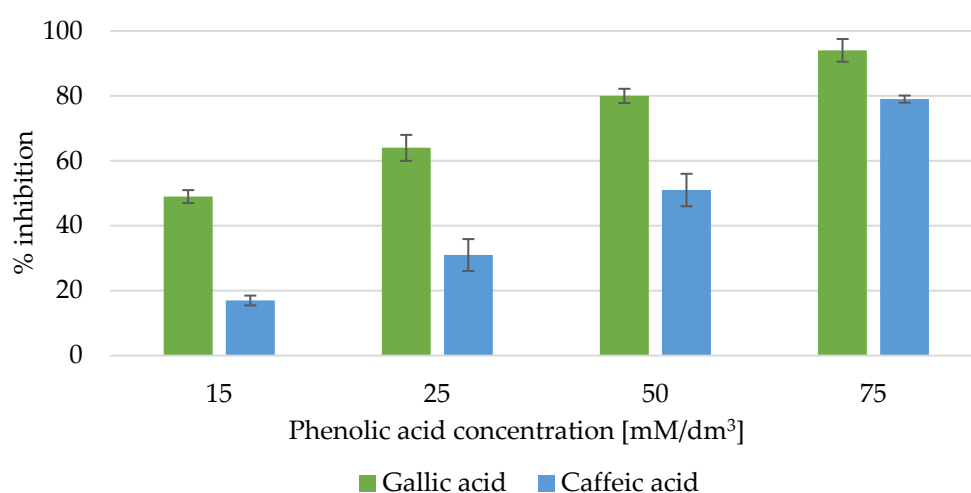


Figure 2. Comparison of the antioxidant activity of substrates containing selected phenolic acids using the DPPH radical reduction method.

The data in Figure 2 confirm that gallic acid has higher antioxidant activity. Regardless of the concentration introduced into the medium, the inhibition level of caffeic acid was approximately 30% lower than that of gallic acid (for concentrations of 15–50 mmol/dm³).

or approximately 15% lower (for a concentration of 75 mM/dm³). This is likely due to differences in the structures of these phenolic acids. The literature suggests that the activity of phenolic acids is influenced by the number of hydroxyl groups attached to the aromatic ring. Zych and Krzepiřko [35] indicate that the antioxidant capacity of flavonoids depends on the position and number of hydroxyl groups—a larger number of them enhances their antioxidant properties. There may be a correlation between antioxidant activity and the ability of polyphenols to inhibit microorganisms, but due to the multitude and complexity of the mechanisms of polyphenol action against microbial cells, it is difficult to definitively determine this.

3.3. The Effect of Coating on the Quality of Pears During Storage

The idea of creating an edible coating containing added phenolic acid arose from the fact that the fresh fruit industry faces significant challenges in maintaining fruit quality and freshness and extending shelf life long enough for distribution to markets. Consumers are already familiar with techniques for extending fruit shelf life, such as modified atmosphere packaging, irradiation, and low-temperature storage. Applying a coating is a relatively innovative method. A thin layer of edible material can act as a semi-permeable barrier, thus creating a modified atmosphere for the coated fruit or its comminuted form [36]. Gallic acid was selected to evaluate its effectiveness in producing an edible coating and maintaining pear quality. It demonstrated the highest antioxidant activity and the strongest inhibitory effect against *B. subtilis*. This part of the research focused on transferring model experiments involving gallic acid to studies on the edible coating of “Konferencja” pear fruit. The initial goal of the research was to produce a pectin coating containing 50 mM/dm³ of gallic acid. At this concentration, complete inhibition of microbial growth was observed (Table 2). Unfortunately, in practice, this concentration of gallic acid, as well as lower concentrations (at 25 mM/dm³), contribute to impaired coating transparency, ultimately deteriorating the fruit’s visual appearance and making it less attractive to consumers. Laboratory experiments have shown that, among the previously tested concentrations, only the addition of gallic acid at 15 mM/dm³ resulted in a transparent, consistent, durable, and smooth coating.

3.4. The Effect of Coating on Fruit Respiration Rate

In order to assess the effect of the produced pectin coating with the addition of gallic acid on the quality of coated pears, the rate of fruit respiration and ripening was analyzed, and the concentration of released carbon dioxide and ethylene was monitored. Fruit respiration rate is an excellent indicator of metabolic activity and is therefore a useful tool in assessing their potential storage life [36]. The results obtained in this study (and summarized in Figure 3) indicate that the respiration rate of coated pears was significantly ($p < 0.05$) lower than that of uncoated samples. Regardless of the storage time (10 days, 24 ± 1 °C, relative humidity $30 \pm 5\%$), the amount of CO₂ released by coated fruit was approximately 4 mg/kg·h lower compared to uncoated pears. It is well known that edible coatings, including those based on polysaccharides, can modify the atmosphere around fruits or vegetables and, consequently, reduce their respiration rate by selectively permeating gases such as O₂ and CO₂ [37].

While analyzing the effect of the pectin coating, the level of ethylene released was also determined. Ethylene (C₂H₄) is a gaseous plant phytohormone. Its activity is stimulated by auxins. One of ethylene’s functions is to accelerate fruit ripening. To verify the effectiveness of the edible coating in reducing gas release, its concentration was measured after 1 h of holding coated and uncoated fruit in a measurement chamber.

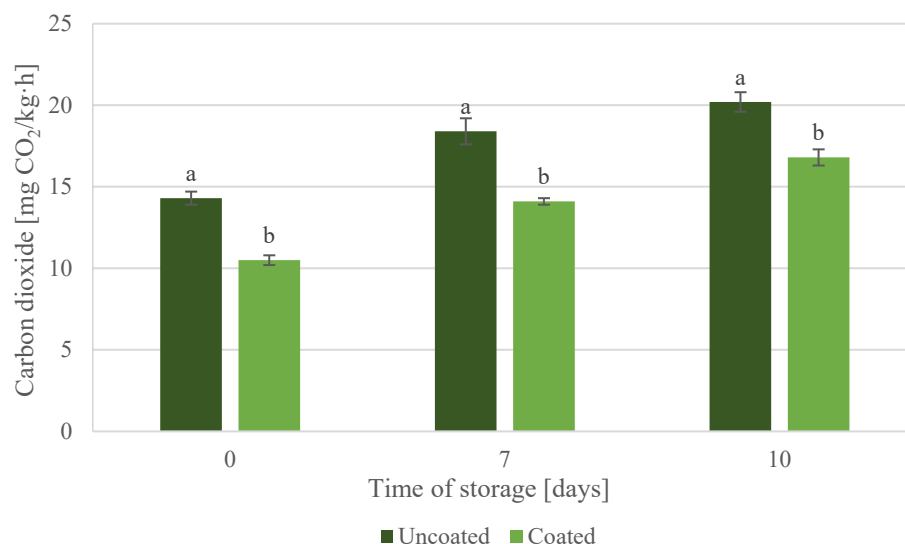


Figure 3. Carbon dioxide production of uncoated and coated pears during storage.

The collected results indicate that the pectin coating with added gallic acid, which covered the pears, inhibits ethylene release. It is natural that, over time, as the fruit ripens, ethylene is released at increasing concentrations, from approximately 20 ppm on the first day of the study to approximately 29 ppm on the tenth day of storage (for uncoated pears). The data in Figure 4 confirm that coating pears with pectin slows ripening, and the level of ethylene released is approximately 6 ppm lower than for uncoated fruit. These results are consistent with those reported by Pham et al. [38], who found that coatings reduce respiration rates and limit ethylene emissions, thereby slowing the aging process. However, it should be remembered that the intensity of these changes depends on the coating composition and the fruit storage conditions.

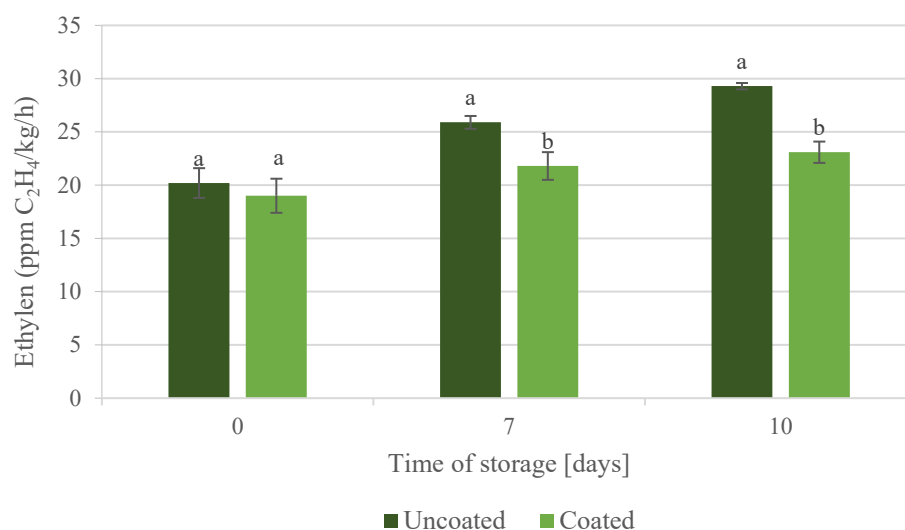


Figure 4. Ethylene production of uncoated and coated pears during storage.

3.5. Effect of Coating on Soluble Solids Content

To verify the effectiveness of the edible coating of pears, water-soluble solids or saccharides were determined in the fruit. Their content was determined by refractometry and reported on the Brix scale. The results of this experiment are presented in Table 3.

Table 3. pH value and total soluble solids of uncoated and coated pears during storage.

Storage Time [days]	pH Value	
	Uncoated	Coated
0	4.67 ± 0.11^a	
7	6.22 ± 0.14^b	4.73 ± 0.23^a
10	6.58 ± 0.17^b	4.91 ± 0.11^b
Storage Time [days]	Total Soluble Solids [°Brix]	
	Uncoated	Coated
0	11.35 ± 0.36^a	
7	12.24 ± 0.46^a	11.76 ± 0.12^a
10	12.94 ± 0.38^a	12.26 ± 0.45^a

Mean values $\pm$ standard deviations. Different superscript letters (^{a–b}) within the same column indicate significant differences between the films ($p < 0.05$).

The results presented in Table 3 indicate that, regardless of the edible coating treatment, the concentration of soluble solids increased slightly over the 10-day storage period—from 11.35 ± 0.36 °Brix to 12.94 ± 0.38 °Brix and to 12.26 ± 0.45 °Brix—for uncoated and coated pears, respectively. This is a consequence of the ripening process, during which polysaccharides are hydrolyzed. Pectin-coated pears showed a slightly slower trend in soluble solids content, but these differences were not statistically significant. The slightly slower rate of increase in °Brix in coated fruit comes from the reduced metabolic activity of these fruits.

3.6. Effect of Coating on the pH Value of Fruit

To assess the impact of the coating on the quality of the coated fruit, changes in the pH of the fruit pulp were also analyzed. Measurements were made using a pH meter. The results summarized in Table 3 show that the pH of the fruit increased in all experimental samples, with a statistically significant increase observed in the uncoated fruit. Over 10 days of storage, the pH of these pulps increased from 4.67 ± 0.11 to 6.58 ± 0.17 . This significant increase in pH during the analyzed storage period of the pears is due to enzymatic activity. In the respiratory metabolism of the fruit, organic acids are utilized in the form of substrates, which translates into a decrease in acidity. By using a protective coating, the respiration-associated metabolism is slowed, resulting in smaller changes in the fruit's acidity. However, it should be noted that the slight increase in pH observed in the coated pears (from 4.67 ± 0.11 to 4.91 ± 0.11) may be due to the influence of gallic acid present in the coating. Phenolic acids added to the coating may partially compensate for the increase in solution pH despite ongoing maturation processes.

3.7. Effect of Coating on Microbial Growth

A crucial study evaluating the pectin coating containing added gallic acid aimed to determine its effectiveness in inhibiting microbial growth on pears. To this end, after 12 days of fruit storage, the total microbial count and yeast and mold counts were determined. The results of this experiment are presented in log CFU/mL and are summarized in Table 4.

The results indicate that a pectin coating containing 15 mM/dm^3 of gallic acid limited the growth of total bacteria, yeasts, and molds. In each case, the coated fruit, after 12 days of storage, showed lower concentrations of these microorganisms—approximately 0.71 log CFU/mL for bacteria and 0.48 log CFU/mL for yeasts and molds, respectively. Therefore, it can be concluded that the addition of phenolic acid to the edible coating serves its intended purpose and acts as a partial microbial inhibitor.

Table 4. The number of microorganisms after 12 days of storage of uncoated and coated pears.

Microorganisms	Uncoated	Coated
	log CFU/mL	
Total bacterial count	2.59 ± 0.04 ^b	1.88 ± 0.22 ^a
Yeast and mold count	2.11 ± 0.09 ^b	1.63 ± 0.10 ^a

Mean values ± standard deviations. Different superscript letters (^{a–b}) within the same column indicate significant differences between the films ($p < 0.05$).

It should be noted that the differences observed between antimicrobial activity in culture media and on the surface of coated pears may be attributed to the significantly greater complexity of the fruit matrix compared with the controlled culture conditions. In culture media, microorganisms are directly exposed to active compounds under homogeneous conditions, which may enhance the inhibitory effects of phenolic acids. In contrast, the pear surface represents a heterogeneous biological environment in which the effectiveness of antimicrobial compounds may be influenced by several factors, including interactions between coating components and fruit tissue, uneven distribution of active substances, limited diffusion, and the availability of nutrients and moisture. Furthermore, the natural physicochemical properties of the fruit surface may affect microbial adaptation and survival, resulting in differences in microbial responses compared with those observed in culture media.

4. Conclusions

Phenolic acids, known for their antioxidant potential, can effectively inhibit microbial growth. This antimicrobial activity depends on the structure of the phenolic acid, the matrix properties, and the specific microorganism. However, when phenolic acids were introduced into a *B. subtilis* culture medium as part of a pectin biofilm, they did not inhibit cell growth; rather, they increased microbial proliferation, resulting in a three- to eight-fold increase in viable *B. subtilis* cells. This is likely due to the strain's amylolytic activity, which degrades the coating's polysaccharide matrix and increases the medium's sugar concentration. The effectiveness of phenolic acids was observed upon their direct addition to the culture medium. Of the six selected phenolic acids—gallic, caffeic, protocatechuic, sinapic, coumaric, and ferulic, added at concentrations ranging from 15 to 75 mM/dm³—antimicrobial activity was observed only for the first two. Complete inhibition of *B. subtilis* cell growth occurred at 50 mM/dm³ for gallic acid and 75 mM/dm³ for caffeic acid. Model studies on coating solutions containing phenolic acids failed to produce a coating suitable for pears that met consumer requirements. While the optimal concentration of gallic acid identified in model studies (50 mM/dm³) resulted in impaired transparency, negatively affecting the fruit's visual quality, a concentration of 15 mM/dm³ produced a transparent, consistent, durable, and smooth coating.

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