TITLE: A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex — Characterization and food application

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A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass oil inclusion complex

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Abstract:	Biopolymer-based films present an ideal matrix for the incorporation of active substances such as antimicrobial agents giving the active packaging in the framework of green chemistry and a step forward in food packaging technology. The chitosan-gelatine active coating has been prepared using lemongrass oil as an antimicrobial compound applying a different approach. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin was used to encapsulate lemongrass oil. The antimicrobial effect was assessed using the dip-coating method on freshly harvested cherry tomatoes artificial contaminated by Pe nicillium aurantiogriseum during 20 days of cold storage. According to the evaluation of the antimicrobial effect of coating formulation on cherry tomato samples was mathematically assessed by predictive kinetic models and digital imaging, and the applied coating formulation was found to be very effective since the development of fungal contamination for active-coated samples was observed for 20 days.
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Cover letter

Tamara Erceg, corresponding author

Dear editors

I am pleased to submit an original research article entitled "**A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex - characterization and food application**" by Tamara Erceg, Olja Šovljanski, Alena Stupar, Jovana Ugarković, Milica Aćimović, Lato Pezo, Ana Tomić, Marina Todosijević, for consideration for publication in *International Journal of Biological macromolecules.*
In this paper, a novel bio-based chitosan/gelatine (CS/Gel) coating with β-cyclodextrin/lemongrass essential oil inclusion complex has been employed for the food application for antimicrobial coating of freshly harvested cherry tomatoes. The used formulation has been shown high antimicrobial and coating effect improving the storage effect of fruit samples for 20 days. Coating of cherry tomato by CS/Gel formulation with β-cyclodextrin/7% lemon grass essential oil inclusion complex results in significantly greater shelf life, considering not only digital imaging results but also mathematically based evaluation of coating antimicrobial effectiveness based on the ratio between contamination area and the surface area of the tomato sample

All figures and tables are the original work of the authors.

Thank you for your consideration!

Sincerely,

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CRediT authorship contribution statement

All authors have a significant intellectual contribution to the work, have read the revised manuscript, and concur with the submission. The article submitted is original work and has not been published elsewhere, either completely, in part, or in another form. All the authors declare no competing interests.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

Tamara Erceg – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Olja Šovljanski – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Alena Stupar – idea, experiment, revising of the manuscript.

Jovana Ugarković – experiments.

Milica Aćimović – experiments, writing, and revising of the manuscript.

Lato Pezo – modeling, calculations, writing of the manuscript.

Ana Tomić – experiments, revising of the manuscript.

Marina Todosijević – experiments, revising of the manuscript.

A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex - characterization and food application

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HIGHLIGHTS

- Framework for obtaining edible active coating system was presented
- Chitosan-gelatine coating has been prepared with β-cyclodextrin - lemongrass oil inclusion complex
- Evaluation of the active role of the coating was done using the dip-coating method
- Cherry tomatoes were coated and artificially contaminated by *P. aurantiogriseum*
- Development of fungal contamination was monitored for 20 days at cold storage

ABSTRACT

Biopolymer-based films present an ideal matrix for the incorporation of active substances such as antimicrobial agents, giving active packaging a framework of *green* chemistry and a step forward in food packaging technology. The chitosan-gelatine active coating has been prepared using lemongrass oil as an antimicrobial compound applying a different approach. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin was used to encapsulate lemongrass oil. The antimicrobial effect was assessed using the dip-coating method on freshly harvested cherry tomatoes artificially contaminated by *Penicillium aurantiogriseum* during 20 days of cold storage. According to the evaluation of the antimicrobial effect of coating formulation on cherry tomato samples, which was mathematically assessed by predictive kinetic models and digital imaging, the applied coating formulation was found to be very effective since the development of fungal contamination for active-coated samples was observed for 20 days.

Keywords: edible coating; chitosan; *Cymbopogon citratus*; antimicrobial coating; cherry tomato; food safety.

1. INTRODUCTION

The increased global demand for fresh natural foods and the high percentage of postharvest loss of fresh fruit and vegetables have imposed a need for the development, production, and consumption of packaging that will ensure the protection of food from physical, chemical, and microbiological contamination, and different environmental influences to maintain food quality and extend shelf life during transport, handling, and storage [1,2]. Massive consumption of conventional polymers in an envelope (wraps, foils) single-use food packaging causes environmental pollution during manufacturing and disposal. Non-degradable petroleum-based polymers persist in nature, leading to waste accumulation, microplastics, and nano-plastic formation, which further pollute water, soil, and food, ending up in living organisms [3]. As a

result of these environmental concerns, there is a greater demand for biodegradable and renewable materials for sustainable, eco-friendly food packaging [4,5]. One of the most studied are biodegradable and edible films and coatings based on biopolymers such as polysaccharides and proteins. Edible coatings/films are a thin layer of edible biopolymers, mostly hydrophilic, intended for coating or wrapping a food product that can be consumed by animals and humans, or easily degraded in the natural environment, without the production of waste or microplastics. Their sustainable nature and the ability to prevent a gas transfer between food and its surrounding medium, enabling food conservation and shelf-life extension, make them a good alternative to conventional polymer-based food packaging wraps and foils [6]. Population growth, market globalization, longer food distribution, increasing environmental awareness of consumers, and modern lifestyles have necessitated the development of functional packaging capable of extending food shelf-life [7]. Edible coatings and films are ideal matrices for the incorporation of active substances such as antimicrobial and antioxidant agents giving the active packaging within a *green* chemistry framework. The selection of an appropriate antimicrobial agent able to prevent or reduce the growth of pathogenic microorganisms that is compatible with a biopolymer matrix enables the use of sustainable active packaging which significantly extends the shelf-life of the food product [8]. The capacity of edible films to substitute conventional polymers and reduce their harmful effects has transformed food packaging. The development of these films with antimicrobial properties has been shown especially important in the packaging of fruits that are subjected to contamination during long-term storage and transportation. Preventing fruit contamination significantly reduces production and distribution costs, increasing the availability of products to a greater number of final consumers. In addition to minimizing the use of non-degradable polymers and their harmful effects on the environment, edible films show advantages as a packaging material by decreasing moisture loss, rates of ripening, and ethylene production, which results in

77 maintaining product quality and storability [9]. They can be formulated as a biopolymer blend,
78 using a combination of polysaccharides and/or proteins [10,11].
79 Chitosan (CS) is a promising material for the preparation of edible coatings/films due to its
80 good properties such as biocompatibility, biodegradability, ability to form films, ability to
81 inhibit moisture, aroma loss, oxygen penetration, and microorganisms' growth [9, 12-14]. The
82 advantages of chitosan also include its low price, inherent antimicrobial nature, and
83 hydrophilicity, which automatically imply the reduced consumption of antimicrobial and
84 antioxidant agents as well as using water instead of organic solvent in the manufacturing of
85 coating films, which contributes to the reduction of production costs. Chitosan is a linear
86 cationic polysaccharide that can be easily obtained by the partial deacetylation of chitin in an
87 alkali medium. Chitin is, along with cellulose, the most abundant biopolymer in nature, which
88 can be extracted simply from biomass such as the exoskeletons of insects, crustaceans, algae,
89 *etc*. [15], which makes this biopolymer economically viable. However, the poor mechanical
90 and barrier properties of chitosan limit its application as self-contained food films and coatings,
91 are reasons why it is combined with other biopolymers such as gelatine (Gel) [16]. Gelatine is
92 a protein obtained from the skin and bones of animals, and as well as chitosan, it is generally
93 recognized as safe and suitable for food packaging [17]. This protein gives edible, transparent,
94 and flexible films [18, 19]. Different approaches have been made to obtain edible films based
95 on chitosan and gelatine with or without additional compounds, which has been confirmed by
96 numerous research papers published in the last decade [20-28]. In recent years, researchers
97 have investigated the development of active food packaging films based on chitosan-gelatine
98 blends with an incorporated essential oil such as garlic essential oil [29], ferulago angulate
99 essential oil [30], ginger essential oil [31], thyme essential oil [32]. Essential oils obtained from
100 plants have been shown to be safe and effective antimicrobial and antioxidant agents. However,
101 these formulations, considering the difference in polarity between biopolymers and essential

oils are mainly prepared in the form of an emulsion, which implies the addition of surfactants to achieve the miscibility of chitosan and gelatine with non-polar essential oils. On the other hand, the tomato, that was selected as a fruit of interest is one of the most economically important plants in the world. The area under tomatoes occupies 4.6 million ha, with a yield of about 130 million tons per year, of which about 88 million tons of fruit are consumed fresh and about 42 million tons are processed. The yearly growth rate for the cherry type of tomato is 36.33% [33]. However, tomatoes are easily contaminated by fungi and are determined to be highly sensitive fruits, while fungal contamination can affect public health worldwide [34]. Therefore, protection and prolongation of the shelf life of raw tomatoes are very important and have led to different approaches and applications for alternative types of storage processes and packaging [10].

The main goal of this work is to obtain active packaging based on a chitosan-gelatine coating system with the addition of lemongrass essential oil as an active antimicrobial compound. Step forward in this paper has been made using cyclodextrins instead of surfactants [10,35], and, therefore, β-cyclodextrin/lemongrass essential oil inclusion complex was used for the final formulation of the mentioned system. A combination of chitosan and gelatine has been made in order to overcome the shortcomings of neat chitosan in terms of mechanical and barrier properties. Except for a comprehensive characterization of the coatings (which included FTIR analysis, thickness measurements, moisture content, total soluble matter, water vapor transmission rate analysis, tensile strength, and elongation at break of biopolymer films, as well as thermal gravimetric analysis), this study involved successive testing of the antimicrobial activity of packaging and, for the first time, modeling of the obtained results using advanced mathematical tools. Finally, application in food packaging was performed, implying that chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex can significantly improve the shelf-life of cherry tomatoes. Considering the high

antimicrobial potential of lemongrass oil, proven by the preliminary assessment, the additional focus of this paper was to optimize the edible film composition in terms of mechanical properties and adequately releasing of essential oil from the inclusion complex to the surface of the food product.

2. EXPERIMENTAL PART

2.1. Materials

Chitosan (M_w = 100000-300000 g/mol) and glacial acetic acid were supplied from Acros Organic B.V.B.A. (USA). Gelatine and glycerol (extra pure) were procured from CENTROHEM (Serbia), while β-cyclodextrin (β-CD) was from Sigma Aldrich (USA). Lemongrass (*Cymbopogon citratus*) essential oil was made from greenhouse-originated aboveground plants of lemon grass plants. The preparation of essential oil was done in July 2021, during which the selected parts were dried in shade and subjected to steam distillation. The distillation process involved the following steps: placement of 100 kg of dry plant material in a vessel (V=0.8 m^3) routing upwards through a plumbing system, and steaming for 20 minutes. The condensed vapor was collected in the Florentine flask, and the distillation process was finished after 3 hours. The obtained essential oil was decanted from the water phase, dried over sodium sulfate, and stored in dark glass bottles at 4 °C.

2.2. Analysis of Volatile Compounds in lemongrass essential oil

The obtained lemongrass essential oil used for CS/Gel formulation was subjected to GC-MS analysis with an Agilent 7890A apparatus equipped with a 5975 C MSD, FID, and an HP-5MS fused-silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The carrier gas was helium, and its inlet pressure was 19.6 psi and linear velocity of 1 mL min^{-1} at 210 °C. The injector temperature was set to 250°C, the injection volume was 1 μL, and the split ratio was 10:1. MS detection was carried out under source temperature conditions of 230 °C and an

interface temperature of 315 °C. The EI mode was set at electron energy, 70 eV with a mass scan range of 40-600 amu. The temperature was programmed from 60 °C to 300 °C at a rate of 3 °C min^{-1}. The components were identified based on their linear retention index relative to C8-C32 n-alkanes, in comparison with data reported in the literature (Adams4 and NIST17 databases). The relative percentage of the oil constituents was expressed as percentages by FID peak area normalization.

2.3. Preparation of edible coating

Chitosan (2.5% w/w) was added to a 5% (v/v) acetic acid aqueous solution and stirred on a magnetic stirrer at 70 °C for 90 min. The same weight of gelatine was added to distilled water at 60 °C and stirred until complete dissolution. The chitosan solution was mixed with the same volume of the gelatine solution for 90 min at 60 °C, after the addition of glycerol in the amount of 30 wt% per total biopolymers weight. The problem of differences in polarity between obtained blend and essential oil was solved by incorporation of β-CD -lemongrass oil inclusion complex in the reaction mixture.

The inclusion complex between β-CD and essential lemongrass oil (β-CD/LG) was prepared by the modified precipitation method described by Kringel et al. [36]. The amount of 2 g of β-CD was dissolved in 50 ml of distilled water at 35 ± 1 °C, by homogenization at a magnetic stirrer. After 3 hours of stirring, 1.6 g of lemongrass oil was slowly added to the reaction mixture. After cooling at room temperature, the mixture was transferred to a refrigerator and left there for 12 hours at 5 °C. The precipitated β-CD/lemongrass oil inclusion complex material was vacuum filtered, rinsed with absolute ethanol, and dried at 40 °C until constant weight was obtained. Different amounts of β-CD/lemongrass oil inclusion complex (3, 5, and 7 wt% per total biopolymers weight) were added to the CS/Gel solution and stirred at 40 °C for 2 h. Different amounts of β-CD/lemongrass oil inclusion complex (3, 5, and 7 wt% per total

biopolymers weight) were added to the CS/Gel solution and stirred at 40 °C for 2 h. The film-forming mixtures with different amounts of β-CD/lemongrass oil inclusion complex were poured into Teflon-coated Petri dishes and dried at 40 °C until a constant weight. One sample, prepared without the addition of complex has served as control.

2.4. Characterisation of coating

2.4.1. Fourier transform infrared spectroscopy (FTIR) analysis

The chemical structure of the obtained samples was investigated by Fourier transform infrared spectroscopy (IRAffinity-1S, Shimadzu). Samples were scanned 24 times with a resolution setting of 16 cm^{-1}. Data were collected in the range of 400 – 4000 cm^{-2} using the Attenuated Total Reflection (ATR) method.

2.4.2. Thickness measurements

The thickness of films was measured using a micrometer Digico 1, with an accuracy of 0.001 mm (Tesa, Renens, Switzerland), at 9 positions, using three samples, and the average value was used.

2.4.3. Moisture content, total soluble matter analysis

For analyzing moisture content (MC) and total soluble matter (TSM), coating samples were cut into squares (20 x 20 mm), put in aluminium dishes, weighted, and dried at 105 ± 2 °C until they reached a constant weight. MC was determined as a percentage of the initial film weight lost during drying. The results were reported as an average of four independent measurements. TSM was determined as a percentage of coating dry matter solubilized for 24 h in 50 ml of distilled water at room temperature, with periodic stirring. Samples were taken out after 24 hours and dried at 105 ± 2 °C until constant weight (m''). The TSM value was determined using Equation 1.

$$TSM = \frac{m' - m''}{m'} \cdot 100\% \quad (1)$$

where *m'* the weight of the sample after drying at 105 ± 2 °C before immersing in distilled water.

2.4.4. Barrier properties - water vapour transmission rate analysis

The water vapor transmission rate (WVTR) for biopolymer films was determined according to the ISO 2528 standard [37] at a temperature of 25 ± 1 °C and relative humidity of 90 ± 2 %. The results were averaged on three independent measurements.

2.4.5. Mechanical properties analysis

Tensile strength and percentage elongation at the break of polymer coatings were investigated using the Instron Universal Testing Machine (Model 4301, Instron Engineering Corp., Canton, MA). The samples were prepared and tested according to the ASTM standard D882-18. Films were shaped in rectangular spaces with dimensions of 80 x 15 mm. The tests were carried out at a temperature of 23 ± 2 °C and relative humidity of 50%, with the initial grip separation set at 50 mm and the crosshead speed set at 50 mm/min. The results were averaged on eight independent measurements, and the extreme values were excluded.

2.4.6. Thermal gravimetric analysis (TGA)

The thermal properties of polymer coatings were investigated using the LECO 701 Thermogravimetric Analyzer (LECO, Germany). Measurements were carried out under the flow of air (50 ml/min) in the temperature range of 25 to 800 °C at a heating rate of 10 °C/min.

2.5. Evaluation of coating antimicrobial effectiveness

2.5.1. Antimicrobial activity of the chitosan-gelatine coating formulations

The referent fungi strain *Penicillium aurantiogriseum* ATCC 16025 was selected for this study. An antimicrobial effect of 7% LG and CS/Gel formulations (control and 7% LG addition) against the strain was done using the disk diffusion method described in detail by Riabov et al. [38]. The used volume of CA/PCL-diol formulations was 10 µL per sterile cellulose disk (analysis was done in triplicate). The sterile distilled water was a negative control, while 3% cycloheximide was a positive control. Additionally, a time-kill kinetics study was done for the evaluation of the fungicide-dynamic pathway of 7% LG, CS/Gel formulations (control and 7% LG addition) against tested strain using the method described by Aćimović et al. [39]. The non-treated, inoculated nutrient medium was a control in the test. For mathematical analysis of the obtained results, the four-parameter sigmoidal model was used, while the data were presented as an S-shaped curve model (Eq. 2).

$$y(t) = d + \frac{a-d}{1+\left(\frac{t}{c}\right)^{b}} \quad (2)$$

The spore concentration (y(t)) during contact time with the extract was the targeted output, while the regression coefficients in Eq. 2 can be described as *a* - minimum of the experimentally obtained values (t = 0), *d* - the maximally obtained value ($t = \infty$), *c* - the inflection point (the point between *a* and *d*, and *b* - the Hill's slope (the steepness of the inflection point c).

2.5.2. Cherry tomato samples

The cherry tomatoes (*Solanum Lycopersicum* var. *cerasiforme*) were obtained from a local organic manufacturer near Novi Sad, Serbia, in May 2022. The selection of the samples was done based on the mass, i.e. each whole mature, fresh, and undamaged fruit had a mass of 15

g ± 0.2 g. After harvesting and selection, fruits were washed with water and sterile distilled water and dried in a laminar chamber. All samples were left with a petiole to make it easier to manipulate the samples during coating and storage. The contamination of the samples was done in minimal manipulation time, and all samples are subjected to immediate storage on harvest day.

2.5.3. Contamination suspension preparation and artificial contamination of tomatoes

The strain was incubated on Sabouraud Dextrose Agar (SDA, HiMedia, Mumbai, India) for 120 hours at 25 °C before being used for antimicrobial testing of CS/Gel formulations, and the initial suspension for contamination was prepared in sterile distilled water with a targeted concentration of 6 log CFU/mL. In each tomato, the sample was injected into one spot with 10 μL of the prepared suspension in such a way that a petiole is turned to the right so that the photographing of the fruit is reproducible in the same way during the storage period. Each scar site sample contained approximately 4 log CFU of mold spores.

2.5.4. Preparation of coating for tomatoes samples

The coating solution was freshly prepared using the previously described protocol and applied to tomatoes by the dip-coating method. Briefly, the samples are directly and individually added to the prepared coating solution and then dried in a laminar chamber (this process did not affect the texture or quality of the sample). In this way, three groups of samples were prepared: artificial contaminated control (samples without coating), artificially contaminated sample with CS/Gel control coating, and artificially contaminated sample with CS/Gel 7% β-CD/LG coating. The tomato samples in individual boxes were stored at 8 °C in a semi-vertical refrigerated display case (model Aruba, Frigo žika, Ruma, Serbia) with sliding doors and lights for 20 days. The experiments were performed in three independent replicates.

2.5.5. Monitoring of the contamination development

The prepared groups of tomatoes were monitored every five days during the cold storage period. Each sample image was recorded with a common digital camera, which captured a region of roughly Ø100 mm (the macro function was used, for a more clear photograph). The obtained pictures were used to determine the contaminated area of the sample using ImageJ 1.53r (used for masking the contaminated area) and Inkscape 1.0 (applied to measure the linear distances on bitmap images and to evaluate the area between lines). The surface area of the tomato was approximated using Knud Thompson's formula, which is presented in Eq (3.) [40].

$$SA = 4 \cdot \pi \cdot \left(\frac{a^p \cdot b^p + a^p \cdot c^p + b^p \cdot c^p}{3} \right)^{\frac{1}{p}} \quad (3)$$

where: *a*, *b*, and *c* are horizontal, vertical, and conjugate radius, while $p \approx 1.6075$.

For evaluation of coating effectiveness (CE), measuring the ratio between the contaminated and the surface area of tomato samples at time *t*, was conducted using Eq. (4)

$$CE(t) = \frac{A_{contaminated}(t)}{SA(t)}. \quad (4)$$

For mathematical analysis of the obtained results, the two-parameter exponential model was used, while the data were presented as an exponential model. The coating effectiveness *CE*(*t*) at time *t* was the targeted output, while *a* and *b* were the regression coefficients.

$$CE(t) = a \cdot \exp(b \cdot t) \quad (5)$$

2.5.6. The accuracy of the model

A numerical verification of the models obtained in the previous step was tested using the coefficient of determination (r^2), reduced chi-squared (χ^2), mean bias error (MBE), root mean square error (RMSE), and mean percentage error (MPE) [41, 42].

$$\chi^2 = \frac{\sum_{i=1}^{N} (x_{\exp,i} - x_{pre,i})^2}{N - n}, \quad (6)$$

290 $$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})^2 \right]^{1/2}, \quad (7)$$

291 $$MBE = \frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i}), \quad (8)$$

292 $$MPE = \frac{100}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left| x_{pre,i} - x_{\exp,i} \right|}{x_{\exp,i}} \right), \quad (9)$$

293 where: $x_{exp,i}$ stands for the experimental values and $x_{pre,i}$ are the predicted values calculated by
294 the model, N and n are the number of observations and constants, respectively.

295 **2. RESULTS AND DISCUSSION**

296 The composite coating was prepared by the creation of a chitosan and gelatine polyelectrolyte
297 complex. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin
298 has been used for the encapsulation of lemongrass oil. Cyclodextrins are cyclic molecules
299 whose inner cavity has a hydrophobic nature, which enables the incorporation of hydrophobic
300 compounds, while the outer surface possesses a hydrophilic nature which makes them soluble
301 in a water medium. A step forward has been made in this study by using cyclodextrins instead
302 of surfactants such as Tween 80 [10, 35]. Cyclodextrins are biocompatible biomolecules used
303 in several times lower amounts than surfactants. In the mentioned papers, the used surfactants
304 are synthetic, can be irritable and their dispersing requires the incorporation of mechanical
305 energy in a biopolymers solution. That implies the use of a high-speed homogenizer, while the
306 addition of cyclodextrins does not require special equipment. The optimal
307 chitosan/gelatine/glycerol ratio was found by performing a series of mechanical tests.
308 Lemongrass oil was used as an antimicrobial compound due to its expressed antimicrobial
309 effect, especially against fungi that contaminate cherry tomatoes. The strong antimicrobial
310 effect of lemongrass essential oil enables the use of its low concentration, which results in
311 economic benefits by avoiding deterioration of the sensor properties of the fruit.

Antifungal activity was determined by analyzing the chemical composition of the essential oil and confirmed by preliminary antimicrobial testing. The consumption of fresh cherry tomatoes has gradually increased in the last two decades, and keeping this fruit healthy and fresh is very challenging considering its perishable nature. According to the authors` knowledge, the preparation and characterization of chitosan/gelatine edible coatings with β-cyclodextrin-lemongrass oil inclusion complex using a simple two-step method have not been studied in the available literature, as well as the determination of the kinetics models for fungal growth and antimicrobial effectiveness of coating based on the ratio between contamination area and the surface area of the cherry tomato sample. Similar approaches in the preparation of active edible films have been applied but using different coating compositions, a higher amount of inclusion complex, and a higher amount of antimicrobial compounds/essential oil, which have resulted in a lower inhibition zone [43]. The model of fungal growth enables the prediction of the level of contamination at certain time intervals. Considering the high antimicrobial potential of lemongrass oil, proven by the preliminary assessment, the focus of this paper was to optimize the active edible film composition in terms of mechanical and antimicrobial properties, providing an effective coating that is capable to prolong the shelf-life of fresh cherry tomatoes. Therefore, the experimental setup can be divided into three main parts: (i) Characterization of the active compound - chemical characterization of selected essential oil; (ii) Characterization of the CS/Gel film properties; (iii) Evaluation of coating antimicrobial effectiveness in *in vitro* experiments and in situ application on food samples.

3.1. Lemongrass essential oil characterization

Table S1 (Supporting Information) shows the chemical analysis of the lemongrass essential oil obtained through the hydrodistillation process. Identification of the components was done according to their linear retention indices (RI), and their equivalence with mass spectral libraries (Wiley and NIST). The relative abundance of each detected compound was calculated

as the percentage area of each peak (only identified compounds are shown). The most abundant compounds in the sample (22 compounds, comprising 99.4%) were geranial (40.8%), neral (31.9%), and myrcene (17.4%). As two dominant compounds that together represent citral, neral (cis-citral) and geranial (trans-citral) have previously been studied as effective agents against different fungi *in vitro*, while the strongest biocide effect of geranial was observed compared with neral as individual substances [44].

According to literature references data (Table S2, Supporting information) on the chemical composition of lemongrass essential oil, as well as cluster analysis performed using this data for the construction an unrooted phylogenetic tree (Figure S1), it is possible to conclude that lemongrass essential oils can be relatedness based on the range of the most dominant compound in all listed essential oil samples - gerinal (Table S1).

Lemongrass essential oil samples can be divided into five subgroups based on chemical composition: with a very high gerinal content (46.6-48.7%) with four samples [45-48], high gerinal content (41.3-44.5%) with five samples [49-51], medium gerinal content (33.9-40.8%) with five samples including the sample from this study [52-54], low gerinal content (33.3 %) with only one sample [55], and very low gerinal content (18.8-32.9%) with four samples [56-59] (Figure S1, Supporting information).

The sample in this study obtained in Serbia is similar to samples obtained from different geographical areas (Vietnam, Sudan, and Egypt) with the content of gerinal in the range between 33.9 and 40.8%. Additionally, this indicates that chemical composition has been attributed to this factor, but also climatic conditions, time and year of harvest, etc., which are in correlation with the hypothesis of Boukhatem et al. [60]. In summary, all the listed essential oil samples in Table S2 have a dominant citral complex, but with various proportions of geranial and neral. Additionally, different percentage of the third dominant compound, i.e.,

myrcene, can be observed in the range between 2.3 and 17.4, with the highest content percentage obtained in this study.

3.2. Characterisation of the CS/Gel film

FTIR

The FTIR spectra of chitosan, gelatine, and their blend are presented in Figure 1. A broad band from 3600 to 3000 cm^{-1} with two peaks at 3380 and 3226 cm^{-1} corresponds to the asymmetrical and symmetrical N-H stretching vibrations overlapping with OH stretching in the FTIR spectrum of pure chitosan (ν_{NH} and ν_{OH}). Two peaks at 2932 and 2862 cm^{-1} are assigned to -CH_2 stretching vibrations (ν_{CH2}). The weak peak at 1705 cm^{-1} corresponds to C=O stretching vibrations ($\nu_{C=O}$) of the residual acetyl group and the band at 1638 cm^{-1} is assigned to amide I. A band at 1539 cm^{-1} is attributed to NH_3^+ bending and C-N stretching (ν_{NH3+} and ν_{C-N}). Bands at 1407 and 1380 cm^{-1} are attributed to CH_2 and C-N bending (δ_{CH2} and δ_{C-N}). A weak peak observed at 1264 cm^{-1} corresponds to the CH_2OH group in the side chain, while a small peak at 1155 cm^{-1} corresponds to C-O-C glycosidic bond. Two peaks at 1069 and 1020 cm^{-1} correspond to the C-C-O stretching and C-O-H bending (ν_{C-C-O} and δ_{C-O-H}). A small peak at 894 cm^{-1} confirms the presence of 1,4-β–glycosidic bond [61]. The peak at 657 cm^{-1} is assigned to O-H bending (δ_{OH}) out of the plane. The FTIR spectrum of pure gelatin shows similar absorption bands as a chitosan spectrum. A broad absorption band between 3600 and 3000 cm^{-1} with a peak at 3303 cm^{-1} is attributed to the overlapping of N-H and OH stretching vibrations of amino acids in gelatine. A weak peak at 3070 cm^{-1} indicates the presence of an aromatic ring originating from a constitutive unit of gelatine–amino acid. Two weak peaks at 2929 and 2854 cm^{-1} correspond to the C-H stretching vibrations. The band at 1631 cm^{-1} (amide-I) appears due to the C=O stretching vibration [62]. The peak at 1543 cm^{-1} is assigned to NH_2 bending and C-N stretching, while the peak at 1451 cm^{-1} corresponds to the stretching of COO^- group. The

bands at 1404 and 1338 cm^{-1} correspond to the CH_2 and C-H bending. Small peaks at 1283 and 1205 cm^{-1} are assigned to O-C stretching from a carboxylic group, while the peak at 1242 cm^{-1} corresponds to C-N stretching from amine. The absorption band at 1081 cm^{-1} corresponds to C-C-O stretching, while at 1030 cm^{-1} is assigned to C-O-H bending. A broad peak with the center at 657 cm^{-1} corresponds to NH_2 bending and OH bending. The spectrum of the chitosan/gelatin blend containing plasticizer glycerol retains the pattern of all compounds in the composition.

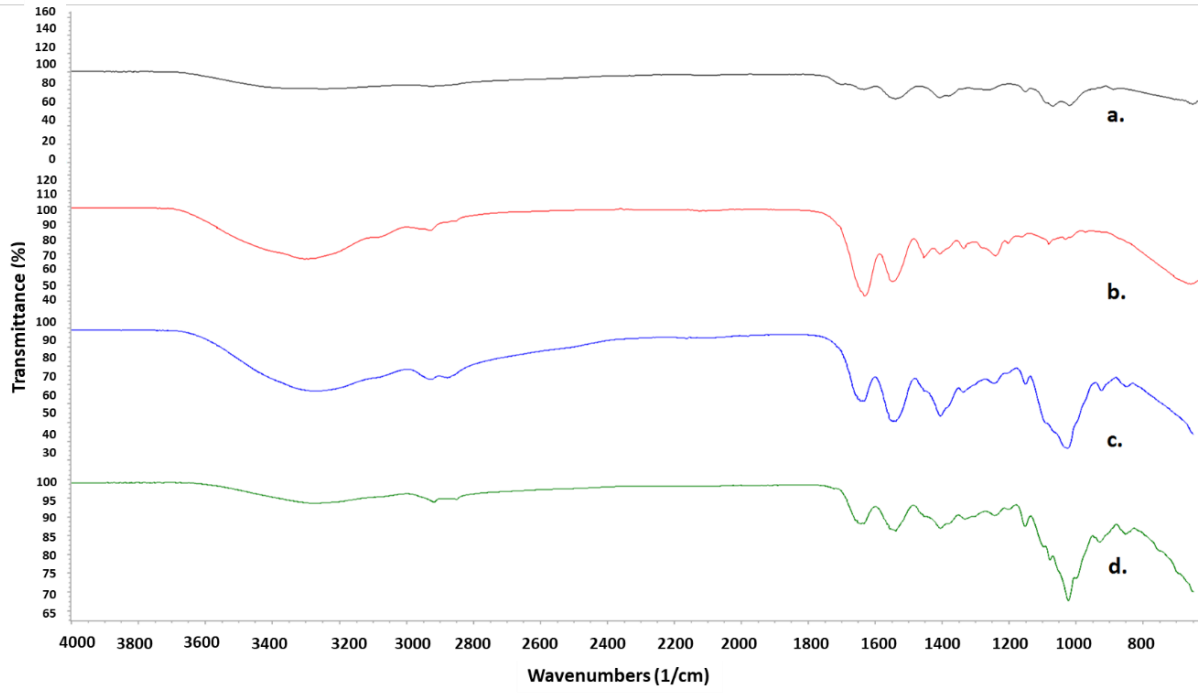


Figure 1. FTIR spectra of: chitosan, b) gelatin, c) chitosan/gelatine blend, d) chitosan/gelatine blend with 5% of β-CD/lemongrass oil.

A broad peak between 3600 and 3000 cm^{-1} with the center at 3372 cm^{-1} in the IR spectra of blends corresponds to OH stretching from chitosan, gelatin, and glycerol, and NH stretching from chitosan, gelatin, and glycerol. Two peaks at 2928 and 2879 cm^{-1}, which correspond to the stretching of CH_2 groups, are presented in the structures of both polymers and the plasticizer

glycerol. The band at 1635 cm^{-1} is assigned to the C=O stretching vibration of amide I. A band at 1538 cm^{-1} is assigned to the N-H bending from the amino group and their protonated form, as well as to the C-N stretching. Peaks at 1405 and 1340 cm^{-1} are attributed to CH_2 and C-H bending. C-N stretching from amine (1245 cm^{-1}) originates from gelatine and appears as a weaker peak than the corresponding peak in the IR spectrum of gelatine. This peak is not visible in the IR spectrum of chitosan. The presence of a glycosidic bond is confirmed by peaks at 1155, 1096 and 860 cm^{-1} in the FTIR spectrum of the blend. Peaks at 1069 and 1026 cm^{-1} are assigned to the C-C-O stretching and C-O-H bending, while the peak at 926 cm^{-1} visible only in the FTIR spectrum of the blend is attributed to the C-O stretching from glycerol. The peak that corresponds to C-O-H bending is more intense in comparison to the same peak in the spectra of neat biopolymers, due to the formation of hydrogen bonds between biopolymers in the blend. FTIR spectrum of blend with β-CD/lemongrass oil inclusion complex in comparison to the spectrum of a control sample (without inclusion complex) has a broader and less intense peak between 3600 and 3000 cm^{-1} and three peaks at 2917, 2977, and 2850 cm^{-1}, which correspond to C-H stretching from chitosan, gelatin, glycerol, and β – cyclodextrin. A peak at 1452 cm^{-1} corresponds to the C=C stretching, which originates from lemongrass oil compounds. This peak appears at 1442 cm^{-1} in the spectrum of neat lemongrass oil. Shifting is a result of the formation of an inclusion complex and its incorporation into the blend.

Moisture content, total soluble matter, and water vapour transmission rate

The results of thickness measurements, MC, TSM, and WVTR are presented in Table 1. The moisture content decreases with an increase in the amount of inclusion complex in blend composition, due to the interaction of hydroxyl groups of β-CD with chitosan and gelatine molecules, via hydrogen bonding, which means that the hydroxyl groups are capable to bond

water molecules and contribute to increasing of MC value are occupied [63]. Moreover, lemongrass essential oil possesses hydrophobic properties, which additionally contribute to reducing the MC content value. The TSM of CS/Gel-based films rises with an increase in the amount of β-CD/LG complex in film composition. Gelatine and glycerol are totally soluble in water, and chitosan solubility demands acidic conditions. However, the inclusion complex which forms hydrogen bonds with glycerol and chitosan can be easily replaced by the water, resulting in an increase in the TSM value [43]. WVTR value for neat chitosan is higher than for the blend, due to the formation of secondary interactions between biopolymers in the blend composition that result in better barrier properties. This confirms the hypothesis about improving chitosan film barrier properties by the formation of blends with gelatine. The addition of complex in a lower amount in blend composition (3 and 5 wt%) results in slight increases in WVTR value, while the addition of inclusion complex in the amount of 7 wt% leads to a greater increase in WVTR. It could be due to a decrease in compatibility between blend compounds (CS, Gel) and the β -CD/LG inclusion complex at higher concentrations of the complex. Decreasing the compatibility leads to the compounds' segregations, which results in easier water vapor permeability [43]. In comparison to the films with a similar composition prepared using the solution-casting method, the WVTR values in this study are higher than those in the investigation of Xu and co-authors [28], but the procedure for the preparation is simpler, avoiding the use of synthetic surfactants as was the case in previous studies [30-32]. However, in comparison to the neat chitosan film, blends possess WVTR values that are increased by 5–9 %. This creates the platform for future improvements in this field, considering the fact that transferring water between the product and its surroundings directly affects the shelf-life of products.

Table 1. Moisture content (MC), total soluble matter (TSM), and water vapour permeability (WVP) with standard deviation values for CS/Gel coating containing different amounts of β-CD/LG inclusion complex

Film	Thickness (μm)	MC (%)	TSM (%)	WVTR (g/m^2h)
CS	0.34 ± 0.2	19.23 ± 1.19	23.12 ± 0.38	99.45 ± 1.9
CS/Gel neat (Control)	0.33 ± 0.02	17.57 ± 1.16	36.53 ± 0.34	90.72 ± 2.3
CS/Gel 3% β-CD/LG	0.34 ± 0.02	16.27± 1.2	37.87 ± 0.5	91.63 ± 3.1
CS/Gel 5% β-CD/LG	0.34 ± 0.02	15.82 ± 1.12	38.35 ± 0.43	92.46 ± 2.7
CS/Gel 7% β-CD/LG	0.34 ± 0.02	14.86 ± 1.23	39.34 ± 0.48	94.59 ± 2.9

Thickness and mechanical properties

The results of the determination of TS and EB are given in Table 2. Blends with gelatine possess better mechanical properties in comparison with a neat CS, which confirms the hypothesis that the formation of polyelectrolyte complexes with gelatine will result in improved mechanical properties. Increasing the content of the inclusion complex in a polymer blend results in an improvement of the mechanical properties – TS and EB, which can be assigned to the secondary interactions (hydrogen bonding, electrostatic forces) between biopolymers and inclusion complex [64]. The formation of a polyelectrolyte complex between CS and Gel results in an improvement of TS by 20.3 % and EB by 22 %, in comparison to the neat chitosan, which is significant. Addition of inclusion complex results in improving TS values for 6.3 to 54.6 % and EB values for 4.9 to 18.3% which present a significant improvement. Incorporation of EOs in biopolymer composition using surfactants leads to a decrease in TS values while the improvement of EB values is much lower in comparison to our study [30, 32] or very similar [31].

Table 2. Tensile strength (TS), and elongation at break (EB) with standard deviation values for CS/Gel coating containing different amounts of β-CD/LG inclusion complex

Film	Tensile strength (TS)	Elongation at break (%)
CS	10.34 ± 0.65	35.34 ± 3.1
CS/Gel neat (Control)	12.44 ± 0.78	43.12 ± 4.5
CS/Gel 3% β-CD/LG	13.23 ± 0.69	45.23 ± 4.1
CS/Gel 5% β-CD/LG	15.45 ± 0.82	47.34 ± 3.9
CS/Gel 7% β-CD/LG	19.23 ± 0.75	51.01 ± 4.2

TGA

Figure 2 shows the TGA curves of a neat blend (control sample) and composite CS/Gel film with β-CD/LG inclusion complex. The results obtained from the derivative thermogravimetric curve (DTG) are summarized in Table 3. The degradation of films is carried out in four steps. Investigated samples expose a very similar degradation pattern - almost identical. The film with inclusion complex has only several degrees higher T5% value, the temperatures of the maximal degradation rate (Table 3), and upper limits of the thermal degradation stage, which is the consequence of the existence of secondary forces between biopolymers and cyclodextrin inclusion complex. The first step, up to 232 °C, with a weight loss of up to 25 % in the thermogram of CS/Gel blend corresponds to the water evaporation, glycerol degradation, and disintegration of smaller side groups. The second, main degradation step (up to 371 °C), with a weight loss of up to 64 %, corresponds to the gelatine decarboxylation, breaking of amide linkages in gelatine, and glycosidic linkages in chitosan [65, 66]. The weight loss in the third (up to 464 °C) and fourth stage (up to 729 °C) are attributed to the complete decomposition of gelatine and chitosan backbone and continuous up to 2.3 % of the residual weight. The first step, up to 234 °C, with a weight loss of up to 23 % in the thermogram of CS/Gel β-CD/LG film corresponds to the loss of the water, glycerol, lemongrass oil, and small side groups disintegration. The second step, up to 373 °C (weight loss of up to 66%) is attributed to the

gelatine decarboxylation, the breaking of amide linkages in gelatine and glycosidic linkages in chitosan, as well as the decomposition of cyclodextrin, which continues in the third step (up to 466 °C) [67]. The fourth stage (up to 800 °C) corresponds to the complete degradation and sample carbonation until 0.12 % of the residual mass.

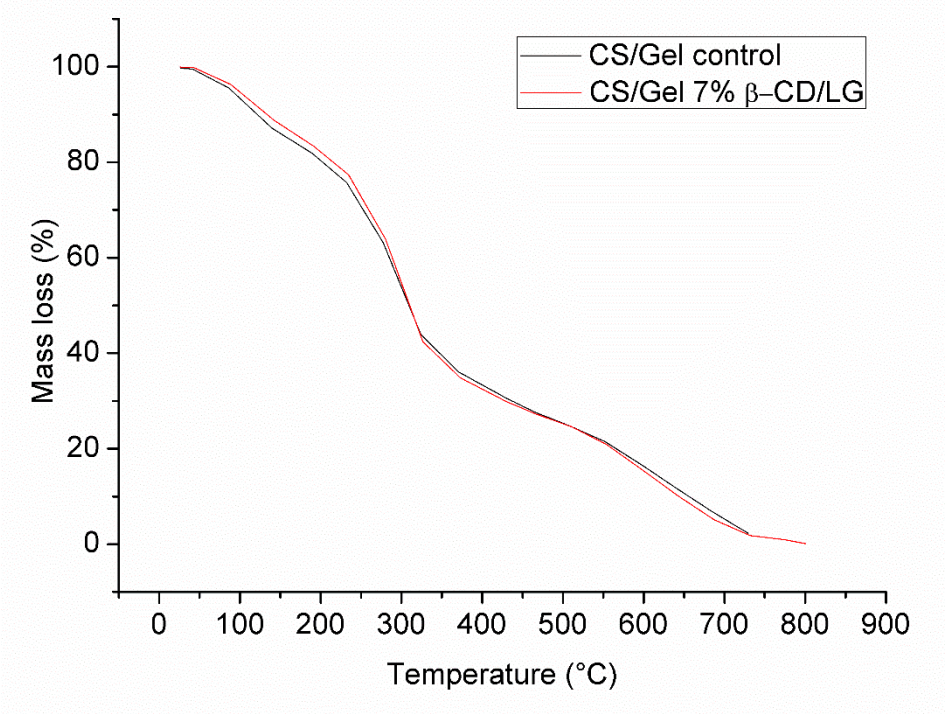


Figure 2. TGA thermograms of control film and film with β-CD/LG inclusion complex.

Table 3. T5% and DTG peak maxima values for control film and film with β-CD/LG inclusion complex

Film	$T_{5\%}$ (°C)	T_{dmaxI} (°C)	T_{dmaxII} (°C)	$T_{dmaxIII}$ (°C)	T_{dmaxIV} (°C)	Residual mass
CS/Gel neat (Control)	86	139	324	429	641	2.3
CS/Gel 5% β-CD/LG	88	142	326	430	643	0.12

3.3. Evaluation of coating antimicrobial effectiveness

Considering the potentiality of lemongrass essential oil as a source of different phytochemicals (Table S1), its antimicrobial activity was tested against a *Penicillium* representative (*P. aurantiogriseum* ATCC 16025) due to the fact that fruits and vegetables are highly susceptible to *Penicillium*-related contamination in the field, during transportation, processing, and storage [68]. The obtained results of antimicrobial screening of 7% LG, as well as CS/Gel control and 7% LG samples, are presented in Table 4 and Figure S2 in Supporting Information (inhibition zone for 7% LG essential oil, CS/Gel control, CS/Gel 7% LG, 3% cycloheximide, and distilled water). Being a good source of antimicrobials, the presence of 7% LG as an individual compound and in the CS/Gel formulation showed a significant fungicide effect compared to the control sample. Lemongrass essential oil's antifungal activity is correlated with its chemical composition (Table S1) and the fact that citral complexes (geranial and neral) have been linked to the inhibition of mycelial growth and spore germination of various *Penicillium strains* [69-72].

Table 4. Assessment of the antimicrobial effect

Sample	**The inhibition zone* (mm) against *Penicillium aurantiogriseum* ATCC 16025**	**Interpretation of the results** (7-22 mm - low activity 22-26 mm - medium activity > 26 mm - high activity)
7% LG essential oil	40.00± 0.00	high activity
CS/Gel control	13.00 ± 0.00	low activity
CS/Gel 7% LG	34.00 ± 1.00	high activity
3% cycloheximide[1]	27.33 ± 0.56	high activity
Distilled water[2]	6.00± 0.00[3]	not exist

* Mean value diameter of the zone including disc (6 mm) ± standard deviation; [1]fungicide -positive control; [2] negative control; [3] the obtained value represents the diameter of the disc (6 mm)

On the other hand, the existence of an inhibition zone in CS/Gel control indicates the potential of chitosan as an antimicrobial agent, but not to the extent that it has a biocide effect. This is in correlation with the previous literature, which deals with chitosan-based films, and the fact that it is always necessary to use a phytochemical-rich source together with a stable concentration

of chitosan in coating formulation [20-23, 73-77]. A similar approach in another study based on using of β-cyclodextrins for encapsulation of different essential oils - carvacrol, trans-cinnamaldehyde, and eugenol has resulted in lower antimicrobial activity against investigated food pathogens, although they were used in higher concentrations [78]. The *in vitro* antimicrobial potential of essential oil and CS/Gel formulations can be further clarified by the time-kill or pharmacodynamic kinetic monitoring. In this way, *in vitro* examination of the antimicrobial substance can be measured in view of the antimicrobial activity path as a function of contact time between sensitive microorganisms and targeted concentrations of antimicrobial agent [79]. Figure 1 shows the kinetics models that were developed.

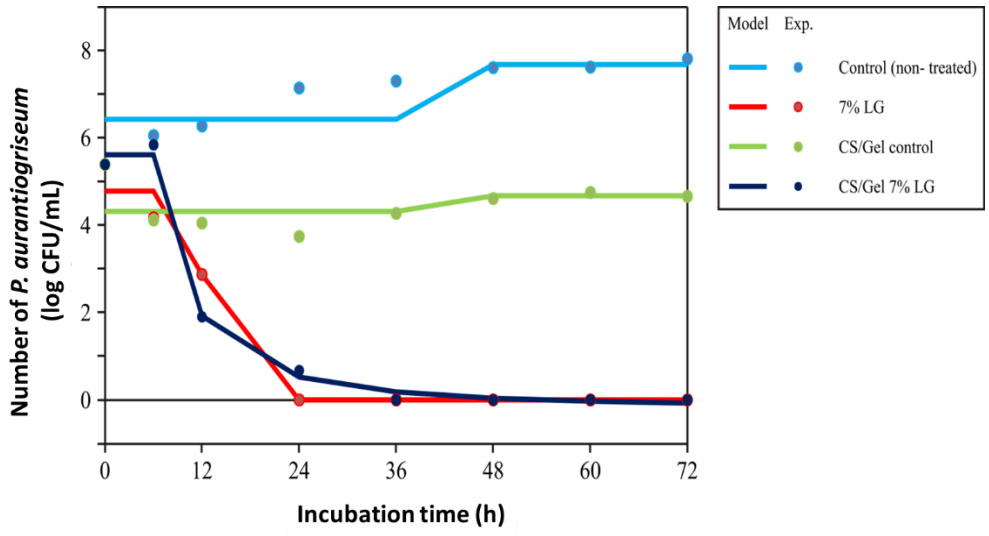


Figure 3. Fungal growth kinetics
(markers signify the experimental data; lines indicate predictive results)

The growth profile curve for *Penicillium aurantiogriseum* ATCC 16025 indicated the number of live bacterial cells over an incubation period that was not treated with any antimicrobial substance. There are noticeable growth phases for the fungi, which were followed by multiplying the fungal concentration (the final concentration was 7.81 log CFU). Figure 4 graphically depicts the pharmacodynamic potential of 7% LG as well as CD/Gel formulation. Kinetics profiles for 7% LG indicate a complete biocide effect for *P. aurantiogriseum* after a contact time of 24 hours, while the same effect was observed for CS/GEL 7% LG for 36 hours.

Interestingly, but in correlation with the obtained results in Table 5, the effect of the CS/Gel control sample did not cause a biocide effect, but an initial decrease in number was detected between 6 and 36 hours of contact time. After that initial phase, a slight increase in the path can be observed until the end of the incubation period, with a final concentration of fungi of 4.66 log CFU. This result confirms once again that the primary structure of the coating is not sufficient to exhibit a biocidal effect, and the addition of a strong biocide agent such as lemongrass essential oil is crucial. Additionally, Table 5 summarizes the regression coefficients of the observed kinetics models for the time-kill study (Figure 4), which explain the speed and intensity of each tested sample.

Table 4. Regression coefficients for fungal growth kinetics

Coefficient	**Fungal concentration (log CFU/mL)**			
	control (non-treated)	**7% LG**	**CS/Gel control**	**CS/Gel 7% LG**
d	7.680	4.781	4.314	37.326
a	6.430	0.000	4.673	-0.196
c	97.620	-12.239	-104.988	-1.614
b	42.177	12.405	41.318	2.096

The goodness of fit between experimental measurements and model calculated results for the time-kill kinetic study is shown in Table 5. The quality of the model fit was also tested and the residual analysis of the developed predictive model was presented in the same table. The presented four-parameter sigmoidal mathematical model appears to be simple, robust, and accurate. Mathematical models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily. A high r^2 indicates that the variation was taken into account and that the data fit the proposed model well.

Table 5. The 'goodness of fit' tests for fungal growth kinetic models

Sample	χ^2	RMSE	MBE	MPE	r^2
Control sample (without any layer)	0.363	0.563	0.000	6.670	0.536
Sample with CS/Gel coating control	0.106	0.305	0.000	3.246	0.979
Sample with CS/Gel coating with 7% LG	0.230	0.448	0.000	6.355	0.131
Control sample (without any layer)	0.023	0.143	0.000	3.880	0.996
	Skew	**Kurt**	**Mean**	**StDev**	**Var**
Control sample (without any layer)	-0.145	0.365	0.000	0.602	0.363
Sample with CS/Gel coating control	-0.001	3.500	0.000	0.326	0.106
Sample with CS/Gel coating with 7% LG	1.786	4.572	0.000	0.479	0.230
Control sample (without any layer)	-0.120	-0.708	0.000	0.153	0.023

Abbreviations: Kurt, kurtosis; MBE, mean bias error; Mean, mean of the residuals; MPE, mean percentage error; r^2, coefficient of determination; RMSE, root mean square error; SD, the standard deviation of the residuals; Skew, skewness; Var, the variance of the residuals; χ^2, reduced chi^2 square.

The antimicrobial effectiveness of three coatings was tested in triplicate using artificially contaminated samples with fungi spores: control (no coating), sample with CS/Gel coating control, and sample with CS/Gel coating with β-CD/7%LG inclusion complex. In brief, the effect of CS/Gel coating on tomato fruits in artificially inoculated cherry tomatoes was evaluated by estimating the affecting area with fungal contamination during the 20-day cold storage period. Selected photographs are presented in Figure 4.

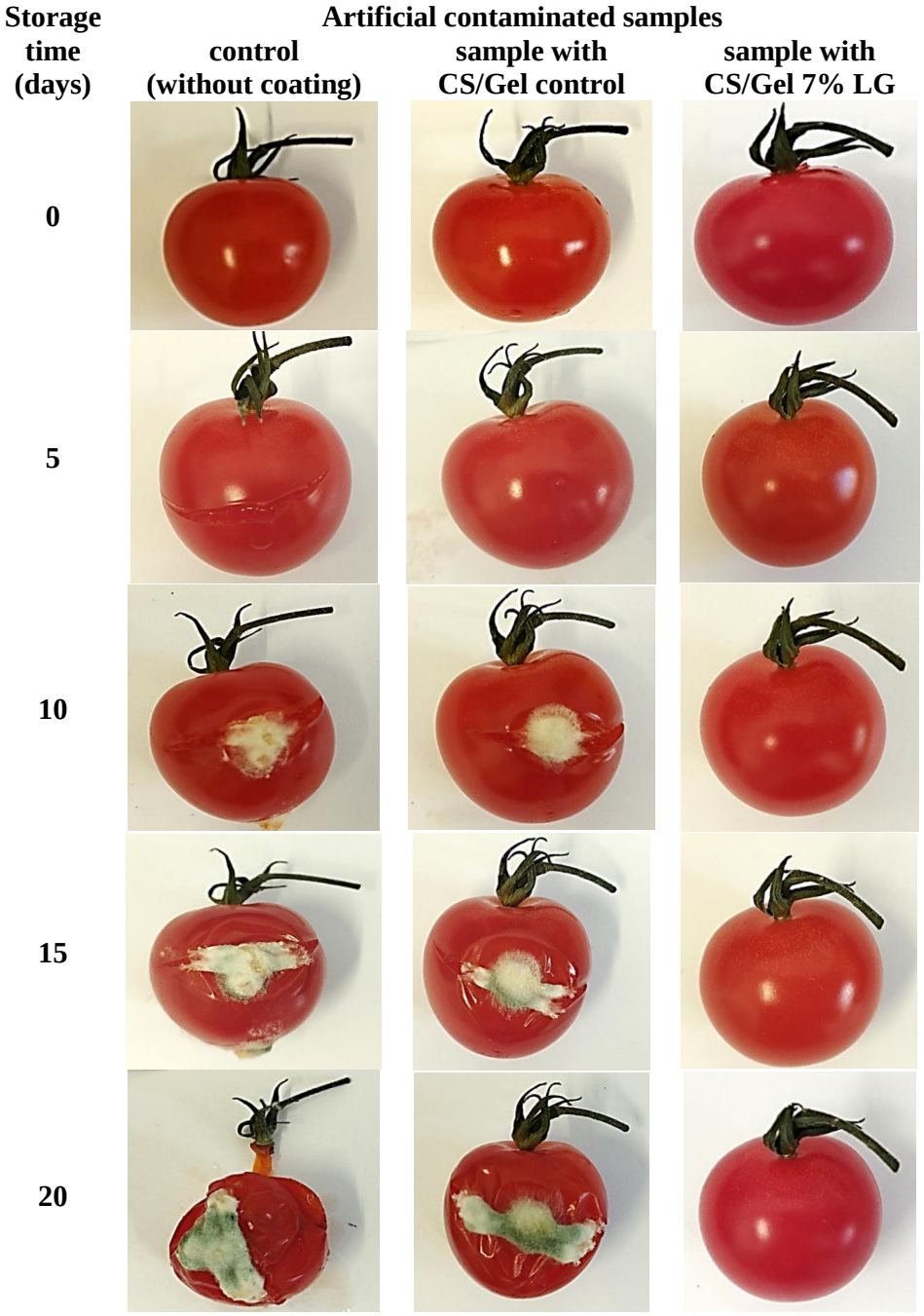


Figure 4. Digital photographs of tomatoes during cold storage

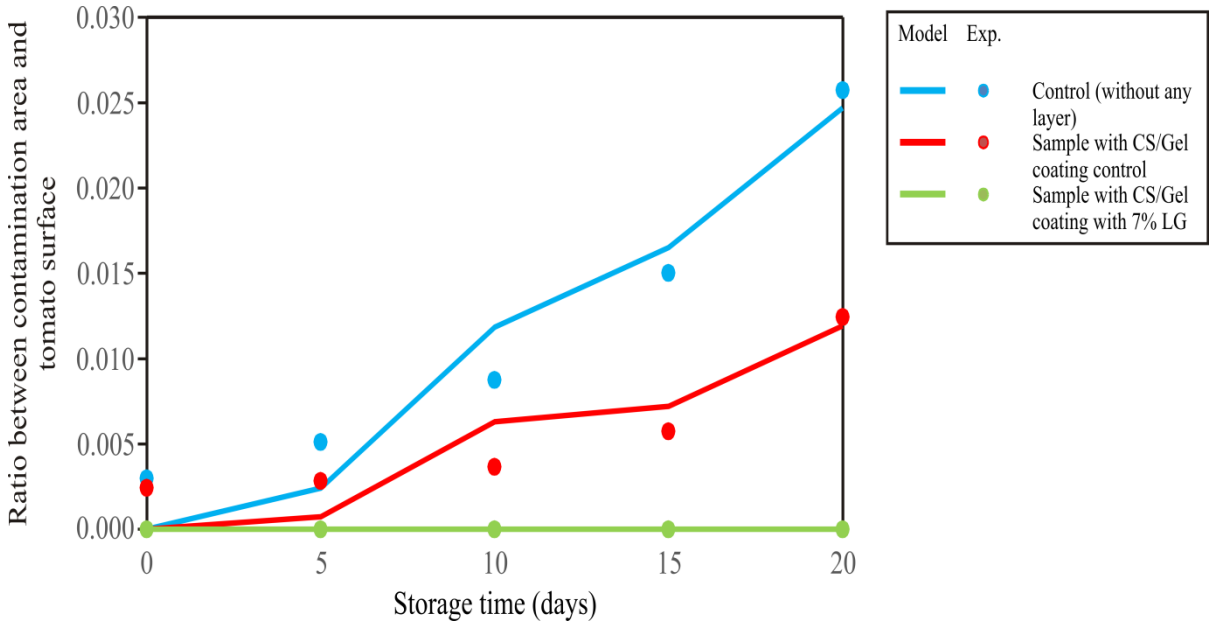
The obtained surface area of tomato samples as well as a ratio between the contamination area and the surface area of the tomato sample (calculated as an ellipsoid) is presented in Table 6. The data is calculated based on digital images. The coating of cherry tomatoes with a 7% LG coating resulted in visually significant differences in appearance compared with control samples.

584 **Table 6.** The ratio between the contamination area and the surface area of the tomato sample

Storage time (days)	Artificial contaminated samples		
	Control sample (without any layer)	Sample with CS/Gel coating control	Sample with CS/Gel coating with 7% LG
0	0.000	0.000	0.000
5	0.002	0.001	0.000
10	0.012	0.006	0.000
15	0.017	0.007	0.000
20	0.025	0.012	0.000

585

586 The antimicrobial effect of the LG in the CS/Gel formulation was found to be prolonged during
587 storage due to the absence of fungal contamination on the surface of these samples (Figure 5,
588 Table 7). A strong fungicide effect on tomatoes was achieved by using a 7% LG in a CS/Gel
589 coating formulation for the first time. The applied coating system can be the solution for the
590 long storage of tomato samples in conditions of commercial application in markets when
591 tomatoes are displayed in refrigerated display cases. Regardless of the obtained results of
592 antimicrobial efficacy, in future steps, it is necessary to examine the impact of the proposed
593 coating system on the physicochemical characteristics of tomatoes as well as the possibility of
594 coating a larger number of samples for commercial use. The obtained kinetics models for the
595 evaluation step are presented in Figure 5, while Table 8 summarizes the regression coefficients
596 of the observed kinetics models (Figure 6), which explain the speed and intensity of each
597 sample.



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599 **Figure 5**. Kinetic study for evaluation of coating antimicrobial effectiveness based on the
600 ratio between contamination area and the surface area of the tomato sample (markers signify
601 the experimental data; lines indicate predictive results).

602

603 **Table 8.** Regression coefficients for evaluation of coating antimicrobial effectiveness based on
604 ratio between contamination area and the surface area of the tomato sample

Coefficient	Control sample (without any layer)	Sample with CS/Gel coating control	Sample with CS/Gel coating with 7% LG
a	7.680	4.781	0.000
b	6.430	0.000	0.000

605

606 The goodness of fit, between experimental measurements and model calculated results is
607 shown in Table 9. The quality of the model fit was also tested, and the residual analysis of the
608 developed predictive model was presented in the same table. The presented four-parameter
609 sigmoidal mathematical model appears to be simple, robust, and accurate. Mathematical
610 models had an insignificant lack of fit tests, which means that all the models represented the
611 data satisfactorily. An r^2 between 0.818 and 1.000 indicates that the variation was taken into
612 account and that the data fit adequately to the proposed model.

613

Table 9. The 'goodness of fit' tests for or evaluation of coating antimicrobial effectiveness based on ratio between the contamination area and the surface area of the tomato sample

Sample	χ^2	RMSE	MBE	MPE	r^2
Control sample (without any layer)	0.000	0.001	0.000	31.709	0.936
Sample with CS/Gel coating control	0.000	0.001	0.000	75.585	0.818
Sample with CS/Gel coating with 7% LG	0.000	0.000	0.000	0.000	1.000
	Skew	**Kurt**	**Mean**	**StDev**	**Var**
Control sample (without any layer)	0.467	-2.255	0.000	0.001	0.000
Sample with CS/Gel coating control	0.235	-2.782	0.000	0.001	0.000
Sample with CS/Gel coating with 7% LG	0.000	0.000	0.000	0.000	0.000

Abbreviations: Kurt, kurtosis; MBE, mean bias error; Mean, mean of the residuals; MPE, mean percentage error; R2, coefficient of determination; RMSE, root mean square error; SD, standard deviation of the residuals; Skew, skewness; Var, variance of the residuals; $\chi 2$, reduced chi^2 square.

4. CONCLUSIONS

The formulation of an innovative bio-based chitosan/gelatine (CS/Gel) coating with a β-cyclodextrin/lemongrass essential oil inclusion complex using a low amount of antimicrobial compounds (lemongrass oil) exemplifies the novelty of this paper. This system has been employed for the food application of an antimicrobial coating on freshly harvested cherry tomatoes. According to the obtained results, the high antimicrobial activity and coating effect improved the shelf-life of fruit samples for 20 days during cold storage. The use of β-cyclodextrin/lemongrass essential oil inclusion complex at an optimized concentration in the coating formulation has created a framework for obtaining an edible coating system whose active function has been proven through step-by-step evaluation protocols during antimicrobial profiling. As the final output of this work, complete growth inhibition of *Penicillium aurantiogriseum*, one of the main causes of fruit spoilage, was demonstrated. For the first time,

predictive capacity and advanced mathematical modeling were used in in situ studies of this type of coating system's antimicrobial evaluation. The obtained results of coating of cherry tomato by CS/Gel formulation with 7 % of β-cyclodextrin/lemon grass essential oil inclusion complex results in a significantly greater shelf-life of cherry tomato samples, but further functionality and universality of the coating system have to be validated in further investigation. In summary, this comprehensive study can be a base for manufacturing edible, bio-based, and cost-effective coating systems for perishable fresh fruits.

CRediT authorship contribution statement

All authors have a significant intellectual contribution to the work, have read the revised manuscript, and concur with the submission. The article submitted is original work and has not been published elsewhere, either completely, in part, or in another form. All the authors declare no competing interests.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

Tamara Erceg – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Olja Šovljanski – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Alena Stupar – idea, experiment, revising of the manuscript.

Jovana Ugarković – experiments.

Milica Aćimović – experiments, writing, and revising of the manuscript.

Lato Pezo – modeling, calculations, writing of the manuscript.

Ana Tomić – experiments, revising of the manuscript.

Marina Todosijević – experiments, revising of the manuscript.

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Declaration of competing interest

The authors declare no competing financial interest.

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A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex - characterization and food application

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HIGHLIGHTS

- Framework for obtaining edible active coating system was presented
- Chitosan-gelatine coating has been prepared with β-cyclodextrin - lemongrass oil inclusion complex
- Evaluation of the active role of the coating was done using the dip-coating method
- Cherry tomatoes were coated and artificially contaminated by *P. aurantiogriseum*
- Development of fungal contamination was monitored for 20 days at cold storage

ABSTRACT

Biopolymer-based films present an ideal matrix for the incorporation of active substances such as antimicrobial agents, giving active packaging a framework of *green* chemistry and a step forward in food packaging technology. The chitosan-gelatine active coating has been prepared using lemongrass oil as an antimicrobial compound applying a different approach. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin was used to encapsulate lemongrass oil. The antimicrobial effect was assessed using the dip-coating method on freshly harvested cherry tomatoes artificially contaminated by *Penicillium aurantiogriseum* during 20 days of cold storage. According to the evaluation of the antimicrobial effect of coating formulation on cherry tomato samples, which was mathematically assessed by predictive kinetic models and digital imaging, the applied coating formulation was found to be very effective since the development of fungal contamination for active-coated samples was observed for 20 days.

Keywords: edible coating; chitosan; *Cymbopogon citratus*; antimicrobial coating; cherry tomato; food safety.

1. INTRODUCTION

The increased global demand for fresh natural foods and the high percentage of postharvest loss of fresh fruit and vegetables have imposed a need for the development, production, and consumption of packaging that will ensure the protection of food from physical, chemical, and microbiological contamination, and different environmental influences to maintain food quality and extend shelf life during transport, handling, and storage [1,2]. Massive consumption of conventional polymers in an envelope (wraps, foils) single-use food packaging causes environmental pollution during manufacturing and disposal. Non-degradable petroleum-based polymers persist in nature, leading to waste accumulation, microplastics, and nano-plastic formation, which further pollute water, soil, and food, ending up in living organisms [3]. As a

result of these environmental concerns, there is a greater demand for biodegradable and renewable materials for sustainable, eco-friendly food packaging [4,5]. One of the most studied are biodegradable and edible films and coatings based on biopolymers such as polysaccharides and proteins. Edible coatings/films are a thin layer of edible biopolymers, mostly hydrophilic, intended for coating or wrapping a food product that can be consumed by animals and humans, or easily degraded in the natural environment, without the production of waste or microplastics. Their sustainable nature and the ability to prevent a gas transfer between food and its surrounding medium, enabling food conservation and shelf-life extension, make them a good alternative to conventional polymer-based food packaging wraps and foils [6]. Population growth, market globalization, longer food distribution, increasing environmental awareness of consumers, and modern lifestyles have necessitated the development of functional packaging capable of extending food shelf-life [7]. Edible coatings and films are ideal matrices for the incorporation of active substances such as antimicrobial and antioxidant agents giving the active packaging within a *green* chemistry framework. The selection of an appropriate antimicrobial agent able to prevent or reduce the growth of pathogenic microorganisms that is compatible with a biopolymer matrix enables the use of sustainable active packaging which significantly extends the shelf-life of the food product [8]. The capacity of edible films to substitute conventional polymers and reduce their harmful effects has transformed food packaging. The development of these films with antimicrobial properties has been shown especially important in the packaging of fruits that are subjected to contamination during long-term storage and transportation. Preventing fruit contamination significantly reduces production and distribution costs, increasing the availability of products to a greater number of final consumers. In addition to minimizing the use of non-degradable polymers and their harmful effects on the environment, edible films show advantages as a packaging material by decreasing moisture loss, rates of ripening, and ethylene production, which results in

77 maintaining product quality and storability [9]. They can be formulated as a biopolymer blend,
78 using a combination of polysaccharides and/or proteins [10,11].
79 Chitosan (CS) is a promising material for the preparation of edible coatings/films due to its
80 good properties such as biocompatibility, biodegradability, ability to form films, ability to
81 inhibit moisture, aroma loss, oxygen penetration, and microorganisms' growth [9, 12-14]. The
82 advantages of chitosan also include its low price, inherent antimicrobial nature, and
83 hydrophilicity, which automatically imply the reduced consumption of antimicrobial and
84 antioxidant agents as well as using water instead of organic solvent in the manufacturing of
85 coating films, which contributes to the reduction of production costs. Chitosan is a linear
86 cationic polysaccharide that can be easily obtained by the partial deacetylation of chitin in an
87 alkali medium. Chitin is, along with cellulose, the most abundant biopolymer in nature, which
88 can be extracted simply from biomass such as the exoskeletons of insects, crustaceans, algae,
89 *etc*. [15], which makes this biopolymer economically viable. However, the poor mechanical
90 and barrier properties of chitosan limit its application as self-contained food films and coatings,
91 are reasons why it is combined with other biopolymers such as gelatine (Gel) [16]. Gelatine is
92 a protein obtained from the skin and bones of animals, and as well as chitosan, it is generally
93 recognized as safe and suitable for food packaging [17]. This protein gives edible, transparent,
94 and flexible films [18, 19]. Different approaches have been made to obtain edible films based
95 on chitosan and gelatine with or without additional compounds, which has been confirmed by
96 numerous research papers published in the last decade [20-28]. In recent years, researchers
97 have investigated the development of active food packaging films based on chitosan-gelatine
98 blends with an incorporated essential oil such as garlic essential oil [29], ferulago angulate
99 essential oil [30], ginger essential oil [31], thyme essential oil [32]. Essential oils obtained from
100 plants have been shown to be safe and effective antimicrobial and antioxidant agents. However,
101 these formulations, considering the difference in polarity between biopolymers and essential

oils are mainly prepared in the form of an emulsion, which implies the addition of surfactants to achieve the miscibility of chitosan and gelatine with non-polar essential oils. On the other hand, the tomato, that was selected as a fruit of interest is one of the most economically important plants in the world. The area under tomatoes occupies 4.6 million ha, with a yield of about 130 million tons per year, of which about 88 million tons of fruit are consumed fresh and about 42 million tons are processed. The yearly growth rate for the cherry type of tomato is 36.33% [33]. However, tomatoes are easily contaminated by fungi and are determined to be highly sensitive fruits, while fungal contamination can affect public health worldwide [34]. Therefore, protection and prolongation of the shelf life of raw tomatoes are very important and have led to different approaches and applications for alternative types of storage processes and packaging [10].

The main goal of this work is to obtain active packaging based on a chitosan-gelatine coating system with the addition of lemongrass essential oil as an active antimicrobial compound. Step forward in this paper has been made using cyclodextrins instead of surfactants [10,35], and, therefore, β-cyclodextrin/lemongrass essential oil inclusion complex was used for the final formulation of the mentioned system. A combination of chitosan and gelatine has been made in order to overcome the shortcomings of neat chitosan in terms of mechanical and barrier properties. Except for a comprehensive characterization of the coatings (which included FTIR analysis, thickness measurements, moisture content, total soluble matter, water vapor transmission rate analysis, tensile strength, and elongation at break of biopolymer films, as well as thermal gravimetric analysis), this study involved successive testing of the antimicrobial activity of packaging and, for the first time, modeling of the obtained results using advanced mathematical tools. Finally, application in food packaging was performed, implying that chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex can significantly improve the shelf-life of cherry tomatoes. Considering the high

antimicrobial potential of lemongrass oil, proven by the preliminary assessment, the additional focus of this paper was to optimize the edible film composition in terms of mechanical properties and adequately releasing of essential oil from the inclusion complex to the surface of the food product.

2. EXPERIMENTAL PART

2.1. Materials

Chitosan (M_w = 100000-300000 g/mol) and glacial acetic acid were supplied from Acros Organic B.V.B.A. (USA). Gelatine and glycerol (extra pure) were procured from CENTROHEM (Serbia), while β-cyclodextrin (β-CD) was from Sigma Aldrich (USA). Lemongrass (*Cymbopogon citratus*) essential oil was made from greenhouse-originated aboveground plants of lemon grass plants. The preparation of essential oil was done in July 2021, during which the selected parts were dried in shade and subjected to steam distillation. The distillation process involved the following steps: placement of 100 kg of dry plant material in a vessel (V=0.8 m^3) routing upwards through a plumbing system, and steaming for 20 minutes. The condensed vapor was collected in the Florentine flask, and the distillation process was finished after 3 hours. The obtained essential oil was decanted from the water phase, dried over sodium sulfate, and stored in dark glass bottles at 4 °C.

2.2. Analysis of Volatile Compounds in lemongrass essential oil

The obtained lemongrass essential oil used for CS/Gel formulation was subjected to GC-MS analysis with an Agilent 7890A apparatus equipped with a 5975 C MSD, FID, and an HP-5MS fused-silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The carrier gas was helium, and its inlet pressure was 19.6 psi and linear velocity of 1 mL min^{-1} at 210 °C. The injector temperature was set to 250°C, the injection volume was 1 μL, and the split ratio was 10:1. MS detection was carried out under source temperature conditions of 230 °C and an

interface temperature of 315 °C. The EI mode was set at electron energy, 70 eV with a mass scan range of 40-600 amu. The temperature was programmed from 60 °C to 300 °C at a rate of 3 °C min^{-1}. The components were identified based on their linear retention index relative to C8-C32 n-alkanes, in comparison with data reported in the literature (Adams4 and NIST17 databases). The relative percentage of the oil constituents was expressed as percentages by FID peak area normalization.

2.3. Preparation of edible coating

Chitosan (2.5% w/w) was added to a 5% (v/v) acetic acid aqueous solution and stirred on a magnetic stirrer at 70 °C for 90 min. The same weight of gelatine was added to distilled water at 60 °C and stirred until complete dissolution. The chitosan solution was mixed with the same volume of the gelatine solution for 90 min at 60 °C, after the addition of glycerol in the amount of 30 wt% per total biopolymers weight. The problem of differences in polarity between obtained blend and essential oil was solved by incorporation of β-CD -lemongrass oil inclusion complex in the reaction mixture.

The inclusion complex between β-CD and essential lemongrass oil (β-CD/LG) was prepared by the modified precipitation method described by Kringel et al. [36]. The amount of 2 g of β-CD was dissolved in 50 ml of distilled water at 35 ± 1 °C, by homogenization at a magnetic stirrer. After 3 hours of stirring, 1.6 g of lemongrass oil was slowly added to the reaction mixture. After cooling at room temperature, the mixture was transferred to a refrigerator and left there for 12 hours at 5 °C. The precipitated β-CD/lemongrass oil inclusion complex material was vacuum filtered, rinsed with absolute ethanol, and dried at 40 °C until constant weight was obtained. Different amounts of β-CD/lemongrass oil inclusion complex (3, 5, and 7 wt% per total biopolymers weight) were added to the CS/Gel solution and stirred at 40 °C for 2 h. Different amounts of β-CD/lemongrass oil inclusion complex (3, 5, and 7 wt% per total

177 biopolymers weight) were added to the CS/Gel solution and stirred at 40 °C for 2 h. The film-
178 forming mixtures with different amounts of β-CD/lemongrass oil inclusion complex were
179 poured into Teflon-coated Petri dishes and dried at 40 °C until a constant weight. One sample,
180 prepared without the addition of complex has served as control.

181 **2.4. Characterisation of coating**

182 **2.4.1. Fourier transform infrared spectroscopy (FTIR) analysis**

183 The chemical structure of the obtained samples was investigated by Fourier transform infrared
184 spectroscopy (IRAffinity-1S, Shimadzu). Samples were scanned 24 times with a resolution
185 setting of 16 cm^{-1}. Data were collected in the range of 400 – 4000 cm^{-2} using the Attenuated
186 Total Reflection (ATR) method.

187 **2.4.2. Thickness measurements**

188 The thickness of films was measured using a micrometer Digico 1, with an accuracy of 0.001
189 mm (Tesa, Renens, Switzerland), at 9 positions, using three samples, and the average value
190 was used.

191 **2.4.3. Moisture content, total soluble matter analysis**

192 For analyzing moisture content (MC) and total soluble matter (TSM), coating samples were
193 cut into squares (20 x 20 mm), put in aluminium dishes, weighted, and dried at 105 ± 2 °C until
194 they reached a constant weight. MC was determined as a percentage of the initial film weight
195 lost during drying. The results were reported as an average of four independent measurements.
196 TSM was determined as a percentage of coating dry matter solubilized for 24 h in 50 ml of
197 distilled water at room temperature, with periodic stirring. Samples were taken out after 24
198 hours and dried at 105 ± 2 °C until constant weight (m''). The TSM value was determined using
199 Equation 1.

$$TSM = \frac{m' - m''}{m'} \cdot 100\% \quad (1)$$

where *m'* the weight of the sample after drying at 105 ± 2 °C before immersing in distilled water.

2.4.4. Barrier properties - water vapour transmission rate analysis

The water vapor transmission rate (WVTR) for biopolymer films was determined according to the ISO 2528 standard [37] at a temperature of 25 ± 1 °C and relative humidity of 90 ± 2 %. The results were averaged on three independent measurements.

2.4.5. Mechanical properties analysis

Tensile strength and percentage elongation at the break of polymer coatings were investigated using the Instron Universal Testing Machine (Model 4301, Instron Engineering Corp., Canton, MA). The samples were prepared and tested according to the ASTM standard D882-18. Films were shaped in rectangular spaces with dimensions of 80 x 15 mm. The tests were carried out at a temperature of 23 ± 2 °C and relative humidity of 50%, with the initial grip separation set at 50 mm and the crosshead speed set at 50 mm/min. The results were averaged on eight independent measurements, and the extreme values were excluded.

2.4.6. Thermal gravimetric analysis (TGA)

The thermal properties of polymer coatings were investigated using the LECO 701 Thermogravimetric Analyzer (LECO, Germany). Measurements were carried out under the flow of air (50 ml/min) in the temperature range of 25 to 800 °C at a heating rate of 10 °C/min.

2.5. Evaluation of coating antimicrobial effectiveness

2.5.1. Antimicrobial activity of the chitosan-gelatine coating formulations

The referent fungi strain *Penicillium aurantiogriseum* ATCC 16025 was selected for this study. An antimicrobial effect of 7% LG and CS/Gel formulations (control and 7% LG addition) against the strain was done using the disk diffusion method described in detail by Riabov et al. [38]. The used volume of CA/PCL-diol formulations was 10 µL per sterile cellulose disk (analysis was done in triplicate). The sterile distilled water was a negative control, while 3% cycloheximide was a positive control. Additionally, a time-kill kinetics study was done for the evaluation of the fungicide-dynamic pathway of 7% LG, CS/Gel formulations (control and 7% LG addition) against tested strain using the method described by Aćimović et al. [39]. The non-treated, inoculated nutrient medium was a control in the test. For mathematical analysis of the obtained results, the four-parameter sigmoidal model was used, while the data were presented as an S-shaped curve model (Eq. 2).

$$y(t) = d + \frac{a-d}{1+\left(\frac{t}{c}\right)^{b}} \quad (2)$$

The spore concentration (y(t)) during contact time with the extract was the targeted output, while the regression coefficients in Eq. 2 can be described as a - minimum of the experimentally obtained values (t = 0), d - the maximally obtained value (t = ∞), c - the inflection point (the point between a and d, and b - the Hill's slope (the steepness of the inflection point c).

2.5.2. Cherry tomato samples

The cherry tomatoes (*Solanum Lycopersicum* var. *cerasiforme*) were obtained from a local organic manufacturer near Novi Sad, Serbia, in May 2022. The selection of the samples was done based on the mass, i.e. each whole mature, fresh, and undamaged fruit had a mass of 15 g ± 0.2 g. After harvesting and selection, fruits were washed with water and sterile distilled

245 water and dried in a laminar chamber. All samples were left with a petiole to make it easier to
246 manipulate the samples during coating and storage. The contamination of the samples was done
247 in minimal manipulation time, and all samples are subjected to immediate storage on harvest
248 day.

249 **2.5.3. Contamination suspension preparation and artificial contamination of tomatoes**

250 The strain was incubated on Sabouraud Dextrose Agar (SDA, HiMedia, Mumbai, India) for
251 120 hours at 25 °C before being used for antimicrobial testing of CS/Gel formulations, and the
252 initial suspension for contamination was prepared in sterile distilled water with a targeted
253 concentration of 6 log CFU/mL. In each tomato, the sample was injected into one spot with 10
254 µL of the prepared suspension in such a way that a petiole is turned to the right so that the
255 photographing of the fruit is reproducible in the same way during the storage period. Each scar
256 site sample contained approximately 4 log CFU of mold spores.

257 **2.5.4. Preparation of coating for tomatoes samples**

258 The coating solution was freshly prepared using the previously described protocol and applied
259 to tomatoes by the dip-coating method. Briefly, the samples are directly and individually added
260 to the prepared coating solution and then dried in a laminar chamber (this process did not affect
261 the texture or quality of the sample). In this way, three groups of samples were prepared:
262 artificial contaminated control (samples without coating), artificially contaminated sample with
263 CS/Gel control coating, and artificially contaminated sample with CS/Gel 7% β-CD/LG
264 coating. The tomato samples in individual boxes were stored at 8 °C in a semi-vertical
265 refrigerated display case (model Aruba, Frigo žika, Ruma, Serbia) with sliding doors and lights
266 for 20 days. The experiments were performed in three independent replicates.

267 **2.5.5. Monitoring of the contamination development**

The prepared groups of tomatoes were monitored every five days during the cold storage period. Each sample image was recorded with a common digital camera, which captured a region of roughly Ø100 mm (the macro function was used, for a more clear photograph). The obtained pictures were used to determine the contaminated area of the sample using ImageJ 1.53r (used for masking the contaminated area) and Inkscape 1.0 (applied to measure the linear distances on bitmap images and to evaluate the area between lines). The surface area of the tomato was approximated using Knud Thompson's formula, which is presented in Eq (3.) [40].

$$SA = 4 \cdot \pi \cdot \left(\frac{a^p \cdot b^p + a^p \cdot c^p + b^p \cdot c^p}{3} \right)^{\frac{1}{p}} \quad (3)$$

where: *a*, *b*, and *c* are horizontal, vertical, and conjugate radius, while $p \approx 1.6075$.

For evaluation of coating effectiveness (CE), measuring the ratio between the contaminated and the surface area of tomato samples at time *t*, was conducted using Eq. (4)

$$CE(t) = \frac{A_{contaminated}(t)}{SA(t)}. \quad (4)$$

For mathematical analysis of the obtained results, the two-parameter exponential model was used, while the data were presented as an exponential model. The coating effectiveness *CE*(*t*) at time *t* was the targeted output, while *a* and *b* were the regression coefficients.

$$CE(t) = a \cdot \exp(b \cdot t) \quad (5)$$

2.5.6. The accuracy of the model

A numerical verification of the models obtained in the previous step was tested using the coefficient of determination (r^2), reduced chi-squared (χ^2), mean bias error (MBE), root mean square error (RMSE), and mean percentage error (MPE) [41, 42].

$$\chi^2 = \frac{\sum_{i=1}^{N} (x_{\exp,i} - x_{pre,i})^2}{N - n}, \quad (6)$$

289 $$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})^2 \right]^{1/2}, \quad (7)$$

290 $$MBE = \frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i}), \quad (8)$$

291 $$MPE = \frac{100}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left| x_{pre,i} - x_{\exp,i} \right|}{x_{\exp,i}} \right), \quad (9)$$

292 where: $x_{exp,i}$ stands for the experimental values and $x_{pre,i}$ are the predicted values calculated by
293 the model, N and n are the number of observations and constants, respectively.

294 **2. RESULTS AND DISCUSSION**

295 The composite coating was prepared by the creation of a chitosan and gelatine polyelectrolyte
296 complex. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin
297 has been used for the encapsulation of lemongrass oil. Cyclodextrins are cyclic molecules
298 whose inner cavity has a hydrophobic nature, which enables the incorporation of hydrophobic
299 compounds, while the outer surface possesses a hydrophilic nature which makes them soluble
300 in a water medium. A step forward has been made in this study by using cyclodextrins instead
301 of surfactants such as Tween 80 [10, 35]. Cyclodextrins are biocompatible biomolecules used
302 in several times lower amounts than surfactants. In the mentioned papers, the used surfactants
303 are synthetic, can be irritable and their dispersing requires the incorporation of mechanical
304 energy in a biopolymers solution. That implies the use of a high-speed homogenizer, while the
305 addition of cyclodextrins does not require special equipment. The optimal
306 chitosan/gelatine/glycerol ratio was found by performing a series of mechanical tests.
307 Lemongrass oil was used as an antimicrobial compound due to its expressed antimicrobial
308 effect, especially against fungi that contaminate cherry tomatoes. The strong antimicrobial
309 effect of lemongrass essential oil enables the use of its low concentration, which results in
310 economic benefits by avoiding deterioration of the sensor properties of the fruit.

Antifungal activity was determined by analyzing the chemical composition of the essential oil and confirmed by preliminary antimicrobial testing. The consumption of fresh cherry tomatoes has gradually increased in the last two decades, and keeping this fruit healthy and fresh is very challenging considering its perishable nature. According to the authors` knowledge, the preparation and characterization of chitosan/gelatine edible coatings with β-cyclodextrin-lemongrass oil inclusion complex using a simple two-step method have not been studied in the available literature, as well as the determination of the kinetics models for fungal growth and antimicrobial effectiveness of coating based on the ratio between contamination area and the surface area of the cherry tomato sample. Similar approaches in the preparation of active edible films have been applied but using different coating compositions, a higher amount of inclusion complex, and a higher amount of antimicrobial compounds/essential oil, which have resulted in a lower inhibition zone [43]. The model of fungal growth enables the prediction of the level of contamination at certain time intervals. Considering the high antimicrobial potential of lemongrass oil, proven by the preliminary assessment, the focus of this paper was to optimize the active edible film composition in terms of mechanical and antimicrobial properties, providing an effective coating that is capable to prolong the shelf-life of fresh cherry tomatoes. Therefore, the experimental setup can be divided into three main parts: (i) Characterization of the active compound - chemical characterization of selected essential oil; (ii) Characterization of the CS/Gel film properties; (iii) Evaluation of coating antimicrobial effectiveness in *in vitro* experiments and in situ application on food samples.

3.1. Lemongrass essential oil characterization

Table S1 (Supporting Information) shows the chemical analysis of the lemongrass essential oil obtained through the hydrodistillation process. Identification of the components was done according to their linear retention indices (RI), and their equivalence with mass spectral libraries (Wiley and NIST). The relative abundance of each detected compound was calculated

as the percentage area of each peak (only identified compounds are shown). The most abundant compounds in the sample (22 compounds, comprising 99.4%) were geranial (40.8%), neral (31.9%), and myrcene (17.4%). As two dominant compounds that together represent citral, neral (cis-citral) and geranial (trans-citral) have previously been studied as effective agents against different fungi *in vitro*, while the strongest biocide effect of geranial was observed compared with neral as individual substances [44].

According to literature references data (Table S2, Supporting information) on the chemical composition of lemongrass essential oil, as well as cluster analysis performed using this data for the construction an unrooted phylogenetic tree (Figure S1), it is possible to conclude that lemongrass essential oils can be relatedness based on the range of the most dominant compound in all listed essential oil samples - gerinal (Table S1).

Lemongrass essential oil samples can be divided into five subgroups based on chemical composition: with a very high gerinal content (46.6-48.7%) with four samples [45-48], high gerinal content (41.3-44.5%) with five samples [49-51], medium gerinal content (33.9-40.8%) with five samples including the sample from this study [52-54], low gerinal content (33.3 %) with only one sample [55], and very low gerinal content (18.8-32.9%) with four samples [56-59] (Figure S1, Supporting information).

The sample in this study obtained in Serbia is similar to samples obtained from different geographical areas (Vietnam, Sudan, and Egypt) with the content of gerinal in the range between 33.9 and 40.8%. Additionally, this indicates that chemical composition has been attributed to this factor, but also climatic conditions, time and year of harvest, etc., which are in correlation with the hypothesis of Boukhatem et al. [60]. In summary, all the listed essential oil samples in Table S2 have a dominant citral complex, but with various proportions of geranial and neral. Additionally, different percentage of the third dominant compound, i.e.,

myrcene, can be observed in the range between 2.3 and 17.4, with the highest content percentage obtained in this study.

3.2. Characterisation of the CS/Gel film

FTIR

The FTIR spectra of chitosan, gelatine, and their blend are presented in Figure 1. A broad band from 3600 to 3000 cm^{-1} with two peaks at 3380 and 3226 cm^{-1} corresponds to the asymmetrical and symmetrical N-H stretching vibrations overlapping with OH stretching in the FTIR spectrum of pure chitosan (ν_{NH} and ν_{OH}). Two peaks at 2932 and 2862 cm^{-1} are assigned to -CH_2 stretching vibrations (ν_{CH2}). The weak peak at 1705 cm^{-1} corresponds to C=O stretching vibrations ($\nu_{C=O}$) of the residual acetyl group and the band at 1638 cm^{-1} is assigned to amide I. A band at 1539 cm^{-1} is attributed to NH_3^+ bending and C-N stretching (ν_{NH3+} and ν_{C-N}). Bands at 1407 and 1380 cm^{-1} are attributed to CH_2 and C-N bending (δ_{CH2} and δ_{C-N}). A weak peak observed at 1264 cm^{-1} corresponds to the CH_2OH group in the side chain, while a small peak at 1155 cm^{-1} corresponds to C-O-C glycosidic bond. Two peaks at 1069 and 1020 cm^{-1} correspond to the C-C-O stretching and C-O-H bending (ν_{C-C-O} and δ_{C-O-H}). A small peak at 894 cm^{-1} confirms the presence of 1,4-β–glycosidic bond [61]. The peak at 657 cm^{-1} is assigned to O-H bending (δ_{OH}) out of the plane. The FTIR spectrum of pure gelatin shows similar absorption bands as a chitosan spectrum. A broad absorption band between 3600 and 3000 cm^{-1} with a peak at 3303 cm^{-1} is attributed to the overlapping of N-H and OH stretching vibrations of amino acids in gelatine. A weak peak at 3070 cm^{-1} indicates the presence of an aromatic ring originating from a constitutive unit of gelatine–amino acid. Two weak peaks at 2929 and 2854 cm^{-1} correspond to the C-H stretching vibrations. The band at 1631 cm^{-1} (amide-I) appears due to the C=O stretching vibration [62]. The peak at 1543 cm^{-1} is assigned to NH_2 bending and C-N stretching, while the peak at 1451 cm^{-1} corresponds to the stretching of COO^- group. The

bands at 1404 and 1338 cm^{-1} correspond to the CH_2 and C-H bending. Small peaks at 1283 and 1205 cm^{-1} are assigned to O-C stretching from a carboxylic group, while the peak at 1242 cm^{-1} corresponds to C-N stretching from amine. The absorption band at 1081 cm^{-1} corresponds to C-C-O stretching, while at 1030 cm^{-1} is assigned to C-O-H bending. A broad peak with the center at 657 cm^{-1} corresponds to NH_2 bending and OH bending. The spectrum of the chitosan/gelatin blend containing plasticizer glycerol retains the pattern of all compounds in the composition.

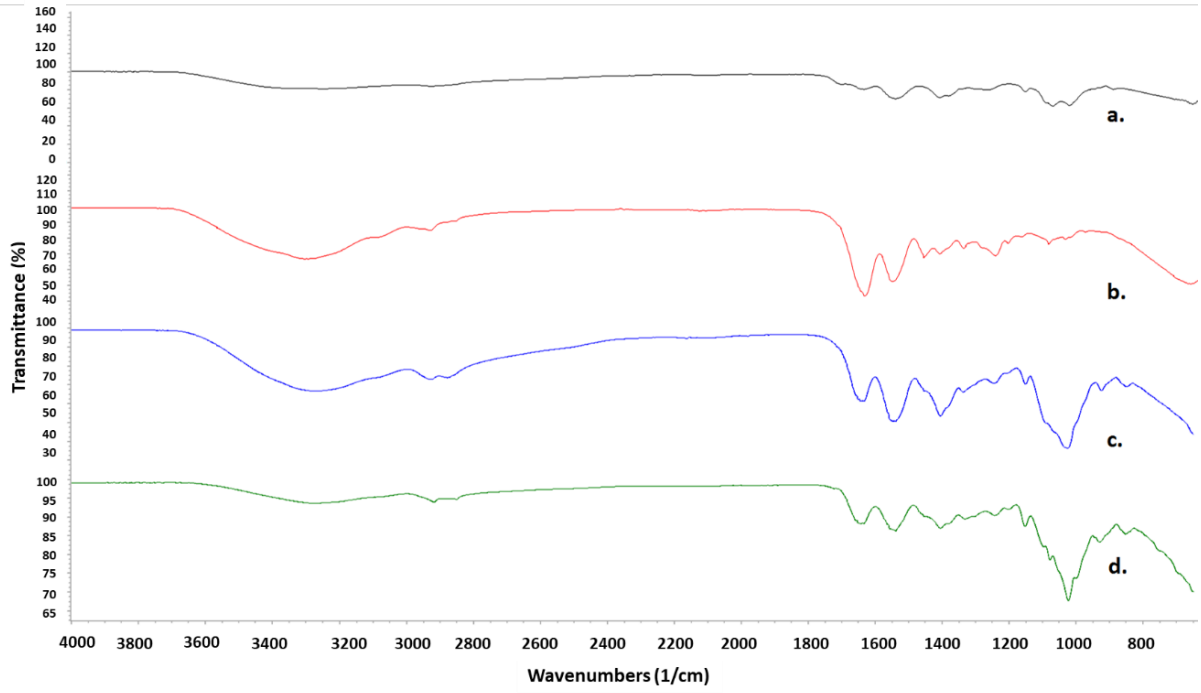


Figure 1. FTIR spectra of: chitosan, b) gelatin, c) chitosan/gelatine blend, d) chitosan/gelatine blend with 5% of β-CD/lemongrass oil.

A broad peak between 3600 and 3000 cm^{-1} with the center at 3372 cm^{-1} in the IR spectra of blends corresponds to OH stretching from chitosan, gelatin, and glycerol, and NH stretching from chitosan, gelatin, and glycerol. Two peaks at 2928 and 2879 cm^{-1}, which correspond to the stretching of CH_2 groups, are presented in the structures of both polymers and the plasticizer

glycerol. The band at 1635 cm^{-1} is assigned to the C=O stretching vibration of amide I. A band at 1538 cm^{-1} is assigned to the N-H bending from the amino group and their protonated form, as well as to the C-N stretching. Peaks at 1405 and 1340 cm^{-1} are attributed to CH_2 and C-H bending. C-N stretching from amine (1245 cm^{-1}) originates from gelatine and appears as a weaker peak than the corresponding peak in the IR spectrum of gelatine. This peak is not visible in the IR spectrum of chitosan. The presence of a glycosidic bond is confirmed by peaks at 1155, 1096 and 860 cm^{-1} in the FTIR spectrum of the blend. Peaks at 1069 and 1026 cm^{-1} are assigned to the C-C-O stretching and C-O-H bending, while the peak at 926 cm^{-1} visible only in the FTIR spectrum of the blend is attributed to the C-O stretching from glycerol. The peak that corresponds to C-O-H bending is more intense in comparison to the same peak in the spectra of neat biopolymers, due to the formation of hydrogen bonds between biopolymers in the blend. FTIR spectrum of blend with β-CD/lemongrass oil inclusion complex in comparison to the spectrum of a control sample (without inclusion complex) has a broader and less intense peak between 3600 and 3000 cm^{-1} and three peaks at 2917, 2977, and 2850 cm^{-1}, which correspond to C-H stretching from chitosan, gelatin, glycerol, and β – cyclodextrin. A peak at 1452 cm^{-1} corresponds to the C=C stretching, which originates from lemongrass oil compounds. This peak appears at 1442 cm^{-1} in the spectrum of neat lemongrass oil. Shifting is a result of the formation of an inclusion complex and its incorporation into the blend.

Moisture content, total soluble matter, and water vapour transmission rate

The results of thickness measurements, MC, TSM, and WVTR are presented in Table 1. The moisture content decreases with an increase in the amount of inclusion complex in blend composition, due to the interaction of hydroxyl groups of β-CD with chitosan and gelatine molecules, via hydrogen bonding, which means that the hydroxyl groups are capable to bond

water molecules and contribute to increasing of MC value are occupied [63]. Moreover, lemongrass essential oil possesses hydrophobic properties, which additionally contribute to reducing the MC content value. The TSM of CS/Gel-based films rises with an increase in the amount of β-CD/LG complex in film composition. Gelatine and glycerol are totally soluble in water, and chitosan solubility demands acidic conditions. However, the inclusion complex which forms hydrogen bonds with glycerol and chitosan can be easily replaced by the water, resulting in an increase in the TSM value [43]. WVTR value for neat chitosan is higher than for the blend, due to the formation of secondary interactions between biopolymers in the blend composition that result in better barrier properties. This confirms the hypothesis about improving chitosan film barrier properties by the formation of blends with gelatine. The addition of complex in a lower amount in blend composition (3 and 5 wt%) results in slight increases in WVTR value, while the addition of inclusion complex in the amount of 7 wt% leads to a greater increase in WVTR. It could be due to a decrease in compatibility between blend compounds (CS, Gel) and the β -CD/LG inclusion complex at higher concentrations of the complex. Decreasing the compatibility leads to the compounds' segregations, which results in easier water vapor permeability [43]. In comparison to the films with a similar composition prepared using the solution-casting method, the WVTR values in this study are higher than those in the investigation of Xu and co-authors [28], but the procedure for the preparation is simpler, avoiding the use of synthetic surfactants as was the case in previous studies [30-32]. However, in comparison to the neat chitosan film, blends possess WVTR values that are increased by 5–9 %. This creates the platform for future improvements in this field, considering the fact that transferring water between the product and its surroundings directly affects the shelf-life of products.

Table 1. Moisture content (MC), total soluble matter (TSM), and water vapour permeability (WVP) with standard deviation values for CS/Gel coating containing different amounts of β-CD/LG inclusion complex

Film	Thickness (μm)	MC (%)	TSM (%)	WVTR (g/m^2h)
CS	0.34 ± 0.2	19.23 ± 1.19	23.12 ± 0.38	99.45 ± 1.9
CS/Gel neat (Control)	0.33 ± 0.02	17.57 ± 1.16	36.53 ± 0.34	90.72 ± 2.3
CS/Gel 3% β-CD/LG	0.34 ± 0.02	16.27± 1.2	37.87 ± 0.5	91.63 ± 3.1
CS/Gel 5% β-CD/LG	0.34 ± 0.02	15.82 ± 1.12	38.35 ± 0.43	92.46 ± 2.7
CS/Gel 7% β-CD/LG	0.34 ± 0.02	14.86 ± 1.23	39.34 ± 0.48	94.59 ± 2.9

Thickness and mechanical properties

The results of the determination of TS and EB are given in Table 2. Blends with gelatine possess better mechanical properties in comparison with a neat CS, which confirms the hypothesis that the formation of polyelectrolyte complexes with gelatine will result in improved mechanical properties. Increasing the content of the inclusion complex in a polymer blend results in an improvement of the mechanical properties – TS and EB, which can be assigned to the secondary interactions (hydrogen bonding, electrostatic forces) between biopolymers and inclusion complex [64]. The formation of a polyelectrolyte complex between CS and Gel results in an improvement of TS by 20.3 % and EB by 22 %, in comparison to the neat chitosan, which is significant. Addition of inclusion complex results in improving TS values for 6.3 to 54.6 % and EB values for 4.9 to 18.3% which present a significant improvement. Incorporation of EOs in biopolymer composition using surfactants leads to a decrease in TS values while the improvement of EB values is much lower in comparison to our study [30, 32] or very similar [31].

Table 2. Tensile strength (TS), and elongation at break (EB) with standard deviation values for CS/Gel coating containing different amounts of β-CD/LG inclusion complex

Film	Tensile strength (TS)	Elongation at break (%)
CS	10.34 ± 0.65	35.34 ± 3.1
CS/Gel neat (Control)	12.44 ± 0.78	43.12 ± 4.5
CS/Gel 3% β-CD/LG	13.23 ± 0.69	45.23 ± 4.1
CS/Gel 5% β-CD/LG	15.45 ± 0.82	47.34 ± 3.9
CS/Gel 7% β-CD/LG	19.23 ± 0.75	51.01 ± 4.2

TGA

Figure 2 shows the TGA curves of a neat blend (control sample) and composite CS/Gel film with β-CD/LG inclusion complex. The results obtained from the derivative thermogravimetric curve (DTG) are summarized in Table 3. The degradation of films is carried out in four steps. Investigated samples expose a very similar degradation pattern - almost identical. The film with inclusion complex has only several degrees higher T5% value, the temperatures of the maximal degradation rate (Table 3), and upper limits of the thermal degradation stage, which is the consequence of the existence of secondary forces between biopolymers and cyclodextrin inclusion complex. The first step, up to 232 °C, with a weight loss of up to 25 % in the thermogram of CS/Gel blend corresponds to the water evaporation, glycerol degradation, and disintegration of smaller side groups. The second, main degradation step (up to 371 °C), with a weight loss of up to 64 %, corresponds to the gelatine decarboxylation, breaking of amide linkages in gelatine, and glycosidic linkages in chitosan [65, 66]. The weight loss in the third (up to 464 °C) and fourth stage (up to 729 °C) are attributed to the complete decomposition of gelatine and chitosan backbone and continuous up to 2.3 % of the residual weight. The first step, up to 234 °C, with a weight loss of up to 23 % in the thermogram of CS/Gel β-CD/LG film corresponds to the loss of the water, glycerol, lemongrass oil, and small side groups disintegration. The second step, up to 373 °C (weight loss of up to 66%) is attributed to the

gelatine decarboxylation, the breaking of amide linkages in gelatine and glycosidic linkages in chitosan, as well as the decomposition of cyclodextrin, which continues in the third step (up to 466 °C) [67]. The fourth stage (up to 800 °C) corresponds to the complete degradation and sample carbonation until 0.12 % of the residual mass.

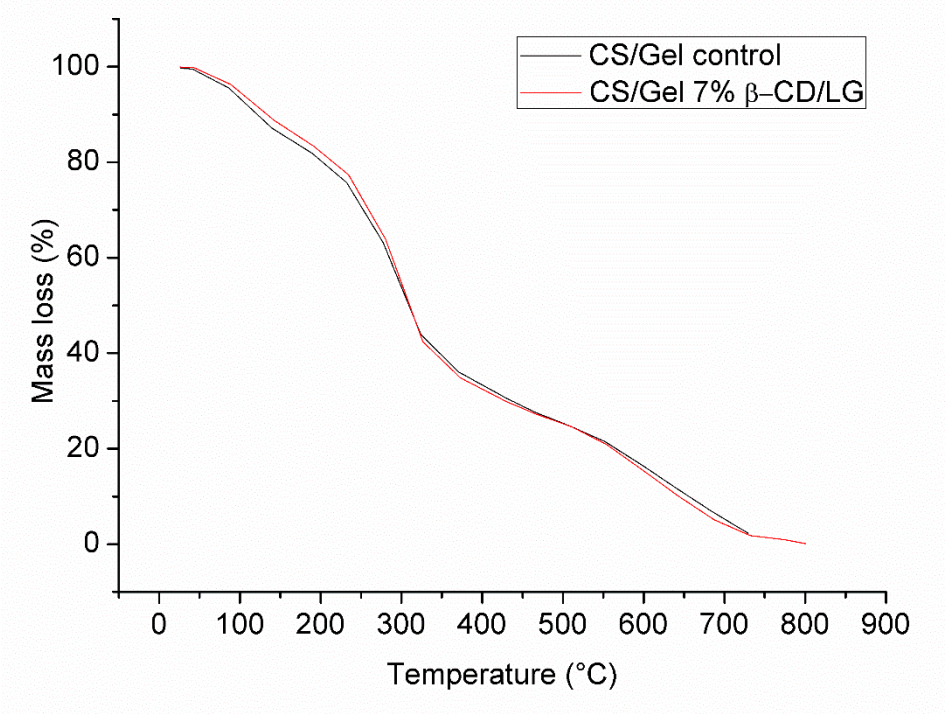


Figure 2. TGA thermograms of control film and film with β-CD/LG inclusion complex.

Table 3. T5% and DTG peak maxima values for control film and film with β-CD/LG inclusion complex

Film	$T_{5\%}$ (°C)	T_{dmaxI} (°C)	T_{dmaxII} (°C)	$T_{dmaxIII}$ (°C)	T_{dmaxIV} (°C)	Residual mass
CS/Gel neat (Control)	86	139	324	429	641	2.3
CS/Gel 5% β-CD/LG	88	142	326	430	643	0.12

3.3. Evaluation of coating antimicrobial effectiveness

Considering the potentiality of lemongrass essential oil as a source of different phytochemicals (Table S1), its antimicrobial activity was tested against a *Penicillium* representative (*P. aurantiogriseum* ATCC 16025) due to the fact that fruits and vegetables are highly susceptible to *Penicillium*-related contamination in the field, during transportation, processing, and storage [68]. The obtained results of antimicrobial screening of 7% LG, as well as CS/Gel control and 7% LG samples, are presented in Table 4 and Figure S2 in Supporting Information (inhibition zone for 7% LG essential oil, CS/Gel control, CS/Gel 7% LG, 3% cycloheximide, and distilled water). Being a good source of antimicrobials, the presence of 7% LG as an individual compound and in the CS/Gel formulation showed a significant fungicide effect compared to the control sample. Lemongrass essential oil's antifungal activity is correlated with its chemical composition (Table S1) and the fact that citral complexes (geranial and neral) have been linked to the inhibition of mycelial growth and spore germination of various *Penicillium strains* [69-72].

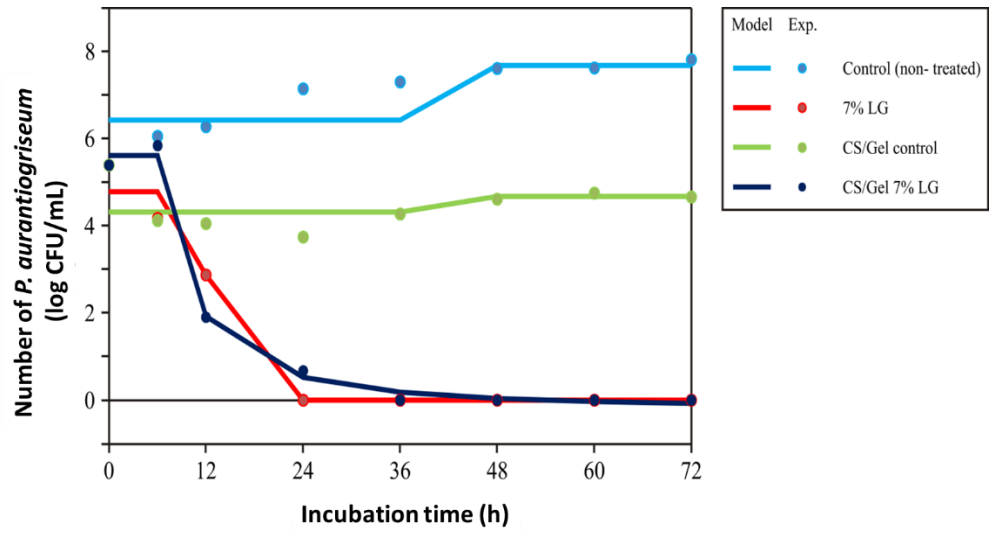
Table 4. Assessment of the antimicrobial effect

Sample	The inhibition zone* (mm) against *Penicillium aurantiogriseum* ATCC 16025	Interpretation of the results (7-22 mm - low activity 22-26 mm - medium activity > 26 mm - high activity)
7% LG essential oil	40.00± 0.00	high activity
CS/Gel control	13.00 ± 0.00	low activity
CS/Gel 7% LG	34.00 ± 1.00	high activity
3% cycloheximide[1]	27.33 ± 0.56	high activity
Distilled water[2]	6.00± 0.00[3]	not exist

* Mean value diameter of the zone including disc (6 mm) ± standard deviation; [1]fungicide -positive control; [2] negative control; [3] the obtained value represents the diameter of the disc (6 mm)

On the other hand, the existence of an inhibition zone in CS/Gel control indicates the potential of chitosan as an antimicrobial agent, but not to the extent that it has a biocide effect. This is in correlation with the previous literature, which deals with chitosan-based films, and the fact that it is always necessary to use a phytochemical-rich source together with a stable concentration of chitosan in coating formulation [20-23, 73-77]. A similar approach in another study based

525 on using of β-cyclodextrins for encapsulation of different essential oils - carvacrol, trans-
526 cinnamaldehyde, and eugenol has resulted in lower antimicrobial activity against investigated
527 food pathogens, although they were used in higher concentrations [78]. The *in vitro*
528 antimicrobial potential of essential oil and CS/Gel formulations can be further clarified by the
529 time-kill or pharmacodynamic kinetic monitoring. In this way, *in vitro* examination of the
530 antimicrobial substance can be measured in view of the antimicrobial activity path as a function
531 of contact time between sensitive microorganisms and targeted concentrations of antimicrobial
532 agent [79]. Figure 1 shows the kinetics models that were developed.



533

534 **Figure 3.** Fungal growth kinetics
535 (markers signify the experimental data; lines indicate predictive results)

536 The growth profile curve for *Penicillium aurantiogriseum* ATCC 16025 indicated the number
537 of live bacterial cells over an incubation period that was not treated with any antimicrobial
538 substance. There are noticeable growth phases for the fungi, which were followed by
539 multiplying the fungal concentration (the final concentration was 7.81 log CFU). Figure 4
540 graphically depicts the pharmacodynamic potential of 7% LG as well as CD/Gel formulation.
541 Kinetics profiles for 7% LG indicate a complete biocide effect for *P. aurantiogriseum* after a
542 contact time of 24 hours, while the same effect was observed for CS/GEL 7% LG for 36 hours.
543 Interestingly, but in correlation with the obtained results in Table 5, the effect of the CS/Gel

control sample did not cause a biocide effect, but an initial decrease in number was detected between 6 and 36 hours of contact time. After that initial phase, a slight increase in the path can be observed until the end of the incubation period, with a final concentration of fungi of 4.66 log CFU. This result confirms once again that the primary structure of the coating is not sufficient to exhibit a biocidal effect, and the addition of a strong biocide agent such as lemongrass essential oil is crucial. Additionally, Table 5 summarizes the regression coefficients of the observed kinetics models for the time-kill study (Figure 4), which explain the speed and intensity of each tested sample.

Table 4. Regression coefficients for fungal growth kinetics

Coefficient	Fungal concentration (log CFU/mL)			
	control (non-treated)	7% LG	CS/Gel control	CS/Gel 7% LG
d	7.680	4.781	4.314	37.326
a	6.430	0.000	4.673	-0.196
c	97.620	-12.239	-104.988	-1.614
b	42.177	12.405	41.318	2.096

The goodness of fit between experimental measurements and model calculated results for the time-kill kinetic study is shown in Table 5. The quality of the model fit was also tested and the residual analysis of the developed predictive model was presented in the same table. The presented four-parameter sigmoidal mathematical model appears to be simple, robust, and accurate. Mathematical models had an insignificant lack of fit tests, which means that all the models represented the data satisfactorily. A high r^2 indicates that the variation was taken into account and that the data fit the proposed model well.

Table 5. The 'goodness of fit' tests for fungal growth kinetic models

Sample	χ^2	RMSE	MBE	MPE	r^2

Control sample (without any layer)	0.363	0.563	0.000	6.670	0.536
Sample with CS/Gel coating control	0.106	0.305	0.000	3.246	0.979
Sample with CS/Gel coating with 7% LG	0.230	0.448	0.000	6.355	0.131
Control sample (without any layer)	0.023	0.143	0.000	3.880	0.996
	Skew	**Kurt**	**Mean**	**StDev**	**Var**
Control sample (without any layer)	-0.145	0.365	0.000	0.602	0.363
Sample with CS/Gel coating control	-0.001	3.500	0.000	0.326	0.106
Sample with CS/Gel coating with 7% LG	1.786	4.572	0.000	0.479	0.230
Control sample (without any layer)	-0.120	-0.708	0.000	0.153	0.023

Abbreviations: Kurt, kurtosis; MBE, mean bias error; Mean, mean of the residuals; MPE, mean percentage error; r^2, coefficient of determination; RMSE, root mean square error; SD, the standard deviation of the residuals; Skew, skewness; Var, the variance of the residuals; χ^2, reduced chi^2 square.

The antimicrobial effectiveness of three coatings was tested in triplicate using artificially contaminated samples with fungi spores: control (no coating), sample with CS/Gel coating control, and sample with CS/Gel coating with β-CD/7%LG inclusion complex. In brief, the effect of CS/Gel coating on tomato fruits in artificially inoculated cherry tomatoes was evaluated by estimating the affecting area with fungal contamination during the 20-day cold storage period. Selected photographs are presented in Figure 4.

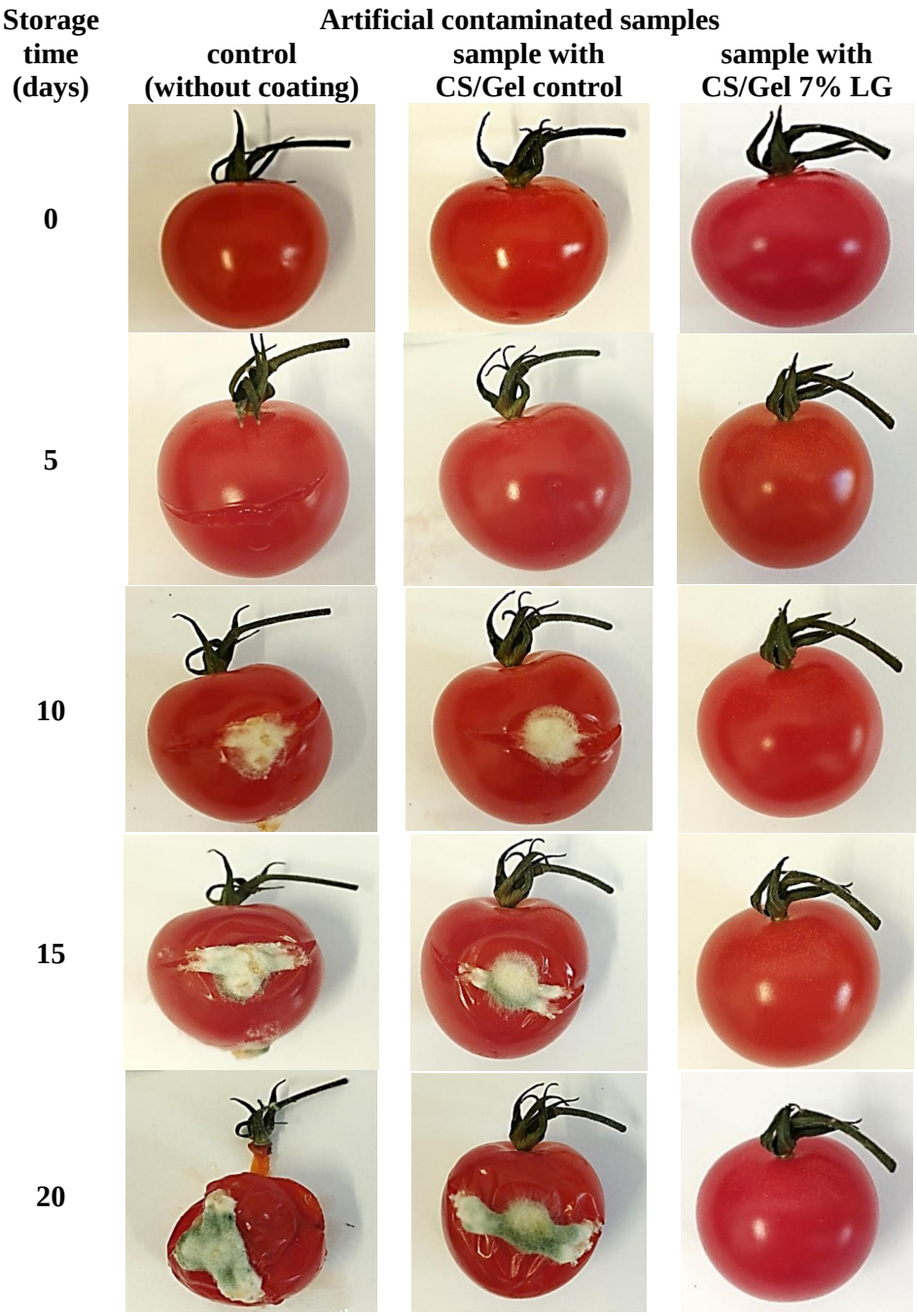


Figure 4. Digital photographs of tomatoes during cold storage

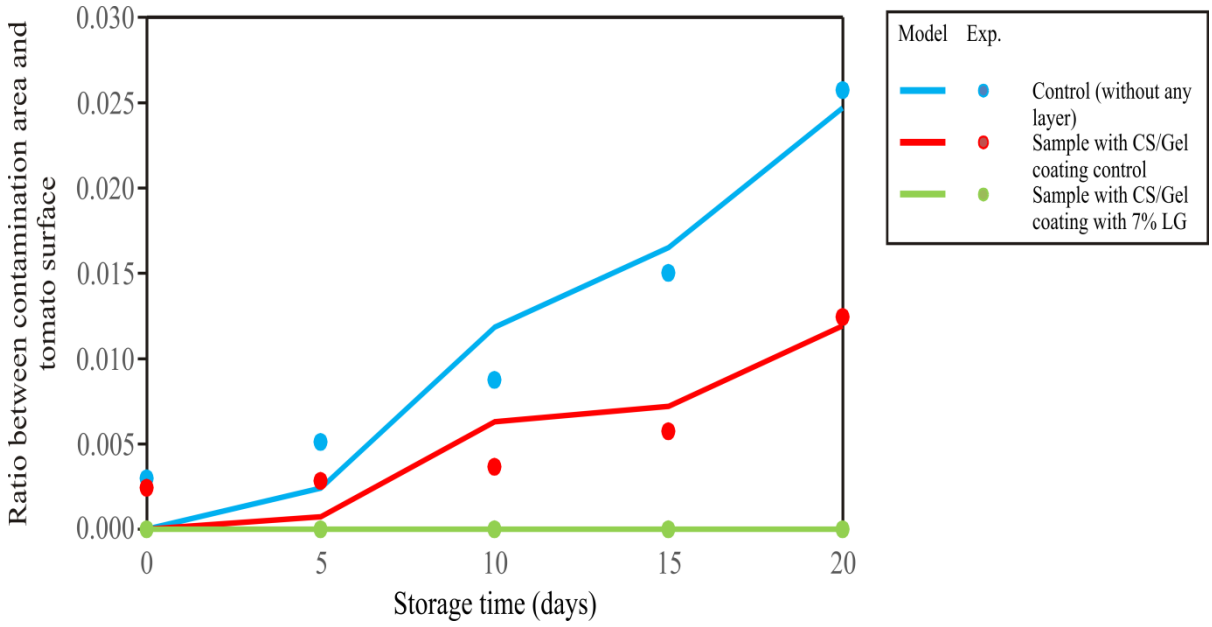
The obtained surface area of tomato samples as well as a ratio between the contamination area and the surface area of the tomato sample (calculated as an ellipsoid) is presented in Table 6. The data is calculated based on digital images. The coating of cherry tomatoes with a 7% LG coating resulted in visually significant differences in appearance compared with control samples.

582 **Table 6.** The ratio between the contamination area and the surface area of the tomato sample

Storage time (days)	Artificial contaminated samples		
	Control sample (without any layer)	Sample with CS/Gel coating control	Sample with CS/Gel coating with 7% LG
0	0.000	0.000	0.000
5	0.002	0.001	0.000
10	0.012	0.006	0.000
15	0.017	0.007	0.000
20	0.025	0.012	0.000

583

584 The antimicrobial effect of the LG in the CS/Gel formulation was found to be prolonged during
585 storage due to the absence of fungal contamination on the surface of these samples (Figure 5,
586 Table 7). A strong fungicide effect on tomatoes was achieved by using a 7% LG in a CS/Gel
587 coating formulation for the first time. The applied coating system can be the solution for the
588 long storage of tomato samples in conditions of commercial application in markets when
589 tomatoes are displayed in refrigerated display cases. Regardless of the obtained results of
590 antimicrobial efficacy, in future steps, it is necessary to examine the impact of the proposed
591 coating system on the physicochemical characteristics of tomatoes as well as the possibility of
592 coating a larger number of samples for commercial use. The obtained kinetics models for the
593 evaluation step are presented in Figure 5, while Table 8 summarizes the regression coefficients
594 of the observed kinetics models (Figure 6), which explain the speed and intensity of each
595 sample.



596

597 **Figure 5**. Kinetic study for evaluation of coating antimicrobial effectiveness based on the
598 ratio between contamination area and the surface area of the tomato sample (markers signify
599 the experimental data; lines indicate predictive results).

600

601 **Table 8.** Regression coefficients for evaluation of coating antimicrobial effectiveness based on
602 ratio between contamination area and the surface area of the tomato sample

Coefficient	Control sample (without any layer)	Sample with CS/Gel coating control	Sample with CS/Gel coating with 7% LG
a	7.680	4.781	0.000
b	6.430	0.000	0.000

603

604 The goodness of fit, between experimental measurements and model calculated results is
605 shown in Table 9. The quality of the model fit was also tested, and the residual analysis of the
606 developed predictive model was presented in the same table. The presented four-parameter
607 sigmoidal mathematical model appears to be simple, robust, and accurate. Mathematical
608 models had an insignificant lack of fit tests, which means that all the models represented the
609 data satisfactorily. An r^2 between 0.818 and 1.000 indicates that the variation was taken into
610 account and that the data fit adequately to the proposed model.

611

Table 9. The 'goodness of fit' tests for or evaluation of coating antimicrobial effectiveness based on ratio between the contamination area and the surface area of the tomato sample

Sample	χ^2	RMSE	MBE	MPE	r^2
Control sample (without any layer)	0.000	0.001	0.000	31.709	0.936
Sample with CS/Gel coating control	0.000	0.001	0.000	75.585	0.818
Sample with CS/Gel coating with 7% LG	0.000	0.000	0.000	0.000	1.000
	Skew	**Kurt**	**Mean**	**StDev**	**Var**
Control sample (without any layer)	0.467	-2.255	0.000	0.001	0.000
Sample with CS/Gel coating control	0.235	-2.782	0.000	0.001	0.000
Sample with CS/Gel coating with 7% LG	0.000	0.000	0.000	0.000	0.000

Abbreviations: Kurt, kurtosis; MBE, mean bias error; Mean, mean of the residuals; MPE, mean percentage error; R2, coefficient of determination; RMSE, root mean square error; SD, standard deviation of the residuals; Skew, skewness; Var, variance of the residuals; $\chi 2$, reduced chi^2 square.

4. CONCLUSIONS

The formulation of an innovative bio-based chitosan/gelatine (CS/Gel) coating with a β-cyclodextrin/lemongrass essential oil inclusion complex using a low amount of antimicrobial compounds (lemongrass oil) exemplifies the novelty of this paper. This system has been employed for the food application of an antimicrobial coating on freshly harvested cherry tomatoes. According to the obtained results, the high antimicrobial activity and coating effect improved the shelf-life of fruit samples for 20 days during cold storage. The use of β-cyclodextrin/lemongrass essential oil inclusion complex at an optimized concentration in the coating formulation has created a framework for obtaining an edible coating system whose active function has been proven through step-by-step evaluation protocols during antimicrobial profiling. As the final output of this work, complete growth inhibition of *Penicillium aurantiogriseum*, one of the main causes of fruit spoilage, was demonstrated. For the first time,

predictive capacity and advanced mathematical modeling were used in in situ studies of this type of coating system's antimicrobial evaluation. The obtained results of coating of cherry tomato by CS/Gel formulation with 7 % of β-cyclodextrin/lemon grass essential oil inclusion complex results in a significantly greater shelf-life of cherry tomato samples, but further functionality and universality of the coating system have to be validated in further investigation. In summary, this comprehensive study can be a base for manufacturing edible, bio-based, and cost-effective coating systems for perishable fresh fruits.

CRediT authorship contribution statement

All authors have a significant intellectual contribution to the work, have read the revised manuscript, and concur with the submission. The article submitted is original work and has not been published elsewhere, either completely, in part, or in another form. All the authors declare no competing interests.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript.

Tamara Erceg – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Olja Šovljanski – idea, experiments, analyzing, writing, and revising of the manuscript, preparation of figures.

Alena Stupar – idea, experiment, revising of the manuscript.

Jovana Ugarković – experiments.

Milica Aćimović – experiments, writing, and revising of the manuscript.

Lato Pezo – modeling, calculations, writing of the manuscript.

Ana Tomić – experiments, revising of the manuscript.

Marina Todosijević – experiments, revising of the manuscript.

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Declaration of competing interest

The authors declare no competing financial interest.

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Thank you very much for all your observations. The changes were incorporated into the text.

4. Could the manuscript benefit from additional tables or figures, or from improving or removing (some of the) existing ones?

Please provide specific suggestions for improvements, removals, or additions of figures or tables. Please number each suggestion so that author(s) can more easily respond.

Reviewer #1: yes

Reviewer #2: it is alright now

The changes have been made and marked in red ink.

5. If applicable, are the interpretation of results and study conclusions supported by the data?

7. Have the authors clearly stated the limitations of their study/methods?

Please list the limitations that the author(s) need to add or emphasize. Please number each limitation so that author(s) can more easily respond.

Limitations along with strengths have been added in each section and marked in red ink.

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Reviewer #1: yes

Reviewer #2: yes

Structure, flow, and writing has been improved (red ink).

9. Could the manuscript benefit from language editing?

Reviewer #1: Yes

Reviewer #2: No

The language has been edited in the whole text.

Reviewer #2:

equation 6,7,8,9 were incorporated but not explained the parameters name, mention the terms properly used in equation 6,7,8,9 etc.

AUTHORS: Thank you very much for this observation, parameter names are listed after equations 6-9.

A comprehensive approach to chitosan-gelatine edible coating with β-cyclodextrin/lemongrass essential oil inclusion complex - characterization and food application

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ABSTRACT

Biopolymer-based films present an ideal matrix for the incorporation of active substances such as antimicrobial agents, giving active packaging a framework of *green* chemistry and a step forward in food packaging technology. The chitosan-gelatine active coating has been prepared using lemongrass oil as an antimicrobial compound applying a different approach. Instead of surfactants, to achieve compatibilization of compounds, β-cyclodextrin was used to encapsulate lemongrass oil. The antimicrobial effect was assessed using the dip-coating method on freshly harvested cherry tomatoes artificially contaminated by *Penicillium aurantiogriseum* during 20 days of cold storage. According to the evaluation of the antimicrobial effect of coating formulation on cherry tomato samples, which was mathematically assessed by predictive kinetic models and digital imaging, the applied coating formulation was found to be very effective since the development of fungal contamination for active-coated samples was observed for 20 days.

Keywords: edible coating; chitosan; *Cymbopogon citratus*; antimicrobial coating; cherry tomato; food safety.