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Effect of Chitosan–Tomato Plant Extract Edible Coating on the Quality, Shelf Life, and Antioxidant Capacity of Pork during Refrigerated Storage

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Abstract: The aim of this study was to evaluate the effectiveness of chitosan–tomato plant extract (C-TPE) edible coating (EC) on the physicochemical, microbiological, sensory, and antioxidant capacity changes of pork during storage. Edible coatings prepared with chitosan 1%, acetic acid 1%, glycerol, and TPE (0.1% and 0.3%) were tested. Slices of pork were submerged in different treatments (T1: C 1%; T2: C 1% + TPE 0.1%; T3: C 1% + TPE 0.3%; T4: control) and stored at 4 °C. The different treatments showed the best results in physicochemical and microbiological analyses, with reduced microbial population relative to the control. The highest antioxidant capacity and total phenolic content were shown in T3, and the overall acceptance was better in T2. The results show that the application of C with the addition of natural extracts, such as the tomato plant with antioxidant and antimicrobial properties, can be an alternative method for preserving pork.

Keywords: chitosan; edible coating; meat products; quality; byproducts

1. Introduction

Consumer demand for food that retains high-quality characteristics has increased rapidly in recent years. Pork is the most highly produced meat in the world [1] and one of the most nutritious foods due to its high contents of protein, lipids, vitamins, and minerals of high biological value [2]. However, these multiple properties make it a highly perishable food, resulting in postmortem changes that modify its characteristics due to bacterial growth and lipid oxidation, which are the major processes leading to the deterioration of meat quality [3]. These problems cause economic losses for the food industry because of the short shelf life of meat. This outcome has led to the development of new technologies for food safety [4,5]. Among these alternative methods is the use of low temperature, active packaging, synthetic antioxidants [6–8], and edible coatings (ECs).

The use of ECs in food preservation offers several advantages over synthetic materials, including that ECs are biodegradable and environmentally friendly. Chitosan, which is derived from chitin, the most abundant polymer in nature, has promising antimicrobial potential and is obtained from crustacean cuticles, arthropod exoskeletons, cell walls of fungi, and mainly from the shells of shrimp [9]. The abilities of ECs include good gel formation, reduced moisture loss and aromas, and oxygen permeability. These abilities can be improved by additives such as antioxidants derived from natural sources [10,11]. Edible chitosan films provide some characteristics that help to preserve the freshness of meat [12–14]; moreover, these types of films present some antimicrobial activity against pathogens and spoilage bacteria in foods and act as antioxidant agents [15,16].

On the other hand, chitosan ECs can act as carriers of different substances; for example, some plant extracts (with antioxidant and antimicrobial properties) can be incorporated into corresponding suspensions to add functionality and obtain chitosan ECs with antioxidant and antimicrobial properties. Leaves and stems of tomato plants represent agro-industrial byproducts because they are discarded after harvesting; however, they possess bioactive substances with interesting properties [17,18]. Tomato plant extracts have been incorporated into edible chitosan coatings to maintain quality in fish and chicken [14,19].

It has been reported that the use of edible coatings with extracts as a protective approach can preserve food products (fruits, vegetables, and meat) with higher quality and safety, while prolonging the shelf life [20–23]. Gelatin EC with grapefruit seed extract (GSE) was found to inhibit *E. coli* O157:H7 and *L. monocytogenes* in pork loin [24]. Moreover, gelatin EC significantly reduced the color deterioration of pork [25]. There are, however, few reports about the use of chitosan EC in pork. Chantarasataporn et al. [26] showed that an oligochitosan concentration of 0.4% reduced the growth of *Enterobacteriaceae* and *Staphylococcus* in minced pork by 1 and 2 Log colony-forming units (CFU)/g, respectively. He et al. [27] reported that chitosan EC with clove oil on sliced pork reduced total viable counts by 3.09 Log CFU/g compared to uncoated samples.

The addition of natural extracts to ECs is intended to potentiate their effects on food conservation by exploiting their properties against bacteria and their use as antioxidants [28]. However, no studies have been found on the efficacy of chitosan EC with tomato plant extract acid in pork. In this study, we evaluated the effect of tomato plant extract as an antimicrobial and antioxidant compound incorporated into an edible chitosan-based coating. Physicochemical, microbiological, sensory, and antioxidant capacity analyses were carried out on coated and uncoated fresh pork loin.

2. Materials and Methods

2.1. Reagents

Potassium persulfate; 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS); 2,2-diphenyl-1picrylhydrazyl (DPPH); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent, sodium carbonate, hydrochloric acid, hydrogen peroxide 3%, acetic acid, red-violet bile agar, trypticase soy agar, phosphate buffer, and sodium chloride were purchased from JT Baker (Baker-Mallinckrodt, Mexico, Mexico). Sodium hydroxide was from Merck (Merck-Darmstadt, Germany), and glycerol was from HYCEL (Zapopan, Jalisco, Mexico).

2.2. Pork and Plant Material

Eight pieces of pork loin were acquired from a local distributor (samples probably came from different animals). Pork loin samples were obtained from local distributors of Cd. Obregon, Sonora. The samples were placed in a sealed cooler layered with ice between the samples and then transported to the Laboratory of Sanitary Microbiology and Food Safety at the Instituto Tecnológico de Sonora, where they were stored for further analysis.

Residues of tomato plants (*Lycopersicon esculentum*) of the Pitenza variety were used in the current study, and they were obtained from greenhouses in the Yaqui Valley, Sonora, México. Twenty fresh sample plants were collected and washed with distilled water. The plants were dried at 45 °C for 24 h, and later were pulverized (by a food processor) and passed through a No. 20 sieve (WS Tyler).

2.3. Chitosan

Chitosan was obtained via the thermo-alkaline deacetylation of chitin as follows: 1 g of chitin was homogenized with 15 mL of 50% w/v NaOH at 95 °C for 2 h as described in Reference [29]. The degree of acetylation of the chitosan used in this study was 34%, with an average molecular weight of 128 kDa, as previously described in Reference [16].

2.4. Preparation of Extracts

Tomato plant extracts were obtained using the methodology described in Reference [18]. First, 35 g of dried sample was mixed with a solution of ethanol 95%, and maceration was carried out via constant stirring for 72 h in complete darkness at room temperature. The sample was subsequently vacuum filtered through Whatman No. 1 paper and concentrated by evaporation using a rotatory evaporator (Buchi R-200 Rotavapor). The extract was lyophilized for 48 h (FreeZone 4.5, Labconco), and then maintained at −20 °C for subsequent analysis.

2.5. Preparation and Application of EC

The 8 pieces of pork loin were sliced (average weight of slices ranged between 100 and 115 g, and thickness was approximately 1.5 cm) and then homogenized to form 4 batches of 21 slices each, corresponding to one batch per treatment. Each treatment was applied to 21 randomized slices from the 8 pieces.

Emulsions were prepared using 1% chitosan (C) with glycerol (1%) as a plasticizer, dissolved using distilled water with acetic acid (1%), and then stirred until the total dissolution of the components. Later, tomato plant extract (TPE) at concentrations of 0.1% and 0.3% was added. The solution was homogenized at 15,500 rpm [11] until homogenization was complete. Four treatments were prepared: T1 (C 1%), T2 (C 1% + TPE 0.1%), T3 (C 1% + TPE 0.3%), and T4 (control). The control was pork slices with no edible coating treatment. Finally, 21 pork loin slices (the average weight of slices ranged between 100–115 g and the thickness was approximately 1.5 cm) were immersed for 1 min in the solution and allowed to dry before storage. Four treatments were prepared: T1 (C 1%), T2 (C 1% + TPE 0.1%), T3 (C 1% + TPE 0.3%) and T4 (control). The control was the pork slice with no edible coating treatment. The pork samples with and without edible coating were packaged and stored in plastic trays with food-grade polyethylene (one slice per plastic tray), and then stored at 4 °C for 21 days. Samples (3 plastic trays, 3 slices) were taken on days 0, 1, 4, 8, 12, 16, and 21 for physicochemical, microbiological, sensory, and antioxidant analyses. All this was done twice.

2.6. Microbiological Analysis

Pork samples (10 g) were homogenized with sterile phosphate buffer (90 mL) using a Stomacher blender (Model 400) for 2 min at 230 rpm. A factor of 10 serially diluted the homogenate. For each dilution, 1 mL was plated on each medium according to Official Mexican Standards. The total microbial count was determined according to the parameters established in Reference [30] for aerobic mesophilic and psychrophilic bacteria, in which the trypticase soy agar (TSA) procedure was performed. The total coliform was determined according to Reference [31] using red-violet bile agar. The plates with mesophilic and total coliform bacteria were incubated at 37 °C for 24–48 h, and the plates with psychrophilic bacteria were incubated at 5 °C for 7–10 days. The results were expressed as Log CFU/g.

2.7. Physicochemical Analysis

pH was determined based on the method described in Reference [32]. A 10 g portion of the sample was mixed with 50 mL of distilled water, and the pH was measured using a digital pH meter (model 213, Hanna Instruments, Woonsocket, RI, USA).

For the color measurement, 3 pork slices of each treatment were chosen, from which 5 mediations were taken. Color was measured by tri-stimulus colorimetry using a system with a colorimeter (model SP6, X-Rite, Grand Rapids, MI, USA). Color coordinates for degree of lightness (L), redness/greenness (+a/–a), and yellowness/blueness (+b/–b) were obtained.

Exudate loss (EL) was determined by measuring the weight before and after a certain period according to each sampling day. The results were expressed in %.

Water retention capacity (WRC) was determined in raw samples using the method described in Reference [33] with modifications. The 5 g sample was finely minced, then 8 mL of NaCl (0.6 M) was added, and the sample was subsequently stirred for 1 min and placed in an ice bath for 30 min. The sample was then centrifuged at 11,500 rpm for 15 min at 4 °C, and the supernatant was recovered. WRC in cooked samples was determined as follows: meat samples were weighed, wrapped in aluminum foil, and subjected to a temperature of 165 °C in an electric grill to an internal temperature of 70 °C (10 min for each side), as measured by a penetration thermometer (Thermco®, Lafayette, NJ, USA). The samples were cooled for 30 min at room temperature (25 °C), and the final weight was recorded. WRC was expressed as the loss of water with respect to the original content (%) in the raw and cooked samples.

Shear force values were expressed as the N of raw and cooked samples, as measured in a texturometer (Food Technology Corp., Sterling, VA, USA). The samples were cut into 30 mm × 10 mm × 10 mm pieces and transverse force in the direction of the muscle fiber was applied.

2.8. Antioxidant Capacity

For the preparation of the extract, 10 g of the sample was homogenized with 50 mL of 10% phosphate buffer (pH 7). The sample was centrifuged at 12,000 rpm for 60 min at 4 °C. The obtained supernatant was used to estimate ABTS, DPPH, and total phenolics.

The ABTS assay was conducted according to the method described in Reference [34] with some modifications. ABTS radical cations were generated in a mixture of 5 mL of a 7 mmol ABTS solution and 88 µL of a 0.139 mmol $K_2S_2O_8$ solution. The mixture was incubated in darkness at room temperature for 16 h (stock solution). After incubation, one mL of stock solution was added to 30 mL of ethanol, and the solution was adjusted at an absorbance of 0.7 ± 0.02 nm at 750 nm using a microplate reader (iMark 168–1135, Bio-Rad, Tokyo, Japan). For the assay, 285 µL of ABTS and 15 µL of the extract were mixed and placed in a microplate, and the absorbance was read after 7 min of incubation in darkness. Trolox was used as a standard, the antioxidant capacity was calculated using a regression equation between the Trolox concentration and the absorbance, and the results were expressed as µmol eq. Trolox/g of meat.

The DPPH assay was conducted according to the method described in Reference [35], with some modifications. For the preparation of the DPPH radical, 0.0025 g was dissolved in 100 mL of methanol, and the solution was adjusted at an absorbance of 0.7 ± 0.02 using a microplate reader at 490 nm (iMark 168–1135, Bio-Rad, Tokyo, Japan). The assay was performed with 20 µL extract placed in the microplate and mixed with 280 µL of DPPH radical and incubated in the dark for 30 min at room temperature. The absorbance was read using a microplate reader at 490 nm. Trolox was used as a standard, and the results were expressed as µmol eq. Trolox/g of meat.

The concentration of total phenol content was determined using the method described in Reference [35] with modifications. The reaction was performed by combining 66 µL of the extract with 134 µL of Folin–Ciocalteau reagent and 200 µL of sodium carbonate. The mixture was stirred manually and then incubated at room temperature for 1 h and filtered. The absorbance was read at 750 nm in a

microplate reader (iMark 168–1135, Bio-Rad, Tokyo, Japan). The total phenolic content was expressed as mg of gallic acid equivalent (GAE)/g of meat.

2.9. Sensory Evaluation

A group of 10 semi-trained panelists scored the samples for odor, flavor, color, texture, and overall acceptability on each day of storage. A 9-point hedonic scale (9 = like extremely; 8 = like very much, 7 = like moderately; 6 = like slightly, 5 = do not like or dislike; 4 = dislike a little; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely) was used to classify the samples. The score of each sample was determined by calculating the mean value. A score of 5 or below was considered to mean that the meat was unsalable. Before sensory evaluation, the pork loin slices of the different treatments were grilled on a hot plate at 190 °C for 4 min on each side cooked previously.

2.10. Experimental Design and Statistical Analysis

The experiment was conducted twice (with 3 replications each time), and each determination was performed in triplicate. The experiment was performed by applying a randomized complete block design in which the day of sampling was considered a block. The statistical significance of the differences between treatments was determined using ANOVA followed by the Tukey–Kramer test to compare mean values for different storage days. The results were expressed as mean values ± SD, and the level of significance was $p < 0.05$. Statistical tests were performed using Statgraphics Plus v. 5.1.

3. Results and Discussion

3.1. Microbiological Analysis

Meat is highly perishable due to characteristics such as pH, nutritional content, and water activity, among others. Additionally, meat products are highly susceptible to microbial growth and pathogenic microorganisms. As shown in Figure 1, the initial count of aerobic mesophilic microorganisms was higher than 1 Log CFU/g of meat for all treatments, decreasing on day 4 for T1 and day 8 for T2 and T3, reaching a value of 0.2 Log CFU/g. However, a progressive increase was observed for T4 until reaching more than 3 Log CFU/g, showing a significant difference ($p > 0.05$) between treatments, with T3 being the most effective treatment for bacterial growth reduction, followed by T2 and T1.

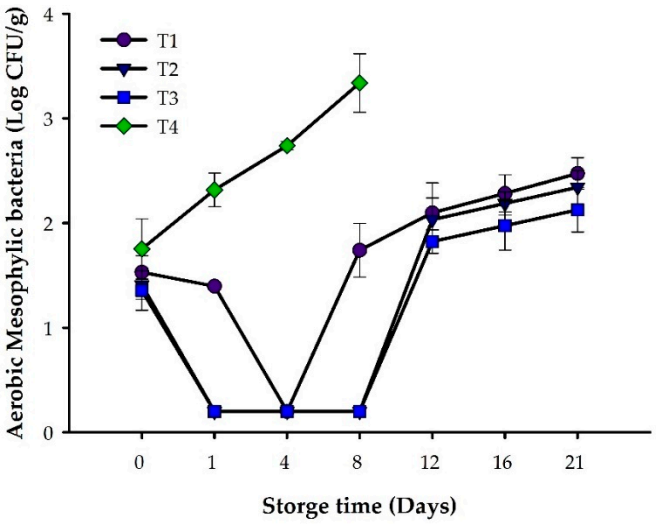

Figure 1. Total aerobic mesophilic bacteria count in pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

Figure 2 shows the effect of treatments on total coliforms, with an immediate effect obtained on day 0 starting at 0.2 Log CFU/g and reaching notable growth on day 4 for T1, and day 8 for T2 and T3, with significant differences between these treatments concerning the control. The control reached values higher than 3 Log CFU/g on the final storage day, while T1, T2, and T3 showed a reduction by 1 Log CFU/g. These results were similar to the findings of Lee et al. [36], who evaluated microperforated films in pork medallions and obtained a minimal reduction of 0.2 Log CFU/g on day 1 in aerobic mesophilic bacteria and total coliforms. This reduction was maintained until day 7 in storage, followed by a significant increase until day 14. However, Chang et al. [37] evaluated edible chitosan coating and glucose concentrations in pork meat over the course of 7 days and found lower values than the present study. The authors observed values of 2.68 Log CFU/g in treatments, that were higher than the control at the initial day of the storage period to aerobic mesophilic, followed by increased growth of the microbial population until the end of the storage time. Meanwhile, a previous study [10] reported higher values than the present study, where values increased during storage time (12 days) using edible chitosan coating with natural extract and obtaining fast growth in the control compared to the coating treatments, which reached a reduction of 3–4 Log CFU/g. This effect could be due to a wide variety of factors, such as molecular weight, concentration, viscosity, deacetylation grade, pH, and temperature, which have been reported to influence chitosan antimicrobial capacity [38].

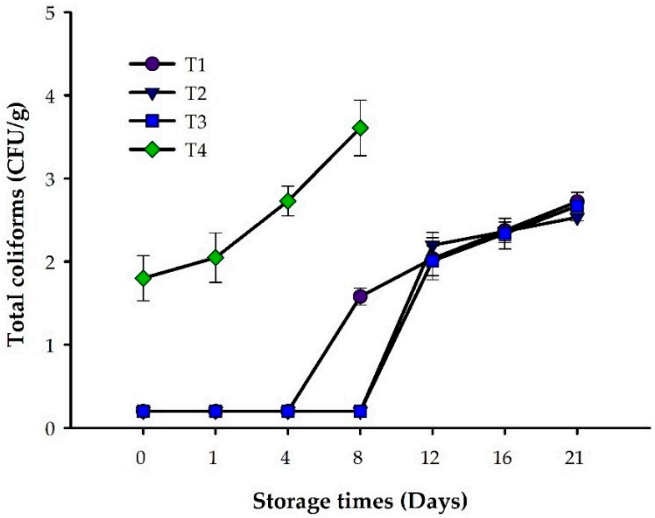

Figure 2. Total coliform bacteria count in pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

Psychrophilic bacterial growth (Figure 3) was initially at 0.2 Log CFU/g for all treatments, and this growth increased as storage time advanced for T4 at day 4, T3 at day 8, and T2 at day 12, showing significant differences ($p > 0.05$) and reaching a reduction of 3 Log CFU/g at the end of storage. T2 obtained the best result, followed by T3 and T1. Chang et al. [28] evaluated edible chitosan coating with glucose concentration and observed a similar behavior, i.e., a reduction of 1 Log CFU/g. They mentioned that the preservation effect of edible chitosan coating in pork could be a consequence of the added antimicrobial compounds thereby improving the attributes of the samples.

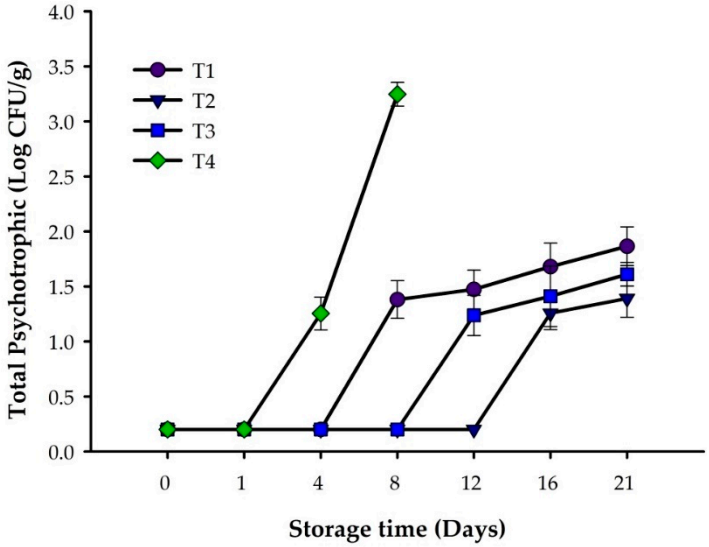

Figure 3. Total psychrotrophic bacteria count in pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

3.2. Physicochemical Analysis

pH measurement in meat is used as a quality indicator. In Table 1, the pH effect shows initial values ranging between 5.23 and 5.49, with a significant difference ($p < 0.05$) between treatments. Meanwhile, at the end of the storage period, all treatments were higher than pH 5.4. T1 was the highest (5.74), followed by T2 and T3 (5.57 and 5.46, respectively), and all treatments were lower than T4 (6.64). A previous study [37] reported similar results in pork, with initial pH values ranging between 5.65 and 5.68, while the addition of 1% chitosan solution with glucose resulted in a slightly lower final value (5.63). However, Reference [10] evaluated the effect of chitosan, ginger, onion, and garlic water extracts in sweetened pork and obtained lower initial values in the treatments (5.47–5.89), with respect to the control (5.92). This indicated that the addition of the extract and chitosan decreased initial pH values in pork, which increased slightly with the storage period. This phenomenon resembled the findings of the present study. Low values can be due to either the lactic acid concentration produced by anaerobic glycolysis or the addition of acetic acid in the edible chitosan coating formulation. Nevertheless, during the latest stages of the storage period, pH values increased due to autolytic activity or microbial metabolism [10].

Color in fresh meat is the principal attribute that consumers pay attention to, considering that color is an indicator of freshness and quality [39]. Table 1 shows the effect of the treatments on pork, indicating L^* values in a range between 55 and 59 during the initial day, with a slight increase during the storage period, reaching final values of 58.34, 59.69, and 58.89 for T1, T2, and T3, respectively. These values were higher than the control (T4), which had an L^* value of 51.59 at the end of its shelf life (day 8). Another study [36] obtained lower values in pork treated with microperforated and nonperforated polypropylene film, with initial L^* values of 46.22 and 49.75, respectively, and at day 14 the values were 40.08 and 45.56, higher than the control (28.59). Meanwhile, Reference [37] reported initial L^* values higher than the control (60.91) in fresh pork treated with 1% chitosan solution in 1%, 1.5%, and 2% concentrations. This increase may be due to the absorption of solutions by the meat during immersion, and this may increase the light reflection on the sample surfaces, subsequently increasing the L^* values [37]. Regarding the a^* parameter, the initial values ranged from −0.19 to −1.39, showing significant differences ($p > 0.05$) between treatments. The increase was significant at day 8, reaching values of 4.12, 3.55, 3.1, and 1.86 for T2, T1, T4, and T3, respectively, which decreased to final values of 0.89 (T1), 1.01 (T2), and 1.88 (T3). Meanwhile, initial b^* values ranged from 5 to 7 for all treatments, showing a progressive increase on day 8, followed by a decrease until day 21, reaching values of 9.39 (T1), 9.22 (T2), and 11.24 (T3), that were lower than the control (13.86). A previous study [40] evaluated

edible coatings with the addition of microencapsulated spicy radish extract in pork oxidation, and it reported higher a^* values (13 for treatment, 17 for control) and lower b^* values (6 for treatment, 7 for control) compared to the present study. These values may be affected by oxidation compounds in the muscle, where low values indicate opacity [41].

Table 1. Effect of chitosan–tomato plant extract coatings on the physicochemical parameters of pork fillet during refrigerated storage for 21 days.

Parameter	Treatment	Storage Time (Days)						
		0	1	4	8	12	16	21
pH	T1	5.34 ± 0.02 ^{a}	5.36 ± 0.01 ^{a}	5.41 ± 0.00 ^{a}	5.54 ± 0.02 ^{a}	5.54 ± 0.02 ^{a}	5.63 ± 0.07 ^{a}	5.74 ± 0.02 ^{a}
	T2	5.23 ± 0.04 ^{b}	5.29 ± 0.00 ^{b}	5.34 ± 0.01 ^{b}	5.41 ± 0.00 ^{b}	5.47 ± 0.03 ^{b}	5.47 ± 0.07 ^{b}	5.57 ± 0.02 ^{b}
	T3	5.23 ± 0.01 ^{b}	5.30 ± 0.01 ^{b}	5.32 ± 0.02 ^{b}	5.33 ± 0.01 ^{c}	5.36 ± 0.01 ^{c}	5.41 ± 0.02 ^{c}	5.46 ± 0.00 ^{c}
	T4	5.49 ± 0.03 ^{c}	5.52 ± 0.05 ^{c}	5.70 ± 0.01 ^{c}	6.64 ± 0.02 ^{d}	NE	NE	NE
L*	T1	55.88 ± 1.93 ^{a}	56.08 ± 1.29 ^{a,c,d}	55.09 ± 1.58 ^{a,c}	54.15 ± 1.34 ^{a}	57.20 ± 1.36 ^{a}	57.96 ± 1.45 ^{a}	58.34 ± 1.24 ^{a,c}
	T2	59.68 ± 1.23 ^{b}	58.84 ± 2.48 ^{b}	59.45 ± 2.00 ^{b}	53.01 ± 1.43 ^{a}	59.81 ± 2.79 ^{b}	60.40 ± 2.07 ^{b}	59.69 ± 1.81 ^{b,c}
	T3	55.94 ± 2.04 ^{a}	56.64 ± 1.18 ^{c}	56.18 ± 1.10 ^{c}	53.00 ± 0.61 ^{a}	58.86 ± 1.20 ^{b}	58.73 ± 1.24 ^{a}	58.89 ± 0.94 ^{c}
	T4	58.19 ± 0.63 ^{c}	55.37 ± 2.00 ^{d}	54.92 ± 1.41 ^{c}	51.59 ± 1.40 ^{b}	NE	NE	NE
a*	T1	−0.93 ± 0.34 ^{a}	0.60 ± 0.38 ^{a}	1.35 ± 0.25 ^{a}	3.55 ± 1.87 ^{a}	1.65 ± 0.25 ^{a}	1.47 ± 0.35 ^{a}	0.89 ± 0.31 ^{a}
	T2	−1.39 ± 0.64 ^{b}	0.29 ± 0.79 ^{a}	0.37 ± 0.80 ^{b}	4.12 ± 0.89 ^{a,b}	1.61 ± 0.77 ^{a}	1.03 ± 0.75 ^{a}	1.01 ± 0.78 ^{a}
	T3	−0.19 ± 0.54 ^{c}	1.04 ± 0.56 ^{a}	1.57 ± 0.70 ^{b,c}	1.86 ± 0.50 ^{b}	1.18 ± 0.70 ^{a}	1.03 ± 0.48 ^{a}	1.88 ± 1.81 ^{a}
	T4	−0.58 ± 0.68 ^{d}	1.72 ± 0.51 ^{b}	2.06 ± 0.44 ^{c}	3.1 ± 1.33 ^{b}	NE	NE	NE
b*	T1	5.30 ± 0.51 ^{a}	7.83 ± 0.67 ^{a}	8.55 ± 0.53 ^{a}	12.36 ± 1.33 ^{a}	9.05 ± 0.38 ^{a}	9.46 ± 0.38 ^{a}	9.39 ± 0.45 ^{a}
	T2	5.95 ± 1.25 ^{a}	5.72 ± 1.21 ^{a}	8.36 ± 0.80 ^{a}	16.41 ± 1.35 ^{b}	9.42 ± 0.97 ^{a}	8.93 ± 0.68 ^{a}	9.22 ± 0.88 ^{a}
	T3	7.10 ± 0.61 ^{b}	10.30 ± 0.82 ^{b}	10.68 ± 0.90 ^{b}	12.28 ± 0.78 ^{a}	11.12 ± 0.92 ^{b}	10.87 ± 0.91 ^{b}	11.24 ± 0.98 ^{b}
	T4	7.77 ± 1.19 ^{b}	8.77 ± 0.77 ^{c}	9.32 ± 0.49 ^{c}	13.86 ± 1.40 ^{a}	NE	NE	NE
Exudate loss (%)	T1	4.65 ± 0.45 ^{a}	4.14 ± 0.59 ^{a}	4.41 ± 0.53 ^{a}	4.16 ± 0.28 ^{a}	4.11 ± 0.59 ^{a}	4.38 ± 0.57 ^{a}	3.68 ± 0.07 ^{a}
	T2	3.52 ± 0.15 ^{b}	4.57 ± 0.70 ^{b}	5.70 ± 0.18 ^{b}	4.47 ± 0.71 ^{b}	4.42 ± 0.40 ^{b}	3.79 ± 0.15 ^{b}	3.76 ± 0.75 ^{b}
	T3	3.92 ± 0.34 ^{c}	4.20 ± 0.42 ^{c}	5.03 ± 0.35 ^{c}	3.54 ± 0.58 ^{c}	3.34 ± 0.46 ^{c}	4.41 ± 0.32 ^{c}	4.91 ± 0.22 ^{c}
	T4	4.94 ± 0.51 ^{d}	3.70 ± 0.39 ^{d}	4.46 ± 0.34 ^{d}	1.90 ± 0.15 ^{d}	NE	NE	NE

Different letters in the same column indicate significant differences ($p < 0.05$) among treatments. NE, not evaluated.

The exudate loss (EL) in pork is shown in Table 1. Initial values of 4.65 (T1), 3.52 (T2), 3.92 (T3), and 3.94 (T4) increased after 4 days of storage, showing a decreasing tendency and reaching values of 3.68 (T1), 3.77 (T2), and 4.93 (T3), while the control reached a value of 1.9 on the last shelf-life storage day. Lahucky et al. [42] evaluated the effect of adding antioxidant compounds on pork exudate loss, and they obtained values ranging from 3.25 to 3.78 for the treatments, while the control showed a value of 3.78. This result indicated no significant differences between the treatments and the control. Meanwhile, samples in T3 showed a higher EL, and this may have been due to the chitosan solution with 0.3% extract being highly absorbed during immersion and being liberated in a better way during refrigerated storage. Chang et al. [37] evaluated chitosan application and glucose at different concentrations in pork EL; wherein the values increased after 3 storage days with respect to day 1. These results showed a higher loss in glucose treatment samples and an initial value of 3.57 for the control, while the treated samples followed the same behavior at the end of storage, reaching values of 6.44 to 7.74. The initial values were similar to the present study, but the final values were higher. Exudate loss change is a good indicator of protein structure. The decreased liberation from muscle seems to be independent of the contraction state after rigor mortis, and this may be due to filament reduction and cellular membrane changes [43].

The WRC in fresh and cooked pork samples after treatment is shown in Figure 4. The initial values for the fresh samples were 81.26, 89.75, 98.13, and 98.21 for T1, T2, T3, and T4, respectively. Day 1 showed an increase in T1 and T2, followed by a decrease during the storage days, reaching values of 90.82 (T1), 81.33 (T2), 80.95 (T3), and 92.30 (T4). A previous study [44] evaluated the effect in pork WRC during storage and obtained similar results to the present study, showing an initial value of 96.2% and a final value of 93.8% during the first 7 days; where these values increased until the final day of storage. This behavior may have been due to there being no changes in the total water content, which was produced during aging in the samples. This result could explain the results in the present study and the findings of other studies reporting that pH values higher than or equal to 5.8 would benefit the capacity of protein binding to water molecules [43]. Regarding the WRC of the cooked pork

(Figure 4b), the values were initially 48.31, 54.69, 51.14, and 50.03 for T1, T2, T3, and T4, respectively. These values showed a variable tendency in all treatments during the storage period, starting at day 12 with constant behavior and finishing at higher values than the initial ones. The control obtained the highest value (69.49), followed by T1 (60.94), T3 (56.57), and T2 (54.06). The same behavior was observed in Reference [45], where values ranging between 54% and 79% using different methods in cooked pork WRC were obtained. Meanwhile, Reference [46] evaluated untreated pork to observe the changes during sample aging, and they obtained an initial value of 60% and a final value of 57% over 14 days. High pH values can protect proteins from denaturalization, indicating a close relationship between quality parameters and other factors, such as cooking method, temperature, type of muscle in samples, species, and maturity state, among others [44,47].

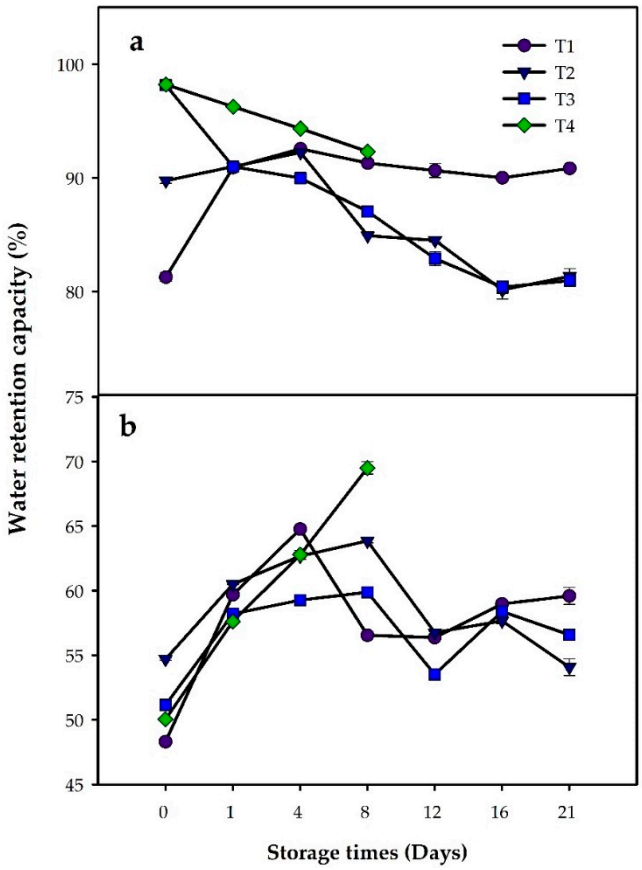

Figure 4. Water retention capacity (WRC) in (**a**) raw and (**b**) cooked pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

Figure 5 depicts the effect of treatment on pork texture. On the initial day, examining fresh samples (Figure 5a), the shear force used ranged from 10 to 12 N for T1, T2, and T4, while T3 required 20 N of shear force, showing a significant difference ($p > 0.05$). During storage, increased WTC was observed until day 8, followed by a decrease on the final storage day, when T1 and T3 showed better texture (17.73 and 15.09 N, respectively) compared to T2, which showed higher texture loss (11.26 N). Cooked samples (Figure 5b) showed a decreasing tendency from the initial storage day in all treatments until day 4, and then later increased until the final day of storage, when T1 and T2 showed the best results (66.95 and 66.44 N, respectively), with a significant difference ($p > 0.05$) between these treatments and T3 (45.91). Yingyuad et al. [48] evaluated the effect of a modified atmosphere and a 2.5% edible chitosan coating in pork for 40 storage days. The values were lower than the ones in this study, without significant differences ($p > 0.05$) between the treatments and control. However, Jayasena et al. [49] evaluated a plasma-based flexible coating on pork, obtaining an initial value of 9.66 N, which was

similar to our findings on day 0. Changes in firmness can be a consequence of water loss during storage, resulting in weight loss due to food surface water evaporation. Likewise, chitosan coating has no protective effect against water loss, probably due to the large numbers of NH_2 side groups caused by a strong affinity to water molecules [48]. T3 showed lower values in both meat preparations (fresh and cooked pork), followed by T2 and T1. This result may be due to the increased extract concentration in the chitosan solution causing decreased gelation capacity, thereby affecting edible coating adherence.

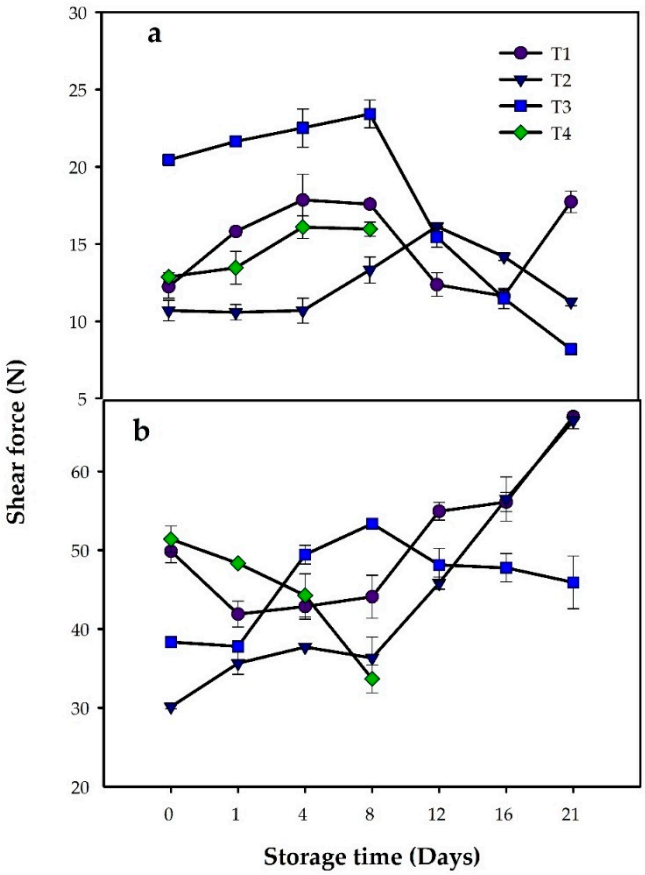

Figure 5. Shear force in (**a**) raw and (**b**) cooked pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

3.3. Antioxidant Capacity and Total Phenolic Compounds

Figure 6 shows the effect of edible chitosan coating on pork from the ABTS (Figure 6a) and DPPH (Figure 6b) antioxidant assays. The antioxidant capacity by ABTS of the treated meat (T1, T2, and T3) was significantly higher ($p < 0.05$) than that of the control (T4) after 21 and 8 days of storage, with values of 0.426–0.431 μmol TE/g and 0.382 μmol TE/g, respectively. No differences were found between the different treatments. On the other hand, at the end of the storage time, T3 obtained a higher value of antioxidant capacity using the DPPH assay, followed by T2 and T1, at 0.443, 0.412, and 0.342 μmol TE/g, respectively, showing a significant difference between the treatments ($p < 0.05$). Moreover, all treatments were significantly different ($p < 0.05$) with respect to the control, which had a value of 0.172 μmol TE/g after 8 days of storage. Huang et al. [50] evaluated the antioxidant capacity in fresh and cooked mutton and pork treated with *Nelumbo nucífera* root and leaf extracts, and they obtained similar results to the present study, showing higher antioxidant capacity with respect to the control.

The effect of edible coatings on the TPC in pork samples is shown in Figure 7. T3 obtained the highest content with initial and final values of 24 mg GAE/g and 17 mg GAE/g, respectively, followed by T2 and T1. These results showed a significant difference ($p < 0.05$) between the treatments and the control, following variable behavior during the storage period. Antioxidant capacity and total phenolic

compounds have been evaluated in some studies using natural extracts in meat preservation [37,50,51] that use edible coatings. The authors related higher antioxidant capacity with TPC in the samples, which provided a tool for counteracting the major deterioration problems.

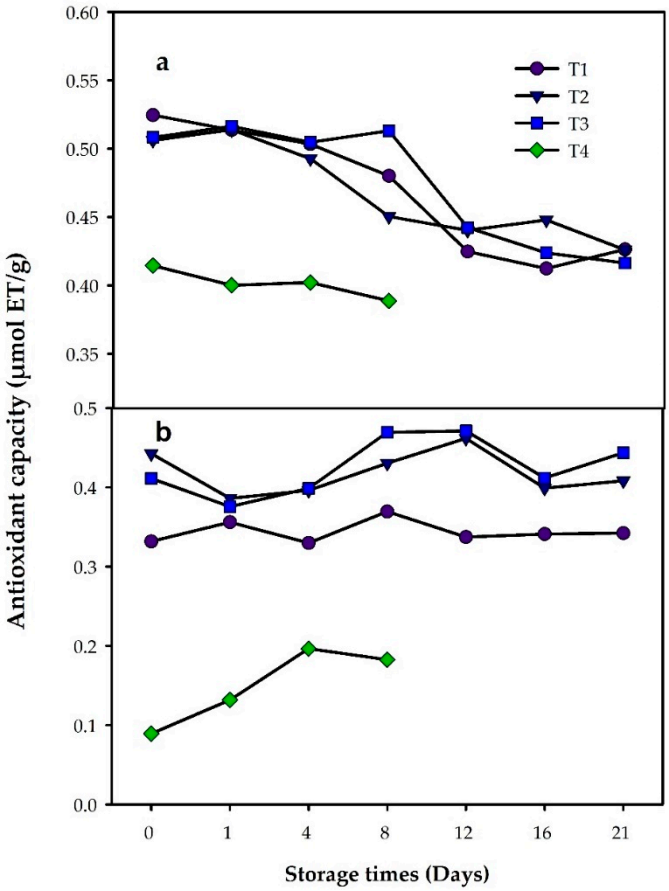

Figure 6. Antioxidant capacity by (**a**) 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and (**b**) 2,2-diphenyl-1picrylhydrazyl (DPPH) in pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

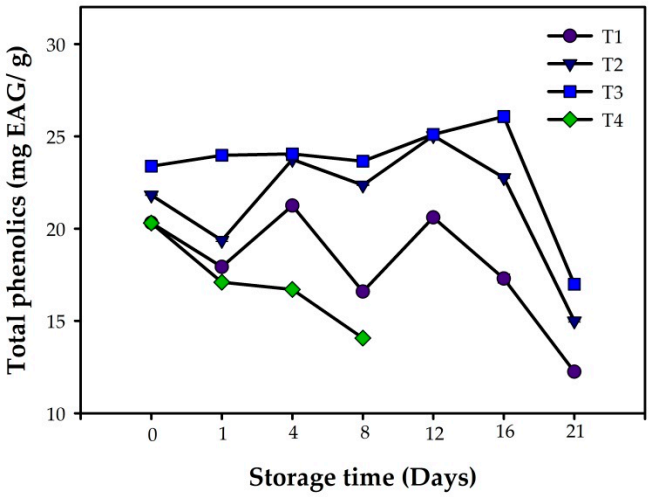

Figure 7. Total phenolic compounds in pork fillet coated with chitosan and tomato plant extract during refrigerated storage for 21 days. Vertical bars show the standard deviations.

3.4. Sensory Evaluation

The sensory qualities of pork stored at 4 °C are shown in Table 2. T2 obtained the best score with respect to all attributes evaluated, being slightly higher than T3, but with no significant difference ($p > 0.05$) between the treatments. At day 0, the color and flavor were good, implying that the edible coating with tomato extracts was acceptable for the panelists. The edible coating helped to preserve a nice fresh color for a longer time, keeping the pork acceptable for human consumption until day 21 (final storage day), where it started to decrease in quality attributes on day 8, thereby reflecting deterioration. According to the scores, low concentrations of extract had no negative effect on the sensory properties; in fact, they significantly improved the properties during the storage period. This result may have been because the antioxidant properties of the tomato extracts affected the color scores when added to the samples. Panelists preferred T2, which showed a significant effect, with a slightly darker color and a nice natural flavor, color, and texture. Meanwhile, the scores for the other treatments were lower as the extract concentration was increased; wherein panelists were most sensitive to flavor [50,52–55].

Table 2. Effect of the chitosan–tomato plant extract coatings on the sensory evaluation of pork fillet during refrigerated storage for 21 days.

Parameter	Treatment	Storage Time (Days)						
		0	1	4	8	12	16	21
Odor	T1	8.4 ± 0.51 ^{a}	8.5 ± 0.52 ^{a}	8.3 ± 0.48 ^{a}	8.0 ± 0.0 ^{a}	6.9 ± 0.31 ^{a}	6.8 ± 0.63 ^{a}	6.0 ± 0.81 ^{a}
	T2	8.4 ± 0.51 ^{a}	8.6 ± 0.51 ^{a}	8.3 ± 0.48 ^{a}	8.0 ± 0.0 ^{a}	7.6 ± 0.51 ^{b}	7.5 ± 0.52 ^{b}	6.4 ± 0.69 ^{a}
	T3	8.2 ± 0.78 ^{a}	8.2 ± 0.63 ^{a}	8.3 ± 0.48 ^{a}	8.0 ± 0.0 ^{a}	7.4 ± 0.51 ^{b}	7.1 ± 0.31 ^{ab}	6.3 ± 0.82 ^{a}
	T4	8.3 ± 0.67 ^{a}	8.3 ± 0.67 ^{a}	8.2 ± 0.42 ^{a}	4.2 ± 0.22 ^{b}	NE	NE	NE
Flavor*	T1	7.8 ± 0.78 ^{a}	7.9 ± 0.87 ^{a}	8.1 ± 0.56 ^{a}	7.5 ± 0.52 ^{a}	6.7 ± 0.48 ^{a}	6.6 ± 0.51 ^{a}	4.6 ± 1.07 ^{a}
	T2	8 ± 0.66 ^{a}	8.2 ± 0.42 ^{a}	8.2 ± 0.42 ^{a}	7.7 ± 0.48 ^{a}	7.5 ± 0.52 ^{b}	7.1 ± 0.31 ^{b}	5.9 ± 0.89 ^{b}
	T3	7.7 ± 0.48 ^{a}	7.7 ± 0.48 ^{a}	7.7 ± 0.48 ^{a}	7.5 ± 0.52 ^{a}	6.5 ± 0.52 ^{a}	6.2 ± 0.63 ^{a}	5.2 ± 0.63 ^{a,b}
	T4	7.8 ± 0.78 ^{a}	7.8 ± 0.78 ^{a}	7.7 ± 0.82 ^{a}	NE	NE	NE	NE
Color	T1	8.9 ± 0.31 ^{a}	9.0 ± 0.0 ^{a}	8.8 ± 0.42 ^{a}	8.0 ± 0.0 ^{a}	7.6 ± 0.51 ^{a}	7.1 ± 0.31 ^{a}	6.0 ± 0.66 ^{a}
	T2	8.9 ± 0.31 ^{a}	9.0 ± 0.0 ^{a}	8.8 ± 0.42 ^{a}	8.0 ± 0.0 ^{a}	7.7 ± 0.48 ^{a}	7.5 ± 0.52 ^{a}	6.3 ± 0.67 ^{a}
	T3	8.9 ± 0.31 ^{a}	9.0 ± 0.0 ^{a}	8.8 ± 0.42 ^{a}	8.0 ± 0.0 ^{a}	7.5 ± 0.52 ^{a}	7.4 ± 0.69 ^{a}	6.0 ± 0.81 ^{a}
	T4	8.8 ± 0.42 ^{a}	8.8 ± 0.42 ^{b}	8.8 ± 0.42 ^{a}	5.9 ± 0.37 ^{b}	NE	NE	NE
Texture	T1	8.3 ± 0.67 ^{a}	8.6 ± 0.51 ^{a}	8.3 ± 0.48 ^{a}	6.9 ± 1.8 ^{a}	6.5 ± 0.84 ^{a}	6.4 ± 0.51 ^{a}	4.5 ± 0.84 ^{a}
	T2	8.4 ± 0.31 ^{a}	8.4 ± 0.51 ^{a,b}	8.5 ± 0.52 ^{a}	7.3 ± 1.05 ^{a}	6.9 ± 0.73 ^{a}	6.3 ± 0.48 ^{a}	5.8 ± 0.63 ^{b}
	T3	8.4 ± 0.51 ^{a}	8.9 ± 0.31 ^{ac}	8.4 ± 0.69 ^{a}	7.3 ± 1.15 ^{a}	6.8 ± 0.78 ^{a}	6.2 ± 0.78 ^{a}	5.8 ± 0.78 ^{b}
	T4	8.3 ± 0.67 ^{a}	8.1 ± 0.73 ^{b}	8.2 ± 0.63 ^{a}	NE	NE	NE	NE
Overall acceptability	T1	8.2 ± 0.42 ^{a}	8.3 ± 0.48 ^{a}	8.1 ± 0.31 ^{a}	7.5 ± 0.97 ^{a}	6.7 ± 0.48 ^{a}	6.5 ± 0.52 ^{a}	5.3 ± 0.67 ^{a}
	T2	8.3 ± 0.48 ^{a}	8.3 ± 0.48 ^{a}	8.2 ± 0.42 ^{a,c}	7.9 ± 0.31 ^{a}	7.5 ± 0.52 ^{b}	7.0 ± 0.47 ^{b}	6.3 ± 0.67 ^{b}
	T3	7.8 ± 0.42 ^{b}	7.8 ± 0.42 ^{b}	7.9 ± 0.31 ^{a}	7.4 ± 0.69 ^{a}	6.9 ± 0.56 ^{a}	6.7 ± 0.48 ^{a,b}	5.9 ± 0.73 ^{a,b}
	T4	8.2 ± 0.42 ^{a}	8.0 ± 0.0 ^{b,c}	7.8 ± 0.42 ^{a,b}	4.52 ± 0.42 ^{b}	NE	NE	NE

Different letters in the same column indicate significant differences ($p < 0.05$) among treatments. NE, not evaluated.

4. Conclusions

All studied coatings showed a high reduction of microbial population growth during storage time compared to the control. Sensory analysis showed a significant difference between the coated and uncoated (control) samples. The shelf life of the control and treatments according to the parameter of overall acceptability was 8 and 21 days, respectively. The principal parameters to determine the shelf life of the product were microbiological deterioration and sensory analysis. Moreover, the edible coatings with the addition of tomato plant extract (T2 and T3) showed higher values of antioxidant capacity and total phenolics, which was a favorable outcome for the application of these coatings. The selected edible coating was treatment 2 (chitosan + tomato plant extract 0.1%), which increased the shelf life of pork loin by 13 days with respect to the control (i.e., a higher value of overall acceptability of 6.3). The edible chitosan-based coatings with the addition of tomato plant extract maintained some quality parameters and improved the microbial safety of pork loin during the 21 days of refrigerated storage.

The results of this study confirm that using edible chitosan-based coatings with the addition of tomato plant extract on pork loin will help food producers to profit from higher manufacturing quality with a longer shelf life and improved product safety. Moreover, consumers will benefit from safe

products and products that are free of chemical additives. Therefore, edible chitosan coatings with the addition of tomato plant extract could represent an effective alternative to improve the safety and quality of pork loin for commercial purposes.

Author Contributions: Performed the experiments, collected data, analyzed the results and wrote the paper: S.C.-H.; Designed the experiments and supplied instrumentation by analysis: G.E.D.-I.; Designed the experiments: L.E.G.-O.; Analyzed and interpreted the results: J.d.J.O.-P.; Interpreted antioxidant capacity results: C.L.D.-T.-S.; Provided reagents and materials: E.M.-R. and V.M.O.-H.; Designed the methodology and interpreted the results: M.A.L.-M.; Conducted the research, analyzed and interpreted results, and wrote the manuscript: S.R.-C.

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