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Changes in Collagen across Pork Tenderloin during Marination with Rosehip Nanocapsules

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Featured Application: In this study, taking advantage of their bioactive properties, rosehip nanocapsules were applied to pork marinating systems to obtain marinated products with added value and improved texture. An evaluation of the changes in collagen is a good reference associated with these enhancements.

Abstract: The objective of this study was to prepare zein–gum Arabic nanocapsules with rosehip oil (NC-RH), apply them to pork tenderloin, and analyze the changes in collagen structure under different conditions (pH 6.5 and 4.0) and temperatures (25 °C and 4 °C). NC-RHs were prepared using the nanoprecipitation method. Nanocapsules had a particle size of 423 ± 4.1 nm, a polydispersity index of 0.125 ± 3.1, a zeta potential value of −20.1 ± 0.41 mV, an encapsulation efficiency of 75.84 ± 3.1%, and backscattering (ΔBS = 10%); the antioxidant capacity of DPPH was 1052 ± 4.2 µM Eq Trolox and the radical scavenging capacity was 84 ± 0.4%. The dispersions exhibited Newtonian behavior at 25 °C and 4 °C. Incorporating NC-RH into acid marination benefited the tenderness, water-holding capacity, and collagen swelling, and favored changes in myofibrillar proteins corroborated with histological tests. The conditions with the best changes in pork tenderloin were a pH of 4.0 at 4 °C with an NC-RH-administered 11.47 ± 2.2% collagen area. Incorporating rosehip nanocapsules modifies collagen fibers and can be applied in pork marinades to increase the shelf life of a functional product.

Keywords: stability; tenderness; marination; infiltration; characterization

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1. Introduction

Pork production is a dynamic and growing industry that produces 33% of the total meat consumption of animal meat in the population's diet, with differences between continents. The main nutrients in pork are proteins with essential amino acids, fats with essential fatty acids, vitamins, minerals, and other health-promoting compounds such as antioxidants [1]. One of the most important proteins is myofibrillar protein, which constitutes 55 to 60% of the total meat proteins or 10% of the weight of skeletal muscle. Meat proteins play a significant role in determining the quality of muscle products because they are responsible for the functional characteristics of meat and meat products, impart textural and emulsifying properties, and have gel-forming and water-holding abilities [2,3]. Another important protein is collagen, which plays a significant role in the tenderness of meat and meat products; it is the major structural protein of connective tissue [4,5].

However, the contribution of collagen characteristics to tenderness may differ between species, age, and gender [6]. It has been reported that the collagen present in muscles is approximately 1.5–10% of its dry weight [7]. The functional characteristics of these proteins are mainly affected by the processing conditions, for example, ionic strength, pH, and temperature [2], affecting the tenderness and biological value of the meat.

Different chemical reactions occur during the processing and storage of meat due to molecular interactions as a result of thermal treatment or the addition of additives. For this reason, other methods, both physical and chemical, have been developed to preserve meat. These methods not only aim to maintain quality but also consider sensory and nutritional features as well as consumer appeal. There is a growing demand for natural-origin preservation systems and minimally processed meat products with longer shelf lives [8].

Marination is one of the chemical methods used for improving meat tenderization [8]. However, acid marination improves the tenderization and flavoring of treated meat, causing meat fibers to swell [4,9] through collagen and myofibrillar solubility and muscle protein swelling [10]. Various ingredients have been incorporated into marinades to preserve and improve tenderness and flavor. Using nanoparticles to enhance the release of antibacterial and antioxidant compounds could be an option to improve meat products' quality. In recent investigations, functional and bioactive nanoparticles in meat and meat products have been used to enhance the nutritional benefits by reducing saturated fats and salts [10].

Rosehip oil is an active compound that can be used as a natural additive in meat during marination. It is obtained from the seeds of the pseudo fruit *Rosa canina* L. It has shown a powerful natural antioxidant capacity due to the content of ascorbic acid, carotenoids, and phenolic compounds [11,12]. In addition, this oil is rich in linoleic (54%), linolenic acid (19.37%), and other polyunsaturated fatty acids (19.50%). Phytosterols (β-sitosterol), in particular, have important functional properties that can potentiate the beneficial effects of consumption. This oil is important because, due to its phenolic compounds, it has a greater antioxidant capacity than other vegetable oils, such as grape seed oil and pomegranate seed oil. Rosehip oil also has a higher level of linolenic acid than vegetable oils, such as canola and soybean, which are the primary dietary sources of linolenic acid [13].

A problem with this oil is its high content of linoleic and linolenic acids, which are vulnerable to lipid oxidation [14]. The entrapment of rosehip oil in nanocapsules increases its stability and can be used for meat marination. Nanoencapsulation protects oil against fatty acid oxidation and modifies its release time. Little information is available on the use of nanoparticles in marination and meat quality preservation [10].

This study aimed to obtain stable zein–gum Arabic nanocapsules with rosehip oil, apply them to pork tenderloin, and evaluate the changes in collagen under different conditions (pH values of 6.5 and 4.0) and temperatures (25 and 4 °C).

2. Materials and Methods

2.1. Materials

Ascorbic acid, ethanol (96°), ethyl acetate, and sodium ascorbate were purchased from the Meyer Co. (Mexico City). Zein, Triton TM X-100, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid were supplied by Sigma-Aldrich, Inc. (St. Louis, MO, USA). Food-grade soy lecithin, gum Arabic, and propylene glycol were obtained from Cosmopolita Drugstore (Mexico City, Mexico). Rosehip oil was purchased from Newsystec S.A. de C. V. (Anonymus Society with Variable Capital) (Mexico City, Mexico); methanol was acquired from Fermont (Monterrey, Mexico); and acid fuchsin, buffered formalin, eosin, hematoxylin, and synthetic resin were obtained from Hycel (Mexico City, Mexico). Xylene was purchased from Quimicos Wöhler Mexico S.A. de C.V. (Anonymus Society with Variable Capital) (Mexico City, Mexico), and paraffin Paraplast plus Leica (Wood Dale, IL, USA), picric acid A.C.S. (Chemical grade) and Weiger's iron hematoxylin were obtained from Fisher Scientific Mexico S. de R.L. de C.V. (Limited Liability Company) (Mexico City, Mexico).

2.2. Zein–Gum Arabic Nanocapsule with Rosehip Oil (NC-RH) Preparation

Nanocapsules were prepared using the nanoprecipitation method. In brief, the organic phase was prepared with 2 g/L of zein, 1660 µg/L of rosehip, 90% ethanol, and 10% ascorbate buffer (pH = 3.0). Then, 4.5 g/L of lecithin was incorporated, and the mixture was stirred at 500 rpm for 4 h at 25 °C. The aqueous phase was prepared with gum Arabic to 50 g/L. Using a Model NE-4000 positive displacement pump (New Era Pump Systems, Farmingdale, NY, USA), the organic phase was added dropwise at 4.0 mL/min into the aqueous phase with continuous stirring (Model BK-HG160 homogenizer, Biobase, Shandong, China) at 200 s^{-1}, with two five-minute cycles of work–rest. The solvent was evaporated in a Model RV10 rotary evaporator (IKA, Wilmington, NC, USA) at 50 °C for 30 min and 66.66 kPa.

2.3. Characterization of the Nanocapsule (NC-RH)

The particle size (PS) and polydispersity index (PDI) were measured using dynamic light scattering (DLS) with a Zetasizer Nano ZS90 (Malvern Ltd., Enigma Business Park, Grovewood Road, UK); the zeta potential (ζ) was obtained using electrophoretic movement with the same instrument. The entrapment of rosehip in zein nanocapsules was measured in the supernatant after centrifugation and quantified using a rosehip calibration curve at λ = 250 nm in a UV–vis Genesys 10S (Thermo Scientific, Waltham, MA, USA). The results were reported as encapsulation efficiency (EE) values. All measurements were performed in triplicate.

2.4. DPPH in NC-RH

This determination was carried out following the method proposed by [15]. Briefly, 0.1 mM of DPPH in methanol was prepared, and the standard curve was obtained using Trolox at a wavelength of 517 nm. The DPPH was measured in the rosehip oil and the NC-RH; the results are reported in µM equivalents of Trolox. The percentage of the scavenging capacity (% SC) was calculated as $\%SC = 1 - (A_{sample}/A_{control}) \times -100\%$, where (A) is the absorbance. All measurements were performed in triplicate.

2.5. Viscosity

The dynamic viscosity was measured in a ReholabQC rotational rheometer (Anton Paar, Graz, Austria) equipped with a concentric cylinder CC39 by applying an up–down interval at a shear rate of 400–700 (s^{-1}). The viscosity of the NC-RH was measured at 25 °C and 4 °C. The graphic of shear stress (τ) (Pa) vs. the shear rate (γ) (s^{-1}) was obtained by fitting the data using a linear regression, where the slope represents the viscosity (µ, (mPa·s)).

2.6. Morphological Characterization

Morphological analysis was performed with a JSM 5600 LV-SEM® LV scanning electron microscope (JEOL, Tokyo, Japan). Sample treatment followed the methodology proposed previously by [16].

2.7. Stability

The stability of the NC-RH was measured in a Tubiscan MA2000 (Formulation, Toulouse, France). The sample was diluted in distilled water and placed in a flat-bottomed cylindrical glass cell up to 5 cm at 25 °C. Measurements were taken every 16 min for 48 h.

2.8. FTIR

A Fourier transform infrared spectrometer with a diamond ATR was used to characterize the presence of specific chemical groups. The materials were analyzed in transmittance mode. FTIR spectra were obtained in the 4000–500 cm^{-1} range in 64 scans, with a wavenumber resolution of 4 cm^{-1} (Spectrum Two, Perkin-Elmer, Waltham, MA, USA). For these measurements, special care was taken to subtract the background in each experimental run after draining the system with dry air. The FTIR spectra were normalized, and the major vibrational bands were associated with chemical groups. All FTIR spectra were measured

at 25 °C. The methodology was based on that of Radulović et al., 2020 [17] with some modifications. The spectra were plotted using Origin Pro 9.0 2012 software.

2.9. Pork Tenderloin Selection

The pork tenderloin was obtained from the local Cuautitlan Romero Rubio (Mexico) market, which receives pig carcasses every 24 h. The sample was taken after 36 h of mandatory post-slaughter pre-cooling.

2.10. pH of Pork Tenderloin

Changes in pH associated with pork tenderloin marination were measured with a meat potentiometer from Hanna Instruments, Model HI99163 (Nusfalau, Romania), using an FC233 electrode.

2.11. Histological Techniques

The pork tenderloin samples were cut transversally to approximately 2 cm in size for the control (10 ± 1 g), where only surfactant solution (30 g/L of Triton and 10 g/L of propylene glycol) (10 mL) was applied with or without NC-RH (5 mL). Both conditions were treated at different pH values (6.5 and 4.0) and temperatures (25 °C and 4 °C) after 48 h of exposure to the tissue. Later, the samples were fixed in 10% buffered formalin at 25 °C for 48 h. Then, the samples were placed in histocassettes, dehydrated, and included using the microwave paraffin-embedding method, with consecutive immersions in increasing solutions of ethanol: R-OH (70%, 80%, 90%, and 96%) (two changes each), R-OH 100% (3 changes), and xylene (2 changes), in each of the changes. The samples were put in the microwave for 1 min (medium–high power) and were kept at rest for 15 min before moving on to the next ethanol. Once the process was finished, they were submerged in two paraffin changes and included in blocks. Tissue sections were cut in microtome (Leica RM 2125 RT, Nussloch, Germany) with a thickness of 5 µm. The paraffin-embedded tissue sections were subjected to deparaffinization and rehydration for subsequent staining and final mounting with synthetic resin.

2.11.1. Hematoxylin and Eosin Stain (H&E)

The tissue sections were immersed in an aqueous solution of oxidized hematoxylin with aluminum salt for 15 min. When a purple-reddish tone was observed, the slides were washed with 0.05% ammonia water for 5 s until the color became bluish. The contrast was performed with a 1% aqueous eosin solution for 1 to 3 min to tint the non-nuclear elements a pinkish tone [18].

2.11.2. Van Gieson stain (VGS)

The slides were immersed for 5 min in Weigert's ferric hematoxylin. Subsequently, they were washed and immersed in a solution saturated with aqueous picric acid and 1% acid fuchsin for 5 min [19]. After staining, the sections were dehydrated in alcohol until they reached xylol and were finally mounted with synthetic resin.

2.11.3. Collagen Fiber Quantification

The amount of collagen fibers in the tissue was detected based on image analysis. A total of six random fields of pork tenderloin sections were captured with the 10× and 40× objectives. The percentage of the area of collagen fibers was obtained by subtracting this from the total area visualized on the field. A Zeiss Mod. Axio A1 microscope, New York, NY, USA, was used; images were captured using a digital camera (Cannon, Mod. EOS Rebel T5i, New York, NY, USA) attached to a microscope adapter. The images were analyzed using specialized ImageJ® software (version 1.53).

2.12. Statistical Analysis

To establish the differences between the treatments, variance analysis (with a significance level of 0.05) and Tukey's test were used to differentiate the means.

3. Results

3.1. Nanocapsule Characterization (NC-RH)

Rosehip oil zein nanocapsules had a PS of 423 ± 4.1 nm, a PDI of 0.125 ± 0.01, ζ of −20.1 ± 0.4 mV, and an EE of 75.84 ± 3.1%, suggesting that the system had a submicronic size with a narrow size distribution. The PS is associated with the rosehip composition on polyunsaturated fatty acid; Pereira Oliveira et al. (2023) [20] prepared rosehip and sunflower oil nanoemulsions, presenting average sizes between 304 and 403 nm. In their research, the obtained PDI was between 0.197 and 0.430; the NC-RH system presented narrower PDI behavior; moreover, the zeta potential value indicates a stable system with a particle repulsion due to surface charge. EE results demonstrated that a significant part of the oil was encapsulated, showing that the components used in the preparation effectively contributed to the entrapment and preservation of the rosehip oil.

3.2. Morphological Characterization of the Nanocapsules

Figure 1 shows the presence of smooth spherical NC-RH with some hollow structures. Similar structures observed in zein–gum Arabic nanoparticles showed that the presence of gum Arabic modifies the surface and shape of zein nanoparticles by forming a compact ternary component associated with gum Arabic's capacity to cover the surface and successfully entrap the free active compound not embedded in the zein. There are also reports that irregular forms are associated with highly hydrophilic polysaccharides because gum Arabic produces severe interparticle agglomeration, and the nanoparticles have irregular shapes [21,22]. This behavior evidences the good encapsulation capacity of rosehip oil determined in this study.

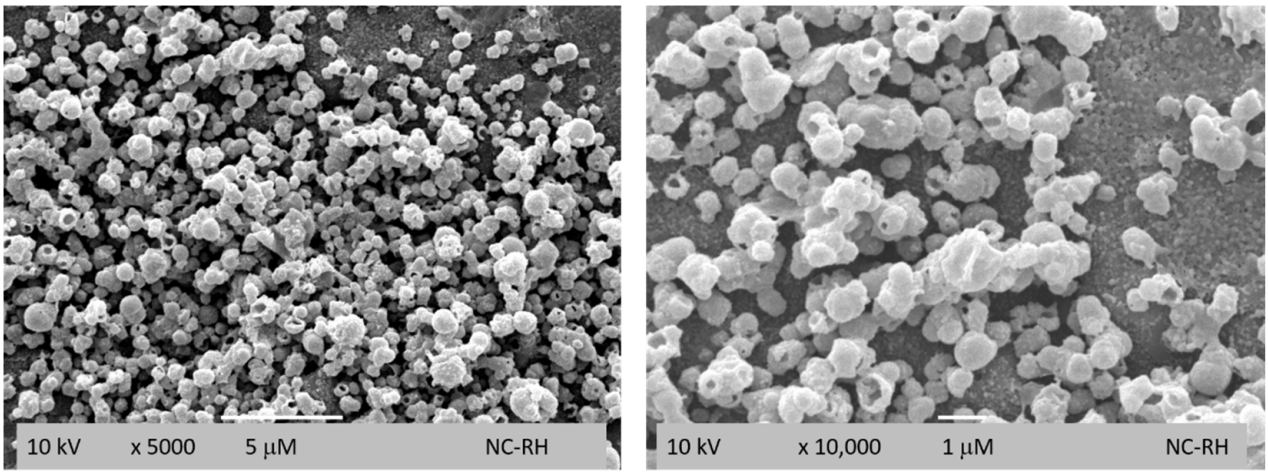

Figure 1. Morphology of the NC-RH.

3.3. Stability

Figure 2 shows the backscattering profile variation (ΔBS) over assay time, presenting a slight difference in the curves, indicating a slow rate of nanocapsule flocculation and a destabilization phenomenon characterized by the aggregation process of one or more particles without the loss of individuality [23]. This aggregation is due to the forces of attraction between the nanocapsules and was also observed in previously obtained morphology (Figure 1). However, the nanocapsules presented good system stability since the ΔBS was 10% after 48 h and constant from a 10–45 mm vial height.

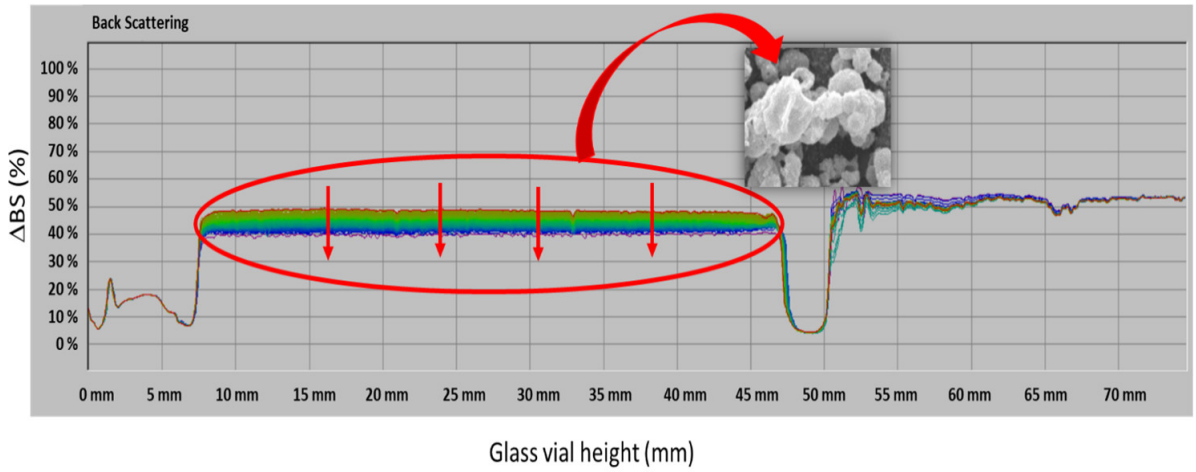

Figure 2. Backscattering of the NC-RH at 48 h.

3.4. DPPH Free Radical and % Scavenging Capacity

The DPPH of NC-RH compared to rosehip oil did not show a statistically significant difference ($p \leq 0.05$) with 1052 ± 4.2 and 1143 ± 22 µM of Trolox Eq/mg per sample, respectively. In addition, the % SC was 84 ± 0.4 for NC-RH and 92 ± 2% for rosehip oil. However, the free oil does not remain stable when applied to meat or meat products, and it is not easy to incorporate it into products with high water content because of its lipidic nature. The antioxidant capacity of rosehip oil is attributable to the level of unsaturation of fatty acids (like linolenic acid) and the number of lipophilic antioxidants (such as tocopherol and carotenoids) [24].

3.5. Viscosity

The NC-RH showed Newtonian fluid behavior and was affected by temperature with a µ of 4.7 ± 0.1 mPa·s at 25 °C and 7.4 ± 0.3 mPa·s at 4 °C, both with $R^2 \geq 0.99$. Viscosity was modified via the particle volume fraction, shear rate, dispersion technique, particle shape, particle size, loading, aggregation, temperature, pH, and use of a surfactant, but temperature was the principal parameter [25]. The fact that the system had Newtonian behavior allows it to be used in the marinating liquid without adhesion and modification in the treated meat.

3.6. FTIR

Figure 3 displays the FTIR spectra of the individual ingredients and the NC-RH. In Figure 3a, the spectra of zein at orf3295 correspond to the N-H bond of the amide group of protein, and 2927 cm^{-1} shows the C-H bond of the methyl groups corresponding to glutamine and asparagine, while the vibration 1444 cm^{-1} belongs to the C-H bond of the alkane structure, and the vibrations at 1237 and 1116 cm^{-1} correlate with the stretching of the N-C bond of the amides. Figure 3b shows the lecithin spectrum at 922 cm^{-1}, which presents N-$(CH_3)_3$ 922 cm^{-1}, P-O at 847 cm^{-1}, and PO_2 at 1233 and 1615 cm^{-1}. These bonds represent phosphatidylcholine, a phospholipid that binds the O atom's N-$(CH_3)_3$ group to its phosphate group [26]. The FTIR of gum Arabic (Figure 3c) shows the stretching vibration of the O-H groups of the glycosidic ring at 3330 cm^{-1} and the C-H(CH_2) bond at 2910 cm^{-1}, representing the vibrational modes of the C-H_2 group. The characteristic C-O-C bonds of the carbohydrate fingerprint correspond to the vibrations of the arabinogalactan ring at 1027 cm^{-1}, while at 771 cm^{-1}, it is assigned to the 1–4 bond of galactose [27].

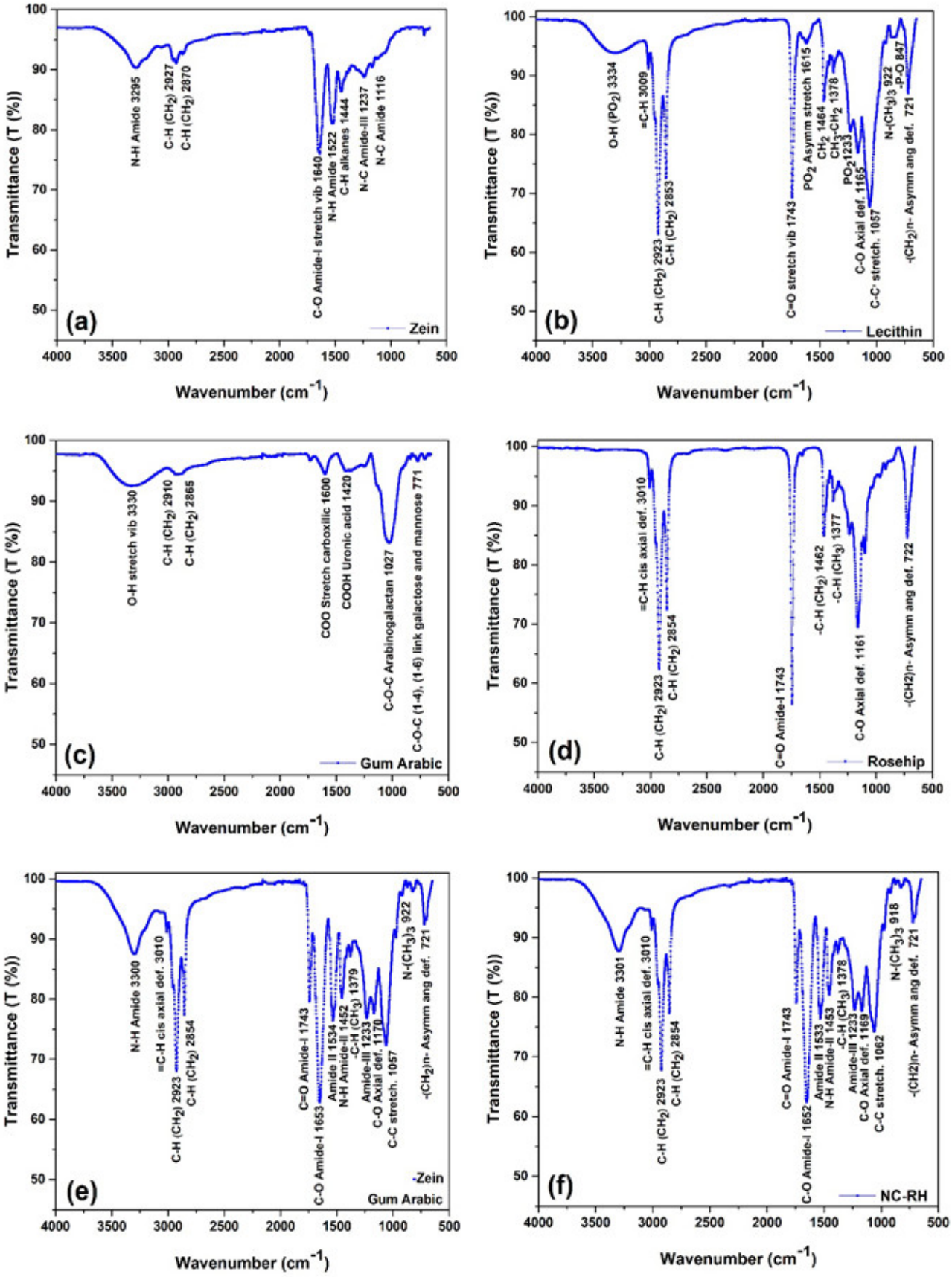

Figure 3. FTIR of (**a**) zein; (**b**) lecithin; (**c**) gum Arabic; (**d**) rosehip; (**e**) zein–gum Arabic; and (**f**) NC-RH.

Figure 3d corresponds to rosehip essential oil. It shows the =C-H bond group at 3010 cm^{-1} as a result of deformation of the aromatic ring. The characteristic C-H bond of the CHs group shows the first peak at 2923 cm^{-1} and the second peak at 2854 cm^{-1}. The vibration at 1743 cm^{-1} corresponds to the axial deformation of the C=O bond, while at 1161 cm^{-1}, it belongs to the C-O bond, both characteristic of the ester group. The vibration at 722 cm^{-1} is characteristic of the deformation of the aromatic rings of phenols [28]. Figure 3e indicates the presence of the N-H bond of the amide group in zein–gum Arabic at 3300 cm^{-1}; this interaction of amide groups and hydroxyl groups facilitates the formation of nanocapsules. The N-H group of amides at 1653 and 1534 cm^{-1} demonstrates the interactions between zein and gum Arabic [29]. Figure 3f shows the presence of the N-H bond of the amide groups in the NC-RH at 3301 cm^{-1}, with the first peak [30] evidencing hydrogen bonds formed between amide groups of the glutamines zein and hydroxyl groups of the gum Arabic. This hydrogen bonding is important because it facilitates nanoparticle formation. The second peak, between 1553 and 1652 cm^{-1}, represents the bending of the N-H bond of amides I and II in the NC-RH. When the zein–lecithin nanoparticles formed and the stretching vibration peak of amide II groups shifted to 1533 cm^{-1}, hydrogen bonding or hydrophobic interactions between lecithin and zein were indicated [31].

3.7. pH Changes in Meat

Acid marinades improve tenderness, make muscle structures more flexible, and increase the water-holding capacity. This is achieved by reducing cooking losses; improving color, juiciness, and flavor; and mitigating off-flavors [32]. Figure 4 shows the changes in meat pH from the beginning until 48 h later. First, the acid marinades decreased the meat pH after marination and before 48 h in the treatment with NC-RH at pH 4.0. It is worth noting that the acid marinade favors pork meat preservation, decreases the vulnerability to microbial growth, and facilitates the action of collagenases alongside enzymes associated with meat tenderization [33].

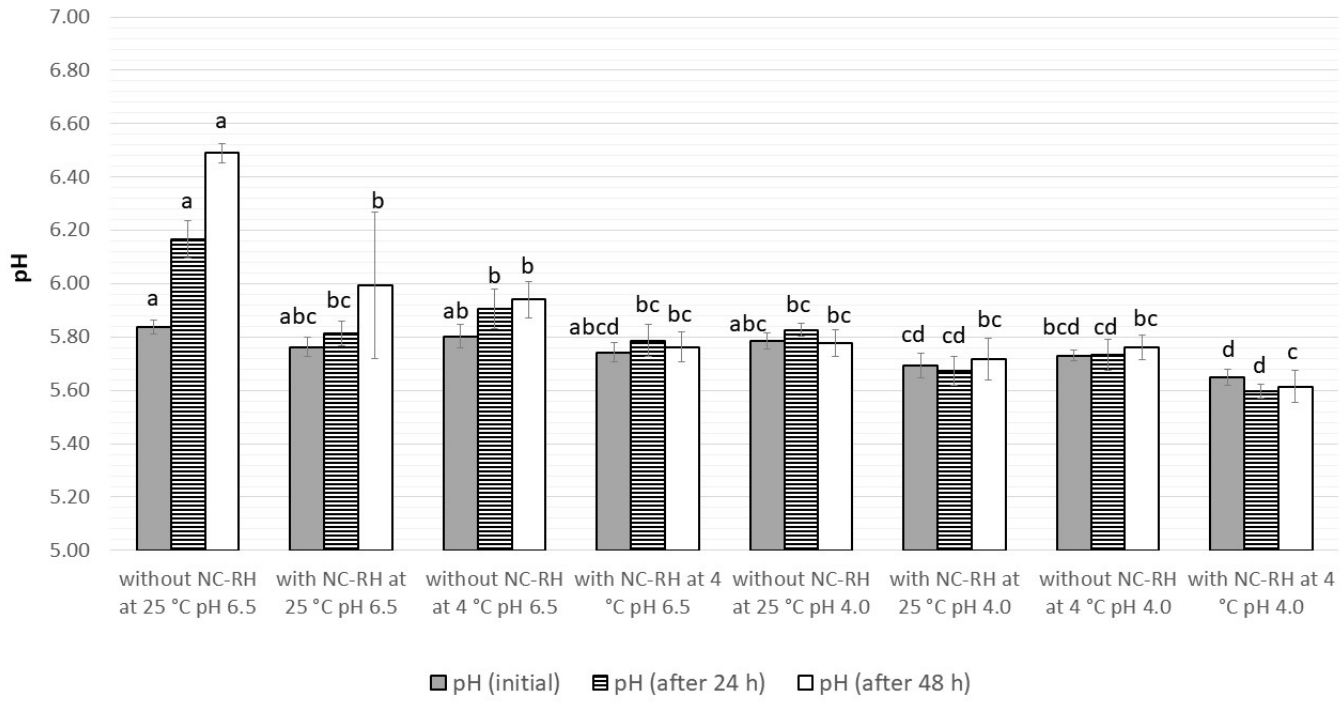

Figure 4. Changes in meat pH (initial, after 24 h, and after 48 h). Different letters indicate statistically significant differences ($p < 0.05$).

The pH also determines the protein net charge, dissociation or aggregation, and the electrostatic repulsive forces between protein molecules [34]. The preference to use an acid pH in the marinade is due to protein denaturation, which makes the meat soft, decreases moisture release and hardness, and improves chewiness [35]. In addition, the tenderness provided by an acidic marinade occurs in the first few days of marinating, and this continuous increment in tenderness is attributed to a more significant release of enzymes. In this case, the greater amount of rosehip oil released was at around 7 h, after which the concentration was constant. On the other hand, the temperature also affects the structure of myofibrillar proteins during the processing and storage of meat and meat products. For example, at 30 °C, myosin tails start to unspin and unfold, enhancing protein interactions. The hydrogen bonds within the myofibrillar protein expose more active sites to the surface, forming a continuous cross-linked three-dimensional gel matrix [34].

Therefore, pH and temperature can modify the structure of the myofibrillar proteins. This can be demonstrated in the amount of rosehip in the pork tenderloin. The advantage of the amount released at 4 °C is that the meat is stored at a refrigeration temperature, and the rosehip oil can be released slowly, favoring its action in the meat and increasing its shelf life. This results in significant improvements in the meat characteristics.

3.8. Histological Test and Collagen Quantification

Connective tissue is the main factor affecting the meat's texture, and its main component is collagen; changes in collagen content and characteristics are essential markers affecting connective tissue strength. Therefore, muscle texture is significantly affected by collagen characteristics and the degree of muscle fiber fragmentation [36–38].

Figure 5 shows the images obtained in the histological test and the different applied treatments. The control samples were only immersed in a surfactant solution, and the other samples were applied with NC-RH, both conditions at different pH levels (6.5 and 4.0) and temperatures (25 °C and 4 °C) after 48 h. In the control (Figure 5a) at pH = 6.5 and 25 °C, the tissue structure is organized and characterized by the presence of muscle fibers delimited by the connective tissue surrounding the muscle fiber that makes up the endomysium (Figure 5a, black arrows). This makes the separations visible, generating a well-organized tissue structure [39].

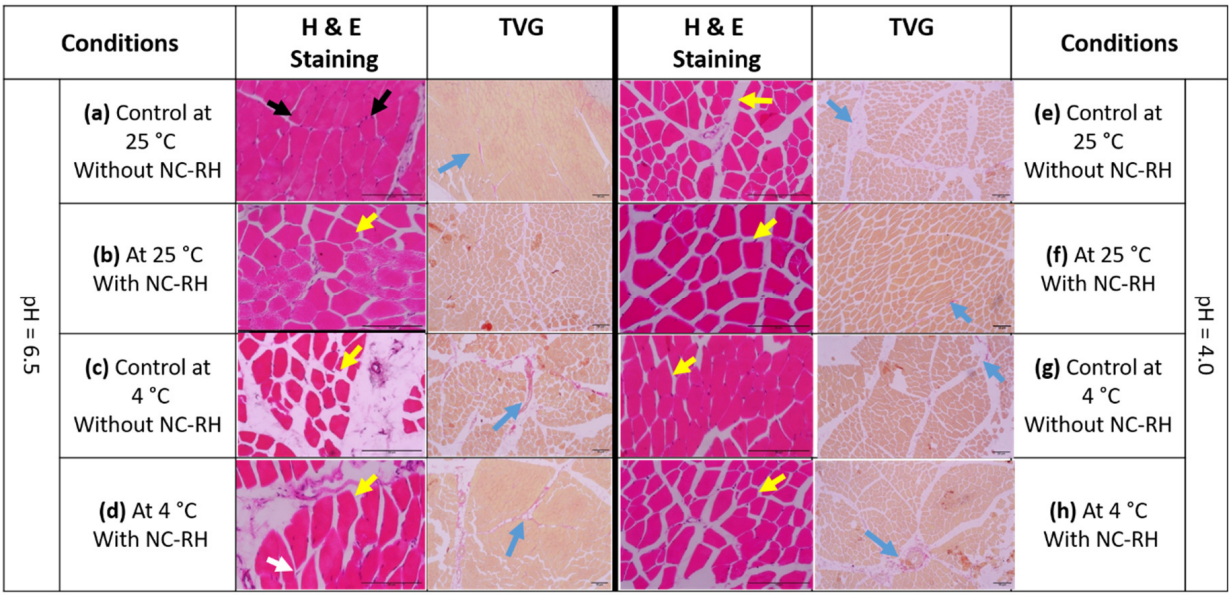

Figure 5. Changes in collagen after 48 h at pH = 6.5: (**a**) control at 25 °C without NC-RH; (**b**) at 25 °C with NC-RH; (**c**) control at 4 °C without NC-RH; (**d**) at 4 °C with NC-RH. Changes in collagen after 48 h at pH = 4.0; (**e**) control at 25 °C without NC-RH; (**f**) at 25 °C with NC-RH; (**g**) control at 4 °C without NC-RH; (**h**) at 4 °C with NC-RH. H&E (40×) TVG (10×), bar 50 µm.

The application of the NC-RH to pork tenderloin generated a tissue change characterized by the swelling of the tissue at both temperatures; the muscle fibers presented greater separation (Figure 5, yellow arrows) with the presence of disordered and lax collagen fibers (Figure 5d, white arrows). The connective tissue at the level of the endomysium and perimysium (Figure 5, yellow and blue arrows) showed a more significant loosening of the arrangements, which increased the distance between the muscle fibers. The above may occur due to the denaturation and contraction of collagen fibers during marinating [40,41].

In addition, it is reported that the distance between cells increased with a pH decrease (to approximately pH 4.3), and the surface hydrophobicity was inversely proportional to the pH. This was due to conformational changes, i.e., the unfolding of myofibrillar proteins and the exposure of non-polar amino acids to the surface of proteins [40]; thus, it is evident that collagen is principally affected by the pH. It has been reported that collagen's isoelectric point is pH 7.0–7.5 [5], but the charge varies little from pH 5 to 9 [5,41]. A pH below the isoelectric point indicates that charge interactions are involved in collagen aggregation and stabilization. Additionally, each collagen molecule possesses 216 hydrophobic residues essential to its stability [5].

Collagen is insoluble in neutral solutions and does not decompose in an aqueous solution, while it can dissolve in the presence of a strong acid or alkali. The muscle fiber proteins started swelling at pH 4.5 [41], with maximum degradation in the region of pH 3.5, the optimal pH range for cathepsins. The maximum degradation was at approximately pH 2.5 [42].

Acid marination improves meat tenderness over a pH range of 4.6 to 4.1 [42], which could be an effect of loosening the structure in collagen connective tissue. The acid solution breaks the transversal bounds of collagen, leading to the unstable structure loss of this connective tissue protein. The effects of acid marinades provoke swelling and the enhanced extraction of myofibrillar proteins due to decreased pH and increased ionic strength, allowing water to penetrate into the muscle [43]. Acid marination also accelerates the proteolysis reaction caused by cathepsins, initiates the weakening of muscle structure due to meat swelling, and causes an increase in the conversion of collagen to gelatin at low pH levels during cooking [8,9,44].

Early studies demonstrated that collagen's solubility is not affected by the temperature and time of conditioning [5]. Later, it was reported that during refrigerated storage, endogenous enzymes in muscle, such as collagenase, could promote collagen degradation or transform insoluble collagen into soluble collagen and reduce the total collagen content. Soluble collagen can be dissolved in water and come out of the muscle as the water-holding capacity of the muscle decreases, causing a decrease in the soluble collagen content [36]. These changes in collagen with thermal properties can be attributed to peptide bond hydrolysis and the low breakage of covalent cross-links in collagen [4,7].

Figure 6 shows the collagen area percentage quantified transversally. When the NC-RH were applied, the highest percentage of collagen was obtained (28.63 $\pm$ 4.6% area of collagen) at pH 6.5 and 25 °C. However, this increment could have the opposite effect on tenderness, making the meat harder. At pH 4 and 4 °C, there was an 11.47 $\pm$ 2.2% area of collagen, representing a better quantity of collagen. It was demonstrated that the transverse breaking stress of meat, which reflects connective tissue strength, decreases in pH, and cannot be explained only by swelling. This suggests the significant weakening of connective tissues at low pH levels [7].

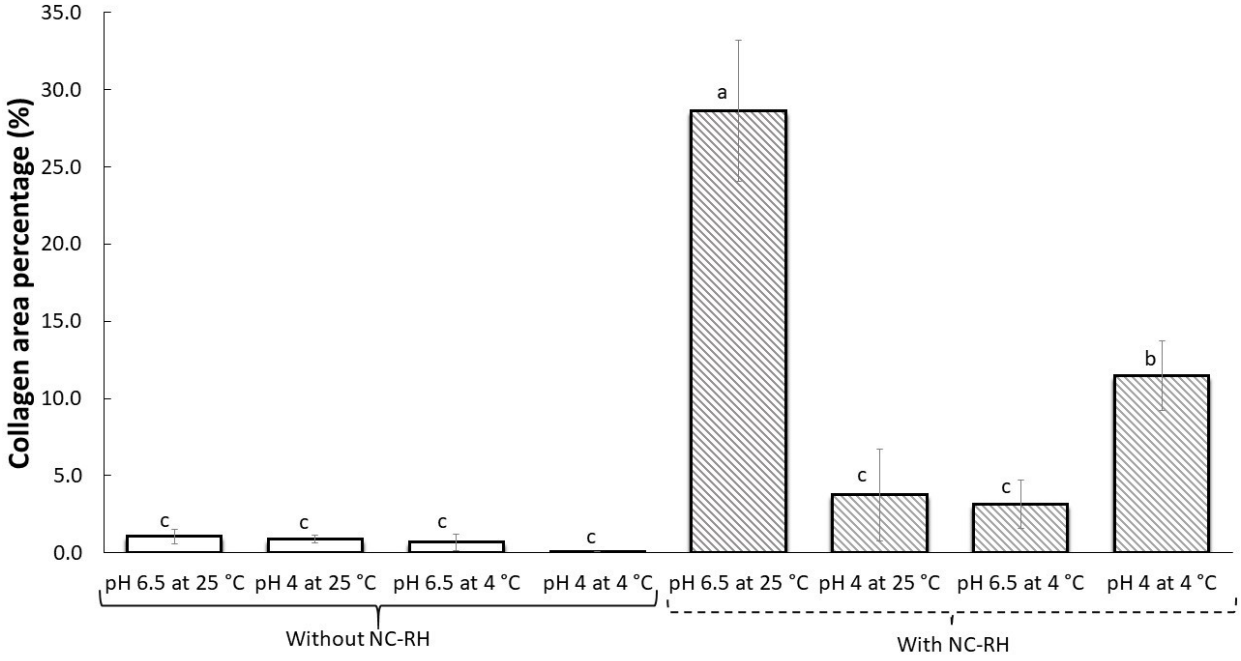

Figure 6. Collagen area percentage in transverse sections in different conditions at 48 h. Different letters indicate statistically significant differences ($p < 0.05$).

4. Conclusions

The nanoprecipitation method was able to obtain physically stable nanocapsules of rosehip oil (NC-RH) with excellent encapsulation efficiency (75.84%), showing that the bonds present in the system facilitate the formation of nanocapsules. In addition to this, the method is environmentally friendly, using only green solvents. Moreover, the Newtonian behavior facilitates the incorporation of the system into the marinade, allowing for the utilization of the effect of fatty acids present in rosehip oil and the antioxidant capacity of the system. Nanoencapsulation helps to protect the active compound from degradation, improves the bioavailability of rosehip oil during its application, and grants a slow release of the oil entrapped into the meat, prolonging its antioxidant activity.

The histological test showed positive changes in collagen structure associated with the swelling of the collagen, disordered tissue structure, and loose fiber arrangement, which increased the distance between them. All these changes are thought to improve the tenderness and water-holding capacity of meat. Therefore, the treatment with NC-RH at pH 4.0 and 4 °C had the most considerable influence on the changes in pork tenderloin, given that the percentage area of collagen was increased 10-fold. The results highlight that this study presents an innovative technology that can be applied to meat. It has shown significant improvements in the characteristics of collagen and pH, which are important for meat tenderness and conservation. For these reasons, it is relevant to continue this study to the next stage, in which the organoleptic properties of the meat and consumer acceptance will be evaluated.

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