American Journal of Biochemistry and Biotechnology

Original Research Paper

Preparation of Antimicrobial Collagen Casings with High Mechanical Properties

[1]Zhike Xie, [1]Shuyan Yu, [1]Ming He, [1]Shaoxuan Yu, [2]Huanying Zhao, [1]Haifang Xiao and [1]Yuanda Song

[1]*Colin Ratledge Center for Microbial Lipids, School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China*
[2]*Zibo Huanghelong Bioengineering Co. Ltd, Zibo, Shandong, 256400, China*

Article history
Received: 10-06-2020
Revised: 04-08-2020
Accepted: 01-09-2020

Corresponding Author:
Haifang Xiao
Colin Ratledge Center for Microbial Lipids, School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China
Email: xiaohaifang@sdut.edu.cn

Abstract: Tea Polyphenols (TP) has received much attention for their excellent antioxidant and antibacterial activities. Some additives (such as sodium alginate and sodium pyrophosphate) have good hydrophobicity which have influence on the mechanical properties of food. The effect of TP on the antibacterial activity of collagen casing was investigated, the effect of addition of TP, sodium pyrophosphate and sodium alginate on mechanical properties including tensile force, tensile strength and elongation at break were also studied. Results from the single factor experiments demonstrated that TP showed strong antibacterial activities against Escherichia coli, Staphylococcus aureus and Salmonella according to the Minimum Inhibitory Concentration (MIC) and the inhibition zone method. Moreover, the mechanical properties of collagen casings were obviously influenced by the addition of TP and two food additives including sodium alginate and sodium pyrophosphate. This work would be useful for application of collagen casings with TP in food package to extend shelf-life of sausage during processing, transportation and storage.

Keywords: Collagen Casings, Tea Polyphenols, Antimicrobial Activities, Mechanical Properties

Introduction

In recent years, tremendous changes have taken place in food packaging with the improvement of consumer requirements for food quality and safety (Priyadarshi *et al*., 2018). Active packaging which has the potential to replace traditional packaging in some cases has got great attention because of its environment-friendly, easily degraded and several extra functions including antimicrobial activity, antioxidant activity, moisture scavenging and so on in addition to barrier action (Riaz *et al*., 2018; Lei *et al*., 2019). Natural biopolymers such as polysaccharides and proteins are prove to be good candidates used in active food packaging because of their biodegradability and edibility (Romani *et al*., 2017; Nouri *et al*., 2018).

Collagen casing, firstly produced in the 1980s, is one type of the active packaging and widely used in the meat industry (Wang *et al*., 2015). It has received much attention due to their high strength, great biocompatibility and biodegradability (Yang *et al*., 2016). Collagen casings also act as a barrier to protect sausages during production, storage and distribution (Conte *et al*., 2012; Mandic *et al*., 2018). Nevertheless, casings base on pure collagen have some limitations such as poor antibacterial properties and unsuitable mechanical performances which restrict their applications in food packaging (Harper *et al*., 2012; Oechsle *et al*., 2014). In recent years, foodborne diseases due to microorganisms contaminated with sausage occurred all over the world. These foodborne outbreaks caused significant economic loss and posed a great threat to people's health. Therefore, it's very necessary to develop collagen films with superior antibacterial activity. Many researchers have made many useful explorations in edible films incorporated with bioactive substances. Chitosan-based active food packaging film incorporated with apple peel polyphenols exhibited good mechanical and antimicrobial properties (Riaz *et al*., 2018). Collagen casing impregnated with nisin has been proved to possess antimicrobial activity against foodborne pathogens and food spoilage bacteria associated with ready-to-eat sausages (Batpho *et al*., 2017). The mechanical properties of collagen casings are important in determining their application to foods because these properties provide an indication of how much stress and strain the casing can withstand before fracture (Simelane and Ustunol, 2005). This is particularly important during sausage manufacturing because a sausage casing must be tender enough to be pliable during stuffing and yet be strong enough to hold the meat batter during the cooking process and mechanical handling. Nevertheless, casings based on pure collagen will expand due to heating, which will lead to collagen casing damage in the process of cooking, minced meat (mixture of meat protein and other ingredients). However, relatively few researches have been carried out on collagen casings.

TP, water soluble natural phenolic compounds, are extracted from tea and mainly composed of several catechins including (-)-Epicatechin (EC), (-)-Epicatechin-3-Gallate (ECG), (-)-Epigallocatechin (EGC), (-)-Epigallocatechin-3-Gallate (EGCG), (+)-gallocatechin and (+)-catechin, etc. (Azam *et al*., 2004). Many epidemiological studies have proved that green tea was closely associated with many physiological and pharmacological health benefits which mainly attributed to TP (Cabrera *et al*., 2006). Moreover, TP were reported to possess strong antioxidant and antibacterial activities (Lagha *et al*., 2017; Zhang *et al*., 2018). In view of the advantages mentioned above as well as low-cost and safety of TP, many researchers have concentrated on the application of TP in developing edible active packaging films in recent years, it was found that TP could interact with chitosan to improve the water vapor barrier performance and endowed the membrane with good DPPH radical scavenging ability (Wang *et al*., 2013). Another research reported that great physical properties and antioxidant activity were exhibited in gelatin-sodium alginate edible films with TP (Dou *et al*., 2018). The incorporation of TP in pectin-konjac glucomannan composite edible films obviously enhanced the antioxidant activity, antimicrobial activity as well as mechanical and water-resistant properties of the films (Lei *et al*., 2019). Additionally, the previous study demonstrated that catechin improved the stabilization of collagen through crosslinking involved of hydrogen bonding and hydrophobic interactions (Madhan *et al*., 2005). Despite so many advantages of TP in edible films reported, few researches have regarded the application of TP in collagen casings.

Sodium alginate is an anionic polysaccharide extracted from brown algae (Bokkhim *et al*., 2016), which is widely used in the emulsion and restructured meat products as gel producing, thickening, suspending and emulsion stabilizing agents because it’s thickening property, high stability and good gelling characteristic, could improve the juiciness, texture, color and flavor of meat products (Chen *et al*., 2014). Sodium pyrophosphate is a most widely used functional phosphate employed in meat processing (Shen and Swartz, 2010). To promote gelation, sodium pyrophosphate is commonly added during meat processing to aid in the extraction of myofibrillar protein that will subsequently aggregate and gel during cooking (Jongberg *et al*., 2015).

Base on those backgrounds, the aim of this work was to investigate the antimicrobial activities of TP and collagen casings against four bacteria including two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Salmonella*), the influence of TP and two food additives (sodium alginate, sodium pyrophosphate) on mechanical properties of collagen casings were also examined. These findings will provide the optimizing formulation of antimicrobial collagen casings applied in food packaging industry.

Materials and Methods

Materials

All the materials used in this study were commercially available. The collagen casings (from cowhide and the aperture is 25 mm) were offered by Zibo Huanghelong Bioengineering Co., Ltd. (Zibo, China). Tea polyphenols were obtained from Freder Biotechnology Co., Ltd (Suzhou, China). Sodium alginate and sodium pyrophosphate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other reagents used in this study were analytical grade. Sterile water was used throughout this study.

Experimental Methods

Strains Preparation

Escherichia coli (ATCC25922), *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC6633) and *Salmonella* (ATCC14028) were preserved in Colin Ratledge Center for Microbial Lipids of Shandong University of Technology.

Preparation of Collagen Casings

Collagen casings were cut into 20 cm lengths and then sprayed with prepared solutions of food additions (sodium alginate, sodium pyrophosphate and TP). After treatment for 10 h at 35°C, collagen casings were kept in a desiccator at 25°C and 50% Relative Humidity (RH) before further analysis.

Evaluation of Minimum Inhibition Concentration (MIC)

MIC of TP was evaluated by diffusion method according to the previously report with slight modifications (Duarte *et al*., 2015). Briefly, serial concentrations of TP, ranging from 2 to 18 mg/mL, sequentially mixed with cooled nutrient broth. The same nutrient broth with sterile distilled water instead of TP was used as the control group. Test microorganisms including Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Salmonella with approximately 1×10^6 CFU/mL colony number used in this study. Test microorganisms were inoculated into the culture medium and cultured at 37°C for 24 h. MIC values were determined as the lowest concentration that caused a visible inhibition zone compared to the control group.

Determination of the Inhibition Zone

The inhibition zones of collagen casings were evaluated by disc diffusion method (Celiktas *et al*., 2007). Gram-positive bacterium (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacterium (*Escherichia coli* and *Salmonella*) were employed in this experiment. Test microorganisms containing a bacterial load of 1×10^6 CFU/mL were cultured using nutrient broth. Collagen casings were cut into small disks of 20 mm diameter and were placed on the surface of solid nutrient broth that was inoculated with the test bacteria. The same nutrient broth with pure collagen casings was served as the control. The plates were then incubated at 37°C for 24 h and the inhibition zone was measured.

Mechanical Properties

The films were cut into strips (15×3 cm in the longitudinal direction, 5×3 cm in the transverse direction) and stored at 25°C and 50% RH for 24 h before measurement. The tensile force, tensile strength and elongation at break of the collagen casings were measured at 25°C with a tensile testing machine (XLW; Labthink, China). The tensile tests were performed with a longitudinal gap of 10 cm, a transverse gap of 3 cm and a crosshead speed of 1 mm/s.

Statistical Analysis

Origin (Version 8.0; Origin Lab, USA) and Excel (Version 2010; Microsoft, USA) were used for statistical analysis. Analysis Of Variance (ANOVA) was employed to determine the significance. All the results were expressed as mean value ± standard deviation (n = 3 independent experiment). P values less than 0.05 were considered to be statistically significant and all determinations were performed in triplicate.

Results and Discussion

MIC of TP

MIC is an indicator to determine the antibacterial activity of antibacterial agents. The MICs of TP against four food-borne pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella*) were investigated in this study. Results in Table 1 showed that MIC values of TP against (*Salmonella*, *Escherichia coli*, *Bacillus subtilis and Staphylococcus aureus*) were 6, 8, 8 and 18 mg/mL, respectively. Above results indicated that *Bacillus subtilis* was the most resistant bacterium and *Salmonella* was the most sensitive bacterium among the tested bacteria to TP used in this study. These results were consistent with previous report that TP showed weaker antimicrobial activity against *Bacillus subtilis* than *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* though there were differences between MIC values (He *et al*., 2016), which could be explained as the strong resistance and vitality of *Bacillus subtilis* (Rao *et al*., 2019). In addition, previous studies revealed that TP exerted its inhibitory effects on *Escherichia coli* through increasing endogenous oxidative stress (Xiong *et al*., 2017) and on methicillin-resistant *Staphylococcus aureus* by regulating expression of extracellular proteins (Cho *et al*., 2008).

Table 1: Minimum Inhibition Concentration (MIC) of TP

		MIC			
NO.	TP concentration (mg/mL)	Escherichia coli	Staphylococcus aureus	Bacillus subtilis	Salmonella
1	18	-	-	-	-
2	16	-	-	+	-
3	14	-	-	++	-
4	12	-	-	++	-
5	10	-	-	+++	-
6	8	-	-	+++	-
7	6	+	+	+++	-
8	4	++	+++	++++	+
9	2	+++	++++	++++	+++
10	0	++++	++++	++++	++++

The blank group was sterile water, "-" was sterile growth, "+" was very weak growth of bacteria, "++" was weak growth of bacteria, "+++" was strong growth of bacteria, "++++" was particularly strong growth of bacteria.

Antimicrobial Property of Collagen Casings

Antimicrobial property of collagen casings after addition of TP was evaluated using the diameter of inhibition zones. As observed in Fig. 1, the diameters of inhibition zone against *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* were increased with increasing addition of TP in collagen casings and ranged from 20 to 33.75 mm, 20 to 29.25 mm and 20 to 28.25 mm, respectively. For *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*, the maximum inhibition zones were 33.75, 29.25 and 28.25 mm at 2.5% of TP addition in collagen casings, respectively. However, collagen casings with TP exhibited weak antimicrobial ability against *Bacillus subtilis* which was consistent with our MIC results. Base on the above, collagen casings with TP showed stronger antimicrobial activities against Gram-negative bacterium than Gram-positive bacterium. Previous studies reported that EGCG, the main active ingredient of TP, exerted stronger inhibitory effect on Gram-positive bacteria than Gram-negative bacteria which was different with our results in this study (Cho *et al*., 2008). The difference of results might be ascribed to the crude extracts of TP (food grade) used in this study which was different with the high purified individual component of TP. Moreover, different mechanisms were involved in inhibition of EGCG against Gram-positive and Gram-negative bacteria (Cui *et al*., 2012).

Effect of Sodium Alginate on Mechanical Properties of Collagen Casings

Sodium alginate with great water retention is widely used in food industry and is excellent material to make edible films (Galus and Lenart, 2013; Liu *et al*., 2017). In this study, sodium alginate was used to improve the mechanical properties of collagen casings which usually become hard and brittle during storage. As shown in Fig. 2, the tensile force, tensile strength and elongation at break of collagen casings longitudinally and transversely increased firstly and then decreased with the increase of sodium alginate concentration. Better mechanical properties were achieved by 2.5-3.5% sodium alginate reinforced collagen casings. These results revealed that sodium alginate played an important role in reinforcing the mechanical properties of collagen casings. The potential explanation of these results might be that a mesh interpenetrating structure formed through the bond cooperation between sodium alginate and collagen and the resulting synergistic effect made the support stronger, thus greatly improved the mechanical properties of collagen casings. Further investigations should be performed to verify this speculation.

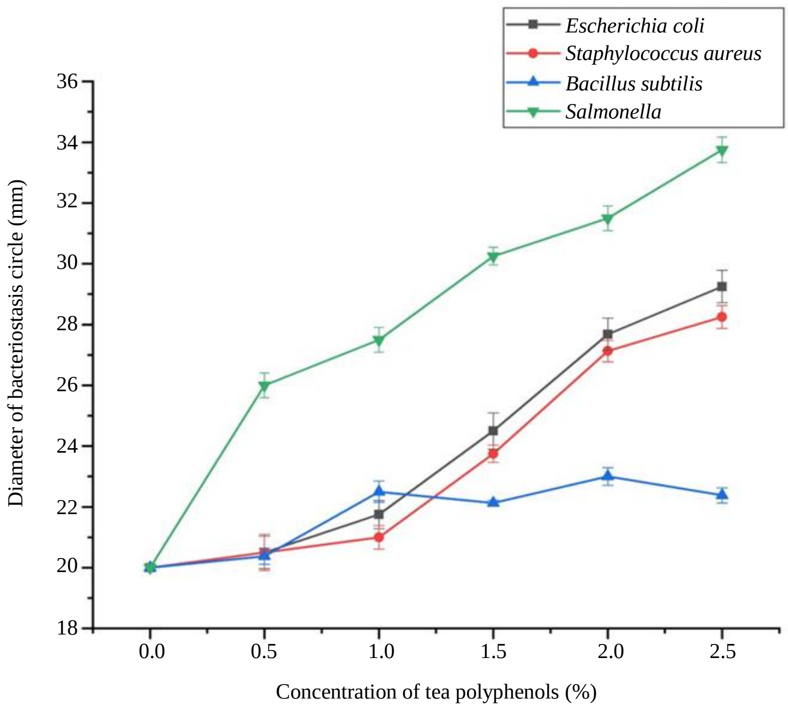


Fig. 1: Effect of TP on bacteriostatic properties of collagen casings

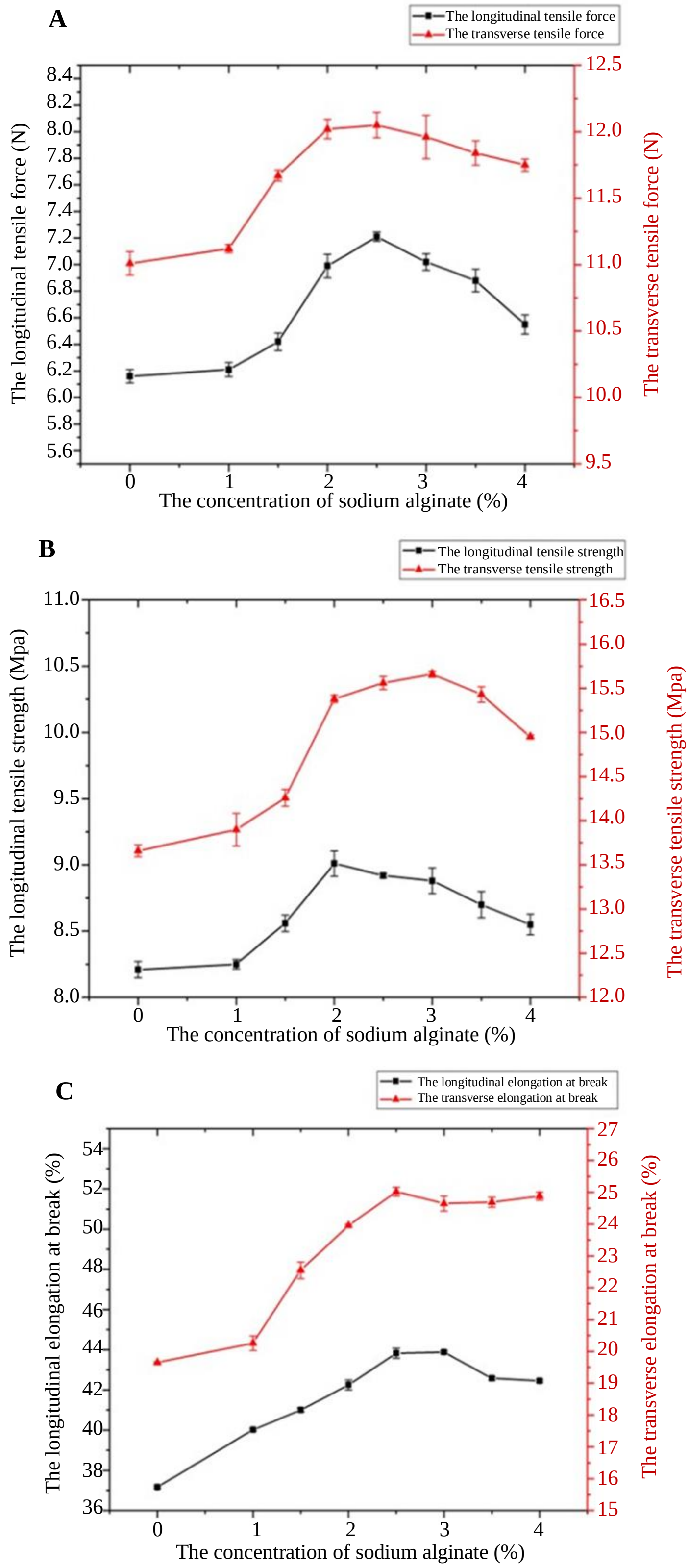


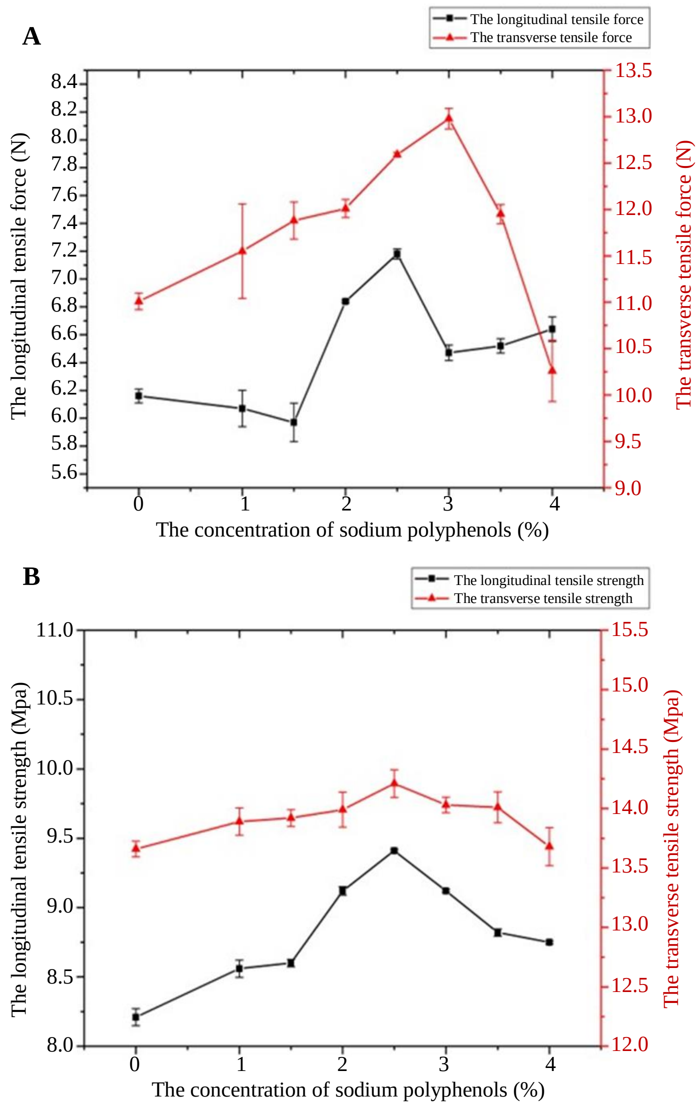
Fig. 2: Effect of sodium alginate on mechanical properties of collagen casings. (A) Effect of sodium alginate on the tensile force of collagen casings. (B) Effect of sodium alginate on the tensile strength of collagen casings. (C) Effect of sodium alginate on the elongation at break of collagen casings

Effect of Sodium Pyrophosphate on Mechanical Properties of Collagen Casings

Sodium pyrophosphate is great food quality improver and widely used in food processing. Previous studies revealed that phosphate improved the structure and properties of edible films (Tkaczyk *et al*., 2001). Therefore, we investigated the effect of sodium pyrophosphate on mechanical properties of collagen casings. As illustrated in Fig. 3, the mechanical properties of collagen casings including the tensile force, tensile strength and elongation at break longitudinally and transversely reinforced firstly and then decreased with increasing sodium pyrophosphate additions up to 4%. Collagen casings added 2.5-3.5% sodium pyrophosphate gained better mechanical properties compared to other samples. The reinforcement of sodium pyrophosphate in a certain addition range on mechanical properties of collagen casings might be explained as its great water retention property. Base on the above, we speculated that the addition of sodium pyrophosphate might help to relieve the embrittlement of collagen casings during storage. We will explore this issue in future research.

Effect of TP on Mechanical Properties of Collagen Casings

In recent years, several researches focused on the application of plant polyphenol in edible films. Thinned young apple polyphenols were reported to enhance the antioxidant and antimicrobial properties of chitosan film though mechanical properties of the film were decreased (Sun *et al*., 2017). Apple peel polyphenols with the concentration of 0.50% significantly improved the mechanical and antimicrobial properties of chitosan film (Riaz *et al*., 2018). In addition to the antimicrobial properties of TP, the effect of TP on mechanical properties of collagen casings was also investigated in this study. Results in Fig. 4 showed that the mechanical properties of collagen casings were changed after the addition of TP and gained better values with 1.5-2.5% of TP.



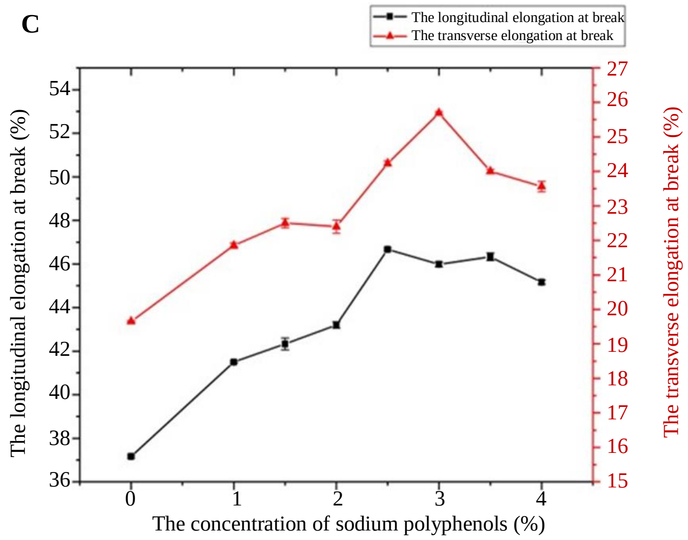
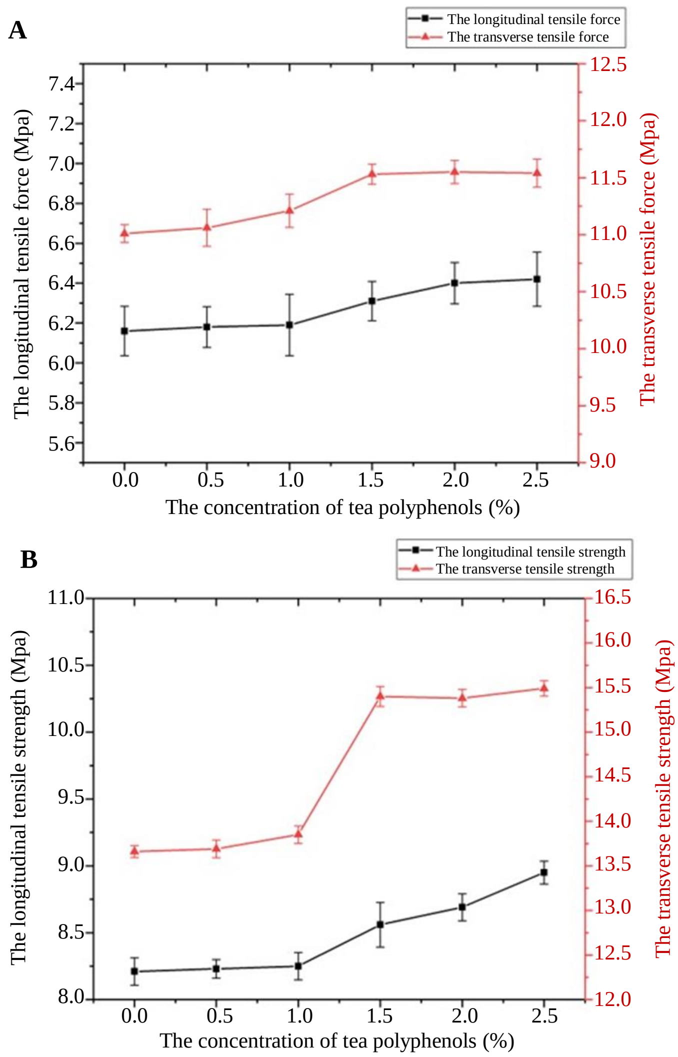


Fig. 3: Effect of sodium pyrophosphate on mechanical properties of collagen casings. (A) Effect of sodium pyrophosphate on the tensile force of collagen casings. (B) Effect of sodium pyrophosphate on the tensile strength of collagen casings. (C) Effect of sodium pyrophosphate on the elongation at break of collagen casings



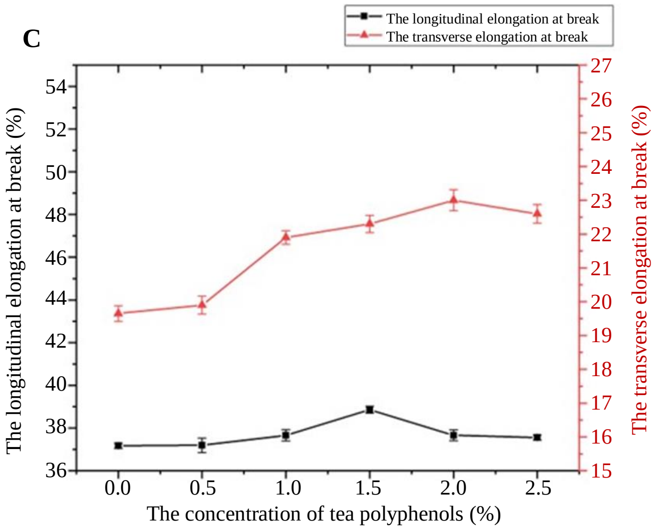


Fig. 4: Effect of TP on mechanical properties of collagen casings. (A) Effect of TP on the tensile force of collagen casings. (B) Effect of TP on the tensile strength of collagen casings. (C) Effect of TP on the elongation at break of collagen casings

Previous studies reported that TP at appropriate levels significantly increased the mechanical properties of composite edible films of pectin and konjac glucomannan (Lei *et al*., 2019) and green tea extract rich in TP was revealed to enhance tensile strength of silver carp skin gelatin film (Wu *et al*., 2013). The effect of TP on edible films probably due to the interaction between TP and other compositions incorporated in films. Moreover, TP exhibited less influence on the mechanical properties of collagen casings compared to sodium alginate and sodium pyrophosphate in this study.

Conclusion

The antimicrobial effect of TP and collagen casings was observed through diameter of inhibition zone and MIC. Meanwhile, the effects of TP and two additives on mechanical properties of collagen casings were investigated. In this study, the findings revealed that advantages of adding TP in collagen casings included improving mechanical properties as well as enhancing antibacterial abilities against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella*. Meanwhile, the mechanical properties of collagen casings were improved by sodium alginate and sodium pyrophosphate in a certain concentration range. Base on the above, the collagen casings added with TP, sodium alginate and sodium pyrophosphate could be applied to coat sausages to extend shelf-life and maintain the adequate mechanical properties of sausages. In a similar type of study, the mechanical and antibacterial properties of chitosan membranes were significantly improved by 0.50% apple peel polyphenols (Riaz *et al*., 2018; Zhang *et al*., 2020), found that zwitterionic chitosan derivative could improve the mechanical properties and antibacterial activity of carboxymethyl cellulose-based films (Tosati *et al*., 2017) found good antimicrobial effect of edible coating blend based on turmeric starch residue and gelatin. Future researches are required to explore the mechanism of interaction between additions used in this study and collagen.

Acknowledgement

This work was supported by SDUT and Zibo City Integration Development Project (2017ZBXC004) and Shandong Provincial Natural Science Foundation, China (No. ZR2014CQ002). Therefore, we are grateful for the funding and support of this research.

Funding Information

This work was supported by SDUT & Zibo City Integration Development Project (2017ZBXC004) and Shandong Provincial Natural Science Foundation, China (No. ZR2014CQ002).

Author's Contributions

Zhike Xie: Participated in the whole experiment process and also contributed to the interpretation of the results and manuscript preparation.

Shuyan Yu and Ming He: Participated in part of the experimental design.

Shaoxuan Yu: Ameliorated the manuscript.

Huanying Zhao: Contributed to the preparation of collagen casings.

Haifang Xiao: Contributed to the study design, the interpretation of the results and manuscript preparation.

Yuanda Song: Contributed to the guidance of experimental design and ameliorated the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Azam, S., Hadi, N., Khan, N. U., & Hadi, S. M. (2004). Prooxidant property of green tea polyphenols epicatechin and epigallocatechin-3-gallate: Implications for anticancer properties. Toxicology in vitro, 18(5), 555-561.

Batpho, K., Boonsupthip, W., & Rachtanapun, C. (2017). Antimicrobial activity of collagen casing impregnated with nisin against foodborne microorganisms associated with ready-to-eat sausage. Food Control, 73, 1342-1352.

Bokkhim, H., Bansal, N., Grøndahl, L., & Bhandari, B. (2016). In-vitro digestion of different forms of bovine lactoferrin encapsulated in alginate micro-gel particles. Food Hydrocolloids, 52, 231-242.

Cabrera, C., Artacho, R., & Gimenez, R. (2006). J Am Coll Nitr. Beneficial effect of green tea–a review, 25(2), 79-99.

Celiktas, O. Y., Kocabas, E. H., Bedir, E., Sukan, F. V., Ozek, T., & Baser, K. H. C. (2007). Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chemistry, 100(2), 553-559.

Chen, X., Li, P. J., Nishiumi, T., Takumi, H., Suzuki, A., & Chen, C. G. (2014). Effects of high-pressure processing on the cooking loss and gel strength of chicken breast actomyosin containing sodium alginate. Food and Bioprocess Technology, 7(12), 3608-3617.

Cho, Y. S., Schiller, N. L., & Oh, K. H. (2008). Antibacterial effects of green tea polyphenols on clinical isolates of methicillin-resistant Staphylococcus aureus. Current microbiology, 57(6), 542-546.

Conte, A., Marino, R., Della Malva, A., Sevi, A., & Del Nobile, M. A. (2012). Influence of different casings on salami produced with meat from buffalo and podolian cattle. Journal of Food Quality, 35(2), 127-136.

Cui, Y., Oh, Y. J., Lim, J., Youn, M., Lee, I., Pak, H. K., ... & Park, S. (2012). AFM study of the differential inhibitory effects of the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. Food Microbiology, 29(1), 80-87.

Dou, L., Li, B., Zhang, K., Chu, X., & Hou, H. (2018). Physical properties and antioxidant activity of gelatin-sodium alginate edible films with tea polyphenols. International journal of biological macromolecules, 118, 1377-1383.

Duarte, A., Alves, A. C., Ferreira, S., Silva, F., & Domingues, F. C. (2015). Resveratrol inclusion complexes: antibacterial and anti-biofilm activity against Campylobacter spp. and Arcobacter butzleri. Food Research International, 77, 244-250.

Galus, S., & Lenart, A. (2013). Development and characterization of composite edible films based on sodium alginate and pectin. Journal of Food Engineering, 115(4), 459-465.

Harper, B. A., Barbut, S., Lim, L. T., & Marcone, M. F. (2012). Microstructural and textural investigation of various manufactured collagen sausage casings. Food research international, 49(1), 494-500.

He, L., Zou, L., Yang, Q., Xia, J., Zhou, K., Zhu, Y., ... & Liu, S. (2016). Antimicrobial activities of nisin, tea polyphenols and chitosan and their combinations in chilled mutton. Journal of food science, 81(6), M1466-M1471.

Jongberg, S., Terkelsen, L. D. S., Miklos, R., & Lund, M. N. (2015). Green tea extract impairs meat emulsion properties by disturbing protein disulfide cross-linking. Meat science, 100, 2-9.

Lagha, A. B., Haas, B., & Grenier, D. (2017). Tea polyphenols inhibit the growth and virulence properties of Fusobacterium nucleatum. Scientific Reports, 7, 44815.

Lei, Y., Wu, H., Jiao, C., Jiang, Y., Liu, R., Xiao, D., ... & Li, S. (2019). Investigation of the structural and physical properties, antioxidant and antimicrobial activity of pectin-konjac glucomannan composite edible films incorporated with tea polyphenol. Food Hydrocolloids, 94, 128-135.

Liu, S., Li, Y., & Li, L. (2017). Enhanced stability and mechanical strength of sodium alginate composite films. Carbohydrate polymers, 160, 62-70.

Madhan, B., Subramanian, V., Rao, J. R., Nair, B. U., & Ramasami, T. (2005). Stabilization of collagen using plant polyphenol: role of catechin. International journal of biological macromolecules, 37(1-2), 47-53.

Mandic, S., Savanovic, D., Velmir, A., Kalaba, V., Savanovic, J., & Jokanovic, V. (2018). Effect of incorporating blackthorn fruit (Prinus spinosa L.) extract in natural casing on quality of Kranjska sausage. Scientific journal" Meat Technology", 59(2), 80-90.

Nouri, A., Yaraki, M. T., Ghorbanpour, M., Agarwal, S., & Gupta, V. K. (2018). Enhanced Antibacterial effect of chitosan film using Montmorillonite/CuO nanocomposite. International journal of biological macromolecules, 109, 1219-1231.

Oechsle, A. M., Wittmann, X., Gibis, M., Kohlus, R., & Weiss, J. (2014). Collagen entanglement influenced by the addition of acids. European polymer journal, 58, 144-156.

Priyadarshi, R., Kumar, B., & Negi, Y. S. (2018). Chitosan film incorporated with citric acid and glycerol as an active packaging material for extension of green chilli shelf life. Carbohydrate polymers, 195, 329-338.

Rao, L., Zhao, L., Wang, Y., Chen, F., Hu, X., Setlow, P., & Liao, X. (2019). Mechanism of inactivation of Bacillus subtilis spores by high pressure CO2 at high temperature. Food microbiology, 82, 36-45.

Riaz, A., Lei, S., Akhtar, H. M. S., Wan, P., Chen, D., Jabbar, S., ... & Zeng, X. (2018). Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. International journal of biological macromolecules, 114, 547-555.

Romani, V. P., Prentice-Hernández, C., & Martins, V. G. (2017). Active and sustainable materials from rice starch, fish protein and oregano essential oil for food packaging. Industrial crops and Products, 97, 268-274.

Shen, Q. W., & Swartz, D. R. (2010). Influence of salt and pyrophosphate on bovine fast and slow myosin S1 dissociation from actin. Meat science, 84(3), 364-370.

Simelane, S., & Ustunol, Z. (2005). Mechanical Properties of Heat-cured Whey Protein-based Edible Films Compared with Collagen Casings under Sausage Manufacturing Conditions. Journal of food science, 70(2), E131-E134.

Sun, L., Sun, J., Chen, L., Niu, P., Yang, X., & Guo, Y. (2017). Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material. Carbohydrate Polymers, 163, 81-91.

Tkaczyk, A. H., Otaigbe, J. U., & Ho, K. L. G. (2001). Bioabsorbable soy protein plastic composites: effect of polyphosphate fillers on biodegradability. Journal of Polymers and the Environment, 9(1), 19-23.

Tosati, J. V., Messias, V. C., Carvalho, P. I., Pollonio, M. A. R., Meireles, M. A. A., & Monteiro, A. R. (2017). Antimicrobial effect of edible coating blend based on turmeric starch residue and gelatin applied onto fresh frankfurter sausage. Food and Bioprocess Technology, 10(12), 2165-2175.

Wang, L., Dong, Y., Men, H., Tong, J., & Zhou, J. (2013). Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food hydrocolloids, 32(1), 35-41.

Wang, W., Zhang, Y., Ye, R., & Ni, Y. (2015). Physical crosslinkings of edible collagen casing. International journal of biological macromolecules, 81, 920-925.

Wu, J., Chen, S., Ge, S., Miao, J., Li, J., & Zhang, Q. (2013). Preparation, properties and antioxidant activity of an active film from silver carp (Hypophthalmichthys molitrix) skin gelatin incorporated with green tea extract. Food Hydrocolloids, 32(1), 42-51.

Xiong, L. G., Chen, Y. J., Tong, J. W., Huang, J. A., Li, J., Gong, Y. S., & Liu, Z. H. (2017). Tea polyphenol epigallocatechin gallate inhibits Escherichia coli by increasing endogenous oxidative stress. Food Chemistry, 217, 196-204.

Yang, S., Wang, J., Wang, Y., & Luo, Y. (2016). Key role of collagen fibers orientation in casing-meat adhesion. Food Research International, 89, 439-447.

Zhang, C., Suen, C. L. C., Yang, C., & Quek, S. Y. (2018). Antioxidant capacity and major polyphenol composition of teas as affected by geographical location, plantation elevation and leaf grade. Food chemistry, 244, 109-119.

Zhang, C., Yang, X., Li, Y., Qiao, C., Wang, S., Wang, X.,... & Li, T. (2020). Enhancement of a zwitterionic chitosan derivative on mechanical properties and antibacterial activity of carboxymethyl cellulose-based films. International Journal of Biological Macromolecules.