Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Food Technology/ Scientific Notes

Antioxidant and antimicrobial potential of tilapia gelatin coating containing pepper extract

Abstract – The objective of this work was to evaluate tilapia gelatin with pepper extract as a sausage coating, applied through immersion and spraying. The treatments were: control (without coating), coating by immersion and spraying, and coating with the extract by immersion and spraying. The pepper extract showed a high phenolic content (217.9 mg gallic acid equivalents per gram) and antioxidant activity. All coated sausages presented a lower peroxide index than the control. The sausages coated with pepper extract showed a lower oxidation rate. However, no significant differences were observed between the application methods and antimicrobial activity of the coatings. Tilapia gelatin with pepper extract as a coating has proven to be an alternative to reduce lipid oxidation in meat products and preserve their quality.

Index terms: food quality, lipid oxidation, meat products.

Potencial antioxidante e antimicrobiano de revestimento de gelatina de tilápia contendo extrato de pimenta

Resumo – O objetivo deste trabalho foi avaliar a gelatina de tilápia com extrato de pimenta como revestimento para salsichas, aplicado por imersão e pulverização. Os tratamentos foram: controle (sem revestimento), revestimento por imersão e pulverização, e revestimento com o extrato por imersão e pulverização. O extrato de pimenta apresentou teor fenólico (217,9 mg de equivalentes de ácido gálico por grama) e atividade antioxidante elevados. Todas as salsichas com revestimento apresentaram menor índice de peróxido do que o controle. Já as salsichas revestidas com extrato de pimenta apresentaram menor índice de oxidação. No entanto, não foram observadas diferenças significativas entre os métodos de aplicação e a atividade antimicrobiana dos revestimentos. A gelatina de tilápia com extrato de pimenta como revestimento tem se mostrado uma alternativa para reduzir a oxidação lipídica em produtos cárneos e preservar sua qualidade.

Termos para indexação: qualidade de alimentos, oxidação lipídica, produtos cárneos.

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Received
July 16, 2025

Accepted
October 29, 2025

How to cite
ALFARO, A. da T.; PIVA, D.; BALBINOT-ALFARO, E.; MACHADO-LUNKES, A. Antioxidant and antimicrobial potential of tilapia gelatin coating containing pepper extract. **Pesquisa Agropecuária Brasileira**, v.61, e04191, 2026. DOI: https://doi.org/10.1590/S1678-3921. pab2026.v61.04191.

For the meat industry, preserving and maintaining product quality remains a critical challenge. To overcome it, packaging technologies are used to protect against deterioration effects such as discoloration, development of off-odors and off-flavors, nutrient loss, and texture changes, often caused by microbial growth, lipid oxidation, and surface dehydration (Sbardelotto et al., 2024).

Among the new packaging technologies, edible films and coatings are effective methods to protect food against microbial growth, protein degradation, and lipid oxidation (Zhao et al., 2022). In this context, tilapia gelatin may be an alternative raw material for the production of coating films due to its high availability, cost-effectiveness, and favorable properties such as a high biodegradability and a low antigenicity and allergenicity (Santos et al., 2024).

The extract from peppers of the genus *Capsicum* is widely used in foods due to its color, flavor, and aroma, as well as antioxidant and antimicrobial properties attributed to its active compounds (Baenas et al., 2019). This extract contains ascorbic acid, carotenoids, phenolic compounds, and alkaloids, among which capsaicin is the main responsible for the pungency of peppers (Lidiková et al., 2021). Since microbial contamination in meat products is a constant concern, incorporating these extracts into coatings can offer an additional barrier against pathogens and spoilage microorganisms, directly influencing food safety and shelf life. Although there are studies on the application of edible coatings with plant extracts to meat products, there are no known reports on the specific combination of tilapia gelatin and pepper extracts.

The objective of this work was to evaluate tilapia gelatin with pepper extract as a sausage coating, applied through immersion and spraying.

For the study, Vienna-type sausages sold in bulk were purchased from local retailers. The sausages were then packaged in plastic bags and put into a thermal box with ice to be transported to the laboratory for immediate processing. The used chili peppers [*Capsicum baccatum* var. *pendulum* (Willd.) Eshbaugh] were acquired from local markets.

To obtain the extract, the peppers were sanitized in 2% sodium hypochlorite and dried in the MA 035 oven (Marconi, Piracicaba, SP, Brazil) at 50°C for 7 hours. Then, they were macerated in 70% (v/v) hydroalcoholic solvent in the BIO-650s shaker (7Lab, Rio de Janeiro, RJ, Brazil) at 30°C and 200 rpm for 24 hours. After that, the solvent was evaporated in a rotary evaporator, resulting in the raw pepper extract.

The concentration of the phenolic compounds and antioxidant activity of the pepper extract were determined to evaluate its antioxidant potential. The analyses were performed on the day the extract was obtained (day zero) and repeated after ten days (day ten). The concentration of phenolic compounds was measured using the Folin-Ciocalteau method described by Singleton et al. (1999). The antioxidant activity of the pepper extract was evaluated by the ferric reducing antioxidant power (FRAP) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods, both according to Rumpf et al. (2023).

To prepare the gelatin, tilapia skins were washed and subjected to alkaline and acid treatments, as in Alfaro et al. (2013). The gelatin was extracted in distilled water at 1:2 (w/v) at 55°C for 9 hours. Afterward, the solution was filtered in a Büchner funnel with Whatman grade 4 filter paper (Cytiva, Wilmington, DE, United States of America), lyophilized, ground, and stored in hermetically sealed plastic pots.

The coating solution was prepared by hydrating the gelatin in distilled water (4% w/w) at room temperature for 30 min, followed by the addition of glycerol (20% w/w). Afterward, 0.10 g of the extract was added to 200 mL of the coating solution, which was subjected to moderate agitation and heating at 40°C for 30 min. The coating solution without the pepper extract was prepared using the same procedure, excluding the addition of the extract.

The coating solution was applied to the sausages using two methods: immersion, by completely submerging the sausages in the coating solution; and spraying, by manually spraying the sausages until fully covered. A total of five treatments were evaluated: control, without coating; coating by immersion; coating by spraying; coating with the extract by spraying; and coating with the extract by immersion. Afterwards, the sausages were hung for dripping during 10 min and then dried in the MA 035 oven (Marconi, Piracicaba, SP, Brazil) with forced-air circulation at 50°C for 24 hours. Subsequently, the sausages were vacuum-sealed using 0.12 mm poly nylon packaging and stored at 4°C.

The lipid oxidation of the sausages was assessed using the peroxide index (Brasil, 2022) and the TBARS method described by Crackel et al. (1988). Following the TBARS method, 5.0 g samples were homogenized with 15 mL of 0.38 mol L^{-1} $HClO_4$ and 0.5 mL of 0.19 mol L^{-1} butylated hydroxytoluene, centrifuged, and filtered. A 0.7 mL aliquot of the extract was mixed with 0.7 mL of 0.02 mol L^{-1} thiobarbituric acid, then heated at 100°C for 30 min, cooled, and centrifuged. Absorbance was measured at 532 nm, and the results were expressed as milligrams of malondialdehyde per

kilogram (MDA kg^{-1}) of sample. All evaluations were performed in triplicates.

In the microbiological evaluation, coliforms at 45°C, coagulase-positive *Staphylococcus*, *Salmonella* spp., and sulfite-reducing *Clostridia* were determined. The analyses were carried out on the fifteenth day after coating application using the protocols of Brasil (2022). All evaluations were performed in triplicates.

The analysis of variance was applied to the data, followed by Tukey's test at a 95% confidence level ($p<0.05$), using the Statistica, version 10.0, software (TIBCO Software Inc., Palo Alto, CA, USA). The antioxidant capacity of the pepper extract, determined by the FRAP method, was 100.6±1.3 μmol L^{-1} of trolox equivalents per gram (TE g^{-1}) on day zero and 95.5±5.8 μmol L^{-1} TE g^{-1} on day ten of the experiment, meaning no significant differences were observed ($p>0.05$). These results are consistent with those of the study by Gomes et al. (2019), who analyzed different *C. baccatum* var. *pendulum* genotypes and found FRAP values between 40.5 and 185.5 μmol L^{-1} TE g^{-1}.

Regarding the ABTS method, antioxidant capacity increased significantly ($p<0.05$) from day zero to ten, rising from 18.7±0.9 to 24.8±1.5 μmol L^{-1} TE g^{-1}. These results are within the range of 15.7 to 65.7 μmol L^{-1} TE g^{-1} of dry matter found by Lidiková et al. (2021) when evaluating extracts of sweet and spicy pepper. According to these authors, sweet pepper showed higher values likely due to a more significant presence of phenolic compounds and vitamin C, which contribute to the antioxidant activity of this variety.

The variations observed in the results of the ABTS and FRAP methods can be attributed to methodological specificity, as evidenced by Rumpf et al. (2023). The ABTS method uses organic radical cations to interact with various antioxidants that donate electrons or hydrogen atoms, while the FRAP method, based on reducing ferric ions, is more sensitive to antioxidants that exclusively donate electrons.

The pepper extract presented a high concentration of phenolic compounds, which showed relative stability over the storage period, with values ranging from 217.9±0.2 to 211.4±1.7 mg gallic acid equivalents per gram (GAE g^{-1}) from day one to ten. Gomes et al. (2019) analyzed different *C. baccatum* var. *pendulum* pepper cultivars and reported concentrations of phenolic compounds from 200 to 400 mg GAE g^{-1}. This variation in the obtained results can be explained by factors such as differences in the chemical forms of the compounds and in the used preparation methods, sample extraction, and quantification techniques, also including genotype diversity, stage of maturation, and use of fresh or dehydrated fruit (Lidiková et al., 2021).

All treatments showed a significantly lower peroxide index ($p<0.05$) than the control. These results are in alignment with those reported by Hamann et al. (2022) when studying the addition of green tea to gelatin films to inhibit the oxidation of fresh sausages. The authors concluded that the application of the gelatin coating alone already exerts a protective action against the oxidation of the meat sausage.

The reduced oxidation verified in the present work was possibly due to the formation of a physical barrier, which reduced the diffusion of oxygen from the environment to the sausage's surface, limiting oxidative reactions in the main substrate. Additionally, the reduced loss of surface moisture may have indirectly influenced the kinetics of the oxidation reactions.

The lowest peroxide levels were found in the treatments in which coating with the extract was applied by spraying and by immersion, showing the antioxidant potential of the pepper extract. However, no significant differences ($p>0.05$) were observed between these treatments (Table 1).

Table 1. Lipid oxidation in sausages subjected to treatments with the application of coating with tilapia gelatin containing pepper extract[(1)].

Analysis	Treatment[(2)]				
	Control	CI	CS	CSE	CIE
Peroxide index (mEq peroxide per kilogram)	3.92±0.28a	3.55±0.15b	3.57±0.13b	2.77±0.13c	2.55±0.23c
TBARS (mg malondialdehyde per kilogram)	4.31±0.37a	3.84±0.16a	4.27±0.30a	3.05±0.26b	2.82±0.12b

[(1)]Means followed by different letters, in the same line, differ significantly by Tukey's test ($p<0.05$). [(2)]CI, coating by immersion; CS, coating by spraying; CSE, coating with the extract by spraying; and CIE, coating with the extract by immersion.

The TBARS method quantifies MDA, a primary decomposition product of polyunsaturated fatty acid hydroperoxides, indicative of oxidation. The control and the treatment with only gelatin coating showed similar oxidation levels ($p>0.05$). Conversely, the treatments in which the pepper extract was applied by spraying and immersion significantly ($p<0.05$) reduced oxidation levels to 3.05 and 2.82 mg MDA kg^{-1}, respectively, highlighting the potent antioxidant action of the extract. The efficacy of the extract in inhibiting lipid oxidation shows the potential of natural antioxidants, such as plant extracts, to preserve the quality and prolong the shelf life of meat products.

All coating application methods reduced oxidation, with no significant difference ($p<0.05$) between them. This indicates that the application of coating with an antioxidant extract is equally effective through spraying and immersion. Therefore, the choice between these methods can be based on operational factors, such as ease of application, cost, and scalability, as long as the effectiveness of the antioxidant is not compromised.

The results obtained for lipid oxidation exceeded the recommended levels, since rancid odors are noticeable at concentrations above 2.5 mg MDA kg^{-1} (Demarco et al., 2022). This increased oxidation is likely primarily caused by the composition and inadequate storage conditions of the meat sausages. According to these findings, sausages are highly susceptible to oxidation due to their elevated lipid content and the addition of raw material with a high surface area-to-volume ratio, such as mechanically separated meat. In the present work, the used Vienna sausages were sold in bulk, i.e., in larger packages that were divided into smaller ones for retail stores, resulting in extra handling, which increases environmental exposure, further accelerating degradative processes such as lipid oxidation.

The results of the microbiological analysis indicated the absence of *Salmonella* spp. and sulfite-reducing *Clostridium* in all treatments (Table 2). Samples tested for coagulase-positive *Staphylococci* showed values lower than $1.0x10^1$ colony forming units per gram (CFU g^{-1}), which complies with the current Brazilian legislation (Brasil, 2019).

The legislation requirements were met by all treatments, except for coliforms at 45°C, which exceeded the established limits despite a proper sample handling. The high amount of coliforms at 45°C in the present study suggest shortcomings in sanitary conditions during processing or post-processing. Although the exact cause has not yet been identified, over handling possibly led to this contamination, as the analyzed sausages were sold bulk and then portioned into smaller packages for retail sale.

Since all treatments presented values similar to those of the control, the influence of gelatin coatings with pepper extract on the microbiological standard of the sausages could not be confirmed. Probably, the evaluation period of 15 days was insufficient to evaluate whether the coating with pepper extract has antimicrobial potential. Other feasible explanations include: an insufficient concentration of pepper extract to reach the minimum inhibitory concentrations to prevent the growth of the evaluated microorganisms; and a slow or insufficient release of the active compounds from the gelatin matrix to the surface of the sausage.

The applied pepper extract showed a high antioxidant potential, likely due to its high concentration of phenolic compounds. The tilapia

Table 2. Microbiological analyses of sausages subjected to treatments with the application of coating with tilapia gelatin containing pepper extract.

Treatment[(1)]	Coliforms at 45°C (MPN per gram)	*Salmonella*	*Staphylococcus* coagulase positive (CFU per gram)	Reducing-sulfite *Clostridium* (CFU per gram)
Control	$1.1x10^5$	Absence	$<1.0x10^1$	Absence
CI	$1.1x10^5$	Absence	$<1.0x10^1$	Absence
CS	$1.1x10^5$	Absence	$<1.0x10^1$	Absence
CSE	$1.1x10^5$	Absence	$<1.0x10^1$	Absence
CIE	$1.1x10^5$	Absence	$<1.0x10^1$	Absence

[(1)]CI, coating by immersion; CS, coating by spraying; CSE, coating with the extract by spraying; and CIE, coating with the extract by immersion. MPN, most probable number; and CFU, colony forming units.

gelatin coating containing the extract effectively reduced lipid oxidation in the sausages regardless of the application method, meaning that both spraying and immersion can be adapted to existing automated processes, offering flexibility to the industry. The assessed approach represents a promising and natural alternative to synthetic additives, in alignment with the growing demand for active packaging and clean-label food. However, the scalability and cost-effectiveness of the extract, the production of gelatin coating in larger volumes, and the potential sensory impact of pepper pungency on sausages should be further evaluated in future studies.

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Author contributions

Alexandre da Trindade Alfaro: conceptualization, data curation, funding acquisition, methodology, project administration, supervision, validation, writing – original draft, writing – review & editing; **Daiane Piva**: formal analysis, investigation, methodology, writing – original draft, writing – review & editing; **Evellin Balbinot-Alfaro**: data curation, formal analysis, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing; **Alessandra Machado-Lunkes**: conceptualization, methodology, supervision, validation, writing – original draft, writing – review & editing.

Chief editor: Edemar Corazza
Edited by: Madalena Rinaldi

Data availability statement

Data available upon request: research data are only available upon reasonable request to the corresponding author.

Declaration of use of AI technologies

No generative artificial intelligence (AI) was used in this study.

Conflict of interest statement

The authors declare no conflicts of interest.

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