Original scientific paper
UDC 637.5'711.1.037:[547.472.3:547.475.2

INFLUENCE OF LACTIC ACID AND ASCORBIC ACID MIXTURE ON THE QUALITY OF WILD BOAR MEAT STORED UNDER VACUUM PACKAGING AT CHILLED STORAGE

Munkhnasan Enkhbold[1*], Attila Lőrincz[2], Majd Elayan[1], László Friedrich[1], Attila Solymosi[1], Balázs Wieszt[1], Jáni Kornél[1], Adrienn Tóth[1]

[1]Department of Livestock and Food Preservation Technology, Hungarian University of Agriculture and Life Sciences, Menesi 44, 1118 Budapest, Hungary
[2]VADEX Mezőföldi Forestry and Wild Management, Petőfi Sándor 275, 8123 Soponya, Hungary

[*]e-mail: munkhnasan.e@gmail.com

Abstract

The wild boar (*Sus scrofa*) is one of the world's most widely dispersed animals. Recently, wild boar meat consumption has been increasing each year. Therefore, in this work, the impact of lactic acid and ascorbic acid mixture treatments was evaluated with the spray method in wild boar meat surfaces on meat quality parameters such as pH and color.

For the experiment, fresh wild boar meat from a local processing plant was used, and stored at 4 ± 1 ^{0}C for 1 day. A mixture of 2% lactic acid with 2% ascorbic acid was sprayed onto meat samples. The samples were vacuum-packed and stored at 4 ± 1 ^{0}C for 21 days. Quality parameters were measured on days: 0, 7, 14, and 21. The pH values were determined with a digital pH meter, and the color of the meat surface was measured using a colorimeter. L*, a*, and b* values were measured and delta E, hue angle, and chroma were calculated. The significance of differences between the treated and non-treated samples was determined by a two-way analysis of variance using the software IBM SPSS27.

Color measurement data indicate that the L* values of treated and non-treated wild boar meat samples did not show a significant ($P > 0.05$) difference from each other. However, treated samples had significantly ($P < 0.05$) higher a* values compared to non-treated samples on days 14 and 21. The a* values are important because a* values show redness and larger values that indicate a redder color. Therefore, spray treatment effected positively for color. For pH measurement, it was established that the lactic acid and ascorbic acid mixture are decreasing the pH of wild boar meat at the beginning of the display period. This initial decline could be caused by the acid treatment. Furthermore, pH values were slightly higher than non-treated samples at the end of the display period.

Therefore, in the conclusion, a 2% lactic acid and 2% ascorbic acid mixture could be an alternative to extend wild boar meat shelf life.

***Key words**: Wild boar meat, Lactic acid, Ascorbic acid, Spray method, Meat quality, Color.*

1. Introduction

Wild boars (*Sus scrofa*) are widely distributed in many parts of the world, including Europe, Asia, and America. Wild boar meat is a highly valued source of protein, with unique flavor and nutritional qualities [1]. Wild boar meat is considered a delicacy due to its distinct flavor and nutritional value. It is rich in protein, vitamins (B_1, B_2, B_6, and B_{12}), and minerals (zinc, iron, and selenium) [2, 3]. Wild boar meat is also known for its higher levels of polyunsaturated fatty acids (PUFAs) compared to conventional pork meat [3]. However, wild boar meat is susceptible to spoilage due to its high protein and fat content, as well as low pH [4]. The microbial growth and oxidation of lipids and proteins are the primary causes of meat spoilage, leading to off-flavors, discoloration, and reduced shelf life. Microbial growth can also occur during storage, leading to a

decline in quality and safety [5]. To address these issues, various preservation methods have been developed, including the use of organic acids and antioxidants, which have been shown to effectively extend the shelf life of meat products by reducing microbial growth and improving color stability [6].

Lactic acid is a widely used organic acid in the food industry for its antimicrobial and antioxidant properties. It is used as a food preservative to extend the shelf life of perishable food products, including meat [7]. It has been shown to be a safe and effective way of extending the shelf life of meat products without altering their sensory qualities. Lactic acid acts by lowering the pH of the meat, creating an unfavorable environment for the growth of microorganisms. Lactic acid has also been reported to have a positive effect on meat tenderness, color, and flavor [4]. The use of lactic acid in meat preservation has been approved by the US Food and Drug Administration (FDA) and the European Union (EU) [8]. It has been successfully used as a preservative in various meat products, such as beef [9], pork [10], and chicken [11]. However, its use in wild boar meat has not been extensively studied.

Ascorbic acid (vitamin C) is an essential water-soluble vitamin that acts as a potent antioxidant in biological systems. It is widely used as a food additive due to its ability to enhance the color stability and shelf life of meat products. Ascorbic acid can protect against lipid oxidation, reduce nitrate to nitrite, and improve the curing process, leading to a better quality of meat products [12]. In meat products, ascorbic acid can increase the oxidative stability of lipids by scavenging free radicals and chelating metal ions, which can catalyze oxidation reactions. It also reacts with nitrite and other nitrogen oxides, preventing the formation of harmful nitrosamines, which can occur during the curing process [13]. Ascorbic acid has been found to have a positive effect on the color stability of meat products, especially in cured meats. It can increase the formation of stable pigments such as myoglobin, which contribute to the red color of meat products. It can also improve the retention of other pigments such as carotenoids, which contribute to the yellow color of meat products [12]. Several studies have investigated the effects of ascorbic acid on the quality and shelf life of meat products. For example, the addition of ascorbic acid to chicken meat has been shown to reduce lipid oxidation and improve color stability during storage [14]. Similarly, the addition of ascorbic acid to beef patties has been shown to improve color stability and reduce lipid oxidation during storage [12]. It has been used as an antioxidant in various meat products, including beef [15], pork, and chicken [16]. There is limited research on the application of ascorbic acid in preserving wild boar meat.

Vacuum packaging is another widely used technique in the meat industry to extend the shelf life of meat products. It creates an oxygen-free environment that reduces the risk of microbial growth and oxidation [17]. However, vacuum packaging alone may not be enough to maintain the quality of wild boar meat during storage, especially if the meat has been subjected to microbial contamination during processing.

Therefore, this study aims to investigate the influence of lactic acid and ascorbic acid mixture on the quality of wild boar meat stored under vacuum packaging at chilled storage. Specifically, we investigated the changes in pH, instrumental color, and microbiological counts over 21 days. The results of this study will provide valuable insights into the potential use of lactic acid and ascorbic acid mixture as a preservative for wild boar meat and contribute to the development of effective meat preservation strategies and improve its shelf life.

2. Materials and Methods

2.1 Sample preparation

Wild boar meat was obtained from a local processing plant, "Vadex" Mezőföldi Zrt., and transported to the laboratory in chilled condition. The meat was cut into steaks of similar size. The samples were randomly divided into two groups, a control group, and a treatment group. The control group samples were vacuum-packed in polyethylene bags and stored in a refrigerated cabinet at 4 ± 1 ^{0}C. The treatment group samples were treated by spraying a mixture of 2% lactic acid and 2% ascorbic acid on both the top and bottom surfaces at room temperature. To create a mixture of lactic acid (Molar Chemicals Kft., Halásztelek, Hungary) and ascorbic acid (VitalTrend Kft., Budapest, Hungary), a 2%+2% (v/v) solution was prepared by diluting lactic acid and ascorbic acid in distilled water. The resulting solution had a pH of 2.48 and a volume of 500 ml. The acid mixture was applied in an amount equal to 10% of the meat weight. After treatment, the samples were vacuum packaged and stored at 4 ± 1 ^{0}C for 21 days. Quality parameters were measured on days 1, 7, 14, and 21.

2.2 pH determination

The pH of the meat samples was measured using a one-hand digital pH meter (Testo, Model 206-pH2, UK). The pH was measured directly from the muscles of the samples, which were chilled before measurement. Measurements were carried out at room temperature, and each sample was measured three times. pH meter was cleaned and calibrated with buffer solutions of pH 4.0 and 7.0 after each measurement, following the manufacturer's instructions.

2.3 Instrumental color measurement

The surface color of the wild boar meat was determined using a Chroma meter CR-400 (Konica Minolta, Inc., Osaka, Japan). The Chroma meter was calibrated with a standard white tile before taking measurements, and 20 replicate measures were performed on each sample representing the whole surface of the meat samples in a vacuum package. The Chroma meter was set to measure L*, a*, and b* values, which correspond to lightness, redness, and yellowness, respectively. The Chroma and hue angle were calculated.

2.4 Microbiological evaluation

To determine the microbiological count, each meat sample was diluted 10 times and subjected to the Aerobic Plate Count (APC) using nutrient agar through the pour plate method with duplicate plates. The plates were then incubated at 37 ^{0}C for 48 hours under aerobic conditions. The colonies were then counted to determine the total CFU per cm^2. The effectiveness of the decontamination procedure in extending the shelf-life of the meat was also evaluated based on the APC results, which served as an indicator of microbial growth. The shelf-life was considered to have reached its limit when the APC reached 7 $\log_{10}$ CFU per cm^2, as specified by the International Commission on Microbiological Specifications for Foods [18].

2.5 Statistical analysis

The data analysis was performed using IBM SPSS27 (Armonk, NY 2020) as a statistical evaluation tool. A two-way analysis of variance (ANOVA) and Tukey's HSD post hoc test were conducted to evaluate the effect of the treated and non-treated methods on the measured quality parameters of the wild boar meat samples. Differences were considered significant at $P < 0.05$.

3. Results and Discussion

3.1 pH

The pH of meat is an important factor that affects its quality, safety, and shelf life. As meat ages, its pH decreases due to the production of lactic acid by bacteria present in the meat [19]. In this study, the pH values of treated and non-treated wild boar meat samples were measured over 21 days. The results (Table 1) showed that the pH of the treated samples was slightly lower than the pH of the non-treated samples on day 1. This could be due to the use of the lactic acid and ascorbic acid mixture solution, which has a lower pH than the meat. The decrease in pH on day 7 for both treated and non-treated samples could be attributed to the natural postmortem changes in the meat.

However, the pH of the treated samples decreased at a slower rate compared to the non-treated samples, indicating that the lactic acid and ascorbic acid mixture solution may have had a protective effect on the meat. On day 14, the pH of the treated samples was almost the same as the pH of the non-treated samples, which could be due to the depletion of the protective effect of the lactic acid and ascorbic acid mixture solution over time. On day 21, the pH of the treated samples was slightly higher than the pH of the non-treated samples, indicating that the treated samples may have had a better shelf life compared to the non-treated samples.

3.2 Instrumental color measurement

Changes in CIE L*, a*, b*, hue angle, and Chroma values throughout the display of deer meat samples are shown in Table 2. The instrumental color measurement of wild boar meat samples revealed a significant decrease in L* values during the storage period for both treated and non-treated samples ($p < 0.05$). However, there was no significant difference between the L* values of treated and non-treated samples during the storage period. The decrease in L* values observed in both treated and non-treated samples during the storage period could be attributed to the natural postmortem changes in the meat, such as the oxidation of myoglobin and the breakdown of muscle tissue. This decrease in L* values indicates a darkening of the meat color, which may negatively affect its visual appeal to consumers.

Table 1. Effect of lactic acid and ascorbic acid mixture and vacuum packaging on pH values of wild boar meat samples during retail display at 4 ± 1 ^{0}C

Day	Treatment	pH
1	Treated	5.59 ± 0.02^b
	Non-treated	5.66 ± 0.05^b
7	Treated	5.39 ± 0.06^b
	Non-treated	5.50 ± 0.20^b
14	Treated	5.11 ± 0.06^a
	Non-treated	5.12 ± 0.02^a
21	Treated	5.01 ± 0.02^a
	Non-treated	4.98 ± 0.07^a

Legend: ^{ab}Different letters are for significantly different groups (Tukey, $p < 0.05$). Data are recorded as Mean ± Standard Error. Treated (sprayed with 2% lactic acid and 2% ascorbic acid mixture).

Table 2. Effect of lactic acid and ascorbic acid mixture on CIE L*, a*, b*, hue angle, and Chroma values of vacuum-packed wild boar meat samples during 21 days of retail display at 4 ± 1 °C

Da	Treatment	L*	a*	b*	Hue angle (°	Chroma
1	Treated	34.73 ± 1.25^{b}	11.54 ± 0.77^{b}	3.06 ± 0.79^{b}	0.26	11.94
	Non-treated	33.12 ± 1.94^{b}	11.54 ± 0.44^{b}	1.72 ± 0.20^{a}	0.15	11.67
7	Treated	32.99 ± 1.35^{ab}	11.89 ± 1.20^{b}	3.92 ± 0.87^{bc}	0.32	12.52
	Non-treated	33.56 ± 2.16^{b}	12.40 ± 0.46^{c}	2.54 ± 0.52^{ab}	0.20	12.65
14	Treated	32.49 ± 2.49^{a}	12.91 ± 1.11^{c}	4.29 ± 0.50^{bc}	0.32	13.61
	Non-treated	32.70 ±3.23^{ab}	11.93 ± 2.51^{b}	3.53 ± 0.59^{b}	0.29	12.44
21	Treated	31.78 ± 1.61^{a}	12.14± 1.30^{bc}	4.86 ± 0.77^{c}	0.38	13.07
	Non-treated	30.31 ± 4.03^{a}	10.14 ± 2.80^{a}	3.80 ± 0.17^{bc}	0.36	10.82

Legend: ^{abc}Different letters are for significantly different groups (Tukey, $p < 0.05$). Data are recorded as Mean ± Standard Error. Treated (sprayed with 2% lactic acid and 2% ascorbic acid mixture). L* Values are a measure of darkness to lightness (larger value indicates a lighter color); a* values are a measure of redness (larger value indicates a redder color); and b* values are a measure of yellowness (larger value indicates a more yellow color). The Hue angle represents the change from the true red axis (a larger number indicates a greater shift from red to yellow). Chroma is a measure of total color (a larger number indicates a more vivid color).

The a* values of non-treated samples significantly decreased ($p < 0.05$), while the treated samples showed an increase in a* values at the end of the storage period. However, there was a significant difference between treated and non-treated samples at all time points except day 1 ($p < 0.05$). The decrease in a* values of non-treated samples during the storage period is likely due to the development of metmyoglobin, a brown pigment that is formed when oxygen is no longer bound to myoglobin. However, the treated samples showed an increase in a* values only at the end of the storage period, suggesting that the lactic acid and ascorbic acid mixture may have delayed the formation of metmyoglobin.

Regarding the b* values, both treated and non-treated samples showed a significant increase during the storage period ($p < 0.05$). The b* values of treated samples were consistently higher than the non-treated samples throughout the storage period. This could be attributed to the oxidation of lipids and pigments in the meat samples during storage [20]. The consistent increase in b* values of both treated and non-treated samples during the storage period may be due to the accumulation of lipid oxidation products, which can contribute to the yellowing of the meat color. The higher b* values of treated samples compared to non-treated samples may be attributed to the presence of ascorbic acid, which can enhance the yellow color of meat by reacting with iron ions and promoting the formation of yellow pigments.

Overall, the use of lactic acid and ascorbic acid mixture did not have a significant impact on the instrumental color measurements of wild boar meat samples during the storage period. The use of lactic acid and ascorbic acid mixture solution can prevent color changes in meat products by inhibiting the growth of microorganisms and reducing oxidation reactions. Similar results were reported by Greene (1969), and Perlo *et al.*, (2018) [21, 22] respectively, in their studies on the effect of lactic acid and ascorbic acid on the color stability of beef and pork, respectively. They found that the treated samples had lower L* values and higher a* and b* values than the non-treated samples, indicating better color stability. The combination of lactic acid and ascorbic acid can work synergistically to prevent the oxidation of lipids and proteins, which can lead to color changes in meat products [22].

3.3 Microbiological evaluation

The microbial growth of the treated and non-treated wild boar meat samples during 21 days of retail display at 4 ± 1 °C was evaluated by measuring the aerobic plate count (APC). As shown in Figure 1, the initial APC of the treated and non-treated samples were not significantly different on day 1. However, the APC of both treated and non-treated samples increased significantly over time, which is expected due to the natural post-mortem microbial growth in the meat.

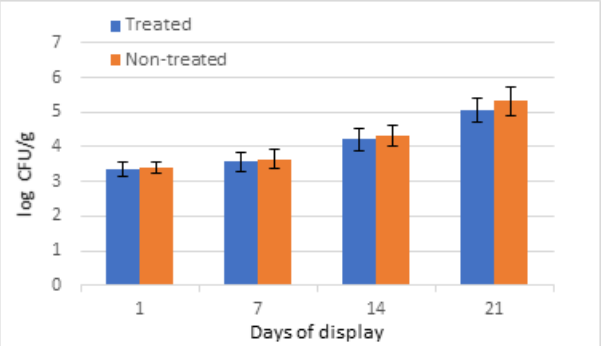

Figure 1. Effect of lactic acid and ascorbic acid mixture on aerobic plate count (Log cfu/g) of vacuum-packed wild boar meat samples during 21 days of retail display at 4 ± 1 °C

On days 14 and 21, the APC of the treated samples was slightly lower than the non-treated samples, indicating that the treated samples may have had a

better microbial shelf life compared to the non-treated samples. This result is consistent with previous studies that reported the antimicrobial activity of lactic acid and ascorbic acid against various bacteria [23, 24].

4. Conclusions

- In conclusion, the use of a lactic acid and ascorbic acid mixture solution can improve the quality and safety of vacuum-packed wild boar meat during retail display.
- The results showed that the lactic acid and ascorbic acid mixture solution may have had a protective effect on the meat by slowing down the decrease in pH and can improve the color stability of wild boar meat during storage.
- This treatment can prevent the oxidation of myoglobin and the growth of microorganisms, which are the main causes of color changes in meat products. These findings may have implications for the meat industry and can be used to guide the development of new preservation methods for meat products.

Acknowledgment

The research was supported by the Hungarian University of Agriculture and Life Sciences's Doctoral School of Food Science.

5. References

[1] Sales J., Kotrba R. (2013). *Meat from wild boar (Sus scrofa L.): A review*. Meat Science, 94, (2), pp. 187-201.

[2] Strazdina V., Jemeljanovs A., Sterna V., Ikauniece D. (2014). *Nutritional characteristics of wild boar meat hunted in Latvia*. Proc. Foodbalt., 1, pp. 32-36.

[3] Strazdina V., Jemeljanovs A., Sterna V. (2012). *Fatty acids composition of elk, deer, roe deer, and wild boar meat hunted in Latvia*. Int. J. Anim. Vet. Sci, 6, (9), pp. 765-768.

[4] Klupsaite D., Buckiuniene V., Sidlauskiene S., Lele V., Sakiene V., Zavistanaviciute P., Klementaviciute J., Viskontaite E., Bartkiene E. (2020). *Comparison studies of the chemical, physical, technological, and microbiological characteristics of the European roe deer, boar, red deer, and beaver hunted wild game meat*. Animal Science Journal, 91, (1). DOI:10.1111/asj.13346. Accessed 24 June 2023.

[5] Marchiori A. F., Felício P. E. D. (2003). *Quality of wild boar meat and commercial pork*. Scientia Agricola, 60, pp. 1-5.

[6] Aminzare M., Hashemi M., Ansarian E., Bimakr M., Hassanzad Azar H., Mehrasbi M. R., Daneshamooz S., Raeisi M., Jannat B., Afshari A. (2019). *Using natural antioxidants in meat and meat products as preservatives: A review***.** Advances in Animal and Veterinary Sciences, 7, (5), pp. 417-426.

[7] Smulders F. J. M., Barendsen P., Van Logtestijn J. G., Mossel D. A. A., Van Der Marel G. M. (1986). *Lactic acid: Considerations in favor of its acceptance as a meat decontaminant*. International Journal of Food Science and Technology, 21, (4), pp. 419-436.

[8] Barcenilla C., Ducic M., López M., Prieto M., Álvarez-Ordónez A. (2022). *Application of lactic acid bacteria for the biopreservation of meat products: A systematic review*. Meat Science, 183. DOI:10.1016/j.meatsci.2021.108661. Accessed 24 June 2023.

[9] Castillo A., Lucia L. M., Roberson D. B., Stevenson T. H., Mercado I., Acuff G. R. (2001). *Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef*. Journal of food protection, 64, (1), pp. 58-62.

[10] Greer G. G., Dilts B. D. (1995). *Lactic acid inhibition of the growth of spoilage bacteria and cold tolerant pathogens on pork*. International journal of food microbiology, 25, (2), pp. 141-151.

[11] Anang D. M., Rusul G., Bakar J., Ling F. H. (2007). *Effects of lactic acid and lauricidin on the survival of Listeria monocytogenes, Salmonella enteritidis, and Escherichia coli O157: H7 in chicken breast stored at 4 C*. Food Control, 18, (8), pp. 961-969.

[12] Nam K. C., Ahn D. U. (2003). *Effects of ascorbic acid and antioxidants on the color of irradiated ground beef*. Journal of food science, 68, (5), pp. 1686-1690.

[13] Varvara M., Bozzo G., Celano G., Disanto C., Pagliarone C. N., Celano G. V. (2016). *The use of ascorbic acid as a food additive: Technical-legal issues*. Italian journal of food safety, 5, (1). DOI:10.4081/ijfs.2016.4313. Accessed 24 June 2023.

[14] Ahn D. U., Nam K. C. (2004). *Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef*. Radiation Physics and Chemistry, 71, (1-2), pp. 151-156.

[15] [15] Zhang H., Zheng Y., Li R. (2022). *Effects of chitosan-based coatings incorporated with ε-polylysine and ascorbic acid on the shelf-life of pork*. Food Chemistry, 390. DOI:10.1016/j.foodchem.2022.133206. Accessed 24 June 2023.

[16] Young J. F., Stagsted J., Jensen S. K., Karlsson A. H., Henckel P. (2003). *Ascorbic acid, alpha-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality*. Poultry Science, 82, (8), pp. 1343-1351.

[17] Gómez I., Janardhanan R., Ibañez F. C., Beriain M. J. (2020). *The effects of processing and preservation technologies on meat quality: Sensory and nutritional aspects*. Foods, 9, (10). DOI:10.3390/foods9101416. Accessed 24 June 2023.

[18] Shamloofar M., Hoseini E., Kamali A., Motalebi Moghanjoghi A. A., Poorgholm R. (2015). *Antibacterial activities of nisin encapsulated in zein and modified atmosphere packaging on rainbow trout (Oncorhynchus mykiss) fillet during chilled storage 4 ^{0}C*. Iranian Journal of Fisheries Sciences, 14. <URL:https://jifro.ir/article-1-1890-en.html. Accessed 24 June 2023.

[19] Jay J. M., Loessner M. J., Golden D. A. (2008). *Modern food microbiology*. Springer Science and Business Media, Berlin, Germany.

[20] Jayas D. S., Jeyamkondan S. (2002). *PH - postharvest technology: Modified atmosphere storage of grains meats fruits and vegetables*. Biosystems Engineering, 82, (3), pp. 235-251.

[21] Greene B. E. (1969). *Lipid oxidation and pigment changes in raw beef*. Journal of Food Science, 34, (2), pp. 110-113.

[22] Perlo F., Fabre R., Bonato P., Jenko C., Tisocco O., Teira G. (2018). *Refrigerated storage of pork meat sprayed with rosemary extract and ascorbic acid*. Ciência Rural, 48, 04.

<URL:https://www.scielo.br/j/cr/a/TR8vL9CgYCMVWVSkGR6bpPN/?lang=en. Accessed 24 June 2023.

[23] Tajkarimi M., Ibrahim S. A. (2011). *Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on Escherichia coli O157: H7 in laboratory medium and carrot juice*. Food Control, 22, (6), pp. 801-804.

[24] [24] Enkhbold M., Lőrincz A., Elayan M., Friedrich L., Surányi J., Tóth A. (2023). *Improvement of shelf-life of beef using lactic acid, ascorbic acid mixture, and potassium sorbate*. Journal of Hygienic Engineering and Design, 42, pp. 45-50.