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EFFECT OF WET-AGING WITH VITAMIN C ON QUALITY BIOMARKERS OF *Biceps femoris* MUSCLES COLLECTED FROM SUSTAINABLE MANAGEMENT OF *Cervus elaphuS* L. POPULATION FROM NORTHERN EASTERN CARPATHIANS, ROMANIA

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Abstract

*This work aims to investigate Wet-Aging with Vitamin C on chemical properties of Biceps femoris muscles collected from sustainable management of Cervus elaphus L. population from Northern Eastern Carpathians, Romania. 2% Alkaline Vitamin C Powder per 0.520 kg muscle sample, 4% Alkaline Vitamin C Powder per 0.504 sample kg, and 6% Alkaline Vitamin C Powder per 0.496 kg sample were used. Samples were protected from light and kept at 2°C for 10 days in order not to accelerate oxidative stress of the muscle samples. The influence of Vitamin C used in the wet-aging method was tested by performing quality biomarkers analyses. Data distribution was evaluated using SPSS Statistics 26.0 software. Non-parametric Independent Samples Kruskal-Wallis test was performed to analyze how the percentage of protein, water, collagen, and fat varied with the concentration of vitamin C in muscle samples. The results suggest that there are significant differences for quality biomarkers (***$p < 0.001$) in the percentage of fat, water, protein, and collagen in particular, between the percentages of 4.00 % and 6.00% Vitamin C introduced. Bonferroni correction was applied to counteract errors for multiple assays and to reduce the chance of an erroneous conclusion.*

Key words*: game meat, quality component, vitamin C.*

INTRODUCTION

In terms of nutritional value and organoleptic properties, game meat meets the demanding expectations of specialists (McMillin & Hoffman, 2009; Boișteanu et al., 2024).

Game meat is a rich source of bioactive compounds (Ciobanu et al., 2023). Conjugated linoleic acid (CLA) is found, as well as carnosine and anserine. It has been shown, that carnosine and its analogs, anserine and N-acetyl carnosine can significantly reduce infarct volume and improve neuronal function (Czarniecka-Skubina et al., 2022; Min et al., 2008). The quality of meat from species resulting from game management is influenced, according to the literature (Wiklund et al., 2010; Węglarz, 2010) by seasonal differences and physiological factors of the species. The color can be attributed to physical activities, stress, or dietary changes caused by the season (Neethling et al., 2017; Frunză et al., 2023). Differences in color between species are due to differences in myoglobin (Mb) content, proportion of muscle fiber type, and intramuscular fat content. Meat with a higher myoglobin (Mb) content also has a higher concentration of Iron, which in turn promotes the oxidation process (Farouk et al., 2007; Neethling et al., 2017). Intramuscular fat contributes to the sensory attributes of meat. The gender of the animal influences fat deposition, with an important impact on the flavor of the meat. Female game species assimilate protein differently and tend to accumulate more fat over time than males, which have a higher fat percentage at any chronological age (Ciobanu et al., 2022a).

Preservation processes in the case of meat from the exploitation of game are carefully chosen to sustain post-mortem quality for a longer time (Postolache et al., 2015). Maturation processes are consistent with improved tenderness and flavour Wet-aging, respectively Dry-aging is the most widely used preservation method in the meat industry (Ha et al., 2019). They influence juiciness, and flavors and ultimately

result in the desired palatability (Anchidin et al., 2023; Ciobanu et al., 2022b).
Velotto et al. demonstrated in 2015 the effects of two methods, Wet-aging and Dry-aging often used in the meat industry. They stated that both methods at 7 days post-mortem resulted in similar palatability. In contrast, sensory tests revealed differences, with the Wet-aging method compared to the Dry-aging method having a higher percentage of consumers as preference. A study of increased interest was that of Yu et al., 2023 who brought to our attention that Wet-Aging compared to Dry-aging resulted in improved water-holding capacity.

MATERIALS AND METHODS

The study included the game mammal species (*Cervus elaphus* L.) which was harvested in accordance with the Romanian National Legislation on Hunting, Hunting and Wildlife Protection (Hunting and Game Fund Protection Law no. 407/2006, 2010) during the hunting season 2022-2023 (winter), to control the population density in the area of hunting ground no. 24 in Frasin, Suceava, Romania. The available food during the cold season is limited, so supplementary food in concentrated (cereals, seeds) and succulent (beetroot, potatoes, and carrots) forms was administered. The samples taken for determination of chemical and physical analyses were m. *Biceps femoris* (duplicate samples/musk). According to the health inspection in the first 24 h postmortem, samples were collected, identified according to gender and age class, sealed in sterile bags, and transported to the laboratory under refrigerated conditions (0-5°C) (Regulation (EC) no. 853/2004 of the European Parliament and of the Council, 2004).
After the samples arrived in the laboratory, 100% natural alkaline Vitamin C powder (bioroots) was used: 100% sodium L-ascorbate. To avoid any conflict of interest for these products, the brand name of the manufacturer has been kept anonymous. We chose to use them in the study to investigate a scenario similar to a real case. According to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, for Ascorbic Acid (E 300) no dose limits are being quantum satis*. The experimental set-up has been designed by the Regulation. 2% Vit C at 0.520 kg sample, 4 Vit C at 0.504 kg Vit C, and 6% Vit C at 0.496 kg were used. Samples were refrigerated and separated from light so as not to accelerate the oxidative stress of muscle samples.
At 48 h post-mortem *Biceps femoris* samples were obtained, weighed, and vacuum packed in vacuum-embossed bags, 2 layers, inner layer 60 µm polyethylene suitable for food contact, outer layer 15 µm polyamide, UV filter. Samples were vacuumed with ATM Machinery, having a 630W chamber, refrigerated at 2°C, and separated from light for 10 days.
For biomarker quality analysis of the experimental samples after 10 days of Wet- Aging the samples were ground and homogenized using an electric grinder. Fat (%), water (%), protein (%), and collagen (%) contents were evaluated using the Omega Bruins Food-Check Near Infrared (NIR) spectrophotometer with infrared light beams (Bruins Instruments GmbH, Puchheim, Germany).
All statistical analyses were performed using the SPSS v.26 software package (SPSS Inc., Chicago, IL, USA). The non-parametric Independent Samples Kruskal-Wallis test was performed to analyse how the percentage of protein, water, collagen, and fat varied with Vitamin C concentration in muscle samples.

RESULTS AND DISCUSSIONS

According to the results presented in Table 1, application of the Independent Samples Kruskal-Wallis test to evaluate differences in the percentage of fat (%) of experimental Biceps femoris muscle samples according to the percentage of vitamin C added using the wet maturation method shows that there are significant differences (***p<.001) between the groups tested.

Table 1. Independent-Samples Kruskal-Wallis Test Summary Fat % with Vitamin C

Total N	**20**
Test Statistic	16.731[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	<.001

[a]The test statistic is adjusted for ties.

As can be seen in Table 2, there are also significant differences (***p<.001) in water content between the groups tested. During the wet-aging process, Vitamin C can form bonds with water molecules, thus contributing to the maintenance of optimal moisture levels in muscle samples subjected to the Wet-Aging method. Vitamin C can form electrostatic interactions with water molecules via partial electric charges in its molecular structure. These interactions may contribute to the stability and cohesion of the system, helping to maintain moisture in muscle (Morrissey et al., 1998).

Table 2. Independent-Samples Kruskal-Wallis Test Summary Water % and Vitamin C

Total N	20
Test Statistic	16.722[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	<.001

[a]The test statistic is adjusted for ties.

Vitamin C is known for its antioxidant properties. During the wet ripening process, vitamin C can protect proteins from oxidative stress in muscle caused by free radicals or other oxidative processes (Morrissey et al., 1998).
According to the data presented in Table 3, there are significant differences in the protein content of the analyzed samples with a high level of significance (***p<0.002).

Table 3. Independent-Samples Kruskal-Wallis Test Summary Protein % and Vitamin C

Total N	20
Test Statistic	14.531[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	.002

[a]The test statistic is adjusted for ties.

These results suggest that Vitamin C in different concentrations (2%, 4%, 6%) had a significant impact on the protein level in the analyzed muscle samples. We can affirm the impact of Vitamin C and how it can protect biomolecules, such as proteins, against oxidative damage.
The results presented in Table 4 indicate that the percentage of vitamin C used in the wet aging process can influence the beffe content of experimental *Biceps femoris* muscle samples. This finding may be of importance in understanding the effects of wet aging and the role of Vitamin C in maintaining the structural integrity of collagen following the experimental process.

Table 4. Independent-Samples Kruskal-Wallis Test Summary Beffe % and Vitamin C

Total N	20
Test Statistic	15.016[a]
Degree Of Freedom	3
Asymptotic Sig. (2-sided test)	.002

a. The test statistic is adjusted for ties.

In the graphical configuration shown in Figure 1 one can see the differences between the level of quality biomarkers (%) as a function of the percentage of Vitamin C (Control Sample (0), 2%, 4%, 6%). In the case of the Fat level, as the percentage of Vitamin C increases, the level of Vitamin C also increases. In the case of water, protein, and collagen the process is reversed, increasing the percentage of Vitamin C up to 6% decreases the amount of water in the test samples significantly.
From Table 5, one can see the significant differences between .00%-4.00% Vitamin C and 0.00%-6.00% Vitamin C at the fat level. The percentage of 6.00% Vitamin C significantly increased the amount of fat.

Table 5. Pairwise Comparisons of Vitamin C-Fat%

Sample1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
.00%-2.00%	-4.900	3.703	-1.323	.186	1.000
.00%-4.00%	-8.900	3.703	-2.403	.016	.098
.00%-6.00%	-14.600	3.703	-3.942	<.001	.000
2.00%-4.00%	-4.000	3.703	-1.080	.280	1.000
2.00%-6.00%	-9.700	3.703	-2.619	.009	.053
4.00%-6.00%	-5.700	3.703	-1.539	.124	.743

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

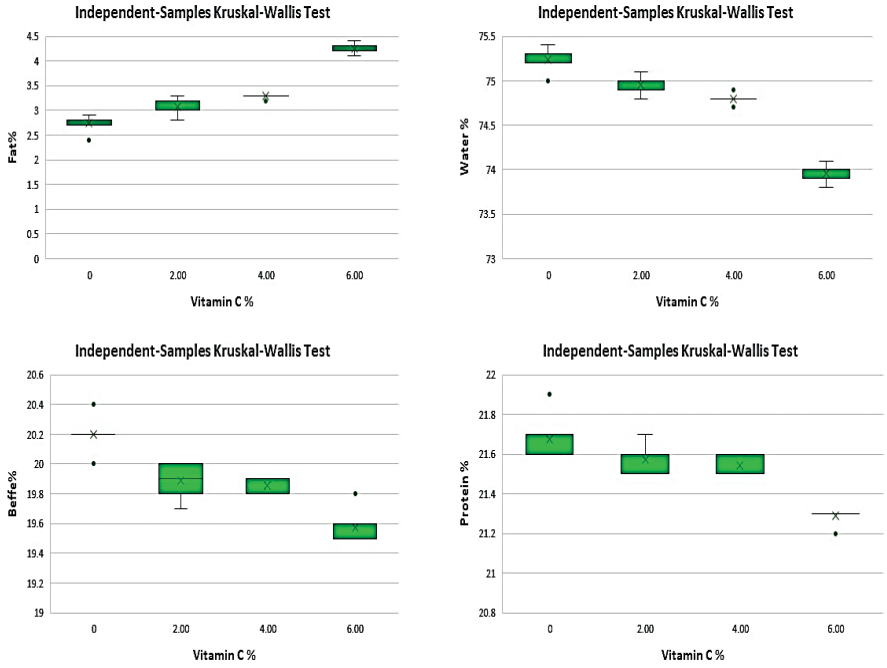


Figure 1. Level of quality biomarkers (Fat %), Water (%), Beffe (%), and Protein (%) as a function of the percentage of added Vitamin C (Control Sample (0), 2%, 4%, 6%)

Table 6. Pairwise Comparisons of Vitamin C-Water%

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig[a]
6.00%-4.00%	5.600	3.718	1.506	.132	.792
6.00%-2.00%	9.800	3.718	2.636	.008	.050
6.00%-.00%	14.600	3.718	3.927	<.001	.001
4.00%-2.00%	4.200	3.718	1.130	.259	1.000
4.00%-.00%	9.000	3.718	2.421	.015	.093
2.00%-.00%	4.800	3.718	1.291	.197	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

In the case of Water level, according to Table 6, the most significant differences are observed between 00%-4.00% Vitamin C and .00%-6.00% Vitamin C. The Water level decreases with increasing percentage of Vitamin C added to the Wet-aging method.

Table 7. Pairwise Comparisons of Vitamin C-Protein%

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig[a]
6.00%-4.00%	7.200	3.643	1.976	.048	.289
6.00%-2.00%	9.200	3.643	2.525	.012	.069
6.00%-.00%	13.600	3.643	3.733	<.001	.001
4.00%-2.00%	2.000	3.643	.549	.583	1.000
4.00%-.00%	6.400	3.643	1.757	.079	.474
2.00%-.00%	4.400	3.643	1.208	.227	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

Protein level as a function of Vitamin C percentage shown in Table 7 varies significantly in the experimental samples between 6.00%-2.00% Vitamin C and .00%-6.00% Vitamin C. Similar case according to Table 8 and at collagen level, significant differences between .00%-6.00% Vitamin C.

Table 8. Pairwise Comparisons of Vitamin C-Collagen (%)

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
6.00%-4.00%	6.400	3.699	1.730	.084	.502
6.00%-2.00%	7.300	3.699	1.973	.048	.291
6.00%-.00%	14.300	3.699	3.866	<.001	.001
4.00%-2.00%	.900	3.699	.243	.808	1.000
4.00%-.00%	7.900	3.699	2.136	.033	.196
2.00%-.00%	7.000	3.699	1.892	.058	.351

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .050. [a]Significance values have been adjusted by the Bonferroni correction for multiple tests.

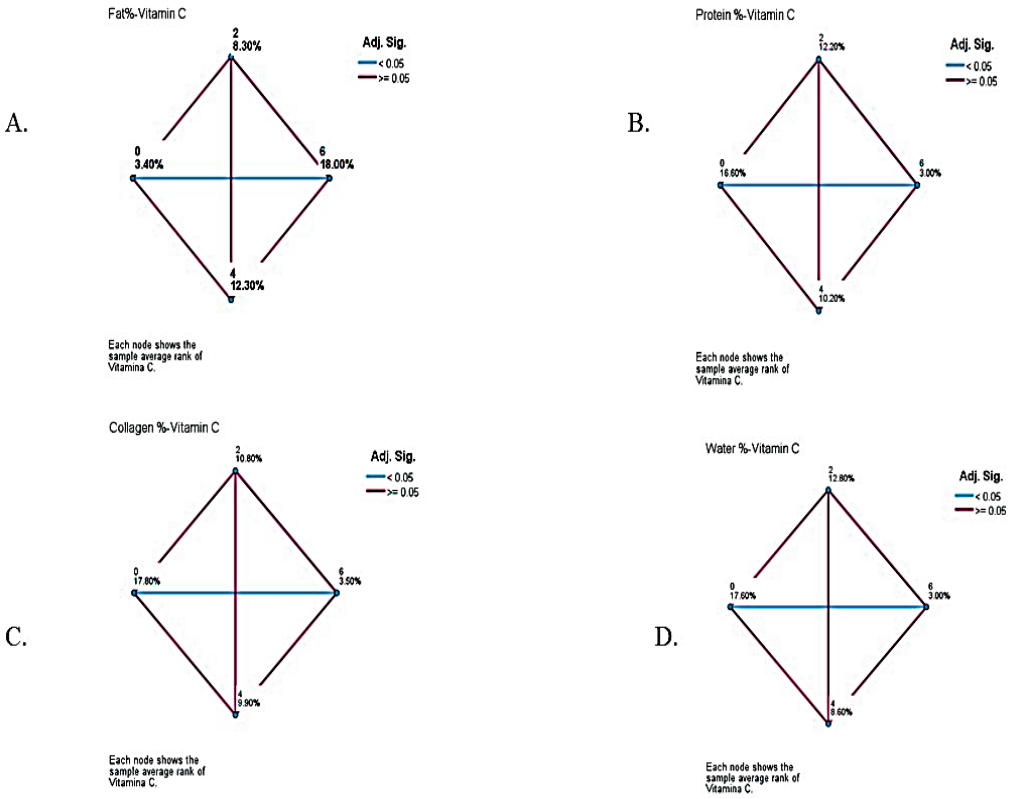


Figure 2. Mean Rank Distribution of the percentages of 0%, 2%, 4%, and 6% Vitamin C in the muscle samples tested to control the level of A (Fat), B (Protein), C (Collagen), and D (Water)

The mean rank of Vitamin C percentages is a statistical measure that indicates the average position of a Vitamin C concentration under study in the whole data set. According to Figure 2, Vitamin C is the variable of interest, which in this study refers to the Vitamin C concentration in the muscle samples. According to the distribution of the Mean Rank

Distribution of the percentages of 0%, 2%, 4%, 6% Vitamin C in the muscle samples tested for controlling the level of A (Fat %), B (Protein %), C (Collagen) and D (Water), for all the parameters tested significant differences are observed between the control sample (0.00% Vitamin C) and the sample with 6.00% Vitamin C.

CONCLUSIONS

The presented results significantly conclude the differences in biomarkers of quality, and the presence of different percentages of Vitamin C in the Wet-Aging method for *Biceps fermoris* muscle samples from *Cervus elaphus* L. The results suggest that there are significant differences for fat, water, protein, and collagen in the percentage (***$p < 0.001$) of in particular, between the percentages of 4.00 % and 6.00% Vitamin C introduced. The Wet-Aging method with Vitamin C is an efficient and sustainable technique for improving the quality of raw material. However, the final quality of the meat depends on other factors such as biological factors of the species and processing conditions.
In future studies, we aim to test the antioxidant activity of vitamin C in the targeted muscle samples.

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