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EVALUATION OF THE IMPACT OF ARTIFICIAL ADDITIVE ON PHYSICOCHEMICAL QUALITY PARAMETERS IN A FUNCTIONAL MEAT PRODUCT WITH HETEROGENEOUS STRUCTURE

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Abstract

This work aims to evaluate the impact of sodium erythorbate ($C_6H_7NaO_6$) in different amounts (0.05%; 0.1%) on physicochemical quality parameters in a functional product with heterogeneous structure. We aimed to test two main anatomical areas used in the process of obtaining the finished product: Musculus gluteus maximus and Musculus longissimus dorsi from Sus scrofa domestica. Data distribution was evaluated using SPSS Statistics 26.0 software. Multivariate Analysis of Variance (MANOVA) is used to determine if there are significant differences between the amounts of $C_6H_7NaO_6$ and anatomical area on physicochemical parameters considering their interactions. Pearson correlation was used to analyse the degree of association between the amounts of $C_6H_7NaO_6$, the anatomical zone, and the physicochemical quality parameters of the finished product. Based on the results obtained, recommendations can be made on the optimal concentration of $C_6H_7NaO_6$ % to achieve the desired effects on physicochemical quality parameters without compromising consumer safety.

Key words*: artificial additive, functional product, physicochemical quality.*

INTRODUCTION

Meat a major basis in the human diet, challenges food industry specialists through consumer perception of meat quality (Boișteanu et al., 2024). Despite emerging dietary trends in Western societies promoting the reduction or replacement of meat in the human diet, global meat consumption is increasing (Bohrer, 2017; Manessis et al., 2020; Boișteanu et al., 2023).

Meat during shelf life is affected by several factors that have negative effects on sensory parameters and not least, nutritional quality (de Carvalho et al., 2019). Some changes related to sensory parameters lead to consumer uncertainty towards the product (Ciobanu et al., 2023a).

Lately, the implementation of strategies in line with a sustainable approach, such as improving lipid profile and nutritional indices has been extensively studied (Velázquez et al., 2022; Anchidin et al., 2023).

Research in this direction has increased since improving the lipid profile of meat products generates technological disadvantages, the main reason being the increase in the amount of monosaturated fatty acids and polyunsaturated fatty acids. These two factors lead to oxidative instability and significantly decrease the shelf life of products (Velázquez et al., 2022; de Carvalho et al., 2019; Domínguez et al., 2019).

Specialists in the food industry have found as a method of stabilization, the use of antioxidants that inhibit oxidation reactions of meat products, in particular, preserving the optimal characteristics for sensory testing. Antioxidants are divided into two categories, natural and synthetic (Ribeiro et al., 2019; Bellucci et al., 2022). The courses of action of antioxidants include inhibition of the chain reaction by scavenging radicals that initiate oxidation, breaking chain reactions that extract hydrogen for prolonged periods, lowering oxygen concentrations, decomposing peroxides, and preventing their conversion to initiating radicals (Manessis et al., 2020). Some researchers argue that the reaction of antioxidants occurs through electron donation to break and stop oxidation in the propagation step, thus preventing the formation of additional radicals, lipids, and

proteins (Lorenzo, 2018; Bellucci et al., 2022). In 2021, Samantha Pfiffner and collaborators demonstrated the effects of tert-butyl hydroquinone (TBHQ) on the tumor suppressor gene p53 in breast cancer cells. TBHQ is one of the aromatic compounds used in food to prevent oxidation and extend shelf life. The present study examined the effects of TBHQ, alone and in combination with hormones and anti-hormones, on the expression of estrogen alpha (ERα) and tumor suppressor gene p53 in MCF-7 and T-47D breast tumor cell lines. Changes in ERα and p53 protein expression were shown after 24 h of treatment with different concentrations of TBHQ (0.005 to 1 mM). P53 levels show a continuous increase in expression by TBHQ concentrations (0.005 to 1 mM) in both MCF-7 and T-47D. Synthetic antioxidants are used due to their low cost and availability.
The study investigates the impact of $C_6H_7NaO_6$ analysed at different amounts (0.05%; 0.1%) on quality biomarkers and color parameters in a functional pork sausage product. Quality biomarkers and color parameters are analysed in the context of the use of this additive in the manufacturing process of the heterogeneous structure product. The results provide insight into how this additive can influence the properties and quality of the final product, providing relevant information for the food industry and the consumer.

MATERIALS AND METHODS

The samples used in this study were obtained in the Meat Microproduction Department (ULS IASI), following ISO quality standards. The raw materials, sourced from a local producer, were stored in cold storage for 24 hours before the start of the study. The samples used in the research consisted of muscle samples taken from *Musculus gluteus maximus* and *Musculus longissimus dorsi*, after the removal of excess fat and connective tissue. Two types of salt were used: NaCl (table/natural salt) for the control group and $NaNO_3$ (nitrate salt) for the experimental groups. These were mixed manually with the muscle samples and then subjected to a maturation period of 12 h at a controlled temperature of 2-4°C. After this stage, the samples were subjected to grinding. After maturation, the samples were coarsely grinded by Grinder WP - 105 using a sieve with a diameter of 3 mm, then the emulsion was obtained using the Cutter Titane V 45L. Subsequently, the samples were with the studied additive ($C_6H_7NaO_6$) in varying amounts depending on the experimental batches and were subjected to a specific heat treatment according to (Table 1).

Tabel 1. Heat treatment stage for meat product with heterogenous structure for the experiment

Heat treatment stage	Time	Temperature inside the cell	Temperature in the thermal center
	minutes	°C	°C
Drying	30	60	50
Smoking	25	64	58
Boiling	-	76	72
Drying	10	76	72

Samples were vacuum bagged in 2-layer vacuum bags, inner layer 60 µm polyethylene suitable for food contact, outer layer 15 µm polyamide, UV filtered using ATM Machinery, having 630W chamber, refrigerated at 2°C and separated from light for 12 h. After 12 hours separated from light, they were transported to the control laboratory for further analysis. The experimental configuration was designed in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives as regards heat-treated meat products. The amounts of $C_6H_7NaO_6$ taken in the study are shown in (Table 2).

Table 2. The amount of $C_6H_7NaO_6$ used in the control and experimental batch from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Sample	$C_6H_7NaO_6$	*Musculus gluteus maximus*	*Musculus longissimus dorsi*
SC	0	3kg	3kg
S1	0.05%	3kg	3kg
S2	0.1%	3kg	3kg

SC-samples control, S1-sample experimental, S2-samples experimental

The final products were subjected to physico-chemical evaluations to test the influence of $C_6H_7NaO_6$ on the heterogeneously structured product. Instrumental color characteristics of the product were determined using a Chroma Meter CR-410 colorimeter from Konica Minolta Inc.,

Japan, on the CIELAB scale. These characteristics were expressed as L*, a*, and b* values. The primary color coordinates L* (brightness), a* (complementary red-green color coordinates), and b* (complementary yellow-blue color coordinates) were evaluated according to the LAB [CIE (Commission Internationale de l'Eclairage)] space over the sample area (presented as the average value of measurements taken at five locations equally distributed over the sample) and section using a Hunter Minolta CM-2600d colorimeter, with an observation angle of 2° and an 8 mm diameter measurement area, illuminating a 50 mm diameter surface, according to (Manoliu et al., 2023). Fat (%), Water (%), Protein (%), Collagen (%), and Salt (%) contents were also monitored using the Omega Bruins Food-Check near-infrared (NIR) spectrophotometer (Bruins Instruments GmbH, Puchheim, Germany). NIR (a region of the electromagnetic spectrum near the infrared spectrum, characterised by wavelengths between about 700 and 2500 nanometres. In this range, visible light is dominated by the colors red, orange, and yellow. These wavelengths allow absorption and emission by different substances, as well as interaction with different materials and biological systems).

Data distribution was evaluated using SPSS Statistics 26.0 software. Multivariate Analysis of Variance (MANOVA) is used to determine if there are significant differences between the amounts of $C_6H_7NaO_6$ and anatomical area on physicochemical parameters considering their interactions. Pearson correlation was used to analyse the degree of association between the amounts of $C_6H_7NaO_6$, the anatomical areas, and the physicochemical quality parameters of the finished product.

RESULTS AND DISCUSSIONS

The results of multivariate analysis (MANOVA) of variance for quality biomarkers in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus* are presented in Table 3.

Table 3. Results of multivariate analysis of variance (MANOVA) for quality biomarkers (Fat, Water, Protein, Collagen, Salt) in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Anatomical Area	Effect		Value	F	Hypothesis df	Error df	Sig.
Musculus gluteus maximus	$C_6H_7NaO_6$	Pillai's Trace	1.981	188.86	10	18	<.001
		Wilks' Lambda	0	190.240[b]	10	16	<.001
		Hotelling's Trace	269.45	188.62	10	14	<.001
		Roy's Largest Root	198.33	356.988[c]	5	9	<.001
Musculus longissimus dorsi		Pillai's Trace	1.797	15.912	10	18	<.001
		Wilks' Lambda	0.002	39.089[b]	10	16	<.001
		Hotelling's Trace	129.44	90.61	10	14	<.001
		Roy's Largest Root	125.32	225.582[c]	5	9	<.001

[b] Exact statistic; [c] The statistic is an upper bound on F that yields a lower bound on the significance level. General linear model with significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root are analytical methods used to assess different aspects of the impact of the independent variable ($C_6H_7NaO_6$) on physicochemical quality parameters. Experimental samples from both the *Musculus gluteus maximus* and *Musculus longissimus dorsi* anatomical areas indicate a significant difference ($p < 0.001$), between the groups tested in these anatomical zones. These results show that $C_6H_7NaO_6$ influences physicochemical quality parameters in both anatomical zones studied, regardless of concentration (0.05% or 0.1%), and significant differences appear between control and experimental samples.

Table 4. Results of Tests of Between-Subjects Effects $C_6H_7NaO_6$ on Fat, Water, Protein, Collagen, and Salt in a product with heterogeneous structure from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*

Anatomical Area	Independent Variable	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Musculus gluteus maximus	**$C_6H_7NaO_6$**	Fat (%)	64.025	2	32.013	108.887	<.001
		Water (%)	70.341	2	35.171	197.588	<.001
		Protein (%)	4.609	2	2.305	138.28	<.001
		Collagen (%)	8.1	2	4.05	418.966	<.001
		Salt (%)	0.497	2	0.249	10.971	0.002
Musculus longissimus dorsi		Fat (%)	51.6	2	25.8	323.849	<.001
		Water (%)	33.937	2	16.969	261.056	<.001
		Protein (%)	2.821	2	1.411	40.692	<.001
		Collagen (%)	2.981	2	1.491	159.714	<.001
		Salt (%)	0.485	2	0.243	16.93	<.001

Tests of Between-Subjects Effects; significance level *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 4 presents the results of the Tests of Between-Subjects Effects carried out to determine the influence of $C_6H_7NaO_6$ on Fat%, Water%, Protein%, Collagen%, and Salt% in a heterogeneously structured product from two different anatomical areas *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*, recording significant differences (p <0.001) between the tested groups for all measured physicochemical quality parameters. $C_6H_7NaO_6$ is known to be used in the food industry for its antioxidant activity. The interaction with protein may be influenced by the ability of sodium erythorbate to protect protein structures against oxidative damage. Thus, it could contribute to maintaining or even increasing protein levels in the product. According to (ANS, 2015) depending on the processing and storage conditions, it could contribute to water retention in the product or reduce water loss by inhibiting fat oxidation.

Table 5. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat, Water, Collagen, Protein, and Salt variables in *Musculus gluteus maximus* samples subjected to the experimental protocol

		$C_6H_7NaO_6$ (%)	**Fat (%)**	**Water (%)**	**Collagen (%)**	**Protein (%)**	**Salt (%)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	.931**	-.981**	-.993**	0.346	.613*
	Sig. (2-tailed)		<.001	<.001	<.001	0.206	0.015
	N	15	15	15	15	15	15
Fat (%)	Pearson Correlation	.931**	1	-.947**	-.925**	0.048	.681**
	Sig. (2-tailed)	<.001		<.001	<.001	0.865	0.005
	N	15	15	15	15	15	15
Water (%)	Pearson Correlation	-.981**	-.947**	1	.973**	-0.276	-.676**
	Sig. (2-tailed)	<.001	<.001		<.001	0.319	0.006
	N	15	15	15	15	15	15
Collagen (%)	Pearson Correlation	-.993**	-.925**	.973**	1	-0.343	-.591*
	Sig. (2-tailed)	<.001	<.001	<.001		0.211	0.02
	N	15	15	15	15	15	15
Protein (%)	Pearson Correlation	0.346	0.048	-0.276	-0.343	1	-0.182
	Sig. (2-tailed)	0.206	0.865	0.319	0.211		0.516
	N	15	15	15	15	15	15
Salt (%)	Pearson Correlation	.613*	.681**	-.676**	-.591*	-0.182	1
	Sig. (2-tailed)	0.015	0.005	0.006	0.02	0.516	
	N	15	15	15	15	15	15

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

According to (Table 5), correlation matrix analysis using Pearson correlation coefficient for the variables $C_6H_7NaO_6$%, Fat%, Water%, Collagen%, Protein%, and Salt% in samples from *Musculus gluteus maximus* subjected to the experimental protocol indicates a strongly significant positive correlation (p<0.001) between $C_6H_7NaO_6$ and fat (Pearson correlation = 0.931**), positively significant correlation with salt (Pearson correlation = 0.613*) and weakly significant with protein (Pearson correlation = 0.346). This indicates that an increase in $C_6H_7NaO_6$ concentration is associated with an increase in fat and salt percentage.

$C_6H_7NaO_6$ correlates negatively (p<0.001) with the amount of water (Pearson correlation = -0.981**) and the amount of collagen (Pearson correlation = -0.993**). Increasing the amount of $C_6H_7NaO_6$ decreases the amount of water and collagen.

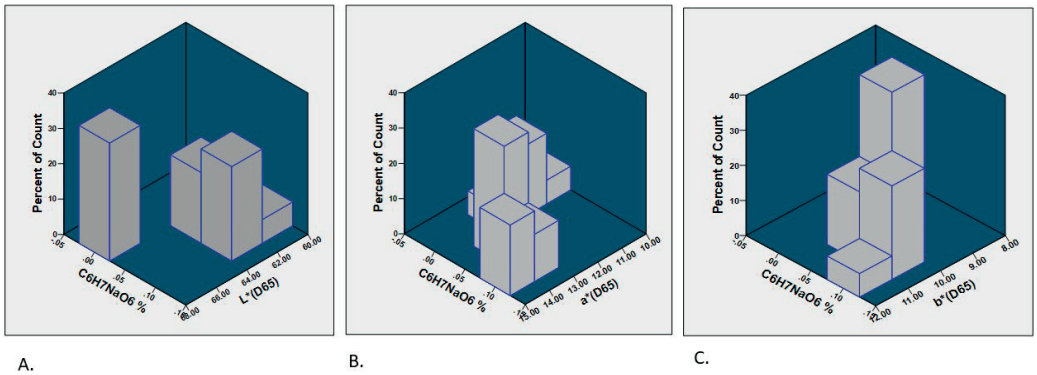
Table 6. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat, Water, Collagen, Protein, and Salt variables in *Musculus longissimus dorsi* samples subjected to the experimental protocol

		$C_6H_7NaO_6$ *(%)*	**Fat (%)**	**Water (%)**	**Protein (%)**	**Collagen (%)**	**Salt (%)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	0.785**	-0.794**	-0.808**	-0.791**	0.702**
	Sig. (2-tailed)		<0.001	<0.001	<0.001	<0.001	0.004
	N	15	15	15	15	15	15
Fat (%)	Pearson Correlation	0.785**	1	-0.999**	-0.931**	-0.997**	0.224
	Sig. (2-tailed)	<0.001		<0.001	<0.001	<0.001	0.422
	N	15	15	15	15	15	15
Water (%)	Pearson Correlation	-0.794**	-0.999**	1	0.929**	0.997**	-0.243
	Sig. (2-tailed)	<0.001	<0.001		<0.001	<0.001	0.382
	N	15	15	15	15	15	15
Protein (%)	Pearson Correlation	-0.808**	-0.931**	0.929**	1	0.928**	-0.240
	Sig. (2-tailed)	<0.001	<0.001	<0.001		<0.001	0.388
	N	15	15	15	15	15	15
Collagen (%)	Pearson Correlation	-0.791**	-0.997**	0.997**	0.928**	1	-0.234
	Sig. (2-tailed)	<0.001	<0.001	<0.001	<0.001		0.402
	N	15	15	15	15	15	15
Salt (%)	Pearson Correlation	0.702**	0.224	-0.243	-0.240	-0.234	1
	Sig. (2-tailed)	0.004	0.422	0.382	0.388	0.402	
	N	15	15	15	15	15	15

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

In Table 6, using Pearson correlation coefficient for $C_6H_7NaO_6$, Fat%, Water%, Collagen%, Protein%, and Salt% variables in *Musculus longissimus dorsi* samples subjected to the experimental protocol compared to *Musculus gluteus maximus*, $C_6H_7NaO_6$ and protein were significantly negative correlations (Pearson correlation = -0.808**). Between $C_6H_7NaO_6$, and fat (Pearson correlation = 0.785**) and salt (Pearson correlation = 0.702**). The color intensity of the finished product can be influenced by the content of fat, water, collagen, protein, and salt (Ciobanu et al., 2023b). Figures 1 and 2 show the distribution of the means of the

primary color coordinates L*(D65), a*(D65), and b*(D65) for control and experimental samples of *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus*.



A. B. C.

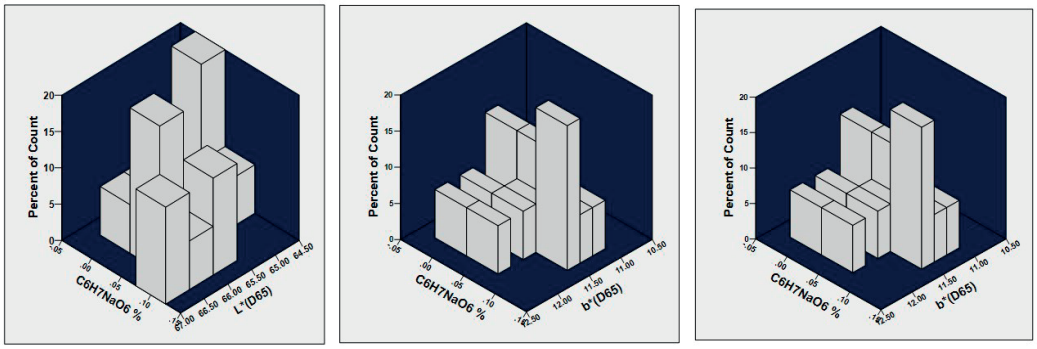
Figure 1. Distribution of mean primary color coordinates (**A**) L*(D65), (**B**) a*(D65), (**C**) b*(D65) for control and experimental samples (0.05% $C_6H_7NaO_6$, 0.1% $C_6H_7NaO_6$) from *Musculus gluteus maximus* of *Sus scrofa ferus*

Table 7. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$ variables L*(D65), a*(D65), b*(D65), in samples from *Musculus gluteus maximus* subjected to the experimental protocol

		***$C_6H_7NaO_6$* (%)**	**L*(D65)**	**a*(D65)**	**b*(D65)**
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	-0.830**	0.897**	0.940**
	Sig. (2-tailed)		<0.001	<0.001	<0.001
	N	15	15	15	15
L*(D65)	Pearson Correlation	-0.830**	1	-0.873**	-0.886**
	Sig. (2-tailed)	<0.001		<0.001	<0.001
	N	15	15	15	15
a*(D65)	Pearson Correlation	0.897**	-0.873**	1	0.840**
	Sig. (2-tailed)	<0.001	<0.001		<0.001
	N	15	15	15	15
b*(D65)	Pearson Correlation	0.940**	-0.886**	0.840**	1
	Sig. (2-tailed)	<0.001	<0.001	<0.001	
	N	15	15	15	15

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

For Table 7, all *p*-values are below 0.01, showing that there are significant and very strong correlations between $C_6H_7NaO_6$ level (%) and all L*(D65), a*(D65), and b*(D65) color values. The correlation between $C_6H_7NaO_6$ and L*(D65) is negative and extremely strong, with a Pearson coefficient equal to -0.830. $C_6H_7NaO_6$ and a*(D65) are positively and very strongly correlated, Pearson coefficient is 0.897, and for $C_6H_7NaO_6$ (%) and b*(D65) are strongly correlated (Pearson coefficient = 0.940.) There are significant and very strong correlations between all L*(D65), a*(D65) and b*(D65). The correlation between L*(D65) and a*(D65) is negative and very strong, with a Pearson coefficient of -0.873. The correlation between L*(D65) and b*(D65) is negative and very strong, with a Pearson coefficient of -0.886. The correlation between a*(D65) and b*(D65) is positive and very strong, with a Pearson coefficient of 0.840.



A. B. C.

Figure 2. Distribution of mean primary color coordinates (**A**) L*(D65), (**B**) a*(D65), (**C**) b*(D65) for control and experimental samples (0.05% $C_6H_7NaO_6$, 0.1% $C_6H_7NaO_6$) from *Musculus longissimus dorsi* of *Sus scrofa ferus*

No significant correlation (p>0.05) between $C_6H_7NaO_6$ and b*(D65). The correlation between $C_6H_7NaO_6$ and L*(D65) is positive and moderate, with a Pearson coefficient of 0.579. The correlation between $C_6H_7NaO_6$ and a*(D65) is positive and strong, with a Pearson coefficient of 0.717. There is a significant correlation between a*(D65) and L*(D65) color values, but it is weaker and does not reach the significance level of 0.01. There is no significant correlation between L*(D65) and b*(D65) color values (Table 8).

Table 8. Correlation matrix analysis using Pearson correlation coefficient for $C_6H_7NaO_6$ variables L*(D65), a*(D65), b*(D65), in samples from *Musculus longissimus dorsi* subjected to the experimental protocol

		$C_6H_7NaO_6$ (%)	L*(D65)	a*(D65)	b*(D65)
$C_6H_7NaO_6$ (%)	Pearson Correlation	1	0.579*	0.717**	-0.151
	Sig. (2-tailed)		0.024	0.003	0.592
	N	15	15	15	15
L*(D65)	Pearson Correlation	0.579*	1	0.483	-0.372
	Sig. (2-tailed)	0.024		0.068	0.172
	N	15	15	15	15
a*(D65)	Pearson Correlation	0.717**	0.483	1	0.072
	Sig. (2-tailed)	0.003	0.068		0.799
	N	15	15	15	15
b*(D65)	Pearson Correlation	-0.151	-0.372	0.072	1
	Sig. (2-tailed)	0.592	0.172	0.799	
	N	15	15	15	15

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

The results of multivariate analysis of variance (MANOVA) for quality biomarkers (Fat, Water, Protein, Collagen, Salt) in the study of the influence of $C_6H_7NaO_6$ in a product with heterogeneous structure from two anatomical zones *Musculus gluteus maximus* and *Musculus longissimus dorsi* from *Sus scrofa ferus* indicate the influence of $C_6H_7NaO_6$ on physicochemical quality parameters in both anatomical zones studied, regardless of concentration (0.05% or 0.1%) and significant differences between control and experimental samples. For the colorimetric control profile of experimental samples from *Musculus gluteus maximus* the results suggest a strong correlation between $C_6H_7NaO_6$ content and color characteristics, and the color values L*(D65), a*(D65) and b*(D65) are also strongly correlated with each other. In

the case of the colorimetric control profile of the experimental samples from *Musculus longissimus dorsi* the results indicate some significant correlations between $C_6H_7NaO_6$ and certain color values L*(D65) and a*(D65), as well as between a*(D65) and L*(D65). Compared to the experimental samples from *Musculus gluteus maximus*, there is no significant correlation between L*(D65) and b*(D65) color values.

Based on the results obtained, recommendations can be made on the optimal concentration of $C_6H_7NaO_6$ to achieve the desired effects on biomarker quality and colorimetric properties of the product without compromising its safety or acceptability. As we study the impact of artificial additives whereby the literature increasingly recommends their elimination, advanced research, acceptability, and sensory testing of natural versus artificially additivated products is needed.

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