

IMPACT ON QUALITY CHARACTERISTICS OF A PLANT-BASED MEAT ANALOGUES ENRICHED WITH BIOACTIVE COMPOUNDS RECOVERED FROM OLIVE MILL WASTE WATER

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Abstract

The paper presents a preliminary study on the effect of phenolic extract obtained from olive mill waste water (OMWW) in meat analogues based on vegetable proteins. For the first time, meat analogues enriched with phenolic extract, recovered from OMWW through filtration systems and spray-dry technology, were made. The antioxidant activity as well as the sensory properties of three different samples (control, ascorbic acid (0,5%) and phenolic extract (3%) from (OMWW), were evaluated. The results obtained are promising, opening up new opportunities for research on the exploitation of the use of olive powder in the production of foods rich in bioactive compounds, contributing to the sustainability of the environment and the circular economy.

Key words: antioxidant activity, phenolic extract, polyphenols, sensory analysis, sustainability.

INTRODUCTION

The negative effects of animal production are driving the development of technologies to find alternatives, such as meat analogues based on plant proteins (Szpicier et al., 2022; Estell et al., 2021; Lai et al., 2017).

Textured plant proteins are the most common ingredients in plant-based meat analogues (Lin et al., 2022). However, proteins from plant products are deficient in at least one of the essential amino acids, such as lysine, methionine or cysteine (Xie et al., 2022).

Functional foods are a growing market segment that requires useful bioactive ingredients, with particular attention nowadays being paid to natural compounds derived from plant extracts and their association with valuable bioactivity (Faustino et al., 2019).

The development of functional foods by adding fiber and antioxidants has positive effects on health. Fiber and antioxidant intakes have a protective role against cardiovascular disease, metabolic syndrome, and chronic diseases (Organization, 2002).

Fruits, vegetables, and other foods are rich sources of dietary carbohydrates and bioactive phytochemicals that, in addition to providing basic nutritional intake, also provide significant health benefits (Dranca et al., 2018).

Olives are small fruits cultivated in the Mediterranean and Middle East, mainly for the production of table olives and olive oil (Ghanbari et al., 2012).

Olives, in their raw and processed form, are rich in bioactive and functional compounds (Bianco et al., 2000). Unprocessed olives are a valuable source of oleuropein, and secoiridoid glycoside (Owen et al., 2000). Olive oil processing generates large quantities of by-products that can lead to environmental pollution (e.g. OMWW, pomace, seeds), but which can be further exploited for their chemical profile, with beneficial effects on health (Dermeche et al., 2013).

OMWW have a penetrating smell of olive oil, being rich in pectin, mucilages, flavonoids and phenolic compounds (De Marco et al., 2007), the phenolic fraction being made up of different groups: phenolic acids, phenolic alcohols,

flavonoids and secoiridoids (Veneziani et al., 2017).

Replacing chemical additives with natural compounds allows not only an increase in the intake of dietary fiber and antioxidants, but also improves the shelf life and rheological properties of supplemented products (Grispoldi et al., 2022).

In this context, paper presents a preliminary study on the effect of phenolic extract obtained from olive mill wastewater (OMWW) in vegetable protein-based meat analogues, which is produced for the first time as meat analogues enriched with phenolic extract (PE), recovered from OMWW through filtration systems and spray-drying technology.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

The ingredients required for the preparation of meat analogues were purchased from a local supermarket in Perugia, Italy.

The phenolic concentrate was obtained from OMWW and dried by Extracta S.n.c. (Muggio (MB), Italy).

The chemical compounds 2,2-Diphenyl-1-picrylhydrazyl (DPPH[•]), 2,4,6-tripyridyl-s-triazine (TPTZ), phosphomolybdic phosphotungstic acid (Folin–Ciocalteu reagent), anhydrous sodium carbonate was provided by Merck (Milan, Italy).

PHENOLIC CONCENTRATE

Obtaining phenolic concentrate obtained from olive mill waste water

Within 24 hours of olive oil extraction process using a three-phase decanter system, the olive vegetation waters are treated with 500 g/ton of enzymatic preparation, at 20°C, for 12 hours. Subsequently, the microfiltration, ultrafiltration and reverse osmosis operations are performed, as described by (Servili et al., 2011), according to Figure 1.

At the industrial level, crude phenolic concentrate (PE) obtained from olive mill wastewater (OMWW) is produced in Italy by the company Extracta S.n.c. Muggio (MB), Italy.

The crude phenolic concentrate (PE) obtained from olive mill wastewater (OMWW), according to Figure 2, is also called olive powder.

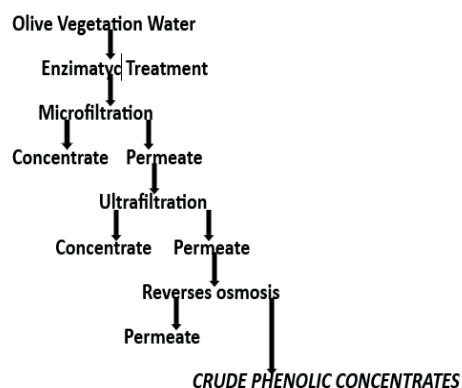


Figure 1. Obtaining crude phenolic concentrate (PE) obtained from olive mill wastewater (original)



Figure 2. Crude phenolic concentrate (PE) obtained from olive mill wastewater (OMWW) (original)

MEAT ANALOGUES

Obtaining meat analogues

A total of ten kilograms of meat analogue paste were produced in the Animal Feed Inspection Laboratory at the University of Perugia's Department of Veterinary Medicine in Italy. The raw materials used for manufacturing the meat analogues were sourced from the local market and consisted of lentils, champignon mushrooms, extra virgin olive oil, and a selection of spices. The meat analogue batter was thoroughly mixed until well combined and then divided into three equal portions: (1) Control (antioxidant-free), (2) C2, with addition of ascorbic acid (0.5%), and (3) C3, with addition of PE (3%). Approximately 100 grams of each sample were shaped into sausages and placed in trays (according to Figure 3). To

simulate retail storage conditions, the trays holding the sausages were kept at chilling temperature of 4°C for 8 days, with the temperature monitored using a portable TESTO 175-T2 device (Testo, Lenzkirch, Germany). For each meat analogue formulation, 9 sausages were sampled after production (T0) and then again after 4 days (T4) and 8 days of chilling storage (T8), respectively. The sausages were cooked on a stovetop grill pan at 200°C for 4 minutes on each side, reaching an internal temperature of 70°C. For each sampling time, five samples of the meat analogues were allocated for sensory analysis, while three samples were vacuum-sealed in a plastic bag after chilling at 20°C for 5 minutes, then stored at in the freezer -30°C until chemical analysis was performed.



Figure 3. Freshly produced meat analogues (T0) to be heat treated for testing (original)

Determination of the moisture content of meat analogues after the application of the final heat treatment, at different time intervals

The moisture content of meat analogues after the application of the final heat treatment (grilling), was determined according to method B described in (Standardization, 1998), the method being adapted to the specific working conditions. The meat analogue samples were distributed on the surfaces of aluminium capsules (± 2 g) and placed in an oven (Binder GmbH, Tuttlingen, Germany) at $103 \pm 2^\circ\text{C}$ for 24 hours. The samples removed from the oven were placed in a desiccator until constant values were obtained according to Figure 4 and subsequently weighed.



Figure 4. Samples stored in a desiccator for temperature stabilization (original)

Determination of antioxidant activity and total phenols

Determination of antioxidant activity in meat analogues after applying the final heat treatment, at different time intervals

The determination of the antioxidant activity of phenolic compounds was performed according to the authors' description (Munteanu et al., 2021). The analyses were performed using a Cary 100 Scan UV-Visible spectrophotometer (Varian, Walnut Creek, CA, USA) according to figure 2.3.3.1.2., and the DPPH test was used to study the antioxidant activity of phenolic compounds. The DPPH[•] solution was prepared by accurately weighing 12.5 mg of DPPH and bringing it to volume in a 50 ml volumetric flask with methanol, followed by a 10-fold dilution. The preparation of meat analogue extract samples was performed according to standard methods for determining antioxidant activity. From each meat analogue extract sample, 200 μL were placed in specific plastic cuvettes with a capacity of 4 ml, to which 3800 μL of DPPH solution diluted 1:10 were added, according to Figures 5a and 5b. The samples were read at the wavelength $\lambda=515\text{nm}$.

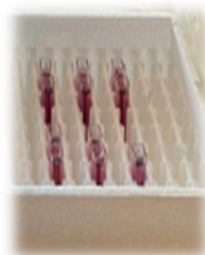


Figure 5a. Samples kept in the dark, before reading with a spectrophotometer (original)



Figure 5b. Sample inserted into the spectrophotometer for reading the absorbance (original)

Determination of total phenols in meat analogues after applying the final heat treatment, at different time intervals

The determination of total phenols was carried out according to the authors' description (Munteanu et al., 2021), by colorimetric methods using a Cary 100 Scan UV-Visible spectrophotometer (Varian, Walnut Creek, CA, USA). The Folin-Ciocalteu test aims to determine the total phenolic content and is based on the reduction of the Folin-Ciocalteu reagent with phenolic compounds in alkaline condition, according to Figure 6. Total polyphenols were determined with the Folin-Ciocalteu reagent, and the results are expressed in mg/L of gallic acid.



Figure 6. Colorimetric reaction of samples with the Folin-Ciocalteu reagent (original)

Sensory evaluation

Sensory evaluation was carried out by performing two tests: the triangular test and the descriptive test.

Triangular test

The antioxidant power of the raw material of the meat analogues (C1) show a slight variation of its concentration after the heat treatment that was not statistically significant.

The variability could be only due to a low homogeneity of the sample. The antioxidant activity of C1 was lower during storage time for all the samples when compared with both C2 and C3. The ascorbic acid showed the highest antioxidant activity that rapidly decreased after 8 days of storage with a reduction of 75.3% after the heat treatment. The meat analogues enriched with phenolic compounds also showed a reduction of its free-radical scavenging activity during storage with a lower reduction (44.3%) if compared with C2 samples.

The antioxidant power of C3 was higher than control samples during the 3 times tested with an activity value that was 5.2, 3.8 and 2.3 times higher respectively for T0, T4 and T8 as shown in Table 1.

Descriptive sensory analysis (QDA)

A quantitative descriptive analysis (QDA) was conducted with a panel of 10 selected and trained assessors according to ISO 13299:2016. This analysis aimed to define the sensory profile of the meat analogue samples and observe their evolution during storage and after cooking.

The panel comprised members from the University of Perugia, ensuring a balance in gender and an age range of approximately 25 to 55 years.

The sensory profile for meat analogue samples of different formulation included 43 attributes of appearance, odour, and texture, as well as off-flavours.

Each attribute was evaluated on a structured continuous scale of 9 cm. As above mentioned, the samples were cut into 2 cm x 2 cm cylinders and served to the panellists in a random order.

Statistical Analysis

All analytical determinations were performed twice, and the results were reported as mean value \pm standard deviation (SD). Statistical analysis was performed with GraphPad Prism, version 6.0 h, for Mac OS X (GraphPad, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by the Tukey's multiple comparisons test was performed considering the product type as the treatment. A p -value < 0.05 was considered to be significant.

RESULTS AND DISCUSSIONS

Determination of the moisture content of meat analogues after the application of the final heat treatment, at different time intervals

After data processing, the moisture content of the plant-based meat analogue samples (after the application of the final heat treatment), at different time intervals (T0 - initial, T4 - after 4 days, T8 - after 8 days of chilling storage), were expressed in percentages according to Figures 7, 8, 9.

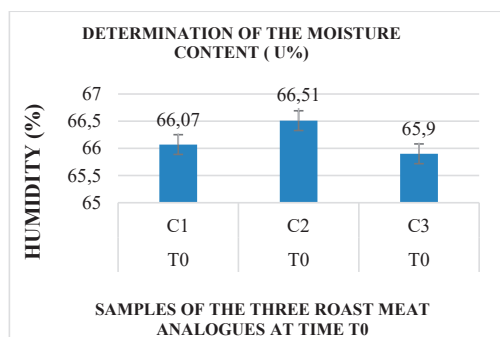


Figure 7. Moisture content of roasted meat analogue samples at time T0 (original)

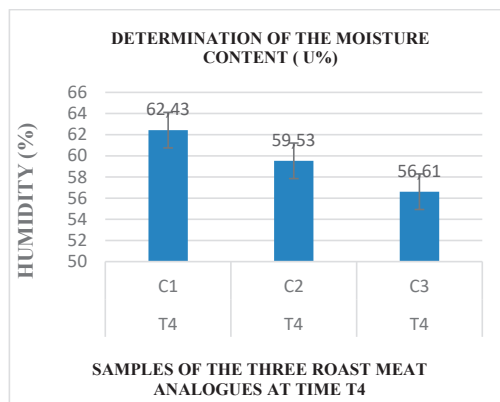


Figure 8. Moisture content of roasted meat analogue samples at time T4 (original)

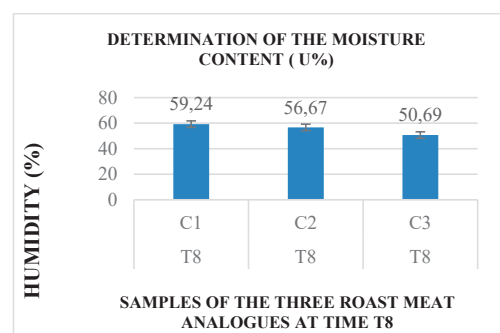


Figure 9. Moisture content of roasted meat analogue samples at time T8 (original)

According to graphs in Figures 7, 8, 9, the variation in the humidity of the control sample (C1) can be observed, the humidity decreases from the initial moment T0 ($U=66.07\%$) to the moment T4 (after four days, $U=62.43\%$), and subsequently reaches a value at the moment T8 (after eight days from the creation of the meat analogue) equal to $U=59.24\%$.

The moisture content of the roast meat analogue sample (to which 0.5% ascorbic acid was added during preparation) decreases from the initial time T0 ($U=66.51\%$) to the time T4 (after four days, $U=59.53\%$), reaching a moisture content value at time T8 equal to $U=56.67\%$. The evolution of the moisture content of the three samples decreases as the storage time increases. The meat analogue with 3% phenolic concentrate presents the lowest moisture content at times T0, T4 and T8, in relation to the other two samples, this is due to the absorption of water by the phenolic concentrate. In the case of the moisture content of the roast meat analogue sample (to which 3% phenolic concentrate obtained from olive mill waste water was added during preparation), decreases from the initial time T0 ($U=65.9\%$) to the time T4 (after four days, $U=56.61\%$), reaching a moisture content value at time T8 equal to $U=50.69\%$. It can be observed according to Figures 7, 8, 9 that at different time intervals (T0, T4, T8) the moisture of the roasted meat analogue (with 3% phenolic concentrate) remains the lowest.

Determination of antioxidant activity and total phenols

Determination of antioxidant activity in meat analogues after final heat treatment, at different time intervals

The antioxidant power of the raw material of the meat analogues (C1) show a slight variation of its concentration after the heat treatment that was not statistically significant.

The variability could be only due to a low homogeneity of the sample. The antioxidant activity of C1 was lower during storage time for all the samples when compared both with C2 and C3. The ascorbic acid showed the highest antioxidant activity that rapidly decreased after 8 days of conservation with a reduction of 75.3% after the heat treatment.

The meat analogues added with phenolic compounds also showed a reduction of its free-radical scavenging activity during storage with a lower reduction (44.3%) if compared with C2 samples. The antioxidant power of C3 was higher than control samples during the 3 times tested with an activity value that was 5.2, 3.8 and 2.3 times higher respectively for T0, T4 and T8 as shown in Table 1.

Table 1. Determination of antioxidant activity of meat analogue samples subjected to final heat treatment at different time intervals.

Sample	Time	DPPH ($\mu\text{mol TE/g d.w.}$)		ANOVA
C ₁	T0	1.97	± 0.04	Aa
	T4	2.48	± 0.13	Aa
	T8	2.51	± 0.26	Aa
C ₂	T0	75.06	± 1.56	Ba
	T4	45.42	± 3.67	Bb
	T8	18.51	± 5.98	Bc
C ₃	T0	10.33	± 0.18	Ca
	T4	9.39	± 0.15	Cb
	T8	5.75	± 0.03	Cc

The data are the mean values of two extraction evaluated two times \pm standard deviation. C₁ = control; C₂ = ascorbic acid; C₃ = phenolic extract; d.w. = dry weight. For each different time, the values having different capital letters are significantly different from one another ($p < 0.05$). For each different sample, the values having different lower-case letters are significantly different from one another ($p < 0.05$).

The graphical representation of the results obtained in Table 2 is presented in Figure 10.

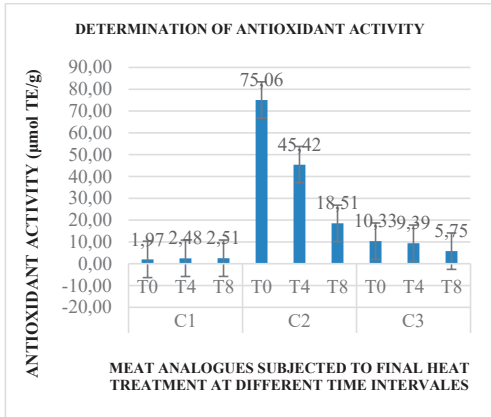


Figure 10. Graphical representation of the antioxidant activity of meat analogues subjected to final heat treatment at different time intervals.

Determination of total phenolic content

The analysis of total phenols (Table 2) partially confirmed the evolution trend of the antioxidant activity of all the three different meat analogues. The C2 showed the highest values that were due to an interference of ascorbic acid with the Folin-Ciocalteu test and not with a real presence of higher phenolic content in the sample. The C3 samples showed a concentration sufficiently stable after the heat treatment for all the storage time tested maintaining a value of about 2.5

g/kg, a content of the phenolic fraction that was significantly increased compared with the control trials: +170%, +110 and + 109 for T0, T4 and T8, respectively.

Table 2. Determination of total phenols of meat analogue samples subjected to the final heat treatment, at different time intervals

Sample	Time	Total phenols (g/kg d.w.)		ANOVA
C1	T0	0.95	± 0.00	Aa
	T4	1.16	± 0.03	Ab
	T8	1.17	± 0.01	Ab
C2	T0	6.53	± 0.05	Ba
	T4	5.71	± 0.02	Bb
	T8	2.70	± 0.01	Bc
C3	T0	2.57	± 0.01	Ca
	T4	2.44	± 0.03	Cb
	T8	2.45	± 0.01	Cb

The data are the mean values of two extraction evaluated two times \pm standard deviation. C₁ = control; C₂ = ascorbic acid; C₃ = phenolic extract; d.w. = dry weight. For each different time, the values having different capital letters are significantly different from one another ($p < 0.05$). For each different sample, the values having different lower case letters are significantly different from one another ($p < 0.05$).

The graphical representation of the results presented in Table 3 is illustrated in Figure 11.

Table 3. Results of triangular tests for meat analogues subjected to final heat treatment (C1, C2, and C3) at different intervals (0, 4, and 8 days) and the maximum number of correct answers required (values extracted from ISO 4120:2021

Days	Meat analogues /Heat treatment	Number of evaluators	Correct answers	Wrong answers	α (0.01)
T0	C1 vs. C3	18	12	6	Significant
T0	C1 vs. C2	18	12	6	Significant
T0	C2 vs. C3	18	12	6	Significant
T4	C1 vs. C3	18	9	9	Not significant
T4	C1 vs. C2	18	12	6	Significant
T4	C2 vs. C3	18	11	7	Not significant
T8	C1 vs. C3	18	12	6	Not significant
T8	C1 vs. C2	18	12	6	Significant
T8	C2 vs. C3	18	14	4	Significant

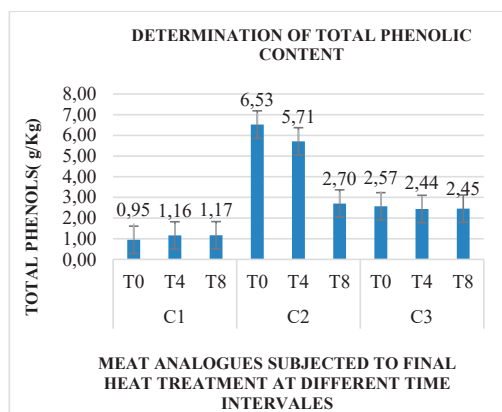


Figure 11. Graphical representation of total phenols of meat analogues subjected to final heat treatment at different time intervals

Sensory evaluation

Sensory analysis is crucial for evaluating the quality of stored-cooked meat analogues enriched with PE (C3 samples) and comparing them with those that contain synthetic additive (C2 samples) and those that do not (C1 samples). In this study, we employed two distinct sensory evaluation methods: discriminative method and descriptive sensory analysis.

1. Triangular test

A triangle test was conducted to compare samples C1, C2, and C3 at various sampling times. Table 3 shows a significant difference among the three formulations at T0 and T8, confirming the findings regarding moisture content. Of the 18 expert panellists, 12 were able to identify the different samples when a factor of $\alpha = 0.01$ was applied ISO 4120:2021. At T4, however, no significant differences were found between C1 and C3, and between C2 and C3.

2. Test descriptive

A trained sensory panel consisting of 10 panellists conducted a quantitative descriptive sensory analysis to investigate the sensory characteristics of stored-cooked meat analogue samples C1, C2, and C3. The evaluation focused on various attributes such as appearance, odor, and texture. Statistical analysis of the sensory data was performed using principal component analysis (PCA). The PCA model explained 91.9% of the total variance with five principal components (55%, 13.2%, 12.6%, 6.7%, and 4.4%, respectively), revealing a distinct clustering of the samples within the PCA biplot.

Figure 12 presents a biplot that illustrates the results of the PCA, visually representing the characteristics of the meat analogues at three different storage time points (T0, T4, and T8). The biplot (PC1/PC2) shows a clear differentiation of the samples as function of storage time along PC1, with T0 samples positioned on the left, and those at T4 and T8 located progressively toward the center and right, respectively. This distribution suggests a clear influence of storage time on the sensory properties of the samples.

The formulation differences are also evident, with samples C1 and C2 situated on the upper part of the biplot, while sample C3 is located on the opposite side along PC2. The sensory attributes most responsible for the distribution of the meat analogues at both the initial stored-cooked period (T0) included: Appetizing, Softness, Rubbiness, Adhesiveness, Clumpiness, Elasticity, Cohesiveness, Brightness, and Juiciness attributes. These attributes typically associate to the food products freshness. In contrast, Hardness, Homogeneity of Colour, Red Colour, Broth Odor, and Spicy Odor were the main sensory attributes that characterized the samples at the end of stored-cooked period. Several authors observed a flavour development and structural changes in food during storage (Haug et al., 2017).

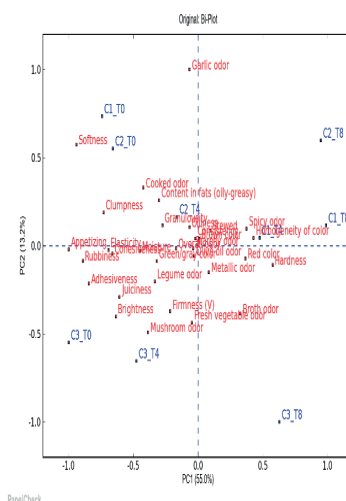


Figure 12. Bi-Plot of objects and variables from PCA on the first two principal components (PC1 and PC2), based on sensory evaluations of stored-cooked meat analogues (objects) and attributes (variables) assessed by 10 panellists. Objects are in blue, variables in red

Further analysis reveals that on the upper side of PC2, the Garlic Odor and Softness attributes were most influential in distinguishing the stored-cooked C1 and C2 samples. Conversely, the stored-cooked meat analogue enriched with PE (C3) was characterized by a more distinctive sensory profile, dominated by Mushroom Odor, Fresh Vegetable Odor, Broth Odor, Brightness, and Firmness.

This suggests that PE formulation may retain some of fresh-like qualities over time.

These findings are consistent with previous studies on plant-based analogues, where the incorporation of different protein sources and flavour enhancers can significantly alter the sensory characteristics during storage (Bhat et al., 2011).

Our results indicate that formulation, storage time and cooking process play a significant role in the sensory attributes of meat analogues.

Specifically, the differences observed among the samples may be attributed to the varying levels of preservation or changes in texture and aroma as a result of ingredient composition and storage conditions (Cai et al., 2020).

CONCLUSIONS

The meat analogues enriched with phenolic extract obtained from olive mill wastewater is an interesting combination for the reduction of meat consumption and the valorization of agri-food by-products, both for a common aim of the sustainability of the production and the reduction of its environmental impact.

The addition of phenolic molecules, characterized by important biological activities, guarantees higher values of phenolic fraction after the heat treatment for all the storage time tested with a significant impact on the safety and health properties of the final product due to the phenolic antioxidant and antimicrobial effects.

Furthermore, the sensory evaluations indicated that adding PE did not negatively affect the pleasantness of meat analogues, preserving their desirable sensory characteristics.

The phenolic extract from olive vegetation water could be a valid and natural alternative to be used as food additives of synthetic origin enhancing the bioactive properties of a plant-based meat analogues.

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