





Article

Effects of Different Natural Additives Alternative to Chemical Ones on Artisanal Bologna-Type Sausages Shelf Life

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Abstract: This study aims to evaluate the effects of mandarin peel (*Citrus reticulata* L.), oregano (*Origanum vulgare*), and common lambsquarter (*Chenopodium album*) powders as natural additives on the shelf lives of artisanal Bologna-type sausages by comparing them with a chemically preserved control formulation. In this regard, four mortadella formulations (MTS1, MTS2, MTS3, and MTC) were produced and analyzed for physicochemical, microbiological, rheological, and sensory properties at days 1 (T0), 15 (T1), 25 (T2), and 30 (T3) after vacuum packaging. The results highlighted greater performances in the experimental samples MTS2 (made with common lambsquarters) and MTS3 (made with oregano), particularly in microbiological stability and antioxidant activity, which were similar to those of the control sample (MTC), with TBAR values extremely low, even at the end of the storage for both MTS2 (0.65 mg MDA/kg) and MTS3 (1.11 mg MDA/kg), reflecting effective lipid oxidation control. The sensory analysis further revealed oregano-containing mortadella (MTS3) as the most preferred sample for appearance and taste. These findings suggest that natural additives, like oregano or lambsquarter powders, could replace or complement nitrites in Bologna-type sausages, ensuring product quality, safety, and shelf life while meeting consumer demand for clean-label and chemical-additive-free products. Further research could optimize these formulations to support commercial applications.

Keywords: artisanal Bologna-type sausages; oregano powder; mandarin peel powder; common lambsquarter powder; natural additives



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1. Introduction

The growing demand for meat has led industries to increasingly invest in processed products. Nowadays, industrialization has become the primary solution for managing raw materials, as it not only extends the shelf life of food but also enhances its value [1]. Numerous are processed meat derivatives, including hams, sausages, and many others [1]. Among these, meat sausages and, in particular, mortadella, which is a cooked, cured, emulsion-type sausage [2], stand out as popular and affordable fast-food options [3]. Specifically, mortadella is an industrialized meat product made from an emulsion of butchered animal meat, such as pork, beef, poultry, lamb, or combinations of these [2], with or without bacon and added ingredients, embedded in natural or artificial casings in different forms, and subjected to the appropriate heat treatment [4]. It is sold either as a cylindrical roll or as vacuum-packed slices [5,6]. Because of its chemical composition (high

water activity (a_w) and notable fat content (around 30%) [4,7], this product is particularly susceptible to oxidative deterioration and microbial contamination, which negatively influence its stability [8]. In fact, these chemical and microbiological processes can lead to the development of unpleasant flavors and odors, as well as a reduction in the product's nutritional quality, ultimately decreasing consumer acceptance [9]. Thus, to minimize and prevent these changes in the nutritional composition or organoleptic properties and to extend the shelf life of mortadella, synthetic chemical preservatives, such as antioxidants, antimicrobials, and curing salts (primarily nitrites and nitrite salts), are commonly used in its production process [10]. However, several studies [11–14] on the safety of these types of additives in the food industry have raised concerns about potential toxicity risks associated with the consumption of such products, as they may contain or generate compounds harmful to human health [15]. A notable example is nitrites, widely used as chemical additives in processed meats, particularly in mortadella, for their strong antimicrobial and antioxidant properties, which can generate human carcinogens under conditions that promote endogenous nitrosation [16].

For this reason, there is a growing demand for healthier meat products, including artisanal mortadella, made with natural additives, such as those extracted from plants and/or vegetables and their wastes [17]. In this regard, plant-based extract powders with significant biological activities, such as parsley (*Petroselinum hortense*) [18], oregano (*Origanum vulgare*) [19], paprika (*Capsicum annuum*) [20], and common lambsquarters (*Chenopodium album*), and vegetable byproducts, like those derived from grapes (*Vitis vinifera*), clementine mandarin oranges (*Citrus reticulata*), and fennel (*Foeniculum vulgare*) [21,22], have sparked considerable interest in the food industry, especially in the meat chain, because of their well-known powerful bioactive properties, including antioxidant, antimicrobial, and antifungal activities [17–22]. Regarding mortadella, in the few scientific studies available in the literature on artisanal mortadellas, i.e., Bologna-type sausages in which chemical additives are replaced by alternative natural substances, some of these plant-derived extracts, primarily essential oils and occasionally powders, have already been used to partially or completely substitute chemical additives, showing promising results in terms of antimicrobial and antioxidant activities [18,23–25].

Building on this knowledge and considering the few studies present in the literature, the present work aimed to evaluate the effects of three different natural additives, which were mandarin (*Citrus reticulata* L.) peel, oregano (*Origanum vulgare*) (already known for their antimicrobial and antioxidant properties [19]), and common lambsquarter (*Chenopodium album*) (never been tested before in mortadella) powders, on the shelf lives of three artisanal mortadellas in terms of physicochemical, microbiological, rheological, and sensory profiles and compare them to a control formulation made with sodium nitrite. The evaluations were conducted at days 1 (T0), 15 (T1), 25 (T2), and 30 (T3) after vacuum packaging, with the final objective of creating a new artisanal product in which nitrites are totally or partially replaced by alternative natural additives having comparable characteristics, including shelf life, without causing any health hazards.

2. Materials and Methods

2.1. Materials

The three tested plant powders (mandarin peel (*Citrus reticulata* L.), common lambsquarter (*Chenopodium album*), and oregano (*Origanum vulgare*) powders) were made by a local business. The artisanal mortadellas were produced at a local specialized meat-processing facility. The analyses were carried out using instruments located in the laboratories of the Department of Veterinary Medicine and Animal Production at University Federico II.

2.2. Methods

2.2.1. Preparation and Characterization of the Tested Powders

The three tested plant powders were mandarin peel (*Citrus reticulata* L.), common lambsquarter (*Chenopodium album*), and oregano (*Origanum vulgare*) powders. According to the method described by Gwak et al. [26], with some modifications, the oregano powder was prepared by drying the entire plant at 23–25 °C for four days, while the production of the other two involved drying approximately 250 g of fresh mandarin peel and 300 g of common lambsquarter leaves at 45–50 °C for 15 h. After drying, each dried plant substance was ground for 5 min to produce a fine powder of approximately 2 × 2 mm², using a chopper (C6 VV, Sirman, Curtarolo, Italy). Finally, to reduce the microbial load while preserving the organoleptic properties, all three powders were subjected to high-temperature steam treatment for 1 min.

2.2.2. The Manufacturing Process of the Mortadellas

One batch (ca. 20 kg) of freshly manufactured artisanal mortadella was formulated using a blend of pork meat from domestic pigs combined with fatback from Cinta Senese breed pigs. A total of 18 kg of meats (shoulder, neck, ground bardello, ham trimmings, pancetta trimmings, demucosed stomachs, and fat) previously stored in specialized cooling chambers at 4 ± 1 °C, was weighed, trimmed, and minced in a meat grinder (Type WD 114, Maschinenfabrik Seydelmann KG, Aalen, Germany) through plate holes with a 2 mm diameter. The previously minced meat and lard were then transferred to a kneader (MAINCA, Granollers, Spain), where 45 g/kg of non-meat ingredients, such as salt, sugar, garlic, black pepper (both ground and whole), beet powder, and pistachios, were added. The mixture was then kneaded for twenty minutes. After mixing, the dough was divided into four equal parts to produce four different mortadella formulations (three experimental (MTS1, MTS2, and MTS3) and one control (MTC)), ca. 5 kg each, using a chemical additive in the control sample (MTC) and three natural preservatives (sourced from a local producer) in powdered form in the other three artisanal products (MTS1, MTS2, and MTS3). In this regard, to the control sample (MTC), was added 150 mg/kg of sodium nitrite, while the three experimental formulations (MTS1, MTS2, and MTS3), were made using a concentration of 1 g/kg (0.1%), respectively, of mandarin peel powder (*Citrus reticulata* L.) for MTS1, common lambsquarter powder (*Chenopodium album*) for MTS2, and oregano (*Origanum vulgare*) powder for MTS3. Each mixture was then blended again to achieve a homogeneous mass. Finally, each homogenized meat emulsion was embedded in a natural envelope and cooked in an oven until it reached 72 °C (in the internal part). Soon after cooking, thermal shock was carried out for 15 min in running water, then the four mortadellas (MTS1, MTS2, MTS3, and MTC) were weighed (a drop in the weight of about 1.2 kg occurred) and conditioned at refrigeration temperature (4 ± 1 °C) in a refrigerator with digital control of the air temperature and relative humidity. The manufacturing process of the tested samples is represented schematically in Figure 1.

2.2.3. Packaging and Storage

Each mortadella (MTS1, MTS2, MTS3, and MTC) was cut into four equal parts of approximately 1 kg each and then vacuum packed, labeled, and stored in a cold room at 4 ± 1 °C on the same day of the production. This storage temperature was chosen following the storage conditions commonly applied by consumers and in the retail sector, respectively.

2.2.4. Sample Collection

To evaluate the impacts of the three plant powders (mandarin peel, common lambsquarter, and oregano powders) on the MTS1, MTS2, and MTS3 shelf lives and to compare

them with that of the control sample (MTC), a vacuum pack of each mortadella formulation was taken at four different time points (T0 = 1 day, T1 = 15 days, T2 = 25 days, and T3 = 30 days after packaging) for carrying out chemical (pH, a_w , color, and lipid oxidation), microbiological, sensorial, and rheological (TPA) analyses.

Physicochemical analyses:

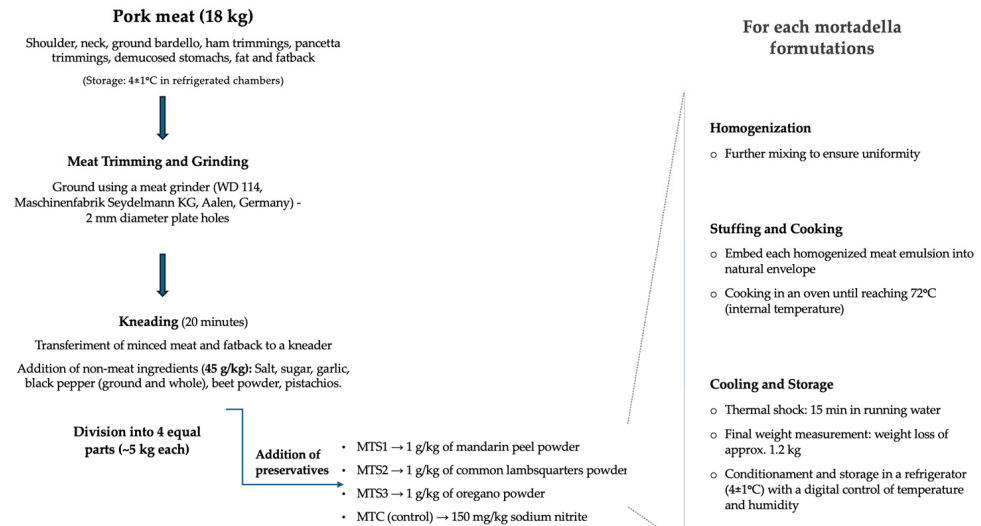


Figure 1. The schematic figure illustrates the manufacturing process of the mortadellas (MTC, MTS1, MTS2, and MTS3).

2.2.5. pH and a_w Measurements

According to Ambrosio et al. [27], pH values were determined using a pH meter (Crison-Micro TT 2022, Crison Instruments, Barcelona, Spain) equipped with an insertion glass electrode and a temperature sensor. The water activity (a_w) was measured using an Aqualab 4 TE (Decagon Devices Inc., Pullman, WA, USA). Each measurement was performed in triplicate ($n = 3$) at room temperature (23 ± 2 °C).

2.2.6. Lipid Oxidation

Lipid oxidation was monitored by determining the thiobarbituric acid ($C_4H_4N_2O_2S$) substance (TBAR) value, expressed as a malondialdehyde ($CH_2(CHO)_2$) concentration (mg/kg), which represents secondary oxidation products. Measurements were performed according to the extraction method proposed by Xiong et al. [28] and modified by Di Paolo et al. [29]. The TBAR value was expressed as milligrams per kilogram of mortadella according to the following formula [29]:

$$\text{TBARs value (mg/kg)} = A_{532} \times 7.8$$

2.2.7. Texture Profile Analysis

Texture profile analysis (TPA) was performed following the procedures described by Ambrosio et al. [27], with an EZ-Test texturometer (Shimadzu Corporation, Kyoto, Japan) measuring the compression force (Newtons) developed. The following parameters were evaluated from the TPA curve: hardness (N), cohesiveness (N), gumminess (N), chewiness (N), resilience, and adhesiveness ($N \times mm$). Three slices of each mortadella were used to obtain an average of seven to ten repetitions, resulting in approximately three repetitions per slice. The average values were used.

2.2.8. Color Analysis

Color determination was measured using a Konica Minolta CR 300 colorimeter (Minolta, Osaka, Japan) according to the CIE L* a* b* system. L* (lightness), a* (red intensity), and b* (yellow intensity) values were determined using operative conditions described by Marrone et al. [30]. In each instrumental test, five replicates were performed for each sample.

2.2.9. Microbiological Analyses

According to UNI EN ISO 18593:2018 [31], samples were transported to the Department of Veterinary Medicine and Animal Production at University of Naples Federico II in a cool box at approximately 4 ± 1 °C. Then, microbiological analyses were carried out using international standard methods at days 1 (T0), 15 (T1), 25 (T2), and 30 (T3) after vacuum packaging to compare the antimicrobial properties of the experimental natural substances to each other and to those of the chemical control additive. In this regard, the following parameters were determined: the total mesophilic bacterial count (TAB at 30 °C) according to UNI EN ISO 4833 [32], enterobacteria according to UNI EN ISO 21528 [33], coagulase-positive staphylococci according to UNI EN ISO 6888-1:2003 [34], *Escherichia coli* according to UNI EN ISO 4831:2006 [35], *Bacillus cereus* according to UNI EN ISO 7932:2020 [36], *Campylobacter* spp. according to UNI EN 10272:2006 [37], *Clostridium perfringens* according to UNI EN ISO 14189:2016 [38], *Listeria monocytogenes* according to UNI EN ISO 11290-1:2017 [39], and *Salmonella* spp. according to UNI EN ISO 6579-1:2017 [40].

2.2.10. MALDI-TOF MS Identification

To confirm the probable positive results, all the presumptive pathogenic bacteria isolated on the agar plates were analyzed using the direct colony identification method with MALDI-TOF MS (MALDI Biotyper[®], Sirius, San Antonio, TX, USA). Briefly, colonies grown on the agar plates were smeared in duplicate on a 96-spot steel plate (Bruker Daltonics, Bremen, Germany). Each spot was then covered with 1 µL of a matrix solution comprising 10 mg/mL of α -cyano-4-hydroxycinnamic acid dissolved in a mixture of acetonitrile, deionized water, and trifluoroacetic acid (50:47.5:2.5, v/v/v). A Bruker bacterial test standard (BTS, Bruker Daltonics) was employed as the reference standard for the mass calibration. The analysis was performed using the software Flex Control 3.4 (Bruker Daltonics) set in the linear positive ion detection mode (Bruker Daltonics) in order to match the collected spectra to those in the Bruker MSP database (MBT Compass Library), using Bruker Compass software Flex Control 3.4 at the default settings. The obtained identification scores were interpreted following criteria adapted from Jeong et al. [41]: scores of ≥ 2.3 indicate highly reliable species-level identification, scores between 2.0 and 2.3 suggest probable species-level identification and genus-level identification, scores between 1.7 and 1.99 indicate probable genus-level identification, and scores of < 1.7 are considered as unreliable for identification. The analysis was repeated when the spots resulted in “no peaks found”.

2.2.11. Sensory Analyses

Sensory analyses were carried out at the beginning (time T0 = day 1) and at the end (time T3 = day 30) of the refrigerated storage to deliberately obtain subjective evaluations to identify the most preferred product by 10 semi-trained tasters. After a preparatory training held to discuss and clarify each attribute to be evaluated in the mortadellas, 2 cm thick slices were cut from each sample and served at room temperature to the 10 tasters, without revealing the nature of the tested samples. At T0 (day 1) and at T3 (day 30), a descriptive evaluation form was used, incorporating 10 relevant sensory attributes to define the primary organoleptic characteristics: color intensity, visible seasoning, homogeneity

perception, presence and size of holes, shine, odor intensity, seasoning smell, pepper smell, and unpleasant odors. Only at T0, the chew resistance, gelatinous texture, juiciness, characteristic flavor intensity, seasoning and pepper flavors, saltiness, and unpleasant taste were evaluated. Each attribute was assessed using a continuous linear scale from 1 (a very low perception of the descriptor) to 5 (a very intense perception), and at the end of the test, the single scores were summed to obtain the total score for each sample. During the sensory evaluation, to cleanse their palate, the tasters received unsalted crackers and water at room temperature between the samples [29].

2.2.12. Statistical Analyses

Statistical analyses were performed using SAS software, version 6.3. The results were analyzed using generalized linear mixed models (GLMMs) to evaluate the influences of natural additive inclusion on the microbiological, rheological, and physicochemical profiles of the three experimental mortadella formulations over time (T0, T1, T2, and T3) and compare them with those of the control sample (MTC). Each measurement was performed in triplicate ($n = 3$), with the mean value calculated and used for the analyses. Comparisons between means were performed using Tukey's test at significance levels of $p < 0.05$ and $p < 0.01$. The results are presented as means (Ms) \pm standard errors (SEs).

3. Results

3.1. Physicochemical Analysis

3.1.1. Effects of the Three Tested Plant Powders on the pH and Water Activity (a_w) Values of the Mortadellas

The mean values and standard deviations of the pH and water activity (a_w) of the three experimental mortadella formulations (MTS1 (containing mandarin peel powder), MTS2 (containing common lambsquarter powder), and MTS3 (containing oregano powder)) and the control one (MTC) are shown in Figures 2 and 3. Further statistical information is reported in the supplementary materials (Table S1).

From day 1 to day 30, corresponding to the beginning and the end of the evaluation period, pH trends were non-linear across all the samples (Figure 2). Notably, MTS1 and MTS3 showed similar trends, with pH values initially increasing, then decreasing, and subsequently rising again. In meat and meat products, an increase in pH values during aging could be attributed to the hydrolysis of meat proteins to amino acids [42]. A similar trend was reported by Júnior et al. [43], who conducted a study comparable to ours, comparing mortadella formulated with synthetic antioxidants to that made of curcumin microcrystals in relation to their physicochemical and sensory characteristics. Conversely, the pH values of MTS2 showed a decrease at 15 days of storage, probably attributed to the antimicrobial activity of the extract [30], followed by an increase from day 15 to day 30. Although at the end of the evaluation period (T3), all the samples showed higher values compared to those recorded at T0, according to Biasi et al. [44], the observed pH levels are still considered as ideal to guarantee the microbiological and structural stability of emulsified products. Furthermore, these values have also contributed to the color stability of the mortadellas, especially the redness values (Table 1). Similar findings were obtained by Lúcia Fernandes Pereira et al. [45], in which mortadella formulations were prepared by adding mango seed extract, and slight increases in pH values were observed, rising from 5.91 ± 0.01 (at day 0) to 6.06 ± 0.02 at the end of a 21-day storage period. Our results align with the findings of Júnior et al. [43], who incorporated curcumin into mortadella formulations. In their study, a pH value of 6.10 was recorded after 30 days of storage. The pH variations of the three experimental mortadella samples were compared to those of

the control sample from day 1 to day 30, highlighting that the trends for MTS1 and MTS3 deviated from that observed in the control.

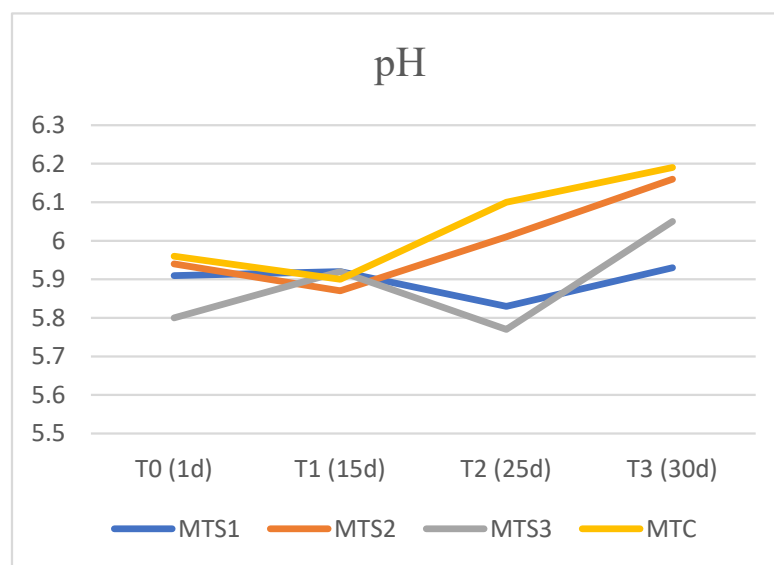


Figure 2. Trends of pH values in the four mortadella formulations during the storage period. MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

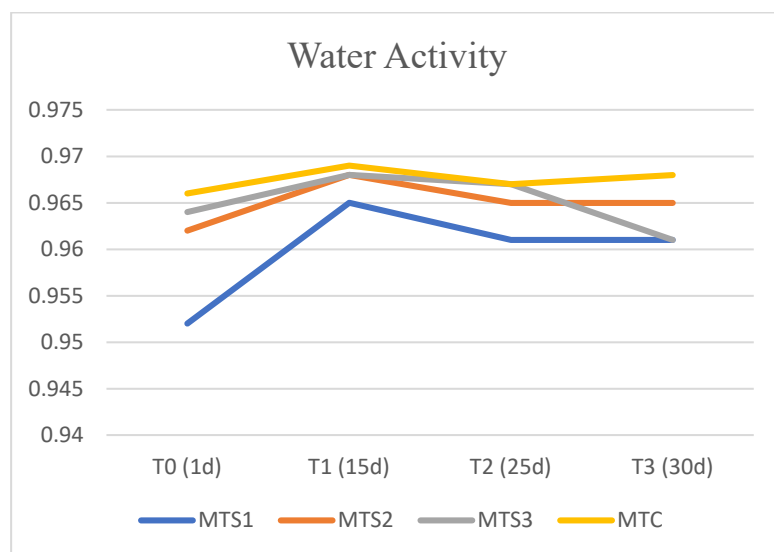


Figure 3. Trends of a_w values in the four mortadella formulations during the storage period. MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

Regarding the water activity (a_w), this is a critical parameter of cooked meat products, as high values promote the development of undesirable microorganisms, while low values negatively impact the texture and flavor [44]. From Figure 3, it is evident that the obtained a_w values remained consistently around 0.95–0.96 across all the samples (MTS1, MTS2, MTS3, and MTC), indicating that this parameter was well controlled by the natural powders. Notably, slight increases in a_w were observed at 15 days of the storage period in all the samples, followed by slight decreases at 30 days of storage (Figure 3). These results agree with the findings of Orsolin et al. [46], who reported similar trends in mortadella formulations stored at 14 °C.

Table 1. Trends of color parameters L^* , a^* , and b^* in mortadella formulations during storage.

Parameter	Sample	T0 (Day 1)	T1 (Day 15)	T2 (Day 25)	T3 (Day 30)
Lightness, L^*	MTS1	55.08 ± 3.23	56.70 ± 0.51	57.27 ± 1.15	56.73 ± 0.27
	MTS2	57.36 ± 0.77	57.27 ± 1.15	57.88 ± 0.80	55.82 ± 0.49
	MTS3	57.55 ± 0.76	57.50 ± 0.69	56.87 ± 1.15	54.99 ± 3.68
	MTC	57.59 ± 0.76	57.63 ± 0.69	57.94 ± 1.15	56.91 ± 3.68
Redness, a^*	MTS1	9.81 ± 0.46 ^A	11.09 ± 0.78 ^Y	9.10 ± 0.75 ^{B,X}	9.89 ± 0.24 ^{A,X}
	MTS2	8.74 ± 1.14 ^A	9.10 ± 0.75 ^{X,a}	9.75 ± 0.60 ^Y	11.04 ± 0.34 ^{B,b,Y}
	MTS3	9.14 ± 0.70	10.11 ± 0.51 ^a	9.59 ± 0.23 ^Y	8.22 ± 0.38 ^{X,b}
	MTC	10.09 ± 0.70	10.98 ± 0.51 ^Y	11.06 ± 0.23	11.20 ± 0.38 ^Y
Yellowness, b^*	MTS1	13.23 ± 0.71 ^{a,A,X,x}	10.52 ± 1.73 ^b	10.59 ± 0.35 ^{X,B}	11.59 ± 0.50
	MTS2	9.64 ± 0.38 ^Y	10.59 ± 0.35	11.30 ± 0.36	11.65 ± 0.66
	MTS3	10.78 ± 1.01 ^Y	10.43 ± 1.13	10 ± 0.82 ^X	11.44 ± 1.07
	MTC	11.80 ± 1.01	11.84 ± 1.13	12.58 ± 0.82 ^Y	12.72 ± 1.07

The data are presented as means ± standard errors. Statistical analyses were performed by comparing the parameters of the same samples along the storage period and those of each mortadella formulation (MTC, MTS1, MTS2, and MTS3) at each storage time (1, 15, 25, and 30 days). The a,b mean values in the same row (storage time) with different letters differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). The x,y mean values in the same column (different samples for the same storage time) differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

3.1.2. Instrumental Color Parameters

The color parameter (L^* , a^* , and b^*) values are given in Table 1. The analysis of the L^* parameter showed that there were neither significant differences between treatments nor over time within the same sample. According to the luminosity results (parameter L^*), the samples containing the common lambsquarter (MTS2) and oregano (MTS3) powders had L^* values similar to those of the control sample (MTC) from days 1 to 15, while the samples containing the mandarin peel powder (MTS1) showed lower luminosities with a fluctuating trend over time. However, at 30 days, decreases in L^* values were observed in all the experimental samples compared to that of the control sample, which exhibited higher luminosity. Our results align with those of Baldin et al. [47], who investigated the addition of jabuticaba (*Myrciaria cauliflora*) extract to fresh sausages at a much higher concentration (4%) compared to that used in our study (0.1%). Similar to our observations, they reported decreases in L^* values after 15 days of storage, from 65.6 at day 0 to 62.5 at day 28.

The table below (Table 1) also presents the results for the redness parameter (a^*), revealing differences between the three experimental samples and the control sample throughout the storage period. Specifically, the MTS1 and MTS3 samples exhibited a fluctuating trend, while MTS2 showed a consistent linear increase, similar to that of the control sample. It was also observed that the a^* value of MTS2 (11.04) was significantly ($p < 0.01$) higher than those of the other two experimental samples MTS1 and MTS3 (9.89 and 8.22, respectively) at 30 days of storage, highlighting that the integration of the common lambsquarter powder appeared to enhance the redness of the mortadella.

Regarding the b^* parameter (yellow content), as shown in Table 1, there were notable differences between the treatments. Higher b^* values compared to those of the control sample were recorded in MTS1 on day 1 of storage, followed by a decrease throughout the storage period. In contrast, MTS2 and MTS3 exhibited increasing trends in b^* values over time, similar to that in the b^* values of the control sample (MTC). The results obtained corroborate those obtained in the study by Almeida et al. [48], who found that b^* values

measured in mortadella made with jaboticaba peel extract (1%) had the same trend as that of our values until the end of the storage.

It is important to note that the addition of plant-derived extracts often influences the color of meat products [49]. These results are consistent with those in several studies reporting that natural antioxidants contribute to the stabilization of coloration in meat and meat products, such as beef patties, mortadella, and beef burgers [30,50,51].

3.1.3. Texture Profile Analysis

Table 2 shows the mean values of the mortadella formulations (MTC, MTS1, MTS2, and MTS3) obtained during the TPA tests for hardness (N), cohesiveness (N), gumminess (N), chewiness (N), resilience, and adhesiveness (N × mm).

For the hardness values, similar trends to that of the control sample (MTC) were observed for the MTS1 and MTS2 samples, with progressive increases from day 1 to day 25, followed by decreases between days 25 and 30. It is important to note that despite following a similar pattern, MTS2 exhibited notably lower hardness values compared to those of MTS1 (Table 2) throughout the entire storage period (7–8 N for MTS1 and 4–5 N for MTS2) and were more similar to the control sample's hardness values. Highly significant differences ($p < 0.01$) between the MTS1 sample and both the other two experimental formulations and the control sample were also observed over time. Similar trends to those observed in MTS1 and MTS2 were reported by Lee et al. [52], who investigated the incorporation of 1% and 2% dried kimchi powder into breakfast sausage formulations, highlighting comparable textural behaviors during storage. In contrast, MTS3 displayed a fluctuating trend in hardness, characterized by an initial decrease, a subsequent increase, and a final decline.

Table 2 shows significant differences in adhesiveness and elasticity values among the experimental samples and the control sample. Notably, MTS1 consistently exhibited higher adhesiveness and elasticity values across all the storage times, while MTS2 and MTS3 displayed similar values to those of the control sample (MTC) from day 1 to day 25. The only exception in the elasticity profile occurred on day 30, where the MTS3 formulation (0.36 mm) diverged from all the other samples. A similar trend was observed in terms of the statistical significance, with the MTS1 sample exhibiting significantly greater differences ($p < 0.01$) compared to those of the other mortadella formulations (Table 2) until the time point T2. However, at T3, MTS3 became the sample that significantly deviated the most from the others.

Regarding the chewiness parameter (Table 2), all the experimental samples (MTS1, MTS2, and MTS3) exhibited increasing trends from day 1 to day 15, followed by decreases at day 25 and further reductions at day 30, with values closely resembling those recorded at T0. The MTS1 sample consistently showed higher chewiness values, with significant differences ($p < 0.01$) compared to the control sample (MTC) and no significant differences compared to the other two experimental samples except for MTS2 at time T0.

Regarding cohesiveness, all three experimental samples exhibited fluctuating trends similar to the control sample's trend, consistently maintaining values below 1 N throughout the storage period, except for MTS3, which, at time T3, displayed the highest cohesiveness value, reaching 1.63 N.

The gumminess parameter (Table 2) exhibited fluctuating trends across all three experimental samples (MTS1, MTS2, and MTS3) compared to the increasing trend of the control sample (MTC) during storage. The sample with the lowest values (approximately 1) was MTS3, while the highest values (approximately 3) were observed in MTS1.

Table 2. Trends of texture parameters measured in mortadella formulations during storage.

Parameter	Sample	T0 (Day 1)	T1 (Day 15)	T2 (Day 25)	T3 (Day 30)
Hardness, <i>N</i>	MTS1	7.09 ± 0.67 ^X	7.49 ± 0.31 ^X	8.06 ± 0.63 ^{X,a}	6.90 ± 0.79 ^{X,b}
	MTS2	4.61 ± 0.67 ^{Y,a}	5.19 ± 0.99 ^Y	5.88 ± 0.74 ^{Y,b}	4.79 ± 0.88 ^Y
	MTS3	4.25 ± 0.28 ^{Y,A}	3.93 ± 0.61 ^{Y,D}	5.84 ± 0.14 ^{Y,B}	4.48 ± 0.52 ^{Z,C}
	MTC	4.06 ± 0.28 ^{a,Y}	4.53 ± 0.61 ^Y	5.36 ± 0.14 ^{b,Y}	4.62 ± 0.52 ^Y
Adhesiveness	MTS1	−15.77 ± 4.43 ^X	−9.45 ± 4.61 ^X	−7.34 ± 2.41	−6.07 ± 1.57 ^Y
	MTS2	−5.55 ± 2.62 ^Y	−6.03 ± 3.53	−5.86 ± 3.04	−4.45 ± 4.30 ^Y
	MTS3	−3.66 ± 1.18 ^{Y,A}	−3.34 ± 0.43 ^{Y,A}	−3.65 ± 1.88 ^A	−3.53 ± 1.28 ^{X,B}
	MTC	−3.84 ± 1.18 ^Y	−3.49 ± 0.43	−3.75 ± 1.88	−4.63 ± 1.28 ^Y
Elasticity	MTS1	0.92 ± 0.33	1.04 ± 0.29 ^{x,X}	1.01 ± 0.21 ^x	0.90 ± 0.12 ^Y
	MTS2	0.89 ± 0.02	0.77 ± 0.09 ^Y	0.81 ± 0.03	0.72 ± 0.21 ^Y
	MTS3	0.85 ± 0.09 ^A	0.82 ± 0.12 ^{y,A}	0.76 ± 0.10 ^{y,A}	0.36 ± 0.07 ^{X,B}
	MTC	0.88 ± 0.09	0.81 ± 0.12 ^y	0.89 ± 0.10	0.84 ± 0.07 ^Y
Chewiness, <i>N</i> × <i>mm</i>	MTS1	2.88 ± 0.18 ^{X,A,a}	3.93 ± 0.22 ^{X,B}	3.54 ± 0.50 ^{X,b}	2.30 ± 0.30
	MTS2	1.74 ± 0.60 ^Y	2.01 ± 0.22	1.99 ± 0.98	1.30 ± 0.47
	MTS3	1.62 ± 0.38	1.97 ± 0.34	1.88 ± 0.32	1.76 ± 0.35
	MTC	1.70 ± 0.38 ^Y	1.84 ± 0.34 ^Y	1.95 ± 0.32 ^Y	1.68 ± 0.35
Cohesiveness	MTS1	0.50 ± 0.04	0.51 ± 0.03 ^x	0.40 ± 0.03	0.42 ± 0.06 ^Y
	MTS2	0.45 ± 0.03	0.43 ± 0.05	0.38 ± 0.05	0.43 ± 0.03 ^Y
	MTS3	0.43 ± 0.02 ^A	0.30 ± 0.04 ^{y,A}	0.46 ± 0.05 ^A	1.63 ± 0.34 ^{Z,B}
	MTC	0.42 ± 0.02 ^A	0.44 ± 0.04 ^A	0.35 ± 0.05 ^A	0.67 ± 0.34 ^{B,X}
Gumminess, <i>N</i>	MTS1	3.48 ± 0.45 ^{x,A,X}	3.76 ± 0.21 ^{Y,A}	3.15 ± 0.50 ^{B,X}	2.88 ± 0.46 ^{B,X}
	MTS2	2.09 ± 0.39 ^Y	2.27 ± 0.62 ^Z	2.27 ± 0.37 ^Y	2.07 ± 0.34 ^{y,Z}
	MTS3	1.83 ± 0.20 ^{Y,A}	1.46 ± 0.21 ^{W,B}	2.69 ± 0.21 ^C	2.43 ± 0.30 ^{Y,D}
	MTC	2.85 ± 0.20 ^{y,X}	2.96 ± 0.21 ^X	3.17 ± 0.21 ^X	2.66 ± 0.30 ^{x,X}

The data are presented as means ± standard errors. Statistical analyses were performed by comparing the parameters of the same samples along the storage period and those of each mortadella formulation (MTC, MTS1, MTS2, and MTS3) at each storage time (1, 15, 25, and 30 days). The a,b,c,d mean values in the same row (storage time) with different letters differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). The x,y,z,w mean values in the same column (different samples for the same storage time) differ significantly for $p < 0.05$ (lowercase) or $p < 0.01$ (uppercase). MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

In summary, the results of the texture profile analysis revealed a greater difference between the MTS1 sample and the control formulation, with higher values observed during the storage period and few differences between the MTS2 and MTS3 experimental samples and the control sample for all the analyzed parameters. These findings indicate minimal or no adverse effects from the use of the oregano or common lambsquarter experimental powders in artisanal mortadella formulations compared to the formulation without the addition of plant powders (MTC).

3.1.4. Lipid Oxidation Measurements

The TBAR values are reported in Figure 4. Further statistical information is reported in the supplementary materials (Table S2). All the samples demonstrated consistently increasing trends over time, similar to that of the control sample (MTC) but with different

values, especially for the MTS1 sample, observed from days 1 to 25 (Figure 4). The most significant differences were noted at T3 (30 days of storage), when MTS1 showed a higher TBAR value (3.7) compared to those of MTS2 (0.65) and MTS3 (1.11). Throughout the storage period, the TBAR values of MTS2 remained similar to those of the control sample (MTC). This trend aligns with the findings of Baldin et al. [47], who reported an inhibitory effect in fresh processed meat after using natural antioxidants (jabuticaba extract). Our results are also consistent with the observations of Almeida et al. [48], who reported data like those obtained for MTS2 and MTS3 at T3 for mortadella formulations prepared by adding jabuticaba peel extract (1%). Therefore, the addition of natural substances to the mortadella formulation, replacing synthetic antioxidants, decreased lipid oxidation reactions. Notably, data have shown that the addition of common lambsquarter and oregano powders to Bologna-type sausages could prevent lipid oxidation more efficiently than the addition of mandarin peel powder.

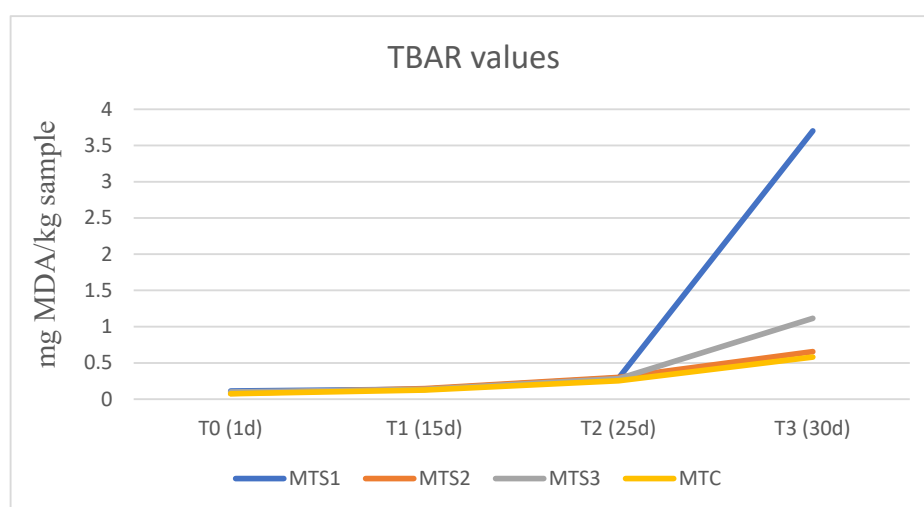


Figure 4. Trends of TBAR values in mortadella formulations during storage. MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

3.2. Microbiological Analyses

The microbiological analysis results obtained at the four time points (T0, T1, T2, and T3) are reported in the table below (Table 3), illustrating the temporal evolutions of the microbial loads across the four tested products (three experimental formulations (MTS1, MTS2, and MTS3) and one control (MTC)).

Specifically, the total aerobic mesophilic bacteria (TAB at 30 °C), well known as a key factor in assessing the microbiological quality and safety of foods, exhibited constant increases over time in MTS1 and MTC (albeit reaching different concentrations) and fluctuating trends in MTS2 and MTS3 (Table 3). However, none of the samples exceeded the acceptability limit set at >6 Log (CFU/g) by regulation EC No. 2073/2005 [53] on microbiological criteria for foodstuffs. The artisanal mortadella produced with mandarin (*Citrus reticulata* L.) peel powder (MTS1), exhibited the highest value of TAB 30 °C, reaching 6.46 Log (CFU/g) at 30 days (T3) after vacuum packaging, approaching the regulatory threshold but not exceeding it. In contrast, lower mesophilic counts and lower growth rates, closely resembling those of the control sample (MTC), were observed in MTS2 (5.94 Log (CFU/g)) and MTS3 (5.65 Log (CFU/g)), likely because of the inclusion of herbs, particularly oregano, which have already been shown in other studies to have strong antimicrobial properties when used as natural additives in meat and meat products [19,24]. Similar results were obtained in the present study, where their use appeared to effectively contribute

to extending the shelf lives of the artisanal mortadellas compared to that of the control sample made with a chemical additive.

Table 3. Microbiological trends of the four mortadella formulations (three experimental (MTS1, MTS2, and MTS3) and one control (MTC)) during the storage period.

Parameter	Sample	T0 (Day 1)	T1 (Day 15)	T2 (Day 25)	T3 (Day 30)
TAB 30 °C	MTS1	1.96	5.49	5.70	6.46
	MTS2	1.96	4.10	3.96	5.94
	MTS3	3.96	2.48	4.73	5.65
	MTC	2.83	3.31	3.89	4.02
<i>Enterobacteriaceae</i>	MTS1	<1	<1	<1	<1
	MTS2	<1	<1	<1	<1
	MTS3	<1	<1	<1	<1
	MTC	<1	<1	<1	<1
<i>β-Glucuronidase-positive E. coli</i>	MTS1	<1	<1	<1	<1
	MTS2	<1	<1	<1	<1
	MTS3	<1	<1	<1	<1
	MTC	<1	<1	<1	<1
<i>Coagulase-positive Staphylococci</i>	MTS1	<2	3.07	5.70	3.49
	MTS2	<2	2.56	3.95	3.47
	MTS3	3.96	<2	2.65	4.8
	MTC	<2	<2	<2	<2
<i>Sulfite-reducing Clostridia</i>	MTS1	<2	<2	<2	<2
	MTS2	<2	<2	<2	<2
	MTS3	<2	<2	<2	<2
	MTC	<2	<2	<2	<2
<i>Campylobacter spp.</i>	MTS1	<2	<2	<2	<2
	MTS2	<2	<2	<2	<2
	MTS3	<2	<2	<2	<2
	MTC	<2	<2	<2	<2
<i>B. cereus</i>	MTS1	<2	<2	2.91	<2
	MTS2	<2	<2	<2	<2
	MTS3	<2	<2	<2	<2
	MTC	<2	<2	<2	<2
<i>Listeria monocytogenes</i>	MTS1	<2	<2	<2	2.25
	MTS2	<2	<2	<2	<2
	MTS3	<2	<2	<2	<2
	MTC	<2	<2	<2	<2

The results are expressed as Log (CFU/g) values. MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

Regarding the other hygiene indicator microorganisms, the detection of enterobacteria and *Escherichia coli* at concentrations below 1 Log (CFU/g) over time indicates that operators adopted proper hygienic practices during the production process. These low microbial loads reflect effective sanitation protocols and contamination control implemented during the early stages of production. Conversely, the consistent presence of coagulase-positive staphylococci at all the time points (T0, T1, T2, and T3) suggests contamination occurred during the processing chain. Notably, considering that, except for the control mortadella MTC (likely due to the presence of the chemical additive), the concentration trends were non-linear for any of the three experimental samples, with a consistently increasing until T2 followed by a decreased at T3 in MTS1 and MTS2 and a more fluctuating pattern in MTS3 with concentrations initially decreasing, then increasing and subsequently decreasing

again, it is plausible that contamination occurred during the packaging process of the product. However, despite there being evident contamination, the oregano and especially the common lambsquarters used as natural antimicrobial agents demonstrated their ability to reduce microbial growth, effectively being able to maintain microbial loads between 2 and 3 Log (CFU/g) over time (except for the oregano at the final stage (T3)). Conversely, the mandarin peel powder did not exhibit comparable efficacy, as highlighted by a staphylococcal concentration of 5.70 Log (CFU/g) detected in the MTS1 sample at T2, exceeding the acceptability limits established by regulation EC No. 2073/2005 [53].

As regards pathogenic microorganisms, no sulfite-reducing clostridia or *Campylobacter* spp. were detected in any of the analyzed samples, with concentrations remaining always below 2 Log (CFU/g), which represents the detection limit of the ISO methods. Similarly, all the samples tested negative for *Salmonella*. Conversely, *Bacillus cereus* and *Listeria monocytogenes* were detected at two different sampling times (T2 and T3, respectively) in the same sample (MTS1). However, the ISO method provides only presumptive positive results, as colonies with characteristic appearances are considered as significant and counted. To confirm the presence of the targeted bacteria, suspect colonies were analyzed using MALDI-TOF/MS, which ultimately did not validate their presence.

In summary, despite all the experimental samples exhibiting pH ranges from 5.9 to 6.16 and water activity (a_w) values consistently between 0.95 and 0.96—conditions known to promote bacterial growth and proliferation in meat—the natural additives employed demonstrated efficacy in inhibiting or reducing microbial growth in the analyzed artisanal mortadellas compared to the control (MTC). Notably, the analysis of the microbial load trends (Table 3) revealed that among the tested additives, the oregano and common lambsquarter powders were more effective than the mandarin peel powder. With the exception of the MTS1 sample, our findings are perfectly consistent with those reported by Viuda-Martos et al. [54], who analyzed the effects of incorporating orange dietary fiber (1%), rosemary essential oil (0.02%), and thyme essential oil (0.02%) on the quality and shelf lives of Bologna-type sausages. No specific studies on mortadella samples made with the other two natural additives investigated in our study were found. Despite this, similar microbiological counts have been reported by Fernández-López et al. [55], who evaluated the effects of another natural substance, which was black quinoa applied either as whole seeds and as the fibre-rich fraction obtained from its wet-milling process, at a concentration of 2.5 g/100 g, on the shelf lives of Bologna-type sausages during cold storage (for 21 days). Except for the MTS1 sample, which microbial count exceeded 4 Log CFU/g, reaching 5.70 Log CFU/g at T2, their findings for microbial counts, ranging between 2 and 4 Log CFU/g over the storage period, are in accordance with the results shown below (Table 3). Comparable microbiological loads during refrigerated storage have also been observed by Ranucci et al. in cooked sausages with almond nuts (15 g/100 g) and emmer wheat (30 g/100 g) added [56]. These comparisons highlight the importance of our findings, as the concentration (0.1 g/100 g) of each natural additive used in our artisanal mortadellas is substantially lower than those reported in the other two works, which ranged from 2.5 [55] to 30 g/100 g [56]. Finally, our data, except for those for the sample made with the mandarin peel powder (MTS1), align also with those reported by Dos Santos et al. [57], who made Bologna-type sausages with different concentrations of jabuticaba peel extract (0.5–1 g/100 g) combined with the natural additive nisin and other chemical additives, including sodium nitrite, sodium tripolyphosphate, and sodium erythorbate. This result is particularly encouraging for the development of a healthier alternative produced exclusively with natural additives. In fact, in our study, we achieved comparable outcomes to those of Dos Santos et al. [57], using only a single untreated natural additive and at a

significantly lower concentration (0.1 g/100 g) for each sample. Moreover, unlike in their approach, we did not combine the additive with any other natural or chemical additives.

3.3. Sensory Evaluation

Table 4 shows the results obtained for the sensorial analysis carried out at the beginning of the assay (T0) by a panel of 10 tasters. During the sensory evaluation, visual (appearance), gustatory (texture and taste), and olfactory (odor) assessments of each mortadella sample were performed. After tasting each one in random order, the 10 semi-trained tasters rated the single parameters using a scale ranging from 1 to 5. The mean scores and standard deviations calculated for each attribute are presented in the table below. Considering the experimental formulations, a preliminary global sensory assessment revealed that the artisanal mortadellas MTS1 and MTS3 were similar, with slight differences in preference for the MTS3 sample, which achieved a total score of 51.55 compared to 50.5 for MTS1. MTS2 received the lowest total score (45.3) among the three innovative formulations and was the least appreciated in all respects. Specifically, both MTS1 and MTS3 achieved the highest scores for appearance (mean = 3.3) and taste (mean = 2.7). Additionally, MTS3 scored better for odor (mean = 2.9), while MTS1 performed better in terms of texture (mean = 2.3).

Table 4. Mean scores and standard deviations of sensory and gustatory descriptors assigned to the four mortadella formulations (MTS1, MTS2, MTS3, and MTC) by a panel of 10 tasters.

Attribute	MTS1	MTS2	MTS3	MTC
External evaluation				
Color intensity	4.1 ± 0.7	3.5 ± 0.4	3.5 ± 0.9	4.2 ± 0.0
Visible seasoning	4.1 ± 0.6	3.2 ± 0.3	4.9 ± 0.1	2.5 ± 0.1
Homogeneity perception	4.5 ± 0.7	4.1 ± 0.5	3.2 ± 0.8	4.8 ± 0.0
Presence of holes	2.1 ± 1.2	2.5 ± 0.3	2.5 ± 0.1	1 ± 0.0
Hole size	2 ± 0.6	1 ± 0.2	2 ± 0.2	1 ± 0.5
Shine	3 ± 0.0	3.5 ± 0.1	3.5 ± 0.0	3.6 ± 0.3
Odor				
Intensity	4 ± 0.0	4 ± 0.5	4.5 ± 0.7	4.75 ± 0.3
Seasoning smell	2.75 ± 0.8	2.75 ± 0.7	3.75 ± 0.9	4.1 ± 0.6
Pepper smell	2.25 ± 0.2	2.25 ± 0.9	2.5 ± 0.7	1 ± 0.2
Unpleasant odors	1 ± 0.5	1 ± 0.0	1 ± 0.3	1 ± 0.0
Texture				
Chew resistance	1.5 ± 0.6	1.25 ± 0.0	1 ± 0.0	1 ± 0.5
Gelatinous texture	2 ± 0.0	1.75 ± 0.2	2 ± 0.8	2.75 ± 0.4
Juiciness	3.5 ± 0.3	3 ± 0.0	3.5 ± 0.1	1.5 ± 0.1
Taste				
Flavor intensity	3.2 ± 0.6	2.5 ± 0.3	3.2 ± 0.6	4.7 ± 0.5
Seasoning flavor	3.5 ± 0.3	3 ± 0.5	3.5 ± 0.2	3.25 ± 0.2
Pepper flavor	2.5 ± 0.0	2 ± 0.3	2.5 ± 0.8	1 ± 0.8
Saltiness	3.5 ± 0.8	3 ± 0.6	3.5 ± 0.3	3 ± 0.3
Unpleasant taste	1 ± 0.0	1 ± 0.5	1 ± 0.0	1 ± 0.0
Total score	50.5	45.3	51.55	46.15

The data are presented as means ± standard errors. MTS1 = mandarin peel, MTS2 = common lambsquarters, MTS3 = oregano, and MTC = sodium nitrite.

Finally, a comparative analysis with the control sample (MTC) revealed that the mortadella formulations containing mandarin peel (MTS1) and oregano (MTS3) powders were also more appreciated than the control sample (total score = 46.15).

At the end of the storage (T3), visual and olfactory evaluations of the tested products were conducted.

The visual assessment revealed browning in the three experimental samples (MTS1, MTS2, and MTS3) compared to the control (MTC). This observation was supported by color analysis, which showed decreases in L^* values for all the experimental formulations at 30 days, indicating lower luminosities compared to that of the control, which maintained higher brightness. Notably, the MTS3 sample appeared darker than the others, likely because of its higher visible seasoning content.

From the olfactory evaluation, it was observed that the MTS1 sample exhibited a rancid odor, most likely associated with increased MDA production (as indicated by its TBAR value of 3.7). Conversely, the MTS2 and MTS3 samples retained a spiced aroma, with no signs of off-odors, a characteristic also observed in the control sample (MTC).

4. Conclusions

In conclusion, the analysis of the three experimental artisanal mortadellas revealed that the samples with the best microbiological, rheological, and physicochemical characteristics were produced with common lambsquarter (MTS2) and oregano (MTS3) powders, in which similar parameters to those of the control sample (MTC) (produced with a chemical additive) were noted. In fact, at the end of the storage period, extremely low TBAR values, similar to those of the MTC formulation, were observed for both MTS2 and MTS3 (MTS2, 0.65 mg MDA/kg; MTS3, 1.11 mg MDA/kg; and MTC, 0.57 mg MDA/kg), reflecting the effective control of lipid oxidation by oregano and common lambsquarter powders. Also, color stability, particularly redness, was maintained in both samples until the end of the storage (11.04 for MTS2 and 8.22 for MTS3 at 30 days) and closely matched that of the MTC formulation (11.20 for MTC), aligning with consumer preferences. This suggests that the addition of plant-derived extracts in processed meat products also contributes to color stabilization. Finally, although the experimental samples showed pH and water activity (a_w) values known to promote bacterial growth and proliferation, the natural additives used effectively reduced microbial growth in the artisanal mortadellas, preserving their shelf lives compared to that of the control (MTC), thereby contributing also to microbial stability. Furthermore, the panel test carried out by the semi-trained tasters suggested that the most appreciated experimental sample was the mortadella containing oregano (MTS3). In addition, from a further comparison with other studies, which utilized natural substances at higher concentrations or in combination with other chemical additives, it was observed that the mentioned natural additives used in their raw, unprocessed forms (i.e., as leaves and whole plant powders rather than as essential oils or bioactive compounds extracted from these sources) and at lower concentrations achieved comparable results. Conversely, the mortadella made with mandarin peel powder (MTS1) showed poorer physicochemical and microbiological characteristics, revealing the low effectiveness of this additive at the tested concentration.

These preliminary findings have highlighted the great antimicrobial and antioxidant properties of oregano and common lambsquarter powders used as potential natural preservatives and have shown that replacing chemical additives with natural alternatives in Bologna-type sausages could be a technologically feasible approach for producing healthier products while maintaining microbiological safety, shelf life, and product quality. Thus, considering the lack of specific studies on these types of natural substances in mortadella production and the growing consumer interest in chemical-additive-free food, future research will focus on exploring their uses at different concentrations and in different forms (both as raw materials and as bioactive compounds extracted from them), studying their

individual effects and interactions to determine if their combined use results in enhanced antimicrobial and antioxidant activities.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app15073571/s1>: Table S1: Mean values (\pm standard errors) of pH and aw in the three experimental mortadella formulations and control test during the storage. Table S2: Lipid oxidation (mg of malonaldehyde per kg of the sample) of samples during the storage.

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