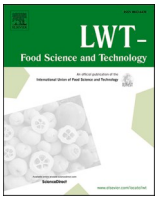
LWT - Food Science and Technology 196 (2024) 115852

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



Physicochemical properties and sensory features of ripened, industrially prepared sausages, enriched with olive leaf extract to replace nitrite and nitrate

Michela Pia Totaro, Graziana Difonzo, Antonella Pasqualone, Carmine Summo *

Department of the Soil, Plant and Food Science DiSSPA, University of Bari Aldo Moro, Via Amendola, 165/a, I-70126, Bari, Italy

ARTICLE INFO

Keywords:
Vegetable extract
Nitrites replacement
Lipid oxidation
Protein oxidation
N-nitrosamines content

ABSTRACT

The use of nitrite and nitrate in meat products has been questioned due to the generation of N-nitroso compounds with carcinogenic effects. Thus, research has been focused on their replacement with several alternatives. This study is focused on evaluating the effects of a commercial olive leaf extract (OLE), marketed as dietary supplement, on protein and lipid oxidation, N-nitrosamine content and sensory properties of industrial sausages at the end of the ripening period. Five different formulations were prepared: F1 (OLE: 1000 mg/kg; NO_2–NO_3: 0 mg/kg), F2 (OLE: 1000 mg/kg; NO_2–NO_3: 75-75 mg/kg), F3 (OLE: 0 mg/kg; NO_2–NO_3: 0 mg/kg), F4 (OLE: 0 mg/kg; NO_2–NO_3: 75-75 mg/kg), F5 (OLE: 500 mg/kg; NO_2–NO_3: 35-35 mg/kg). The use of OLE in combination with a reduced dose of nitrite and nitrate (F5) significantly reduced the lipid and protein oxidation ($p < 0.05$) compared to the formulation with synthetic additives alone (F4). No differences were observed in terms of textural properties with the samples without the extract. The OLE did not affect the microbiological quality and enabled a significant reduction in the N-nitrosamines content. However, the addition of OLE decreased the intensity of red colour and determined the insurgence of a slight bitter taste in the sausages.

1. Introduction

The consumption of meat and meat products is one of the most questioned aspects in public debates on sustainable food systems, climate change and healthy eating. Public attention on these products is related to human health and ethical aspects of meat production (Parlasca & Qaim, 2022). Indeed, several studies (Turner & Lloyd, 2017; Zhang et al., 2023) highlighted the association between red and processed meat with the insurgence of cardiovascular diseases and colorectal cancer. These risks are mainly due to the high content of saturated fatty acids and cholesterol as well as to the carcinogen compounds generated from processing, arising from some additives included in the formulation (Shpaizer, Nussinovich, Kanner, & Tirosh, 2018). Among meat additives, nitrite and nitrate are the most commonly used. Their efficiency makes them indispensable for this category of products, especially for ripened ones (Zhang et al., 2023). They play a major role in the development of typical reddish-pink meat colour and in inhibiting the growth of undesirable bacteria (e.g., *Clostridium botulinum* and its toxin production, *Listeria monocytogenes*). Furthermore, these compounds, especially nitrite, act against oxidation phenomena (e.g., lipid and protein oxidation) and contribute to the improvement of the oxidative stability (Nader et al., 2022). Nevertheless, the discovery of N-nitroso compounds (NOCs) raised concerns regarding their safety when employed in meat processing (Zhang et al., 2023). Indeed, N-nitrosamines are associated with genotoxicity and metabolic disturbances within the large intestine mucosa, posing a potential risk for the development of colorectal cancer (Zhang et al., 2023). However, due to the multiple functions of nitrite and nitrate in meat and meat products, their complete restriction in meat products may not be acceptable to producers and consumers (Karwowska & Kononiuk, 2020). Thus, to guarantee the safety of their use, many countries have implemented regulations and directives (Zhang et al., 2023). Indeed, specific restrictions have been imposed by the European Union as defined by Reg. (EU) 1333/2008 and its modifications and integrations. The latter regulation, issued on October 6, 2023, defines new limits aimed at reducing consumers' exposure to N-nitrosamines while simultaneously maintaining a protective effect against pathogenic bacteria of meat products. For raw meat products like ripened ones, the maximum allowed amounts of nitrite decrease from 150 to 80 mg/kg, while those of nitrate drop from 150 to 90 mg/kg (EU Commission, 2023). As results,

* Corresponding author. Università degli Studi di Bari Aldo Moro, Via Amendola 165/a, I-70126, Bari, Italy.
E-mail address: carmine.summo@uniba.it (C. Summo).

https://doi.org/10.1016/j.lwt.2024.115852
Received 22 September 2023; Received in revised form 7 February 2024; Accepted 10 February 2024
Available online 13 February 2024

a shifting of the consumption towards plant-based meat alternatives has been observed in recent years (Parlasca & Qaim, 2022).

One of the strategies currently used during processing of meat products is principally based on lowering the amount of additives (e.g., nitrite and nitrate) and harmful compounds formed during processing (e.g., N-nitrosamines) (Karwowska, Stadnik, Stasiak, Wójciak, & Lorenzo, 2021). Many studies evaluated the addition of essential oils, vegetables spices, extracts from fruits or plant wastes and by-products in meat formulations, to reduce the amount of the commonly used synthetic additives and inhibit the formation of N-nitrosamines (Tian et al., 2020; Zhou, Wang, & Wang, 2020), thanks to their high content of highly valuable bioactive substances such as polyphenols (Sharma, Usmani, Gupta, & Bhat, 2021). To this regard, several studies reported the positive effect of Olive Leaf Extract (OLE) on the oxidative (Caponio, Difonzo, Calasso, Cosmai, & De Angelis, 2019) and microbial stability (Testa et al., 2019), thus on the shelf-life extension (Khemakhem et al., 2019) of several food products. Similarly, in a preliminary study (Difonzo, Totaro, Caponio, Pasqualone, & Summo, 2022), the effectiveness of using OLE to reduce the addition of nitrite and nitrate in the formulation of ripened pork sausages was evaluated on a lab-scale production. As a result of this preliminary research, an effective reduction of the nitrate and nitrite residual in the final products was observed, with no impact on the hygiene and safety parameters of the samples (Difonzo et al., 2022). Based on these preliminary results, an industrial-scale-up was needed to further validate the feasibility of reducing nitrite and nitrate in ripened sausages by following the proposed strategy.

The aim of this study was therefore to assess the physicochemical properties and sensory features of ripened, industrially prepared sausages, enriched with olive leaf extract as a substitute to nitrite and nitrate. Unlike the previous study, a commercial OLE, commonly marketed as dietary supplement, with a standardized oleuropein content, was used, and samples were manufactured following the standard industrial production process. Thus, all stages of sausage production were carried out mechanically and vacuum filling of the casings was applied. Moreover, the analytical determinations have been deepened compared to the preliminary study, focusing on protein and lipid oxidation, N-nitrosamine content, and sensorial properties. This approach was adopted to standardise the production process and facilitate the immediate practical application of the findings at industrial level.

2. Material and methods

2.1. Sausages production and experimental design

Ripened sausages were produced at a local company (Salumi Martina Franca S.r.l., Martina Franca, Italy) where the ripening phases also took place, following the common industrial production process. Fresh lean pork meat (shoulder pork and lean trimmings) and fatty tissue (pork belly) (85/15, *w/w*) were trimmed and minced with an industrial grinder equipped with a pre-mixer (TCA-10, Omet Foodtech, Poggibonsi, Italy). Pork meat was mechanically mixed and kneaded in a mixer (K-400, Omet Foodtech, Poggibonsi, Italy) and the following ingredients were added: commercial olive leaf extract (Olive Leaf Extract, Hepatica, Germany) commonly marketed as dietary supplement, 40% oleuropein; potassium nitrate (E252; SolMar, Italy); sodium nitrite (E250; SolMar, Italy); salt and pepper. Five different combinations of nitrite and nitrate (NO_2–NO_3) and OLE have been formulated as reported in Table 1. The quantities of OLE incorporated into the various formulations were determined by considering to the reported amounts of vegetable extracts used in sausages as substitutes for nitrite and nitrate in existing literature (Kurt & Ceylan, 2017; Pateiro, Bermúdez, Lorenzo, & Franco, 2015; Xiang, Cheng, Zhu, & Liu, 2019). Additionally, the results of our previous lab-scale study results were taken into account during this selection process (Difonzo et al., 2022). The level of nitrite and nitrate was defined according to the current law (Reg. EU 2018/2023) and a trial (F5) with reduced level of nitrate and nitrites and OLE has been also performed to assess the possibility for a partial replacement of the synthetic additives.

Table 1
Combinations of nitrite and nitrate and OLE in the formulation (mg/kg of raw meat mixture).

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)
F1	1000	0
F2	1000	75–75
F3	0	0
F4	0	75–75
F5	500	35–35

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate.

The mixture was mechanically vacuum-filled (continuous vacuum filler F20, Omet Foodtech, Poggibonsi, Italy) into 40 mm diameter natural pork casings and submitted to stewing (23 °C, RH 95%) for 24 h, drying (17–20 °C, RH 60–75%) for 96 h and ripening (15–18 °C, RH 80%) (80 SM-DX, Forix Index, Conselice, Italy) for 30 days.

For each formulation 10 different sausages, each one approximatively weighting 500 g, were produced in order to enable replicated analyses.

2.2. Microbial determinations

The microbial safety of the sausages was evaluated considering the following microbiological parameters: mesophilic lactic bacteria count, *Clostridium* sulphite reducers and spores count, coliform count, *Escherichia coli* beta-glucuronidase positive count, coagulase-positive *Staphylococci* count, and *Listeria monocytogenes* detection, as reported by Difonzo et al. (2022). Two samples of each formulation were analysed.

2.3. Physico-chemical determinations

2.3.1. Weight loss, moisture content, water activity and pH determination

Weight loss, moisture content, water activity (a_w) and pH were determined according to the methods reported by Difonzo et al. (2022).

2.3.2. Oxidation of the lipid and protein fraction

The lipid oxidation was assessed by measuring thiobarbituric acid reactive substances (TBARs) as reported in Rosmini et al. (1996). The lipid oxidation level of the samples was expressed as mg of malondialdehyde (MDA) per kg of ripened sausages.

The carbonyl content was considered to determine the protein oxidation level of ripened sausages. Carbonyl groups were obtained by derivatisation with DNPH (dinitrophenylhydrazine) as described in Ganhão, Morcuende, and Estévez (2010). The carbonyl concentration (nmol/mg protein) was calculated from the absorbance at 280 nm and 370 nm of the samples using equation (1) as reported in Berardo et al. (2016):

$$\frac{C_{hydrazone}}{C_{protein}} = \frac{A_{370}}{\varepsilon_{hydrazone,370}\ x\ (A_{280} - A_{370}\ x\ 0,43)}\ x\ 10^6 \quad (1)$$

where $\varepsilon_{(hydrazone,370)}$ is 22,000 M/cm. The determinations were performed in three analytical replicates on three sausages for each formulation.

2.3.3. Free fatty acids analysis

Lipid extracts from sausages were obtained according to Folch method (Folch, Lees, & Sloane Stanley, 1957) and free fatty acids (FFA) were separated through solid phase extraction (SPE) columns (NH2-aminopropyl mini-columns) (Supelco, Bellefonte, PA, USA) according to Lorenzo, Cittadini, Bermúdez, Munekata, and Domínguez (2015). After separation, FFA were methylated according to the AOCS (American Oil

Chemists Society) method Ch 1–91. Nonadecanoic acid ($C_{19:0}$) at 0.3 mg/mL was used as internal standard and added to each sample before methylation. Fatty acid profile of FFA was analysed using a gas-chromatograph 7890B (Agilent Technologies, Palo Alto, CA, USA) equipped with a FID detector and a capillary column SP2340 (60 m × 250 μm × 0.2 μm film thickness) (Supelco Park, Bellefonte, PA, USA) as reported in Difonzo et al. (2022). Identification and quantitative calculation of free fatty acids were carried out by comparing the retention time of the free fatty acid with that of the corresponding methyl ester, using a standard mixture (C_4–C_{24}) (Supelco 37 component FAME Mix Bellefonte, PA, USA). The results were expressed as g of fatty acid/100 g of fat. The determinations were performed in three analytical replicates on three sausages for each formulation.

2.3.4. Nitrite and nitrate residual content and N-nitrosamines analysis

The residual nitrate content was carried out by spectrophotometric method (Baldini et al., 1996), while the nitrite residual content was determined by the ion-exchange chromatographic method as reported (UNI EN12014–4:2005). For the determination of N-nitrosamines, the extraction was performed according to the method described by Cintya, Silalahi, Putra, and Siburian (2019). The N-nitrosamines level was determined using High Performance Liquid Chromatography (HPLC) (Thermo Fischer Scientific, Waltham, MA, USA) as reported in Al-Kaseem, Al-Assaf, and Karabeet (2014) with some modifications. The filtered samples were injected into an AcclaimTM 120 Å C18 column (150 × 3 mm) (Thermoscientific, Waltham, MA, USA). A 1% formic acid aqueous solution was used as phase A and acetonitrile as phase B. Wavelengths of 231 nm was used for absorbance detection and the quantification of the N-nitrosamines was carried out using the EPA 521 standard (Nitrosamine Mix, Supelco, Bellefonte, USA). The results were expressed in μg/kg of ripened sausage. The determinations were performed in three analytical replicates on three sausages for each formulation.

2.3.5. Colour measurement

The International Commission on Illumination (CIE) lightness (L*), redness (a*), and yellowness (b*) were determined using a colorimeter CM-600d (Konica Minolta, Tokyo, Japan) calibrated using white calibration plate after 30 min blooming at room temperature (25 ± 5 °C). For the determination, sausages slice with thickness ranged between 15 and 20 mm were prepared, and the measurement was performed in three different points on the central part of three different slices. The colour determination was performed on three sausages for each formulation.

2.3.6. Texture profile analysis

Texture profile analysis (TPA) of the samples was performed at room temperature (25 ± 5 °C). Hardness (N), springiness, chewiness (N), and cohesiveness values were evaluated using a Texture analyser Z1.0 TN (ZwickRoell, Ulm, Germany) equipped with a cylindrical probe of 36 mm in diameter. Sausages were cut into 1-cm-thick portions, having a diameter of 2 cm, compressed twice up to 50% of recorded deformation at a speed of 1 mm/s. Four different portions of each sausage were considered, and the determination was performed on three sausages for each formulation.

2.4. Sensory analysis

Quantitative descriptive analysis (QDA) of the ripened sausages, was performed by a sensory panel consisting of 14 semi-trained members. The sensory panellists (7 males; 7 females; age range 35–52) were recruited, based on their previous experience with sensory evaluation of meat-based, among the employees of the sausage manufacturing company (Salumi Martina Franca S.r.l., Martina Franca, Italy), the technicians and the researchers of the laboratory of the Food Science and Technology unit of the Department of Plant, Soil, and Food Sciences of the University of Bari, Italy. All panel members had neither food allergies nor intolerances and were regular consumers meat-based products. Pre-test sessions were carried out to: (i) define the list of descriptors to be evaluated in the samples object of the study; (ii) define the intensity range of each descriptor; (iii) fix the scale anchors of each descriptor; (iv) verify reliability, consistency, and discriminating ability of panellists when testing meat-based products. The study protocol followed the ethical guidelines of the laboratory. Panellists were informed about study aims, and written informed consent was individually acquired from each participant. All tested samples were food-grade. The tests were conducted using a structured scale from 0 to 9. Samples cut into thin slices approximately 5 mm thick were coded with three-digit number and presented in small plates to each participant in a randomized order. The sensory parameters evaluated were: colour intensity (0 = light pink; 9 = brown), typical odour of ripened sausage (0 = absence of perception; 9 = extremely intense perception), rancid odour (0 = absence of perception; 9 = extremely intense perception), consistency (0 = soft; 9 = extremely hard), bitterness (0 = absence of perception; 9 = extremely intense perception), saltiness (0 = absence of perception; 9 = extremely intense perception). The sensory analysis was of sausages were performed in one session and considering two samples for each formulation.

2.5. Statistical analysis

Data were subjected to One-way ANOVA and Tukey's HSD test was used as a post-hoc test to determine statistically significant differences among means ($p < 0.05$) using Minitab Statystical Software (Minitab Inc., State College, PA, USA). Means ± standard deviations were calculated with the same software.

3. Results and discussion

3.1. Microbial count

All the parameters considered for microbial counts of the sausages at the end of ripening period, (mesophilic lactic bacteria, *Clostridium* sulphite count reducers and spores, coliforms, beta-glucuronidase positive *Escherichia coli* beta-glucuronidase positive, coagulase-positive Staphylococci, *Listeria monocytogenes*) were within the legal limits of Reg. (EU) no. 1441/2007 (EU Commission, 2007) (data not shown). Differences among the formulations were observed only for the mesophilic lactic bacteria, which were found in the highest concentration (10^7 ufc/g) in the F3 sample, prepared without any additives, and in concentration of 10^6 ufc/g in all the other formulations, highlighting the possible role of OLE, comparable with that of nitrite and nitrate, in exerting an antimicrobial effect due to the presence of polyphenols (Caponio et al., 2019; Difonzo et al., 2022). These outcomes agreed with our preliminary study carried out on a lab-scale (Difonzo et al., 2022), as well as with studies aimed at substituting synthetic additives with green tea and grape seed polyphenols (Li, Shao, Zhu, Zhou, & Xu, 2013) or with natural extracts of fruits and vegetables (Martínez-Zamora, Peñalver, Ros, & Nieto, 2021b).

3.2. Weight loss, moisture content, water activity and pH determination

The results of weight loss, moisture content, water activity (a_w) and pH determination are summarized in Table 2. Regarding moisture and a_w, the F2, F4 and F5 samples, all containing nitrite and nitrate, showed significantly lower values than F1 (with OLE alone) and F3 (without any additives). No significant differences emerged among F2 and F5 samples, containing both types of additives at different doses. This could be due to the osmotic dehydration induced by nitrite and nitrate (Martín-Sánchez et al., 2014). These outcomes also agree with the weight loss during the ripening period: these parameters are indeed known to be correlated (Puolanne & Petäjä-Kanninen, 2014). In fact, the F1 and F3 samples showed significantly lower values of weight loss than the

Table 2
Mean value, standard deviation and results of the statistical analysis (One-Way ANOVA) of the weight loss (%), moisture (%), pH and a_w of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	Moisture (%)	a_w	pH	Weight loss (%)
F1	1000	0	29.85 ± 0.16 a	0.85 ± 0.01 a	6.04 ± 0.02 c	41.61 ± 0.42 b
F2	1000	75–75	24.60 ± 0.27 b	0.83 ± 0.00 b	6.04 ± 0.03 c	48.56 ± 0.44 a
F3	0	0	30.51 ± 0.50 a	0.86 ± 0.00 a	6.13 ± 0.02 b	42.03 ± 1.04 b
F4	0	75–75	22.46 ± 0.38 c	0.83 ± 0.00 b	6.19 ± 0.01 a	48.08 ± 1.18 a
F5	500	35–35	23.88 ± 0.89 b	0.82 ± 0.01 b	6.02 ± 0.01 c	47.85 ± 1.00 a

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate. Different letters denote significant differences at $P < 0.05$ among the formulations.

samples with nitrite and nitrate, confirming lower loss of water during the ripening period. In line with other similar studies (Tang et al., 2021; Sarıçoban & Unal, 2022), the presence of the OLE in the F1, F2, and F5 samples resulted in significantly lower pH values than F3 (OLE: 0 mg/kg; NO_2–NO_3: 0 mg/kg) and F4 (OLE: 0 mg/kg; NO_2–NO_3: 75-75 mg/kg). This could be due to the increased availability of fermentable carbohydrates present in the OLE (glucose: 55.83 ± 2.38 mg/g; fructose: 32.52 ± 2.26 mg/g) (Flamminii et al., 2019), which can promote the metabolism of lactic acid bacteria (Pexara, Metaxopoulos, & Drosinos, 2002). Indeed, a study of Pexara et al. (2002) demonstrated that the pH reduction of meat and meat products is linked to an increased availability of fermentable carbohydrates. The decrease of the pH value is very important for ripened meat products as it inhibits growth of undesired bacteria, minimises the rate of colour change, and is responsible for the formation of the desired flavours (Li et al., 2013).

3.3. Lipid and protein oxidation

Considering the TBARs (Fig. 1A), F3, without any type of additives, was the most oxidised, with a mean MDA content of 0.66 mg/kg. Significant differences were observed comparing the F4 sample, containing only nitrite and nitrate (0.37 mg MDA/kg), and the sample F1, with OLE alone (0.29 mg/kg), highlighting the better effect of OLE than nitrate and nitrite. In the F2 and F5 samples, a synergistic effect of the combined use of OLE and nitrite and nitrate, even at reduced doses, was observed. Thus, these results highlight the effectiveness of the OLE, both alone and in combination with nitrite and nitrate, in retarding the oxidation process of ripened sausages. This could be due to the antioxidant effect of OLE, rich in polyphenols, and specifically in oleuropein which could prevent the formation of free radicals by chelating metals, such as copper and iron, which catalyse free radical generation reactions, such as lipid oxidation (Dua, Bhat, & Kumar, 2015). The observed results agreed with data previously reported by other authors (Kurt & Ceylan, 2017; Martínez-Zamora, Peñalver, Ros, & Nieto, 2021a; Zhou et al., 2020), who observed a significant decrease in the lipid oxidation of sausages after the addition of natural antioxidants. A similar trend was also observed for the protein oxidation of ripened sausages (Fig. 1B). F3, without any additives, exhibited the highest value of protein oxidation. The lowest values were found in the F2 and F5 samples (containing both OLE and nitrite and nitrate), highlighting the synergic effect already observed against lipid oxidation. Overall, the presence of OLE, alone and in combination with nitrite and nitrate, protected the proteins from oxidation, since significantly lower content of carbonyl groups were found in samples with OLE compared to the samples without any additives (F3). However, the only addition of the OLE in the F1 sample was not able to ensure a reduction of carbonyl content compared to the F4 sample, with only nitrite and nitrate. These results could be associated with a different antioxidant effect of nitrite and nitrate for protein and lipid oxidation (Berardo et al., 2016; Feng et al., 2016). According to Feng et al. (2016), the presence of nitrite might decrease carbonyl presence by forming active carbonyl-NH_2 group interactions, potentially explaining the reduction in sausage carbonyl content at the end of the ripening period.

3.4. FFA (free fatty acids)

The amount of the main free fatty acids (FFAs) determined in the sausage samples at the end of the ripening period is reported in Table 3. The F2 sample, with both types of additives added at the highest dose, showed the lowest total FFAs content, with a significant difference compared to all the other samples. At the same time, even if without significant differences, F3 (without additives) and F4 (OLE: 0 mg/kg; NO_2–NO_3: 75-75 mg/kg) showed the highest contents of FFAs, followed by, F5 (OLE: 500 mg/kg; NO_2–NO_3: 35-35 mg/kg) and F1 (OLE: 1000 mg/kg; NO_2–NO_3: 0 mg/kg). The most represented FFAs, in decreasing order, were oleic, palmitic, linoleic and stearic acids. A similar FFA profile was observed in other studies for dry-ripened sausages (Fonseca, Gómez, Domínguez, & Lorenzo, 2015; Munekata et al., 2017). Lipolysis in meat products is mainly caused by the endogenous lipases and

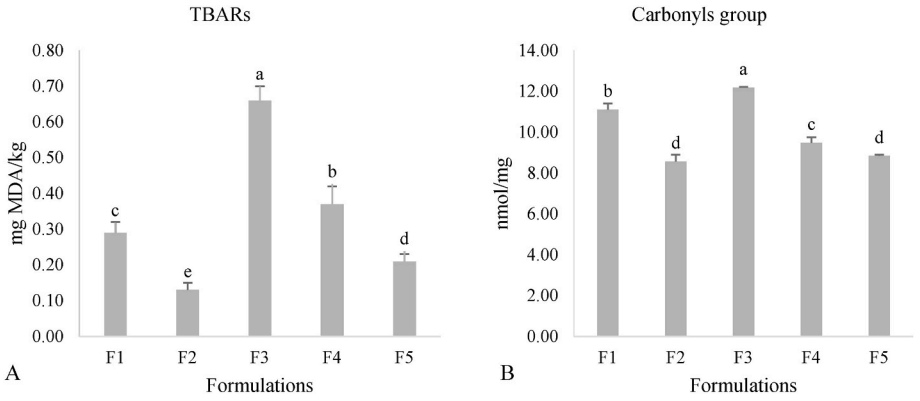


Fig. 1. Mean value, standard deviation and results (One-Way ANOVA) of the statistical analysis of the lipid (A) and protein oxidation (B) of the ripened sausages. F: formulation; F1 (OLE: 1000 mg/kg; NO_2–NO_3: 0 mg/kg), F2 (OLE: 1000 mg/kg; NO_2–NO_3: 75-75 mg/kg), F3 (OLE: 0 mg/kg; NO_2–NO_3: 0 mg/kg), F4 (OLE: 0 mg/kg; NO_2–NO_3: 75-75 mg/kg), F5 (OLE: 500 mg/kg; NO_2–NO_3: 35-35 mg/kg). Different letters denote significant differences at $P < 0.05$.

Table 3
Mean value, standard deviation and results of the statistical analysis (One-Way ANOVA) of the free fatty acids content (g fatty acid/100 g fat) of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	Myristic C14:0	Palmitic C16:0	Palmitoleic C16:1	Stearic C18:0	Oleic C18:1	Linoleic C18:2 n-6	Linolenic C18:3 n-3	SFA	MUFA	PUFA	Total FFAs
F1	1000	0	0.09 ± 0.01a	1.26 ± 0.14a	0.14 ± 0.02a	0.35 ± 0.04a	1.88 ± 0.11c	0.53 ± 0.04b	0.05 ± 0.00a	1.70 ± 0.18a	2.02 ± 0.12c	0.58 ± 0.05 ab	4.31 ± 0.32b
F2	1000	75–75	0.05 ± 0.01b	0.71 ± 0.02b	0.10 ± 0.01b	0.23 ± 0.01b	1.18 ± 0.01d	0.58 ± 0.02a	0.06 ± 0.01a	0.99 ± 0.07b	1.28 ± 0.07d	0.64 ± 0.02a	2.61 ± 0.17c
F3	0	0	0.09 ± 0.00a	1.42 ± 0.04a	0.15 ± 0.01a	0.40 ± 0.00a	2.24 ± 0.09a	0.28 ± 0.01c	0.03 ± 0.00b	1.91 ± 0.05a	2.39 ± 0.09b	0.31 ± 0.02c	4.91 ± 0.16a
F4	0	75–75	0.08 ± 0.00a	1.29 ± 0.02a	0.14 ± 0.01a	0.38 ± 0.01a	2.22 ± 0.35b	0.48 ± 0.02b	0.05 ± 0.01a	1.75 ± 0.03a	2.63 ± 0.10a	0.53 ± 0.02b	4.96 ± 0.18a
F5	500	35–35	0.08 ± 0.00a	1.38 ± 0.02a	0.14 ± 0.01a	0.40 ± 0.03a	2.06 ± 0.02bc	0.52 ± 0.05b	0.05 ± 0.00a	1.87 ± 0.05a	2.20 ± 0.02bc	0.57 ± 0.06 ab	4.60 ± 0.01 ab

OLE: Olive Leaf Extract; NO_2–NO_3: Nitrite and nitrate. Different letters denote significant differences at $P < 0.05$.

phospholipases, which cause the release of FFAs. The lowest amount of FFAs, detected in the F2 sample (containing OLE and nitrite and nitrate at the highest dose) can be explained by the inhibition of lipolytic bacteria as a result of the antimicrobial properties of OLE (Kurt & Ceylan, 2017) and nitrates. The same trend was also observed by Kurt and Ceylan (2017) and Libera, Latoch, and Wójciak (2020), who reported the ability of OLE and grape seed extracts to decrease the FFAs in dry-ripened meat products. Other authors suggested that an increase of FFAs promotes lipid oxidation because free fatty acids oxidise more readily thank the linked ones (Wu, Xiao, Yin, Zhang, & Richards, 2021). The most abundant fatty acids at the end of ripening were monounsaturated fatty acids (MUFA), followed by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA).

3.5. Residual nitrite and nitrate and N-nitrosamines

The content of residual nitrite and nitrate in meat products is crucial for the formation of N-nitrosamines (Xiang et al., 2019). At the end of ripening period, no residues of nitrate and nitrite were found in any of the samples under investigation (data not shown). Therefore, the incorporation of OLE in the formulation of sausages did not influence this parameter. Also, in the sample containing only nitrite and nitrate (F4) no residual content was observed, due to the observance of good manufacturing practices by the company where the sausages were produced. Indeed, processing technology, pH value and the presence of reducing agents are the main factors that affect the level of residual nitrite and nitrate in meat products (Zhang et al., 2023). Table 4 shows the content of N-nitrosamines in the five different sausage formulations, together with the results of the statistical analysis. Five different N-nitrosamines were detected: NMEA (N-nitrosomethylethylamine), NDEA (N-nitrosodiethylamine), NDPA (N-nitrosodi-*n*-propylamine), NDBA (N-nitrosodi-*n*-butylamine), NPYR (N-nitroso-pyrrolidine). These N-nitrosamines were all present in the F4 sample, prepared using only nitrite and nitrate. The F2 and F5 samples, which also contained OLE, did not show NDEA. On the contrary, in the F1 sample (prepared exclusively using OLE), only NDPA, NDBA and NPYR were found, in significantly lower amounts than in F2, F4 and F5. Finally, in F3, without any type of additive, only NPYR was found, in a significantly lower amount compared to all other samples. Unlike other meat products, ripened sausages are particularly susceptible to the formation of N-nitrosamines due to their production process. Indeed, the direct addition of ingredients in raw meat products leads to a higher concentration of nitrosating agents compared to products prepared by dissolving the ingredients in a brine solution. Furthermore, fermentation and dehydration processes promote the obtaining of a meat product with low water activity, reduced pH, and high biogenic amines content, contributing to the formation of N-nitrosamines (De Mey, De Maere, Paelinck, & Fraeye, 2017). Furthermore, the trituration of meat to produce sausages increases the surface area, and consequently enhances contact between nitrosating exogenous agents and amines present in the meat, compared to meat product prepared in whole pieces (Scheeren, Sabik, Gariépy, Terra, & Arul, 2015). However, the presence of the OLE in the formulation of ripened sausages (F1, F2, F5) contributed to a significant decrease of N-nitrosamines compared to F4, containing nitrite and nitrate alone. According to the literature (Karwowska & Kononiuk, 2020; Ribeiro, Silva, & da Silva, 2020) this phenomenon could be due to the presence of phenols in OLE. In fact, it has been stated that phenolic compounds could reduce nitrite and release hydrogen which in turn could react with the free radicals that are involved in the reactions of N-nitrosamines formation (Gao et al., 2022). Among the N-nitrosamines found in our trials, NDEA, found in the F4 sample, containing only nitrite and nitrate, is the most potent carcinogenic and mutagenic nitrosamines found in meat products (Scheeren et al., 2015). Gao et al. (2022) observed that sausages with the addition of tea polyphenols extract presented a significantly lower N-nitrosamines content than the control sample. The addition of rosemary extract, green tea and grape

Table 4
Mean value, standard deviation and results (One-Way ANOVA) of the statistical analysis of the N-nitrosamines content (μg/kg) of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	NMEA (μg/kg)	NDEA (μg/kg)	NDPA (μg/kg)	NDBA (μg/kg)	NPYR (μg/kg)
F1	1000	0	nd	nd	3.28 ± 0.07 a	0.20 ± 0.00 c	4.57 ± 0.01 b
F2	1000	75–75	12.45 ± 0.23 b	nd	2.96 ± 0.01 b	0.22 ± 0.00 b	5.23 ± 0.25 a
F3	0	0	nd	nd	nd	nd	3.90 ± 0.20 c
F4	0	75–75	15.76 ± 0.92 a	0.92 ± 0.01	3.00 ± 0.01 b	0.26 ± 0.01 a	5.33 ± 0.20 a
F5	500	35–35	10.75 ± 0.14 c	nd	2.92 ± 0.01 b	0.22 ± 0.00 b	5.13 ± 0.09 a

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate; nd, not detected; NMEA, N-nitrosomethylethylamine; NDEA, N-nitrosodiethylamine; NDPA, N-nitrosodi-*n*-propylamine; NDBA, N-nitrosodi-*n*-butylamine; NPYR, N-nitrosopyrrolidine. Different letters denote significant differences at $P < 0.05$.

seeds also reduced the amount of N-nitrosamines in sausage samples compared to the control sample (Zhou et al., 2020). However, although no nitrite and nitrate were added to F1 and F3, the presence of N-nitrosamines in these samples could be due to the naturally occurrence of nitrite and nitrate in the extract (Flores & Toldrá, 2021) and to contaminations during the production process (De Mey et al., 2017). The presence of N-nitrosamines in elastomer or rubber teats and soothers, in cosmetic products and in toys is regulated by the European Union. However, there is no available EU legislation regulating the presence of N-nitrosamines in food or drinking water (Schrenk et al., 2023). In our study, the F4 sample, prepared with nitrite and nitrate alone, showed the highest content of total N-nitrosamines, equal to 25.27 μg/kg, followed by F2 and F5 (20.86 and 19.02 μg/kg, respectively), containing both OLE and nitrite and nitrate. Lower values were found in F1 (8.05 μg/kg) and F3 (3.90 μg/kg), containing respectively, OLE alone and no additives. These results fitted with the maximum N-nitrosamines level allowed for cured meat and meat products in the USA, equal to 10 μg/kg (United States Department of Agriculture, 2023), and in Chile, equal to 30 μg/kg (Ministerio de Salud, Republica de Chile MINSAL, 1997). In addition, the contents of N-nitrosamines found were similar to those reported after 28 days of ripening in dry-cured sausages with plant polyphenols and ascorbic acid (Li et al., 2013) and in western style smoked sausages with rosemary extract, grape seed extract and green tea polyphenols (Zhou et al., 2020).

3.6. Colour parameters

Table 5 shows the results of colour analysis (lightness L*, redness a*, yellowness b*) of the sausages at the end of the ripening period. The addition of nitrite and nitrate positively influenced the colour parameters. As known, they determine a brilliant red by the formation of nitroso-myoglobin (Ozaki et al., 2021). As can be seen in Table 5, the lowest value of a* was found in samples F1 and F3 (without nitrites and nitrate), probably due to the lack of nitroso-myoglobin. On the contrary, the highest value was found for the samples F4 and F2, containing the highest doses of nitrite and nitrate. These findings agree with the results of our previous study (Difonzo et al., 2022). Moreover, also Bologna sausage (Djeri & Williams, 2014) and cold-smoked sausages (Eisinaitė, Tamkutė, Vinauskienė, & Leskauskaitė, 2020) enriched with natural antioxidants showed lower a* index compared to control samples containing sodium nitrite.

Considering yellowness, the samples containing OLE, F1, F2 and F5 showed lower values compared to F4, containing only nitrite and nitrate, even if significant differences emerged only by comparing F2 and F5 *vs* F4. The yellowness parameter is associated with oxidation processes of the lipid fraction, in fact an increase of this parameter is linked to a more oxidised lipid fraction (Wang, Tu, Zhou, Lu, & Xu, 2021). In our study, the highest b* value was observed in F3, prepared without any additives, that shown the highest level of oxidation degree of the lipid fraction (Fig. 1A). Moreover, all the differences observed among the formulations could be linked with the different degree of lipid oxidation, in fact a significant correlation $p < 0.05$ between the parameters b* and TBARs has been found (data not shown). The presence of OLE may have limited the increase of b* index, thanks to its antioxidant effect. The increase of b* value in meat is related to the non-enzymatic browning reactions between lipid oxidation products and the protein's or phospholipid's amines (Wang et al., 2021). The observed values of yellowness agreed with those found by other authors for sausages prepared with natural antioxidants (Martínez-Zamora et al., 2021a; Çağlak, Kobya, Öğretmen, Karslı, & Kara, 2022).

Lightness (L*) is highly related to the moisture content, thus lighter-coloured meat products are associated with higher moisture content (Fernández-López et al., 2019). The trend found for lightness values, indeed, agreed with the moisture content reported in Table 2. F1 (UR% = 29.85%) and F3 (UR% = 30.51%) samples, with the highest moisture content, also showed significantly higher values for L*. On the contrary, F4 showed the lowest UR% and L* values. In addition, no significant differences emerged between F2 and F5, containing both additives at different concentration.

Table 5
Mean value, standard deviation and results of the statistical analysis (One-Way ANOVA) of lightness (L*), redness (a*) and yellowness (b*) of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	Lightness (L*)	Redness (a*)	Yellowness (b*)
F1	1000	0	36.61 ± 0.24 a	6.43 ± 0.69 d	6.85 ± 0.42 bc
F2	1000	75–75	34.18 ± 0.42 b	8.07 ± 0.18 b	6.64 ± 0.27 c
F3	0	0	36.37 ± 0.07 a	5.74 ± 0.16 d	8.10 ± 0.92 a
F4	0	75–75	33.59 ± 0.29 c	8.78 ± 1.39 a	7.65 ± 0.64 b
F5	500	35–35	34.68 ± 0.09 b	6.87 ± 1.05 c	6.63 ± 0.81 c

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate. Different letters denote significant differences at $P < 0.05$.

3.7. Texture profile analysis (TPA)

The TPA results for sausages at the end of ripening period are summarized in Table 6. Regarding hardness, the F4 sample, containing only nitrite and nitrate, showed the highest value, with significant differences compared to all the other samples except for F2. On the contrary, F1 (exclusively containing OLE) and F3 (without any additives), were significantly less hard. This outcome is in line with our previous study (Difonzo et al., 2022). In general, hardness is related to moisture content and weight loss (Ekici et al., 2015). The shrinkage occurring during ripening promotes a closer contact between proteins and the formation of new interactions, leading to increased hardness (Li, Zhuang, Qiao, Zhang, & Wang, 2016). Indeed, F1 and F3 showed the highest values of moisture content (F1 = 29.85%; F3 = 30.51%), while F4 the lowest (22.46%). Regarding springiness and chewiness no significant differences emerged by comparing samples containing OLE alone (F1) and in combination with nitrite and nitrate (F2, F5). A similar trend was also found for cohesiveness, although F4 and F5 showed a significantly lower value compared to F1 and F2. Furthermore, for all these parameters, the F3 sample (without any type of additive) showed the lowest values compared to the other samples. These outcomes could be related to the different level of protein oxidation, in fact as reported in Fig. 1B, F3 was the sample with the highest content of carbonyl groups. The formation of crosslinks between oxidised proteins may affect the texture of ripened sausages, in particular with respect to gelation (Berardo et al., 2016). Zhou, Zhao, Zhao, Sun, and Cui (2014) demonstrated that the textural parameters decreased with the increase of oxidation since the protein carbonylation leads to fast and severe reduction of water-holding and gelling capacities.

3.8. Sensory analysis

Table 7 shows the results of the sensory analysis carried out on the sausages at the end of the ripening period. Considering the intensity of colour, no significant differences were observed among the samples (Table 7). Thus, unlike redness (a*) highlighted differences among the samples (Table 5), the lack of nitroso-myoglobin synthesis, caused by the absence of nitrite and nitrate, was not visually perceivable by the panellists. At the same time, the F3 sample showed a significantly lower score of the typical odour compared to the other samples. This could be due to the absence of nitrite and nitrate, which generally contribute to the development and maintenance of the characteristic odour of this category of meat products (Perea-Sanz, Montero, Belloch, & Flores,

Table 6
Mean value, standard deviation and results of the statistical analysis (One-Way ANOVA) of hardness, springiness, chewiness and cohesiveness of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	Hardness (N)	Springiness	Chewiness (N)	Cohesiveness (N)
F1	1000	0	75.89 ± 2.59 c	0.50 ± 0.02 a	13.88 ± 0.43 a	0.32 ± 0.05 a
F2	1000	75–75	106.20 ± 1.40 ab	0.50 ± 0.00 a	12.74 ± 2.83 a	0.34 ± 0.01 a
F3	0	0	82.00 ± 0.30 c	0.41 ± 0.01 b	10.07 ± 0.70 c	0.21 ± 0.00 c
F4	0	75–75	110.86 ± 3.86 a	0.48 ± 0.01 a	12.09 ± 0.25 b	0.24 ± 0.05 b
F5	500	35–35	99.90 ± 3.93 b	0.50 ± 0.01 a	13.82 ± 0.05 a	0.24 ± 0.00 b

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate. Different letters denote significant differences at $P < 0.05$.

Table 7
Mean value, standard deviation and results (One-Way ANOVA) of the statistical analysis of the sensory analysis of the ripened sausages.

Formulation	OLE (mg/kg)	NO_2–NO_3 (mg/kg)	Colour intensity	Typical odour of ripened sausages	Rancid odour	Consistency	Bitterness	Saltiness
F1	1000	0	6.83 ± 0.29 a	7.35 ± 0.13 a	0.58 ± 0.02 c	5.25 ± 0.00 cd	0.87 ± 0.12 a	5.37 ± 0.37 ab
F2	1000	75–75	7.12 ± 0.13 a	7.55 ± 0.05 a	0.37 ± 0.12 d	6.37 ± 0.62 b	0.63 ± 0.12 ab	4.87 ± 0.37 ab
F3	0	0	6.89 ± 0.45 a	5.87 ± 0.38 b	0.87 ± 0.37 a	4.62 ± 0.37 d	0.32 ± 0.11 b	4.50 ± 0.25 b
F4	0	75–75	7.18 ± 0.08 a	7.57 ± 0.02 a	0.71 ± 0.07 b	7.25 ± 0.25 a	0.33 ± 0.07 b	6.25 ± 0.75 a
F5	500	35–35	7.15 ± 0.02 a	7.55 ± 0.12 a	0.48 ± 0.03cd	5.87 ± 0.37 c	0.45 ± 0.02 ab	5.12 ± 0.87 ab

OLE, Olive Leaf Extract; NO_2–NO_3, Nitrite and nitrate. Different letters denote significant differences at $P < 0.05$.

2018). The use of OLE, both alone (F1) and in combination with nitrite and nitrate (F2, F5) led to colour and odour similar to those of F4 sample, where only nitrite and nitrate were added. A similar result was also reported by Sucu and Turp (2018) as fermented beef sausages with beetroot extracts were like those of the control samples with only nitrites. In line with the TBARs-test (Fig. 1A), the panellists perceived a rancid odour with a higher score in the sample F3 ($p < 0.05$), followed by the samples F4 and F1. The F2 sample (OLE:1000 mg/kg; NO_2–NO_3: 75-75 mg/kg) showed the lowest score for rancid odour ($p < 0.05$). The 50% reduction of the dose of OLE and nitrite and nitrate (F5) did not cause a significant variation of this sensorial parameter. A similar trend was reported by Nieto, Martínez, Castillo, and Ros (2017). In fact, sensory analysis revealed that sausages processed with olive oil and nut extracts had a significantly lower rancid odour than control sample, demonstrating that the addition of the natural extracts delayed lipid oxidation (Nieto et al., 2017). Focusing on consistency, the results agreed with those instrumentally obtained by the texture profile analysis, confirming that F4 was the hardest sample. The sensory evaluation of consistency also confirmed that F1 (OLE only) and F3 (no additives) samples were not significantly different. However, the panellists assigned a significantly higher score to the F2 sample (containing both types of additives at the highest dose) compared to F5 (with a 50% reduced dose). As previously discussed, these outcomes could be linked to the different moisture content of the samples. Of particular interest were the scores related to bitterness, as the oleuropein (and OLE in general) is characterised by a clearly perceivable bitter taste. The F1 sample, with 1000 mg/kg of OLE, showed the highest scores for bitterness, while samples F3 and F4, without OLE, had the lowest scores. It must be considered, however, that the bitterness intensity was never very high, being the maximum value observed equal to 0.87 on a 0–9 scale (F1 sample). In fact, Martínez-Zamora et al. (2021a) reported that a purified antioxidant extract without phenolic derivatives from olive did not cause bitter perception in samples of "Fuet", a Spanish-type dry-cured sausage. However, the combination of the OLE with nitrite and nitrate could mask this perception since no significant differences emerged by comparing the samples F2–F5 and the samples F3–F4, which did not contain OLE. Finally, considering saltiness, samples F4 (with only nitrate and nitrite) and F3 (no additives) showed significantly higher and lower value, respectively. At the same time, no significant differences emerged among samples with OLE added, both alone (F1) and in combination with nitrite and nitrate (F2, F5).

4. Conclusion

The results demonstrated the efficacy of OLE in reducing nitrite and nitrate in the preparation of ripened pork sausages at industrial scale. The inclusion of the OLE in the sausage formulation and in combination with highest (F2) a reduced dose of nitrite and nitrate (F5), successfully limited lipid and protein oxidation in the final product, without modifications in terms of textural properties of the finished product. The microbiological safety of sausages was also ensured, accompanied by a significant decrease in N-nitrosamines content. The intensity of red colour and the bitter taste of the sausages were the main parameters negatively affected by the OLE addition. However, also in this case, the use of OLE in combination with the highest (F2) and a reduced amount of nitrite and nitrate (F5) masked these inconveniences. Therefore, this study demonstrates another effective use of OLE, a derivative of the olive leaves discarded during olive oil production, already known as a useful antioxidant in plant-based foods such as baked goods, oils, and in-brine table olives. The findings demonstrate that OLE stands out as a valuable alternative to nitrate and nitrite, demonstrating its effectiveness in the manufacturing of ripened sausages. Its integration into industrial processes underscores its immediate applicability on a large scale, emphasizing the practical feasibility and benefits of repurposing this waste from the agro-industry. However, it is important to note that OLE has not yet obtained official authorisations a food additive. Therefore, in the prospect of a possible authorisation of OLE as novel food additive, this study, together with many other studies that tested the use of OLE in different food formulations, provides valuable data available for companies and food safety authorities to evaluate the effect of this new additives in foods.

CRediT authorship contribution statement

Michela Pia Totaro: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Graziana Difonzo:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Antonella Pasqualone:** Writing – review & editing, Methodology. **Carmine Summo:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to Francesco Carriero of "Salumi Martina Franca S.r.l. (Martina Franca, Italy)" for the execution of the production trials and the availability of raw meats, ingredients and equipment. This work was supported by POR Puglia 2014/2020-Asse X-Azione 10.4 Research for Innovation- REFIN code n. E65BAEEE.

References

Al-Kaseem, M., Al-Assaf, Z., & Karabeet, F. (2014). A rapid, validated RP-HPLC method for the determination of seven volatile N-Nitrosamines in meat. *Pharmacology & Pharmacy, 5*, 298–308. https://doi.org/10.4236/pp.2014.53037

Baldini, M., Fabietti, F., Giammarioli, S., Onori, R., Orefice, L., & Stacchini, A. (1996). *Metodi di Analisi Utilizzati per il Controllo Chimico Degli Alimenti—Rapporti ISTISAN 96/34*. Rome, Italy: Istituto Superiore di Sanità (ISS).

Berardo, A., De Maere, H., Stavropoulou, D. A., Rysman, T., Leroy, F., & De Smet, S. (2016). Effect of sodium ascorbate and sodium nitrite on protein and lipid oxidation in dry fermented sausages. *Meat Science, 121*, 359–364. https://doi.org/10.1016/j.meatsci.2016.07.003

Çağlak, E., Kobya, O., Öğretmen, Ö. Y., Karslı, B., & Kara, B. (2022). Effect of pomegranate peel extract as a natural antioxidant on sausage production from rainbow trout fillet waste. *Food Bulletin, 1*, 11–15. https://doi.org/10.29329/foodb.2022.495.03

Caponio, F., Difonzo, G., Calasso, M., Cosmai, L., & De Angelis, M. (2019). Effects of olive leaf extract addition on fermentative and oxidative processes of table olives and their nutritional properties. *Food Research International, 116*, 1306–1317. https://doi.org/10.1016/j.foodres.2018.10.020

Cintya, H., Silalahi, J., Putra, E. D. L., & Siburian, R. (2019). Analysis of nitrosamines in processed meat products in medan city by liquid chromatography-mass spectrometry. *Open Access Macedonian Journal of Medical Sciences, 7*, 1382. https://doi.org/10.3889/oamjms.2019.261

De Mey, E., De Maere, H., Paelinck, H., & Fraeye, I. (2017). Volatile N-nitrosamines in meat products: Potential precursors, influence of processing, and mitigation strategies. *Critical Reviews in Food Science and Nutrition, 57*, 2909–2923. https://doi.org/10.1080/10408398.2015.1078769

Difonzo, G., Totaro, M. P., Caponio, F., Pasqualone, A., & Summo, C. (2022). Olive leaf extract (OLE) addition as tool to reduce nitrate and nitrite in ripened sausages. *Foods, 11*, 451. https://doi.org/10.3390/foods11030451

Djeri, N., & Williams, S. K. (2014). Celery juice powder used as nitrite substitute in sliced vacuum-packaged Turkey bologna stored at 4°C for 10 weeks under retail display light. *Journal of Food Quality, 37*, 361–370. https://doi.org/10.1111/jfq.12102

Dua, S., Bhat, Z. F., & Kumar, S. (2015). Effect of oleuropein on the oxidative stability and storage quality of Tabaq-Maz, fried mutton ribs. *Food Bioscience, 12*, 84–92. https://doi.org/10.1016/j.fbio.2015.08.002

Eisinaitė, V., Tamkutė, L., Vinauskienė, R., & Leskauskaitė, D. (2020). Freeze-dried celery as an indirect source of nitrate in cold-smoked sausages: Effect on safety and color formation. *Lebensmittel-Wissenschaft und -Technologie, 129*, Article 109586. https://doi.org/10.1016/j.lwt.2020.109586

Ekici, L., Ozturk, I., Karaman, S., Caliskan, O., Tornuk, F., Sagdic, O., et al. (2015). Effects of black carrot concentrate on some physicochemical, textural, bioactive, aroma and sensory properties of sucuk, a traditional Turkish dry-fermented sausage. *LWT–Food Science and Technology, 62*, 718–726. https://doi.org/10.1016/j.lwt.2014.12.025

EN 12014-4:2005. (2005). *Foodstuffs—determination of nitrate and/or nitrite content—Part 4: Ion-exchange chromatographic (Ic) method for the determination of nitrate and nitrite content of meat products*. Brussels, Belgium: CEN-CENELEC Management Centre.

2007 EU Commission. (2007). Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union L, 322*, 2–29.

EU Commission. (2023). Commission Regulation (EC) No 2108/2023 of 6 October 2023 amending annex II to regulation (EC) No 1333/2008 of the European parliament and of the council by establishing a union list of food additives. *Official Journal of the European Union L, 295*, 12, 11.

Feng, X., Li, C., Jia, X., Guo, Y., Lei, N., Hackman, R. M., et al. (2016). Influence of sodium nitrite on protein oxidation and nitrosation of sausages subjected to processing and storage. *Meat Science, 116*, 260–267. https://doi.org/10.1016/j.meatsci.2016.01.017

Fernández-López, J., Lucas-González, R., Viuda-Martos, M., Sayas-Barberá, E., Navarro, C., Haros, C. M., et al. (2019). Chia (Salvia hispanica L.) products as ingredients for reformulating frankfurters: Effects on quality properties and shelf-life. *Meat Science, 156*, 139–145. https://doi.org/10.1016/j.meatsci.2019.05.028

Flamminii, F., Di Mattia, C. D., Difonzo, G., Neri, L., Faieta, M., Caponio, F., et al. (2019). From by-product to food ingredient: Evaluation of compositional and technological properties of olive-leaf phenolic extracts. *Journal of the Science of Food and Agriculture, 99*, 6620–6627. https://doi.org/10.1002/jsfa.9949

Flores, M., & Toldrá, F. (2021). Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products. *Meat Science, 171*, Article 108272. https://doi.org/10.1016/j.meatsci.2020.108272

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.

Fonseca, S., Gómez, M., Domínguez, R., & Lorenzo, J. M. (2015). Physicochemical and sensory properties of Celta dry-ripened "salchichón" as affected by fat content. *Grasas Y Aceites, 66*. https://doi.org/10.3989/gya.0709142. e059-e059.

Ganhão, R., Morcuende, D., & Estévez, M. (2010). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science, 85*, 402–409. https://doi.org/10.1016/j.meatsci.2010.02.008

Gao, X., Xia, L., Fan, Y., Jin, C., Xiong, G., Hao, X., et al. (2022). Evaluation of coloration, nitrite residue and antioxidant capacity of theaflavins, tea polyphenols in cured sausage. *Meat Science, 192*, Article 108877. https://doi.org/10.1016/j.meatsci.2022.108877

Karwowska, M., & Kononiuk, A. (2020). Nitrates/nitrites in food—risk for nitrosative stress and benefits. *Antioxidants, 9*, 241. https://doi.org/10.3390/antiox9030241

Karwowska, M., Stadnik, J., Stasiak, D. M., Wójciak, K., & Lorenzo, J. M. (2021). Strategies to improve the nutritional value of meat products: Incorporation of bioactive compounds, reduction or elimination of harmful components and alternative technologies. *International Journal of Food Science and Technology, 56*, 6142–6156. https://doi.org/10.1111/ijfs.15060

Khemakhem, I., Fuentes, A., Lerma-García, M. J., Ayadi, M. A., Bouaziz, M., & Barat, J. M. (2019). Olive leaf extracts for shelf-life extension of salmon burgers. *Food Science and Technology International, 25*, 91–100. https://doi.org/10.1177/1082013218795816

Kurt, Ş., & Ceylan, H. G. (2017). Effects of olive leaf extract on the oxidation stability and microbiological quality of dry fermented sausage (sucuk). *Carpathian Journal of Food Science & Technology, 9*.

Li, L., Shao, J., Zhu, X., Zhou, G., & Xu, X. (2013). Effect of plant polyphenols and ascorbic acid on lipid oxidation, residual nitrite and n - nitrosamines formation in dry - cured sausage. *International Journal of Food Science and Technology, 48*, 1157–1164. https://doi.org/10.1111/ijfs.12069

Li, F., Zhuang, H., Qiao, W., Zhang, J., & Wang, Y. (2016). Effect of partial substitution of NaCl by KCl on physicochemical properties, biogenic amines and N-nitrosamines during ripening and storage of dry-cured bacon. *Journal of Food Science and Technology, 53*, 3795–3805. https://doi.org/10.1007/s13197-016-2366-x

Libera, J., Latoch, A., & Wójciak, K. M. (2020). Utilization of grape seed extract as a natural antioxidant in the technology of meat products inoculated with a probiotic strain of LAB. *Foods, 9*, 103. https://doi.org/10.3390/foods9010103

Lorenzo, J. M., Cittadini, A., Bermúdez, R., Munekata, P. E., & Domínguez, R. (2015). Influence of partial replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ on proteolysis, lipolysis and sensory properties during the manufacture of dry-cured lacón. *Food Control, 55*, 90–96. https://doi.org/10.1016/j.foodcont.2015.02.035

Martín-Sánchez, A. M., Ciro-Gómez, G., Vilella-Esplá, J., Ben-Abda, J., Pérez-Álvarez, J.Á., & Sayas-Barberá, E. (2014). Influence of fresh date palm co-products on the ripening of a paprika added dry-cured sausage model system. *Meat Science, 97*, 130–136. https://doi.org/10.1016/j.meatsci.2013.12.005

Martínez-Zamora, L., Peñalver, R., Ros, G., & Nieto, G. (2021a). Innovative natural functional ingredients from olive and citrus extracts in Spanish-type dry-cured sausage "fuet". *Antioxidants, 10*, 180. https://doi.org/10.3390/antiox10020180

Martínez-Zamora, L., Peñalver, R., Ros, G., & Nieto, G. (2021b). Substitution of synthetic nitrates and antioxidants by spices, fruits and vegetables in Clean label Spanish chorizo. *Food Research International, 139*, Article 109835. https://doi.org/10.1016/j.foodres.2020.109835

Ministerio de Salud, Republica de Chile (MINSAL). (1997). *Reglamento Sanitario de los Alimentos DTO. N○ 977/96. Diário Oficial de la Republica de Chile*. Santiago, Chile: Ministerio de Salud Republica de Chile. Retrieved from https://www.minsal.cl/sites/default/files/files/DECRETO_977_96%20actualizado%20a%20Enero%202015(1).pdf.

Munekata, P. E. S., Domínguez, R., Franco, D., Bermúdez, R., Trindade, M. A., & Lorenzo, J. M. (2017). Effect of natural antioxidants in Spanish salchichón elaborated with encapsulated *n*-3 long chain fatty acids in konjac glucomannan matrix. *Meat Science, 124*, 54–60. https://doi.org/10.1016/j.meatsci.2016.11.002

Nader, M., Hosseininezhad, B., Berizi, E., Mazloomi, S. M., Hosseinzadeh, S., Zare, M., et al. (2022). The residual nitrate and nitrite levels in meat products in Iran: A systematic review, meta-analysis and health risk assessment. *Environmental Research, 207*. https://doi.org/10.1016/j.crfs.2023.100470

Nieto, G., Martínez, L., Castillo, J., & Ros, G. (2017). Hydroxytyrosol extracts, olive oil and walnuts as functional components in chicken sausages. *Journal of the Science of Food and Agriculture, 97*, 3761–3771. https://doi.org/10.1002/jsfa.8240

Ozaki, M. M., Dos Santos, M., Ribeiro, W. O., de Azambuja Ferreira, N. C., Picone, C. S. F., Domínguez, R., et al. (2021). Radish powder and oregano essential oil as nitrite substitutes in fermented cooked sausages. *Food Research International, 140*, Article 109855. https://doi.org/10.1016/j.foodres.2020.109855

Parlasca, M. C., & Qaim, M. (2022). Meat consumption and sustainability. *Annual Review of Resource Economics, 14*, 17–41. https://doi.org/10.1146/annurev-resource-111820032340

Pateiro, M., Bermúdez, R., Lorenzo, J. M., & Franco, D. (2015). Effect of addition of natural antioxidants on the shelf-life of "Chorizo", a Spanish dry-cured sausage. *Antioxidants, 4*, 42–67. https://doi.org/10.3390/antiox4010042

Perea-Sanz, L., Montero, R., Belloch, C., & Flores, M. (2018). Nitrate reduction in the fermentation process of salt reduced dry sausages: Impact on microbial and physicochemical parameters and aroma profile. *International Journal of Food Microbiology, 282*, 84–91. https://doi.org/10.1016/j.ijfoodmicro.2018.06.004

Pexara, E. S., Metaxopoulos, J., & Drosinos, E. H. (2002). Evaluation of shelf life of cured, cooked, sliced Turkey fillets and cooked pork sausages - 'piroski'- stored under vacuum and modified atmospheres at +4 and +10 C. *Meat Science, 62*, 33–43. https://doi.org/10.1016/S0309-1740(01)00224-8

Puolanne, E., & Petäjä-Kanninen, E. (2014). Principles of meat fermentation. *Handbook of Fermented Meat and Poultry*, 13–17.

Ribeiro, J. S., Silva, L. K. R., & da Silva, M. V. (2020). Natural antioxidants used in meat products. *Plant Antioxidants and Health*, 1–29. https://doi.org/10.1007/978-3-030-45299-5_10-2

Rosmini, M. R., Perlo, F., Pérez-Alvarez, J. A., Pagán-Moreno, M. J., Gago-Gago, A., López-Santoveña, F., et al. (1996). TBA test by an extractive method applied to 'paté'. *Meat Science, 42*, 103–110.

Sarıçoban, C., & Unal, K. (2022). Influence of pre-treated bitter orange albedo on the physicochemical, textural and sensory properties of fermented sausages (sucuk). *Journal of Food Science and Technology, 59*, 1478–1486. https://doi.org/10.1007/s13197-021-05158-6

Scheeren, M. B., Sabik, H., Gariépy, C., Terra, N. N., & Arul, J. (2015). Determination of N-nitrosamines in processed meats by liquid extraction combined with gas chromatography-methanol chemical ionisation/mass spectrometry. *Food Additives & Contaminants: Part A, 32*, 1436–1447. https://doi.org/10.1080/19440049.2015.1066037

EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., del Mazo, J., & Grasl-Kraupp, B. (2023). Risk assessment of N-nitrosamines in food. *EFSA Journal, 21*, Article e07884. https://doi.org/10.2903/j.efsa.2023.7884

Sharma, M., Usmani, Z., Gupta, V. K., & Bhat, R. (2021). Valorization of fruits and vegetable wastes and by-products to produce natural pigments. *Critical Reviews in Biotechnology, 41*, 535–563. https://doi.org/10.1080/07388551.2021.1873240

Shpaizer, A., Nussinovich, A., Kanner, J., & Tirosh, O. (2018). S-nitroso-N-acetylcysteine generates less carcinogenic N-nitrosamines in meat products than nitrite. *Journal of Agricultural and Food Chemistry, 66*, 11459–11467. https://doi.org/10.1021/acs.jafc.8b04549

Sucu, C., & Turp, G. Y. (2018). The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative. *Meat Science, 140*, 158–166. https://doi.org/10.1016/j.meatsci.2018.03.012

Tang, R., Peng, J., Chen, L., Liu, D., Wang, W., & Guo, X. (2021). Combination of Flos Sophorae and chili pepper as a nitrite alternative improves the antioxidant, microbial communities and quality traits in Chinese sausages. *Food Research International, 141*, Article 110131. https://doi.org/10.1016/j.foodres.2021.110131

Testa, B., Lombardi, S. J., Macciola, E., Succi, M., Tremonte, P., & Iorizzo, M. (2019). Efficacy of olive leaf extract (*Olea europaea* L. cv Gentile di Larino) in marinated anchovies (*Engraulis encrasicolus*, L.) process. *Heliyon, 5*. https://doi.org/10.1016/j.heliyon.2019.e01727

Tian, Y., Du, H., Wang, L., Li, S., Zhang, L., & Zhang, L. (2020). Nitrite scavenging and inhibition of N-Nitrosamines formation by phenolic extracts from *Diospyros lotus* L. leaves and active ingredients. *Natural Product Communications, 15*. https://doi.org/10.1177/1934578X20961186, 1934578X20961186.

Turner, N. D., & Lloyd, S. K. (2017). Association between red meat consumption and colon cancer: A systematic review of experimental results. *Experimental Biology and Medicine, 242*, 813–839. https://doi.org/10.1177/1535370217693117

United States Department of Agriculture (USDA). Food Safety and Inspection Service, USDA § 424.22. Certain Other Permitted Uses. 9 CFR Ch. III. Retrieved from https://www.law.cornell.edu/cfr/text/9/424.22. Accessed August 11, 2023..

Wang, Z., Tu, J., Zhou, H., Lu, A., & Xu, B. (2021). A comprehensive insight into the effects of microbial spoilage, myoglobin autoxidation, lipid oxidation, and protein oxidation on the discoloration of rabbit meat during retail display. *Meat Science, 172*, Article 108359. https://doi.org/10.1016/j.meatsci.2020.108359

Wu, H., Xiao, S., Yin, J., Zhang, J., & Richards, M. P. (2021). Mechanisms involved in the inhibitory effects of free fatty acids on lipid peroxidation in Turkey muscle. *Food Chemistry, 342*, Article 128333. https://doi.org/10.1016/j.foodchem.2020.128333

Xiang, R., Cheng, J., Zhu, M., & Liu, X. (2019). Effect of mulberry (*Morus alba*) polyphenols as antioxidant on physiochemical properties, oxidation and bio-safety in Cantonese sausages. *Lwt, 116*, Article 108504. https://doi.org/10.1016/j.lwt.2019.108504

Zhang, Y., Zhang, Y., Jia, J., Peng, H., Qian, Q., Pan, Z., et al. (2023). Nitrite and nitrate in meat processing: Functions and alternatives. *Current Research in Food Science.* , Article 100470. https://doi.org/10.1016/j.crfs.2023.100470

Zhou, Y., Wang, Q., & Wang, S. (2020). Effects of rosemary extract, grape seed extract and green tea polyphenol on the formation of N-nitrosamines and quality of western-style smoked sausage. *Journal of Food Processing and Preservation, 44*, Article e14459. https://doi.org/10.1111/jfpp.14459

Zhou, F., Zhao, M., Zhao, H., Sun, W., & Cui, C. (2014). Effects of oxidative modification on gel properties of isolated porcine myofibrillar protein by peroxyl radicals. *Meat Science, 96*, 1432–1439. https://doi.org/10.1016/j.meatsci.2013.12.001