

# The Effect of a Combination of Nitrite with Lactate, and Sorbate on Physical, Chemical, Antioxidant and Sensory Properties of Beef Mortadella during Storage

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Received on 8/6/2022 and Accepted for Publication on 22/11/2022.

## ABSTRACT

This study aimed to evaluate the effect of a combination of nitrite with lactate and/or sorbate on the physical, chemical antioxidant, and sensory properties of beef mortadella during storage. Five treatments (Tr1, Tr2, Tr3, Tr4, and Tr5) of beef mortadella were prepared and stored at 4°C. Tr1 and Tr2 with 120ppm and 60ppm of nitrite (NaNO<sub>2</sub>) respectively, were controls. Tr3 was prepared with the addition of Na-nitrite and Na-lactate, Tr4 with Na-nitrite and K-sorbate, and Tr5 with a combination of all of them. Samples were tested for chemical composition, pH, hunter color, thiobarbituric acid values (TBA), residual nitrite concentration, and sensory characteristics during storage. Additionally, the residual nitrite content of 12 different meat products was estimated. Nitrite had a slight effect on the chemical composition. At the end of storage, TBA values did not exceed 2mg/Kg malondialdehyde in all treatments. However, Tr2 and Tr5 had lower TBA values. The highest pH value recorded was for Tr5 (6.33). A sharp decrease in nitrite level was found in all treatments. During storage, Tr3, Tr4, and Tr5 were more stable in terms of color than the control samples. Treatments with 60ppm nitrite only, or combined with an organic acid, were well-accepted from a sensorial aspect. Flavor and aroma scores were statistically similar with ratings between 5.89-6.47/9. Nitrite reduction in meat products is considered a markedly healthier practice.

**Keywords:** Beef Mortadella, Nitrite Level, Sodium Lactate, Potassium Sorbate, Antioxidant Activity, Sensory Properties, Chemical Composition.

## INTRODUCTION

The prevailing diets of industrialized countries consist of nutritionally poor foods (Viuda-Martos *et al.*, 2010). In the meat industry, manufacturers are continuously developing new technologies to improve the quality and productivity of their products (Yang *et al.*, 2021). All emulsified food products, such as meat products, deserve to be highlighted, as they are one of the main protein sources for human nutrition (Lise *et al.*, 2021; Horita *et al.*, 2011).

Mortadella is one of the most consumed cooked sausages by the world population and can be made from the meat of various species (do Santos Junior *et al.*, 2020). In developing countries including Jordan, the average per capita consumption of sausage (mortadella) is 31.1kg/person per year, while globally, the consumption is 42.1kg/person per year (FAO, 2008).

Mortadella is a popular meat product in Jordan and neighboring countries because of its pleasant taste and texture, high nutritional value, and ease of incorporation into sandwiches (Abdullah, 2004). Mortadella is an emulsified meat product that is developed by chopping or comminuting one or more types of meat, which is

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seasoned and cured with sodium nitrite and other ingredients such as soya protein, starch, salt, phosphate, ascorbate, ice water, and spices (Jordanian Standard JS: 816/2008). Mortadella is usually sold as cylindrical rolls in casing or sliced and vacuum-packed (Abdullah, 2004).

It is critical to keep the product safe throughout the food chain, this is because any chemical, physical, or microbial substance can reduce its safety or quality and thus lead to food spoilage (Sharma *et al.*, 2020). Therefore, using techniques to ensure the quality and safety of meat products during processing is of great importance (Cichoski *et al.*, 2021). Sodium nitrite is most commonly used in the curing of meat (Honikel, 2008). Nitrite is a multifunctional food additive used in the meat processing industry (Wójciak *et al.*, 2019). The main roles of nitrite in meat products include color fixation, flavor enhancement, antioxidant activity, and antimicrobial (*Clostridium botulinum*) preservation. Therefore, all attempts remained unsuccessful in identifying an effective single replacement material possessing all the properties of nitrite (Sindelar & Milkowski, 2011). Nitrite breakdown into nitric oxide (NO) can occur in several stages, which is then reliably combined with the heme pigment (myoglobin) in the meat, allowing for the maintenance of a stable pink color in cooked meat products. The most important factors influencing meat color are myoglobin content and chemical state (Lee *et al.*, 2021).

On one hand, a previous study found that nitrite should be strictly used due to its potential negative impact on human health (Wójciak *et al.*, 2019), and due to the potential interaction of nitrite and amines to produce nitrosamines (Abdullah, 2004), which have potential mutagenic and carcinogenic effects (e.g. leukemia in children) on human health (Al Marazeeq *et al.*, 2015). On the other hand, some studies found that nitrite has beneficial effects on human health (Wójciak *et al.*, 2019) such as lowering blood pressure and enhancing cardiovascular function, in addition to playing an important role in vasoregulation when used at low doses (Govari *et al.*, 2015).

Cassens (1995) suggested two alternatives to control the health problems related to nitrite: the use of agents that partially or completely replace nitrite or agents that inhibit the formation of nitrosamines in products containing conventional nitrite concentrations.

Organic acids, such as lactate and sorbate, had many different applications in a wide variety of foods for several years (Kim *et al.*, 2006). Lactate serves as an antioxidant agent and has been shown to improve fresh meat color stability (Alahakoon *et al.*, 2015). Sorbate has been widely used as a preservative ingredient in various food products around the world over the last 30 years (Sofos & Busta, 1981). It is currently receiving increased attention as a potential nitrite replacer for botulism control in processed meat products (Sofos & Busta, 1981). Previous research, however, suggested that sorbate could be used as a selective agent for clostridia in laboratory media (Sofos & Busta, 1981). Potassium sorbate is generally recognized as safe (GRAS), and it has relatively low toxicity in humans (Hoang & Vu, 2016). Additionally, lactate was permitted by the USDA-Food Safety and Inspection Service (USDA-FSIS).

The objectives of this study were to evaluate the effect of the combination of nitrite with lactate, sorbate, or lactate-sorbate on the physical, chemical, shelf-life, and sensory properties of low-nitrite beef mortadella during storage, as well as to study the synergistic effect of the additives. In addition, to examine nitrite concentration in selected cured meat products from the Jordanian market.

## Materials and Methods

### Experiment 1: Assessment of Nitrite Level in Some Processed Meat Products

This test was conducted for comparative purposes to quantify the nitrite level differences among the market's meat products and we subsequently tested experimental samples. Four types of cured products (salami, chicken, beef mortadella, and corned beef) were collected from a local market in Amman, Jordan, and analyzed for residual nitrite (AOAC, 2000). Three samples from each type of product were collected from three different manufacturers and brands from the Amman area. The production date of

salami, chicken, and beef mortadella was 21/2/2021, and the expiration date was 24/2/2021. For corned beef, the date of production was 20/3/2018, and the expiration ranges between 2021 and 2024. These products were from Siniora and Nabil products.

### **Experiment 2: Mortadella Manufacture**

Mortadella was prepared at a local meat factory (Siniora Factory Food Industries Co. Ltd. in Sahab Industrial City, Jordan) as described by Abdullah (2004). The prepared mortadella composition was as follows: for beef mortadella, 25 kg of frozen beef meat was used for each treatment. Frozen meat was tempered at 2°C for 24 hours before processing. The tempered meat was coarsely ground, chopped, and blended in two stages: first, ground meat was blended in a bowl chopper (Alpina, Geneva, Switzerland) at low speed for 2 min at a temperature of 0°C. Sodium chloride (NaCl: 0.375kg), ascorbate (0.0125kg), sodium tripolyphosphate (0.0125kg), and ice-water (2.5kg) (KMC, Denmark) were added and blended for 2 min at high speed (cutter size 150 L is 2200rpm, Siniora company, Jordan), at which stage the temperature of the mixture reached -2°C. Finally, starch (0.75kg; KMC, Denmark), soya (0.5kg) (KMC, Denmark), and spices (0.25kg; Gewurze country, Germany) (All these ingredients belong to the allowed range of food additives, and were selected from the usual types used in the meat industry) were added and the mixture and blended for 3 min at low speed (2200rpm; Siniora company, Jordan). Afterward, the temperature of the final meat blend was 8°C. Immediately after chopping, the batter was loaded into a trolley and divided into five batches of 25 kg.

Formulations of the five mortadella treatments were as follows: Treatment 1 (Tr1) and 2 (Tr2) were controlled, with the addition of 120 and 60 ppm of sodium nitrite, respectively, and treatment 3 (Tr3) was formulated with the addition of 60ppm sodium nitrite and 2% sodium lactate, while, treatment 4 (Tr4) was made with the addition of 60ppm sodium nitrite and 1500ppm potassium sorbate, and finally, treatment 5 (Tr5) included 60ppm sodium nitrite, 2% sodium lactate, and 1500 ppm potassium sorbate.

Then, the treatments were transferred to the filling machine (Handtmann, Biberach, Germany), loaded into the funnel of the machine, and vacuum stuffed into the polyethylene bags. The bags were laminated with polyethylene terephthalate and polyethylene dichloride. This is a high-barrier film with good resistance to permeation by oxygen and water vapor. After stuffing, the casing was closed and sealed with a metal clip. The vacuum was created by a vacuum pump, which constitutes part of the filling machine. Mortadella batches were thermally processed in a steam oven as follows: first, cooked at an oven temperature of 60°C, achieved within the first 3 hrs (internal product temperature 50°C), then the oven temperature was increased to 85°C (within the next 2 hrs). After that, the product temperature was reduced to 55°C using cold water spray in the oven. The mortadella was removed from the oven to temper at room temperature for 2hrs at 20°C, and then refrigerated, achieving a temperature of 4°C within 2 hrs. The cooling regime was designed to achieve a core temperature of 4°C within 6 hours. The cooked mortadella was retained in its original casing and held at 4°C throughout the experiment.

### **Proximate Analysis**

The contents of protein, moisture, fat, ash, and salt of all samples of beef mortadella were determined using the InfraLab NIR meat analyzer (LIMAB UK Ltd Wellington, England). The proximate analysis was conducted immediately after processing.

### **Determination of pH**

The pH values were determined as described by (Al Assoly *et al.*, 2019). Briefly, 10 g of mortadella was blended and combined with 90 ml of distilled water and homogenized in a stomacher for 30 seconds. The homogenate pH was measured in duplicate runs by a pH meter (model 340, Mettler-toledo GmbH, Schwarzenbach, Switzerland).

### **Lipid Oxidation Determination by Thiobarbituric Acid Number (TBARs)**

The extent of lipid oxidation was determined by the thiobarbituric acid reactive substances values (TBARS) method of Faustman *et al.* (1992). Ten grams portions of

the homogeneous sample (mortadella) were combined with 25 ml of 20% trichloroacetic acid (TCA) (Labchem, USA) and 20 ml of warmed distilled water and homogenized using a stomacher (Model AES, France, Laboratory) for 2 minutes. The homogenate was filtered through Whatman No.1 filter paper and 2 ml of the filtrate was combined with 2 ml of 0.02 M aqueous 2-thiobarbituric acid (TBA; Labchem, USA) in a test tube. The tubes were then incubated at 22°C in the dark for 20 hours. Measurements of lipid oxidation were made during 12 weeks of refrigerated storage at 4°C in triplicates. The absorbance was measured at 532 nm using a Spectrophotometer (Elico, SL 150, India). The TBARS number was expressed as mg of malondialdehyde per kg of sample and calculated as follows:

TBA value = Abs. (532)  $\times$  7.8 (7.8 is a constant conversion factor)

#### **Determination of Residual Nitrite in Mortadella**

The nitrite content of the mortadella was determined according to the AOAC method No. 973.31 (AOAC, 2000). Briefly, 5 g of the prepared sample was mixed in a 50 ml beaker by a glass rod with 40 ml heated distilled water (80 °C), and then the volume was completed to 300 ml in a 500 ml volumetric flask. Then, the flask was transferred to a steam bath and let stand for 2 hrs, then it was cooled to room temperature and the volume was diluted with distilled water and filtered through a filter paper. An aliquot of 10 ml sample filtrate was transferred to a 50 ml volumetric flask. Then sulfanilamide (Sigma-Aldrich, USA) reagent (0.5 g sulfanilamide in 150 ml 15% (v/v) acetic acid) was added and mixed. After 5 minutes, 2.5 ml of NED (0.2g N-(1-naphthyl) ethylenediamine; Sigma-Aldrich, USA) reagent in 150 ml 15% (v/v) acetic acid was added and then diluted to the volume, mixed and held 15 minutes for color development. Absorbance was read at 540 nm using a UV/Visible Spectrophotometer against a blank of 45 ml water, 2.5 ml sulfanilamide reagent, and 2.5 ml NED reagent. The concentrations of 10, 20, 30, 40, and 50 ppm of sodium nitrite were used as a standard for establishing the calibration curve.

#### **Color Determination**

Color measurements of the prepared mortadella samples ( $L^*$ ,  $a^*$ , and  $b^*$  values) were measured at room temperature (15-20°C) in duplicate runs using a colorimeter Hunter Lab Colour Flex (Chroma Meter, CR-400, Konica Minolta, Sensing, INC., Japan) according to the method described by Maskan (2001).

#### **Sensory Evaluation**

The sensory evaluation was conducted by non-trained panelists from 50 students, teaching staff, and technicians of the Department of Nutrition and Food Technology at the University of Jordan (because they are more experienced than others). The panelists were of both sexes and different ages, they were requested to taste each sample separately without comparing it with other samples. The samples of all formulations were evaluated for desirability in terms of color and appearance, aroma and flavor, texture, juiciness, and overall acceptability using a 9-point hedonic scale test as described by Larmond (1991), varying from 9, which means "like extremely" to 1, which means "dislike extremely". Each sample was coded with a randomly selected three-digit number.

#### **Statistical Analysis**

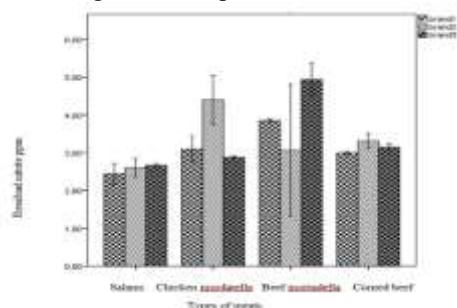
Analysis of variance (ANOVA) using JMP (release 10, SAS Institute, Cary NC) was carried out to determine any significant differences in the parameters of the treatments associated with the developed mortadella properties. The Least significant differences (LSD) at the 5% level of probability were determined to separate differences in the properties among treatments.

#### **Results and Discussion**

##### **Experiment 1: Determination of Residual Nitrite in Selected Cooked Products**

The residual nitrite (ppm) of salami, chicken, beef mortadella, and corned beef randomly selected from a local supermarket in Amman, Jordan, is shown in Figure 1. The results showed that none of the samples had a high residual nitrite, and all different types of meat were relatively close to each other. This agrees well with Lee *et al.* (2021) who revealed that the sodium nitrite content

of commercially processed meat products was low, confirming that these products are safe to eat.



**Figure 1.** Residual nitrite in some selected types of meat products in Jordan.

Data are expressed as means of triplicate determinations  $\pm$  standard deviations (SD)

The samples' low nitrite content could be attributed to nitrite depletion during product storage from the time of manufacture to the time of analysis (Paudel *et al.*, 2021). Furthermore, Oh *et al.* (2015) revealed that the sodium nitrite content of processed meat products decreased over time as a result of processing, distribution, and storage. Additionally, a similar finding was found by Keeton *et al.* (2009) who reported that the residual nitrite amounts in meat products sold in major cities in the United States were found to be 7 ppm in hot dogs, bacon, and ham products, which is an 80% decrease from the 1980s. Also,

Novelli *et al.* (1998) reported a relatively low amount of residual nitrite in commercial mortadella and Salame Milano.

The results are also in agreement with Cassens (1997) who found a low residual nitrite level in some retail packages products purchased from a nearby supermarket. The author explained that the properties of the biologically complex meat matrix, as well as manufacturing conditions, have an impact on the final product. Furthermore, subsequent product changes may occur during storage, distribution, retail display, and final preparation of the cured meat.

### Proximate Analysis

The results of the proximate composition of beef mortadella treatments are displayed in Table 1. Among all treatments, data ranged from 18.36 to 19.15% protein, 0.77 to 1.46% fat, 70.23 to 71.73% moisture, 1.92 to 2.04% salt, and 3.13 to 3.27% ash. These results are considered satisfactory for good quality mortadella in terms of the composition following the relevant Jordanian Standard for Meat and meat products - Sausage products (JS 816:2008 third edition) except for fat content.

**Table 1:** Proximate composition of manufactured beef mortadella prepared with the replacement of nitrites with sodium lactate and potassium sorbate

Treatments	Protein (%)	Fat (%)	Moisture (%)	Salt (%)	Ash (%)
Tr1	18.36 <sup>d</sup> $\pm$ 0.015	1.46 <sup>a</sup> $\pm$ 0.153	70.23 <sup>b</sup> $\pm$ 0.040	2.04 <sup>a</sup> $\pm$ 0.026	3.13 <sup>a</sup> $\pm$ 0.060
Tr2	18.46 <sup>cd</sup> $\pm$ 0.053	0.86 <sup>bc</sup> $\pm$ 0.055	71.65 <sup>a</sup> $\pm$ 0.141	1.92 <sup>a</sup> $\pm$ 0.098	3.14 <sup>a</sup> $\pm$ 0.075
Tr3	18.63 <sup>b</sup> $\pm$ 0.078	1.03 <sup>b</sup> $\pm$ 0.026	71.69 <sup>a</sup> $\pm$ 0.250	1.95 <sup>a</sup> $\pm$ 0.046	3.27 <sup>a</sup> $\pm$ 0.067
Tr4	18.61 <sup>bc</sup> $\pm$ 0.042	0.81 <sup>bc</sup> $\pm$ 0.010	71.52 <sup>a</sup> $\pm$ 0.083	2.00 <sup>a</sup> $\pm$ 0.006	3.14 <sup>a</sup> $\pm$ 0.050
Tr5	19.15 <sup>a</sup> $\pm$ 0.050	0.77 <sup>c</sup> $\pm$ 0.031	71.73 <sup>a</sup> $\pm$ 0.062	1.92 <sup>a</sup> $\pm$ 0.045	3.20 <sup>a</sup> $\pm$ 0.265

Values are expressed as averages of triplicate determinations  $\pm$  SD

Levels not connected by the same letter are significantly different.

Small letters within the same column with different subscript letters have significant differences between treatments using LSD ( $p \leq 0.05$ ).

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2% sodium lactate, Tr4=60 ppm sodium nitrite + 1500ppm potassium sorbate, and Tr5=60 ppm sodium nitrite+ 2% sodium lactate+ 1500ppm potassium sorbate.

Referring to the data in Table 1, it is obvious that all mortadella samples exhibited nearly similar composition, which indicates that the inclusion of lactate or sorbate had

little effect on the proximate analysis. Similarly, Moawad *et al.* (2012) found the proximate composition of sausage samples after one day of processing contained 62.9%

moisture, 16.1% protein, and 1.8% ash. In this regard, Pereira *et al.* (2000) reported that most sausage formulas fall within the following specifications: moisture 50-70%, protein 11-15%, and ash contents 1.5-2.8% (on a fresh weight basis).

Results indicated that there were significant differences ( $p \leq 0.05$ ) between Tr5 and the remaining samples regarding their protein content. However, despite the presence of significant differences among treatments, they were numerically analogous. Every single one of them met the minimum 12% value stipulated by the Jordanian standards. Protein content ranged from 18.36% to 19.15%, this proves that Jordanian mortadella is a good source of high-quality protein. Tr1 had the lowest value (18.36 %), a similar result was observed by Dharmaveer *et al.* (2007). Tr5 had the highest protein level (19.15 %). This finding is in agreement with Al Marazeeq *et al.* (2015). The variation may be due to nitrite which can react with the protein in meat (mainly referred to as myosin) and affect the water-holding capacity (WHC) of cured meat, which resulted in a slight increase in the WHC of samples. Consequently, this could be the reason the protein content was increased and the fat content was decreased in the present study (Deng *et al.*, 2021).

In the case of fat content, there were significant differences among treatments ( $p \leq 0.05$ ), where the highest value was in Tr1 (1.46%), followed by Tr3 (1.08%), and the remaining three treatments ranged from 0.77% to 0.86%. Results agreed with the findings of Maayah *et al.* (2016) who reported 1.8% fat content in packaged roast beef.

Similar findings were reported by dong *et al.* (2007) who stated that nitrite, when added to food systems, undergoes complex chemical interconversions and metabolism. Because of the reduction reaction of nitrite to NO in the curing meat, red oxygenated myoglobin was oxidized to brown met-myoglobin, then to bright pink NO myohaemochromogen during cooking. According to previous studies, after nitrite was added into the meat system, about 1–10% nitrite was oxidized into nitrate; 5–10% reacted with myoglobin; 5–15% with sulfhydryl group; 1–5% with fat; 20–30% with protein; and 1–5%

transformed into gas. As a result, these complex reactions concerning nitrite might contribute to the textural variation of meat products.

In general, protein and moisture have a proportional relationship which is evident in Tr5 which had the highest value of protein (19.15%) and moisture (71.73%). Alternatively, the latter is inversely related to fat content with the lowest value of 0.77% in Tr5. The low amount of fat was attributed to the fact that no beef fat was added to the mortadella sample at the factory. This can be demonstrated by comparing the fat content of 10.23% by Al Marazeeq *et al.* (2015) in beef mortadella.

There were moderate differences in moisture content between Tr1 and the remaining treatments ( $P \leq 0.05$ ), in which Tr1 had the lowest moisture value of 70%, while the other four treatments exhibited values of around 71%. This is in agreement with Deng *et al.* (2021) who found the sample with high nitrite had a low moisture content as they noted that these results are typical of meat salting and curing. Additionally, the authors agree with the results obtained in the present study showing that the decrease in water content is probably due to osmotic dehydration induced by the addition of nitrites. Nitrite penetration takes place simultaneously with the decrease in water-holding capacity, ultimately leading to reduced water content.

Moreover, our results were in agreement with Pietrasik & Janz (2010), who reported moisture content ranges between 61.40-71.60% in low-fat bologna-type mortadella. The authors have declared that the moisture content is proportional to the amount of water added to these products; which was higher due to fat removal.

Obtained protein and moisture data were in agreement with the findings of Al-Shuibi and Al-Abdullah (2002). The authors reported higher levels of moisture and protein, in addition to a lower fat content due to comparatively higher batter stability, which led to increased water binding via the formation of a three-dimensional protein network during heat processing.

There were no significant differences among treatments ( $p \leq 0.05$ ) regarding salt content (1.92-2.04%). The highest value detected was in Tr1 (2.04%), followed by Tr4

(2.00%), whereas the remaining three treatments ranged from 1.92% to 1.95%. Similarly, Hallerbach and Potter (1981) reported a salt level of 2.1% in frankfurters. As noted by Cestari *et al.* (2015), the salt content in chicken is typically 2%, to maintain and extract myofibrillar protein, which aids in linking meat to keep the product shape after cooking and flavoring.

In the present study, salt and protein contents were within the range specified in the Jordanian standards, i.e., 12% minimum for protein content and salt content does not exceed 3%. Fat content, however, was lower than that specified in the standards.

Regarding ash content, there were no significant differences among all treatments ( $p \geq 0.05$ ). All samples were within the value of 3%. This finding is similar to that reported by Saldaña *et al.* (2015) who obtained an ash level of 3.24% in mortadella-type products. However, lactate-containing Tr3 and Tr5 had slightly higher ash percentages (3.27% and 3.20%, respectively), but they were not statistically different from the other treatments. Naveena *et al.* (2006) explained that it might be because of the incorporation of sodium salts.

### pH Measurement

The pH value for meats is critical, not only in influencing the microbiota that may develop in the

product but also in indicating its conservation status based on reference value considerations (Frey, 1983). Measured pH readings for all mortadella samples stored in refrigeration at 4°C for 12 weeks are illustrated in Table 2.

As illustrated in Table 2, the pH values of Tr1, Tr2, Tr3, Tr4, and Tr5 samples after one week of storage were 6.21, 6.29, 6.29, 6.25, and 6.25, respectively. This complies with Moawad *et al.* (2012) who stated that neither nitrite concentration nor lactate or sorbate addition alters the acidity of mortadella formulas.

The results indicated that there were significant differences both between treatments and during the storage period. The highest pH was observed in Tr1 with a mean value of 6.47, while the lowest value was in Tr3 and Tr4 (i.e., 5.84) throughout storage. Despite the presence of significant differences ( $p \leq 0.05$ ) between treatments in the same storage period, values were numerically analogous. At first, the lowest pH was observed in Tr1 (6.21) and the highest was in Tr2 and Tr3 (6.29). This is similar to data reported by Karwowska *et al.* (2019) who detected significantly lower pH in the sample with the highest concentration of nitrite.

**Table 2:** Measured pH values of prepared mortadella samples made with the replacement of nitrites with sodium lactate and potassium sorbate during the storage period of 12 weeks at 4°C.

Treatments	Storage time (weeks)							
	1	4	5	8	9	10	11	12
Tr1	<sup>BC</sup> 6.21 <sub>c</sub> ±0.010	<sup>B</sup> 6.25 <sub>d</sub> ±0.005	<sup>A</sup> 6.47 <sub>a</sub> ±0.11	<sup>C</sup> 6.18 <sub>a</sub> ±0.040	<sup>D</sup> 6.04 <sub>a</sub> ±0.005	<sup>E</sup> 5.93 <sub>a</sub> ±0.005	<sup>B</sup> 6.25 <sub>c</sub> ±0.015	<sup>B</sup> 6.25 <sub>bc</sub> ±0.015
Tr2	<sup>B</sup> 6.29 <sub>a</sub> ±0.005	<sup>BC</sup> 6.29 <sub>b</sub> ±0.00	<sup>A</sup> 6.38 <sub>ab</sub> ±0.005	<sup>E</sup> 6.16 <sub>ab</sub> ±0.010	<sup>G</sup> 5.88 <sub>c</sub> ±0.005	<sup>F</sup> 5.92 <sub>ab</sub> ±0.005	<sup>CD</sup> 6.27 <sub>b</sub> ±0.005	<sup>D</sup> 6.26 <sub>b</sub> ±0.25
Tr3	<sup>B</sup> 6.29 <sub>a</sub> ±0.005	<sup>D</sup> 6.20 <sub>c</sub> ±0.010	<sup>A</sup> 6.36 <sub>b</sub> ±0.01	<sup>E</sup> 6.13 <sub>ab</sub> ±0.015	<sup>F</sup> 6.00 <sub>b</sub> ±0.005	<sup>G</sup> 5.84 <sub>c</sub> ±0.005	<sup>C</sup> 6.22 <sub>d</sub> ±0.00	<sup>C</sup> 6.23 <sub>c</sub> ±0.025
Tr4	<sup>D</sup> 6.25 <sub>b</sub> ±0.010	<sup>C</sup> 6.27 <sub>c</sub> ±0.005	<sup>A</sup> 6.37 <sub>b</sub> ±0.00	<sup>E</sup> 6.12 <sub>b</sub> ±0.005	<sup>F</sup> 5.88 <sub>c</sub> ±0.010	<sup>G</sup> 5.84 <sub>c</sub> ±0.005	<sup>C</sup> 6.28 <sub>b</sub> ±0.005	<sup>B</sup> 6.32 <sub>a</sub> ±0.005
Tr5	<sup>D</sup> 6.25 <sub>b</sub> ±0.005	<sup>B</sup> 6.33 <sub>a</sub> ±0.005	<sup>A</sup> 6.43 <sub>ab</sub> ±0.01	<sup>E</sup> 6.14 <sub>ab</sub> ±0.030	<sup>F</sup> 6.00 <sub>b</sub> ±0.005	<sup>G</sup> 5.9 <sub>b</sub> ±0.005	<sup>C</sup> 6.30 <sub>a</sub> ±0.005	<sup>B</sup> 6.33 <sub>a</sub> ±0.010

Data are expressed as means of triplicate determinations ± standard deviations (SD)

Data were subjected to two-way ANOVA.

Levels not connected by the same letter are significantly different.

Small latter within the same column with different subscript letters have significant differences between treatments using LSD ( $p \leq 0.05$ ).

Capital latter within the same row with different superscript letters have significant differences between storage periods using LSD ( $p \leq 0.05$ ).

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2% sodium lactate, Tr4=60 ppm sodium nitrite + 1500ppm potassium sorbate, and Tr5=60 ppm sodium nitrite+ 2% sodium lactate+ 1500ppm potassium sorbate

Moreover, the authors found on day 15 that the pH of the sample with sodium nitrite reduced to  $50 \text{ mg kg}^{-1}$  and did not significantly differ from the sample with traditional nitrite use ( $150 \text{ mg kg}^{-1}$ ).

At the beginning of storage, results indicated a slight increase of pH values in all treatments until the 5th week, after that, values decreased significantly ( $p \leq 0.05$ ) in each treatment at the 8<sup>th</sup> week of storage. The change of pH was in a similar pattern for all samples, as it started decreasing slowly until it reached the lowest value recorded at the tenth week of storage.

Additionally, Table 2 shows that during storage, there were significant differences regarding pH values among all treatments. Choi & Chin (2003) noticed a decrease in the pH values of their treatments owing to storage time.

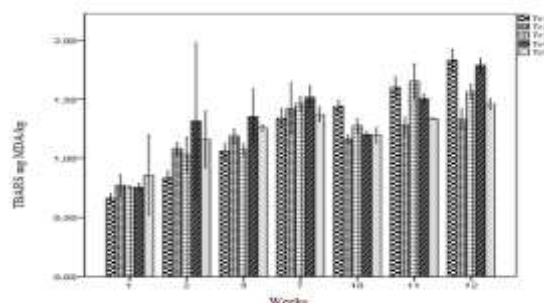
However, data showed that the pH decrease was followed by a slight increase after the tenth week of storage in all treatments. According to Bozkurt and Erkmen (2002), the pH of all sausage samples decreased ( $p \leq 0.05$ ) from 5.98 to around 4.53. This was attributed to lactic acid bacteria producing lactic acid. Following that, the pH increase ( $p \leq 0.05$ ) is caused by the decomposition of acids and becomes almost constant. Also, they declared that the additives increased the pH values of sausages especially those with buffering capacity (sodium polyphosphate). Moreover, as declared by Cenci *et al.* (2018), a decline in pH is caused by the action of lactic acid bacteria and the storage temperature that can produce acidification of the product while the pH rise is due to the production of basic amines and the medium's buffer capacity.

These results are in agreement with Moawad *et al.* (2012) who declared when the concentration of nitrite increased in the formula, the pH values decreased. In addition, they found a decrease in pH values of sausage samples during storage, which could be explained by the basis of the formation of lactic acid (Viuda-Martos *et al.*, 2009). After 20 days of chilling storage, all pH values of sausages increased reaching higher values than the initial values after processing and they attributed that to protein denaturation and/or accumulation of basic substance.

However, the pH values of sausage samples were below the critical limit value of 7.0 during chilling storage (Pearson, 1981), which could be due to the ability of nitrite and/or lactate and sorbate to inhibit or reduce the development of spoilage microorganisms (Moawad *et al.*, 2012).

### Lipid Oxidation Measurement

The TBARS test was used to assess the extent of fat oxidation in all treatments during refrigerated storage at  $4^\circ\text{C}$  for 12 weeks, and the results are depicted in Figure 2. Results showed that at the beginning of the storage period, the sample with the highest content of sodium nitrite (i.e., Tr1) had the lowest content of secondary fat oxidation products reacting with thiobarbituric acid. However, there were no significant differences between other treatments, where TBA values ranged from 0.75 to  $0.85 \text{ mg mda kg}^{-1}$  with Tr5 being the highest.



**Figure 2:** TBARS values MDA (mg/kg) in mortadella samples made with the replacement of nitrites with sodium lactate and potassium sorbate during a storage period of 12 weeks (w) at  $4^\circ\text{C}$ .

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2%sodium lactate, Tr4=60 ppm sodium nitrite + 1500ppm potassium sorbate, and Tr5=60 ppm sodium nitrite+ 2%sodium lactate+ 1500ppm potassium sorbate.

Data are expressed as means of triplicate determinations  $\pm$  standard deviations (SD)

According to the TBA values of mortadella samples illustrated in Figure 2, values of Tr1, Tr2, Tr3, Tr4, and Tr5 in the 1<sup>st</sup> week of mortadella processing were 0.75,



0.77, 0.77, 0.75, and 0.85, respectively. During storage, however, lipid oxidation values increased significantly ( $p \leq 0.05$ ) in all treatments. Within the same storage time, there was no or slightly significant difference between treatments.

Karwowska *et al.* (2020), who studied the lipid oxidation of sausage samples with varying doses of nitrite, declared that the sample with the highest content of sodium nitrite ( $150 \text{ mg kg}^{-1}$ ) showed the lowest content of TBARs. The authors have further reported that the TBARs value increased in tandem with the decrease in the share of nitrite in the samples, where they found at the beginning of storage a TBARs value of 0.84 in the sample containing  $50 \text{ mg kg}^{-1}$  of nitrite, and a value of 0.64 in the sample containing  $100 \text{ mg kg}^{-1}$  of nitrite. This is similar to the present study at different doses of nitrite.

The primary mechanism of antioxidant activity of nitrite is related to NO. Nitrite inhibits lipid oxidation primarily through oxygen deletion. NO, which is formed from nitrite, can be oxidized to form  $\text{NO}_2$ , which causes oxygen sequestration. The oxidation of meat lipids is inhibited under these conditions. On the other hand, nitrite can cause lipid oxidation by chelating free radicals. NO reacts with other radicals (hydroxyl, alkoxy, and peroxy radicals) breaking down radical chains. NO can also bind to transitional metals, which is important because binding to ferrous heme complex reduces the likelihood of producing hydroxyl radicals in the Fenton reaction.

The increase in the TBARs values in all treatments was due to the cooking process of mortadella. As described by Amaral *et al.* (2018), cooked meat products are more susceptible to lipid oxidation than raw meat, because cooking temperature causes the release of heme iron, which causes the production of free radicals.

Results indicated that the storage time had an effect ( $p \leq 0.05$ ) on TBARs, resulting in higher values with increased storage time (Figure 2). These findings agreed with those of Domínguez *et al.* (2019) who reported that storage time has enormous relevance in the promotion of lipid oxidation, and the possibility of radicals causing lipid damage grows over time.

From the beginning of the storage period until the 7<sup>th</sup> week, it was observed that the sample with 120ppm of nitrite was more stable and had a slower rate of oxidation than that with 60ppm only or those combined with lactate and sorbate. However, at the end of the storage period, mortadella samples with 60ppm sodium nitrite only or combined with organic acid (sorbate and lactate) presented a close amount of TBARs, and Tr1 (120ppm nitrite) had the highest value of 1.83, followed by Tr3 (containing a combination of nitrite and sorbate) with a value of 1.78. This was in agreement with Al Marazeeq *et al.* (2015) who found a higher value of TBA in the treatment containing 120ppm nitrite alone during 12 weeks of storage, whereas the lowest value was reported in the samples containing a reduced amount of nitrite combined with olive leaf extract.

The lowest TBA value at the end of storage was in Tr2 followed by Tr5, this indicated that a low quantity of nitrite or a combination of nitrite with sorbate and lactate enhanced the oxidative stability by decreasing TBA values. This conforms with Mohamed (2011) who studied the antioxidant synergistic effect of rosemary aqueous extract and green tea extract in buffalo meatloaves. Furthermore, Jin and Park (2013) found that adding Schisandra Chinensis powder (0.5, and 1%) and 100 ppm sodium nitrite enhanced antioxidant activity measured by TBARs of pork sausages.

Al-Shuibi & Al-Abdullah (2002) revealed that 40 and 80ppm nitrite, with lower TBA values, had a greater antioxidant effect than 120ppm nitrite. They also declared that nitrite had an antioxidant effect even at 40 ppm, implying that reducing nitrite levels in mortadella from current levels is feasible. Additionally, the results of Karwowska *et al.* (2019) indicated that nitrite reduction in cooked meat products is possible. The Reduction of nitrite to the level of  $50 \text{ mg kg}^{-1}$  would seem to be comparable with the traditional use of nitrite in meat products ( $150 \text{ mg kg}^{-1}$ ) in terms of the properties related to lipid oxidation.

### Residual Nitrite Measurement

The results of residual nitrite of mortadella samples are shown in Table 3. Values showed that during the early product life, there was a steep and rapid initial decrease ( $p \leq 0.05$ ) in nitrite from the point of sodium nitrite

addition (120 and 60 ppm). The nitrite reduction continued, albeit at a much slower rate, in the later stages of product life.

**Table 3:** Residual nitrite values of prepared mortadella samples made with the replacement of nitrites with sodium lactate and potassium sorbate during storage for up to 100 days at 4°C

Treatments	Storage time (days)				
	5	15	37	45	100
Tr1	<sup>A</sup> 5.98 <sub>a</sub> ±0.030	<sup>B</sup> 3.81 <sub>a</sub> ±0.165	<sup>B</sup> 3.63 <sub>a</sub> ±0.511	<sup>C</sup> 2.93 <sub>a</sub> ±0.049	<sup>C</sup> 3.04 <sub>a</sub> ±0.263
Tr2	<sup>A</sup> 5.40 <sub>b</sub> ±0.050	<sup>BC</sup> 2.86 <sub>bc</sub> ±0.049	<sup>B</sup> 3.06 <sub>bc</sub> ±0.049	<sup>C</sup> 2.63 <sub>a</sub> ±0.345	<sup>C</sup> 2.72 <sub>b</sub> ±0.117
Tr3	<sup>A</sup> 5.05 <sub>d</sub> ±0.00	<sup>B</sup> 2.96 <sub>b</sub> ±0.049	<sup>C</sup> 2.78 <sub>b</sub> ±0.065	<sup>D</sup> 2.55 <sub>a</sub> ±0.197	<sup>D</sup> 2.40 <sub>c</sub> ±0.049
Tr4	<sup>A</sup> 5.12 <sub>c</sub> ±0.033	<sup>B</sup> 2.78 <sub>bc</sub> ±0.00	<sup>B</sup> 2.73 <sub>bc</sub> ±0.016	<sup>C</sup> 2.60 <sub>a</sub> ±0.115	<sup>D</sup> 2.39 <sub>c</sub> ±0.033
Tr5	<sup>A</sup> 4.67 <sub>c</sub> ±0.016	<sup>B</sup> 2.75 <sub>c</sub> ±0.131	<sup>B</sup> 2.58 <sub>c</sub> ±0.174	<sup>B</sup> 2.65 <sub>a</sub> ±0.296	<sup>B</sup> 2.57 <sub>bc</sub> ±0.049

Levels not connected by the same letter are significantly different. ± standard deviation (SD)

Capital letter within the same row with different superscript letters have significant differences between storage periods using LSD ( $p \leq 0.05$ )

Small letter within the same column with different subscript letters have significant differences between treatments using LSD ( $p \leq 0.05$ ).

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2%sodium lactate, Tr4=60 ppm sodium nitrite + 1500ppmpotassium sorbate, and Tr5=60 ppm sodium nitrite+ 2%sodium lactate+ 1500ppm potassium sorbate.

According to Pereira *et al.* (2014), a rapid decline of residual nitrite level during storage even at the initial measurement (week 0) was much lower than the ongoing amount of nitrite (385 mg kg<sup>-1</sup>) in their formulations. They claimed that the pasteurization process and the addition of reducing agents reduce the added amount of nitrite in sausages, leaving only about 10mg/kg<sup>-1</sup> after storage.

Tr1 with 120ppm nitrite only showed the highest level of residual nitrite ( $p \leq 0.05$ ) in the first week after processing and during the entire storage period. According to Zahran & Kassem (2011), the treatment that had the highest residual nitrite was the one to which the highest amount of nitrite was added to the product during processing. The findings are in disagreement with Al-Shuibi & Al-Abdullah (2002), as they found that 120ppm nitrite added to mortadella leaves a residual nitrite of 32ppm after processing. This could be due to the difference in the processing temperature, where they used lower temperature and less time in the cooking of

mortadella (Heating for 30 min at 55°C, then cooking at 80°C) in comparison with the current study (as mentioned above). As reported by Paudel *et al.* (2021), the higher the cooking temperature and duration, the greater the reduction in residual nitrite content of the final product.

Similarly, Novelli *et al.* (1998) found that the residual nitrites were consistently low (6.86-6.45mg/kg<sup>-1</sup>) in mortadella with 100 ppm of added nitrite. They have further found residual nitrite around 4 mg/kg<sup>-1</sup> in Salame Milano (with 125ppm of nitrite added initially). The reason for this decrease is cooking at an elevated temperature in a dry oven for mortadella and the long cooking time of salame Milano.

Merino *et al.* (2016) found a sharp decrease in nitrite levels between the point of adding to the product and the first sampling of the product 24 hours later in four types of meat products. Then residual nitrite levels continued to decline, but at a much slower rate. They found the decrease in nitrite in pork/beef was faster than in chicken, this is probably because red meat contains much more

haem than chicken. Nitrite-induced formation of nitrosyl haem may cause greater and faster loss of added nitrite in red meat products. Additionally, heating during the initial processing phase, pH, temperature, and ascorbate addition have been shown to influence nitrite levels in meat products.

As presented in Table 3, each Tr2, Tr3, Tr4, and Tr5 had lower residual nitrite than Tr1. The lowest value at the beginning of the analysis was in Tr5 (4.67ppm), then in Tr3 (5.05ppm), this may be due to lactate interaction with sodium nitrite. As suggested by McClure (2009), lactate facilitates nitrite reactions in cured meat products. The author found in their study a significant difference between treatments with and without lactate. This is supported by the findings of Kim *et al.* (2006) who proposed that lactate increases lactic dehydrogenase activity by converting lactate to pyruvate and regenerating NADH. NADH regeneration would have converted metmyoglobin to deoxymyoglobin, resulting in a higher concentration of reduced myoglobin in the system to subsequently react with nitrite.

The results are comparable to those reported by Ferysiuk & wójciak (2020) as they found that nitrite residues were only detected in the sample containing 100 mg/kg of sodium nitrite. They concluded that the decrease in residual nitrite in treatment containing 100 mg/kg was 10 mg/kg and <10 mg/kg in treatment containing 50 mg/kg of added nitrite. Nitrite is a reactive substance that can be bound with other substances in meat, indicating a tendency to disappear in meat products. No nitrite has

been found in frankfurters after the first day of storage (sample with 40 mg/kg of nitrite addition).

The rate of decrease from the first analysis in the first week to the fifteenth day ranged from 37% to 47% in all treatments. Bardhi *et al.* (2014) observed a decrease in nitrite level from 116 to 9 ppm residual nitrite after 30 days of storage. Pérez-Rodríguez *et al.* (1998) reported residual nitrite levels of 8–10 mg kg<sup>-1</sup> in frankfurters after 12 days of storage.

The nitrite added to meat products could be recovered completely as nitrate, nitrosyl myoglobin, gaseous nitrogen compounds, and residual nitrite. Alahakoon *et al.* (2015) explained the rapid decline in detectable amounts during storage due to the combination of nitrite with meat pigments and other compounds. As a result, the residual nitrite level is substantially lower than the ongoing nitrite level. Furthermore, because the residual and added amount of nitrite in processed meat tends to decrease gradually with time, it can be assumed that the risk of nitrite ingestion from processed meat is further reduced (Lee *et al.*, 2021).

### Color Determination

Color is one of the first and most important factors in the consumer's decision to select and accept food (Al Marazeeq *et al.*, 2015). Table 4 summarizes the effect of sodium nitrite and organic acid on the chromatic coordinates (L\*, a\*, and, b\*) of mortadella samples during the refrigerated storage period at 4°C.

**Table 4:** Changes in L\*, a\*, and, b\* values of prepared mortadella samples made with the replacement of nitrites with sodium lactate and potassium sorbate during storage at 4°C.

Treatment	Lightness(L*)		Redness(a*)		Yellowness(b*)	
	1 week	12 week	1 week	12 week	1 week	12 week
Tr1	<sup>B</sup> 42.06 <sub>a</sub> ±1.534	<sup>A</sup> 51.87 <sub>a</sub> ±0.021	<sup>B</sup> 15.29 <sub>b</sub> ±0.311	<sup>A</sup> 17.65 <sub>a</sub> ±0.098	<sup>B</sup> 12.51 <sub>a</sub> ±0.318	<sup>A</sup> 14.31 <sub>a</sub> ±0.134
Tr2	<sup>B</sup> 41.87 <sub>a</sub> ±0.735	<sup>A</sup> 49.37 <sub>a</sub> ±0.091	<sup>B</sup> 15.38 <sub>b</sub> ±0.183	<sup>A</sup> 17.62 <sub>a</sub> ±0.282	<sup>A</sup> 12.50 <sub>a</sub> ±0.226	<sup>A</sup> 12.21 <sub>b</sub> ±0.197
Tr3	<sup>A</sup> 43.49 <sub>a</sub> ±3.895	<sup>A</sup> 44.32 <sub>bc</sub> ±0.530	<sup>A</sup> 15.78 <sub>ab</sub> ±1.258	<sup>A</sup> 16.28 <sub>b</sub> ±0.113	<sup>A</sup> 10.78 <sub>b</sub> ±0.975	<sup>A</sup> 10.95 <sub>c</sub> ±0.134
Tr4	<sup>A</sup> 45.76 <sub>a</sub> ±1.065	<sup>A</sup> 47.88 <sub>ab</sub> ±2.913	<sup>A</sup> 17.26 <sub>a</sub> ±0.091	<sup>A</sup> 17.24 <sub>a</sub> ±0.424	<sup>A</sup> 12.49 <sub>a</sub> ±0.165	<sup>A</sup> 12.35 <sub>b</sub> ±0.827
Tr5	<sup>A</sup> 44.68 <sub>a</sub> ±4.292	<sup>A</sup> 43.10 <sub>c</sub> ±2.100	<sup>A</sup> 16.92 <sub>ab</sub> ±0.626	<sup>A</sup> 15.88 <sub>b</sub> ±0.410	<sup>A</sup> 11.77 <sub>ab</sub> ±0.092	<sup>A</sup> 11.00 <sub>c</sub> ±0.311

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Levels not connected by the same letter are significantly different  $\pm$  standard deviation (SD)

Data were subjected to two-way ANOVA

Capital letter within the same row with different superscript letters have significant differences between storage periods using LSD ( $p \leq 0.05$ )

Small letter within the same column with different subscript letters have significant differences between treatments using LSD ( $p \leq 0.05$ ).

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2%sodium lactate, Tr4=60 ppm sodium nitrite + 1500ppm potassium sorbate, and Tr5=60 ppm sodium nitrite+ 2%sodium lactate+ 1500ppm potassium sorbate

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As for the lightness ( $L^*$ ) index, no significant differences ( $p \geq 0.05$ ) were observed among all formulations in the first week. In general, the experimental treatments that contained lactate, sorbate, and nitrite were lighter than the control that only contained nitrite (in both concentrations 120 and 60 ppm) at the beginning of the hunter  $L^*$  measurement. This is in agreement with Al Marazeeq *et al.* (2015) who substituted sodium nitrite with olive oil leaf extract in beef mortadella. The author found the combination treatments that contained olive leaves and nitrite was lighter than treatments that contained only 120ppm of sodium nitrite.

Massingue *et al.* (2018) noted that when the fat content is reduced, sausages become darker (i.e., lower  $L^*$  values). This is consistent with the present study where the fat content was very low in all samples. This was proved by Pietrasik & Janz (2010) who found in low-fat bologna (type sausages) a significant difference ( $p \leq 0.05$ ) between high-fat and low-fat treatments. The researchers explained that the darker appearance was most likely caused by a reduction in the overall light-scattering properties associated with fat.

During storage, the ( $L^*$ ) value increased significantly in Tr1 and Tr2 ( $p \leq 0.05$ ). Similarly, Ferysiuk & Wójciak (2020) found a significant increase in the  $L^*$  parameter after 180 days of storage in all treatments with different concentrations of nitrite, indicating that the meat color was fading systematically during storage. The breakdown of heme pigments during storage might have led to the lower lightness value. Whereas Tr3, Tr4, and Tr5 which contained nitrite with a combination of lactate and sorbate showed an increase and decrease of  $L^*$  value during storage but without significant difference, This could be

due to the presence of lactate and sorbate which might have contributed to the stability of lightness.

The  $a^*$  value measures the amount of redness in the meat, which is an important factor influencing consumer acceptability. It was found that the highest values were for the share of treatment containing a mixture of nitrite and sorbate. In the sensory evaluation results (as will be demonstrated in the next section), the highest color score obtained by panelists was in Tr4 (7.11) which had a high  $a^*$  value of 17.26. In previous research by Heaton *et al.* (2000), the authors confirmed that the  $a^*$  value correlated well with sensory (visual) meat color scores. The importance of nitrite as a curing agent alone or combined with other food additives (organic acids in the current study) is in the development of the pink-red color of mortadella. A Similar finding was demonstrated by Holownia *et al.* (2003) as the presence of sodium nitrite, alone or in combination with other ingredients, significantly increased the  $a^*$  value of the cooked fillets.

During storage, there was a significant ( $p \leq 0.05$ ) increase in  $a^*$  value for Tr1 and Tr2, while other treatments experienced a decrease and an increase but were not significantly different ( $p \geq 0.05$ ). Brook *et al.* (2011) reported that the  $a^*$  value increases with time in treatments containing nitrite which is typical of nitrite effects on meat color.

In the case of the hunter yellowness ( $b^*$ ) values, the highest ( $p \leq 0.05$ ) value at the beginning and during storage was found in Tr1 (120ppm nitrite). According to Al Marazeeq *et al.* (2015), the control (containing only 120ppm of nitrite) had a  $b^*$  value of around 12, and other treatments ranged from 12 to 14 during the storage period. However, the authors found that the lowest  $b^*$  value was in treatment containing only 120 ppm sodium nitrite

compared with other treatments combined with nitrite and olive leaves. During storage, there was a significant increase in the  $b^*$  value in treatment one ( $p \leq 0.05$ ). Also, Sedghi *et al.* (2014) found that the other samples exhibited a change in the  $L^*$  value during the storage period.

### Sensory Evaluation

Sensory evaluation scores of the developed mortadella are shown in Table 5. The difference in appearance and color acceptability among reformulated treatments was not statistically different ( $p \geq 0.05$ ). Scores given by panelists ranged from 'like slightly' to 'like moderately' for all treatments, indicating that these products have a high potential for success based on appearance and color after minor changes based on consumer feedback. The panelists' preference for the appearance of all treatments

is due to the desired color of mortadella (red) (Al-Slaihat, 2014).

Nitrite by itself does not fix the pigment responsible for cured meat color. Rather, it forms nitrosylating agents through a variety of mechanisms, which can transfer NO, which then reacts with myoglobin to produce the cured meat color (Sindelar & Milkowski, 2011).

According to Al-Shuibi and Al-Abdullah (2002), there was no significant difference between the treatment that contained only 120ppm nitrite and other treatments that contained nitrite and sorbate at various concentrations (typical and lighter red color). The most noticeable effect of nitrite addition is the retention of a desirable red color, shaded pink, which is frequently regarded as a critical attribute for consumer acceptance (Sindelar & Milkowski, 2011).

**Table 5:** Sensory evaluation scores (based on a 9-point hedonic scale) of manufactured beef mortadella made with the replacement of nitrites with sodium lactate and potassium sorbate

Treatments	Appearance	Color	Aroma and flavor	Juiciness	Texture	Overall liking
Tr1	6.15 <sup>a</sup> ±1.922	6.73 <sup>a</sup> ±1.627	5.89 <sup>a</sup> ±2.492	6.21 <sup>a</sup> ±1.988	7.05 <sup>a</sup> ±1.899	6.15 <sup>b</sup> ±1.463
Tr2	6.94 <sup>a</sup> ±1.899	7.10 <sup>a</sup> ±1.523	6.47 <sup>a</sup> ±2.220	6.47 <sup>a</sup> ±1.982	6.89 <sup>a</sup> ±2.157	7.31 <sup>a</sup> ±1.765
Tr3	6.31 <sup>a</sup> ±1.600	6.26 <sup>a</sup> ±2.130	6.05 <sup>a</sup> ±2.223	6.00 <sup>a</sup> ±1.666	6.21 <sup>a</sup> ±2.043	6.57 <sup>ab</sup> ±1.070
Tr4	6.73 <sup>a</sup> ±1.820	7.11 <sup>a</sup> ±1.629	6.15 <sup>a</sup> ±2.217	5.36 <sup>a</sup> ±2.241	6.31 <sup>a</sup> ±1.857	5.89 <sup>b</sup> ±2.052
Tr5	6.47 <sup>a</sup> ±2.294	6.78 <sup>a</sup> ±2.347	6.21 <sup>a</sup> ±2.439	5.94 <sup>a</sup> ±1.899	6.36 <sup>a</sup> ±1.738	6.63 <sup>ab</sup> ±1.832

Data are means ± standard deviation.

Levels not connected by the same letter are significantly different.

Small letter within the same column with different subscript letters have significant differences between treatments using LSD ( $p \leq 0.05$ ).

Tr1=control with 120ppm sodium nitrite, Tr2= control with 60ppm sodium nitrite, Tr3=60 ppm sodium nitrite+ 2%sodium lactate, Tr4=60 ppm sodium nitrite + 1500 ppm potassium sorbate, and Tr5=60 ppm sodium nitrite+ 2%sodium lactate+ 1500 ppm potassium sorbate

In the current study, treatments 3, 4, and 5 were comparable as they contained a combination of sodium nitrite, sodium lactate, and potassium sorbate, while in the case of Tr1 and Tr2, they were different. The scores for both color and appearance ranged from 'like slightly' to 'like moderately' which means that the combination of nitrite with organic acid was well received by the

panelists. As described by Zepeda *et al.* (1994), a combination of lactic and gluconic acid minimized color defects in vacuum-packaged beef. Friedrich *et al.* (2008) also reckoned that additives, when combined with an organic acid, alleviate meat discoloration. In addition, though potassium sorbate improved the visual appearance of minced beef, its use at such high concentrations is not

advised due to the undesirable off-flavors produced. The lactate appears to promote meat color stability.

The formulation of mortadella samples with different nitrite concentrations in Tr1 and Tr2, or those combined with lactate and sorbate in the other three treatments, under investigation, had no significant effect ( $p \geq 0.05$ ) on their acceptability in terms of aroma and flavor. Similar findings were reported by Maaya and Al-Abdullah (2016).

The juiciness of the prepared mortadella did not significantly vary ( $p \geq 0.05$ ) among all treatments. This may be because the same formula was used for all treatments except for the nitrite concentration. As reported by Abdullah (2007), the juiciness of the formulations is related to the type of meat used rather than the chemical composition. It is worth noting that the juiciness may be related to the moisture content of meat products, where the author stated that juiciness arose as a result of moisture released from the meat during chewing.

Regarding texture scores, results showed that there was no significant difference among treatments, where ratings ranged from "like slightly" to "like moderately". Tr1 (120ppm) had a softer texture, which made it preferable. Moreover, the other four treatments were considered desirable by panelists, since the inclusion of nitrite, even at a low level (60ppm), has also improved the texture. This agrees well with Al-Shuib and Al-Abdullah (2002) since they reported that the addition of nitrite, even at a low level, enhanced the texture.

Overall acceptability (i.e., the acceptance of the product in general) scores showed that there was a significant difference among treatments, where they ranged between 'Neither like nor dislike' and 'like moderately'. The panelists preferred Tr2, Tr5, and Tr3 giving scores of 7.31, 6.63, and 6.57, respectively. This means that treatments with nitrite only or combined with lactate and sorbate are accepted and received by panelists without any sensory problems.

### Conclusion

The results of the present study showed a rapid decline in nitrite level that occurred initially after the addition of

nitrite during the production of mortadella, as a result, the residual nitrite in all samples was low, and this level of nitrite will not pose health risks to humans. Among the 12 analyzed samples of meat chosen from a supermarket, none of them had a high content of residual nitrite, referring to the results it is clear that the residual nitrite level of all samples was found to be accepted and below the critical limit value of 125 ppm stated as the maximum permissible set by Jordanian standard No. 816. The reduction in residual nitrite levels is beneficial because it reduces the possibility of nitrosamine formation. The results of the present study suggest that aside from the slight variability observed between different brands, there were no perceptible differences between commercial and experimentally produced mortadella. The results also showed that the reduction of nitrite to the level of 60ppm seems to be comparable with the traditional use of nitrite in meat products in terms of the physicochemical properties and properties related to lipid oxidation. The treatments containing lactate and sorbate combined with nitrite had a more stable Hunter L\*, a\*, and b\* color than the control (with nitrite only) during storage. The treatment with reduced nitrite to 60ppm only, or combined with an organic acid, was accepted and well received by panelists without any sensory problems. It is recommended and possible to reduce the added level of nitrite in mortadella up to 60ppm without affecting other quality aspects of the products. The results of this study could be utilized by the meat industry to reduce the nitrite level in cured meat products, which could be considered a healthier practice. As for the limitation of the study, in the application of nitrite reduction in mortadella, all other measurements and practices to preserve the product's safety and quality should have been taken into consideration and applied properly.

### Acknowledgments:

This work was financially supported by the University of Jordan – Scientific Research Deanship.

### Conflict of Interests

The authors have no conflict of interest to declare.

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## تأثير مزيج النيترايت مع اللاكتات والسوربات على الخصائص الفيزيائية والكيميائية ومضادات الأكسدة والحسية لمرتديلا البقر أثناء التخزين.

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تاريخ استلام البحث: 2022/6/8 وتاريخ قبوله: 2022/11/22.

### ملخص

هدفت هذه الدراسة إلى تقييم تأثير مزيج النيترايت مع اللاكتات و / أو السوربات على الخصائص الفيزيائية والكيميائية والحسية لمرتديلا اللحم البقري أثناء التخزين. تم تحضير خمسة معالجات من مرتديلا البقر وتخزينها عند 4 درجات مئوية. كل من المعاملات الشاهدة 1 (Tr1) و 2 (Tr2) اضيف لها 120 جزء في المليون و 60 جزء في المليون من النيترايت ( $\text{NaNO}_2$ )، على التوالي. وللمعاملة 3 (Tr3) باستخدام نيترايت الصوديوم ولاكتات الصوديوم ، والمعاملة 4 (Tr4) باستخدام نيترايت الصوديوم وسوربات البوتاسيوم، و المعاملة 5 (Tr5) مزيجا من كل منهم. تم قياس التركيب الكيميائي مبدئياً، وتم تمييز العينات برقم الأس الهيدروجيني، اللون، وقيم حمض الثيوبارببتيوريك (TBA) وتركيز النيترايت المتبقي and sensory properties طوال فترة التخزين. وقد تم اجراء التقييم الحسي، ضافة الى ذلك تم قياس محتوى النيترايت المتبقي لـ 12 منتجاً مختلفاً من منتجات اللحوم. كان للنيترايت تأثير طفيف على التركيب الكيميائي. وفي نهاية التخزين لم تتجاوز قيم TBA المتحصل عليها 2 مغ / كغ مالونالدهيد في جميع المعالجات، بينما كان Tr5 و Tr2 اقل قيمة TBA . كان أعلى اس هيدروجيني 6.33 في Tr5. ووجد انخفاض حاد في مستوى النيترايت في جميع المعاملات المدروسة. كانت Tr3 و Tr4 و Tr5 أكثر استقراراً من حيث اللون من العينات الشاهدة. كانت المعالجات بـ 60 جزء في المليون من النيترايت فقط، أو مجتمعة مع حمض عضوي مقبولة من الناحية الحسية. كانت درجات النكهة والرائحة متشابهة إحصائياً بتصنيفات بين 9/5.89 و 9/6.47 . يعتبر تقليل النيترايت في منتجات اللحوم ممارسة صحية أكثر.

**الكلمات الدالة:** مرتديلا البقر، مستوى النيترايت، صوديوم اللاكتيت، بوتاسيوم السوربيت، نشاط مضاد للأكسدة، الخصائص الحسية، التركيب الكيميائي

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