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# A study on the application of natural extracts as alternatives to sodium nitrite in processed meat

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#### Abstract

Consumers are increasingly interested in purchasing meat products with low food additives content or even without the, since these kind of foodstuffs are perceived as more natural and healthier. Nitrites are the most commonly used additives in the meat industry to prevent the growth of pathogenic bacteria, keep red color (secondary effect on myoglobin = iron and oxygen-binding), and improve flavor. In this scenario, meat processors are challenged to produce nitrite-free products guaranteeing the microbial quality and sensory characteristics. The objective of the present study was to determine the effect of various natural extracts against the color of thermal processed beef, manufactured without nitrites. A total of fourteen natural alternatives have been evaluated: capsicum extract liquid phase (capsanthin), paprika oleoresin liquid phase, monascus yellow powder (Monascus purpureus), red yeast rice powder (Monascus purpureus) from three different producers, lycopene powder, red beet juice powder (Beta vulgaris), rosemary extract (Rosmarinus officinalis), capsicum extract powder (capsanthin), carmine pigment powder (cochineal extract), sorghum red pigment powder (Sorghum bicolor), and two factory-supplied recipes. For the first trial, extracts were added at a concentration of 0.3% in canned meat without nitrite. Samples were analyzed by colorimetric measurements before and after sterilization. The aim was to find natural extracts that provide similar color characteristics as canned meat with nitrite (used as reference). After color analysis, the extracts that did not show statistically significant differences (p > .05) from the positive control were chosen for the second trial, consisted of sample preparation at three different concentrations of extract (0,1%, 0,2%, and 0,3%) following factory manufacture procedures to ensure that the results were as accurate as possible. Results showed that sorghum red pigment powder (Sorghum bicolor) provides stable pigments and can be added as a natural additive to the manufacture of traditional canned meat recipe to maintain a similar red/pink color as same as provided by sodium nitrite.

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**Novelty impact statement:** Producers are trying to obtain meat products without nitrites that maintain the same level of microbial safety and the typical color. In this study, we analyzed the effect of adding different natural extracts on the color of canned meat made without nitrites. The powder extracted from sorghum husks (*Sorghum bicolor*) can be added to the classic canned meat recipe to obtain a product that maintains the typical red/pink color.

# 1 | INTRODUCTION

Meat color influenced the consumer buying behavior. Thus, consumer's perception of color variation in meat influences the likelihood of purchase (Carpenter et al., 2001). Meat and other perishable products are commonly cured with nitrates and nitrites. Foods are preserved with preservatives due to their ability to inhibit the growth of harmful microorganisms and their antioxidant properties (Cenci-Goga et al., 2020; Sindelar & Milkowski, 2012). Their addition in meat batter during meat products manufacturing has the added benefit of keeping the bright red color that is appreciated by consumers (Karwowska et al., 2019). According to food policy (Regulation 1333/2008), nitrates and nitrites are listed as preservatives: potassium nitrite (E249), sodium nitrite (E250), sodium nitrate (E251), and potassium nitrate (E252). Although nitrates/ nitrites inhibit the growth of Clostridium botulinum spores (Lebrun et al., 2020), other research studies have demonstrated that maximum acceptance levels in foods allowed by law are not enough to inhibit the growth of concern foodborne pathogens such as Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens or Pseudomonas spp. (Cenci-Goga et al., 2014; Grispoldi et al., 2019; Hospital et al., 2017; King et al., 2016; Rossi et al., 2016). The characteristic reddish color of meat products made with nitrates/nitrites is caused by myoglobin, an iron and oxygen-binding protein found in skeletal muscles (Suman et al., 2016). It is a globular protein with a heme-group containing a ferrous iron ( $Fe^{2+}$ ) atom in the center. The iron atom is linked to globin and four nitrogen atoms, with a sixth coordination site used to bind oxygen or water (Silverstein et al., 2015). There are three types of myoglobin: oxymyoglobin (oxyMb), deoxymyoglobin (deoxyMb), and metmyoglobin (metMb). The physiologically active forms in living tissues are oxymyoglobin and deoxymyoglobin (Fe<sup>2+</sup>), whereas metmyoglobin is found in dead muscles and characterized by the presence of iron in the trivalent state ( $Fe^{3+}$ ) making it unable to bind to oxygen. The oxidation degree of myoglobin determines the color of meat after seasoning, cooking, and other processes (Bernofsky et al., 1959). Because the iron atom present in myoglobin has lost an electron, it is present in the ferric oxidation state (Fe<sup>3+</sup>) displaying a brown color in meat. Nitric oxide (NO) is formed when nitrites are added to meat products and preparations, which reacts with the iron in myoglobin ( $Fe^{2+}$ ) and metmyoglobin ( $Fe^{3+}$ ) to form the nitrosyl-myoglobin complex, a bright red but extremely unstable compound. It is converted to nitrohemochrome, a red/pink, stable compound, during

the heat treatment process (Parthasarathy & Bryan, 2012). Meat nitrates can also act as nitrosating agents, forming nitroso compounds (Hiramoto et al., 1993). Some epidemiological studies (Alexander & Cushing, 2011) suggested a possible link between N-nitroso compound consumption and cancer risk. Even though the general health risks associated with high nitrite and nitrate overdoses are well understood (Gangolli et al., 1994; Sanchez-Echaniz et al., 2001), the direct link between processed meat consumption and cancer risk remains unknown. In 2015, the International Agency for Research on Cancer (IARC) convened a working group to assess the carcinogenicity of red and processed meat consumption. They concluded that "On the basis of the large amount of data and the consistent associations of colorectal cancer with the consumption of processed meat across studies in different populations, ... there is sufficient evidence in human beings for the carcinogenicity of the consumption of processed meat" (Bouvard et al., 2015). Numerous epidemiological studies corroborate these findings (Corpet et al., 2014; Kim et al., 2013; Norat et al., 2005). However, the same research group noticed that the experimental evidence of the biochemical mechanisms of this correlation in animal models was inadequate (Turesky, 2018).

Nevertheless, the industry is becoming more interested in natural alternatives that can reduce the addition of nitrates/nitrites during the manufacture with lower nitrites content in the final product aimed to reduce the consumer's nitrite intake (Mahgoub et al., 2017; Saad et al., 2020). We have already analyzed the microbial safety of canned meat produced without nitrites in a previous study on risk assessment to evaluate the HACCP system (Grispoldi et al., 2019). The aim of this study was to assess to what extent it is possible to replace sodium nitrite with natural ingredients in the preparation of canned meat yet maintaining the desired color.

# 2 | MATERIALS AND METHODS

#### 2.1 | Experimental design

Fourteen natural alternatives to the addition of sodium nitrate to canned meat were selected. All of them were evaluated for their effect on the color of the meat before and after heat treatment. Six samples were prepared on the same day for each extract: three replicates for the trial without sterilization and three replicates for the trial with sterilization. Colorimetric measurements were carried out in triplicate for each sample, and the mean was calculated.

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Then, the selected extract was tested at three different concentrations according to the factory recipe procedures to obtain similar results that were as close as possible to the model (made with nitrates).

# 2.2 | Natural extracts selection

Twelve natural extracts were chosen based on data from the literature, some in powder form and others in liquid form, with pigmentation ranging from yellow/orange to red/purple. The natural extracts are as follows: capsicum extract liquid phase (capsanthin, CAPI), paprika oleoresin liquid phase (PAP), monascus yellow powder (*Monascus purpureus*, MON), red yeast rice powder (*Monascus purpureus*) from three different producers (RYR1, RYR2, RYR3), lycopene powder (LYC), red beet juice powder (*Beta vulgaris*, RBJ), rosemary extract (*Rosmarinus officinalis*, ROS), *Capsicum* extract powder (capsanthin, CAPp), carmine pigment powder (cochineal extract, CAR), and sorghum red pigment powder (*Sorghum bicolor*, SOR). Two canned meat factory-supplied recipes were also evaluated (for contractual and patent reasons, only the primary ingredients can be disclosed), bringing the total number of alternative recipes evaluated to fourteen.

## 2.3 | Experimental trials

The canned nitrite meat was used as a positive control during all experimental tests and the canned meat without nitrite was a negative control. All tests used frozen, cooked beef. All tests were performed in triplicate, and each replicate was subjected to three color measurements. All natural additives were initially tested at the same concentration. Briefly, the extracts were added at a concentration of 0.3% in 20 gr of canned meat without nitrite. The correct amount of extract (0.06 g) was placed in a falcon tube and vortexed with 2 ml of physiological solution. The nitrite-free canned meat was placed in a sterile bag and homogenized with the dissolved extract using a Stomacher 400 (PBI International, Milan, Italy). Before the heat treatment, the samples were placed in glass jars for colorimetric measurements. The jars were then sterilized for 15 min at 121°C in an autoclave. After heating, the samples were returned to room temperature, protected from direct light, and allowed to cool. The jars were opened the next day, and the contents were poured onto plastic dishes in preparation for the colorimetric measurements. After color analysis, the extracts that did not show statistically significant differences (p > .05) from the positive control were chosen for the second trial, consisted of sample preparation followed factory manufacture to ensure that the results were as accurate as possible. The meat was defrosted and cut into small pieces before being placed in empty cans (31.5 g in each). The aspic was prepared using a traditional factory recipe. The exact composition cannot be disclosed due to contractual and privacy considerations. The extracts were added in various

concentrations (0.1%, 0.2%, and 0.3%) to the aspic and homogenized. Fifty-eight milliliters of the aspic, obtained as described above, was added to each can to.

reach a final weight of 90 g (). Sterilization of samples was performed in an autoclave at 121°C for 15 min. Following heating, the samples were brought to room temperature, protected from direct light, and allowed to cool. The jars were opened the next day, and the contents were poured onto plastic dishes in preparation for the colorimetric measurements.

#### 2.4 | Colorimetric analysis

The «ColorMeter RGB Colorimeter» app (White Marten GmbH, Stuttgart, Germany) was used to measure color three times on the entire surface of the meat patties placed on a white dish using an iPhone XS running iOS 13.7. The conventional colorimeters (such as the one described below) are designed to determine the color of a single point in a uniform area. In this case, we chose to measure the average color of the entire meat patty in the dish in order to replicate how the consumer perceives his/her portion of meat. The «ColorMeter RGB Colorimeter» app's color measurement was calibrated against a reference colorimeter, a Minolta CR 200 Chroma Meter (Konica Minolta Inc. Tokyo, Japan), in order to determine the CIELAB  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (yellowness) color spaces. Briefly, the Minolta CR 200 Chroma Meter was used to measure a series of red/reddish calibration plates (specifically, the CR-A47 DP, CR-A47 R, and CR-A47 B) in conjunction with a standard white plate in order to determine the CIELAB  $L^*$  (lightness),  $a^*$  (redness), and  $b^*$  (vellowness) color spaces, and the results were used to calibrate the readout the «ColorMeter RGB Colorimeter». The Minolta CR 200 Chroma Meter was set to measure under the CIE Standard Illuminant D65. D65 is approximately equivalent to the average midday light in Western Europe/Northern Europe, which includes both direct sunlight and diffused light from a clear sky. Hence, it is also referred to as a daylight illuminant and has a correlated color temperature of approximately 6500 K. The light used to illuminate the calibration plates for the «ColorMeter RGB Colorimeter» app was, therefore, a source of 6500 K light (Godox Led 64, Godox, Shenzhen, China) under controlled conditions in a photographic light box. The CIELAB system describes colors visible to the human eye according to their hue and chroma (position on the  $a^* b^*$  plane) and their lightness, L\*, which corresponds to a position on a black-to-white scale (MacDougall, 1994).

## 2.5 | Statistical analysis

Color parameters were analyzed separately and together using respectively a univariate and multivariate GLM procedure in SAS (SAS Italy, Milan, Italy), due to the independence of data, considering one or two factors: different treatments as a fixed factor and the meat batch as a random factor. Parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) within the pre and poststerilization preparation were analyzed according to the following univariate model:

$$\mathbf{x}_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where  $x_{ij}$  is the *L*, *a* or *b* measured parameter;  $\alpha_i$  is the fixed effect of the *i* = 16 treatments (control with and without nitrite plus 14 different extracts); *j* the repeated measures.

For the selected final extract data were analyzed according to the following mixed model:

$$\mathbf{x}_{ijr} = \boldsymbol{\mu} + \boldsymbol{\alpha}_i + \boldsymbol{\beta}_j + \boldsymbol{\varepsilon}_{ijr}$$

where  $x_{ijr}$  is the *L*, *a*, or *b* measured parameter;  $\alpha_i$  is the fixed effect of the *i* = 5 treatments (control with and without nitrite plus three different concentrations of the selected extract); *j* the random effect of the two batches of canned meat; *r* the repeated measures. Tukey tests for multiple mean comparisons were then performed. Including in the MODEL statement more than one dependent variable, a multivariate analysis of variance with the MANOVA statement to measure the differences in the color characteristics was performed (SAS, 2021).

# 3 | RESULTS AND DISCUSSION

Consumer demand safe and high-quality meat products, increasing the tendency to adhere to all-natural and free-additives label concepts (Alahakoon et al., 2015). Currently, meat processors investigate how to remove the use of nitrates/nitrites to meet the consumers' demands without compromising the safety and sensory characteristics of meat products. Thus, the removal of nitrates/nitrites in meat products must be based by risk assessments. As sterilization might ensure itself product's microbiological safety in this study. Additionally, a previous evaluation of the behavior of enterotoxin-producing Staphylococcus aureus strains in canned meat demonstrated that nitrite was ineffective against both S. aureus growth and enterotoxin production (Grispoldi et al., 2019). Most of the literature regarding potential natural alternatives to the addition of nitrates and nitrites in processed meat is aimed at vegetable juice and extracts (e.g., celery, lettuce, radish, and spinach), which are well-known by its high levels of nitrate contents (Santamaria, 2006). According to a Food Standards Agency [FSA] (2015) "...the indirect addition of nitrates to foods via nitrate-rich extracts of vegetables, such as spinach or celery, should be considered an additive use, and not a food use. In such cases, the extract is being added for preservation, as it contains a standardised level of nitrate and consequently, such use would not be permitted by Regulation 1333/2008, as these extracts have not been approved as preservatives...".

A few years later, the Food Safety and Inspection Service [FSIS] (2019) indicated that "...the Centre for Science in the Public Interest (CSPI) petitioned FSIS to amend its labelling regulations to prohibit the statements "No Nitrate or Nitrite Added" and "Uncured" on meat products that have been processed using any sources of nitrates or nitrites, including non-synthetic sources, such as celery powder...Additionally, the

	L* coordinate		a* coordinate		b* coordinate	
	Mean	SE	Mean	SE	Mean	SE
СР	18.3	1.5	17.8	1.3	16.7	1.3
CN	26.7*	1.3	12.2	1.4	19.2	0.4
CAPI	20.4	2.2	20.2	0.4	23.4	1.2
PAP	16.4	1.3	18.3	1.1	19.7	1.8
MON	27.3*	2.3	11.9	3.3	33.5*	1.9
RYR1	22.4	3.6	16.7	2.6	20	2.2
RYR2	22.5	6.7	13.9	0.8	20.9	3.7
RYR3	26.6	2.4	8.7*	0.7	17.9	1.4
LYC	19	2.5	14.4	2.8	21.6	1.7
RBJ	23.2	1.5	8.7*	1.8	19.7	1.3
ROS	24.9	2.4	7.7*	4	18.5	1.8
HER	20.6	2.1	2.8*	1.6	16.6	1.3
CHI	21.9	2.7	11.5	1.5	19.2	1.7
CAPp	19.9	0.3	13	0.2	16.1	0.2
CAR	27.2*	0.4	7*	0.1	15.4	0.2
SOR	30.4*	0.4	13.6	0.2	20	0.3

Note: Means in the same row with the superscript asterisk differ significantly from the positive control (p < .05).

Abbreviations: CAPI, capsicum extract liquid phase (capsanthin); CAPp, *Capsicum* extract powder (capsanthin); CAR, carmine pigment powder (cochineal extract); CHI, canned meat with chilli; CN, negative control; CP, positive control; HER, canned meat with herbs; LYC, lycopene powder; MON, monascus yellow powder (*Monascus purpureus*); PAP, paprika oleoresin liquid phase; RYR1, RYR2, RYR3, red yeast rice powder (*Monascus purpureus*) from three different producers; RBJ, red beet juice powder (*Beta vulgaris*); ROS, rosemary extract (*Rosmarinus officinalis*); SOR, sorghum red pigment powder (*Sorghum bicolor*).

petition requested that ingredients used as a source of nitrates or nitrites be declared as such in the product labelling, e.g., "celery powder (source of nitrates or nitrites for curing)." In light of these recent developments, we focused on natural products capable of coloring meat without the use of nitrates or nitrites in any form. Tables 1 and 2 present the colorimetric measurement data and the statistical analysis results for the data obtained prior to and following heat treatment, respectively.

Before the heat treatment, the negative control (CN) presented average color values with a statistically significant difference from the positive control (CP) in  $L^*$  parameter; monascus yellow powder (*Monascus purpureus*, MON) in  $L^*$  and  $b^*$  parameter; red yeast rice powder (*Monascus purpureus*, RYR3) in  $a^*$  parameter; red beet juice powder (*Beta vulgaris*, RBJ), rosemary extract (*Rosmarinus officinalis*, ROS) and spiced canned meat (HER) in  $a^*$  parameter; carmine pigment powder (cochineal extract, CAR) in  $L^*$  and  $a^*$  parameter; sorghum red pigment powder (*Sorghum bicolor*, SOR) in  $L^*$  parameter.

After the heat treatment, the negative control (CN) presented average color values with a statistically significant difference from the positive control (CP) in  $L^*$  and  $a^*$  parameter; *Capsicum* extract liquid phase (capsanthin, CAPI) in  $b^*$  parameter; paprika oleoresin liquid phase (PAP) in  $L^*$  parameter; monascus yellow powder (*Monascus* 

TABLE 2 Color measurements and results of the statistical analysis after heat treatment

	L* coordi	L* coordinate		a* coordinate		b* coordinate	
	Mean	SE	Mean	SE	Mean	SE	
СР	24.4	1.3	15.8	1.6	18.5	1.8	
CN	33*	2.6	8.3*	2.2	21.4	1.8	
CAPI	19.8	0.6	16.8	0.7	23.7*	0.7	
PAP	17.3*	1.3	15.2	0.5	21	1.3	
MON	26.4	0.4	11.3	0.8	32.3*	0.4	
RYR1	17.4*	0.7	13.2	1.2	18.3	1	
RYR2	13.3*	0.5	16.7	1	15.4	0.8	
RYR3	23	1.3	8.8*	0.7	18.3	0.3	
LYC	20	1.7	15.2	1.2	22.2	0.9	
RBJ	23.2	1.7	7.5*	1.2	19.4	1.6	
ROS	19.8	1.5	4.8*	0.6	17.3	1.3	
HER	16.7*	1.8	6.4*	0.3	14.7	0.6	
CHI	21.5	2.7	8.8*	2	19	0.9	
CAPp	36.4*	0.5	16.6	0.2	23	0.3	
CAR	28.7	0.4	14.4	0.2	23.3	0.3	
SOR	25.6	0.4	15.3	0.2	19.2	0.3	

*Note*: Means in the same row with the superscript asterisk differ significantly from the positive control (p < .05).

Abbreviations: CAPI, capsicum extract liquid phase (capsanthin); CAPp, *Capsicum* extract powder (capsanthin); CAR, carmine pigment powder (cochineal extract); CN, negative control; CHI, canned meat with chilli; CP, positive control; HER, canned meat with herbs; LYC, lycopene powder; MON, monascus yellow powder (*Monascus purpureus*); PAP, paprika oleoresin liquid phase; RBJ, red beet juice powder (*Beta vulgaris*); ROS, rosemary extract (*Rosmarinus officinalis*); RYR1, RYR2, RYR3, red yeast rice powder (*Monascus purpureus*) from three different producers; SOR, sorghum red pigment powder (*Sorghum bicolor*).

purpureus, MON) in  $b^*$  parameter; red yeast rice powder (*Monascus purpureus* RYR1 and RYR2) in  $L^*$  parameter; red yeast rice powder (*Monascus purpureus*, RYR3) in  $a^*$  parameter; red beet juice powder (*Beta vulgaris*, RBJ) and rosemary extract (*Rosmarinus officinalis*, ROS) in  $a^*$  parameter; canned meat with herbs (HER) in  $L^*$  and  $a^*$  parameter; spiced canned meat (CHI) in  $a^*$  parameter; *Capsicum* extract powder (capsanthin, CAPp) in  $L^*$  parameter.

Data obtained after thermal treatment were essential because color thermal stability is essential for a sterilized product. Although nitrites can react to myoglobin and produce heat-stable compounds (Honikel, 2008), this capability has not been known for any of the natural extracts tested. The results obtained during the experiments were considered together with the requests from the factory, and the sorghum red pigment powder was chosen for the second trial. Sorghum red pigment powder is produced from the seed husks of the *Sorghum bicolor* species.

Sorghum husks are an abundant, safe and cheap by-product of sorghum cultivation. Studies have proved that extracts from sorghum husks are nontoxic and not cause health issues (Olifson et al., 1978). From a chemical viewpoint, the pigment extracted from the sorghum husks consists mainly of apigenin ( $C_{15}H_{10}O_5$ ) and 7-O-glucoside

TABLE 3Color measurements and results of the statisticalanalysis after second trial following factory procedures

	L* coordinate		a* coordinate		b* coordinate	
	Mean	SE	Mean	SE	Mean	SE
СР	25.4	3.4	14.7	2.3	19.7	2.2
CN	41.6*	2.6	16.1	0.8	23.2	1.4
SOR 0,1%	26.9	2.3	15.1	0.7	25.6	1.7
SOR 0,2%	25.4	2.9	16.7	1.1	23.3	0.7
SOR 0,3%	22.4	4.2	14.5	1.4	21.7	1.9

*Note*: Means in the same row with the superscript asterisk differ significantly from the positive control (p < .05).

Abbreviations: CN, negative control; CP, positive control; SOR 0,1%, sorghum red pigment powder, 0,1% concentration; SOR 0,2%, sorghum red pigment powder, 0,2% concentration; SOR 0,3%, sorghum red pigment powder, 0,3% concentration.

(C<sub>21</sub>H<sub>20</sub>O<sub>12</sub>). Apigenin in particular is the aglycone of numerous natural glycosides, for which it provides high stability (Awika et al., 2004). In comparison to similar molecules, the increased stability is derived to the lack of a hydroxyl group in the C3 position (Mazza & Brouillard, 1987; Sweeny & Iacobucci, 1981). These molecules are distinguished by resistance to acidulants and pH variations, resistance of their dimeric forms to nucleophilic and hydrophilic attacks by sulfites, improved color stability in the presence of co-pigments, slow ring opening and the formation of chalcones upon exposure to heat, and antioxidant activity. On an industrial scale, sorghum seed husks are used in the extraction process. China is the world's largest producer, producing around two billion kilograms per year. Sorghum husks were pulverized and passed through meshes with 0.8 mm-diameter holes under optimized extraction conditions (Barros et al., 2013). To the powder obtained in this manner, a 70% (v/v) solution of ethanol and water with a 50:1 liquid-solid ratio was added. The extraction was carried out at 80°C for 120 min in a thermostatic water bath equipped with an agitator. The extracted liquid was filtered through a 200 mesh per square inch stainless steel sieve and cooled to room temperature. The filtrate was then centrifuged at 8,000 rpm for 60 min in a high-speed, refrigerator-cooled centrifuge. The supernatant was concentrated using a rotary evaporator under reduced pressure, dried at 80°C in an oven, and finally sterilized. The yield was approximately 6%. The resulting powder was dark brownish-red in color and soluble in ethanol and water but not in solvents or nonpolar oils. The product's aqueous solution was brownish-red in color. The color became lighter in acidic environments and darker in basic environments. Due to the fact that it formed complex compounds with metal ions, it was prudent to avoid contact with them, particularly iron ions. It was resistant to both heat and light (Hou et al., 2017). The results of the color analysis of canned meat produced using sorghum extract at various concentrations (0.1%, 0.2%, and 0.3%) in accordance with factory procedures are shown in Table 3.

Consumer perceptions of changes in the color of canned meat due to the addition of various ingredients have been investigated by several authors. Slowinski et al. (Słowiński et al., 2020) investigated the effect of wheat fiber preparations on the quality of canned meat. While this addition had no effect on the products' fundamental chemical composition (water, protein, fat, collagen, and salt content), water activity, or pH, it did result in a color lightening. The colorimetric analysis revealed that when compared to the control, the  $L^*$  parameter values were significantly ( $p \le .05$ ) higher, the  $a^*$  parameter values (redness) were slightly lower, and the  $b^*$  parameter values (yellowness) were significantly ( $p \le .05$ ) higher. Choi et al. reported a similar reduction in the redness of meat with the addition of dietary fibers (Choi et al., 2008). Another study examined the color of canned pork meat produced without nitrates. The parameter of redness in nitrite-free canned meat was found to be significantly lower than in nitrite-containing products (Ferysiuk & Wójciak, 2020). Additionally, the addition of ginger rhizome resulted in a lighter color and a lower redness contribution to pork canned meat (Draszanowska et al., 2020).

# 4 | CONCLUSIONS

Food additives, such as nitrate and nitrites, are extremely attractive to the meat industry since they are cheap and they keep meat products stable both microbiological and sensory. Indeed, consumers are becoming more aware of the benefits of natural, clean-label products. Producers face numerous obstacles in order to accomplish this goal. More precisely, even without nitrites, the product shall remain safe and the characteristics (color, flavor, taste) must be preserved. The findings of this study show that a powder extracted from sorghum husks (*Sorghum bicolor*) produced heat stable pigments that could be added to a classic canned meat recipe to achieve a product with the typical red/pink color obtained with sodium nitrite.

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#### CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

#### AUTHOR CONTRIBUTIONS

Luca Grispoldi: Conceptualization; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft. Musafiri Karama: Supervision; Validation; Writing – review & editing. Saeed El-Ashram: Supervision; Validation; Writing – review & editing. cristina saraiva: Supervision; Validation; Writing – review & editing. Juan García-Díez: Supervision; Validation; Writing – review & editing. Juan García-Díez: Supervision; Validation; Writing – review & editing. Athanasios Chalias: Formal analysis; Investigation. Matteo De Gennis: Formal analysis. Andrea Vannuccini: Formal analysis. Giusi Poerio: Formal analysis. Paolo Torlai: Funding acquisition; Resources. Giuseppina Chianese: Funding acquisition; Resources. Anna Giovanna Fermani: Supervision; Validation. Salvatore Barbera: Data curation; Software. Beniamino Cenci-Goga: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – review & editing.

# DATA AVAILABILITY STATEMENT

All the data that support the findings of this study are available in the manuscript itself.

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#### REFERENCES

- Alahakoon, A. U., Jayasena, D. D., Ramachandra, S., & Jo, C. (2015). Alternatives to nitrite in processed meat: Up to date. *Trends in Food Science & Technology*, 45(1), 37–49. https://doi.org/10.1016/j.tifs.2015.05.008
- Alexander, D. D., & Cushing, C. A. (2011). Red meat and colorectal cancer: A critical summary of prospective epidemiologic studies. *Obesity Reviews*, 12(5), e472–493. https://doi.org/10.1111/j.1467-789X.2010.00785.x
- Awika, J. M., Rooney, L. W., & Waniska, R. D. (2004). Properties of 3-deoxyanthocyanins from sorghum. *Journal of Agriculture and Food Chemistry*, 52(14), 4388–4394. https://doi.org/10.1021/jf049653f
- Barros, F., Dykes, L., Awika, J. M., & Rooney, L. W. (2013). Accelerated solvent extraction of phenolic compounds from sorghum brans. *Journal of Cereal Science*, 58(2), 305–312. https://doi.org/10.1016/ j.jcs.2013.05.011
- Bernofsky, C., Fox, J. B., Jr., & Schweigert, B. S. (1959). Biochemistry of myoglobin. VII. The effect of cooking on myoglobin in beef muscle. *Journal of Food Science*, 24(4), 339–343. https://doi.org/10.1111/ j.1365-2621.1959.tb17281.x
- Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., Ghissassi, F. E., Benbrahim-Tallaa, L., Guha, N., Mattock, H., & Straif, K. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology*, 16(16), 1599–1600. https://doi.org/10.1016/ s1470-2045(15)00444-1
- Carpenter, C. E., Cornforth, D. P., & Whittier, D. (2001). Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science*, *57*(4), 359–363. https://doi.org/10.1016/S0309 -1740(00)00111-X
- Cenci-Goga, B. T., Karama, M., Hadjichralambous, C., Sechi, P., & Grispoldi, L. (2020). Is EU regulation on the use of antioxidants in meat preparation and in meat products still cutting edge? *European Food Research and Technology*, 246(4), 661–668. https://doi. org/10.1007/s00217-020-03433-y
- Cenci-Goga, B. T., Karama, M., Sechi, P., Iulietto, M. F., Novelli, S., & Mattei, S. (2014). Evolution under different storage conditions of anomalous blue coloration of Mozzarella cheese intentionally contaminated with a pigment-producing strain of Pseudomonas fluorescens. *Journal of Dairy Science*, 97(11), 6708–6718. https://doi. org/10.3168/jds.2014-8611
- Choi, Y.-S., Jeong, J.-Y., Choi, J.-H., Han, D.-J., Kim, H.-Y., Lee, M.-A., Kim, H.-W., Paik, H.-D., & Kim, C.-J. (2008). Effects of dietary fiber from rice bran on the quality characteristics of emulsion-type sausages. *Korean Journal for Food Science of Animal Resources*, 28(1), 14–20. https://doi.org/10.5851/KOSFA.2008.28.1.14
- Corpet, D. E., De Smet, S., & Demeyer, D. (2014). Epidemiological evidence for the association between red and processed meat intake and colorectal cancer. *Meat Science*, 98(2), 115. https://doi. org/10.1016/j.meatsci.2014.05.008
- Draszanowska, A., Karpińska-Tymoszczyk, M., & Olszewska, M. A. (2020). The effect of ginger rhizome and refrigerated storage time on the quality

of pasteurized canned meat. *Food Science and Technology International*, 26(4), 300–310. https://doi.org/10.1177/1082013219889439

- Ferysiuk, K., & Wójciak, K. M. (2020). The possibility of reduction of synthetic preservative E 250 in canned pork. *Foods*, 9(12), 1869. https://doi.org/10.3390/foods9121869
- Food Safety and Inspection Service. (2019). 2019 Petition no nitrates added CSPI CU\_COMPLETE. https://www.regulations.gov/docum ent/FSIS-2019-0022-0001
- Food Standards Agency (FSA). (2015). Food additives legislation guidance to compliance.
- Gangolli, S. D., van den Brandt, P. A., Feron, V. J., Janzowsky, C., Koeman, J. H., Speijers, G. J. A., Spiegelhalder, B., Walker, R., & Wishnok, J. S. (1994). Nitrate, nitrite and N-nitroso compounds. *European Journal of Pharmacology*, 292(1), 1–38. https://doi. org/10.1016/0926-6917(94)90022-1
- Grispoldi, L., Popescu, P. A., Karama, M., Gullo, V., Poerio, G., Borgogni,
  E., Torlai, P., Chianese, G., Fermani, A. G., Sechi, P., & Cenci-Goga,
  B. (2019). Study on the growth and enterotoxin production by Staphylococcus aureus in canned meat before retorting. Toxins (Basel), 11(5), 291. https://doi.org/10.3390/toxins11050291
- Hiramoto, K., Kato, T., & Kikugawa, K. (1993). Generation of DNAbreaking activity in the Maillard reaction of glucose-amino acid mixtures in a solid system. *Mutation Research*, 285(2), 191–198. https://doi.org/10.1016/0027-5107(93)90106-p
- Honikel, K.-O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science*, 78(1), 68–76. https:// doi.org/10.1016/j.meatsci.2007.05.030
- Hospital, X. F., Hierro, E., Arnau, J., Carballo, J., Aguirre, J. S., Gratacós-Cubarsí, M., & Fernández, M. (2017). Effect of nitrate and nitrite on Listeria and selected spoilage bacteria inoculated in dry-cured ham. *Food Research International*, 101, 82–87. https://doi.org/10.1016/ j.foodres.2017.08.039
- Hou, X., Fang, F., Guo, X., Wizi, J., Ma, B., Tao, Y., & Yang, Y. (2017). Potential of sorghum husk extracts as a natural functional dye for wool fabrics. ACS Sustainable Chemistry & Engineering, 5(6), 4589– 4597. https://doi.org/10.1021/acssuschemeng.6b02969
- Karwowska, M., Kononiuk, A., & Wójciak, K. M. (2019). Impact of sodium nitrite reduction on lipid oxidation and antioxidant properties of cooked meat products. *Antioxidants (Basel)*, 9(1), 9. https://doi. org/10.3390/antiox9010009
- Kim, D., Lee, Y.-J., Ryu, H.-Y., Lee, J.-H., Kim, H.-K., Kim, E., Moon, J.-D., Chang, D. D., & Yoon, H.-S. (2013). Genetic polymorphisms in metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine. *Journal of Applied Toxicology*, 33(1), 63–70. https://doi.org/10.1002/ jat.1712
- King, A. M., Glass, K. A., Milkowski, A. L., Seman, D. L., & Sindelar, J. J. (2016). Modeling the impact of ingoing sodium nitrite, sodium ascorbate, and residual nitrite concentrations on growth parameters of Listeria monocytogenes in cooked, cured pork sausage. *Journal of Food Protection*, 79(2), 184–193. https://doi.org/10.4315/0362-028x.jfp-15-322
- Lebrun, S., Van Nieuwenhuysen, T., Crèvecoeur, S., Vanleyssem, R., Thimister, J., Denayer, S., Jeuge, S., Daube, G., Clinquart, A., & Fremaux, B. (2020). Influence of reduced levels or suppression of sodium nitrite on the outgrowth and toxinogenesis of psychrotrophic *Clostridium botulinum* Group II type B in cooked ham. *International Journal of Food Microbiology*, 334, 108853. https://doi. org/10.1016/j.ijfoodmicro.2020.108853
- MacDougall, D. (1994). Colour of meat quality attributes and their measurement in meat, poultry and fish products (pp. 79–93). Springer.
- Mahgoub, S. A. M., Osman, A., & Ramadan, M. F. (2017). Inhibitory effect of Nigella sativa oil against *Listeria monocytogenes* and *Salmonella Enteritidis* inoculated in minced beef meat. *Journal of Food Measurement and Characterization*, 11(4), 2043–2051. https:// doi.org/10.1007/s11694-017-9587-1
- Mazza, G., & Brouillard, R. (1987). Color stability and structural transformations of cyanidin 3,5-diglucoside and four 3-deoxyanthocyanins

in aqueous solutions. Journal of Agricultural and Food Chemistry, 35(3), 422-426. https://doi.org/10.1021/jf00075a034

- Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., Overvad, K., Olsen, A., Tjønneland, A., Clavel, F., Boutron-Ruault, M.-C., Kesse, E., Boeing, H., Bergmann, M. M., Nieters, A., Linseisen, J., Trichopoulou, A., Trichopoulos, D., Tountas, Y., ... Riboli, E. (2005). Meat, fish, and colorectal cancer risk: The European Prospective Investigation into cancer and nutrition. *Journal of the National Cancer Institute*, 97(12), 906–916. https://doi.org/10.1093/jnci/dji164
- Olifson, L. E., Osadchaia, N. D., Chaĭkovskaia, E. V., & Semchenko, I. P. (1978). Novyĭ pishchevoĭ krasitel' iz plenki zerna sorgo i ego toksikologicheskaia kharakteristika [New food dye from sorghum grain hull and its toxicological characteristics]. Voprosy pitaniia, 76–80. Russian. PMID: 629038.
- Parthasarathy, D. K., & Bryan, N. S. (2012). Sodium nitrite: The "cure" for nitric oxide insufficiency. *Meat Science*, 92(3), 274–279. https://doi. org/10.1016/j.meatsci.2012.03.001
- Rossi, C., Chaves-López, C., Serio, A., Goffredo, E., Goga, B. T. C., & Paparella, A. (2016). Influence of incubation conditions on biofilm formation by *Pseudomonas fluorescens* isolated from dairy products and dairy manufacturing plants. *Italian Journal of Food Safety*, 5(3), 5793. https://doi.org/10.4081/ijfs.2016.5793
- Saad, A. M., Osman, A. O. M., Mohamed, A. S., & Ramadan, M. F. (2020). Enzymatic hydrolysis of phaseolus vulgaris protein isolate: Characterization of hydrolysates and effect on the quality of minced beef during cold storage. *International Journal of Peptide Research and Therapeutics*, 26(1), 567–577. https://doi.org/10.1007/s10989-019-09863-x
- Sanchez-Echaniz, J., Benito-Fernández, J., & Mintegui-Raso, S. (2001). Methemoglobinemia and consumption of vegetables in infants. *Pediatrics*, 107(5), 1024. https://doi.org/10.1542/peds.107.5.1024
- Santamaria, P. (2006). Nitrate in vegetables: Toxicity, content, intake and EC regulation. Journal of the Science of Food and Agriculture, 86(1), 10–17. https://doi.org/10.1002/jsfa.2351
- Silverstein, T. P., Kirk, S. R., Meyer, S. C., & Holman, K. L. (2015). Myoglobin structure and function: A multiweek biochemistry laboratory project. *Biochemistry and Molecular Biology Education*, 43(3), 181–188. https://doi.org/10.1002/bmb.20845
- Sindelar, J. J., & Milkowski, A. L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide*, 26(4), 259–266. https://doi.org/10.1016/j.niox.2012.03.011
- Słowiński, M., Miazek, J., & Chmiel, M. (2020). Influence of the dose and length of wheat fiber on the quality of model sterilized canned meat products. *Foods*, 9(8), 1001. https://doi.org/10.3390/foods9081001
- Suman, S. P., Nair, M. N., Joseph, P., & Hunt, M. C. (2016). Factors influencing internal color of cooked meats. *Meat Science*, 120, 133–144. https://doi.org/10.1016/j.meatsci.2016.04.006
- Sweeny, J. G., & lacobucci, G. A. (1981). Synthesis of anthocyanidins-III. Tetrahedron, 37(8), 1481–1483. https://doi.org/10.1016/S0040-4020(01)92086-
- Turesky, R. J. (2018). Mechanistic evidence for red meat and processed meat intake and cancer risk: A follow-up on the International Agency for Research on Cancer Evaluation of 2015. *Chimia (Aarau)*, 72(10), 718–724. https://doi.org/10.2533/chimia.2018.718

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