Effect of Fermentation Time and Vegetable Concentrate Addition on Quality Parameters of Organic Botifarra Catalano, a Cured−Cooked Sausage

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JUNE 2012

Impact Factor: 2.91 · DOI: 10.1021/jf301218k · Source: PubMed

5 AUTHORS, INCLUDING:

Nuria Magrinya
University of Barcelona
5 PUBLICATIONS **20** CITATIONS
SEE PROFILE

Ricard Bou
IRTA Institute of Agrifood Research and Techn…
52 PUBLICATIONS **713** CITATIONS
SEE PROFILE

Núria Rius
University of Barcelona
27 PUBLICATIONS **313** CITATIONS
SEE PROFILE

Rafael Codony
University of Barcelona
100 PUBLICATIONS **2,240** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Fermentation Time and Vegetable Concentrate Addition on Quality Parameters of Organic *Botifarra Catalana*, a Cured−Cooked Sausage

Núria Magrinyà,[†] Ricard Bou,*[,†] Núria Rius,[‡] Rafael Codony,[†] and Francesc Guardiola[†]

[†]Nutrition and Food Science Department−XaRTA-INSA and [‡]Department of Health Microbiology and Parasitology, Faculty of Pharmacy, University of Barcelona, Avinguda Joan XXIII s/n, 08028 Barcelona, Spain

ABSTRACT: The effects of the addition of two different sources of nitrite (pure $NaNO_2$ or a nitrate-rich vegetable concentrate) and three different fermentation times with nitrate-reducing cultures (6, 12, or 24 h at 16 °C) on microbial counts, pH, residual nitrate and nitrite amounts, and susceptibility to oxidation of *botifarra catalana* sausage were studied. Moreover, curing efficiency, color, tocopherol and tocotrienol contents, oxidative status, and consumer acceptability of these sausages were assessed after vacuum packaging and storage at 4 °C for up to 180 days. Residual nitrate and nitrite amounts were lower than the limits established by the European Union for organic meat products. Longer periods of fermentation produced higher meat curing efficiency ratios, whereas consumer acceptability scores were highest for sausages with added vegetable concentrate. Storage of the sausages caused small quality changes. Therefore, these results indicate that vegetable concentrate is a useful alternative for organic cured−cooked meat products.

KEYWORDS: *botifarra catalana sausage, cured-cooked meat, organic meat, nitrate and nitrite reduction, Staphylococcus carnosus*

INTRODUCTION

Botifarra catalana is a cured−cooked meat product typical of Catalonia (northeastern Spain); its process of manufacturing resembles that of cooked−cured ham. In August 2008, European Union (EU) and Spanish regulations prohibited the trade in and use of heat-treated meat products containing nitrate additives. Therefore, the only chemical curing agent currently permitted is nitrite.[1,2] Before that, pork butchers traditionally mixed diced or coarse ground pork meat with jowl fat, salt, pepper, and nitrate and nitrite as curing agents to produce botifarra catalana. This mixture was stored in a cool place for a period of time and, thereafter, stuffed into natural casings and finally cooked.[3]

The past practice of storing the mixed ingredients in a cool place allowed the fermentation and reduction of nitrate to nitrite. This resulted in the development of the typical cooked−cured meat pigment, as well as the characteristic aroma and flavors of this sausage. The current conventional production of botifarra catalana does not require a period of fermentation to reduce nitrate to nitrite. Therefore, the time needed to produce botifarra catalana has been considerably shortened, although some sensory differences may exist between the products obtained by the different procedures.

The addition of either nitrite or nitrate prevents the growth of spoilage and pathogenic bacteria, such as *Clostridium botulinum*, and prevents lipid oxidation.[4] However, nitrate and nitrite must be used with caution because they can produce nitroso compounds under certain conditions, some of which are specific and potent carcinogens.[4] For this reason, the EU has established the maximum amount of nitrite that can be added to organic and conventional cooked−cured meat products, as well as the maximum amounts of residual nitrate and nitrite that organic cooked−cured meat products can contain.[1,5]

Traditional and organic products are more expensive than their conventional counterparts. Moreover, organic food is one of the fastest growing areas of the food market in Europe, North America, Australia, and Japan. Some consumers are willing to pay more for organic food because they perceive these products to be healthier and of higher quality. Moreover, animal welfare, better taste, and food free of additives are other reasons for buying organic meat products.[6,7] However, the withdrawal or reduction of nitrite sources as chemical additives from cooked−cured meats may result in products with a beige color, thus possibly lowering consumer acceptance.[8,9] Therefore, it is important to find a substitute for nitrite that reproduces the characteristic cured-meat color while still providing clean-label products without chemical additives. Vegetables and their concentrates contain high amounts of nitrate. Once they are added to sausages, nitrate can be reduced to nitrite, and they offer the greatest potential of introducing natural sources of nitrite into processed meats.[10]

Thus, not only do nitrate-rich vegetable concentrates represent an alternative to conventional production but their use also mimics the former production of botifarra catalana. This enables the recovery of old practices as well as the traditional aromas and flavors. However, regardless of the nitrate source, it has to be first reduced to nitrite and then reduced again to nitric oxide to form the coordinate−covalent complex with heme pigments that produces the pink color of cooked−cured meats.[11] Therefore, it is necessary for nitrate-reducing cultures, which have been commercially available for several years, to reduce nitrate to nitrite for meat curing.

Received: March 20, 2012
Revised: May 21, 2012
Accepted: June 13, 2012
Published: June 13, 2012

dx.doi.org/10.1021/jf301218k | *J. Agric. Food Chem.* 2012, 60, 6882−6890

Staphylococcus species with nitrate reductase activity are commonly used as starter cultures for dry-fermented sausages.[10,12]

The ripening of dry-fermented sausages ensures the complete reduction of nitrate, but for this conversion to take place in cooked−cured products, an incubation period is required, normally achieved immediately before thermal processing.[10] Hence, it is important to determine the proper conditions of early and rapid fermentation for the correct development of the cured color and sensory properties.

Thus, the objective of this study was to determine the effects of a nitrate-rich vegetable concentrate compared to the conventional nitrite addition on various quality characteristics of botifarra catalana sausages over an extended storage period. Different cultures, including *Staphylococcus carnosus*, which possess intense nitrate reductase activity, were added to the sausage formulation to allow the reduction of nitrate to nitrite. Due to this, different fermentation times were also studied to determine the optimum time for this conversion to take place.

MATERIALS AND METHODS

Reagents and Standards. A meat starter culture for bioprotection containing *Lactobacillus sakei* and *Staphyloccocus xylosus* (B-FM SafePro), a starter culture containing *S. carnosus* with intense nitrate reductase activity (CS-300 BactoFerm), and a vegetable concentrate (celery and carrot) rich in nitrates (NATASY CC 227) were obtained from CHR Hansen (Hørsholm, Denmark). Sodium nitrite used as pure sodium nitrite source (99.6%) was from Merck (Darmstadt, Germany). Sodium ascorbate and dextrose were obtained from Espècies Teixidor (Manresa, Spain). Tocopherol standards were obtained from Calbiochem (San Diego, CA, USA). All chemicals used were of ACS grade except the solvents used in the induced ferrous oxidation−xylenol orange (FOX) method, Hornsey's method, and tocopherol plus tocotrienol determination, which were all of HPLC grade.

Experimental Design. Six treatments resulted from a 2 × 3 factorial design to study the effects of two different sources of nitrite (either pure $NaNO_2$ or a nitrate-rich vegetable concentrate providing the equivalent of 80 mg of $NaNO_2$/kg) and three different times of fermentation (6, 12, or 24 h at 16 °C) on various cooked−cured meat quality parameters. The amount of nitrite used was the maximum level of ingoing sodium nitrite allowed in organic meat products.[5] Two different batters (one for each nitrite source) were prepared from the same homogenized common ingredients. After storage at 4 °C for 72 h, mixed batters were stuffed into natural casings, fermented (6, 12, or 24 h at 16 °C and 95% humidity), cooked, vacuum-packed, and pasteurized as described later. For each treatment, two sausages from different casings were subjected to chemical analyses, treated as replicates, and stored at 4 °C for 0, 60, 120, and 180 days, thus resulting in 48 samples. The remaining sausages were subjected to sensory analysis and stored under the same conditions for 60 days, with sausages corresponding to each treatment being treated as a single sample.

Sausage Preparation and Sampling. Sausages were manufactured according to a traditional formula: 850 g/kg of lean organic pork meat; 100 g/kg of organic pork jowl fat; 50 g/kg of cold spring water; 0.25 g/kg of each starter culture; 0.5 g/kg of sodium ascorbate; 3 g/kg of dextrose; 18 g/kg of salt; 3 g/kg of ground black pepper, and 80 mg/kg of pure $NaNO_2$ or 3.345 g/kg of vegetable concentrate rich in nitrates. Salt, black pepper, dextrose, each nitrite source, the two starter cultures, and sodium ascorbate were mixed in cold spring water before being added to improve ingredient homogenization.

To prepare each of the two batters (one for each nitrite source), a mixture of diced pork meat plus diced jowl fat was first minced (8 mm in diameter). After mixing, salt, ground black pepper, and dextrose were added to the batter. Then, the nitrite source was added to the mixture and homogenized for 1 min. After homogenization, the meat starter culture for bioprotection (B-FM SafePro) and the culture with intense nitrate reductase activity (CS-300 Bactoferm) were added before homogenization for 2 min. Finally, sodium ascorbate was added to the mixture, which was further homogenized for 4 min. As for batter characterization, a 275 g sample was collected and finely ground (Retsch knife mill model Grindomix GM200; Haan, Germany) and vacuum-packed into high-barrier multilayer bags (Cryovac BB325; approximately 20 g of meat/bag) and stored at −25 °C until chemical analyses. Unless specified, batter samples were analyzed twice.

The remaining two batters containing different sources of nitrite were stored for 72 h at 4 ± 2 °C. After storage, each batter was subdivided into three batters, which were then stuffed into seven different natural casings (50−55 mm in diameter) and string-tied. Five sausages were made to weigh about 500 g and were subjected to sensory and microbiological analyses. Two more sausages, treated as duplicates, were made to weigh about 1200 g to analyze chemically. The seven resulting sausages from each batter corresponded to a single period of fermentation (6, 12, or 24 h) at 16 °C and 95% humidity. After this fermentation period, a sample of about half of the weight of one 500 g sausage was aseptically taken for microbiological analysis and the casing string tied again. The seven sausages of each treatment were cooked in a cooking pot containing 50 L of tap water, 200 g of salt, and 1 L of commercial vegetable broth and heated as follows: first, heating at 40 °C for 2 h, then at 60 °C for 2 h, and, finally, at 75 °C until a temperature of 68 °C was reached inside the sausage. After cooking and cooling, the sausages undergoing chemical analyses were cut into four pieces, and these plus the ones for sensory analyses were then individually placed in a barrier bag (Cryovac HT3050; 325 × 550 mm; permeability to oxygen = 15 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 °C and 0% RH; approximately 500 g of sausage/bag), vacuum-packaged, and pasteurized at 80 °C for 25 min. Finally, pasteurized sausages destined for sensory analysis were stored at 4 °C for 60 days, and those for chemical analyses were stored at 4 °C for 0, 60, 120, and 180 days. Following the storage period, the sausages were finely ground (Robot Coupe mixer model BX3; Jackson, MS, USA), vacuum-packed in high-barrier multilayer bags (Cryovac BB325; 130 × 180 mm; permeability to oxygen = 25 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 °C and 0% RH; approximately 15 g of meat/bag), and stored at −25 °C until chemical analysis. Unless otherwise specified, each replicate was analyzed twice.

Moisture Determination. The ISO 1442 procedure was used to determine the level of moisture of the samples.[13] Moisture was used to express some results on a dry weight basis.

Determination of Crude Fat Content and Fatty Acid Composition. The fat content of the raw mix batters was measured according to AOAC Official Method 991.36.[14] The fatty acid composition of the raw mix batters was determined by gas chromatography. First, lipid extraction was carried out with 20 mL of chloroform/methanol (2:1, v/v) in 1.5 g of raw meat, which was subsequently re-extracted twice by using 10 mL of the same solvent mixture each time. Then, fatty acid methyl esters were prepared from the lipid fraction using sodium methoxide and BF_3.[15] Fat content was expressed on a fresh weight basis, whereas fatty acid composition was expressed as a percentage of area normalization.

Microbiological Analysis and pH Determination. From one sausage weighing approximately 500 g, half of the weight was aseptically collected from the casing after each fermentation period. Only about 50 g was stored at 4 °C until the analysis, which was started the day after and analyzed as described elsewhere.[16] Microbiota is destroyed by cooking and hence, lactobacilli and total staphylococci were not analyzed at the different storage time points. After fermentation, the leftovers of the aseptically collected samples were used to measure pH in quintuplicate using a Crison pH 25 model pH-meter, and the average was treated as a single measurement (Crison Instruments, S.A., Alella, Barcelona, Spain). The pH of the raw mix batters before fermentation was also measured in quintuplicate.

Nitrate and Nitrite Determination. The determination of the nitrate and nitrite contents in raw mix batters and sausages was made as described elsewhere.[16] Results were expressed on a fresh weight basis.

Total and Cured Pigment Analysis. Mononitrosylhemochrome and total pigment concentrations were measured after extraction in 80% acetone and acidified acetone, respectively, using Hornsey's method.[17]

Color Measurements. Color was measured by a Konica Minolta Chroma-meter (model CR-410; Konica Minolta Sensing Inc., Osaka, Japan) based on the CIE $L^*a^*b^*$ color space. CIE (Commission International de l'Eclairage) L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) were determined from five different random surfaces of the ground samples, and the average of each parameter was treated as a single measurement. The CIE $L^*a^*b^*$ color space was transformed into the L^*C^*h color space, where L^* represents lightness, C^* represents chroma, and h represents the hue angle, by the following formulas:

$$h = \tan^{-1}\left(\frac{(b^*)}{(a^*)}\right)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

The instrument was set at illuminant D-65 and a 2° observer angle and standardized using a standard white plate.

Tocopherol and Tocotrienol Determination. Four grams of vegetable concentrate or 2 g of raw mix batters or sausages samples were weighed and saponified at 70 °C for 30 min with methanolic KOH.[18] The unsaponifiable matter was subsequently extracted with petroleum ether, filtered, evaporated, and dissolved in *n*-hexane prior to HPLC determination.[19] Results for the vegetable concentrate were expressed as milligrams of each tocopherol and tocotrienol per kilogram on a fresh weight basis. In raw mix batters and sausages, α-tocopherol was the only analyte found above the limit of quantification. Results for raw mix batters were expressed as milligrams of α-tocopherol per kilogram on a fresh weight basis, whereas results for sausages were expressed on a dry weight basis.

Oxidative Status and Susceptibility to Oxidation. Lipid hydroperoxide (LHP) levels were determined by means of the ferrous oxidation−xylenol orange (FOX) method.[20] This method is useful for measuring LHP content and the susceptibility of samples to oxidation. The LHP content was determined after 30 min of incubation, whereas the time course of LHP formed after incubation over 378.5 h was used to calculate susceptibility to oxidation by using various parameters described elsewhere.[21] The induced FOX assay to measure oxidation susceptibility was carried out only once.

Secondary oxidation was determined by means of thiobarbituric acid (TBA) values through third-derivative spectrophotometry after acid aqueous extraction.[22]

Sensory Analysis. A consumer test was carried out to measure the overall acceptability of sausages after storage for 60 days at 4 °C in vacuum packaging. All treatments were randomly presented to an untrained panel of 34 consumers aged between 20 and 60 years who regularly consumed cooked−cured meat products (≥1 per week), botifarra catalana being one of them. Consumer panelists were asked to rank the overall acceptability of the product on a 9-point scale (1 = very bad; 9 = very good). Each consumer had several slices of sample sausage that were placed on white plastic dishes, identified by random three-digit numbers and served to the consumer panel at room temperature. Water and unsalted crackers were provided to panelists to cleanse their palates between tasting the samples.

Statistical Analysis. A multifactor ANOVA determined significant differences produced by the different factors on microbiological counts, pH, residual nitrate and nitrite contents, mononitrosylhemochrome and total pigment concentrations, color measurements, tocopherol and tocotrienol concentrations, LHP content, induced FOX assay parameters, TBA values, and overall acceptability. The studied factors were nitrite source (pure $NaNO_2$ or a vegetable concentrate rich in nitrates), fermentation time (6, 12, or 24 h), and storage time (0, 60, 120, and 180 days), except for microbiological analyses, pH, residual nitrate and nitrite contents, induced FOX assay parameters, and overall acceptability, where only the nitrite source and fermentation time were the studied factors. Interactions between more than two factors were ignored. When significant interactions were found between two factors, series of one-way ANOVAs (for factors with more than two levels) or *t* tests (for factors with two levels) were performed for each factor by fixing the other factor at each specific level. In all cases, $P \leq 0.05$ was considered to be significant. When significant differences were found through the multifactor or one-way ANOVAs, the least-squares means and means were separated using Scheffé's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Moisture, Crude Fat, Fatty Acid Composition, and pH of Raw Mix Batters. The average moisture level of the raw mix batters containing pure sodium nitrite and vegetable concentrate were 68.61 ± 0.08 and 68.18 ± 0.06%, respectively. The crude fat contents of the raw mix batter with pure sodium nitrite was 10.1 ± 0.20% and that of the vegetagle concentrate, 10.6 ± 0.25%. The relative percentages of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids of the raw mix batter containing pure sodium nitrite were 35.72, 48.94, and 15.34%, respectively, and 34.93, 48.83, and 16.24%, respectively, for the batter with vegetable concentrate. The average pH values of the raw mix batters containing pure sodium nitrite and vegetable concentrate were 5.88 ± 0.05 and 5.92 ± 0.06, respectively. Therefore, the moisture level, crude fat content, fatty acid composition, and pH were similar in both raw mix batters.

Microbiological Analyses and pH Determination. Lactobacilli and total staphylococci were analyzed in sausages just after fermentation, and the results are shown in Table 1.

Table 1. Effect of Fermentation Time and Nitrite Source on Microbial Counts and pH in Fermented Botifarra Catalana before Cooking[a]

	lactobacilli (CFU/g)	staphylococci (CFU/g)	pH
fermentation time (h)			
6	1.28×10^9	3.19×10^7	5.72 z
12	6.76×10^8	2.24×10^7	5.50 y
24	7.49×10^8	2.03×10^7	5.33 x
SEM[b]	7.15×10^8	1.19×10^7	0.014
nitrite source[c]			
pure $NaNO_2$	1.16×10^9	2.98×10^7	5.51
vegetable concentrate	6.42×10^8	1.99×10^7	5.52
SEM	5.11×10^8	8.48×10^6	0.012

[a]Values given in this table correspond to the least-squares means obtained from multifactor ANOVA ($n = 6$). Least-squares means within the same column for the same factor with different letters differ significantly ($P \leq 0.05$). [b]SEM, standard error of the mean. [c]Both the pure $NaNO_2$ and the vegetable concentrate were used at a concentration equivalent to 80 mg of $NaNO_2$/kg raw mix batter.

There were no differences in the levels of lactobacilli and total staphylococci between treatments. The recorded microbial growths were in agreement with those reported by Scannell et al.[23] in hams to which *L. sake* and *S. carnosus* cultures had been added followed by incubation at 18 °C for either 3 or 7 days. Moreover, the pH drop compared to raw mix batters indicated that these microorganisms participated in the fermentation.

Nitrate and Nitrite Amounts. Nitrate and nitrite amounts were analyzed in both nitrite sources (the pure $NaNO_2$ and the vegetable concentrate). The pure sodium nitrite source contained a 99.6 ± 0.85% of $NaNO_2$, whereas the vegetable concentrate contained 21493 ± 63 mg NO_3^-/kg and 3.2 ± 1.8

Table 2. Effect of Fermentation Time and Nitrite Source on Residual Nitrate and Nitrite Amounts, Susceptibility to Oxidation (AUC), and Consumer's Overall Acceptability of Cured−Cooked Botifarra Catalana[a]

	residual nitrate[b] (mg/kg)	residual nitrite[c] (mg/kg)	AUC[d] ((mmol CHP equiv kg^{-1}) × h)	overall acceptability[e]
fermentation time (h)				
6	5.1	11.2 z	930	5.5
12	5.2	4.3 y	920	5.5
24	4.5	1.5 x	990	5.2
SEM[f]	0.86	0.24	44	0.24
nitrite source[g]				
pure $NaNO_2$	5.6	5.9	880 x	5.1 x
vegetable concentrate	4.3	5.5	1010 y	5.7 y
SEM	0.70	0.20	36	0.20

[a]Values given in this table correspond to the least-squares means obtained from multifactor ANOVA (n = 12, 12, 204, and 12 for residual nitrate, residual nitrite, overall acceptability, and AUC, respectively). Least-squares means within the same column for the same factor with different letters differ significantly ($P \leq 0.05$). [b]Residual nitrate is expressed as mg of $NaNO_3$ per kg of sausage. Storage time = 0 days. [c]Residual nitrite is expressed as mg of $NaNO_2$ per kg of sausage. Storage time = 0 days. [d]Results are the area under the curve (AUC) of lipid hydroperoxide formation determined by means of the induced ferrous oxidation−xylenol orange (FOX) assay (incubation for 378.5 h) and expressed as mmol of cumene hydroperoxide equivalents per kg of sausage as dry weight × hours. Storage time = 0 days. [e]Overall acceptability was ranked using a 9-point scale (where 1 = very bad and 9 = very good). Storage time = 60 days at 4 °C. [f]SEM, standard error of the mean. [g]Both the pure $NaNO_2$ and the vegetable concentrate were used at a concentration equivalent to 80 mg $NaNO_2$/kg raw mix batter.

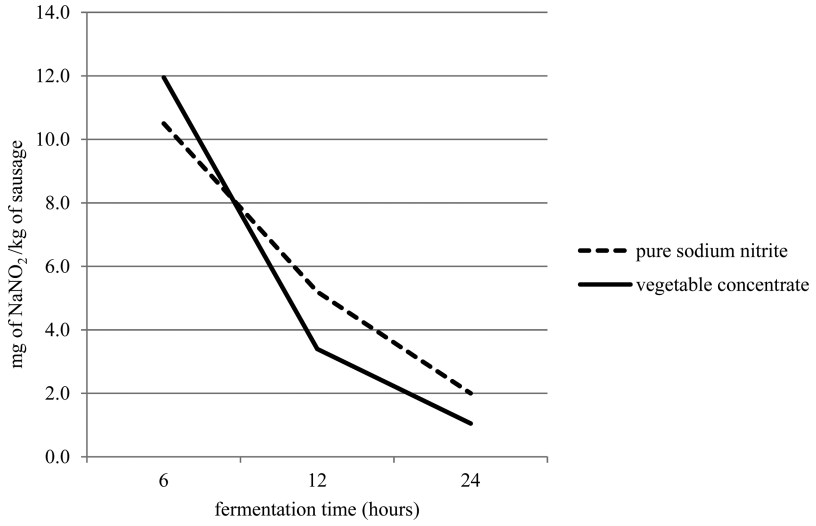

Figure 1. Interaction between fermentation time and nitrite source for the residual nitrite content in sausages.

mg NO_2^-/kg. After the addition of 3.345 g/kg of vegetable concentrate, the raw mix batter, expressing the content of NO_3^- and NO_2^- as sodium salts, contained 97 ± 1 mg sodium nitrate/kg and 1 ± 0.1 mg sodium nitrite/kg. Therefore, the sum of NO_3^- and NO_2^- in this raw mix batter, expressed as sodium nitrite, is equivalent to the intended dose of 80 mg $NaNO_2$/kg/kg. The other raw mix batter, after the addition of 80 mg of pure $NaNO_2$/kg, contained 61.7 ± 0.6 mg sodium nitrate/kg and 20.5 ± 0.4 mg sodium nitrite/kg. Nitrite is very reactive and is quickly reduced to NO and also oxidized to NO_3^-. In fact, various authors have found residual nitrate contents in fermented sausages formulated with nitrite.[24−26] This oxidative reaction has been attributed to the presence of various compounds in the meat.[9,27−31] First, the presence of endogenous enzymatic (i.e., catalase and xanthin oxidase) or nonenzymatic oxidizing (hydrogen peroxide) agents can convert nitrite into nitrate. Second, the presence of oxymyoglobin has also been reported to play a role in forming nitrate from nitrite in a simultaneous redox reaction. Finally, the most convincing explanation involves the participation of myoglobin (Mb), ascorbate, and oxygen.[9] In this reaction, metmyoglobin (metMb) in the presence of ascorbate is reduced as follows:

$$2\text{metMb} + \text{ascorbate} \leftrightarrow 2\text{Mb} + \text{dehydroascorbate}$$

Mb and nitrite are then simultaneously oxidized to metMb and nitrate, respectively. Finally, ascorbate is regenerated from the reduction of dehydroascorbate as shown below:

$$2\text{Mb} + \text{O}_2 + \text{NO}_2^- \leftrightarrow 2\text{metMb} + 2\text{NO}_3^- + 2\text{e}^-$$

$$\text{dehydroascorbate} + 2\text{e}^- \rightarrow \text{ascorbate}$$

Residual nitrate amounts in sausages were determined after pasteurization, and the results are given in Table 2. Nitrate residual amounts were far below the limit established for organic production in all cases.[5] Despite the vegetable concentrate being rich in nitrate, this content was reduced most likely due to the intense nitrate reductase activity of *S. carnosus*.[32] Provided that this activity increases at higher temperatures, the microorganism, during the cooking procedure in which the temperature is initially held for 2 h at 40 °C, may be responsible for the almost complete nitrate reduction.[12,33] These results were in agreement with other

dx.doi.org/10.1021/jf301218k | J. Agric. Food Chem. 2012, 60, 6882−6890

Table 3. Effect of Fermentation Time, Nitrite Source, and Storage Time on Mononitrosylhemochrome, Curing Efficiency, Instrumental Color, Tocopherol Content, LHP Content, and TBA Values on Cured−Cooked Botifarra Catalana[a]

			instrumental color[d]						
	mononitrosylhemochrome[b] (mg/kg)	curing efficiency[c] (%)	L^*	a^*	C^*	h	α-tocopherol[e] (mg/kg)	LHP[f] (μmol CHP kg^{-1})	TBA[g] (μg MDA/kg)
fermentation time (h)									
6	197.3 x	78.2 x	60.52 x	15.96 y	17.98 y	27.22	11.8	234	343
12	204.6 y	80.6 y	61.12 y	15.76 x	17.72 x	27.01	12.0	225	354
24	202.5 y	81.5 y	61.56 y	15.77 x	17.77 x	27.25	11.5	217	313
SEM[h]	1.11	0.41	0.141	0.051	0.045	0.128	0.16	7.0	28
nitrite source[i]									
pure $NaNO_2$	200.1 x	79.8	60.63 x	15.94 y	17.83	26.44 x	10.4 x	229	316
vegetable concentrate	202.8 y	80.4	61.49 y	15.72 x	17.81	27.88 y	13.1 y	221	358
SEM	0.90	0.34	0.115	0.042	0.037	0.105	0.13	5.7	23
storage time (days)									
0	201.5	79.7	60.00 x	15.77 xy	18.47 z	31.39 y	11.64	263 z	273 x
60	203.4	80.6	61.30 y	15.94 y	17.67 y	25.52 x	11.93	233 y	288 x
120	200.6	79.4	61.14 y	15.96 y	17.74 y	25.85 x	11.74	198 x	332 xy
180	200.4	80.6	61.81 y	15.67 x	17.42 x	25.89 x	11.70	208 x	452 y
SEM	1.28	0.48	0.162	0.059	0.052	0.148	0.19	8.1	32

[a]Values given in this table correspond to the least-squares means obtained from multifactor ANOVA ($n = 48$). Least-squares means within the same column for the same factor with different letters differ significantly ($P \leq 0.05$). [b]Results are expressed as mg of mononitrosylhemochrome per kg of sausage as dry weight. [c]Curing efficiency expressed as the percentage of the concentration of mononitrosylhemochrome divided by the concentration of total heme pigments; both concentrations are expressed per kg as dry weight. [d]L^*, lightness; a^*, redness; b^*, yellowness; chroma (C^*), root of the sum of the squares of a^* and b^* used to express color saturation; hue angle (h), arctangent of the quotient of b^*/a^* used to express color hue ($h = 0$, true red; $h = 90$, true yellow). [e]Results are expressed as mg of α-tocopherol per kg of sausage as dry weight. [f]Results are expressed as μmol of cumene hydroperoxide equivalents per kg of sausage as dry weight. [g]Results are expressed as μg of malondialdehyde per kg of sausage as dry weight. [h]SEM, standard error of the mean. [i]Both the pure $NaNO_2$ and the vegetable concentrate were used at a concentration equivalent to 80 mg $NaNO_2$/kg raw mix batter.

studies using this microorganism[16,34,35] and also explained why the nitrite source had no effect on the residual nitrate content.

Residual nitrite amounts in sausages were determined after pasteurization, and the results are shown in Table 2. Similar to nitrate, nitrite residual amounts were also far below the limit established for organic production.[5] The addition of pure sodium nitrite or vegetable concentrate had no influence on residual nitrite content. However, longer fermentation times led to lower residual nitrite contents because of the complete reaction of nitrite into nitric oxide, which subsequently reacted with the heme moiety to form colored nitrosylheme complexes characteristic of cured meat pigments.[9]

A significant interaction between fermentation time and nitrite source was found for the residual nitrite content in sausages. A higher reduction of residual nitrite was observed in sausages to which vegetable concentrate was added (Figure 1). In fact, by statistically analyzing each factor separately, it was found that fermentation time decreased residual nitrite content for both nitrite sources and that there were no differences between the nitrite sources at 6 or 24 h of fermentation, whereas at 12 h the pure sodium nitrite addition produced sausages with higher residual nitrite amounts. The formation of nitric oxide from nitrite is facilitated by reductants such as ascorbate, but other factors such as pH or the redox potential could be modified by the vegetable concentrate, thus governing the reaction and explaining this interaction.[10,36]

Total and Cured Pigment Analyses. The mononitrosylhemochrome concentrations of the sausages are given in Table 3. This table also shows the efficiency of meat curing expressed as follows: curing efficiency (%) = (mg/kg mononitrosylhemochrome)/(mg/kg total heme pigments) × 100. The majority of cured meat products are considered to be acceptable when the pigment conversion ratio is 80% or higher.[17] Increasing the time of fermentation generated sausages with higher concentrations of mononitrosylhemochrome and, as a consequence, higher curing efficiencies. Cured pigment concentrations increasing with fermentation time have also been observed by other authors.[35,37] The relatively high residual nitrite amounts found after 6 h of fermentation may partly explain the lower mononitrosylhemochrome level and curing efficiency. Overall, after 12 h of fermentation, pigment conversion can be considered as acceptable because the curing efficiency obtained at that time was not different from that after 24 h of fermentation.

In relation to the nitrite source, analysis of mononitrosylhemochrome concentration revealed that concentrations were higher in sausages with vegetable concentrate. Although there were no differences for this factor in the curing efficiency, an acceptable curing efficiency of 80% was observed when vegetable concentrate was added. Vegetable concentrate may slightly change the pH and/or redox potential, thus positively affecting the conversion of nitrite into nitric oxide and, consequently, nitrosylhemochrome content (Table 3). Accordingly, Terns et al.[38] suggested that the presence of ascorbic acid or any other reductant in frankfurter-style-cooked sausages with nitrate-rich vegetable juice powder (0.2%) favored the formation of the cured meat pigment.

Neither the nitrosylhemochrome concentration nor the curing efficiency changed in vacuum-packaged sausages stored at 4 °C for up to 180 days (Table 3). Terns et al.[38] studied nitrosylhemochrome content in frankfurters, which were immediately vacuum-packaged after being cooked and stored for 84 days at 2 °C. They found that both the cured pigment concentration and the conversion percentage of total to cured pigment decreased with increased storage times. Conversely, in a preceding study using nitrate-rich vegetable juice powder (0.2−0.4%) in frankfurters, cured pigment concentrations increased over time (stored vacuum-packaged for 90 days at 0−2 °C). The authors explained that the maintenance of residual nitrates and nitrites could have been used as a reservoir for nitrite-related reactions during storage,[35] whereas the depletion of the nitrite source would explain the pigment decrease over the storage period.[38] In both studies, residual nitrite levels on day 0 of storage were much higher than those reported here. However, in our conditions, in which the initial levels of residual nitrate and nitrite amounts were low, the lack of changes in these parameters suggests that this pigment was relatively stable during storage (vacuum-packaged for 180 days at 4 °C).

Color Measurements. Instrumental color was measured in cooked−cured sausages (Table 3). Overall, sausages presented minimal color differences between treatments. However, fermentation time influenced lightness (L^*), redness (a^*), and chroma (C^*), with higher fermentation times producing sausages with higher lightness (L^*) and lower chroma (C^*).

Although lightness has been reported to be affected by various factors in raw meat, cooked−cured, and dry-cured meat products,[39−44] there are few publications reporting the effect of fermentation conditions on the final color of the product. However, the general trend in raw meat indicates that decreased moisture content and water-holding capacity correlate with increased lightness and decreased pH. Thus, the decrease in pH with longer fermentation times of raw sausages (Table 1) may explain the L^* increase in cooked−cured sausages with longer fermentation times (Table 3).

Sausages fermented for 6 h were redder and had higher chroma than those fermented for 12 or 24 h. The addition of vegetable concentrate produced significantly lighter and yellower sausages compared to those made with pure sodium nitrite. Several authors[16,26,38] have found similar results in fermented sausages produced with vegetable concentrate as an indirect source of nitrate and nitrite. These authors reported the intrinsic color of the powder as responsible for these changes in color.

For the study of changes in color values during storage, the literature gives inconsistent results. Overall, in cooked−cured meat products such as ham, bologna sausage, frankfurters, and other emulsified sausages, Hunter's a and CIE a^* values have been reported to decrease with longer storage times.[34,38,45−47] A reasonable explanation involves the nitrosylhemochrome formed during curing, which has been reported to be lost during storage, causing color fading.[38,48] Indeed, Sindelar et al.[34] reported decreased a^* values with increased storage times in a comparable work in which cured ham had been cooked, vacuum-packaged, and stored at 2 °C for up to 90 days. Conversely, the same authors found that in vacuum-packaged frankfurter type sausages, a^* increased with storage time.[35] Despite this, the authors in the latter study found significant interactions that were attributed to the residual nitrate and/or nitrite that served as a reservoir for nitrite-related reactions. In the present study, the highest a^* values were found after 60 or 120 days of storage and the lowest at 0 and 180 days of storage (Table 3).

The sausages that had been stored for 0 days were also significantly darker and had a higher color intensity (C^*) than those stored for longer periods (Table 3). Various authors[34,35,37,38] have also observed an increase in L^* values with storage time in cooked−cured sausages. This increase could result from small decreases in the concentrations of the darkish pigmented myoglobin. Consistent with our results, other authors[49] have also reported a decrease in the C^* value of vacuum-packaged bologna sausages stored at 4 °C for 28 days.

Tocopherol and Tocotrienol Contents. In raw mix batters and sausages, the quantification was possible for only the α-tocopherol analogue, whereas the other tocopherol and tocotrienol analogues were not detected or were at trace levels. Sausage α-tocopherol contents are shown in Table 3 and, ranging from 9.3 to 14.1 mg/kg on a dry weight basis, were slightly lower than reported for dry-cured sausages produced from organic pig meat without tocopherol addition (18 mg/kg dry weight),[16] but similar to or slightly higher than reported for cooked ham and frankfurters (10 mg/kg dry weight) produced from pigs fed conventional diets.[49,50] Although various factors (i.e., the amount of this antioxidant provided by the feed, the amount of fat added to the meat product, and processing) may have important effects on meat product α-tocopherol content, the results seem to be consistent with those of other authors.

The addition of vegetable concentrate increased α-tocopherol content (Table 3), which could be caused by the intrinsic α-tocopherol content of the mix batters used (2.5 and 3.6 mg of α-tocopherol/kg for the pure sodium nitrite and vegetable concentrate batters, respectively). The different times of fermentation had no effect on sausage α-tocopherol content. In relation to the storage time, the α-tocopherol content was stable, thus suggesting that after 180 days of storage, there were few losses of this antioxidant during vacuum packing and refrigeration.

Oxidative Status and Susceptibility to Oxidation. The LHP contents are given in Table 3. Overall, LHP contents were low compared to other meat products.[16,51] Neither the fermentation time nor the nitrite source affected the content of primary oxidation products. However, LHP content decreased with longer storage times, which may be explained by the predominance of LHP breakdown over formation in sausages that had been vacuum-packed and stored at 4 °C for a long time. Moreover, the lack of oxygen may have been responsible for the relatively low LHP formation, thus explaining the unchanging α-tocopherol content during storage. In addition, nitrite is an efficient antioxidant, which could have contributed to the low levels of LHP found.[9]

Susceptibility to oxidation was measured according to the same method used to determine LHP content but after a period of incubation, as described elsewhere.[21] The time of fermentation did not affect susceptibility to oxidation when assessed through the different parameters described by Tres et al.[21] The most useful of these parameters was the area under the curve that described LHP formation during the incubation time (AUC); the results of this parameter are shown in Table 2. The addition of vegetable concentrate increased susceptibility to oxidation (Table 2). As reported above, this ingredient was rich in nitrate. However, the composition of the vegetable concentrate is complex. For instance, it was found to contain 4.54 ± 0.18 mg/kg of α-tocopherol, 1.57 ± 0.05 mg/kg of β-

tocotrienol, and 0.44 ± 0.02 mg/kg of γ-tocotrienol, but other antioxidants (i.e., carotenoids, ascorbic acid) and prooxidants (i.e., transition metals) are also likely to be present. Therefore, the vegetable concentrate composition may be responsible for the increased susceptibility to oxidation. However, it is noteworthy to comment that different vegetable concentrates may have different compositions and, therefore, behave differently. This explains why a citrus fiber byproduct, associated with bioactive compounds such as flavonoids, polyphenols, and carotenes, prevented oxidation in bologna sausages,[47] whereas a vegetable concentrate similar to the one used in the present study, in which celery was the only ingredient, did not increase susceptibility to oxidation in dry-cured sausages.[16]

In line with the reported LHP content, TBA values of cooked−cured sausages did not change with either fermentation time or nitrite source (Table 3). However, TBA values increased with storage time, and those sausages that had been stored for 180 days had the highest malondialdehyde concentration (Table 3). This may be explained by the action of various prooxidants such as heme species, which are important contributors to lipid oxidation.[52,53] Despite this, the formation of stable heme proteins through the reaction with nitric oxide, thus tying up catalytically active trace metals present in meat,[9,54] could explain why the levels of oxidation were low in food products with added nitrite or nitrate.

Sensory Analysis. Conventional botifarra catalana and other similar products present in retail markets have an average shelf life of 90 days under the storage conditions assayed. The sausages' overall acceptability test was carried out after they had been stored for 60 days at 4 °C under vacuum conditions to determine whether trading of this product was possible (Table 2). In general, consumers gave scores above 5, suggesting that these sausages may be accepted by regular consumers even after a relatively long storage time.

Consumer scores for overall acceptability were similar for the different times of fermentation, indicating that there is no need for relatively long fermentation periods to produce this sausage. However, consumers gave higher acceptability scores for those with the vegetable concentrate (3.34 g/kg) (Table 2). In hams, Sindelar et al.[34] reported that the vegetable aroma of celery powder at 3.5 g/kg could be detected. Because botifarra catalana is sometimes cooked with a kind of natural broth, consumers might prefer sausages using vegetable concentrate. Therefore, the addition of vegetable concentrate not only produces more acceptable sausages but is also a useful alternative for producing cooked−cured meat products with a "clean label", without chemical nitrite.

Overall, the addition of vegetable concentrates can be a useful strategy for producing organic meat products with low residual nitrate and nitrite amounts that are accepted by consumers. However, the addition of starter cultures with intense nitrate reductase activity is necessary to reduce the large amounts of nitrates present in these vegetable concentrates. In relation to this, a fermentation period of 12 h at 16 °C can be enough for the appropriate development of the characteristic pink color of cooked−cured meat products. It is also remarkable that this concentrate showed minimal effects on oxidative stability. Moreover, sausages stored under the studied storage conditions (4 °C and vacuum packaged) showed only minor changes in their oxidative status, even for extended periods of storage.

AUTHOR INFORMATION

Corresponding Author

*Phone: (+34) 93 402 4508. Fax: (+34) 93 403 5931. E-mail: ricard_bou@ub.edu.

Funding

N.M. obtained financial support from an FPU research grant from the Ministry of Science and Innovation.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to the Gremi de Carnissers-Cansaladers-Xarcuters de Barcelona i Comarques for the use of their facilities and to Josep Dolcet and Pere Durán for their technical advice during preparation of the sausages. We thank CHR-Hansen and Espècies Teixidor for kindly providing the starter cultures and vegetable concentrate and some additives and ingredients, respectively.

ABBREVIATIONS USED

FOX, ferrous oxidation−xylenol orange; LHP, lipid hydroperoxide; TBA, thiobarbituric acid; Mb, myoglobin; metMb, metmyoglobon; AUC, lipid hydroperoxide formation area under the curve.

REFERENCES

(1) European Comission Directive 2006/52/EC of the European Parliament and of the Council of 5 July 2006 amending Directive 95/2/EC on food additives other than colours and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs. *Off. J. Eur. Union* **2006**, *L204*, 10−22.

(2) Real Decreto 1118/2007, de 24 de agosto, por el que se modifica el Real Decreto 142/2002, de 1 de febrero, por el que se aprueba la lista positiva de aditivos distintos de colorantes y edulcorantes para su uso en la elaboración de productos alimenticios, así como sus condiciones de utilización. *BOE* **2007**, *221*, 37533−37544.

(3) Dolcet, J.; Pons i Argimon, I. *Els Embotits de Catalunya*; Generalitat de Catalunya, Departament d'Agricultura i Acció Rural: Barcelona, Spain, 2010.

(4) Cassens, R. G. Composition and safety of cured meats in the USA. *Food Chem.* **1997**, *59*, 561−566.

(5) European Comission Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Off. J. Eur. Union* **2008**, *L250*, 1−84.

(6) Makatouni, A. What motivates consumers to buy organic food in the UK? Results from a qualitative study. *Br. Food J.* **2002**, *104*, 345−352.

(7) Krystallis, A.; Chryssohoidis, G. Consumers' willingness to pay for organic food − factors that affect it and variation per organic product type. *Br. Food J.* **2005**, *107*, 320−343.

(8) Froehlich, D. A.; Gullett, E. A.; Usborne, W. R. Effect of nitrite and salt on the color, flavor and overall acceptability of ham. *J. Food Sci.* **1983**, *48*, 152−154.

(9) Pegg, R. B.; Shahidi, F. *Nitrite Curing of Meat: The N-Nitrosamine Problem and Nitrite Alternatives*; Food and Nutrition Press: Trumbull, CT, 2000.

(10) Sebranek, J. G.; Bacus, J. N. Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Sci.* **2007**, *77*, 136−147.

(11) Fox, J. B. Chemistry of meat pigments. *J. Agric. Food Chem.* **1966**, *14*, 207−210.

(12) Casaburi, A.; Blaiotta, G.; Mauriello, G.; Pepe, I.; Villani, F. Technological activities of *Staphylococcus carnosus* and *Staphylococcus*

simulans strains isolated from fermented sausages. *Meat Sci.* **2005**, *71*, 643−650.
(13) *Meat and Meat Products: Determination of Moisture Content (Reference Method; ISO 1442:1997)*; International Organization for Standardization: Geneva, Switzerland, 1997.
(14) AOAC Official Method 991.36. In *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, 2000.
(15) Bou, R.; Codony, R.; Tres, A.; Baucells, M. D.; Guardiola, F. Increase of geometrical and positional fatty acid isomers in dark meat from broilers fed heated oils. *Poult. Sci.* **2005**, *84*, 1942−1954.
(16) Magrinya, N.; Bou, R.; Tres, A.; Rius, N.; Codony, R.; Guardiola, F. Effect of tocopherol extract, *Staphylococcus carnosus* culture, and celery concentrate addition on quality parameters of organic and conventional dry-cured sausages. *J. Agric. Food Chem.* **2009**, *57*, 8963−8972.
(17) Wrolstad, R. E. *Handbook of Food Analytical Chemistry:Pigments, Colorants, Flavors, Texture, And Bioactive Food Components*; Wiley-Interscience: Hoboken, NJ, 2005.
(18) Bou, R.; Guardiola, F.; Tres, A.; Barroeta, A. C.; Codony, R. Effect of dietary fish oil, α-tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poult. Sci.* **2004**, *83*, 282−292.
(19) Nuchi, C.; Guardiola, F.; Bou, R.; Bondioli, P.; Della Bella, L.; Codony, R. Assessment of the levels of degradation in fat co-and byproducts for feed uses and their relationships with some lipid composition parameters. *J. Agric. Food Chem.* **2009**, *57*, 1952−1959.
(20) Bou, R.; Codony, R.; Tres, A.; Decker, E. A.; Guardiola, F. Determination of hydroperoxides in foods and biological samples by the ferrous oxidation−xylenol orange method: a review of the factors that influence the method's performance. *Anal. Biochem.* **2008**, *377*, 1−15.
(21) Tres, A.; Daniela Nuchi, C.; Bou, R.; Codony, R.; Guardiola, F. Assessing rabbit and chicken tissue susceptibility to oxidation through the ferrous oxidation-xylenol orange method. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 563−573.
(22) Grau, A.; Guardiola, F.; Boatella, J.; Barroeta, A.; Codony, R. Measurement of 2-thiobarbituric acid values in dark chicken meat through derivative spectrophotometry: influence of various parameters. *J. Agric. Food Chem.* **2000**, *48*, 1155−1159.
(23) Scannell, A.; Kenneally, P.; McCarthy, D.; Schwarz, G.; Arendt, E. Optimisation of fermentation conditions for the production of a novel cooked fermented ham product. *Eur. Food Res. Technol.* **2002**, *215*, 183−188.
(24) Marco, A.; Navarro, J. L.; Flores, M. The influence of nitrite and nitrate on microbial, chemical and sensory parameters of slow dry fermented sausage. *Meat Sci.* **2006**, *73*, 660−673.
(25) Sindelar, J. J.; Terns, M. J.; Meyn, E.; Boles, J. A. Development of a method to manufacture uncured, no-nitrate/nitrite-added whole muscle jerky. *Meat Sci.* **2010**, *86*, 298−303.
(26) Tsoukalas, D. S.; Katsanidis, E.; Marantidou, S.; Bloukas, J. G. Effect of freeze-dried leek powder (FDLP) and nitrite level on processing and quality characteristics of fermented sausages. *Meat Sci.* **2011**, *87*, 140−145.
(27) Heppel, L. A.; Porterfield, V. T. Metabolism of inorganic nitrite and nitrate esters. 1. The coupled oxidation of nitrite by peroxide-forming systems and catalase. *J. Biol. Chem.* **1949**, *178*, 549−556.
(28) Chance, B. On the reaction of catalase peroxides with acceptors. *J. Biol. Chem.* **1950**, *182*, 649−658.
(29) Cassens, R. G.; Greaser, M. L.; Ito, T.; Lee, M. Reactions of nitrite in meat. *Food Technol.* **1979**, *33*, 46−57.
(30) Honikel, K. O. The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.* **2008**, *78*, 68−76.
(31) Silanikove, N.; Shapiro, F.; Silanikove, M.; Merin, U.; Leitner, G. Hydrogen peroxide-dependent conversion of nitrite to nitrate as a crucial feature of bovine milk catalase. *J. Agric. Food Chem.* **2009**, *57*, 8018−8025.
(32) Gøtterup, J.; Olsen, K.; Knöchel, S.; Tjener, K.; Stahnke, L. H.; Møller, J. K. S. Relationship between nitrate/nitrite reductase activities in meat associated staphylococci and nitrosylmyoglobin formation in a cured meat model system. *Int. J. Food Microbiol.* **2007**, *120*, 303−310.
(33) Krause, B. L.; Sebranek, J. G.; Rust, R. E.; Mendonca, A. Incubation of curing brines for the production of ready-to-eat, uncured, no-nitrite-or-nitrate-added, ground, cooked and sliced ham. *Meat Sci.* **2011**, *89*, 507−513.
(34) Sindelar, J. J.; Cordray, J. C.; Sebranek, J. G.; Love, J. A.; Ahn, D. U. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *J. Food Sci.* **2007**, *72*, S388−S395.
(35) Sindelar, J. J.; Cordray, J. C.; Sebranek, J. G.; Love, J. A.; Ahn, D. U. Effects of vegetable juice powder concentration and storage time on some chemical and sensory quality attributes of uncured, emulsified cooked sausages. *J. Food Sci.* **2007**, *72*, S324−S332.
(36) Chasco, J.; Lizaso, G.; Beriain, M. J. Cured colour development during sausage processing. *Meat Sci.* **1996**, *44*, 203−211.
(37) Terns, M. J.; Milkowski, A. L.; Claus, J. R.; Sindelar, J. J. Investigating the effect of incubation time and starter culture addition level on quality attributes of indirectly cured, emulsified cooked sausages. *Meat Sci.* **2011**, *88*, 454−461.
(38) Terns, M. J.; Milkowski, A. L.; Rankin, S. A.; Sindelar, J. J. Determining the impact of varying levels of cherry powder and starter culture on quality and sensory attributes of indirectly cured, emulsified cooked sausages. *Meat Sci.* **2011**, *88*, 311−318.
(39) Perez-Alvarez, J. A.; Sayas-Barbera, M. E.; Fernandez-Lopez, J.; Aranda-Catala, V. Physicochemical characteristics of Spanish-type dry-cured sausage. *Food Res. Int.* **1999**, *32*, 599−607.
(40) Brewer, M. S.; Zhu, L. G.; Bidner, B.; Meisinger, D. J.; McKeith, F. K. Measuring pork color: effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Sci.* **2001**, *57*, 169−176.
(41) Freise, K.; Brewer, S.; Novakofski, J. Duplication of the pale, soft, and exudative condition starting with normal postmortem pork. *J. Anim. Sci.* **2005**, *83*, 2843−2852.
(42) Schilling, M. W.; Marriott, N. G.; Acton, J. C.; Anderson-Cook, C.; Alvarado, C. Z.; Wang, H. Utilization of response surface modeling to evaluate the effects of non-meat adjuncts and combinations of PSE and RFN pork on water holding capacity and cooked color in the production of boneless cured pork. *Meat Sci.* **2004**, *66*, 371−381.
(43) Sanabria, C.; Martin-Alvarez, P. J.; Carrascosa, A. Colour and moisture changes during the manufacture of Iberian dry-cured ham caused by some biotic and abiotic factors. *Food Sci. Technol. Int.* **2004**, *10*, 269−275.
(44) Olivares, A.; Luis Navarro, J.; Salvador, A.; Flores, M. Sensory acceptability of slow fermented sausages based on fat content and ripening time. *Meat Sci.* **2010**, *86*, 251−257.
(45) Villegas, R.; O'Connor, T. P.; Kerry, J. P.; Buckley, D. Effect of gelatin dip on the oxidative and colour stability of cooked ham and bacon pieces during frozen storage. *Int. J. Food Sci. Technol.* **1999**, *34*, 385−389.
(46) Dineen, N. M.; Kerry, J. P.; Lynch, P. B.; Buckley, D. J.; Morrissey, P. A.; Arendt, E. K. Reduced nitrite levels and dietary α-tocopheryl acetate supplementation: effects on the colour and oxidative stability of cooked hams. *Meat Sci.* **2000**, *55*, 475−482.
(47) Fernandez-Gines, J. M.; Fernandez-Lopez, J.; Sayas-Barbera, E.; Sendra, E.; Perez-Alvarez, J. A. Effect of storage conditions on quality characteristics of bologna sausages made with citrus fiber. *J. Food Sci.* **2003**, *68*, 710−715.
(48) Sindelar, J. J.; Cordray, J. C.; Olson, D. G.; Sebranek, J. G.; Love, J. A. Investigating quality attributes and consumer acceptance of uncured, no-nitrate/nitrite-added commercial hams, bacons, and frankfurters. *J. Food Sci.* **2007**, *72*, S551−S559.
(49) Sarraga, C.; Guardia, M. D.; Diaz, I.; Guerrero, L.; Garcia Regueiro, J. A.; Arnau, J. Nutritional and sensory quality of porcine raw meat, cooked ham and dry-cured shoulder as affected by dietary enrichment with docosahexaenoic acid (DHA) and α-tocopheryl acetate. *Meat Sci.* **2007**, *76*, 377−384.

(50) Estevez, M.; Ventanas, S.; Ramirez, R.; Cava, R. Influence of the addition of rosemary essential oil on the volatiles pattern of porcine frankfurters. *J. Agric. Food Chem.* **2005**, *53*, 8317−8324.
(51) Bou, R.; Codony, R.; Baucells, M. D.; Guardiola, F. Effect of heated sunflower oil and dietary supplements on the composition, oxidative stability, and sensory quality of dark chicken meat. *J. Agric. Food Chem.* **2005**, *53*, 7792−7801.
(52) Bou, R.; Hanquet, N.; Codony, R.; Guardiola, F.; Decker, E. A. Effect of heating oxyhemoglobin and methemoglobin on microsomes oxidation. *Meat Sci.* **2010**, *85*, 47−53.
(53) Bou, R.; Guardiola, F.; Codony, R.; Faustman, C.; Elias, R. J.; Decker, E. A. Effect of heating oxymyoglobin and metmyoglobin on the oxidation of muscle microsomes. *J. Agric. Food Chem.* **2008**, *56*, 9612−9620.
(54) MacDonald, B.; Gray, J. I.; Gibbins, L. N. Role of nitrite in cured meat flavor: antioxidant role of nitrite. *J. Food Sci.* **1980**, *45*, 893−897.