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Effect of Tocopherol Extract, *Staphylococcus carnosus* Culture, and Celery Concentrate Addition on Quality Parameters of Organic and Conventional Dry-Cured Sausages

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The effects of the addition to sausage mix of tocopherols (200 mg/kg), a conventional starter culture with or without *Staphylococcus carnosus*, celery concentrate (CP) (0.23% and 0.46%), and two doses of nitrate (70 and 140 mg/kg expressed as $NaNO_3$) on residual nitrate and nitrite amounts, instrumental CIE Lab color, tocol content, oxidative stability, and overall acceptability were studied in fermented dry-cured sausages after ripening and after storage. Nitrate doses were provided by nitrate-rich CP or a chemical grade source. The lower dose complies with the EU requirements governing the maximum for ingoing amounts in organic meat products. Tocopherol addition protected against oxidation, whereas the nitrate dose, nitrate source, or starter culture had little influence on secondary oxidation values. The residual nitrate and nitrite amounts found in the sausages with the lower nitrate dose were within EU-permitted limits for organic meat products and residual nitrate can be further reduced by the presence of the *S. carnosus* culture. Color measurements were not affected by the CP dose. Product consumer acceptability was not affected negatively by any of the factors studied. As the two nitrate sources behaved similarly for the parameters studied, CP is a useful alternative to chemical ingredients for organic dry-cured sausage production.

KEYWORDS: Dry-cured sausages; organic production; nitrate and nitrite reduction; celery concentrate; *Staphylococcus carnosus*

INTRODUCTION

Ancient Greeks and Romans used salt to preserve fish and meat through curing, and these kinds of food products are still present in our diets. Though salt was historically believed to be responsible for obtaining cured meat products, it has now been demonstrated that nitrate impurities were crucial to the curing process (*1*).

Nowadays, it is understood that sausages like Spanish salchichón or Italian salame are dry-fermented cured meat products requiring several ingredients, such as salt, a nitrate or nitrite source, and a bacterial culture to develop their distinctive color, flavor, and texture (*2*). The addition of nitrate, which is reduced to nitrite, or the direct addition of nitrite is necessary to develop their characteristic color and flavor. In addition, it acts as an antimicrobial to control *Clostridium botulinum* and helps to prevent oxidation (*1*).

However, the formation of carcinogenic nitrosamines from nitrate and nitrite sources is a health concern. It has led to the reduction of their content in cured meats since the mid-1970s (*3*,*4*) and to regulations on the amounts of nitrate and nitrite that can be added to, or found in, the cured product (*5*). The endogenous and/or exogenous microbiota present in the sausage mix are very relevant to the industry since nitrate and nitrite amounts can be reduced by the action of certain specific bacteria, while allowing the curing process. In relation to this, many starter cultures include *Staphylococcus* sp., since nitrate reductase activity is present in *Micrococcaceae* (*2*). The formation of nitrite exerts an antioxidative effect by preventing the release of iron from the porphyrin molecule, protecting unsaturated lipids within membranes against oxidation, interaction of nitrite as a metal chelator, and formation of nitroso and nitrosyl compounds acting as radical scavengers (*1*). Therefore, the reduction of nitrite levels may increase the susceptibility of sausages to oxidation, which would make it necessary to protect these meat products with antioxidants.

However, most of the nitrite formed is reduced to nitric oxide which reacts with myoglobin to produce nitrosylmyoglobin, but this can also be formed indirectly by nitrites oxidizing myoglobin into metmyoglobin and, subsequently, reacting with nitric oxide to produce nitrosylmetmyoglobin (*1*, *6*). In the presence of exogenous and endogenous reductants, nitrosylmetmyoglobin

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reduces to the more stable form, nitrosylmyoglobin (*1*). The formation of this heme complex gives dry-cured meat products their characteristic color.

Nowadays, there is increased demand for healthier and organic food products, without chemical preservatives. In traditional cured meat products, however, consumers tend to dislike those to which nitrite has not been added (*7*). Consequently, the EU has regulated the maximum amounts of nitrate and nitrite to be added and the maximum residual amounts to be found in organic meat foods (*8*). An alternative to the addition of chemical grade nitrates or nitrites is the addition of natural sources containing nitrates or nitrites, which act like the curing salts used in ancient times. Some vegetable sources such as celery powder concentrates (CP) are known to be rich in nitrate (*9*), which means that they can be used instead of nitrate or nitrite in the manufacture of cured sausages (*10*, *11*).

The aim of this study was to assess the possibility of reducing nitrate and nitrite amounts, by examining how various quality parameters of sausages produced under differing conventional and organic strategies are affected.

MATERIALS AND METHODS

Reagents and Standards. Tocopherol extract (Guardian, 70% of mixed tocopherols) was obtained from DANISCO (Copenhagen, Denmark). Conventional starter culture containing *Lactobacillus sakei* and *Staphyloccocus xylosus* (SM-181 Bactoferm), a nitrate reductase-active culture containing *Staphylococcus carnosus* (CS 299 Bactoferm) and CP, was obtained from CHR Hansen (Hørsholm, Denmark). Potassium nitrate (Suprapur), cadmium (coarse powder), copper(II) sulfate pentahydrate, sodium nitrite, and *N*-1-napthylethylenediamine dihydrochloride (NED) were obtained from Merck (Darmstadt, Germany). Sulphanilamide was obtained from Carlo Erba (Milan, Italy). Sodium ascorbate, dextrose, and lactose were obtained from Espècies Teixidor (Manresa, Spain). Tocopherol standards were obtained from Calbiochem (San Diego, CA). All chemicals used were of ACS grade except the solvents used in the induced ferrous oxidation−xylenol orange (FOX) method and the tocol determination, which were of HPLC grade.

Experimental Design. Sixteen treatments resulted from a $2 \times 2 \times 4$ factorial design (**Table 1**) to study the effects of the addition of a tocopherols extract to the sausage mix formula (0 and 200 mg of mixed tocopherols/kg meat), of two starter cultures (conventional and conventional plus *Staphylococcus carnosus*), and of 4 different sources of nitrate (either KNO_3 or CP sources providing 70 and 140 mg of nitrate/kg expressed as $NaNO_3$) on several cured meat quality parameters. These two nitrate levels were at the maximum level of ingoing sodium nitrate allowed in conventional and organic meat products (*5*, *8*). Meat with the ingredients was stuffed into natural casing and dry-cured for 48 days. Finally, the storage time factor of the dry-cured sausage, sliced and packed in modified atmosphere for 0 or 45 days, was added to this design, thus resulting in 32 treatments.

Sausage Preparation. A meat mix consisting of 91.7% diced pork loin meat plus 8.3% diced back fat from organic pigs was used to prepare the ground meat. After homogenization, the raw mix batter was then divided into 2 batches of 24 kg. Then, the following common ingredients were added to each batch: 0.5 g/kg of sodium ascorbate, 3 g/kg of dextrose, 5 g/kg of lactose, 3 g/kg ground black pepper, 22 g/kg of salt, and 0.25 g/kg of a conventional starter culture. Natural spring water was used to deliver the sodium ascorbate (100 mL) and the starter culture (100 mL) to the mix. In each batch, 100 mL of sunflower oil with or without tocopherol extract supplementation were added. After the addition of all these ingredients, samples were mixed for 4 min. Samples from these 2 mixes, with and without tocopherols extract, were finely ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; approximately 20 g of meat/bag) and stored at −25 °C until analysis.

Each batch was divided again into two more subsets, resulting in four different batches of 12 kg of meat, to which 100 mL of natural spring water with or without the *Staphylococcus carnosus* culture (1.33 g) was added according to the experimental design (**Table 1**). The resulting mixes were homogenized for 2 min. According to the experimental design, mixes were subdivided into 16 batters of 3 kg in which CP (added at the following amounts: 6.9 or 13.8 g) or chemically pure KNO_3 (>99.99%) (added at the following amounts: 253 mg or 506 mg) were added after being dissolved previously in 50 mL of double deionized water. These amounts were added to provide the doses of 70 and 140 mg of nitrate/kg, respectively, expressed as $NaNO_3$. The resulting 16 different raw mix batters were mixed manually for 2 min and stuffed into natural casings (40−45 mm diameter). To check the nitrate dose, samples from these 16 mix batters were finely ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; approximately 20 g of meat/bag) and stored at −25 °C until nitrate and nitrite analysis. In addition, samples from these mix batters were aseptically taken for microbiological analysis and stored at 4 °C until the analysis, which was started the same day.

Table 1. Sausage Formula Treatments

treatments	tocopherols (mg/kg)[a]	starter culture[b]	nitrate source[c] (origin and dose in mg/kg)
1	0	conventional	celery conc. powder 70
2	0	conventional	celery conc. powder 140
3	0	conventional	chemical grade 70
4	0	conventional	chemical grade 140
5	0	*S. carnosus*	celery conc. powder 70
6	0	*S. carnosus*	celery conc. powder 140
7	0	*S. carnosus*	chemical grade 70
8	0	*S. carnosus*	chemical grade 140
9	200	conventional	celery conc. powder 70
10	200	conventional	celery conc. powder 140
11	200	conventional	chemical grade 70
12	200	conventional	chemical grade 140
13	200	*S. carnosus*	celery conc. powder 70
14	200	*S. carnosus*	celery conc. powder 140
15	200	*S. carnosus*	chemical grade 70
16	200	*S. carnosus*	chemical grade 140

[a] Expressed as average sum of tocopherol analogues. The tocopherol extract contains α-, β-, γ-, and δ-tocopherol analogues at the concentrations of 109 ± 4, 12.7 ± 0.3, 476 ± 12, and 189 ± 5 g/kg, respectively. [b] Conventional starter culture includes *Lactobacillus sakei* and *Staphylococcus xylosus*. The conventional starter culture was also added in the *Staphylococcus carnosus* treatments. [c] Addition of chemically pure KNO_3 or celery concentrate powder providing different doses of nitrate, 70 or 140 mg, expressed as $NaNO_3$/kg.

Sausage Dry-Curing and Sample Preparation. Sausages were hung for 48 days in a ripening chamber at 14 ± 2 °C and 75−85% moisture. At this time (storage time 0), half of the sausages were ground and vacuum-packed in high-barrier multilayer bags (Cryovac BB325; 180 × 200 mm; permeability to oxygen, 25 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 °C and 0% RH, DIN 53380; approximately 20 g of meat/bag) and stored at −25 °C until analysis. The remaining sausages were sliced (2 mm thick) and packaged in sealed metallized polyester/polyethylene bags (Termopack PETM/PE; 300 × 200 mm; permeability to oxygen, nitrogen and carbon dioxide was 50, 10, and 150 $cm^3 \cdot m^{-2} \cdot day^{-1} \cdot bar^{-1}$ at 23 °C and 0% RH, DIN 53380, respectively; approximately 20 slices/bag) containing 80% N_2 and 20% CO_2 for 45 days at 4 °C (storage time 45). After this period, samples were ground, vacuum-packed in high-barrier multilayer bags, and stored until analysis, the same as at time 0. For the microbiological analysis, samples were aseptically taken at the different storage times, 0 and 45 days, and stored at 4 °C before analysis, which was started the same day.

Moisture Determination. The ISO 1442 procedure (*12*) was used to determine the moisture of the samples. Moisture was used to express results as dry weight unless otherwise specified.

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 (*13*), whereas fatty acid composition was as described elsewhere (*14*). Fat content was expressed on a fresh weight basis, whereas fatty acid composition was expressed as a percentage of area normalization.

Nitrite and Nitrate Determination. Ten grams of sample was weighed in a 250 mL beaker, and approximately 80 mL of distilled water was added. Then, Carrez I and Carrez II solutions (3 mL each) were added, and the solution was filled up to 100 g. Subsequently, this solution was

homogenized with a high speed homogenizer (Ultraturrax T25 basic with a dispersing tool S25N-18G, IKA-Werke GmbH, Germany) at 3500 rpm for 75 s. The homogenate was then centrifuged at 4350*g* for 20 min, and the supernatant was used for nitrate and nitrite analyses.

Sum of nitrate plus nitrite and nitrite analyses were performed on two segmented continuous flow systems (AutoAnalyzer 3 model, SEAL Analytical, UK). Nitrate plus nitrite content of the clarified samples was found by first reducing nitrate to nitrite with a copperized cadmium reduction column. Subsequently, the pre-existing nitrite plus the nitrite formed after the reduction step reacted with sulfanilamide for diazotization and coupling with NED formed a purple azo dye. The dye absorbance was then read at 550 nm. With an independent continuous flow system, only nitrite was determined, using the same reaction of nitrate but omitting the previous reduction step. Nitrate amounts were calculated by the difference between the two analyses. Results for raw mix batter were expressed as $NaNO_3$ or $NaNO_2$ per kg on a fresh weight basis.

Microbiological Analyses. Twenty-five grams of either raw batter or sausage samples, the latter previously diced in small pieces, was aseptically taken and homogenized with 75 mL of buffered peptone water (BPW; OXOID, Basingstoke, UK) for 2 min in an IUL masticator (IUL S.A., Barcelona, Spain). Serial decimal dilutions were made in sterile Ringer 1/4 solution (Scharlau, Barcelona, Spain). The following food-borne pathogens were determined in raw sausages. *Escherichia coli* was enumerated on McConkey agar (OXOID, Basingstoke, UK) and the population of sulfite-reducing clostridia by counting in SPS agar (Scharlau, Barcelona, Spain) anaerobically. Both agars were incubated at 37 °C for 48 h. The absence of *Salmonella* was determined by pre-enrichment in BPW for 16 h at 37 °C, enrichment in Selenite Cystine broth (OXOID, Basingstoke, UK) for 24 h at 37 °C, and in Rappaport Vassiliadis broth (OXOID, Basingstoke, UK) for 24 h at 42 °C, and isolation on SS agar (OXOID, Basingstoke, UK) and DCLS agar (OXOID, Basingstoke, UK). Both agars were incubated for 48 h at 37 °C. Kligler Iron agar (OXOID, Basingstoke, UK), Lysine Iron agar (OXOID, Basingstoke, UK), urease broth (OXOID, Basingstoke, UK) and API 20E system (bioMérieux España, Madrid, Spain) were used to identify colonies grown on SS agar and/or DCLS agar. Starter bacteria were analyzed by spread plating on MRS agar (OXOID, Basingstoke, UK) for lactic acid bacteria and on Mannitol Salt agar (Cultimed, Barcelona, Spain) for staphylococci. Both cultures were incubated at 30 °C for 3 days.

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) lightness L*, redness a*, and yellowness b* values were determined from four different random surfaces of the ground samples. The instrument was set for illuminant D-65 and at a 10° observer angle, and standardized using a standard white plate.

Oxidative Status and Susceptibility to Oxidation. As reviewed elsewhere, the ferrous oxidation−xylenol orange (FOX) method measures lipid hydroperoxides (LHP) (*15*). Using this method and with the same assay both the content in these primary oxidation compounds and the susceptibility to oxidation by means of different parameters can be determined (*15*).

The time course of lipid hydroperoxides formed after incubation over 210 h was used to calculate the susceptibility to oxidation parameters of the induced FOX assay as well as the content in LHP (LHPC), which was determined after 30 min of incubation. These oxidation parameters were maximum lipid hydroperoxide value (MAXLHP), the time in which the maximum lipid hydroperoxide value was achieved (TMAX), oxidation rate (OR), the lipid hydroperoxide value obtained at the end of the incubation period (Final LHP), and the area under the curve (AUC), and were calculated as described elsewhere (*16*).

Secondary oxidation compounds were determined by means of the thiobarbituric acid (TBA) values through third-derivative spectrophotometry after acid aqueous extraction (*17*).

Tocopherol and Tocotrienol Analogue Determination. Tocopherol and tocotrienol analogues were determined as described elsewhere (*18*). Results for raw mix batter were expressed as mg of each tocol per kg on a fresh weight basis, whereas results for sausages were expressed as mg of each tocol per kg.

Sensory Analyses. The following tests were carried out on different days in single sessions:

Table 2. Effect of Sausage Formulation Factors and Processing Points (after Inoculation in the Raw Mix Batter, after Curing at 0 Days and after 45 Days Storage) on Microbial Counts[a]

	Microbial counts (log CFU/g)	
	Lactobacilli[b]	Staphylococci[c]
Tocopherols[d]		
0	8.4	6.9
200	8.2	7.0
SEM[e]	0.085	0.082
Starter Culture[f]		
conventional	8.2	6.9
S. carnosus	8.4	7.1
SEM	0.085	0082
Nitrate Source (Origin and Dose)[g]		
chemical 70	8.3	7.0
chemical 140	8.3	7.1
celery 70	8.2	6.9
celery 140	8.3	6.9
SEM	0.12	0.12
Time		
raw mix batter	8.2 x	7.5 y
0 days	8.6 y	7.2 y
45 days	8.1 x	6.2 x
SEM	0.10	0.10

[a] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 48, 48 for lactobacilli and staphylococci). Least-squares means within the same column with different letters differ significantly ($P \leq 0.05$). [b] Microbial counts expressed as the logarithm of lactobacilli colony-forming units per g of dried sample. Significant interactions between tocopherols × storage time for lactobacilli ($P = 0.005$) were found. [c] Microbial counts expressed as the logarithm of staphylococci colony-forming units per g of dried sample. A significant interaction between starter culture × nitrate source ($P = 0.001$) for staphylococci was found. [d] Tocopherol dose expressed in mg of tocopherol analogues/kg meat. [e] SEM means standard error of the mean. [f] Conventional starter culture includes *Lactobacillus sakei* and *Staphylococcus xylosus*. The *Staphylococcus carnosus* starter culture also includes the conventional starter culture. [g] Origin of nitrate and dose of nitrate expressed in mg $NaNO_3$/kg meat.

Overall Acceptability. The 16 treatments were randomly presented to the consumers in a balanced incomplete block design (*19*): 16 blocks, 6 samples per block and 6 replicates for each treatment. This design was duplicated by using 32 consumers to evaluate the overall acceptability of the product. In addition, each panelist evaluated the acceptability of a blind control (total samples given to each panelist = 7), which was treatment number 4. Consumer panelists were asked to rank the overall acceptability of the product on a 9-point scale (1 = very bad; 9 = very good). The scores of the blind control given by each panelist were subtracted from their respective sample acceptability scores.

Color Triangle Test. Samples of treatments 3 and 14 (see **Table 1** for sausage formula factors) were used to perform this test to assess whether a difference existed in the color of the samples (*20*). Twenty-four panelists were used to perform this test.

Color Intensity Ranking Test. Samples of treatments 1, 2, 3, 4, 8, 12, and 16 (**Table 1**) were randomly presented to the panelists, who were asked to rank color intensity (*20*). Thirty panelists were used to perform this test. Along with this test, panelists were asked to select their preferred sample.

In each test, several slices of sample sausages were placed in 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 samples.

Statistical Analyses. A multifactor ANOVA determined significant differences produced by the different factors on sample moisture, microbial determinations, nitrate and nitrite content, color measurements, tocopherol and tocotrienol analogues, TBA values, LHPC, and induced

Table 3. Effect of Formula Factors and Storage Time on Sausage Moisture, Residual Nitrate, Residual Nitrite, and CIE L* a* b* Color Values[a]

	moisture (%)[b]	residual nitrate (mg/kg)[c]	residual nitrite (mg/kg)[d]	L* [e]	a* [f]	b* [g]
Tocopherols[h]						
0	25.3 x	27	0.36	37.52 x	15.38 x	8.32 x
200	26.0 y	26	0.33	38.36 y	16.21 y	8.61 y
SEM[i]	0.19	1.5	0.012	0.063	0.054	0.019
Starter Culture[j]						
conventional	25.8	53 y	0.35	37.85 x	15.71 x	8.43 x
S. carnosus	25.6	Tr[l] x	0.34	38.00 y	15.88 y	8.50 y
SEM	0.19	1.5	0.012	0.063	0.054	0.019
Nitrate Source (Origin and Dose)[k]						
chemical 70	25.6	9 x	0.36	37.18 x	15.78 x	8.36 x
chemical 140	25.3	48 y	0.35	37.85 x	15.77 x	8.43 x
celery 70	25.7	Tr x	0.34	37.82 x	15.70 x	8.43 x
celery 140	26.0	49 y	0.33	38.34 y	15.93 x	8.66 y
SEM	0.27	2.1	0.017	0.091	0.077	0.027
Storage Time						
0 days	25.8	25	0.38 y	38.17 y	16.30 y	8.52 y
45 days	25.6	28	0.31 x	37.71 x	15.29 x	8.42 x
SEM	0.19	1.5	0.012	0.063	0.054	0.019

[a] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 32, 32, 32, and 128 for moisture, nitrate, and nitrite analyses and color measurements, respectively). Least-squares means within the same column for the same factor with different letters differ significantly ($P \leq 0.05$). [b] Significant interactions between nitrate source × starter culture ($P = 0.005$) and between nitrate source × tocopherols ($P = 0.007$) for moisture were found. [c] Residual nitrate is expressed as mg of $NaNO_3$ per kg of sausage as dry weight. A significant interaction between starter culture × nitrate source ($P \leq 0.001$) for residual nitrate was found. [d] Residual nitrite is expressed as mg of $NaNO_2$ per kg of sausage as dry weight. A significant interaction between nitrate source × storage time ($P = 0.0042$) for residual nitrite was found. [e] Significant interactions between starter culture × nitrate source ($P \leq 0.001$), starter culture × storage time ($P = 0.002$), nitrate source × tocopherols ($P \leq 0.001$), and nitrate source and storage time ($P \leq 0.001$) for L* values were found. [f] Significant interactions between starter culture × tocopherols ($P = 0.003$), starter culture × nitrate source ($P \leq 0.001$), starter culture × storage time ($P = 0.014$), nitrate source × tocopherols ($P \leq 0.001$), and nitrate source × storage time ($P = 0.001$) for a* values were found. [g] Significant interactions between starter culture × tocopherols ($P = 0.002$), starter culture × nitrate source ($P \leq 0.001$), nitrate source × tocopherols ($P \leq 0.001$), and nitrate source × storage time ($P \leq 0.001$) for b* values were found. [h] Tocopherol dose expressed in mg of tocopherol analogues/kg meat. [i] SEM means standard error of the mean. [j] Conventional starter cultures include *Lactobacillus sakei* and *Staphylococcus xylosus*. The *Staphylococcus carnosus* starter culture also includes the conventional starter culture. [k] Origin of nitrate and dose of nitrate expressed in mg $NaNO_3$/kg meat. [l] Tr stands for traces. The analyte amounts were found between the limits of detection and quantification of the method used.

FOX assay parameters. Factors were tocopherols extract addition (0 and 200 mg of tocopherol analogues/kg), starter culture (conventional and conventional plus *S. carnosus*), nitrate source (70 mg of $NaNO_3$/kg and 140 mg $NaNO_3$/kg, each dose provided by the addition of CP or chemical grade KNO_3), and time (after ripening, hereafter referred to as day 0 or time 0, and after 45 days of storage under a modified atmosphere). Because microbiological determinations were carried out at three different periods (after starter culture inoculation in raw mix batter and at 0 and 45 days), these periods were included for the time factor. Interactions between more than two factors were ignored. When main effects were significant, the least-squares means were separated using the Scheffé test ($\alpha = 0.05$).

The storage time factor was not studied in the consumers' sensory analysis of acceptability. The significance estimation in triangle and ranking tests was analyzed using tables (*21*, *22*).

Interactions were examined, and whenever the interaction involving the nitrate source was present, we ran an ANOVA in which the dose of nitrate added (70 and 140 mg/kg) and the origin of nitrate (chemical and CP) were taken into account separately, instead of as a combination. In addition, storage time can also be a confounding factor in various interactions. Therefore, to understand the interactions better, the data file was split into two groups, and an ANOVA was run to study the interactions' effects on each storage time.

Spearman correlation coefficients between sausage TBA values and color measurements, and between tocol analogues and FOX parameters, were calculated. In all cases, $P \leq 0.05$ was considered significant.

RESULTS

Moisture, Crude Fat Content, and Fatty Acid Composition of Raw Mix Batters. The moisture averages of the raw mix batters with and without the tocopherol extract were 61.86% ± 0.13 and 62.19% ± 0.07, respectively. The crude fat content averages of the raw mix batters with and without the tocopherols extract were 17.2% ± 0.29 and 16.7% ± 0.27, respectively. The relative percentages of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids of the raw mix batter without the addition of the tocopherol extract were 40.2%, 40.8%, and 19.0%, respectively, whereas for the raw mix batter with the addition of the tocopherols extract, they were 40.5%, 40.7%, and 18.8%, respectively. A table including all of the quantified fatty acids in these mixes is available as Supporting Information. These results demonstrate the homogeneity of these two mixes.

Microbiological Analyses. The 16 raw mix batter samples were checked for presence of food poisoning bacteria. *E. coli* was less than 100 CFU/g; *Salmonella* sp. was absent in 25 g; and sulfite-reducing clostridia were less than 10 CFU/g for the 16 sausages. All samples met microbiological standards for raw minced meat.

Lactobacilli and total staphylococci were analyzed in raw mix batter samples just before stuffing as well as after curing (0 days) and after 45 days of storage in sealed bags (**Table 2**).

The study of the interactions showed that they were only present at the initial time (data not shown). Provided that several factors affect the initial microbiota rather than just the factors studied, these interactions were considered not relevant.

The lactobacilli and staphylococci were only affected by time. During fermentation, the population of lactic acid bacteria increased at the initial stages and then began to drop slowly as the fermented meat product was processed (*2*, *23*), which explains why at time 0 the levels of *Lactobacillus* sp. were higher than that in raw mix batter samples. In both cases, a reduction in

Table 4. Effect of Formula Factors and Storage Time on Sausage Tocol Analogues[a]

	α-tocopherol (mg/kg)[b]	β-tocopherol (mg/kg)[c]	γ-tocopherol (mg/kg)[d]	δ-tocopherol (mg/kg)[e]	α-tocotrienol (m/kg)[f]
Tocopherols[g]					
0	18.8 x	0.3 x	1 x	Tr[h] x	0.77 x
200	74.5 y	7.3 y	260 y	47.3 y	1.22 y
SEM[i]	0.91	0.08	3.0	0.38	0.04
Starter Culture[j]					
conventional	45.0 x	3.7 x	126 x	23.0 x	0.96
S. carnosus	48.2 y	3.9 y	136 y	24.3 y	1.04
SEM	0.91	0.08	3.0	0.38	0.04
Nitrate Source (Origin and Dose)[k]					
chemical 70	43.3 x	3.5 x	123	22.4	0.83 x
chemical 140	47.4 xy	3.9 xy	132	24.0	1.10 y
celery 70	47.2 xy	3.8 xy	130	23.7	1.00 xy
celery 140	48.7 y	4.0 x	139	24.6	1.06 xy
SEM	1.3	0.11	4.3	0.54	0.06
Storage Time					
0 days	52.6 y	4.1 y	140 y	24.8 y	1.16 x
45 days	40.7 x	3.5 x	122 x	22.5 x	0.83 x
SEM	0.91	0.08	3.0	0.38	0.04

[a] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (each analogue $n = 64$). Least-squares means within the same column for the same factor with different letters differ significantly ($P \leq 0.05$). Since β-, γ-, and δ-tocotrienols were normally below the quantification limits, they were not reported. [b] Results are expressed as mg of α-tocopherol per kg of sausage as dry weight. Significant interactions between starter culture × nitrate source ($P = 0.030$), starter culture × storage time ($P = 0.023$), and storage time × tocopherols ($P = 0.015$) for α-tocopherol were found. [c] Results are expressed as mg of β-tocopherol per kg of sausage as dry weight. Significant interactions between starter culture × tocopherols ($P = 0.046$), starter culture × nitrate source ($P \leq 0.001$), tocopherols × nitrate source ($P = 0.045$), and tocopherols × storage time ($P \leq 0.001$) for β-tocopherol were found. [d] Results are expressed as mg of γ-tocopherol per kg of sausage as dry weight. Significant interactions between starter culture × nitrate source ($P = 0.021$), starter culture × tocopherols ($P = 0.031$), and storage time × tocopherols ($P \leq 0.001$) for γ-tocopherol were found. [e] Results are expressed as mg of δ-tocopherol per kg of sausage as dry weight. Significant interactions between starter culture × tocopherols ($P = 0.032$), starter culture × nitrate source ($P \leq 0.001$), and storage time × tocopherols ($P \leq 0.001$) for δ-tocopherol were found. [f] Results are expressed as mg of α-tocotrienol per kg of sausage as dry weight. Significant interactions between starter culture × nitrate source ($P = 0.003$) and starter culture × storage time ($P = 0.043$) were found for α-tocotrienol. [g] Tocopherol dose expressed in mg of tocopherol analogues/kg meat. [h] Tr stands for traces. The analyte amounts were found between the limits of detection and quantification of the method used. [i] SEM means standard error of the mean. [j] Conventional starter cultures include *Lactobacillus sakei* and *Staphylococcus xylosus*. The *Staphylococcus carnosus* starter culture also includes the conventional starter culture. [k] Origin of nitrate and dose of nitrate expressed in mg $NaNO_3$/kg meat.

population during storage time was observed. These results corroborate those reported by Marco et al. (*24*). The environmental conditions during storage, such as low temperature, moisture, nitrate concentration, and a reducing atmosphere, could have enhanced the decrease from the starter population.

Nitrate and Nitrite Residual Amounts. Nitrate amounts in raw mix batters, expressed as fresh weight, averaged 127 ± 13 mg NO_3/kg and 66 ± 5.8 mg NO_3/kg for those treatments containing the higher and lower doses of nitrate, respectively. Therefore, the nitrate dosage was appropriate. Raw mix batters contained trace amounts of nitrite.

A significant interaction was found between nitrate source and starter culture for nitrate content in sausages ($P \leq 0.001$). This interaction was examined and was due to the dose of nitrate added in the sausage formula since the origin of nitrate had no effect.

This fact explained why the higher dose of nitrate added to the raw mix batters led to sausages with higher residual nitrate content (**Table 3**). In addition, *S. carnosus* decreased the residual nitrate content in sausages because of its reported nitrate reductase activity (*2*). As expected, tocopherol addition had no effect on residual nitrate content. Likewise, storage time under modified atmosphere did not affect the residual nitrate content (**Table 3**).

Neither the various ingredients added in the raw mix batter formula nor the storage under modified atmosphere affected the residual nitrite content (**Table 3**).

Sausage Color. Several interactions were found between various factors for L*, a*, and b* values (**Table 3**). An ANOVA examining dose of nitrate added and origin of nitrate separately, instead of together, was run to check whether these two new factors were responsible for the interaction. This seemed to be the case for L* and b* values which were affected significantly by the dose and the origin of nitrate. Conversely, neither the dose nor the origin explained the interactions found for a* values, although they were affected by the tocopherol extract, the nitrate source, and, only at 0 days, also the starter culture. Therefore, the combination of these latter significant factors at the same time explained the multiple interactions found.

Meat lightness (L*), redness (a*), and yellowness (b*) were increased by the addition of tocopherols (**Table 3**). Lightness and yellowness also increased in those sausages in which the higher dose of CP had been added to the sausage formula. In addition, the presence of *S. carnosus* led to sausages with increased L*, a*, and b* values, whereas these values decreased after 45 days of storage under a modified atmosphere at 4 °C.

Tocol Content. The tocol content was determined in those raw mix batters with and without the tocopherol extract. The α-tocopherol, β-tocopherol, γ-tocopherol, and α-tocotrienol content in the mix without the addition of the tocopherol extract averaged 7.8 ± 0.93, 0.069 ± 0.011, 0.18 ± 0.015, and 0.36 ± 0.051 mg/kg, expressed as fresh weight, respectively. However, the α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, and α-tocotrienol in the mix containing the tocopherol extract averaged 26.2 ± 2.9, 1.8 ± 0.23, 61.3 ± 2.0, 9.2 ± 0.69, and 0.4 ± 0.28 mg/kg, expressed as fresh weight, respectively. In both cases, those tocols below the quantification limits were not reported.

Several interactions were found between different factors for the content of the different tocopherol and tocotrienol analogues

in sausages (**Table 4**). The study of the interactions showed that the factor dose of nitrate was significant, leading to higher content in tocol analogues when the nitrate dose was 140 mg/kg, whereas there were no differences between origins. In addition, various factors showed significant effects at 0 days but not at 45 days, explaining the interactions involving storage time.

The addition of the tocopherol extract to the formula led to significant changes in the content of the different analogues found in sausages (**Table 4**). This extract was rich in γ-tocopherol (**Table 1** footnote), which explained the high content of this analogue in sausages. The β-, γ-, and δ-tocotrienol analogue amounts were normally below the quantification limits and, as a result, were not reported. The content of α-tocopherol, β-tocopherol, and α-tocotrienol was lower in those sausages with the lower dose of chemical nitrate added. There was decreased content in all tocopherol analogues because of either the addition of conventional starter culture or storage time under modified atmosphere (**Table 4**).

Oxidative Status and Susceptibility to Oxidation. TBA determination measures malondialdehyde, which is a typical secondary oxidation product derived from lipid oxidation (*25*), whereas the FOX method measures LHP, which are primary oxidation products (*15*). However, it should be noted that this method was used to measure the LHPC but was also used to assess the susceptibility of samples to oxidation by means of various parameters described elsewhere (*15*, *16*).

Interactions between tocopherols × nitrate source ($P = 0.006$), tocopherols × storage time ($P = 0.007$), and nitrate source × storage time ($P = 0.047$) were found for the LHPC (**Table 5**). These interactions were examined, and we found that the origin of nitrate was a significant factor ($P = 0.005$). The LHPC of sausages containing chemical and CP origin of nitrate were 293 and 132 mmol cumene hydroperoxide eq/kg, respectively, which indicated that CP may have some antioxidant properties. In addition, this antioxidant activity was only exhibited after 45 days. Despite the fact that the presence of all of these significant factors acting together may confound some main effects and explain some interactions, the addition of the tocopherol extract reduced the LHPC because of its antioxidant activity, whereas the storage time caused an increase in the LHPC (**Table 5**).

As for secondary oxidation, TBA values showed significant interactions between storage time × tocopherols ($P \leq 0.001$), storage time × starter culture ($P = 0.013$), and starter culture × nitrate source ($P = 0.002$). Interactions were examined, and results indicated that the *S. carnosus* culture reduced TBA values after 0 days, but with further storage, it promoted oxidation. This can be due to the fact that tocopherol extract ($P = 0.007$), nitrate source ($P = 0.011$), and the starter culture ($P = 0.033$) were significant factors after 0 days storage, whereas only the tocopherol extract ($P \leq 0.001$) and starter culture ($P = 0.042$) showed to be significant factors after 45 days storage.

The combination of these effects that were taken together in the ANOVA explained several interactions but, as expected, the addition of the tocopherols led to sausages with an overall lower secondary oxidation values because of their antioxidant activity (**Table 5**). In addition, lipid oxidation increased during storage, and thus, higher TBA values were recorded when samples had been stored for 45 days (**Table 5**). Neither the starter culture nor the different nitrate sources had any effect on TBA values.

Several significant interactions were found between the different factors for the induced FOX parameters (**Table 6**). These interactions were examined, and higher doses of nitrate and CP addition reduced the susceptibility to oxidation. In addition, all of the factors studied were significant for all of the FOX assay parameters at both storage times, with the exception of the starter culture at 0 days of storage. All of these significant effects taken together explain why we found many interactions.

Table 5. Effect of Formula Factors and Storage Time on Sausage Lipid Hydroperoxide Content (LHPC), Thiobarbituric Acid (TBA) Values, and Overall Acceptability to Consumers[a]

	LHPC (mmol CHP eq/kg)[b]	TBA (μg MDA/kg)[c]	overall acceptability
Tocopherols[d]			
0	352 y	300 y	−0.3
200	73 x	30 x	0.1
SEM[e]	38	22	0.33
Starter Culture[f]			
conventional	203	170	−0.3
S. carnosus	222	170	0.1
SEM	38	22	0.33
Nitrate Source (Origin and Dose)[g]			
chemical 70	348 x	220	−0.5
chemical 140	237 x	130	0.4
celery 70	129 x	140	0.4
celery 140	134 x	180	−0.7
SEM	54	32	0.46
Storage Time			
0 days	138 x	90 x	N.A.[h]
45 days	287 y	240 y	
SEM	38	22	

[a] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (n = 64, 64, and 192 for LHPC, TBA values, and acceptability, respectively). 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 mmol of cumene hydroperoxide equivalents per kg of sausage as dry weight. Significant interactions between tocopherols × nitrate source ($P = 0.006$), tocopherols × storage time ($P = 0.007$) and nitrate source × storage time ($P = 0.047$) for LHPC were found. [c] Results are expressed as μg of malondialdehyde per kg of sausage as dry weight. Significant interactions between storage time × tocopherols ($P \leq 0.001$), storage time × starter culture ($P = 0.013$), and starter culture × nitrate source ($P = 0.002$) for TBA were found. [d] Tocopherol dose expressed in mg of tocopherol analogues/kg meat. [e] SEM means standard error of the mean. [f] Conventional starter cultures include *Lactobacillus sakei* and *Staphylococcus xylosus*. The *Staphylococcus carnosus* starter culture also includes the conventional starter culture. [g] Origin of nitrate and dose of nitrate expressed in mg $NaNO_3$/kg meat. [h] N.A. means not analyzed at 0 days.

The results in **Table 6** indicate that tocopherols were efficient in delaying lipid oxidation onset. In addition, the lower dose of chemical nitrate showed significant differences with other nitrate sources for all of the defined FOX parameters, whereas no differences were observed between the other 3 nitrate source combinations. The addition of *S. carnosus* showed increased values for MAXLHP, final LHP, and AUC, thus showing that sausages to which this culture had been added were more prone to oxidation. All of the induced FOX parameters increased after 45 days of storage under modified atmosphere at 4 °C.

Sensory Characteristics. The results for the overall acceptability test carried out after 45 days storage under a modified atmosphere in sealed bags are shown in **Table 5**. Consumers found no significant differences between sausage formulas.

In addition to this sensory test, a triangle test was carried out to examine whether there were significant differences in color between samples 3 and 14 (see **Table 1** for formula factors) since these two samples showed the maximum differences in the data obtained through the colorimeter. In this triangle test, 14 out of 24 panelists ($P \leq 0.05$) matched the similar samples. When these panelists were also asked which overall color they preferred, 11 out of 14 indicated the sample without the tocopherols added. On

Table 6. Effect of Formula Factors and Storage Time on Sausage Maximum Lipid Hydroperoxide Value (MAXLHP), Time to Reach the Maximum Lipid Hydroperoxide Value (TMAX), Oxidation Rate (OR), Final Lipid Hydroperoxide Value (Final LHP) ,and Area under the Curve (AUC)[a]

	MAXLHP (mmol CHP eq kg $^{-1}$)[b]	TMAX (h)[c]	OR (μmol CHP eq kg^{-1} h^{-1})[d]	final LHP (mmol CHP eq kg $^{-1}$)[e]	AUC (mol CHP eq kg^{-1} h)[f]
Tocopherols[g]					
0	2900 y	48	86 y	2190 y	500 y
200	1500 x	54	26 x	980 x	251 x
SEM[h]	104	2.2	6.6	90	19
Starter Culture[i]					
conventional	2100 x	50	54	1390 x	343 x
S. carnosus	2400 y	53	59	1790 y	402 y
SEM	104	2.2	6.6	90	19
Nitrate Source (Origin and Dose)[j]					
chemical 70	2900 y	39 x	105 x	1900 y	478 y
chemical 140	2000 x	54 y	51 y	1500 xy	344 x
celery 70	1900 x	56 y	31 y	1400 x	312 x
celery 140	2200 x	56 y	38 y	1600 xy	357 x
SEM	147	3.2	9.4	127	28
Storage Time					
0 days	1700 x	44 x	39 x	1120 x	296 x
45 days	2700 y	59 y	74 y	2050 y	450 y
SEM	104	2.2	6.6	90	19

[a] Values given in this table correspond to least-squares means obtained from multifactor ANOVA (each parameter $n = 64$). 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 mmol of cumene hydroperoxide equivalents per kg of sausage as dry weight. Significant interactions between tocopherols × starter culture ($P = 0.040$), tocopherols × nitrate source ($P = 0.005$), tocopherols × storage time ($P \leq 0.001$), starter culture × nitrate source ($P = 0.004$), starter culture × storage time ($P = 0.006$), and storage time × nitrate source ($P = 0.007$) for MAXLHP were found. [c] Significant interactions between nitrate source × tocopherols ($P = 0.001$), nitrate source × storage time ($P = 0.015$), and storage time × tocopherols ($P = 0.017$) for TMAX were found. [d] Results are expressed as μmol of cumene hydroperoxide equivalents per h and kg of sausage as dry weight. Significant interactions between nitrate source × tocopherols ($P \leq 0.001$), nitrate source × starter culture ($P = 0.008$), nitrate source × storage time ($P = 0.007$), and storage time × tocopherols ($P \leq 0.001$) for OR were found. [e] Results are expressed as mmol of cumene hydroperoxide equivalents per kg of sausage as dry weight. Significant interactions between starter culture × tocopherols ($P \leq 0.001$), starter culture × nitrate source ($P = 0.030$) and starter culture × storage time ($P = 0.001$) for Final LHP were found. [f] Results are expressed as mol of cumene hydroperoxide equivalents and h per kg of sausage as dry weight. Significant interactions between tocopherols × nitrate source ($P = 0.009$), tocopherols × starter culture ($P = 0.039$), tocopherols × storage time ($P \leq 0.001$), starter culture × nitrate source ($P = 0.009$), and starter culture × storage time ($P = 0.009$) for AUC were found. [g] Tocopherol dose expressed in mg of tocopherol analogues/kg meat. [h] SEM means standard error of the mean. [i] Conventional starter cultures include *Lactobacillus sakei* and *Staphylococcus xylosus*. The *Staphylococcus carnosus* starter culture also includes the conventional starter culture. [j] Origin of nitrate and dose of nitrate expressed in mg $NaNO_3$/kg meat.

a different day, in a sensory test panelists were asked to rank samples by overall color intensity using sausages from the following treatments: 1, 2, 3, 4, 8, 12, and 16 (see **Table 1** for formula factors). Treatment 12 was ranked in the first position as the least dark one, followed by treatment 16 in second position, treatments 3 and 4 in third and fourth positions indistinctly, treatment 8 in fifth position, treatment 1 in sixth position, and finally treatment 2 as the darkest sample. With the exception of positions 3 and 4, the other positions were significant at $P \leq 0.05$. Thus, the addition of tocopherols to the formula led to sausages with lower color intensity, which was consistent with colorimeter values. Nevertheless, the addition of *S. carnosus* led to darker sausages when tocopherols had not been added. Finally, according to the panelists, the presence of CP in the sausage formula led to a darker color. Along with this test, panelists were also asked to indicate their preferred sample by appearance (30% of panelists preferred sample 3, whereas 23%, 17%, and 13% preferred samples 12, 1, and 4, respectively), which was not related to the darkness ranking.

DISCUSSION

The results showed that, when the dose added to the sausage raw mix batter met the EU regulation for organic meat products (*8*), the residual nitrate and nitrite amounts were far below the limits (each at 50 mg/kg expressed as $NaNO_3$ and $NaNO_2$, respectively) (**Table 3**). In addition, no differences were observed in the residual content of nitrate or nitrite when comparing the CP or the chemical origin.

S. xylosus possesses nitrate and nitrite reductase activity, but this is not as intense as that of *S. carnosus* (*26*). Therefore, the addition of *S. carnosus* culture to the mix caused a very efficient reduction of nitrate to nitrite, which ensured optimal color formation during initial fermentation stages (*10*, *27*). This activity seems to disappear with storage time, which may be due to the environmental conditions after the curing process (**Table 3**). The nitrite formed is a reactive compound that can be further reduced after reacting with the heme moiety and various endogenous and exogenous reductants such as ascorbate (*1*, *6*). This reactivity explains the recorded residual nitrite decrease after 45 days of storage. Alternatively, Ahn et al. (*28*) found lower residual nitrite in vacuum-packed sausages than those stored under aerobic conditions: they argued that the reducing environment allowed the conversion of nitrite to nitric oxide.

Among various functions, nitrite contributes to the characteristic color of cured meat (*1*, *29*). This seems to be ensured at the dose of 70 mg/kg regardless of the nitrate source because measurement of the sausage color was not significantly different from those sausages that received conventional doses (chemical at 140 mg/kg) of nitrate (**Table 3**). These results corroborate other studies on CP in cooked ham processing (*10*). However, in the present study, the addition of CP at high doses led to sausages with greater lightness and yellowness, which could be due to the intrinsic color of the concentrate powder.

Table 7. Spearman Correlation Coefficients between Lipid Hydroperoxide Content, Induced FOX Parameters and Tocol Content in Sausages, after Storage for 45 Days[a]

	LHPC	MAXLHP	TMAX	OR	Final LHP	AUC	α-T	β-T	γ-T	δ-T	α-T3
LHPC	1[b]	0.75	−0.43	0.73	0.78	0.77	−0.77	−0.75	−0.78	−0.81	−0.69
		0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	16	16	16	16	16	16	16	16	16	16	16
MAXLHP		1.00	−0.37	0.97	0.98	0.97	−0.79	−0.74	−0.78	−0.69	−0.83
			0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		16	16	16	16	16	16	16	16	16	16
TMAX			1.00	−0.50	−0.42	−0.45	0.45	0.14	0.41	0.33	0.49
				0.05	0.10	0.08	0.08	0.61	0.11	0.21	0.05
			16	16	16	16	16	16	16	16	16
OR				1.00	0.97	0.97	−0.82	−0.75	−0.82	−0.73	−0.86
					0.00	0.00	0.00	0.00	0.00	0.00	0.00
				16	16	16	16	16	16	16	16
final LHP					1.00	1.00	−0.80	−0.78	−0.84	−0.72	−0.83
						0.00	0.00	0.00	0.00	0.00	0.00
					16	16	16	16	16	16	16
AUC						1.00	−0.80	−0.77	−0.84	−0.72	−0.84
							0.00	0.00	0.00	0.00	0.00
						16	16	16	16	16	16
α-T							1.00	0.87	0.91	0.90	0.84
								0.00	0.00	0.00	0.00
							16	16	16	16	16
β-T								1.00	0.95	0.93	0.78
									0.00	0.00	0.00
								16	16	16	16
γ-T									1.00	0.93	0.86
										0.00	0.00
									16	16	16
δ-T										1.00	0.82
											0.00
										16	16
α-T3											1.00
											16

[a] LHPC = lipid hydroperoxide content, MAXLHP = maximum lipid hydroperoxide value, TMAX = time the maximum lipid hydroperoxide value was achieved, OR = oxidation rate, final LHP = the final lipid hydroperoxide value, AUC = area under the curve, α-T = α-tocopherol, β-T = β-tocopherol, γ-T = γ-tocopherol, δ-T = δ-tocopherol, and α-T3 = α-tocotrienol. [b] Spearman correlation coefficient, *P* value, and number of samples are given in order one below the other.

Isabel et al. (*30*) found that a higher α-tocopherol concentration in dry-cured hams reduced color fading and weight loss, thus explaining the greater moisture content found in those sausages receiving the tocopherol extract. Therefore, it is possible that the higher water content found in those sausages enriched with tocopherols provoked the increase in L*, a*, and b* values (**Table 3**) since the addition of 200 mg/kg of tocopherol extract in a sunflower oil matrix did not significantly increase these instrumental color values (data not shown). Although other authors reported no effect on the color stability of cured pork products from animals that received diets rich in tocopherol (*31*, *32*), the latter's addition may protect from oxidation, thus maintaining color properties during fermented sausage ripening. Meat discoloration because of oxidation and lipid oxidation is a major drawback (*33*, *34*), and the protective effect of tocopherols against lipid oxidation during ripening is clearly observed by looking at primary and secondary oxidation values (**Table 5**).

The decrease in redness values and the increase in yellowness values are often related to increased lipid oxidation (*32*, *35*). After 45 days of storage under a modified atmosphere, all color parameters (L*, a*, and b*) were lower than those found after ripening (**Table 3**). Rubio et al. (*35*) studied the effect of storage time on color stability in a conventional dry-cured sausage stored under the same modified atmosphere (20% CO_2 and 80% N_2). These authors found that, on comparing instrumental color values after 0 and 120 days of storage, L* and a* values increased, whereas yellowness decreased. However, they also found that these trends changed with different storage periods. In addition, the recorded interactions we found between nitrate source and storage time (**Table 3**) probably confuse some effects on color since significant Spearman correlations between TBA values and yellowness ($r_s = 0.500$, $P = 0.049$), and between TBA values and redness ($r_s = -0.724$, $P = 0.002$) were found only when using the data obtained from samples stored for 45 days.

Redness is used as an indicator of color stability since oxidative discoloration of cured meats converts nitrosylmyoglobin to nitrate and metmyoglobin (*36*, *37*). This phenomenon explains the recorded decrease in redness during storage (**Table 3**). Red was more stable over a storage period, and lipid oxidation was lower when low nitrite-cured pork products (50 mg/kg) came from animals that received 500 mg α-tocopheryl acetate/kg feed supplement (*38*).

Color influences consumers' decisions: differences in sausage color are associated with the product's quality and freshness (*39*). In a triangle test, panelists were able to differentiate a sausage to which neither tocopherols nor *S. carnosus* had been added from another sausage to which both were added. This suggests that consumers were able to find differences in color between samples. To confirm this, a ranking test was also carried out. The results of this test confirmed that there were differences between samples when assessing the sausages' overall darkness. However, consumers' preferences seemed to be neither positively nor negatively correlated with darker-colored sausages.

Overall acceptability was studied after 45 days of storage, and consumers showed no differences in preference between treatments for any of the factors studied (**Table 5**). It should be taken into account that Catalan consumers because of the big quantity of small- and large-scale producers are used to a broad variability in these types of dry-cured sausages in their local markets. This indicates that the panelists differ in their color preferences and/or appreciate other characteristics apart from color. Consumers did not show differences in their acceptability scores when CP was added at 0.23% and 0.46% (**Table 5**). In cured cooked ham produced using the same vegetable extract, the addition of CP at 0.2% had no effect on sensory attributes, whereas at 0.3%, the panel described an increased vegetable aroma (*10*).

The addition of the tocopherols extract reduced the oxidative status (LHPC and TBA values) of the sausages but also their susceptibility to oxidation (**Tables 5** and **6**). Therefore, the addition of tocopherols to the formula could be a useful strategy for preventing lipid oxidation in dry-fermented sausages since lipid oxidation increased with storage time (**Table 5**). The LHPC and the induced FOX parameters correlated closely with each other. In addition, LHPC, final LHP, MAXLHP, and AUC correlated closely with the tocol amounts found in sausages after 0 (data not shown) and after 45 days of storage (**Table 7**), which supports the finding that these parameters are good markers of lipid oxidation.

Studying the addition of nitrite instead of nitrate, Walsh et al. (*38*) found that the dose of 100 mg of nitrite/kg meat in cured pork products reduced TBA values from values in products with only 50 mg/kg. The interaction between starter culture and nitrate source found for TBA values ($P = 0.002$) could explain why we found no differences between TBA values for nitrate source in our study. The nitrate reductase activity of *S. carnosus* caused a rapid formation of nitrite, which because of its antioxidant properties may have protected all tocol analogues from oxidation from the early stages of the curing process (**Tables 3** and **4**). Besides, interactions involving tocol analogues may also be explained by the dose of nitrate added since it may affect the nitrite supply during curing.

The presence of carotenoids in the CP, which may act as radical scavengers during storage, reduced these oxidation parameters, whereas the low tocopherol content when conventional starter cultures were combined with low ingoing nitrate amounts caused their increase. The nitrate reductase activity of *S. carnosus* exhausted nitrate rapidly, which explains that this culture showed greater susceptibility to oxidation. In fact, this indicates the protective role of residual amounts of nitrate and/or nitrite against oxidation rather than the pro-oxidative activity of *S. carnosus* (**Table 6**). This reasoning involves a reduced nitrite supply during long-term ripening and/or storage, which is consistent with the significant interactions involving starter culture and nitrate source found when assessing oxidation.

Taken together, these results indicate that organic sausages can be produced without significantly affecting quality or consumer acceptability. Moreover, the addition of *S. carnosus* reduces the residual levels of nitrate without affecting TBA values after 45 days of storage in an atmosphere containing 20% CO_2 plus 80% N_2 at refrigeration. However, according to the induced FOX values, the combination of low doses of nitrate with the addition of *S. carnosus* may lead to increased susceptibility to oxidation. The replacement of chemical grade nitrate source by CP is a useful strategy for organic production and, at the lower dose, does not affect color formation or any other parameter, when compared with the conventional procedures.

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Supporting Information Available: All of the quantified fatty acids in the raw mix batter. This material is available free of charge via the Internet at http://pubs.acs.org.

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