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Properties of reformulated hot dog sausage without added nitrites during chilled storage

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Abstract

The aim of this study was to assess the effect of a complete nitrite replacement strategy using celery, carmine, sodium lactate and orange dietary fibre combined with vitamins C and E, on the quality characteristics (technological, sensorial and safety properties) of hot dog sausages (five samples) during chilled storage ($2 \pm 1^{\circ}$C 60 days). Nitrite replacers (combined with vitamins C and E) presented antioxidant activity, reducing lipid oxidation in reformulated samples. At the end of storage redness (a^*) was similar in the control sample (with added nitrite) and in the sample without added nitrite. Sensory evaluation detected no significant difference between samples with and without added nitrite. All the reformulated samples were judged acceptable by the panellists. At the end of storage, the control sample contained more than four times as much residual nitrite as the reformulated samples. Growth of presumptive *Clostridium perfringens* was not observed in any of the samples. Samples without added nitrite had longer shelf-lives than control sausage. Samples containing 0.1% vitamin C registered the lowest microbiological levels. This strategy could be a good alternative to reduce and/or eliminate added nitrite in hot dog sausages.

Keywords

Hot dog, residual nitrite, L-ascorbic acid, vitamin E, orange dietary fibre, sodium lactate

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INTRODUCTION

Hot dog sausage is one of the most important and popular meat products worldwide. The processing for this product consists of mixing meat with different curing ingredients, flavourings and colourings to obtain a product with acceptable, technological and sensory characteristics.

The use of curing ingredients, mainly NO_2 (sodium nitrite), is one of the most effective means for controlling microbial spoilage, mainly from *Clostridium botulinum,* in hot dog sausage, but in addition to acting as an antioxidant it can significantly affect the colour, odour and flavour of the sausage (Cassens, 1997; European Food Safety Authority (EFSA), 2003). However, during cooking the nitrites can combine with amines in the meat to form carcinogenic *N*-nitroso compounds.

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In response to current consumer demands for products that are healthier and safer but retain their sensory characteristics (colour, taste, etc.), the industry, researchers and official bodies are striving to reduce or eliminate the nitrite present in meat products with as little modification as possible. In this context, various ingredients have been used to replace added nitrites to ensure that these additives perform their functions in the end product while maintaining quality (Coutinho de Oliveira et al., 2012; EFSA, 2003; Jafari and Emam-Djomeh, 2007; Viuda-Martos et al., 2009; Zarringhalami et al., 2009). Celery, beet sugar and other ingredients have been assayed as natural sources of nitrites, and particularly nitrates which are reduced to nitrites by microorganisms e.g. *Staphylococcus* (EFSA, 2003; Gotterup et al., 2008).

Different concentrations of sodium lactate (SL) (3–3.3%) have also been added to low fat sausage as an antibacterial agent, with sensorially acceptable results (Lin and Lin, 2002).

Colour is one of the most important criteria for consumers when purchasing sausages. Traditionally, nitrites lend frankfurter or hot dog sausages the pink colour characteristic of meat products (Cassens, 1997; Ruiz-Capillas and Jiménez-Colmenero, 2008), but other natural ingredients such as cochineal (Madsen et al., 1993) or fibres such as orange dry fibre (ODF) have been used to achieve a similar result in bologna sausage (Viuda-Martos et al., 2010a, 2010b).

Then again, the NO_2 in the product is important for its antioxidant activity. Lipid oxidation causes undesirable odours and flavours, as well as reducing the nutritional value of the food. Questions of safety and consumer preference have led to increased interest and research into natural antioxidants (Georgantelis et al., 2007). Vitamin E (vit E; tocopherol) is a typical natural antioxidant used directly in meat product formulation or as an animal feed supplement (Estévez and Cava, 2007; Georgantelis et al., 2007; Guidera et al., 1997; Mercier et al., 1998). Vitamin C (vit C; ascorbic acid) is also a reducing agent which inhibits oxidation chains and the development of browning in beef patty and sausage (Pourazrang et al., 2002). Combinations of vit C and tocopherol are effective in reducing oxidative reactions and the production of sulphur volatiles in meat by scavenging free radicals and can enhance meat product stability (Lee et al., 1999).

Studies aimed at eliminating or reducing nitrite in meat processing have generally tended to test individual alternatives (ingredients/additives) for specific purposes (colour, flavour and safety). However, there has been little research dealing with a global strategy for producing a cooked meat product (hot dog) without added nitrite, including different ingredients/additives to reproduce the functions (colour, antioxidant, antimicrobial, etc.) of nitrite. Therefore, the aim of this study was to evaluate the technological, sensory and microbiological properties, during chilled storage, of hot dog sausage reformulated with different ingredients (celery, SL, ODF and cochineal) along with different combinations of vit C and vit E, as a global strategy for replacing added chemical nitrites in these products and so offering healthier products to the consumer.

MATERIALS AND METHODS

Raw meat materials, non-meat ingredients and additives

A representative amount of fresh beef (flank steak and shank from different animals) obtained from a local meat market was passed through a grinder with a 0.4 cm plate (Mainca, Model PM70, Granollers, Spain). Batches of approx. 1 kg were vacuum packed, frozen and stored at $-20^{\circ}C$ until used, within 2 weeks. The meat contained $19.98 \pm 0.43\%$ protein and $13.49 \pm 1.46\%$ fat.

The ingredients used for formulation of the hot dogs were: egg white and fresh celery (from a local market, Spain), potato starch (Trades S.A., Barcelona, Spain), garlic powder (Novelda, Alicante, Spain), sunflower oil (Koipesol SOS, Cuetara S.A., Madrid, Spain), milk powder (Anvisa, Madrid, Spain) and ODF (Anvisa, Madrid, Spain). Other additives used in product formulation included sodium chloride (Panreac Química S.A., Barcelona, Spain), sodium tripolyphosphate (TPP) (Manuel Riesgo S.A., Madrid, Spain), sodium nitrite (Fulka Chemie GmbH, Buchs, Germany), species-flavouring (Gewürzmüller, GmbH, Münichingen, Germany), SL (E325, Manuel Riesgo, Madrid, Spain), vit E (Manuel Riesgo S.A., Madrid, Spain), vit C (Manuel Riesgo S.A., Madrid, Spain), colourings: carmine (E120) (Trades S.A., Barcelona, Spain) and annatto (E160b) (CHR Hansen, Madrid, Spain).

Hot dog sausage preparation

Five different formulations (all with 50% meat) were studied. A control sample (Control) was prepared traditionally with chemical nitrite added (120 mg/kg). Reformulated samples were prepared with celery (1%), SL (3%), carmine (0.05%) and orange dietary fibre (ODF) (1%) instead of nitrite, and different combinations of vitamins C and E depending on the sample. C5E5: 0.05% vit C and 0.05% vit E; C0E10: 0.0% vit C and 0.1% vit E; C10E0: 0.1% vit C and 0.0% vit E; C10E10: 0.1% vit C and 0.1% vit E. The following ingredients were also added to all samples: 3.8% starch, 0.5% spices/flavouring, 1.5% garlic powder, 1.9% egg white, 17% corn oil, 1.2%

milk powder, 1.4% sodium chloride and 0.46% sodium TPP.

Preparation of the sausages (hot dog) was as described by Delgado-Pando et al. (2010). The specific preparation was briefly as follows: raw meat material (previously thawed at $2 \pm 1^{\circ}C$ for 18 h) was comminuted and homogenized (at the same time) for 1 min in a chilled cutter (2°C) (Stephan Model UM5 Universal, Stephan u. Söhne, Hameln, Germany). Half of the ingredients/additives used for each formulation were added to the ground meat and the sample mixed again for 1 min. The rest of the ingredients/additives were added and the whole was homogenized for 1 min. Finally, the whole meat batter was homogenized under vacuum for 2 min. Mixing time was standardized at 5 min. The final batter temperature was below 13°C in all cases. The meat batter was stuffed into 20 mm diameter Nojax cellulose casings (Viscase S.A., Bagnold Cedex, France) and hand-linked. Hot dog sausages were heat processed in an Eller smokehouse (model Unimatic 1000, Micro 40, Eller, Merano, Italy) until the core of the product reached 72°C. Heat processing conditions were established beforehand, and the internal temperature was monitored throughout heating by means of thermocouples inserted in each frankfurter (thermal centre) and connected to a temperature recorder (Yokogawa Hokuskin Electric YEM, Model 3087, Tokyo, Japan). When heating was complete, the sausages were cooled (at room temperature) and kept in a cold room (2°C for 14 h), after which the casing was removed and the sausages were vacuum packed (Cryovac1 BB3050, Madrid, Spain) and stored at 2°C (±1°C).

Proximate composition

Moisture and ash contents of the sausages were determined in triplicate using the standard Association of Official Analytical Chemists (AOAC) (2000) method. Fat content was evaluated (in triplicate) according to Bligh and Dyer (1959). Protein content was measured in quadruplicate by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI).

Processing and cooking loss

Water and fat binding properties were evaluated by measuring processing and cooking loss. Processing loss was calculated, in sextuplicate, as the weight loss (expressed as % of initial sample weight) occurring after heat processing and chilling overnight at 2°C.

Cooking loss (water and fat binding properties) was determined (in quadruplicate) as percent total, water and fat loss (Jiménez-Colmenero et al., 2010a). Briefly, around 32 g from each formulation was placed in containers (27 mm diameter) and hermetically closed, then heated (70°C/30 min) in a water bath (GRANT, OLS 200, Grant instruments, Cambridge, Ltd., England). When heating was complete, the containers were opened and left to stand upside down (for 30 min) to release the exudate onto a plate that had been previously weighed. Total fluid release (TFR) was expressed as g 100/g of initial sample weight. Water release (WR) was determined as weight loss after heating the total released fluid for 16 h on an oven (Model IDL-AI-36, Labolan SL, Navarra, Spain) at 105°C and expressed as g 100/g of initial sample weight. Fat release (FR) was calculated as the difference between TFR and WR.

pH

The pH was determined on a pH-meter (Meterlab, Denmark) at room temperature on sausage homogenates in water in a ratio 1:10 (w/v). Three determinations were performed for each formulation.

Colour measurement

Colour, CIE-LAB tristimulus values, lightness L^*, redness a^* and yellowness b^* of sausage cross-sections were evaluated on a CR-400 Chroma Meter (Konica Minolta Business Technologies, Tokyo, Japan). Ten determinations were performed for each formulation.

Texture profile analysis (TPA)

TPA was performed at room temperature in a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) following the method of Bourne (1978). Eight cylindrical cores (diam = 20 mm, height = 20 mm) from each formulation were axially compressed to 40% of their original height. Force–time deformation curves were obtained with a 30 kg load cell, applied at a crosshead speed of 1 mm/s. TPA is a compression test based on two compression cycle (known as the ''two bite test''). This analysis measures textural parameters (hardness (Hd), cohesiveness (Ch) springiness (Sp) and chewiness (Cw)) which correlate well with sensory evaluation parameters.

Thiobarbituric acid value (TBARs)

Lipid oxidation was evaluated on the basis of changes in thiobarbituric acid-reactive substances (TBARs) during the chilled storage. The resulting colour was measured at 532 nm in a UV/VIS Spectrophotometer (Perkin–Elmer Lambda 15, Boston). The results were expressed as mg malonaldehyde/kg of sample. TBARs determinations were performed in triplicate.

A standard curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO). The procedure for measurement of TBARs was based on methods used by Delgado-Pando et al. (2011).

Microbiological analysis

For each sample, 10 g (in replicate) was taken and placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 ml of peptone water (0.1%). After 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on the following media: Plate Count Agar (PCA) (Merck, Germany) for total viable count (30°C for 72 h); De Man, Rogosa, Sharpe Agar (MRS) (Merck, Germany) for lactic acid bacteria (LAB) (30°C for 3–5 days); and Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for *Enterobacteriaceae* (37°C for 24 h). The results were expressed as logarithms of colony forming units per gram (Log cfu/g).

Presumptive Clostridium perfringens. For each sample, 10 g (in replicate) was homogenized with 90 ml of peptone water, followed by heat treatment at 80°C for spore activation. Spore counts of *C. perfringens* were performed by the pour plate technique in tryptose-sulphite-cycloserine (TSC) agar (Oxoid), which was incubated in an anaerobic jar with an anaerobic kit (Oxoid) at 37°C for 20 h. The counts were performed according to French Standard NF-V-08-056. All microbiological analyses were performed in duplicate.

Residual nitrite content

Residual nitrite contents were determined using flow injection analysis (FIA) as described by Ruiz-Capillas et al. (2007). Results, expressed as mg/kg of sample, were averages of three replications per sample.

Sensory evaluation

The sensory properties of the hot dogs were evaluated by a tasting panel of at least 18 members from the Institute (ICTAN-CSIC) who consumed hot dogs regularly. The panel received preliminary training with the products and terminology. The sensory analysis was performed on day 1 of storage. Samples 2.5 cm long from each formulation were heated in a microwave oven for 30 s, then immediately presented to panellists in random order. Panellists were instructed to evaluate the colour (0 = very bad, 10 = very good), juiciness (0 = very dry, 10 = very juicy), firmness (0 = very soft, 10 = very hard) and overall acceptability (0 = dislike extremely, 10 = like extremely) on a non-structured scale without fixed extremes.

Statistical analysis

Analysis of variance (ANOVA one way) and Tukey's multiple range test were carried out to evaluate the statistical significance ($p < 0.05$) of the effect of hot dog formulation. The normal distribution of samples was checked using the Shapiro Wilks test. The Kruskal-Wallis test was used to test samples that did not fit the normal distribution. Statistical analysis was performed using SPSS Statistics 17.0 (SPSS Inc, Chicago).

RESULTS AND DISCUSSION

Proximate composition

Ash levels ranged from 2.49% to 3.95%. Significant differences were observed between the control sample and the reformulated samples, with higher ash levels ($p < 0.05$) in the reformulated sausage (>3.50%) than in the control sample (2.49%). The differences in ash content between control and reformulated samples can probably be explained by the addition of celery powder to the latter. Protein levels in the samples ranged from 12.19% to 13.91%, with no significant differences between samples. Similarly, fat levels ranged from 23.50% to 24.12%, with no significant differences between the control and reformulated samples. Humidity values were higher ($p < 0.05$) in the control (57.68%) than in the reformulated samples, which presented values ranging from 55.43% to 56.15%. These results agree with those of other authors for frankfurter reformulation (Jiménez-Colmenero et al., 2010a; Viuda-Martos et al., 2010a, 2010b).

Processing loss, cooking loss and pH

Reformulation produced no significant ($p > 0.05$) differences in processing loss between treatments; values ranged from 7% to 10%. TFR (combination of water and fat losses) was <1 throughout storage, again without significant differences between samples. Similar results were reported by Jiménez-Colmenero et al. (2010a) for low-fat sausages with an emulsion to replace pork backfat, where again water- and fat-binding properties during cooking were unaffected by differences in formulation.

pH values were between 5.93 and 6.50, with significant differences between treatments (Figure 1). The initial pH was higher than 6 in all samples, and the highest pH (6.50) was recorded in the control. Other authors have reported similar levels in frankfurters (Delgado-Pando et al., 2011). The samples reformulated without added nitrite presented lower levels ($p < 0.05$) than the

control sample, with values between 6.07 and 6.24. The lower pH in these samples may be due to the use of SL in their reformulation. This has been reported by other authors in connection with the reduction of pH by lactate in low-fat Chinese-style sausage (Lin and Lin, 2002). In our study, pH was also significantly affected by vit C. The pH of samples treated with 1% vit C (C10E0 and C10E10) (6.07–6.08) was lower ($p < 0.05$) than the other samples, where levels ranged from 6.20 to 6.24. This could be the result of acidification due to the high concentration of vit C.

All samples registered a significant general reduction in pH during storage. This was less significant in samples formulated with high concentrations of vit C (C10E0 and C10E10), but they still registered the lowest ($p < 0.05$) pH values (6.01–5.93). The decrease in pH may have been due to a high rate of microbial growth, mainly LAB (Dave and Ghaly, 2011). The microbial results in this experiment (Table 3) could also explain this behaviour of pH (Figure 1). The decrease in pH was more pronounced in the control sample than in the samples formulated with high vit C levels, where there was less microorganism growth.

Colour

The colour of the sausage was significantly affected by formulation and storage time (Table 1). The control sample (with added nitrite) had the highest ($p < 0.05$) initial lightness (L^*) values (69.98), followed by the sample (C10E0) containing 1% vit C (63.65), while the samples containing vit E (C5E5, C0E10, C10E10) presented significantly lower L^* values (56–59). Higher lightness (L^*) has been linked to the reactions of the added nitrite with the meat (Cassens, 1997; Coutinho de Oliveira et al., 2012). Other authors have also reported that meat products prepared with nitrite presented higher L^* levels than those prepared without nitrite (Li et al., 2013; Viuda-Martos et al., 2009; Zarringhalami et al., 2009).

Replacing nitrite with vit C, carmine, vit E and the other ingredients increased ($p < 0.05$) redness (a^*), particularly in the samples with vit C (C5E5, C10E0, C10E10), while no significant differences in yellowness (b^*) were observed between samples. This could be due to the colorant effect of these ingredients, especially carmine.

During storage, L^* and b^* values were lower ($p < 0.05$) in all samples up to day 40, with an increase at the end of the storage period (Table 1). These findings are similar to reports by other authors for frankfurters (Delgado-Pando et al., 2011). However, the a^* values remained more or less unchanged until the end of storage, when an increase was observed in parameters L^* and b^* only (Table 1).

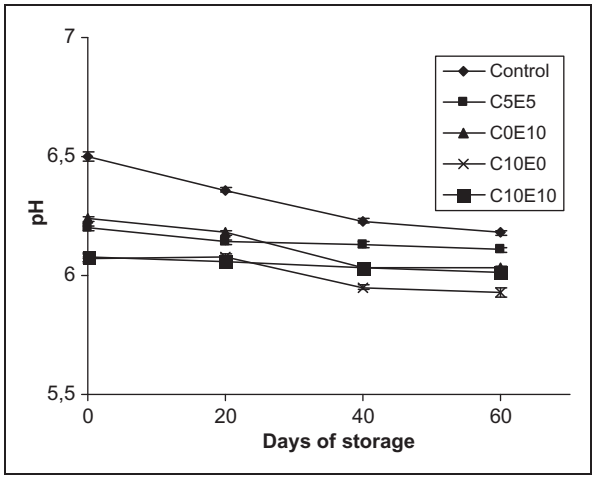

Figure 1. pH values of hot dog samples during chilled storage (2 ± 1°C). Data expressed as mean ± SD. ($n = 6$).

Texture profile analysis

Textural behaviour was affected by formulation (Table 2). Results showed lower ($p < 0.05$) hardness and chewiness in the control sample, but higher ($p < 0.05$) cohesiveness than reformulated samples (C5E5, C0E10, C10E0 and C10E10) (Table 2). However, springiness was similar ($p > 0.05$) irrespective of the formulation. In particular, control samples had a lower protein/moisture ratio than reformulated samples (C5E5, C0E10, C10E0 and C10E10). This would imply a lower effective protein concentration in control samples (in terms of less possibilities of forming a gel/emulsion matrix), and so the structures should be less firm. In addition, the reformulated samples had a higher protein/moisture ratio (higher effective protein concentration), which in the presence of other ingredients such as ODF should provide firmness in the end product. Other authors report that the addition of ODF to cooked meat sausages produces an increase in hardness associated with the inclusion of particles in the protein matrix, which would presumably reinforce binding during cooking (Viuda-Martos et al., 2009, 2010a, 2010b). Other authors have also reported increased hardness with the addition of various kinds of fibre (soy, wheat, cereal or fruit) to cooked meat emulsions (Fernández-Ginés et al., 2003; Jiménez-Colmenero et al., 2010b; Viuda-Martos et al., 2010a, 2010b).

Chilled storage affected ($p < 0.05$) some TPA parameters (Table 2). Hardness had increased ($p < 0.05$) by day 60 in the control and reformulated samples (Table 2). The increase in the intensity of hardness was different in each sample type. Nevertheless, at the end of storage, all samples registered similar ($p > 0.05$)

Table 1. Colour parameters of hot dog samples during chilling storage ($2 \pm 1^{\circ}C$).[a]

Parameters	Samples	Days of storage			
		0	20	40	60
*L**	Control	69.98 ± 0.51c5	67.38 ± 0.83b4	59.89 ± 0.57a4	66.61 ± 0.31b3
	C5E5	61.98 ± 1.63c3	61.16 ± 0.48c2,3	52.431 ± 0.32a3	59.72 ± 0.38b2
	C0E10	59.43 ± 0.70b2	60.74 ± 0.71c2	51.325 ± 0.21a2	59.89 ± 0.75b2
	C10E0	63.65 ± 0.35d4	61.87 ± 0.63c3	53.05 ± 0.54a3	60.51 ± 0.56b2
	C10E10	56.69 ± 0.32b1	57.39 ± 0.53b1	48.01 ± 0.34a1	56.60 ± 0.69b1
*a**	Control	9.65 ± 1.04a1	11.12 ± 0.18c2	10.14 ± 0.19b3	11.35 ± 0.25c2
	C5E5	10.64 ± 0.21c2	10.04 ± 0.26b1	9.37 ± 0.19a2	11.51 ± 0.22d2
	C0E10	9.99 ± 0.36b1	10.05 ± 0.20b1	8.29 ± 0.11a1	10.77 ± 0.35c1
	C10E0	10.81 ± 0.18c2	10.20 ± 0.17b1	9.38 ± 0.07a2	11.24 ± 0.17d2
	C10E10	12.03 ± 0.20c3	11.37 ± 0.34b2	9.87 ± 0.09a3	12.82 ± 0.50d3
*b**	Control	11.43 ± 0.53c2	9.41 ± 0.14b2	8.43 ± 0.07a2	9.12 ± 0.33b2,3
	C5E5	11.43 ± 0.55d2	9.24 ± 0.35b2	8.39 ± 0.23a2	10.02 ± 0.38c4
	C0E10	11.48 ± 0.46c2	9.09 ± 0.30b2	8.55 ± 0.09a2	8.70 ± 0.39a,b2
	C10E0	11.41 ± 0.25c2	9.04 ± 0.27b2	8.53 ± 0.11a2	9.44 ± 0.41b3
	C10E10	10.33 ± 0.29c1	7.36 ± 0.52a,b1	7.22 ± 0.11a1	7.79 ± 0.41b1

Means ± standard deviation. Different letters (a.b.c) in the same row and different numbers (1.2.3) in the same column indicate significant differences for each parameter ($p < 0.05$) ($n = 20$).

[a]Sample denomination: Control sample prepared only with sodium nitrite. Other reformulated samples were prepared without nitrite and with different ingredients: celery (1%), sodium lactate (3% of 60% solution), carmine (0.05%) and orange dietary fibre (1%), in combination with different % of vitamin C (vit C) and E (vit E). C5E5 (0.05% vit C and 0.05% vit E); C0E10 (0.0% vit C and 0.1% vit E); C10E0 (0.1% vit C and 0.0% vit E); C10E10 (0.1% vit C and 0.1% vit E).

hardness and chewiness (Table 2). Results also showed that cohesiveness and springiness were not affected ($p > 0.05$) by storage time (Table 2).

Lipid oxidation (TBARs)

The lipid oxidation levels indicated by TBARs values of hot dog sausage during storage are shown in Figure 2. TBARs values were lowest in the control and C5E5 sample at the start of storage, and the antioxidant effect of the control (NO_2) was similar to that of the samples with combinations of vitamins E and C (C5E5) in low concentrations (0.5%). The antioxidant action of the nitrite and of the vitamins has been widely reported (Cassens, 1997; Pourazrang et al., 2002; Shahidi and Pegg, 1991).

TBARs values increased during storage in all samples up to day 20, except in the samples with vit C (C10E0 and C10E10) (Figure 2). Thereafter TBARs values decreased until the end of storage in all samples (Figure 2). It has been suggested that this decrease is result of malonaldehyde/protein reactions (Delgado-Pando et al., 2011; Jiménez-Colmenero et al., 2010a). After 20 days storage, the control sample presented the highest ($p < 0.05$) TBARs values, followed by sample COE10. The lowest TBARs values ($p < 0.05$) during storage were observed in the samples containing vit C (C10E0 and C10E10). The combination of vit C and E (C10E10) also retarded TBARs values significantly ($p < 0.05$). Many authors have reported an antioxidative effect produced by synergy between vit C and other antioxidants (Estévez and Cava, 2007). The results in the present study also suggest a synergy between vitamins C and E, which is consistent with the results reported in beef by Schaefer et al. (1995).

Microbiology

Presumptive *C. perfringens* was not observed in any sample. The microbial count (Table 3) clearly shows the effects of reformulation and storage time on levels of the microorganisms studied. Microorganism levels were initially very low in all samples (<3 log cfu/g of total viable aerobics-TVC), and levels of LAB and *enterobacteriaceae* were lower than 1 log cfu/g. During storage, the control sample was the first to exceed levels of 6 log cfu/g of both TVC and LAB. After 40 days storage, the control sample reached TVC levels of 6.95 log cfu/g, and LAB levels of 6.13 log cfu/g. In the same period, the reformulated samples presented TVC and LAB levels lower than 5 log cfu/g, indicating a major increase in shelf-life of the reformulated samples with no added nitrite. Levels in these samples exceeded the legal limit after 60 days storage,

Table 2. Texture profile analysis (TPA) parameters of hot dog samples during chilling storage ($2 \pm 1^{\circ}C$).[a]

Parameters	Samples	Days of storage			
		0	20	40	60
Hardness (N)	Control	12.38 ± 0.50a1	13.60 ± 0.55a1,2	13.50 ± 0.81a1	15.99 ± 0.38b1,2
	C5E5	14.07 ± 0.80a2	14.32 ± 0.24a2	14.16 ± 0.50a1	15.44 ± 0.45b1
	C0E10	14.86 ± 0.87a2	15.41 ± 0.32a3	16.19 ± 0.63b3	16.39 ± 1.59b1,2
	C10E0	14.22 ± 0.46a2	14.60 ± 0.59a2	14.53 ± 0.68a2,3	15.56 ± 0.22b1
	C10E10	14.09 ± 0.79a2	14.26 ± 1.02a1	13.76 ± 1.11a1,2	15.74 ± 0.51b1
Cohesiveness (dimensionless)	Control	0.77 ± 0.03a1	0.78 ± 0.01a4	0.79 ± 0.01a4	0.78 ± 0.00a3
	C5E5	0.72 ± 0.01a,b2	0.72 ± 0.01a,b3	0.74 ± 0.02a,b3	0.71 ± 0.02a2
	C0E10	0.72 ± 0.03b2	0.67 ± 0.01a2	0.72 ± 0.03b2,3	0.69 ± 0.02a,b2
	C10E0	0.70 ± 0.02a2	0.69 ± 0.02a2,3	0.71 ± 0.02a2	0.71 ± 0.01a2
	C10E10	0.70 ± 0.03b2	0.64 ± 0.04a1	0.65 ± 0.03a,b1	0.66 ± 0.02a,b1
Springiness (mm)	Control	11.43 ± 0.53c2	9.41 ± 0.14b2	8.43 ± 0.07a2	9.12 ± 0.33b2,3
	C5E5	11.43 ± 0.55d2	9.24 ± 0.35b2	8.39 ± 0.23a2	10.02 ± 0.38c4
	C0E10	11.48 ± 0.46c2	9.09 ± 0.30b2	8.55 ± 0.09a2	8.70 ± 0.39a,b2
	C10E0	11.41 ± 0.25c2	9.04 ± 0.27b2	8.53 ± 0.11a2	9.44 ± 0.41b3
	C10E10	10.33 ± 0.29c1	7.36 ± 0.52a,b1	7.22 ± 0.11a1	7.79 ± 0.41b1
Chewiness (N mm)	Control	8.37 ± 0.15a1	9.79 ± 0.47b2	9.72 ± 0.34b2,3	10.91 ± 0.43b1,2
	C5E5	9.38 ± 0.40a,b2	9.40 ± 0.62a,b2	8.82 ± 0.71a2	10.23 ± 0.54b1
	C0E10	9.41 ± 0.56a,b2	9.68 ± 1.11a,b2	10.29 ± 0.95a,b3	10.69 ± 0.86b1,2
	C10E0	9.19 ± 0.38a,b2	9.19 ± 0.57a2	9.75 ± 1.07a2,3	10.11 ± 0.60b1
	C10E10	9.25 ± 0.41a2	9.12 ± 0.98a,b1	9.28 ± 0.69a,b1	10.21 ± 0.81b1

Means ± standard deviation. Different letters (a.b.c) in the same row and different numbers (1.2.3) in the same column indicate significant differences for each parameter ($p < 0.05$) ($n = 16$).

[a]Sample denomination: Control sample prepared only with sodium nitrite. Other reformulated samples were prepared without nitrite and with different ingredients: celery (1%), sodium lactate (3% of 60% solution), carmine (0.05%) and orange dietary fibre (1%), in combination with different % of vitamin C (vit C) and E (vit E). C5E5 (0.05% vit C and 0.05% vit E); C0E10 (0.0% vit C and 0.1% vit E); C10E0 (0.1% vit C and 0.0% vit E); C10E10 (0.1% vit C and 0.1% vit E).

except for sample C10E0, which still registered 5 log cfu/g, when levels in the control sample had reached 8.85 log cfu/g. This sample also had the lowest levels of LAB (Table 3).

The enterobacteria levels in the control sample were also high ($p < 0.05$) at the end of storage, with behaviour similar to sample C5E5, while the other reformulated samples with no added nitrite presented counts of 1 log cfu/g, with no significant differences from the start of storage (Table 3).

These results suggest that the ingredients used to substitute added nitrite produced a greater antimicrobial effect than the nitrite itself. Some authors have already reported the antimicrobial power of SL and celery (Lin and Lin, 2002), although what these authors assayed was a combination of these two ingredients with low levels of added NO_2. In this study, however (Table 3), the combination of these ingredients only produced an effect when the formulation contained no added nitrite. In addition, the antimicrobial effect of vit C can be clearly seen. The samples with vit C presented lower microorganism levels ($p < 0.05$) throughout storage (Table 3), and most particularly ($p < 0.05$) when vit C was used alone and not combined with vit E. The antimicrobial effect of the vit C was also proportional to the amount used, with significantly lower microbiological counts in the reformulation with 0.1% vit C (Table 3). These results are consistent with a previous report by other authors (Dave and Ghaly, 2011).

Residual nitrite

Residual nitrite was affected ($p < 0.05$) by formulation and storage time. The control sample contained 88.69 mg/kg, similar to levels reported by other authors in frankfurters (Delgado-Pando et al., 2011). The reformulated samples without nitrite contained around 23–33 mg/kg, with higher levels in the samples containing 0.1% vit C (C10E0 and C10E10). The nitrite in the samples reformulated without added nitrite may have come from other ingredients, such as celery, which

Table 3. Microbiological counts (log cfu/g) of different hot dog samples during chilling storage ($2 \pm 1^{\circ}C$).[a]

Microorganisms	Samples	Days of storage			
		0	20	40	60
Total viable count (TVB)	Control	$2.38 \pm 0.08a^{1}$	$3.10 \pm 0.10b^{2}$	$6.95 \pm 0.04c^{4}$	$8.85 \pm 0.02c^{4}$
	C5E5	$2.89 \pm 0.04b^{1}$	$2.24 \pm 0.24a^{1}$	$4.54 \pm 0.09c^{2,3}$	$7.07 \pm 0.16d^{2}$
	C0E10	$2.67 \pm 0.18a^{1}$	$3.00 \pm 0.00a^{2}$	$5.02 \pm 0.18b^{3}$	$7.70 \pm 0.27c^{3}$
	C10E0	$2.54 \pm 0.54a^{1}$	$2.85 \pm 0.15a^{2}$	$3.67 \pm 0.67b^{1}$	$5.01 \pm 0.17c^{1}$
	C10E10	$2.92 \pm 0.08a^{1}$	$3.28 \pm 0.02a^{2}$	$4.37 \pm 0.05b^{2}$	$6.90 \pm 0.30c^{2}$
Lactic acid bacteria (LAB)	Control	$2.06 \pm 0.06a^{1}$	$3.32 \pm 0.02b^{2}$	$6.13 \pm 1.13c^{3}$	$8.29 \pm 0.33d^{4}$
	C5E5	$2.65 \pm 0.05a^{1}$	$2.65 \pm 0.35a^{1}$	$4.39 \pm 0.09b^{2}$	$6.93 \pm 0.07c^{2,3}$
	C0E10	$2.16 \pm 0.16a^{1}$	$2.89 \pm 0.11a^{1}$	$5.05 \pm 0.12b^{2}$	$7.59 \pm 0.25c^{3,4}$
	C10E0	$2.00 \pm 0.00a^{1}$	$2.80 \pm 0.20ab^{1}$	$3.48 \pm 0.80b^{1}$	$4.87 \pm 0.03c^{1}$
	C10E10	$2.40 \pm 0.10a^{1}$	$2.80 \pm 0.20a^{1}$	$4.40 \pm 0.14b^{2}$	$6.73 \pm 0.39c^{2}$
Enterobacteriaceae	Control	$1.00 \pm 0.00a^{1}$	$1.50 \pm 0.00a^{1}$	$1.15 \pm 0.15a^{1}$	$4.25 \pm 0.05b^{2}$
	C5E5	$1.00 \pm 0.00a^{1}$	$1.50 \pm 0.00a^{1}$	$1.15 \pm 0.15a^{1}$	$5.91 \pm 0.00b^{3}$
	C0E10	$1.00 \pm 0.00a^{1}$	$1.50 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$
	C10E0	$1.00 \pm 0.00a^{1}$	$1.50 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$
	C10E10	$1.00 \pm 0.00a^{1}$	$1.50 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$	$1.00 \pm 0.00a^{1}$

Means $\pm$ standard deviation. Different letters (a.b.c) in the same row and different numbers (1.2.3) in the same column indicate significant differences ($p < 0.05$) ($n = 4$).

[a]Sample denomination: Control sample prepared only with sodium nitrite. Other reformulated samples were prepared without nitrite and with different ingredients: celery (1%), sodium lactate (3% of 60% solution), carmine (0.05%) and orange dietary fibre (1%), in combination with different % of vitamin C (vit C) and E (vit E). C5E5 (0.05% vit C and 0.05% vit E); C0E10 (0.0% vit C and 0.1% vit E); C10E0 (0.1% vit C and 0.0% vit E); C10E10 (0.1% vit C and 0.1% vit E). Presumptive *Clostridium perfringens* was not observed in any sample.

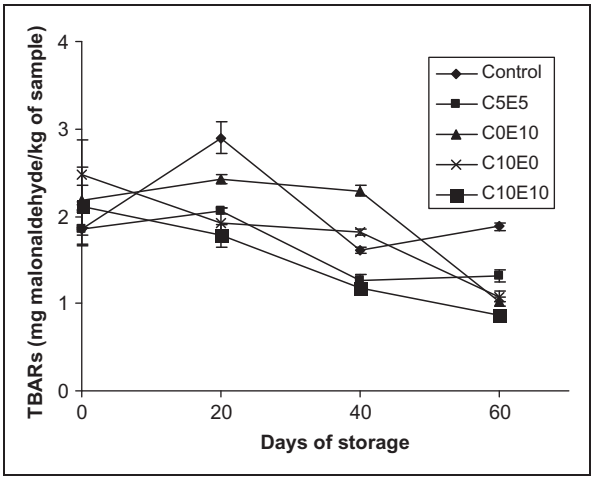

Figure 2. Thiobarbituric acid values (TBARs) of hot dog samples during chilling storage ($2 \pm 1^{\circ}C$). Data were expressed as mean $\pm$ SD ($n = 4$).

contain high concentrations of nitrites and nitrates, as reported by other authors (Cassens, 1997; Sindelar et al., 2007a, 2007b). Nitrite levels decreased over storage in all samples. The decrease was greatest ($p < 0.05$) in the control sample (67%), followed by the samples without added nitrite, where the decrease was around 2.5–3% (data not shown). This behaviour in the reformulated sausage could be due to lower microbial growth in these samples (Table 3), which has been associated with reduced depletion of NO_2 and NO_3 (Cassens, 1997; Gotterup et al., 2008). In fact decreasing residual nitrite during storage has been reported elsewhere, the explanation being that the added nitrite is also rapidly depleted in meat products since nitrite reacts with or binds to constituents of the meat (lipids, proteins, etc.) (Ruiz-Capillas and Jimenez-Colmenero, 2008).

Sensory analysis

The sensory evaluation data for hot dog samples at day 0 of the assay are presented in Figure 3. In general, there were no significant differences between the reformulated and control samples in the sensory parameters evaluated. This was attributed to the use of lactate in the formulation and the absence of nitrite, given the way in which this affects flavour and juiciness. In this connection, Jafari and Emam-Djomeh (2007) reported that differences in levels of nitrite (120–50 mg/kg) were not perceived by consumers. Given the absence of significant differences between the lots reformulated with and without added nitrite, this reformulation strategy would appear to be effective in sensory terms.

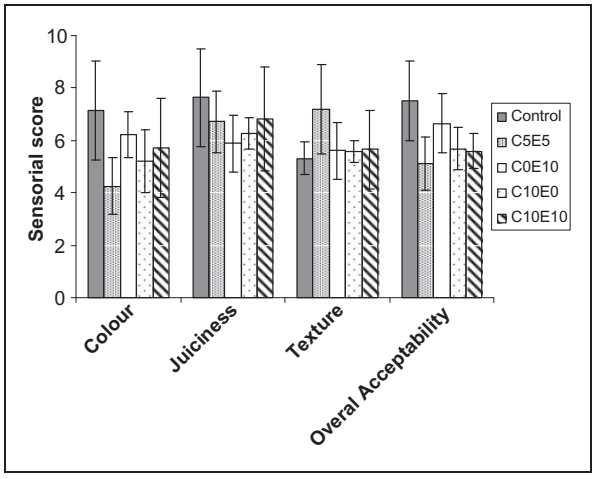

Figure 3. Sensory evaluation of different hot dog samples during chilling storage ($2 \pm 1°C$). Data were expressed as mean $\pm$ SD ($n = 36$).

CONCLUSION

The proposed reformulation strategy for hot dog sausage without added nitrite using a combination of celery powder, SL, ODF, carmine and vitamins E and C as nitrite replacers affected some technological, sensory and safety properties of the experimental meat product. The tasting panel awarded all samples similar scores for acceptability, and colour values were maintained with no significant differences among the treatments. The results (microbial growth and antioxidant stability) for the samples with vit C (C10E0) were significantly better than those of the other treated/reformulated samples. This effect was proportional to the levels of vit C used. The reformulated samples achieved higher levels of microbiological safety than samples containing nitrite.

The results of this study suggest that the reformulation of hot dog sausage with no added nitrite and with combinations of other ingredients, mainly vit C, is a viable strategy for producing healthier hot dog sausages, and one that solves some of the chemical problems associated with nitrite-free hot dog sausages.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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