J Food Sci Technol (July 2015) 52(7):4333–4341
DOI 10.1007/s13197-014-1460-1

ORIGINAL ARTICLE

# Nitrite-free Asian hot dog sausages reformulated with nitrite replacers

**C. Ruiz-Capillas · S. Tahmouzi · M. Triki · L. Rodríguez-Salas · F. Jiménez-Colmenero · A.M. Herrero**

Revised: 18 June 2014 / Accepted: 27 June 2014 / Published online: 9 July 2014


**Abstract** This research deals with the application of a global strategy designed to produce a nitrite-free Asian hot dog. Different ingredients such as annatto, cochineal, orange dietary fibre, vitamins E and C, lactate and celery were combined in order to study the appearance (colour), lipid oxidation stability and microbial stability of the nitrite-free formulations. The control sample contained much more ($P<0.05$) residual nitrite (88.7 mg/kg) than the samples without added nitrite (23–24 mg/kg). Generally, no formulation-dependent variations were observed in fat and water binding properties. Control sample had the highest L* and a* values, while the product with annatto (RA) had the lowest a* values. Lipid oxidation levels were similar irrespective of formulation. The hot dog reformulated with cochineal (RC) scored higher for overall acceptability than RA, mainly due to its colour.

**Keywords** Hot dog · Nitrite · Annatto · Cochineal · Colour · Sensory analysis

## Introduction

Hot dogs are a generally popular frankfurter made from beef, pork, turkey, chicken, or a combination of some of these. The processes of formulation and marketing of meat products generally differ in terms of flavour/taste according to the country where they are manufactured (McCarthy et al. 2001). Traditional Asian hot dogs are formulated with beef, a high proportion (around 17 %) of sunflower oil (as it is cheap and flavourless) and variable quantities of nitrite. Nitrite prevents the growth of *Clostridium botulinum* and it may also have preservative effects on other harmful and spoilage bacteria. In addition, nitrite develops the flavour and colour of cured meat and retards the development of rancidity and off-odours during storage of final products (Cassens 1997; Pegg and Shahidi 2000; EFSA 2003; Ruiz-Capillas and Jiménez-Colmenero 2011; Sindelar and Milkowski 2012).

In recent years, dietary nitrite has been associated with methemoglobinemia and the formation of nitrosamines, which are chemical agents considered to exert carcinogenic, mutagenic and teratogenic effects. To limit this unhealthy effect, there have been proposals for the omission or lowering of nitrite/nitrate (by means of different alternatives for colour, lipid oxidation and safety purposes) in meat processing, and the application of strategies to inhibit damage due to nitros(yl)ation reactions (Pegg and Shahidi 2000; EFSA 2003; Zarringhalami et al. 2009). The use of non-meat ingredients which by nature contain high nitrate levels, such as celery, has been proposed to avoid direct addition of nitrite to meat products (Sindelar et al. 2007a, b; Magrinya et al. 2009, 2012; Tahmouzi et al. 2013). However, the modification and processing conditions needed to replace it may affect the technological properties and quality of meat products in different ways depending on the type of product.

From a commercial standpoint, the colour of meat products is a very important factor influencing the customer's decision to purchase. A number of colourings have been tested as nitrite replacers in cooked meat products in order to reproduce as closely as possible the characteristics and the stable pink pigments produced after the thermal treatment (Pegg and Shahidi 2000). Carmine is a bright red pigment obtained from aluminium salt of carminic acid, which is produced by some

C. Ruiz-Capillas (✉) · M. Triki · L. Rodríguez-Salas · F. Jiménez-Colmenero · A. Herrero
Institute of Food Science, Technology and Nutrition (ICTAN-CSIC) (Formerly Instituto del Frío), Consejo Superior de Investigaciones Científicas (CSIC), C/José Antonio Novais 10, Ciudad Universitaria 28040, Madrid, Spain
e-mail: claudia@ictan.csic.es

S. Tahmouzi
Department of Food Science & Engineering, Faculty of Agricultural Engineering and Technology, University of Ramin, Mollasani, Ahwaz, Iran

scale insects such as cochineal and Polish cochineal and is used as a general term for a particularly deep red colour of the same name (Marmion 1984). Carmine is used in the manufacture of artificial flowers and cosmetics and is routinely added to food products such as yoghurt and certain kinds of juice, most notably those of the ruby-red variety (Díaz Medina et al. 2007). This colouring has been used in meat products and in some cases for partial reduction of nitrite in them (Madsen et al. 1993; Bloukas et al. 1999). The new trend is to use natural substances to replace synthetic colourings. Of these, annatto is the most widely used because of the possibilities that it offers. Annatto has been used in various foods for antimicrobial, antioxidant and colouring purposes and can be used as a nitrite replacer in sausages (Galindo-Cuspinera et al. 2003; Zarringhalami et al. 2009). Other ingredients, such as orange dietary fibre (ODF), have also been used to improve colour in meat products and to retard oxidative changes in bologna sausages, and also to take advantage of their useful functional and technological properties (Viuda-Martos et al. 2010a, b). It is well known that nitrite is a strong antioxidant in cured meat, thus preventing lipid oxidation. To reproduce the antioxidant efficacy of nitrite, a number of antioxidants, sequestrants and combinations of these have been studied (Pegg and Shahidi 2000). In this regard, vitamin E and ascorbic acid (vitamin C) have also been used as natural antioxidants in meat products (Sammet et al. 2006; Estévez and Cava 2007). Besides their antioxidant effects, these substances can limit nitrosamine formation (Pourazrang et al. 2002; EFSA 2003). Alternatives to nitrite that have been tested include some salts such as lactate and lactic acid (Tahmouzi et al. 2013). These compounds have also been used for their inhibitory effects on microbial growth in sausages (low-fat Chinese style) during storage, as well as to improve the flavour (Lin and Lin 2002) and colour of meat products (Bradley et al. 2011).

Studies to achieve the omission or lowering of nitrite in meat processing have generally tended to test individual alternatives (ingredients/additives) for specific purposes (colour, flavour and safety). The objective of this research was therefore to devise a global strategy for producing a cooked meat product (hot dog) without added nitrite. This strategy includes the use of different ingredients/additives in order to reproduce the colour (including natural substances such as annatto, cochineal and orange dietary fibre), to promote oxidative stability (orange dietary fibre, vitamins E and C) and induce microbial stability (lactate). Celery was also used as a non-meat ingredient because of its high natural nitrate/nitrite content. Characteristics such as composition, pH, colour, water and fat binding properties, texture, sensory properties, lipid oxidation, residual nitrite and microbial population were analysed to evaluate the impact of reformulation on the product.

## Materials and methods

### Materials and ingredients for preparation of hot dogs

Fresh beef (flank steak and shank meat) was obtained from a local meat market and was passed through a grinder with a 0.4 cm plate (Mainca, Granollers, Spain). Lots of approx. 1 kg were vacuum packed, frozen and stored at −20 ºC for 2 weeks until use. Protein and fat contents of meat samples were 19.98 ±0.43 and 13.49±1.46 respectively.

The ingredients used for the formulation of the Asian hot dog 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 SA, Madrid, Spain), milk powder (Anvisa, Madrid, Spain), orange dietary fibre (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), sodium lactate (E325, Manuel Riesgo, Madrid, Spain), vitamin E (DL Alpha-Tocopherol Acetate, Manuel Riesgo S.A., Madrid, Spain), vitamin C (ascorbic acid L Manuel Riesgo, S.A., Madrid, Spain), carmine colourings (E120) (Trades SA) and annatto (E160b) (CHR Hansen, Spain).

### Asian hot dog preparation

Three different hot dog formulations were assayed (Table 1). A control sample (CN) was prepared with a traditional Asian hot dog formulation containing 120 mg/kg product of sodium nitrite. Two other sausages were formulated without adding nitrite, but with two types of traditional natural colourings: cochineal (RC) and annatto (RA). These hot dogs also contained celery (1 %), as a natural source of nitrite/nitrate, sodium lactate (3 % of solution) for its antimicrobial functions, ODF (1 %), vitamin E (0.05 %) and vitamin C (0.05 %) (to retard lipid oxidation, stabilize colour and improve water holding capacity). All lots (including the control) contained 0.5 % species-flavouring, 1.5 % garlic, 1.9 % egg white, 17 % sunflower oil, 1.2 % milk powder, 1.4 % salt and 0.46 % TPP. The formulations including annatto or carmines were derived from previous studies at our laboratories (ICTAN-CSIC, Spain and University of Teheran).

Preparation of the sausage (hot dog) was as described by Delgado-Pando et al. (2010). Briefly, raw meat material (previously thawed for 18 h at 2±2 ºC) was homogenized and ground for 1 min in a chilled cutter (2 ºC) (Stephan Universal Machine UM5, Stephan u. Söhne, Hameln, Germany). Half of the ingredients/additives (Table 1) used for each formulation were added to the ground

**Table 1** Formulation (%) of Asian hot dog sausages

| Sample | Meat | Annatto | Carmine | Nitrite | ODF | Celery | SL | Vit E | Vit C | Water |
|---|---|---|---|---|---|---|---|---|---|---|
| CN | 50 | 0 | 0 | 0.012 | | 0 | 0 | | | 22.23 |
| RC | 50 | 0 | 0.05 | 0 | 1 | 1 | 3 | 0.05 | 0.05 | 17.09 |
| RA | 50 | 0.025 | 0 | 0 | 1 | 1 | 3 | 0.05 | 0.05 | 17.11 |

Sample denomination: control sample (CN) was prepared with sodium nitrite. The reformulated samples were prepared without nitrite and with two different colourings, carmine (RC) and annatto (RA), and similar % of celery, sodium lactate (SL) (3 %), orange dietary fibre (ODF) and vitamins C and E. The following ingredients were also added to all samples: 3.8 %, potato starch, 0.5 % species/flavouring, 1.5 % garlic powder, 1.9 % egg white, 17 % sunflower oil, 1.2 % milk powder, 1.4 % sodium chloride, 0.46 % sodium tripolyphosphate

meat and mixed again for 1 min. The rest of the ingredients/additives were added and then the whole 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 ºC 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 ºC. 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, Mod. 3087, Tokyo, Japan). Once heating was complete, the sausages were cooled (at room temperature), kept in a cold room (14 h at 2ºC), packed (Cryovac1 BB3050) and stored at 2ºC (±1 ºC) until analysis the next day.

### Proximate composition

Hot dog samples from each lot were first homogenized in a blender to obtain a homogeneous sample. Moisture and ash contents were determined in triplicate using the standard AOAC (2005) 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, USA).

### Residual nitrite content

Residual nitrite contents were determined (in sextuplicate) by flow injection analysis (FIA) as described by Ruiz-Capillas et al. (2007), based on the reaction with sulphanylamide to form a diazonium salt. This was added to NED to form azo dye compounds whose absorbance was measured spectrophotometrically at 540 nm.

### Processing loss and emulsion stability

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

Determination of thermal emulsion stability of hot dogs (in quadruplicate) was calculated as total, water and fat loss (Jiménez-Colmenero et al. 2010). Briefly, around 32 g from each formulation was placed in containers (27 mm diameter), which were hermetically closed and 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 a stove at 105 °C (Model IDL-AI-36, Labolan SL, Navarra, Spain) and expressed as g 100/g of initial sample weight. Fat release (FR) was calculated as the difference between TFR and WR.

### pH determination

The pH was determined on a Radiometer model PHM 93 pH-meter (Meterlab, Copenhagen, Denmark) at room temperature. Four determinations were performed for each formulation.

### Texture profile analysis (TPA)

Texture Profile Analysis (TPA) was performed in a TA.XTplus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Eight cylindrical cores (diameter = 20 mm, height = 20 mm) from each hot dog formulation were axially compressed to 30 % of their original height (Bourne 1978). Force-time deformation curves were obtained with a 30 kg load cell, applied at a crosshead speed of 1 mm/s. Attributes were calculated as follows: hardness (Hd) = peak force (N) required for first compression; cohesiveness (Ch) = ratio of active work done under the second compression curve to that done under the first compression curve (dimensionless); springiness (Sp) = distance (mm) the sample recovers after the first compression; Chewiness (Cw): Hd x Ch x Sp (N x mm). Measurement of samples was carried out at room temperature.

### Colour measurement

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

### Thiobarbituric acid value (TBARs)

Lipid oxidation was evaluated on the basis of levels of thiobarbituric acid-reactive substances (TBARS) in hot dogs. The procedure for measurement of TBARS was based on methods used by Delgado-Pando et al. (2011). Briefly, 5 g of each sample was homogenized for 90 s in 35 ml of 7.5 % trichloroacetic acid at high speed in an Ultraturrax blender (Ika-Werke, GmbH & Co, Staufen, Germany). The blender sample was centrifuged (3,000 g, 2 min, Solvall BA, RTB6000B, Dupont, USA) and 5 ml of the supernatant was mixed with 5 ml of 20 mM thiobarbituric acid; finally the solution was mixed and kept in the dark for 20 h at 20±1.5 °C. Pinkness was measured spectrophotometrically (Lambda 15UV/VIS spectrophotometer, Perkin-Elmer, USA) at 532 nm. A calibration curve was plotted with 1,1,3,3-tetraethoxypropane (Sigma Chemical Co., St. Louis, MO, USA) to measure the malonaldehyde (MDA) concentration and results were expressed as mg malonaldehyde/kg of sample. TBARS were determined in triplicate.

### Microbiological analysis

Samples were prepared in a vertical laminar-flow cabinet (model AV 30/70, Telstar, Madrid, Spain). For each sample, 10 g (in replicate) were 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 (30ºC for 72 h) 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).

### Sensory evaluation

The sensory properties of the hot dogs were assessed by an 18-member (minimum) tasting panel recruited from employees of the Institute (ICTAN-CSIC) who consumed hot dog regularly. Preliminary sessions were conducted with the products and terminology to train the panel. Sensory analysis was performed on day 1 of storage. Samples (length = 2.5 cm) from each formulation were heated in a microwave 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 = extremely dislike, 10 = extremely like) on a non-structured scale without fixed extremes. The sensory analysis was carried out three times for the different samples.

### Statistical analysis

Analysis of variance (ANOVA one way) and Tukey's multiple range tests 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. Kruskal-Wallis was used to test samples that did not fit the normal distribution. Statistical analysis was performed using SPSS Statistics 17.0 (SPSS Inc, Chicago, USA).

## Results and discussion

### Proximate composition

Proximate analysis showed some differences between control and modified hot dogs (Table 2). Results were consistent with product formulation (Table 1). There were no significant differences between samples as regards protein and fat contents (Table 2). Sausages were formulated with the same target muscle protein content (Table 1). Fat content was generally close to the target level (around 24 %) (Table 2). Samples were made according to the formulation (Table 1), basically for non-animal fat (>70 % sunflower oil), with less than 30 % beef fat.

The addition of vegetable oils to meat products has been found to be an efficient and successful strategy to enhance the nutritional value of muscle foods by reducing saturated fatty acid (SFA) levels and by adding natural antioxidants such as tocopherols. The large amount of polyunsaturated fatty acids (PUFA) in sunflower oil could increase the oxidative instability of a meat product and hence seriously affect its sensory quality. Yilmaz et al. (2002) reported no adverse sensory effects on frankfurters formulated with 15 % sunflower oil, while Pennisi-Forell et al. (2010) added natural antioxidants to

**Table 2** Proximate composition (%) of Asian hot dog sausages

| Sample | Moisture | Ash | Protein | Fat |
|---|---|---|---|---|
| CN | 58.32±0.35[a] | 2.76±0.14[a] | 12.42±0.48[a] | 23.48±0.86[a] |
| RC | 56.24±0.13[b] | 3.21±0.23[b] | 13.15±0.56[a] | 24.29±1.64[a] |
| RA | 57.68±0.77[b] | 3.03±0.14[b] | 13.54±0.40[a] | 23.58±1.94[a] |

For sample formulations see Table 1

Means±Standard deviation. Different letters in the same column indicate significant differences ($P<0.05$)

beef burger patties to prevent oxidative instability associated with the addition of sunflower oil. Different procedures have been used in meat products to replace animal fat with plant or marine lipids (Jiménez-Colmenero 2007), among them direct addition in the form of liquid oils as in this experiment. Liquid oils have been directly added in different proportions (5-40 %) to products like beef patties, fermented sausages, "salami" and gel/emulsion products (frankfurters) (Bloukas et al. 1997; Yilmaz et al. 2002; Zarringhalami et al. 2009; Pennisi-Forell et al. 2010; Viuda-Martos et al. 2010a, b; Coutinho de Oliveira et al. 2012). Moreover, it has been suggested that replacing fatty meat raw materials with vegetable oils (in this case sunflower oil) will reduce cholesterol, since cholesterol is strictly associated with animal cells. These dilution strategies have made it possible to significantly reduce cholesterol contents with respect to conventional meat products. There were significant differences ($P<0.05$) in moisture and ash contents, with the highest and lowest values respectively registered in control samples. Besides the modified products, the formulation included some components such as dietary fibre which have been added to meat products for their known technological effects (water and fat binding properties, texture, etc.) and physiological properties (reduction of risk of diabetes, promotion of blood lipid regulation, prevention of cardiovascular disease (CVD), colon cancer and constipation, regulation of intestinal transit). Besides having considerable antioxidant potential in the meat matrix, the increased plasma concentrations of vitamins E and C in the formulation (Table 1) have been associated with various human health benefits (Jiménez-Colmenero et al. 2012).

## Residual nitrite

As expected, residual nitrite was affected ($P<0.05$) by formulation. Compared with reformulated samples (RC 23.2±1.80 and RA 24.8±1.55 mg/kg), CN had the highest ($P<0.05$) residual nitrite with levels of 88.7±1.28 mg/kg. In this sample almost 74 % of the added nitrite was detectable in the final product after processing. Many studies have demonstrated that the added nitrite is rapidly depleted in meat products since nitrite reacts with constituents of the meat (lipids, proteins, etc.) or binds to them (Carballo et al. 1991; EFSA 2003). The two reformulated samples (RC and RA) had similar ($P>0.05$) residual nitrite contents. In our experimental conditions, since these samples were free of added nitrite, the concentration found there was presumably supplied by the celery. As noted earlier, celery contains considerable amounts of nitrates and nitrites, which can naturally reach levels of 1.5 g/kg (nitrates) and 0.1 mg/kg (nitrites) (Chung et al. 2004). Various authors have studied the effect of powdered celery juice concentrates with different levels of nitrate (70–140 mg) on cooked emulsified sausages and dry-cured sausages (Sindelar et al. 2007a, b; Magrinya et al. 2009; Tahmouzi et al. 2013).A similar behaviour pattern was observed in this study, and logically this relatively low nitrite concentration could act as a curing agent, affecting product colour levels, antimicrobial activity and oxidation processes (oxidative stability).

## Emulsion stability, processing loss and pH

Generally, there were no formulation-dependent variations in fat and water binding properties. All samples displayed appropriate emulsion stability (Table 3). Levels of water release, fat release and total release were lower than 0.5 %, with no significant differences ($P>0.05$) between treatments. Processing loss of Asian hot dogs was close to 12 % with no significant differences between formulated samples (Table 3). Processing losses ranging between 12 and 20 % have been reported in reformulated frankfurter sausages with added plant or marine oils (Márquez et al. 1989; Bloukas et al. 1997; López-López et al. 2009).

pH values ranged between 5.82 and 6.55, and there were significant differences between treatments in the control sample (CN), which had the highest ($P<0.05$) pH (6.55). The low pH in the reformulated hot dogs (RC and RA) is probably mainly due to the use of sodium lactate (Lin and Lin 2002), since no variations of pH have been reported in reformulated systems as the effect of other ingredients in modified sausages such as citric fibre (Viuda-Martos et al. 2009), colourings (Mercadante et al. 2010) or tocopherols (Georgantelis et al. 2007). These results suggest that there are a variety of

**Table 3** Emulsion stability (water, fat and total fluid release) (%) and processing loss (%) of Asian hot dog sausages

| Sample | Water release | Fat release | Total fluid release | Processing loss |
|---|---|---|---|---|
| CN | 0.10±0.06[a] | 0.07±0.02[a] | 0.16±0.06[ab] | 12.96±1.31[a] |
| RC | 0.004±0.001[a] | 0.18±0.10[a] | 0.19±0.10[ab] | 11.35±0.61[a] |
| RA | 0.04±0.03[a] | 0.28±0.03[a] | 0.32±0.06[b] | 12.09±1.20[a] |

For sample formulations see Table 1

Means±Standard deviation. Different letters in the same column indicate significant differences ($P<0.05$)

mechanisms that can explain the excellent fat and water binding properties of the three samples. In this respect, the effect of pH reduction on modified Asian hot dogs (Table 3), which would logically have caused some loss of fat and water binding properties, seems to have been balanced basically by the presence of ODF (Table 1), which is well known for its technological effects (water and fat binding properties, texture, etc.).

## Texture

Texture profile analysis (TPA) parameters are shown in Table 4. Textural behaviour was affected by formulation. Reformulated Asian hot dogs (RC and RA) were hardness and chewiness ($P<0.05$) but less cohesiveness ($P<0.05$) than control sample (CN) (Table 4). Springiness was similar ($P>0.05$) in all the hot dogs. It is important to note that since muscle protein content was very similar, the differences in textural properties between hot dogs (Table 4) must have been determined mainly by the different ingredients in the formulations, particularly fibre content (ODF), and their action in the meat protein matrix. Similarly, the addition of orange dietary fibre to cooked meat sausages is reported to cause an increase in hardness which is associated with particles added to the protein matrix that would strengthen the binding that occurs during cooking (Viuda-Martos et al. 2009, 2010a, b). Moreover, other authors have indicated that the addition of different kinds of fibre (soy, wheat, cereal or fruit) to cooked meat emulsions increased hardness (Fernández-Ginés et al. 2003; López-López et al. 2009). An increase in hardness has also been reported in low-fat meat emulsion systems with added rice bran fibre and in frankfurters with added carrageenan (Choi et al. 2009).

## Colour

Table 5 shows the effect ($P<0.05$) of hot dog reformulation on colour parameters. Control sample (CN) registered the highest ($P<0.05$) values of L* and a*, while the products made with annatto (RA) registered the lowest ($P<0.05$) redness and the highest ($P<0.05$) yellowness. Asian hot dog prepared with carmine (RC) had the lowest L* values (Table 5). CN colour parameters are therefore presumably determined by nitrite reactions commonly occurring in this type of products. In the case of reformulated sausages, other factors have to be taken into account. In the given conditions, the colour that developed in RC and RA samples is attributable to the action of the added colouring in all cases, to the effect of the nitrifying agents (nitrate which is reduced to nitrite) in the celery (Chung et al. 2004; Sindelar et al. 2007a, b; Magrinya et al. 2009, 2012), and to a lesser extent to the ODF.

Various studies have reported that meat products made with nitrite registered higher L* levels than the ones prepared without nitrite (Viuda-Martos et al. 2009; Zarringhalami et al. 2009; Li et al. 2013). Coutinho de Oliveira et al. (2012) also observed a minor effect exerted on colour by other ingredients (such as oil) in bologna samples made without nitrites. Zarringhalami et al. (2009) observed no significant differences in L* when all the nitrite was replaced by annatto. Increased lightness has been reported in bologna made with fibre (Viuda-Martos et al. 2010b); however, that effect seems to be associated with many factors, including the concentration and type of pigments, fibre content and type, and water content and hygroscopicity of the materials dissolved in the water matrix (Fernández-Ginés et al. 2003; Viuda-Martos et al. 2010a). The increase in redness ($P<0.05$) of the sample with added nitrite (CN) as compared with reformulated sausages (RC and RA) is consistent with the role played by additives in the redness/pinkness of products (Coutinho de Oliveira et al. 2012). Zarringhalami et al. (2009), observed no significant differences in a* when annatto was used as a nitrite replacer in hot dogs, similar to the ones formulated in our experiment. The lower redness values ($P<0.05$) observed in RA sample as compared with the product containing cochineal (RC) are attributable basically to the different colourings since the formulation conditions were similar in the rest of the product ingredients. Also, it is worth noting the increase of b* (15.95) when annatto was used (RA). Similar behaviour was observed by Zarringhalami et al. (2009) in their study of nitrite replacement by annatto as a colour additive in sausage. These authors reported that yellowness (b*) increased as nitrite content decreased, a fact that was also associated with the beef used in the sausages.

**Table 4** TPA parameters of Asian hot dog sausages

| Sample | Hardness | Cohesiveness | Springiness | Chewiness |
|---|---|---|---|---|
| CN | $9.20\pm0.44^{a}$ | $0.74\pm0.02^{b}$ | $0.89\pm0.02^{a}$ | $5.8\pm0.46^{a}$ |
| RC | $10.68\pm0.65^{b}$ | $0.68\pm0.04^{a}$ | $0.86\pm0.04^{a}$ | $6.29\pm0.57^{b}$ |
| RA | $11.67\pm0.32^{b}$ | $0.70\pm0.03^{a}$ | $0.85\pm0.04^{a}$ | $6.87\pm0.29^{b}$ |

For sample formulations see Table 1

Means±Standard deviation. Different letters in the same column indicate significant differences ($P<0.05$)

**Table 5** Colour parameters of Asian hot dog sausages

| Samples | L* | a* | b* |
|---|---|---|---|
| CN | $63.66\pm0.86^{b}$ | $12.76\pm0.37^{c}$ | $10.32\pm0.12^{a}$ |
| RC | $60.01\pm0.22^{a}$ | $8.59\pm0.15^{b}$ | $10.72\pm0.26^{a}$ |
| RA | $62.22\pm0.41^{c}$ | $7.11\pm0.17^{a}$ | $15.95\pm0.20^{c}$ |

For sample formulations see Table 1

Means±Standard deviation. Different letters in the same column indicate significant differences ($P<0.05$)

### Lipid oxidation

CN, RC and RA hot dog formulations registered similar ($P$>0.05) TBARs values (around 1.72 mg MDA/kg of product). These TBARs values are lower than reported by Coutinho de Oliveira et al. (2012) in bologna sausage prepared with different levels of sodium nitrite. Minimum reported TBARs values needed to detect objectionable flavours ranged between 0.3 and 3.0 depending on the product, the meat source, etc. (Coutinho de Oliveira et al. 2012). Similar oxidation lipid levels were observed in all samples after processing. This indicates that the strategy adopted to counterbalance the absence of nitrite for the prevention of lipid oxidation in RC and RA samples was effective. The antioxidative system used to control rancidity is based on the combined action of various compounds with demonstrated antioxidant activity, such as vitamins E and C (Shahidi and Pegg 1991; Cassens 1997; Estévez and Cava 2007), ODF (Viuda-Martos et al. 2010a) and nitrite as supplied by celery (Magrinya et al. 2009). One important aspect is the effect of that strategy during chilling storage, which is a common procedure in these meat products.

### Microbiology

Total viable counts (TVC) and *Enterobacteriaceae* did not differ significantly among the three samples (CN, RC and NA), which registered levels of <3 Log cfu/g and <1 Log cfu/g in TVC and *Enterobacteriaceae* respectively. These values were similar to the ones observed in heat-treated products such as frankfurters (Jiménez-Colmenero et al. 2010; Delgado-Pando et al. 2011; Tahmouzi et al. 2013), indicating that the reduction in bacterial counts was mainly a consequence of the thermal treatment applied during processing. Since the determinations were made less than 24 h after sample preparation, the effect of the antimicrobial strategy adopted in nitrite free samples could not be observed clearly. The effectiveness of that strategy, as in the case of antioxidant system, can only be assessed during chilling storage (work in progress).

### Sensory evaluation

The results of the sensory evaluation of hot dog samples (24 h after preparation) are presented in Table 6. Sensory evaluation indicated that reformulation affected ($P$<0.05) some of the sensory attributes of the Asian hot dogs. There were no significant differences in colour evaluation between control samples and nitrite-free samples containing carmine (RC), although the latter was awarded a lower score. Similar results have been reported by other authors (Tahmouzi et al. 2013). Samples prepared with annatto (RA) scored less ($P$<0.05) for colour than the control (CN), but about the same ($P$>0.05) as RC sample (Table 6). As indicated in the colour analysis, the colour of CN sample is attributable to the reaction of the nitrites usually present in this type of products, while in the case of reformulated sausages, the colour in RC and RA samples may be due to the added colouring in all cases, to nitrifying agents (nitrate reduced to nitrite) in the celery, and to a lesser extent to the ODF. Thus, the sensory perception of the colour follows a similar trend to the variations in a*. There were no significant differences among any of the samples in terms of juiciness, although juiciness tended to decrease as a result of the absence of added nitrite (Table 6). The pattern was similar for firmness scores, although these diverged from the hardness values (Table 4). The panellists considered all products acceptable. Control sample scored higher ($P$<0.05) than RA, but similar ($P$<0.05) to RC sample. No differences ($P$>0.05) were registered in overall acceptability of reformulated samples (Table 6). Of the sensory parameters considered, colour seemed to be the one that counted most towards overall acceptability.

**Table 6** Sensory evaluation of Asian hot dog sausages

| Samples | Colour | Juiciness | Firmness | Overall acceptability |
|---|---|---|---|---|
| CN | $7.51 \pm 2.37^{b}$ | $7.36 \pm 1.85^{a}$ | $5.13 \pm 0.63^{a}$ | $7.15 \pm 1.53^{b}$ |
| RC | $4.71 \pm 2.34^{ab}$ | $5.82 \pm 1.96^{a}$ | $4.75 \pm 1.48^{a}$ | $4.85 \pm 1.67^{ab}$ |
| RA | $4.02 \pm 2.21^{a}$ | $5.27 \pm .61^{a}$ | $4.56 \pm 1.11^{a}$ | $4.46 \pm 1.80^{a}$ |

For sample formulations see Table 1

Means±Standard deviation. Different letters in the same column indicate significant differences ($P$<0.05)

## Conclusion

Hot dog reformulation with cochineal and other ingredients (ODF, celery, SL, vitamins C and E) as nitrite replacers seems a good alternative to the use of chemical nitrite in this kind of sausages. From the standpoint of product colour, the samples reformulated with cochineal, although with a slightly less intense colour than control sample, gave better results in technological and sensory terms than the ones made with annatto. Hot dogs made with cochineal scored better for acceptability and had the virtue of being more similar to sausages formulated with nitrite. There was no observable effect of reformulation on lipid oxidation and microbial stability of Asian hot dogs; in any case the effectiveness of these strategies can only be assessed during chilling storage, which is a common procedure in this kind of meat products. Further studies are therefore needed to address these aspects of the nitrite-free hot dogs.

**Acknowledgments** This research was supported by projects AGL 2011-29644-C02-01, AGL2008-04892-CO3-01, AGL2010-19515/ALI of the Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) and the Consolider-Ingenio 2010:CARNISENUSA (CSD2007-00016), Ministerio de Ciencia y Tecnología. The authors wish to thank the University of Tehran for partial support of Mr. Saeid Tahmouzi's study assistance, and the AECID-MAE for Mr. Mehdi Triki's outstanding scholarly assistance. Also we thank CHR Hansen for kindly providing the annatto. The authors are indebted to Cristina Navarro for her cooperation.

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