Optimization of oxidative stability, color and sensory properties of uncured (nitrite-free) Asian hot dogs (Jigo) using response surface methodology (RSM)

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Abstract This work investigated the effect of natural antioxidants (ascorbic acid (AA), α -tocopherol (TOC) and orange dietary fibre (ODF)) on oxidative stability, color and sensory properties in uncured hot dogs during chilled storage (3 ± 1 °C 4 weeks). A box-behnken design was employed for analysis of the responses (TBARS, peroxide value, pH, colour, taste and aroma) to obtain optimal conditions. Sausages containing TOC (20 mg/kg) and AA (0.1 %) had lower (0.11 mg malonaldehyde (MAD)/kg) TBARS values than those other combinations. This treatment also showed a peroxide value of 1.53 meq/kg when the experiment was finished. Lightness, redness and yellowness values varied among variables. Treatment with AA (0.1 %) resulted in lower lightness, yellowness and pH values than other treatments. Based on analysis, AA (0.1 %), TOC (20 mg/kg) and ODF (5 %), gave the optimum results. Under these conditions, the actual values were in close agreement with the values predicted by the model.

Keywords Optimization · Uncured · Sausage · Oxidation stability · Orange dietary fibre · Meat quality

Introduction

In many parts of the world, organic foods have been experiencing an explosive market growth. Demand for

healthier food products, without chemical preservatives, is increasing. Numerous studies have reported that curing is an important technique for preserving meat and fish. The processes of formulation and marketing of Asian sausages generally differ in terms of flavour/taste according to the country where they are produced (Sindelar and Milkowski 2012).

Nitrite as a curing agent is widely used because it develops the flavor of the cured meat, acts as an antioxidant to delay the development of oxidative rancidity, acts as an antimicrobial agent against outgrowth and neurotoxin formation by *Clostridium botulinum* and contributes to control of other microorganisms such as *Listeria monocytogenes* (Ruiz-Capillas and Jiménez-Colmenero 2011).

However, increased concerns about the potential health risks associated with the consumption of nitrite-cured foods have led the meat industry to search for alternative curing methods. Specifically, they have sought to produce meats that are free of added nitrites, yet maintain the color and oxidative characteristics of nitrite-cured meat products. An alternative source 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 conventional curing. Sebranek and Bacus (2007) have investigated a “natural curing” process, it uses a relatively high content of naturally occurring nitrate in conjunction with a bacterial starter culture to reduce nitrates to nitrites. Clearly, vegetable juices or powders, such as celery, offer the greatest potential as natural sources of nitrate; they allow normal curing chemistry, yielding color development and other properties typical of a product cured with added nitrites (Fujihara et al. 2001; Tahmouzi et al. 2012).

The meat industry is faced with the challenge of developing strategies for preventing lipid oxidation. The use of antioxidants, synthetic or natural, is one of the major strategies for reducing lipid oxidation. However, questions regarding their

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safety, together with consumers' preferences, have led to increased interest and research on natural additives (Georgantelis et al. 2007). Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers as being safer than synthetics (Georgantelis et al. 2007).

Several natural extracts – for example, vitamin E (α -tocopherol), and vitamin C (ascorbic acid) – have also been investigated to retard meat products' lipid oxidation and stabilize their color characteristics (Arnold et al. 1991; McCarthy et al. 2001). However, there is limited information on their effect when used in uncured meat products. Some authors have reported that α -tocopherol is as effective as butylhydroxytoluene (BHA) in retarding lipid oxidation when used as a processing ingredient in cooked and sliced beef (Aksu and Kaya 2005).

Isolation of citrus-fibre compounds can be of interest to the food industry, as they can retard oxidative changes in food and thereby improve the quality and nutritional value of food. Besides the efficiency of these compounds in oxidation reduction, some of them can also contribute to the color of cured products. Comprehensive research on the combination of all permitted natural additives on the color and lipid-oxidative properties of nitrite-free hot dogs has not been carried out. Additionally, no reports have been published to date on the effect added citrus fibre on the processing of hot dogs.

Various levels of vitamin C or vitamin E or the effects of their use on hot dog color stability are not so well known. The results of this research could be very valuable to the meat industry in selecting the most appropriate natural additives for improving product quality. The aim of this study was to assess the possibility of replacing nitrite with natural ingredi-

ents such as ascorbic acid, α -tocopherol and ODF, providing naturally cured hot dog characteristics similar to those of a nitrite-added control, determining their optimum added levels using response surface methodology and examining how the addition of different natural ingredients affects various quality parameters of uncured hot dogs.

Materials and methods

Natural ingredients and chemicals

Ascorbic acid was purchased from Sigma (St. Louis, MO, USA). α -tocopherol extract (Guardian, 70 % of mixed tocopherols) was obtained from DANISCO (Copenhagen, Denmark). Stabilizer, paprika and season were purchased from a commercial manufacturer (Robertet, France). All other chemicals used were of analytical grade or the highest grade available and were obtained either from Sigma-Aldrich or Merck (Darmstadt, Germany).

Experimental design

To determine the effect of AA, TOC and ODF concentrations on various quality parameters of uncured Asian hot dogs, response surface methodology was applied with a Box and Behnken. This design led to studying the effects of three factors in a single block of 17 sets of test conditions. The order of the experiments was fully randomized. Three levels were attributed to each factor, coded as -1, 0, +1 (Table 1). Statistical analysis was performed with the software package 'Design-Expert version 7.0.' Quadratic and cubic models were defined to fit the responses (TBARS, PV, a*, taste and odor):

$$Y_{\text{quadratic}} = \beta_0 + \beta_1 X_{AA} + \beta_2 X_{TOC} + \beta_3 X_{ODF} + \beta_{11} (X_{AA})^2 + \beta_{22} (X_{TOC})^2 + \beta_{33} (X_{ODF})^2 + \beta_{12} X_{AA} X_{TOC} + \beta_{13} X_{AA} X_{ODF} + \beta_{23} X_{TOC} X_{ODF}$$

$$Y_{\text{cubic}} = \beta_0 + \beta_1 X_{AA} + \beta_2 X_{TOC} + \beta_3 X_{ODF} + \beta_{11} (X_{AA})^2 + \beta_{22} (X_{TOC})^2 + \beta_{33} (X_{ODF})^2 + \beta_{12} X_{AA} X_{TOC} + \beta_{13} X_{AA} X_{ODF} + \beta_{23} X_{TOC} X_{ODF} + \beta_{112} (X_{AA})^2 X_{TOC} + \beta_{113} (X_{AA})^2 X_{ODF} + \beta_{122} X_{AA} (X_{TOC})^2 + \beta_{111} (X_{AA})^3 + \beta_{222} (X_{TOC})^3 + \beta_{333} (X_{ODF})^3$$

Where β_0 is a constant coefficient of the models. The regression coefficients (β_1 , β_2 and β_3), (β_{11} , β_{22} and β_{33}), (β_{111} , β_{222} and β_{333}) and (β_{12} , β_{13} , β_{23} , β_{112} , β_{113} and β_{122}) respectively represent linear, quadratic, cubic and interaction effects of the model estimated by multiple regression analysis. X_{AA} (ascorbic acid), X_{TOC} (α -tocopherol) and X_{ODF} (orange dietary fiber) are coded variables ranging from -1 to +1.

Asian hot dog manufacturing

Hot dogs were prepared in a pilot plant in conditions resembling those of commercial processing. Partially thawed meat (70 % lean beef) was used to prepare the ground meat. The meat was chopped at low speed and mixed with all the seasonings and ingredients for about 2 min in a cutter (Berkel Type, SEL300, Germany). The following recipe was used for

Table 1 Variables and levels used for the box-behnken design

Levels	Ascorbic acid (%)	Variables	
		Tochopherol (mg/kg)	ODF* (%)
−1	0.05	10	2
0	0.075	15	3.5
+1	0.10	20	5

*Orange dietary fiber

all hot dog treatments: 0.88 kg sunflower oil, 1 kg ice, 0.10 kg wheat flour, 0.06 kg milk powder, 0.1 kg salt, 0.08 kg spice (black and red pepper) and 0.10 kg garlic for 5.24 kg paste. 50 % of the ground ice was added and mixed at high speed. When the temperature of the mixture decreased to 2 °C, sunflower oil was added and chopped until the temperature of the mixture reached 8 °C. The remaining 50 % of the ice and other ingredients were added, and mixed until the temperature of the mixture reached 12 °C (for about an additional 3 min). Total emulsification time was about 8 min, and the processing-room temperature was about 17 °C. Once the homogenization was completed, mixes were subdivided into 17 batters of 1.5 kg (Table 2). 100 ml natural spring water was used to deliver the AA, TOC or ODF, depending on the experiment, to the mix. In the control sample, the lean meat was cured with sodium nitrite (120 mg/kg sample). The resulting 17 raw mix samples were mixed for 2 min and stuffed into 24-mm diameter high-barrier polyamide bags (Arta, Tabriz, Iran). After stuffing, the

hot dogs were heated with steam at 80 ± 1 °C for an hour with the aim of achieving a core temperature of 75 °C. The hot dogs were then showered with cold water (12 °C) for about 8 to 10 min to a core temperature of 30 °C, drained and transferred to an ice bank to reduce their temperature to 5 to 6 °C. The hot dogs were stored under refrigeration (3 ± 1 °C 30 days) to ensure that their temperature did not rise above 5 °C; this temperature limit was maintained throughout their shelf life (Tahmouzi et al. 2012).

Proximate composition

Hot dog samples were homogenized using a blender (Brockville, Ontario, Canada) until a uniform paste was obtained. The proximate properties of the samples were determined using the standard AOAC (1995) method. Moisture content was determined by weight loss after 12 h at 105 °C in a drying oven (SW-90D., Bucheon, Korea). Fat content was determined by the Soxhlet method with a solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Höganäs, Sweden) and total protein content was determined by the Kjeldahl method with an automatic Kjeldahl nitrogen analyzer (Höganäs, Sweden).

Thiobarbituric acid reactive substance (TBARS) values

Lipid oxidization was assessed by measuring thiobarbituric acid reactive substances during storage. Each hot-dog sample (10 g) was mixed with 25 ml of trichloroacetic acid solution

Table 2 Box-behnken experimental design for with the observed responses for ascorbic acid, tochopherol and ODF

Trial	Ascorbic acid (%)	Tochoferol (mg/kg)	ODF (%)	TBARS (mg/kg)	PV (mg/kg)	pH	L*	a*	b*	Taste	Odor
1	0.075	15	3.5	0.22	3.14	6.66	62.78	10.04	10.19	7.55	6.99
2	0.075	10	2	0.44	5.13	6.70	63.39	10.43	10.52	6.33	7.56
3	0.075	15	3.5	0.25	3.24	6.63	62.98	10.33	10.33	7.34	7.86
4	0.075	15	3.5	0.20	3.08	6.86	63.13	10.65	10.56	7.76	7.65
5	0.05	15	2	0.43	6.13	7.01	68.86	8.95	14.67	6.45	6.55
6	0.05	10	3.5	0.53	6.48	6.98	69.09	8.66	13.98	7.38	6.99
7	0.10	20	3.5	0.11	1.53	6.34	57.37	12.53	7.77	7.19	8.76
8	0.075	20	5	0.19	2.98	6.67	64.43	9.98	10.23	8.66	7.45
9	0.075	15	3.5	0.40	6.12	6.73	63.98	10.33	10.21	7.54	7.68
10	0.075	20	3.5	0.27	3.65	7.01	70.25	8.54	14.23	7.54	6.84
11	0.10	15	5	0.28	3.33	6.44	56.69	12.67	8.01	8.65	8.67
12	0.075	10	5	0.47	6.19	6.66	63.23	10.42	11.03	8.56	7.33
13	0.075	15	3.5	0.38	5.59	6.72	62.98	10.44	10.54	7.43	7.67
14	0.05	15	5	0.50	2.24	6.38	71.28	8.54	13.67	8.93	6.19
15	0.10	15	2	0.25	3.67	6.28	58.08	12.33	7.98	6.43	8.42
16	0.075	20	2	0.14	1.64	6.65	63.65	10.32	10.42	6.44	7.33
17	0.10	10	3.5	0.39	5.67	6.47	56.89	12.56	7.77	7.80	8.56
control	–	–	–	0.10	1.67	6.59	55.72	12.76	9.32	8.64	8.45

(200 g/l of TCA in 135 ml/l phosphoric acid solution) and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate was added to 2 ml TBA solution (3 g/l) in a test tube. The test tubes were incubated at room temperature in darkness for 20 h, after which the absorbance was measured at 538 nm against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a UV–Vis spectrophotometer (model UV-1200, Shimadzu, Japan). TBARS value in each treatment was calculated and expressed as milligram of malonaldehyde per kilogram of hot dog samples. For each treatment, measurements were performed in triplicate.

Peroxide values (PVs)

The peroxide values (PVs) of all samples were determined according to the method of Sallam et al. (2004). A 10 g sample was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated in a water bath at 50°C for 3 min to melt the fat, then thoroughly mixed for 3 min with 30 ml acetic acid-chloroform solution (3:2 v/v) to dissolve the fat. After filtration through Whatman filter paper, saturated potassium iodide solution (0.5 ml) was transferred into the burette and added to the filtrate. The titration was allowed to run against a standard solution of sodium thiosulfate (25 g/l). PV was expressed as milliequivalent (meq) peroxide per kilogram of sausage sample and calculated as follows:

$$PV(\text{meq/kg}) = \frac{S \times N}{W} \times 1000$$

Where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N = 0.01) and W the sample weight (kg).

pH measurement

The pH was measured by blending 10 g of sample with 90 ml distilled deionized water for 2 min. The pH of the resulting suspension was measured with a Crison pH meter (Model 507, Crison, Barcelona, Spain) calibrated with phosphate buffers 4.0 and 7.0. For each treatment, three replications were used for analysis.

Instrumental color measurement

The instrumental color analyses of the samples were conducted at 12 ± 2 °C using a Konica Minolta Chromameter (model CR-410, Konica Minolta Sensing Inc., Osaka, Japan). The instrument was set for illuminant D-65 and at a 10° observer angle, and calibrated against a white standard tile supplied by the manufacturer before every session. The following color coordinates were determined: lightness (L^*), redness (a^*) and yellowness (b^*). The color values were measured on the

sample surfaces and data were taken in triplicate for each sample.

Sensory evaluation

The sensory properties of the cooked hot dogs were investigated by a 20-member taste panel. A preparatory session was held prior to testing so that each panel member could thoroughly discuss and clarify each attribute to be evaluated. Testing was initiated after the panelists agreed on the specifications. Panelists evaluated the samples in isolated booths and red incandescent lighting was used inside each booth to reduce the influence of color on the sensory response. Panelists were instructed to cleanse their palates between samples using unsalted crackers and tap water. Prior to sensory analysis, samples were removed from the refrigerator and kept for 20 min to reach room temperature. Panelists were at first served with a section (about 10 cm long, cut across its axis) of the fresh hot dogs, and asked to evaluate the samples' odor (from imperceptible to extremely intense) and taste (from imperceptible to extremely intense). Each sample was given a random two-digit alphabetical code for identification and each panelist evaluated three replicates of all the treatments; the order of the presentation during the sessions was randomized for each panelist. Panelists were provided with areas on printed sheets of paper for sensory rating of all samples. The two questions were: "Please indicate your opinion of the odor of this sample" and "Please indicate your opinion of how this sample tastes." For each question, 9 boxes were provided, with a scale of "Dislike Extremely" on the left and "Like Extremely" on the right. The form contained the instruction, "Rate the hot dog sample for each question asked by marking an 'X' in the box." Each of the 9 boxes was given a numerical value upon compilation of results, ranging from 1 = "Dislike Extremely" to 9 = "Like Extremely" (Tahmouzi et al. 2013).

Statistical analysis

The proximate composition, pH, color, TBARS values, POVs and sensory results were analyzed using two-way analysis of variance (ANOVA) with treatments. Data was analysed using the General Linear Model (GLM) procedure of SAS software (Version 9.1; Statistical Analysis System Institute Inc., Cary, NC, USA).

Results and discussion

Model fitting

The "fitness" of the models was evaluated through the coefficients of determination (R^2), probability values (p) and lack-of-fit values. The fit quality of the quadratic and cubic models

was good, since the R^2 ranged from 0.76 (TBARS values) to 0.93 (a^*). Moreover, all models were significant ($p < 0.05$). F-values ranged from 7.5 (L^*) to 10.1 (pH) (Table 3). X_1 , X_2 and X_3 were AA, TOC and ODF, respectively.

Proximate composition

The average chemical composition of the control hot dogs was 60.6 ± 0.5 g moisture, 13.5 ± 1.2 g protein and 13.8 ± 1.6 g lipids/100 g of hot-dog sample. The added ingredients (AA, TOC and ODF) did not influence the chemical composition of the uncured hot dogs (data not shown).

TBARS

The values of the response (TBARS) obtained under the different experimental conditions are presented in this work. The application of RSM offers, based on parameter estimates, an empirical relationship between the TBARS value and the independent variables (AA, TOC and ODF) under consideration. For TBARS values the quadratic term of test variables was the only significant ($p = 0.00076$) factor. Due to the value of the coefficient the increase in variables concentration had a negative effect on the lipid oxidation. The R^2 of the predicted model (eq. (1)) was good (0.87). To examine the adequacy of the predicted model, ANOVA test for the quadratic model was carried out and the results are summarized in Table 3. The model F-value of 9.6 with a low p -value ($p < 0.05$) implies

that this model was statistically significant at 95 % confidence level.

The optimal pretreatment conditions for minimum TBARS value were AA = 0.09 %, TOC = 19.58 mg/kg and ODF = 3.14 %, which would result in a predicted TBARS value of 0.1004 mg/kg. To confirm the result of the predicted value, experiments were conducted at the optimal conditions, displaying the TBARS value of 0.112 mg/kg (Table 4).

$$\text{TBARS} = 72x_1^2 + 0.01x_3^2 - 0.26x_1x_3 + 1.3 \quad (1)$$

Tarladgis et al. (1960) found that the TBA value at which rancid odor was first perceived was between 0.5 and 1.0 (mg malonaldehyde/kg meat). This threshold has served as a guide for interpreting TBA test results. Table 2 shows the effect of adding AA, TOC and ODF on the TBA value of uncured hot dogs. In the present study, both control and treated hot dog samples had TBA values less than 0.6. TBARS values were low for the control sample, as expected, because sodium nitrite had been added. Nitrite has been shown to be an effective antioxidant. Low TBARS numbers may be the result of malonaldehyde reactions with proteins (Zapata et al. 1990). Such competing reactions could account for the variations seen in Table 2. TBARS values for hot dogs containing 20 mg/kg α -tocopherol (TOC) or 0.1 % ascorbic acid (AA) did not differ significantly ($p > 0.05$) with control. When both AA (0.07 %) and TOC (20 mg/kg) were used, TBARS values were significantly ($p < 0.05$) decreased 0.11 (mg MAD/kg

Table 3 ANOVA for the response surface models determined between response variables (y_1 , y_2 , y_3 , y_4 , and y_5) and independent variables

Source of variation	Degree of freedom	Sum of squares	Mean square	F _{statistic}	P-value
TBARS values (mg/kg) (y_1)					
Model	3	0.031	0.0045	9.6	0.00076
Residual	4	0.036	0.0004		
Total	17	0.044			
Peroxide values (meq/kg) (y_2)					
Model	3	0.081	0.0035	8.2	0.00432
Residual	4	0.36	0.0044		
Total	17	0.044			
pH (y_3)					
Model	3	0.054	0.0054	10.1	0.00561
Residual	4	0.060	0.0269		
Total	17	0.448			
L^* (y_4)					
Model	3	0.214	0.0177	7.5	0.01876
Residual	4	0.065	0.0460		
Total	17	0.095			
Taste (y_5)					
Model	3	0.046	0.0267	8.3	0.00684
Residual	4	0.054	0.0064		
Total	17	0.068			

Table 4 Optimum (maximum or minimum) levels of responses obtained from the response optimizer option of RSM program

Response	Optimal condition (variable concentrations)	Y (Predicted)	Y (observed)
TBARS values (mg/kg)	AA (0.09 %), TOC (19.58 mg/kg) and ODF (3.14 %)	Min: 0.1004	0.112
PV (meq/kg)	AA (0.10 %), TOC (18.69 mg/kg) and ODF (2.13 %)	Min: 1.44	1.49
a*	AA (0.10 %), TOC (10 mg/kg) and ODF (2 %)	Max: 12.54	11.93
taste	AA (0.05 %), TOC (10 mg/kg) and ODF (5 %)	Max: 8.73	8.55
odor	AA (0.10 %), TOC (10 mg/kg) and ODF (2.03 %)	Max: 8.56	8.32
All objectives (TBARS, PV, a*, taste and odor)	AA (0.10 %), TOC (20 mg/kg) and ODF (5 %)	TBARS (min): 0.14	0.14
		PV (min): 2.11	2.26
		a* (max): 12.24	12.12
		taste (max): 8.61	8.43
		odor (max): 8.47	8.68

meat) (Fig. 1a). Many authors have reported that vitamin C, when used in combination with other antioxidants, functions synergistically to promote their antioxidative effect (Naveena et al. 2006). In fact, various natural antioxidants (such as α -tocopherol or citrus fibre) have been used together with vitamin C for preventing food oxidation. Mitsumoto et al. (1991) reported that treatment of ground beef with 500 mg/kg ascorbic acid decreased lipid peroxidation. However, Benedict et al. (1975) reported that adding 50 mg/kg ascorbic acid to ground beef caused increased lipid peroxidation. The results in the present study suggested a synergy between vitamins C and E, in agreement with the results for beef reported by Schaefer et al. (1995). It was observed in the present study that treatments with ODF maintained the initial TBARS values. This behavior could indicate that ODF protects against the oxidation process. The level of TBARS was found to be lowest in the hot dogs formulated with TOC, AA and ODF together. ODF treatment alone was not as effective as the control treatment (Fig. 1b-c); however, when it was used in combination with the other antioxidants, lipid oxidation was retarded. The oxidation-retarding effect of ODF may be due to the presence of bioactive compounds, mainly polyphenol. The effect of the polyphenols contained in citrus fibre on lipid oxidation in both meat- and fish-based products was expected and has been discussed in the literature (Fernández-Ginés et al. 2003; Fernández-López et al. 2007).

As can be seen in Fig. 1-c, the quadratic effects of AA and TOC exhibited a significant ($p < 0.0001$) negative effect on the TBARS value (y_1); in contrast, the variation of TBARS value was not influenced by the concentrations of ODF ($p < 0.0001$). The mutual interaction of AA content with ODF concentration was also significant ($p < 0.001$). Fig. 1b shows the interaction between AA and ODF contents. When AA concentration was increased from 0.05 to 0.1 %, the TBARS value decreased in a parabolic manner. The TOC concentration revealed a nonlinear decrease for the hot dog's TBARS value. The results suggested that

an uncured sausage containing 0.09 % AA, 19.58 mg/kg TOC and 3.14 % ODF would result in the minimum TBARS value ($y_1 = 0.1004$ mg/kg).

Peroxide value

By using ANOVA test on the estimated parameter, we found statistical significance with quadratic and cubic coefficient for estimation of PV the treatments. A summary of the estimated parameters for coded variables with significances is given in Table 3. The simplified model is shown as Eq. 2.

The coefficient of determination of the predicted model was equal to 0.93 ($p = 0.001$). Thus, the predicted model can successfully represent the observed values, being the responses sufficiently explained by the model extracted. Based on three-dimensional plots, AA (0.10 %), TOC (18.69 mg/kg) and ODF (2.13 %), gave the optimum results (1.44 meq/kg) for PV.

Peroxide value

$$= -0.39x_1x_2^2 - 1.66x_1^2 - 0.068x_1^2x_2 - 0.37x_3^2 + 4.23 \quad (2)$$

Peroxide values (PVs) are the most common measure of lipid oxidation, assessing the production of hydroperoxides, which are the primary oxidation products (Ólafsdóttir et al. 1997). Table 2 shows the changes in PVs for hot dogs with various ingredients. Significant differences were observed in the hot dogs containing 0.05 % AA and 10 mg/kg TOC (6.48 meq/kg) compared to the control (1.67 meq/kg). The PV for hot dogs treated with AA (0.075 %) + TOC (20 mg/kg) + ODF (2 %) was significantly lower than that for the control; nonetheless, the PV for all samples was well below the proposed acceptable level of 10 to 20 meq peroxide/kg. Regarding ODF, the AA and TOC had a significant effect ($p < 0.05$), followed by the cubic term of variables concentration (Fig. 1d-f).

Fig. 1 Effect of increasing (a and d) tocopherol and ascorbic acid, (b and e) ODF and ascorbic acid and (c and f) ODF and tocopherol content on TBARS (a, b and c) and peroxide values (d, e and f) of uncured hot dog

As illustrated in Fig. 1, the quadratic and cubic effect of all independent variables on the hot dogs' peroxide value (y_2) was highly significant. The quadratic effect of AA concentration and ODF content was significant at the 0.075 % and 2 % level, respectively. The inhibitory influence of AA with ODF on lipid oxidation in uncured sausages can probably be attributed to the synergistic properties of vitamin C and dietary fiber (Arnold et al. 1991). The results also indicated that among the interactions, only the interaction effect of AA with TOC was significant ($p < 0.0001$).

Increasing scavenging activity against free radicals in the uncured hot dogs by adding AA and TOC inhibits diffusion of oxygen, hence decreasing the rate of lipid oxidation (Naveena et al. 2006). As Fig. 1d shows, the peroxide value of hot dogs was significantly decreased by increasing AA content. The

sausages with low ODF contents had lower peroxide values compared to the high-ODF hot dogs (Fig. 2e and f). It may also be due to the high contents of some fatty acids (C18:1 and C18:2) at higher ODF levels. The results of the present study showed that low contents of ODF not only protect the hot dogs against oxidation, but also act as an excellent prooxidant to the diffusion of oxygen. These findings are in contrast to those obtained by Viuda-Martos et al. (2010), but agree with those recorded previously by Georgantelis et al. (2007).

pH

Table 2 presents the pH values of the uncured hot dog samples. The pH values ranged from 6.28 to 7.01 for all

Fig. 2 Effect of increasing (a and d) tochoferol and ascorbic acid, (b and e) ODF and ascorbic acid and (c and f) ODF and tochoferol content on a^* (a, b and c) and b^* (d, e and f) values of uncured hot dog

treatments. pH values of hot dogs treated with both 0.1 % ascorbic acid, 20 % tochoferol and 3.5 % ODF were lower than those of the other treatments ($p < 0.05$).

Color stability

From the variables present in Table 2, the only that had significant effect ($p < 0.05$) on redness (a^*) was the quadratic term of AA concentration. As such, higher concentrations of AA originated higher final redness (a^*) (Eq. (3)). On the other hand, the other variables (TOC and ODF) did not make any important contribution towards final redness. Even though the determination coefficient of the mathematical model was the lowest, the model was still significant ($p = 0.022$). The maximum redness was obtained and recommended as a practical

optimum: AA concentration (0.10 %), TOC concentration (10 mg/kg), and ODF concentration (2 %).

$$\text{Redness } (a^*) = 0.29x_1^2 - 0.049x_2^2 + 0.19x_1x_3 + 1.94x_1 + 10.36 \quad (3)$$

Color formation and its stability are very important quality attributes in meat products (Gómez et al. 1998). Color measurement is an important parameter because brightness and a particular pink color are expected in this type of product (Viuda-Martos et al. 2010). Table 2 shows the effects of AA, TOC and ODF on the color of the hot dogs under refrigerated conditions.

Lightness in food color, especially in meat products, is associated with many factors, including the concentration

and type of pigments present, water content and hygroscopicity of the material dissolved in the water matrix (Viuda-Martos et al. 2010). As the results of the present study show, L^* decreased to a statistically significant ($p < 0.05$) extent as the percentage of additives increased. This could be explained by the increased water retention of hygroscopic materials; also, because they may have absorbed free water within the product, thereby decreasing lightness values (Fernández-López et al. 2005).

Several authors have found reductions in redness when vitamins used (McCarthy et al. 2001; Nuñez de Gonzalez et al. 2008). However, our observations did not agree with these findings (Fig. 2a-c): we observed that a^* values in general increased or remained similar over time (despite their reduction for some treatments). This may be because the decrease in redness associated with the oxidation process is being compensated for by the presence of red component pigments such as carotene in these natural additives (Zarringhalami et al. 2009). In our study, color changes of treated hot dogs were shown as increases of redness, leading to a slightly redder appearance more appealing to visual inspection. The reason suggested for color changes is the scavenging of oxidant agents of sausage (Viuda-Martos et al. 2010). Combined use of independent variables prevented intense discoloration of final product by avoiding pigment denaturation.

The use of AA led to a significant decrease ($p < 0.05$) in b^* relative to the control (Fig. 2d-f). The behavior of b^* depends to a great extent on the food matrix, and it is recognized that changes in the matrix have the greatest influence on b^* in many foods (Viuda-Martos et al. 2010).

Sensory evaluation

Tables 2 presents the sensory evaluation data for hot dog samples. There were significant differences in overall appearance among the treatments with the addition of vitamins and ODF. For taste and odor, the ODF and AA concentration were the most significant, respectively ($p = 0.0068$). The R^2 of the predicted models (cubic for taste and quadratic for odor) was good (0.92 and 0.88), indicating that the predicted model can reasonably represent the observed values (Eqs. (4) and (5)).

$$\text{Taste} = 0.093x_1x_2^2 - 0.16x_1^2x_2 - 0.19x_1x_2 + 1.11x_3 + 7.52 \quad (4)$$

$$\text{Odor} = -0.23x_3^2 + 0.12x_1^2 + 0.15x_1x_3 + 0.97x_1 + 7.57 \quad (5)$$

Adding ODF resulted in no significant changes ($p > 0.05$) compared to the control in the hot dogs' taste. However, panel members clearly preferred the hot dogs processed with high ascorbic acid concentration (0.1 %) compared to the high ODF samples, even though they were unable to distinguish the former from the control.

As shown in Table 2, the AA + TOC + 5 % ODF sample scored best for taste and the AA (0.1 %) + TOC + ODF sample scored best for odor, while the TOC samples alone scored lowest. These results coincided with the chemical analysis described above. When AA, ODF or TOC alone were used, no differences in the panel members' preferences were observed compared to the control ($p > 0.05$). Panelists generally preferred samples containing AA and ODF over the control. Perceived taste and odor preferences increased compared to the control at all concentrations of AA and ODF ($p < 0.05$). When we added 5 % ODF along with 0.1 % AA and 15 mg/kg TOC, panelists preferred the odor over the control ($p < 0.05$).

Optimization of responses using response surface methodology

Optimum levels of TBARS, PV, a^* , taste and odor were studied Using the “response optimizer” option of RSM program. This option helps to identify the combination of AA, TOC and ODF that optimize a single response or a set of responses. The combined optimization must satisfy all responses included. This parameter is a measure of how the solution obtained satisfies the combined objectives for all responses. In order to optimize uncured hot dog production, the objectives specified in the present work (Table 4).

Confirmation experiments

To validate the model, confirmation experiments were conducted under the optimized conditions and then responses were measured. TBARS values, PV, a^* , taste and odor scores were obtained as 0.14 mg/kg, 2.26 meq/kg, 12.12, 8.43 and 8.68 respectively (which is the same as that predicted using RSM). These values are very close to the corresponding predicted values (Table 4). This validated that the RSM approach was effective for optimizing the operational conditions for the uncured Asian sausage process.

Conclusion

Consumers' interest in meat products formulated with natural ingredients has motivated the researchers to evaluate the effectiveness of naturally occurring compounds. The authors concluded in this study that natural antioxidant compounds such as ascorbic acid and α -tocopherol could act as effectively as nitrite in preserving the quality of cured meat products. Additionally, we demonstrated that ascorbic acid, when used in combination with α -tocopherol, functions synergistically to promote the latter's antioxidative effect. The addition of ascorbic acid seems to be a technologically viable alternative for enhancing the “natural” image of cooked meat products, and can potentially substitute for nitrite in the production of

uncured Asian hot dogs. ODF improved consumer acceptance (taste) of hot dogs, and had desirable effects in retarding oxidation, which contributes to prolonging shelf life. RSM was applied to determine the optimum AA, TOC and ODF concentrations to be used in uncured hot dog production. Quadratic and cubic models were used for predicting all the responses, namely, TBARS values, PV, pH, color, taste and aroma of sausages. The optimum conditions based on all responses were an AA concentration of 0.1 %, a TOC concentration of 20 mg/kg and an ODF concentration of 5 %. Our results showed that experimental and predicted values were quite similar. Therefore, the models obtained can be used to optimize uncured hot dogs.

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