Quality attributes and retention of vitamin E in reduced salt chicken sausages fortified with vitamin E

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Abstract The effect of salt reduction and vitamin E (α -tocopherol) fortification, at a level in excess of that required for a European Food Safety Authority (EFSA) health claim, on the quality of chicken sausages was investigated. Four formulations of chicken sausages were prepared: C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Salt or α -tocopherol level did not significantly affect the fat content, protein content, moisture content, lipid oxidation, microbial growth, cook loss or texture profile analysis values of raw or cooked sausages. There was a significant effect of salt level on the ash content of raw and cooked sausages. Colour of cooked sausages was affected by salt reduction, with fried low salt sausages being darker and yellower than control, while grilled low salt sausages were lighter and redder than control. Cooking and storage did not significantly affect α -tocopherol retention, and enough was retained to meet both the EFSA nutrient and health claims. The sensory properties of the sausages were unaffected by the reduction in salt, and by fortification with α -tocopherol.

Keywords Vitamin E · Salt · Chicken · Sausages · α -Tocopherol

Introduction

In many countries the World Health Organisation (WHO) recommended daily allowance (RDA) of less than 5 g/day salt (NaCl) or 2 g/day sodium is exceeded (IUNA 2011; DEFRA 2012; USDA 2015). In the UK (DEFRA 2012) and in Ireland (IUNA 2011), processed meat products are the greatest and second greatest contributor, respectively, to salt to the diet making processed meat products attractive targets for salt reduction.

Salt has an important functional role in the production of processed meat products as it contributes to taste, texture, consumer acceptability and microbial safety (Ruusunen and Puolanne 2005). Thus, reducing salt can lead to a significant reduction in the quality of meat products. Methods of salt reduction which have previously been investigated include changing the physical form of salt (Frasch-Melnik et al. 2010) or substituting salt with other ingredients which have a similar functional role in processed meat products (Desmond 2006). The major limitations of these methods is that they maintain the high salt taste threshold of consumers and several government agencies, such as the UK Food Safety Authority, have recommended that manufacturers reduce the amount of salt added to their products over time, with the objective of lowering the consumer's salt taste threshold (British Meat Processors Association 2010). Pork sausages currently have a salt range between 0.5 and 2.5 g salt/100 g (Sheard et al. 2010; Bolger et al. 2015) in the UK and Ireland, and a salt range between 1.05 and 3.6 g salt/100 g (Walmart 2015) in the US. Poultry sausages currently have a salt range between 1.0 and 1.4 g salt/100 g (Bolger et al. 2015) in the UK and Ireland, and a salt range between 1.2 and 2.6 g salt/100 g (Walmart 2015) in the US.

Vitamin E is a strong antioxidant. There are eight isoforms of vitamin E, and α -tocopherol is the most biological

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active isoform (Brigelius-Flohe 2006). α -Tocopherol has been shown to have a functional role in the production of meat products by decreasing lipid oxidation and thereby increasing shelf life and reducing off-flavour formation in the products. This effect has been shown when α -tocopherol is added to the diets of animals in order to increase the α -tocopherol content of their meat (Sammet et al. 2006) or when α -tocopherol is added directly to processed meat products (Georgantelis et al. 2007a, b; Kim and Choi 2012). Vitamin E has also been shown to have a positive effect on human health and EFSA has approved the use of the health claim that “vitamin E contributes to the protection of cells from oxidative stress” when food contains enough vitamin E to meet the “source of vitamin E” claim which is 1.8 mg per 100 g or 100 mL or a single portion of food or beverage (European Commission 2012).

While the effect of α -tocopherol on the quality of meat products has previously been studied, there is little research on the stability of α -tocopherol following cooking or storage, particularly if α -tocopherol is added exogenously for fortification purposes. Harms et al. (2003) and Gerling and Ternes (2014) have shown that in salami-type sausages there was no decrease in the α -tocopherol content over storage of up to 56 days, but Rosli et al. (2006) showed a 58% decrease in the α -tocopherol content of beef burgers and a 26% decrease in the α -tocopherol content of chicken frankfurters over 6 months of frozen storage. Another study by the same group also showed a 46% decrease in the α -tocopherol content of beef frankfurters over 6 months storage (Rosli et al. 2010). However, to our knowledge the stability of α -tocopherol in breakfast style poultry sausages has not previously been investigated.

The aim of this study was to evaluate the effect of simultaneously reducing an undesirable ingredient, salt (reduced by 60%), and increasing a desirable ingredient, α -tocopherol, on the physical and sensory quality of chicken sausages. The stability of α -tocopherol during refrigerated storage and cooking, frying and grilling, was also explored. α -Tocopherol was added at the concentration of 200 mg/kg, an amount considered necessary to reduce lipid oxidation in processed meat products (Du and Ahn 2002; Kim and Choi 2012) and in excess of the amount needed to meet both EFSA nutrient and health claims (EFSA 2013).

Materials and methods

Materials

Fresh chicken thigh meat and skin were obtained from Manor Farm Chicken (Clonee, Co. Meath). The thigh meat was deboned and skinned before being stored under vacuum at -20°C and was used within a month of storage.

Natural sheep casing was sourced from a local butcher (Dunnes of Donnybrook, Dublin 4, Ireland). Rusk, tetrasodium pyrophosphate, sodium metabisulphite, yeast extract, whey protein isolate, dextrose, ascorbic acid, salt, pepper and nutmeg were obtained from All-in-All Ingredients Ltd. (Parkwest, Dublin 12, Ireland) and olive oil was sourced from a local supermarket (Tesco, Blanchardstown, Dublin, Ireland). Vitamin E in the form of d- α -tocopherol (872 mg/g, 1300 IU/g) was obtained from Xi'an Healthful Biotechnology Co. Ltd (China). All reagents for analysis and α -tocopherol standards were analytical grade and sourced from Sigma-Aldrich.

Sausage manufacture

The chicken sausage formulations are shown in Table 1. The sausages were prepared in 1 kg batches by first thawing the chicken thigh meat and chicken skin at room temperature for 3 h. The meat and skin were then minced separately using an 8 mm plate (Kenwood AT950A multi food mincer chef attachment). The meat was mixed with the dry ingredients and half of the iced water, for 3 min at setting 1 in a Kenwood Mayor Classic KM800 with a K-beater. Chicken skin and olive oil were added and mixed for a further 3 min. When α -tocopherol was included in the formulation, it was mixed with the olive oil prior to addition to the meat matrix, to improve distribution in the batter. The rusk was added and mixed for 1.5 min, after

Table 1 Raw chicken sausage product formulations

Ingredient (%)	Formulation			
	C	L	CE	LE
Dark meat	60.43	60.94	60.42	60.93
Chicken skin	4.65	4.69	4.65	4.69
Rusk	9.30	9.38	9.29	9.37
Ice/water	18.59	18.75	18.59	18.75
Olive oil	0.56	0.56	0.56	0.56
Tetrasodium pyrophosphate	0.28	0.28	0.28	0.28
Sodium metabisulphite	0.05	0.05	0.05	0.05
Yeast extract	1.86	1.88	1.86	1.87
Whey protein isolate	1.21	1.22	1.21	1.22
Dextrose	0.82	0.83	0.82	0.82
Ascorbic acid	0.82	0.83	0.82	0.82
Salt	1.30	0.47	1.30	0.47
α -Tocopherol	0.00	0.00	0.02	0.02
Pepper	0.09	0.09	0.09	0.09
Nutmeg	0.05	0.05	0.05	0.05
Total	100	100	100	100

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol)

which the remaining water was added and mixed for 1.5 min. The batter was stuffed into natural sheep casing using the Kenwood AT950A multi food mincer chef attachment. Sausages were hand linked at about 7 cm intervals. The sausages were placed on PET trays (Versatile Packaging, Co. Monaghan, Ireland) and overwrapped with PVC film ($15,500\text{--}16,275\text{ cm}^3\text{ O}_2/\text{m}^2/24\text{ h}$ at $23\text{ }^\circ\text{C}$) (McDonnells, Blackhall Street, Dublin 7, Ireland) before being stored at $4\text{ }^\circ\text{C}$ in the dark for 10 days. For microbiological analysis, lipid oxidation and colour analysis samples were taken at days 0, 3, 7 and 10 of storage for raw sausages and at days 0, 2 and 5 for cooked sausages. For α -tocopherol analysis and proximate analysis samples were frozen ($-20\text{ }^\circ\text{C}$) on the relevant days and thawed prior to analysis.

Cook loss

The cook loss of both fried and grilled sausages was determined. Sausages were fried using a NEFF induction hob which was preheated and maintained at $130\text{ }^\circ\text{C}$. Sausages were removed from refrigerated storage and allowed to reach $10\text{ }^\circ\text{C}$ before frying. They were cooked without oil, while being continuously turned and without pricking, for 15 min after which an internal temperature of $77\text{ }^\circ\text{C}$ was reached.

Sausages were grilled using a NEFF oven preheated to $130\text{ }^\circ\text{C}$. Sausages were removed from refrigerated storage and allowed to reach $10\text{ }^\circ\text{C}$ before grilling. The grill tray was placed 12.5 cm below the grill element. Sausages were turned every 5 min and cooked for 15 min until an internal temperature of $77\text{ }^\circ\text{C}$ was reached.

Cook loss during frying and grilling was determined using the following formula (Sikes et al. 2009):

Cook loss (%)

$$= \frac{[\text{raw sausage weight (g)} - \text{cooked sausage weight (g)}]}{\text{raw sausage weight (g)}} \times 100$$

α -Tocopherol extraction and analysis

α -Tocopherol was extracted and analysed using a modified method of Buttriss and Diplock (1984). At each storage time three sausages per batch were analysed. In brief, 1 mL of a 25% sausage homogenate in water was saponified using 2 mL of 1% ethanolic pyrogallol and 0.3 mL of 50% potassium hydroxide, and incubation in a shaking water bath at $70\text{ }^\circ\text{C}$ for 30 min. Samples were cooled in an ice-bath and 2 mL of hexane was added; samples were vortexed for 30 s and centrifuged at 2500 rpm for 10 min. The hexane was removed and transferred to an Eppendorf and dried under nitrogen at $30\text{ }^\circ\text{C}$. The extraction and drying

were repeated twice. The residue was redissolved in 200 μL methanol and centrifuged for 9 min at 15,000 rpm. The supernatant was stored at $-20\text{ }^\circ\text{C}$ and retained for HPLC.

α -Tocopherol was quantified by reverse-phase HPLC using an Agilent 1200 series autosampler system and a Zorbax Eclipse XDB-C18 $4.6 \times 150\text{ mm } 5\text{ }\mu\text{m}$ column with 100% methanol as the mobile phase at a rate of 1 mL/min; peaks were detected at 292 nm. The amount of α -tocopherol was quantified by comparing peak areas of samples with α -tocopherol standards (Sigma-Aldrich, Steinheim, Germany) made up to the following concentrations: 0, 2, 4, 8, 16, 32, 64, 128 and 256 $\mu\text{g/mL}$ in methanol. The calibration curve had a consistent $R^2 \geq 0.99$ and the percentage recovery of α -tocopherol from the sausage meat was 90%. The results were expressed as mg α -tocopherol per 100 g sausage.

Texture profile analysis (TPA)

Instrumental texture was evaluated by using an Instron (Model No. 5940 Single Column Tabletop Testing Systems) and Bluehill software using a modified procedure as described by Savadkoochi et al. (2014). Cooked sausages were allowed to come to room temperature ($20\text{ }^\circ\text{C}$) after cooking and cored into cylindrical sections (20 mm length and 15 mm diameter). The samples were kept at room temperature before being compressed twice to 50% of their original height at a crosshead speed of 1 mm/s. The following parameters were determined: hardness (the maximum initial force required to compress the product, N), cohesiveness (ratio of active work done under the second compression curve to that done under the first compression curve, dimensionless), springiness (ability to recover shape after initial compression, mm), gumminess (force required to masticate the sample – hardness $\times$ cohesiveness, N) and chewiness (work required to masticate the sample – hardness $\times$ cohesiveness $\times$ springiness, N mm).

Lipid oxidation

Lipid oxidation was determined using the distillation method as described by Tarladgis et al. (1960) and expressed as 2-thiobarbituric acid-reactive substances (TBARS) in mg malondialdehyde (MDA)/kg sausage sample.

Colour analysis

Colour was analysed using a reflectance Minolta CR-400 colorimeter (Osaka, Japan) according to CIE (1986). The measurement was carried out under illuminant D65, 10° observer angles and following instrument calibration using

a white tile. The chromaticity coordinates recorded were L^* (Lightness), a^* (redness) and b^* (yellowness). The value for each of these parameters was calculated as the average of three measurements.

Microbiological analysis

Microbial analysis was carried out as described by Higenyi et al. (2014). In brief, 20 g sausage samples were weighed and placed in stomacher bags with 180 mL of buffered peptone water under aseptic conditions. Serial dilutions were made accordingly and the following microbes were tested for: (1) total aerobic count (plate count agar) and (2) *E. Coli* (McConkey agar). The plates were incubated for 48 h at 37 °C and results expressed as $\log_{10}$ colony forming units per gram (CFU/g).

Proximate composition

Protein, fat, moisture and ash in sausages were determined according to AOAC (1995) methods on the day of manufacture for raw and on the day of cooking for fried and grilled sausages.

Sensory analysis

Sensory analysis was approved by the Human Research Ethics Committee of University College Dublin, Ireland, LS-15-14-Bolger-Monahan. All participants signed a consent form agreeing to voluntarily participate in the study. Sensory analysis was conducted by 105 panellists, with ages ranging from 19 to 56 years. Sensory analysis was carried out on grilled sausages, cooked after 4 days of raw storage at 4 °C, and served immediately after cooking. Samples from each formulation (C, L, CE, LE) were labelled with random, four-digit codes and presented in disposable white cups with water and cream crackers to cleanse the palate between samples. Participants were asked to evaluate the sausages for colour, flavour, texture, aroma and overall liking using a 10 cm unlabelled line scale ranging from 0 (disliked extremely) to 10 (liked extremely).

Statistical analysis

Three repetitions of the experiment were carried, apart from sensory analysis which was performed on a separate repetition. All instrumental analyses were performed on three sausages per measurement per time point. The main and interactive effects of salt level, vitamin E level, storage time and cooking method were evaluated using a three or four-way ANOVA with differences between mean values being tested using Tukey's test (significance $p \leq 0.05$).

A Kruskal–Wallis analysis was applied to sensory data (significance $p \leq 0.05$). All analyses were conducted using the SPSS 18 (SPSS Inc., Chicago, IL, USA). Table 2 displays the p values obtained from the analysis of variance of independent variables on vitamin E retention and measurements of quality of chicken sausages.

Results and discussion

Proximate composition

The proximate composition of the raw chicken sausages is shown in Table 3. There were no differences in fat, protein or moisture between formulations. The ash content was significantly lower ($p \leq 0.05$) in the reduced salt formulations.

Cooking led to a significant increase ($p \leq 0.05$) in the protein and ash content of the sausages with a corresponding decrease in the moisture content but no change in the fat content. There was no significant difference ($p \geq 0.05$) in composition between fried and grilled sausages, similar to the findings of Sheard et al. (1998).

Cook loss and TPA

Cook loss was unaffected ($p \geq 0.05$) by cooking method, the level of α -tocopherol (C vs CE and L vs LE) or the level of salt inclusion (C vs L and CE vs LE) (Table 3). The lack of a salt effect on cook loss was in contrast to reports for beef (Sikes et al. 2009) and pork (O'Flynn et al. 2014) sausages which showed a significant increase in cook loss when salt was reduced below 2 and 1%, respectively. Our results agreed with those of Hongprabhas and Barbut (1999) for poultry batters where no difference in cook loss was observed between products at salt inclusion levels of 0–1.5%. Although studies in chicken batters (Jimenez-Colmenero et al. 1998) and chicken nuggets (Verma et al. 2010, 2012) have shown a significant difference in cook loss between low and high salt meat products, the levels of salt added were higher (1.5–2.5% and 1.2–2%, in the chicken batters and nuggets, respectively) than in our current study. Cooking method (frying vs grilling) had no significant effect ($p \geq 0.05$) on cook loss, consistent with the findings of Sheard et al. (1998).

There was no significant effect of cooking method, α -tocopherol level or salt level on hardness, springiness, cohesion, gumminess or chewiness (Table 3). This is in contrast to studies in pork sausages with similar formulations. O'Flynn et al. (2014) showed that in pork sausages a reduction in salt level below 1% led to a significant reduction in hardness, probably due to a decrease in the extraction of salt-soluble proteins, while springiness,

Table 2 *P* values obtained from the analysis of variance of independent variables on vitamin E retention and measurements of quality of chicken sausages

Variables	Vit E	MDA	L* value	a* value	b* value	TVC	E.Coli	Cook loss	H1	H2	Sprin	Coh	Gum	Chew	Fat	Pro	Mois	Ash
Raw																		
Storage	0.30	0.69	0.00	0.00	0.51	0.50	0.00											
Salt	0.45	0.28	0.79	0.10	0.46	0.76	0.85											
Vitamin E	0.00	0.45	0.73	0.34	0.86	0.48	0.55											
Storage × salt	0.74	0.85	0.99	0.87	0.95	0.96	0.08											
Storage × vitamin E	0.30	0.11	0.93	0.80	0.97	0.93	0.11											
Salt × vitamin E	0.48	0.95	0.31	0.21	0.70	0.67	0.57											
Storage × salt × vitamin E	0.75	0.93	0.97	0.84	0.77	0.58	0.37											
Cooked																		
Method (fried vs grilled)	0.53	0.93	0.00	0.00	0.00			0.48	0.00	0.00	0.78	0.82	0.05	0.12	0.01	0.00	0.00	0.00
Salt	0.20	0.29	0.02	0.76	0.84			0.60	0.29	0.41	0.22	0.23	0.16	0.15	0.32	0.00	0.66	0.00
Vitamin E	0.00	0.24	0.32	0.67	0.47			0.10	0.29	0.35	0.20	0.20	0.73	0.63	0.80	0.93	0.41	0.44
Storage	0.07	0.00	0.00	0.00	0.00			0.41	0.6	0.16	0.84	0.81	0.20	0.31	0.34	0.56	0.06	0.40
Method × salt	0.84	0.22	0.02	0.13	0.19			0.55	0.4	0.03	0.18	0.19	0.40	0.05	0.93	0.29	0.06	0.76
Method × vitamin E	0.40	0.86	0.85	0.95	0.53			0.58	0.35	0.61	0.69	0.76	0.76	0.72	0.03	0.30	0.02	0.13
Method × Storage	0.76	0.41	0.00	0.00	0.00			0.26	0.15	0.18	0.29	0.30	0.72	0.55	0.19	0.37	0.18	0.39
Salt × vitamin E	0.15	0.22	0.36	1.00	0.72													
Salt × storage	0.86	0.84	0.02	0.98	0.34													
Vitamin E × storage	0.07	0.97	0.85	0.96	0.78													
Method × salt × vitamin E	0.91	0.19	1.00	0.37	0.21													
Method × salt × storage	0.09	0.45	0.78	0.11	0.41													
Method × vitamin E × storage	0.73	0.30	0.22	0.99	0.24													
Salt × vitamin E × storage	0.96	0.74	0.28	0.78	0.61													
Method × salt × vitamin E × storage	0.10	0.66	0.24	0.80	0.67													
Raw (day 0) vs cooked (day 0)																		
State (raw, fried, grilled)	0.29														0.00	0.00	0.00	0.00
Salt	0.65														0.31	0.00	0.66	0.00
Vitamin E	0.00														0.80	0.93	0.41	0.44
State × salt	0.13														0.34	0.58	0.06	0.40
State × vitamin E	0.36														0.93	0.28	0.06	0.76
Salt × vitamin E	0.49														0.03	0.30	0.02	0.13
State × salt × vitamin E	0.15														0.19	0.37	0.18	0.89

* *Vit E* vitamin E, *MDA* lipid oxidation, *TVC* total viable count, *E. coli* *E. coli* count, *H1* hardness 1, *H2* hardness 2, *Sprin* springiness, *Coh* cohesion, *Gum* gumminess, *Chew* chewiness, *Pro* protein, *Mois* moisture

Table 3 Proximate composition (%), cook loss (%) and the texture profile analysis of the chicken sausages

State	Formulation	Fat (%)	Protein (%)	Moisture (%)	Ash (%)	Cook loss (%)	Hardness 1 (N)	Hardness 2 (N)	Springiness (mm)	Cohesion	Gumminess (N)	Chewiness (N mm)
Raw	C	8.1 ± 1.1	14.2 ± 0.4 ^x	65.4 ± 1.5 ^y	2.7 ± 0.3 ^{ax}							
	L	7.4 ± 1.1	14.6 ± 0.7 ^x	66.6 ± 0.9 ^y	1.7 ± 0.3 ^{bx}							
	CE	7.3 ± 1.0	14.3 ± 0.5 ^x	65.5 ± 0.58 ^y	2.7 ± 0.1 ^{ax}							
	LE	8.4 ± 1.2	14.8 ± 0.7 ^x	66.4 ± 0.3 ^y	1.9 ± 0.1 ^{bx}							
Fried	C	7.6 ± 0.8	17.1 ± 0.6 ^y	60.0 ± 0.7 ^x	3.4 ± 0.1 ^{ay}	13.4 ± 1.2	2.9 ± 0.6	2.1 ± 0.5	4.3 ± 0.6	43.3 ± 5.3	0.7 ± 0.2	3.0 ± 1.0
	L	8.2 ± 0.7	17.6 ± 0.5 ^y	60.1 ± 0.9 ^x	2.4 ± 0.2 ^{by}	13.5 ± 1.2	3.0 ± 0.7	2.2 ± 0.5	4.8 ± 0.7	48.2 ± 7.3	0.8 ± 0.2	3.8 ± 1.4
	CE	7.5 ± 0.7	17.4 ± 0.5 ^y	59.3 ± 1.5 ^x	3.3 ± 0.2 ^{ay}	12.8 ± 1.2	3.5 ± 0.7	2.5 ± 0.6	4.8 ± 0.6	48.4 ± 6.3	0.8 ± 0.2	3.8 ± 1.5
	LE	8.1 ± 0.4	17.7 ± 0.8 ^y	58.7 ± 1.6 ^x	2.6 ± 0.4 ^{by}	13.1 ± 1.4	4.1 ± 1.2	2.9 ± 0.8	5.3 ± 0.7	53.4 ± 7.0	0.9 ± 0.3	5.0 ± 1.8
Grilled	C	8.8 ± 0.9	17.5 ± 1.1 ^y	57.4 ± 2.3 ^x	3.1 ± 0.4 ^{ay}	14.1 ± 2.0	3.5 ± 0.8	2.6 ± 0.6	4.8 ± 0.7	47.8 ± 7.6	0.9 ± 0.3	4.2 ± 1.7
	L	8.1 ± 0.9	17.8 ± 0.8 ^y	58.5 ± 1.5 ^x	2.4 ± 0.2 ^{by}	14.6 ± 1.3	3.8 ± 1.1	2.7 ± 0.9	4.8 ± 0.6	47.6 ± 6.2	0.9 ± 0.4	4.6 ± 2.5
	CE	8.4 ± 0.7	16.7 ± 1.3 ^y	59.3 ± 1.3 ^x	3.1 ± 0.6 ^{ay}	13.2 ± 1.1	3.3 ± 0.7	2.5 ± 0.6	4.6 ± 0.5	46.1 ± 5.1	0.7 ± 0.2	3.5 ± 1.3
	LE	8.8 ± 0.5	17.9 ± 0.7 ^y	57.8 ± 1.9 ^x	2.3 ± 0.2 ^{by}	14.1 ± 2.3	3.7 ± 1.0	2.7 ± 0.8	5.0 ± 0.8	49.8 ± 7.9	0.8 ± 0.3	4.1 ± 1.8

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Table shows the means ($\pm$ standard deviation)

Values within a column with different superscript ^{xy} are significantly ($p \leq 0.05$) different between raw, fried and grilled treatments

Values within a column with different superscript ^{ab} are significantly ($p \leq 0.05$) different between formulations within raw, fried and grilled treatments

cohesiveness, gumminess and chewiness decreased significantly when the salt level was below 1.5%. Other studies in chicken type products also showed a decrease in the texture profile parameters, with Jimenez-Colmenero et al. (1998) showing a decrease in the hardness of chicken meat batters when the salt content was reduced from 2.5 to 1.5%. The reason why no change in the texture profile of chicken sausages was observed in our study may be due to the control salt level being lower (1.3 g salt/100 g) than that used in other studies (above 2 g salt/100 g). The level we used is, however, representative of the salt content in poultry sausages currently on sale in a number of Irish and English supermarket outlets (range 1.0–1.4 g salt/100 g) (Bolger et al. 2015). The difference in the effect of salt reduction on poultry (chicken and turkey) and pork sausages may also be due to poultry meat having increased amounts of extractable salt-soluble protein compared to pork (Keever 2011). Cooking method had no significant effect ($p \geq 0.05$) on the texture of the sausages, which was not surprising as no significant change in composition or cook loss was observed between fried and grilled sausages.

α -Tocopherol retention

Table 4 shows the retention of α -tocopherol in the chicken sausages during storage and cooking. The mean α -tocopherol contents of fortified sausages ranged between 17.4 and 18.3 mg α -tocopherol per 100 g of raw sausage on day 0 of storage and, since the endogenous α -tocopherol content of the sausages was approximately 1 mg α -tocopherol per 100 g (Table 4), this indicates that approximately 82–86% of the added α -tocopherol was retained during the manufacture of the sausages. In the cooked (fried or grilled) sausages, while there were numerical differences between mean values of day 0 samples and samples stored for 5 days the differences were not significant ($p \geq 0.05$). This is similar to what was observed by Harms et al. (2003) and Gerling and Ternes (2014) in salami-type sausages, with no significant changes in the α -tocopherol

concentration for up to 56 days of storage at 9 °C. In contrast, Sammet et al. (2006) showed a significant decrease, approximately 37%, in α -tocopherol in salami-type sausages following storage at 9 °C over 84 days (12 weeks). The reduction in α -tocopherol content may be due the utilisation of α -tocopherol in bringing about a reduction in lipid oxidation, between weeks 4–6 of storage, in fortified salamis when compared to control (Sammet et al. 2006). In the studies of Harms et al. (2003) and Gerling and Ternes (2014) no effect on lipid oxidation was observed.

The α -tocopherol content was not significantly affected ($p \geq 0.05$) by cooking method. This result is similar to what was observed by Rosli et al. (2006) in chicken frankfurters, where no difference in α -tocopherol content was observed between raw and cooked (oven or retort cooked) immediately after cooking; however, they did observe a reduction in α -tocopherol after 6 months of frozen (−18 °C) storage.

Lipid oxidation

Lipid oxidation (TBARS values) in raw and cooked chicken sausages, during refrigerated storage, is shown in Tables 5 and 6, respectively. Lipid oxidation was unaffected ($p \geq 0.05$) by salt level, α -tocopherol level and cooking method. In raw sausages, lipid oxidation only increased significantly ($p \leq 0.05$) in CE sausages over time, but there was no significant difference ($p \geq 0.05$) between formulations. The minimal effect of storage on the TBARS value in raw sausages is similar to what was observed by Narciso-Gaytán et al. (2010) in raw chicken breast, thigh and skin, with lipid oxidation only increasing after 5 days of storage in chicken meat with low (33 mg/kg) dietary supplementation of vitamin E and only increasing after 10 days of storage in chicken meat with high (200–400 mg/kg) dietary supplementation of vitamin E. The stability of TBARS values over storage for chicken breast and thigh meat was also observed in an animal industry report from Iowa State University

Table 4 The α -tocopherol content (mg/100 g) of raw and cooked sausages during refrigerated storage at 4 °C

Formulation	Raw		Fried		Grilled	
	Day 0	Day 10	Day 0	Day 5	Day 0	Day 5
C	0.9 ± 0.1 ^b	1.0 ± 0.1 ^b	1.1 ± 0.1 ^b	1.2 ± 0.1 ^b	1.0 ± 0.2 ^b	1.1 ± 0.1 ^b
L	1.0 ± 0.1 ^b	1.1 ± 0.2 ^b	1.2 ± 0.2 ^b	1.2 ± 0.2 ^b	1.2 ± 0.1 ^b	1.1 ± 0.2 ^b
CE	17.4 ± 1.7 ^a	18.7 ± 2.5 ^a	18.1 ± 1.1 ^a	19.0 ± 1.2 ^a	19.8 ± 1.8 ^a	18.8 ± 1.6 ^a
LE	18.3 ± 0.6 ^a	21.0 ± 3.2 ^a	19.4 ± 2.5 ^a	17.3 ± 1.1 ^a	19.8 ± 2.0 ^a	18.7 ± 1.5 ^a

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Table shows the means (±standard deviation)

Values within a column with different superscript ^{ab} are significantly ($p \leq 0.05$) different between formulations

Table 5 Lipid oxidation (TBARS, mg MDA/kg), colour (lightness, L*; redness, a*; yellowness, b*) parameters and microbial growth (log CFU/g) in raw chicken sausages during refrigerated storage (4 °C)

Parameters	Storage (days)	Formulations			
		C	L	CE	LE
Lipid oxidation (MDA/kg)	0	1.0 ± 0.2	1.2 ± 0.4	0.8 ± 0.2 ²	0.9 ± 0.4
	3	1.1 ± 0.6	1.1 ± 0.4	0.8 ± 0.2 ²	1.3 ± 0.6
	7	1.2 ± 0.1	1.1 ± 0.4	1.2 ± 0.2 ¹	1.3 ± 0.5
	10	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.1 ¹	1.1 ± 0.1
L*	0	67.1 ± 3.1 ¹	66.1 ± 4.1 ¹	66.6 ± 2.5 ¹	67.5 ± 3.6 ¹
	3	65.4 ± 4.4 ¹²	64.1 ± 3.3 ¹²	65.0 ± 3.5 ¹²	64.1 ± 3.3 ¹²
	7	62.2 ± 3.7 ²	61.9 ± 3.7 ¹²	61.8 ± 4.2 ²	62.2 ± 3.8 ²
	10	61.4 ± 3.0 ²	60.8 ± 2.7 ²	60.7 ± 3.4 ²	60.6 ± 2.8 ²
a*	0	6.8 ± 0.8 ¹	7.3 ± 1.1 ¹	6.7 ± 0.8	6.9 ± 0.8
	3	6.6 ± 0.6 ¹²	6.6 ± 0.6 ¹²	6.8 ± 0.6	6.8 ± 0.6
	7	5.9 ± 0.7 ¹²	6.3 ± 0.6 ¹²	6.1 ± 0.4	5.9 ± 0.9
	10	5.9 ± 0.7 ²	6.2 ± 0.6 ²	6.1 ± 0.4	6.1 ± 0.4
b*	0	18.6 ± 2.3	18.3 ± 2.4	18.4 ± 2.3	18.9 ± 2.4
	3	18.1 ± 3.2	17.9 ± 2.9	18.6 ± 2.8	17.9 ± 2.9
	7	18.3 ± 2.6	18.4 ± 2.0	19.3 ± 4.3	17.7 ± 2.3
	10	18.6 ± 2.6	18.2 ± 2.6	18.3 ± 2.3	18.0 ± 2.1
Total viable count (log CFU/g)	0	3.2 ± 0.4	3.6 ± 0.3	3.3 ± 0.4	3.3 ± 0.4
	3	3.1 ± 0.4	3.3 ± 0.5	3.1 ± 0.6	3.3 ± 0.5
	7	3.2 ± 0.7	3.5 ± 0.4	3.0 ± 0.3	3.5 ± 0.4
	10	3.5 ± 0.1	3.8 ± 0.3	3.5 ± 0.1	3.8 ± 0.3
<i>E. Coli</i> count (log CFU/g)	0	3.0 ± 0.3	3.2 ± 0.4	3.0 ± 0.3 ²	2.7 ± 0.3 ²
	3	3.4 ± 0.3	3.1 ± 0.4	3.5 ± 0.3 ¹	3.2 ± 0.5 ¹²
	7	3.0 ± 0.2	3.1 ± 0.3	2.9 ± 0.4 ²	3.2 ± 0.4 ²
	10	3.4 ± 0.3	3.5 ± 0.3	3.6 ± 0.1 ¹	3.7 ± 0.2 ¹

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Table shows the means ($\pm$ standard deviation)

Values within a column with different superscript ¹² are significantly ($p < 0.05$) different between days

(2008) which reported little change in TBARS over 7 days of refrigerated storage. Our results are also similar to those of Marcinčák et al. (2005) and Jayawardana et al. (2015) who observed no change in lipid oxidation in chicken sausages over 7 days and 5 weeks, respectively, of refrigerated storage.

In cooked sausages, lipid oxidation significantly increased ($p \leq 0.05$) with storage time in fried L and CE sausages but increase was not significant ($p \geq 0.05$) for C and LE sausages. Lipid oxidation significantly increased ($p \leq 0.05$) with storage time in all the formulations of grilled sausages; however, there was no significant difference ($p \geq 0.05$) in lipid oxidation between cooked sausage formulations. This is in contrast to previous studies which have observed that the addition of α -tocopherol to chicken sausages (Shin et al. 2011), turkey sausages (Du and Ahn 2002), fresh pork sausages (Georgantelis et al. 2007a), burgers (Georgantelis et al. 2007b) and salami (Sammet et al. 2006) led to a reduction in lipid oxidation. In contrast,

other studies, similar to ours, have observed no change in TBARS values when α -tocopherol was added to cured pork sausages (Harms et al. 2003), coarse ground pork sausage and pork roast (Channon and Trout 2002). A possible explanation for the lack of effect, may be that ingredients, such as white pepper (Martinez et al. 2006), nutmeg (El-Alim et al. 1999; Vasavada et al. 2006) and sodium metabisulphite (Mathenjwa et al. 2012), have proven antioxidant activity in meat products and therefore may mask any potential effect of added α -tocopherol.

Colour analysis

There was a general reduction in the external product lightness and redness values of raw sausages with storage (Table 5). No significant effect of salt level was observed in raw sausages; this was similar to what was observed by O'Flynn et al. (2014) in pork sausages, where a reduction in the salt content from 1 to 0.5% produced no changes in

Table 6 Lipid oxidation (mg MDA/kg) and colour parameters (lightness, L*, redness, a*, yellowness, b*) of the cooked chicken sausages during refrigerated storage

Formulation	Lipid oxidation (MDA/kg)					L*					a*					b*				
	0	2	5	2	5	0	2	5	0	2	5	0	2	5	0	2	5			
<i>Fried</i>																				
C	1.0 ± 0.3	1.4 ± 0.3	1.5 ± 0.5	64.3 ± 1.6 ^{2a}	67.5 ± 2.2 ^{1ay}	67.2 ± 2.7 ^{1by}	6.7 ± 0.5 ^{1x}	5.7 ± 0.8 ²	5.7 ± 1.4 ^{2y}	22.0 ± 2.2 ^{ably}	21.2 ± 1.5 ^{2y}	21.4 ± 2.4 ²								
L	1.0 ± 0.3 ²	1.0 ± 0.3 ²	1.8 ± 0.4 ¹	62.5 ± 2.3 ^{2b}	65.5 ± 3.5 ^{1by}	66.8 ± 1.4 ^{1by}	6.9 ± 1.1 ^{1x}	5.9 ± 1.4 ²	5.2 ± 0.3 ^{2x}	23.5 ± 2.6 ^{aly}	20.9 ± 1.0 ^{2y}	19.3 ± 1.2 ²								
CE	0.9 ± 0.2 ²	1.4 ± 0.3 ²	1.6 ± 0.6 ¹	66.1 ± 3.8 ^{2a}	68.5 ± 1.2 ^{1ay}	68.0 ± 1.6 ^{1ay}	6.3 ± 1.1 ^{1x}	5.2 ± 0.3 ²	5.0 ± 0.8 ^{2x}	21.6 ± 2.2 ^{by}	20.2 ± 1.6 ^{2y}	19.9 ± 2.0 ²								
LE	1.2 ± 0.3	1.6 ± 0.3	1.5 ± 0.2	62.8 ± 2.8 ^{2b}	67.9 ± 0.8 ^{1ay}	67.7 ± 0.8 ^{1by}	7.5 ± 0.8 ^{1x}	4.9 ± 0.3 ²	4.2 ± 1.1 ^{2x}	25.4 ± 1.8 ^{aly}	20.1 ± 1.0 ^{2y}	20.0 ± 1.3 ²								
<i>Grilled</i>																				
C	1.0 ± 0.2 ²	1.4 ± 0.6 ²	2.2 ± 0.6 ¹	61.3 ± 4.6 ^{a2}	64.2 ± 4.3 ^{1x}	65.3 ± 3.7 ^{1x}	6.7 ± 1.4 ^{a2y}	5.4 ± 0.6 ¹²	4.9 ± 0.7 ^{1x}	22.5 ± 2.3 ^{1x}	20.0 ± 0.9 ^{2x}	18.7 ± 1.9 ²								
L	1.0 ± 0.4 ²	1.4 ± 0.5 ¹²	2.1 ± 0.9 ¹	57.0 ± 2.1 ^{b2}	63.3 ± 3.5 ^{1x}	63.3 ± 3.7 ^{1x}	8.8 ± 1.6 ^{b1y}	5.3 ± 0.7 ²	5.3 ± 0.8 ^{2y}	22.6 ± 1.5 ^{1x}	18.8 ± 1.4 ^{2x}	19.3 ± 1.8 ²								
CE	0.9 ± 0.1 ²	1.5 ± 0.3 ¹	1.7 ± 0.7 ¹	61.8 ± 4.3 ^{a2}	64.3 ± 3.6 ^{1x}	64.5 ± 2.8 ^{1x}	7.6 ± 1.1 ^{aely}	5.5 ± 0.6 ²	5.1 ± 0.8 ^{2y}	22.1 ± 2.8 ^{1x}	19.8 ± 1.8 ^{2x}	18.9 ± 1.4 ²								
LE	0.9 ± 0.3 ¹	1.4 ± 0.4 ¹²	1.8 ± 0.6 ¹	58.5 ± 1.8 ^{ab2}	63.4 ± 3.2 ^{1x}	63.8 ± 2.7 ^{1x}	8.3 ± 1.2 ^{bely}	5.4 ± 0.6 ²	5.2 ± 0.4 ^{2y}	22.7 ± 3.5 ^{1x}	19.2 ± 2.8 ^{2x}	18.8 ± 1.7 ²								

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Table shows the means ($\pm$ standard deviation)

Values within a column with different superscript ^{ab} are significantly ($p < 0.05$) different between formulations

Values within a row with different superscript ¹² are significantly ($p < 0.05$) different between days

Values within a row with different superscript ^{xy} are significantly ($p < 0.05$) different between cooking methods

Table 7 Acceptance values for colour, flavour, texture, juiciness, and overall acceptance for the four difference chicken sausages formulations

Formulation	Colour	Flavour	Texture	Aroma	Overall acceptance
C	4.0 ± 2.2	3.8 ± 2.4 ^b	3.3 ± 2.1 ^b	4.7 ± 2.1	3.6 ± 2.2
L	4.5 ± 2.3	4.5 ± 2.5 ^a	4.1 ± 2.4 ^a	5.2 ± 2.0	4.1 ± 2.3
CE	4.4 ± 2.4	4.0 ± 2.5 ^{ab}	3.5 ± 2.2 ^{ab}	4.8 ± 2.1	3.8 ± 2.4
LE	4.4 ± 2.3	4.3 ± 2.4 ^{ab}	3.9 ± 2.3 ^{ab}	5.0 ± 2.1	3.9 ± 2.2

C (control, 1.3% NaCl, 0 mg/kg added α -tocopherol), L (low salt, 0.47% NaCl, 0 mg/kg added α -tocopherol), CE (1.3% NaCl, 200 mg/kg added α -tocopherol), LE (0.47% NaCl, 200 mg/kg added α -tocopherol). Table shows the means ($\pm$ standard deviation)

Values within a column with different superscript ^{ab} are significantly ($p < 0.05$) different between formulations

colour. Also, no significant difference ($p \geq 0.05$) between control and α -tocopherol fortified sausages was observed; this is similar to the findings of Du and Ahn (2002), who observed that the addition of α -tocopherol to raw turkey sausage did not significantly effect their colour. Even though no significant difference between formulations was observed, the addition of vitamin E did prevent a significant change ($p \geq 0.05$) in the a^* value over storage.

For fried sausages (Table 6), salt level had a significant effect on L^* values with the high salt sausages (C and CE) being lighter ($p \leq 0.05$) than the low salt sausages (L and LE). This difference in lightness between the salt levels decreased with storage, and on day 2 of storage only L was significantly darker and on day 5 of storage only CE was significantly lighter than the other formulations. A corresponding change in the b^* value was also observed, with low salt sausages (L and LE) being yellower ($p \leq 0.05$) than high salt sausage (C and CE), but this difference was not observed after 2 days of storage. There was no significant difference ($p \geq 0.05$) observed between formulations for the a^* value. Storage time significantly affected ($p \leq 0.05$) all formulations with L^* values increasing over time, while a^* and b^* values decreased over time.

For grilled sausages (Table 6), the only significant difference in colour was observed on day 0 with low salt sausages (L) being significantly ($p \leq 0.05$) darker and redder than high salt sausages (C). However, this difference in colour was not observed between CE and LE sausages, where it appears that the addition of α -tocopherol to the sausages is able to offset the changes in colour due to the reduction in salt. Storage time significantly affected ($p \leq 0.05$) all formulations with L^* values increasing over time, while a^* and b^* values decreased over time.

The cooking method also had a significant effect ($p \leq 0.05$) on colour, with fried sausages being lighter, yellower and less red than grilled sausages.

Microbiological analysis

Reducing the salt level of the sausages did not significantly affect ($p \geq 0.05$) the total viable count or *E. Coli* count in

raw sausages (Table 5). This is similar to what was observed by Aaslyng et al. (2014), who also found that microbial growth was not significantly affected by reducing the amount of salt added to hotdog sausages (0, 0.5 and 1% salt) and salami (1.5, 2.25 and 3% salt). The lack of an effect on microbial counts may have been due to the antimicrobial activities of the other ingredients added, such as sodium metabisulphite or the various spice constituents (Owino and Muroki 1999).

The addition of α -tocopherol did not significant effect ($p \geq 0.05$) microbial growth (Table 5). This concurs with a study by Georgantelis et al. (2007a), in pork sausages, who showed that the addition of α -tocopherol (115 mg/kg) had no significant effect on the growth of *Enterobacteriaceae*, *Pseudomonas*, total viable count, lactic acid bacteria or yeasts/moulds. No microbial growth was observed in cooked sausages (results not shown).

Sensory analysis

The results of sensory analysis are presented in Table 7. Neither salt nor α -tocopherol level had a significant effect ($p \geq 0.05$) on colour, aroma and overall acceptance. However, salt level had a significant effect ($p \leq 0.05$) on flavour and texture, with participants preferring low salt (L) sausages over control (C) sausages, but the values were not significant in the α -tocopherol (CE and LE) fortified sausages. This is an interesting result as the addition of salt is thought to improve the texture and flavour of processed meat products and other studies have shown significant decreases in sensory parameters with reductions in salt. O'Flynn et al. (2014) observed a significant decrease in consumers liking for the juiciness, flavour, texture and overall acceptability of pork sausages with a salt content of 0.5% when compared to sausages containing 1.5% salt. However, our findings are in line with a study of Pietrasik and Gaudette (2015) who observed no significant difference in the appearance, colour, flavour, saltiness, texture, aftertaste or overall acceptability when the salt content of turkey sausages was reduced from 1.8 to 0.9%.

Conclusion

Our results suggest that it is possible to reduce the salt content of chicken sausages, by up to 60%, without having a significant effect on quality attributes, microbial growth or sensory attributes. Chicken sausages were also found to be an appropriate matrix for the addition of α -tocopherol at a level in excess of that at which an EFSA claim could be made as there is no significant loss in α -tocopherol content following cooking or during storage. Overall the study showed that it is possible to produce low salt chicken sausages fortified with enough α -tocopherol to meet the EFSA nutrient and health claims, without adverse effects on the quality or shelf life of the sausages.

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