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ORIGINAL ARTICLE

Effect of conjugated linoleic acid enrichment on the quality characteristics of Turkish dry fermented sausage

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Abstract The effects of conjugated linoleic acid (CLA) enrichment on lipid oxidation, the fatty acid profile, physicochemical, sensory and microbiological features of sucuk were investigated. The control sucuk did not contain CLA, however, other groups contained 0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, and 3 % CLA, respectively. The results indicated that CLA concentration was increased in sucuk with CLA addition ($p<0.05$). CLA concentration decreased ($p<0.05$) during the fermentation period, but did not alter during the storage. Lipid oxidation gradually increased with the increasing of storage in all treatments ($p<0.05$). However, addition of CLA decreased lipid oxidation compared to control ($p<0.05$). With regard to color, higher L* values were observed in 2 % CLA treatment group compared to other treatments during storage ($p<0.05$). Moreover, CLA addition resulted in increased a* values in sucuk ($p<0.05$). With the addition of CLA, saturated fatty acids in sucuk decreased and poly-unsaturated fatty acids and conjugated linoleic acids increased ($p<0.05$).

Keywords Conjugated linoleic acid (CLA) · Sucuk · Oxidation · Fatty acid profile

Introduction

Conjugated linoleic acid (CLA) is a mixture of linoleic acids that are geometric and positional isomers of linoleic acid with a conjugated double bond system. CLA recently has gained attention because of its possible human health benefits. Anticarcinogenic, anti-oxidant, anti-atherosclerotic and improvements of immune systems effects have been reported for CLA (Pariza et al. 2001).

Animal source foods, especially red meat and meat products, which play an important role as one of the meat essential protein source of human diet, have a negative image because of its saturated fatty acid (SFA) content, especially SFA in fat which have been implicated in diseases associated with modern life such as coronary hearth diseases. However, mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) reduce the level of plasma low-density lipoproteins-cholesterol and nutritionists are recommending a higher intake of PUFA (Matson and Grundy 1985). The estimated daily human CLA intake ranges from 200 to 1,000 mg per day. However, these concentrations are probably not high enough to exert the potential health effects of CLA (Schmid et al. 2006). Ip et al. (1995) estimated that a 70 kg human should consume 3.0 g CLA/day to obtain beneficial effects from CLA. The CLA content in meat products varies from 1.2 to 12.5 mg/g fat (Schmid et al. 2006). Therefore, there is an increasing demand for meat and meat products higher levels of PUFA, as well as enriched in fatty acids with potentially positive health effects such as omega-3 fatty acids or CLA. Thus, there has been an increased interest in recent years in ways to manipulate the fatty acid composition of meat and meat products.

Even though animal source foods such as beef and dairy products naturally have CLA, the concentration of CLA in beef is low (Schmid et al. 2006). Therefore, studies about enhancing CLA level in the meat and meat products by animal dietary supplementation or direct addition have increased to improve human health, productivity of carcass and meat quality (Martin et al. 2008). Supplementation of animal diets with CLA has been proven as a successful strategy for CLA

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enrichment in meat; however, there is limited study about the direct addition of CLA into meat products (Hah et al. 2006; Hur et al. 2004). Hur et al., (2004) reported that the CLA concentration significantly increased ($p<0.05$) by substituting CLA sources for fat and it improved the color stability possibly by inhibition of lipid oxidation. Because of limited information about the direct addition of CLA, more research is needed to determine the effects of substituted CLA lipid oxidation, fatty acid composition, quality and sensory characteristics of meat and meat products.

One of the most important and widely consumed traditional Turkish meat products is sucuk, a dry, uncooked, cured, fermented sausage, produced from beef or water buffalo meat. It consists of ground meat and sheep tail fat, and curing ingredient (nitrite or nitrate), with various spices including cumin, garlic, salt, and black and red pepper. This mixture is stuffed into a natural sausage casing (mostly cattle small intestines), hung for fermentation (ripening period) at 22–23 °C by either microorganisms naturally present or added starter cultures and allowed to dry for several weeks at ambient temperature and humidity (Kilic 2009). In regard to healthy food concept, it is important to develop an approach that can be implemented into traditional sucuk processing and provides enhanced health benefits for the consumers. Therefore, the aim of the present study was to investigate the effects of CLA on lipid oxidation, fatty acid composition, water activity, color, textural, chemical, microbiological and sensory properties of sucuk enriched with CLA through direct addition.

Materials and methods

Sucuk preparation

A 24 h post-mortem boneless beef cuts (*M. Longissimus dorsi*) and beef fat was purchased from Gülköy Meat Plants (Isparta, Turkey) for each of 3 replications on separate production days. The powder CLA was obtained from commercial company (Met food inc., Denizli, Turkey), consisted of 73 % (w/w) of the two main CLA isomers (trans-9, cis-11 and cis-10, trans-12). Other ingredients were obtained from local markets in Isparta. The production of sucuk (approximately 500 g each) was carried out in our laboratories according to the traditional method (Bozkurt and Erkmen 2004). The sucuks contained 70 % lean meat and 22 % fat. Other ingredients were added as follows: 2.5 % NaCl, 1.5 % garlic, 0.5 % sucrose, 1.5 % red pepper, 0.5 % black pepper, 0.8 % cumin, 0.5 % allspice and 150 ppm $NaNO_2$. During the mixing, the starter culture mixture (*Lactobacillus curvatus*, *Staphylococcus xylosus*, *Staphylococcus carnosus and Pediococcuspentosaceus*) was added at a dose of 10^8 cfu/kg of sucuk dough. Seven batches were prepared from ground beef. The control group was prepared without CLA. Other treatments were prepared with: 0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, and 3 % CLA respectively. Sucuks were ripened at 95–70 % relative humidity (RH) at 25–18ºC during 7 days; 24 h at 95 % RH at 24ºC; 24 h at 90 % RH at 22ºC; 12 h at 85 % RH at 20ºC; 12 h at 80 % RH at 20ºC; 48 h at 75 % RH at 18ºC; and 48 h at 70 % RH at 18ºC. Sucuks were vacuum-packaged and then stored at 4ºC for 30 days.

Fatty acid analysis

Total lipid was extracted by the method of Bligh and Dyer (1959), and extracted lipid was stored at –80ºC until methylation was carried out as reported by others (He et al. 2009). Briefly, lipid samples were placed in methylation tubes and then, 2 ml of sodium methoxide (0.5 mol/l in methanol) was added, vortexed for 1 min and placed in a 50ºC oven for 10 min. Then, 1 ml boron triflouride (14 % in methanol) was added and vortexed for 1 min. This was placed in a 50ºC oven for a further 10 min. After removal from the oven, 5 ml water was added and vortexed, followed by 2 ml hexane and vortexed. After allowing the layers to separate, an aliquot of the upper hexane layer was removed and placed in a 2 ml GC vial, capped and stored at −80ºC.

Fatty acids methyl esters were measured with a Agilent 7820A gas chromatograph (Agilent Technologies, USA) equipped with a flame ionization detector. Separation of the fatty acid methyl esters was carried out on a silica capillary column CP-Sil88 (100 m×0.25 mm i.d.) (Chrompack Inc., Middleburg, Netherlands). Oven temperature was maintained at 140ºC for 5 min, then increased at 5ºC/min to 225ºC. Injector and detector temperatures were respectively 250ºC and 260ºC. Hydrogen was the carrier gas with a split ratio of 50:1 and a 20 ml/min column flow. Fatty acids were identified by comparing retention times with fatty acid methyl ester standards (Sigma-Aldrich Inc., Oakville, ON) and are reported as % fatty acids. Fatty acid profile of sucuks was determined at manufacturing day, at the end of fermentation and storage.

TBARS analysis

Evaluation of oxidative stability was performed by measuring the formation of thiobarbituric acid reactive substances (TBARS). TBARS values of sucuk samples were determined as described by Kilic and Richards (2003) at manufacturing day, at the end of fermentation and during 5, 10, 15 and 30 days of storage.

Chemical composition

pH measurements were carried out using Orion Model 420 digital pH meter (Orion, Boston, USA). The pH was determined after mixing a 10 g sample with 90 ml distilled water

and equilibrating for 10 min. Fat, ash, protein and moisture content of sucuks were determined at manufacturing day, at the end of fermentation and during 15 and 30 days of storage (Anonymous 1995, 2000).

Water activity analysis

Water activity measurements were carried out using a Thermoconstanter Novasina TH200 water activity meter (Axair Ltd., Switzerland). The meter was calibrated using the manufacturer's standards.

Color measurement

Color measurement was taken with a Hunterlab model Precise Color Reader TCR 200 (BAMR Ltd, Claremont, South Africa) colorimeter. Three readings were taken and averaged for each of the three replications. L*, a*, b* values were determined at manufacturing day, at the end of fermentation and during 15 and 30 days of storage.

Microbiological analysis

Sucuk samples (10 g) were aseptically weighed, added to sterile buffered peptone water (90 ml) and homogenized in a stomacher at room temperature. Decimal dilutions in buffered peptone water were prepared and duplicate 0.1 ml samples of appropriate dilutions were spread on the following media: Plate Count Agar (PCA; Merck, Darmstadt, Germany) for total viable aerobic count (TVAC), incubated at 30 °C for 48 h; Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany) for yeast and moulds, incubated at 25 °C for 72 h; Eosin Metilen Blue Agar (EMB; Merck, Darmstadt, Germany) for coliforms, incubated at 37 °C for 48 h.

Texture profile analysis

For texture profile analysis (TPA), sucuk samples were cut into cylinders with height 10±0.5 mm, wrapped with plastic, and held for equilibration to room temperature (20ºC). TPA tests were performed using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, UK) to determine hardness (N), adhesiveness (Ns), springiness, cohesiveness, gumminess (N), chewiness, and resilience. Test conditions were: aluminum rectangular probe (5 cm·4 cm); test speed 5 mm/s; pre-test speed 2 mm/s, post-test speed 2 mm/s; compression 70 % and 50 kg load cell.

Sensory evaluation

The degree of difference and descriptive sensory analysis were performed at the Department of Food Sciences at the Suleyman Demirel University by a group of twenty, non-smoker panelists (8 males and 12 females, between 23 and 45 years old) experienced in the sensory evaluation of foods, using procedures described in the IFT Guideline (1981). Each panelist was seated in individual booth with white illumination and water was provided for rinsing the mouth between samples. Sucuk sample from each treatment was randomly chosen, presented in dishes coded with random three-digit numbers, reheated 40 s at microwave oven and served to the panelists. The panelists evaluated the appearance attributes (integrity, color and color intensity), juiciness, ease of fracture, firmness, greasiness, flavor, off-flavor, meat flavor intensity, off-odor and the overall acceptability of the sucuks. Sucuk attribute intensities were rated on 9 point scale.

Statistical analysis

The entire experiments were replicated three times. Data collected for chemical composition, physicochemical properties and sensory attributes were analyzed by the statistical analysis system. The statistical evaluation of the results was performed using the SPSS 18.0.0 (SPSS Inc., Chicago, USA). The generated data were analyzed by analysis of variance (ANOVA). Differences among mean values were established using the Duncan test and were considered significant when $p<0.05$. Differences among mean values obtained from sensory observations were established using the Kruskal-Wallis test which is non-parametric tests. In this test, Bonferroni-Dunn method was used to determine differences between group means.

Results and discussion

Chemical composition

The average fat, ash, moisture, water activity and protein content of sucuk samples before and after fermentation had shown non-significant differences among treatment groups (Table 1). While the initial moisture and water activity of sucuk dough decreased, fat, protein and ash content increased during 7 days of fermentation period in all treatment groups due to drying ($p<0.05$). Similar changes in chemical composition of traditional sucuk during fermentation were reported previously (Bozkurt and Erkmen 2004).

Chemical composition of sucuks produced in this study was in accordance with sucuks reported by previous studies (Ercoşkun 2006) and Turkish standards for sucuk (TS 1070 2002). In general, using CLA in formulation of sucuk had no significant effect on the chemical composition of sucuks ($p>0.05$).It is not possible to compare our results with previous studies since most of the previous studies about CLA

Table 1 Effect of CLA addition on the chemical composition of sucuks

Treatments	Fat %		Ash %		Moisture %		Protein %		Water activity	
	Man. day	End ferm. per	Man. day	End ferm. per	Man. day	End ferm. per	Man. day	End ferm. per	Man. day	End ferm. per
Control	23.73 ± 1.34^{aB}	30.43 ± 1.84^{aA}	2.89 ± 0.27^{aAB}	3.19 ± 0.27^{aA}	58.12 ± 0.23^{aA}	37.62 ± 0.56^{aB}	16.31 ± 1.03^{aB}	21.63 ± 1.20^{aA}	0.979 ± 0.003^{aA}	0.910 ± 0.001^{aB}
CLA 0.5 %	24.56 ± 1.01^{aB}	32.47 ± 2.16^{aA}	2.76 ± 0.22^{aAB}	3.19 ± 0.18^{aA}	59.26 ± 0.16^{aA}	35.54 ± 1.67^{abB}	17.16 ± 1.27^{aB}	22.24 ± 1.86^{aA}	0.978 ± 0.002^{aA}	0.902 ± 0.003^{bB}
CLA 1 %	23.32 ± 1.67^{aB}	32.65 ± 1.88^{aA}	2.69 ± 0.36^{aAB}	3.01 ± 0.25^{aA}	60.44 ± 0.37^{aA}	35.94 ± 1.77^{abB}	16.25 ± 1.64^{aB}	20.17 ± 2.08^{aA}	0.978 ± 0.004^{aA}	0.911 ± 0.004^{aB}
CLA 1.5 %	23.09 ± 2.04^{aB}	30.89 ± 2.34^{aA}	2.80 ± 0.34^{aAB}	3.01 ± 0.34^{aA}	58.86 ± 0.62^{aA}	36.12 ± 2.23^{abB}	17.38 ± 0.95^{aB}	21.28 ± 1.71^{aA}	0.976 ± 0.001^{aA}	0.896 ± 0.005^{bcB}
CLA 2 %	24.11 ± 1.98^{aB}	32.78 ± 1.77^{aA}	2.82 ± 0.44^{aAB}	3.00 ± 0.13^{aA}	60.10 ± 0.46^{aA}	36.24 ± 0.79^{abB}	16.47 ± 1.12^{aB}	22.03 ± 1.54^{aA}	0.978 ± 0.001^{aA}	0.895 ± 0.002^{cB}
CLA 2.5 %	23.87 ± 0.88^{aB}	31.13 ± 1.43^{aA}	2.71 ± 0.27^{aAB}	3.14 ± 0.31^{aA}	57.73 ± 0.39^{aA}	34.03 ± 0.79^{bB}	16.68 ± 1.23^{aB}	21.78 ± 1.36^{aA}	0.980 ± 0.002^{aA}	0.911 ± 0.002^{aB}
CLA 3 %	23.65 ± 1.60^{aB}	31.93 ± 2.29^{aA}	2.64 ± 0.47^{aAB}	3.09 ± 0.19^{aA}	59.27 ± 0.26^{aA}	36.39 ± 0.83^{aB}	16.74 ± 1.30^{aB}	22.83 ± 2.14^{aA}	0.977 ± 0.001^{aA}	0.907 ± 0.002^{abB}

a, b, c, d (↓) Different letters within a column are significantly different ($p<0.05$)

A,B,C,D (→)Different letters within a row are significantly different ($p<0.05$)

addition have examined the chemical composition of cooked products.

pH

pH plays a major role on meat quality the meat and meat products because of the pH value through the impact on meat proteins is the main determinant of product quality, influencing on water holding capacity, color, tenderness, taste and durability (Wasilewski et al. 2009). pH of the sucuks decreased during fermentation period ($p<0.05$). pH of sucuks before and after fermentation ranged from 5.61 to 5.69 and from 4.49 to 4.61 respectively (Fig. 1). This reduction in pH was found to be statistically significant and the lowest pH among all treatments was determined in sucuks containing 1.5 % CLA ($p<0.05$). These results were in agreement with literature that pH values of sucuk decreased sharply during the ripening period due to the production of organic acids (Bozkurt and Erkmen 2004). Organic acids, mainly lactic acid, are formed in fermented sausages as a result of carbohydrate breakdown during fermentation giving rise to the reduction in pH.

Results show that there was no significant pH difference among treatment groups which means that the addition of CLA had no observable effect on pH in sucuk. Similarly, Baublits et al. (2007) reported that there were no differences in beef pH among the CLA treatments.

Fatty acid composition

The fatty acid profiles of CLA source used in this study contained 41.58 % cis-9,trans-11 and 32.90 % trans-10,cis-12 isomers. Additionally, tridecanoic acid (0.82 %), palmitic acid (8.41 %), oleic acid (0.32 %) and linoleic acid (0.43 %) were the minor components found in this CLA source.

As shown in Table 2, CLA level in sucuk gradually increased with increasing the amount of added CLA. Sucuk groups enriched with CLA showed significantly higher CLA content compared to the control ($P<0.05$). Control had a low concentration of cis-9,trans-11 CLA (0.98 %) and trans-10,cis-12 CLA (0.07 %) as expected. However CLA level in sucuk enriched with CLA ranged from 1.29 to 4.82 % of total fatty acids. Sucuks containing 3 % added CLA had concentrations of both isomers of 4.82 %, which were similar to concentrations reported by Chae et al. (2004) in 4 % CLA added beef patties. The total amount of CLA in sucuk increased approximately 400 % by addition of 3 % CLA. The cis-9,trans-11 and trans-10,cis-12 CLA isomers increased from 0.98 to 3.56 % and from 0.07 to 1.25 %, respectively, in sucuks containing 3 % added CLA ($P<0.05$; Table 2). Baublits et al. (2007) reported that amount of cis-9,trans-11 and trans-10,cis-12 isomer of CLA in steak similarly could be increased by injection of powder and oil form of CLA. The

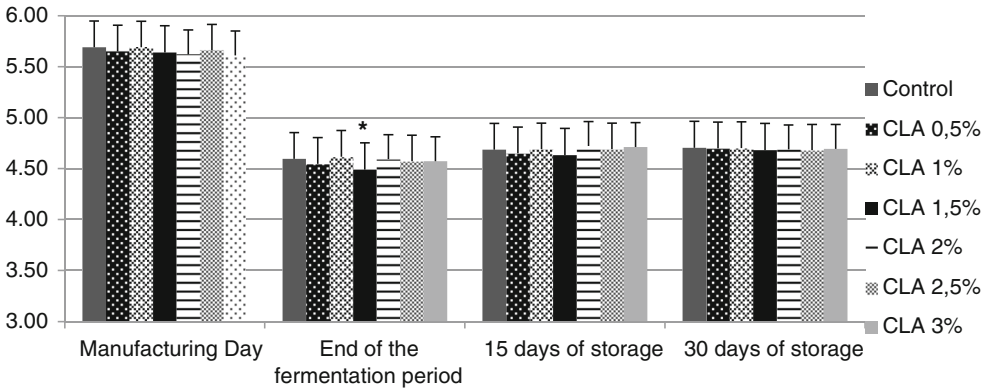

Fig. 1 Effect of CLA addition on pH value during the ripening and storage periods

authors stated that the increase in cis-9,trans-11 and trans-10,cis-12 isomer of CLA in beef strip loin ranged from 1,600 to 2,500 % and from 16,000 to 23,000 % respectively. Similarly, CLA has been added to ground beef and emulsion type sausage formulations and CLA levels were successfully heightened in the final product (Chae et al. 2004; Hur et al. 2004; Hah et al. 2006). Moreover, no significant changes were observed in the content of cis-9, trans-11 and trans-10, cis-12 CLA isomers of the samples during the storage period in those studies. Hah et al. (2006) and Martin et al. (2008) also detected no change in the CLA concentrations with the storage. From a nutritional point of view, since treatment groups in this study

Table 2 Fatty acid composition of sucuks

Fatty acid		Control	CLA 0.5 %	CLA 1 %	CLA 1.5 %	CLA 2 %	CLA 2.5 %	CLA 3 %
C14:0		1.19a	1.17b	1.11c	1.10c	1.07c	1.04d	1.04d
C14:1		0.15a	0.15a	0.15a	0.15a	0.15a	0.15a	0.15a
C15:0		0.22a	0.21a	0.20a	0.21a	0.20a	0.19a	0.19a
C16:0		21.98a	21.56b	21.04c	21.01d	20.79e	20.68f	20.44 g
C16:1		2.56a	2.54a	2.50b	2.58a	2.50b	2.50b	2.49b
C17:0		0.81a	0.81a	0.81a	0.81a	0.80a	0.80a	0.81a
C17:1		0.17a	0.17a	0.17a	0.17a	0.17a	0.17a	0.17a
C18:0		26.47a	26.29b	26.08c	25.96d	25.79e	25.36f	25.33 g
C18:1		38.36a	38.35a	38.06b	37.94b	37.76c	37.62d	37.54d
C18:2		1.54a	1.54a	1.53a	1.54a	1.52a	1.50a	1.50a
C18:3		0.39a	0.38a	0.38a	0.38a	0.39a	0.39a	0.38a
C20:1		2.23a	2.22a	2.22a	2.22a	2.21a	2.22a	2.22a
C20:2		2.52a	2.51a	2.50a	2.50a	2.50a	2.49a	2.49a
C22:0		0.19a	0.18a	0.18a	0.18a	0.17a	0.18a	0.18a
∑SFA		51.05	50.43	49.64	49.48	49.04	48.49	48.21
∑UFA		48.87	49.08	49.86	49.85	50.43	51.50	51.61
∑MUFA		43.47	43.44	43.11	43.06	42.80	42.65	42.58
∑PUFA		5.40	5.64	6.75	6.79	7.64	8.85	9.04
SFA/UFA		1.04	1.03	1.00	0.99	0.97	0.94	0.93
CLA c9.t11	Manufacturing day	0.980^{Ag}	1.060^{Af}	1.869^{Ae}	2.096^{Ad}	2.725^{Ac}	3.124^{Ab}	3.566^{Aa}
	End of the fermentation	0.923^{Bg}	0.979^{Bf}	1.856^{Be}	2.093^{Bd}	2.689^{Bc}	3.028^{Bb}	3.521^{Ba}
	30 days of storage	0.934^{Bg}	0.975^{Bf}	1.844^{Be}	2.078^{Bd}	2.671^{Bc}	3.005^{Bb}	3.519^{Ba}
CLA t10.c12	Manufacturing day	0.070^{Ag}	0.230^{Af}	0.556^{Ae}	0.423^{Ad}	0.674^{Ac}	1.161^{Ab}	1.253^{Aa}
	End of the fermentation	0.067^{ABg}	0.208^{Bf}	0.551^{Be}	0.394^{Bd}	0.659^{Bc}	1.122^{Bb}	1.217^{Ba}
	30 days of storage	0.063^{ABg}	0.193^{Bf}	0.548^{Be}	0.395^{Bd}	0.646^{Bc}	1.108^{Bb}	1.207^{Ba}

a,b,c,d,e(→) Different letters within a column are significantly different ($p<0.05$)

A,B,C,D (↓) Different letters within a row are significantly different ($p<0.05$)

contain higher CLA level compared to control, sucuks enriched with CLA can be considered to be healthier food source for consumer.

CLA isomers in sucuks significantly decreased during the fermentation period ($p<0.05$). This suggests that the CLA isomers were oxidized with extended ripening period. CLA also may be consumed in reactions in which it serves as an antioxidant. Yurawecz et al. (1995) reported that furan fatty acids, formed during the autooxidation of CLA, can protect against peroxide attack. However, a decrease in CLA level was non-significant during storage. Shantha et al. (1995) reported that CLA was a stable component compared with PUFA in beef patties. Hur and others (2004) also reported that storage did not affect the CLA concentration. Our result was confirmed with the above findings that the concentration of CLA isomers in sucuk did not change during storage.

As shown in Table 2, results of fatty acid composition analysis indicated that myristic acid (C14:0) content increased, palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2) contents decreased in sucuk samples with CLA enrichment ($P<0.05$). Furthermore, CLA addition decreased SFA and MUFA levels in sucuk as well as increasing CLA levels in the product. PUFA levels of the sucuks with the added CLA were higher than control sucuks ($p<0.05$). These results are in agreement with Du et al. (2000), who found that CLA supplemented diet resulted in similar increasing trend in fatty acid composition of chicken meat. Hur et al. (2004) reported that a significant increase in the amount of unsaturated fatty acid for meatball produced with added CLA. In another study, it has reported that liver patties produced CLA showed a three times higher PUFA and significantly lower level MUFA and SFA content compared to control and liver patties produced with olive oil. Similarly, CLA injection into beef loin strip was reported to increase the amount of CLA and PUFA of samples (Baublits et al. 2007). Moreover Juàrez et al. (2009) reported that CLA supplementation was able to get high CLA levels in sausages. There was no significant change in fatty acid profile during storage in all treatment groups (data is not presented). Our results showed that these results are in accordance with those found by Hah et al. (2006) and Martin et al. (2008) for emulsion-type sausages and liver patties with added CLA.

From a nutritional point of view, a result of fatty acid composition analysis indicated that increased PUFA and CLA levels can be accomplished in sucuk with CLA addition. Therefore, sucuk enriched with CLA may serve healthy food source for human diet.

TBARS

The oxidative stability of sucuks was measured throughout fermentation and the storage and TBARS values are presented in Table 3. There were no differences in TBARS among treatment groups at the manufacturing day (Table 3). The TBARS values of the sucuk samples increased during fermentation and storage period. However, TBARS values were significantly lowered by CLA treatments at end of 30 days storage period ($p<0.05$). Sucuk samples with 2 % CLA had lower TBARS than that of other treatments on 1, 5, 10 and 30 days of storage ($p<0.05$). TBARS values were reduced by 13.3 %, 16.3 %, and 12.8 % for 0, 5, and 30 days of storage in sucuks produced with 2 % CLA, respectively. Similarly, Baublits et al. (2007), Hur et al. (2004) and Chae et al. (2004) reported lower TBARS for ground beef patties and beef with CLA than control. In another study a decrease in TBARS of emulsion type sausage as substituted CLA source was also reported (Hah et al. 2006).

Joo et al. (2002) also reported that dietary CLA reduced TBARS levels and lipid oxidation of meat. Yu (2001) reported that CLA has free radical-scavenging properties, which would protect against lipid oxidation. Shantha et al. (1995) postulated that high relative levels of CLA could reduce the formation of fatty acid free radicals and subsequent oxidation reactions. They also suggested that effective antioxidant activity of CLA can be accomplished because of the lack of changes in CLA content during storage. A 2.5 % and 3 % CLA treatment groups in our study showed lower antioxidant effect than 2 % CLA ($p<0.05$). This result may be explained with possible prooxidant effect of cis-9, trans-11 CLA isomer at these concentrations. Leung and Liu (2000) demonstrated that the trans-10, cis-12 CLA isomer possessed antioxidant properties over a wide range of concentrations, whereas the cis-9, trans-11 CLA isomer was actually a pro-oxidant at high concentrations. They indicated that at lower concentrations (2 and 20 μM) cis-9, trans-11 CLA isomer possesses weak antioxidant activity, whereas at a concentration of 200 μM it acts as a strong pro-oxidant. Researchers suggested that a mixed effect of the antioxidant properties of trans-10, cis-12 CLA isomer and the pro-oxidant properties of cis-9, trans-11 CLA isomer may occur when a mixture of CLA isomers is used. Similarly, Chae et al. (2004) have stated that there was insufficient benefit from 4 % CLA over 2 % CLA in reducing lipid oxidation to justify the higher level unless the primary focus is to increase the consumption of CLA isomers. Therefore, a lower antioxidant effect of CLA in 2.5 % and 3 % CLA treatment groups compared to 2 % CLA group in our study may be explained with dominated pro-oxidant effect of cis-9, trans-11 CLA isomer over antioxidant effect of trans-10, cis-12 CLA isomer.

The oxidative stability of CLA is another important concern. It has been reported that CLA was oxidized rapidly, because a conjugated double bond was vulnerable to auto-oxidation (Chen et al. 1997; Van den Berg et al. 1995; Zhang et al. 1994). Therefore, CLA must be protected from oxidation when it is to be used in food systems as additive. In an effort to maintain oxidative stability, microencapsulation of CLA with cyclodextrins has been suggested (Park et al. 2002; Kim et al. 2000).

Table 3 TBARS values of sucuk with CLA addition (μmol/kg)

Treatments	Manufacturing day	End of the fermentation period	Storage (days)			
			5	10	15	30
Control	2.28 ± 0.15^{aE}	3.15 ± 0.11^{aD}	3.60 ± 0.19^{aC}	3.70 ± 0.21^{aC}	3.94 ± 0.18^{aB}	6.09 ± 0.19^{aA}
CLA 0.5 %	2.27 ± 0.09^{aE}	3.08 ± 0.15^{aD}	3.46 ± 0.16^{abC}	3.70 ± 0.32^{aBC}	3.87 ± 0.07^{aB}	5.81 ± 0.20^{bA}
CLA 1 %	2.23 ± 0.06^{aE}	2.96 ± 0.12^{aD}	3.59 ± 0.15^{aC}	3.64 ± 0.25^{aC}	3.87 ± 0.11^{aB}	5.51 ± 0.10^{cdA}
CLA 1.5 %	2.26 ± 0.12^{aE}	3.10 ± 0.18^{aD}	3.47 ± 0.19^{abC}	3.57 ± 0.11^{aC}	3.85 ± 0.06^{aB}	5.65 ± 0.11^{bcA}
CLA 2 %	2.27 ± 0.07^{aF}	2.73 ± 0.10^{bE}	3.01 ± 0.13^{cD}	3.19 ± 0.13^{bC}	3.62 ± 0.07^{abB}	5.31 ± 0.13^{dA}
CLA 2.5 %	2.26 ± 0.09^{aE}	2.97 ± 0.11^{aD}	3.28 ± 0.12^{bC}	3.23 ± 0.16^{bC}	3.76 ± 0.20^{abB}	5.77 ± 0.15^{bA}
CLA 3 %	2.21 ± 0.12^{aF}	3.09 ± 0.07^{aE}	3.27 ± 0.13^{bD}	3.63 ± 0.18^{aC}	3.80 ± 0.09^{aB}	5.84 ± 0.14^{bA}

a, b, c, d (↓) Different letters within a column are significantly different ($p<0,05$)

A, B, C, D, E, F(→) Different letters within a row are significantly different ($p<0.05$)

Color analysis

Addition of CLA influenced L* and a* values (Table 4) in sucuk ($p<0.05$). It was determined that the control group has the lowest L* value at the end of the ripening period. Moreover, higher L* and a* values were observed in sucuk with 2 % CLA compared to other groups during storage. Especially, L* and a* values of sucuk with 2 % CLA were the higher than others at the beginning and at the end of storage. Du et al. (2000) suggested that dietary CLA treatment reduced oxidation and improved color stability during storage. Similarly, Hur et al. (2004) reported that substituted CLA for fat significantly influenced L* and a* values in beef patties. The authors observed higher L* and a* values in patties produced with 2 % CLA at the 7 days of storage compared to control. They also reported that CLA improved the oxymyoglobin stability due to its antioxidant effect. Previous study showed that CLA treatments in beef strip loin resulted in higher L*

Table 4 L*, a* and b values of sucuk with CLA addition

	Treatments	Manufacturing day	End of the fermentation period	15 days of storage	30 days of storage
L*	Control	51.31 ± 2.51^{aA}	47.12 ± 1.04^{cB}	45.98 ± 1.43^{dB}	40.69 ± 1.66^{cC}
	CLA 0,5 %	53.32 ± 2.67^{aA}	49.63 ± 1.07^{bB}	49.62 ± 1.32^{cB}	41.65 ± 1.53^{cC}
	CLA 1 %	52.44 ± 0.91^{aA}	49.50 ± 1.18^{bB}	52.57 ± 2.20^{abA}	46.24 ± 4.28^{bC}
	CLA 1.5 %	51.62 ± 2.32^{aA}	50.12 ± 1.05^{bAB}	50.08 ± 2.63^{bcAB}	46.76 ± 1.05^{bC}
	CLA 2 %	53.60 ± 2.69^{aA}	52.18 ± 1.03^{aAB}	54.29 ± 1.63^{aA}	50.36 ± 1.16^{aC}
	CLA 2.5 %	53.63 ± 2.47^{aA}	50.32 ± 1.83^{bAB}	50.24 ± 1.65^{bcAB}	47.21 ± 1.08^{bC}
	CLA 3 %	52.35 ± 1.73^{aA}	50.27 ± 0.98^{bAB}	49.67 ± 0.89^{cB}	45.91 ± 2.03^{bC}
a*	Control	10.28 ± 0.25^{dAB}	10.45 ± 0.16^{cA}	9.61 ± 0.43^{dB}	7.88 ± 0.22^{cC}
	CLA 0,5 %	10.38 ± 0.50^{dAB}	10.80 ± 0.15^{bA}	9.81 ± 0.15^{cdB}	8.92 ± 0.57^{bC}
	CLA 1 %	11.50 ± 0.75^{bA}	11.56 ± 0.69^{abA}	11.13 ± 0.69^{aA}	8.97 ± 0.54^{bBC}
	CLA 1.5 %	$12,36\pm0.62^{abA}$	12.64 ± 0.45^{aA}	10.83 ± 0.45^{abB}	8.67 ± 0.21^{bC}
	CLA 2 %	12.86 ± 0.57^{aA}	12.31 ± 0.34^{aA}	11.47 ± 0.36^{aB}	9.41 ± 0.60^{abC}
	CLA 2.5 %	10.58 ± 0.22^{cdB}	11.36 ± 0.41^{abA}	10.36 ± 0.41^{bcB}	8.73 ± 0.23^{bC}
	CLA 3 %	11.30 ± 0.63^{bcA}	11.13 ± 0.27^{abA}	10.03 ± 0.27^{cB}	9.27 ± 0.15^{bC}
b*	Control	5.69 ± 0.48^{abcA}	5.33 ± 0.63^{abAB}	4.74 ± 1.11^{aB}	4.57 ± 0.47^{abB}
	CLA 0,5 %	5.98 ± 0.20^{aAB}	5.64 ± 0.32^{aAB}	6.14 ± 0.64^{aA}	5.07 ± 1.18^{aB}
	CLA 1 %	5.10 ± 0.82^{bcAB}	5.18 ± 0.47^{bcAB}	5.55 ± 0.99^{aA}	3.19 ± 0.23^{cC}
	CLA 1.5 %	5.82 ± 0.37^{abA}	5.76 ± 0.51^{aA}	5.87 ± 0.67^{aA}	3.67 ± 0.61^{bcB}
	CLA 2 %	5.36 ± 0.58^{abcAB}	5.39 ± 0.48^{abAB}	5.49 ± 1.04^{aA}	3.80 ± 0.28^{bcC}
	CLA 2.5 %	4.86 ± 0.24^{cA}	4.75 ± 0.27^{cAB}	4.68 ± 0.94^{aB}	3.24 ± 0.34^{cC}
	CLA 3 %	6.02 ± 0.65^{aA}	5.87 ± 0.52^{aAB}	4.86 ± 1.12^{aAB}	3.56 ± 0.42^{cC}

a, b, c, d (↓) Different letters within a column are significantly different ($p<0.05$)

A, B, C, D (→) Different letters within a row are significantly different ($p<0.05$)

values than control group (Baublits et al. 2007). In addition, Hah et al. (2006) reported similar results in emulsion type sausage.

It is well demonstrated that meat discoloration is related to the rate of myoglobin oxidation formed by lipid oxidation (Yin and Faustman 1993). Therefore, higher L* and a* values of sucuk produced with CLA compared to control group in our study may be explained with possible antioxidant effects of CLA on lipid oxidation. Thus, the rate of myoglobin oxidation formed by lipid oxidation might be reduced. Contrary to our results Hah et al. (2006) reported that addition of CLA showed significantly higher b* values than the control group. However, we did not determine significant differences for b* values among sucuk treatment groups.

Texture profile

Textural characteristics of all treatments have similarly evolved due to the coagulation of protein at low pH and decreasing moisture content due to drying of sucuks. Bozkurt

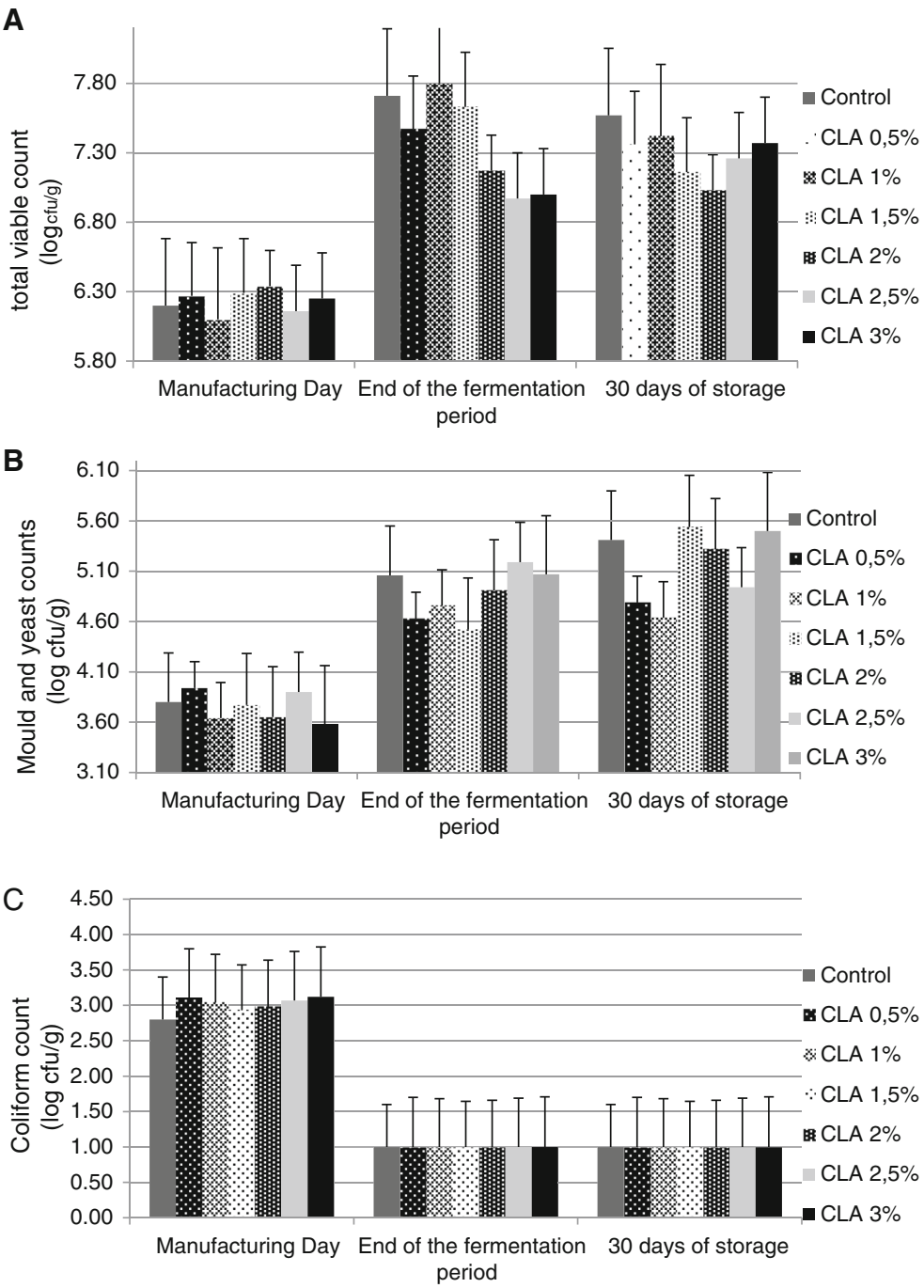

Fig. 2 Effect of CLA level on microbial count in sucuk during the ripening and storage periods

and Bayram (2005) indicated that textural parameters such as hardness, gumminess and chewiness closely related to ripening conditions. In general, statistical analysis results showed that there was no significant effect of CLA enrichment on textural properties of sucuks (data is is not presented). However, increasing the amount of CLA added into sucuk dough resulted in an increase in cohesiveness values ($p<0.05$).

Microbiological analysis

The microbial changes during ripening and storage are shown in Fig. 2. The initial bacterial counts of sucuks were between 6.1 and 6.3 log cfu/g for total viable, 3.6 and 3.9 log cfu/g for mould and yeast and 2.8 and 3.1 log cfu/g for coliform bacteria, respectively. Total viable counts increased during fermentation period. However, total viable counts showed decreasing trend during the storage period due to drying, the low ambient temperature and the decrease in pH. Early studies about sucuk indicated similar trends for total viable counts during fermentation and storage periods (Bozkurt and Erkmen 2004).

Yeast and mold counts in all sucuk groups increased during fermentation and storage periods. On the other hand, coliform bacteria counts decreased due to the effect of nitrite and the fall of pH. Coliform bacteria counts determined in this study were under the limits required by Turkish standards (TS 1070 2002) for all groups in the final product. The addition of CLA did also not affect yeast, mold and coliform counts during fermentation and storage.

The results of this study showed that the addition of CLA did not have any effect on total viable, coliform bacteria and yeast and mould during fermentation and storage period.

Sensory taste panel

Sucuk samples were evaluated for integrity, color, color intensity, firmness, juiciness, greasiness, ease of fracture, flavor, off-flavor, meat flavor intensity, odor and the overall acceptability. Sensory evaluation of sucuk groups showed that addition of CLA had no effect on sensory properties compared to the control group. Concerning overall acceptability, there were no differences among groups and all treatment groups received high overall acceptability scores ranging 6–8 score from panelists.

Some research indicated that substituted and added oil into meat products can be affect sensorial properties of samples (Liu et al. 1991). Hah et al. (2006) reported that substituted CLA source for fat decreased overall acceptability than those of control in emulsion type sausage. However, CLA treatments in beef strip loin were rated tenderer than control group for myofibrillar, connective tissue and overall tenderness (Baublits et al. 2007).

Conclusion

CLA enrichment in sucuk manufacture was accomplished. Results have demonstrated that lipid oxidation was efficiently inhibited in sucuk manufactured with 2 % CLA. It was assumed that lipid oxidation of sucuk might be affected by the levels of CLA addition. Furthermore, this study showed that addition of CLA into sucuk improves the color properties because of increased L* and a* values. It can be concluded that CLA addition in sucuk manufacture has nutritionally positive effects due to an increase in PUFA and CLA and decrease in SFA levels in final product. This research suggests that CLA could be used for the manufacture of sucuk for the inhibition of lipid oxidation and improvement of color and nutritional properties.

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