Effect of Substituted Conjugated Linoleic Acid for Fat on Meat Qualities, Lipid Oxidation and Residual Nitrite Content in Emulsion-type Sausage

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ABSTRACT : An experiment was carried out to evaluate the effects of substituted conjugated linoleic acid (CLA) for fat on qualities, lipid oxidation and residual nitrite contents in emulsion-type sausage (ETS) after different periods of storage. The control sausage contained 20% fat, however, the substituted CLA for fat sausages contained 17.5% fat+2.5% CLA, 15% fat+5% CLA, 12.5% fat+7.5% CLA and 10% fat+10% CLA, respectively. CLA concentration was significantly increased ($p<0.05$) by substitution of CLA sources for fat, and storage did not affect the CLA concentration. TBARS were significantly increased with the increasing of storage time in all treatments ($p<0.05$). Significantly higher L* values were observed in the 10% CLA treatment compared to other treatments during storage. Moreover, a* values in CLA treatments significantly ($p<0.05$) increased compared to the control. Again, b* values were significantly ($p<0.05$) higher in CLA treatments than those of the control during storage. The concentration of residual nitrite in ETS was significantly ($p<0.05$) different between the treatments during storage and the results were in the following order: control >2.5% CLA>5% CLA and 7.5% CLA>10% CLA. Overall acceptability of CLA treatments was significantly ($p<0.05$) lower than the control during storage, whereas no significant differences were found in overall acceptability between the treatments after 28 days of storage. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 744-750)***

Key Words : Conjugated Linoleic Acid (CLA), Emulsion-type Sausage, TBARS, Residual Nitrite, Meat Quality

INTRODUCTION

Current meat research is focusing on improving safety and quality food for human consumption. Functional foods contain dietary components that have beneficial properties beyond their traditional nutrient value (Corl et al., 2001). CLA is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds. CLA has been recognized as having anticarcinogenic and antioxidative properties in several animals' models (Ha et al., 1990). Also, CLA has been shown to have beneficial effects on human health (Ip et al., 1995). Du et al. (2000) reported that color stability in fresh chicken meat was improved by dietary CLA. Mullan et al. (2002) reported that under commercial conditions dietary CLA can improve growth performance and decrease backfat in pigs. Also, Hur et al. (2003) reported that lipid oxidation of egg yolk during cold storage could be inhibited by dietary CLA due not only to changes in fatty acid composition but also to the high concentration of CLA in egg yolk. Nicolosi et al. (1997) reported that animals fed the CLA containing diets collectively had significantly reduced levels of plasma total cholesterol, low density lipoprotein cholesterol, and triglycerides, but with no effect on high density lipoprotein cholesterol. Daily intake of CLA is not well documented but has been estimated to be under several hundred mg/g (Jones et al., 2000). Data from animal research indicates that 3.0 g/day of CLA may be necessary for beneficial effects in human (Ip et al., 1994). Reevaluation of these data on the basis of total dietary energy consumption suggests that 600 mg CLA/day may have anticancer effects (Jones et al., 2000). CLA fortification could contribute to diet-based cancer prevention in human populations (Jiang et al., 1993). Additive CLA to meats could be easy method to obtain foods containing CLA for human consumption.

Nitrites are important antimicrobial agents against *Clostridium botulism*, as well as food preservatives, flavoring and coloring agents in meat products. A high dietary intake of nitrite has been implicated as a risk factor for human cancer, and formation of nitroso-compounds in the stomach during inflammation is correlated with nitrate in food and water (Mirvish, 1995). However, the reported association between the risk of cancer and nitrite intake are inconsistent (Weyer et al., 2001). Experimental data suggest that antioxidants may be inhibited the formation of carcinogenic substances such as nitroso-compounds (Mirvish, 1995). Wagner et al. (1985) have shown that dietary ascorbic acid and α-tocopherol blocked synthesis of nitroso-compounds. Similarly, Chow and Hong (2002) reported that dietary antioxidants reduce the nitrite and nitrate toxicity and formation of nitric oxide. CLA also has been shown to antioxidative properties in several animals' models (Ha et al., 1990). Thus, CLA possibly can affect the residual nitrite contents. However, there is little information on the effect of CLA on residual nitrite contents and qualities of ETS. The purpose of this study was to determine the effect of substituted CLA sources for fat on

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Table 1. Composition (%) of emulsion-type sausage

Ingredient (g)	Treatments				
	0% CLA	2.5% CLA	5% CLA	7.5% CLA	10% CLA
Lean pork	60	60	60	60	60
Pork back fat	20	17.5	15	12.5	10
CLA	0	2.5	5	7.5	10
Cornstarch	5.6	5.6	5.6	5.6	5.6
Sausage seasoning (contained 0.4% nitrite)	3	3	3	3	3
Phosphate	0.2	0.2	0.2	0.2	0.2
NaCl	1.2	1.2	1.2	1.2	1.2
Water	10	10	10	10	10
Total	100	100	100	100	100

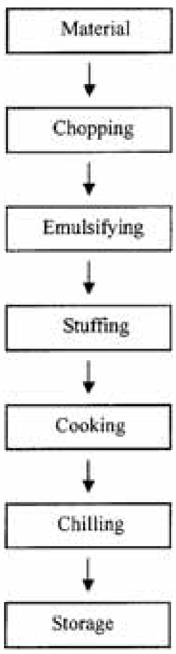

Figure 1. Flow diagram of sausage preparation.

fatty acid, lipid oxidation, residual nitrite, color and sensory evaluation in ETS.

MATERIALS AND METHODS

Sausage formulation and processing

Lean pork and backfat were purchased from local meat processing plant. Excess fat was trimmed from the meat and then ground in a grinder twice through a 7-mm plate. Ingredient composition of the sausages was presented in Table 1, while the flow diagram of sausage preparation was shown in Figure 1. The emulsified meat batters were stuffed into PVDC casings (30-mm diameter) and cooked in a cooking chamber (programmed at 65°C for 30 min, increased to 75°C and held for 30 min, and then increased to 80°C and held for 20 min). After cooling in ice water for 20 min, the emulsified cook meat was storage at 5°C.

Synthesis of CLA

The CLA used in this study was chemically synthesized from linoleic acid (99.9%) by alkaline isomerization method and purified by the low-temperature precipitation method of Ha et al. (1990). Purity of the CLA was 95%, consisting of four major CLA isomers (46% *cis*, 9-*trans*, 11, 45% *trans*, 10-*cis*, 12, 2% *trans*, 9-*trans*, 11, and 2% *trans*, 10-*trans*, 12 CLA). The purity and composition were confirmed by using the method of Kim et al. (2000).

Fatty acid analysis

Lipids were extracted with chloroform and methanol as described by Folch et al. (1957). The extracts were concentrated using an evaporator (Zymark turbovap 500, Hopkinton, MA, USA) at 40°C under nitrogen and stored at -40°C until required for analysis. For lipid hydrolysis, an aliquot of lipid extract (30 mg) and 3 ml of 4% H_2SO_4 in methanol were combined in a screw-capped test tube. The test tube was placed in boiling water (100°C) for 20 min and subsequently cooled at room temperature. The resulting free fatty acids were methylated with 1 ml of 14% boron trifluoride in methanol at room temperature for 30 min and then water (1 ml) and hexane (5 ml) were added. Samples were vortexed and centrifuged at 500×g for 10 min. The upper organic solvent layer was used to determine fatty acids composition. Fatty acid methyl esters were analyzed on a gas chromatography (Shimadzu GC-14A; Tokyo, Japan) equipped with an on column injector port and flame-ionization detector. A Silar capillary column (30 m×0.32 mm×0.25 µm; Shimadzu, Japan) was used for the separation of the fatty acid methyl esters. The gas chromatography oven temperature was 140°C, and increased at a rate of 2°C/min to a final temperature of 230°C. The temperatures of injector port and detector temperatures were set at 240°C and 250°C, respectively. Fatty acid methyl ester (1 ml) was injected onto the split injection port (100:1 split ratio). The flow rate for He carrier gas was 50 ml/min. Each fatty acid was detected by the standards' retention time.

Table 2. Changes in fatty acids composition of emulsion-type sausage with CLA during 28 days of storage at 4°C

Fatty acid (%)	Storage period (days)									
	0 day					28 day				
	Control	2.5% CLA	5% CLA	7.5% CLA	10% CLA	Control	2.5% CLA	5% CLA	7.5% CLA	10% CLA
Myristic acid (C14:0)	1.93^{A}	1.62^{B}	1.59^{B}	1.58^{B}	1.47^{B}	1.92^{A}	1.68^{B}	1.66^{B}	1.64^{B}	1.56^{B}
Palmitoleic acid (C16:0)	2.19	2.21	2.21	2.14	2.18	2.20	2.23	2.17	2.18	2.16
Palmitic acid (16:0)	25.14^{A}	24.82^{B}	24.17^{C}	23.78^{D}	23.61^{D}	25.15^{A}	24.76^{B}	24.24^{C}	24.21^{C}	23.59^{D}
Linoleic acid (18:2)	13.28	12.71	12.49	12.64	12.44	13.28^{A}	12.68^{B}	12.60^{B}	11.32^{D}	12.33^{C}
Oleic acid (18:1)	45.61^{A}	44.53^{B}	43.64^{C}	43.23^{D}	42.89^{E}	45.74^{A}	44.67^{B}	43.44^{C}	43.48^{C}	42.88^{D}
Linolenic acid (18:3)	1.17^{A}	0.95^{AB}	0.86^{AB}	0.69^{B}	0.56^{B}	1.15^{A}	1.05^{A}	0.94^{A}	0.92^{A}	0.69^{B}
Stearic acid (18:0)	10.11	9.75	10.12	9.64	9.65	10.13	9.71	10.08	10.13	9.57
CLA	-	3.10^{D}	4.64^{C}	6.02^{B}	6.97^{A}	-	2.91^{D}	4.57^{C}	5.83^{B}	6.97^{A}
Arachidonic acid (20:4)	0.57^{A}	0.31^{B}	0.27^{B}	0.27^{B}	0.24^{B}	0.45^{A}	0.31^{B}	0.29^{B}	0.28^{B}	0.26^{B}
SFA[1]	37.18	36.19	35.89	35.00	34.72	37.19	36.16	35.97	35.98	34.72
USFA[2]	62.82	63.81	64.11	65.00	65.28	62.81	63.84	64.03	64.02	65.28

[A, B, C] Different letters within a row are significantly different (p<0.05).
[1] SFA: Saturated fatty acid, [2] USFA: Unsaturated fatty acid.

TBARS analysis

Five grams of sausage sample was weighed into a 50 ml test tube and homogenized with 15 ml of deionized distilled water using the Polytron homogenizer (IKA Labortechnik T25-B, Selangor, Malaysia) for 10s at the highest speed. One ml of meat homogenate was transferred to a disposable test tube (3×100 mm), and butylated hydroxyanisole (50 µl, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA, 2 ml) were added. The mixture was vortexed and then incubated in a boiling water bath for 15 min to develop color. The sample was cooled in cold water for 10 min, vortexed again, and centrifuged for 15 min at 2,000×g. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 ml of double distilled water and 2 ml of TBA/TCA solution. The amounts of TBARS were expressed as milligrams of malondialdehyde per kilogram of meat.

Residual nitrite analysis

The residual nitrite was determined in triplicate on emulsion-type sausage according to the colorimetric method of AOAC, Method No.973.31 (AOAC, 1990).

Color analysis

The surface color (CIE L*, a*, b*) of emulsion-type sausage was measured using a Minolta Chromameter (Minolta CR 301, Tokyo, Japan). Three random reading were made from the surface of samples.

Sensory evaluation

Sensory evaluation (overall acceptability) was conducted using ten panelists who had experience on ETS. For evaluation of overall acceptability, samples in coded, sliced samples were presented to each panelist in isolated booths. Panelists were asked to evaluate the substituted CLA for fat on overall acceptability of ETS. Each point marked was converted to a numerical value from 1 (dislike) to 5 (like) according to location.

Statistical analysis

Data of the different parameters were mean values from three replications. Analysis of samples was conducted in triplicate. The design of the experiment was Completely Randomized Design. The effects of substituted CLA for fat on fatty acid, lipid oxidation, residual nitrite, color, and sensory evaluation in ETS were analyzed using one-way ANOVA with SAS (SAS, 1996) at 5% level of significance and Dancun's Multiple Range test was used to determine the statistical significance among means.

RESULTS AND DISCUSSION

Fatty acid analysis

As shown in Table 2, CLA concentration significantly increased by substitution of CLA sources for fat, and storage did not affect the CLA concentration. Shantha et al. (1995) reported that CLA was a stable component compared with polyunsaturated fatty acids. Chin et al. (1992) also reported that contents of CLA were not changed in cooked meat products during storage. Our result was confirmed with the above findings that the concentration of CLA in ETS did not change during storage.

TBARS analysis

The TBARS of ETS decreased as substituted CLA sources increased (Table 1). After 7 days of storage, TBARS were significantly lowered following CLA treatments (p<0.05), however, no significant difference was found between 0% and 2.5% CLA treatment. As a result of lipid oxidation, complex mixtures of aldehydes, ketones, hydrocarbons, esters, lactones, and alchohols can be produced and oxidative off-odor can be generated.

Table 3. Changes in TBARS of emulsion-type sausage with CLA during 28 days of storage at 4°C

Treatment	Storage (days)				
	0	7	14	21	28
	mg MA[1]/kg meat				
Control	0.41±0.06^{c}	0.44±0.02^{Abc}	0.44±0.03^{ABbc}	0.47±0.02^{Ab}	0.53±0.04^{Aa}
2.5% CLA	0.41±0.08^{b}	0.41±0.04^{ABb}	0.46±0.01^{Aab}	0.47±0.02^{Aab}	0.53±0.06^{Aa}
5% CLA	0.40±0.01^{c}	0.42±0.02^{ABc}	0.43±0.01^{ABc}	0.47±0.01^{Ab}	0.50±0.02^{ABa}
7.5% CLA	0.40±0.01^{b}	0.40±0.02^{ABb}	0.42±0.01^{ABb}	0.45±0.05^{ABab}	0.47±0.02^{Ba}
10% CLA	0.38±0.02^{b}	0.38±0.01^{Bb}	0.40±0.03^{Bb}	0.41±0.06^{Bb}	0.46±0.03^{Ba}

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).
1 Malondialdehyde.

Table 4. Changes in residual nitrite (mg/kg) of emulsion-type sausage with CLA during 28 days of storage at 4°C

Treatment	Storage (days)				
	0	7	14	21	28
Control	7.03±0.24^{Aa}	6.97±0.62^{Aa}	5.27±0.01^{Ab}	5.03±0.05^{Ab}	5.17±0.01^{Ab}
2.5% CLA	6.09±0.48^{Ba}	5.89±0.73^{Ba}	4.69±0.08^{Bb}	3.75±0.26^{Bb}	4.63±0.31^{Bb}
5% CLA	5.30±0.21^{Ca}	4.68±0.12^{Cb}	3.00±0.02^{Cc}	2.43±0.12^{Cd}	2.47±0.14^{Cd}
7.5% CLA	5.73±0.37^{BCa}	3.94±0.18^{Cb}	2.92±0.05^{Cc}	2.07±0.23^{Dd}	2.21±0.06^{CDd}
10% CLA	5.31±0.15^{Ca}	4.38±0.34^{Cb}	2.51±0.09^{Dc}	1.81±0.05^{Dd}	1.98±0.07^{Dd}

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).

Apparently, the proportion of polyunsaturated fatty acids in fat significantly influences the rate of lipid oxidation. Joo et al. (2002) also reported that dietary CLA reduced TBARS levels and lipid oxidation of pork loin. The results of these studies show the same trend as in our study. The CLA is a stable component (Shantha et al., 1995), it was postulated that high relative levels of CLA could reduce the formation of fatty acid free radicals and subsequent oxidation reactions. However, the lack of changes in CLA contents during storage could be due to the greater stability of CLA compared with other polyunsaturated fatty acids (Shantha et al., 1994). It is also possible that tissue CLA could slow lipid oxidation during storage. Ha et al. (1990) suggested that CLA might have an antioxidant effect. The autooxidation of CLA forms furan fatty acids that can protect cells against peroxide attack (Yurawecz et al., 1995). As a result of this study, we found that using CLA sources as a fat source had an antioxidant effect in ETS during the storage. Thus, substitutes CLA for fat can improve the lipid stability in ETS.

Residual nitrite analysis

Residual nitrite contents were significantly (p<0.05) decreased with storage periods and the lower residual nitrite contents were observed in all CLA treatment compared to control during storage. The concentration of residual nitrite in ETS was, in the following order: control >2.5% CLA>5% CLA and 7.5% CLA>10% CLA. Sodium nitrite is used in the preparation of cured meats as a potent antibacterial agent (Shahidi et al., 1987). The nitrite is converted to nitric oxide, which on chemical interaction with myoglobin, produces nitric oxide myoglobin, the characteristic pink color of cured meat (Gray and Pearson, 1984). Nitrite can also act as an antioxidant maintaining meat flavour. Nitrites may react with certain amines in foods to produce carcinogenic N-nitroso compound nitrosamines, and many of which are known to cause cancer (Van maanen et al., 1998; Mirvish et al., 2000). A high dietary intake of nitrite has been implicated as a risk factor for human cancer. The result has been increased interest in and movement toward a more vegetarian type of diet. However, the reported association between the risk of cancer and nitrite/nitrate intake are inconsistent (Weyer et al., 2001). In this study, we found that residual nitrite contents were significantly (p<0.05) decreased when substituted CLA for fat increased in ETS, and residual nitrite contents were significantly (p<0.05) decreased with storage periods. Cassens (1997) also reported that less than 50% added nitrite can be detected analytically when the heat processing is finished; and its depletion continues during storage. Chow and Hong (2002) suggested that direct chemical interaction between antioxidants and nitrites/nitrates has long been recognized and they suggested that antioxidants have reduced the nitrite toxicity. We also assume that antioxidants and residual nitrite in meat are both closely related. Delaying lipid oxidation may similarly reduce the residual nitrite. Walsh et al. (1999) stated that a combination vitamin E supplementation with nitrite may be used to reduce the levels of nitrite in cured porcine products, thereby reducing the potential for nitrosamine formation. If CLA has an antioxidant effect (Ha et al., 1990), substituted CLA could reduce the residual nitrite. However, the mechanism for reducing the residual

Table 5. Changes in color of emulsion-type sausage with CLA during 28 days of storage at 4°C

Treatment		Storage (days)				
		0	7	14	21	28
L*	Control	66.48±0.62^{Bd}	68.11±0.28^{Bcd}	68.68±0.19^{Cc}	69.23±0.35^{Aa}	69.27±0.24^{ABa}
	2.5% CLA	63.87±0.37^{Cc}	68.17±0.58^{Cab}	67.46±0.62^{Cb}	68.27±0.72^{Ba}	68.27±0.72^{Ba}
	5% CLA	66.78±0.20^{ABc}	68.91±0.35^{Bb}	69.30±0.88^{Bab}	69.95±1.50^{Aa}	68.69±0.98^{Bb}
	7.5% CLA	64.57±0.62^{Cd}	65.92±0.72^{Dc}	68.13±0.42^{Ca}	67.64±1.54^{Bab}	67.01±0.77^{Cb}
	10% CLA	67.35±0.78^{Ab}	70.56±0.38^{Aa}	70.25±1.05^{Aa}	69.80±0.56^{Aa}	69.98±8.69^{Aa}
a*	Control	8.31±0.18^{C}	8.30±0.08^{D}	8.30±0.22^{B}	8.32±0.27^{B}	8.29±0.20^{C}
	2.5% CLA	8.49±0.29^{BC}	8.53±0.12^{BC}	8.54±0.25^{AB}	8.52±0.12^{A}	8.47±0.15^{B}
	5% CLA	8.62±0.14^{BC}	8.68±0.12^{CD}	8.69±0.12^{A}	8.67±0.13^{A}	8.63±0.32^{AB}
	7.5% CLA	8.67±0.15^{AB}	8.78±0.30^{AB}	8.79±0.20^{A}	8.72±0.28^{A}	8.71±0.07^{AB}
	10% CLA	8.90±0.21^{A}	8.92±0.26^{A}	8.89±0.18^{A}	8.87±0.19^{A}	8.88±0.11^{A}
b*	Control	7.57±0.13^{Ba}	7.54±0.09^{Cab}	7.39±0.12^{Dc}	7.42±0.08^{Dbc}	7.39±0.04^{Cc}
	2.5% CLA	7.59±0.23^{B}	7.71±0.07^{B}	7.71±0.10^{C}	7.75±0.01^{C}	7.69±0.10^{B}
	5% CLA	8.02±0.12^{A}	8.09±0.10^{A}	7.99±0.10^{B}	7.98±0.11^{B}	8.04±0.18^{A}
	7.5% CLA	7.93±0.09^{Ab}	8.04±0.07^{Aab}	7.97±0.11^{Bb}	7.89±0.13^{Bc}	8.09±0.18^{Aa}
	10% CLA	8.10±0.10^{A}	8.07±0.07^{A}	8.17±0.09^{A}	8.17±0.13^{A}	8.11±0.07^{A}

$^{A, B, C, D}$ Different letters within column a are significantly different (p<0.05).
$^{a, b, c, d}$ Different letters within a row are significantly different (p<0.05).

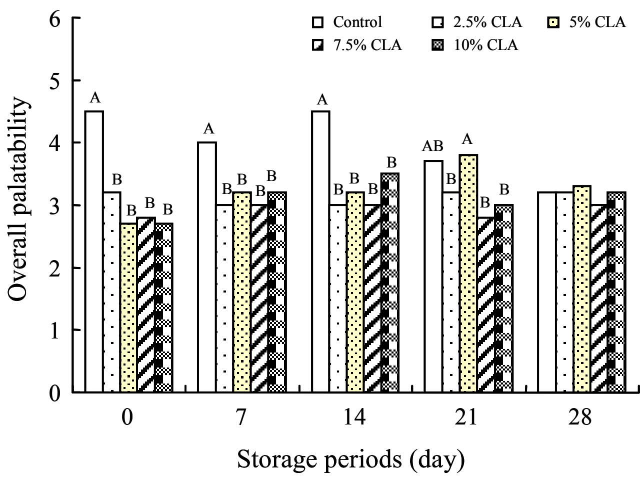

Figure 2. Changes in overall palatability of emulsion-type sausage with CLA during 28 days of storage at 4°C (Different letters in the same day differ significantly at p<0.05).

nitrite by CLA is not yet clear, because no study was conducted on the effect of CLA on the amount of residual nitrite in meats products. More research is needed to determine the effects of substituted CLA for fat on residual nitrite in ETS.

Color analysis

Substituted CLA sources for fat influenced L* values in ETS. Higher L* values were observed in 10% CLA treatment compared to other treatments during storage. However, a* values in CLA treatments significantly (p<0.05) increased compared to control. Especially, a* values of 10% substituted CLA sources treatments were highest than others at 28 days of storage. Similarly, b* values were significantly (p<0.05) higher in CLA treatments than those of control during storage. Du et al. (2000) suggested that dietary CLA treatment not only reduced oxidation, but also improved color stability during storage. Thiel et al. (1998) also reported increases in a* values with increasing levels of CLA, from 0 to 1.0% in the diet, suggesting that dietary CLA may protect meat color. The mechanism for the improvement color stability by CLA is not yet clear. The rate of meat discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation (Yin and Faustman, 1993). Lipid oxidation can produce a wide range of secondary aldehyde products such as 4-hydroxynonenal, which accelerated equine myoglobin oxidation *in vitro* (Faustman et al., 1999). If CLA has an antioxidant effect (Ha et al., 1990), substituted CLA sources could reduce the color deterioration. However, we can not suggest that substituted CLA for fat in ETS improve the color because not only a* value but also b* value were increased. Sensory panels also did not distinguish significantly difference of color among the treatments (data are not shown). Substituted CLA have located of different positions compared with incorporated CLA by dietary CLA. Therefore, some functions of CLA could be different as a result of CLA supplementation in diet compared to CLA added to meat. More research is needed to determine the effects of substituted CLA source for fat on lipid oxidation and color in ETS.

Sensory evaluation (overall acceptability)

Overall acceptability of CLA treatments were significantly (p<0.05) lower than control during storage. Whereas, no significantly different among the treatments were shown at the 28 days of storage. Liu et al. (1991) also reported that substituted plant oil for beef fat was lower overall acceptability than those of control in low-fat patty.

However, Paneras and Bloukas (1994) suggested that substituted plant oil for fat was received more good point by sensory panel compared with control in frankfurter. As a result of this study, we found that substituted CLA source for fat influenced properties of sensory in ETS. Because substituted CLA source have own flavor that decreased overall acceptability than those of control in ETS. Thus studies on how to prevent this decrease acceptability and improve the taste in ETS are needed.

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