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A METHOD FOR DETERMINING *9cis, 11trans* CONJUGATED LINOLEIC ACID AND SOME FACTORS INFLUENCING ITS CONCENTRATION IN MEATS

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Keywords: Conjugated linoleic acid (CLA), Preparation of CLA methyl ester, GLC analysis, Meat CLA content, Type of feed, Refrigerated storage, Lipid oxidation

Background:

Conjugated linoleic acids (CLAs) are isomers of linoleic acid which have recently gained importance as one of the biologically active substances in food. Among the various CLA isomers, the *9cis, 11trans* CLA (*9c, 11t* CLA) found in beef (Pariza et al., 1979) has been shown to have beneficial effects such as antimutagenic activity (ha et al., 1991) and serum cholesterol-lowering activity (Lee et al., 1994). The *9c, 11t* CLA content is higher in products from ruminants, including meats and dairy products, as compared with other foods from nonruminants (Chin et al., 1992). Hughes et al. (1982) suggested that *9c, 11t* CLA is produced by linoleate *Δ12-cis, Δ11-trans* isomerase which is an enzyme synthesized by a rumen bacteria, and this is believed to be a major contributor of *9c, 11t* CLA in foods derived from ruminants. On the other hand, it is also proposed that *9c, 11t* CLA is chemically formed during the process of lipid peroxidation (Ha et al., 1989). It is well known that *9c, 11t* CLA is easily isomerized and oxidized during the preparation of its methyl ester (Shantha et al., 1993).

Objectives:

The objectives of this study are (1) to develop a precise and reproducible method for determining *9c, 11t* CLA, (2) to determine the *9c, 11t* CLA contents in various meats by using this developed method, (3) to evaluate the *9c, 11t* CLA contents in meats from ruminants fed different feeds, and then (4) to investigate the effects of heating, storage and lipid oxidation on *9c, 11t* CLA contents in meats.

Methods:

Total lipids from meat were extracted according to the method of Folch et al. The following five methods were used for the preparation of *9c, 11t* CLA methyl ester, including total fatty acid methyl esters (FAMEs): in Method Ⅰ, 0.5 M KOH / MeOH + 14 % BF_3 / MeOH; in Method Ⅱ, 8 % BF_3 / MeOH; in Method Ⅲ, 4 % HCl / MeOH; in Method Ⅳ, tetramethylguanidine / MeOH; and in Method Ⅴ, 0.5 M KOH / MeOH + aqueous HCl / MeOH. FAMEs were analyzed on a GC-17A gas chromatograph (Shimadzu, Tokyo) equipped with a capillary column (SUPELCOWAX™10, 60 m × 0.32 mm, i.d., 0.25 μm film-thickness, SUPELCO). The *9c, 11t* CLA contents were determined for samples of meats and adipose tissues from ruminants and nonruminants (beef, mutton, goat meat, pork and chicken meat) by using our proposed method. In addition, effects on meat *9c, 11t* CLA content were investigated by the type of feed(high roughage or high concentrate), heating(at 75 ℃ for 1 hour), refrigerated storage (at 4 ℃ for 1 week) and salting (2 % NaCl + 0.01 % ascorbate) by which lipid oxidation of meat is accelerated. Degree of lipid oxidation was expressed as the TBA value (mg MDA / kg meat) by using the steam-distillation method of Yamauchi et al.(1980).

Results and Discussions:

In this experiment, *9c, 11t* CLA isomer was separated and identified from beef lipids. However, Methods Ⅲ and Ⅳ resulted in lower yields of total FAMEs, and Methods Ⅰ and Ⅱ had remarkably decomposed *9c, 11t* CLA isomer. These four methods were unsuitable for *9c, 11t* CLA isomer analysis in terms of the yield of total FAMEs and the decomposition of the *9c, 11t* CLA isomer. From the standpoint of the yield of total FAMEs and the decomposition of the *9c, 11t* CLA isomer, Method Ⅴ involved hydrolysis of total lipids with 0.5 M KOH / MeOH, followed by esterification with aqueous HCl(35%) / MeOH (4 : 4, v / v) was most suitable for determining the *9c, 11t* CLA content in meat as compared with the other methods used. The resulting *9c, 11t* CLA methyl ester was separated by means of gas-liquid chromatograph equipped with a capillary column. The *9c, 11t* CLA content in meat was determined by dividing the value estimated using tricosanoic acid ($C_{23:0}$) as an internal standard by 0.9261, which was the correction factor determined on the basis of both the recovery of total FAMEs and the ratio of decomposition of *9c, 11t* CLA.

The *9c, 11t* CLA contents in meats from ruminants were four to ten times greater than those in meats from nonruminants (Table 1). The results were similar to those of Chin et al.(1992). The respective *9c, 11t* CLA contents in meat and fat from ruminants fed a relatively higher roughage ration were higher than those from ruminants fed a relatively higher concentrate ration (Table 2). The results suggested that the type of feed may lead to changes in the microflora of rumen, resulting in varying *9c, 11t* CLA concentrations in tissues.

The *9c, 11t* CLA concentrations in unheated / heated meats (goat meat, mutton and pork) with or without salt (2 % NaCl + 0.01 % ascorbate) did not change during refrigerated storage (Tables 3 and 4). However, the TBA values for meats remarkably increased by refrigerated storage, heating and salting. These findings suggested that the *9c, 11t* CLA isomer might be highly stable against heating, and its yield may be only a little even if the *9c, 11t* CLA isomer could be formed during the process of lipid oxidation of meat.

Conclusions:

In the present study, we developed a precise and reproducible method for determining *9c, 11t* CLA content in meat. Meats from ruminants contained considerably more *9c, 11t* CLA than meats from nonruminants. The *9c, 11t* CLA content was also affected by the type of feed. The TBA values of meats remarkably increased due to treatments of heating, refrigerated storage and salting, but the *9c, 11t* CLA concentrations in meats did not change. These results suggested that the *9c, 11t* CLA yield may be only a little even if it could be formed during the process of lipid oxidation of meat.

Pertinent literature:

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Table 1 *9c, 11t* CLA contents in meats

Meat	*9c, 11t* CLA
Beef	31.62(3.21)
Mutton	4.79(2.28)
Goat Meat	11.79(6.35)
Pork	1.94(0.63)
Chicken	3.65(0.56)

Values represent mg/100g meat with mg/g lipid in parenthesis

Table 2 *9c, 11t* CLA contents in meat and fat from ruminants fed different feeds

Animal/Meat	Loin	Round	Short Plate	Depot Fat
Cattle 1	19.18(2.58)	31.62(3.21)	210.69(6.09)	
Cattle 2	12.18(1.26)	20.32(2.19)	59.01(2.46)	
Sheep 1		15.78(4.17)		460.25(5.20)
Sheep 2	8.20(2.17)	4.79(2.28)		166.32(1.80)
Goat 1	17.03(6.82)	11.79(6.35)		425.08(5.19)
Goat 2	10.65(4.53)	9.59(4.58)		461.09(5.08)
Goat 3	4.60(1.65)	2.69(1.45)		115.66(1.28)

Values represent mg/100g samples with mg/g lipid in parenthesis

Table 3 TBA value and *9c, 11t* CLA content of refrigerated goat meat

	Loin		Round	
Treatments	0days	7days	0days	7days
Unheated				
TBA value (mg MDA/kg)	0.22	0.27	0.94	2.23
CLA content (mg/g lipid)	4.82	4.69	4.03	4.25
Heated				
TBA value (mg MDA/kg)	0.95	4.89	1.33	7.00
CLA content (mg/g lipid)	4.68	4.66	4.27	3.97

Table 4 Effect of lipid oxidation on *9c, 11t* CLA contents of pork and mutton during refrigerated storage

	Pork				Mutton			
Treatments	Unheated		Heated		Unheated		Heated	
	0days	7days	0days	7days	0days	7days	0days	7days
Unsalted								
TBA value (mg MDA/kg)	0.13	0.13	1.69	4.76	0.16	0.30	0.22	1.87
CLA content (mg/g lipid)	0.52	0.65	0.49	0.53	4.17	4.96	3.57	4.08
Salted								
TBA value (mg MDA/kg)	0.18	3.54	1.91	7.93	0.17	0.85	0.21	1.83
CLA content (mg/g lipid)	0.51	0.56	0.56	0.59	4.12	4.20	3.53	4.51

Salted: 2 % NaCl + 0.01 % Ascorbate