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Research Article

Investigation of the Effect of Different Cooking Methods on Conjugated Linoleic Acids in Red Meat

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Abstract

This study was carried out to investigate the effect of different cooking techniques applied to meats on total CLAs, cis-9,trans-11-CLA (c9,t11-CLA) and trans-10,cis-12-CLA (t10,c12-CLA). . In the study, four different cooking methods such as boiling, frying, baking and grilling were applied to meat samples taken from the bovine carcasses. In raw and cooked meat samples, CLA, cis-9, trans-11 and trans-10, cis-12 isomer amounts were examined in Gas Chromatography-Flame Ionization Detector (GC-FID) device.

It was determined that the meat samples with frying treatment had the highest average value in total CLAs and c9,t11-CLAc9,t11-CLA. The difference between the total CLA values was found to be significant at the level of $P < 0.01$ and the difference between the mean values of the c9,t11-CLA at the level of $P < 0.001$. In other cooking methods, the difference between the mean values of total CLA, c9,t11-CLA and t10,c12-CLAwas not significant ($P > 0.05$). According to the findings obtained, it was concluded that thermal processes do not have a significant enhancing effect on the amount of CLAs in the meat and therefore, meat and products should be supplemented with CLAs to benefit from its positive effects.

Keywords: Red Meat, Cooking, CLAs and Bioactive Isomer

Farklı Pişirme Yöntemlerinin Kırmızı Etlerdeki Konjuge Linoleik Asit (KLA) Üzerine Etkisinin Araştırılması

Öz

Konjuge linoleik asit (KLA), linoleik asidin pozisyonel ve geometrik izomerlerinden oluşan grup için kullanılan terimdir ve bazı izomerlerin, sağlık açısından yararlı olduğu bilinmektedir. Bu çalışma, etlere uygulanan farklı pişirme tekniklerinin konjuge linoleik asit (KLA) ve cis9-trans11 ile trans10-cis12 izomerleri üzerine etkisini araştırmak amacıyla yapıldı. Çalışmada büyükbaş hayvan gövdesinden alınan et numunelerine haşlama, kızartma, fırınlama ve ızgara olmak üzere dört farklı pişirme yöntemi uygulandı. Çiğ ve dört farklı yöntem ile pişirilen et numunelerinde KLA, cis-9, trans-11 ve trans-10, cis-12 izomer miktarları GC-FID (Gas Chromatography-Flame Ionization Detector) cihazında incelendi.

Kızartma işlemi uygulanmış et numunelerinin, toplam konjuge linoleik asit ve c9-t11 izomerinde en yüksek ortalama değere sahip olduğu saptandı. Toplam KLA değerleri arasındaki fark $P<0.01$ düzeyinde, c9-t11 izomeri ortalama değerleri arasındaki fark ise $P<0.001$ düzeyinde önemli bulundu. Uygulanan diğer pişirme yöntemlerinde toplam KLA, c9-t11 izomeri ve t10- c12 izomeri ortalama değerleri arasındaki fark önemli bulunmadı ($P>0.05$). Elde edilen bulgulara göre, ısısal işlemlerin etlerdeki konjuge linoleik asidin miktarı üzerine önemli bir arttırıcı etkisinin olmadığı ve bu nedenle olumlu etkilerinden yararlanabilmek için et ve ürünlerinin konjuge linoleik asid ile desteklenmesi gerektiği sonucuna varıldı.

Keywords: Kırmızı Et, Pişirme, Konjuge Linoleik Asit, Biyoaktif İzomer

Introduction

Meat and meat products are considered high-quality food because they contain nutrients such as protein, vitamin B, minerals (especially iron, zinc, and phosphorus), essential fatty acids, etc. (Djordjevic et al., 2016 ; Doulgeraki et al., 2012). On the other hand, high saturated fatty acids (SFA) and trans fats in Ruminant meats are known to have various negative effects on human health (Kiralan et al., 2005; Pattanayak, 2019). In contrast, CLAs (CLA), which is a bioactive fatty acid naturally found in the meat and fat of ruminant animals, draws much attention due to its nutritional and therapeutic properties (Park and Pariza, 2007; Serra et al., 2009; Suresh et al., 2018).

CLAs, consisting of the positional and geometric isomers of linoleic acid, a polyunsaturated essential fatty acid, is mostly found in meat, milk and their products derived from ruminants. CLAs is produced by biohydrogenation of unsaturated fatty acids in the rumen of ruminant animals and is absorbed through the intestine and spread to other tissues (Schmid et al., 2006; Kurban and Mehmetoğlu, 2006).

Biologically active isomers of CLAs (CLA) are cis-9, trans-11 and trans-10, cis-12. The cis-9, trans-11 isomer, which is called rumenic acid, constitutes approximately 80-90% of the total CLAs in beef (Ercoşkun et al., 2005; Mulvihill, 2001). It is reported that the two main isomers (cis9, trans11 and trans10, cis12) of CLA have anticarcinogenic, antiatherogenic, antiobesitic and antidiabetic effects on health (Aydın 2005, Hartigh, 2019; Park and Pariza, 2007). Besides the ability of CLA to reduce body fat levels, its beneficial effects on glycemic profile, atherosclerosis and cancer have also been proven in experimental models (Lehnen et al., 2015).

The variety of useful properties of CLAs is due to the separate or common effects of each or a few of its isomers (Ercoşkun et al., 2005). It is reported that the main factors affecting the amount of CLA in meat and meat products are the animal's diet, seasonal change, species, race, gender, bacterial population in rumen and enzyme activation level (Mulvihill, 2001). Despite its known positive biological effects, CLAs exists in low quantities in the human body, and therefore, studies of enrichment of meat and meat products with CLAs have been increasing recently (Çelebi and Kaya, 2008; Juarez et al., 2009).

It is known that thermal processes can affect the meat composition, especially the fat content and change the meat quality, and some research has shown that the cooking method can alter the fatty acid content and oxidize fats by altering the nutritional value of the products compared to raw samples (Alfaia et al., 2010; Juárez et al., 2010; Oz et al., 2017). Different studies focused on the effect of cooking methods on the fatty acid composition (Lorenzo et al., 2015; Maranesi et al., 2005), but the research for the effect of cooking on CLA and its isomers is still limited.

In the current studies, it is seen that the processing and storage methods applied do not have a significant effect on the amount of CLA in meat, and different results are obtained in cooking processes. (Campo et al., 2013; Herzallah et al., 2005; Hur et al., 2004; Li et al. al., 2016). In the first studies, it has been suggested that oxidation reactions occurring during heat treatments increase the amount of CLA in meat, but dry matter and fat increases during these processes were not taken into account (Ercoşkun et al. 2005; Zarow et al., 2017). It is determined that heat treatments are effective on meat composition, especially fat content, product final quality and physicochemical properties in meat,. However its effect on CLA in meat is variable (Oliveira et al., 2015; Serrano et al., 2007).

In many studies, it has been revealed that various cooking methods applied in meat do not cause any significant change in the CLA content of meat (Alfaia et al., 2010; Kamal et al., 2019; Li et al., 2016; Rant et al., 2019). In a study on the effect of cooking on fatty acids in CLA-enriched breakfast pork sausages, there was a slight increase in CLA and cis-9, trans-11 and trans-10, cis-12 isomers in cooked meat (Juarez et al., 2009). The CLA content of a ruminant-derived product increased little or did not change as a result of heat treatment, and the CLA content slightly increases as a result of oxidation (Ercoşkun et al. 2005).

Some studies have reported that heat application causes undesirable changes such as loss of essential fatty acids and decreased nutritional value of meat due to the lipid oxidation that usually occurs (Ahmed, 2015; Amaral et al., 2018; Dominguez et al., 2014). In other studies, it has been shown that unsaturated fatty acids are more prone to oxidation and therefore the high unsaturation index in meat can affect oxidative stability (Bou et al 2001; Hur et al., 2004; Yang et al., 2000).

The effects of four different cooking methods (boiling, frying, baking and grilling) on the nutritional and quality criteria of beef *Longissimus dorsi* muscle were investigated by Aşçıoğlu and Şevik (2019). The amount of polyunsaturated fatty acids (PUFA) increased in all cooking modes, while the amount of saturated fatty acids (SFA) decreased in all applications. In another study, it was found that processes such as frying, grilling, microwave and oven cooking did not cause any change in the CLA content of meat, and also, keeping the meat in the refrigerator caused oxidative changes in the meat, but not the CLA content of the meat (Cristina et al., 2010).

However, in many studies, it has been concluded that there is great instability in the changes in each fatty acid in the different cooking methods applied and there is not sufficient information about whether the existing CLA in meat is sensitive to the thermal processes applied household cooking methods (Badiani et al., 2002; Lorenzo et al. et al., 2015; Nikmaram et al., 2011). This study was carried out to investigate the effect of different cooking techniques applied to meat such as boiling, frying, baking and grilling on CLA (CLAs) and cis9 trans11 and trans10-cis12 isomers.

Material and Methods

Material

2 kg of meat samples taken from the *M.longissimus dorsi* muscle of the bovine body and frozen into cubes were used as materials in the study. Raw and cooked meat samples of 300 g each were first divided into 3 groups of 100 g each and then into 2 groups of 50 g each. Thus, a total of 30 samples were examined, 6 for each group. Methanol, potassium hydroxide, hexane, CLAs analytical standard (O5507) and other chemicals used in the analysis were all supplied from Sigma Aldrich.

Methods

Material Preparation and Cooking Process

After the frozen meat samples were thawed at + 4°C, fat, nerve and membrane were cleaned and divided into 5 groups, each containing 300 grams of meat. The first group was left raw for control (internal temperature 8°C), the second group was boiled (105 °C and 45 minutes, internal temperature 71.33°C), the third group was frying (220°C and 15 minutes, internal temperature 88.83°C), the fourth group was baking (200°C and 30 minutes, internal temperature 80.58°C) and the fifth group was grilled (250°C and 15 minutes, internal temperature 77.83°C) were processed and the internal temperatures of the meat samples were measured with an IR thermometer (Cem DT-8862).

Fat Extraction and Esterification Process

Fat determination of raw meat and meat-applied meat was done by the Soxhlet method (Anonymous, 2002). 0.1 g of the fat obtained was weighed and dissolved in 10 ml of n-Hexane. The dissolved samples were mixed for 30 seconds on the vortex device and 100 µl of 2 N KOH (prepared in methanol) solution was added and mixed again for 30 seconds. Then, it was centrifuged at 40 rpm / 5 min, taken from the liquid phase into 2 ml vials and made ready to be read in the GC-FID device. The methyl esterification process was carried out in an analytical standard (Anonymous, 2005; Roach et al., 2002).

Detection of CLA bioactive Isomers

Bioactive isomers of CLAs in esterified fat samples were analyzed on the Thermo Scientific, Trace 1310 model instrument. Accordingly, the TR-FAME (30 m x 0.25 mm ID x 0.25 µm, Part No: 260 M142P, Serial No: 0035744) column was used. Injection volume was 1 µl and split ratio 1:20, column furnace temperature (gradient increased from 180°C to 210°C by 5°C / min) at 210°C, 15°C injection temperature was set at 250°C and detector temperature at 260°C. Helium gas with a flow rate of 30 ml/min was used as the carrier gas (Alonso et al., 2004; Christie et al., 2001; Steinhart et al., 2003).

Statistical Analysis

The samples analyzed in the groups were determined using the kruskal wallistest. SPSS 13.0 analysis package program was used in this analysis.

Results and Discussion

Although studies on the effect of cooking on CLAs in meat are limited, some researchers point out that cooking methods are effective in changing the nutritional value of meat, especially in the fat composition of meat (Badiani et al., 2004; Bravo- Lamas, 2018; Maranesi and al., 2005; Sarriés et al., 2009).

CLAs includes 28 geometric and positional isomers of linoleic acid, omega-6 essential fatty acid. Among these isomers, the biological properties of t10,c12 -CLA, which are called rumenic acid and which are found in high amounts in foods (C18: 2) and of the (C18: 2) t10,c12 -CLA following this isomer have been determined (Aydın, 2005; Kurban and Mehmetoğlu, 2006; Schmid et al., 2006). The biologically active isomers c9-t11 and t10-c12 of CLAs constitute approximately 80-90% of the total isomers in beef (Mulvihill, 2001, Ercoşkun et al., 2005).

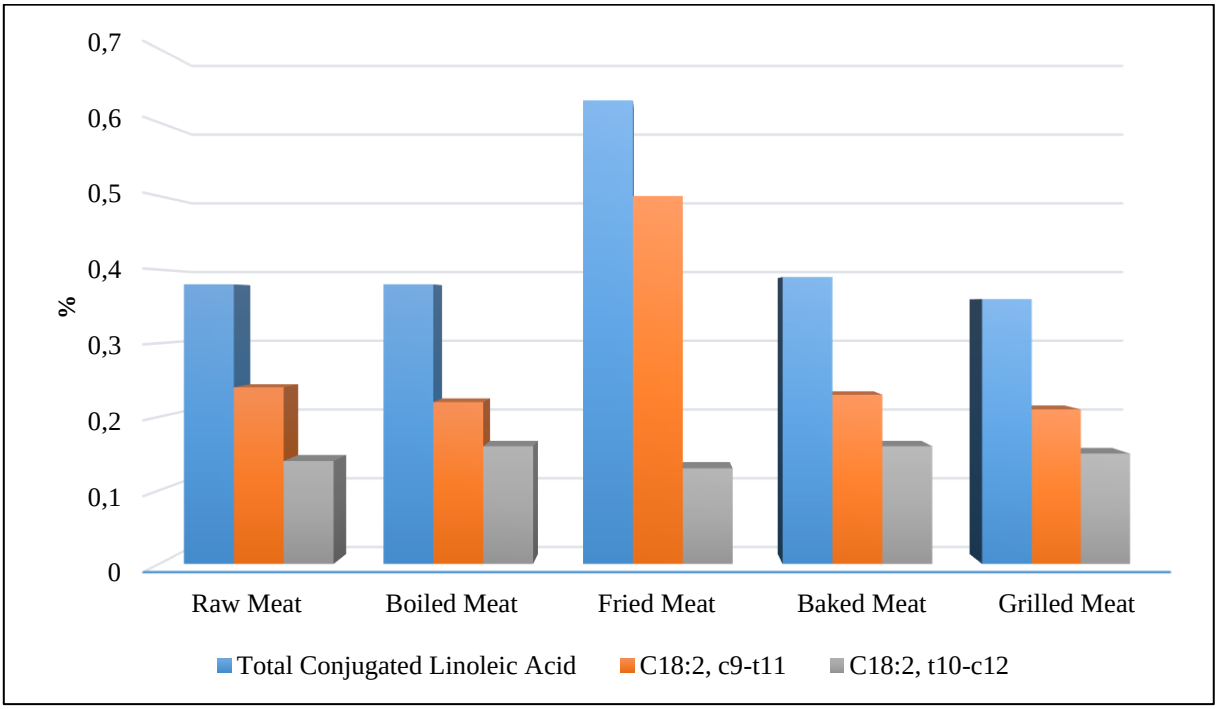

Figure 1. *The amount of CLAs and isomer detected in raw and cooked meat samples*

In our study, the amounts of c9-t11 and t10,c12-CLA determined from meat fat in the samples and the amounts of CLAs, which is their sum, are given in table 1 and Figure 1. Accordingly, the total CLAs amount was calculated as 0.3836% (3.8 mg / g fat) from the amount of fat which was determined as 1.2850 g per 100 grams of raw meat whose internal temperature was measured as + 8 ºC in our study. It is formed of this amount 0.2353% (2.3 mg / g fat) was c9-t11 and 0.1483% (1.5 mg / g fat)

t10-c12 bioactive isomers. The total amount of CLAs in our study was evaluated over the c9-t11 and t10,c12-CLA, which are the most abundant of 28 isomers.

The average amount of CLAs obtained from the different muscles of cattle and calves is reported by Schmid et al. (2006) as 2.9-4.3 mg / g fat. In a study of Cristina et al. (2010), 3.59 mg total CLAs was determined in grams of meat fat examined. Suresh et al. (2018) detected 2.5 to 8.5 mg / g fat and 1.4 to 3.7 mg / g meat CLAs in goat and buffalo fat and muscle tissues. The amounts of CLAs reported in these studies were found to be compatible with the findings obtained in our study. It is reported by other studiesthat the major isomer found in natural resources is c9-t 11 and that c9-t11 and t10,c12-CLA are found in similar amounts in commercial preparations (Schmid et al. 2006; Wang and Jones. 2004).

0.2353% (2.3 mg / g fat) of c9,t11-CLAand 0.1483% (1.5 mg / g fat) of t10,c12-CLA were found in raw meat samples. The c9,t11 -CLA was calculated as 61.34% and the t10,c12-CLA was 38.66% of the total CLAs, (Table 1).The fact that the amount of t10,c12-CLAis so high in raw meat samples gives the idea that the animal whose meats are examined is fed with commercial preparations. As a matter of fact, in a study conducted by Cristina et al. (2010), the c9,t11-CLA was detected at a rate of 67.35% and the t10,c12-CLAat a rate of 1.12% in animals treated with natural pasture fattening, and it was found to be significantly different from our findings.

Some authors point out that the applied heat treatments can be effective on meat composition and especially fat and fatty acid content, affecting the final product quality (Badiano et al., 2004; Campo et al., 2013; Oz et al., 2017; Serrano et al., 2007). In our study, the amount of fat in 100 grams of raw meat is 1,285 grams. This amount was determined as 5.1380 grams in frying process, 4.6620 grams in baking, 1.1843 grams in boiling and 1.0430 grams in grilling process. Accordingly, the intramuscular fat content increased in the frying and baking process and decreased in the boiling and grilling process. It is thought that the increase in the frying and baking process is due to sunflower oil added before the process. Similarly, in a study by Kamal et al. (2019), the intramuscular fat content of 6.14% to raw meat decreased to 5.31% and 4.83%, respectively, after the grilling and boiling, but significant on the desired fatty acids of cooking. it had no effect.

The effect of applied heat treatments on fatty acids in meats has been investigated in many studies and it has been concluded that heat treatments reduce the amount of saturated fatty acid due to the conversion to trance, but increase the amount of unsaturated fatty acid. In the study conducted by Li et al. (2016), prolonged slow cooking with pre-frying and different heating methods increased the polyunsaturated fatty acid values in pork, but decreased the fat percentage of pork. In the pre-frying process combined with flame heating, lower total saturated fatty acid percentages were determined, while higher mono and polyunsaturated fatty acids were obtained.

A study on the effect of heat treatments on the fatty acid composition of chicken meat enriched by Zarow et al. (2017) was conducted. As a result, the fatty acid content in raw meat has not changed, but the amounts of total monounsaturated and polyunsaturated fatty acids have increased significantly after frying and roasting. The effects of four different cooking methods (boiling, frying, baking and grilling) on the nutritional and quality criteria of beef *Longissimus dorsi* muscle were investigated by Aşçıoğlu and Şevik (2019). Accordingly, the amount of polyunsaturated fatty acids increased in all cooking methods, while the amount of saturated fatty acids decreased in all applications.

Linoleic acid turns into conjugate due to the heat treatment applied and causes an increase in the amount of CLAs in the environment (Wang, 2004; Kurban & Mehmetoğlu, 2006). For example, in a study conducted by Juarez et al. (2009), a decrease in the amount of linoleic acid and an increase in the amount of CLAs was detected in the grilled breakfast sausages at 200°C for 30 minutes.

Table 1. *The amounts of fat, conjugated linoleic acid and isomer detected in raw and cooked meat samples*

Raw and Cooked Meat Samples	**Quantity of Fat (g/100 g meat)**	**Total Conjugated Linoleic Acid**		**C18:2, c9-t11**		**C18:2, t10-c12**	
		mg/g fat	**(%)**	**mg/g fat**	**(%)**	**mg/g fat**	**(%)**
Raw Meat	1.28	3.83	0.38	2.35	0.24	1.48	0.14
Boiled Meat	1.18	3.78	0.38	2.16	0.22	1.62	0.16
Fried Meat	5.14	6.35	0.63	5.04	0.50	1.30	0.13
Baked Meat	4.66	3.91	0.39	2.34	0.23	1.57	0.16
Grilled Meat	1.04	3.64	0.36	2.13	0.21	1.51	0.15

In Table 1, the total amount of CLAs detected in grams of meat has decreased slightly in the boiling and grilling process compared to raw meat, while it has increased in the frying process and the baking process in small amount. The total amount of CLAs determined as 3.83 mg/g fat in the raw meat sample was determined as 3.78 mg/g fat in the lowest boiled meat sample and 6.34 mg/g in the highest fried meat sample.

On the other hand, bovine *Longissimus dorsi* muscle, which was subjected to heat treatment at 140°C for 30 minutes, was examined by Sarries et al. (2009) and it was determined that the CLAs content was not affected by the thermal process. Maranesi et al. (2005) reported that there was no significant difference in the total CLAs amounts of lean meat taken from the waist region before and after the microwave and frying process.

According to our study and the studies above, there is an indecision in the effect of different cooking methods applied to meat on the amount of CLAs. In some cooking methods, the amount of CLAs increases due to the conversion of linoleic acid, while in other methods it is not affected.

c9,t11-CLA, which is 2,36 mg / g fat in raw meat, 2,16 mg / g fat in boiled meat, 5,04 mg / g fat in fried meat, 2,34 mg / g fat in baked meat, and 2,13 mg / g fat in grilled meat was determined. The t10,c12 -CLA, which was 1,48 mg / g in raw meat, 1,62 mg / g in boiled meat, 1,30 mg / g in fried meat, 1,57 mg / g in baked meat and 1,51 mg / g in grilled meat. Accordingly, it was observed that the c9,t11-CLA increased significantly in fried meat compared to raw meat, decreased slightly in other cooking techniques and t10,c12 -CLA decreased in fried meat compared to raw meat and increased slightly in other cooking techniques (Table 1).

Rant et al. (2019) investigated the effect of cooking processes such as microwave and roasting on fatty acid composition in lamb *Longissimus dorsi* muscle. When compared with raw meat, it was concluded that the heat treatments applied did not change the saturated fatty acid and polyunsaturated fatty acid content of the meat and the CLAs did not cause a significant change in the content of the C18: 2 c9,t11-CLA.

Alfaia et al. (2010) reported that cooking processes such as microwave, baking, boiling and grilling cause higher saturated fatty acid, monounsaturated fatty acid and lower polyunsaturated fatty acid ratios in beef, whereas CLAs isomers against heat treatment. It has been reported to show stability.

It has been reported by Alfaia et al. (2010) that cooking processes such as microwave, baking, boiling and grilling cause higher saturated fatty acid, monounsaturated fatty acid and lower polyunsaturated fatty acid ratios in beef, while CLAs isomers show stability.against heat treatment.

Juanéda et al. (2003), in their study to determine the effect of heat on the formation of CLAs isomers in sunflower oil, two temperatures (180 and 220°C) were used for heating and the level of CLAs was positively affected by the temperature. In addition, it was observed that more CLAs isomers were formed in 180°C application compared to 220°C application.

Some studies have reported that the application of heat generally causes the loss of essential fatty acids due to lipid oxidation and theunsaturated fatty acids are more prone to oxidation (Amaral et al., 2018; Bou et al., 2001; Dominguez et al., 2014). In the study of Bou et al. (2001), it was reported that unsaturated fatty acids are more prone to oxidation, and therefore high unsaturation index in meat can affect oxidative stability. In a study conducted by Cristina et al. (2010), it was found that oxidative changes that were not important occurred immediately after application in grilled and microwaved meat, and a slight oxidative increase in boiled meat.

In the study conducted by Sarriés et al. (2009), the effect of cooking on the fatty acid and CLAs content of bovine *Longissimus dorsi* muscle was investigated. It was concluded that there was no change in the relative distribution of fatty acids during cooking (140 ° C for 30 minutes) and cooking did not cause thermal degradation or oxidative synthesis of polyunsaturated fatty acid or CLAs.

The statistical analysis results with the total average values of the total CLAs and bioactive isomers found in the raw and cooked meat samples are given in Table 2.

Table 2. *Statistical analysis results with the mean values of the total CLAs and bioactive isomers detected in the samples* (n = 6 for each group).

Groups	C18:2, c9-t11 (%)		C18:2, t10-c12 (%)		Total CLA (%)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Raw Meat	0.24[b]	0.01	0.15	0.02	0.39[b]	0.02
Blanched Meat	0.22[b]	0.02	0.16	0.01	0.38[b]	0.03
Fried Meat	0.50[a]	0.03	0.13	0.01	0.63[a]	0.03
Baked Meat	0.23[b]	0.01	0.16	0.00	0.39[b]	0.01
Grilled Meat	0.21[b]	0.01	0.15	0.01	0.36[b]	0.02
Importance	***		NI		**	

a, b: the difference between columns bearing different letters is significant.

NI: Not important (P> 0.05); **: P <0.01; ***: P <0.001

Accordingly, the difference between the mean values of the total CLAs and the mean values of the c9,t11-CLA was found to be significant at the P <0.001 level, and the difference between the mean values of the t10,c12-CLA was found to be insignificant (P> 0.05).

In the study conducted by Alfaia et al. (2010), the percentage of CLAs C9,t11-CLA increased significantly after boiling (p <0.05), but there was no change in cooking with microwave or grill. The other bioactive isomer t10-c12 was not affected by heating processes (p> 0.05). In a study conducted by Juarez et al. (2009), 3 groups of breakfast sausages containing different amounts of CLAs were grilled at 200^0C for 30 minutes, and the differences between c9-t11 and t10,c12-CLA values were not found to be statistically significant.

Similarly, in another study by Cristina et al., (2010), the effect of cooking methods on CLAs of bovine intramuscular fat was examined and it was concluded that there was no significant change in the amount of CLAs, c9,t11-CLA and t10,c12-CLA detected in raw meat in boiling, microwave cooking and grilling processes.

Among the cooking methods applied in our study, the frying process applied meat samples had the highest average value in total CLAs and c9,t11-CLA. The idea that factors such as the amount and structure of sunflower oil used in the frying process,

the temperature and duration applied, the internal temperature reached and thus the oxidation formed played a role in this. In Table 2, no significant difference was found between the total CLAs and c9,t11-CLA and t10,c12-CLA average values in other cooking methods (P> 0.05).

Conclusion

The amounts and proportions of CLAs bioactive isomers in the beef samples examined in the study were found to be close to each other. This suggests that the beef samples were obtained from animals fed commercial feed in a closed fattening environment.

Although the CLAs and its bioactive isomers detected in the samples showed a slight increase or decrease in the applied boiling, baking and grilling processes, it showed that these changes were not statistically significant.

The increase detected in the frying process occurred due to the sunflower oil used. As a result, the CLAs and bioactive isomers present in meat are not adversely affected by the heat treatment applied and this does not create a negative situation in terms of health.

On the other hand, it is an important advantage that CLAs and bioactive isomers are not negatively affected by various processes applied to meats. However, CLAs and its bioactive isomers are not available in meat and meat products in an amount that can show their biological effects. For this reason, the amount of CLAs in meat and meat products should be increased in order to meet the amount required for a healthy life.

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