Evaluation of conjugated linoleic acid and other health-related lipid components in raw, boiled and smoked beef

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Abstract

Conjugated octadecadienoic ($C_{18}:2$) Linoleic Acid (CLA) if not destroyed by heat during processing can be beneficial to health. About 100g of Longissimus dorsi Beef Muscle (LDBM) each was trimmed of external fat and connective tissues and used for this study. Samples were spiced, group into 3 of $N=10$ samples per group and treated as raw (control), boiled and smoked to internal temperature of 76.1°C . Samples did not show significant difference ($p > 0.05$) in the amount of CLA content which had values of 0.60% and 0.40% for boiled and smoked samples respectively. However, these values were different from the raw sample with a value of 2.39 ± 0.01 . Boiled samples preserved the total beneficial lipids of CLA, PUFA, MUFA and omega 3 and 6 fatty acids more than smoked samples. The major fatty acids found in the muscle analyzed are palmitic ($C_{16}:0$), stearic ($C_{18}:0$) and octadecenoic ($C_{18}:1$). Omega 3 fatty acids are 1.05%, 1.16% and 1.30 % for boiled, smoked and raw samples respectively and 4.21%, 6.39% and 12.87% for omega-6 fatty acids in the same order. Ratios of fl -3/fl -6 are 0.25, 0.18 and 0.10 for boiled, smoked and raw samples respectively. By preserving the beneficial CLA, the Nigerian traditional high temperature moist-cooking method should not pose any risk in processing beef. Our internal cooking temperature of 76.1°C for the boiled and smoked beef conforms to USDA cooking temperature for a 'well done' beef.

Key words: Conjugated Linoleic Acid (CLA), Longissimus dorsi muscle, Boiled & Smoked Beef, Omega 3, 6 Fatty Acids.

Introduction

Traditionally, and particularly in Southwest Nigeria, meat is moist cooked for immediate consumption. The left over is usually stored at room temperature and intermittent warming and cooling prevent spoilage giving a life span of about 5 days (Ockerman and Agboola, 1991).

Conjugated linoleic acid (CLA) supplemented diet was reported as an effective method to decrease fat deposition and increase growth performance in pigs (Eggert *et al.*, 1998, Quinn *et al.*, 2000,

Pieszka *et al.*, 2004 and Wasilewski *et al.*, 2011). Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid containing conjugated unsaturation. The *cis-9, trans-11-octadecadienoic* acid isomer is produced in ruminant animals as a first intermediate in the biohydrogenation of dietary linoleic acid by a linoleic acid isomerase from the rumen *Butyriflavobacter fibrisolvans* and hence CLA is most abundant in ruminants than non ruminants. Roles of *B. fibrisolvans* on lipids profiles have been well discussed

(Kunsman, 1970). Another intriguing interest in CLA is that it has been a subject of research as an anti-carcinogenic, anti-atherogenic when applied subcutaneously in rat (Ha *et al.* (1987) and Pariza *et al.* (1979) but Shantha *et al.* (1994) noted that cooking temperatures and methods could influence CLA concentrations in beef if CLA is formed as a result of thermal oxidation of C_{18:2} (n-6) 9, 12 octadecadienoic acid (Linoleic acid). Therefore, it should be a concern in Nigeria where cooking always involves high temperature.

Thus this study was designed to:

- i) determine the proximate analysis of raw, boiled and smoked beef products;
- ii) investigate the fatty acid compositions and CLA contents of the three samples;
- iii) assess the palatability attributes of boiled and smoked beef using sensory trained panelist;

Materials and Methods

Longissimus dorsi beef muscles (LDBM) were used for this study as its intramuscular fat had been reported to have high concentration of CLA (Wasilewski *et al.* 2011). Thirty samples of about 100g each for the production of raw, boiled and smoked beef were cut into chunks after trimming off connective tissues and subcutaneous fat. The chunks were further sliced into sheets of average thickness of 0.7 cm. The sheets were dusted with a mixture of groundnut cake powder (kulikuli), salt, ginger, red pepper (barkono), melegueta (chilla mai kwaya), magi (dadawa) and spices (masoro) and arranged around a glowing smokeless fire about 35cm distance from the fire as described for 'Suya' (Igene and Ekanem,

1985). They were then skewered on to slender wooden sticks (30cm long) with the average weight of beef per stick as 110 +/- 0.2g. With intermittent turning of the product, the temperature at the distance described was about 75.5°C to 77.7°C For moist cooked samples, separate chunks were moist cooked to internal temperature of about 76.1°C using a conventional meat internal-probe thermometer. The cooking time is about 30 mins. Except for the samples minced for the sensory evaluation which was served warmed, all samples were allowed to cool to room temperature, wrapped in freezer papers and stored at -20°C until further analyzed.

Lipids were extracted using the dry column procedure of Marmer and Maxwell (1981) as modified by Agboola (1984) and separated lipids were re-extracted in hexane solution and purified using microfiltration procedure of Miller *et al.* (1979). The extracted lipid in hexane solution was quantitatively transferred into 25mm Teflon-lined screw cap test tubes and stored (-20 °C) until further analyzed. For fatty acids methyl esters (FAME) preparation, the frozen tubes were allowed to warm at room temperature and vortexed vigorously. Aliquots of the solution were transferred into 25mm culture tubes and 10ul of heneicosanoic acid (C_{21:0}) added as internal standard for long chain fatty acids (LCFA) and hexane evaporated under vacuum (Miller *et al.*, 1979). Three millimeters of ethanol were added to the tubes and the tubes were placed in a water bath at a temperature of 90 °C for 1 hour with periodic vortexing. The tubes were then cool, after which 3ml hexane and 5 ml of de-ionized water were added. The tubes were wrist-shaken for 10 minutes, centrifuged at 2000 rpm for 10 minutes and the upper hexane layer was discarded. The extraction was repeated but no water was

added. One ml of concentrated hydrochloric acid (HCl) was added to each tube followed by 3ml hexane and shaken and centrifugation was repeated. Most of the hexane solution of free fatty acid (FFA) was transferred to 12 x 100mm culture tubes and hexane evaporated under nitrogen vacuum. About 800ul of boron trifluoride methanol (14%, Supolco, Bellenfonte, PA, U. S. A.) were added to each tube, and the tubes were incubated at 50 °C in a water bath for 20 minutes with frequent vortexing. After cooling to room temperature, 3ml hexane, 5 ml of water were orderly added and tubes wrist-shaken again for 10 minutes, centrifuged at 2000rpm for 10 minutes and the upper phase which is the Fatty Acid Methyl Ester (FAME) were transferred to 10 x 75mm test tubes and corked. FAME were stored at –20 °C until analyzed by GC-MS chromatography. The chromatographic conditions of Miller *et al.* (1986) were used for fatty acids analysis while the conjugated linoleic acid in the extracted lipid was analyzed as described by Shantha *et al.* (1994).

Statistical Analysis

Statistical analyses were performed using the ANOVA procedure and means separated using Duncan-protected test when a significant ($p < 0.05$) differences existed as described by Steel and Torrie (1980).

Results and Discussion

Proximate results of raw, boiled and smoked beef are presented in Table 1. Smoked beef had the highest protein content of 26.48% followed by the boiled beef (23.58%). Highest carbohydrate value of 16.28% was recorded in smoked beef as against the 14.38% in cooked ones. Raw beef has the lowest amount of carbohydrate. As expected values of moisture and lipids are inversely related as increase in moisture was accompanied by a decrease in total lipid content. Lipid and moisture values in our study are expressed on a basis of 100g fresh weight of the *longissimus dorsi* muscle and resemble the procedure used to describe a 100g portion of a well trimmed steak of Miller *et al.* (1981).

Fatty acids composition of the raw, boiled and cooked longissimus muscle samples are presented in Table 2. The major fatty acids are palmitic ($C_{16:0}$), stearic ($C_{18:0}$) and oleic ($C_{18:1}$) which would be expected in tissue of ruminant animals (Miller *et al.* 1981, 1986). The level of 9,11 CLA with the chromatograph of retention time of 28.01mins across all treatments are 2.39% in the raw sample and significantly ($p < 0.05$) reduced by boiling and smoking as shown in the table. In this study, boiled and smoked beef were processed to the internal temperature of 76.1°C using internal probe thermometer as recommended by the USDA (1975). This conforms to the USDA safety recommendation of internal

Table 1. Proximate composition (%) and Internal temperature (IT) of raw, boiled and smoked longissimus dorsi muscle samples

Samples	Protein	Fat	Moisture	Ash	CHO	IT (°C)
A	26.48 ± 0.014 ^c	8.53 ± 0.014 ^c	46.76 ± 0.014 ^a	1.95 ± 0.014 ^c	16.28 ± 0.014 ^c	70.5
B	23.57 ± 0.028 ^b	7.36 ± 0.028 ^b	58.77 ± 0.028 ^b	1.78 ± 0.014 ^b	14.38 ± 0.014 ^b	70.5
C	19.86 ± 0.014 ^a	2.48 ± 0.014 ^a	74.56 ± 0.014 ^c	1.68 ± 0.014 ^a	1.40 ± 0.014 ^a	70.1

Means with different alphabets within the same column are significantly different ($p < 0.05$).

A = boiled beef; B = smoked beef and C = raw beef.

temperature of 73.8 ° C for cooked beef which is beyond the 'Temperature Danger Zone' of 7.2 – 60 ° C. (Prescott *et al.*, 1999). Temperature greater than 60° C will kill most vegetative cells but the spores and thermophiles species may survive while temperature below 7.2 ° C may temporarily cease growth that may surge when temperature rises. With the high temperature ways of cooking in Nigeria, and if cross-contamination and temperature abuse can be avoided, such products described herein should be safe. USDA (2003, 2013 and 2014) classified cooking temperature for beef with 60°C as medium rare, 70°C, as medium; of which the latest 75°C as 'well done' being the acceptable cooking stage for meat in the Nigerian culture. Shanta *et al.* (1994) cooked their patties to 60°C at internal temperature which is lower than the one being reported in this study. Such difference of 16.1°C we suggest is sufficient to cause the difference in amount of CLA reported in both studies but does not present significant concern. Arachidonic acid, a polyenoic acid (C_{20:4}) found to be higher in raw beef (8.17%) but considerably lower in boiled (1.49%) and smoked (1.72%) can only be attributed to

effect of heat and hence cooking loss. An important polyunsaturated fatty acid, C_{20:4} has been elucidated to be nutritionally important in production of cholesterol, an important precursor in biosynthesis of sex hormones in mammal (Marieba, 1995). Fatty acids by classes reported as % are presented in Table 3. Of considerable interest is the level of omega-3 fatty acids being reported. Klimis-Zacas (2006) reported that omega-3-fatty acids found in fish and some plant oils can reduce the likelihood of ventricular fibrillation a heart rhythm disturbance that causes sudden death. Sensory evaluation using fifteen experienced food scientists shown on the scale of 1-7 (where 7 stands for most preferred and 1 least preferred) did not (p.>0.05) show any dislike or preference for the boiled (5.3 vs 5.10 ± 0.16) and smoked samples in this study.

Implications

Due to arrays of factors that may affect food safety during processing, the findings that traditional cooking methods involving high heat preserve beneficial fatty acids such as CLA is encouraging. Particularly that high internal temperature often used in traditional cooking method of meat in

Table 2. Relative fatty acid compositions, conjugated linolenic acid (CLA) of the raw, boiled and smoked longissimus dorsi muscle samples

Fatty Acids (%)	A	B	C
C _{14:0}	2.96 ± 0.014 ^a	4.40 ± 0.014 ^c	3.71 ± 0.014 ^b
C _{16:0}	22.40 ± 0.014 ^a	31.89 ± 0.014 ^c	26.86 ± 0.014 ^b
C _{18:0}	24.09 ± 0.014 ^c	19.55 ± 0.014 ^b	18.57 ± 0.014 ^a
C _{18:1}	44.73 ± 0.014 ^c	37.27 ± 0.014 ^b	35.00 ± 0.028 ^a
C _{18:2}	2.67 ± 0.014 ^a	3.51 ± 0.014 ^c	3.40 ± 0.014 ^b
C _{18:3}	1.05 ± 0.014 ^a	1.16 ± 0.014 ^b	1.34 ± 0.014 ^c
9, 11 CLA	0.60 ± 0.014 ^b	0.49 ± 0.014 ^a	2.39 ± 0.014 ^c
C _{20:4}	1.49 ± 0.014 ^a	1.72 ± 0.014 ^b	8.17 ± 0.014 ^c

Means with different alphabets within the same row are significantly different (p < 0.05).

A = boiled beef; B = smoked and C = raw beef.

Table 3. Percent Fatty acids grouping by degree of saturation, CLA content of raw, boiled, and smoked longissimus dorsi muscle samples

Classes of Fatty Acids	SAMPLE		
	A	B	C
SFA	49.45 ± 0.014 ^b	55.84 ± 0.014 ^c	49.14 ± 0.014 ^a
MUFA	37.27 ± 0.014 ^b	44.73 ± 0.014 ^c	18.57 ± 0.014 ^a
CLA	0.60 ± 0.014 ^b	0.49 ± 0.014 ^a	2.39 ± 0.014 ^c
PUFA	5.21 ± 0.014 ^a	6.39 ± 0.014 ^b	5.21 ± 0.014 ^a
OMEGA-3	1.05 ± 0.014 ^a	1.16 ± 0.014 ^b	1.30 ± 0.014 ^c
OMEGA-6	4.21 ± 0.014 ^a	6.39 ± 0.014 ^b	12.87 ± 0.014 ^c
OMEGA-3/OMEGA-6	0.25 ± 0.014 ^b	0.18 ± 0.014 ^a	4.17 ± 0.014 ^c

Means with different alphabets within the same row are significantly different (p < 0.05).

A = boiled beef; B = smoked beef and C = raw beef. SFA = saturated fatty acid, MUFA = monounsaturated fatty acid; PUFA = polyunsaturated; OMEGA-3 = omega-3; OMEGA-6 omega 6 and OMEGA-3:OMEGA-6 = ratio of omega-3 to omega-6 fatty acids. CLA is Conjugated linoleic acid.

Nigeria is microbiologically safe as long as cross-contamination by the 3 Fs: fingers, flies, and feces are avoided.

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