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# Changes of Fatty Acids Composition in Beef under Different Thermal Treatment

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**Abstract** Beef semitendinosus muscles were collected from ten bulls' carcasses and used to determine fatty acids changes with different thermal treatment, boiling or microwave cooking. The results obtained show variabilities of fatty acids profiles in neutral lipid (NL), polar lipid (PL) and total lipid (TL) fraction under different internal temperature (60°C, 70°C or 80°C). Generally, in NL fraction (mainly beef intramuscular fat), content of polyunsaturated fatty acids (PUFA) increased ($P < 0.05$) significantly with boiling compared with raw beef, however, content of monounsaturated fatty acids (MUFA) and PUFA unchanged ($P > 0.05$) with the method of microwave cooking. On considering health benefit, it is proposed that beef with abundant intramuscular fat were more suitable for being treated with boiling, not microwave cooking. In PL and TL fraction, content of PUFA decreased ($P < 0.05$) with boiling and microwave cooking comparing with raw beef. Ratios of P/S (PUFA to saturated fatty acid (SFA)) decreased in TL with boiling or microwave cooking, whistle, ratios of M/S (MUFA to SFA) did not change under two thermal treatments in TL. Values of n-6/n-3 (n-6 PUFA to n-3 PUFA) increased significantly when beef internal temperature reached 80°C comparing with 60°C or 70°C despite being boiled or microwave cooked. Based on these observations, we considered that beef should not be over-heated when treated with boiling or microwave cooking to achieve premium ratio of n-6/n-3, the terminal core temperature of around 70°C was more appropriate.

***Keywords:*** *beef, fatty acids, microwave cooking, boiling, thermal treatment*

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## 1. Introduction

In the past, food quality was more related to sensory, shelf-life and safety aspects of food products, but more recently it is associated with nutrition and health [1]. Meat and meat products are generally recognized as highly nutritious foods that provide valuable amounts of vitamins, minerals, fatty acids (FA) and other bioactive compounds [2], and consumers consider meat to be a healthy and important component of the diet [3].

Moreover, beef consumption become more important in China due to the improving economy and dietary habits change [4]. Beef consumption is promoted for the health benefits because of its nutritional value including vitamin and mineral content, but criticized for the high proportion of saturated fatty acid (SFA) in fat fraction especially in subcutaneous and intermuscular fat [5]. SFA increases plasma cholesterol and low density lipoprotein (LDL) levels [6,7,8], promotes development of atherosclerosis leading to coronary heart disease (CHD) or cardiovascular disease (CVD) [8,9,10], whereas polyunsaturated fatty acids (PUFA), especially n-3 serial PUFA, reduces the risk of CHD [11,12]. Monounsaturated fatty acids (MUFA) as well as n-3 PUFA are recognized as benefit for health [13,14,15]. Previous studies confirm that FA profiles can be influenced by numerous factors such as breed [16,17,18], diet [19,20] and different regions [21]. Duckeet also reported that cooking can increase stearic acid and total SFA contents of intramuscular lipid and reduce total PUFA content [22].

On the other hand, the use of the microwave oven for cooking has increased considerably during the past few decades[23]. There are numerous reports on the cooking of meat using microwaves. During microwave cooking, the air surrounding the meat is cold and water evaporating from meat which makes the meat condensed [24]. Microwave cooking also changes flavor qualities of food in a lesser extent as opposed to conventional heating [25]. Microwave cooking is caused by the ability of the food materials (mainly the moisture or water in food) to absorb microwave energy and convert it into heat. However, microwave can generate high temperature may affect the oxidation of the lipids and quantitatively change the FA composition [26,27]. García-Arias, Álvarez Pontes [28] found SFA increased, but n-3 PUFA reduced in sardine with microwave processing. Maranesi, Bochicchio [29] reported FA composition changed in lamb due to microwave treated, however limited research is available

to document changes in FAs composition of beef with microwave cooking.

Previous studies have focused on nutritional value related to changes in FA composition during cooking [29,30,31]. However, few studies have examined the effects of different thermal treatment on FA of different lipid fractions [22,32]. The objective of this study was to assess changes in FAs composition of the neutral lipid (NL), polar lipid (PL), and total lipid (TL) with boiling and microwave cooking, and evaluate the implications to human health.

## 2. Materials and Methods

### 2.1. Preparation of Animals and Beef

Ten bulls of Chinese Yellow Cattle (live weight, 463 ± 35kg) ranging from 18 to 24 months of age were used in current experiment. Animals were pen-housed, fed with the common diet pattern including alfalfa, maize silage, and long-stem hay, followed by a diet consisting of maize for three months. The animals were slaughtered in a commercial meat processing abattoir (Changchun Haoyue Islam Meat Co. Ltd.) in northeastern China. After 2 days of chilling (0-2°C), carcasses were segmented, then semitendinosus muscles were obtained from left side. All samples were prepared 25mm thickness each, vacuum packed, and stored at -20°C until use.

Beef samples were thawed in room temperature (about 10°C) for 24 h and one steak sampled was used directly as the control (uncooked raw beef steak with room temperature about 10°C). Samples were individually placed inside polyethylene bags and cooked in a boiled water bath for approximate 60s, 130s, and 250s to get the final internal temperatures of 60°C, 70°C and 80°C respectively. Internal temperature was determined using thermocouples piercing probes attached to a thermometer. In microwave cooking treatment, preliminary testing was conducted first to attain the final internal temperature needed. Beef samples were placed in separate glass dishes in a pan placed in the centre of the carousel of a 2450 MHz, 700W variable power oven (G70F20N2L-DG(SO), Galanz, China). The power control was set at 350W for 30s, then shut down the microwave oven for 10s to permit the internal heat equilibration, and measured the internal temperature of beef samples with digital thermocouples piercing probes outside the microwave oven, and then went on with the repeated procedure of microwave cooking (30s) - shutting down (10s) - measuring internal temperature until the internal temperature reaching 60°C, 70°C or 80°C. Each treatment was conducted with three replications.

### 2.2. Lipid Extraction

Beef samples were dissected free of intermuscular fat and the epimysium. The content of NL, PL and TL were extracted by Dry Column method [33], as described briefly below. Beef samples (2g) were ground in a 750ml porcelain mortar with granular anhydrous sodium sulfate (8g) and then with diatomaceous earth (6 g), and the resulting mixture was packed above an 1:9 $CaHPO_4$/diatomaceous earth trap (4g) in a glass chromatography column (16mm id×25cm with 8mm id×5cm drip tip) and charged first with 80ml of dichloromethane, when the last of the dichloromethane reached the top of the column packing, the volumetric flask (100ml) containing the collected eluate (NL) was replaced by a second 100ml flask, and the column was charged with 80ml of the 9:1 (v/v) $CH_2Cl_2$/MeOH solvent mixture. The first (NL) and second flasks (PL) were vacuum distilled in relatively low temperature (25°C). Another 2g of the sample was processed in the same manner, instead of charging with 80ml of the 9:1 solvent mixture directly, the third flask (TL) was collected and vacuum distilled.

### 2.3. Fatty Acids Analyses

The saponification, methylation, and FAs analyses of NL, PL and TL were based on the methods of [34,35], and the AOCS [36]. Lipids were saponified using methanolic NaOH and methylated using a freshly prepared methanolic $BF_3$ solution. The dry methanol solution of FA methyl esters was stored at 0°C in a refrigerator until analysis. To determine the FA composition, 100 mg of the extracted lipid was dissolved in 2ml of hexane and added with 50μg of heneicosanoic acid methyl ester (21:0, Augsburg, Germany) as an internal standard. For all steps in the procedure the extract was blanketed with nitrogen. Methylated lipid samples were analyzed using a flame ionization detector (FID) on a gas chromatograph (Shimadzu GC14A, Kyoto, Japan) equipped with a 50m × 0.32mm capillary column (CP-Sil 88, Varian Inc., CA, USA). Both injector and detector temperatures were set at 280°C. The initial oven temperature was 160°C, held for 2min, raised by a gradient of 5°C/min to 220°C, and held for 30min, for a total run time of 44 min. The pressures of the gases were 0.8kg/cm$^2$ for the carrier gas (nitrogen), 0.6kg/cm$^2$ for the hydrogen, 0.6kg/cm$^2$ for make-up gas (nitrogen), and 0.5kg/cm$^2$ for the combustion air. Chromatograms were recorded with a computing integrator (Shimadzu Chromatopac C-R6A). Identification of sample FAs was made by comparing the relative retention times of standard FA methyl-esters, and the relative proportions were determined as percentages of summed peak areas.

### 2.4. Ratios and Indices of Fatty Acids

The group of SFA consists of 12:0, 14:0, 15:0, 16:0, 17:0 18:0 and 20:0, while group of MUFA consists of 14:1 *cis*-9, 16:1 *trans*-9, 16:1 *cis*-9, 18:1 *trans*-11, 18:1 *cis*-9, and 22:1 *cis*-13. The group of PUFA includes n-6 PUFA (omega-6 PUFA, including 18:2*cis*-9,12, 18:3 *cis*-6,9,12, 20:3 *cis*-8,11,14, 20:4 *cis*-5,8,11,14, 22:4 *cis*-7,10,13,16) and n-3 PUFA (omega-3 PUFA, including 18:3 *cis*-9,12,15, 20:5 *cis*-5,8,11,14,17, 22:5 *cis*-7,10,13,16,19, 22:6 *cis*-4,7,10,13,16,19). Ratios of PUFA to SFA (P/S), MUFA to SFA (M/S) and n-6 PUFA to n-3 PUFA (n-6/n-3) were calculated. Attempts were conducted to develop better indices of the potential health attributes of foods containing a mixture of FAs with their indices of atherogenicity (IA) and thrombogenicity (IT) [12]. The mg FA of 100g meat was used to calculate the indices.

$$IA = \frac{(12:0 + 4\times 14:0 + 16:0)}{(MUFA + PUFA)}$$

$$IT = \frac{(12:0 + 16:0 + 18:0)}{0.5 \times (MUFA + n\text{-}6PUFA) + 3 \times (n\text{-}3PUFA) + \frac{(n\text{-}3PUFA)}{(n\text{-}6PUFA)}}$$

## 2.5. Statistical Analyses

Ratios and indices were calculated individually of each beef sample before (raw) or after thermal treatment with different internal temperature. Mean differences of FAs from different lipid fractions were analyzed as a 2×3 factorial experimental design, by GLM-ANOVA model, with the factors of thermal treatment (boiling and microwave cooking) and terminal temperature (60°C, 70°C and 80°C), and using the raw meat data as a covariate. The statistical analysis was performed using SPSS 20.0.

# 3. Results and Discussion

**Table 1. Changes in fatty acid composition (%) in the neutral lipid (NL) of beef (n = 10) †**

| Fatty acid | Raw | Boiling | | | Microwave cooking | | | SEM | Effect Sig. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 60°C | 70°C | 80°C | 60°C | 70°C | 80°C | | HT | TT | HT×TT |
| 12:0 | 0.18$^{a}$ | 0.08$^{bc}$ | 0.10$^{b}$ | 0.08$^{bc}$ | 0.07$^{bc}$ | 0.05$^{c}$ | 0.06$^{c}$ | 0.01 | * | NS | NS |
| 14:0 | 2.4 | 2.1 | 2.1 | 2.0 | 2.2 | 2.3 | 2.2 | 0.07 | NS | NS | NS |
| 14:1 *cis*-9 | 0.67$^{a}$ | 0.37$^{b}$ | 0.40$^{b}$ | 0.39$^{b}$ | 0.43$^{b}$ | 0.43$^{b}$ | 0.46$^{ab}$ | 0.03 | NS | NS | NS |
| 15:0 | 0.07$^{c}$ | 0.08$^{c}$ | 0.08$^{c}$ | 0.08$^{c}$ | 0.30$^{bc}$ | 0.83$^{a}$ | 0.51$^{b}$ | 0.36 | *** | ** | NS |
| 16:0 | 21.5 | 21.8 | 22.2 | 21.7 | 23.2 | 22.6 | 22.9 | 0.38 | NS | NS | NS |
| 16:1 *trans*-9 | 0.36$^{b}$ | 0.43$^{a}$ | 0.41$^{ab}$ | 0.42$^{a}$ | 0.43$^{a}$ | 0.43$^{a}$ | 0.43$^{a}$ | 0.01 | NS | NS | NS |
| 16:1 *cis*-9 | 3.0$^{ab}$ | 2.6$^{b}$ | 2.9$^{ab}$ | 2.7$^{ab}$ | 3.1$^{a}$ | 2.5$^{b}$ | 3.1$^{a}$ | 0.11 | * | NS | NS |
| 17:0 | 1.1 | 1.1 | 1.0 | 0.9 | 0.9 | 0.8 | 0.9 | 0.03 | NS | NS | NS |
| 17:1 *cis*-9 | ND$^{c}$ | ND$^{c}$ | ND$^{c}$ | ND$^{c}$ | 0.36$^{ab}$ | 0.44$^{a}$ | 0.28$^{b}$ | 0.05 | *** | * | NS |
| 18:0 | 16.1 | 17.5 | 17.4 | 17.8 | 16.6 | 17.2 | 16.2 | 0.47 | NS | NS | NS |
| 18:1 *trans*-11 | 2.4$^{a}$ | 2.2$^{ab}$ | 2.2$^{ab}$ | 2.2$^{ab}$ | 2.2$^{ab}$ | 1.8$^{b}$ | 1.9$^{b}$ | 0.12 | NS | NS | NS |
| 18:1 *cis*-9 | 36.9$^{ab}$ | 34.8$^{b}$ | 36.5$^{ab}$ | 34.3$^{b}$ | 39.2$^{a}$ | 36.3$^{ab}$ | 39.6$^{a}$ | 0.78 | * | NS | NS |
| 18:2 *cis*-9, *trans*-11 | 0.18$^{a}$ | 0.18$^{a}$ | 0.13$^{a}$ | 0.17$^{a}$ | ND$^{b}$ | ND$^{b}$ | ND$^{b}$ | 0.02 | *** | NS | NS |
| 18:2 *cis*-9,12 | 3.2$^{c}$ | 5.4$^{a}$ | 5.0$^{ab}$ | 6.4$^{a}$ | 4.4$^{b}$ | 5.5$^{a}$ | 4.8$^{b}$ | 0.44 | * | * | * |
| 18:3 *cis*-6,9,12 | 0.82$^{a}$ | 0.70$^{ab}$ | 0.57$^{b}$ | 0.22$^{c}$ | ND$^{d}$ | ND$^{d}$ | ND$^{d}$ | 0.04 | *** | ** | NS |
| 18:3 *cis*-9,12,15 | 1.1$^{ab}$ | 1.2$^{a}$ | 1.2$^{a}$ | 0.8$^{bc}$ | 0.5$^{c}$ | 0.7$^{c}$ | 0.6$^{c}$ | 0.07 | ** | * | * |
| 20:0 | 0.65$^{a}$ | 0.29$^{c}$ | 0.42$^{b}$ | 0.35$^{bc}$ | 0.14$^{d}$ | 0.17$^{d}$ | 0.18$^{d}$ | 0.09 | * | NS | NS |
| 20:3 *cis*-8,11,14 | 0.39$^{a}$ | 0.19$^{b}$ | 0.21$^{b}$ | 0.17$^{b}$ | ND$^{c}$ | ND$^{c}$ | ND$^{c}$ | 0.03 | ** | NS | NS |
| 20:4 *cis*-5,8,11,14 | 0.6$^{c}$ | 1.3$^{ab}$ | 1.0$^{bc}$ | 1.5$^{a}$ | 0.7$^{c}$ | 0.9$^{bc}$ | 0.9$^{bc}$ | 0.02 | * | NS | NS |
| 20:5 *cis*-5,8,11,14,17 | 0.13$^{b}$ | 0.34$^{a}$ | 0.31$^{a}$ | 0.27$^{a}$ | 0.07$^{c}$ | 0.05$^{c}$ | 0.04$^{c}$ | 0.10 | *** | NS | NS |
| 22:1 *cis*-13 | 0.17$^{ab}$ | 0.12$^{b}$ | 0.11$^{b}$ | 0.13$^{b}$ | 0.10$^{b}$ | 0.11$^{b}$ | 0.22$^{a}$ | 0.03 | * | * | * |
| 22:4 *cis*-7,10,13,16 | 0.19$^{c}$ | 0.26$^{b}$ | 0.26$^{b}$ | 0.37$^{a}$ | 0.13$^{d}$ | 0.22$^{bc}$ | 0.17$^{c}$ | 0.02 | * | * | * |
| 22:5 *cis*-7,10,13,16,19 | 0.35$^{ab}$ | 0.44$^{a}$ | 0.35$^{ab}$ | 0.40$^{a}$ | 0.19$^{d}$ | 0.25$^{c}$ | 0.32$^{bc}$ | 0.04 | * | * | NS |
| 22:6 *cis*-4,7,10,13,16,19 | 0.14$^{a}$ | 0.12$^{a}$ | 0.08$^{b}$ | 0.04$^{c}$ | 0.06$^{bc}$ | 0.06$^{bc}$ | 0.07$^{bc}$ | 0.01 | NS | * | NS |
| Unknown - <C16 | 1.4$^{a}$ | 1.3$^{ab}$ | 1.2$^{ab}$ | 1.3$^{ab}$ | 1.1$^{b}$ | 1.2$^{ab}$ | 1.1$^{b}$ | 0.12 | NS | NS | NS |
| Unknown - C16~C18 | 2.6$^{a}$ | 2.6$^{a}$ | 2.2$^{ab}$ | 2.7$^{a}$ | 2.1$^{b}$ | 2.2$^{ab}$ | 1.9$^{b}$ | 0.13 | * | NS | NS |
| Unknown - >C18 | 3.6$^{a}$ | 2.6$^{ab}$ | 1.8$^{b}$ | 2.7$^{ab}$ | 1.6$^{bc}$ | 2.1$^{b}$ | 1.2$^{c}$ | 0.27 | * | * | NS |

$^{abcd}$ Comparisons within a row without a common superscript are significantly different ($P < 0.05$).

† ND, not detected; HT, heating treatment; TT, terminal temperature; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS, not significant.

**Table 2. Changes in fatty acid composition (%) in the polar lipid (PL) (n = 10) †**

| Fatty acid | Raw | Boiling | | | Microwave cooking | | | SEM | Effect Sig. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 60°C | 70°C | 80°C | 60°C | 70°C | 80°C | | HT | TT | HT×TT |
| 12:0 | 0.12$^{a}$ | 0.04$^{bc}$ | 0.04$^{bc}$ | 0.03$^{c}$ | 0.06$^{b}$ | 0.05$^{bc}$ | 0.05$^{bc}$ | 0.01 | NS | NS | NS |
| 14:0 | 0.16$^{b}$ | 0.18$^{b}$ | 0.09$^{c}$ | 0.08$^{c}$ | 0.13$^{bc}$ | 0.17$^{b}$ | 0.27$^{a}$ | 0.02 | * | ** | ** |
| 14:1 *cis*-9 | 0.55$^{a}$ | 0.42$^{b}$ | 0.45$^{b}$ | 0.41$^{b}$ | 0.43$^{b}$ | 0.49$^{ab}$ | 0.40$^{b}$ | 0.02 | NS | NS | NS |
| 15:0 | 8.2$^{a}$ | 7.1$^{ab}$ | 6.8$^{b}$ | 7.6$^{ab}$ | 7.9$^{ab}$ | 8.0$^{a}$ | 7.6$^{ab}$ | 0.19 | * | NS | NS |
| 16:0 | 10.2$^{b}$ | 11.5$^{ab}$ | 12.1$^{ab}$ | 12.4$^{a}$ | 11.6$^{ab}$ | 11.4$^{ab}$ | 12.4$^{a}$ | 0.22 | NS | NS | NS |
| 16:1 *trans*-9 | 0.51 | 0.46 | 0.48 | 0.46 | 0.46 | 0.44 | 0.48 | 0.02 | NS | NS | NS |
| 16:1 *cis*-9 | 0.90$^{a}$ | 0.81$^{ab}$ | 0.90$^{a}$ | 0.75$^{b}$ | 0.77$^{b}$ | 0.85$^{ab}$ | 0.91$^{a}$ | 0.06 | * | * | * |
| 17:0 | 0.49$^{a}$ | 0.36$^{b}$ | 0.44$^{a}$ | 0.46$^{a}$ | 0.36$^{b}$ | 0.37$^{b}$ | 0.38$^{b}$ | 0.02 | NS | * | NS |
| 17:1 *cis*-9 | 7.8$^{ab}$ | 8.6$^{a}$ | 7.8$^{ab}$ | 7.6$^{ab}$ | 7.3$^{ab}$ | 7.3$^{ab}$ | 7.1$^{b}$ | 0.31 | * | NS | NS |
| 18:0 | 11.5 | 12.1 | 11.8 | 12.3 | 12.2 | 12.0 | 12.0 | 0.20 | NS | NS | NS |
| 18:1 *trans*-11 | 0.51$^{bc}$ | 0.57$^{b}$ | 0.68$^{ab}$ | 0.60$^{b}$ | 0.59$^{b}$ | 0.48$^{c}$ | 0.79$^{a}$ | 0.04 | * | * | * |
| 18:1 *cis*-9 | 12.7$^{b}$ | 9.6$^{c}$ | 12.0$^{bc}$ | 12.2$^{bc}$ | 13.9$^{ab}$ | 15.4$^{a}$ | 15.5$^{a}$ | 0.97 | * | NS | NS |
| 18:2 *cis*-9, *trans*-11 | 0.18$^{bc}$ | 0.14$^{bc}$ | 0.23$^{b}$ | 0.53$^{a}$ | 0.06$^{c}$ | 0.06$^{c}$ | 0.05$^{c}$ | 0.03 | ** | * | * |
| 18:2 *cis*-9,12 | 22.6$^{ab}$ | 23.4$^{a}$ | 20.4$^{b}$ | 20.6$^{b}$ | 22.0$^{ab}$ | 21.4$^{ab}$ | 20.9$^{ab}$ | 1.06 | NS | NS | NS |
| 18:3 *cis*-6,9,12 | 0.39$^{a}$ | 0.03$^{c}$ | 0.04$^{c}$ | 0.03$^{c}$ | 0.11$^{b}$ | 0.10$^{b}$ | 0.11$^{b}$ | 0.05 | ** | NS | NS |
| 18:3 *cis*-9,12,15 | 1.7$^{a}$ | 1.3$^{b}$ | 1.3$^{b}$ | 0.9$^{c}$ | 1.5$^{ab}$ | 1.3$^{b}$ | 1.0$^{c}$ | 0.15 | NS | * | NS |
| 20:0 | ND | ND | ND | ND | ND | ND | ND | 0.00 | NS | NS | NS |
| 20:3 *cis*-8,11,14 | 0.29$^{a}$ | 0.13$^{b}$ | 0.05$^{c}$ | 0.04$^{c}$ | ND$^{d}$ | ND$^{d}$ | 0.19$^{b}$ | 0.03 | ** | * | ** |
| 20:4 *cis*-5,8,11,14 | 7.5 | 7.6 | 7.4 | 8.0 | 8.4 | 8.4 | 8.5 | 0.27 | NS | NS | NS |
| 20:5 *cis*-5,8,11,14,17 | 0.72$^{ab}$ | 0.71$^{ab}$ | 0.62$^{b}$ | 0.60$^{b}$ | 0.70$^{ab}$ | 0.80$^{a}$ | 0.84$^{a}$ | 0.8 | * | NS | * |
| 22:1 *cis*-13 | 0.17$^{c}$ | 0.52$^{a}$ | 0.17$^{c}$ | 0.25$^{b}$ | 0.18$^{c}$ | 0.17$^{c}$ | 0.14$^{c}$ | 0.06 | * | * | * |
| 22:4 *cis*-7,10,13,16 | 1.0$^{b}$ | 1.0$^{b}$ | 1.0$^{b}$ | 1.4$^{a}$ | 1.2$^{ab}$ | 1.0$^{b}$ | 1.2$^{ab}$ | 0.07 | NS | * | NS |
| 22:5 *cis*-7,10,13,16,19 | 1.6$^{ab}$ | 1.9$^{a}$ | 1.7$^{ab}$ | 1.4$^{b}$ | 1.6$^{ab}$ | 1.8$^{a}$ | 1.7$^{ab}$ | 0.09 | NS | * | * |
| 22:6 *cis*-4,7,10,13,16,19 | 0.18$^{a}$ | 0.03$^{b}$ | 0.03$^{b}$ | 0.02$^{b}$ | 0.14$^{a}$ | 0.14$^{a}$ | 0.15$^{a}$ | 0.01 | *** | NS | NS |
| Unknown - <C16 | 1.5$^{bc}$ | 2.0$^{a}$ | 2.2$^{a}$ | 1.6$^{ab}$ | 1.4$^{bc}$ | 1.3$^{c}$ | 1.3$^{c}$ | 0.12 | * | NS | NS |
| Unknown - C16~C18 | 2.2$^{ab}$ | 2.3$^{a}$ | 2.4$^{a}$ | 2.1$^{ab}$ | 2.0$^{ab}$ | 1.7$^{b}$ | 1.6$^{b}$ | 0.16 | * | NS | NS |
| Unknown - >C18 | 6.4$^{b}$ | 7.4$^{ab}$ | 8.9$^{a}$ | 8.5$^{a}$ | 5.3$^{c}$ | 4.9$^{cd}$ | 4.7$^{d}$ | 0.57 | * | * | ** |

$^{abcd}$ Comparisons within a row without a common superscript are significantly different ($P < 0.05$).

† ND, not detected; HT, heating treatment; TT, terminal temperature; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS, not significant.

Fatty acids profiles changed differently between boiling and microwave cooking. There was no interaction ($P < 0.05$) between thermal treatment (boiling or microwave cooking) and terminal temperature (60°C, 70°C, and 80°C) of the main FA composition in NL (Table 1), PL (Table 2), and TL (Table 3). Briefly, in TL, boiling treatment affected 15:0, 18:1 *cis*-9, 18:2 *cis*-9,*trans*-11, 18:3 *cis*-6,9,12, 20:0, 20:3 *cis*-8,11,14, 22:4 *cis*-7,10,13,16, 22:6 *cis*-4,7,10,13,16,19 (Table 3) and M/S ratio (Table 4); whistle, terminal temperature affect the FA of 15:0, 16:1 *cis*-9, 17:1 *cis*-9, 18:2 *cis*-9,*trans*-11, 18:3 *cis*-6,9,12, 18:3 *cis*-9,12,15, 20:0, 20:3 *cis*-8,11,14, 20:5 *cis*-5,8,11,14,17, 22:1 *cis*-13, 22:4 *cis*-7,10,13,16, 22:5 *cis*-7,10,13,16,19, the group of MUFA, n-3 PUFA (Table 3) and ratio of n-6/n-3 (Table 4). However, thermal treatment or terminal temperature did not affect ($P > 0.05$) SFA, PUFA, n-6 PUFA, P/S, IA and IT index.

**Table 3. Changes in fatty acid composition (%) in the total lipid (TL) (n = 10) †**

| Fatty acid | Raw | Boiling | | | Microwave cooking | | | SEM | Effect Sig. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 60°C | 70°C | 80°C | 60°C | 70°C | 80°C | | HT | TT | HT×TT |
| 12:0 | 0.14$^{a}$ | 0.03$^{b}$ | 0.04$^{b}$ | 0.04$^{b}$ | 0.05$^{b}$ | 0.04$^{b}$ | 0.05$^{b}$ | 0.01 | NS | NS | NS |
| 14:0 | 0.9 | 1.0 | 1.0 | 1.0 | 1.0 | 1.1 | 1.2 | 0.06 | NS | NS | NS |
| 14:1 *cis*-9 | 0.61$^{a}$ | 0.39$^{c}$ | 0.44$^{bc}$ | 0.39$^{c}$ | 0.43$^{bc}$ | 0.47$^{b}$ | 0.44$^{bc}$ | 0.02 | NS | NS | NS |
| 15:0 | 5.5$^{a}$ | 4.2$^{b}$ | 3.8$^{c}$ | 3.8$^{c}$ | 4.8$^{ab}$ | 5.4$^{a}$ | 4.2$^{b}$ | 0.20 | * | * | * |
| 16:0 | 14.0$^{c}$ | 16.0$^{b}$ | 16.5$^{ab}$ | 16.9$^{ab}$ | 16.4$^{ab}$ | 16.4$^{ab}$ | 17.4$^{a}$ | 0.37 | NS | NS | NS |
| 16:1 *trans*-9 | 0.46 | 0.45 | 0.46 | 0.44 | 0.46 | 0.43 | 0.45 | 0.01 | NS | NS | NS |
| 16:1 *cis*-9 | 1.6$^{ab}$ | 1.5$^{b}$ | 1.8$^{ab}$ | 1.7$^{ab}$ | 1.7$^{ab}$ | 1.5$^{b}$ | 2.0$^{a}$ | 0.08 | NS | * | NS |
| 17:0 | 0.7 | 0.6 | 0.7 | 0.7 | 0.6 | 0.6 | 0.6 | 0.02 | NS | NS | NS |
| 17:1 *cis*-9 | 5.3$^{a}$ | 4.9$^{ab}$ | 4.5$^{bc}$ | 4.0$^{c}$ | 4.5$^{bc}$ | 4.3$^{bc}$ | 3.9$^{c}$ | 0.23 | NS | * | NS |
| 18:0 | 13.0$^{b}$ | 14.6$^{a}$ | 14.0$^{ab}$ | 15.0$^{a}$ | 14.1$^{ab}$ | 14.3$^{ab}$ | 14.0$^{ab}$ | 0.29 | NS | NS | NS |
| 18:1 *trans*-11 | 1.1$^{ab}$ | 1.3$^{a}$ | 1.3$^{a}$ | 1.3$^{a}$ | 1.3$^{a}$ | 1.0$^{b}$ | 1.3$^{a}$ | 0.06 | NS | NS | NS |
| 18:1 *cis*-9 | 20.7$^{b}$ | 20.0$^{b}$ | 22.6$^{ab}$ | 22.7$^{ab}$ | 23.9$^{ab}$ | 24.2$^{a}$ | 26.7$^{a}$ | 0.91 | * | NS | NS |
| 18:2 *cis*-9, *trans*-11 | 0.18$^{a}$ | 0.16$^{a}$ | 0.19$^{a}$ | 0.12$^{b}$ | 0.04$^{c}$ | 0.03$^{c}$ | 0.04$^{c}$ | 0.02 | *** | * | * |
| 18:2 *cis*-9,12 | 15.7 | 15.4 | 13.8 | 14.0 | 14.8 | 14.7 | 13.5 | 0.80 | NS | NS | NS |
| 18:3 *cis*-6,9,12 | 0.53$^{a}$ | 0.26$^{b}$ | 0.24$^{b}$ | 0.10$^{c}$ | 0.06$^{d}$ | 0.06$^{d}$ | 0.06$^{d}$ | 0.02 | ** | * | * |
| 18:3 *cis*-9,12,15 | 1.5$^{a}$ | 1.3$^{ab}$ | 1.2$^{ab}$ | 0.8$^{c}$ | 1.1$^{b}$ | 1.0$^{bc}$ | 0.8$^{c}$ | 0.11 | NS | * | NS |
| 20:0 | 0.30$^{a}$ | 0.12$^{bc}$ | 0.16$^{b}$ | 0.17$^{b}$ | 0.06$^{d}$ | 0.07$^{d}$ | 0.09$^{cd}$ | 0.03 | ** | * | * |
| 20:3 *cis*-8,11,14 | 0.32$^{a}$ | 0.15$^{b}$ | 0.08$^{c}$ | 0.09$^{c}$ | ND$^{d}$ | ND$^{d}$ | 0.10$^{c}$ | 0.02 | * | * | * |
| 20:4 *cis*-5,8,11,14 | 5.1$^{a}$ | 4.9$^{ab}$ | 4.6$^{b}$ | 4.8$^{ab}$ | 5.3$^{a}$ | 5.1$^{ab}$ | 4.8$^{ab}$ | 0.16 | NS | NS | NS |
| 20:5 *cis*-5,8,11,14,17 | 0.53$^{ab}$ | 0.60$^{a}$ | 0.47$^{b}$ | 0.43$^{b}$ | 0.48$^{b}$ | 0.47$^{b}$ | 0.46$^{b}$ | 0.04 | NS | * | NS |
| 22:1 *cis*-13 | 0.17$^{b}$ | 0.37$^{a}$ | 0.15$^{b}$ | 0.18$^{b}$ | 0.15$^{b}$ | 0.14$^{b}$ | 0.18$^{b}$ | 0.04 | NS | * | * |
| 22:4 *cis*-7,10,13,16 | 0.73$^{b}$ | 0.65$^{b}$ | 0.70$^{b}$ | 0.90$^{a}$ | 0.72$^{b}$ | 0.67$^{b}$ | 0.69$^{b}$ | 0.07 | * | * | * |
| 22:5 *cis*-7,10,13,16,19 | 1.1$^{bc}$ | 1.4$^{a}$ | 1.1$^{bc}$ | 0.9$^{c}$ | 1.1$^{bc}$ | 1.1$^{bc}$ | 1.0$^{bc}$ | 0.10 | NS | * | * |
| 22:6 *cis*-4,7,10,13,16,19 | 0.17$^{a}$ | 0.05$^{c}$ | 0.04$^{c}$ | 0.03$^{c}$ | 0.11$^{b}$ | 0.11$^{b}$ | 0.10$^{b}$ | 0.01 | ** | NS | NS |
| Unknown - <C16 | 1.8$^{ab}$ | 1.7$^{ab}$ | 1.9$^{a}$ | 2.0$^{a}$ | 1.6$^{b}$ | 1.6$^{b}$ | 1.4$^{b}$ | 0.10 | * | NS | NS |
| Unknown - C16~C18 | 2.1$^{a}$ | 2.1$^{a}$ | 2.2$^{a}$ | 1.9$^{ab}$ | 1.5$^{bc}$ | 1.4$^{c}$ | 1.2$^{c}$ | 0.11 | * | NS | NS |
| Unknown - >C18 | 5.7$^{a}$ | 6.1$^{a}$ | 6.0$^{a}$ | 5.5$^{a}$ | 4.0$^{b}$ | 4.1$^{b}$ | 3.3$^{c}$ | 0.23 | * | * | NS |

$^{abcd}$ Comparisons within a row without a common superscript are significantly different ($P < 0.05$).

† ND, not detected; HT, heating treatment; TT, terminal temperature; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS, not significant.

**Table 4. Changes in ratios and indices in the total lipid (TL) (n = 10) †**

| Ratios and indices | Raw | Boiling | | | Microwave cooking | | | SEM | Effect Sig. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 60°C | 70°C | 80°C | 60°C | 70°C | 80°C | | HT | TT | HT×TT |
| P/S | 0.75$^{a}$ | 0.69$^{ab}$ | 0.63$^{b}$ | 0.59$^{b}$ | 0.58$^{b}$ | 0.62$^{b}$ | 0.65$^{b}$ | 0.03 | NS | NS | NS |
| M/S | 0.87$^{ab}$ | 0.79$^{b}$ | 0.86$^{ab}$ | 0.81$^{b}$ | 0.93$^{a}$ | 0.85$^{ab}$ | 0.88$^{ab}$ | 0.02 | * | NS | NS |
| n-6/n-3 | 6.7$^{c}$ | 8.4$^{b}$ | 7.9$^{b}$ | 11.2$^{a}$ | 9.5$^{b}$ | 9.1$^{b}$ | 10.5$^{a}$ | 0.81 | NS | * | NS |
| IA | 0.32$^{b}$ | 0.39$^{ab}$ | 0.38$^{ab}$ | 0.40$^{a}$ | 0.38$^{ab}$ | 0.38$^{ab}$ | 0.42$^{a}$ | 0.02 | NS | NS | NS |
| IT | 0.8$^{b}$ | 1.0$^{a}$ | 0.9$^{a}$ | 1.0$^{a}$ | 1.0$^{a}$ | 1.0$^{a}$ | 1.0$^{a}$ | 0.05 | NS | NS | NS |

$^{abc}$ Comparisons within a row without a common superscript are significantly different ($P < 0.05$).

† HT, heating treatment; TT, terminal temperature; * $P \leq 0.05$; NS, not significant.

## 3.1. SFA

In FA composition of NL, percentage of total SFA did not change with boiling or microwave cooking. However, boiling or microwave cooking increased ($P < 0.05$) percentage of total SFA more than 6% in PL when heated to 80°C (Figure 1), which mainly caused by significant increase ($P < 0.01$) of 16:0 percentage. In TL, percentages of 16:0, 16:0 + 18:0 and total SFA were increased ($P < 0.05$) significantly after boiling and microwave cooking treatment. It was noted that SFA increased ($P < 0.05$) 9.09% in TL with boiling at the internal temperature of 80°C, while SFA only increased about 5% ($P > 0.05$) when internal temperature reached 60°C or 70°C. SFA increased ($P < 0.05$) 7%~9% in TL when beef microwave cooking to internal temperature from 60°C to 80 °C. It is suggested that beef overcooked (internal temperature ≥ 80°C) was not good for human health as SFA increased significantly.

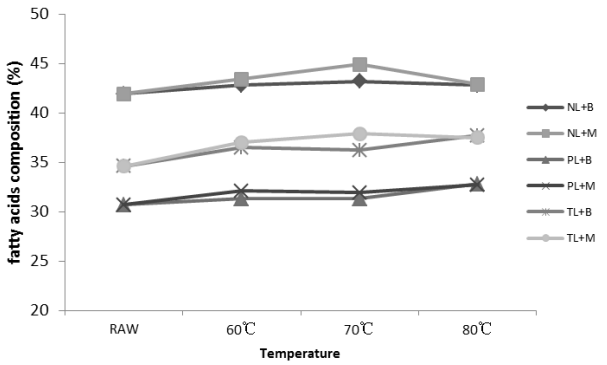


**Figure 1.** Changes in SFA composition (%) in beef different fraction (NL, PL and TL) before (RAW) and after boiling (B) or microwave cooking (M)

## 3.2. MUFA

The MUFA composition of NL (Figure 2) was slightly reduced ($P > 0.05$) after the beef cooked to an internal temperature of 80°C compared with control, which mainly due to a reduction in 18:1*cis*-9 percentage. However, MUFA composition was increased ($P > 0.05$) conversely after the beef microwave heated compared with raw beef in NL. The MUFA composition of PL was reduced ($P > 0.05$) after the beef cooked to an internal temperature of 80°C. However, percentage of 18:1*cis*-9 was increased ($P > 0.05$) slightly with beef microwave cooking compared with raw, and percentage of 17:1*cis*-9 did not change ($P > 0.05$). These changes caused the MUFA of PL increased ($P > 0.05$) conversely after the beef microwave cooked compared with raw. The proportion of MUFA in TL (Figure 2) did not differ significantly ($P > 0.05$) after boiling. However, percentage of 18:1*cis*-9 was increased ($P < 0.05$) which caused the proportion of MUFA in TL increased ($P < 0.05$) with microwave cooking.

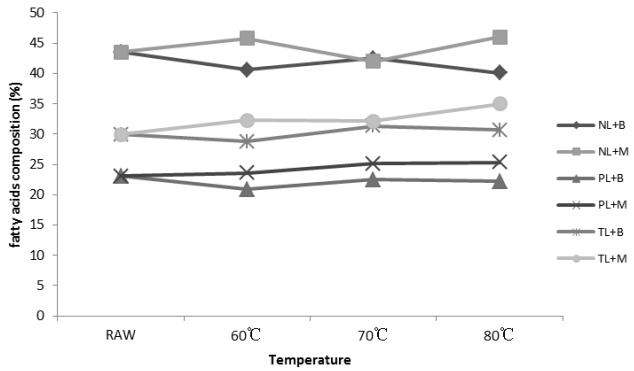


**Figure 2.** Changes in MUFA composition (%) in beef different fraction (NL, PL and TL) before (RAW) and after boiling (B) or microwave cooking (M)

## 3.3. PUFA

The fatty acids of PUFA, especially n-3 serial PUFA, reduces the risk of CHD [12], thus, changes of n-3 PUFA deserve particular attention during beef boiled or microwave cooked. In NL (Figure 3), total PUFA and n-6 PUFA composition increased ($P < 0.05$) after boiling to the internal temperature of 60°C or 80°C (mainly caused by increase of 18:2 *cis*-9,12 and 20:4 *cis*-5,8,11,14). Salcedo-Sandoval also found that cooked pork samples had higher concentrations of MUFAs and PUFAs (both n-3 and n-6) compared with raw products [31]. Stephen investigated the chemical changes involved during cooking (boiling), frying, canning and microwave heating of skipjack tuna (Katsuwonus pelamis), and found that the cholesterol content and the health beneficial n-3 PUFA of heat processed tuna fish were increased comparing with raw fish, thus recommended that boiling and microwave heating could be used to process tuna to retain n-3 fatty acids [37]. Composition of PUFA did not change ($P > 0.05$) after microwave cooking. Moreover, composition of n-3 PUFA was reduced ($P < 0.05$) with beef microwave heated in NL. In PL (Table 2), percentage of 18:3 *cis*-9,12,15 was reduced ($P < 0.05$), which caused total PUFA and n-3 PUFA composition reduced ($P < 0.05$) after boiling to the internal temperature of 70°C and 80°C. Compositions of total PUFA and n-3 PUFA did not change ($P > 0.05$) after microwave cooking in PL. Fatty acid of 20:3 *cis*-8,11,14 was not detected after microwave cooking in the PL fraction. In TL (Table 3), percentage of 18:2 *cis*-9,12 was slightly reduced ($P > 0.05$), however, 18:3 *cis*-9,12,15 and 20:4 *cis*-5,8,11,14 decreased with two thermal treatment. These changes caused total PUFA and n-3 PUFA compositions reduced ($P < 0.05$) in TL after the internal temperature of boiling or microwave cooking over 70°C.

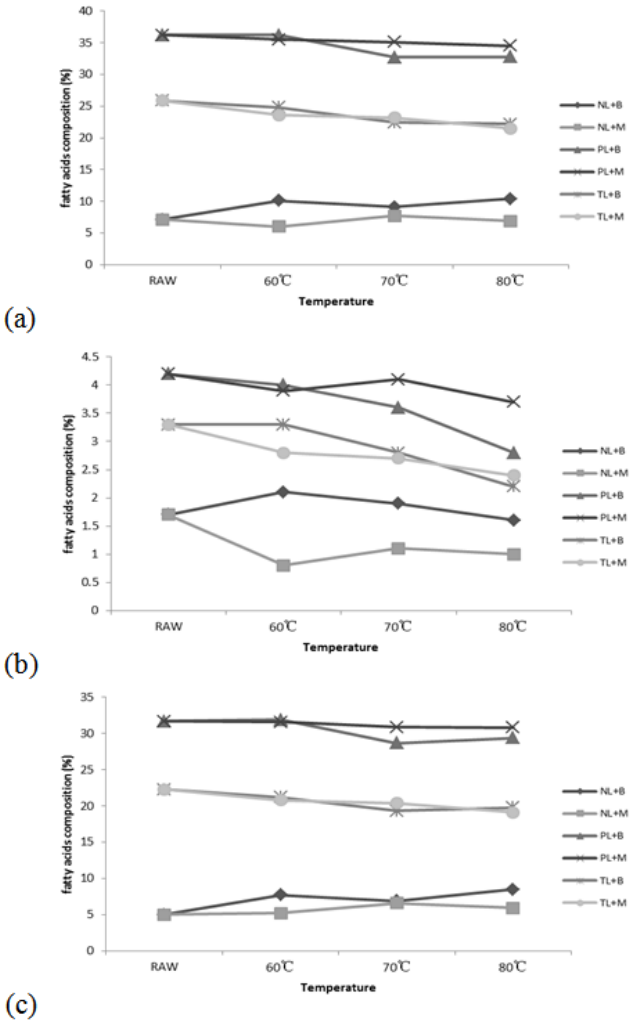


**Figure 3.** Changes in total (a), n-3 (b), and n-6 (c) PUFA composition (%) in beef different fraction (NL, PL and TL) before (RAW) and after boiling (B) or microwave cooking (M)

PUFA had relatively lower melting points and were more susceptible to oxidation comparing with MUFA and SFA [32]. Intramuscular lipids were composed of the NL primarily consisting of triglycerides and the PL containing mostly phospholipids. The FA composition of the PL fraction contained a large proportion of PUFA. Polar lipids were known to be more susceptible to oxidation and the PUFA content of PL was the primary substrate of rancidity development. The PUFA in the PL exhibited similar susceptibility to thermal oxidation where cooking affected the PL to a greater extent than it does the NL. Intramuscular fat content was increased primarily through accumulation of FA in the NL [38]. An increase in beef intramuscular fat content in Longissimus lumborum beef steaks had been associated with increased flavor liking by consumers or flavor intensity rated by trained panelists [32,39].

### 3.4. Trans Fatty Acids and Unknown Fatty Acids

The undesirable effects of dietary trans fatty acids (TFA) on human health had been reported[40], but a distinction had often been made between trans FA from undesirable industrial sources and those that occurred naturally in food derived from animals such as milk and meat. TFA in diet from animal source were always considered acceptable [41,42]. In current study, percentage of 18:1 *trans*-11 did not change ($P > 0.05$) with beef cooked to internal temperature of 60°C, 70°C and 80°C comparing with raw beef. However, with microwave cooking, percentage of 18:1 *trans*-11 was reduced ($P < 0.05$) in NL at internal temperature of 70°C and 80°C. However, percentage of 18:1 *trans*-11 was increased ($P < 0.05$) in PL at internal temperature of 80°C, which resulted in no changes ($P > 0.05$) in TL when microwave heated to internal temperature of 80°C.

Boiling reduced the unknown FAs in NL, but increased the unknown FAs in PL, which result in percentage of unknown FAs did not change in TL (Table 1-Table 3). However, microwave cooking reduced percentage of unknown FAs (< C16, C16 - C18, or > C18) significantly comparing with raw beef in NL, PL and TL.

Boiling or microwave cooking lean beef without external fat resulted in changes in the FA composition between the lipid fractions. The NL fraction represents the storage component of the lipid, whereas the PL fraction is the membrane component of the cell. Fatty acid compositions of the fractions differ greatly, with storage (neutral) lipids contained high composition of SFA [33] and MUFA [43] and PUFA located predominately in the membrane fraction [44]. Different distributions of FAs between storage and membrane fraction results in different responses with boiling [22] or microwave cooking [29] treatment. However, Smith, Savell [30] reported no changes in the FA changes of TL of beef steak after boiling, this mainly caused that only the TL fraction was investigated, therefore changes of FAs composition been overlooked.

The primary TFA in current study was trans vaccenic acid (18:1 *trans*-11), a precursor of the conjugated linoleic acid (18:2 *cis*-9 *trans*-11), for which there is evidence of desirable health effects [45]. Conjugated linoleic acids (CLA) are intermediates in the ruminal bio-hydrogenation, and appear in the lipid fractions of ruminant's meat in higher concentrations than in non-ruminant meat. CLA had the potential to reduce the risk of cancer, CVD, diabetes, and obesity, as well as to boost the immune system [46]. Efforts had been made to increase amounts of PUFA in beef, particularly n−3 PUFA, which may have health benefits for consumers. Of the isomers of CLA, 18:2 *cis*-9,*trans*-11 was the most abundant FA in present experiment, which agreed with the conclusion of Rule, Broughton [47]. In storage lipid (NL), the percentages of 18:2 *cis*-9,*trans*-11 were less than 0.2% in raw and cooked beef, and not detected in beef with microwave cooking. However, 18:2 *cis*-9,*trans*-11 detected in raw, cooked and microwave heated beef membrane fraction (PL), and microwave cooking reduced ($P < 0.01$) percentage of 18:2 *cis*-9,*trans*-11 significantly in PL. It is considered that instant high energy microwave cooking changes the spatial structure of CLA and produces other FAs we did not identified. Fatty acid of 18:2 *cis*-9,*trans*-11 did not change ($P > 0.05$) with beef cooked to 60°C and 70°C in TL, these agreed with reports from Scheeder, Casutt [48] that CLA unchanged with beef cooked, however, 18:2 *cis*-9,*trans*-11 percentage decreased ($P < 0.05$) when cooked to internal temperature of 80°C in TL. Percentage of 18:2 *cis*-9,*trans*-11 decreased ($P < 0.01$) significantly with beef microwave cooking in TL.

### 3.5. Ratios and Indices

The P/S ratios (0.58 ~ 0.75) in present experiment were higher than the recommended value (0.45) [49], which was desirable and good for health. P/S ratio decreased ($P < 0.05$) with beef cooked and microwave heated to the internal temperature of 70°C (0.62 vs. 0.75) in TL (Table 4). However, when the neutral and polar fractions were investigated, FAs change did not consistent as that of TL. PUFA of neutral fraction was increased ($P < 0.05$), while SFA did not change ($P > 0.05$) with beef boiled, which caused P/S ratio increased ($P < 0.05$) in storage (neutral) lipid. Conversely, P/S ratio of neutral fraction was slightly decreased ($P > 0.05$) with beef microwave cooked. In membranes (polar) lipid, SFA was increased ($P < 0.05$) with beef boiled or microwave cooked, which caused P/S ratio decreased slightly ($P > 0.05$). Thus, it was supposed that beef with intramuscular fat (marbling) affluent shall be treated with boiling as P/S ratio increase in storage lipid.

Although the consumers concerned about the health aspect, they also wanted a product that was highly palatable. The concentration of MUFAs (and specifically oleic acid) in beef was positively correlated with beef or beef fat flavors, indicating that as the concentration of oleic acid increases, so did beefy flavors. Therefore, increasing the M/S ratio would increase the palatability as well as the healthfulness of beef and beef products.[50] However, both SFA and MUFA increased with beef boiling or microwave cooking comparing with raw in current study, which caused ratios of M/S did not change ($P > 0.05$) significantly in TL when internal temperature up to 70°C (Table 4).

Among n-3 PUFA, the nutritional importance of α-linolenic acid was unclear, since α-linolenic acid was not as bioactive as longer chain of n-3 FAs, such as eicosapentaenoic acid (EPA) and docosaesaenoic acid (DHA) [51]. The long chain n-3 PUFA, such as EPA (C20:5), DPA (C22:5) and DHA (C22:6) were widely recognized for their beneficial effects on health [14,15]. Recommended intake by humans of n-6 FAs was suggested to be about 4% of dietary energy with a minimum of 1.5% and intake of n-3 FAs should be about 0.75% of dietary energy to avoid essential fatty acid (EFA) deficiency. Enser, Hallett [49] recommended that the n-6/n-3 PUFA value should be less than 4. In present research, n-6/n-3 PUFA value was of 6.72 in raw beef (Table 4) and increased with boiling and microwave cooking, and when the internal temperature reached 80°C, n-6/n-3 ratios increased ($P < 0.01$) significantly to 11.15 and 10.46 with boiling or microwave cooking.

The IA (index of atherogenicity) values of 0.32-0.42 in raw, boiled or microwave cooked beef in the present experiments (Table 4) were less than 0.70-0.72 reported by Ulbricht and Southgate [12] and 0.56-0.69 reported by

Knight [52]. Value of IA increased ($P < 0.05$) from 0.32 to 0.40 or 0.42 at the internal temperature of 80°C with boiling or microwave cooking comparing with raw beef. Value of IT (index of thrombogenicity) increased ($P < 0.05$) stepwise when beef boiled or microwave cooked at the internal temperature of 60°C, 70°C and 80°C comparing with raw beef.

## 4. Conclusion

Fatty acids profiles changed with different thermal treatment (boiling or microwave cooking). Based on the above-mentioned results, it is proposed that beef with abundant intramuscular fat should be treated with boiling, as ratios of P/S increased with boiling comparing with microwave cooking in neutral lipid (mainly intramuscular fat) and the thermal treatment of microwave cooking decreased CLA content substantially. Beef was not recommended to be over-cooked (boiling or microwave cooking), the core temperature of beef should be around with 70°C as value of some indices linked with the potential health attributes of foods including n-6/n-3 PUFA, IA and IT increased significantly when beef internal temperature reaching 80°C compared with 70°C.

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