Chemical composition and fatty acid profile of Bangladeshi beef at retail

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<u>Abstract</u>

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Keywords

Bangladesh Beef Chemical composition Fatty acid profile n-6/n-3 fatty acids ratio In an attempt to develop the food composition database for Bangladesh, chemical composition and fatty acid profile of beef in Bangladesh purchased at retail were analysed. The experiment was carried out on 10 boneless beef samples of Longissimus dorsi portion from male indigenous non-descript deshi breed were purchased from local butcher shop in Dhaka on separate occasions. The average chemical composition of the beef was as follows: moisture $75.56 \pm 0.55\%$, protein $19.66 \pm 0.70\%$, intramuscular fat $3.72 \pm 0.38\%$ and ash $1.06 \pm 0.04\%$. The average muscle fatty acid content was 3.52g/100g of beef sample. Total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) content in the intramuscular fat was on average of $42.69 \pm 1.97\%$, $40.50 \pm 1.28\%$ and 16.82 \pm 1.05% of total fatty acids, respectively. The PUFA/SFA ratio for beef was around 0.4 which falls into the recommended range. The level of total n-6 and n-3 PUFA in the beef sample were $15.56 \pm 0.91\%$ and $1.26 \pm 0.05\%$ respectively. The total n-6/n-3 fatty acids ratio in this study was 12.35 ± 2.22 , which is higher compared to values presented in some literatures. Overall results suggest that, the local beef is not favorable for human consumption and health, and therefore further studies are needed to improve the nutritional quality of beef by addressing factors- such as feeding strategy, sex, breed, age and weight and level of fatness, that influence the fatty acid composition in beef.

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Introduction

Currently, in developing countries like Bangladesh the concern about the fat and fatty acid (FA) composition of beef is increasing because some FA can adversely affect the human health and lead to the development of modern chronic diseases. The demand for beef is really high in Bangladesh because of its rich flavours and tastiness, and beef is widely consumed after poultry meat. Beef is mainly consumed by middle to upper class family, while the level of beef consumption is relatively low in poor families.

Beef is widely considered as a good sources of high biological value protein and essential amino acids, vitamins (Vitamin A, vitamin B2, vitamin B6, vitamin B12, pantothenic acid and niacin) and minerals (iron, zinc, phosphorus and selenium) (Daley *et al.*, 2010). In addition, beef is a source of other bioactive substances (such as conjugated linoleic acid and essential omega-3 polyunsaturated fatty acids)- and contains endogenous antioxidants (such as coenzyme Q10, glutathione, lipoic acid, etc.) (Williams, 2007). However, beef is generally seen as disease promoting food because of its higher levels of cholesterol and few saturated fatty acids (SFA) content, which are considered to have negative effects on human health by raising the total and low-density lipoprotein (LDL) cholesterol and can lead to cardiovascular diseases (CVD) (Scollan *et al.*, 2006). On the contrary, oleic acid (18:1), which is a monounsaturated fatty acid (MUFA), can lower cholesterol together with other health benefits, including reduction of the incidence of stroke and favorable effects on blood pressure (Daley *et al.*, 2010).

Polyunsaturated fatty acids (PUFA) are generally known to have positive effects on human health. Linoleic acid (LA, 18:2 n-6), α -linolenic acid (ALA, 18:3 n-3) and long chain PUFA (especially C20 and C22 PUFA) present in the tissue of phospholipids are involved in certain important physiological functions in nerve tissue and retina. Although human has the enzymatic ability to synthesize the long chain PUFA from their n-6 and n-3 precursors, LA and ALA, respectively, an increase consumption of C20 and C22 n-3 PUFA has been recommended (Vatansever and Demirel, 2009). This recommendation was made to overcome the so-called 'Diseases of Western Civilization' due to the imbalance of n-6 to n-3 ratio (James *et al.*, 1992).

Among n-3 PUFA, the nutritional significance of ALA is not clear, because ALA is not as bioactive

as long chain n-3 PUFA (n-3 LCPUFA) - such as eicosapentaenoic acid (EPA) and docosaesaenoic acid (DHA) (Decker and Park, 2010). The n-3 LCPUFA, such as EPA (C20:5), DPA (C22:5) and DHA (C22:6) are widely known for their favorable effects on several physiological actions- such as reduced incidence of heart attack, depression and cancer, and lowering of the inflammation caused by rheumatoid arthritis (RA) (Daley et al., 2010; McAfee et al., 2010). n-6 PUFA have the potential to decrease the level of LDL-cholesterol whereas n-3 PUFA have partial effects on the level of blood cholesterol and n-3 LCPUFA are efficient in lowering the level of blood triacylglycerol (Chizzolini et al., 1999). Moreover, a balanced n-6/n-3 ratio is desired to reduce the risk of developing condition related to CVD (Aldai et al., 2006).

Considering this scenario, numerous studies were carried out in many countries to produce meat of low fat and high PUFA content especially n-3 PUFA (Scollan *et al.*, 2001; Demirel *et al.*, 2006). However, FA composition of beef is influenced by several factors, such as diet, sex, breed, age, weight and level of fatness (Daley *et al.*, 2010). Several countries in the world have their own database for fat and FA content in beef but information about fat and FA content of the beef in Bangladesh is relatively scarce. Therefore, the aim of this study was to analyse the chemical composition and FA content of beef at retail.

Materials and Methods

Sample collection and preparation

In total, 10 fresh boneless beef muscle samples of Longissimus dorsi portion (between the 12th and 13th ribs) from male indigenous non-descript deshi breed were collected from local butcher shops during May and June 2013 in Dhaka on several occasions and carried in polythene bags to the Institute of Nutrition and Food Science laboratory. Samples were collected from different animals and individual samples were analysed from each animal. All visible fat and connective tissue were trimmed off as much as possible. Immediately following fat removal, samples were homogenized in a blender and prepared for chemical and FA composition analysis.

Analysis of chemical composition

Analysis of the chemical composition of the samples was carried out according to the AOAC method of analysis described by (Gul and Safdar, 2009). The moisture content was determined by drying meat samples at 105°C to the constant weight.

The nitrogen content was determined by the standard Kjeldahl procedure, and expressed as protein content (nitrogen content multiplied by 6.25). Ash content in the beef sample was estimated by heating the dried sample in a Muffle furnace at 600°C for 3h. Ash content was calculated from weight difference. The intramuscular fat content was determined using Chloroform-methanol extraction (Folch *et al.*, 1957). Total fatty acids were calculated according to the method described by Greenfield and Southgate, 2003.

Analysis of fatty acids profile

The FAs obtained after extraction were converted to the corresponding FAMEs by transesterification using methanolic solution of potassium hydroxide (2 mol/L). The solution was then shaken vigorously for around 30 seconds. The solution was neutralized by addition of salt (sodium hydrogen sulphate monohydrate). After the salt had settled, 100 μ L of upper phase was transferred into a GC vial containing insert and analysed (Petrović *et al.*, 2010).

FAME were analysed by gas chromatography equipped with flame ionization detection (GC-FID; HP 6890 chromatograph, Hewlett-Packard, Avondale, PA, USA) using capillary column (100 m \times 0.25 mm i.d., 0.2 μ m df). Briefly, the oven temperature was initially 100°C (held for 5 minute), then increased at 3°C min⁻¹ to 140°C (held for 20 minute), then increased at 8°C min⁻¹ to 230 °C (held for 10 minute) and finally increased at 6°C min⁻¹ to 240 °C (held for 8 minute). Hydrogen was used as the carrier gas at a flow rate of 1.0 ml.min⁻¹. The injector and detector temperatures were maintained at 250°C and 300°C respectively. 1 µl was taken up by the GC-FID from the vial. Identification of common FA was accomplished by comparison of sample peak retention times with those of known FAME standard mixtures (SupelcoTM 37 component FAME Mix, Supelco-18919-1amp, USA). Quantification of total FAME was done using 5-Dodecenoic acid (C12:1) as internal standard (NU-CHEK PREP, USA) which was added prior to salt addition. The result of the evaluation was the percentage (%) of total fatty acids (TFA). The calculation of Desaturation index for palmitic acid (C16:0) and stearic acid (C18:0) was carried out according to the formula described by Aldai et al., 2006.

Statistical analysis

The experiments were performed with three replicates. SPSS software package (version 20.0 SPSS Inc. Chicago, IL, USA) was used to analyse the nutrient data. Descriptive statistics were used for all of the variables. Values were expressed as mean

and standard deviation (SD).

Results and Discussion

Chemical composition of beef

Chemical composition (moisture, protein, fat, ash and TFA) of the beef sample is summarized in Table 1. Considering the present study was first attempt at estimating the chemical and FA composition of Bangladeshi beef at retail, no data was available for national level comparison. In many countries the Intramuscular fat (IMF) content <5% is considered as being "low in fat" meat (Scollan et al., 2006). Therefore, according to our findings (Table 1) beef Longissimus dorsi muscle of Bangladesh could be classified as lean meat. In concomitant with this, muscle tissue after removal of any visible fat (<5% fat) would normally satisfy for incorporation into a healthy diet. The chemical composition of Bangladeshi retail beef (Table 1) is close to most of the European, Australian and American beef at retail (Enser et al., 1996; Rhee, 2000; Raes et al., 2003; Realini et al., 2004; Nuernberg et al., 2005; Williamson et al., 2005; Almeida et al., 2006; Droulez et al., 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia et al., 2014).

Fatty acids composition of beef

The FA composition, expressed as percentage (%) of TFA, is presented in Table 2. From the Table 2, it can be seen that IMF contained on average of $42.69 \pm 1.97\%$, $40.50 \pm 1.28\%$ and $16.82 \pm 1.05\%$ of TFA as SFA, MUFA and PUFA, respectively. According to Scollan *et al.* (2006), the major SFA are C14:0 (myristic acid), C16:0 (palmitic acid) and C18:0 (stearic acid), which correlates to our findings and accounts for $1.55 \pm 0.10\%$, $24.02 \pm 0.71\%$ and $16.37 \pm 1.16\%$ of TFA respectively.

Among the total SFA, C16:0 showed the highest proportion (Table 2), which is an agreement with Enser et al. (1996), who studied UK beef FA composition at retail level. Several studies also reported similar results where they analysed the beef FA composition at retail level (Droulez et al., 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia et al., 2014). SFA especially C12:0, C14:0 and C16:0 significantly influences the plasma level of cholesterol. These FA are termed as hypercholesterolemic, whereas C18:0 is believed to have neutral effects on plasma cholesterol level in humans despite being a SFA (Scollan et al., 2006). C14:0 in red meat is believed to increase cholesterol levels more greatly than C16:0 (Fink-Gremmels, 1993). Recently, it has been reported that C12:0 and

Table 1. Chemical composition (%) of beef sample

Nutrients	Mean	SD
Moisture	75.56	0.55
Protein	19.66	0.70
IMF ¹	3.72	0.38
Ash	1.06	0.04

¹Intramuscular fat

C14:0 may have positive effects on health as they both helps to reduce the total/HDL-C ratio while C16:0 have the opposite effect (Givens, 2010). The content of C14:0 in our study was lower than those reported in other studies of retail beef (Enser *et al.*, 1996; Droulez *et al.*, 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia *et al.*, 2014) whereas the level of C18:0 was higher than that reported in UK, Turkish and Australian Beef (Enser *et al.*, 1996; Droulez *et al.*, 2006; Vatansever and Demirel, 2009) and lower than the Italian beef (Brugiapaglia *et al.*, 2014).

MUFA content found in this study was mainly consisted of palmitoleic (C16:1) and C18:1 FAs, in agreement with the results obtained by other authors (Enser et al., 1996; Droulez et al., 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia et al., 2014). The main MUFA component was C18:1n9cis and accounts for $33.60 \pm 1.38\%$. C18:1n9cis content in Bangladeshi beef was lower than that of the European, American and Australian beef (Enser et al., 1996; Droulez et al., 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia et al., 2014). The degree of hydrogenation of C18:1 in the rumen, as well as the breed difference can influence these phenomena (Piasentier et al., 2009). An elevated proportion of C18:1 in red meat is desirable because of its cholesterol lowering ability. Steroyl-CoA desaturase (Δ 9-desaturase) is the prime enzyme in FA metabolism and regulate the desaturation of SFAs, especially C16:0 and C18:1, to its corresponding MUFA (C16:1 and C18:1). It introduces the double bond in Stearoyl-CoA and subsequently MUFA is generated from the SFA in the adipocyte of mammals. FA from the diet are degraded by ruminal microorganisms and absorbed as SFA. The Δ 9-desaturase activity can be influenced by genetic factors as well as by other factors such as the interaction between breed type, age and diet (Brugiapaglia et al., 2014).

Total PUFA content was higher in our study compared to those reported in European, American and Australian beef (Enser *et al.*, 1996; Raes *et al.*, 2003; Droulez *et al.*, 2006; Vatansever and Demirel, 2009; USDA, 2013; Brugiapaglia *et al.*, 2014). The n-3 PUFA content in beef is dependent on the feeding

total fatty acids) of the beef muscle sample		
Total fatty acid and Fatty acids	Mean	SD
Total fatty acid	3.52	0.39
Fatty acids	0.02	0.00
C8:0	0.004	0.0001
C10:0	0.019	0.003
C12 ^{.0}	0.029	0.001
C13:0	0.003	0.001
C14:0	1.55	0.10
C14:1	0.53	0.07
C15:0	0.36	0.06
C15:1	0.12	0.03
C16:0	24.02	0.71
C16:1	2.45	0.55
C17:1	0.40	0.08
C18:0	16.37	1.16
C18:1n9trans	2.26	0.58
C18:1n11trans	1.09	0.07
C18:1n9cis	33.60	1.38
C18:2n6trans	0.08	0.01
C18:2n6cis	11.25	0.92
C18:3n6	0.07	0.002
C18:3n3	0.97	0.04
C20:0	0.07	0.003
C20:2	0.18	0.001
C20:3n6	1.12	0.02
C20:3n3	0.05	0.001
C20:4n6	3.12	0.05
C20:5n3	0.088	0.002
C21:0	0.013	0.001
C22:0	0.25	0.05
C22:2n6	0.23	0.07
C22:6n3	0.15	0.01
C24:1	0.05	0.01
∑SFA ¹	42.69	1.97
ΣMUFA ²	40.50	1.28
∑PUFA [«]	16.82	1.05
ΣPUFA/ΣSFA	0.39	0.08
∑n3 [*]	1.26	0.05
∑n6°	15.56	0.91
∑n6/∑n3	12.35	2.22
Desaturation Index	9.26	0.38

Table 2. Total fatty acid (%) and Fatty acid profile (% of

¹Sum of saturated fatty acids: C8:0 + C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C21:0 + C22:0;

67.24

1.01

²Sum of monounsaturated fatty acids: C14:1 + C15:1 + C16:1 + C17:1 + C18:1 n9t + C18:1n11t + C18:1n9cis + C24:1;

3Sum of n-3 and n-6 fatty acids;

(C16)⁶ Desaturation Index

(C18)

⁴Sum of n-3 fatty acids: C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3;

⁵Sum of n-6 fatty acids: C18:2n6trans + C18:2n6cis + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:2n6;

⁶Desaturation index (C16) = 100*[(C16:1)/(C16:0 + C16:1)];

⁷Desaturation index (C18) = 100*[(C18:1n9cis)/(C18:0 +C18:1n9cis)]

of either n-3 series precursor ALA or the preformed FA. Higher ALA level in meat is desired because humans are capable of synthesizing n-3 LCPUFA (C20 n-3 PUFA and C22:6 n-3) from it (Enser et al., 1996). These n-3 PUFA, in conjunction with C20:4 n-6, contribute to several important metabolic actions, such as preventing atherosclerosis, heart attack, depression, cancer and reducing the inflammation of RA through the production of eicosanoid (Wood et al., 2007; Daley et al., 2010). Moreover, higher proportion of n-3 PUFA is desired in meat to create a more favorable n-6 to n-3 ratio. We observed higher value of LA (11.25 \pm 0.92%) and lower proportion of ALA (0.97 \pm 0.04%). In our study we found relatively higher LA concentration in local beef compared to other studies (Enser et al., 1996; Raes et al., 2003; Nuernberg et al., 2005; Droulez et al., 2006; Vatansever and Demirel, 2009; Brugiapaglia et al., 2014).

The total PUFA/SFA ratio for beef was around 0.4, different from that stated by other authors (Scollan et al., 2006). The minimum recommended value is 0.45 for human consumption (British Department of Health, 1994). The PUFA/SFA ratio for beef is typically low at around 0.1 and the value is about 0.5-0.7 for double muscled animals as meat from these animals generally have very low IMF (<1%) (Raes et al., 2003). Besides the IMF, FA composition is also different in double muscled animals than nondouble muscled animals. Typically, double muscled animals have higher concentration of PUFA and lower concentration of SFA, thereby have higher PUFA/SFA ratio compared to non-double muscled counterparts (Raes et al., 2003). Double muscled animals also showed different pattern of metabolism of n-6 and n-3 fatty acids, especially the deposition rate of n-3 fatty acids in these animals is greater than the non-double muscled animals.

In addition to the PUFA/SFA ratio, greater attention has been given on the type of PUFA and the balance between n-3 and n-6 PUFA in the diet. The recommended n-6/n-3 ratio is <4 (British Department of Health, 1994). The n-6 to n-3 ratio found in this study was 12.35 ± 2.22 , a value much higher than the recommended value (4.0) and this ratio could considered to be a risk factor for the development of coronary heart disease. The total PUFA to SFA and n-6 to n-3 ratio, and desaturation indexes of C16:0 and C18:0 FA are the important indicators of the FA nutritional value. Regarding n-6/n-3 ratio, we found much greater value than the recommended value (<4) (British Department of Health, 1994). This value is much higher compared to the value observed in UK, German, Turkish and Australian beef (Enser et al., 1996; Nuernberg et al., 2005; Droulez et al., 2006; Vatansever and Demirel, 2009) whereas this value is lower than the Italian beef (Brugiapaglia et al., 2014).

Although it was not possible to obtain information about the diet animals received, it can be assumed that the animals were finished on high concentrate diet (such as bran or oilcakes) which contributed to an imbalance in the ratio of n-6 to n-3. It has been suggested that n-6/n-3 ratio could be a marker to differentiate the animals to identify whether they were grass fed or grain (concentrate) fed. Several

studies reported that grain fed animals have significantly higher value of n-6/n-3 ratio than the grass fed counterparts (Daley *et al.*, 2010). Several studies also reported the higher value of LA than the ALA in concentrate fed animals (Enser *et al.*, 1998; Daley *et al.*, 2010). Animals grazing on pasture based diets accumulated 2 to 3 times higher concentrations of total n-3 fatty acids in their muscle fat compared to those fed concentrates due to high bioavailability of ALA in the diet (Wood *et al.*, 2008).

Like Western Europe and North America, the n-6/n-3 ratio in the upper middle and rich families of Bangladesh is extremely unbalanced. This aberrant ratio represents a risk factor for modern diseases such as CVD, cancer, inflammatory and autoimmune diseases. Moreover, a surplus of particular class of FA can hamper the metabolism of another, lowering its inclusion into tissue fats and change their overall physiological activity in human health (Daley *et al.*, 2010).

Conclusion

The results from this study showed the chemical composition and FA profile of the Longissimus dorsi muscle of retail beef in Bangladesh. Although we found that the beef is low in fat (<5% IMF), as well as the PUFA/SFA ratio was within the recommended value, the higher value of total n-6 PUFAs and n-6/n-3 ratio makes it unsuitable for human consumption as n-6 PUFA are pro-inflammatory. As FA composition in IMF is dependent on the diet of the animals, feeding strategy needs to be improved in order to produce the beef which will have low total fat, SFA and n-6/n-3 PUFA ratio and high concentrations of MUFA and n-3 PUFA. Besides the diet, other factors (i.e. age or body weight, degree of fattening, sex and breed) can influence the body fat and IMF content of the animal. So, to make the beef more suitable for human health further studies are needed to address those above mentioned parameters that influence the FA composition. As little information was available on the nutrient content and FA composition of Bangladeshi beef, the results of this study contributes considerably to much needed information on the FA composition of beef in Bangladesh. This was the first comprehensive and systematic analysis of the FA composition of beef samples in Bangladesh.

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