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Effect of boiling and frying on nutritional value and *in vitro* digestibility of rabbit meat

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Effect of boiling and frying on nutritional value and *in vitro* digestibility of rabbit meat were investigated. It was observed that boiling for 15 min increased the digestibility and nutritional value of rabbit meat, while boiling for 5 and 40 min led to their loss. Although, frying for 2 and 4 min produced an acceptable digestibility and nutritional value, the values were lower than those of the boiled rabbit meats. These results were supported by the analysis of amino acid and SDS-PAGE. Boiling or frying for longer time led to decrease in whiteness and protein content of rabbit meat. Micrographs showed that the meat myofibrils became clearer with the increase in boiling time, but a fuzzier surface appeared with the increase in frying time. From these results, it was concluded that a proper boiling or frying time is necessary to acquire a nutritious and high quality rabbit meat.

Key words: Rabbit meat, boiling, frying, amino acid, protein, essential amino acid index (EAAI).

INTRODUCTION

Rabbit meat has a high protein and low sodium contents (Combes, 2004). Its cholesterol and fat contents are much lower than chicken, turkey, beef and pork (Combes, 2004; Cavani et al., 2010). The unsaturated fatty acids content of rabbit meat is 63% (w/w) of its total fatty acids. The United States Department of Agriculture (USDA) has stated that the rabbit meat is the most nutritious meat available to man (Cavani et al., 2010). The rabbit meat was highly accepted for its appearance and color, juiciness and taste when boiled and fried as comparing to the Giant African Land Snail (*Archachatina marginata*) meat (Malik et al., 2011). All these features make rabbit meat a very healthy, nutritious and popular food suitable for general consumption and as a special diet including heart disease patients diet, low sodium diet, weight reduction diet, diets for the aged, etc (Dalle, 2002; Cavani and Petracci, 2004).

Cooking is necessary to prepare palatable and microbially safe foods including rabbit meat. Suitable cooking minimizes the nutrient loss and also improves the digestibility of food (Kimura and Itokawa, 1990). However, improper cooking will terribly harm the nutrients present in the food, and sometimes even produce toxic and hazardous substances. Investigations have demonstrated that there were positive associations between red processed meat and proximal colon cancer, pan-fried red meat and colorectal cancer (Miller et al., 2013), consumption of fried and/or well-done red meat was associated with increased risk of lung cancer in women (Sinha et al., 1998). Carcinogenic compounds formed by high-temperature cooking techniques like heterocyclic amines and polycyclic aromatic hydrocarbons were reported to contribute to the risk of developing colorectal tumors (Sinha et al., 1999; Wong et al., 2012). Meat cooked with high temperature techniques produced mutagenic compounds such as heterocyclic amines, which are strongly associated with the risk of colorectal adenomas (Sinha et al., 1999; Wong et al., 2012). The degree of browning that occurred during deep-frying of meat was related to the pathogenesis of breast cancer (Dai et al., 2002; Lyn-Cook et al.,

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2011) and a high intake of processed meat (pork chops, sausages and hotdogs) was found to increase the risk of lung cancer in men (Tasevska et al., 2009). Fried meat was associated with a higher risk of cancer of oral cavity, pharynx and esophagus, roasting/grilling meat increased risk of prostate cancer (Di Maso et al., 2013).

Due to the health concerns caused by improper cooking of meat, cooking method has been paid special attention and many studies have been done to investigate the effects of heating on meat quality and its nutrients. It was found that the conventional and microwave heating of ground beef samples increased the nonheme iron concentrations (Schricker and Miller, 1983) and the fried ground beef patties had the highest flavor intensity (Berry and Leddy, 1984). The amount of heterocyclic amines increased with frying or boiling time increase during the production of braised chicken (Yao et al., 2013). Cooking had no significant effect on riboflavin content of dark meat, while frying significantly decreased the riboflavin content of light meat (Al-Khalifa and Dawood, 1993). The combination of roasting and microwave heating of hamburgers increased the lipid oxidation (Rodriguez-Estrada et al., 1997). Heat cooking considerably affected the proximate composition and mineral contents and baking and grilling were reported as the best cooking methods for rainbow trout (Gokoglu et al., 2004).

Effect of cooking on rabbit meat quality had been investigated in the past. Some of the investigations include the effect of electric oven cooking on oxidative stability of meat of rabbit fed with fodder containing α-tocopheryl acetate (Monahan et al., 1990). The effect of boiling (in a vacuum sealed polyethylene bag at 100±1°C for 8 min), frying (in sunflower oil at 175±5°C for 3 min), roasting (in an electric oven preheated to 200±10°C for 15 min) on pH, shear force, lightness, color, and water holding capacity of rabbit meat (Bosco et al., 2001), was also investigated. The effect of cooking (immersed in a constant temperature water-bath) temperature (50-90 °C) and time (10-120 min) on tenderness of rabbit muscle had been investigated (Combes et al., 2004). Recently, the effect of cooking methods and the related processes on nutrition and quality of chicken (Das et al., 2013), silver carp (Naseri et al., 2013), beef muscles, rainbow trout fillets (Choubert and Baccaunaud, 2010), and beef roast (Modzelewska-Kapituła et al., 2012; Walsh et al., 2010), were investigated. However, to our knowledge, the effect of cooking methods on the protein content and quality of protein present in the cooked rabbit meat have not been investigated or reported previously. Better knowledge of effect of cooking methods on the nutritional value of protein of rabbit meat will contribute to the development of nutrient rich rabbit meat products.

Heat cooking is being used as a general term for various processes including boiling, frying, roasting, baking, etc. Boiling and frying are most frequently used for cooking of meat (Fillion and Henry, 1998; Chen, 2006). However, the effect of doneness or cooking levels on protein qualities of rabbit meat was rarely investigated. This research is focused on investigating the effect of two commonly used heat cooking methods (boiling and frying) and different doneness levels on nutritional value of protein and *in vitro* digestibility of rabbit meat.

MATERIALS AND METHODS

Reagents

Amino acid standards used in this study were obtained from Sigma-Aldarich Corporation (St. Louis, MO). HPLC grade acetonitrile and methanol were used in this study. The trichloroacetic acid (TCA) was purchased from Jiangsu Yonghua Fine Chemical Co., Ltd (Shanghai, China). Sodium dodecyl sulfate (SDS), urea, Tris (hydroxymethyl) aminomethane (Tris), β-mercaptoethanol (β-ME) and other electrophoresis reagents were purchased from Shanghai Bio Life Science and Technology Co., Ltd (Shanghai, China). All chemicals used in this study were of analytical grade.

Meat samples

Totally, 140 numbers of 10 week old Sichuan Rex Rabbits, having a body weight of 2.35±0.1 kg were used in the experiments. The rabbits were slaughtered by the butchers of Hage Rabbit Industry Co. Ltd, in Sichuan Yilong County and brought to our laboratory within 2 h. The rabbit meat samples for cooking experiments (boiling and frying) were taken from hind legs. The meat was chopped into dice having uniform size of 20 ± 3 mm in both length and width, and 10 ± 3 mm in height. A total weight of 300 g hind leg meat was used for each treatment. Six treatments (boiling for 5, 15 and 40 min; frying for 2, 4 and 6 min) were performed for 9 times each.

Cooking methods

In order to investigate effect of heat cooking methods on nutritional value and *in vitro* digestion of rabbit meat, the diced meat was boiled and also fried using the procedure shown below. The boiling of the rabbit meat was done with six times volume of hot water in a temperature-controlled media MK2102 electromagnetic furnace (Media Group, China) at temperature of 95±3°C for a predetermined time of 5 (equals to medium), 15 (equals to well done) and 40 min (equals to very well done). The rabbit meat was fried with 1/3 times soybean oil in a temperature controlled Media MK2102 electromagnetic furnace (Media Group, China) set at 175±3°C for 2 (equals to medium), 3 (equals to well done) and 6 min (equals to very well done).

Determination of moisture and protein

Moisture contents of samples were analyzed according to the Association of Official Analytical Chemists method (AOAC 950.46, 1990). The crude proteins (N = 6.25) and dry matter contents were analyzed according to Association of Official Analytical Chemists (AOAC, 2006). All the tests were conducted in triplicate.

Color analysis

CIELAB color (L*, a* and b*) of cooked muscles were tested using a portable chroma meter CR-400 (Minolta Cameras, Osaka, Japan). The instrument was calibrated using the white plate

(Calibration Plate CR-A43, Minolta Cameras) at the beginning of each session. The chroma meter has a measuring area of 8 mm in diameter and 8 mm in thickness and illuminates the sample area with the diffused illumination from a pulsed xenon arc lamp. After choosing the Commission International illuminant (CIE) lighting conditions, the calibration channel selection was set at auto select mode. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included. Color is defined by the three CIE coordinates, L* (lightness), a* ((+) redness/ (-) greenness) and b* ((+) yellowness/ (-) blueness). The whiteness (W) is calculated from the L*, a*, b* values using the equation (Lanier et al., 1991).

$$W = 100-((100-L^*)^2+a^{*2}+b^{*2})^{0.5}$$

The cooked samples were dried, milled and filled in a 40 × 25 mm weighing bottle. The powder in the weighing bottle was placed smoothly in the plates and the color was determined using the chroma meter. Six repeated determinations were done for each sample.

Analysis of the micro-structure of meat in optical microscopy

The surface micro-structure of cooked and uncooked rabbit meat was observed with a XSP-15 reading optical microscope (Jiangsu Optical Instrument Factory, Nanjing). In order to maintain the natural state, the meat samples were not treated with any reagents. The meat sample was fixed with the stage clips and was observed under diffused reflection light with a 10 times magnifying eyepiece lens and 16 times objective lens. The optical micro surface structure was obtained by an IXUS 60 6.0 MP Digital Camera.

Sequential *in vitro* protein digestion procedure

The *in vitro* digestibility of the proteins was evaluated by the method used by Fu et al. (2002), with some modification. Pepsin (Sigma, P7000, 1:10,000, 600-1000 units/mg) and trypsin (Genview, DH355-1, 1:250) were used for the *in vitro* digestion study. Five grams of meat sample from each treatment (boiled or fried meat were converted as the weight of uncooked) was chopped into pieces and dipped into 250 mL solution of hydrochloric acid (HCl) of pH 1.5. The dipped samples were pre-incubated in a water bath at 37°C for 3-5 min. Then, an amount of pepsin (20 mg of pepsin per mL of 0.1 M KH_2PO_4, pH 2, buffer) was added, and the ratio of enzyme to protein substrate was maintained at 1:100 (w/w). The pepsin and protein solutions were mixed well and incubated at 37°C. After the predetermined incubation periods: 0, 60, 120, 180, and 300 min, aliquots of the mixtures were taken, and the pH was adjusted to pH 7.0 by adding 1.0 mol/L NaOH to stop the enzymatic reactions. Additionally, the final pepsin-digested hydrolysates (pH 7.0) were further digested by the addition of trypsin (20 mg of trypsin per mL of 0.1 M Tris-HCl buffer, pH 7.0) at 37°C for 60, 120, 180, and 300 min. The amount of enzyme to initial protein substrate ratio was maintained at 1:20 (w/w).

Nitrogen release during digestion

The nitrogen release (%, w/w) during digestion was determined by the TCA-NSI method used by Iwami et al. (1986), with some modifications. 10 mL of the digested mixtures were mixed with 10 mL of 10% (w/v) TCA (and the final concentration of TCA was 5%, w/v). The mixtures were then centrifuged (20°C, 8000 g, 30 min) to obtain the precipitates. After washing with 10 mL of TCA (10%, w/v), the precipitates were obtained again by centrifugation at the same condition. The N content of the samples was determined by Kjeldahl method (N×6.25). The nitrogen release (%, w/w) during the digestion was calculated as:

$$\text{Nitrogen release} = (N_0-N_t)\times 100/N_{total}$$

Where, t is the digestion time (min), N_t (mg) is the TCA-insoluble N after digestion for t (min), N_0 (mg) is the TCA-insoluble N in the protein sample and N_{total} (mg) is the total N of protein sample. Triplicate determinations were done for each sample.

Amino acid analysis

The amino acid composition of the rabbit meat samples were determined using the method used by Bidlingmeyer et al. (1984). The samples were hydrolyzed with a 6 M HCl solution in a vacuum-sealed tube for 24 h at 110°C. The samples were subsequently centrifuged (1500 rpm for 5 min) and dried under vacuum for 1.5-2 h. The pH was adjusted by adding 20 µL of an ethanol : water : triethylamine (2:2:1) solution and the samples were dried for 1.5-2 h. The resulting sample was derivatised by adding 20 µL of ethanol : water : triethylamine : phenylisothiocyanate (7:1:1:1) derivatising solution which was then allowed to react at room temperature for 10 min prior to drying under vacuum (minimum of 3 h). The samples were re-suspended in 200 µL of Picotag sample diluent (Waters, Millford, MA, USA) and 8 µL sub-sample was injected for separation by HPLC under gradient conditions. Buffer A was a sodium acetate buffer (pH 6.4) containing 5000 ppm ethylenediaminetetraacetic acid (EDTA), 1:2000 triethylamine and 6% acetonitrile and buffer B consisted of 60% acetonitrile with 5000 ppm EDTA. A waters high performance liquid chromatography system (1525 HPLC with a binary gradient delivery, 717 auto-sampler and Injector, 1500 column heater, 2487 dual wavelength UV detector) and a Breeze data workstation (Waters, Millford, MA, USA) were used for the determination of amino acid composition. Triplicate determinations were done for each sample.

Nutrition analysis of amino acid

Biological values of cooked rabbit meat was analyzed by 5 parameters; they are total amino acid (TAA) content, essential amino acid ratio (EAAR), protein chemical score (PCS), essential amino acid index (EAAI) (FAO., 1985) and protein efficiency ratio (PER) (Alsmeyer et al., 1974). The specific calculation formula of those parameters as follow:

$$TAA = \sum_{i=1}^{n} a_i$$

$$EAAR = \sum_{j=1}^{m} b_j \Big/ \sum_{i=1}^{n} a_i$$

$$PCS = Min\{(aa/AA)_1,\cdots,(aa/AA)_k\}$$

$$EAAI = \sqrt[k]{(aa/AA)_1\times\cdots\times(aa/AA)_k}$$

$$PER = -0.468+0.454\times leucine-0.105\times tyrosine$$

where a - amino acid in meat sample; b - essential amino acid in meat sample; n - the number of amino acid; m - the number of essential amino acid; AA - the content of amino acid in FAO (1985)

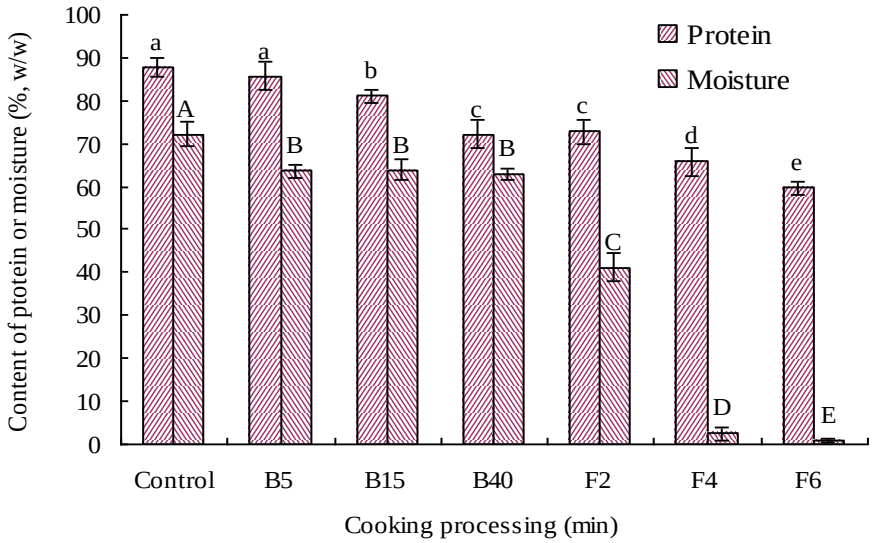

Figure 1. Effect of heat cooking on content of protein and water of rabbit meat. Protein content was calculated on dry basis; Means (n=3) without a common letter differ significantly (P<0.05); "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15, and 40 min, respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

suggested pattern of protein requirement; aa - the content of amino acid as compared to FAO (1985) suggested pattern of protein requirement and k- the number of amino acid type (FAO, 1985, Alsmeyer et al., 1974).

SDS–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE analysis was performed according to the method of Laemmli (1970). Two grams of cooked meat sample was added to 18 mL of SDS solution (5 g/100 mL). The mixture was then homogenized using a FJ-200 homogenizer (Shanghai Specimen and Model Factory, Shanghai, China) at a speed of 10,000 rpm for 1 min. The homogenate was incubated at 85°C in a temperature-controlled water bath for 1 h to dissolve total proteins. The sample was centrifuged at 12,000 g for 6 min. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 mole/L Tris-HCl, pH 6.8, containing SDS [4 g/100 mL], glycerol [20 g/100 mL] and β-ME [10 g/100 mL]), and the mixture was boiled for 3 min. The samples (20 mg protein) were loaded into the polyacrylamide gel made with 10% running gel and 4% stacking gel, and were subjected to electrophoresis at a constant current of 15 mA. After separation, the proteins were stained with Coomassie Brilliant Blue R-250 (0.02 g/100 mL) in methanol (50 mL/100 mL) and acetic acid (7.5 mL/100 mL), and destained with methanol (50 ml/100 ml) and acetic acid (7.5 mL/100 mL), followed by methanol (5 mL/100 mL) and acetic acid (7.5 mL/100 mL). The electrophoresis gel was scanned with a Bio-Rad imaging scanning densitometer (Versa Dco 3000, Bio-Rad, Milan, Italy).

Analysis of electrophoresis gel

The scanned electrophoresis gel image (300 dpi) was subjected to the optical density (O.D) analysis. Optical density is the measure of the transmission of an optical medium for a given light. Higher O.D indicates lower transmittance and vice versa. The O.D of the electrophoresis gel image was determined by a Gel-Pro Analyzer 4.0 (Media Cybernetics, L. P., USA). The O.D is calculated using the equation given below:

$$OD(x, y) = \frac{-\log(INTENSITY(x, y) - BLACK)}{INCIDENT - BLACK}$$

Where: INTENSITY (x, y) is the intensity at pixel (x,y); BLACK is the intensity generated when no light goes through the material; INCIDENT is the intensity of the incident light.

Statistical analysis

The results were analyzed by ANOVA at a significance level of 5% (H0: P<0.05). The comparison of means was analyzed by Fisher's LSD tests using the SAS statistical package (Statistical Analysis Systems Institute (SAS), 2000).

RESULTS AND DISCUSSION

Effect of heat cooking on protein and moisture contents of rabbit meat

The protein and moisture contents of the raw (control) and the cooked rabbit meats are shown in Figure 1. Both boiling and frying reduced the moisture content of rabbit meat significantly (P<0.05) as compared to the control, but the rate of moisture reduction is significantly (P<0.05) higher for frying than boiling. Boiling of rabbit meat for 5 (B5), 15 (B15) and 40 min (B40) caused a moisture reduction of 12.03, 11.66 and 12.98%, respectively. The frying of rabbit meat for 2 (F2), 4 (F4) and 6 min (F6) resulted in the moisture reduction of 49.92, 96.56 and

Table 1. Effect of cooking on color of rabbit meat.

	Control	B5	B15	B40	F2	F4	F6
L*	64.82±0.07a	64.54±0.39a	63.31±1.46b	62.20±0.44c	47.38±0.25d	36.26±0.83e	21.50±0.14f
a*	-1.95±0.12d	-2.85±0.11e	-1.64±0.58d	-2.82±0.48e	4.40±0.15b	12.52±0.14a	3.49±0.19c
b*	25.48±0.34c	24.32±0.05 d	24.26±0.44d	26.04±0.52c	37.54±0.79a	28.18±0.35b	2.21±0.18e
W	56.52±0.26 a	56.90±0.33a	55.97±1.01a	54.01±0.30b	35.21±0.59c	29.19±0.73d	21.40±0.14e

Each data is a mean of three replicate; Means (n=3) without a common letter differ significantly (P<0.05); “Control” is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15 and 40 min, respectively; F2, F4 and F6 represents meat fried for 2, 4 and 6 min, respectively; L* (lightness), a* ((+)redness/(-)greenness) and b* ((+)yellowness/(-)blueness), W (whiteness).

98.88%, respectively. The above results showed that the boiling has less influence on moisture content of rabbit meat as compared to frying which led to severe moisture loss to the extent of 98.88% depending on the frying time. Similar results were reported for boiling and frying of rainbow trout meat by Gokoglu et al. (2004), who found that frying and boiling had considerable effect on the proximate composition and mineral contents of the cooked fish comparing with the raw meat.

All the heat cooking treatments performed in this study resulted in significant loss of protein content of rabbit meat as compared to the control except the boiling for 5 min (B5). The loss of protein content of rabbit meat after boiling for 5 (B5), 15 (B15) and 40 min (B40) were 2.51, 7.75 and 17.78%, respectively. These results are in agreement with the results obtained by Bosco et al. (2001) for the loss of crude protein of rabbit meat during boiling. The protein loss during frying treatments of 2 (F2), 4 (F4) and 6 min (F6) were 17.20, 25.17 and 32.06%, respectively. The loss of protein content increased with an increase in both boiling and frying times and the loss caused by frying is greater than that of boiling. Frying resulted in rabbit meat with a low protein content similar to the results obtained by Oz et al. (2007). New chemicals, such as heterocyclic amines and polycyclic aromatic hydrocarbons, were formed during the frying might be the reason of the low protein content of fried rabbit meat. Those chemicals have been confirmed to contribute to the risk of developing colorectal tumors (Sinha et al., 1999; Wong et al., 2012), cancer of oral cavity and pharynx and esophagus (Di Maso et al., 2013).

Water molecules are highly polar and attracted to the muscle proteins by ionizable basic and acidic groups such as arginine, histidine, lysine, glutamic acid, etc (Wierbicki and Deatherage, 1958). The meat protein was denatured by heating and a part of its ionized basic or acid groups were broken. This led to the release of the water molecules in meat, which resulted in low moisture content of cooked meats than that of the control. The moisture loss of fried meats was more than those of boiled ones. The possible reason for this might be that the high temperature frying (175±3°C) caused more and rapid denaturation of meat protein when compared with low temperature boiling, resulting in more loss of water molecules (Skog et al., 1995). Oil absorption during frying might be another reason for low moisture content of fried meats than that of the boiled meats.

Prolonged boiling caused hydrolyzes of part of connective tissue and other protein in meat (Mutilangi et al., 1996) and leaking out of sarcoplasmic proteins from the muscle fibers channel (Murphy and Marks, 2000). Hydrolysis of proteins and leaking out of sarcoplasmic proteins might be the reason for the higher loss of protein content from the meat boiled for 15, 40 min than the meat boiled for 5 min and the control. The increased loss of protein content with an increase in frying time was related to the loss of nitrogen in the form of volatile nitrogenous compounds during frying (Yu et al., 1993).

Effect of heat cooking on color of rabbit meat

The CIELAB color (L*, a*, b*) values obtained from the raw and cooked rabbit meat samples are shown in Table 1. The L* value of rabbit meat boiled for 5 min (B5) was similar to that of control, whereas the L* values of meat boiled for 15 (B15) and 40 min (B40) and fried for 2 (F2), 4 (F4) and 6 min (F6) were significantly (P<0.05) lower than that of control. The L* value decreased in the order of B15>B40>F2>F4>F6. The L* values showed that the lightness of the rabbit meat was less affected by boiling than the frying.

The a* values of meat obtained from frying treatments F2, F4 and F6 were significantly (P<0.05) higher than that of control, and the boiling treatments B5, B15 and B40 (Table 1). It was also found that a* of control, B5, B15 and B40 were negative, but the a* of F2, F4 and F6 were positive. The positive a* values indicated that the frying made the cooked meat to become red and boiling treatments maintained the greenness (-a*) of meat similar to that of control. There was no significant (P>0.05) difference observed in b* values for the boiling treatments B5, B15 and B40 as compared to control whereas the b* values of meat samples from frying treatments, F2, F4, F6 were found to be significantly (P<0.05) different from the control. The highest b* value was obtained for F2 and b* decreased with increase in frying time. The b* values

indicated that the frying had a greater influence on b* of meat than that of the boiling. No significant ($P>0.05$) difference in the whiteness values was observed for meat samples of control and treatments B5 and B15, but the whiteness values of meat samples of B40, F2, F4 and F6 were found to be significantly ($P<0.05$) different than the control. The whiteness values are found to be in the ascending order of B40>F2>F4>F6. These results showed that either boiling for longer time or frying for even shorter time (2 min) caused the meat to become darker and the frying severely affected the whiteness of cooked meat. These results are consistent with the findings of Lien et al. (2002) for heat processing of pork patties.

Higher moisture content in the meat increased the lightness of meat gels (Park, 2006). The boiled meat samples had a higher moisture contents than the fried meats, which might be the reason for the higher L* values of boiled meats than those of the fried ones. Previous findings showed that the higher moisture content decreased yellowness (b*) (Park, 2006) and higher cooking temperature decreased the b* value of cooked meat (Brewer and Novakofski, 1999). These findings did not help much to explain the changes in b* values for the boiled or fried meat samples. The low a* values of meat samples of low temperature treatments can be corroborated to the prior findings that red color in cooked meat diminished due to a masking of the myoglobin by aggregation/co-precipitation of other myofibrillar and sarcoplasmic proteins at lower temperatures (Brewer and Novakofski, 1999). The darker color of fried meat was also observed by Sosa-Morales et al. (2006), who reported that frying resulted in a darker crust color of pork meat. Higher redness values in fried products could also be attributed to concentration of meat pigments as moisture content was decreased and the Maillard reaction happened during meat frying, which resulted in the formation of heterocyclic amines (Wong et al., 2012) and have been confirmed to contribute to the risk of developing colorectal tumors (Sinha et al., 1999), deep-frying of meat resulted in a browning color (Dai et al., 2002; Lyn-Cook et al., 2011). All these might be a possible reason why the a* of the fried meat samples had positive a* values and also higher than those of the boiled meats.

The equation used for calculating the whiteness values from the observed L*, a*, and b* values illustrated that L* influences the whiteness more than a* and b*. Therefore, the whiteness of meat samples changed similarly to the change in the lightness value.

Effect of heat cooking on surface feature of rabbit meat

In order to differentiate surface feature of cooked meat samples, the micro structure of raw rabbit meat (control) and the cooked rabbit meats were determined (Figure 2). When compared with the control, the myofilament of the boiled meats (B5, B15 and B40) were clearly seen with the increase in boiling time, but the myofilament of the fried meats (F2, F4, F6) became fuzzier with the increase in frying time, and a black coagulation on the surface was observed, especially on the meat fried for 6 min.

Boiling and frying caused moisture loss of meat samples (Figure 1). Water molecules were attracted to the muscle proteins (Wierbicki and Deatherage, 1958) and heating led to denaturation of meat protein as discussed earlier. The water molecules bounded at myofilament were transferred from myofibrils to the extracellular space (Bertram et al., 2002). This transformation of water within the meat myofibrils and the denaturation of meat protein caused the shrinkage of meat within the tissue matrix, and resulted in the myofibrils standing out (Murphy and Marks, 2000). Therefore, the myofibrils of the boiled meats were seen clearly with increase in boiling times. The surface of the fried meat samples looked fuzzier with the increase in frying time. The possible reason for this could be that the high temperature during frying caused severe denaturation of the myofibrillar protein and some of the frying oil is absorbed in fried meat replacing water (Fillion and Henry, 1998). Maillard reaction happened during frying and formation of dark color heterocyclic amines (Wong et al., 2012) is another possible reason causing the natural surface of the rabbit meat to look tougher and fuzzier.

Effect of heat cooking on *in vitro* digestibility of rabbit meat

The *in vitro* digestibility of cooked rabbit meats were evaluated by TCA-soluble nitrogen release during digestion of pepsin and trypsin in simulated gastric fluid (Figure 3). The nitrogen release rate for boiled (B5, B15 and B40) and fried (F2, F4, F6) meats in pepsin section showed a similar trend during 30 to 60 min, but the differences appeared after 60 min. The nitrogen release rates for B15, B40 was higher than that of B5, F2, F4 and F6, especially after 120 min. The nitrogen release rate for longer duration frying (F6) was the lowest and the nitrogen release rate for B5, F2 and F4 were in between. Similar trend was observed during the trypsin digestion, but the nitrogen release rate for B5, B15 and B40, was found to be higher than that of F2 and F4. The nitrogen release rate for 6 min (F6) frying had the lowest values.

The *in vitro* digestibility of boiled and fried rabbit meat samples implied that, the boiled meats were easy to be digested than the fried ones. The longer boiling was found to be beneficial to the digestibility of meat protein while longer frying made the meat protein hard to be digested. The possible reason is that the frying leads to formation of heterocyclic aromatic amines in protein-rich

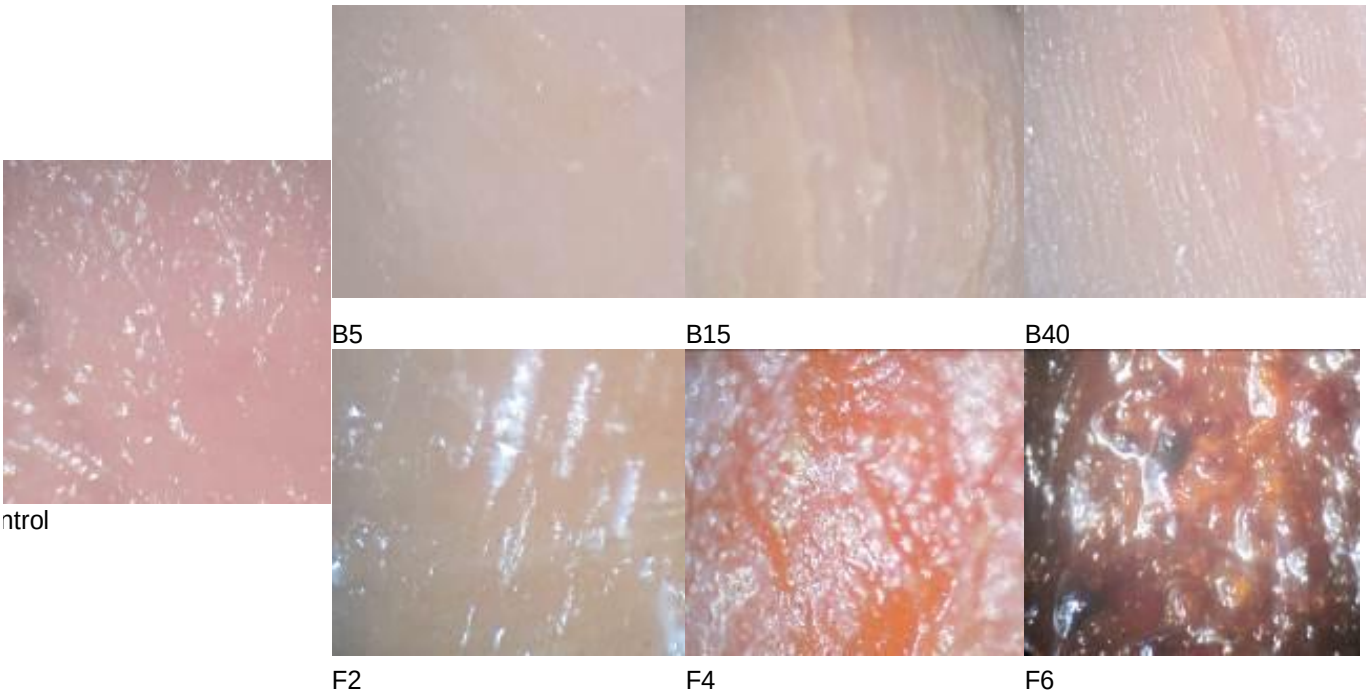

Figure 2. Effect of cooking on micro-surface of rabbit meat. "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

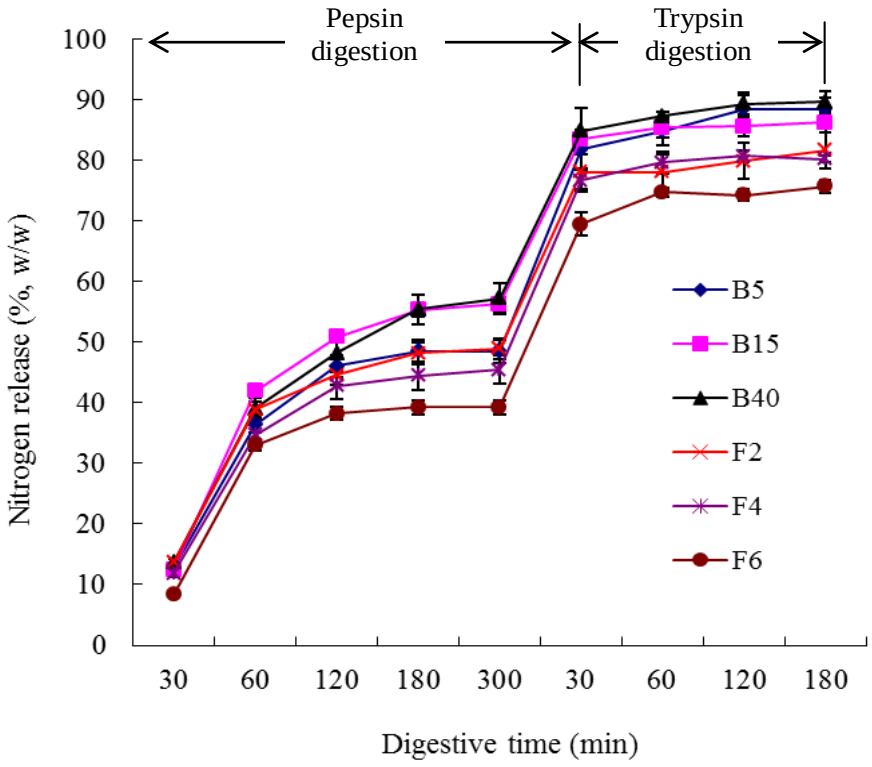

Figure 3. Effect of cooking on digestibility of rabbit meat *in vitro*. Means (n=3) without a common letter differ significantly (P<0.05); "Control" is uncooked rabbit meat, B5, B15 and B40 represents meats boiled for 5 min, 15 min, and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

Table 2. Effect of cooking methods on amino acid of rabbit meat (mg/g protein).

Amino acid	B5	B15	B40	F2	F4	F6
Phenylalanine	9.012±0.018^{d}	11.414±0.020^{a}	10.427±0.131^{b}	9.096±0.006^{cd}	9.174±0.011^{c}	8.686±0.009^{e}
Alanine	18.922±0.067^{d}	21.145±0.034^{a}	21.227±0.094^{a}	18.157±0.075^{e}	19.698±0.094^{b}	19.300±0.057^{c}
Methionine	7.624±0.010^{c}	8.424±0.010^{b}	8.538±0.004^{a}	6.945±0.019^{e}	7.269±0.006^{d}	6.267±0.011^{f}
Proline	11.440±0.003^{d}	12.721±0.010^{a}	12.279±0.057^{c}	11.222±0.037^{e}	10.782±0.007^{f}	12.381±0.009^{b}
Glycine	14.736±0.160^{c}	16.257±0.086^{a}	16.106±0.075^{a}	15.311±0.094^{b}	15.323±0.037^{b}	14.640±0.057^{c}
Arginine	15.857±0.048^{c}	19.122±0.172^{a}	17.641±0.008^{b}	15.853±0.007^{c}	15.478±0.016^{d}	10.364±0.006^{e}
Lysine	20.829±0.058^{c}	25.043±0.064^{a}	22.817±0.075^{b}	20.066±0.031^{d}	16.667±0.008^{e}	12.453±0.004^{f}
Leucine	19.990±0.016^{c}	24.384±0.005^{a}	22.706±0.037^{b}	19.810±0.094^{d}	19.909±0.004^{c}	18.691±0.057^{e}
Tyrosine	8.554±0.005^{c}	10.584±0.052^{a}	9.632±0.094^{b}	8.550±0.057^{c}	8.277±0.037^{d}	7.877±0.024^{e}
Serine	10.913±0.144^{c}	12.267±0.052^{b}	12.478±0.094^{a}	10.278±0.094^{d}	9.184±0.057^{e}	3.318±0.112^{f}
Threonine	11.041±0.067^{c}	13.460±0.104^{a}	12.763±0.094^{b}	10.862±0.043^{d}	10.365±0.037^{e}	5.642±0.131^{f}
Glutamic acid	40.524±0.032^{d}	47.270±0.059^{a}	46.020±0.057^{b}	39.732±0.057^{e}	41.124±0.037^{c}	38.638±0.057^{f}
Aspartic acid	25.607±0.113^{b}	28.978±0.521^{a}	28.671±0.373^{a}	23.948±0.301^{c}	22.233±0.057^{d}	16.529±0.094^{e}
Isoleucine	8.521±0.006^{f}	11.630±0.031^{a}	9.732±0.013^{b}	9.097±0.075^{c}	8.836±0.004^{d}	8.686±0.006^{e}
Histidine	10.091±0.064^{e}	10.436±0.068^{d}	10.688±0.094^{d}	11.371±0.037^{c}	13.628±0.038^{a}	13.173±0.388^{b}
Valine	9.675±0.032^{e}	13.108±0.039^{a}	11.235±0.022^{b}	10.576±0.094^{c}	10.141±0.037^{d}	10.166±0.057^{d}
Cystine	0.609±0.010^{e}	0.582±0.012 ^{f}	1.567±0.008^{b}	0.674±0.009^{d}	1.197±0.011^{c}	2.126±0.008^{a}

Means (n=3) without a common letter differ significantly (P<0.05); B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min, respectively.

foods such as meat and fish at temperatures mostly over 150°C (Oz et al., 2007), which are considered difficult to be decomposed and harmful as they are carcinogenic (Meng et al., 2009, Knize and Felton, 2005). Frying formed a hard dry layer on the surface of meat which prevented loss of internal nutrients of meat (Fillion and Henry, 1998) and also frying led to loss of meat protein (Yu et al., 1993). Harmful and hard to hydrolyzed chemicals, such as heterocyclic amines, formed during meat frying (Wong et al., 2012). All these facts could be cited as the possible reason for the slow nitrogen release rate of fried rabbit meats than those of the boiled meats.

Effect of heat cooking on amino acids content of rabbit meat

The mean amino acid contents of the boiled and fried rabbit meats are presented in Table 2 and the nutrition analysis of amino acids are presented in Table 3. All amino acids, except cystine, increased significantly (P<0.05) when rabbit meat was boiled for 15 min (B15) than boiled for 5 min (B5). The highest increase rate of 36.49% was observed for isoleucine, followed by valine with 35.48% increase. Meat boiled for 40 min also resulted in significant increase in the amino acid contents but at a lower rate when compared with 15 min boiling. These results are in acceptance with our findings on the digestibility of boiled meat which showed that the digestibility of B15 and B40 were easier than that of B5.

Most amino acids decreased significantly after frying for 4 and 6 min while frying for 2 min caused an insignificant (P>0.05) decrease in amino acids contents. Amino acids, which decreased during frying include proline, arginine, lysine, tyrosine, serine, threonine, aspartic acid, isoleucine and valine. Amino acids, lysine and serine had a higher decrease rate than other amino acids. Frying for 6 min caused a severe reduction in the amino acid content than the other two frying treatments, similar results were reported as four marine fishes commonly consumed in Nigeria (*Clupea harengus, Scomber scombrus, Trachurus trachurus* and *Urophycis tenuis*) were fried (Oluwaniyi et al., 2010). Though the boiling of meat for 15 and 40 min showed an increase in amino acid contents when compared with that of 5 min, meat boiled for 15 min contained more amino acids than the meat boiled for 40 min. Boiling resulted in denaturation of proteins in meat and the denatured meat proteins could be easily hydrolyzed (Adler-Nissen, 1976). When dissolved in hydrochloric acid for determination of amino acids, the meat boiled for longer time with high content of denatured proteins were easily hydrolyzed resulting in high amino acid contents than the meat boiled for shorter time. This is the reason for the increase in amino acid contents with the increase in boiling time. However, the prolonged boiling for 40 min resulted in more protein loss in meat and therefore the amino acid contents in meat boiled for 40 min were less than that of meat boiled for 15 min. This speculation agreed with that of Murphy et al. (2008), who reported that an endothermic transition at a temperature

Table 3. Effect of cooking methods on protein nutrition of rabbit meat.

Parameter	B5	B15	B40	F2	F4	F6
Total amino acids (mg/g, w/w)	243.9453±0.1403c	286.8250±0.8239a	274.5260±0.6699b	241.5478±0.1699d	239.2860±0.0980e	208.9370±0.0140f
Essential amino acid (% w/w)	35.5378±0.0621c	37.4667±0.0816a	35.7773±0.0281b	35.7906±0.0795b	34.4197±0.0289d	33.7858±0.1075e
*Chemical score	0.3043±0.0002e	0.4154±0.0011a	0.3476±0.0005b	0.3249±0.0027c	0.3156±0.0001d	0.2015±0.0047f
**Chemical score	0.6307±0.0040d	0.6523±0.0043c	0.6680±0.0059b	0.6998±0.0057a	0.6797±0.0003b	0.6269±0.0145d
*Essential amino acid index	0.3346±0.0005c	0.4041±0.0005a	0.3784±0.0009b	0.3354±0.0005c	0.3269±0.0003d	0.2871±0.0010e
**Essential amino acid index	0.6238±0.0008c	0.7535±0.0009a	0.7054±0.0017b	0.6253±0.0010c	0.6095±0.0006d	0.5353±0.0019e
Protein efficiency ratio	7.7091±0.0068 c	9.4911±0.0048a	8.8290±0.0227b	7.6279±0.0417d	7.7018±0.0022c	7.1908±0.0280e

Means (n=3) without a common letter differ significantly (P<0.05); B5, B15 and B40 represents meats boiled for 5, 15 and 40 min respectively; and F2, B4 and B6 represents meats fried for 2, 3 and 6 min respectively. *Based on amino acid requirements of school children (10-12 years); **Based on amino acid requirements of adults.

range of 59.6 to 68.4°C for collagen in chicken breast patties which resulted in an increase of soluble protein. The soluble protein dissolved in water caused decrease of protein content in chicken breast patties. Similar results were also obtained by Winegarden et al. (1952) for strips of collagenous material heated in distilled water.

The amino acid content of rabbit meat decreased with an increase in frying time. The possible reason is that frying led to formation of heterocyclic aromatic amines (Oz et al., 2007), and a hard and dry surface on the meat (Fillion and Henry, 1998), which made the meat proteins difficult to hydrolyze during amino acid analysis (Yu et al., 1993) and caused the meats fried for longer duration to have a low amino acid content. This result is consistent with that of longer frying time which resulted in low protein of cooked rabbit meat. Heterocyclic amines and polycyclic aromatic hydrocarbons formed during the frying cook, were found to contribute not only to the risk of developing colorectal tumors (Sinha et al., 1999), but also to lung adenocarcinoma (Butler et al., 2013).

The protein nutritional value of boiled and fried rabbit meats are shown in Table 3. The total and essential amino acid contents of boiled rabbit meats (B5, B15 and B40) are significantly (P<0.05) different from each other. The rabbit meat boiled for 15 min (B15) had the highest total and essential amino acid contents followed by meats boiled for 40 (B40) and 5 min (B5). The boiled rabbit meats (B5, B15 and B40) are also found to be significantly (P<0.05) different in the values of chemical score of school children (10-12 years) and adults, essential amino acid index of school children (10-12 years) and adults and the protein efficiency ratio. These results suggest that the boiling time is closely related to the nutritional facts of rabbit meat and there is an optimum time (15 min) to get maximum nutritional value in boiled meat. Boiling for a shorter or longer duration than the optimum time (15 min), resulted in decrease in the nutritional value.

When the protein nutritional value of fried meats were compared, it was found that the total amino acid contents in the meat fried for 4 min (F4) was significantly (P<0.05) lower than that for 2 min (F2), and the total amino acid content of F2 was significantly (P<0.05) lower than that for 6 min (F6). Similar significant (P<0.05) difference was discerned when the essential amino acid content, chemical score and essential amino acid index of F2, F4 and F6 were compared. Exceptionally, the protein efficiency ratio of F4 was found to be significantly (P<0.05) higher than that of F2, and the protein efficiency ratio of F6 was significantly (P<0.05) lower than that of both F2 and F4. The differences of protein nutritional value of F2, F4 and F6 implied that a proper and optimum frying time is essential to maintain the protein nutritional value of rabbit meat and frying for longer time resulted in a severe loss of protein nutritional value.

When comparing the protein nutritional value of boiled and fried rabbit meat, it can be found that the total amino acids, the essential amino acid index of both school children (10-12 years) and adults, and the protein efficiency ratio of boiled meats (B5, B15 and B40) are significantly (P<0.05) higher than that of fried meats (F2,F4 and F6). There is no similar significant (P>0.05) differences observed when the chemical score values of boiled and fried meats were compared. Except for chemical score of adults, all the protein nutritional value parameters of F6 is significantly (P<0.05) lower than B5, B15, B40, F2 and F4. This difference reiterated that frying rabbit meat for

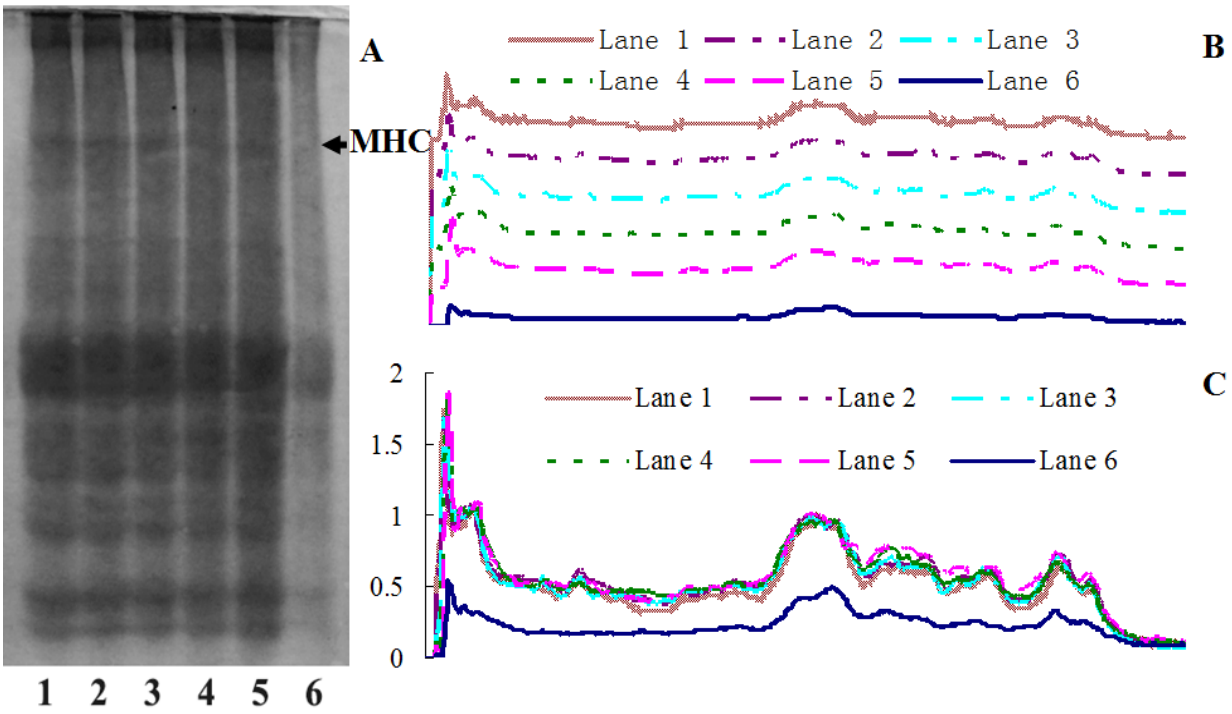

Figure 4. SDS-PAGE of the cooked rabbit meat. Lane 1, 2, 3 represents boiled 5, 15, 40 min, respectively; Lane 4, 5, 6 represents fried 2, 4 and 6 min, respectively; MHC is myosin heavy chains

longer time caused a severe nutrition loss. Beside the protein nutrition was decreased by frying, other investigations showed that frying process caused a significant alteration in the fatty acid profiles of hammour fish lipid and formation of harmful compounds in hammour fish fillets (Ganbi, 2011), heterocyclic aromatic amines were produced during pork frying, and their levels increased with increasing frying time and temperature (Zhang et al., 2013).

SDS-PAGE of cooked rabbit meat

In order to demonstrate the effect of boiling (B5, B15 and B40) and frying (F2, F4, F6) on protein pattern of rabbit muscle meat, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. The results of SDS-PAGE analysis is shown in Figure 4A, along with the optical density (O.D) analysis of the gel (Figure 4B and C). From the Figure 4B and C, it was concluded that there is no difference between O.D values of B5, B15 and B40, but a notable difference is observed between the O.D curves of F2, F4 and F6. The O.D curve of F6 lies below the F2 and F4 (Figure 4 C) and also the O.D curve of F6 is different from all other heat treatments as shown in Figure 4 B.

The O.D values are proportional to the grey value of the bands in the electrophoresis image and a higher O.D value indicated larger grey value (Zhang et al., 2011). The difference of O.D in Figure 4B and C indicate that the protein pattern of the rabbit muscle meat was mildly affected by boiling for 5, 15 and 40 min and the protein pattern of rabbit muscle meat was moderately affected by frying for 2 and 4 min. But the protein pattern was seriously decreased by frying for 6 min. Boiling also resulted in the decrease of myosin heavy chains (MHC) (Figure 4A, B and C), which are polypeptides with a molecular mass of about 200 KDa (Sandri et al., 1999). This result agrees with the findings of Murphy and Marks (2000), who reported the decrease in the MHC of ground chicken breast after boiling.

Conclusions

The nutritional value of rabbit meat was significantly influenced when it was cooked by boiling or frying for different times. Boiling for 15 min enhanced the *in vitro* digestibility and nutritional value of rabbit meat, whereas boiling for 5 or 40 min led to their loss. Frying for 2 and 4 min helped to obtain an acceptable *in vitro* digestibility and nutritional value; even though the values were lower than that of boiled rabbit meat. The frying of rabbit meat for 6 min resulted in a severe impairment to the protein content, color, muscle surface structure, *in vitro* digestibility and protein nutrition. All these results strongly suggest that a proper boiling or frying duration is necessary to acquire a nutritious and high quality cooked rabbit meat.

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