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Amino acid and fatty acid profiles in raw and cooked swamp buffalo meat (*Bubalus bubalis*)

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Abstract: The purpose of this study was to assess the amino acid and fatty acid profiles of swamp buffalo meat (*Bubalus bubalis*) obtained from a local market. The extractable free amino acids and free fatty acids of raw and cooked loin (*Longissimus lumborum*: LL) and round (*Semimembranosus*: SM) buffalo meat were analysed. These experiments were performed in a 2 × 2 factorial in randomised complete block design (RCBD) and there were four treatments combinations, including raw-loin, cooked-loin, raw-round and cooked-round with five replications. The results revealed the amino acid composition for the raw-loin included leucine [4.51 mg·(100 g)$^{-1}$ sample) and isoleucine [4.56 mg·(100 g)$^{-1}$ sample], whereas methionine, aspartic acid and asparagine were the least common amino acids found in both raw and cooked swamp buffalo meat [0.002, 0.020 and 0.034 mg·(100 g)$^{-1}$] of the sample. The amino acid composition of the cooked meat decreased by 50% as compared to raw meat. Swamp buffalo meat contains 10 fatty acids, including saturated fatty acids (SFAs) such as myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and docosanoic acid (C22:0); monounsaturated fatty acids (MUFAs) such as palmitoleic acid (C16:1, *n*-7) and oleic acid (C18:1); and polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2, *n*-6, ω6), gamma-linolenic acid (C18:3, *n*-6, ω6), eicosatetraenoic acid (C20:5, *n*-3, ω3) and docosahexaenoic acid (C22:4, *n*-6, ω6). Stearic acid (C18:0) was the most abundant saturated fatty acids found in the lipid component of buffalo meat. Other medium- and long-chain saturated fatty acids (C14:0, C16:0, C22:0) contributed to around 3–4% of the total fatty acid composition. The most abundant MUFAs and PUFAs were oleic acid (C18:1) and eicosapentaenoic acid (C20:5). The PUFA/SFA ratio, total polyunsaturated fatty acids *n*-3, total polyunsaturated fatty 64 acids *n*-6, and *n*-6/*n*-3 ratios were not significantly different between raw and cooked buffalo meat.

Keywords: meat quality; cooking effect; amino acid composition; fatty acid composition

Swamp buffalo (*Bubalus bubalis*) is highly adapted to swampy areas and low-fertility soils, and it is able to convert high-fibre plants into high-value proteins in geographic areas that are not appropriate for other ruminant species (Joele et al. 2017). Buffalo meat has become an interesting alternative for healthier meat for the consumers, as it is rich in high biological-value proteins, zinc, iron, monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids (Heck et al. 2021). Moreover, several studies have indicated that buffalo meat is leaner and includes less fat, less saturated fatty acids (SFAs), fewer calories, less cholesterol, more minerals, and more protein compared to beef (Gecgel et al. 2018; Hassan et al. 2018). Therefore, buffalo meat is a good choice for people with heart and circulatory system diseases that need to consume red meat. However, buffalo meat's properties have not been properly identified in terms

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of their quality, and the meat also does not receive a fair price given its high nutritional value. In Thailand, swamp buffalo meat is consumed in both cooked and fresh forms; in particular, raw buffalo meat has been accepted by consumers in pickled form and also in salads due to its texture profiles, such as its tenderness and firmness. In addition, some consumers believe that fresh meat has a better nutritional quality than cooked meat.

Biochemical and physical changes occur during meat cooking that affect the meat's sensory characteristics, its flavour and its tenderness (Juárez et al. 2010). The increased temperature during cooking which results in the denaturation of sarcoplasmic, actin, and myosin proteins and the transverse shrinkage of the myofibrils in meat induces changes in both the structure and water distribution of meat (Bejerholm et al. 2014). Heating induces water loss as cooking continues, increasing the meat's lipid content, while only some fat melts and drips out changing the texture and flavour of the meat (Yenrina et al. 2015). Moreover, it has been proposed that heating induces lipid oxidations, leading to undesirable modifications in the nutritional fatty acids of meat. In this regard, the long-chain omega-3 polyunsaturated fatty acids (≥ C20) docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which provide various health benefits, are known to be the most susceptible (Flakemore et al. 2017), and some essential amino acids and other beneficial nutrients are also lost (Abraha et al. 2018).

The aim of this study is to evaluate the differences in the analysis of amino acid and fatty acid compositions of raw and cooked buffalo meat.

MATERIAL AND METHODS

This study used swamp buffalo meat obtained from a local market in Sakon Nakhon province, Thailand. Meat samples of the loin (*Longissimus lumborum*: LL) and round (*Semimembranosus*: SM) were trimmed of their visible fat and stored at 0–4 °C for 24 h before analysis. Ground meat was minced to 1 mm. The meat was then placed in aluminium cups and baked in a hot air oven at 200 °C for 20 min. The experiment was carried out in a 2 × 2 factorial randomised complete block design (RCBD). The four treatment combinations were as follows: T1 = raw meat-loin, T2 = cooked meat-loin, T3 = raw meat-round, T4 = cooked meat-round.

Determination of free amino acid analysis. The amino acids extracted from buffalo meat and 10% trichloroacetic acid (TCA) were mixed in a 1 : 1 ratio by volume to precipitate peptides and proteins, and the homogenate was placed in an ultrasonic bath (Model SK5210HP, Shanghai Kudos Co. Ltd., P.R. China) for 20 min. The solution was filtered through Whatman filter paper No. 4, and the filtrate was centrifuged at 10 000 × g for 10 min (Scientific Instrument Co., Ltd., P.R. China). The supernatants were collected for further detection (Zhang et al. 2018). The free amino acids in the samples were analysed using the LC/MS-8030 Ultra-Fast Triple Quadrupole. Quantification and identification were carried out on the basis of standard compounds (Sigma, USA). The amino acid analysis involved the use of an LC-MS/MS (LCMS-8030, Shimadzu, Japan) (Ratseewo et al. 2020). The experimental conditions were as follows: 0.2 $mL \cdot min^{-1}$ was the flow rate; the temperatures of the autosampler and column oven were set at 4 °C and 38 °C, respectively. The mobile phases used were as follows: (A) demineralised ion (DI) water : formic acid 0.1%; (B) methanol 50% in DI water : formic acid 0.1% (v/v). The amino acids were compared following the twenty authentic amino acid standards, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine.

Determination of free fatty acid analysis. Fat from 5 g of buffalo meat was extracted with 50 mL of chloroform : methanol (2 : 1 v/v) containing 10 $mL \cdot L^{-1}$ of butylated hydroxytoluene (BHT) as the antioxidant, following the procedure described by Folch et al. (1957). The samples were then kept in a fume hood overnight. Each sample was filtered and transferred to a separate funnel, to which 15 mL of physiological saline solution was added. The samples were thoroughly agitated to allow the phases to separate; the lower phase was then evaporated. The fatty acids were converted into methyl esters using the acid catalysed methylation technique (Yang et al. 2006). In detail, 3 mL of HCl–methanol reagent and 1 mL of toluene reagent were applied to 100 mg of isolated lipids. They were then cooked at 70 °C for 2 h. Fatty acid methyl esters were extracted in 2 mL of hexane and kept at 25 °C before the chemical analysis was carried out. The fatty acid methyl esters were evaluated using a Shimadzu (GC-2014, Japan) gas chromatography system with a flame ionisation detector (FID). The esters were separated using a 30 m 0.25 mm ID wall-coated open tubular fused silica capillary column (Agilent, USA)coated in DB-WAX.

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The column injector and detector temperatures were 250 °C and 270 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.27 mL·min^{-1}. The temperature program was set at 150–180 °C at 20 °C·min^{-1}, then it span from 180 °C to 220 °C at 2.5 °C·min^{-1}, it was held at 220 °C for 3 min, it then ranged from 220 °C to 230 °C at 10 °C·min^{-1}, was maintained at 230 °C for 3 min, and finally it spanned from 230 °C to 235 °C at 5.0 °C·min^{-1} and was then held at 235 °C for 10 min. Individual methyl esters were identified using the retention duration of standard methyl esters (Fluka, Switzerland; Sigma, USA) (Thammapat et al. 2010).

Statistical analysis. All samples were examined in duplicate. SAS ver. 9.0 (2003, SAS Institute Inc., USA) was used to perform the analysis of variance (ANOVA) of the Amino acid (AA) and Fatty acids (FA) data. Duncan's multiple range test revealed any significant differences in the results (P < 0.05).

RESULTS AND DISCUSSION

Amino acid composition. The amino acid concentrations in the raw and cooked buffalo meat ranged from 0.002 to 4.558 mg per 100 g sample (Table 1). Raw loin had a higher amount of important amino acids, namely arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine, than any of the other experimental groups. In all the groups, leucine and isoleucine had the highest concentrations [4.558 and 4.506 mg·(100 g)$^{-1}$ of sample], while methionine, aspartic acid, and asparagine had the lowest concentrations in both raw and cooked swamp buffalo meat [0.002, 0.020, and 0.034 mg·(100 g)$^{-1}$ of the sample, respectively]. Additionally, cooked meat lost 50% more amino acids than raw meat. High temperatures can cause amino acids to undergo chemical structural changes or degradation, leading to a loss of nutritional value. Oxidation can alter the structure of amino ac-

Table 1. Protein and amino acid (essentials/non-essentials) composition in raw and cooked swamp buffalo meat (*Bubalus bubalis*)

Composition	Treatments				SEM	P-Value
	T1	T2	T3	T4		
Protein (%)	21.091^{b}	33.461^{a}	21.488^{b}	34.459^{a}	1.511	0.000 1
Essential amino acid [mg·(100 g)$^{-1}$ sample]						
Arginine	2.244^{a}	1.140^{b}	1.118^{b}	1.070^{b}	0.196	0.063
Histidine	1.590^{a}	0.912^{b}	0.766^{b}	0.762^{b}	0.125	0.043
Isoleucine	4.506^{a}	2.100^{b}	2.472^{b}	1.922^{b}	0.424	0.042
Leucine	4.558^{a}	2.126^{b}	2.464^{b}	1.954^{b}	0.423	0.042
Lysine	0.590	0.632	0.260	0.186	0.110	0.360
Methionine	0.016^{a}	0.004^{b}	0.012^{a}	0.002^{b}	0.002	0.003
Phenylalanine	2.660^{a}	1.076^{b}	1.436^{b}	0.944^{b}	0.245	0.016
Threonine	0.396	0.454	0.304	0.446	0.035	0.331
Tryptophan	0.202	0.164	0.108	0.168	0.023	0.369
Valine	3.030^{a}	1.362^{b}	1.564^{b}	1.244^{b}	0.289	0.042
Non-essential amino acids [mg·(100 g)$^{-1}$ sample]						
Alanine	0.208^{a}	0.110^{b}	0.110^{b}	0.106^{b}	0.017	0.076
Asparagine	0.092	0.038	0.034	0.038	0.011	0.238
Aspartic acid	0.062	0.026	0.022	0.020	0.007	0.177
Cysteine	0.274^{a}	0.048^{b}	0.122^{b}	0.040^{b}	0.030	0.010
Glutamic acid	2.406	0.650	0.802	0.516	0.333	0.122
Glutamine	2.534	0.614	0.530	0.274	0.457	0.213
Glycine	2.114^{a}	1.064^{b}	1.138^{ab}	0.974^{ab}	0.197	0.059
Proline	0.616^{a}	0.196^{b}	0.268^{b}	0.194^{b}	0.073	0.046
Serine	0.524^{a}	0.068^{b}	0.154^{b}	0.078^{b}	0.069	0.024
Tyrosine	3.980^{a}	1.038^{b}	1.614^{b}	0.928^{b}	0.441	0.012

^{a,b}different letters in the same row show a statistically significant difference (P < 0.05); T1 – raw meat-loin; T2 – cooked meat-loin; T3 – raw meat-round; T4 – cooked meat-round

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ids, while the loss of a carboxyl group (-COOH) transforms these acids into other compounds. Similarly, the removal of an amino group ($-NH_2$) results in the compound no longer being classified as an amino acid. Mottram (1998) found that heating meat lowered the amounts of leucine, isoleucine, serine, threonine, valine, and phenylalanine by 30–50%. These amino acids are important in aroma formation, providing Strecker aldehydes, as well as other aroma compounds, such as pyrazines.

Fatty acid (FA) composition. Table 2 shows the fatty acid composition (as % of total FAs) in the raw and cooked swamp buffalo meat. This experiment included the following contents in the meat: saturated fatty acids (SFAs) such as myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and docosanoic acid (C22:0); monounsaturated fatty acids (MUFAs) such as palmitoleic acid (C16:1, *n*-7) and oleic acid (C18:1); and polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2, *n*-6, ω6), gamma linolenic acid (C18:3, *n*-6, ω6), eicosatetraenoic acid (C20:5, *n*-3, ω3), and docosahexaenoic acid (C22:4, *n*-6, ω6). The proportions of saturated, monounsaturated, and polyunsaturated fatty acids in buffalo meat were not different between the raw and cooked treatments ($P > 0.05$). Stearic acid (C18:0) was the most abundant saturated fatty acid in the lipid component of buffalo meat. Other medium- and long-chain saturated fatty acids (C14:0, C16:0, C22:0) made up around 3–4% of the total fatty acids. The most abundant MUFAs and PUFAs were oleic acid (C18:1) and eicosapentaenoic acid (C20:5). The PUFA/SFA ratio, total polyunsaturated fatty acids *n*-3, total polyunsaturated fatty acids *n*-6, and *n*-6/*n*-3 ratio were not significantly different ($P > 0.05$) between the raw and cooked buffalo meat. Overall, fatty acids in meat are mainly saturated and monounsaturated fatty acid, generally represented by palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids. PUFAs are found more in pork and poultry than in ruminant meat. Linoleic acid (C18:2) and α-linolenic acid (C18:3) are the predominant PUFAs (Bassam et al. 2022). Changes in the fatty acid profile during cooking can be caused

Table 2. Fat and fatty acid composition in raw and cooked swamp buffalo meat (*Bubalus bubalis*)

Composition	Treatment				SEM	*P*-value
	T1	T2	T3	T4		
Fat (%)	2.320	2.290	2.882	2.335	0.023	0.846
Fatty acids [g·(100 g)$^{-1}$ of sample]						
C14:0 (SFA)	2.905	2.442	2.882	3.242	0.241	0.407
C16:0 (SFA)	0.813^{a}	0.767^{ab}	0.800^{ab}	0.711^{b}	0.022	0.102
C18:0 (SFA)	66.831	67.233	68.082	67.601	0.996	0.980
C22:0 (SFA)	0.468	0.613	0.293	0.370	0.075	0.509
Total (SFA)	70.669	70.933	72.058	71.925	1.131	0.962
C16:1 *n*-7 (MUFA)	1.395^{a}	1.144^{ab}	1.005^{b}	0.976^{b}	0.083	0.014
C18:1 (MUFA)	8.570	7.454	7.804	10.144	0.668	0.544
Total (MUFA)	9.965	8.599	8.809	11.120	0.661	0.552
C18:2 *n*-6 (ω6) (PUFA)	2.288	2.430	3.267	3.125	0.240	0.488
C18:3 *n*-6 (ω6) (PUFA)	6.279	6.391	5.294	5.467	0.449	0.729
C20:5 *n*-3 (ω3) (PUFA)	9.856	10.467	9.515	7.593	0.669	0.186
C22:4 *n*-6 (ω6) (PUFA)	1.177^{a}	1.179^{a}	1.055^{a}	0.769^{b}	0.060	0.007
Total (PUFA)	19.365	20.468	19.132	16.956	1.178	0.628
Total *n*-6	9.509	10.001	9.617	9.362	0.579	0.981
PUFA/SFA	0.116	0.118	0.144	0.151	0.011	0.646
n-6/*n*-3	0.527	0.440	0.536	0.765	0.065	0.413

^{ab}different letters in the same row showed a statistically significant difference ($P < 0.05$); T1 – raw meat-loin; T2 – cooked meat-loin; T3 – raw meat-round; T4 – cooked meat-round; SFA – saturated fatty acid from C4:0 to C20:0; MUFA – monounsaturated fatty acid from C14:1–C22:1; PUFA – polyunsaturated fatty acids from C18:2–C22:6

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by losses of melted lipids in adipose tissue or result from the oxidation of PUFAs (Ortuño et al. 2021). This observation showed that the heating of the meat as a result of cooking did not affect the fatty acid profile, which may be attributed to the absence of the subsequent and rendering loss of intramuscular fat in the lean meat, through losses of melted adipose lipids, the emigration deficiency of fatty acid from intramuscular fat to the lean meat, or the deficiency of oxidative degradation on the double bone of PUFAs within the lean meat (Badiani et al. 2004). Moreover, dry heat and/or conventional cooking methods have been reported to have a small impact on fatty acid oxidation in ruminant meat (Ortuño et al. 2021).

CONCLUSION

Raw meat-loin was determined to be composed of the amino acids leucine [4.558 mg·(100 g)$^{-1}$ sample] and isoleucine [4.506 mg·(100 g)$^{-1}$ sample], while methionine, aspartic acid, and asparagine were the least common amino acids in both raw and cooked swamp buffalo meat [0.002, 0.020, and 0.034 mg·(100 g)$^{-1}$ of the sample]. The amino acid content of cooked meat was reduced by 50% compared to raw meat. High temperatures can cause amino acids to undergo chemical structural changes or degradation, leading to a loss of nutritional value. Oxidation can alter the structure of amino acids, while the loss of a carboxyl group (-COOH) transforms them into other compounds. Similarly, the removal of an amino group ($-NH_2$) results in the compound no longer being classified as an amino acid. The fatty acids found in the meat include the following: unsaturated fatty acids such as myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and docosanoic acid (C22:0); monounsaturated fatty acids such as palmitoleic acid (C16:1, *n*-7) and oleic acid (C18:1); and polyunsaturated fatty acids such as linoleic acid (C18:2, *n*-6, ω6), gamma-linolenic acid (C18:3, *n*-6, ω6), eicosatetraenoic acid (C20:5, *n*-3, ω3), and docosahexaenoic acid (C22:4, *n*-6, ω6). Stearic acid (C18:0) was the most abundant saturated fatty acid in the lipid component of buffalo meat. Other medium- and long-chain saturated fatty acids (C14:0, C16:0, C22:0) made up around 3–4% of the total fatty acids. The most abundant MUFAs and PUFAs were oleic acid (C18:1) and eicosapentaenoic acid (C20:5). The PUFA/SFA ratio, total polyunsaturated fatty acids *n*-3, total polyunsaturated fatty acids *n*-6, and *n*-6/*n*-3 ratios were not substantially different between raw and cooked buffalo meat.

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