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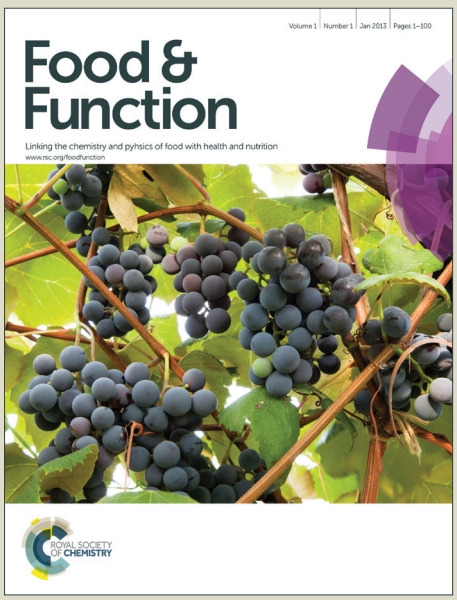

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The *in vitro* digestibility of beef varies with its inherent ultimate pH

Mustafa M. Farouk,[a*] Guojie Wu,[a] Deborah A. Frost,[a] Stefan Clerens[b] and Scott O. Knowles[c]

[a]Food Assurance & Meat Quality Team; [b]Proteins and Biomaterials Team; [c]Food Nutrition & Health Team

AgResearch Ltd, Ruakura Research Centre, Hamilton, New Zealand

*Email: mustafa.farouk@agresearch.co.nz

Abstract

Animal carcasses and cuts of meat are usually differentiated and valued according to size and compositional attributes. An underappreciated variable of red meat is its inherent ultimate pH (pH_u) value, which affects organoleptic and processing characteristics. This study tests the hypothesis that high pH_u aged meat would be more digestible than low pH_u unaged (fresh) meat. *Longissimus dorsi* muscles collected from 59 bull carcasses had pH_u values of 5.6–6.9. These were aged for 21 days at –1.5°C, then raw and cooked (72°C) samples were enzymatically digested at 37°C with pepsin (pH 1.9 for 90 min) followed by pancreatin (pH 8.0 for an additional 120 min) to simulate conditions in the stomach and small intestine, respectively. Meat proteins and peptides in the digests were separated by 1D SDS PAGE. Regardless of pH_u, ageing or cooking, most sarcoplasmic and myofibrillar proteins were rapidly digested by pepsin, with concomitant release of products identified by LC-MS/MS as mainly myosin-1, -2 and -7, α-actinin-2 or -3 and tropomyosin beta and alpha chains. These products were resistant to further digestion for the entire 210 min duration of the incubation. In terms of rate and extent of digestibility of these resistant products, high pH_u > low pH_u ($P < 0.001$), whereas aged > unaged ($P < 0.003$), with the effect of cooking dependent on pH_u and varying somewhat by protein. Overall, the digestibility of meat samples increased with increasing pH_u ($P < 0.001$). Beef meat was highly digestible but could be further differentiated on the basis of its pH_u and the ease of digestibility of proteins. Specific carcasses or cuts could be targeted to consumer groups in order to provide benefits and add value.

1. Introduction

The quality of red meat is typically classified and valued according to rather narrow definitions. Taste, texture and tenderness (and the visual perceptions by which these are inferred) are paramount when pricing meat cuts[1]. For instance fresh is preferred over frozen, bright cherry-red colour over dark brown, and middle cuts from the carcass such as tenderloins (*Psoas major*) over those from the forequarter, which tend to be less shapely and contain more connective tissue. An indicator of meat quality that is invisible to consumers is the inherent ultimate pH (pH_u), which affects organoleptic and processing characteristics. The pH_u is a consequence of muscle metabolism in the animal, related to the availability of glycogen that can be converted to lactate during rigour processes that occur post-mortem. Animal diets, farm management and physiological factors combine to generate a range of meat pH_u values in beef, particularly among young bulls[2].

Meat tenderness is associated with pH_u, usually in a U-shaped curvilinear relationship, with meat that attains low ($pH \leq 5.8$) pH_u or high ($pH \geq 6.2$) being acceptably tender after ageing[3-6]. Also, the rate at which meat tenderises is affected by pH_u, with high pH_u meat tenderising more rapidly[7, 8]. The effect is attributed to a weakening of the highly organised myofibrillar structure, caused post-mortem by proteolytic degradation of key proteins by calpain and cathepsin enzyme systems[9-12].

Digestion of meat in the gastrointestinal tract (GIT) also involves proteolysis and may be similarly influenced by pH_u and post-mortem ageing. Meat nutritional value is realised when the proteins are broken down by enzymes into shorter peptides and free amino acids. Most fragments are efficiently absorbed in the upper GIT to meet the physiological needs of the consumer[13]. However incompletely lysed fragments can reach the lower GIT, where they may be fermented by microbiota. Some of the resulting metabolites may contribute to different types of bowel ailments[14-16]. Therefore, the rate and extent of meat digestibility and the factors affecting those processes are important determinants of the utilisation, differentiability and value of meat.

To facilitate the study of digestion, many *in vitro* models have been developed. These vary in sophistication from test tube chemistry to mechanical anatomical simulation systems[17, 18]. Although none is equivalent to *in vivo* physiology, most attempts to mimic the sequence of digestion conditions encountered by food as it passes from the mouth to the bowel. One of the simplest systems has proven useful and has been widely applied to the study of proteins. It involves proteolysis at 37°C with pepsin at pH 1.9 followed by pancreatin at pH 8.0 to simulate conditions in the stomach and upper small intestine, respectively.

We used an *in vitro* model to evaluate the digestibility of protein in samples of beef having a wide range of pH_u. We hypothesised that pH_u would interact with the effects of meat aging and cooking. To our knowledge this is the first published study to measure the effects of pH_u on digestibility. Our ultimate aim is to differentiate beef on this basis and add value to the meat classes and cuts currently considered low premium.

2. Materials and methods

2.1 Muscle collection and sample preparation

Longissimus dorsi (LD) muscles were collected from 59 dairy bulls (18–24 months old, primarily Holstein-Friesian and the similar KiwiCross™ breed) at a local commercial abattoir. Muscles were converted to meat during 48 h storage at 15°C, at which time the pH_u was measured. A portion of each LD was vacuum-packed and immediately frozen at –30°C; these samples served as unaged meat and are referred to in this paper as 'fresh'. The remainder of each muscle was aged for 21 days at –1.5°C, its pH_u value reconfirmed, and thereafter stored at –30°C. Thirteen of the meat samples having pH_u ranging from 5.6 to 6.9 were selected for further study. Portions of these fresh and aged samples were cooked by placing 15–20 g into a sealed glass bottle in a water bath for 20 min at 72°C. Thus the complete sample set for our study comprised LD-derived meat from 13 bulls, with portions of each prepared as fresh or aged and raw or cooked, for a total of 52 meat samples subjected to *in vitro* digestion and analysis.

2.2 In vitro digestion

Enzymes used in this study were pepsin (P6887, Sigma) and pancreatin (P8096, Sigma). Sodium dodecyl sulphate (SDS), di-sodium hydrogen orthophosphate and hydrochloric acid were analytical grade from Fisher Scientific, UK. Tris (ultrapure Bioreagent) and 2-mercaptoethanol were products of JT Baker (USA) and BDH, respectively.

Two-stage *in vitro* digestion was carried out as described by Kaur et al.[19] with modifications, utilising pepsin and acid to simulate gastric conditions and subsequently pancreatin and weak alkali to simulate conditions in the upper small intestine. For each meat sample, a 4.5 g portion was minced and suspended in 34 ml of 0.1 M HCl, then homogenised (small rotor disperser, IKA Labortechnik) at 22000 rpm for 10 sec twice. The pH was adjusted to 1.9 with NaOH and made up to 36 ml with Milli-Q ultrapure water. Samples were incubated in a water bath at 37 ± 0.2°C equipped with a horizontal shaker at 30 rpm (Thermo Haake DC 10, Karlsruhe, Germany). Pepsin solution was added to each flask to start the proteolysis (enzyme:substrate ratio 1:278 in 0.1 M HCl). At 0, 30, 60 and 90 min, aliquots of 0.5 ml were removed and immediately inactivated with NaOH to increase the pH to 8.0. These aliquots were mixed with SDS sample buffer (0.5 ml, 3) and heated in water bath at 95–100°C for 5 min then stored at –20°C until analysed.

After 90 min, the digestion solutions were adjusted to pH 8.0 with 6 M of sodium hydroxide (approximately 0.6 ml) to inactivate the pepsin enzyme, then pancreatin solution was added (enzyme:substrate ratio 1:100 in 0.1 M phosphate buffer pH 8.0). At 150 and 210 min, aliquots of 0.5 ml were removed and immediately inactivated with HCl to reduce pH to approximately 1.9. These aliquots were treated as described above.

Three digestion controls were run to determine the extent to which endogenous meat enzymes contributed to the release of proteins and peptides during incubation, and the contribution of the enzyme preparations to the total production of digested proteins and peptides. These were fresh meat containing no pepsin or pancreatin; fresh meat with pepsin enzyme only; and pepsin and pancreatin containing no meat.

2.3 Electrophoresis

Proteins and peptides in the meat digests were separated and quantified by 1D SDS PAGE using Criterion TGX gels or Tris-Tricine gels (10–20%, Bio-Rad). Aliquots collected from the digestion solutions were thawed, well-mixed, then centrifuged at 9300 × *g* for 5 min at ambient temperature. The supernatants were loaded on the gel at 40 µg protein per well and electrophoresis conducted at a constant voltage of 150 V. Gels were stained using Coomassie Blue R250, washed thrice with Milli-Q water, then scanned using a GS800 Calibrated Densitometer Scanner (Bio-Rad) and analysed with Quantity One software (version 4.6.5, Bio-Rad). The results were expressed as Relative Quantity (RQ%).

Gels were run to visualise the time-course of digestion of each meat sample, utilising the aliquots collected from 0 min through 210 min. Representatives are shown in Figs. 1 and 2. A second series was run to compare the final products of digestion (i.e. the contents of the aliquots collected at 210 min) from all 52 meat samples across the full range of pH_u values (presented in Figs. 3, 4, 5 and 6). The majority of residual material from 210 min digestion

was found in bands B2/3, B8/9, B11 and B12, so these were further analysed. The meat was categorised as either low or high pH_u (cut-off value 6.2) then mean RQ values charted by 2 factors at 2 levels (± ageing and ± cooking) in Fig. 7. For one of those bands, B3, the relationships between pH_u and RQ were plotted in Fig. 8.

2.4 LC-MS/MS analysis of digested proteins

Protein bands separated by SDS PAGE were identified by LC-MS/MS as described previously[20, 21]. Briefly, bands were destained, reduced with 50 mM TCEP, and alkylated with 100 mM iodoacetamide. The bands were crushed in microcentrifuge tubes using pipette tips and digested using 400 ng sequencing grade porcine trypsin (Promega, USA) with overnight incubation at 37˚C. After digestion, the peptides were extracted from the gel slurry and concentrated in a vacuum centrifuge until near dryness.

The samples were reconstituted in 25 µl loading solvent (2% ACN, 0.2% FA). LC-MS/MS was carried out on an Ultimate nanoflow HPLC (LC-Packings, The Netherlands) coupled to a QSTAR pulsar *i* mass spectrometer (AB Sciex). Ten µL of sample was loaded on a C18 precolumn (300 µm ID, 5 µm particles, 300 Å pore size) and eluted over the analytical column (C18, 20 cm, 75 µm ID, 5 µm particles, 300 Å pore size), at 150 nl/min, with a gradient from 2% to 55% B in 50 min. Solvent A was HPLC-grade water with 0.2% formic acid, solvent B was LCMS-grade acetonitrile with 0.2% formic acid.

Peak lists were extracted from the data files and submitted to an in-house Mascot server. The search engine was Mascot 2.4.0 and the database was SwissProt. NCBInr was also used as a database to further confirm results and the accession numbers of the identified proteins/peptides. The following search parameters were used: Taxonomy Bos taurus; Enzyme trypsin; Fixed modifications Carbamidomethyl (C); MS and MS/MS mass tolerance 0.6 Da; Peptide tolerance 0.3 Da; 1 missed cleavage; Accept proteins with score > 80.0 and peptides with score > 30.0. Data identified as originating from keratin and trypsin were removed, and only identifications corresponding to bovine sequences with a minimum of two unique peptides were accepted.

2.5 Statistical analysis

The experimental design was a factorial with 2 factors at 2 levels (± ageing and ± cooking). The influence of pH_u was considered as either a continuous or binary variable. The latter was created by collapsing the range of inherent meat pH_u values to two categories ('high' being pH_u 6.9–6.2 and 'low' being pH_u less than 6.2). ANOVA repeated measures analysis was performed for each selected gel band, with other bands from the same gel lane included as blocking variables. The pH category and treatment were the explanatory variables, where treatment had 4 levels (unaged raw, unaged cooked, aged raw, and aged cooked; n = 13 each). For the analysis underlying Fig. 7, the overall standard error was calculated from ANOVA as (sqrt(mean square error)/sqrt(min rep)). With pH_u considered as a continuous variable, linear regression could be computed. For the analysis of gel band B3 in Fig. 8, each treatment was allowed to have its own intercept and slope coefficients. Software used for all analyses was Genstat 16 (version 16.2.0).

3. Results

3.1 Effect of digestion duration

The effect of incubation time on the digestion of proteins in a representative sample of unaged cooked beef of low pH_u is shown in Fig. 1. The LC-MS/MS identification of peptides from individual bands of that gel is compiled in Table 1. Meat was highly digestible under these *in vitro* conditions. Sarcoplasmic proteins (generally small, water soluble, intracellular) were quickly hydrolysed, as were most of the myofibrillar proteins (comprising myosin heavy chain (MHC), actin, myosin light chain-1 (MLC1) and -2 (MLC2), troponin, tropomyosin, actin and actinin). Proteins in bands B1, B2, B10 and B14 resisted digestion by pepsin but were ultimately lysed by pancreatin. These included breakdown products of MHC and α-actinin-2 and -3. Tropomyosin underwent rapid partial digestion by pepsin, releasing its breakdown products as bands B10, B11 and B14. The fragments in B10 and B14 appeared to be fully digested by pancreatin while those in B11 were not.

3.2 Effects of pH_u, post-mortem ageing and cooking

Meat pH_u influenced how proteins were digested over time, as shown in Fig. 2 with three representative samples of unaged cooked beef. Proteins from the higher pH_u meats tended to be more completely digested, particularly during the pancreatin stage. An exception was band B13, which was little changed over time in the highest pH_u meat but disappeared quickly from the digest of low pH_u meat.

The proteins and peptides that remained after the full duration of pepsin and pancreatin digestion (i.e. at 210 min) were further analysed to determine the effects of pH_u, ageing and cooking on beef digestibility. This residual material was mainly found in bands B2/3, B8/9, B11 and B12 (Fig. 7). Overall, the low pH_u meat was more resistant to digestion (i.e. higher RQ values). In bands B3 and B8/9, this difference was much greater among the cooked samples. In bands B11 and B12, the unaged meat was more resistant to digestion than the aged meat, as was the raw meat compared to the cooked.

The data for band B3 of Fig. 7 is expanded in Fig. 8 to show by linear regression how pH_u affects digestibility and how this relationship interacts with ageing and cooking. There was more digestion-resistant protein (higher RQ) remaining from the unaged meat than from the aged regardless of cooking. The influence of inherent pH_u was much greater on cooked meat than on raw regardless of ageing, with low pH_u meat being least digestible.

Repeated measures analysis indicated that the effect of pH_u on beef digestibility differed across gel bands ($P < 0.004$), meaning that pH_u is a more important factor for some proteins compared to others. There were significant ($P < 0.05$) interactions between the effects of pH_u versus cooking and between ageing versus cooking ($P < 0.05$), but not between pH_u versus ageing ($P > 0.05$).

High pH_u or aged beef was more digestible compared to low pH_u or unaged (Fig. 2, 3, 4, 5 and 6), and overall digestibility increased with the increase in pH_u ($P < 0.05$) (Fig. 8). Cooking had variable effects. It tended overall to increase the digestibility of proteins and peptides particularly from high pH_u meat (Fig. 3, 4, 5, 6 & 8).

4. Discussion

Our implementation of a two-stage *in vitro* protocol successfully digested samples of beef muscle meat (Fig. 1). It produced fragments of proteins similar to those reported for beef[19] and for pork[22]. The major proteins and their breakdown peptides were identified by LC-MS/MS (Table 1). The bulk of these were derived from the myofibrillar (structural and contractile) proteins that comprise 50–60% of total muscle protein. Most were hydrolysed within 90 min by acidic pepsin, although some were resistant to both pepsin and subsequent pancreatin. These were products of myosin, followed by α-actinin, actin and then tropomyosin. The rapid disappearance of some gel bands and concomitant appearance of others (e.g. MHC and α-actinin versus B2, B3 and B8/9, respectively) illustrates how sequential proteolysis can create high molecular weight fragments (30–90 kDa) from meat parent proteins that are not completely digested in the upper GIT. This might also occur *in vivo*.

The animal species, tissue type, composition and processing of meat are known to affect the digestibility of protein. In this study we demonstrated a role for pH_u - an inherent characteristic of red meat that had not been appreciated as a factor affecting digestion. We observed greater digestibility of high pH_u beef, a phenomenon that could be due to the endogenous breakdown of protein and consequent tenderisation that has been reported for high pH_u meat compared to its low pH_u equivalent [3-5, 23]. The shortened proteins and peptides and the larger protein surface area may have been more accessible to pepsin, making further breakdown easier. Escudero et al.[22] studied the *in vitro* digestion of pork and concluded that pepsin digestion affects meat protein structure, resulting in more open protein chains with more accessible sites for further digestion by pancreatin.

Greater digestibility of cooked aged meat compared to unaged (Fig 4, 5, 6, 7 & 8) might be similarly explained[24], emphasising the importance of meat structural integrity prior to exposure to digestive enzymes. The length and conditions of post-mortem ageing make a difference. Compared to the 21 days of ageing at –1.5°C used for meat in this study, the *in vitro* digestibility of pork was not affected by 4 days at 4°C[25].

Cooking has variable effects on meat digestibility depending on both temperature and time[19, 25-27]. For instance, cooking beef quickly to 100°C lessened pepsin- and enhanced pancreatin-proteolysis, but longer cooking at the same temperature reduced overall susceptibility to proteolytic enzymes. Cooking pork at a mild 70°C enhanced peptic digestion due to protein unfolding and greater accessibility to cleavage sites, while 100°C slowed peptic digestion due to protein aggregation and reduced hydrolyzability. Cooking beef enhanced the digestibility of larger peptides, i.e. those > 25 kDa, while reducing the digestibility of peptides < 10 kDa. When beef meals were digested by pigs *in vivo*[28], cooking affected the speed of protein digestion, but not the overall efficiency. This relationship was U-shaped, with the intermediate temperature showing fastest digestion. Changes to the macro- and microstructure of the meat were suggested as altering accessibility to digestive enzymes. Unfortunately, none of the previous studies reported the pH_u of the meat used.

Our results show that the effect of cooking at 72°C on beef digestibility varied with pH_u and ageing. Cooking mostly improved the digestibility of high pH_u beef ($pH \geq 6.2$) regardless of

ageing, but reduced the digestibility of some proteins from low pH_u beef, particularly those in the unaged samples in band B8/9. Cooking might have changed the conformation and denatured the low pH_u proteins, causing more extensive crosslinking and aggregation that impaired digestibility. Proteins at lower pH are more susceptible to denaturation than native proteins or proteins at near-neutral pH[29]. Based on the mechanism of pepsin action on the digestibility of raw and cooked meat proposed by Bax et al.[25], high pH_u meat cooked to 72°C could be considered as unfolded protein and the low pH_u cooked to the same temperature as aggregated protein.

5. Conclusions

The digestibility of beef assessed using a simple *in vitro* system was affected by the duration of incubation with proteolytic enzymes, the meat pH_u, post-mortem ageing, and cooking at 72°C. High pH_u or aged beef was more digestible compared to low pH_u or unaged beef. Cooking typically improved the digestibility of high pH_u meat but had the opposite effect on some proteins in low pH_u meat. If these phenomena can be verified *in vivo*, meat producers, butchers and chefs could exploit the relationships between pH_u and ageing by targeting beef carcasses and cuts to specific groups of consumers who might benefit from different levels of digestibility and tenderness. Other implications include:

- Marketing meat with extra-high digestibility might be an attractive value proposition for the elderly, those with compromised gastrointestinal function, or those trying to avoid protein fermentation in the lower GIT.
- Chefs could start measuring the pH_u of the meat they offer for the purpose of tailoring the choice of cut and the doneness of the finished dish to suit their customers' desires for digestibility or tenderness.
- Butchers and retailers could start identifying meat products based on its inherent-but-invisible functionalities, such as digestibility, rather than solely on aesthetic-gustatory considerations. This could transform the way that meat is valued, prepared and consumed.

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Table 1. Proteins identified by LC-MS/MS from 14 bands separated by 1D SDS PAGE from a representative sample of unaged cooked beef of low pH_u digested *in vitro* by pepsin and pancreatin.

Gel position Band (MW)	Original protein	Sequence number (GenInfo Identifier, gi)	Score:	Peptides		Proteins	
				Sequence coverage (%)	No. of peptides	MW (kDa)	pI
B1 (100kDa)	Myosin-2	75055812	969	9.9	18	223.2	5.52
	Alpha-actinin-2	77736221	896	18.2	13	103.7	5.19
	Alpha-actinin-3	115495613	538	11.9	9	103.1	5.19
	Glycogen phosphorylase	28461197	214	3.7	3	97.2	6.7
B2 (75kDa)	Myosin-2	261245063	2706	19.2	43	223.2	5.52
	Myosin-7	41386711	2260	16.7	34	223.1	5.47
B3 (74kDa)	Myosin-2	261245063	2075	15.8	34	223.2	5.52
	Myosin-1	41386691	1642	12.3	28	222.9	5.47
	Myosin-7	296483595	1424	11.3	24	223.1	5.47
B4 (70kDa)	Myosin-1	41386691	1580	12.7	27	222.9	5.47
	Myosin-2	75055812	1570	12.8	26	223.2	5.52
	Myosin-7	41386711	697	5.9	11	223.1	5.47
B5 (48kDa)	Myosin-2	75055812	464	4	10	223.2	5.52
	Myosin-1	41386691	312	2.6	7	222.9	5.47
B6 (46kDa)	Myosin-7	41386711	824	7.8	18	223.1	5.47
	Myosin-2	75055812	695	5.9	13	223.2	5.52
	Alpha-enolase	4927286	115	6.7	2	47.3	6.41
B7 (43kDa)	Myosin-2	75055812	1177	10.4	21	223.2	5.52
	Myosin-7	41386711	1169	10.4	20	223.1	5.47
	Myosin-1	41386691	1042	9.2	19	222.9	5.47
	Actin, aortic smooth muscle	4501881	85	5.6	2	42	5.12
B8 (42kDa)	Myosin-7	41386711	516	4.1	8	223.1	5.47
	Myosin-2	75055812	414	3.6	7	223.2	5.52
	Myosin-1	41386691	398	2.7	5	222.9	5.47
B9 (40kDa)	Myosin-2	75055812	2642	19.4	42	223.2	5.52
	Myosin-1	41386691	2089	15.6	32	222.9	5.47
	Myosin-7	41386711	1563	11.2	25	223.1	5.47
B10 (37kDa)	Myosin-2	75055812	2431	18.1	37	223.2	5.52
	Myosin-7	41386711	2323	18.2	37	223.1	5.47
	Myosin-1	41386691	2269	17	34	222.9	5.47
	Tropomyosin beta chain	11875203	1109	52.1	17	32.8	4.51
	Tropomyosin alpha-1 chain	61888866	729	43.7	13	32.7	4.54
	GAPDH	77404273	97	8.7	2	35.8	9.26
B11 (32kDa)	Myosin-2	75055812	2368	18.4	37	223.2	5.52
	Myosin-7	41386711	2145	17.1	35	223.1	5.47

	Myosin-1	41386691	1976	16	33	222.9	5.47
	Tropomyosin alpha-3 chain	58652133	815	46.5	14	32.8	4.53
	Tropomyosin alpha-1 chain	61888866	532	26.1	8	32.7	4.54
B12 (30kDa)	Myosin-7	296483595	2237	15.6	35	223.1	5.47
	Myosin-2	75055812	1509	12.1	24	223.2	5.52
B13 (28kDa)	Myosin-2	75055812	562	4	10	223.2	5.52
	Myosin-7	296483595	375	2.6	7	223.1	5.47
B14 (18kDa)	Myosin-7	41386711	1004	6.5	15	223.1	5.47
	Myosin-2	75055812	647	4.8	11	223.2	5.52
	Myosin-1	41386691	625	4.8	10	222.9	5.47
	Tropomyosin alpha-1 chain	61888866	365	18	5	32.7	4.54
	Tropomyosin beta chain	11875203	329	19	5	32.8	4.51
	Tropomyosin alpha-3 chain	58652133	258	10.6	3	32.8	4.53

Figure Legends

Fig. 1. SDS PAGE gel showing the effect of incubation time on the digestibility of proteins in a representative sample of **unaged cooked** beef of low pH_u by pepsin during 0–90 min followed by pancreatin during 150–210 min.

Fig. 2. SDS PAGE gels showing the effects of incubation time and pH_u on the digestibility of proteins in three representative samples of **unaged cooked** beef by pepsin during 0–90 min followed by pancreatin during 150–210 min.

Fig. 3. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **unaged raw** beef incubated with pepsin for 90 min followed by pancreatin for an additional 120 min.

Fig. 4. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **unaged cooked** beef incubated with pepsin for 90 min followed by pancreatin for an additional 120 min.

Fig. 5. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **aged raw** beef incubated with pepsin for 90 min followed by pancreatin for an additional 120 min.

Fig. 6. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **aged cooked** beef incubated with pepsin for 90 min followed by pancreatin for an additional 120 min.

Fig. 7. Interactions between ageing and cooking on the *in vitro* digestibility of beef samples categorised as having high pH_u (dark bars) or low pH_u (hatched bars). The RQ of gel bands B3, B8/9, B11 and B12 was measured after 210 min of digestion. Protein and peptide composition of those bands is described in Table 1. The mean value for each of the eight categories is shown (n = 6 to 7). The overall standard error of the means is 0.03 RQ.

Fig. 8. Regression analysis of the interactions between ageing and cooking on the *in vitro* digestibility of beef samples having a range of pH_u values. The RQ of gel band B3 was measured after 210 min of digestion for each of the 52 meat samples. Symbols are ▲ unaged cooked; □ unaged raw; ● aged cooked; ▽aged raw.

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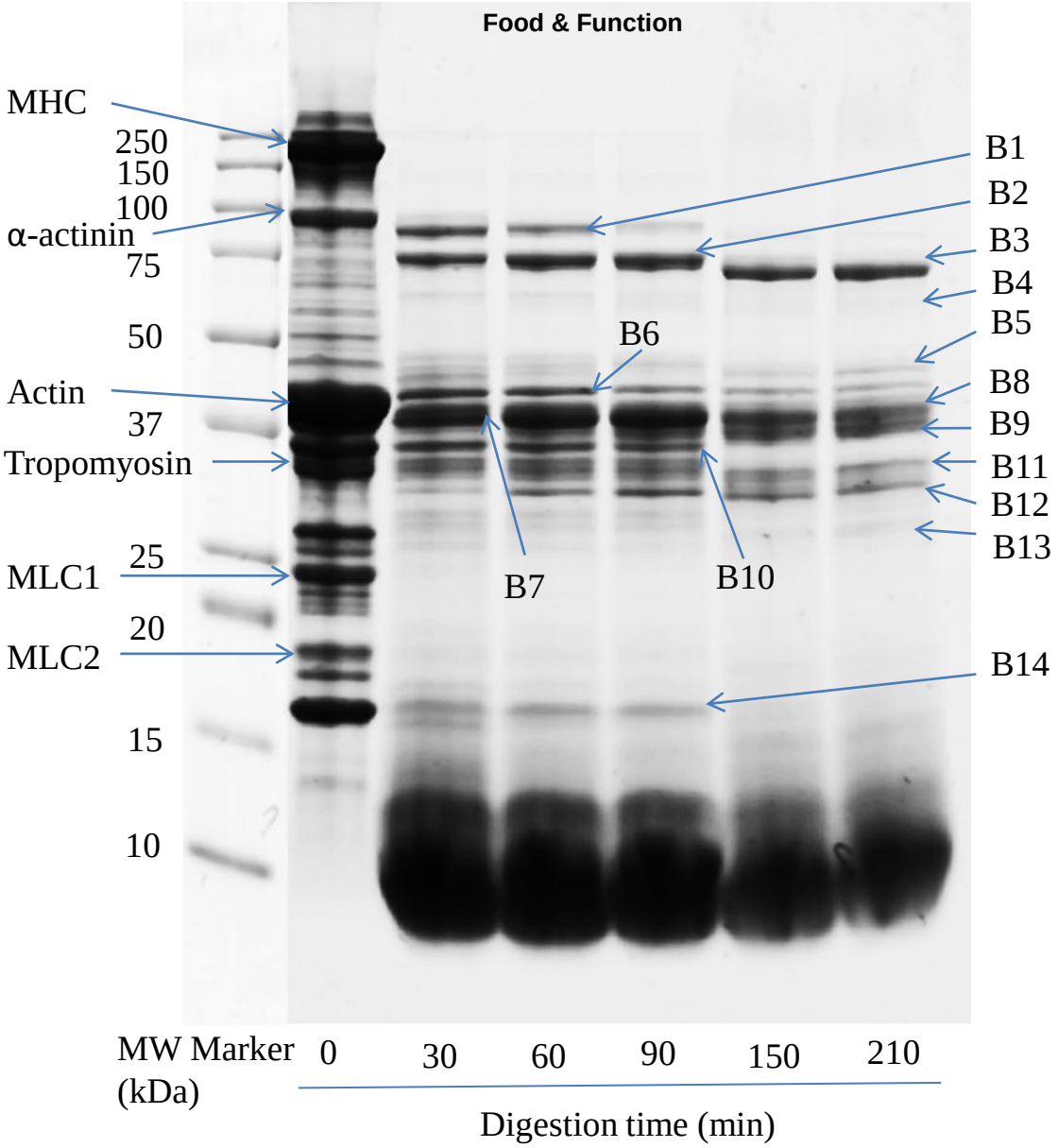


Fig. 1. SDS PAGE gel showing the effect of incubation time on the digestibility of proteins in a representative sample of **unaged cooked** beef of low pH_u by pepsin during 0–90 min followed by pancreatin during 150–210 min

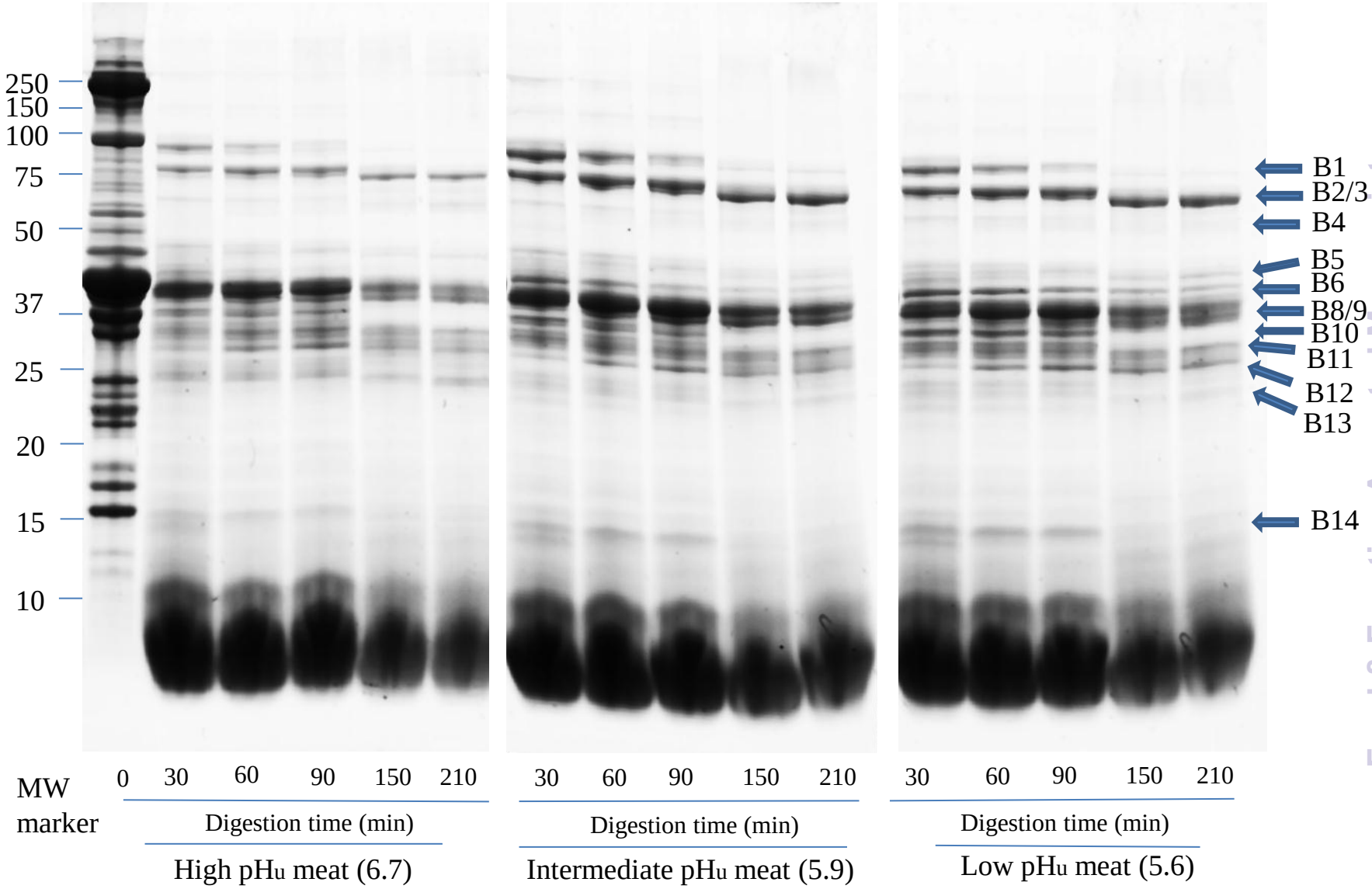


Fig. 2. SDS PAGE gels showing the effects of time and pH_u on the digestibility of proteins in three representative samples of **unaged cooked** beef by pepsin during 0–90 min followed by pancreatin during 150–210 min.

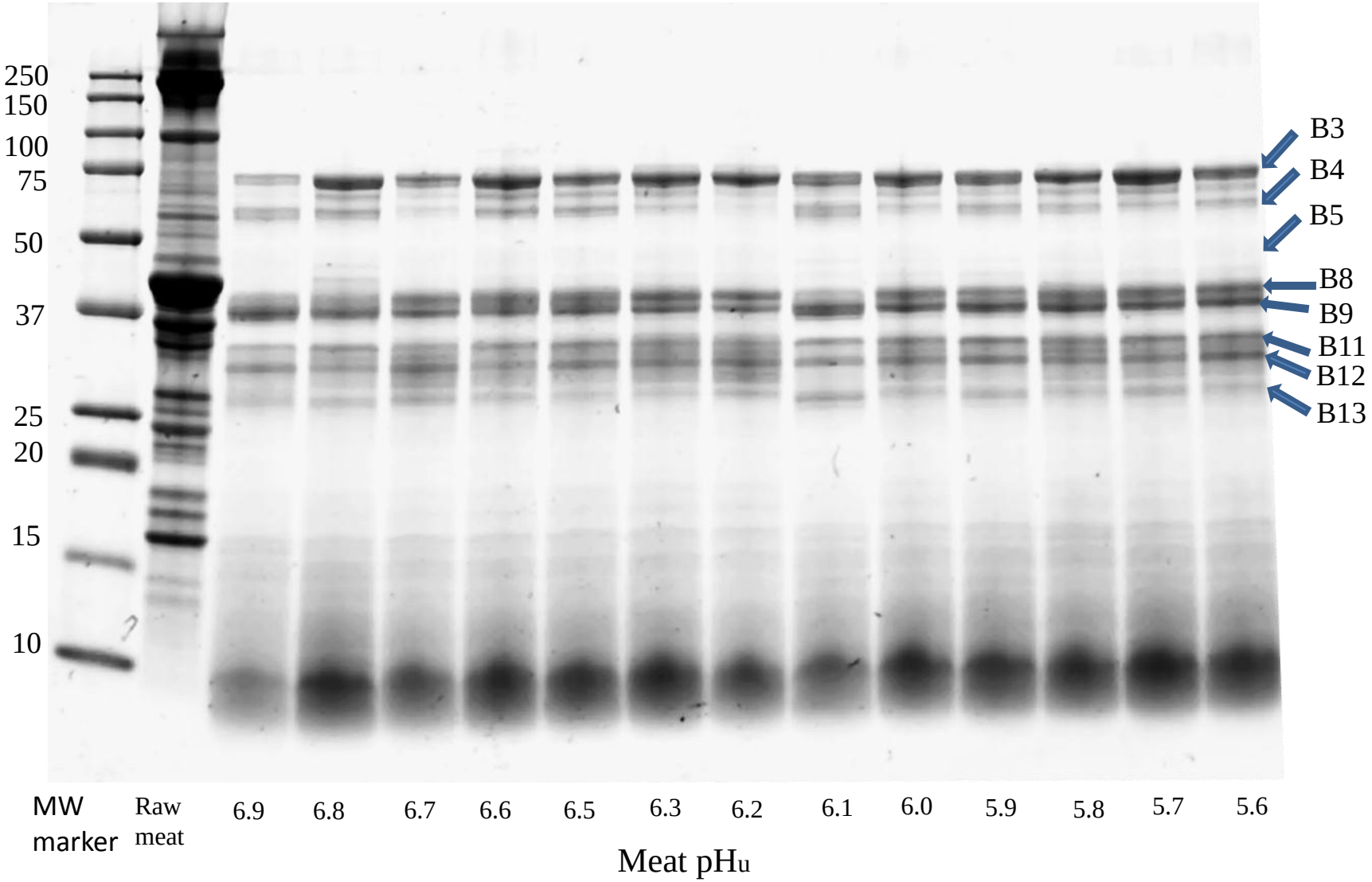


Fig. 3. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **unaged raw** beef by pepsin for 90 min followed by pancreatin for an additional 120 min.

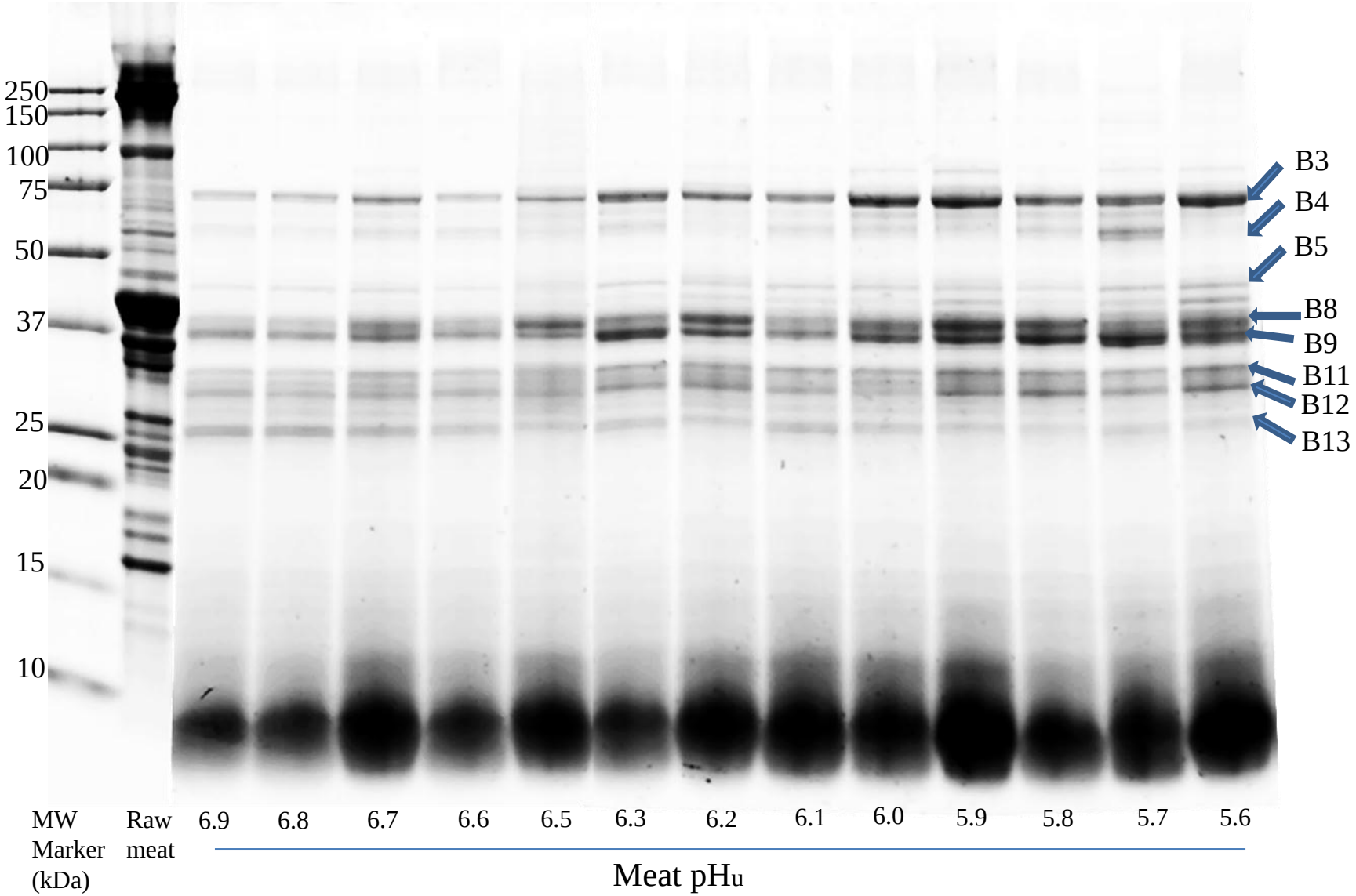


Fig. 4. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **unaged cooked** beef by pepsin for 90 min followed by pancreatin for an additional 120 min.

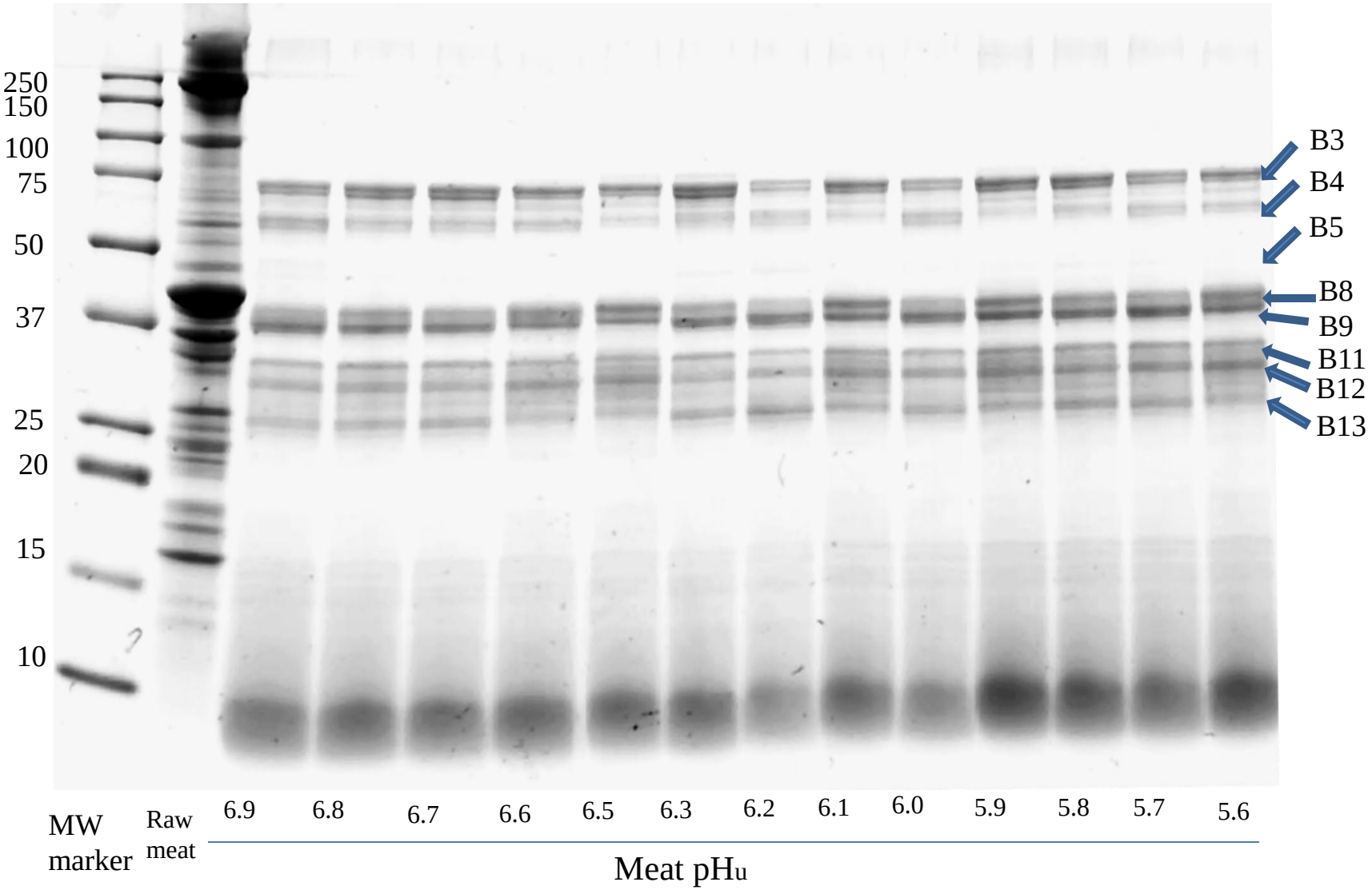


Fig. 5. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **aged raw** beef by pepsin for 90 min followed by pancreatin for an additional 120 min.

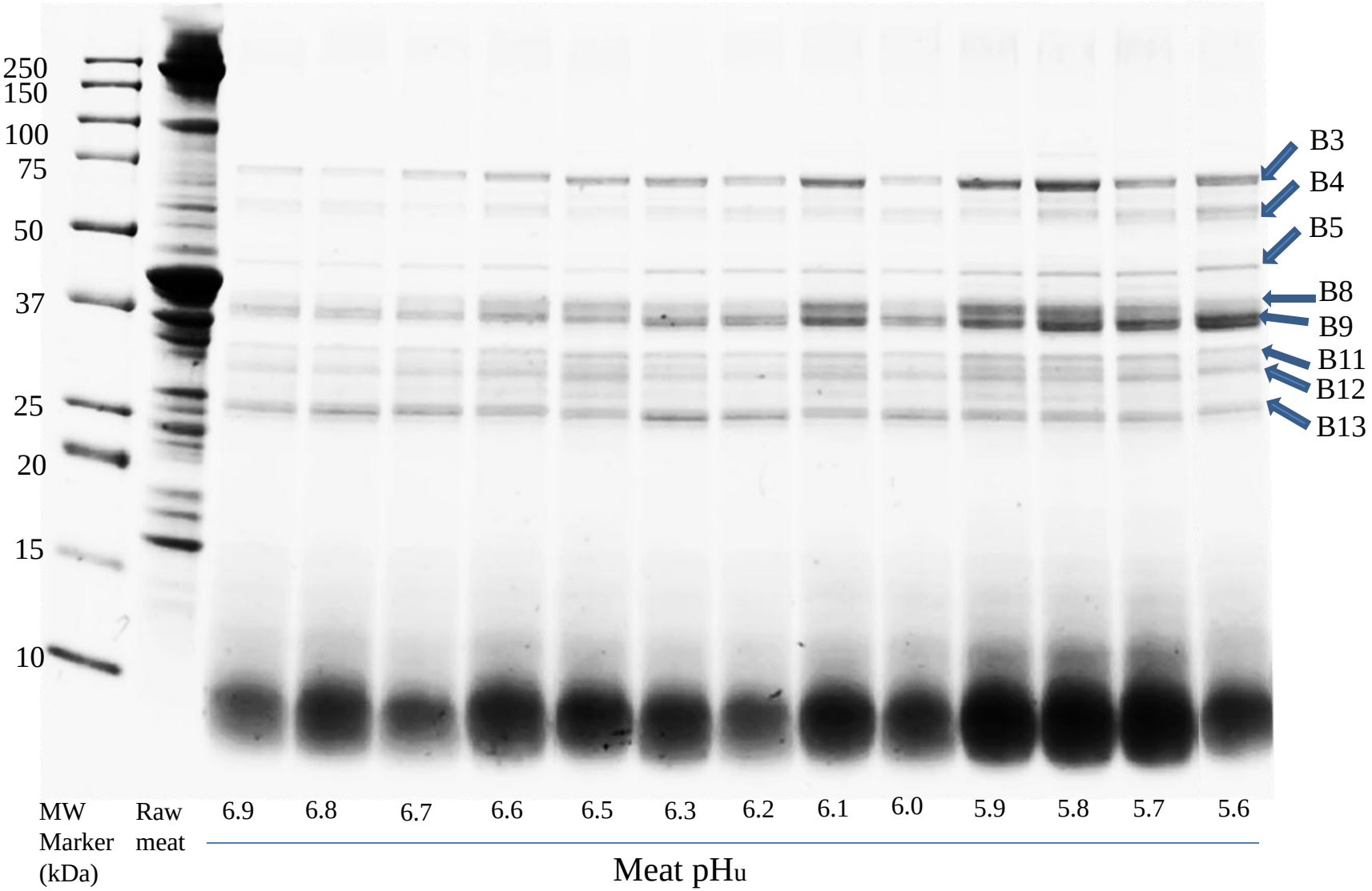
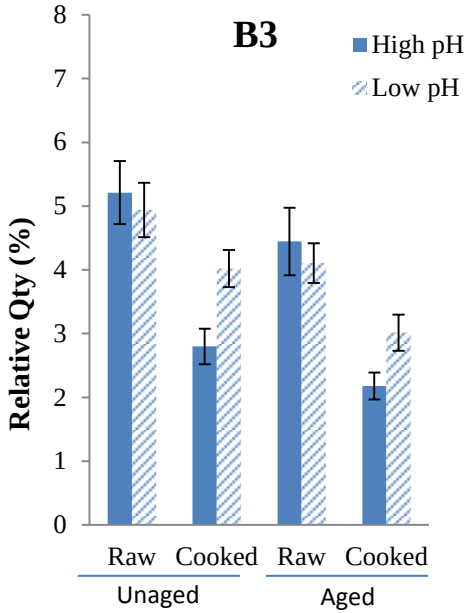
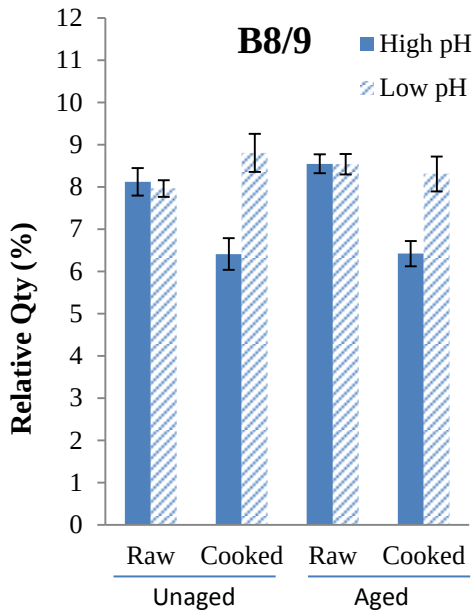
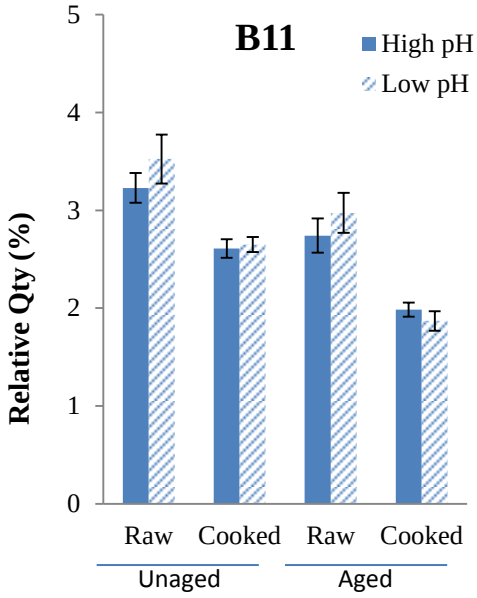


Fig. 6. SDS PAGE gel showing the effect of pH_u on the digestibility of proteins in 13 samples of **aged cooked** beef by pepsin for 90 min followed by pancreatin for an additional 120 min.





Bar: Mean ± SE



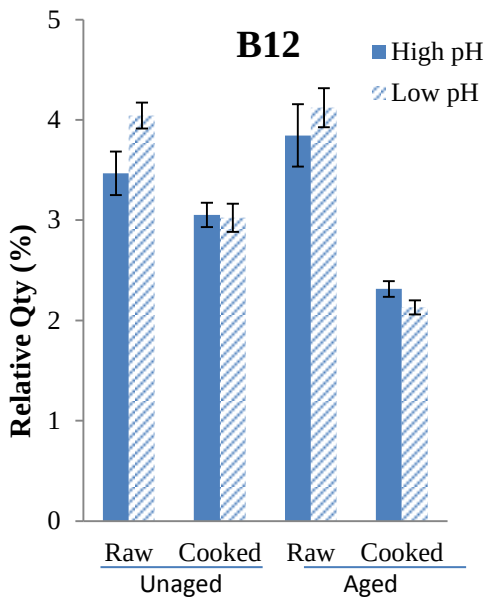


Figure 7

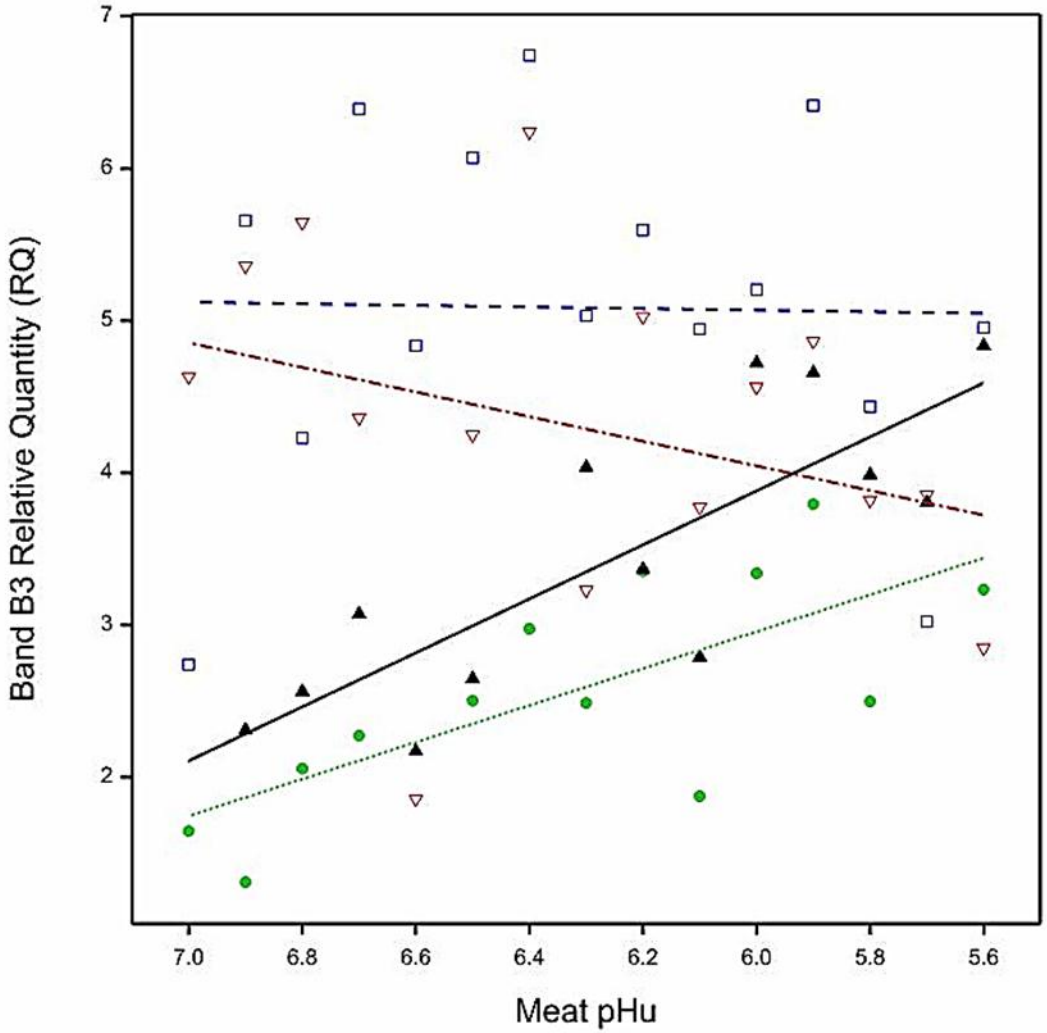


Figure 8