Effect of calpain-calpastatin system in meat tenderness

ASSIGN BUSTER

1. 0 Introduction

Meat quality is the freshness of the meat. This is the most crucial things which supplier always find and think in order to fulfill the high demand from the customer. This shows researcher play an important role in increasing the quality of meat because of the high demand from the wholesalers or consumer. The critical point of appraisal of meat quality occurs when the consumer eats the products and they comment on the colour, nutritional value, and price determines the decision to repurchase (Boleman et al., 1997). In addition, consumer evaluation of eating quality is the most determination of meat quality as tenderness, juiciness and flavor of meat are the most important elements (Tarrant, 1998; Bindon & Jones, 2001). The variability in tenderness cause by a lot of factors before post mortem, like the feeding types and the environment (French et al., 2001) and after post-mortem, like temperature, pH, sarcomere length and proteolysis (Charlotte Maltin et al., 2003).

In this study it focusing majorly on the role of genetic traits which play an important function in order to get the high quality of meat (Williams, 2008). Interest of this study is to identify the relationship between the microsatellite repetition and the calpastatin type1 promoter region effects in meat tenderness. In mid 1980s (Mullis & Faloona, 1987; Saiki et al., 1985) as the advent of Polymerase Chain Reaction(PCR), microsatellites were detected in eukaryotes genome and they are the most promising PCR-based markers. Microsatellites are simple sequence tandem repeats (SSTRs) of variable length that distributed throughout the eukaryotic nuclear genome in both coding and non-coding region (Jarne & Lagoda, 1996). This can be amplify

and identify by the PCR method (Sunnucks, 2000, Strassmann et., 1996, Shriver et., 1995). Due to the high mutation rate of microsatellites, they are potentially the most informative marker with advantages of easy and low-cost detection. Thus, the microsatellites repeat in calpastatin can influence the tenderness of meat because of the different types can produce with different role.

The aim of this findings is to characterise the expression of microsatellite repeat in calpastatin type I promoter region in bovine, to identify the regulation of CAST gene inhibitory calpain system in affecting the tenderization of meat and to develop a mechanism that can control the calpastatin gene in maintaining the tenderization of meat.

2. 0Literature review

2. 1 Meat Quality and consumer perception

Meat quality is a term used to describe a range of attributes of meat. Those factors such as post mortem factors, pH, temperature, proteolysis, sarcomere length, and the most important elements is tenderness and juiciness that affect the consumer to repurchase the meat (Warris, 2000). Besides that, meat quality also determine by color, flavor and texture which influence the consumer to enjoy the meat product (Glitsch, 2000). However, the main cause of failure of consumer complain to repurchase is the variability in eating quality, especially in tenderness. Some of the consumer that has more knowledge will concern on the safety of consuming meat. They will think of the health implication like the composition of the

polyunsaturated fat and saturated fat, and the microbial contamination especially during handling the meat products.

According to the statistical of meat consumption in Ninth Malaysian Plan, the Malaysian government targets to increase the production of beef in order to reduce the import dependence. As per capita consumption which 0. 5 kg in 2003 of mutton is very low, more attention is paid to the beef market which increased from 2. 3 kg to 5. 8 kg (FAO, 2007). Due to the high demand, the qualities of meat need to be increased in order to make sure consumer will repurchase. Anderson and Ferguson (2001) emphasize that quality as the top priority in making decision to buy and consume more meat. Similarly , factors that effect the consumer to repurchase red meat other than economic one is meat quality (Taljaard et al., 2006).

2. 2 Tenderness

Tenderness is a primary factor that influencing the consumers reaction (Glitsch, 2000). Tenderness is an integrated textural property made up of mechanical, particulate and chemical components (Paerson and Young, 1989). The appreciation of tenderness when eating is not explained by the force required to cut through a piece of meat, but is affected by the way the muscle fibers breakdown and the release of juices and flavor while chewing. Several independent studies have identified a locus on bovine chromosomes 29 with affect on tenderness. The caplain1 (CAPN 1) gene that codes for a calcium dependent protease involved in meat tenderization post- mortem.

According to the research Miller et al., 2001, meat tenderness (texture) is the most important organoleptic characteristics that influence the

acceptability for consumer. Tenderness is the consequences of postmortem physicochemical and biochemical changes in muscle of myofibrillar. After slaughtering, muscle is extensible and elastic until the onset of the rigor mortis, when the energy for muscle relaxation is depleted (Alberle et al., 2001).

2. 3 Tenderization phase

2. 3. 1 Pre-rigor phase

The duration of pre-rigor phase is dependent on the animal species. After the slaughtering of animal, blood, oxygen and nutrient supply are cut to the muscle and these triggers the pre-rigor phase to start (Lawrie, 1998). For chicken is less than 0. 5 to 1. 0 h and for beef 4 to 6 h (Aberle et al., 2001). The muscle will becomes gradually stiff and its tension reaches maximum on the completion of rigor. This is due to the formation of an irreversible actomysin complex in muscle which lead to the shorten sarcomere length. This will cause the toughening of muscle at the beginning of the post-mortem process (Koohmaraie et al., 1996).

2. 3. 2 Rigor phase

At this phase, muscles maintain the homeostasis by metabolize muscle glycogen by aerobic glycolysis. Thus, it will continue supply of ATP. During this phase, the depletion of ATP will increase the concentration of calcium ion in sarcoplasma. Sarcoplasma reticulum functioning in removing of calcium ion across the membrane utilizing the calcium ATPase pump and dependent on ATP for this active process (Robbins et al., 2003). In the meat

process, anaerobic glycosis is take place in order to maintain the production of ATP. From this the lactic acid will produced and decrease in pH value and lead to the depletion of creatine phosphate because of lack of ATP. Thus, the availability of substrate required to maintain the contractile proteins actin or myosin in relaxation state. The irreversible cross bridge and rigor mortis occur because of actin and myosin and these will made the muscle reaches to the maximum toughness as the consequences of shortening the sarcomere length (Goll et al., 1995).

2. 3. 3 Post-rigor phase

In the post-rigor, the proteolytic enzyme system are responsible in continuing the tenderness (Kemp et al., 2010; Koohmaraie et al., 1996). This phase started about 24 hours to 14 days of meat storage. The rate change is variable due to the proteolytic degradation of myofibrillar and cytoskeletal proteins cause the loss of structural integrity of myofibrils which enhancing the meat tenderization (Koohmaraie et al., 1996). The calpain/calpastatin(calcium-dependent), proteosomal and lysosomal systems have been extensively investigated for their involvement in post-rigor proteolytic degradation and meat tenderization (Kemp et al., 2010; Koohmaraie et al., 1996).

2. 4 Factors that affect the meat tenderness

2. 4. 1 Muscle pH

After the bovine is being slaughter, they need to maintain their homeostasis. So, the muscle will undergoes anaerobic respiration and regenerate the

production of ATP by aerobic respiration. The amount of ATP produced is less than normal. During anaerobic, the glycogen is metabolized into pyruvate and then converts into lactic acid. The lactic acid will gradually decrease the pH value of the muscle tissue (Maltin et al., 2003). This level of of pH will give varies effects on glycogen level, ATP turn over and the metabolic characteristic of muscle tissue (Lawrie, 1998). The high level of pH which is greater than 7. 5 , typically dark and easy to bacteria to survive on it. This will shorten the shelf life of the meat and this bring to the variability if the tenderness as the low of glycogen substrate (Watanabe et al., 1996).

2. 4. 2 Temperature

Temperature during the pre-rigor and post-rigor phase will affect on the metabolism of the muscle tissue of meat(Hertzman et al., 1993). Meat toughness will increase during the higher temperature (Bruce and Ball, 1990). The declination of muscle temperature will lead to the shortening of muscle. This is because of the reduced calcium sequestering ability by the sarcoplasmic reticulum as a result of the depletion of energy compounds which cause the muscle to contract and increase the toughness of meat (Huff Lonergan et al., 2010). There are a researched found that, at 15 Celsius is the best temperature for maintaining meat tenderization (Geesink et al., 2000).

2. 4. 3 Juiciness

Juiciness is defined as the feeling in the mouth of moisture from cooking meat and chewing. The juiciness is closely related to the attribute of flavor as this latter attribute is also affected by the level of IMF in the meat. The

high the intramuscular fat content (IMF) , the higher the meat quality (Kerry et al., 2002).

2. 4. 4 Proteolysis

Proteolysis is a conversion of muscle to meat entrains changes in tenderness due to changes in the properties of muscle fibre and connective tissue. The steps are toughness increase into rigor, proceed with proteolysis and last the rigor is resolve. Proteolytic system is divided into four which, first, cathepsin-lysomal system second, ATP-dependent ubiquitin –proteasome system, third, calpain-calpastatin system and last is matrix Metalloproteinases (MMP) (Thompson and Palmer, 1998). Tenderization increasing during ageing and it is primarily a result of calpain-mediated degradation of myofibrillar and cytoskeleton proteins.

Most of researcher doing the investigation on proteolytic system and the have a A1QWdebate on these. But most of the studies agreed that the calpain system has play the major role in post-mortem tenderization (Boehm et al., 1998; Koohmaraie. 1992b; Taylor et al., 1995a). Proteolysis involve calpain occurs between 3-14 d post mortem when activity of μ-calpain low , μ-calpain maybe bound to the myofibril and inactivated during post mortem storage but the m-calpain active when the level of calcium arise. Calpain is calcium-dependent which function in softening the muscle tissue of the meat. In proteolysis it involve the calpain proteases and caplain-specific inhibitor, calpastatin. When the low level of calpastatin produce, the more calpain protease produce . Then, the tenderness of meat will increase.

2. 5 Microsatellite

Microsatellites are simple sequence tandem repeats (SSTRs). The repeat units are generally di-, tri-, tetra- or pentanucleotides (Powell et al., 1996) . Like repetition in birds is ACn, where it`s means two nucleotides A and C are repeated in bead-like fashion a variable number of times. The *n* could be range from 8 to 50. This always occur on a non-coding region of DNA. On the each side of the repeat unit are flanking regions which consist of unordered DNA. This flanking region is dangerous because they will allow the development of locus-specific primers to amplify the microsatellites with PCR. By having a forward and reverse primer on each side of microsatellites it will be able to amplify a fairy short (100 to 500bp) locus-specific microsatellite region(Sunnucks, 2000, Strassmann et., 1996, Shriver et., 1995).

Microsatellites were designed for generative neurology disease in human but it shows a great applicability in other species. Microsatellites act as markers was classified based on the number of bases like short repeats are microsatellites while longer repeats are minisatellites. Besides that, it also classified by the type of repeated sequence present whether it is perfect, imperfect or composite. Imperfect means the repeated sequence was interrupted by different nucleotides which are not repeated while composites when two or more different motifs in tandem (Selkoe &Toonen, 2006). In addition, microsatellites is co-dominant and it is widely distributed throughout the genome and transferable between species. These features provide their successful function in these fields (Chistiakov et al., 2006).

2. 5. 1 Microsatellites mutation

Microsatellites are useful genetics markers because they tend to be polymorphic. Normally, human microsatellites with 20 or more alleles ad heterozygosities. This is because their mutation occurs different from the “ classical” point mutations, where the substitution of one nucleotide to another occurs. The mutation in microsatellites occur through slippage replication where two strands could slip relative position a bit but still manage to get the zipper going down the beads. One strand can be lengthened or shortened by the addition or excision of the nucleotides. So, the repeat unit can be one longer and the other is shorter than the original (Selkoe et., 2006).

2. 6 Calpastatin

Study of calpastatin gene promoter activity had been done by some of the researcher . Calpastatin is proteinase inhibitor for calpain which family of calcium-activated neutral proteases that regulate the of Ca2+. It is encoded by single gene in mammal which produces proteins isoforms through the alternating splicing, There are four types of CAST which are Type I, Type II, Type III has been characterized in porcine with the study of the three promoters directing expression(Parr et al., 2004) while in bovine calpastatin transcripts including Type IV had been characterized with the studied of four functional promoters in the gene (Raynaud et al., 2005). This four types of CAST can bind to the calpain and inhibit proteolytic activity. A single calpastatin can inhibit several caplain molecules *in vitro* .

Several isoforms of calpastatin exist due to the alternative promoter usage and differential splicing (Parr et al., 2001; Raynaud etal., 2005). Increasing

response on calpastatin expression to ß- adrenergic stimulation has been associated with skeletal muscle hypertrophy in livestock (Parr et al., 1992; Killefer and Koohmaraie, 1994) and related inversely with the tenderization rates(Koohmaraie, 1996). ß- adrenergic stimulation act by the cyclic adenosine monophosphate (cAMP) responsive elements in calpastatin promoter regions(Cong et al., 1998a, b). three types of promoters located in the 5′ region of gene upstream of exons 1xa, 1xb, and 1u generate calpastatin mRNA transcripts the types I, II, and III respectively (Takano et al., 2000; Parr et al., 2004). In pig, these promoters have putative motifs for another transcription factors that will imply other signaling pathways of calpastatin expression(Parr et al., 2001; Raynaud etal., 2005).

2. 6. 1 The types of calpastatin genes

From the previous studies, there were found calpasatin has four types of repetitive-inhibitor domains which are Type I, Type II, Type III and Type IV. The isolated cDNAs from the various mammalian species have conspicuous differences in the regions encoding the N-terminal sequences. These four different types has different function and from the different sources. The Type I and Type II in mouse and bovine respectively also differ from each other in the uttermost N-terminal sequences, possess longer domain L sequences than those of rabbit, pig and human inhibitors which are Type III. The previous obtained mouse calpastatin cDNA is encoded by as many as 31 exons including the first exon. The other three additional exons specifying the N-terminal sequences of the types were identified in the mouse genomic DNA sequence. The mRNAs for Type I and Type III were expressed in the liver, the Type II high in heart and skeletal muscle . Besides , the Type IV

abundance in testis. These findings show that the calpastatin isoforms possessing different N-terminal sequences are generated by the alternative transcription initiation from their own promoters and skipping of the mutually exclusive exons (Takano et al., 2000).

Cong et al. (1998), reported cAMP-dependent transactivation of the bovine calpastatin gene whose promoter located on the upstream of exon . They identified a sequence GTCA which was important for the cAMP responsiveness and corresponded to the half site of the full CRE(a consensus palindromic cAMP-responsive cis-element; TGACGTCA). They demonstrated that mutation of GTCA at -76 nt to ATCT completely abolished the dibutyryl-cAMP . Comparison of the nucleotide sequences of the mouse and bovine genomic DNAs did not show a high similarity but little similar sequence G T GCGG T GT – CAGCCGG (identical residues are underlined) containing GTCA was found. The differential expression patterns of the type I, II, III mRNAs among different animal suggests that the presence of different transcriptional regulatory elements upstream of the respective promoters. Besides that, the differences in N-terminal sequences might affect the intracellular distribution of the action calpain-calpastatin system in stimulation of meat tenderness (Takano et al., 1999).

2. 7 Calpain

Calpain were intracellular calcium-dependent cysteine proteinases which present in all mammalian(Goll et al., 2003; Sorimachi et al., 2001). In catalysing the limited proteolysis of cytoskeletal and membrane protein , the calpain were play a big role. This regulation occur with help of specific

protein inhibitor calpastatin. In striated muscle, the calpain/calpastatin system has been proved in regulation protein turnover especially in meat texture development (Sensky et al., 2001).

2. 8 The effect of calpain-calpastatin system in meat tenderness

The calpain and calpastatin proteolytic enzyme system is believed to be the main contributor to the tenderness of meat at post mortem. The present of calpastatin in meat influence the calpain by acting as inhibitor. Calpastatin is a marker in order to determine the tenderness of meat. The researcher found the activity of the calpastatin in meat at 24 hours was highly related to shear force value after 14 th day after post mortem. It showed that an early event after the animal being slaughter could be predictive of ultimate shear force because of the low activity of calpastatin (Whipple et al., 1990).

The findings was repeated in pork. The higher level of calpastatin after 2 hours of post mortem is increasing the toughness (parr et al., 1999). We can conclude that the activity of calpastatin was responsible in variation of tenderness of meat by the differences in proteolytic rate of the animals. A more complex study is performed by the Shackleford et al.(1994) that correlate between both calpastatin level and meat toughness and the possibility of using these for selection purposed to improve the meat quality.