The contribution of meat science to the development of a PACCP-based grading scheme for beef

DJ Troy, AM Mullen, P Stapleton, K Brandon, AM White

Ashtown Food Research Centre (formerly the National Food Centre), Teagasc, Ashtown, Castleknock, Dublin 15, Ireland

Introduction

According to McIlveen and Buchanan (2001) flavour, tenderness and juiciness appear to be the three most important determinants of sensory enjoyment for the UK consumer. Of the three attributes mentioned Ouali (1990), Warkup et al., (1995), Szczesniak (1998), Koohmaraie (1998) among others all concluded that beef tenderness was the primary determinant of satisfaction among beef consumers. In Norway, a recent study found that beef consumers were willing to pay 50 % more for very tender beef and 25 % more for tender beef compared with less tender beef (Alfnes et al., 2005). Therefore providing consistently tender beef should be key priority for the beef industry.

While there have been many successful efforts at improving the tenderness of beef research has shown that an unacceptable level of variability still remains in beef tenderness (Maher et al., 2004a). Many factors are known to influence eating quality of beef are now established and many of these have recently been reviewed by Thompson (2002). Breed, sex, feed, handling, environment, finishing weight and age at slaughter are among the production factors that affect palatability. While from a processing point of view pH/temperature regime, hanging methods, days of maturation, and whether or not the carcass has been subjected to electrical stimulation, cooking method and ‘degree of doneness’ all affect beef palatability. Meat Standards Australia (MSA) identified all the factors listed above as critical control points within a palatability assurance scheme.

Development in genomics have offered the opportunity to revolutionise the manner in which we research muscle properties, their interaction with environmental factors and how this impacts on quality traits in the meat. This research may aid the identification of further signatures from the muscle/environment which influence quality and which could be tested for inclusion in the MSA model.

Breed and Sex

The influence of Bos Indicus content on meat quality is well documented and forms a critical parameter in the MSA model. However because of the interfering influences within and between breeds such as feed, growth rates etc., it is difficult to determine the effects of breed on meat quality. Wulf et al., (1996) and Maher et al., (2004b) both reported that breed did not have and effect on WBSF, while in a recent study Dikeman et al., (2005) found that considerable variation in WBSF of M. longissimus muscle steak exists between breeds, while selecting for marbling to improve tenderness would be expected to result in only subtle improvements in tenderness in most breeds. Maher (2003) found heifers to be more variable for tenderness than steers (mixed breed), however this maybe have been some what confounded by age. However, Purchas et al., (2002) reported that bull beef was tougher than steer beef.

Feeding regime, finishing weight and age at slaughter

Feeding, finishing weight and the age of the animal at slaughter all have an important role to play in the determination of meat quality. Feeding regime is thought to have an indirect effect on tenderness. Leaner animals require more precise chilling control than fatter carcasses in order to reduce the risk of cold shortening (Troy, 1995). A number of studies have examined the effect of growth rate on tenderness by altering the feeding regime before slaughter with mixed results. Sazili et al., (2003) reported that animals placed on a restricted diet for 30 days had lower shear force values than fast growing animals. However, Sazili et al., (2003) also reported that after 45 days on a restricted diet the shear values were similar to those for animals on the high plane of nutrition. While Thompson et al., (1999) and Purchas et al., (2002) reported that steers on a faster growing regime were more tender than those finished on a restricted diet. Age at slaughter is reported to effect beef tenderness. Shackelford et al., (1995) compared the tenderness scores of yearling heifers and 2 year old cows. They reported that there was a very slight difference in the tenderness scores between the two age groups, however, there was greater variation in tenderness within each group that there was between age groups. Wulf et al.,
(1996) reported that beef from cattle slaughtered at 15 months was more tender than from those slaughtered at 18 months.

**Hanging Method and Ageing**

Methods for stretching or restraining pre-rigor single muscles or muscles in a carcass have been given increasing attention due to their ability to improve tenderness and reduce variation in tenderness of meat (Sorheim et al., 2002). Various methods of hanging carcases have been tried as an alternative to the conventional Achilles tendon method (Troy, 1995). Among the different hanging techniques developed to improve meat tenderness are ‘tenderstretch’, ‘tendercut’, forequarter hanging (Filho et al., 2005) and the Pi-Vac TenderBound packaging system. The most popular of these techniques is the ‘tenderstretch’ method. For this technique the hanging position is switched from the Achilles tendon to the aitch bone thereby allowing the hind legs to hang freely. This method improves tenderness in the longissimus, semimembranosus and gluteus medius muscles. However, the tenderising effect is none or slight for biceps femoris, semitendinosus and psoas major muscles, while there is no tenderising effect on the forequarter muscles.

Researchers at Virginia Polytechnic Institute and State University have examined pre-rigor skeletal cuts to improve beef tenderness. This procedure is referred to as ‘tendercut’ and requires and additional input of making cuts in the skeleton of the pre-rigor carcass shortly after slaughter while maintaining the Achilles tendon suspension. The weight of the carcass below the points of cutting stretches many of the major loin and round muscles. As with ‘tenderstretch’ the ‘tendercut’ method does not benefit all muscles. Shanks et al., (2002) reported that the use of the ‘tendercut’ technique resulted in increased tenderness in some muscles with decreased tenderness in others. Filho et al., (2005) examined the effect of forequarter hanging on the longissimus and biceps femoris muscles. Hanging by the forequarter caused a significant improvement in tenderness of the longissimus muscles without any detrimental effect on the biceps femoris.

**Electrical Stimulation, hot boning and Pi-Vac**

Due to factors such as physical location on the carcass, fat cover, physiology/biochemistry of the muscle etc., different muscles can vary in their response to a whole carcass treatment. A technique such as hot boning provides a significant advantage in that it enables individual muscles to be processed in a specific tailored fashion. The pros and cons of this approach have been highlighted (Pisula & Tyburgh, 1996) however a major advantage is that it allows muscles to be treated according to their individual optimal conditions. Hot boning needs to be combined with other intervention techniques to ensure tender beef.

Post-mortem electrical stimulation (ES) has received considerable attention as a possible procedure for improving muscle tenderness. It is generally agreed that ES is beneficial in terms of quality, however many authors have reported conflicting results on the effectiveness of ES when used to enhance beef tenderness (Rodbotten et al., 2001; Strydom et al., 2003). Work carried out by White et al., (2006) reported that ES in combination with hot-boning significantly improved beef tenderness scores. Pi-Vac Elasto-Pack system (Maixner & Karnitzschky, 2001) is a system which has been developed to work in conjunction with hot-boning to improve beef tenderness. Hot boned muscles are removed in the pre-rigor state and are more prone to contract as the muscle is not held in a stretched state, as provided by the skeletal framework of the carcass. When hot-boned muscle is chilled quickly before the onset of rigor ‘cold shortening’, severe contraction of the muscle fibres, will occur and significantly reduce the tenderness. The Pi-Vac packaging system involves stretching tubes of elastic film to the inside walls of the packaging chamber, after the muscle is inserted into the chamber pressure is released and the elastic film returns to its original dimensions. The elastic film then hinders the diametrical expansion of the muscle, which restricts muscle contraction (White and Troy, 2006). Work carried out in AFRC (Ashtown Food Research Centre, Dublin) concluded that Pi-Vac packaging of hot-boned beef allowed for fast chilling of this beef without the risk of cold shortening, ensuring consistent quality and improved tenderness (White and Troy, 2006). Sorheim and Hildrum, (2002) found that combining stretching or restraining methods with other tenderising techniques like slow chilling or electrical stimulation usually yield little additional benefits in tenderness.

**Cooking method and degree of doneness**

Behrends et al., (2005) conducted a study examining among other attributes the effects of cooking method and degree of doneness on US consumer evaluation of top round steak. It was determined that the most popular method of cooking was outdoor grilling while the preferred degree of doneness was “medium well and more”. This confirmed results obtained by Lorenzen et al., (1999) who also found that consumers had a preference for outdoor grilled top sirloin steaks cooked to medium doneness or more. Cooking methods which allowed moist heat to be generated (stir frying, stewing etc.) achieved higher consumer
ratings for top round steak (Neely et al., 1999) than grilling, however consumers preferred cooking method was grilling.

Other factors
Pre-slaughter stress, pre-slaughter nutritional status, time/pH window and muscle type all influence palatability but will not be elaborated in this paper.

Future opportunities
DNA polymorphisms
During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or genomic regions that affect quantitative traits. This has provided opportunities to enhance response to selection, in particular for traits that are difficult to improve by conventional selection (low heritability or traits for which measurement of phenotype is difficult, expensive, only possible late in life, or not possible on selection candidates). Although beef quality is influenced by environmental effects, e.g., nutrition and by post-mortem carcass chilling and meat processing, the heritability of meat quality traits is between 0.15 – 0.35 (e.g., Wheeler et al., 2004). This is a substantial level of genetically controlled variation, and should enable genetic markers for these traits to be detected and used to increase beef quality through marker-assisted selection (MAS) (Dekkers, 2004). Improvement of beef quality by marker-assisted selection requires a set of genetic markers thoroughly characterised for their effects in the target population. Presently, only a limited number of genetic markers with confirmed effects is available, however some of these have formed the bases of genetic testing for eating quality such as those provided by Genetic Solutions.

DNA markers, (regions of chromosomes showing polymorphisms in the nucleic acid sequence) can be used to detect quantitative trait loci (QTL), or regions on a chromosome which have a substantial effect on quantitative traits such as tenderness, growth rate, intramuscular fat etc. This is referred to as QTL mapping. DNA markers linked to the QTL can be used to develop strategies for marker assisted selection (MAS) breeding programmes (for review read Dentine, 1999). QTL for carcass and growth traits have been identified (Stone, et al., 1999). Several studies reported QTL for intramuscular fat (marbling) and tenderness (WBSF) (Kuhn, et al., 2005). In pork QTL for intramuscular fat and backfat have been identified (for review see De Vries et al., 2000, Rothschild, 1997).

Before whole genome scan experiments were used to detect QTLs, studies looking for genetic markers associated with beef quality were performed by investigating polymorphisms in candidate genes, which are chosen because of their central role in biological processes with impact on meat quality (Kuhn et al., 2005). Candidate genes are genes which are related physiologically or biochemically to the selected trait and are assumed to have an effect on trait performance. The recent description of QTL regions has further encouraged the search for candidate genes that may underlie the variations seen in the traits. However, at the present time, just a few markers and/or genes have been identified, which has a confirmed association with meat tenderness or marbling. Polymorphisms identified in the CAPN1 (Calpain 1) (Smith et al., 2000, Page et al., 2002), CAST (Calpastatin) (Barendse, 2002) and LOX (Lysyl oxidase) (Barendse, 2002) have been associated with tenderness and polymorphisms in the TG (Thyroglobulin) (Barendse, 1999), DGAT1 (Diacylglycerol-O-acyltransferase) (Thaller et al., 2003) as well as microsatellites have been associated with marbling (Kuhn et al., 2005). While, double muscling in some beef breeds is caused by a mutation of a gene located on bovine chromosome 2 that produces the protein myostatin (Grobet, et al., 1997; Kambadur, et al., 1997; Smith, et al., 1997). The availability of new molecular tools for functional genome analysis and of comprehensive genomic information (DNA sequence, expressed sequence tags (ESTs), genes) that is emerging for cattle enables new approaches for identifying functional polymorphisms within candidate genes with an effect on beef quality (Eggen and Hoqueyte, 2004).

Gene expression and microarray technology
Functional genomics aims to provide further insight to the complex interplay of gene expression events involved in the development of meat quality. Genomics is the study of an organisms whole genetic blueprint and the variations within that blueprint which make every individual in a population unique. Functional genomics seeks to relate differences in the genetic blueprint to physiology and phenotype, i.e addresses the structure and function of genes. Differences in gene expression or changes in the sequence of expressed genes contribute to the various phenotypes in any animal population. Traditional methods allow only one gene to be studied at a time. However, there is much excitement at present over the use of microarrays or biochips, and their potential contribution to the study of genomics, because the expression of thousands of genes can be studied simultaneously by quantifying the levels of their transcripts (Zhao et al., 1995; Duggan et al., 1999 & Sinclair, 1999). This large-scale analysis of mRNA levels is called
The technique, which involves laying down an ordered array of probes (cDNA from EST libraries or oligonucleotides) onto a solid substrate for hybridisation with labelled targets, which originate from the biological samples of interest. The resulting images of hybridisation are captured and analysed using software that quantifies the signal of each spot. The intensity of each spot is proportional to the amount of mRNA of the corresponding gene expressed in the studied sample. The two major applications are (i) identification of differentially expressed genes between different biological samples and (ii) expression monitoring in different physiological conditions.

Limits of functional genomics include the availability of biological molecular material, the use of cross-species hybridisation generating spurious signals, spurious results and technical difficulties (Hocquette et al., 2005).

One important benefit of high-throughput techniques of genomics will be the identification and the practical use of polymorphisms within DNA sequences of those genes (Garnier et al., 2003). Applications, such as DNA-based tests of generic merit for beef quality traits, have been slow to develop (Smith et al., 2003) however companies such as Genetic Solutions have made progress in establishing tests for tenderness and marbling in beef. The application of any of these techniques to an on-line situation requires large scale industrial based trials to verify and confirm their ultimate usefulness. Novel genetic tests will be developed and commercialised to identify cattle bearing traits that satisfy consumer demands for consistency and quality in meat, resulting in superior meat for consumers (Hocquette et al., 2005). Long-term aims of genome analysis in domestic animals is to obtain genetic screening tests that will improve the health and welfare by selective breeding (Kutzer et al., 2003). In the future, SNP chips would probably be a tool of choice for genotyping animals (Hocquette et al., 2005). This is an exciting and revolutionary area with much potential for real applications to improving the consistency of the quality of meat. While methodology requires investment in terms of time and development of specific tools, the benefits in the medium to long term are potentially great.

**Proteomics**

Complementary to the functional genomics approach is proteome analysis (Anderson et al., 2000). The use of proteomics allows the characterisation of expressed protein within any given cell type. This is a very powerful technique as the phenotypic traits of an organism are ultimately manifested through the interaction of the environment and the various proteins expressed in its tissues (structural, enzymatic, metabolic and regulatory proteins) (Pandey and Mann, 2000). Proteomics can be used to describe the function of individual genes, and how heredity and environment interact to control cellular functions and consequently the physiological traits that are relevant for production of farm animals (Bendixen et al., 2005). Characterising the function of genes is a major challenge of the post-genomic era, and within this functional genomics area, a wide range of tools are currently being developed. Currently, there are two classic approaches to proteome characterisation, namely comparative and mapping proteomics. Mapping proteomics is in many ways comparable to genome sequencing projects, where the goal is to characterise and make comprehensive databases of “cellular proteomes”. The aim of comparative proteomics is to characterise the biological mechanisms that form the link between observable phenotypes and the genotypes (Hunter et al., 2002). The most frequently used methods and tools for extraction, separation, quantification and characterisation of proteomes include 2DE (two dimensional electrophoresis), Mass spectrometry, comparative mass spectrometry and new developments in protein arrays (Bendixen et al., 2005). Many research groups world wide, including NFC, have dynamic programmes in place to determine the relationship between patterns of gene expression (and protein expression) and meat quality traits. Proteolytic degradation of key myofibrillar proteins has been shown to contribute to post mortem tenderisation (Troy and Tarrant, 1987; Troy et al., 1987; O’Halloran et al. 1996; Boyer-Berri and Greaser, 1998). Degradation of many structurally important myofibrillar proteins, during the post-mortem ageing of meat, has been observed by many research groups including NFC. Of these troponin T and its 30kDa myofibrillar proteolytic fragment have been related to meat tenderness (Buts et al., 1986; Troy and Tarrant, 1987; Troy et al., 1987). It has been suggested that the appearance of this 30kDa fragment could serve as an early post-mortem indicator of meat tenderness. A soluble 1734.8Da fragment of the troponin T molecule has recently been isolated (Stoeva et al., 2000; Mullen et al., 1998b; Nakai et al, 1995) which appears to be related to meat tenderness (Mullen et al, 2000). Due to its solubility this fragment is more easily extracted from meat than the myofibrillar 30kDa and, therefore, it may be a more suitable candidate for routine factory analysis. Thecalpastatin/calpain proteolytic system which has been implicated in the tenderisation process has also been targeted for the development of an immunoassay test (Doumit et al., 1996; Koohmaraie, 1996b). The analytical performance of these assays needs to be thoroughly validated and convenient sample preparation procedures designed for application of the assays to meat extracts. Evaluation of the efficacy of the
assays in the prediction of meat tenderness is also necessary and the resulting data can then be used to select the assay or combination of assays which give the best correlation with alternative indices of tenderness.

Conclusions

Although our understanding of the scientific factors underpinning meat quality has increased much remains unknown. Precise factors influencing quality such as proteases and their substrates remain elusive. As our ability to study meat science on a molecular basis increases the possibility to apply this knowledge into a future PACCP-based grading system will increase.

References


McIlveen, H., Buchanan, J. (2001) The impact of sensory factors on beef purchase and 101
consumption. Nutrition and Food Science, 31 (6), 286-292.


