Foreword

The aims of this research were:

1. To develop relationships between genotype, sex and age of kangaroo at slaughter with the key meat quality parameters of tenderness, flavour and juiciness.

2. To establish an understanding of the effect of harvesting and post-slaughter carcass storage methods on the rate of change of muscle pH, the ultimate pH, muscle shortening, losses due to drip from meat, cooking losses and ultimately consumer evaluation of the product.

3. To develop a series of recommendations will be developed in close collaboration with the industry partners to establish the major factors controlling kangaroo meat quality for human consumption.

With the increase in trade in kangaroo meat the need to understand factors influencing the quality of product is overdue. The key factors for consumer acceptance identified for other commercial meats traditionally have been tenderness, flavour and juiciness. The need to explore the importance of these parameters for kangaroo meat and mechanisms associated with the variability in these parameters will contribute to the refinement of promotional material for both the domestic and export market places.

The report covers results from a series of trials conducted in both South Australia and in the Northern Tablelands region of NSW designed to define the major factors influencing consumer acceptance of the product and the reasons for variability in the quality descriptors defined through this study.

The project has been funded from RIRDC Core funding associated with a Research and Development levy established for the kangaroo industry. It adds to the portfolio of projects established under RIRDC New Animal Products initiative.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

This report, an addition to RIRDC’s diverse range of over 1000 research publications, forms part of our Sub-Program 1.2 NAP – New Animal Products R&D program, which aims to facilitate R&D investments for the growth and profitability of prospective animal industries, producing in a viable and innovative way quality goods for national and international customers.

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Simon Hearn
Managing Director
Rural Industries Research and Development Corporation
Acknowledgments

The collaborative support of the KIAA is gratefully acknowledged.

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Many thanks are extended to the highly professional Kangaroo Harvesters we have collaborated with throughout the project; of specific note, the irreplaceable support and all-encompassing guidance of Phillip Williamson (also known as Herbie, or Axel) ensured the projects thorough completion, along with many laughs.

Finally, the enormous level of support enthusiastically provided by Prof. John Thompson of the Department of Meat Science, UNE, must be both acknowledged and highlighted. His noble offers of support through infrastructure, guidance and holistic advice have in so many ways allowed for this research project to not only reach successful completion, but attain many high quality research outcomes.
## Abbreviations

### List of kangaroo muscles utilised in this research

<table>
<thead>
<tr>
<th>Muscle name</th>
<th>Predominant Cut</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. vastus lateralis</td>
<td>knuckle/round</td>
<td>VL</td>
</tr>
<tr>
<td>M. biceps femoris</td>
<td>silverside</td>
<td>BF</td>
</tr>
<tr>
<td>M. caudofemoralis (cranial head)</td>
<td>rump</td>
<td>CF</td>
</tr>
<tr>
<td>M. adductor</td>
<td>topside</td>
<td>AD</td>
</tr>
<tr>
<td>M. gluteus medius</td>
<td>rump</td>
<td>GM</td>
</tr>
<tr>
<td>M. semimembranosus</td>
<td>topside</td>
<td>SM</td>
</tr>
<tr>
<td>M. semitendinosus</td>
<td>silverside</td>
<td>ST</td>
</tr>
<tr>
<td>M. longissimus dorsi</td>
<td>loin fillet</td>
<td>LD</td>
</tr>
<tr>
<td>M. cranial dorsolateral sacrocaudalis</td>
<td>long fillet</td>
<td>CrS</td>
</tr>
<tr>
<td>M. caudal dorsolateral sacrocaudalis</td>
<td>striploin</td>
<td>CaS/SAC</td>
</tr>
<tr>
<td>M. psaos minor</td>
<td>tenderloin</td>
<td>PM</td>
</tr>
</tbody>
</table>

### Abbreviations for materials, methods and results

- **Alc**: 100% ethanol (ethyl alcohol)
- **Comp**: Compression, objective measure relating to meat tenderness
- **DDF**: Denominator degrees of freedom, statistical term
- **DWr**: Dressed, or carcass weight (kg)
- **EG**: Eastern Grey kangaroo species: *Macropus giganteus*
- **Flav**: Flavour, as judged by consumer taste panel assessment – scale from 0-100
- **GPD**: Glycerophosphate dehydrogenase
- **Juicy**: Juiciness, as judged by consumer taste panel assessment – scale from 0-100
- **LSMean**: Least squares mean, model based best prediction of a treatment average adjusted value
- **mAbs**: Monoclonal antibodies
- **mATPase**: Myofibrillar adenosine triphosphatase
- **MHC**: Myosin heavy chain
- **n.s.**: (effect) not significant (at a given probability level)
- **NADH**: Nicotinamide adenosine dehydrogenase
- **NDF**: Numerator degrees of freedom, statistical term
Overall acceptability, as judged by consumer taste panel assessment – scale from 0-100

Palatability, combined eating quality descriptive score, based on weighted tenderness, flavour, juiciness and overall acceptability results – scale from 0-100

Paraffin based embedding wax

Warner-Bratzler based peak shear force determination, kg-force value for subjective meat toughness

Initial pH

Ultimate pH

Red kangaroo species: *Macropus rufus*

Statistical Analysis System, statistical data analysis software package

Standard deviation

Standard error of the mean

Sarcomere length

Standard error

Tenderness, as judged by consumer taste panel assessment – scale from 0-100

Western Grey kangaroo species: *Macropus fuliginosus*

Xylene
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Executive Summary

The identification of the major desirable attributes of kangaroo meat will lead to the identification of factors that can be modified to improve these attributes. In these studies we have investigated the importance of the major factors identified by consumers in the evaluation of meat from the domestic commercial species in an evaluation of kangaroo meat quality. Identification of these key factors will enable us to identify the strategic points in the harvesting and processing chain for kangaroo meat that can be manipulated to improve meat quality and therefore product acceptance by the consumers in both the domestic and export marketplace.

The sensory evaluation of commercially important muscles obtained from Red and Western Grey kangaroos identified flavour as the predominant factor influencing the acceptability of grilled kangaroo, showing a high correlation (r = 0.89) with overall acceptability. This contrasts with the results from the evaluation of other commercial meats in which tenderness is considered by consumers as the predominant determinant of meat quality. Muscles derived from the leg were significantly tougher in heavier carcasses, but loin muscles associated with the spinal column such as the loin fillet were not affected by carcass weight. Since carcass weight is highly correlated with age it is likely that this is associated with changes in the ultrastructure and content of connective tissue in the musculature. In general Red kangaroo cuts were more palatable than Western Grey cuts, while heavier carcasses yielded tougher meat from the leg muscles, but not from the loin fillet.

Since tenderness is related to the connective tissue component of meat, we have measured the collagen content of various cuts. Our results suggest that leg cuts derived from lighter, younger male animals should be of higher eating quality than those from older animals, whereas the quality of the loin fillet was independent of carcass weight and therefore age.

The conditioning of carcasses at 24°C for up to 24 hours increased tenderness of the topside, round and striploin, suggesting that methods for the storage of cuts both processing will have an impact on product quality. The importance of attaining an ultimate pH (pH_u) of around 5.5 to minimise productivity variability and microbial spoilage is well established in the beef, pork and lamb industries. Despite glycogen concentrations of 60µmol/g or above (normal values for bovine muscle) being measured in our samples, the pH_u rarely fell below 5.7, suggesting a difference in the regulation of glycolytic pathways in kangaroo muscle when compared with other species. Muscle glycogen concentrations differed significantly between species and sex of kangaroo, suggesting that further research is required to establish the mechanisms for these differences and the implications for meat quality. The difference may be related to the distribution of muscle fibre types in muscles. Our studies have demonstrated that there are differences between the kangaroo and the bovine musculature type and IIB-fast-glycolytic myofibres predominate in a range of commercially important muscles. The implications for muscle glycolysis and the attainment of pH_u remains to be elucidated.

Perhaps the most practical observation made in these studies was that the musculature associated with a suspended leg is tougher than that from the “free” leg on the same carcass. Thus field harvesters who hang carcasses by one leg post-slaughter are in effect adding to the variability in product quality even from the same carcass. Our recommendation of hanging by the tail or from the pelvic bone so that both legs swing freely to stretch muscles of the hind limb is a technology easily adopted by the industry.

Although we have identified a number of important factors associated with consumers perception of meat quality, our identification of flavour as the most important determinant of product quality assessed by consumers highlights an important area of research for the
kangaroo meat industry of the future. This may relate to the vegetation grazed by animals: thus, the potential remains for product from some regions being more acceptable than that from others. Any preferential harvesting from geographic zones, however will have to be balanced against the sustainability of the population and the environment in that region. Clearly the kangaroo meat industry has great potential for development: this project highlights the importance of research required to further increase consumer acceptance of the range of health products that are already available in the commercial marketplace.
1. Introduction

Aims

- develop relationships between genotype, sex and age of kangaroo at slaughter with the key meat quality parameters of tenderness, flavour and juiciness
- establish an understanding of the effect of harvesting and post-slaughter carcass storage methods on the rate of change of muscle pH, the ultimate pH, muscle shortening, losses due to drip from meat, cooking losses and ultimately consumer evaluation of the product
- develop a series of recommendations will be developed in close collaboration with the industry partners to establish the major factors controlling kangaroo meat quality for human consumption.

Background

With the increase in trade in kangaroo meat the need to understand factors influencing the quality of product is now apparent. The key factors for consumer acceptance identified for other commercial meats have been traditionally tenderness, flavour and juiciness. The need to explore the importance of these parameters for kangaroo meat and mechanisms associated with the variability of these parameters in these products is important in their promotion in both the domestic and export market places. This project addresses these issues.

What this report covers

The report covers the results, discussion and relevant conclusions, with directed recommendations. The numerous trials have involved the utilisation of an impressive array of meat science methods and techniques; thus, culminating in a (relatively) broad information base for industry and for further research endeavours. Trials were conducted in South Australia, South Queensland and the Northern Tablelands/North West and Central regions of NSW: this includes samples from a variety of genotypes which widens the relevance of results across the industry. This report is designed to provide the information required to meet the aims of this project as outlined above and serve as a detailed and coherent information source for both industry and future research groups.

Kangaroo population dynamics and harvesting

Aboriginals have been utilising the kangaroo population in sustainable ways for thousands of years (Croft 1992). By far the greatest effect upon numbers of kangaroos and wallabies (and many other native Australian species) was the settlement of Australia by Europeans: clearing forests, native grassland modification to pasture, increased availability of inland water sources for livestock (Anon 2003).

Kangaroos were seen to be a serious threat to European-based agricultural development as early as the 1850’s (Anon 2003), to the extent of laws being implemented requiring landholders to destroy kangaroos on their land. The modification of habitat and general interaction with European settlers resulted in the reduction in populations of the smaller kangaroos and wallabies, while the numbers of the larger species of kangaroos increased
(Croft 1992). The government and society in general, slowly began to realise that wildlife needed to be conserved, in conjunction with sustainable agricultural production. During the 1950’s and 1960’s, these concerns eventually lead to the passing of state and territory government laws controlling the destruction of kangaroos and wallabies. Kangaroo population management was thus implemented, requiring the issuing of permits for the destruction and/or harvest of kangaroos and wallabies (and also all other native fauna). The responsibility was then taken by federal government, passing national law for the protection of all species of native fauna, which also encompassed controls on export of wildlife or wildlife derived products.

Agricultural practices throughout Australia have modified the environment within which kangaroos live in many ways, both positively and negatively. Pastoral activities occurring in the more fragile arid and semi-arid rangelands indirectly support elevated populations of kangaroos, primarily due to increased available water sources such as dams and tanks, and also increased availability of feed (Grigg 1988; Overton 1988; Norbury 1992). Without adequate control of kangaroo numbers through regulated harvesting, the economic viability of pastoral activities and the environmental sustainability of vast areas of land would be seriously threatened (Caughley, Sheppard et al. 1987; Grigg 1987; Grigg 1988; Overton 1988; Norbury 1993; Anon 2003).

The harvesting of kangaroos in Australia is regulated through detailed ‘Kangaroo Management Programs’, defined uniquely for each state and territory by the relevant National Parks Authority (NPWS 2003). Annually, each of the kangaroo and/or wallaby species, with the potential to be harvested, is surveyed to determine the estimated total species population via aerial manual survey (Grigg 1988; Kelly 2002; Anon 2003). Based on the total species population estimate, a maximum allowable take (quota) is set depending on feed availability as well as current and future rainfall predictions, usually between 10-15% of the total population. The National Parks Authority then controls the total harvested numbers though the issuing of sequential individually numbered, single use plastic lockable tags. Every kangaroo or wallaby (legally) harvested requires the application of one of these tags after slaughter, including those animals destroyed by landholders through pest destruction approval, animals harvested for skin only and those used for both meat and skin.

The issuing of kangaroo harvest tags is only possible to accredited, licensed kangaroo harvesters and, in certain clearly defined and unusual circumstances, to landholders with proof that there are excessive numbers of animals that require destruction. Professional kangaroo harvesters must complete thorough training in the form of TAFE accreditation covering animal welfare, hygienic harvesting practices and initial carcass inspection. Firearm competency is tested annually, ensuring the continued adherence to the strict guidelines set out in the federal government’s ‘Code of Practice for the Humane Shooting of Kangaroos’ (Anon 2003). The level of accuracy required by this code is very high, requiring animals to be only head shot. This slaughtering practice has been reviewed by the RSPCA of Australia, and was concluded to be one of the most humane, low stress slaughter methods possible (RSPCA 1985; RSPCA 2002).

This preamble is important in that it establishes the environmental and legislation constraints within which the industry operates. Thus many of these factors have an impact on how meat can be harvested, stored and then processed, all of which contribute to the quality of product available to the consumer.
Human consumption of kangaroo meat – past and present

The first human consumers of kangaroo meat were the indigenous Australians, the Aborigines. The coexistence of Aborigines and kangaroos for so many thousands of years undoubtedly resulted in a wealth of Aboriginal knowledge of kangaroo meat quality, as well as an appreciation of meat quality differences within carcass. Within the larger kangaroo species, and where Greys, Euros and Red kangaroos coexisted, the Red kangaroo was more highly favoured by Aborigines than any other species in the western region of NSW (Dawson 1995). Meat from the Euro (or Inland Wallaroo) for instance, was known to the Aborigines to be comparatively darker, and the fat more yellow: the taste of Euro derived meat was generally regarded as being inferior to the meat from Reds. Western grey kangaroo meat was not favoured by this region’s Aboriginal population due to its unfavourable cooking aroma (Tunbridge 1991; Dawson 1995).

The early settlement and exploration of Australia by Europeans saw the first non-Aboriginal ‘consumer evaluation’ of kangaroo meat. Initial consumption of kangaroo meat was perhaps biased by the fact that the months of travel by ship to Australia only allowed for the consumption of heavily salted meat products and fresh fish during transit – the fresh kangaroo meat on arrival was thus a ‘luxury’. The first recorded kangaroo (not wallaby) harvested by Europeans was on the 14\textsuperscript{th} of July, 1770 (Dawson 1995), during the exploration of the east coast of Australia by the Endeavour, captained by Cook. A journal entry by Cook himself was recorded the next day: “\textit{Today we din’d of the animal shott yesterday & thought it excellent food}” – clearly, some of the first impressions of kangaroo meat by Europeans were good. However, initial European evaluation of kangaroo meat was not all positive, with Governor Phillip regarding the meat as being ‘coarse and lean’ and only fit for provisions when no alternative was available (Dawson 1995). The initial settlement of Eastern Australia by convicts saw Eastern Grey Kangaroo meat being an important meat source, with convicts being employed as kangaroo hunters for public kangaroo meat consumption. Kangaroo meat was initially accepted as a food due, if not due to anything else, to necessity (as well as other native species such as Emu). However the consumption of traditional European domestic meat species such as beef, sheep, pork and poultry predominated and the consumption of kangaroo was denigrated by society. Anecdotal evidence suggests that this bias persists currently and especially in rural areas. This socially based bias against kangaroo meat consumption is still encountered anecdotally to this day, and still persists especially in many rural areas, where kangaroo meat is regarded as fit for pet consumption only.

Strict guidelines exist for the harvesting of product. The animal must be head shot, not only to ensure a quick kill but also to prevent damage to the skin, carcase and internal organs which are required for inspection purposes. Once killed the animal must be immediately bled, the abdomen opened and the stomach and intestines removed and discarded. The carcase with heart, lungs, liver, spleen and kidneys intact and still attached must then be placed under refrigeration within two (2) hours of the animal being shot where the animal is shot after sunset and before sunrise, not more than two (2) hours after sunrise. Once placed under refrigeration, the carcase must be reduced to a deep muscle temperature of no more than 7 degree Celsius within 12 hours, and maintained at that level until presented for inspection at a registered export game establishment. Standards for the construction, equipment, maintenance and operation of export game establishments are identical to those for any other establishment preparing meat from domestic animals for export. On arrival at the game establishment, kangaroo carcasses are subject to the same level of inspection by qualified meat inspectors as are the carcasses of domestic animals at export abattoirs, and are passed as
suitable for human consumption or rejected according to findings. Once passed by the
inspector, the carcase may be boned out, packed and exported as game meat.

Industry guidelines and carcass inspection procedures, (for example refer to Andrew 1988),
have been refined but not greatly modified through to the currently valid industry guideline
publication (ARMCANZ 1997). The major addition was temperature bracketing for carcass
chiller placement, rather than only being based on sunrise and set times. The incidence of
zoonoses and other public health risks from kangaroo meat has also been investigated, and
summarised by (Andrew 1988). In brief summary, kangaroo meat presents little or no risk as
a source of either disease or illness due to consumption, provided that the meat is derived
from carcasses that have been harvested and handled according to the prescribed protocols.
Andrew 1988 gathered records of all carcasses presented for inspection at export game
establishments from 1980 to early 1988: 204,052 total data records with only 0.7% of these
found to have some sort of pathological condition. Of these rejected carcasses, 96% were
rejected due to ‘stifle-joint worm’ *Dirofilaria roemeri*.

Emotive issues also have an impact on the utilisation of kangaroo meat. Globally, the
kangaroo is renowned as an important icon of Australia. The kangaroo is present on
Australia’s Coat of Arms, and on Australian currency. Such emotional and patriotic beliefs
are, for many Australians, the main reasons for their non-acceptance of the consumption of
kangaroo meat, (RIRDC 1998). The lack of acceptence of kangaroo meat due to such
moralistic beliefs is commonly quoted as the ‘Skippy-syndrome’, based on the much loved
television series which focuses on the adventures of a fictitious, extremely intelligent young
grey doe kangaroo. Further to its poor social image, it’s acceptability in the consumer market
is also influenced by its inherent darker colour when compared with other red meat from
domestic species. Australian consumers generally select against darker meat, preferring
brighter red meat cuts. Colour based meat preference may be influenced by cultural
background since, for example, Germans do not discriminate against darker coloured meat to
the extent that Australians do (as reviewed by Grunert 1997).
2. Methodology

The methodology used in this project is outlined in the following sections. In depth description has been provided in covering the many aspects of successful investigation of the processes of harvesting carcasses, subsequent post-harvest handling and processing, with meat quality evaluation through both sensory, objective and biochemical means.

Sample acquisition

Slaughtering methods

All animals utilised throughout this thesis were at all times opportunistically gained as a result of the commercial harvesting of wild kangaroos, as part of the regulated human-consumption kangaroo meat industry of Australia. Briefly, the harvesting process involved the field-slaughter by head shot (using spotlights to stun animals) of specific species of wild ranging kangaroos (and a limited number of wallaby species), by a field processor.

Post-harvest (slaughter) handling

Once the animal was slaughtered, within a short time it was suspended by one leg from a metal spike on the side of the field-processing rig (typically a four-wheel drive utility vehicle) by the opening of a small slit by knife, between the Achilles tendon and distal fibula. The carcass was then immediately bled by thoracic stick or transverse cut of the ventral region of the neck (depending on State prescribed regulation, and destined end-use of the meat). The carcass then remained in this state of suspension for up to 30 minutes, to facilitate bleeding of the carcass.

The carcasses were then field-dressed, usually in a batch of animals harvested within a period of maximum 30 minutes. Field-dressing involved a clearly defined short-gutting process, or partial evisceration, where the viscera not including the kidneys, liver, heart or lungs, were removed very carefully. Any spillage of gut contents resulted in an immediate rejection of the carcass by the field-processor: this process was strictly controlled, and heavily policed by processing plant inspectors. The remaining gut cavity and thoracic cavity organs, excluding the kidneys, were then (depending on the particular states prescribed procedure and also the particular processing plant’s specifications) suspended by their original attachments outside of the animal to increase the chilling potential of the carcass by allowing air circulation within the thoracic and to some extent abdominal cavities. Some processing plants stipulated that the abdominal opening (allowing for evisceration) be minimised as much as possible, and that the remaining viscera after short-gutting be left in place within the carcass.

Both methods were effectively utilised for various trials during this research project, and, surprisingly, there was not perceived or measured difference in the chilling rates between the two evisceration/carcass post-gut dressing procedures. The two divergent methodologies stem from the lack of investigation of the effect of the exterior-hanging of remaining organs on the chilling rate of carcasses and on the potential for carcass microbial contamination and the accumulation of dust and other air-borne debris. In all experiments the time to transfer carcasses to chillers was minimised and air space on the back of rigs was maximised to prevent heat shortening of carcasses. This risk was most obvious on hot summer evenings.
**Skin removal**

Skins were left in place from the time after evisceration in the field to the transfer of carcasses to field-chillers, onto chilled transport container/trucks and into holding chillers at processing plants.

**Carcass chilling, cold chain specifications and hygiene**

All carcasses used in the current experiments were harvested and eviscerated according to the regulations for these processes. Field-processed carcasses were transferred to chillers as soon as possible after slaughter and certainly within the prescribed two hours after dawn. They reached a deep muscle temperature of less than 7ºC within 24 hours prior to trucking to the processing plant.

**Carcass suspension possibilities**

Carcasses destined for human-consumption meat processing are most often suspended only by a pelvic spike within the field harvesting rig. Effectively, pelvic spike suspension is a form of “tenderstretching” assessed extensively for use in the beef industry where both of the hind-legs are free, pivoting around the hip as the fulcrum: this places tension on the major leg muscles thus preventing muscle shortening during rigor. Typical, however, carcasses are suspended by one leg when loading into the field chiller leaving the possibility of shortening in the suspended leg side of the carcass, during the onset and resolution of rigor. Few processors use tail (or pelvic hook) suspension of carcasses as part of their standard operating procedures, suggesting that the effect of carcass suspension may have a significant influence on the quality of kangaroo meat.

We have investigated the impact of this procedure in one of the studies reported herein. Figure 1 (below) depicts the two extremes in conformation of carcasses that occurs through one leg suspension, with location of some muscles indicated for reference.
Figure 1  Representation of freely hanging and suspended kangaroo carcass configurations (viewed from left side) with a selection of some trial muscle locations. For illustration purposes only, and note that kangaroo hides are not removed until processing. Both configurations occur within each carcass with one leg suspension.

Figure 2  A typical, almost capacity, night’s yield of kangaroo carcasses, suspended by pelvic spike on the field harvesting rig.
Figure 3  Tail S-hook suspension of kangaroo carcasses within a field chiller, allowing for continued tension upon the major hindlimb muscles through rigor mortis, known as ‘tenderstretching’, due to the pivoting weight of the freely hanging hindlimb. Note the coloured tags easily visible on the right carcasses, which were the research tags used for unmistakable sample animal identification.

Animal and/or carcass field measurements

As part of the routine data collection procedures for this research project, a number of animal or carcass measurements were taken in the field during the harvesting events. Carcass identification (and ultimately muscles upon boning) was of paramount importance, with complete assurance necessary in positive and permanent carcass tagging system, independent of the Nation Parks and Wildlife Service (NPWS) mandatory tag. With field-proven infallible integrity were tags based on either plastic coated (laminated) card, or plasticised tear-proof cardboard, printed with a Laser printer and attached to the carcass with a cable tie through a slit cut in the hide (as prescribed practice for NPWS tags). Thus, even if the laminate surface was compromised in any way, liquid contact could not then interfere with the printed information below. Animal ID, researcher details (with field accessible mobile contact details) and research institution details were included on each carcass tag.
Figures 4a and b  ‘Field-proof’ research carcass identification, providing a method for (practically) indestructible unique carcass identification and researcher contact details. Bright and vivid coloured tag material was coupled with large high contrast print to allow for easy and positive identification of sample carcasses within dimly lit and potentially fully loaded field chillers. The system has proven 100% effective, and was integral in allowing for the positive tracking of carcasses from field through the cold chain to processing plant and finally carcass breakdown.

**Tail circumference / length and dressed weight**

Measurements of the circumference of the base of the kangaroo’s tail, as well as the total length of the tail from the ventral base to the tip were routinely made to establish a data set of easily measured variables which may be useful for covariate analysis.

Tail circumference was measured ante-mortem, prior to bleeding and evisceration, while the animal is still on the ground at the site of slaughter. A fabric-construction ‘tailors’ tape with millimetre/cm graduations was utilised, with the tape wrapped around the base of the tail firmly, as close to the body as possible while keeping all of the tape perpendicular to the tail axis. Tail length was measured with the same tape, aligning the tape along the tail ventrally, working from the beginning of the tail at the tail-butt to the tip, ensuring that the tape followed the tail surface and that the tail was resting in a natural configuration on the ground.

As kangaroos are wild harvested, the age of the animals is unknown, leaving anatomical measures such as these as indicators of a specimen’s approximate age. The most commonly measured anatomical variable relating to age is the animals live weight, or in the case of field harvested and dressed animals, the dressed weight. Dressed weights were always measured upon delivery to the field chiller, with carcasses being identified clearly with securely fastened carcass indicator tags. Standard trade-accredited spring scales were usually employed on a carcass rail at the entrance or loading ramp to field chillers, and were calibrated by the chiller manager or a processing plant representative.

**Temperature logging**

The automated logging of temperature formed an integral part of many trials during this research project. This included the gathering of temperature data from carcasses, excised samples, ambient temperatures in-field and on-rig, positions within field and holding chillers, and also included the monitoring of equipment conditions such as portable refrigerator/freezer units and field-water baths.
While the pH meters utilised in this research included programmable automated temperature logging facilities, the size of the physical size base-unit, as well as the comparative difficulties involved with separate leads and logging units, lead to the use of smaller, longer term and more robust logging units.

Initially, ‘TinyTag’ dedicated temperature loggers were utilised which are programmable for frequency and duration of total logging time, and have the added feature of being able to be programmed for a delayed start, saving valuable logging storage memory as the units were usually configured in the laboratory as a batch rather than in the field. These units were calibrated against a standard laboratory thermometer, and tested under laboratory conditions for accuracy, which was considered to be ± 0.5°C.

**Figure 5** Infra-red based non-contact temperature monitoring formed an important method for measurement of many aspects of trials and laboratory procedures. For example, meat samples from frozen through to cooking/cooked, grill plate temperatures, chiller/freezer operation.

**Musculature definition and nomenclature**

Muscles and meat cuts were typically described as per the RIRDC publication, “Kangaroo: specifications and selected meat cuts”. Specific anatomical details were provided by Hopwood, 1974, and most significantly through direct tuition by Associate Professor Paul Hopwood, Faculty of Veterinary Science, USYD.

A comprehensive list of all muscles forming part of various trials during this project is included in the ‘Abbreviations’ section on page V. Figure 6 below, depicts the high level of precision and accuracy that was maintained throughout the research to best ensure high-quality results.
Figure 6  Sampling example - a high level of precision and accuracy was maintained for the various types of muscle samples required.

Objective methodology

Cooking procedures and cook loss measurement

Due to the constraints based on the physical size of kangaroos, and hence, the relative sizes of the target muscles for the trials in this research project, cook blocks were typically prepared for a target weight of 65g pre-cooking. Some initial work involved the use of 100g and 250g cook blocks where muscle size allowed. The attainment of the correct target core temperature of 70°C immediately before the cessation of cooking in the different sized cook blocks was tested using thermocouples (Pico TC-08, Esis Pty Ltd, Sydney, Australia) in a preliminary trial in order to establish the correct cooking times. The total weight of muscle placed in the water bath (with a capacity of 80 litres), was kept constant throughout these studies.

Frozen whole muscle samples were thawed at 4°C for 24 hours prior to analysis, with samples spaced out on wire racks to allow for air-flow around the muscles. After thawing, muscles were then cut into the correct cook block size (65g, with the exception of “Carcass suspension trial I” where block sizes of 100g and 250g were chosen). Care was taken to remove the epimysial connective tissue and any adipose tissue (very scare or non-existent in the target kangaroo muscles analysed), with the cook blocks being formed to similar dimensions.

Cooking times were: 65g block – 30 minutes, 100g block - 35 minutes and for 250g blocks - 60 minutes, all block sizes having attained an internal maximum peak temperature of 70°C upon removal from the water-bath. The cooking method employed for all objective tenderness measurements (peak shear force and compression determinations) was by total immersion water-bath cooking with the temperature pre-heated to 70°C (Bouton, Fisher et al. 1973). Samples were weighed pre-cook to two decimal places before being placed in plastic heat-stable bags, and then all samples were simultaneously suspended in the water bath, their
arrangement allowing the water to bathe the samples from all sides (with heat transferred through the plastic bag).

After the prescribed cooking time, samples were then immersed in cool, running tap water for 30 minutes. Samples were then drained of cook juices, washed carefully in tap water and gently patted dry with absorbent cloth. The cooked meat block, free from any cook juice residue, was weighed to two decimal places, to give the post-cook weight. The difference between the pre- and post-cook weights therefore comprised the total cook loss for each sample.

Once the post-cook weight was recorded, samples were placed back in their respective bags (residual cook juices drained) and stored at 1°C overnight before the analysis of peak force and/or compression.

**Peak shear force measurement**

The methodology employed for both peak shear force and compression measurement was based on the work of (Bouton, Harris et al. 1971; Bouton and Harris 1972), as described by (Perry, Shorthose et al. 2001).

From each muscle sample, (after measurement of post-cook weight and storage at 1°C overnight), 6 individual slices were cut as replicates of each cook block. The slices were cut so as to offer a 10mm² cross sectional area to the Warner-Bratzler shear blade (Bouton, Harris et al. 1971) of the Lloyd Instruments LRX Materials Testing Machine (Lloyd Instruments Limited, Hampshire UK) fitted with a 500N load cell. This cross sectional area was achieved by accurately cutting the slices 15mm wide by 6.66mm deep, with the fibre direction oriented parallel to the long axis (allowing the loaded slice to be sheared perpendicular to the shear blade direction). Care was taken to always trim off the outer edges of the block (~2mm depth), utilising only the internal block mass for measurement. The shear blade was of a triangulated design, being 0.64 mm thick, with the shear blade’s shearing surface being horizontal, i.e. meeting the entire loaded slice 15mm wide edge upon shearing. The shear blade was pulled through the clamped muscle sub-sample slice at a constant speed of 100mm per minute, with the force required to shear the sample being logged constantly throughout the process of shearing (producing a real-time force-deformation curve). After the completion of the sub-sample shear, the peak shear force value (or the greatest force measured on the force deformation curve) was stored for each sub-sample, with the six values being then combined into a mean peak shear force value with units of kilograms force (per 10mm² cooked meat sample). Each mean value was tested for coefficient of variation (COV), to test the variance of the individual sub-sample values that make up the mean. A COV value greater than 0.25 indicated potential outliers, and any significantly different outliers were excluded manually from the data.

**Compression measurement**

The same sample preparation procedures as detailed for peak shear force measurement (see above) were used for determination of compression force. In brief, the term compression is defined as the product of hardness and cohesiveness, where hardness is the peak force of the first force deformation curve, and cohesiveness is the total area under the second force deformation curve (which is the work done) divided by the total area under the first force deformation curve (Perry, Shorthose et al. 2001).
At least three wedge shaped slices were cut, with a centre thickness of 10mm for compression measurement. The fibre direction of the sample followed the same orientation as the longer axis of the sub-sample slice, and was loaded onto the compression plate with the fibres horizontal, so that the compression rod was perpendicular to the fibres. The compression plate was simply a stainless steel square plate with a smooth, horizontal face upon which the sub-sample slice was placed. The compression rod was positioned perpendicular and exactly 10mm above the plate. The rod was 6.3mm in diameter, with a blunt end that was driven twice into the slice to a depth of 8mm as identical motions without moving the slice, at a speed of 50mm per minute. Repeated measures were possible on each slice, avoiding irregular areas such as major blood vessels, with each sample then yielding 6 individual compression measurements. These measurements were tested for variance (as described for peak shear force in the preceding section) and combined to form the mean compression value, with units of kilogram force. The apparatus for compression determination is shown in action in Figure 7 below.

![Figure 7](image)

**Figure 7** The Lloyd LRX Materials Testing Machine, configured for compression measurement.

**Measurement of meat colour**

The measurement of sample colour was always conducted as part of the objective tenderness procedure, immediately before the measurement of muscle sample pH (see section below). After cook blocks were prepared, they were covered with typical kitchen plastic cling-wrap (food packaging film permeable to oxygen) to avoid any moisture loss and allowing the samples to ‘bloom’ for a minimum of 60 minutes at 1-4°C. ‘Blooming’ is the development of oxymyoglobin on all of the exposed cook block surfaces from which colour measurements were taken. Post blooming, samples were taken out of the chiller and unwrapped, before the measurement of colour at three separate sites per cook block. The apparatus used for colour measurement was a Minolta Chroma Meter, which recorded the colour of the samples in the Hunter L* a* b* colour space, automatically calculating the average of the three measurements per sample. The meter was calibrated at the beginning of each measurement procedure by cross-checking the measured values of an off-white ceramic tile of known colour description. This colour communication principle was initially defined by the ‘Commission Internationale de l’Eclairage’ in 1976 (Anon 1998)).
Figure 8 below shows colour determination of kangaroo muscle cook blocks.

Figure 8  The Minolta Colour Meter, determining bloomed kangaroo muscle colour pre-cooking

Measurement of pH

Routine ultimate pH measurement – laboratory based

The measurement of meat ultimate pH was usually undertaken after the thawing of samples, with the preparation of cook blocks. After colour measurement, the sample blocks were measured at four separate sites for pH and temperature by stab-probe (see Figure 9 below). The measurement sites were always towards the extremities of the blocks to ensure that there was no damage to the internal areas destined for the cutting of slices for peak shear force or compression determinations.

The pH probe was an Ionode-IJ42 glass-epoxy composite, configured as a spear-tip combination electrode. The identical model of digital pH meter (a number of units were utilised in the various investigations) was utilised both in the laboratory and in the field (or processing plant), being the TPS model WP80. The meters were calibrated with 6.88 buffer against 4.00 buffer (with the buffers equilibrated to ambient temperatures), with the meter calculating the slope and intercept of the calibration automatically, and retaining the calibration details between sessions.

As the measurement of pH is highly dependent upon the temperature of the sample, the automatic temperature compensating (ATC) probe of each of the units were individually calibrated against a standard laboratory certified thermometer, on a regular basis.
Measurement of muscle pH and temperature under field conditions

As mentioned in the preceding section, the same pH meters, and pH / ATC probes, were used for the measurement of muscle pH in locations other than in the laboratory (Figure 10). These locations included: directly during the harvesting process ante-mortem and during the pH decline period, within the field chiller, at the holding chillers of the processing plants and on the chain up until packaging at the plant. These versatile and robust meters offered the best combination of portability and accuracy, combined with internal high-capacity rechargeable batteries, totally waterproof meter housing with pressure-sensitive membrane keypad, simplified data entry and calibration procedures, large internal data storage capability and a reliable PC-interface for data extraction. Additionally, the pH and ATC probes were of a robust construction suitable for measurement under field conditions.

At all times, buffer temperature was monitored and kept within the typical room-temperature variable range of temperatures (from around 15 to 25ºC), as the ambient measurement temperatures varied considerably throughout the seasons during the years of this research project. Therefore, the methodology was identical to that employed in the objective laboratory, except for the target muscle being at times (depending on specific trial methodology), still within the carcass. When this situation occurred, care was taken to remove any skin, fat or epimysium occluding the sample site to expose the muscle, prior to pH measurement.
Figure 10 An example of one (initial) method of ante-mortem field data collection for carcass pH/temperature.

**Measurement of metabolically active, or pre-rigor pH muscle samples**

As the measurement of pH is only possible on thawed muscle samples, glycolysis must be inhibited so that the pH measurement is indicative of the stage of glycolysis of the muscle at the time of sample excision and freezing. This was achieved through the use of buffered iodoacetic acid, which is a metabolic/glycolytic inhibitor effective in halting muscle post mortem glycolysis *in vitro*.

**In-field cryostorage of samples**

Some trial methodology and/or sampling locations limited the possibility of stab probe pH measurement. In these cases, where possible, representative samples of the target muscles (from constant, anatomically defined areas) were excised and placed into capped 5ml plastic tubes and frozen immediately by immersion in liquid nitrogen. The tubes were labelled with laser printed labels, which withstood storage in liquid nitrogen without peeling from the tube surface. Liquid nitrogen was safely transported and used in the field within a 10litre storage tank, which was securely strapped onto the rear tray of the field harvesting rig.

The ultra-rapid freezing that occurs throughout the small samples in these tubes (less than ~5 seconds to reach a stable -196°C), and the subsequent storage at extremely low temperatures (either -196°C in liquid nitrogen, or -80°C in a standard laboratory CO₂ storage freezer), facilitated muscle sample storage in a metabolically inert state. Therefore, gathering samples
for pH measurement (as well as for other enzymic or metabolite parameters) pre-rigor, during the period of pH decline, was possible in-field since the measurements could be made subsequently in the laboratory under standardised conditions.

**Sarcomere length measurement**

Sarcomere length was determined utilising an adapted version of the method originally described by (Bouton et al. 1973). This measurement procedure utilises the phenomenon of light diffraction where light is diffracted away from its original path when it passes through thin sections of muscle, cut with the grain so that the light-path meets the muscle fibres (sarcomeres) at 90° (see Figure 11 for apparatus used in this procedure). The magnitude of diffraction is proportional to the sarcomere length of the muscle section, as described in the following equation.

\[
SL \text{ (in } \mu\text{m)} = \frac{\text{Wavelength}}{\sin \left( \arctan \left( \frac{X_1 - X_2}{75} \right) \right)}
\]

Where
- the measurement wavelength is 635nm
- \(X_1\) is the diffraction distance from the centre to the outside band in mm
- \(X_2\) is the diffraction distance from the centre to the inside band in mm

The distances \(X_1\) and \(X_2\) are dependent on the physical dimensions of the particular sarcomere length diffraction unit. The distance from the glass slide holding the muscle slice to the frosted glass viewing area is proportional to the distance that the light source will diffract – therefore accuracy is important in construction, as well as initial and ongoing calibration – to determine any necessary correction factor.

The mean of a minimum of 5 separately sampled shavings from whole, frozen muscles were taken as the sarcomere length of the particular muscle sample. If a sample had inordinately variable measurements (with differences of more than 0.2µm considered unacceptable), then the measurement procedure was repeated. Outliers were discarded from the measurements when their inclusion within the mean lead to an increased COV, above the threshold of 0.25.

The shavings were gained from the whole muscle samples by initially removing any fat and/or epimysium from the muscle surface sites. A scalpel was used to produce fine muscle shavings which followed the muscle fibre direction, and were placed on a labelled microscope slide. Once the required number of shavings (6) was collected (from various sites around the muscle sample), the slide was covered with another glass slide, and then could be measured immediately or held at room temperature for up to around 30 minutes. If a longer storage time for the slides was required, the slides (with cover-slide) were carefully placed in a zip-lock plastic bag, the internal air was removed, with the slides then stored at -20°C pending measurement. Thawing of the frozen-stored slides was achieved at room temperature for approximately 30mins within the zip-lock bags, after which they were measured as usual.
**Determination of intramuscular fat content**

Unlike most other domestic species, the measurement of intramuscular fat percentage content was not included in the suite of routine objective measures of kangaroo meat. Kangaroo muscle contains a consistently low percentage of intramuscular fat (less than 2% wet weight), which was confirmed in initial experimental work.

The determinations that were completed for kangaroo meat utilised the standard procedures of SOXHLET lipid extraction from freeze dried and ground muscle samples, as described by (Perry, Shorthose et al. 2001).

**Measurement of muscle total collagen content**

Estimation of total collagen in muscle is usually achieved through the assay of hydrolysed muscle samples for hydroxyproline content, as the amino acid hydroxyproline is almost only found as a constituent of the collagens of mammals and basically not found in other constituents.

Most assays of hydroxyproline are based on the original methodology developed in the late 1950’s and 1960’s, with minor modifications.

Briefly, muscle is acid-hydrolysed to yield hydroxyproline in solution, which is then oxidised by chloramine-T. The product thus formed (a pyrrole) is reacted with p-dimethylaminobenzaldehyde (PAB, or Ehrlich’s reagent) to form a chromophore, of which the absorbance can be measured (at ~560nm) relative to a standard curve to indicate the concentration of hydroxyproline in the muscle sample. As intramuscular collagen contains 14% hydroxyproline by weight (as an average across the predominant collagen types occurring in muscle), and is found only in low concentrations in other muscle tissues. Thus, total intramuscular collagen is then estimated by multiplication of the hydroxyproline concentration by 8.
The assay procedure used for the estimation of total intramuscular collagen in this research project were based on ISO3496:1994(E). Many derivations of the hydroxyproline assay were trialled with mixed success before refining and utilising the method outlined in ISO3496:1994(E). Attempts to improve reproducibility of results were relatively unsuccessful, although we were able to establish a relatively reliable and repeatable assay for use in these studies. This is outlined below.

**Determination of total muscle collagen (hydroxyproline assay)**

**Reagents**

~3M Sulphuric acid solution

320ml of concentrated sulphuric acid was diluted to 2 litres with distilled water.

Buffer solution – pH 6.8

26g of citric acid monohydrate, 14g of sodium hydroxide and 78g of anhydrous sodium acetate were dissolved in ~500ml of distilled water. 250ml of propan-1-ol was then added and the solution made up to a total of 1 litre. Solution was stored in a light-proof bottle retaining stability for several weeks.

Chloramine-T reagent

1.41g of sodium N-chloro-p-toluene-sulfonamide trihydrate (chloramine-T) was dissolved in 100ml of the buffer solution. Solution was prepared immediately before use and not stored.

Colour reagent

10.0g of p-dimethylaminobenzaldehyde was dissolved in 35ml of 60% perchloric acid, to which 65ml of propan-2-ol was slowly added. Solution prepared on the day of use and not stored.

Standard hydroxyproline solutions

A stock solution was made by dissolving 50mg of 4-hydroxypyrrolidine-α-carbonic acid and one drop of the sulphuric acid solution in distilled water to 100ml. Stock solution was stable for 1 month at 4°C.

Standard solutions of 0.5, 1, 1.5 and 2µg/ml concentrations were prepared the day of use by diluting 10, 20, 30 and 40ml of the stock solution to 100ml with distilled water.

**Sampling**

Whole muscle samples were denuded (epimysium and any extra-muscular adipose tissue removed) and either homogenised as a complete muscle, or multiple sub-samples from a number of sites throughout the muscle were taken as being representative of the whole muscle and then homogenised in a high speed blade food-processor. The resulting homogenate was mixed by hand and then again homogenised in the food-processor,
ensuring that the whole sample was thoroughly and completely homogenous and of a paste-like consistency.

The homogenised sample was then stored in a vacuum-packed plastic bag at -20°C pending analysis.

**Hydrolysis**

From the frozen homogenate, a ~4g sub-sample was weighed to two decimal places into a 20ml Pyrex screw-capped culture tube, which sufficed as an hydrolysis flask due to heat and elevated pressure resistant properties.

30±0.1ml of the sulphuric acid solution was then added to the flask, and the cap tightly screwed on (checking that the polyurethane pressure seal was intact).

The tubes were then placed in a reciprocating high temperature liquid temperature controlled bath filled with polyethylene glycol 600 (supplier), was set to a stable 105°C for 16 hours (usually overnight), allowing for complete hydrolysis of the samples.

The hydrolysates were then filtered, while still hot, through filter paper to remove the residual ash and other insoluble products. The filter paper was rinsed with 30ml of the sulphuric acid solution in three 10ml washes, and the combined filtrate diluted to 250ml with distilled water. The diluted filtrate was then stored at 4°C for up to 2 weeks, pending further analysis.

**Hydroxyproline assay procedure**

From the diluted filtrate, a 25ml aliquot was diluted further to 250ml with distilled water. The diluted 25ml aliquot resulted in a solution of 0.5 - 2µg/ml of hydroxyproline. The aliquot volume may be reduced to a minimum of 5ml where the hydroxyproline concentration is found to be high (for example in higher connective tissue muscle samples).

4ml of the diluted aliquot solution was transferred, in duplicate, to a test tube. 2ml of the chloramine-T reagent was then added to each tube before low speed vortex mixing. Oxidation proceeded for 20mins at ambient temperatures without any further agitation.

2ml of the colour reagent was then added and each tube and then quickly vortex mixed before capping each tube with either aluminium foil or a glass marble, and placing in a pre-heated 60°C water-bath, for exactly 20 mins (±5 seconds).

Immediately upon removal from the 60°C water-bath, the tubes were placed in running, cool tap water for 3 minutes and then allowed to settle at room temperature for 30 minutes.

The absorbance of each sample was then measured by spectrometer at 558nm in a quartz cuvette relative to distilled water with an absorbance of 0.00.

The hydroxyproline concentration of each sample was then calculated from the standard curve from the mean absorbance value of the duplicates. The standard curve was constructed using the same assay procedures as described above, substituting the diluted hydroxyproline standards for the 4.00 ml aliquots of the diluted hydrolysates.

A new calibration curve was constructed for each hydrolysis batch.
Histological procedures for collagen fibre observation

Sample acquisition and preparation

To microscopically observe collagen proliferation within muscle, as with most histology investigations, a sample was removed from the whole muscle at a pre-defined and constant position and depth, with the excised sample having a constant fibre direction.

Initially, fresh muscle samples were excised up to 2 hours post mortem (pre-rigor) and subsequently at 24 hours (post-rigor) and placed in 10% neutral buffered formalin immediately upon excision, and stored at 4ºC. The excised muscle samples were approximately cylindrical in shape (~6mm diameter) with a length of ~10mm. The fixative was replaced two times, at 24 hours and 48 hours to allow for complete infiltration of the formalin within the entire muscle sample, to effectively preserve the sample.

These procedures followed standard fixing procedures as prescribed for muscle and tissue fixation, prior to wax embedding and sectioning.

After fixation (minimum time of 1 week in the final solution) samples were then either wax embedded or further stored at 4ºC indefinitely.

Paraffin wax embedding of samples

An automated dehydration/wax-infusion apparatus was used for paraffin embedding of tissue. Upon removal from the final heated paraffin solution, the muscle samples were then mounted upon plastic embedded media cassettes using a paraffin embedding station, with due care taken to ensure that the fibre direction was perpendicular to the cassette face. Upon cooling the mounted samples were then ready for sectioning using a standard bench-top microtome at room temperature.

Failed paraffin-mounted sectioning

Due to reasons that remain unknown, any attempts to yield successful sections from the paraffin mounted samples failed with the muscle flaking into many shards from the microtome blade or showing signs of possible incomplete fixing, dehydration or infiltration of paraffin. Numerous attempts were made to rectify the problem with replicate samples; however, as the trial samples were already collected and at the stage of storage in the last neutral buffered formalin solution, only the steps subsequent to this could be manipulated. Due to the limited number of replicate samples available, ultimately this methodology for the histological part of kangaroo muscle collagen investigation was abandoned, with the section below detailing the successful methodology employed in a much smaller histological study.

Frozen, unfixed sectioning and staining procedures

In a smaller study, muscle samples were taken pre-rigor from identical sites (as mentioned in the methodology above), and were mounted in the fresh state on 10 x 10mm cork blocks using gum tragacanth. The excised muscle samples were approximately cylindrical (~6mm diameter) with lengths of ~10mm, orientated so that the fibres were perpendicular to the cork block face. This mounting media was used between the muscle sample and the cork block, and was also used to build up and ‘protect’ the sides and face of the muscle sample, before the entire mounted sample was rapidly frozen in isopentane (2 methyl butane) sitting in liquid N2. Immediately prior to freezing the mounted muscle sample, long forceps were pushed into
the solidified isopentane to thaw a ‘well’ into which the muscle sample was gradually introduced. The mounted muscle sample was removed from the isopentane when the entire block was frozen, taking care not to allow the isopentane immediately around the now frozen muscle sample to re-freeze prevent the removal of the sample.

The frozen samples were then stored at -80°C, pending sectioning. The sectioning of the mounted muscle samples was completed at -25°C, with a freezing microtome, yielding 10µm thick sections air-dried on glass microscope slides. Multiple serial sections were cut for each block. After the sections were completely air-dried (minimum of 20 minutes in low-humidity and typical room temperature conditions), the slides were stored in air-tight slide storage boxes at -20°C until staining.

**Differential staining procedure for intramuscular collagen observation**

As the sections were air-dried upon glass slides, the slides did not need to be cleared in xylene prior to the staining procedure. The slides were removed from -20°C storage and allowed to come up to room temperature for approximately 10 minutes, with positive ventilation within a fume hood (exhaust fan turned on). The air movement effectively prevented any condensation from forming on the cold slides and/or sections during thawing. As the slide rack was placed in, or taken out of, each of the following washes/stains, care was taken to not loosen the sections from the slides through vigorous washing. Slides were then placed in 10% neutral buffered formalin for a minimum of 10 minutes, to fix the sections. Following a wash with tap water slides were dipped for 5 minutes in ‘Weigert’s Iron Haematoxylin’, which stains cell nuclei. The slides were then placed in a tap water wash, and then very quickly (≤2 seconds) in a 1% acid alcohol wash, before being washed in tap water and then distilled water. The slides were then allowed to stain for 5 minutes in ‘Van Gieson’s’ solution, then drained and the excess stain solution was blotted from the slides without a water rinse. A final rapid dehydration wash (≤2 seconds) in 99% ethanol was performed prior to clearing in Histolene (a less hazardous xylene alternative). The stained sections were then mounted using ‘Histoclear’ and cover-slipped. The stained sections were then ready for examination. Generally, after staining, the sections showed dark brown muscle cell walls with much lighter orange-brown cell contents, which contrasted against the pink-red seams of connective tissue.

**Microscopic digital image capture**

Slides were observed under a dissecting microscope with a 2x magnification. After alignment and focusing of the entire muscle section within view, a digital image was captured through the use of a digital camera coupled with appropriate imaging software. The digital images were then optimised using Adobe Photoshop (Version 6.0, Adobe Systems Incorporated), with modification of brightness and contrast and overlaying other filters and factors such as gamma correction, to enhance the visibility and contrast of the collagen seams above the myofibre cell area (Figure 12).
Figure 12  A kangaroo muscle section (M. adductor) stained for connective tissue observation.

Subjective methodology – consumer taste panels

Sample Preparation

For all taste panel evaluation trials, ‘composite’ steaks were produced through the use of a protein binder to effectively ‘glue’ together the left and right sides of any particular type of muscle. This procedure was used for all samples destined for taste panel evaluation, irrespective of size so that any influence that the binder may have had upon eating quality was constant for all samples and trials.

Various ageing times (as fresh chilled meat) were implemented depending on trial, with muscle samples then always stored in the frozen state before the preparation of steaks for taste panel assessment.

At all times strict controls were enforced regarding food safety, with specific focus on cleanliness of equipment and benches, and personal hygiene. Careful note was always taken of the temperatures of samples and the time that any particular sample was at temperatures above -20ºC to eliminate any potential compromise in food safety.

While in the frozen state, sarcomere length was measured on the whole muscle samples, as described above.

On the day of taste panel sample preparation, whole frozen muscles were brought to an internal muscle-core temperature of -5ºC by microwave. Due to the inherent variation in efficiency and settings that occur between microwaves, the particular setting (usually ‘medium-low’) and the time per weight of muscle to be thawed (typically 30sec per 500g at this setting) was pre-determined. The samples were then further thawed to above 0ºC at 4ºC, ensuring adequate air flow around each bag for effective thawing. Samples were usually thawed at 4 hours.

Once thawed, the epimysium and any residual adipose tissue was removed and then multiple sub-samples were removed, bulked, homogenised and then stored at -20ºC in vacuum-pack plastic bags for the subsequent analysis of sample collagen. The remaining muscle was then trimmed to the required shape/s and then combined with the matching muscle from the other side of the carcass.
Composite muscle fabrication: Protein binding of muscle samples

Identical preparation and binding procedures were employed for all samples destined for taste panel analysis, so that any eating quality effects that preparation or binding may have had were constant across all samples.

The protein binder was Pearl “E” Protein Active Meat Binder, (Earlee Products Pty. Ltd., Brisbane Australia) which effectively bound muscle proteins through contact at temperatures greater than 0°C. The preparation and binding procedures lead to ultimate muscle temperatures rising to a maximum of 10°C, before the bound, composite muscles were then re-frozen to -20°C.

Bound composite samples were set in a soluble collagen film casing within a PVC plastic mould while the binder set. The anatomical orientation of the trimmed muscle pieces (from the left and right sides of the carcass) was known at all times during trimming, so that the composite could be formed with opposing ends of the muscle being joined, so-called ‘top-and-tail’. This was undertaken to minimise the effect of anatomical position on eating quality. Muscle pieces were also aligned so that the muscle fibres were aligned along the longitudinal axis. The correctly shaped muscle pieces were then simply rolled in the protein binding powder and then shaken off, ensuring all surfaces received a light coat of the binder and that no areas retained excessive amounts. The muscle pieces were then placed in the PVC former, which was lined with the collagen film, taking care to correctly orientate the muscle pieces. The collagen film was then completely wrapped around the composite mass, before being lightly vacuum packaged in exact-fitting vacuum bags which, in combination with the collagen film, ensured that the correct cylindrical shape of the muscle composite was retained and that the binding sites were securely braced from movement. The composite muscles were then allowed to set for a maximum of 30 minutes before being rapidly refrozen to -20°C.

Steak slicing and ‘The Pick’

The composite muscles were taken directly from -20°C storage and sliced with a commercial meat band saw, perpendicular to the long axis (cut across the fibre direction) to produce the required number of 20mm thick steaks. The ends of the composite were trimmed to produce a straight surface ensuring uniformity of steaks. A minimum of either 4 or 5 replicate steaks (depending on trial design) were sliced from each composite, and then placed in a zip lock bag within a labelled drawer, ready for the randomisation procedure, named ‘The Pick’ (Figure 13). At all stages, the composite muscles and the steaks gained after slicing were monitored for temperature, ensuring that all samples remained frozen (less than ~-10°C).

The Pick involved the placing of individual steaks in predetermined positions upon labelled meat trays, with each tray corresponding to one load of the grill during a particular taste panel. The thorough randomisation is completed prior to The Pick, with care taken to ensure randomisation of animals, treatments, muscle types and steaks across the entire number of taste panel sessions and also across the serves and panellist position within each panel. Each tray is loaded with the corresponding steaks into their cross-checked designated positions, before being carefully vacuum-packaged. The vacuum packaging surrounded the upper surfaces of each steak upon the meat tray, ensuring that the samples remained in their designated positions on the tray in the frozen state while stored, during the thawing procedure prior to use and in the completely thawed state immediately prior to grilling during transport to the taste panel site.
Production of ‘starter’ steaks

As part of the standard consumer taste panel evaluation procedures, a generalised ‘starter steak’ was the first steak that any particular consumer evaluated at the beginning of the taste panel. These steaks are formed using identical procedures as detailed above, but were all sliced from composites formed from kangaroo *M. gluteus medius* (rump). This muscle was chosen as it typically has intermediate rankings for eating quality, and is assumed to be relatively constant across classes of animal. The steaks sliced from the rump composites were then randomly allocated across all taste panels and participants. The starter steak initial evaluation familiarised consumers to the taste panel evaluation procedures, and helped standardise the participants’ palates for grilled kangaroo steak. Although the results for the starter steak are not analysed, the participants were not informed as to the difference between the initial starter serve and the subsequent six sample serves, so that no bias was introduced through the order of sample serving.

Thawing and grilling protocol

The seven trays of frozen steaks corresponding with a full number of taste panel samples (1 starter tray and 6 sample trays) were thawed no longer than 4 hours prior to the time of the taste panel. The pack of trays was removed from -20°C storage and placed in lukewarm tapwater (≤ 30°C), ensuring that all the packs were submerged and free to move. At 30 minute intervals the trays were shuffled underwater to ensure that all the steaks were subjected to the thawing action of the water. Thawing to approximately 0°C (where the steaks still felt ‘icy’ to the touch) was achieved in 2 hours, after which the steaks were either stored at 4°C, or in a passive insulated cooler-box with ice-bricks, depending on the site of the taste panel and length of time until the commencement of grilling.
Grilling was conducted in a separate room/location adjacent to the taste panel participant’s seating area, so that cooking aromas and other distractions did not affect the panellist’s evaluations (Figure 26). The grills used for the taste panels were of a double-clam grill configuration (Silex) with flat, thick heat plates top and bottom allowing the grilling surface to attain a repetitive temperature cycle, corresponding with the cooking protocol cycle. Although the plate temperature fluctuated up to 50ºC around the plate set point of 200ºC (as monitored by an infra-red indirect temperature probe), the temperature cycle was consistent between grill loadings where the loading/unloading and grilling times were constant.

Initial trials were conducted in the laboratory to determine the required total grilling and resting times necessary to attain a maximum internal grilled steak temperature of 65ºC (judged as a medium degree of doneness). A number of factors were found to influence the total time required to cook the samples to the required internal 65ºC internal temperature: grill plate temperature, times and nature of cycling (including grilling times where the grill is shut, unloading/loading times where the grill is open, and closed without samples between grill times), and the mass of meat to be cooked per grill (i.e. number of steaks as the diameter and thickness were constant). Real-time, multi-channel monitoring of internal steak temperature was achieved through the use of internally lodged thermocouples (Pico TC-08, Esis Pty Ltd, Sydney, Australia). Where the protocol required 8 steaks to be grilled per serve, the grilling time (at a 200ºC average plate temperature) was 2.5 minutes: where 10 steaks were required however, the grilling time increased to 3 minutes while samples were stored for 2 min prior to serving.

The grills were pre-heated to the required 200ºC plate temperature for 30 minutes to allow for complete stabilisation, and left cycling until the commencement of grilling. An initial grill, immediately prior to the starter grill, was performed with a similar amount of scrap sample to initiate the temperature cycling of the plates to ensure consistent cooking of the steaks between serves.

A very light spray of olive oil was applied to the plates between grills to aid in preventing the steaks from sticking to the plate surfaces: this was especially necessary for kangaroo meat due to the consistent low intramuscular fat content. The plate surfaces were scraped of any steak residue between grills with a plastic, heat resistant spatula.

![Figure 14](image.png) Grilling of samples for consumer taste panel evaluation. Note the ‘Silex’ clam grill, appearance of cooked (resting) sample steaks and the labelled plates for serving.
The process of consumer evaluation

As the initial preparatory steps were occurring in the kitchen, the consumers were ushered to their seats and received a brief introductory talk including the following key points: participation in the taste panel was completely voluntary and remained so at all times and steaks were from kangaroo.

The demographic detail questionnaire was then described, and after this was filled out and collected, the process of sample evaluation was discussed. The importance of evaluating the sample as it was rather than how it was thought to be, the need to minimise discussion with other panellists, the need to randomise allocation of samples so as to encourage individual sample assessment and the requirement to cut the sample at least once and chew the steak mass a number of times prior to swallowing were all emphasized.

Corresponding with the final points of the evaluation description, each of the starter grill steaks were quickly halved by knife to produce two sub-steaks (after the 2 minute resting period), before each sub-steak was then placed on its respective labelled paper-plate to be served without delay to the consumers. Thus, if 8 steaks were cooked per grill (i.e. per serve), then 16 sub-steaks were cut post-resting, to serve the 16 consumers present. The larger protocol involving 10 steaks per grill which was sufficient to serve 20 consumers in a taste panel.

The sub-steak average internal temperature upon serving was randomly checked by indirect infra-red temperature measurement pending the particular panellists permission: this ranged from 30-40ºC.

The process of serving the samples to the consumers was completed as quickly as possible by trained servers, with serving order within the taste panel room changed between serves to eliminate any consumer seating position bias (for example the possible lower temperature of the last served steak). Consumers were given adequate time to thoroughly evaluate each serve, (with a minimum of 3 minutes found to suffice). A sample evaluation form is included in the appendices along with the demographic details questionnaire and consent form.

The process of consumer sample steak evaluation

Consumers marked each of the four eating quality measures (tenderness, juiciness, flavour and overall acceptability) with a perpendicular line, indicating their preference or judgement for each sample. The sample was then ranked by choosing one of the following rank descriptions (in increasing order): from unsatisfactory to good everyday, followed by better then everyday to the highest possible ranking of premium product. A space at the bottom of the evaluation sheet allowed for a brief comment where applicable.

As each of the four eating quality measure vertical lines were 100mm in length, the point at which the consumers mark bisected the vertical line corresponded with the sample’s particular score as a number out of 100. A clear ruler, or printed plastic cover sheet was used to determine the scores, with data entry cross-checked between people with a random number of duplicate score sheets to check that there was no data entry operator bias.
Ethical committee approval and restrictions

Throughout the entire research project, all animals associated with any investigation were gained opportunistically, with an accredited kangaroo harvester as part of their routine harvesting process. As all investigations involved only the manipulation or measurement of carcasses and not the live animal, ethical approval from neither the University of Sydney nor the University of New England Animal Ethics committees was needed.

The investigations that involved taste panel evaluation did require human ethics committee approval, from the University of New England human ethics committee as this was the site from which all of the taste panel investigations were based. At all times, taste panels were only conducted where a relevant and current human ethics committee approval had been granted.

Human ethics committee approvals for taste panels stipulated that the consumers were to be informed of the species (kangaroo at all times) and preparation procedures (always grilled to a medium degree of doneness) as part of the recruitment process and also during the introduction talk preceding the evaluation. Every participant’s identity was suppressed. All collected data (sample assessment sheets and demographic detail) were only assigned a randomly produced unique identifier number, which corresponded with the seating position for each taste panel and the samples to be served to that position. Any photographs taken any taste panel could only be taken where the photograph could in no way compromised the anonymity of the participants.

At all times it was stressed that the consumer’s presence at the panel, and their continued presence and consumption of the samples was entirely voluntary. Consumers were under no pressure to consume any samples and were free to leave at any time.

A consent form detailing the nature of the investigation and the relevant researchers and ethics committee details was signed by each participant and a designated researcher prior to the commencement of each taste panel. Again, no names or other details were recorded, only the panellists signature. As a further safeguard for anonymity, the signed consent forms did not contain any seating position details and were collected separately as a bundle, so that the signature could not be linked with either the demographic details of the participants nor their evaluations.
Statistical software and procedures used

The statistical analysis software utilised for all analyses was ‘The SAS System for Windows’ (SAS Institute Incorporated, Cary N.C.): beginning with version 6.12, with subsequent software updates leading through to version 8.02 for the final analyses found within this thesis.

Data collation, manipulation and archiving were always accomplished using Microsoft Excel for Windows (Microsoft Corporation). Some very minor statistical procedures were occasionally carried out using the functions available in Excel, amounting simply to means calculations and time format modification.
3. Benchmarking kangaroo meat quality

The evaluation of the impact of sex, genotype and age of kangaroo on the key meat quality parameters tenderness, flavour and juiciness

Summary

Grilled kangaroo meat was evaluated by consumer taste panel to determine the magnitude of pre-harvest effects on meat quality. Discriminant analysis resulted in the weightings of 0.20 for tenderness, juiciness and flavour, and 0.40 for overall acceptability. These weightings were used to combine the results into the one dimension of palatability. Flavour was found to be the predominant factor influencing the acceptability of grilled kangaroo, showing a high correlation (r = 0.89) with overall acceptability. A muscle by species interaction was seen to be significant (p < 0.01) for palatability, as well as muscle by dressed weight. Red kangaroo cuts were evaluated as being more palatable than Western Grey cuts, while heavier carcasses yielded tougher meat from the leg muscles, but not from the loin fillet.

Introduction

Kangaroo meat for human consumption is an emerging Australian industry that is currently based on the field harvesting of wild populations of animals. The very nature of field harvesting introduces a number of variables that can potentially influence the resultant meat quality of the product: these factors have an impact both pre- and post-harvest. This trial focused on the pre-harvest factors influencing kangaroo meat quality, as judged by consumer based taste panel assessment of the product. Post-harvest handling and procedures were kept as constant as possible for all the sample animals. Kangaroo meat is currently gaining in popularity on the Australian domestic market, a market in which consumers are increasingly discriminating against poor quality meat.

Materials and Methods

Sample animals were harvested from the Hallett region of South Australia, (2 hours north of Adelaide). Vegetation was relatively sparse, and total rainfall for the 3 months preceding this experiment was less than 100mm. Ambient late summer temperatures (both nights) ranged from 17 to 23°C.

Animals (n=36) were field harvested over two nights using standard procedures for the harvesting of kangaroo game meat. Muscles were collected from the processing plant after 9 days of ageing. Two species of kangaroo were sampled (n=18 per species), the Western Grey (WG: *Macropus fuliginosus*) and the Red (R: *Macropus rufus*), (dressed weights - WG: mean 28kg, range 14-45kg; R: mean 21kg, range 15-30kg). Equal numbers of each sex were obtained within each species (n=9 for each).
Sample preparation and evaluation procedures

The sample preparation and sensory procedures followed those used by Meat Standards Australia (MSA) for beef and lamb (Thompson et al. 1999). Three muscles were collected for sensory assessment from each animal (M. biceps femoris - silverside, M. adductor - topside and M. longissimus dorsi - loin fillet), plus a fourth which was used as an initial sample for the sensory panels (M. gluteus medius - rump). As many of the muscles were too small to form a sensory sample, muscle binding enzyme powder was used (Pearl “E” Protein Active Meat Binder, Earlee Products Pty. Ltd., Brisbane Australia) to combine L and R side muscles from each animal as described above. For binding, muscles were thawed rapidly (microwave) to -5°C and then thawed to above 1°C within 5 hours. The binding procedure was then carried out without delay, and the samples quickly refrozen to -20°C. After binding, muscles were then sliced across the fibre direction into steaks of a consistent 20mm thickness, all of similar diameter (~50mm), in preparation for grilling.

Sensory Evaluation Procedures

The results of MSA with beef and lamb sensory trials, showed sensory evaluation using untrained consumers to be an effective alternative to trained taste panels (Thompson et al. 1999). Consumer taste panels require many more participants due to the higher level of variation associated with untrained consumers, but yield results that are relatively unbiased and directly relevant to industry. The details of the methods are given above. In brief a total of 7 samples were offered to each consumer (6 plus the starter muscle) per session. Each session/panel consisted of 12 people with 12 panels conducted in total. Therefore, 144 people-assessments were conducted, totalling 864 individual samples (without the starter steak).

Steaks were randomised across all panels and tasters. Panellists were informed of the randomised nature of samples and that the samples were grilled kangaroo, but further details were not disclosed until after the panels had been completed.

Sample cooking was by grilling 6 steaks simultaneously on a Silex double clam grill, at a temperature of 200°C. All samples were cooked for 2.25 min, with 2 min rest at room temperature before halving each steak for serving.

Sample evaluation

Each steak was evaluated separately for tenderness, juiciness, flavour and overall acceptability by vertically marking unstructured 100mm horizontal lines (each converting to a score out of 100). The lines were anchored by the words not tender/very tender for tenderness, not juicy/very juicy for juiciness and dislike extremely/like extremely for both flavour and overall acceptability. Consumers were also requested to rate the eating quality of their steak on a four level ranked scale, from unsatisfactory, good everyday, better than everyday to premium product. A blank box was included at the foot of the evaluation sheet, allowing separate comments to be made on any steak if desired.
Statistical analysis

The four sensory dimensions were combined to give a single dimension of palatability, or the ‘KMQ4’ score, through the use of the discriminant analysis function in SAS. This formulated weightings for each dimension of eating quality based on the rank score given to each sample (Thompson et al. 1999). The mixed model covariance-test procedure was used to model the data, with palatability, tenderness, flavour, juiciness or overall acceptability as the dependent variable. All possible variables and logical interactions were tested. In all models, both animal and taster (within session) were included as random effects.

Results

Discriminant analysis resulted in the weightings of 0.20 for tenderness, juiciness and flavour, and 0.40 for overall acceptability. These weightings were used to multiply each dimension, which, upon addition of the four, resulted in the palatability score. Testing the four original dimensions with discriminant analysis, it was calculated that 67% of the results were effective in determining the ranking score for each sample. Palatability alone correctly ranked 60% of the rankings. Tenderness tested alone correctly ranked 41% of samples, juiciness 32%, flavour 53% and overall acceptability 65%.

Scores for all four eating quality dimensions ranged from 0 to 100, and ranking scores for overall eating quality ranged from 1 to 4. This is a reflection of the increased variability associated with the use of untrained panellists.

Table 1. F ratios for the effect of muscle, species, dressed weight, species x muscle and dressed weight x muscle, after adjustment for session and serving order effects. Results are also adjusted for the random effects of animal and taster (within session). Values in bold have a p < 0.05.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Tenderness</th>
<th>Juiciness</th>
<th>Flavour</th>
<th>Overall Acceptability</th>
<th>Palatability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF/ DDF</td>
<td>F ratio</td>
<td>NDF/ DDF</td>
<td>F ratio</td>
<td>NDF/ DDF</td>
</tr>
<tr>
<td>Muscle</td>
<td>2/644</td>
<td>3.75</td>
<td>2/647</td>
<td>0.97</td>
<td>2/646</td>
</tr>
<tr>
<td>Species</td>
<td>1/644</td>
<td>51.46</td>
<td>1/647</td>
<td>16.46</td>
<td>1/646</td>
</tr>
<tr>
<td>Dress wt</td>
<td>1/644</td>
<td>3.94</td>
<td>1/647</td>
<td>4.81</td>
<td>1/646</td>
</tr>
<tr>
<td>Species*Muscle</td>
<td>2/644</td>
<td>17.48</td>
<td>2/647</td>
<td>2.65</td>
<td>n.a.</td>
</tr>
<tr>
<td>D.wt.*Muscle</td>
<td>2/644</td>
<td>3.2</td>
<td>n.a.</td>
<td>n.a.</td>
<td>2/646</td>
</tr>
</tbody>
</table>

The interaction of dressed weight by muscle was not included in the model describing juiciness, and the interaction of species by muscle was not included in the model describing flavour.
Table 2. Significant effects least squares means (units from 0 to 100 points)

<table>
<thead>
<tr>
<th>Sp.</th>
<th>Muscle</th>
<th>LSmean</th>
<th>std.err</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juicy R</td>
<td>61.84</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>55.37</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Flav. R</td>
<td>60.60</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>55.90</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>Flav.</td>
<td>add</td>
<td>57.44</td>
<td>1.44</td>
</tr>
<tr>
<td>b. fem</td>
<td>59.39</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>l. dorsi</td>
<td>57.93</td>
<td>1.44</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Significant interactions least squares means (units from 0 to 100 points)

<table>
<thead>
<tr>
<th>M. adductor</th>
<th>M. biceps femoris</th>
<th>M. l. dorsi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>WGrey</td>
<td>Red</td>
</tr>
<tr>
<td>Tender.</td>
<td>70.1</td>
<td>41</td>
</tr>
<tr>
<td>Overall</td>
<td>63.6</td>
<td>50.9</td>
</tr>
<tr>
<td>Palat.</td>
<td>64.5</td>
<td>50.1</td>
</tr>
</tbody>
</table>

Table 4. Significant effects and interaction regression estimates for dressed weight by muscle

<table>
<thead>
<tr>
<th>add</th>
<th>b. femoris</th>
<th>l. dorsi</th>
<th>St.err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender</td>
<td>-0.43</td>
<td>-0.23</td>
<td>0</td>
</tr>
<tr>
<td>Flav</td>
<td>-0.07</td>
<td>-0.38</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>-0.28</td>
<td>-0.43</td>
<td>0</td>
</tr>
<tr>
<td>Palat</td>
<td>-0.29</td>
<td>-0.32</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5. Correlation matrix of the four dimensions of eating quality and palatability

<table>
<thead>
<tr>
<th>Tender</th>
<th>Juicy</th>
<th>Flavour</th>
<th>Overall</th>
<th>Palatability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juicy</td>
<td>0.48</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flav</td>
<td>0.44</td>
<td>0.39</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.56</td>
<td>0.48</td>
<td>0.89</td>
<td>1.00</td>
</tr>
<tr>
<td>Palat</td>
<td>0.75</td>
<td>0.68</td>
<td>0.86</td>
<td>0.94</td>
</tr>
</tbody>
</table>

-The regression coefficient for juiciness with respect to dressed weight was 0.29 (st.err 0.13).
-Serve order was significant for tenderness and palatability models. Scores decreased over the duration of each session (~7% for tenderness, ~4% for palatability).
Discussion

Table 5 shows the correlations between the different dimensions of eating quality including palatability. This correlation matrix is thus a measure of the sensitivity that consumers have shown for each of the eating quality dimensions. Between tenderness, juiciness and flavour correlations were all <0.5, indicating that consumers were able to discriminate between these dimensions when judging samples. Flavour and overall acceptability were, however, more highly correlated (r=0.89). Consumers’ acceptability of kangaroo meat is therefore strongly driven by the flavour attributes of the product, in contrast to beef studies where tenderness is of greatest importance (pers. comm. Thompson, J.M. 2002).

The decrease of 7% of correct allocation into ranking groups using palatability alone is quite acceptable, considering that palatability is a combination of four separate dimensions. Similar results have been obtained with beef using untrained consumers (pers. comm. Thompson, J.M. 2002).

Analysis of the palatability scores showed there to be a significant species by muscle interaction (p < 0.01). Palatability scores for Western Greens were lower for all muscles as compared with Reds (see table 3). Palatability scores were similar for all cuts in the Red kangaroo, but the palatability score for the loin fillet was higher in the Western Grey. This species by muscle interaction was also observed for tenderness and overall acceptability when each was analysed in isolation, with Reds scoring higher for both dimensions across all muscles. Differences in tenderness between muscles in the carcass may be explained by such differences as connective tissue content.

Palatability was significantly affected by the interaction between dressed weight and muscle (p < 0.05). Palatability scores for the silverside and topside cuts decreased in heavier dressed carcasses (around 0.3 units decreased palatability with every kg increase in dressed weight, see table 4). Not surprisingly a similar relationship was found for tenderness, flavour and overall acceptability. However, there was a negligible effect of dressed weight upon the palatability score for the loin fillet. For tenderness, topside had the highest negative correlation (-0.43) with dressed weight, followed by silverside (-0.23). Again, the effect was not apparent in the loin fillet, a result that could be explained by the presumed lower content of connective tissue in this muscle. Flavour was reduced in the silverside with increased dressed weight, whereas the topside and loin fillet showed negligible differences. In terms of overall acceptability, silverside showed the strongest effect, followed by topside, with a large part of this evaluation being due to the high negative tenderness correlations.

The observations of decreased tenderness with increasing dressed weight may be explained by increased levels of connective tissue or increased cross-linking of collagen in the leg muscles of the kangaroo as the animal matures, thus increasing the strength and resistance to damage of the muscles. A heavier animal needs to deliver a greater force through its locomotive muscles to move; in the kangaroo this is directed through hopping, and through the need for muscles to resist the strain of landing. The explanation of the interaction of dressed weight with flavour is likely to be more complex: possible biochemical changes occurring in the more heavily exercised leg muscles may influence flavour relative to that observed in the more supportive musculature of the back. The decrease in flavour with increasing dressed weight could be based on the assumption that heavier animals are generally older. In other species, meat flavours usually intensify with increasing age (Lawrie 1991), and in many cases this leads to reduced consumer acceptance. The flavour of Red kangaroo cuts was evaluated more favourably than those from Western Greens. This difference may be due to differences in dietary selection between the species.
Analysis of juiciness found neither the interaction between muscle and species nor dressed weight and muscle to be significant. The most important effectors of juiciness were species and dressed weight. Table 2 shows the effect of species, with Reds scoring over 6 points higher in the evaluation for juiciness than Western Greys (p<0.01). This parameter increased with greater dressed weight (males and females pooled), with scores increasing by ~0.3 units per kg increase in carcass weight. Using dressed weight as a crude indicator of kangaroo age, we concluded that older animals were more likely to yield juicy cuts of meat. The increased juiciness of larger carcasses may result from a decrease in evaporative loss from the larger carcass mass; however, leaving the skin on the carcasses in the chiller minimises this loss.

Although a significant difference in flavour existed between species, the relative importance of this flavour difference was quite low (table 1). Flavour is an attribute of eating quality that is traditionally difficult to link with causal factors, as these are usually subtle in nature. The added complexity with kangaroo is that many consumers compare the flavour with other meats and as such it is considered at times to be stronger in flavour. This leads to some consumers defining the product as having a ‘gamey’ type flavour, which is often discriminated against. No significant difference was found in flavour between sexes, in contrast to other species (Lawrie 1991).

The fact that taste panel session was found to influence all modelled dimensions (p < 0.01) showed that groups scored differently over the 12 taste panel three month period. However, this effect is minor, and is adjusted for in the models. The serving order of steaks exerted a significant effect on tenderness and palatability (p < 0.05). Scores decreased slightly within session. Consumers were possibly becoming slightly fatigued, potentially due to the novel aspect of kangaroo meat. This effect was also adjusted for in the models.

**Conclusion**

The particular muscle, species and dressed weight of the carcass, all influenced the eating quality of grilled kangaroo meat significantly. Significant species by muscle interactions were found for the characteristics of tenderness, overall acceptability and also for the integrated dimension of palatability. Tenderness, flavour, overall acceptability and palatability were all influenced significantly by the interaction between dressed weight and muscle. The overall acceptability score was seen to be the most important aspect influencing the consumer ranking of product, with flavour contributing most to overall acceptability, suggesting that the potential of kangaroo meat as a viable product is strongly dependent on the unique flavour characteristics of the muscle.
4. Post-harvest handling effects upon objective meat quality

The relationship between harvesting and post-slaughter carcass storage methods and the rate of change of muscle pH, the ultimate pH, muscle shortening, losses due to drip from meat and cooking losses.

The effect of pre-rigor temperature on the ageing potential of kangaroo meat

Background
Kangaroo is becoming an important meat source for the Australian domestic consumer and also as an export product into European and other markets. Field processors harvest kangaroos at night under spotlight, the bodies are eviscerated and the carcasses (skin-on) hung at ambient temperatures for up to 8 hours prior to chilling. Given the seasonal variation in ambient night temperatures (from below 0°C to 30°C), muscle temperatures during the early pre-rigor period can vary widely throughout the year. Hwang and Thompson (2001) have shown that the temperature of muscles at pH 6 (rigor) can influence the ageing potential of beef muscles, and that hotter or colder muscle temperatures than optimum (~27°C) can have deleterious effects upon meat quality. This study reports on the impact of the temperature of storage of carcasses pre-rigor on the tenderness of meat assessed objectively.

Objectives
The effects of delayed or rapid chilling upon the ageing potential of various commercially important muscles from the kangaroo carcass were investigated by subjecting carcasses to fast and slow chilling regimes representative of the normal range encountered in industry conditions and comparing shear force at 7 or 21 days in different muscles.

Methods
Eight kangaroo carcasses were obtained under typical industry conditions from NW New South Wales, Australia. Bodies (eviscerated with the skin on) were suspended by the tail, and either chilled as soon as possible post-harvest (chiller air temp ~2°C), or held in ambient temperature conditions (15-20°C, no air movement) for 12 hrs post mortem. Directly after harvest and at 1, 2, 3, 6 and 12 hours post-mortem (PM), samples of the M. vastus lateralis were excised and placed in liquid nitrogen for the subsequent determination of pH by homogenisation of muscle in iodoacetate-KCl buffer (pH 7.00) (Bendall, 1973). Temperature decline was measured using temperature loggers (Gemini Dataloggers (UK) Ltd.) with a thermocouple inserted in the M. adductor. Tail suspension allowed samples to be prepared from both left and right sides of the animal, which was necessary due to the small muscle size in some animals. Five females and three males were used, with 4 animals placed in each temperature environment (randomised for sex and carcass weight) as soon as practical post-harvest (within 2 hrs). The harvest was completed over two nights, with 4 animals sourced
per night. Mean carcass dressed weights (±SD) were 20.4±3.4 and 23.3±3.5 kg for the female and male animals, respectively.

The *M. adductor* (topside), *M. vastus lateralis* (round or knuckle) and *M. sacrocaudalis dorsalis lateralis* (striploin) were excised from the carcasses at 12 hrs post mortem, and vacuum packed for storage at 1°C. The left side muscles were aged for 7 days and then frozen at -20°C, while muscles from the right side were aged for 21 days prior to freezing. Sarcomere length was determined from the 7 day aged frozen muscle samples as described by Perry *et al.*, (2001). Peak force at 7 and 21 days ageing was measured using the modified method of Bouton *et al.*, (1971), (as described by Perry *et al.*, 2001).

The rate of decline in both pH and temperature was modelled for each animal to produce a rate of pH (pHk) and temperature change (tempk), as described by Bruce *et al.*, (2001). Parameters from these functions were used to estimate the temperature at pH 6 for the individual animals.

The effect of rigor temperature on shear force and sarcomere length was analysed using PROC MIXED (SAS), in a model that contained fixed effects for muscle, sex, ageing treatment and collection night, covariates for estimated temperature at pH 6 (as both linear and curvilinear terms) and significant (P<0.10) first order interactions. Animal was included as a random effect.

**Results and Discussion**

A significant quadratic relationship was found between shear force and temperature at pH 6 (p<0.01). There was also an interaction between the quadratic effect of temperature at pH 6 with ageing time (p=0.056), so that the curve for 21 day aged meat was more pronounced than for 7 day aged meat (Fig. 1). This effect occurred across all three muscles sampled. Day of harvest and sex both significantly affected shear force (p<0.01). The harvest on day 1 yielded muscles with higher shear force than those of day 2, and the muscles from females had higher shear force values than those from males. However there was no interaction between these factors and pHk in their effect on shear force, so that all results are presented adjusted for these terms. Shear force also differed between muscles (P<0.01). The values for the *M. vastus lateralis* were higher than for the *M. adductor* and *M. sacrocaudalis dorsalis lateralis*, which were not significantly different from each other.

Previous studies into the effects of accelerated and delayed chilling regimes upon ageing potential of muscle have shown that hotter conditions at rigor can lead to reduced protease activity during the subsequent chilled storage (Pike *et al.*, 1993; Hwang and Thompson, 2001), possibly through the denaturation of proteolytic enzymes at the higher temperatures. Thus the ageing potential of the meat is compromised. Hwang and Thompson (2001) found the optimum temperature for minimum shear force to be around 27°C at pH 6, which is in agreement with the results from this study for kangaroo meat.

Sarcomere length of muscle aged for 7 days exhibited a significant curvilinear relationship with temperature at pH 6 (p<0.05, data not shown). This pattern was similar to that for shear force, with sarcomere length decreasing as temperature diverged higher or lower from 24°C at the standard pH of 6.0. Colder muscle temperatures at rigor can shorten sarcomeres to the extent to which proteases may not be able to gain access to the myofibres (Whipple *et al.*, 1990).
As shear force is correlated with the consumer perception of cooked meat tenderness (Perry et al., 1998), these results suggest that consumers should find 21 day aged kangaroo meat, chilled under a regime which results in a muscle temperature of around 24°C at pH 6, to be more acceptable in terms of tenderness than meat aged for 7 or 21 days chilled under sub-optimal conditions. The relatively flat response of the 7 day ageing curve relative to temperature at pH 6 (Figure 16) indicated that the chilling regimes of kangaroo carcasses had less impact on tenderness when the product was not destined to be aged more than 7 days. In addition to the ageing effect, the potential detrimental effects of heat shortening must be considered, with warmer temperatures than optimal at pH 6 leading to increased drip losses and decreased shelf life of product (Hwang et al., 1999).

Kangaroos are generally harvested from early evening until near sunrise. The proximity of field chillers to harvesting sites does not always allow for carcasses to be placed quickly under refrigeration. Thus achieving a muscle temperature of 24°C at pH 6 is not easy under field conditions. In addition, the vast differences in ambient temperatures that occur between seasons, and even within a particular night’s harvest, make the attainment of optimum muscle temperatures at rigor difficult.

**Conclusions**

Kangaroo meat shows potential for value adding through the extended chilled ageing of boned, vacuum packaged product. Significant decreases in peak shear force were found in 21 day aged product as compared with ageing for 7 days when muscle temperatures were at ~24°C upon commencement of rigor (pH 6). A curvilinear response of peak force with the temperature at pH 6 indicated that ageing potential was adversely affected when muscles entered rigor at temperatures higher or lower than the optimum of 24°C.

![Figure 16. The effect of ageing and rate of chilling/glycolysis upon shear force after adjustment for muscle, day of harvest and sex (vertical bars are ± standard error).](image)
The relationship between muscle glycogen stores and ultimate pH in commercially harvested kangaroos

Background

Following slaughter, the pH of meat declines due to the continued anaerobic catabolism of muscle glycogen to form lactic acid. Assuming that adequate levels of muscle glycogen are present at the time of slaughter, this process continues until the changing cellular environment inhibits the activity of glycolytic enzymes, usually occurring at an ultimate pH (pH\textsubscript{u}) of around 5.5. This will occur in healthy, well-fed animals, subjected to minimal stress. In beef cattle a depletion of glycogen reserves pre-slaughter will result in limited lactic acid production post mortem, and subsequently high pH\textsubscript{u} (Howard and Lawrie, 1956). This is associated with dark coloured beef and a reduced shelf-life of the product, which in turn leads to consumer resistance to purchasing the product (Shorthose, 1989).

Kangaroos are field harvested at night while grazing in their natural feeding habitat, with the animals being dazed with a strong spotlight before being shot. Therefore minimal stress is imposed prior to slaughter, presumably resulting in adequate glycogen stores to ensure a low and desirable pH\textsubscript{u}.

Objectives

The objective of this study was to establish the relationship between muscle glycogen concentration at the time of slaughter and pH\textsubscript{u} of various muscles of commercial importance from the kangaroo carcass.

Methods

Sample acquisition and laboratory analyses

Kangaroos were field harvested over 2 nights in the Hallett district of South Australia (February, 2001) by an accredited commercial kangaroo harvester. Four muscles were collected: \textit{M. adductor} (n=28), \textit{M. biceps femoris} (n=26), \textit{M. vastus lateralis} (n=50), and \textit{M. longissimus dorsi} (n=10), sourced from male and female Western Grey (\textit{Macropus fuliginosus}) or Red kangaroos (\textit{Macropus rufus}). The dressed weight of the carcasses ranged from 12 to 45kg, with a mean (±SD) weight of 22±9kg. Carcasses were stored at 1°C for 9 days before boning, and excised muscles were frozen at −20°C for later analysis. For glycogen determination 0.25g of frozen muscle (free of epimysium and adipose tissue) was homogenised with 30mM HCL (1:10). This homogenate was then assayed for glycogen and lactate concentrations according to the enzymatic methods of Kunst \textit{et al} (1983) and Marbach and Weil (1967). Glycogen concentration at slaughter was represented by the sum of residual glycogen plus free glucose plus lactate concentrations determined in the post-rigor muscle. Muscle pH\textsubscript{u} was measured using a combination electrode (TPS Brisbane, Australia).
**Statistical analyses**

Data were analysed using the PROC MIXED (SAS). The model for total glycogen included muscle, sex and species as fixed effects, and the interaction of muscle with sex and species. The pH<sub>u</sub> model included the same fixed effects, plus total glycogen and its interactions with muscle and sex. Animal was included as a random effect in both models.

**Results and discussion**

**Glycogen levels in kangaroo muscle**

The mean (±SD) glycogen concentration at slaughter for muscles across species and sex were 86.1±17.73 (M. adductor), 88.0±18.84 (M. biceps femoris), 63.6±13.41 (M. vastus lateralis) and 90.9±22.04µmol/g (M. longissimus dorsi). Shorthose (1980) and McVeigh and Tarrant (1982) reported that in healthy, well-fed domestic animals, slaughtered without undue stress, the muscle glycogen concentrations range between 60-100µmol/g wet weight. The majority of kangaroo muscles were seen to lie within this range (Figure 17).

There was a significant interaction of muscle with both species (p<0.01) and sex (p<0.01). The predicted means for each muscle within species and sex are presented in Table 6. Overall, muscles from female kangaroos contained almost 19µmol/g less glycogen than muscles from male kangaroos (p<0.01). The greatest effect was observed in the M. longissimus dorsi with over 30µmol/g less glycogen in females than in males. In this study all females had at least one suckling offspring, which may have limited substrate storage at the muscular depot due to the competing demands of pregnancy and lactation for this substrate. Alternatively, males may naturally have higher glycogen concentrations to meet the anaerobic demands of aggressive interactions between other males.

Estimated glycogen concentrations at slaughter were influenced by species, with the Western Grey exhibiting higher levels. The impact of environmental factors, such as nutrition, in which the two species were harvested may have contributed to this difference.

**The pH<sub>u</sub> of kangaroo muscle**

The mean (±SD) pH<sub>u</sub> values for muscles across species and sex were 5.78±0.09 (M. adductor), 5.80±0.10 (M. biceps femoris), 5.88±0.12 (M. vastus lateralis) and 5.77±0.09 (M. longissimus dorsi). These values are high compared to muscle from well-fed domestic ruminant animals maintained under conditions of adequate nutrition and minimal stress prior to slaughter. Under these conditions values for pH<sub>u</sub> for beef and sheep meats are expected to be around 5.5. Although the international standard for “dark-cutting” is around a pH<sub>u</sub> value of 6.0, values of 5.7 in yearling cattle are considered to be dark-cutting both from an industry and consumer perspective (Shorthose, 1989). The Meat Standards Australia grading scheme has a cut off at 5.7 for beef (Ferguson et al., 1999). Using these standards, there is little doubt that the values measured within this study fall within the range generally considered as being “dark-cutters”.

**The relationship between muscle glycogen and pH<sub>u</sub>**

Tarrant (1989) reported that below a critical glycogen level (at slaughter) of 57µmol/g the pH<sub>u</sub> of meat will be higher, resulting in reduced carcass quality characteristics such as dark
coloured meat and poor keeping quality. Shorthose (1980) reported this critical threshold level as 40µmol/g. Above these glycogen concentrations, pH_u is independent of muscle glycogen concentration and generally should fall to 5.5. This study showed that kangaroo glycogen levels at slaughter were predominantly above these critical concentrations described in beef, yet the pH_u values were high, and equivalent to values of “dark-cutters” in beef (Figure 17). The relationship between total glycogen and pHu differed between muscles and sexes (p<0.01). Irrespective, the results indicate that there seems to have been adequate glycogen stores at slaughter to ensure a low pH_u in kangaroo muscle. Furthermore, residual glycogen values ranged from 6.5 – 91.8µmol/g (pooled for muscle, sex and species), with a mean (±SD) of 49.5±18.3µmol/g, indicating that muscle glycogen levels at slaughter were not exhausted during post-mortem glycolysis. Thus cessation of pH fall post-mortem occurred under conditions when glycogen was not limiting. Various factors have been suggested to influence pH_u when glycogen is not limiting. These include initial metabolite concentrations of creatine phosphate, ATP, and lactate, as well as the buffering capacity of muscle (Vetharaniam and Daly, 2000), and the availability of adenosine monophosphate (Scopes, 1971). Given the species differences, in particular the anaerobic exercise involved in the locomotion of the kangaroo compared to the less strenuous, aerobic, nature of locomotion in sheep and cattle, it is possible that buffering capacities of the muscles would differ. Figure 1 supports this notion, with the glycogen concentrations in excess of 60µmol/g demonstrating little relationship with pH_u.

Conclusions
Muscle glycogen concentrations differed significantly between species and sex within the carcass of the kangaroo. In general the glycogen concentrations were above 60µmol/g, which should have been adequate to achieve an pH_u of 5.5, as seen in sheep and cattle. However, pH_u rarely fell below 5.7, suggesting a difference in the regulation of glycolytic pathways in kangaroo muscle when compared with the close negative correlation between glycogen content and the ultimate pH of ovine and bovine muscle.
Table 6 Predicted means for glycogen concentrations (μmol/g wet weight ± std. err.) in different muscles from male and female Red (Macropus rufus) and Western Grey (Macropus fuliginosus) kangaroos

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Western Grey</th>
<th>Red</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. adductor</td>
<td>88.35 ± 3.00</td>
<td>81.65 ± 2.99</td>
<td>74.28 ± 3.13</td>
<td>95.72 ± 2.85</td>
</tr>
<tr>
<td>M. biceps femoris</td>
<td>88.91 ± 3.00</td>
<td>82.39 ± 3.03</td>
<td>78.34 ± 3.18</td>
<td>92.97 ± 2.85</td>
</tr>
<tr>
<td>M. longissimus dorsi</td>
<td>89.88 ± 3.16</td>
<td>74.10 ± 3.09</td>
<td>65.72 ± 3.38</td>
<td>98.26 ± 2.91</td>
</tr>
<tr>
<td>M. vastus lateralis</td>
<td>65.56 ± 3.06</td>
<td>61.27 ± 2.95</td>
<td>60.39 ± 3.12</td>
<td>66.44 ± 2.89</td>
</tr>
</tbody>
</table>

Figure 17. The relationship of total glycogen and resulting ultimate pH in the M. vastus lateralis of the kangaroo.

Since the nexus between glycogen content and ultimate pH was not apparent in kangaroo muscles it was thought that this may be related to differences in muscle fibre metabolism. For this reason the following study was undertaken to define the muscle fibre types in a range of kangaroo muscles and compare the distribution with that found in bovine muscles.

Myofibre types in eight skeletal muscles from the eastern grey kangaroo (*Macropus giganteus*)

Summary

A selection of muscles from male Eastern Grey kangaroos was assessed for myofibre type. Myofibres were classified as slow-oxidative (type I), fast-oxidative-glycolytic (type IIA), fast-glycolytic (type IIB) and intermediate types (IIC and IIAB) based on staining with monoclonal antibodies specific to myosin heavy chain isoforms. Classification using these antibodies was validated with myofibrillar adenosine triphosphatase, nicotinamide adenine dehydrogenase, and α-glycerophosphate dehydrogenase staining. The most abundant myofibre type in the muscles studied was type IIB-fast-glycolytic, with the exception of *M. psoas minor*. Staining characteristics for myofibres were similar to other mammalian species apart from type IIA, which did not react with the type II (fast) antibody used in this study.
Introduction

Skeletal muscle is an extremely heterogeneous tissue composed of a variety of fast and slow fibre types and subtypes. Muscle fibres can be classified according to their metabolic, contractile and colour properties and there exists an array of classification schemes and nomenclature. According to Pette and Staron (2000), the most informative methods to delineate muscle fibre types are based on specific myosin profiles, especially the MHC isoform complement. The classification of kangaroo muscle has been previously carried out by Zhong et al. (2001) and Dennington and Baldwin (1988) using mAbs and histochemical techniques, respectively. Collectively, these papers classified the *M. tibialis cranialis* and the major extensor muscles of the macropod tibio-tarsal articulation.

In the present study a selection of antibodies against MHC isoforms typically used to study muscle in livestock species was chosen to facilitate the classification of kangaroo muscle fibres as type I, IIC, IIA, IIAB and IIB. These findings were validated using mATPase, NADH, and GPD staining. Six hindquarter muscles (*M. gluteus medius, M. vastus lateralis, M. biceps femoris, M. adductor, M. semitendinosus, M. semimembranosus*), one tail muscle (*M. sacrocaudalis dorsalis lateralis*), and one supportive lumbar muscle (*M. psoas minor*), were examined. These muscles were studied because of their potential economic importance for meat production.

Materials and methods

**Animals, muscles and sample preparations**

Five male Eastern Grey kangaroos were field harvested between 23:00 and 02:00h over two nights using an accredited commercial kangaroo harvester. The dressed weight (bodyweight minus the contents of the gastrointestinal tract) ranged from 16 to 30kg, with a mean (±SD) weight of 23±5kg. Eight muscles were sampled from each of the 5 animals approximately 7 hrs post-mortem. For each muscle, a specific sampling site was chosen (Table 7). Muscle blocks (1 to 2cm³) were trimmed of epimysium and adipose tissue and mounted on cork tiles using 5% gum tragacanth (w/v in ddH₂O). They were immediately frozen in isopentane, which was frozen in liquid nitrogen, and then stored at –70°C. Transverse 10µm serial sections were cut from the frozen muscle blocks using a cryostat microtome at -25°C, and mounted on glass slides. Sections were air dried at room temperature and stored at –20°C.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Site of sampling within the muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. psoas minor</em> (PM)</td>
<td>Central portion</td>
</tr>
<tr>
<td><em>M. sacrocaudalis dorsalis lateralis</em> (SAC)</td>
<td>Dorsal portion from the sacral part of the muscle</td>
</tr>
<tr>
<td><em>M. semitendinosus</em> (ST)</td>
<td>Proximal portion</td>
</tr>
<tr>
<td><em>M. vastus lateralis</em> (VL)</td>
<td>Central portion</td>
</tr>
<tr>
<td><em>M. biceps femoris</em> (BF)</td>
<td>Proximal portion</td>
</tr>
<tr>
<td><em>M. adductor</em> (AD)</td>
<td>Central portion</td>
</tr>
<tr>
<td><em>M. gluteus medius</em> (GM)</td>
<td>Cranio-distal portion</td>
</tr>
<tr>
<td><em>M. semimembranosus</em> (SM)</td>
<td>Central portion</td>
</tr>
</tbody>
</table>
**Immunohistochemistry**

Immunohistochemical analysis was carried out on serial sections using mAbs against MHC (Picard et al., 1998). Anti-slow MHC mouse monoclonal antibody (clone MHCs; Novacastra, Newcastle-upon-Tyne) was used to identify type I fibres; MY-32 anti-fast MHC antibody (Sigma Chemical Co. St. Louis, Missouri) for type II fibres; and mAb S5 8H2 (gift from Dr Brigitte Picard, INRA, Thiex, France) for the identification of type I and IIB myofibres. Antibodies were detected using a labeled-strept-avidin-biotin (LAB-SA) system with the substrate-chromagen DAB (Zymed Laboratories, South San Francisco, California). Individual myofibres were compared across serial sections and classified as type I, IIC, IIA, IIAB or IIB (Picard et al., 1998). Table 8 summarises the classification system used based on staining characteristics for kangaroo muscle.

Table 8 Differential staining using three monoclonal antibodies. ++ indicates strong staining, + indicates intermediate staining, and - indicates no staining.

<table>
<thead>
<tr>
<th>Type</th>
<th>I</th>
<th>IIC</th>
<th>IIA</th>
<th>IIAB</th>
<th>IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-I</td>
<td>MHCs</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-II</td>
<td>MY-32</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Anti-I+IIB</td>
<td>S5 8H2</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

*No staining, but for sheep (Greenwood et al., 2000) and cattle (Greenwood, unpublished results) a strong reaction occurs for type IIA MHC.*

**Histochemistry**

Histochemical analyses were undertaken to validate MHC immunostaining classification. The contractile properties of myofibres were determined by staining for mATPase activity (Padykula and Herman, 1955; Guth and Samaha, 1970). This activity was revealed at pH 9.4 following acid pre-incubations (range pH 4.1 to 4.8 at increments of 0.1 pH units) for five minutes, or alkaline pre-incubation (range pH 10.2 to 10.4) for 10 minutes. Myofibres that showed mATPase activity at an optimal acidic pH value of 4.3 were classified as slow-twitch fibres, those reacting strongly at an optimal alkaline pH value of 10.3 or 10.4 were classified as fast-twitch fibres, and fibres reacting positively following acid or alkali pre-incubation as type IIC. The metabolic properties of myofibres were determined using staining characteristics for NADH (Novikoff et al. 1961) and GPD (Wattenburg and Leong, 1960).

**Image Analysis**

Muscle sections were viewed with a Leica compound microscope (DMLB, Germany) using bright field microscopy. Images were captured using a Spot RT colour camera (Diagnostic Instruments, Michigan) and analysed using Spot RT Software v3.1 (Diagnostic Instruments, Michigan). The prevalence of each fibre type as a percentage of myofibres was measured manually from the serial sections using two randomly selected regions, each containing a minimum of 100 cells.
**Statistical analysis**

The statistical package SAS v8.2 (SAS Institute) was utilised. The differences between muscles within fibre type were analysed using a mixed model procedure with muscle as the fixed effect and animal as a random effect. These differences were then analysed using the differences of least squares means.

**Results and discussion**

*Myofibre staining characteristics*

*Figure 18* Myofibre staining characteristics
Type I myofibres stained positively for the anti-type I MHC and anti-types I+IIB MHC. Type IIC stained positively for all three mAbs. Type IIB stained positively for the anti-type II and type I+IIB MHC, as did type IIAB myofibres but with reduced staining intensity. Type IIA myofibres were negative for all 3 mAbs and required histochemical staining profiles to prescribe functionality to this fibre type. This class of myofibre showed weak GPD staining and a strong reaction to the NADH stain, which is indicative of strong oxidative potential. These fibres also showed weak reactivity following acidic pre-incubation but strong reactivity following basic pre-incubation, thereby characterising them as fast-twitch myofibres. Hence, these myofibres were classified as type IIA. The finding of non-specificity of the fast MHC antibody suggests a unique type IIA isoform in macropod muscle compared to other mammalian species including livestock. This finding is not consistent, however, with those of Zhong et al. (2001), who showed that kangaroo limb muscles express the same fast MHC sub-types as the cat, baboon, and rodent species. Irrespective, the findings of the present study highlight the importance of validating immunostaining with histochemical techniques.

**Myofibre Type Distribution**

Table 9 shows the fibre type distribution for each muscle. The eight muscles studied contained predominantly type IIB fibres followed by type IIA; the exception was the *M. psoas minor*. This indicates that kangaroos display considerable potential for both aerobic and anaerobic ATP production. The *M. psoas minor* displayed a significantly higher proportion of type I fibres (p<0.01) compared to the other muscles analysed in this study. It therefore has a higher oxidative potential, consistent with this muscle being a supportive/postural muscle in the kangaroo, as muscles involved in posture are more oxidative than those involved in movement (Totland and Kryvi, 1991).
Table 9 Muscle fibre type percentages in 8 kangaroo muscles. Values are means ± SD for 5 animals.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>I (SO)</th>
<th>IIC (FOG/SO)</th>
<th>IIA (FOG)</th>
<th>IAB (FOG/FG)</th>
<th>IIB (FG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM</td>
<td>31.9 ± 3.2</td>
<td>__</td>
<td>30.2 ± 7.5</td>
<td>3.0 ± 3.4</td>
<td>34.9 ± 3.6</td>
</tr>
<tr>
<td>SAC</td>
<td>8.1 ± 2.0</td>
<td>0.1 ± 0.1</td>
<td>35.3 ± 14.2</td>
<td>10.7 ± 6.8</td>
<td>45.9 ± 14.0</td>
</tr>
<tr>
<td>ST</td>
<td>7.2 ± 1.1</td>
<td>__</td>
<td>28.0 ± 3.3</td>
<td>5.3 ± 3.1</td>
<td>59.5 ± 4.2</td>
</tr>
<tr>
<td>VL</td>
<td>6.1 ± 2.4</td>
<td>0.3 ± 0.6</td>
<td>27.0 ± 9.5</td>
<td>10.6 ± 6.2</td>
<td>56.1 ± 13.4</td>
</tr>
<tr>
<td>BF</td>
<td>4.4 ± 2.6</td>
<td>0.5 ± 1.2</td>
<td>34.8 ± 13.2</td>
<td>11.4 ± 4.5</td>
<td>48.9 ± 12.9</td>
</tr>
<tr>
<td>AD</td>
<td>2.8 ± 1.2</td>
<td>__</td>
<td>32.6 ± 10.0</td>
<td>12.4 ± 3.0</td>
<td>52.3 ± 12.3</td>
</tr>
<tr>
<td>GM</td>
<td>2.1 ± 0.8</td>
<td>0.1 ± 0.2</td>
<td>24.5 ± 7.2</td>
<td>10.8 ± 5.0</td>
<td>62.5 ± 11.0</td>
</tr>
<tr>
<td>SM</td>
<td>1.5 ± 1.2</td>
<td>0.1 ± 0.2</td>
<td>32.0 ± 10.1</td>
<td>7.5 ± 3.9</td>
<td>59.0 ± 11.8</td>
</tr>
</tbody>
</table>

1See Table 7 for details of muscles studied; 2SO=Slow-oxidative, FOG=Fast-oxidative-glycolytic, and FG=Fast-glycolytic

Conclusion

The results from this study add to the knowledge base on muscle metabolism in macropods by providing a detailed classification of kangaroo muscle fibre types in an array of muscles of potential economic importance. This information is important in assisting with our understanding of factors that impact on post-mortem metabolism, and hence, eating quality of kangaroo muscle. This study also highlights the importance of validating classification techniques when determining myofibre characteristics using MHC antibodies.

Since the impact of ageing will be influenced by the connective tissue content of muscles, we have also investigated their collagen content.

Kangaroo total muscle collagen as influenced by carcass weight and muscle location

Background

The collagen within muscle forms the structural framework around which muscular structure and function is based, allowing for the translation of myofibrillar contraction into movement and the maintenance of muscle conformation. Total intramuscular collagen measurement can be a valuable indicator of meat quality. This paper illustrates the effects of carcass weight and muscle within the carcass on total collagen content of kangaroo meat.

The animals comprised 50 male Eastern Grey (Macropus giganteus) kangaroos, commercially harvested over 10 nights from north-west NSW. Animals were sampled at the extremes in body size; to produce low (mean wt 13.3kg, range 10-15) or high (mean wt 40.8, range 36-48). Animals meeting both classes were taken randomly on each night to reduce any harvest night bias. The muscles investigated were the M. adductor (topside) M. biceps femoris (silverside) and M. longissimus dorsi (loin fillet).

Carcasses were suspended by the tail. Ambient conditions were mild to cool (night temperature range 5-15°C). Carcasses were kept at ambient for a minimum of 4 hours before being placed in a 1°C chiller. At 24 hours post mortem, the three muscles were excised from both sides of the carcass. The muscles were trimmed of the epimysium and multiple sub-samples were collected from each sample to be bulked, homogenised and subsequently stored...
at -20°C. Total intramuscular collagen was determined indirectly by assaying for hydroxyproline.

Table 10. Effect of muscle by dressed weight (DW) on total muscle collagen (% dried muscle (s.e. mean))

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Low DW</th>
<th>High DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. adductor</td>
<td>1.87 (0.13)</td>
<td>3.09 (0.13)</td>
</tr>
<tr>
<td>M. biceps femoris</td>
<td>1.94 (0.13)</td>
<td>2.83 (0.13)</td>
</tr>
<tr>
<td>M. l. dorsi (Loin)</td>
<td>1.33 (0.13)</td>
<td>1.52 (0.13)</td>
</tr>
<tr>
<td>Leg muscles pooled</td>
<td>1.90 (0.10)</td>
<td>2.96 (0.07)</td>
</tr>
</tbody>
</table>

*a,b* Matching superscripts (within row) depict no significant difference between means (p<0.01)

* Matching subscripts (within column, contrasting DW groups) depict no significant difference between means (p<0.01)

The results show that as the animals become heavier (which also would have been older), total collagen content increases. There was an interaction between muscle and weight group (P<0.05) whereby the rate of collagen increase was greater in the leg compared with the loin muscle. The physical strength of the connective tissue matrix within live muscle facilitates efficient maintenance of integrity and function. It is hypothesised that the greater increase in collagen in leg muscles was a response to the need for greater structural resilience in locomotory muscles of the leg, particularly during hopping. In contrast, the loin, which is a postural muscle, no significant relationship between collagen content and live weight was found, despite the increased physical stress of locomotion as animals become heavier.

These results have important meat quality and industry implications. Increased tenderness of meat cuts has commonly been associated with lower collagen (or connective tissue) content of muscles irrespective of species. These results suggest that leg cuts derived from lighter, younger male animals should be of higher eating quality, where as the quality of the loin fillet should be independent of age.

Total intramuscular collagen provides a good indication of the impact that collagen will have upon ultimate muscle tenderness. Further investigations of the chemical cross-linkages and therefore solubility and fibre morphology, currently in progress, will provide further insight into the contribution of this important meat component to the quality of kangaroo meat.

Any manipulations of the carcass that can be readily adopted by industry to improve meat quality will be very attractive to processors. The following study resulted from an astute observation of the method for hanging carcasses on the back of harvesting field rigs.
Improving the quality of kangaroo meat: effects of carcass suspension by one leg.

Summary

Field harvested kangaroo was investigated for differences in tenderness and sarcomere length between sides of animals suspended from one leg from harvest until processing. Significant differences (p<0.05) were found in peak force and sarcomere length measurements for hind limb Mm. biceps femoris (silverside), caudofemoralis cranial head (a rump muscle), and adductor (topside). The free leg of the carcass had longer sarcomere lengths and lower peak force values than the corresponding muscles of the suspended leg, indicating improved eating qualities. This research shows that tenderstretching methods can improve the quality of the major hind limb muscles of the kangaroo.

Introduction

Research in beef, lamb and pork has shown that suspension methods that place tension on (or stretch) the muscles pre-rigor can have a substantial impact on tenderness of the major leg muscles (Hostetler et al., 1970; Bouton et al., 1973; Davey and Gilbert, 1973; Dransfield et al., 1991). Current industry practices for field-harvested kangaroo include a variety of suspension methods, including one leg, pelvic and tail suspension, which could potentially alter the tension on muscles pre-rigor and therefore have an impact on meat quality.

This paper reports the results of a preliminary investigation on the effect of a suspension method on meat quality in field-harvested kangaroo.

Materials and Methods

Carcass acquisition and experimental design

Ten Eastern Grey Kangaroos (Macropus giganteus) were field harvested at night by an accredited field processor from the Moree region, NSW. These included 3 does and 7 bucks, with average dressed carcass weights of 18kg (17-19kg range) and 27kg (23-33kg range), respectively. Carcasses remained at ambient temperatures (range 8°C to 14°C) for approximately 5 hours before refrigeration in a field chiller (range -3°C to +3°C).

All carcasses were suspended by one leg, five by the left leg and five by the right, from harvest until processing (a total time of 60 hours). After evisceration carcasses are usually placed on a pelvic spike within the field-processor’s rig for transport to the chiller, but for experimental purposes, the carcasses remained suspended by one leg.

Sample preparation

Muscles sampled in this experiment included the hind limb Mm. vastus lateralis (knuckle/round), biceps femoris (silverside), caudofemoralis cranial head (part of the rump), and adductor (topside). Two back muscles were also sampled, including the Mm. longissimus (Loin fillet) and sacrocaudalis dorsalis lateralis (Long fillet).

These muscles were dissected 60 hours after harvest, and the epimysial connective tissue removed. The individual samples were then vacuum packed, labelled and stored at –20°C until required for analyses.
**Cooking procedure**

Frozen muscle samples were thawed at 4°C for 24 hours prior to analysis. They were then cut into 100g blocks, except for 8 *vastus lateralis* and 15 *biceps femoris* blocks of 250g each, placed in individual polyethylene bags, and cooked for 35 minutes (100g blocks) or 60 minutes (250g blocks) by total immersion in a water-bath (pre-heated to 70°C), according to the method of Bouton *et al.* (1973). Differences in cook block size were due to the initial differences in muscle size. Samples were then immersed in running tap water for 30 minutes, after which they were stored in their respective bags (after the cook juices were poured out) in a 1°C chiller overnight before analysis of peak force.

**Measurement of peak force**

Muscles strips were prepared as described by Perry *et al.*, 2001. The objective measurement of peak force was performed with a Lloyd Instruments™ LRX Materials Testing Machine, fitted with a 500N load cell (Lloyd Instruments Ltd, Hampshire, UK). Six measurements were made from each muscle sample (usually from six individual sub-samples), with the average peak force value (kg force) calculated for each sample.

**Measurement of sarcomere length**

Samples used for measuring sarcomere length were taken from the whole frozen muscle samples. Sarcomere lengths were determined using the Helium-Neon laser diffraction technique (utilising a diode laser at 635nm wavelength) on unfixed, frozen muscle as described by Bouton *et al.*, (1973). Five readings were taken per muscle sample from replicate shavings. Each was converted to a sarcomere length (µm) with an average of these results reported.

**Statistical methods**

Peak force and sarcomere length results were analysed using a mixed model procedure in SAS (SAS, 1997). Models contained fixed effects for muscle, suspension method, side of the animal, sex and carcass weight, and all first order interactions, with animal as a random term. Non-significant interactions were sequentially deleted from the model (P<0.05). Due to the different block size and cooking times used for the *Mm. vastus lateralis* and *biceps femoris*, a term for block size nested within muscle was included in the analysis, but was not significant (P>0.05) and was excluded.
**Results**

For peak force and sarcomere length, the effects of muscle by suspension method, muscle and suspension method were significant (p<0.05, Table 11).

Suspension method affected both sarcomere length and peak force, with significantly lower peak force values for the *Mm. Adductor, biceps femoris* (p<0.05) and *M. caudofemoralis* cranial head (p<0.01) of the free leg. Sarcomere length was also significantly (p<0.05) longer in these muscles of the free leg. There was no significant difference in sarcomere length or peak force between legs for the *Mm. vastus lateralis, longissimus* and *sacrocaudalis dorsalis lateralis*. As percentage differences, the *Mm. adductor, biceps femoris* and *caudofemoralis* cranial head were 34%, 39% and 45% more tender, respectively.

| Table 11. Predicted means for the effect of carcass suspension method upon peak force (kg) and sarcomere length (microns) measurements in leg (Mm. adductor and biceps femoris, M. caudofemoralis cranial head, and vastus lateralis) and back muscles (Mm. longissimus and sacrocaudalis dorsalis lateralis) after adjustment for side, dressed weight and a random animal effectootnote{Means are adjusted for random animal effect. *=5% confidence, **=1% confidence, n.s. = no significant difference at 5% level, based on the Student’s *t* distribution.} |
|---|---|---|---|---|---|---|---|---|---|---|
| Muscle | Mean Peak Force (kg) | Mean Sarcomere Length (µm) | Suspended | Free | std. err | sig. | Suspended | Free | std. err | sig |
| Adductor (topside) | 5.43 | 3.61 | 0.287 | * | 1.73 | 2.53 | 0.05 | * |
| B. femoris (silverside) | 6.44 | 3.96 | 0.287 | * | 1.72 | 2.51 | 0.05 | * |
| Caudofemoralis cranial head (rump) | 6.36 | 3.50 | 0.287 | ** | 1.74 | 2.43 | 0.06 | * |
| V. lateralis (knuckle/round) | 6.06 | 5.37 | 0.287 | n.s. | 1.88 | 2.17 | 0.06 | n.s. |
| Longissimus (Loin fillet) | 4.35 | 5.63 | 0.287 | n.s. | 1.90 | 1.77 | 0.05 | n.s. |
| Sacrocaudalis dorsalis ventralis (Long fillet) | 7.06 | 7.36 | 0.287 | n.s. | 1.79 | 1.73 | 0.06 | n.s. |
Fig 19 shows peak force values as a function of sarcomere length for Mm. Adductor, Biceps femoris and Caudofemoralis cranial head. There is a clear division in the spread of data, at approximately 1.9µm sarcomere length. Samples with sarcomere length greater than 1.9µm, generally had lower peak force values with lower variation. Those samples with a sarcomere length less than 1.9µm generally showed higher peak force values and greater variation.

Discussion

This experiment has indicated clearly that the suspension of a kangaroo carcass by a single leg, until rigor mortis is complete, can cause significant side-to-side variation in meat tenderness. This was due to more tension being placed on the major leg muscles (Mm. adductor, caudofemoralis cranial head, biceps femoris) of the free leg as it went into rigor, resulting in increased sarcomere lengths and lower peak force values.

The muscles that were affected and showed significant variation in tenderness included the Mm. adductor and biceps femoris (p < 0.05 for peak force and sarcomere length), and M. caudofemoralis cranial head (p < 0.01 for peak force and p<0.05 for sarcomere length). Such muscle specific effects may be explained by the anatomical musculature of the kangaroo, where the relative positions of the hind limb muscles and the effect of different suspension configurations becomes important. For instance, the Mm. adductor, biceps femoris and caudofemoralis cranial head are located on the caudal side of the hind limb, relative to the M. vastus lateralis. In the case of a free hanging leg, these more caudally positioned leg muscles are under considerable tension and are stretched, whereas the M. vastus lateralis was not affected. This resulted in an increase in sarcomere length and lower peak force values, therefore minimising post-slaughter muscle shortening associated with rigor post-slaughter. In the case of a suspended leg, the Mm. adductor, biceps femoris and caudofemoralis cranial head were not placed under tension and were therefore allowed to shorten, as reflected by the higher peak force values and shorter sarcomere lengths. Again, the M. vastus lateralis was not affected. For the back Mm. longissimus and sacrocaudalis dorsalis lateralis there was no significant variation in tenderness between sides.
Similar results have been found in tenderstretching (pelvic suspension) experiments in domestic species (Bouton et al., 1973; Dransfield et al., 1991; Hostetler et al., 1970), where carcasses were suspended in such a way to provide maximum tension on particular muscles to prevent them from shortening.

Peak force is commonly used in meat science as an objective measure of meat tenderness. In beef, values ranging from about 6.0 upwards are classed as very tough, 4.3-6.0 as slightly tough, 3.9-4.2 as medium, 3.6 – 3.9 as slightly tender, and below this to be very tender (Ramsbottom and Strandine, 1948 in Judge et al., 1989; Perry et al., 2001). Using this arbitrary ranking of tenderness for the results of this experiment, suspension by one leg can have a marked effect upon the eating quality of meat from kangaroo. For instance, the *M. caudofemoralis* cranial head from the free hanging leg had a peak force value of 3.50 (very tender) compared to 6.36 (very tough) in the suspended leg.

A clear division in the data is seen in Figure 19, which corresponds with a sarcomere length of about 1.9µm. This shows that samples having sarcomere lengths below 1.9µm attain lower tenderness scores. Above 1.9µm, tenderness was not influenced by sarcomere length, so the samples in this range had correspondingly lower and less variable peak forces. These muscles were not shortened enough for sarcomere length to influence tenderness.

The shortening that did occur in the suspended leg was not due to cold shortening as the muscle temperature was above 30°C when rigor commenced. Heat shortening is the most probable cause of this shortening in this case, which was effectively avoided in the *Mm. adductor, biceps femoris* and *caudofemoralis* cranial head of the free leg by stretching. Further study into this shortening effect needs to be undertaken.

Kangaroo carcass suspension from a single leg is one method used by the industry. Another method involves suspending the carcass by the tail in the chiller, resulting in both legs hanging freely. This method would favour the minimisation of variation in meat quality between sides of the carcass and would potentially have a positive ‘tenderstretch’ effect on most leg muscles. Further research into suspension methods that are practical for the industry need to be carried out in order to recommend the best method of carcass suspension that will minimise problems of muscle shortening. The magnitude and transportability of these results needs to be tested under a variety of processing temperatures and be confirmed through consumer based taste panels.
5. Concise recommendations for the kangaroo industry based on findings of the ‘kangaroo meat quality’ project

**Standardisation of tenderstretching practices**

Our observation of the use of a single leg for carcass suspension by field harvesters has lead to the finding that this practice results in increases in toughness of up to 50% in commercially important hind limb muscles in the suspended leg relative to the free limb. This practice should therefore be replaced with tail or pelvic-spike suspension, to allow both legs to hang freely; thus increasing tension within these muscles in both legs as the process of rigor resolves post-slaughter.

**Product differentiation based on species, sex and dressed weight**

Irrespective of the species of kangaroo harvested, superior quality meat is obtained from smaller carcasses which in turn equates with the age of the animal. Sex also plays a role here, with females being smaller than males with age. The exception to this rule could be the use of small drought affected animals in years like 2004 for commercial production, which may be the reality in some regions used for commercial production at present. We did not use such animals for the present studies. Those effects described in our studies, that kangaroo species places upon various meat qualities, provides further opportunity for product differentiation.

**Characterisation of kangaroo meat flavour components: improving the acceptability of kangaroo meat products in both domestic and export markets.**

Since flavour is the most significant attribute influencing palatability and therefore consumer acceptance of kangaroo meat products, a greater emphasis should be placed on identifying these unique flavour components through research. This will lead to production strategies focusing on the directed utilisation, or differentiation, of product displaying the most desirable sensory characteristics. This may be related to species, sex or age of animal, or even to the nature of the constituents of their diet. Thus, meat products of known flavour profile may be directed toward different markets, much in the same way as beef is currently, based on its quality characteristics.

**Extending the time of chilling and ageing of boned kangaroo meat to promote tenderness**

There is much potential for value adding through vacuum package storage of kangaroo meat through to at least 21 days. Our research has shown that very substantial increases in tenderness can be achieved through such methodology. Associated potential problems with shelf-life may be alleviated through further development of successful Modified Atmosphere Packaging (MAP) methodologies, with associated minimisation the risk of microbial contamination.
**Optimising fresh meat colour and stability: enhancing consumer acceptance of kangaroo products**

Fresh, chilled meat colour is a very significant factor influencing consumer acceptance of fresh meat cuts on retail display. While colour has not been a major focus of the present studies, a large database of colour values has been accumulated through the project, as this parameter was measured routinely with ultimate pH, peak shear force, compression, and other related quality parameters. Our studies concluding currently, suggest that potential exists to further our understanding of those factors responsible for kangaroo meat colour, and of the regimes leading to colour desired by the consumer. The elucidation of those factors influencing the stability of this colour could be realised through further targeted investigation.

**Adoption of strategies to optimise kangaroo carcass chilling regimes for increased product quality**

We have identified the importance of temperature of muscle through rigor mortis (coupled with the manipulation of intramuscular tension i.e. tenderstretching) and its effect on the potential for increasing tenderness through subsequent ageing. An optimum muscle temperature of around 20-25°C during rigor maximises ageing potential, with a reduction in ultimate tenderness resulting from the storage of carcasses at temperatures above or below this range. As the timing of the onset and dynamics of rigor mortis through to its completion are yet to be characterized in kangaroo meat, additional studies are required to optimise chilling regimes for maximum tenderness.
6. Publications arising as a result of the ‘Kangaroo Meat Quality’ research

Harvesting the Australian kangaroo population for meat production: a self-sustaining wildlife industry

Improving the quality of kangaroo meat: effects of carcass suspension by one leg

The effect of pre-rigor temperature on the ageing potential of kangaroo meat

Myofibre types in eight skeletal muscles from the Eastern Grey kangaroo (*Macropus giganteus*)

The relationship between muscle glycogen stores and ultimate pH in commercially harvested kangaroos

Sensory evaluation of kangaroo meat

Kangaroo total muscle collagen as influenced by carcass weight and muscle location.
Appendix 1 – Consumer evaluation sheets

Figure 20  Consumer taste panel sample evaluation sheet. A separate sheet is filled out for each served steak by a bisecting vertical line for each of the four dimensions of eating quality and a tick for the samples perceived ranking score.
Figure 21  Consumer demographic detail questionnaire. Data collection was strictly voluntary, with complete assurance of privacy of personal information.
References


Ramsbottom JM, Strandine EI. (1948) Food Res. 13, 315.


