# Colorimetric determination of muscle glycogen in slaughter animals

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# ATTESTATION OF AUTHORSHIP

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Signed: (Yuanyuan DENG)

Date:

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# ABSTRACT

This project aimed to refine and validate an optical remote sensing method to predict the ultimate pH of slaughter beef animals. An existing commercial method converts muscle glycogen in a known mass of muscle sample into glucose that is determined by a diabetic's personal meter. The method is expensive in terms of consumables and results are fraught with inadequate operator skill levels. Pilot studies showed that it may be possible to measure the mass of the muscle sample and the concentration of glucose by colorimetry. Redness was a measure of muscle mass in acetate-buffered slurry, and after addition of Fehlings solution and heating, yellowness was a measure of glucose. This was the starting point for the study. Phase 1 determined the value of individual Hunter colour a*, b* and L* values for predicting mass of meat samples by linear equations. Hunter a* was a useful predictor of meat mass, but only within animals, probably due to the different muscular origins of the meat cuts selected. However, it was proposed that if samples were taken from a single muscular site, as in the existing commercial method, among animal variability might be much reduced. In Phase 1, a digital camera was also used to extract colour data, but it proved much less useful than the Hunter meter. Its use was thus discontinued. Phase 2 showed that different concentrations of glucose did not affect the colour due to meat mass, which was a necessary condition for using colour as a predictor of meat mass. Phase 3 explored the broad relationship between glucose concentration and meat mass on colour change due to the Fehlings reaction induced in a microwave oven. As expected from prior research, the concentration of glucose strongly affected the heat-induced colour, but meat mass also affected colour presumably through the Maillard reaction which would compete with the Fehlings reaction for the available glucose. However, if the mass of meat were known, colour values could be adjusted for this effect. In Phase 4, randomly chosen but defined masses of meat, and similarly randomly chosen, defined concentrations of glucose were used with the Fehlings reaction to test the predictive value of equations relating concentration of glucose/mass of meat to various Hunter colour values. The ratio was well predicted by Hunter b* and L*, unexpectedly implying that information about meat mass and glucose could be simultaneously extracted from the same colour data. This result suggests that there may be no need to measure meat mass, gravimetrically or by colour, to get useful results. In a limited way, Phase 5 extended the Phase 4 work by using the ratio of colour values before heating (no Fehlings added) colour values after heating (Fehlings added) to see

if this would improve the predictive values established in Phase 4. It did not. The results are discussed with a focus on future work required to confirm the results in Phase 4, and also describe the steps required in a hypothetical semi-automated application of the technology.

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# CHAPTER 1

# INTRODUCTION

## The high pH problem and its current solution

In many New Zealand meat processing plants, it is often required to measure the ultimate pH of the meat. Ultimate pH refers to the final pH attained by any carcass muscle after the rigor state has been achieved. Attainment of rigor takes up to 24 hours, when the pH can be read with a conventional pH probe that is inserted below the surface of the muscle. In so-called high quality meat cuts, from specific muscle or muscle groups, the pH values range from 5.5 to 5.8. Within this range, the meat is resistant to microbial spoilage, has a highly acceptable flavour, and is more often than not tender. If the pH is higher than 5.8, the colour becomes dark red rather than bright red, microbial spoilage can occur easily, and the cooked flavour becomes bland (Dransfield, 1981; Braggins, 1996). The higher pH condition occurs particularly in bulls, where about half the bulls slaughtered each year in New Zealand have this condition (Graafhuis and Devine, 1994; Young and others, 2004).

Of the approximately 2 million bulls slaughtered in New Zealand each year, about half have this high pH condition. The meat is not wasted however. Bull meat is lean, and is mostly exported frozen to the U.S.A. where it is minced with fatty beef trim from domestic beef cattle finished on grain-based diets to yield hamburger patties among other processed meat uses, such as emulsion sausages. In this context it is called manufacturing bull beef. Because the cold chain between abattoir and fast food outlet is maintained by freezing at nearly all points, spoilage is not an issue. Neither is flavour because the flavour of cooked fatty trim swamps the blandness of the bull beef. Colour is not an issue either because the consumer sees only the finished hamburger.

The problem for the New Zealand beef industry is that all bull meat is destined for the manufacturing bull beef trade, which commands a lower averages price than so-called premium cuts that are usually exported and sold chilled rather than frozen. If the normal pH carcasses could be cost-effectively identified and segregated from the high pH carcasses, then the normal pH meat could enter the higher value chilled meat trade. At the same time, other classes of animals can also yield higher pH meat, but these are all destined for the chilled meat trade, thus posing spoilage risk and flavour problems.

There is also another advantage for being able to segregate high pH meat from normal. High pH meat has better water holding capacity than normal pH meat, and is much more suited processed to meat products that so often depend on this attribute. Moreover, the advantage of high pH cannot be duplicated by simply raising the pH of normal meat with edible alkalis; there are biochemical reasons for this beyond the scope of this thesis (Young and others, 2004). Thus, each category (high pH and normal) has its advantages that can be fully capitalised on only when each category is fully segregated.

In view of the importance of ultimate pH it seems obvious that pH testing should be applied to all cattle if testing is cheap enough. In many cases ultimate pH testing is routinely applied, but a technological change in slaughter procedure has rendered conventional meter testing useless in an increasing number of abattoirs in New Zealand. This change is called hot-boning, where the meat is cut from carcasses and boxed within one hour of slaughter. pH at 24 hours can no longer be determined. About half of New Zealand's beef abattoirs now use this technology, first adopted in New Zealand in the19thcentury.

A method had to be found to determine the ultimate pH and that method would have to apply within one hour of slaughter. Young and others (2004ab) developed an internationally patented method that circumvents the problem imposed by hot boning. This will be described in more detail later. Before that, it is useful to describe the events occurring in ruminant muscle after slaughter and how these relate to the high pH condition. After that section, the existing method will be described, followed by a description of the options available for improving the technology. One of these options has been selected for the research that is the main subject of this thesis.

## Postmortem events in ruminant muscle

### *The biochemistry of post-mortem metabolism*

Muscle ceases to function normally when an animal is slaughtered. Metabolic functions are greatly altered principally because the flow of blood stops when the heart stops. Blood is a supply of nutrients, and critically for normal muscle metabolism, is the supply of oxygen. To maintain normal ATP concentrations, normal muscle depends on the combination of glycolysis, and the Krebs cycle and oxidative phosphorylation in mitochondria. In the absence of oxygen the Krebs cycle and oxidative phosphorylation can no longer occur, but glycolysis still can (Figure 1 and Figure 2).

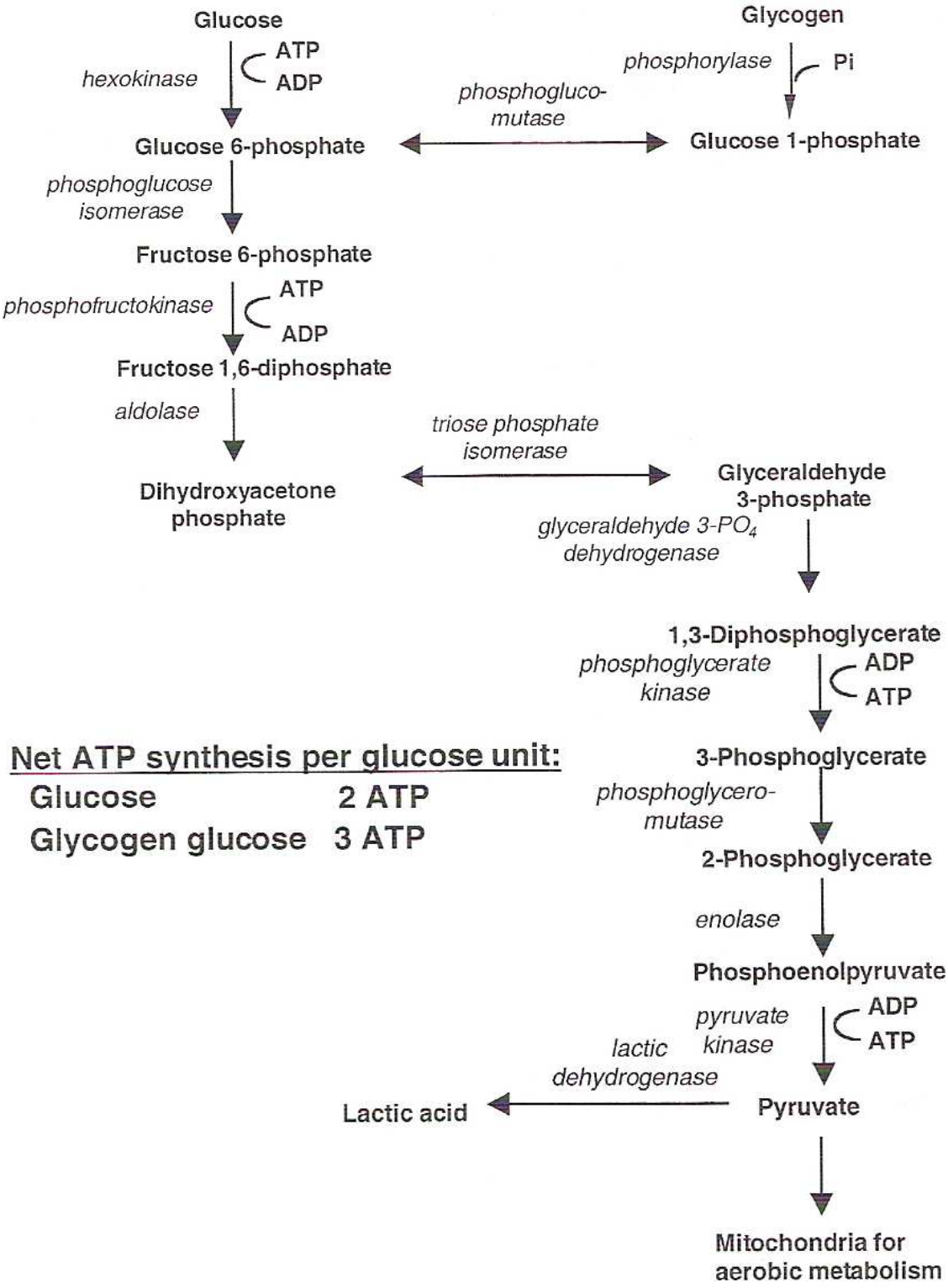


Figure 1. The glycolytic pathway showing the accumulation of lactic acid when the oxygen supply to the muscle is lost after slaughter. From Greaser (2001)

The pyruvate created as product of glycolysis is converted to lactic acid. Lactic acid cannot be removed without a blood flow, so the lactic acid accumulates in the muscle and the pH falls from the typical live pH of 7. Although the animal is dead the nervous system is

still active, at least to the point that the sarcoplasmic reticulum calcium pump and the cell membrane sodium potassium pump continue to work moving their respective ions against the concentration gradients. These pumps require ATP as an energy source (Figure 2), which is generated by glycolysis, but not nearly to the same extent as oxidative phosphorylation.

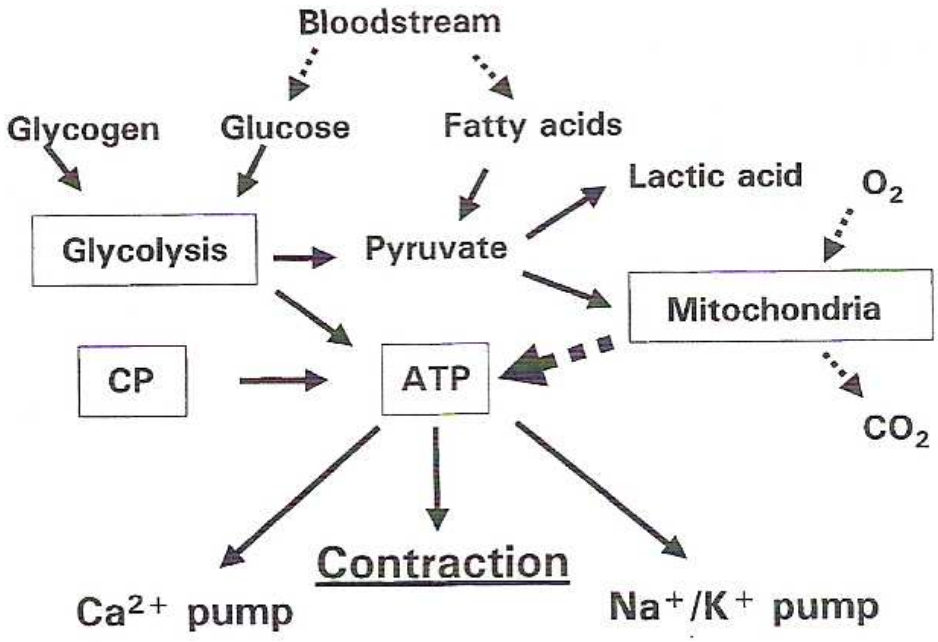


Figure 2. An overview of muscle metabolism. CP is creative phosphate. From Greaser (2001).

Glycogen becomes exhausted and/or pH falls to the point that glycolytic enzymes no longer work. ATP concentration falls close to zero and myosin and actincombine in the rigor state (Bendall, 1951). The pH attained is the ultimate pH to which the pH falls during rigor development. The pH finally reached is normally between 5.4 and 5.7 in high value bovine meat cuts.

For reasons to be discussed later, the glycogen content of muscle is sometimes lower than the normal 0.8 to 1.5% of wet muscle weight. In this situation, the pH cannot fall to the normal pH because there are not enough glucose equivalents in glycogen to generate enough lactate in the form of lactic acid (Figure 3). In this figure a normal starting content (more accurately concentration) is assumed to be 100 µmol equivalents of lactate per g of muscle. Each glucose equivalent in glycogen yields two lactate molecules, so the muscle contains 50 µmole equivalents of glucose. This in turn is equivalent to approximately 0.9% glycogen in wet muscle. Lower concentration in Figure 3 yield has lower concentrations of lactate as

lactic acid, so the ultimate pH becomes progressively higher as glycogen concentration falls. pH values above 5.8 increasingly approach the high pH condition. An ultimate pH above 6 usually defines high pH meat, although definitions vary from researcher to researcher.

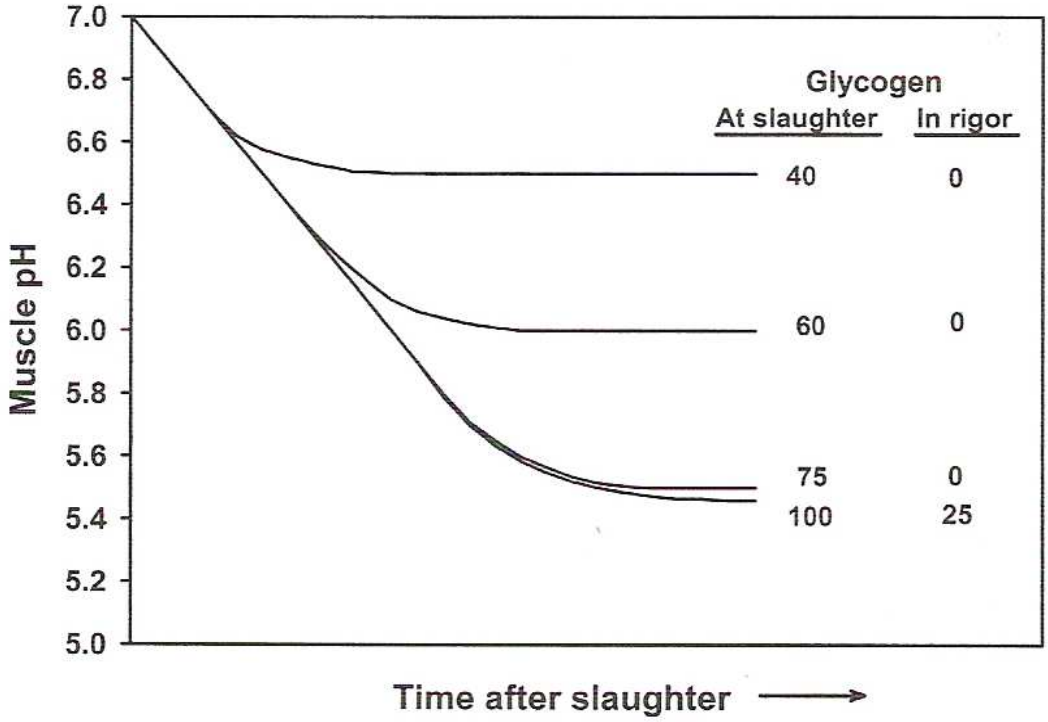


Figure 3. Hypothetical time course of pH fall for muscle containing different concentrations of glycogen. Low concentration result in a high ultimate pH and no residual glycogen. (From Young and Gregory (2001).

***The properties of high pH meat***

Other names for the condition are dark-cutting meat, and DFD meat, dark, firm, and dry to the touch.

The dark colour has two causes. First, at normal pH the muscle fibres occupy a minimal volume, creating gaps between the fibres that cause an increase in refracted of ambient light. Light is therefore scattered more than at higher pHs. At higher pHs ambient light penetrates the meat more deeply and is more likely to be absorbed than scattered. The meat thus appears darker. Second, at higher pH values the mitochondria remain more active than at normal meat pH values. Oxygen consumption rates are high in high pH meat (Figure 4; Faustman and Cassens, 1990) leaving little to convert the dark deoxymyoglobin to the bright red oxymyoblobin.

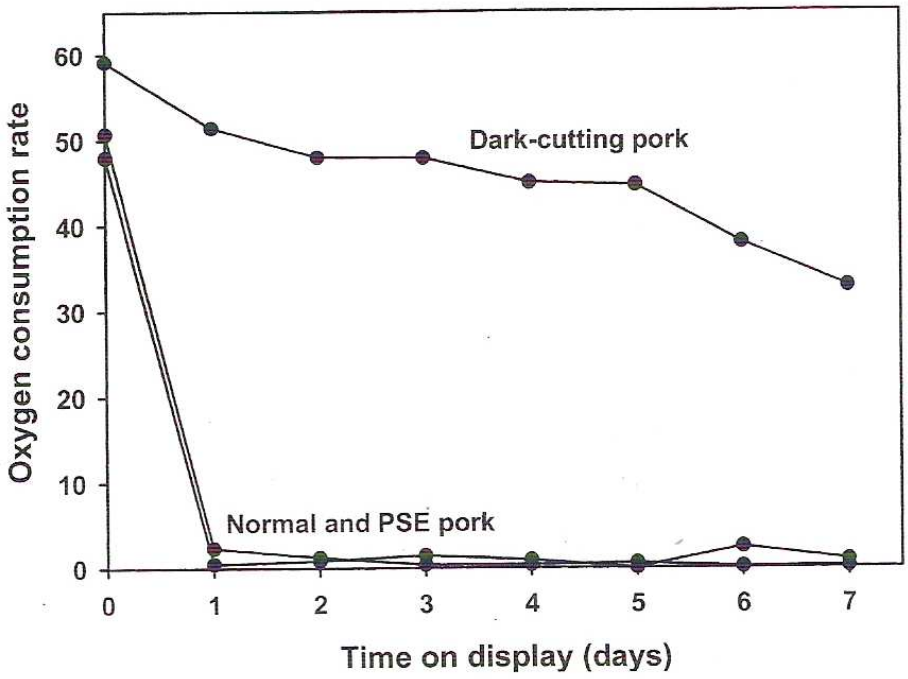


Figure 4. Relative oxygen consumption rates in beef with three rigor conditions during display for 7 days. (From Zhu and Brewer, 1998).

The apparent firmness and dryness arise from the fact that at higher pH values, the muscle proteins are far above their isoelectric pH, between 5 and 5.5. The water-holding capacity is near minimal in normal pH meat, and rises markedly as pH increases. When water is tightly bound the meat is dry to the touch.

Glucose is important for the microbiological stability of meat (Young and Gregory, 2001). According to these authors, the storage life of high pH meat is compromised for two reasons. One is that a putrefactive microflora develops in high pH meat, and it achieves a higher log occurrence value than in normal pH meat (Barnes and Impey, 1968; Grau, 1981). Another reason is that the residual glucose concentration decreases as pH increases. Microbial growth requires a carbon source. If glucose concentration is low or almost absent, the microbes begin to use free amino acids as the energy source. When these are decarboxylated, putrid amines are generated. The most common solution to storing high pH meat is freezing. This prevents microbial growth and solid blocks of lean high pH New Zealand meat are exported to USA where they are minced (as a tempered block) with fatty US trim to ultimately yield hamburger patties, other food service products and emulsion sausages. In many of these products high water-holding capacity is an advantage. As noted earlier the high water-holding capacity of high pH meat cannot be achieved by simply raising the pH of

normal meat with edible alkalis; there are biochemical reasons for this and other positive attributes of high pH meat that are beyond the scope of this thesis (Young and others, 2004).

***Incidence of the high pH condition in New Zealand slaughter bovines***

A surly by Graafhuis and Devine (1994) indicated that nearly 30 percent of cattle had the ultimate pH above 5.8 in New Zealand slaughterhouse (Figure 5). The figure shows that bulls are more prone to develop the high pH condition, and this is caused by beef production systems and pre-slaughter animal handling that have not significantly changed in New Zealand in a decade, as shown by Young and others (2004).

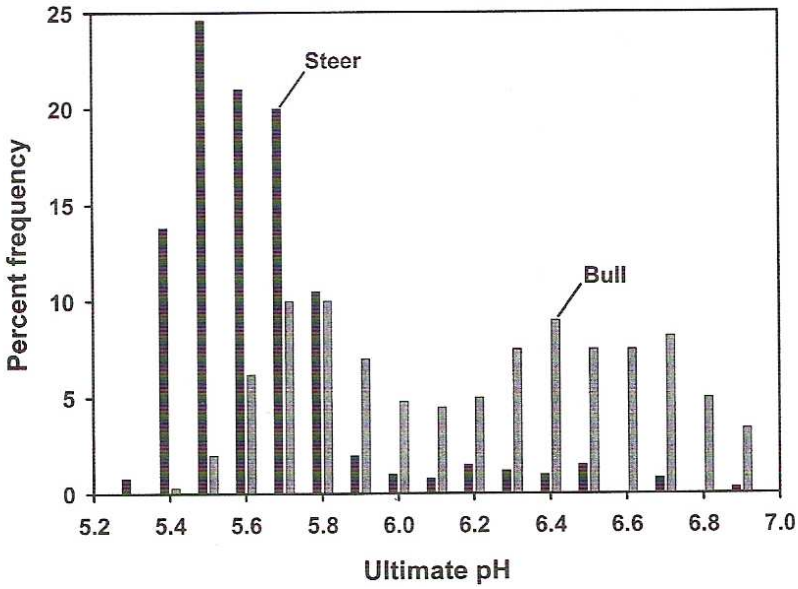


Figure 5. Frequency of ultimate pH values in different animal muscle from pasture finished steers and bulls. (From Graafhuis and Devine, 1994).

Using a different pH assessment technology (that is the subject of the present study), Young and others (2004) conducted a comprehensive survey in one abattoir on 13,700 cattle. Young and others (2004) showed that the glycogen content of muscle at slaughter (expressed in this method as a 'glucose value') was much lower on average in bulls than in so-called prime cattle (Figure 6). This necessarily leads to a higher mean ultimate pH.

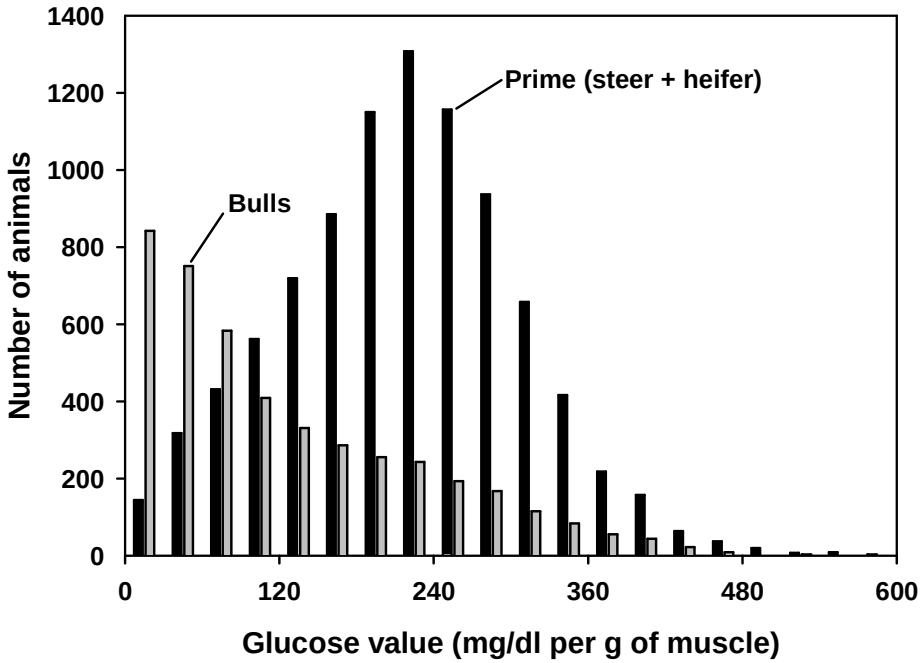


Figure 6. Glucose values in 13,700 pasture-finished animal slaughtered in one abattoir (From Graafhuis and Devine, 1994).

Since the Young and others survey, production, transport and slaughter practices have not changed, so there is almost certainly a continuing high incidence of the pH problem, providing motivation to segregate the high and normal categories to take advantages of each category's positive attributes.

In this thesis, a further development of the technology described by Young et al (2004) is reported on detecting high pH meat within 20 minutes of slaughter are reported. Both depend on determination of glycogen in a small muscle sample excised immediately after the pelt is removed in the abattoir process. For reasons that will be discussed in the final discussion, Chapter 3, another abnormal post-mortem condition could compromise the accuracy of the method. Therefore this condition – the pale, soft exudative condition – is now described in anticipation of the final discussion

***PSE, the pale, soft and exudative condition***

In this condition, post-mortem muscle becomes pale in colour, develops a soft texture and exudes excess volumes of fluid. For reasons outside the scope of this thesis, the post-mortem metabolic rate is greatly increased. ATP depletion, pH decline, and attainment of rigor mortis is very rapid and occurs when the carcass is still warm (Bendall and

Wismer-Pedersen, 1962; Young and others, 2001). The high muscle temperature can lead to denaturation of some muscle proteins. This reduces the water holding activity of the muscle and results in excess drip loss. Thus the PSE condition is caused by the denaturation of muscle proteins that takes place when muscles simultaneously experience a low pH, from post-mortem metabolism, and high temperature.

## Measurement of muscle and meat pH

### *pH probes*

Young and other (2002) reported that pH probes are the standard method of determining ultimate pH in New Zealand abattoirs. This involves insertion of the probe into the longissumus dorsi muscle at the beef carcass quartering point. This has to be done when the carcasses are in rigor at their ultimate pH which can take up to 24 hours. If the test is performed earlier pH values can be erroneously high. In busy abattoirs the pressure on storage space is high so the natural tendency is for the measurement to be made too early (it cannot be made too late). This coupled with poor calibration, and clogging of the probe with fat, returns a high error rate. Moreover, the abattoir workers who perform this task are generally poorly trained and have 'blind faith' in the values returned by the meter. This has been confirmed by unpublished trials comparing abattoir results with equivalent validated results; in one abattoir the data generated amounted to random numbers between 5.00 and 7.00 (O.A. Young, personal communication).

The other problem is that with the increasingly popular technique of hot-boning and early packaging, the meat is in cartons and being chilled to freezing long before the ultimate pH has been reached (Young and others 2004). pH probes yield meaningless data at these times. pH has to be predicted before hot-boning. The RapidpH method was developed to do this.

### *RapidpH*

A small sample of muscle is taken from an indicator muscle of the slaughter animal at 15 minutes after slaughter. At this time, the muscle glycogen that will ultimately cause the decrease in pH is substantially intact, representing a maximum of 2% of the muscle mass. The sample is weighed and dispersed in a small volume of an acidic buffer that also contains a hydrolytic fungal enzyme, amyloglucosidase. This rapidly and completely hydrolyses the glycogen to glucose. The concentration of glucose is conveniently measured with a diabetic's personal meter. In outline, the glucose concentration divided by the mass of muscle indicates the concentration of glycogen in the indicator muscle. If the value is high and above a critical value, pH is predicted to fall to normal, less than 5.8. If the value is

lower than the critical value, the pH will be higher and the carcass could be validly classed as higher pH destined for the lower value hamburger market (Young and others, 2004).

The method works and is in routine industrial use in many New Zealand beef abattoirs. The patent is owned by AgResearch Limited, Hamilton (WO 00112844) and royalties accrue to that company.

The method is however not without its problems. The method requirs two labour units for each beef processing line, the diabetic test strips cost about $1 each per animal, and the method requires a degree of skill not always found in the low-paid labourers that do this job. It is easy to take shortcuts that compromise accuracy.

***Near infrared spectroscopy***

A recent AUT MAppSc student, Dominic Lomiwes, explored the use of near infrared (NIR) spectroscopy as a means of measuring glycogen concentration fifteen minutes after slaughter (Lomiwes and others 2009). This was always going to be challenging because of the inherent variability of muscle. The attempt was unsuccessful, although curiously NIR was able to distinguish different beef classes based on gender. It is possible that with a much larger data base that useful algorithms could be developed, but that is for the future.

## Options for improving the RapidpH method

The RapidpH depends on a labour-intensive mass determination and homogenisation step, followed by a labour-intensive glucose determination with a diabetic meter. To avoid some of the costs associated with this manual method, AgResearch Limited developed a semi-automated method in the form of a dedicated machine that required only that the operator manually inserts the muscle sample in a receiving hole (Figure 7). However, the complexity and estimated price in excess of NZ$200,000 meant that no machines were ever sold.

Figure 7. An exposed view of a semi-automatic machine to measure liberated glucose in a small pre-rigor muscle sample. The sample is placed in the hole on the fascia of the machine. (Picture courtesy of Dr. O.A. Young.)

There may be opportunities to simplify the existing manual method such that only one operator is required and that the diabetic meters are not needed. The meters are designed for domestic use and have a continuing cost for each test on each animal.

It is proposed that optical methods might be used to obviate the need to weigh meat and determine glucose concentration.

***Determination of muscle mass by muscle colour***

Myoglobin is the main characteristic red protein in muscle and meat. It comprises a proteinaceous globin and a non protein part, a porphyrin ring structure. That holds iron. The porphyrin ring has six coordination sites, four of which bind iron one binds to a histidine residue on the globin, and the sixth can bind oxygen (Figure 8).

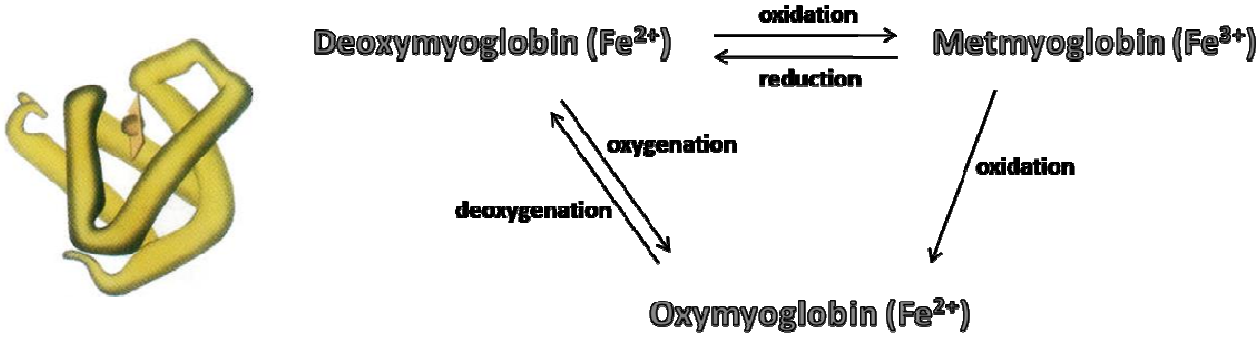


Figure 8. The myoglobin molecule (left) consists of a helical polypeptide chain and a haem group within the folded chain (Garret & Grisham, 2005). The different forms of the myoglobin molecule are shown (right). The colour of meat is regulated by the oxidation state of iron in the haem group within the molecule.

Whether myoglobin is binding oxygen or not, the higher the myoglobin concentration in muscle the redder the muscle is. It follows that within one muscle of a single animal the redness of a constant-volume muscle slurry made from a sample would be a guide to the mass of the sample. Thus, if a muscle sample for RapidpH were dispersed in a slurry and the reflected colour of incident white light were measured, the sample mass might be estimated by a suitable calibration curve. Clearly the sample would have to be taken from one muscle in a carcass, and the calibration would assume that that muscle was equally red in all bovines slaughtered in a particular abattoir. The former constraint is not a problem because the RapidpH samples are routinely taken from one site of one muscle (Figure 9). This site is a prominent bulge lateral to the spine in the lumbar region.

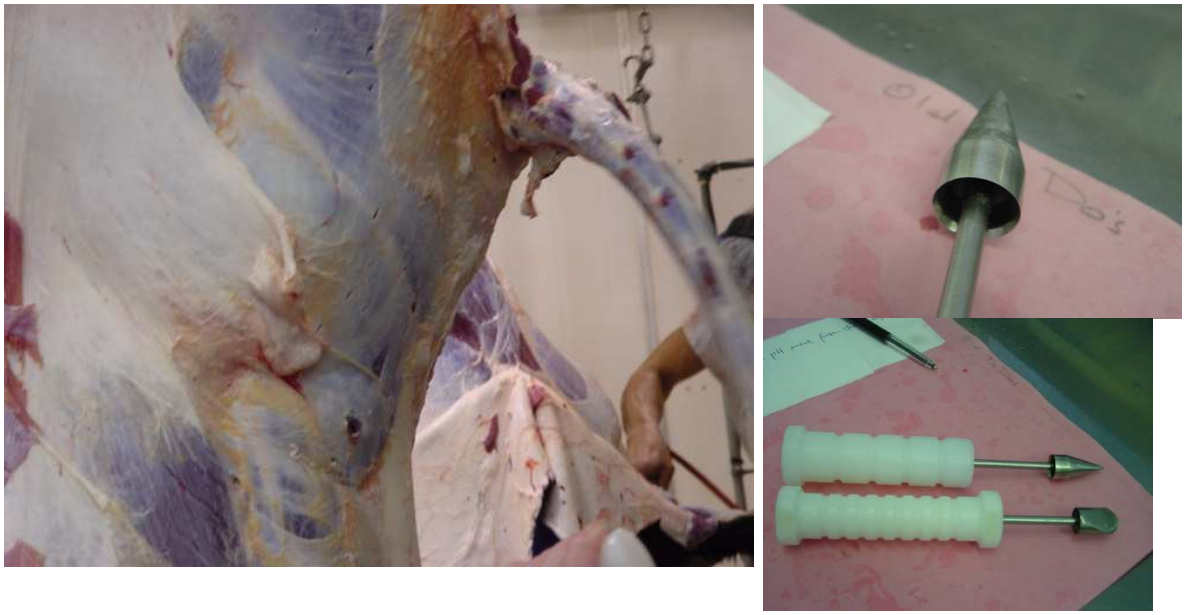

Figure 9. Tools for sampling pre-rigor muscle. The tool is inserted into the muscle bulge (arrowed), then rapidly extracting taking a sample reliably between 0.8 and 2.2 g, The rim of the tool is sharp in order to cut the cut the muscle sample. (Picture courtesy of Dr. O.A. Young.)

The second constraint is more serious due to between-animal variation in myoglobin concentration. This constraint will be examined in detail in subsequent chapters, but is ignored for the moment.

Tan (2007) set out to devise a way of measuring muscle sample mass without the need to handle the sample on a balance. Bovine muscle is red and the proposition was that redness in muscle slurry in the acid buffer would be a measure of muscle mass. Tan showed this was a fair assumption. Figure 10 is drawn directly from her report. She has fitted a least squares straight line. The a* value (a measure of redness) could be used to predict mass. a* is a measure of redness-greenness in the so-called Hunter colour space. Other Hunter values might also have been useful in deriving a line of best fit, but she did not pursue that.

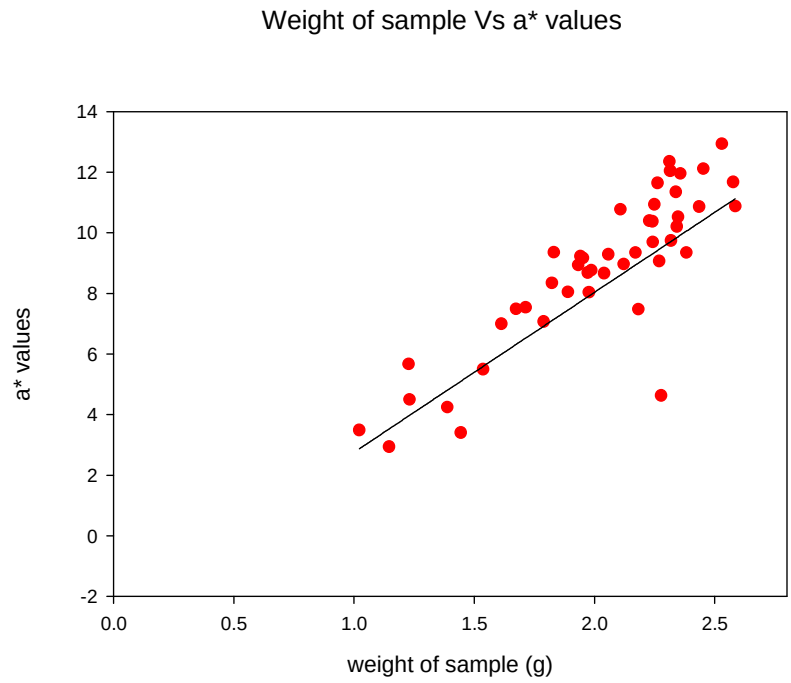


Figure 10. Relationship between muscle mass in a buffered slurry and Hunter a* values. From Tan (2007).

***Determination of glucose by colour***

There are several potential methods for determination of glucose by colour, the most obvious being exploitation of the enzyme glucose oxidase, which in the presence of oxygen generates gluconic acid and $H_2O_2$:

Equation:

$$\text{Glycogen} \xrightarrow{\text{Amyloglucosidase}} \text{Glucose} \xrightarrow{\text{Glucose oxidase (GOD)}} \text{Gluconic acid} + H_2O_2$$

$H_2O_2$ is a metabolite that is the basis of oxidoreductive methods of measuring the original analyst, in this case glucose. Peroxidase is an industrially-available enzyme that catalysis the oxidation of a peroxidases substrate with $H_2O_2$.

Many of these substrates change colour when oxidised. Unpublished work by Dr. O.A. Younghas shown that peroxidase is active under acid conditions and will generate coloured (or luminescent) products from a range of peroxidase substrates. In the case of opaque slurry, the colour could be measured as reflected light in Hunter colour space. However, it was often difficult to dissolve sufficient mole equivalents of the substrates in the slurry. Moreover, the sequence from glucose to oxidised peroxidase substrate requires that a mole of $O_2$ is present to react with each mole of glucose. At the concentrations of glucose commonly

encountered in these slurries, there was insufficient $O_2$ dissolved in the aqueous slurry to react with all the available glucose (data not shown). Oxygen was thus limiting. The problem could be overcome by shaking to encourage aeration, but this requirement added unwanted complexity. Also, the peroxidase method requires the routine purchase of three biochemicals, glucose oxidase, peroxidase and the chosen peroxidase substrate. This would add to cost. Finally, catalase in the meat slurry might compete with the intended reaction. This possibility was never investigated.

Another approach was explored by Tan (2007). In the presence of nitrite, $H_2O_2$ turns myoglobin a vivid green. If nitrite were added at the same time as glucose oxidase, the green colour might be proportional to the amount of glucose present. Conceptually, the redness of the muscle slurry would indicate the mass of muscle (Figure 10), and the greenness would indicate the mass of glucose. The higher the ratio of greenness to redness, the more glucose – originally as glycogen – there would be per g of muscle sample. However, calculations showed that at the concentrations of glucose commonly encountered in these slurries, there were insufficient mole equivalents of myoglobin (Young and West, 2001) dissolved in the aqueous slurry to react with the nitrite/ $H_2O_2$. At the same time the problem of insufficient oxygen applied as it did in the peroxidase concept. Experiments by Tan (2007) showed that green colour could be generated but a positive and significant correlation between glucose concentration and greenness could not be produced, presumably in response to the limiting myoglobin.

A way was sought to measure glucose by colour without the use of oxygen and enzymes. A classic test for glucose, or more accurately any reducing sugar, is the Fehlings test. Under alkaline conditions, $Cu^{2+}$ is reduced to $Cu^{1+}$, which is visible as a clear colour change. This is shown in the following series of pictures (Lei, 2009). Figure 11 shows the colour of muscle slurries containing 0 to 6.67 mM. This concentration range matches the range that would be derived from muscle tissue in a typical RapidpH trial.

Figure 11. Colour of muscle slurries containing different concentrations of glucose, but no Fehlings' solution. The glucose concentrations were, from left to right, 6.67, 3.34, 1.22 and 0 mM. (From Lei, 2009).

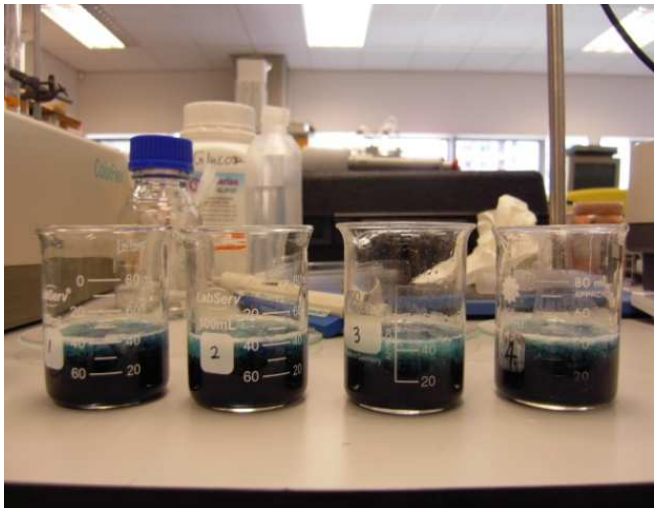

Figure 12. The muscle slurries with the Fehlings solution added. (From Lei, 2009).

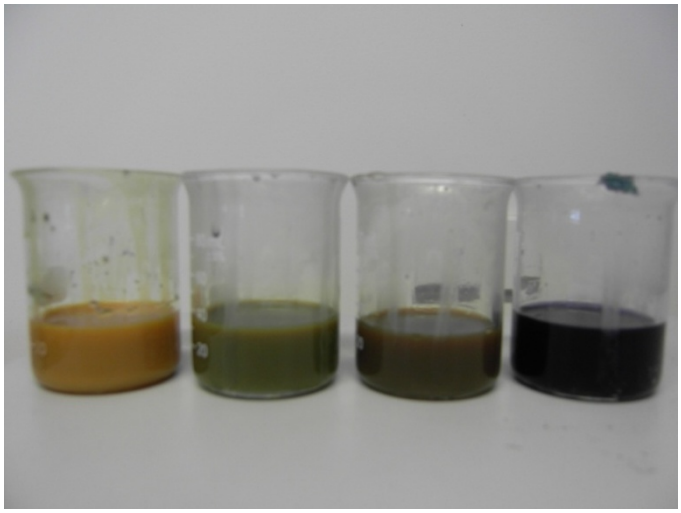

Figure 13. The muscle slurries with the Fehlings solution after heating in a microwave oven for 60 seconds. The glucose concentrations were, from left to right, 6.67, 3.34, 1.22 and 0 mM. (From Lei, 2009).

The colour changes were measured by Lei (2009) and a clear relationship was established between several Hunter colour variables and glucose concentration (data not shown).

The concept of determining relative glucose concentration (representing glycogen) in an unknown mass of muscle is simple. The greater the colour changes due to the Fehlings reaction (Figure 12, 13), the higher the concentration of glucose: call this value G. The greater the colour of the meat slurry before Fehlings addition (Figure 11) the greater the mass of muscle: call this value M. The relative concentration of glycogen is thus given by the expression G/M.

If the process were semiautomated only one operator would be required and moreover, Fehlings solution is cheap, as are microwave ovens. Thus the model method presented here could solve the cost and skill problem associated with the existing method.

## The planned research

The research described in this thesis formally explores the effect of different muscle sample masses on slurry colour, the effects of different glucose concentrations on Fehlings colour, and their interaction, if any. This is done within the volume and mass constraints of the existing pH method, with a future goal to adapt what is learned here to a commercial industrial environment.

The ideal meat to work with in developing a novel method of determining muscle glycogen is to use pre-rigor, harvested from a slaughtered animal within 20 minutes of

slaughter. Outside the meat industry this is impracticable to achieve. However, it is possible to simulate the pre-rigor condition by adding glycogen or glucose equivalents to rigor muscle. If glycogen were to be added, amyloglucosidase would also have to be added to hydrolyse the glycogen to glucose, as is the situation with the existing RapidpH method. This hydrolysis step is very well defined in that the glycogen is rapidly hydrolysed 100% to glucose. It is far easier to simply add glucose to rigor meat, and this was the strategy adopted here.

The research to date has employed a Hunter meter to measure colour (HunterLab, 2001). Hunter meters are expensive research instruments. The modern digital camera offers a cheap and versatile alternative. The proposed research will determine colour in parallel with both systems. There will be no attempt to design a semiautomated procedure but the design concept will be defined.

# CHAPTER 2

# MATERIALS AND METHODS

## Chemicals, equipment and meat sampling

### *Chemicals*

Sodium acetate buffer (200mM, pH 5.0) was prepared from reagent-grade acetic acid. A dilution series of D (+)-glucose was prepared in this buffer between 0 to 6.67 mM.

Fehlings solutions 1 and 2 were prepared by dissolving 69.3 g of $CuSO_4.5H_2O$ in water and making up to one litre for Fehlings 1, and dissolving 100 g sodium hydroxide and 345 g $KNaC_4O_6.4H_2O$ in water and making up to one litre for Fehlings 2. In the experiments, 4 mL each of Fehlings 1 and 2 were added to meat slurry in a ratio to be described later.

### *Equipment*

A balance with a vibration compensation mechanism was used to weigh the muscle samples from 0.8 g to 2.2 g. An Ika (Staufen, Germany) T25 Ultra-Turrax drive fitted with a S25 dispersing element 18mm in diameter was modified in a confidential way to make muscle slurry (Figure 14). The assembly was mounted on a heavy-weight laboratory stand so that dispersion was accomplished by moving vials containing samples about the dispersing element.

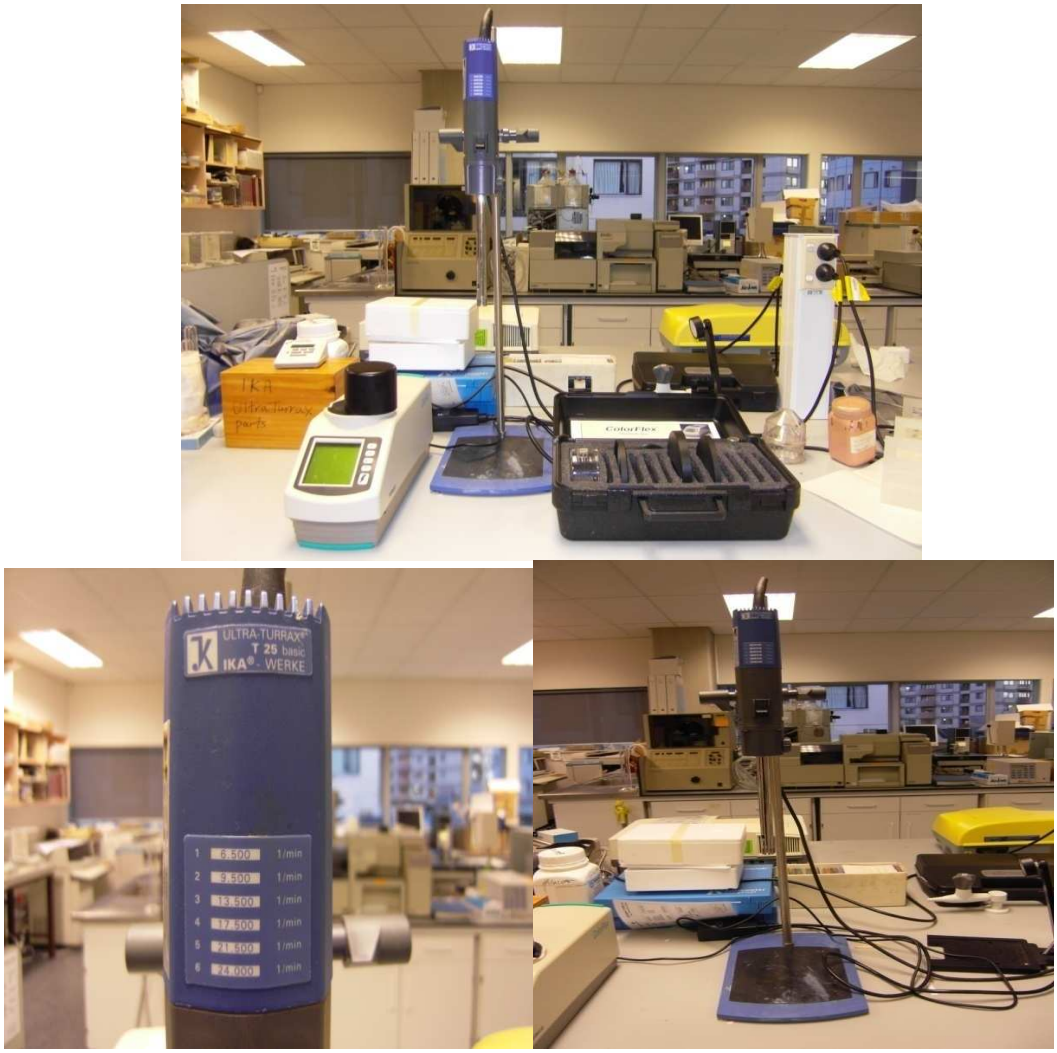


Figure 14. Homogenizer (Ika T25) basic Ultraturrax fitted with a dispersing element.

A disperser (Ultraturrax T25 basis) used to create the slurries. This was done before measuring colour or testing glucose concentration. The dispersing process was completed within ten seconds and then different concentrations of glucose added.

A microwave oven was used to heat the slurry with Fehlings solution in Phase 3. The brand is National and model is NE-6770, made in Japan. Input is 1200w 5.2a 240v ~50Hz, output is 600w 2450MHz.

The sample was in a glass reaction dish under a black shroud lid. To measure colour, the L*, a*, and b* readings were recorded by using a Hunter lab ColorFlex colorimeter set (Figure 15).

Figure 15. Hunter colour meter, Model 45/0.

A Canon SX110 digital camera was used to identify the surface colours of slurries. The illumination system used a compact fluorescent lamp (10W D65 1000 lamp) as shown in Figure 16. In all trials, the distance between the base of the interior the box was white box and the front of the lens was 19 cm, and the two lamps were 5 cm above the camera screen. The fluorescent laboratory room lights were off, but there was some ambient room light from large, south-facing windows 8 m from the photographic assembly. The field of view was shown in Figure 16 and was achieved by using the macro focus function. With the lighting level achieved, satisfactory exposures were achieved with an ISO setting of 100, an F stop of 6.3 and an exposure time of 2.5 seconds.

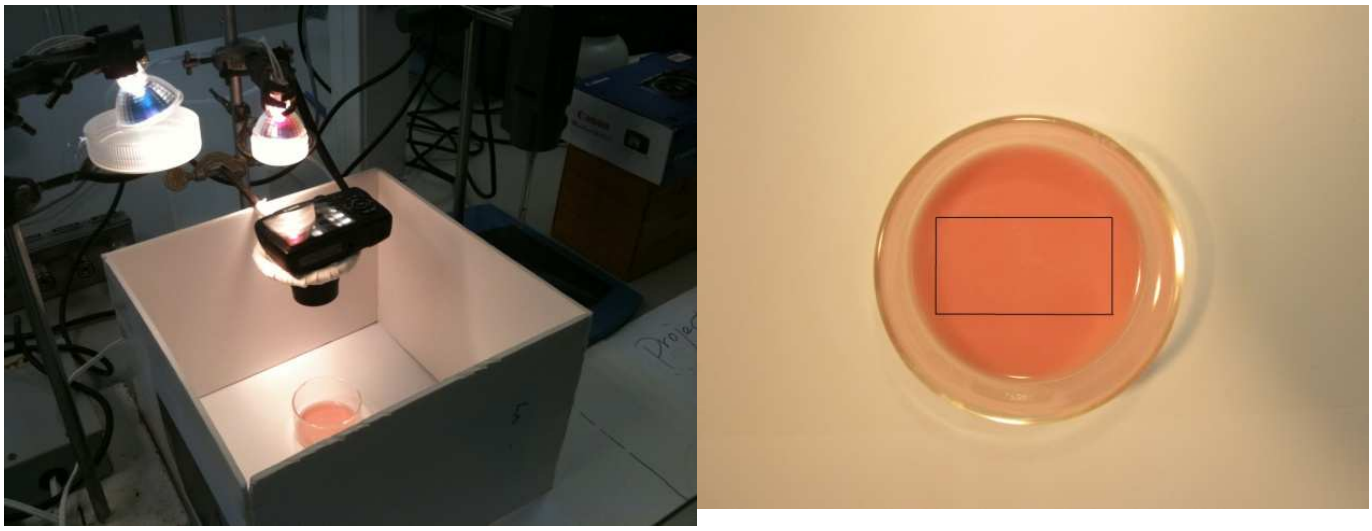

Figure 16. The digital camera system under standard illumination conditions. The rectangle in the right-side photograph was the image analysed by the ImageAnalysis software.

***Meat selection***

Chilled rump steaks from beef, were purchased from local supermarkets, such as Countdown and New World in Auckland. Rump steak includes the muscle from which the industrial samples are routinely taken (Figure 9), but the rump obviously includes many more muscles than the RapidpH sample muscle (Figure 39). Thus there was no control over age, breed and position within the rump.


Figure 17. A typical piece of rump steak bought for one day of experimentation

The samples for dispersion were always taken by scalpel from the core of meat, to avoid meat that was 'bloomed' due to atmospheric oxygen.

## Description of the colorimetric methods

The intensity or colour characteristic of an object depends upon the amount of light which it reflects (HunterLab, 2001). A colorimeter is able to represent this as numbers. The colorimeter used for most of the work in this thesis was a Hunter lab ColorFlex colorimeter (Figure 15). Colour is represented as 3-dimensional rectangular colour space which stems from the opponent-colours theory (HunterLab, 2001). Theory is based on opponent pairs of colour which are red-green, blue-yellow and black-white, and states that colours cannot be seen or perceived "as both reddish and greenish at the same time, but they can be perceived as reddish-yellow or reddish-blue" (Figure 18).

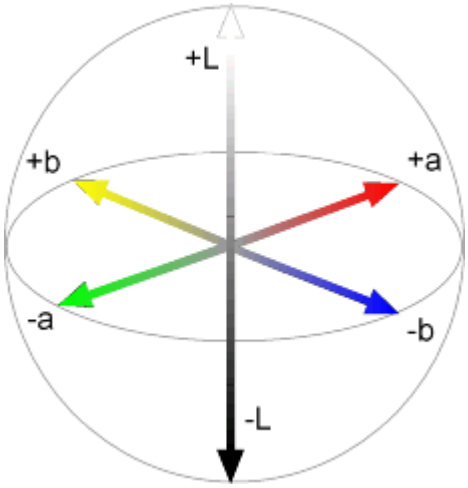


Figure 18. 3-dimensional rectangular colour space. (From http://www.tasi.ac.uk/images/cielab.gif)

The L* (lightness) axis refers to the degree of lightness in a colour, ranging from 0 (-L) which is black, to 100 (+L), which is white. The a* (red-green) axis measures the change from green to red as the values move from the negative range into the positive range. 0 is the neutral midpoint. The b* (blue-yellow) axis measures the change from blue to yellow as the values move from the negative end into the positive end. Again, 0 is the neutral midpoint.

Two public domain software programs were tested for image analysis. These were ImageAnalyst Version 1.0, apparently sourced from China and written by Long Xiao Li, and the prorgram Image J 1.42q (http://rsb.info.nih.gov/ij). They both gave identical results from the same model image. As seen in Figure 16, the camera focused on the beaker, and the operator-selected rectangle was analysed by ImageAnalyst to yield RGB colour values. A

color in the RGB color model is described by how much red, green, and blue light is included in the image. The color is expressed as an RGB triplet, each component of which can vary from zero to a defined maximum value, commonly 255 for each. If all the components are at zero the result is black; if all are at maximum, the result is the brightest representable white (Wikipedia, 2011). Zero to 255 inclusive represents a numerical value of $256 = 2^8$, 8-bit binary code.

## The experimental method

**Phase 1** was to validate the relationship between mass of meat and colour, where the latter was measured in two ways. The first way was with the Hunter meter to measure the colour as L*, a*, and b* through the base of a glass beaker. The second way was by digital photography under controlled white light conditions. In the digital camera method, the field of view was the maximum area of surface slurry that did not include the glass walls of the beaker. Data was extracted from the image by programs such the free software ImageAnalyst.

The main factor of interest was obviously the relationship between colour and mass, but a major thrust of Phase 1 was to explore the effect of animal. That is to say, does the relationship vary from animal to animal (within a muscle)? To explore this effect, a standard cut of beef (rump) was bought at retail on six occasions over three weeks. Masses of meat between 0.8 g and 2.2 g were cut from the core (supposed to be at a low oxygen partial pressure)of the meat sample, and dispersed in 7.5 mL of sodium acetate-acetic acid buffer (pH 5.0) plus 0.5 mL of the same buffer to simulate addition of the amyloglucosidase enzyme solution used in the existing industrial process. Glucose was included in the acetate buffer to achieve a final concentration of 3.34 mM. This glucose concentration represents the median concentration of glycogen, as glucose, that would be derived from a 1.5 g muscle sample. The reason for this addition is simply to simulate the industrial process as closely as possible. Analysis of the colour data will be discussed after the description of Phase 3.

An issue in these Phase 1 experiments was the concentration of acetate in the buffer. The colour development method planned (see Phase 3) involves the reaction of glucose in a strongly alkaline solution containing copper sulphate (Fehlings solution). If the acetate buffer were too strong, it could reduce the alkalinity to a level where the reaction from $Cu^{2+}$ to $Cu^{1+}$ might be inhibited. At the same time the buffer must be strong enough to hold the pH

within a range where amyloglucosidase activity is constant. This issue required some experiments to determine the maximum acetate concentration that could be tolerated.

**Phase 2** was to confirm or otherwise that different concentrations of glucose would not affect the colour due to meat (without application of the Fehlings reaction). It was not expected to do so. This experiment required equal masses of meat (1.5 ± 0.05 g) to be dispersed in 8 mL of acetate buffer (7.5 mL + 0.5 mL as before) containing varying glucose concentrations between 0 and 6.67 mM. The highest value, 6.67 mM, represents the very highest concentrations of glycogen that are likely to be encountered in the industrial situation.

**Phase 3** was to explore the relationship between added glucose concentration – 0, 3.34 and 6.67 mM – and colour change due to the Fehlings reaction induced in a microwave oven in the presence of four levels of meat mass. These were 1.00, 1.33, 1.66 and 2.00 g to a precision of ± 0.05 g. mass of meat was dispersed in 8 mL of acetate buffer, to which was added 8 mL of Fehlings solution, making a final volume between 17 and 18 mL. Prior work by Lei (2009) showed that 30 seconds in the microwave oven on full power was sufficient to generate a potentially useful colour change. This heating regime was used here. Colour was measured after addition of Fehlings solution and heating.

In the execution of Phase 3, linear regression models were developed between concentration of glucose/mass of meat after microwave heating.

On one of the four days that this experiment was done with replicate rump steak purchases, the colour of the slurries was also recorded before addition of Fehlings solution. This particular data set was used for Phase 5 (see below).

In **Phase 4** a widely varying range of meat masses (0.8 to 2.2 g) were randomly mixed with varying concentrations of glucose in the slurry (0 to 6.67 mM) to generate colours that were substituted into the single relationship described in Phase 3 to predict the relationships' accuracy and precision. In this work only Hunter values after heating were considered because it was technically difficult to measure them before heating (no Fehlings solution added) when it was required to do 30 tests on one rump steak per day.

In **Phase 5** a single trial was performed to test the hypothesis that colour values obtained from slurries before Fehlings addition could be used to improve regression models between colour values and concentration of glucose/mass of meat after microwave heating. This contrasts with Phase 4 where only values after heating were considered. The masses of meat were 1.00, 1.33, 1.66 and 2.00 g, and the concentrations of glucose were 0, 3.34 and

6.67 mM. Data were analysed to compare the predictive value of absolute Hunter values after heating, with the predictive value of Hunter value ratios before and after heating.

## Data analysis

Basic data handling functions in Microsoft Excel were central to data analysis throughout this study, and the linear regression function in Excel was used in Phase 1 to correlate colour data and meat mass in the buffered slurries. The one-way analysis of variance routine in Minitab Release 14.2 (Minitab, Stale College, Pennsylvania) was used to test for statistically significant differences among various treatments throughout the study. The multiple linear regression function in Minitab was used in Phases 4 and 5.

# CHAPTER 3

# RESULTS AND DISCUSSION

## Phase 1

As described in the Chapter 2 the aim of Phase 1 was to validate the relationship between mass of meat and colour, where the latter was to be measured in two ways. The first way was with a Hunter meter to measure colour in L*, a*, and b* space through the base of a glass beaker. The second way was to measure colour by digital photography under controlled white light conditions.

The main factor of interest is the relationship between colour and mass with an emphasis on the effect of animal. That is to say, does the relationship (if any exists) vary from animal to animal? To explore the effect of animal, a standard cut of beef (rump) was bought at retail on nine occasions over three weeks. Masses of meat between 0.8 g and 2.2 g were cut from the oxygen-free core of the meat, and dispersed in 8 mL of 200 mM sodium acetate-acetic acid buffer (pH 5.0) containing 3.34 mM glucose to simulate an average concentration in the existing industrial process.

Also as noted in Chapter 2, an important issue in later phases is the concentration of acetate in the buffer. The colour development method planned (see Phase 3) involves the reaction of glucose in a strongly alkaline solution containing copper sulphate (Fehlings solution). If the acetate buffer is too strong, it would reduce the alkalinity perhaps to a level where the reduction of copper from $Cu^{2+}$ to $Cu^{1+}$ may be inhibited. At the same time the buffer must be strong enough to hold the pH within a range where amyloglucosidase activity is constant. This issue required a preliminary experiment to determine the maximum acetate acetate-acetic acid buffer concentration that could be tolerated.

### *Effect of acetate-acetic acid buffer concentration on pH of Fehlings solution*

The initial concentration of acetate-acetic acid buffer was set at 200 mM because that is the concentration used routinely in the current industrial RapidpH method (Young and others 2004).

Tables 1 and 2 shows the effect of different concentrations of acetate-acetic acid buffer at approximately pH 5 on the pH of a mixture with Fehlings solution. In these two experiments the volumes are different but the ratios of components are the same.

Table 1. Effect of acetate-acetic acid buffer concentration on pH of Fehlings solution where water was added to simulate meat.

| Volume of acetate-acetic acid buffer (mL) | Volume of water added to simulate meat (mL) | Initial concn. acetate-acetic acid buffer (mM) | Final concn. acetate-acetic acid buffer (mM) | Measured pH of buffer | Volume Fehlings (mL) | Measured pH of final mixture |
|---|---|---|---|---|---|---|
| 16 | 2.4 | 200 | 174[†] | 5.0 | 10 | 14.5 |
| 16 | 2.4 | 150 | 131 | 4.9 | 10 | 14.5 |
| 16 | 2.4 | 100 | 87 | 4.9 | 10 | 14.5 |
| 16 | 2.4 | 50 | 44 | 4.9 | 10 | 14.5 |
| 16 | 2.4 | 30 | 26 | 4.9 | 10 | 14.6 |
| 16 | 2.4 | 20 | 17 | 4.9 | 10 | 14.6 |
| 16 | 2.4 | 10 | 9 | 4.9 | 10 | 14.7 |
| 16 | 2.4 | 0 | 0 | 4.8 | 10 | 14.7 |
| 16 | 2.4 | 0 | 0 | 4.8 | 10 | 14.7 |

[†]This calculation was based on a moisture content of meat of 80%

Table 2 Effect of acetate-acetic acid buffer concentration on pH of Fehlings solution where meat was added.

| Volume of acetate-acetic acid buffer (mL) | Mass of meat sample (g) | Initial concn. acetate-acetic acid buffer (mM) | Final concn. acetate-acetic acid buffer (mM) | Measured pH of buffer | Volume Fehlings (mL) | Measured pH of final mixture |
|---|---|---|---|---|---|---|
| 40 | 7.5 | 200 | 174[†] | 4.8 | 25 | 14.5 |
| 40 | 7.5 | 150 | 130 | 4.9 | 25 | 14.6 |
| 40 | 7.5 | 100 | 87 | 4.8 | 25 | 14.6 |
| 40 | 7.5 | 50 | 43 | 4.9 | 25 | 14.7 |
| 40 | 7.5 | 30 | 26 | 5.0 | 25 | 14.8 |
| 40 | 7.5 | 20 | 17 | 5.0 | 25 | 14.8 |
| 40 | 7.5 | 10 | 9 | 5.0 | 25 | 14.8 |
| 40 | 7.5 | 0 | 0 | 4.8 | 25 | 14.8 |
| 40 | 7.5 | 0 | 0 | 4.8 | 25 | 14.8 |

[†]This calculation was based on a moisture content of meat of 80%

Whether meat was added or not, the alkalinity of the Fehlings solution was high enough to swamp any effect of the buffer. The pH of the final mixtures was never below 14.5.

At the time this experiment was performed, it was anticipated that the ratio of the buffer/meat slurry to Fehlings solution (A and B combined) would be about 1.8 in the main body of this thesis. That is the ratio set in Tables1 and 2, and was based on the ratio used by Lei (2009). In Phases 2 to 5 of the present study the ratio used was about 1.14 (see later). That is to say the buffer concentration relative to Fehlings was higher in these pH trials (Tables 1 and 2) than in the work reported in Phases 2 to 5. Thus the volume of Fehlings in

Phases 2 to 5 – and thus the ratio used – was more than enough to swamp the buffer effect from 200 mM acetate-acetic acid buffer.

To summarise, this experiment showed that the strength of buffer in the existing RapidpH method had no significant effect on the required high pH of a Fehlings solution mixture.

Therefore 200 mM acetate-acetic acid at pH 5.0 was used in all subsequent work and is referred to simply as 'buffer'.

***Relationship between mass of meat and Hunter colour values***

In the nine trials the relationship between meat mass in buffer slurry and Hunter a* values was statistically variable, as exemplified in Figures 19 and 20, which were the worst and best results, with $r^2$ values of 0.215 and 0.911 respectively.

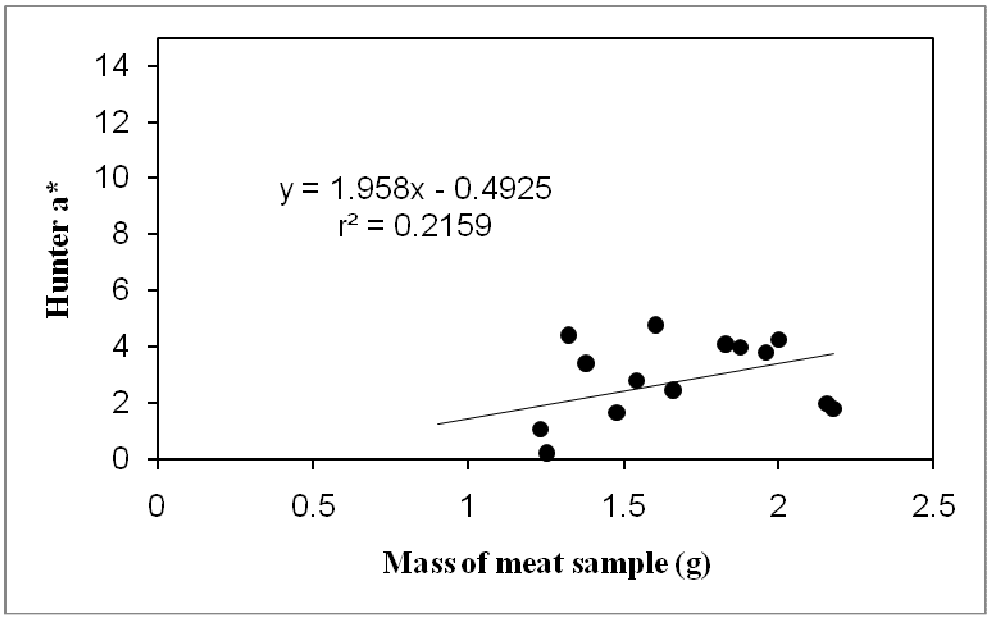


Figure 19. A poor linear relationship between mass of meat sample and Hunter a* value

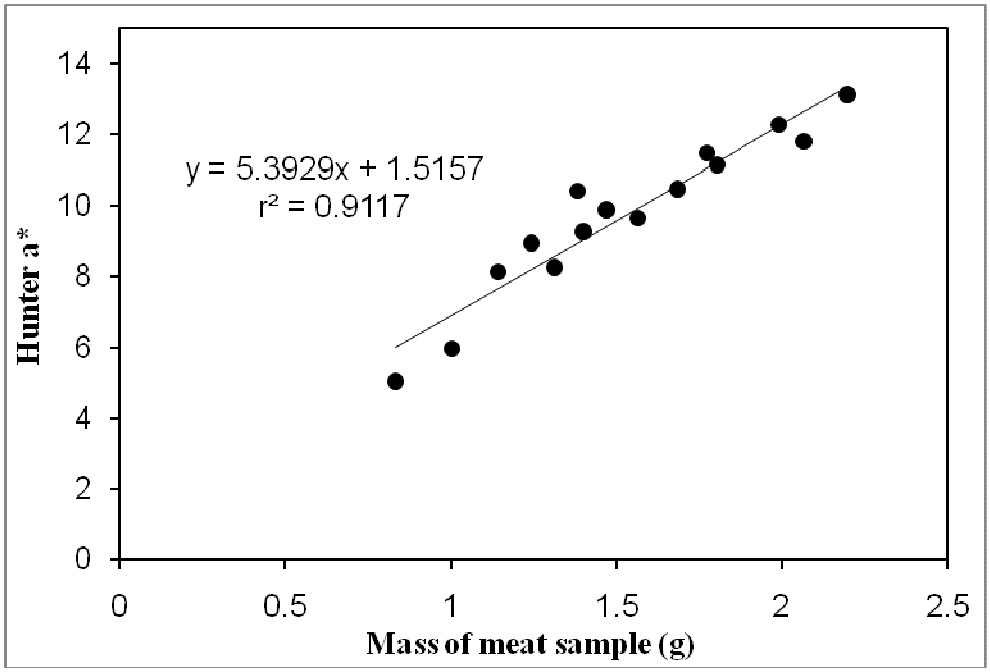


Figure 20. A good linear relationship between mass of meat sample and Hunter a* value

The $r^2$ value of the other seven trials lay between these extremes. The data are summarised in Figure 21, shown without data points, and in Table 3.

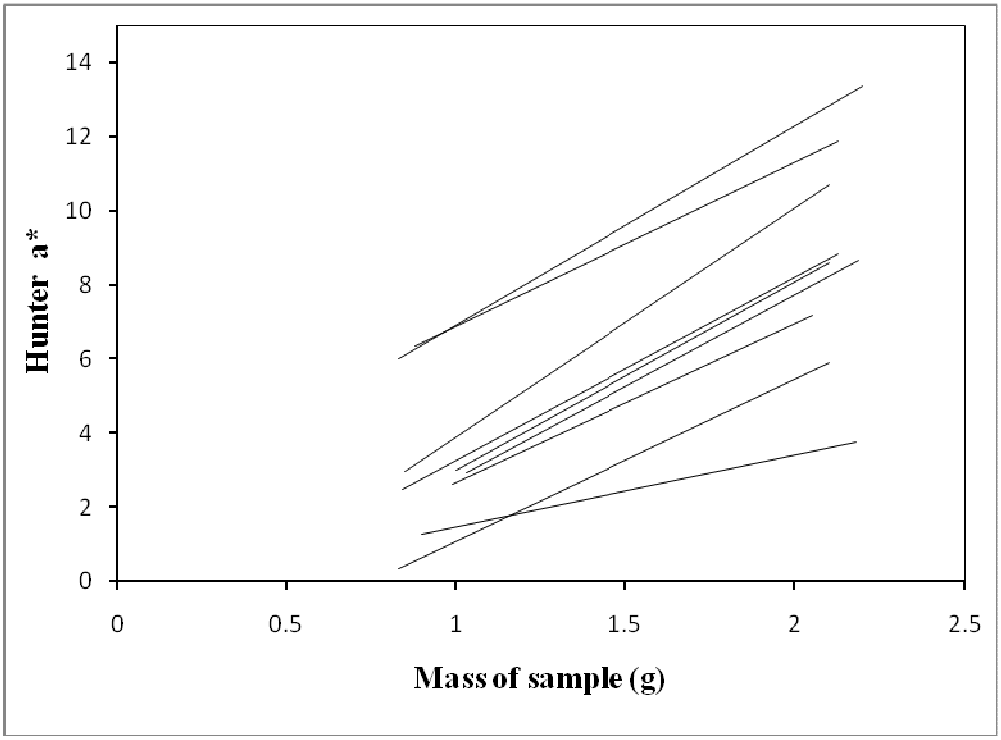


Figure 21. Nine linear relationships between Hunter a* values and mass of meat sample in a buffered slurry

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| Table 3. | Linear relationships between mass of meat sample and Hunter a* value in nine trials. | |
|---|---|---|
| Trial number | Predicted value of Hunter a* where x is mass of meat sample | $r^2$ |
| 1 | 4.297x - 1.640 | 0.462 |
| 2 | 4.937x - 1.659 | 0.436 |
| 3 | 6.179x - 2.271 | 0.615 |
| 4 | 5.392x + 1.515 | 0.911 |
| 5 | 4.425x + 2.460 | 0.738 |
| 6 | 4.405x - 3.331 | 0.897 |
| 7 | 1.958x - 0.492 | 0.215 |
| 8 | 4.949x – 2.177 | 0.841 |
| 9 | 5.088x – 2.101 | 0.805 |

Figure 21 shows that there was always a positive relationship between Hunter a* value and mass of meat in the buffer slurry. However, the slopes and particularly the y axis intercepts were variable, as were the $r^2$ values. At first sight these two result indicate that Hunter a* values could not be used to predict meat mass, because the apparent among-animal variability (slopes, intercepts) is too high as is within-animal variability ($r^2$). For example, a Hunter a* value of 4 in Figure 21 could represent masses anywhere between about 0.3 and 2.3 g of meat depending on which one of nine equations was chosen. However, as is explained later, the results for Hunter a*are necessarily as useless as appears at first sight.

It was anticipated that Hunter b* would less useful than Hunter a* because meat is fundamentally red. Hunter b* values represent blueness/yellowness. This proved to be the case (Figures 22, 23, 24, Table 4). The slopes of the lines were lower than for Hunter a*, and the $r^2$ values ranged from 0.017 to 0.688, well below the range for Hunter a*. The worst case relationship (Figure 23) would be entirely useless at predicting mass. Overall, Hunter b* on its own is of no value in predicting meat mass.

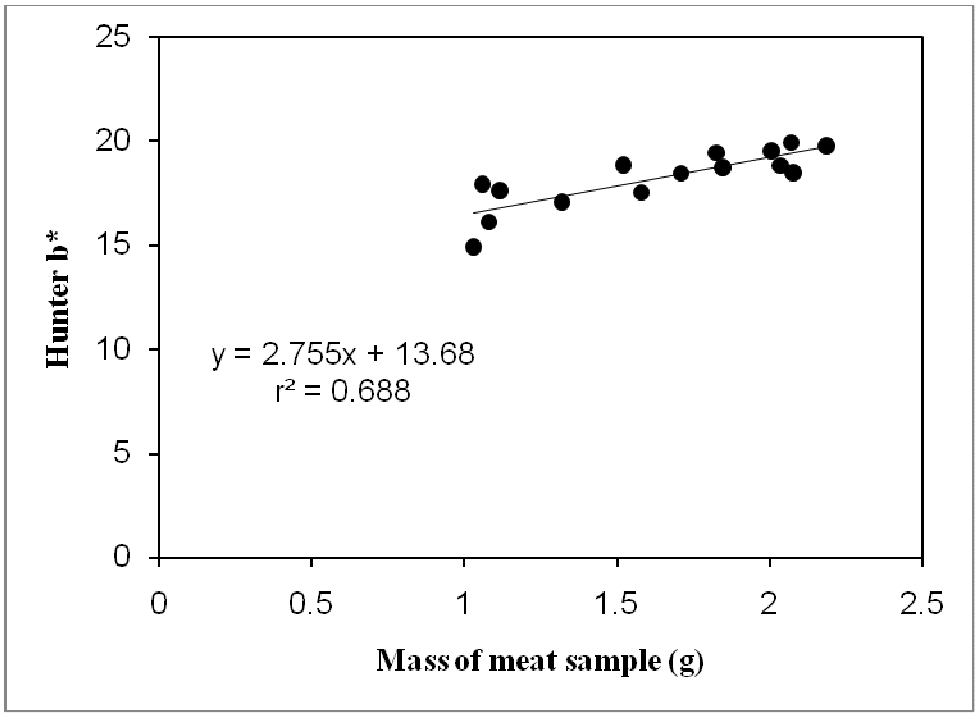



Figure 22. A good linear relationship between mass of meat sample and Hunter b* value

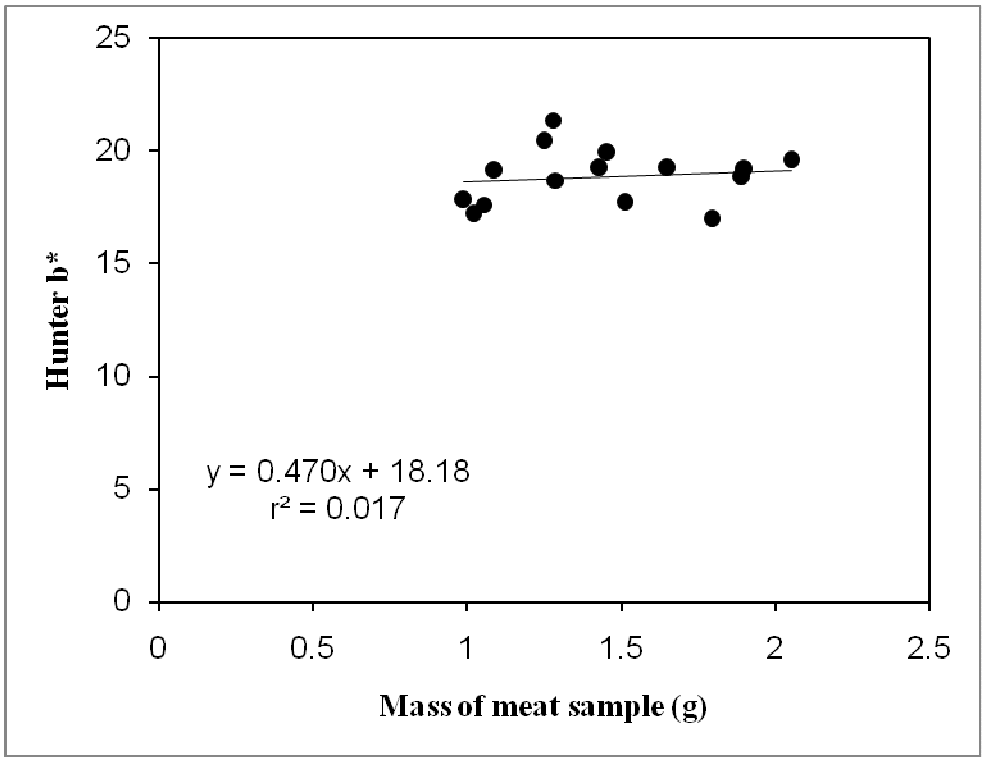



Figure 23. A poor linear relationship between mass of meat sample and Hunter b* value

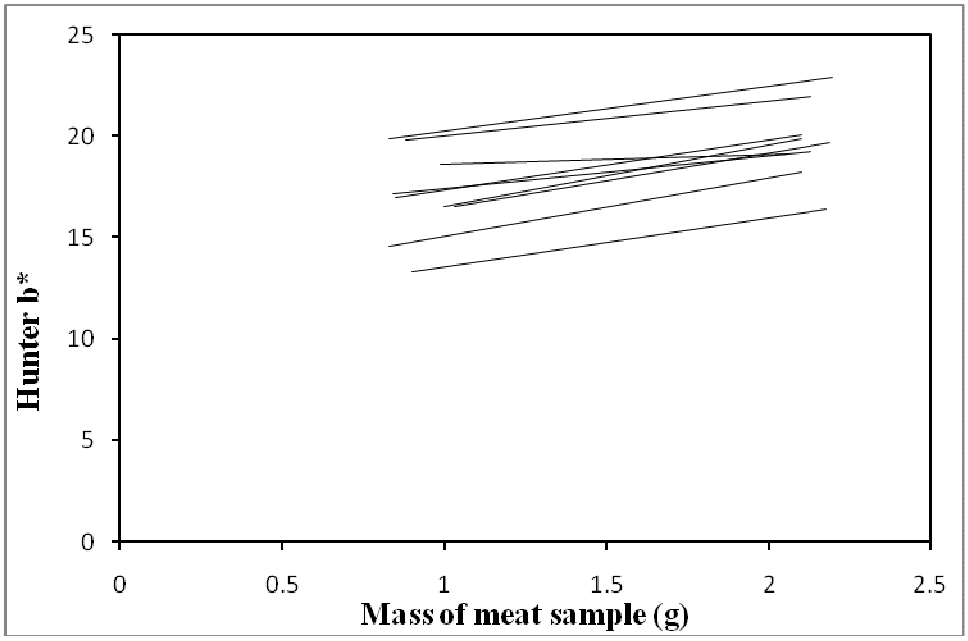



Figure 24. Nine linear relationships between Hunter b* values and mass of meat sample in a buffered slurry

Table 4. Linear relationships between mass of meat sample and Hunter b* value in nine trials.

| Trial number | Predicted value of Hunter b* where x is mass of meat sample | $r^2$ |
|---|---|---|
| 1 | 1.697x + 18.33 | 0.481 |
| 2 | 2.238x + 18.01 | 0.631 |
| 3 | 3.064x + 13.45 | 0.682 |
| 4 | 1.586x + 15.84 | 0.224 |
| 5 | 2.880x + 12.20 | 0.577 |
| 6 | 0.470x + 18.16 | 0.017 |
| 7 | 2.755x + 13.68 | 0.688 |
| 8 | 2.461x + 14.88 | 0.423 |
| 9 | 2.380x + 11.20 | 0.342 |

Turning now to Hunter L*, the linear relationships in all nine trials were essentially flat (Figure 25) and most $r^2$ vales approached zero (Table 5). As for Hunter b*, Hunter L* on its own is of no value in predicting meat mass.

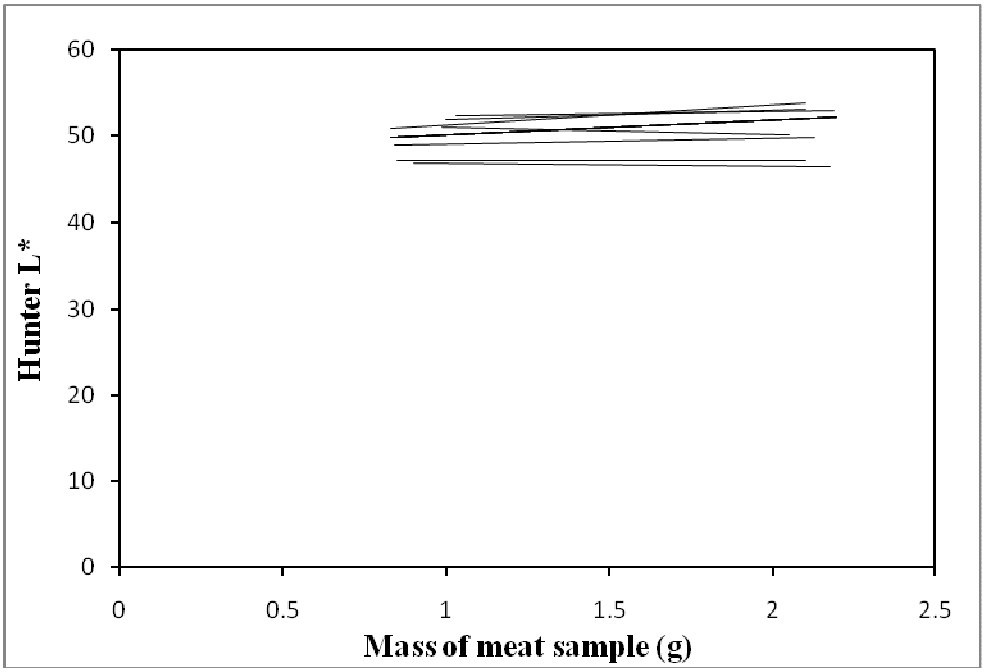



Figure 25. Nine linear relationships between Hunter L* values and mass of meat sample in a buffered slurry

Table 5. Linear relationships between mass of meat sample and Hunter L* value in nine trials

| Trial number | Predicted value of Hunter L* where x is mass of meat sample | $r^2$ |
|---|---|---|
| 1 | 2.388x + 48.81 | 0.193 |
| 2 | 0.443x + 51.91 | 0.007 |
| 3 | 1.092x + 50.73 | 0.021 |
| 4 | -0.892x + 51.90 | 0.013 |
| 5 | 1.739x + 48.38 | 0.092 |
| 6 | 0.670x + 48.36 | 0.004 |
| 7 | -0.062x + 47.17 | 0.00005 |
| 8 | 2.461x + 14.88 | 0.423 |
| 9 | -0.325x + 47.13 | 0.00005 |

***Relationship between mass of meat and R, G and B values of digital images***

The alternative method of measuring colour was by analysis of digital photographs. This was done in five trials. The images were processed by the software program ImageAnalyst.

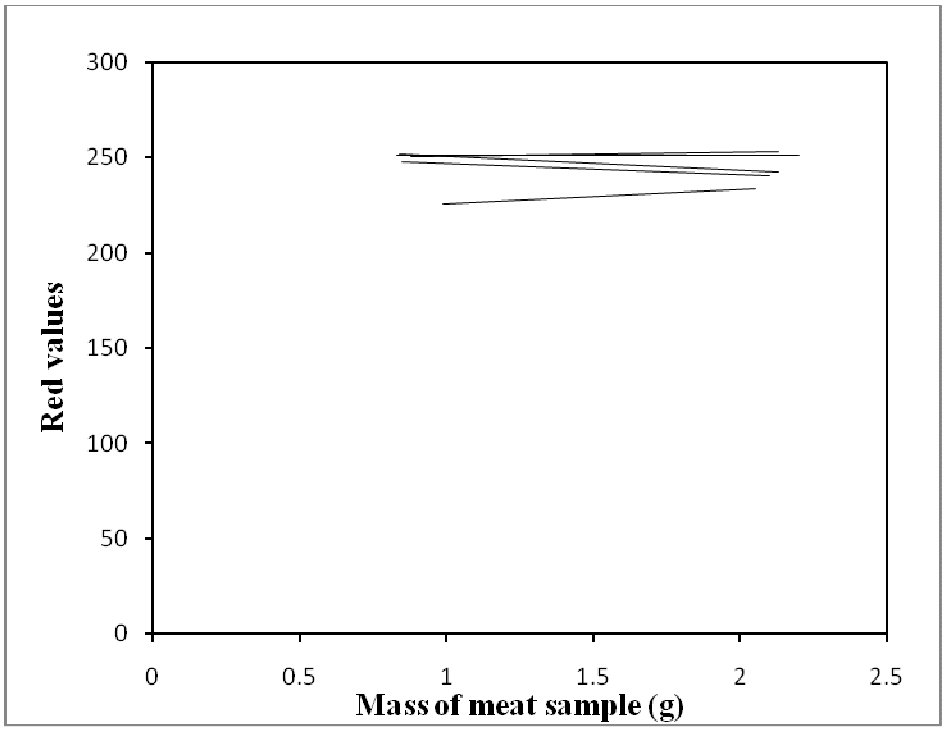



Figure 26. Five linear relationships between R (red) values and mass of meat sample in a buffered slurry

Table 6. Relationships between mass of meat sample and R values in five trials

| Trial number | Predicted value of R where x is mass of meat sample | $r^2$ |
|---|---|---|
| 1 | -1.996x + 249 | 0.097 |
| 2 | 7.832x + 218 | 0.050 |
| 3 | -7.584x + 258 | 0.339 |
| 4 | -5.973x + 253 | 0.288 |
| 5 | -0.255x + 251 | 0.000 |

As Figure 26 shows, the trend lines are close to flat and therefore useless to predict meat mass (by application of inverse equations). The $r^2$ values were low, ranging from 0 to 0.339. However, it is important to note that the values encompassed by the data were all close to 255, the upper limit of redness in RGB colour space. The importance of this is discussed later in this section.

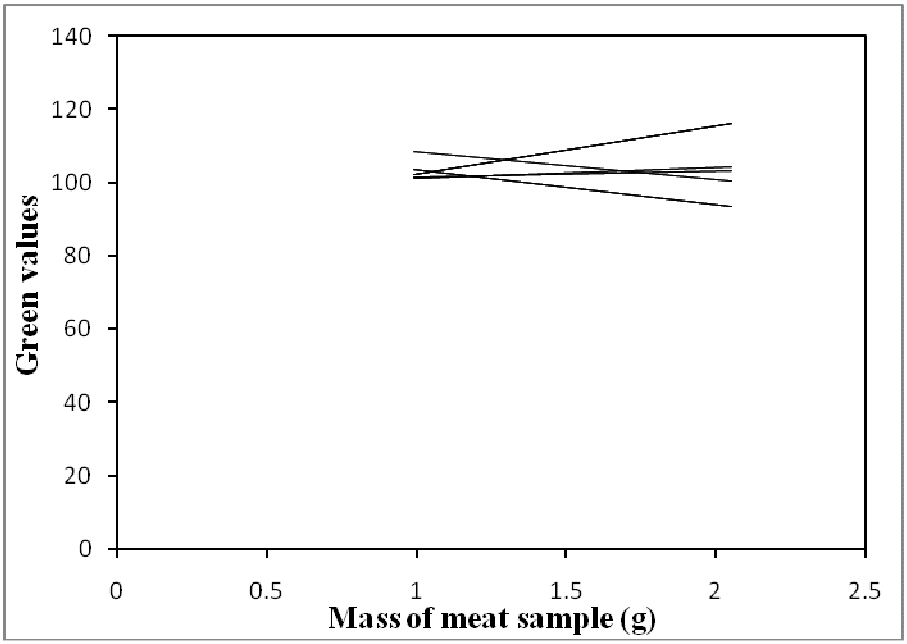



Figure 27. Five linear relationships between G (green) values and mass of meat sample in a buffered slurry

Table 7. Relationships between mass of meat sample and G values in five trials

| Trial number | Predicted value of G where x is the mass of meat sample | $r^2$ |
|---|---|---|
| 1 | -7.457x + 115.8 | 0.050 |
| 2 | 13.38x + 88.88 | 0.065 |
| 3 | 2.819x + 98.39 | 0.013 |
| 4 | -9.492x + 112.9 | 0.059 |
| 5 | 1.709x + 99.79 | 0.001 |

As for Figure 26, Figure 27 shows a flat relationship between G values and mass, and a very low range of $r^2$ values 0.001 to 0.059 (Table 7). Thus, G as recorded in this optical system is of no value in predicting meat mass. The equivalent values for B are shown in Figure 28 and Table 8, with the same outcome: B is of no value.

Colour saturation, which is mathematically derived from R, G and B was similarly of no value (Figure 29, Table 9), as expected.

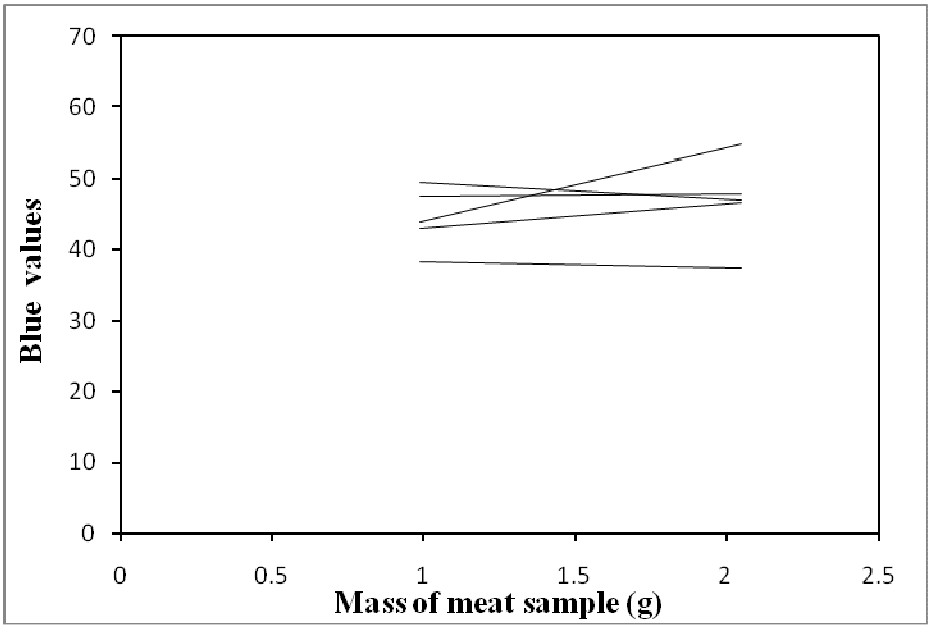



Figure 28. Five linear relationships between B (blue) values and mass of meat sample in a buffered slurry

Table 8. Relationships between mass of meat sample and B value in five trials

| Trial number | Predicted value of B where x is mass of meat sample | $r^2$ |
|---|---|---|
| 1 | -0.919x + 39.26 | 0.002 |
| 2 | 0.330x + 47.32 | 0.000 |
| 3 | 10.17x + 33.84 | 0.159 |
| 4 | -2.234x + 51.69 | 0.032 |
| 5 | 3.446x + 39.62 | 0.031 |

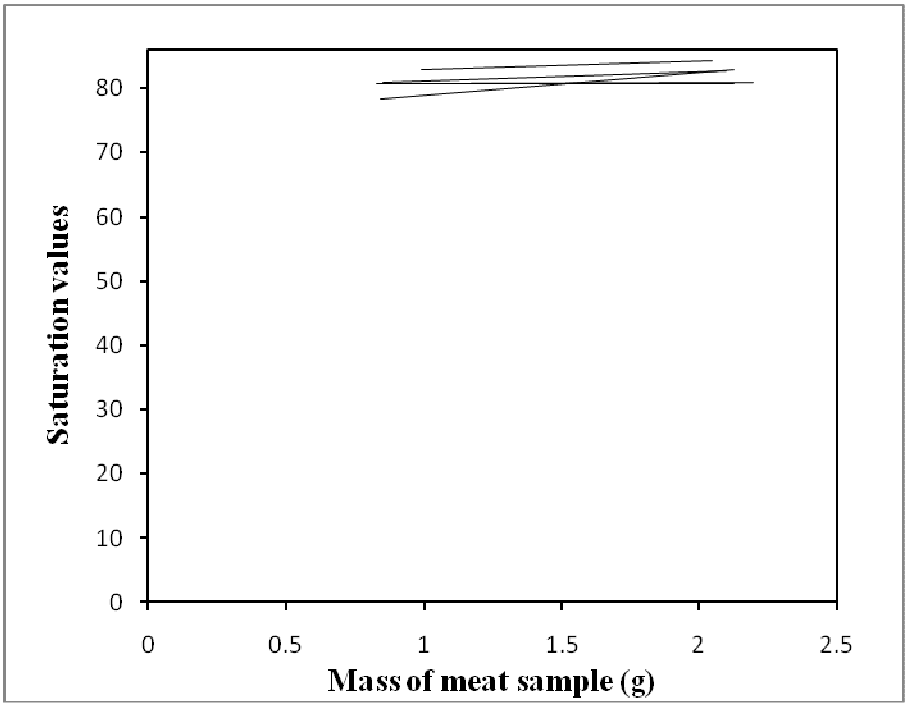



Figure 29. Five linear relationships between saturation values and mass of meat sample in a buffered slurry

Table 9. Relationships between mass of meat sample and Saturation values in five trials

| Trial number | Predicted value of Saturation where x is mass of meat sample | $r^2$ |
|---|---|---|
| 1 | -0.153x + 80.85 | 0.001 |
| 2 | 1.249x + 81.59 | 0.027 |
| 3 | 3.677x + 75.07 | 0.226 |
| 4 | 1.542x + 79.45 | 0.066 |
| 5 | 0.177x + 80.52 | 0.002 |

It should be noted that Figures 26 to 29 are all scaled with zero on the ordinate, which is arguably the most honest way to present these data. It also emphasised the fact that R values are usually around 250, close to the upper limit of 255 (0 to 255 represents $2^8 = 256$). It is possible that the R values, which intuitively would be the most useful in predicting mass, are saturating around 250, and thus might be insensitive to changes in meat mass. If R values were closer to the middle of the range, say 125, sensitivity might have been better and might be manifest as a positive slope. One way of achieving this might be to under expose the image gathering by reducing exposure time from 2.5 seconds to 1.25 seconds. This possibility remains unexplored.

With the existing data set, the digital images would be entirely useless at predicting mass.

***Summary of main outcomes of Phase 1***

Of all the data collected in Phase 1, Hunter a* values were potentially the most useful, because the slopes were positive and $r^2$ were mostly high. However, the values of intercepts were also unacceptably variable between meat samples bought on different days. At first sight, the high variability in intercept values would rule out Hunter a* values as mass predictors. However, as Figure 9 shows in Chapter 1, the extracted sample in the industrial application of the RapidpH method is always taken from one site on the carcass rump, a distinct bulge, and therefore from one site of a single muscle. By contrast, the rump steaks used in the present study were taken from anywhere in the rump (Figure 51 in Chapter 4). Myoglobin content responsible for the red colour of meat varies from muscle to muscle, and this could account for much of the variability in intercept. To test this hypothesis would require sampling from an unvarying site in an unvarying muscle, as is done industrially (Figure 9). This cannot be done without access to an abattoir slaughter line.

## Phase 2

In Phase 1, a constant amount of glucose was included in each slurry. The aim of Phase 2 was to confirm or otherwise that different concentrations of glucose will not affect the colour due to meat. As discussed in Chapter 1, a single muscle from different animals has different concentrations of glycogen (the cause of the high pH condition), which when hydrolysed by the RapidpH enzyme amyloglucosidase yields different concentrations of glucose. Different concentrations of glucose were not expected to affect colour, because glucose is colourless in aqueous solution, and in the high moisture environment of the slurry it would be very unlikely to react with free amine groups in the Maillard reaction at ambient temperature. However, the possibility that glucose could affect colour had to be formally tested.

### *Hunter L*, a* and b* values where meat mass was constant and glucose concentration was variable*

The hypothesis was tested by having equal masses of meat (1.5 ± 0.05 g) dispersed in 8 mL of acetate buffer (7.5 mL + 0.5 mL as before) containing varying glucose concentrations, 0, 3.34 and 6.67 mM. The highest value, 6.67 mM, represents the very highest concentrations of glycogen that is likely to be encountered in the industrial situation.

Trials were done on four days with a different meat purchase on each, each sourced from a different supermarket. On each day five replicate meat masses were dispersed at each of the three glucose concentrations.

Table 10 shows the means and standard deviations for each of the four days testing for significant differences between glucose concentrations.

Table 10. Effect of glucose concentration on Hunter colour values in the presence of 1.5 g of meat. Data are means and standard deviations.

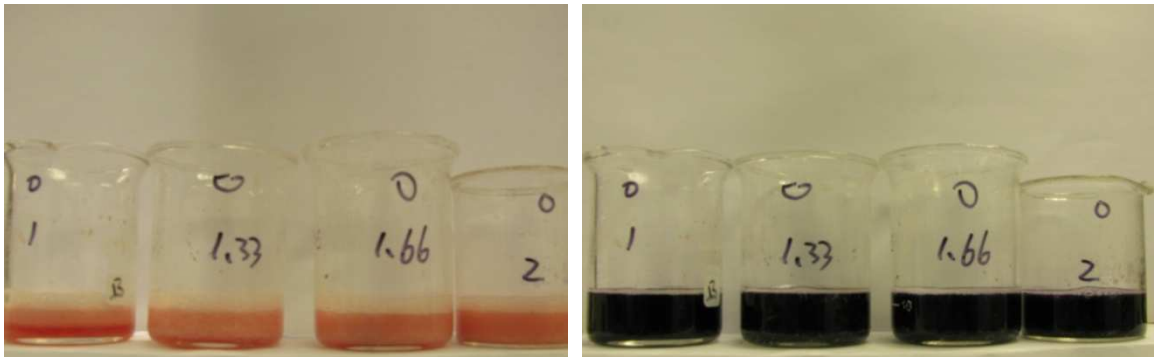
| Hunter values | Day | Glucose concn. (mM) 0 | 3.34 | 6.67 | Statistical effect of glucose concn. (*P*) |
|---|---|---|---|---|---|
| a* | 1 | 3.14 ± 0.49 | 3.38 ± 1.60 | 3.49 ± 1.03 | 0.88 |
| | 2 | 6.32 ± 0.23 | 6.82 ± 0.37 | 7.40 ± 1.05 | 0.07 |
| | 3 | 4.38 ± 0.97 | 4.38 ± 0.17 | 4.55 ± 1.69 | 0.96 |
| | 4 | 5.14 ± 1.02 | 5.30 ± 0.82 | 4.37 ± 0.94 | 0.28 |
| b* | 1 | 15.48 ± 0.81 | 15.42 ± 1.18 | 15.59± 1.04 | 0.96 |
| | 2 | 19.43 ± 0.83 | 19.35 ± 1.00 | 19.15± 1.46 | 0.92 |
| | 3 | 16.81 ± 2.02 | 15.42 ± 1.29 | 17.42± 2.52 | 0.31 |
| | 4 | 18.72 ± 0.49 | 18.90 ± 0.93 | 18.68± 1.23 | 0.93 |
| L* | 1 | 50. 79 ± 2.37 | 49.66 ± 2.37 | 49.66± 2.37 | 0.65 |
| | 2 | 51.45 ± 2.92 | 48.33 ± 1.48 | 46.92± 0.99 | 0.10 |
| | 3 | 52.39 ± 2.06 | 49.04 ± 2.33 | 47.16± 0.95 | 0.20 |
| | 4 | 46.30 ±2.34 | 46.44 ± 2.34 | 47.76± 2.21 | 0.51 |

In all cases' *P* is more than 0.05, so the hypothesis that varying glucose concentrations (0, 3.34 and 6.67mM) do not affect colour readings where meat mass was constant is supported.

## Phase 3

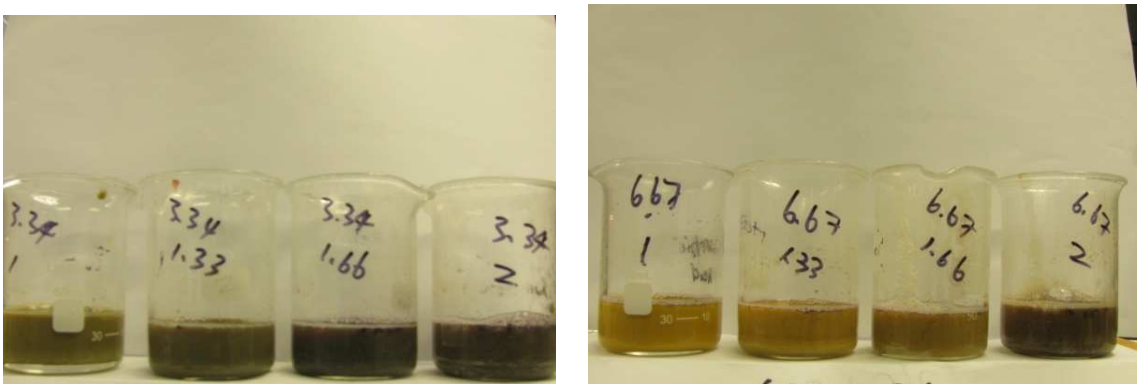
Phase 1 established that although there was considerable variation among to animals and in the position of sampling with the rump cut, Hunter a* values showed some potential in predicting mass, because in the industrial application sampling is always taken from one site in one muscle within the rump. Phase 2 indicated that the concentration of glucose did not affect meat colour. It remained to develop a colorimetric method to quantify glucose concentration and thus glycogen concentration. This is the aim of Phase 3: to explore the relationship between glucose concentration – 0 to 6.67 mM – and colour change due to the Fehlings reaction induced in a microwave oven. (Zero to 6.67 mM covers the glucose concentration range derived from glycogen expected in the industrial application.)

Lei (2009) showed that a final volume of around 15 mL (meat + 8 mL buffer + 8 mL Fehlings) would require about 30 seconds of microwave heating to a temperature that would usefully accelerate the reaction. Subsequently, four mass categories of meat from four supermarket purchases (from different supermarkets, different days) were selected: 1.00, 1.33, 1.66 and 2.00 g to a precision of ± 0.05 g. In each of these categories, single slurries were prepared each containing three concentrations of glucose as in Phase 2: 0, 3.34 and 6.67 mM. Colours were developed with Fehlings by microwave heating for 30 seconds on full power and the colour measured by the Hunter meter.




0mM 0mM

Figure 30. The relationship between zero concentration of glucose with 1.00, 1.33, 1.66 and 2.00 g meat samples and colour change due to the Fehling reagent. The left photograph shows the original slurries, and the right photograph shows the result after addition of Fehlings solution and microwave heating.




3.34mM 6.67mM

Figure 31. The relationship between 3.34 and 6.67 mM concentrations of glucose with 1.00, 1.33 1.66 and 2.00 g meat samples and colour change due to the Fehling reagent after heating. The left photograph shows the colour development with 3.34 mM glucose and the right with 6.67 mM.

Figure 30 (right) shows that in the absence of added glucose, the colour due to different masses of meat and Fehlings solution after heating was essentially constant. When glucose was present however (Figure 31), the blue Fehlings colour was lost, yielding a $Cu^{+1}$ yellow pigment, particularly when the quantity of mass of meat added was low (1.00 g in Figure 31, left and right). This means that the colour development due to glucose and Fehlings was affected by the quantity of meat in the slurry.

The following graphs (Figures 32, 33 and 34) show the effect of glucose concentration after heating on Hunter values when meat mass was 1.00, 1.33, 1.66 and 2.00 g.

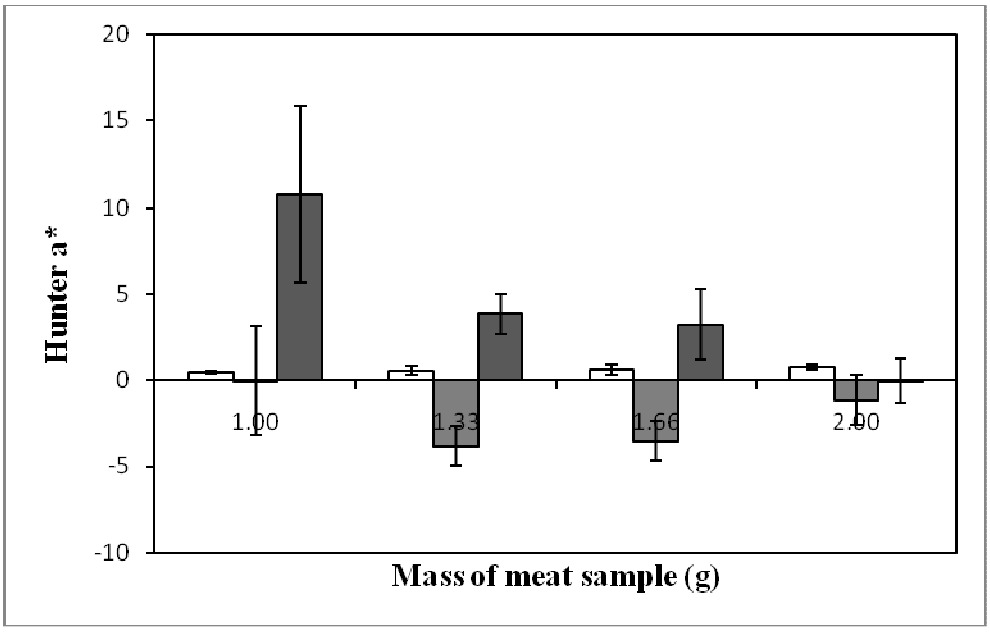



Figure 32. Effect of glucose concentration on Hunter a* values when meat mass was 1.00, 1.33, 1.66 and 2.00 g. Column heights are means of four meat replicates and bars are standard deviations. The white bar displays 0.00 mM glucose, the light grey bar 3.34 mM and the dark grey bar displays 6.67 mM.

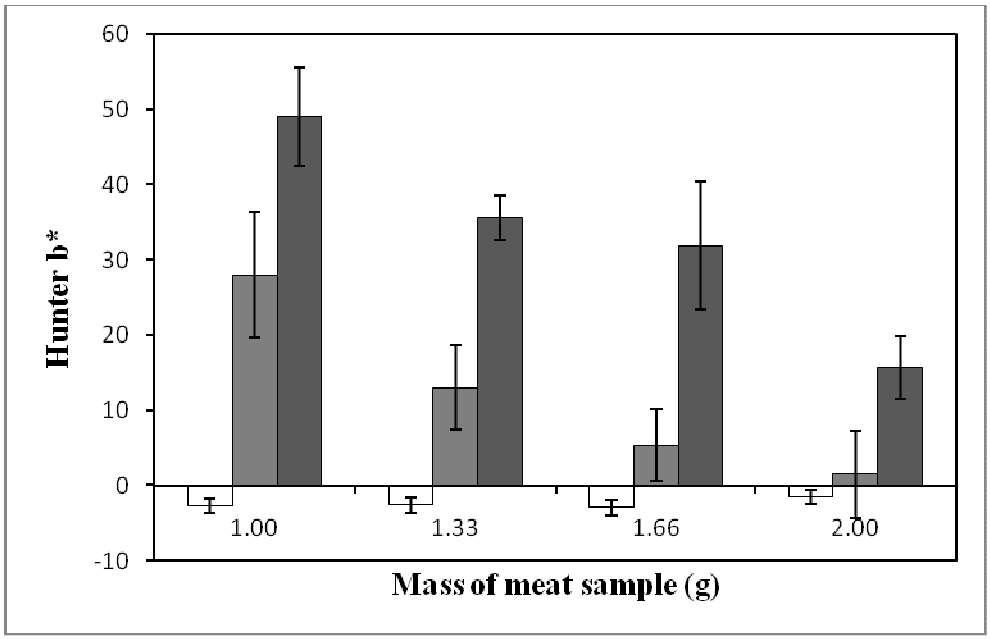



Figure 33. Effect of glucose concentration on Hunter b* values when meat mass was 1.00, 1.33, 1.66 and 2.00 g. Column heights are means and bars are standard deviations. The white bar displays 0.00 mM glucose, the light grey bar 3.34 mM and the dark grey bar displays 6.67 mM.

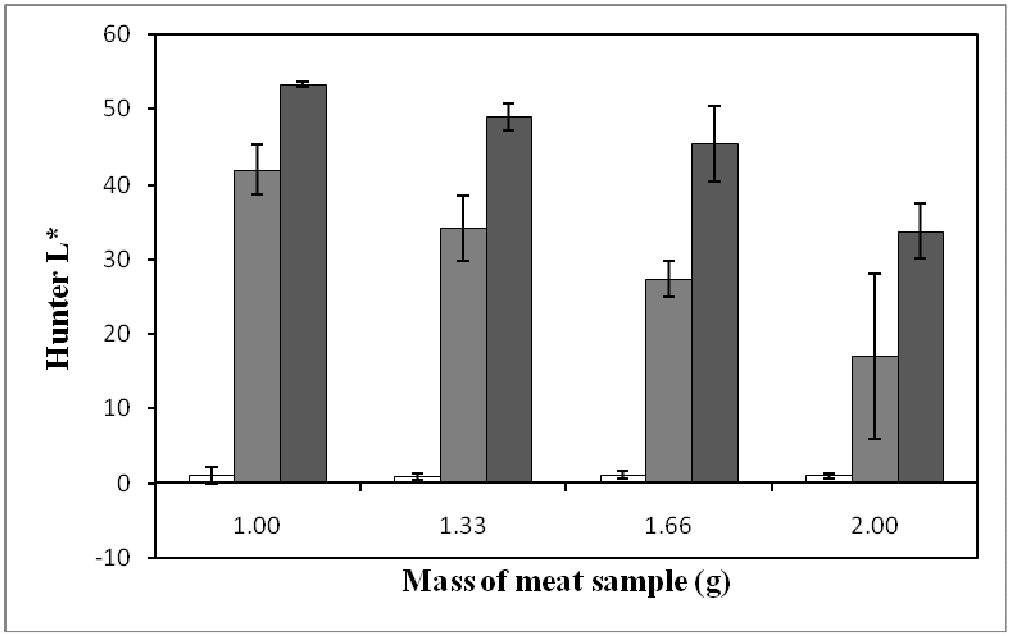



Figure 34. Effect of glucose concentration on Hunter L* values when meat mass was 1.00, 1.33, 1.66 and 2.00 g. Column heights are means and bars are standard deviations. The white bar displays 0.00 mM glucose, the light grey bar 3.34 mM and the dark grey bar displays 6.67 mM.

At the two glucose concentrations above zero, Hunter b* and L* decreased with increased mass of meat. At 0.00 mM the situation was essentially static. At the highest concentration of glucose (6.67 mM), Hunter a* values decreased with increased mass of meat, as was observed for Hunter b* and L*. However, 0 and 3.34 mM glucose, the situation was more complicated. Overall the data show that on heating under these very alkaline conditions, some component(s) in the meat competes with $Cu^{2+}$ for reaction. That component(s) is likely to be amine groups, abundant in meat proteins, which can participate in the first stages of the Maillard reaction (Hodge 1953).

It was initially hoped that the quantity of meat in the slurry would not affect colour development due to glucose but clearly that was not the case. However, it might be possible to linearly relate the colour values to the parameter of fundamental interest, mass of glucose per mass of meat (Young and others 2004). Possible relationships were explored with the multiple regression function in Minitab (Table 11). Table 11 is summary of the Minitab output and the full analysis is shown in the Appendix.

In the industrial situation the predicted value of commercial interest is mass of glucose (glycogen) per mass of meat. This value is dimensionless. In Table 11, the solutions to the equations – which are not shown – have units of mM $g^{-1}$. This does not matter because volumes are very close to identical in all trials (Figure 32, 33 and 34).

Table 11. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0361 | | | 0.203 | 6.4 | 0.030 | 39.1 |
| b* alone | | 0.0119 | | 0.0688 | 157 | < 0.001 | 94 |
| L* alone | | | 0.0103 | -0.0255 | 130 | < 0.001 | 92.9 |
| a*, b* and L* combined | 0.0325 | -0.0101 | 0.0160 | -0.0580 | 392 | < 0.001 | 99.3 |

What is important in Table 11 are the statistics. a* alone was not a particularly good indicator of concentration of glucose/mass of meat. F was a low 6.4, and although the P value was significant at 0.030, only 39.1% of the variation in concentration of glucose/mass of meat could be explained by Hunter a*. This was not surprsing because a* measures redness/greenness, a range that is not obvious to the eye in Figures 30 and 31. b* was much more useful as was expected because of the yellow/blue colour range in Figures 30 and 31. The surprise was L*. The purported $Cu_2O$ precipitate was highly reflective, yielding an F value of 130, a $P < 0.001$ and an $r^2$ (%) is 92.9. Of some interest was the colour of the precipitate. The literature describes $Cu_2O$ as being red. However, it was clearly more yellow than red in this particular situation as is obvious from Figure 31 and the predictive value of b* in Table 11. Combining all the Hunter values was the most useful: F was 392, *P* was < 0.001 and $r^2$ (%) was 99.3.

In this approach the mass of meat was known to high precision, 1.00 to 2.00 g, because it was weighed rather than predicted by colour. Phase 1 showed that colour was not particularly useful in predicting mass so if redness had been used instead of known mass 1.00, 1.33 etc., then the predictive values of the equations would have been worse.

Accepting this limitation in the meantime, Phase 4 extends Phase 3 work by selecting randomly chosen but defined masses of meat, and similarly chosen defined concentrations of glucose to test the predictive value of linear equations such as in Table 11.

## Phase 4

The aim of Phase 4 was to randomly choose defined masses of meat between 0.8 and 2.15 g, and similarly defined concentrations of glucose between 0 and 6.67 mM to test the predictive value of equations between concentration of glucose/mass of meat and various Hunter colour values. Concentration of glucose/mass of meat after heating is directly proportional to the value of commercial interest: mass of glycogen/mass of meat). This was done of each of four rump steaks bought on different days.

For a given steak there were 30 concentration-mass combinations. On each of four days two columns of random numbers were generated in Excel using the function RANDBETWEEN (1, 7) for glucose concentration and RANDBETWEEN(1,10) for meat mass (Figure 35). These random numbers dictated each concentration-mass combination. For example, in Combination 1, the concentration of glucose was 0.111 mM and the mass of meat was 1.10 ± 0.05 g. The mixtures were dispered with the Ultraturrax, the slurry mixed with Fehlings solution and the colour developed in the microwave oven.

| Concn (final in 8 mL) (mM) | Code number glucose | Meat mass (g) | Code number meat | Combination number | Random code number glucose | Random code number meat |
|---|---|---|---|---|---|---|
| 0.000 | 1 | 0.80 | 1 | 1 | 2 | 3 |
| 0.111 | 2 | 0.95 | 2 | 2 | 5 | 6 |
| 0.222 | 3 | 1.10 | 3 | 3 | 1 | 10 |
| 0.334 | 4 | 1.25 | 4 | 4 | 3 | 2 |
| 0.445 | 5 | 1.40 | 5 | 5 | 1 | 10 |
| 0.556 | 6 | 1.55 | 6 | 6 | 6 | 2 |
| 0.667 | 7 | 1.70 | 7 | 7 | 1 | 1 |
| | | 1.85 | 8 | 8 | 6 | 5 |
| | | 2.00 | 9 | 9 | 7 | 8 |
| | | 2.15 | 10 | 10 | 5 | 10 |
| | | | | 11 | 7 | 8 |
| | | | | 12 | 7 | 5 |
| | | | | 13 | 5 | 3 |
| | | | | 14 | 6 | 8 |
| | | | | 15 | 5 | 1 |
| | | | | 16 | 5 | 4 |
| | | | | 17 | 3 | 2 |
| | | | | 18 | 2 | 7 |
| | | | | 19 | 6 | 1 |
| | | | | 20 | 7 | 5 |
| | | | | 21 | 1 | 9 |
| | | | | 22 | 7 | 5 |
| | | | | 23 | 3 | 5 |
| | | | | 24 | 6 | 2 |
| | | | | 25 | 7 | 4 |
| | | | | 26 | 5 | 9 |
| | | | | 27 | 3 | 10 |
| | | | | 28 | 3 | 6 |
| | | | | 29 | 4 | 4 |
| | | | | 30 | 1 | 3 |

Figure 35. Excel spreadsheet output used to select 30 combinations of concentration and meat mass. The random numbers generated in Excel were different for each of the four days. See the text for more details

Preliminary inspection of the data by plotting colour values against concentration of glucose/mass of meat, exemplified by Figures 36 to 41, showed that a* and b* plots were possibly linear, and that L* plots were possibly curvilinear. For simplicity all the relationships were considered as linear to a first approximation. Tables 12, 13, 14 and 15 show the coefficients of linear equations developed between a*, b* and L* (and their linear combination) and concentration of glucose/mass of meat.

Table 12. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating (Day 1)

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0189 | | | 0.2240 | 29.6 | <0.001 | 51.4 |
| b* alone | | 0.00936 | | 0.0954 | 262.3 | <0.001 | 90.4 |
| L* alone | | | 0.00965 | -0.0034 | 104.8 | <0.001 | 78.9 |
| a*, b* and L* combined | 0.0165 | -0.00345 | 0.0105 | -0.0105 | 286.5 | <0.001 | 97.1 |

Table 13. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating (Day 2)

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0281 | | | 0.2190 | 12.9 | 0.001 | 31.6 |
| b* alone | | 0.00946 | | 0.0999 | 210.7 | <0.001 | 88.3 |
| L* alone | | | 0.00916 | -0.0089 | 243.6 | <0.001 | 89.7 |
| a*, b* and L* combined | 0.00816 | 0.00119 | 0.00746 | 0.0110 | 121.4 | <0.001 | 93.3 |

Table 14. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating (Day 3)

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0214 | | | 0.232 | 18.3 | <0.001 | 39.6 |
| b* alone | | 0.00883 | | 0.137 | 133.9 | <0.001 | 82.7 |
| L* alone | | | 0.00959 | 0.0193 | 116.0 | <0.001 | 80.6 |
| a*, b* and L* combined | 0.0135 | -0.00156 | 0.00943 | 0.0210 | 74.1 | <0.001 | 89.5 |

Table 15. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating (Day 4)

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0239 | | | 0.223 | 19.8 | < 0.001 | 36.8 |
| b* alone | | 0.00888 | | 0.126 | 115.8 | < 0.001 | 77.3 |
| L* alone | | | 0.00896 | 0.0103 | 100.9 | < 0.001 | 74.8 |
| a*, b* and L* combined | 0.0149 | -0.00142 | 0.00874 | 0.0186 | 54.4 | < 0.001 | 83.6 |

Inspection of the *P* and $r^2$ values in these tables shows that Hunter b* was, with one exception (Table 13), the single most useful colour parameter to predict concentration of glucose/mass. Hunter a* was the worst predictor, and L* $r^2$ values were close to those of b*. The combined linear function with a*, b* and L* was always the best predictor. The best data were obtained on Day 1, and the worst on Day 4. These are illustrated in Figures 36 to 41.

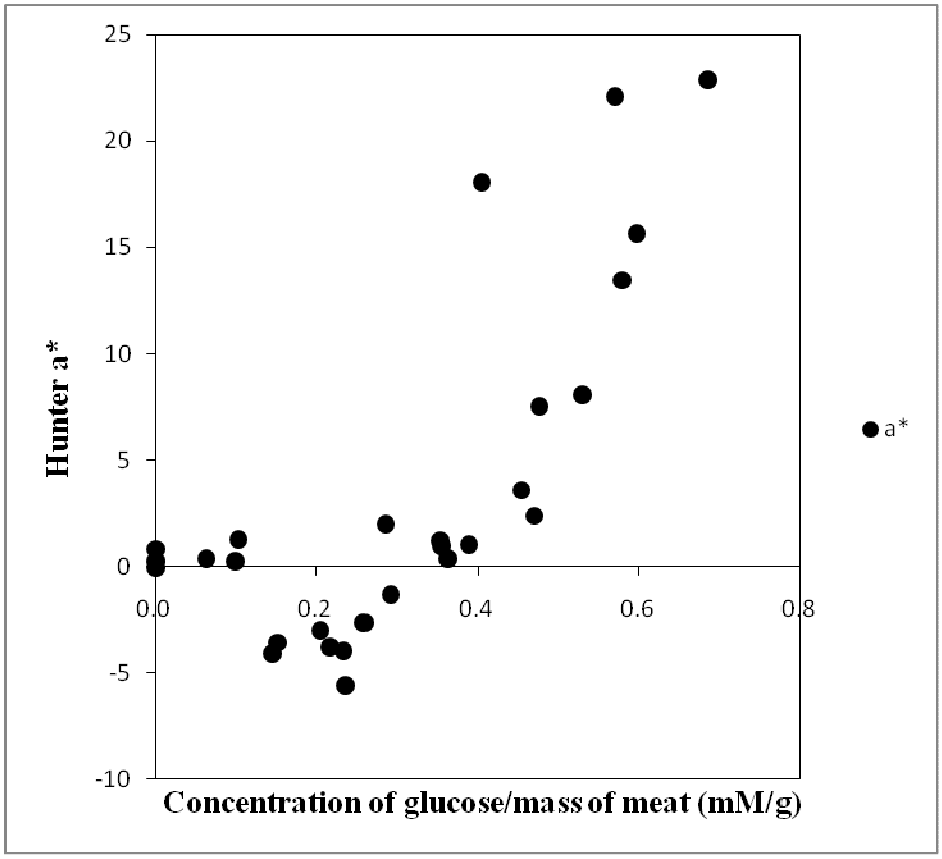



Figure 36. The relationship between Hunter a* and concentration of glucose/mass of meat after microwave heating (Day 1)

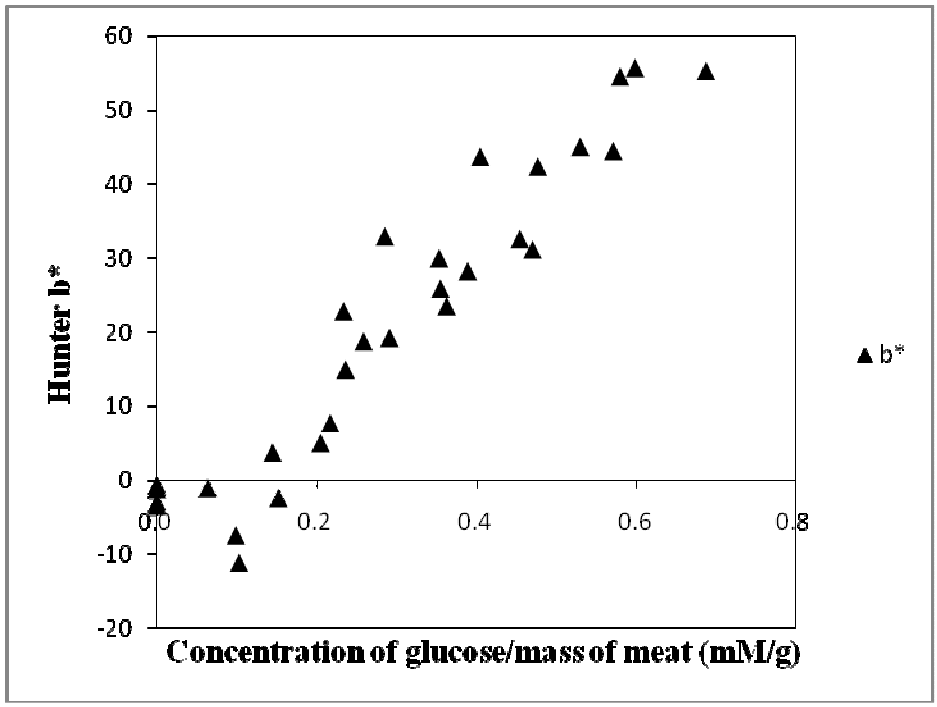



Figure 37. The relationship between Hunter b* and concentration of glucose/mass of meat after microwave heating (Day 1)

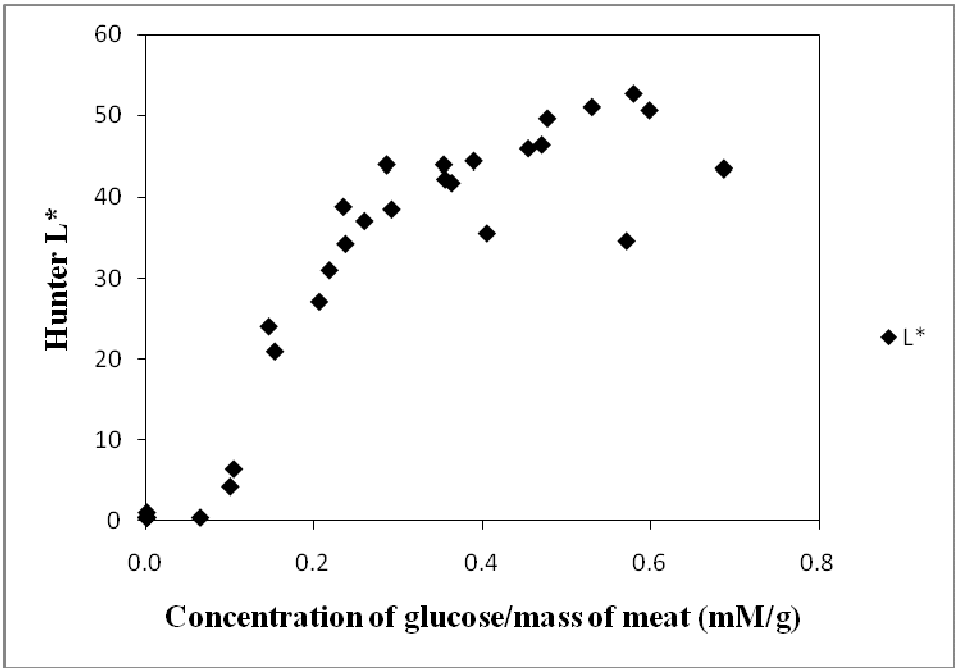



Figure 38. The relationship between Hunter L* and concentration of glucose/mass of meat after microwave heating (Day 1)

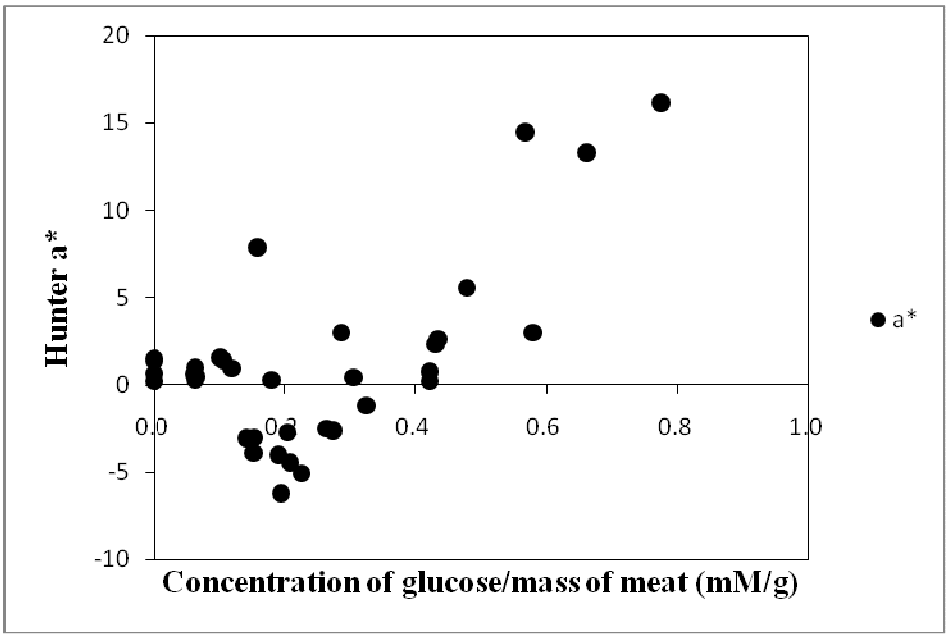



Figure 39. The relationship between Hunter a* and concentration of glucose/mass of meat after microwave heating (Day 4)

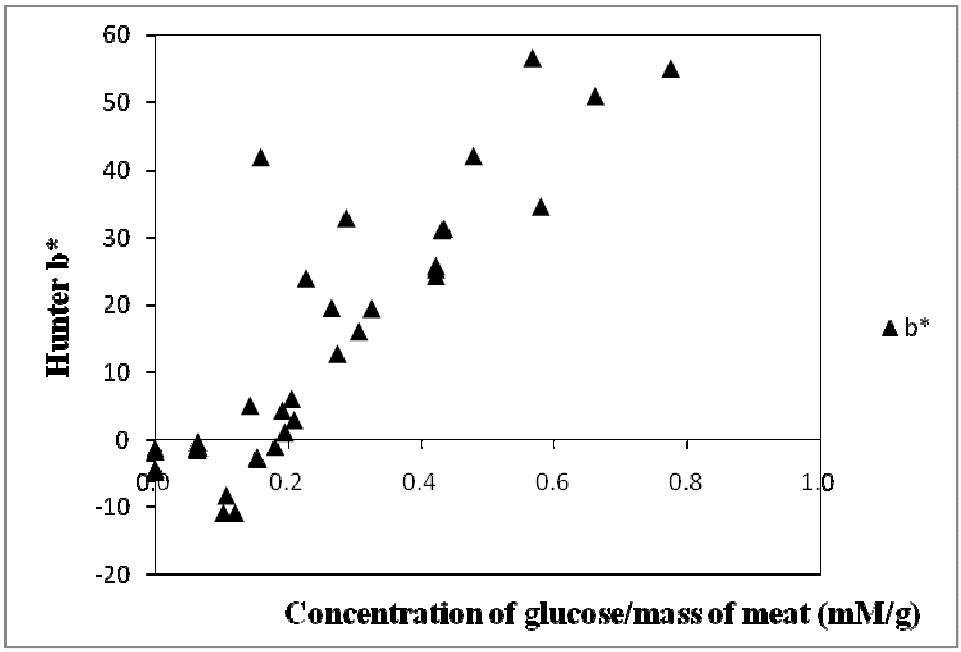



Figure 40. The relationship between Hunter b* and concentration of glucose/mass of meat after microwave heating (Day 4)

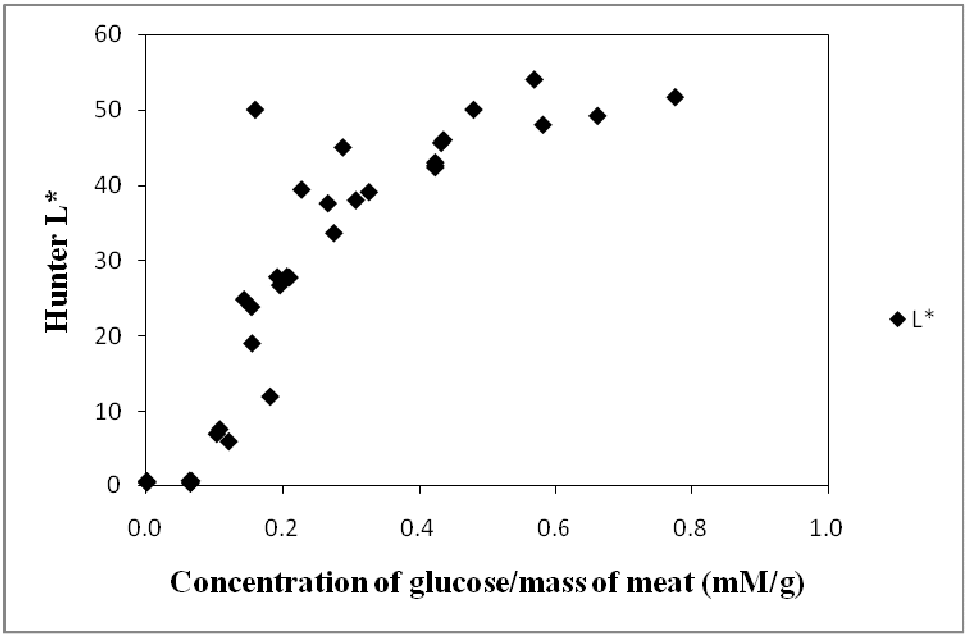



Figure 41. The relationship between Hunter L* and concentration of glucose/mass of meat after microwave heating (Day 4)

Figures 42 to 44 show plotted data combined across all four days and Table 16 shows the regression with the Hunter values and their combination. Unlike the results for a* values to predict meat mass in Phase 1 (Figure 21), the data in Figure 42 to 44 were tightly clustered, suggesting that it may not be required to measure the mass of meat by colour or direct weighing before the development of colour due to glucose and the Fehlings' reagent.

(Recall that the between-animal variation was high in Figure 21, probably arising in part from different locations of muscle in the rump.) In other words, information about the mass of meat in each slurry may be inherent in the colour of the heated slurries. However, there is no obvious physical model to explain how this could be the case. Equally, the choice of meats on the four days in Phase 4 may have by chance resulted in very similar reflectance for equal mass. These issues are further discussed in Chapter 4 as they are fundamentally important to the development of this method.

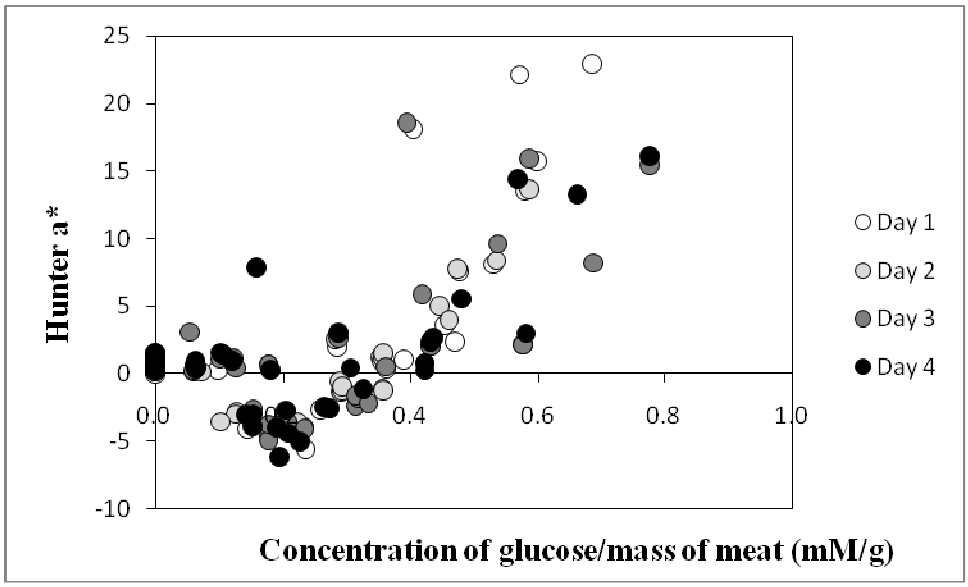



Figure 42. The relationship between Hunter a* and concentration of glucose/mass of meat after microwave heating, all four days combined

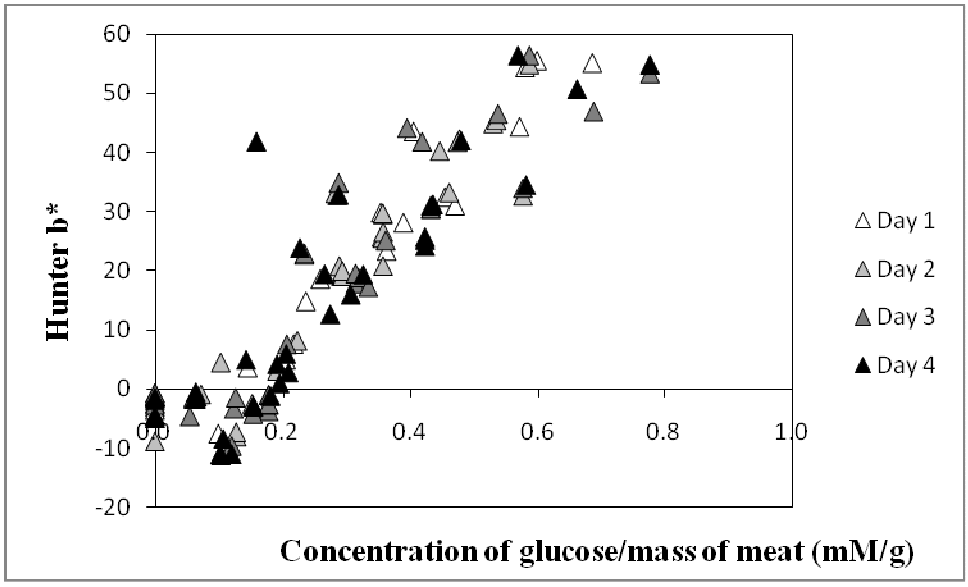



Figure 43. The relationship between Hunter b* and concentration of glucose/mass of meat after microwave heating, all four days combined

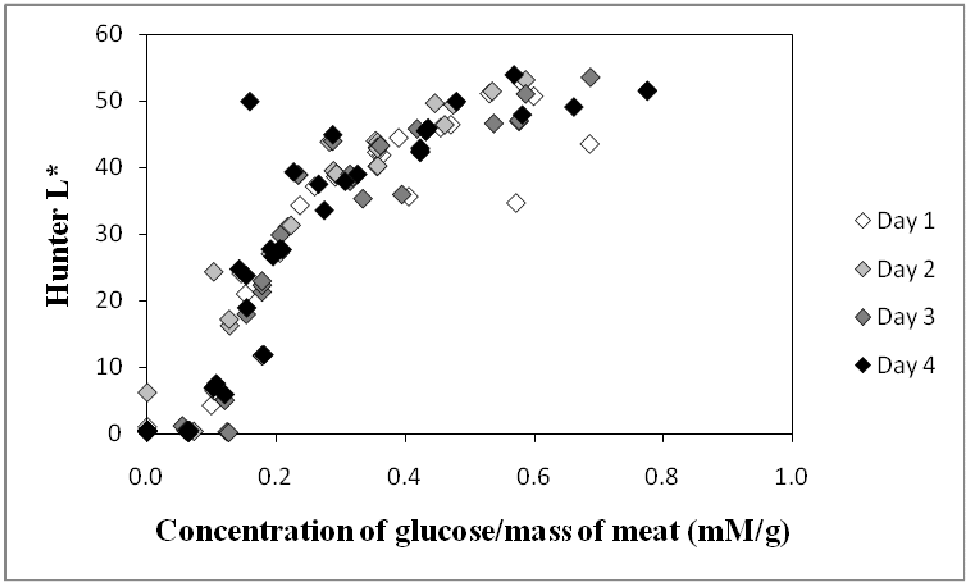



Figure 44. The relationship between Hunter L* and concentration of glucose/mass of meat after microwave heating, all four days combined

Table 16. Usefulness of linear equations with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating, all 4 days combined

| Value used | Coefficients | | | | Statistical values | | |
|---|---|---|---|---|---|---|---|
| | a* | b* | L* | Constant | F | *P* | $r^2$ (%) |
| a* alone | 0.0215 | | | 0.225 | 81.2 | < 0.001 | 39.6 |
| b* alone | | 0.00905 | | 0.117 | 643.7 | < 0.001 | 83.8 |
| L* alone | | | 0.00930 | 0.0054 | 494.9 | < 0.001 | 80.0 |
| a*, b* and L* combined | 0.0139 | -0.00148 | 0.00910 | 0.00097 | 369.6 | < 0.001 | 90.1 |

Inspection of the preceding graphs in Phase 4 shows that when glucose concentration/mass of meat is 0.0, there is often a 'kink' or 'tail' in the distribution of data points. This is particularly obvious in Hunter L* values as can be seen in Figure 38 for example, and possibly contribute to lower $r^2$ values than if those 0.0 data points were ignored. It is valid to ignore these points if required because the situation of zero glucose concentration can never exist in the real world of slaughter animals. Therefore the regression statistics were recalculated ignoring the 0.0 data and compared with the results in Table 16 (Table 17).

| Table 17. | $r^2$ with a*, b* and L* to predict concentration of glucose/mass of meat after microwave heating, all 4 days combined with and without 0.00 data |
|---|---|

| Value used | Statistical values | |
|---|---|---|
| | $r^2$ (%) with 0.00 data | $r^2$ (%) without 0.00 data |
| a* alone | 39.6 | 40.9 |
| b* alone | 83.8 | 83.7 |
| L* alone | 80.0 | 78.4 |
| a*, b* and L* combined | 90.1 | 89.4 |

Table 17 shows that $r^2$ values remained similar for all values used, and thus there was no advantage to be gained by ignoring 0.00 values. However, while that is true for linear equations, there may be an advantage for ignoring 0.00 values if non-linear equations are fitted, in particular for Hunter L* where the true line of best fit is probably curvilinear . This can be seen by comparing Figures 44 and 45.

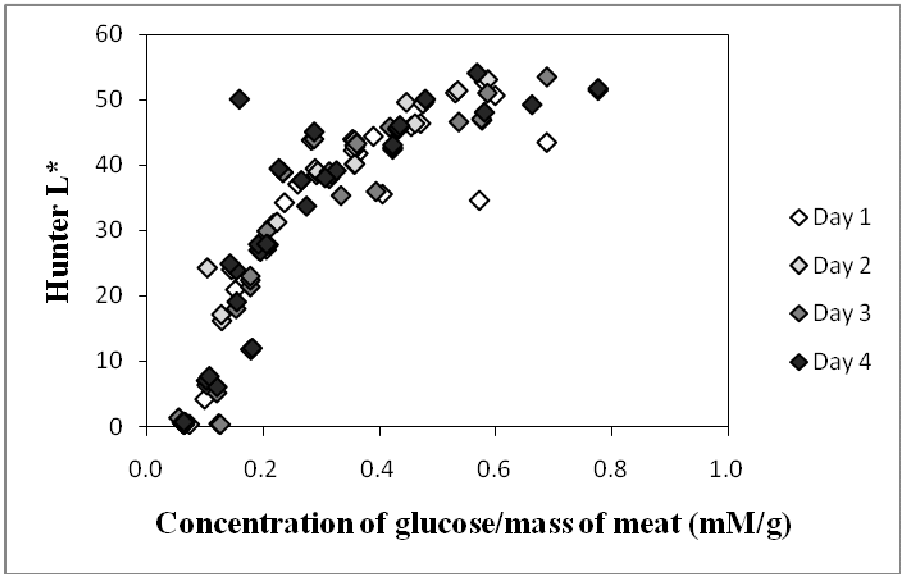



Figure 45. The relationship between Hunter L* and concentration of glucose/mass of meat after microwave heating ignoring 0mM data, all four days combined

**Phase 5**

Phase 4 showed that over four days b*, L* and a linear combination of a*, b* and L* after heating were useful predictors of concentration of glucose/mass of meat, and implied that it may not be necessary to measure the mass of meat. However, it seems intuitive that colour information from before heating could add to predictive value. In particular it was thought that ratios of Hunter colour values might compensate for variation and thus error in mass prediction. For example, the function 'b* after heating/a* before heating' might contain information about mass of meat in both parts of the function, and would cancel this variation by division.

Limited data were available to test this hypothesis. In Phase 3 where meat from four days was used to measure colour of slurries after heating, on Day 1, colour before heating was also measured. This limited data set was used in Phase 5.

The aim of Phase 5 was to choose defined masses of meat, 1, 1.33, 1.66 and 2 g, and similarly defined concentrations of glucose between 0 and 6.67 mM to compare the predictive value of absolute Hunter values after heating, with the predictive value of Hunter value ratios before and after heating. In the case of absolute Hunter values after heating there are three basic equations: a*, b* and L* each regressed on concentration of glucose/mass of meat (Table 18). There are many more possibilities with ratios. These can be calculated in two fundamentally different ways: after heating/before heating, and before heating/after heating. For the ratios after heating/before heating, there are nine possible ratios from the three primary values L*, a* and b*. However, a*/b* for example is simply the inverse of b*/a*. Thus the possibilities contract to six (Table 19). Likewise for before heating/after heating there are six possibilities.

Table 18, from the one meat selection, shows that Hunter values L* and b* were potentially useful in predicting concentration of glucose/mass of meat. Hunter a* was not useful, and these results general concur with the outcomes of Phase 4.

| Table 18. | Predictive $r^2$ values from linearly regressing ratios of Hunter values on concentration of glucose/mass of meat after heating |
|---|---|

| Hunter values | Statistics |
|---|---|
| | $r^2$ (%) |
| L* | 0.89 |
| a* | 0.05 |
| b* | 0.87 |

The results in Table 18 can be compared with the ratio results in Table 19 where ratios containing a* also had poor predictive value compared to ratios with L* and b* but no a*. However, there was no improvement in $r^2$ values by using the ratios, after heating/before heating and before heating/after heating. Further, only after heating/before heating ratios were useful.

Figures 46 to 50 show the four best correlations and the worst, the latter showing how useless some ratios were.

| Table 19. | Predictive $r^2$ values from linearly regressing ratios of Hunter values on concentration of glucose/mass of meat |
|---|---|

| Ratio of values | Statistics | |
|---|---|---|
| | $r^2$ (%) from after heating/before heating | $r^2$ (%) from before heating/after heating |
| L*/b* | 0.86 | 0.36 |
| a*/b* | 0.05 | 0.24 |
| L*/a* | 0.67 | 0.03 |
| b*/b* | 0.86 | 0.33 |
| a*/a* | 0.04 | 0.01 |
| L*/L* | 0.88 | 0.28 |

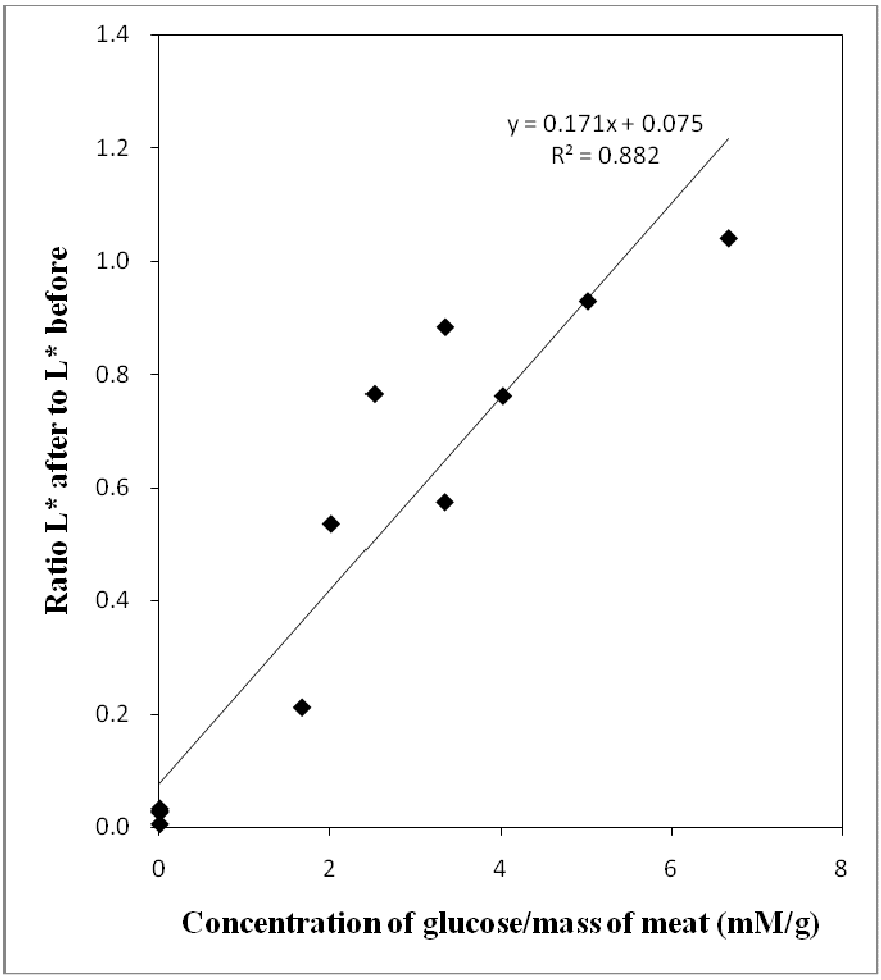



Figure 46. The relationship between Ratio L* after heating /L* before heating and concentration of glucose/mass of meat

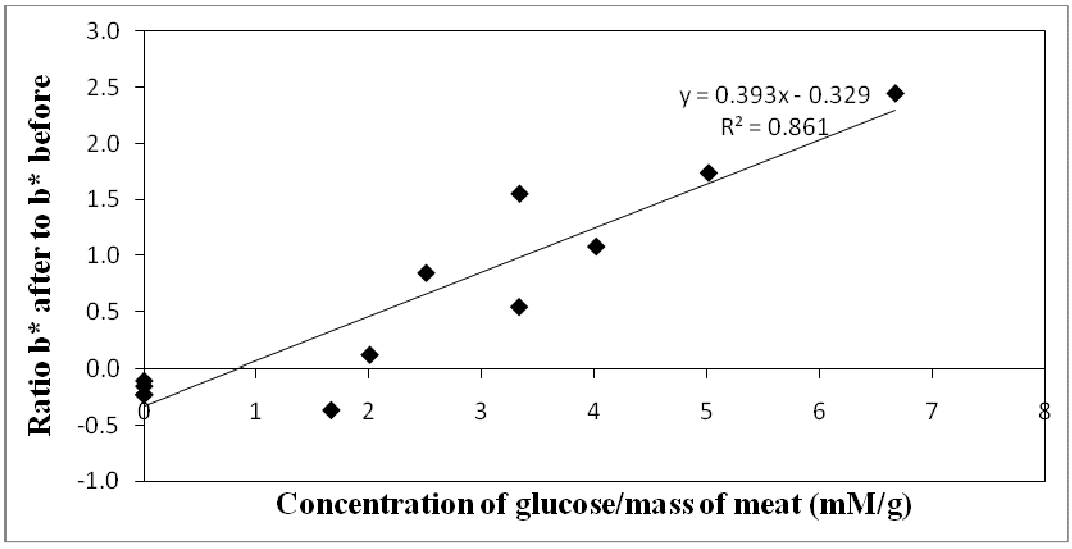



Figure 47. The relationship between Ratio b* after heating /b* before heating and concentration of glucose/mass of meat

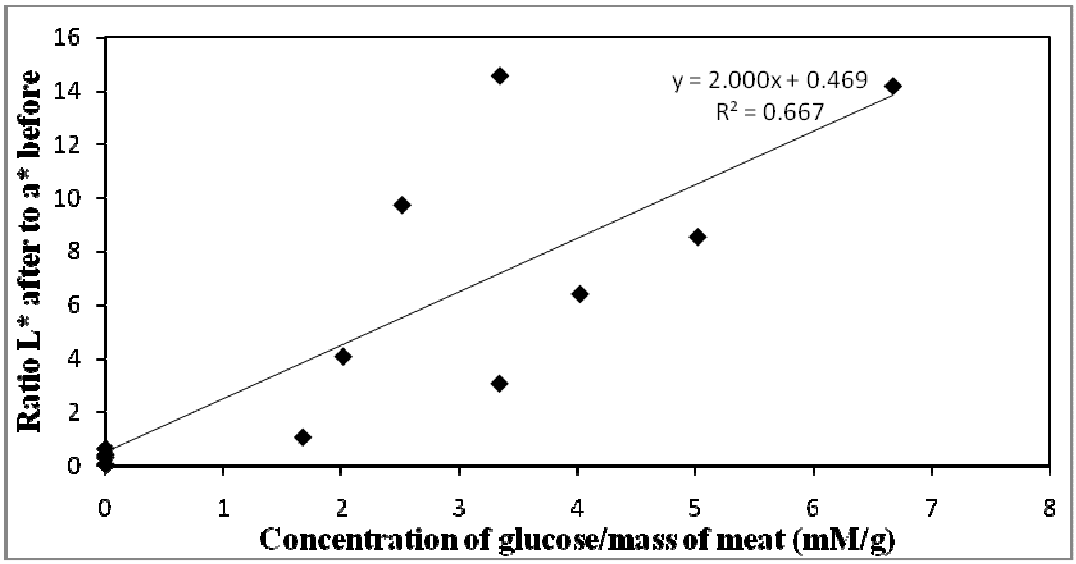



Figure 48. The relationship between Ratio L* after heating /a* before heating and concentration of glucose/mass of meat

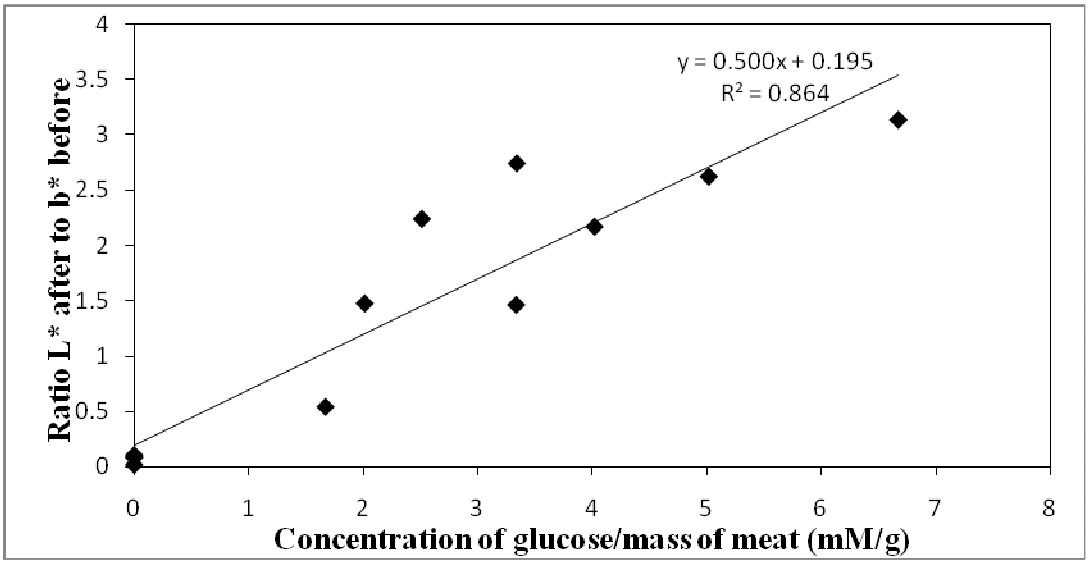



Figure 49. The relationship between Ratio L* after heating /b* before heating and concentration of glucose/mass of meat

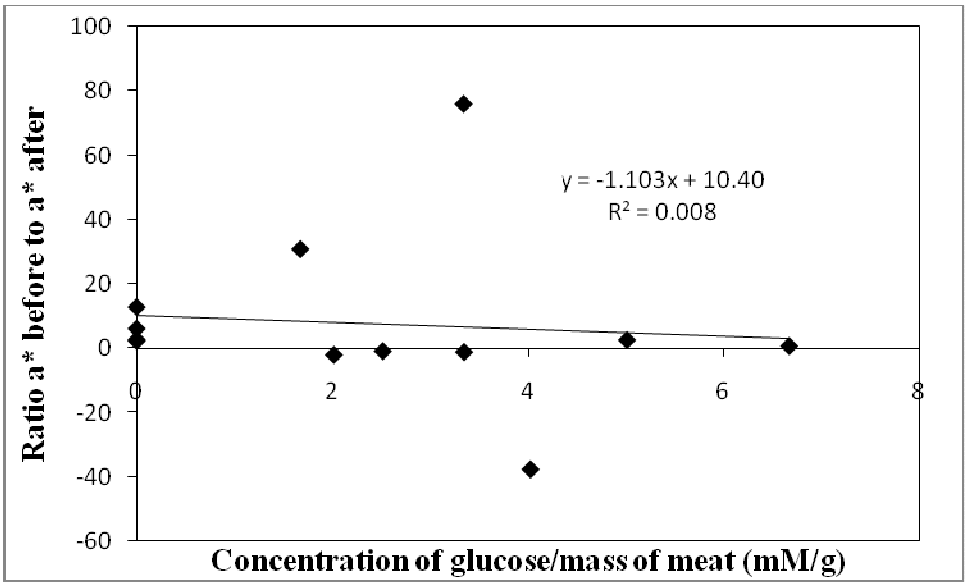



Figure 50. The relationship between the ratio: a* before heating /a* after heating and concentration of glucose/mass of meat

# CHAPTER 4

# OVERALL DISCUSSIONS AND CONCLUSION

In this work the ideal meat source would be pre-rigor muscle obtained from the commercial sampling site (Figure 9). It would be weighed to record mass, and colour development work would be performed after total glycogen hydrolysis with amyloglucosidase and subsequent determination of glucose concentration by a validated method. These data would yield mass (or concentration) of glucose/mass of meat, which is the ratio of commercial importance. Hunter colour data would be used to predict this ratio. Pre-rigor muscle was not available, so the first part of the commercial glycogen test was simulated by adding different known amounts of glucose in the form of different concentrations of glucose in 8 mL of acetate dispersion buffer. Thus in this approach the mass of meat was accurately known as was the concentration of glucose. (It must be noted however that this addition would not represent the total amount of glucose in a given slurry because rigor meat contains low concentrations of glucose.)

In Phase 1, the aim was to determine the value of individual Hunter colour values for predicting mass of meat samples by linear equations, where mass was gravimetrically known. Hunter a* was the best predictor as might be expected from the red colour of meat. Hunter b* was less useful, and Hunter L* was the poorest predictor. Even for a*, the $r^2$ values varied between 0.215 and 0.911, and the intercepts of the positively sloped regressions were variable, suggesting high variation among-animal. However, much of this intercept variation might be due to differences between sites within the rump, which is a large cut that would yield many steaks each derived from different combinations of muscles (Figure 51). Restated, the rump steaks on offer in supermarkets do not describe the site of origin in the rump. If the muscle sample were taken only from a single site – ideally the commercial sampling point – then the intercept variation would likely be much reduced. But at the same time the wide range in $r^2$ values suggests that the predictive value might be poor, requiring a weighing step by the operator in the abattoir.

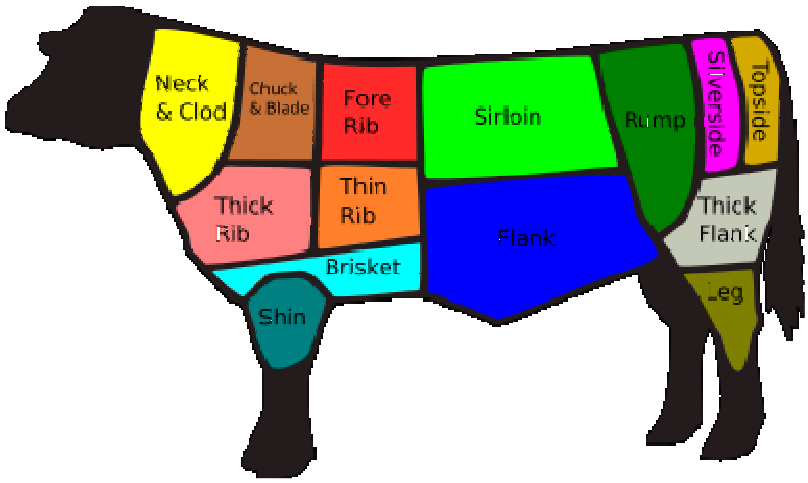



Figure 51. British beef cut diagram. The rump is a hindquarter cut. (From: http://upload.wikimedia.org/ British_Beef_Cuts.svg)

Phase 1 also established that with the optical systems used, the digital camera method of measuring colour was much less useful than the Hunter meter. However, the digital camera was not applied to heated slurries where colour differences were much more obvious. The camera method is further discussed later in respect of future research directions.

Phase 2 confirmed that different concentrations of glucose would not affect the colour due to meat. Different concentrations of glucose were not expected to affect colour, because glucose is colourless in aqueous solution, and in the high moisture environment of the slurry it would be very unlikely to react with free amine groups in the Maillard reaction at ambient temperature. However, the possibility that glucose could affect colour had to be formally tested.

Phase 3 was to explore the broad relationship between glucose concentration and meat mass and the Hunter colour change due to the Fehlings reaction induced in a microwave oven. It was hoped that the quantity of meat in the slurry would not affect colour development due to glucose but clearly that was not the case. It seemed likely that the glucose could not only reduce the $Cu^{2+}$ of the Fehlings' reagent, but could also react with a meat component(s), presumed to be protein by way of the Maillard reaction. However, the fate of glucose mixed with meat was not further examined.

Phase 4 was to randomly choose defined masses of meat between 0.8 and 2.15 g, and similarly defined concentrations of glucose between 0 and 6.67 mM to test the predictive value of equations indicating a relationship concentration of glucose/mass of meat and various Hunter colour values. Concentration of glucose/mass of meat after heating is directly proportional to the value of commercial interest: mass of glucose (glycogen)/mass of

meat. Plots between L* and concentration of glucose/mass of meat were curvilinear, but linear between a*, b* and that ratio. As a first approximation, all regressions were declared linear. Strong correlations were obtained between the ratio and b*, L*, and a*, b* and L* combined, but not a* alone. When the data for the four days the work was replicate were combined, the correlations remained strong, implying that information about meat mass and glucose concentration was simultaneously inherent in the colour-developed slurries. This is a very important result because – subject to repeat experiments – it suggests that meat mass may not have to be known, by a* values for example or direct weighing, to get accurate estimates of the commercially important ratio.

Phase 5 extended the Phase 4 work by using colour values before heating (no Fehlings added) in ratios with colour values after heating (Fehlings added) to see if this would improve the predictive values established in Phase 4. Fortunately, limited data were available from Phase 3 where the colour values before addition of Fehlings solution were recorded as well as after heating. There were four defined masses of meat, 1.0, 1.33, 1.66 and 2.0 g, and four defined concentrations of glucose, 0, 3.34 and 6.67 mM, to first compare the predictive value of absolute Hunter values after heating for concentration of glucose/mass of meat, with the predictive value of Hunter value ratios before and after heating. The absolute Hunter L* and b* were useful predictors but a* was not, as was demonstrated in Phase 4. Turning now to the predictive values of Hunter value ratios, only the after heating/before heating ratios were useful. However, the best three correlations, L* (after heating)/b* (before heating), b*/b*, and L*/L* were no better than those obtained for absolute Hunter values after heating. This result reinforces the idea that that meat mass may not have to be known to get accurate estimates of the commercially important ratio: mass of glucose (glycogen)/mass of meat.

Thus, the directions for future statistical work are clear. Phase 5 will have to be repeated with many steak replicates to confirm or deny the prospect of not having to determine meat mass colour or weighing. In the data analysis part of that work, it is likely that regressions will involve curvilinear functions for L*.

At a practical level, the results suggest several processes that need to be further developed or optimised.

First, a digital camera records reflected light in a similar manner to a Hunter ColorFlex meter, and offers the advantages of low cost, convenience and remote recording. The Hunter meter requires a transparent container, and direct contact with the light housing. However data analysis indicated that the camera approach needs improvement. There are many factors affecting the result. With the Hunter meter, the internal flash light is at the bottom of the

machine, and the black cover avoids problems with external light sources. Light is let directly into the internal slurry and may penetrate to a deeper level than the continuous halogen light used to illuminate the slurry container and the surrounding light box. A better arrangement might be to have an intense pulse of directed white light and a short exposure time to ensure the useful colour values – R,G, or B as the case may be – are in the middle to the dynamic range 0 to 255. However, the camera work did not extend to Phases 3 to 5, and the existing light box arrangement and exposure time should be used as a starting point for further work. It is likely that the camera will be more useful where the colour differences are marked, as they are after heating.

Table 20. Steps of a semi-automated procedure to determine the value of mass of glucose as glycogen/mass of pre-rigor muscle

| Step | Action | Comment |
|---|---|---|
| 1 | Sample excised from carcass | Weighs between 0.8 and 2.2 g |
| 2 | Add 8 mL acetate buffer with amyloglucosidase | |
| 3 | Disperse meat to a slurry | Disperser must be clog-free |
| 4 | Incubate to hydrolyse glycogen | This takes about 3 min |
| 5 | Record colour with camera or other colour meter | This is to determine mass if Step 8 is inadequate to simultaneously determine mass |
| 6 | Add Fehlings solution | Optimum ratio applied |
| 7 | Microwave heat | Optimum conditions applied |
| 8 | Record colour with camera or other colour meter | This is to determine glucose and possibly mass |
| 9 | Compute ratio of interest | Mass of glucose as glycogen/mass of pre-rigor muscle |
| 10 | Discard entire volume and rinse | |
| 11 | Go to Step 1 | |

Second, the 30 seconds microwave heating at the chosen power setting yielded good results. The temperature of the resulting coloured slurry was not measured but was felt to be warm to hot. Further experimentation is required to optimise the heating.

Third, the ratio of slurry volume to Fehlings solution volume was arbitrarily chosen to be approximately 1:1. This ratio needs to be optimised.

Fourth, at some point an abattoir trial will have to be held where pre-rigor meat and the amyloglucosidase enzyme will be used to generate the glucose and colour-derived results will be compared with the results from the existing technology.

After all this work has been done, the engineering for a semi-automated procedure could begin. Table 20 is an outline of the steps involved which could be arranged in a carousel.

# APPENDIX

## Phase 3

### Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = - 0.0255 + 0.0103 L*


Predictor      Coef    SE Coef      T     P
Constant    -0.02553    0.02917  -0.88  0.402
L*        0.0103442  0.0009071  11.40  0.000


S = 0.0616239   R-Sq = 92.9%   R-Sq(adj) = 92.1%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      1  0.49381  0.49381  130.04  0.000
Residual Error  10  0.03798  0.00380
Total          11  0.53179


Unusual Observations

Obs    L*    mM/g    Fit  SE Fit  Residual   St Resid
  3  53.3  0.6670  0.5257  0.0309    0.1413     2.65R

R denotes an observation with a large standardized residual.
```

### Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.203 + 0.0361 a*


Predictor    Coef  SE Coef    T     P
Constant   0.20324  0.05375  3.78  0.004
a*        0.03607  0.01424  2.53  0.030


S = 0.180000   R-Sq = 39.1%   R-Sq(adj) = 33.0%


Analysis of Variance

Source         DF      SS      MS    F     P
Regression      1  0.20779  0.20779  6.41  0.030
Residual Error  10  0.32400  0.03240
Total          11  0.53179


Unusual Observations

Obs    a*    mM/g    Fit   SE Fit  Residual   St Resid
  3  10.8  0.6670  0.5912  0.1488    0.0758     0.75 X

X denotes an observation whose X value gives it large leverage.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

```
The regression equation is
mM/g = 0.0688 + 0.0119 b*


Predictor      Coef    SE Coef     T     P
Constant    0.06880   0.02113   3.26  0.009
b*        0.0119072  0.0009487  12.55  0.000


S = 0.0563400   R-Sq = 94.0%   R-Sq(adj) = 93.4%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      1  0.50005  0.50005  157.53  0.000
Residual Error  10  0.03174  0.00317
Total          11  0.53179
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

```
The regression equation is
mM/g = - 0.0580 + 0.0160 L* + 0.0325 a* - 0.0101 b*


Predictor      Coef   SE Coef     T     P
Constant   -0.05795   0.01809  -3.20  0.013
L*         0.016007  0.002145   7.46  0.000
a*         0.032540  0.005699   5.71  0.000
b*        -0.010082  0.003111  -3.24  0.012


S = 0.0211918   R-Sq = 99.3%   R-Sq(adj) = 99.1%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      3  0.52819  0.17606  392.05  0.000
Residual Error   8  0.00359  0.00045
Total          11  0.53179


Source  DF   Seq SS
L*       1  0.49381
a*       1  0.02966
b*       1  0.00472


Unusual Observations

Obs    L*     mM/g     Fit   SE Fit  Residual  St Resid
  9  45.4  0.40181  0.45257  0.00884  -0.05076    -2.64R

R denotes an observation with a large standardized residual.
```

## Phase 4

## Day 1

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = - 0.0105 + 0.0105 L* + 0.0165 a* - 0.00345 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | -0.01049 | 0.01714 | -0.61 | 0.546 |
| L* | 0.010461 | 0.001359 | 7.70 | 0.000 |
| a* | 0.016518 | 0.002471 | 6.68 | 0.000 |
| b* | -0.003449 | 0.001719 | -2.01 | 0.055 |

S = 0.0368704  R-Sq = 97.1%  R-Sq(adj) = 96.7%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 1.16840 | 0.38947 | 286.49 | 0.000 |
| Residual Error | 26 | 0.03535 | 0.00136 | | |
| Total | 29 | 1.20375 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L* | 1 | 0.94988 |
| a* | 1 | 0.21305 |
| b* | 1 | 0.00547 |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 14 | 44.0 | 0.28500 | 0.36975 | 0.01088 | -0.08475 | -2.41R |
| 16 | 35.6 | 0.40500 | 0.51001 | 0.01517 | -0.10501 | -3.12R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

The regression equation is
mM/g = - 0.0034 + 0.00965 L*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | -0.00341 | 0.03302 | -0.10 | 0.918 |
| L* | 0.0096519 | 0.0009430 | 10.24 | 0.000 |

S = 0.0952197  R-Sq = 78.9%  R-Sq(adj) = 78.2%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.94988 | 0.94988 | 104.76 | 0.000 |
| Residual Error | 28 | 0.25387 | 0.00907 | | |
| Total | 29 | 1.20375 | | | |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 17 | 34.7 | 0.5710 | 0.3311 | 0.0180 | 0.2399 | 2.57R |
| 23 | 43.5 | 0.6860 | 0.4166 | 0.0217 | 0.2694 | 2.91R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

The regression equation is
mM/g = 0.224 + 0.0189 a*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.22375 | 0.02861 | 7.82 | 0.000 |
| a* | 0.018941 | 0.003480 | 5.44 | 0.000 |

S = 0.144528  R-Sq = 51.4%  R-Sq(adj) = 49.7%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.61888 | 0.61888 | 29.63 | 0.000 |
| Residual Error | 28 | 0.58487 | 0.02089 | | |
| Total | 29 | 1.20375 | | | |

Unusual Observations

| Obs | a* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 17 | 22.1 | 0.5710 | 0.6424 | 0.0709 | -0.0714 | -0.57 X |
| 23 | 22.9 | 0.6860 | 0.6577 | 0.0736 | 0.0283 | 0.23 X |

X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.0954 + 0.00936 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.09541 | 0.01654 | 5.77 | 0.000 |
| b* | 0.0093605 | 0.0005779 | 16.20 | 0.000 |

S = 0.0643910  R-Sq = 90.4%  R-Sq(adj) = 90.0%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.0877 | 1.0877 | 262.33 | 0.000 |
| Residual Error | 28 | 0.1161 | 0.0041 | | |
| Total | 29 | 1.2037 | | | |

## Day 2

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = 0.0110 + 0.00746 L* + 0.00816 a* + 0.00119 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.01100 | 0.02492 | 0.44 | 0.663 |
| L* | 0.007458 | 0.001985 | 3.76 | 0.001 |
| a* | 0.008157 | 0.005610 | 1.45 | 0.158 |
| b* | 0.001191 | 0.002606 | 0.46 | 0.651 |

S = 0.0513616  R-Sq = 93.3%  R-Sq(adj) = 92.6%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 0.96067 | 0.32022 | 121.39 | 0.000 |
| Residual Error | 26 | 0.06859 | 0.00264 | | |
| Total | 29 | 1.02925 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L* | 1 | 0.92315 |
| a* | 1 | 0.03696 |
| b* | 1 | 0.00055 |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 18 | 43.8 | 0.28200 | 0.39783 | 0.01480 | -0.11583 | -2.35R |
| 25 | 53.1 | 0.58500 | 0.58368 | 0.03373 | 0.00132 | 0.03 X |
| 28 | 46.8 | 0.57500 | 0.41700 | 0.01351 | 0.15800 | 3.19R |

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = - 0.0089 + 0.00916 L*


Predictor      Coef    SE Coef     T     P
Constant    -0.00889    0.02007  -0.44  0.661
L*        0.0091581  0.0005868  15.61  0.000


S = 0.0615579   R-Sq = 89.7%   R-Sq(adj) = 89.3%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      1  0.92315  0.92315  243.62  0.000
Residual Error  28  0.10610  0.00379
Total          29  1.02925


Unusual Observations

          mM/g
Obs    L*            Fit  SE Fit  Residual  St Resid
 28  46.8   0.5750  0.4201  0.0156    0.1549     2.60R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.219 + 0.0281 a*


Predictor     Coef   SE Coef     T     P
Constant   0.21865   0.03029  7.22  0.000
a*        0.028080  0.007808  3.60  0.001


S = 0.158573   R-Sq = 31.6%   R-Sq(adj) = 29.2%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.32518  0.32518  12.93  0.001
Residual Error  28  0.70407  0.02515
Total          29  1.02925


Unusual Observations

          mM/g
Obs    a*            Fit  SE Fit  Residual  St Resid
 25  13.7   0.5850  0.6025  0.1020   -0.0175    -0.14 X

X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.0999 + 0.00946 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.09994 | 0.01586 | 6.30 | 0.000 |
| b* | 0.0094594 | 0.0006517 | 14.51 | 0.000 |

S = 0.0656710  R-Sq = 88.3%  R-Sq(adj) = 87.8%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.90850 | 0.90850 | 210.66 | 0.000 |
| Residual Error | 28 | 0.12075 | 0.00431 | | |
| Total | 29 | 1.02925 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 18 | 33.2 | 0.2820 | 0.4137 | 0.0164 | -0.1317 | -2.07R |
| 28 | 32.7 | 0.5750 | 0.4093 | 0.0162 | 0.1657 | 2.60R |

R denotes an observation with a large standardized residual.

## Day 3

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

```
The regression equation is
mM/g = 0.0210 + 0.00943 L* + 0.0135 a* - 0.00156 b*


Predictor      Coef   SE Coef     T     P
Constant    0.02105   0.03238  0.65  0.521
L*         0.009434  0.002330  4.05  0.000
a*         0.013467  0.005006  2.69  0.012
b*        -0.001558  0.002828 -0.55  0.587


S = 0.0704008   R-Sq = 89.5%   R-Sq(adj) = 88.3%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      3  1.10176  0.36725  74.10  0.000
Residual Error  26  0.12886  0.00496
Total          29  1.23062


Source  DF   Seq SS
L*       1  0.99133
a*       1  0.10893
b*       1  0.00150


Unusual Observations

          mM/g
Obs    L*            Fit  SE Fit  Residual  St Resid
 22  36.0   0.3940  0.5425  0.0414   -0.1485    -2.61R
 27  51.5   0.7760  0.6319  0.0333    0.1441     2.32R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = 0.0193 + 0.00959 L*


Predictor      Coef    SE Coef     T     P
Constant    0.01933    0.02905   0.67  0.511
L*        0.0095906  0.0008905  10.77  0.000


S = 0.0924459   R-Sq = 80.6%   R-Sq(adj) = 79.9%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      1  0.99133  0.99133  116.00  0.000
Residual Error  28  0.23929  0.00855
Total          29  1.23062


Unusual Observations

          mM/g
Obs    L*            Fit  SE Fit  Residual  St Resid
 27  51.5   0.7760  0.5130  0.0279    0.2630     2.98R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.232 + 0.0214 a*


Predictor     Coef   SE Coef     T     P
Constant   0.23160   0.03136  7.39  0.000
a*        0.021415  0.005002  4.28  0.000


S = 0.162981   R-Sq = 39.6%   R-Sq(adj) = 37.4%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.48686  0.48686  18.33  0.000
Residual Error  28  0.74376  0.02656
Total          29  1.23062


Unusual Observations

          mM/g
Obs    a*            Fit  SE Fit  Residual  St Resid
 22  18.6   0.3940  0.6306  0.0884   -0.2366    -1.73 X
 25  16.0   0.5850  0.5734  0.0760    0.0116     0.08 X
 27  15.5   0.7760  0.5631  0.0738    0.2129     1.47 X

X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.137 + 0.00883 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.13672 | 0.01985 | 6.89 | 0.000 |
| b* | 0.0088272 | 0.0007627 | 11.57 | 0.000 |

S = 0.0871741  R-Sq = 82.7%  R-Sq(adj) = 82.1%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.0178 | 1.0178 | 133.94 | 0.000 |
| Residual Error | 28 | 0.2128 | 0.0076 | | |
| Total | 29 | 1.2306 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 27 | 53.5 | 0.7760 | 0.6085 | 0.0330 | 0.1675 | 2.08R |

R denotes an observation with a large standardized residual.

**Day 4**

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

```
The regression equation is
mM/g = 0.0186 + 0.00874 L* + 0.0149 a* - 0.00142 b*


Predictor      Coef   SE Coef     T     P
Constant    0.01859   0.03547   0.52  0.604
L*         0.008739  0.002493   3.50  0.001
a*         0.014906  0.005950   2.51  0.018
b*        -0.001417  0.003141  -0.45  0.655


S = 0.0839414   R-Sq = 83.6%   R-Sq(adj) = 82.1%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      3  1.15025  0.38342  54.42  0.000
Residual Error  32  0.22548  0.00705
Total          35  1.37573


Source  DF   Seq SS
L*       1  1.02906
a*       1  0.11976
b*       1  0.00143


Unusual Observations

          mM/g
Obs  L*              Fit  SE Fit  Residual  St Resid
 12   50.0   0.1590  0.5136  0.0258   -0.3546    -4.44R
 36   51.7   0.7760  0.6329  0.0450    0.1431     2.02R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

The regression equation is
mM/g = 0.0103 + 0.00896 L*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.01028 | 0.02932 | 0.35 | 0.728 |
| L* | 0.0089578 | 0.0008917 | 10.05 | 0.000 |

S = 0.100976  R-Sq = 74.8%  R-Sq(adj) = 74.1%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.0291 | 1.0291 | 100.93 | 0.000 |
| Residual Error | 34 | 0.3467 | 0.0102 | | |
| Total | 35 | 1.3757 | | | |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 12 | 50.0 | 0.1590 | 0.4582 | 0.0266 | -0.2992 | -3.07R |
| 27 | 49.2 | 0.6620 | 0.4507 | 0.0260 | 0.2113 | 2.17R |
| 36 | 51.7 | 0.7760 | 0.4730 | 0.0277 | 0.3030 | 3.12R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

The regression equation is
mM/g = 0.223 + 0.0239 a*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.22332 | 0.02738 | 8.16 | 0.000 |
| a* | 0.023905 | 0.005367 | 4.45 | 0.000 |

S = 0.159851  R-Sq = 36.8%  R-Sq(adj) = 35.0%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.50695 | 0.50695 | 19.84 | 0.000 |
| Residual Error | 34 | 0.86878 | 0.02555 | | |
| Total | 35 | 1.37573 | | | |

Unusual Observations

| Obs | a* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 26 | 14.4 | 0.5670 | 0.5685 | 0.0760 | -0.0015 | -0.01 X |
| 27 | 13.3 | 0.6620 | 0.5410 | 0.0703 | 0.1210 | 0.84 X |
| 36 | 16.2 | 0.7760 | 0.6094 | 0.0847 | 0.1666 | 1.23 X |

X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.126 + 0.00888 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.12644 | 0.01975 | 6.40 | 0.000 |
| b* | 0.0088793 | 0.0008251 | 10.76 | 0.000 |

S = 0.0958300  R-Sq = 77.3%  R-Sq(adj) = 76.6%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.0635 | 1.0635 | 115.81 | 0.000 |
| Residual Error | 34 | 0.3122 | 0.0092 | | |
| Total | 35 | 1.3757 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 12 | 41.9 | 0.1590 | 0.4984 | 0.0280 | -0.3394 | -3.70R |

R denotes an observation with a large standardized residual.

**Without Zero:**

**Day 1**

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

```
The regression equation is
mM/g = 0.0128 + 0.00927 L* + 0.0153 a* - 0.00243 b*


Predictor      Coef   SE Coef     T     P
Constant    0.01277   0.03708   0.34  0.734
L*         0.009271  0.002278   4.07  0.001
a*         0.015309  0.003300   4.64  0.000
b*        -0.002426  0.002446  -0.99  0.333


S = 0.0402453   R-Sq = 95.3%   R-Sq(adj) = 94.6%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      3  0.68614  0.22871  141.21  0.000
Residual Error  21  0.03401  0.00162
Total          24  0.72015


Source  DF   Seq SS
L*       1  0.46636
a*       1  0.21818
b*       1  0.00159


Unusual Observations

          mM/g
Obs    L*            Fit   SE Fit  Residual   St Resid
  2   0.4  0.06300  0.02562  0.03310   0.03738     1.63 X
  9  44.0  0.28500  0.37182  0.01240  -0.08682    -2.27R
 11  35.6  0.40500  0.51367  0.01718  -0.10867    -2.99R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = - 0.0005 + 0.00958 L*

Predictor     Coef   SE Coef     T     P
Constant   -0.00050   0.05653  -0.01  0.993
L*        0.009582  0.001474   6.50  0.000

S = 0.105045   R-Sq = 64.8%   R-Sq(adj) = 63.2%

Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.46636  0.46636  42.26  0.000
Residual Error  23  0.25379  0.01103
Total          24  0.72015

Unusual Observations

          mM/g
Obs    L*            Fit  SE Fit  Residual   St Resid
  2   0.4   0.0630  0.0036  0.0559    0.0594     0.67 X
 12  34.7   0.5710  0.3316  0.0211    0.2394     2.33R
 18  43.5   0.6860  0.4165  0.0240    0.2695     2.64R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.278 + 0.0166 a*

Predictor     Coef   SE Coef     T     P
Constant   0.27849   0.02334  11.93  0.000
a*        0.016592  0.002592   6.40  0.000

S = 0.106099   R-Sq = 64.0%   R-Sq(adj) = 62.5%

Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.46124  0.46124  40.97  0.000
Residual Error  23  0.25891  0.01126
Total          24  0.72015

Unusual Observations

          mM/g
Obs    a*            Fit  SE Fit  Residual   St Resid
  2   0.4   0.0630  0.2851  0.0229   -0.2221    -2.14R
 12  22.1   0.5710  0.6452  0.0521   -0.0742    -0.80 X
 18  22.9   0.6860  0.6586  0.0540    0.0274     0.30 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.137 + 0.00829 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.13719 | 0.01761 | 7.79 | 0.000 |
| b* | 0.0082878 | 0.0005620 | 14.75 | 0.000 |

S = 0.0547264  R-Sq = 90.4%  R-Sq(adj) = 90.0%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.65127 | 0.65127 | 217.45 | 0.000 |
| Residual Error | 23 | 0.06888 | 0.00299 | | |
| Total | 24 | 0.72015 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 9 | 32.9 | 0.2850 | 0.4095 | 0.0119 | -0.1245 | -2.33R |

R denotes an observation with a large standardized residual.

## Day 2

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = 0.0487 + 0.00575 L* + 0.00730 a* + 0.00247 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.04870 | 0.04053 | 1.20 | 0.243 |
| L* | 0.005746 | 0.002602 | 2.21 | 0.038 |
| a* | 0.007298 | 0.006284 | 1.16 | 0.259 |
| b* | 0.002471 | 0.003091 | 0.80 | 0.433 |

S = 0.0550465  R-Sq = 90.2%  R-Sq(adj) = 88.8%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 0.58852 | 0.19617 | 64.74 | 0.000 |
| Residual Error | 21 | 0.06363 | 0.00303 | | |
| Total | 24 | 0.65215 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L* | 1 | 0.54624 |
| a* | 1 | 0.04034 |
| b* | 1 | 0.00194 |

Unusual Observations

| Obs | L | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 13 | 43.8 | 0.2820 | 0.4008 | 0.0163 | -0.1188 | -2.26R |
| 23 | 46.8 | 0.5750 | 0.4145 | 0.0146 | 0.1605 | 3.02R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = - 0.0136 + 0.00927 L*


Predictor      Coef    SE Coef     T     P
Constant   -0.01358    0.03190  -0.43  0.674
L*        0.0092710  0.0008512  10.89  0.000


S = 0.0678584   R-Sq = 83.8%   R-Sq(adj) = 83.1%


Analysis of Variance

Source         DF      SS      MS      F     P
Regression      1  0.54624  0.54624  118.63  0.000
Residual Error  23  0.10591  0.00460
Total          24  0.65215


Unusual Observations

          mM/g
Obs   L*             Fit  SE Fit  Residual   St Resid
 23  46.8   0.5750  0.4207  0.0175    0.1543     2.35R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.268 + 0.0275 a*


Predictor     Coef   SE Coef     T     P
Constant   0.26846   0.02534  10.59  0.000
a*        0.027528  0.006002   4.59  0.000


S = 0.121694   R-Sq = 47.8%   R-Sq(adj) = 45.5%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.31153  0.31153  21.04  0.000
Residual Error  23  0.34062  0.01481
Total          24  0.65215

Unusual Observations

          mM/g
Obs   a*             Fit  SE Fit  Residual   St Resid
  3   1.4   0.0000  0.3078  0.0244   -0.3078    -2.58R
 20  13.7   0.5850  0.6448  0.0788   -0.0598    -0.64 X
 23   2.2   0.5750  0.3282  0.0251    0.2468     2.07R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.134 + 0.00845 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.13439 | 0.01752 | 7.67 | 0.000 |
| b* | 0.0084490 | 0.0006580 | 12.84 | 0.000 |

S = 0.0589183  R-Sq = 87.8%  R-Sq(adj) = 87.2%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.57231 | 0.57231 | 164.87 | 0.000 |
| Residual Error | 23 | 0.07984 | 0.00347 | | |
| Total | 24 | 0.65215 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 13 | 33.2 | 0.2820 | 0.4146 | 0.0147 | -0.1326 | -2.33R |
| 23 | 32.7 | 0.5750 | 0.4107 | 0.0146 | 0.1643 | 2.88R |

R denotes an observation with a large standardized residual.

## Day 3

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = 0.0531 + 0.00765 L* + 0.0112 a* + 0.00011 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.05307 | 0.04548 | 1.17 | 0.255 |
| L* | 0.007647 | 0.002970 | 2.58 | 0.017 |
| a* | 0.011191 | 0.005655 | 1.98 | 0.060 |
| b* | 0.000115 | 0.003356 | 0.03 | 0.973 |

S = 0.0730967  R-Sq = 87.5%  R-Sq(adj) = 85.8%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 0.85754 | 0.28585 | 53.50 | 0.000 |
| Residual Error | 23 | 0.12289 | 0.00534 | | |
| Total | 26 | 0.98043 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L* | 1 | 0.74347 |
| a* | 1 | 0.11406 |
| b* | 1 | 0.00001 |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 19 | 36.0 | 0.3940 | 0.5417 | 0.0430 | -0.1477 | -2.50R |
| 24 | 51.5 | 0.7760 | 0.6260 | 0.0350 | 0.1500 | 2.34R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = 0.0290 + 0.00935 L*


Predictor     Coef   SE Coef    T     P
Constant   0.02903   0.03630  0.80  0.431
L*        0.009350  0.001056  8.86  0.000


S = 0.0973567   R-Sq = 75.8%   R-Sq(adj) = 74.9%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.74347  0.74347  78.44  0.000
Residual Error  25  0.23696  0.00948
Total          26  0.98043


Unusual Observations

          mM/g
Obs  L*             Fit   SE Fit  Residual  St Resid
 24  51.5   0.7760  0.5103  0.0299    0.2657     2.87R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.261 + 0.0202 a*


Predictor     Coef   SE Coef    T     P
Constant   0.26085   0.03021  8.63  0.000
a*        0.020204  0.004573  4.42  0.000


S = 0.148404   R-Sq = 43.8%   R-Sq(adj) = 41.6%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.42984  0.42984  19.52  0.000
Residual Error  25  0.55059  0.02202
Total          26  0.98043


Unusual Observations

          mM/g
Obs  a*             Fit  SE Fit  Residual  St Resid
 19  18.6   0.3940  0.6372  0.0806   -0.2432    -1.95 X

X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.160 + 0.00825 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.16043 | 0.01987 | 8.08 | 0.000 |
| b* | 0.0082487 | 0.0007245 | 11.39 | 0.000 |

S = 0.0796252  R-Sq = 83.8%  R-Sq(adj) = 83.2%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.82193 | 0.82193 | 129.64 | 0.000 |
| Residual Error | 25 | 0.15850 | 0.00634 | | |
| Total | 26 | 0.98043 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 15 | 34.9 | 0.2870 | 0.4482 | 0.0199 | -0.1612 | -2.09R |
| 24 | 53.5 | 0.7760 | 0.6013 | 0.0302 | 0.1747 | 2.37R |

R denotes an observation with a large standardized residual.

**Day 4**

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

```
The regression equation is
mM/g = 0.0519 + 0.00726 L* + 0.0141 a* - 0.00035 b*


Predictor      Coef   SE Coef     T     P
Constant    0.05192   0.04671   1.11  0.276
L*         0.007264  0.002935   2.48  0.020
a*         0.014102  0.006353   2.22  0.035
b*        -0.000348  0.003472  -0.10  0.921


S = 0.0873390   R-Sq = 80.4%   R-Sq(adj) = 78.3%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      3  0.87763  0.29254  38.35  0.000
Residual Error  28  0.21359  0.00763
Total          31  1.09122


Source  DF   Seq SS
L*       1  0.74582
a*       1  0.13174
b*       1  0.00008


Unusual Observations

          mM/g
Obs   L*              Fit  SE Fit  Residual  St Resid
  8   50.0   0.1590  0.5117  0.0269   -0.3527    -4.25R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

```
The regression equation is
mM/g = 0.0175 + 0.00878 L*


Predictor     Coef   SE Coef    T     P
Constant   0.01752   0.03804  0.46  0.648
L*        0.008779  0.001091  8.05  0.000


S = 0.107300   R-Sq = 68.3%   R-Sq(adj) = 67.3%


Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.74582  0.74582  64.78  0.000
Residual Error  30  0.34540  0.01151
Total          31  1.09122


Unusual Observations

          mM/g
Obs  L*              Fit  SE Fit  Residual  St Resid
  8   50.0   0.1590  0.4564  0.0287   -0.2974    -2.88R
 23   49.2   0.6620  0.4492  0.0280    0.2128     2.06R
 32   51.7   0.7760  0.4710  0.0301    0.3050     2.96R

R denotes an observation with a large standardized residual.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

```
The regression equation is
mM/g = 0.254 + 0.0236 a*

Predictor     Coef   SE Coef    T     P
Constant   0.25436   0.02556  9.95  0.000
a*        0.023643  0.004736  4.99  0.000

S = 0.140960   R-Sq = 45.4%   R-Sq(adj) = 43.6%

Analysis of Variance

Source         DF      SS      MS     F     P
Regression      1  0.49513  0.49513  24.92  0.000
Residual Error  30  0.59609  0.01987
Total          31  1.09122


Unusual Observations

          mM/g
Obs  a*              Fit  SE Fit  Residual   St Resid
  8   7.9   0.1590  0.4407  0.0403   -0.2817    -2.09R
 22  14.4   0.5670  0.5958  0.0675   -0.0288    -0.23 X
 23  13.3   0.6620  0.5686  0.0624    0.0934     0.74 X
 32  16.2   0.7760  0.6362  0.0750    0.1398     1.17 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.
```

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.149 + 0.00826 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.14885 | 0.02154 | 6.91 | 0.000 |
| b* | 0.0082575 | 0.0008496 | 9.72 | 0.000 |

S = 0.0936363  R-Sq = 75.9%  R-Sq(adj) = 75.1%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 0.82819 | 0.82819 | 94.46 | 0.000 |
| Residual Error | 30 | 0.26303 | 0.00877 | | |
| Total | 31 | 1.09122 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 8 | 41.9 | 0.1590 | 0.4948 | 0.0274 | -0.3358 | -3.75R |
| 32 | 54.9 | 0.7760 | 0.6022 | 0.0368 | 0.1738 | 2.02R |

R denotes an observation with a large standardized residual.

**All 4 days:**

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = 0.0097 + 0.00910 L* + 0.0139 a* - 0.00148 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.00974 | 0.01421 | 0.69 | 0.494 |
| L* | 0.009096 | 0.001044 | 8.71 | 0.000 |
| a* | 0.013899 | 0.002228 | 6.24 | 0.000 |
| b* | -0.001484 | 0.001304 | -1.14 | 0.257 |

S = 0.0628710  R-Sq = 90.1%  R-Sq(adj) = 89.8%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 4.3829 | 1.4610 | 369.61 | 0.000 |
| Residual Error | 122 | 0.4822 | 0.0040 | | |
| Total | 125 | 4.8652 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L* | 1 | 3.8905 |
| a* | 1 | 0.4873 |
| b* | 1 | 0.0051 |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 17 | 34.7 | 0.57100 | 0.56623 | 0.02351 | 0.00477 | 0.08 X |
| 23 | 43.5 | 0.68600 | 0.64205 | 0.02241 | 0.04395 | 0.75 X |
| 58 | 46.8 | 0.57500 | 0.41742 | 0.00837 | 0.15758 | 2.53R |
| 82 | 36.0 | 0.39400 | 0.53027 | 0.01836 | -0.13627 | -2.27R |
| 87 | 51.5 | 0.77600 | 0.61374 | 0.01470 | 0.16226 | 2.65R |
| 89 | 53.5 | 0.68800 | 0.54165 | 0.01031 | 0.14635 | 2.36R |
| 90 | 47.1 | 0.57500 | 0.41807 | 0.00883 | 0.15693 | 2.52R |
| 102 | 50.0 | 0.15900 | 0.51189 | 0.00940 | -0.35289 | -5.68R |
| 113 | 45.0 | 0.28700 | 0.41211 | 0.00793 | -0.12511 | -2.01R |
| 125 | 48.0 | 0.58000 | 0.43676 | 0.00840 | 0.14324 | 2.30R |
| 126 | 51.7 | 0.77600 | 0.62262 | 0.01522 | 0.15338 | 2.51R |

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

The regression equation is
mM/g = 0.0054 + 0.00930 L*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.00542 | 0.01407 | 0.38 | 0.701 |
| L* | 0.0092976 | 0.0004179 | 22.25 | 0.000 |

S = 0.0886596  R-Sq = 80.0%  R-Sq(adj) = 79.8%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 3.8905 | 3.8905 | 494.94 | 0.000 |
| Residual Error | 124 | 0.9747 | 0.0079 | | |
| Total | 125 | 4.8652 | | | |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 17 | 34.7 | 0.57100 | 0.32767 | 0.00840 | 0.24333 | 2.76R |
| 23 | 43.5 | 0.68600 | 0.41005 | 0.01026 | 0.27595 | 3.13R |
| 87 | 51.5 | 0.77600 | 0.48397 | 0.01264 | 0.29203 | 3.33R |
| 89 | 53.5 | 0.68800 | 0.50321 | 0.01333 | 0.18479 | 2.11R |
| 102 | 50.0 | 0.15900 | 0.47030 | 0.01217 | -0.31130 | -3.54R |
| 117 | 49.2 | 0.66200 | 0.46258 | 0.01191 | 0.19942 | 2.27R |
| 126 | 51.7 | 0.77600 | 0.48573 | 0.01270 | 0.29027 | 3.31R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

The regression equation is
mM/g = 0.225 + 0.0215 a*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.22493 | 0.01440 | 15.62 | 0.000 |
| a* | 0.021486 | 0.002384 | 9.01 | 0.000 |

S = 0.153973  R-Sq = 39.6%  R-Sq(adj) = 39.1%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.9254 | 1.9254 | 81.22 | 0.000 |
| Residual Error | 124 | 2.9397 | 0.0237 | | |
| Total | 125 | 4.8652 | | | |

Unusual Observations

| Obs | a* | mM/g | Fit | SE Fit | Residual | St Resid | |
|---|---|---|---|---|---|---|---|
| 16 | 18.1 | 0.4050 | 0.6132 | 0.0411 | -0.2082 | -1.40 | X |
| 17 | 22.1 | 0.5710 | 0.6998 | 0.0502 | -0.1288 | -0.88 | X |
| 23 | 22.9 | 0.6860 | 0.7172 | 0.0521 | -0.0312 | -0.22 | X |
| 24 | 15.7 | 0.5980 | 0.5618 | 0.0357 | 0.0362 | 0.24 | X |
| 82 | 18.6 | 0.3940 | 0.6252 | 0.0423 | -0.2312 | -1.56 | X |
| 85 | 16.0 | 0.5850 | 0.5678 | 0.0364 | 0.0172 | 0.11 | X |
| 87 | 15.5 | 0.7760 | 0.5575 | 0.0353 | 0.2185 | 1.46 | X |
| 126 | 16.2 | 0.7760 | 0.5719 | 0.0368 | 0.2041 | 1.36 | X |

X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.117 + 0.00905 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.116648 | 0.009176 | 12.71 | 0.000 |
| b* | 0.0090544 | 0.0003569 | 25.37 | 0.000 |

S = 0.0796102  R-Sq = 83.8%  R-Sq(adj) = 83.7%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 4.0793 | 4.0793 | 643.65 | 0.000 |
| Residual Error | 124 | 0.7859 | 0.0063 | | |
| Total | 125 | 4.8652 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 58 | 32.7 | 0.57500 | 0.41273 | 0.00919 | 0.16227 | 2.05R |
| 87 | 53.5 | 0.77600 | 0.60061 | 0.01503 | 0.17539 | 2.24R |
| 102 | 41.9 | 0.15900 | 0.49594 | 0.01156 | -0.33694 | -4.28R |
| 126 | 54.9 | 0.77600 | 0.61374 | 0.01549 | 0.16226 | 2.08R |

R denotes an observation with a large standardized residual.

**Without Zero:**

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*, a*, b*

The regression equation is
mM/g = 0.0151 + 0.00881 L* + 0.0136 a* - 0.00122 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.01514 | 0.01589 | 0.95 | 0.343 |
| L* | 0.008810 | 0.001123 | 7.85 | 0.000 |
| a* | 0.013569 | 0.002312 | 5.87 | 0.000 |
| b* | -0.001224 | 0.001371 | -0.89 | 0.374 |

S = 0.0639917  R-Sq = 89.4%  R-Sq(adj) = 89.1%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 3 | 4.0222 | 1.3407 | 327.41 | 0.000 |
| Residual Error | 117 | 0.4791 | 0.0041 | | |
| Total | 120 | 4.5013 | | | |

| Source | DF | Seq SS |
|---|---|---|
| L | 1 | 3.5273 |
| a | 1 | 0.4917 |
| b | 1 | 0.0033 |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 12 | 34.7 | 0.57100 | 0.56596 | 0.02395 | 0.00504 | 0.08 X |
| 18 | 43.5 | 0.68600 | 0.64178 | 0.02282 | 0.04422 | 0.74 X |
| 53 | 46.8 | 0.57500 | 0.41720 | 0.00853 | 0.15780 | 2.49R |
| 77 | 36.0 | 0.39400 | 0.53071 | 0.01870 | -0.13671 | -2.23R |
| 82 | 51.5 | 0.77600 | 0.61318 | 0.01498 | 0.16282 | 2.62R |
| 84 | 53.5 | 0.68800 | 0.54121 | 0.01050 | 0.14679 | 2.33R |
| 85 | 47.1 | 0.57500 | 0.41810 | 0.00899 | 0.15690 | 2.48R |
| 97 | 50.0 | 0.15900 | 0.51126 | 0.00960 | -0.35226 | -5.57R |
| 120 | 48.0 | 0.58000 | 0.43641 | 0.00856 | 0.14359 | 2.26R |
| 121 | 51.7 | 0.77600 | 0.62217 | 0.01550 | 0.15383 | 2.48R |

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus L*

The regression equation is
mM/g = 0.0069 + 0.00926 L*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.00692 | 0.01532 | 0.45 | 0.652 |
| L* | 0.0092608 | 0.0004461 | 20.76 | 0.000 |

S = 0.0904716  R-Sq = 78.4%  R-Sq(adj) = 78.2%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 3.5273 | 3.5273 | 430.94 | 0.000 |
| Residual Error | 119 | 0.9740 | 0.0082 | | |
| Total | 120 | 4.5013 | | | |

Unusual Observations

| Obs | L* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 12 | 34.7 | 0.57100 | 0.32790 | 0.00861 | 0.24310 | 2.70R |
| 18 | 43.5 | 0.68600 | 0.40996 | 0.01048 | 0.27604 | 3.07R |
| 82 | 51.5 | 0.77600 | 0.48358 | 0.01297 | 0.29242 | 3.27R |
| 84 | 53.5 | 0.68800 | 0.50275 | 0.01370 | 0.18525 | 2.07R |
| 97 | 50.0 | 0.15900 | 0.46997 | 0.01247 | -0.31097 | -3.47R |
| 112 | 49.2 | 0.66200 | 0.46228 | 0.01220 | 0.19972 | 2.23R |
| 121 | 51.7 | 0.77600 | 0.48534 | 0.01304 | 0.29066 | 3.25R |

R denotes an observation with a large standardized residual.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus a*

The regression equation is
mM/g = 0.235 + 0.0210 a*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.23533 | 0.01428 | 16.47 | 0.000 |
| a* | 0.021047 | 0.002318 | 9.08 | 0.000 |

S = 0.149489  R-Sq = 40.9%  R-Sq(adj) = 40.4%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 1.8420 | 1.8420 | 82.43 | 0.000 |
| Residual Error | 119 | 2.6593 | 0.0223 | | |
| Total | 120 | 4.5013 | | | |

Unusual Observations

| Obs | a* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 11 | 18.1 | 0.4050 | 0.6157 | 0.0399 | -0.2107 | -1.46 X |
| 12 | 22.1 | 0.5710 | 0.7005 | 0.0488 | -0.1295 | -0.92 X |
| 18 | 22.9 | 0.6860 | 0.7175 | 0.0506 | -0.0315 | -0.22 X |
| 19 | 15.7 | 0.5980 | 0.5654 | 0.0347 | 0.0326 | 0.22 X |
| 77 | 18.6 | 0.3940 | 0.6274 | 0.0411 | -0.2334 | -1.62 X |
| 80 | 16.0 | 0.5850 | 0.5712 | 0.0353 | 0.0138 | 0.09 X |
| 82 | 15.5 | 0.7760 | 0.5611 | 0.0343 | 0.2149 | 1.48 X |
| 121 | 16.2 | 0.7760 | 0.5752 | 0.0357 | 0.2008 | 1.38 X |

X denotes an observation whose X value gives it large influence.

## Regression Analysis: Glucose (mM) / mass of meat (g) versus b*

The regression equation is
mM/g = 0.124 + 0.00886 b*

| Predictor | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 0.124067 | 0.009392 | 13.21 | 0.000 |
| b* | 0.0088595 | 0.0003580 | 24.74 | 0.000 |

S = 0.0784563  R-Sq = 83.7%  R-Sq(adj) = 83.6%

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Regression | 1 | 3.7688 | 3.7688 | 612.28 | 0.000 |
| Residual Error | 119 | 0.7325 | 0.0062 | | |
| Total | 120 | 4.5013 | | | |

Unusual Observations

| Obs | b* | mM/g | Fit | SE Fit | Residual | St Resid |
|---|---|---|---|---|---|---|
| 53 | 32.7 | 0.57500 | 0.41377 | 0.00907 | 0.16123 | 2.07R |
| 82 | 53.5 | 0.77600 | 0.59761 | 0.01485 | 0.17839 | 2.32R |
| 97 | 41.9 | 0.15900 | 0.49519 | 0.01140 | -0.33619 | -4.33R |
| 121 | 54.9 | 0.77600 | 0.61045 | 0.01531 | 0.16555 | 2.15R |

R denotes an observation with a large standardized residual.

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