Chapter 17
EVALUATING WATER/FAT BINDING AND COLOUR

The Science of Poultry and Meat Processing
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Preface

The aim of The Science of Poultry and Meat Processing book is to provide students and industry personnel with a comprehensive view of the modernized primary poultry meat industry and further processing of both red meat and poultry. An emphasis is placed on basic concepts as well as recent advancements such as automation (e.g. increasing poultry line speed from 3,000 to 13,000 birds per hour over the last 40 years) and food safety (e.g. HACCP in primary and the further processing areas). The book also includes chapters explaining basic muscle biology, protein gelation, heat and mass transfer, microbiology, as well as meat colour and texture to help the reader understand the underlying scientific concepts of meat processing. The Science of Poultry and Meat Processing book is based on over two decades of university teaching experiences, and is designed to be used as a course textbook by students, as well as a resource for professionals working in the food industry. The book is available online, at no cost, to any interested learner. Using this format has also allowed me to include many colour pictures, illustrations and graphs to help the reader.
The book is dedicated to my past and current students who have inspired me to learn more and conduct challenging research projects. I see this as an opportunity to give back to the field that I have received so much from as a student and as a faculty member. Looking back, I have learned a great deal from my MSc and PhD advisor, Dr. A. Maurer, who was the student of Dr. R. Baker - the father of poultry processing in North America. I would also like to thank Dr. H. Swatland with whom I worked for almost 20 years, for the many challenging scientific discussions.

Writing The Science of Poultry and Meat Processing book was a long process, which also included having all chapters peer reviewed. I appreciate the help of my colleagues, but I still take responsibility for any inaccuracy in the book. If you have comments or suggestions, I would appreciate hearing from you (sbarbut@uoguelph.ca), as I am planning to revise and update a few chapters on a yearly basis.

I would like to thank the many people who have helped me during the writing process. To Deb Drake who entered all of the material for the book, to Mary Anne Smith who assisted in editing, and to ArtWorks Media for the design and desktop publishing of the book. I greatly appreciate the help of my colleagues who reviewed chapters and provided useful discussions. They include Mark B., Ori B., Sarge B., Gregoy B., Joseph C., Mike D., Hans G., Theo H., Melvin H., Myra H., Walter K., Roland K., Anneke L., Massimo M., Johan M., Erik P., Robert R., Uwe T., Rachel T., Jos V., Keith W., and Richard Z. I would also like to thank my family for their love and support during the entire process.

About the Author

Shai Barbut is a professor in the Department of Food Science at the University of Guelph in Ontario, Canada. He received his MSc and PhD at the University of Wisconsin in meat science and food science. He specializes in primary and further processing of poultry and red meat. His research focuses on factors affecting the quality of meat, as well as protein gelation with an emphasis on structure / function relationships, rheological properties and food safety aspects. He has published over two hundred peer reviewed research papers and is the author of the Poultry Products Processing – An Industry Guide textbook. He is a fellow of the Institute of Food Technologists and has received awards from the Meat Science Association, Poultry Science Association, and the Canadian Institute of Food Science and Technology. He is involved in a number of government committees as well as academic and industrial research projects.
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**EVALUATING WATER/FAT BINDING AND COLOUR**

### 17.1 Introduction

Consumers look for food products with an appealing appearance, texture, and flavour. Meeting consumers’ expectations is a critical factor, especially when a large selection of products is available on the market. There are obviously other factors that influence the buying decision (e.g., price, brand recognition), but if, for example, the colour of the product is off or there is free liquid in the package, then the consumer will most likely not buy it. The fact that many products are prepacked puts more emphasis on presentation. Fresh meat cuts and processed products rely on their proteins (i.e., salt-soluble proteins; see Chapter 3) to assist in holding water/fat and water-soluble colour pigments. Therefore, studying and understanding the relationships between protein and water/fat holding and colour are very important. This chapter will discuss the principles and methods used to study and influence water/fat holding and colour. Various methods have been developed in the past, but their standardization would permit the test results, from different laboratories, to be compared more easily. Examples will be provided.

### 17.2 Water Holding Capacity

**17.2.1 Water Holding of Fresh and Cooked Meats**

Lean meat contains about 75% water that is held within the muscle structure (muscle fibers and their associated components). This large amount of water is held both by chemical bonds (e.g., hydrogen bonds) and physical forces (e.g., capillary forces). In further processed products moisture usually ranges from 55-80% and protein from 10-18%. In certain countries, such as Canada, a minimum of 11% protein is required; otherwise, words like “imitation” must be included in the product’s name. Because proteins are responsible for holding water,
ensuring that they have highly functional properties is extremely important. The water holding capacity (WHC) of proteins is influenced by factors such as muscle type, rigor conditions (e.g., pale, soft exudative meat; see Chapter 16), processing conditions (e.g., storage time and temperature, freezing, tumbling), and additives (e.g., alkaline phosphate, salt). Determining the WHC is important for both fresh meat cuts sold directly to the consumer and products that will be further processed by the industry. In both cases, increased yield is a highly desired outcome.

In the scientific literature there are a variety of terms that describe the phenomenon of WHC such as water binding, water retention, hydration capacity, water absorption, suction potential and swelling. In this chapter, the term WHC will be used. The specific molecular structure and conformation of the protein can have a significant effect on WHC as has been described in various reviews (Mohsenin, 1986; Kinsella et al., 1989; Huff-Lonergan and Lonergan, 2005). Over the years, different methods have been suggested to estimate the water holding capacity of meat and non-meat protein systems (Hamm, 1960; Honikel and Hamm, 1994; Honikel, 1998; Hermansson, 1986; Trout, 1988; Barbut, 1996; Tomberg, 2013).

In this section, the major methods used for meat and meat products will be discussed and some will be highlighted as potential standard methods for specific applications. As indicated in the introduction, the existence of different methods and the use of different test conditions (e.g., centrifugal force, speed, and time) have made it difficult to compare results from different laboratories.

Location of water in meat – Fennema (1985), Kinsella et al. (1989) and Puolanne and Halonen (2010) describe in detail the types of water in a food protein system. They distinguished between six basic categories:

a. Structural water – tightly bound to protein molecules and unavailable for chemical reactions
b. Hydration water – found around the apolar residues of amino acids
c. Monolayer water – first layer of water absorbed to the protein groups; it may be available for some reactions
d. Unfreezable water – does not freeze at the first sharp transition temperature
e. Capillary water – held by surface tension forces
f. Hydrodynamic hydration water – loosely surrounds the proteins

For practical reasons, water held within a protein matrix such as meat can be divided into three major categories:
a. **Structural/bound water** — includes water directly attached to the protein molecules that is no longer available as a solvent. In muscle food, it usually amounts to 5-10 g water per 100 g of protein. In this case, the polar water molecules bond with the charged amino acid side chains (Fennema, 1985). In practice this represents about 10% of tightly bound water as a monomolecular layer to the thin and thick filament structure (Zayas, 1996).

b. **Immobilized water/hydration** — represents only a few layers of water molecules that are attached to the bound water (usually by hydrogen bonds). The attachment becomes successively weaker as the distance from the charged protein groups increases. In muscle food, immobilized water usually amounts to 20-60 g water per 100 g protein (Fennema, 1985). In practice this represents about 10-20% of a second layer outside the first layer (Zayas, 1996).

c. **Bulk/free water** — is mainly held by surface forces and can be squeezed out of the meat with relative ease. The processor’s goal is to keep this water in the product as it is of major importance in meat processing and usually amounts to 50-60% of the water in the muscle. Overall, Zayas (1996) indicated that only 40-80 g out of the 280-380 g of water/100 g protein is directly bound to protein and the remaining 240-300 g water/100 g protein is found in the thick and thin filament lattice. This points out to the fact that a substantial amount of water is only ‘trapped’ within the filament structure, and during the conversion of muscle to meat this water can come out as drip loss, purge, etc. Therefore, processors must be very conscientious about conserving as much of the “trapped” water.

Factors affecting binding of water — different aspects influence the amount of water and degree of binding for each water category; e.g., molecular structure and properties of meat proteins, pH, protein type and concentration, number of exposed charged groups, salt concentration, and temperature. The pH is a very important factor influencing the fresh meat, and later during further processing (e.g., adding salt/alkaline phosphate affects the pH and the charges on the amino acid side chains). To a certain extent, the processor can control the pH as will be explained in more detail below (see Fig. 17.2.1.1).

There is no doubt that the basic molecular protein structure as it is related to WHC. Proteins consist of a folded chain of amino acids attached by peptide bonds. The linear order of amino acids represents the primary structure of the protein. The three dimensional folds in the chain represent the secondary and tertiary structures. Finally, quaternary structure refers to the geometric arrangement of various amino acid chains that are bound, most often, by non-covalent bonds (e.g., see the 3D structure of myoglobin in this chapter). The side chains of the individual
amino acids “stick out” from the main strand of the protein molecule and may be positively, negatively, or neutrally charged depending on the amino acid and the pH of the environment. The pH of the living muscle is close to 7.0.

![Figure 17.2.1.1 Effect of pH on the amount of immobilized water present in meat. The pH effect is due to its impact on the distribution of charged groups on the myofilaments and the amount of space between them. (A) Excess positive charges on the filaments. (B) Balance of positive and negative charges. (C) Excess negative charges on the filaments. Adapted from Price and Schweigert (1987).](image)

However, after slaughter, the pH drops due to the accumulation of lactic acid in the muscle (see Chapter 3). This pH decline results in a decrease in the number of reactive, charged groups in the proteins that otherwise would be available for water binding. The shift in pH causes a reduction in WHC (Fig. 17.2.1.1), which can be explained as the result of three main factors (Aberle et al., 2001):
a. **Net-charge Effect** – refers to the number of charged amino acid groups that are available for water binding. During the conversion of muscle to meat, lactic acid formation results in a pH reduction that approaches the isoelectric point (pI) of the muscle which is about 5.1. Note this is an average value obtained for the major muscle proteins (myosin - pI of 5.4, and actin pI of 4.7; Zayas, 1996). At the pI, the numbers of negatively and positively charged groups are equal and the net charge of the protein is zero. As a result, the side chains have fewer groups available for water attachment; this is known as the net charge effect (also see next section regarding the steric effect. Thus, at the pre rigor muscle pH (around 7.0) more water will be bound to the muscle proteins than at the post rigor pH of about 5.4 (see also Huff-Lonergan and Lonergan, 2005).

b. **Steric Effect** – most of the water inside living muscle cells is located within the myofibrils (i.e., up to 85%). Much of this water is held by capillary forces that arise from the unique layout of the thick and thin filaments (see Chapter 3). As the muscle goes into rigor, cross bridges between the thick and thin filaments are formed, which causes a reduction in the space available for water (Offer and Trinick, 1983). More recent studies using nuclear magnetic resonance (NMR) have improved our understanding of the relationship between cell structure and water distribution (Bertram et al., 2002). It has been suggested that loss of volume in the myofibrillar region combined with pH induced lateral shrinkage of the myofibril could lead to water expulsion from the myofibril into the extramyofibrillar space (i.e., this can be used to explain drip loss of muscle going into rigor). The steric effect refers to the repulsion of similarly charged side chains (i.e., like charges repel). Understanding the charge repulsion spacing of proteins can be beneficial to the processor, who can later add ingredients such as an alkaline phosphate to shift the pH, add charges, and hence increase WHC. By doing this, larger spaces are created for water molecules to reside. This can take place on both sides of the isoelectric point, where a high proportion of negatively or positively charged groups will result in more repulsion.

c. **Ion exchange** – takes place during the meat aging process (after rigor mortis has been completed). Enzymatic degradation of the cellular structure results in a redistribution of ions and as divalent ions (e.g., calcium) are replaced with monovalent ions (e.g., sodium), charged amino acid side groups are freed and hence WHC increases. The calcium ion is mentioned here because it is released during the post mortem process and is capable of attaching to and thereby neutralizing two negatively charged side groups. Once calcium is replaced by monovalent ions, the proteins can bind more water.
When meat is further processed, sodium chloride is the most common ingredient used. One of the major reasons for this is to enhance WHC (Fig. 17.2.1.1; see the WHC curve shift to the left) as myofibrillar proteins are solubilized (also so-called salt soluble fraction of the muscle proteins; see Chapter 3) and negative chloride ions are added to the system. There is, however, a maximum salt level that can be used to increase WHC. Increasing salt concentration from 0 to 5% dramatically increases the WHC, but at salt concentrations above 5% the reverse is seen. This is due to the “salting-out” effect where proteins will aggregate in such a way that their amino acid side groups are not available for water binding water.

17.2.2 Measuring Water Holding Capacity (WHC)

It is important that the food/meat industry be able to measure WHC and predict food/meat behavior in specific applications (e.g., storage, cooking). As a matter of fact, several of the least cost formulation programs (i.e., computer programs used for calculating ingredients; see Chapter 13) have a value assigned for water binding. Over the years, various methods to measure WHC have been developed and used by industry personnel and university scientists. The methods can be basically divided into:

a. Monitoring meat sample behavior
b. Pressure application
c. Microstructure evaluation
d. Optical sensors
e. Studying water molecule behavior – NMR and DSC

a. Meat sample behavior – a simple and inexpensive way to evaluate how fresh meat will behave in terms of WHC. To evaluate WHC during storage, a small sample (e.g., 10-100 g) is usually placed in a closed plastic bag where drip loss is collected (e.g., tissue hung on a hook and placed inside a plastic bag; note that sample geometry and the direction of the cut applied relative to the orientation of the myofibers are also important). A possible disadvantage of this method is that the analytical data are extremely affected by meat quality and storage time, thus the results will not predict the behavior of the batch on hand unless all the data collection is at standardized times. That said, this is a very popular test and drip loss values are reported in numerous research papers. For evaluating WHC during cooking, the raw meat/meat batter is placed in a closed jar/test tube and heated to the desired temperature at a specified rate while cooking loss is monitored (e.g., during heating or after cooling the sample).
b. **Pressure application** – this is one of the most popular approaches for quickly obtaining a WHC estimate. An external force, low to high, is applied by a press or centrifuge to extract a certain volume of water. However, it should be noted that when different conditions are used, it is difficult to compare results among different research groups. An example of the effect of employing different test conditions for the centrifugation test can be seen in Table 17.2.2.1 (note: a recommendation for a standard test will be presented at the end of this section).

<table>
<thead>
<tr>
<th>Test conditions</th>
<th>Mean value of WHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Test time (min)</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15.0</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>22.5</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>-1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salt concentration (M)</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>-2.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.3</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.6</td>
<td>6.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Centrifugal force (g)</td>
<td></td>
</tr>
<tr>
<td>959</td>
<td>25.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8,630</td>
<td>-1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>34,500</td>
<td>-17.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values (n = 5) followed by the same superscript letter in a column are not significantly different at the 95% level

Centrifugal force (low to high) is applied by placing a meat sample in a test tube. Various test conditions (g-force, time, temperature) have been reported for different foods and also for the same food. Meat sample sizes have ranged from 1.5 to 20 g (a 13 fold variation) and forces of 1,500 to 190,000 g (a 127 fold variation) have been reported. These variations allow relative comparisons of treatment parameters but
not a proper comparison of the results between different laboratories. Zhang et al. (1995) set up an experiment to illustrate the difference in results obtained using low, medium, and high g-forces at three different times, temperatures, and salt levels. Their test was based on a fairly common test procedure (Wardlaw et al., 1973) that is used by meat scientists around the world. According to this test, 16 ml of a salt solution (0.6 M) is added to a 10 g meat sample and incubated for 30 min at refrigeration temperature prior to centrifugation. The WHC is expressed as the percent of added water retained (positive value) or the amount of the original water expelled (negative value) by the sample after centrifugation. The salt solution is added to solubilize the major meat proteins (myosin, actin) and also to evaluate the potential ability of the meat to hold added moisture during further processing (i.e., salt is the most common additive used by the industry to enhance water retention).

At a high g-force, water was expelled from the sample (Table 17.2.2.1), but at low g-force some of the added brine was retained. Based on a statistical analysis, the following conditions were suggested for analyzing a fresh meat sample: 8,630 g for 7.5 min at 20°C at all the salt concentrations tested (0 - 0.6 M). Their study and other research showed that an increase in g-force does not cause a linear increase in the WHC values obtained.

The press method has also been used, for a long time, to evaluate the amount of “squeezable water” in a product. In this test, the meat sample (fresh/cooked) is compressed between two parallel plates; force is applied by putting a certain weight on top of the upper plate or by using a hydraulic press/texture analyzer. The released moisture is commonly collected on a pre-weighed, dehumidified filter paper (Trout, 1988; Zhang et al., 1993). Depending on the pressure, the sample is compressed into a thin layer from which most or all of the free water has been squeezed out. Various test conditions have been reported in the literature for evaluating meat samples. They range from a force of 0.01 to 44 kN, a sample size of 0.3 to 1.5 g, a temperature of 4 to 23°C, a compression time of 1 to 20 min, and different filter paper types. Zhang et al. (1993) evaluated test conditions such as applied force, sample size, compression time, and salt in common ground beef meat samples. After analyzing the data, they proposed the following conditions: sample size of 1 g, compression force of 20 kN, compression time of 2 min. The authors showed that these conditions can also be used to study processing parameters such as salt addition (i.e., 0-2% salt level commonly used by the meat industry).

A note of caution is that the units of expression can be confusing as confirmed by differences of expression used in reported literature. Depending on what is used for the numerator and denominator and other parts of the calculations, it is possible that “high” WHC values actually indicate a low WHC and vice versa. Choose
your formulas for calculating WHC considering how the data will be used and what relationships you want to show.

When it comes to evaluating the WHC of cooked meat protein gels, one should be careful not to destroy the gel structure during the test (e.g., high compressive pressure or g force will cause the sample to break/collapse). It is also important to prevent water reabsorption at the end of the test (i.e., while waiting for the centrifuge to slow down and stop). Hermansson and Lucisano (1982) studied the effect of g force on heat induced gelled plasma proteins (5%, at pH 9.0, heated to 82°C). They measured the amount of exudate from 1, 2, and 5 g samples centrifuged at 5,100, 9,750, and 30,000 g. Higher force resulted in the higher moisture loss. For example, the 2 g sample lost 3.8, 6.6 and 38.3% moisture when centrifuged at 5,100, 9,750 and 30,000 g, respectively. Low g forces (465, 790, 1045 and 1290 g) were also examined, but in a set up that included a net to hold the sample above the bottom of the test tube; i.e., preventing water reabsorption at the end of the test. This was called the net (or basket) test. These samples showed a water loss of 20-22% at all four g forces. The state of the sample after applying a high centrifugation force was also investigated by Hermansson and Lucisano (1982) and later by Kocher and Foegeding (1993). They showed that a permanent sample deformation indicates structural breakdown. Therefore, the two groups recommended using a low g force net test so there would be no damage to the gel structure. Overall, low speed centrifugation that will not cause permanent deformation to protein samples refers to a force of 100 - 1,000 g (Wierbicki et al., 1957; Hermansson and Lucisano, 1982; Barbut, 1996). As outlined above, the main advantages are that sample deformation is minimal and no or minimal structural damage occurs. In this current and commonly used test, a small sample is centrifuged while placed on a net at about 750 g for 10 min (Kocher and Foegeding, 1993).

c. Microstructure evaluation is an indirect measure of WHC that provides a more basic understanding of the science of WHC, but it is not as good as other methods for predicting WHC. Studies using low resolution light microscopy (Oroszvári et al., 2006) or by high resolution scanning/transmission electron microscopy (see Chapter 16; SEM of meat gels) can reveal relationships between structure and functionality that will help provide food scientists with a better understanding of the factors that affect water holding during structure forming (e.g., gelation), pressure application (e.g., compression, freezing), use of different protein concentration, pH, ionic strength, and temperature. In general, protein can form two distinct categories of gels. The first is a fine-stranded gel made up of small-diameter molecules forming an order network. The second is an aggregate gel comprised of relatively large particles bound to one another to form a network (Hermannsson, 1986; Barbut, 1996). In between these two categories there are
mixed gels and/or gels showing different degrees of small and large aggregates. It should be mentioned that the same protein can form both types of gels. For example, egg white proteins can form the typical white large aggregated gel, but also a transparent gel with thin strands at a low pH.

Information obtained by microscopy can be used to show how microstructure affects WHC. Increasing pore size above 0.5 μm seems to have the greatest effect on WHC. Capillary forces exhibit a significant holding force at a small pore diameter. Hermansson (1986) provided a table showing the calculated height of a water column drawn by capillary forces: a 0.1 μm radius capillary draws water up to 150 m, a 1.0 μm radius up to 15 m, a 10 μm radius up to 1.5 m, and a 100 μm radius up to 0.15 m. The corresponding water activities have been calculated as 0.90, 0.99, 0.999, and 0.9999, respectively.

Changing the pH or salt level in a meat batter can also result in the formation of a finer stranded protein structure. Wang and Smith (1992) showed that salt soluble proteins (0.6 M NaCl) formed a finer structure at pH 6.5 and 7.5 than at pH 4.5; the latter showed a large aggregate structure. Such a change also affects the WHC. In finely comminuted meat batters, Gordon and Barbut (1992) showed that adding urea caused a reduction in pore size as compared to a control (2.5% salt) and resulted in a much higher WHC (0.4 vs 4.8%, respectively). Overall, studying the relationship between protein gel structure and WHC is an active research area where more work would help develop better modeling of food systems.

d. Optical sensors – an indirect method to assess water binding in different meats. For example, meats that are categorized as pale, soft, and exudative (PSE), or dark, firm, and dry (DFD) are known to have very poor and very good WHC, respectively. The distinction of pale and dark indicates that humans can detect a visual difference even without the use of an instrument. PSE has been reported by many researchers to affect poultry, turkey, pork, beef, lamb, bison, deer and African game animal meat. Overall, the relationship between colour and WHC in meat is very complex and not fully understood. However, for practical application, it has been established that the lightness value can be used to predict PSE (Bendall and Swatland, 1988) and meat reflectance, used to predict WHC, correlates well with the violet and red spectrum (Swatland, 1995). Numerous researchers have used the International Commission on Illumination (CIE) system, but the results have not been impressive. This may be because the CIE system puts much more emphasis on green light, which is the region of the spectrum where the human eye is most sensitive (CIE, 1976). In addition, some misuse of the CIE data, by disregarding inherent differences in L*a*b* values between illuminants.
When developing probes it is also important to understand the relationships between the different meat components. Bendall and Swatland (1989) discussed the effect of measuring water from myofibrillar spaces compared to water from inter-fibre and inter-fissiculae spaces, and the relationship between WHC and pH. Depending on the “type” of water, the curve can either be linear (increasing from pH 5 to 7), or step-wise with a change at around pH 6. In order to compare results, they summarized data from various studies and showed that the physical location of the water determines the apparent behavior of the curve. Therefore, when optical measurement is developed/used, one should be careful to understand the origin of the water as water within myofibrillar spaces is highly pH-dependent, while inter-fibrillar water is more dependent on capillary forces.

The use of near-infrared (NIR) birefringence was later shown to correlate well with WHC of raw turkey breast meat samples \( (r = 0.85, P < 0.0005) \), and with fluid losses during cooking \( (r = -0.82, P < 0.005; \text{Swatland and Barbut, 1995}) \). This was the basis for developing a NIR birefringence probe, which was almost as useful as a pH probe and more accurate than a visible colourimeter paleness measurement \( (L^* \text{ value}) \) at predicting WHC and cooking losses of the turkey meat samples. Optical probes are also used to monitor specific components of meat products that are important to WHC (Prieto et al., 2009). For example, high levels of collagen can be detrimental to WHC because its conversion to gelatin during cooking can result in excessive cooking losses. Collagen in meat batters can be assessed using a quartz probe to evaluate fluorescence intensity and comparing that to a characteristic fluorescence pattern. Assessing collagen content this way has been used to successfully predict the cooking losses \( (r = 0.99, P < 0.005) \) and WHC of meat batters (Swatland and Barbut, 1991). When compared to a common centrifugation method (i.e., raw meat mixed with salt and centrifuged at 7,000 g; Wardlaw et al., 1973; described earlier in the chapter), a good correlation was obtained \( (r = -0.92, P < 0.005) \). Overall, the NIR fiber optic probe measurement was much faster and easier. Today, there are many more opportunities for developing optical sensors with quick response times in the food industry. Such fast reacting probes help optimize production and product quality while increasing automation and reducing production cost.

e. **Studying water molecule behaviour** - instruments such as nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) can be used to indirectly measure the WHC in various foods. These instruments provide information on water molecule relaxation time (e.g., NMR) and the degree of hydration (Bertram et al., 2002; 2006) and/or amount of freezable water in a protein system (e.g., DSC). Fundamental information on the relationship between proteins and WHC can be provided, as well as basic information about chemical
bonding, etc. However, the equipment is more complex and expensive than the previously described methods and therefore trained employees are required.

NMR spectroscopy measures water binding indirectly by scanning a small, homogeneous sample (e.g., 1 to 5 g in a special tube) several hundred times to produce a representative value for the “relaxation times” of the water molecules within the sample (Bertram et al., 2002; Pearce et al., 2011). The relaxation time is the time required for the magnetic nuclei of the water molecules to return to their original energy level after being excited to a higher energy level by high frequency radio waves in the presence of a magnetic field. The three nuclei with magnetic moments that can be used in an NMR study of water are the proton, the deuteron, and oxygen$^{17}$; the latter two are most commonly used. After excitation, the nuclei show two relaxation times ($T_1$ and $T_2$). These represent the times required for the longitudinal (rotational) and transfer (gyrational) motions of the nuclei to return to normal. Usually the $T_2$ values are used to determine WHC in food systems such as meat because they show greater changes (Bertram et al., 2002). In pure water, $T_2$ values are in the order of 1-2 sec. When compounds such as proteins are present, however, the time is reduced by a factor of 10-150. This is because proteins can absorb some of the energy from the water, which results in a faster return to the original low energy state. In a meat system, a large portion of the water is held in pores (e.g., spaces between the muscle filaments); therefore, it has a shorter distance to diffuse to the water-protein interface than in a pure open water system. As a result, the water in the pores shows a much lower $T_2$ value.

### 17.3 Fat Holding Capacity

Fat is another major component in many meat products and ranges between 4-40%. It contributes to the texture and mouth feel of the product but, unlike protein (ranges from 10-20%), it does not contribute to water holding capacity. Sometimes high fat products have problems holding water, fat, or both as will be discussed later in this section (note: the importance of fat in providing flavour and juiciness is discussed in Chapter 16).

Fat holding capacity is important in all products (whole muscle, ground meat, and finely comminuted products). Similar to WHC, losing too much fat during processing can result in products with an unacceptable product while also creating a net loss for the processor. In whole muscle and ground products fat is usually trapped within the adipose tissue cells that are in the confines of the connective tissue network, which provide an envelope and hold a considerable amount of the fat. In finely comminuted products the fat is usually extracted from the adipose
tissue cells (i.e. by chopping the meat batter). The ability to retain fat in a finely comminuted product (e.g., a frankfurter that contains 25% fat) is a challenge and of great importance to the meat industry. This is especially important during cooking when animal fat is converted to liquid before the meat proteins coagulate (40-50°C and 50-60°C, respectively). After melting, the liquid fat can flow out of the product if not properly confined. In some whole muscle products partial fat exudation can be desirable (e.g., while roasting a whole chicken or barbequing a steak some of the fat drips out and helps provide unique “roasty” flavours). However, in comminuted products, such as frankfurters and bologna, fat losses would leave voids that are considered defects in the product and adversely affect texture, mouth feel and appearance as exuded fat, commonly referred to as “fattening out”, “fat caps”, “fat streaking”, would accumulate in the casings and later appear as white pools of fat.

Various methods to predict fat holding capacity in processed products have been developed. The fat holding values are often used in least cost formulation programs as mentioned in the WHC section. In such programs, raw materials are assigned numerical values indicating their functional properties such as general binding and fat holding capacity.

The principles of the methods currently used to predict fat holding capacity are fairly similar to WHC methods and involve:

a. Monitoring meat sample behavior
b. Pressure application
c. Microstructure evaluation
d. Emulsion capacity testing
e. Chemical extraction
f. Optical sensors

**a. Monitoring meat sample behavior:** Numerous methods can be used during processing to access quality issues of the final product. For example, sausage may be monitored while they are cooked in a smoke house. Alternatively, product samples can be processed in small batches (e.g., test tubes) while fat and moisture losses are captured and measured. An example of measuring fat and moisture losses that occur while cooking finely comminuted meat batters formulated with different levels of animal fat and vegetable oil is provided in Chapter 13. The example uses a common test where 34 g samples were stuffed into 50 ml plastic test tubes and cooked in a water bath (Youssef and Barbut, 2011). The closed system allows precise collection of fat and moisture (cooking exudates). The test allows the processor to see if and where problems occur and to specifically
address processing/formulation issues. Another common test in this category is monitoring exudates during cooking of a ground meat product (Fig. 17.3.1; fat loss is plotted against fat content). The figure also provides an example of an emulsion type product. The results show that fat loss increased as fat content was raised from 5 to 35%. In that study the goal was to look at the mechanism(s) of fat holding rather than to optimize the meat formulation (e.g., by adding salt) or processing conditions (e.g., frying temperature). Overall, observing a sample while cooking and monitoring the amount of fat/water loss is a common practice. Such monitoring can reveal the advantages/disadvantages of using certain meats, processing parameters (e.g., slow versus fast heating rate) and different ingredients. The test is easy to perform and does not require sophisticated equipment. However, this specific test is not designed to determine the upper limit of fat holding capacity (e.g., needed for Least Cost Formulation programs).

Whiting (1987) monitored meat samples to study the effect of adding a broad spectrum of chemical compounds (salts, alcohols, monoglycerides, nonionic detergents, chelating agents, etc.) on fat holding in a meat protein system. He showed that cations from groups IA and IIA of the periodic table equaled or surpassed the stability obtained of meat batters prepared with sodium chloride, while the inclusion of cations such as zinc greatly reduced fat holding. Nonionic detergents, alcohol, and monoglycerides were detrimental to both fat and water holding. Other compounds such as urea, which stabilizes hydrophobic and peptide groups, improved fat holding. In that study, the cooking test was very beneficial and provided information about the mechanisms associated with fat binding. Olsson and Tornberg (1991) used the cooking test (frying in this case) to study the relationship between fat level and fat retention. In low fat products, fat loss was minimal when regular animal fat was used. However, when rendered fat was used (fat that has been heated to 80°C for 30 min and then filtered to remove cell wall material) fat losses were much higher than when regular fat was used, even at the low fat level. The reason for this was the absence of cell wall structure to hold the fat in place during frying. Understanding this relationship is important to the industry in terms of achieving reasonable yield and product acceptability.

b. **Pressure application** to evaluate fat holding capacity can be employed by using low/high centrifugation to using a hydraulic press. Olsson and Tornberg (1991) compared results of fat holding capacity in hamburger patties between the cooking test and the net test described in the WHC section. In the frying/cooking test, fat losses were minor in low fat products, and increased linearly with fat content \( r = 0.98 \). In the corresponding net test, centrifuged samples also showed a linear increase with raising fat content \( r = 0.88 \) but the fat losses were significantly higher than for the frying test. The authors suggested that the higher fat losses were
due to the centrifugal force being applied after cooking. They used a force of 500 g to centrifuge cooked (77°C for 35 min), 10 g samples. The authors also indicated that the longer cooking time in the net test could allow more fat to coalesce and, therefore, separate more easily from the product. The authors indicated that the net test was useful in predicting fat holding in ground hamburgers.

High centrifugation force (18,000 g) was also used to separate some of the fat from raw meat batters prepared with different chloride salts (NaCl, MgCl₂, CaCl₂; Gordon and Barbut, 1990). This high centrifugation force was required to evaluate raw stable and semi-stable meat products, since low force would not remove any fat from the raw batters. The test showed a significantly higher fat release from raw batters formulated with the two divalent salts (MgCl₂, CaCl₂) as compared to monovalent salts. There was also a significant difference between the two divalent salts in their influence on fat holding. Later, during cooking, both divalent salts showed a detrimental effect on fat holding capacity that resulted in almost total fat loss (note: this is why calcium-reduced milk powder should be used in emulsion-type meat products instead of regular milk powder). Overall, the high centrifugation force predicted fat stability during cooking. Although such high g forces are not commonly used by the industry to check raw meats, they are useful in studying the mechanisms of fat binding.

c. Microstructure evaluations are often used to study fresh and cooked meats. Understanding the relationship between microstructure and fat/water holding has proven useful. As previously indicated, in ground meat products like hamburger the fat is held within the original fat cell structure. Figure 17.3.1 shows fat loss from the product as a function of fat content. The authors also provided light microscopy pictures (not shown here) that display the distribution of fat clusters within the raw product. To the consumer, these fat clusters appear as white dots in a hamburger or a salami type product. In the cooked product some of the fat stayed in clusters, while other fat came out via fat channels. The authors indicated that when regular fat trimmings were used, intact fat cells were dispersed within the protein matrix. In the micrographs of the rendered fat treatments (stained with Aniline blue), hardly any connective tissue was observed around the fat clusters and this resulted in much higher fat losses compared to using regular fat trimmings. The losses were determined by both a frying test and a hexane extraction test (see additional discussion on chemical extraction, below).
In a finely comminuted meat product (e.g., bologna), fat is removed from cells during the chopping operation. The fat globules are then coated with meat proteins, which help stabilize the fat within the meat protein matrix. Overall, the protein gel matrix has an open structure (Fig. 17.3.2) that has embedded fat globules. The thin protein coat around the fat globule serves as an emulsifying agent that separates the fat and aqueous phases. Assuming that there is enough protein extraction and the surface area of the fat is not too large, this usually provides good fat stabilization. The addition of ingredients such as caseinate and polyphosphates can greatly enhance fat holding as they can increase emulsifying capacity (see Chapter 13). In the examples shown in Figure 17.3.1, fat stabilization was much better in the emulsion type product compared to the ground hamburger sample (note: fat losses reach 80% on the left graph and only 40% on the right). Examining the microstructure provides a better understanding of the mechanisms responsible for fat holding in these two distinct systems. Using microscopy (low/high magnification) has been shown to be very beneficial in studying the interactions between fat and proteins, fat distribution (see also micrographs in Chapter 13), pore size, and the extent of the interfacial protein film.
Figure 17.3.2 Transmission electron micrographs of fat globules in a cooked meat batter produced with KCl (ionic strength = 0.43). Showing a high magnification (a) and a lower magnification (b) of the interfacial protein film surrounding the fat globules within the protein matrix of the batter. f = fat; m = matrix; p = thick diffuse protein coat; im = internal membrane; i = interconnecting diffuse region; x = unidentified particle. Bar = 1 um. From Gordon and Barbut (1990).
d. Emulsion capacity tests are commonly done in model systems by overloading the food/meat system with liquid oil and determining the maximum emulsification point. These tests have been used for many years and the results have helped develop numerical systems for scoring meat using cost and formulating programs, such as the Least Cost Formulation program (previously discussed). The meat/protein sample is placed in a high speed homogenizer that is used to emulsify the oil. The oil (e.g., vegetable oil) is slowly added to the sample at a constant rate and is gradually emulsified until the system is overloaded and reaches a "breakdown" point where the oil and protein phases separate. This separation is observable by an abrupt change in viscosity, a change in the sound of the mixer, or changes in the electrical conductivity of the product. The latter basically shows the transformation to a continuous fat phase, which has a much higher resistance to electrical conductivity than before. Maurer et al. (1969) used the test to characterize salt soluble proteins from chicken breast muscle and showed that emulsifying capacity decreased when salt was removed by dialysis or even removed and then added back. Common test parameters reported in the literature include the slow addition (1 ml/sec) of room temperature vegetable oil (cotton seed oil) to a high speed blender (Maurer et al., 1996).

e. Chemical extraction is used to estimate fat holding by removing fat that is not well held within the product and can escape during conventional processing (e.g., cooking). This fat is known as the unbound fat portion. In whole muscle and ground products this is usually the fat that is not surrounded by a cellular membrane structure. In finely comminuted products it is fat that is not properly surrounded by an interfacial protein film. Andersson et al. (2000) used hexane to extract unbound fat from hamburgers and emulsion sausages formulated with increasing fat levels: 10 to 35% and 18 to 35%, respectively (Fig. 17.3.1). The results were compared to fat losses achieved from frying the products and showed that the hamburgers lost more fat than the sausages. In the hamburgers, fat losses were related to fat content. In the sausages, however, fat loss was independent of fat content and the hexane extraction test values actually decreased with increasing fat content (Andersson et al., 2000). The authors also noted that fat instability in the sausages was related to water loss (results not shown here), which also reflected the properties of the protein matrix. They concluded that the physical entrapment of fat within the protein matrix was more important in the emulsion type sausages than in the ground hamburger product (see also discussion of the emulsion theory in Chapter 13).
f. Optical sensors and spectrophotometry are not often used to assess fat binding in food products but are commonly used to assess fat content via infrared spectroscopy (Prieto et al., 2009). However, the need to develop rapid/online methods to monitor food processing conditions has sparked interest in looking for optical methods including fiber optic sensors. An example is the development of a fiber optic probe to predict lipid content and processing losses from finely comminuted meat batters (Swatland and Barbut, 1990). A bifurcated light guide was produced to measure reflectance of different mixtures of lean beef and fat. The authors looked at a range of 400-1,000 nm and found that reflectance at 1,000 nm correlated best with lipid content of the meat/fat mixtures ($r = 0.99, P < 0.005$). At 930 nm, fluid loss, which was determined by centrifugation, was significantly correlated with fat content ($r = 0.77, P < 0.005$). Such a probe could be calibrated against the spectrum of an ideal reference meat batter and provide the processor with a rapid response to control meat batter composition on a sausage manufacturing line (i.e., feed forward control). Later, a fiber optic probe to determine the optimal time for meat batter chopping was developed (Barbut, 1998a). This probe measures light reflectance as fat globule size first decreases and then increases again due to coalescence. At this point the chopping process should be stopped, because too much coalescence will cause a meat batter breakdown. The probe can be calibrated to indicate when the desired fat globule size has been reached. Data for calibrating the probe is initially obtained from cooking test results. Later a few other researchers worked on this concept and improved the prediction value of the probe.

These examples illustrate the way optical sensors can be developed to obtain measures to optimize processing parameters. The meat batter/emulsion probe can be used to determine the chopping time endpoint when no visible signs are available to operators. Most people in the industry rely on temperature to determine the chopping endpoint but it cannot be used to truly optimize the process. Other very experienced operators use stickiness and/or viscosity changes but these are not always accurate and this skill cannot be easily transferred to a new employee. Despite published papers about such probes, so far there has not been a large scale adoption of fiber optic probes to monitor sausage production. As described in the WHC section, the advantage of a light measuring device/probe is its fast response, convenient use for online measurements, durability in a processing plant (e.g., fiber optics inside a stainless steel sleeve), and its relatively simple operation at the plant level.
17.4 Colour

17.4.1 Colour – Introduction

Vision is an important sense for our survival as it helps us make choices (about food/other items) and communicate with others. The way we see and interpret colour is complex and beyond the scope of this book. However, a few basic explanations and references are provided, below. Briefly, humans can detect different wavelengths and translate them into either black and white or colour images. The visible spectrum for humans is presented in Figure 17.4.1.1.

![Visible Spectrum](image)

**Figure 17.4.1.1** Use of a prism to separate the white sunlight into its components. Note that by using a second prism, one can combine the colours to reproduce the white light. From Wikipedia.

Healthy humans can sense electromagnetic waves in the wavelength range of 400 to 700 nm whereas insects, such as bees, can sense shorter wavelengths in the ultraviolet (UV) range (e.g., cameras with special UV sensitivity show unique patterns on flowers that are not visible to humans). In the animal kingdom, colour also plays an important role in both warning other animals and/or attracting animals from the same species. For example, a male peacock tail feather shows an impressive colour presentation (Fig. 17.4.1.2) that actually requires a lot of energy to grow and maintain.
When it comes to acceptance or rejection of food by humans, colour plays an important role. Adding purple food colouring to a scrambled egg mix, for example, will make the product unacceptable to consumers even though there are no deviations in flavour, texture, odour, and safety. This can be verified by presenting the purple eggs under red light, which masks the colour differences (see Chapter 16). It is also important to note that we use colour to make strong assumptions about the flavour of a product. For example, when the colour of an ice cream is switched from red to yellow, people are easily tricked into believing that the flavour has also changed.

The colour of meat primarily arises from the red myoglobin molecules present in the tissue. However, it should be emphasized that meat colour is also affected by factors such as the breed, nutrition and feed/forage antioxidants, animal age, muscle type, post mortem changes (e.g., see later discussion on PSE meat), processing methods (e.g., cooking, frying), use of additives (e.g., nitrite), lighting conditions, and packaging. Interactions between these factors can make evaluating a specific meat product’s colour fairly complicated.
17.4.2 Vision and Colour Perception

Light is a key component in our ability to see. A simple example is entering a dark room where we cannot perceive the items inside. As the light level is slowly increased, one will first start to see the outline of the items but without colour. Then, as the light intensity is increased further, colours will gradually start to appear, indicating that a minimum level of light is required to see colour. The colours we see are the result of light reflected from different objects, which will also absorb and scatter some light. Light is a form of radiant energy produced by a hot object such as a candle, lightbulb, or the sun. Light waves radiate in all directions from their originating source and vibration occurs at right angles to the direction of the wave’s travel (Fig. 17.4.2.1). The high points of a light wave are called crests and the low points, troughs. The distance from crest to crest is called wavelength and the number of vibrations, or cycles per second is called frequency. When the wavelength (λ) is multiplied by the frequency (ν), the result is the speed of light (c):

\[ c = \lambda \cdot \nu \]

This relationship indicates that as wavelength increases, frequency decreases, since the speed of light is constant. This can be used to show why blue light (see Fig. 17.4.2.2; \( \lambda = 400 - 425 \text{ nm} \); \( \nu = 75 \times 10^7 \text{ cycles/sec} \)) has a shorter wavelength and is more penetrating than red light (\( \lambda = 650 - 700 \text{ nm} \); \( \nu = 40 \times 10^7 \text{ cycles/sec} \)). The higher penetration of blue light makes it potentially more damaging to our skin (i.e., closer to the UV zone), and explains why it can cause more problems with meat colour and fading (see later discussion on storage). White sunlight can be split into its components naturally by water droplets (as seen in rainbows) or by using a prism (Fig. 17.4.1.1). The Gage Dictionary definition of colour is, “the sensation produced by the different effects of waves of light striking the retina of the eye. Different colours are produced by rays of light having different wavelengths”.


Figure 17.4.2.1 Wavelength is the distance from one crest to the next and frequency is the number of wavelengths per second as shown in (a). Section (b) shows that light is a three dimensional electromagnetic wave, vibrating at right angles to its direction of travel. From Wikipedia http://en.wikipedia.org/wiki/Light.

Figure 17.4.2.2 Wavelength of blue vs green vs red light – relationship between $\lambda$ and frequency
Figure 17.4.2.3 shows the light reflected from meat. It absorbs all/most of the blue and green light and reflects back small amounts of yellow, moderate amounts of orange, and a large amount of red light. Therefore, the overall colour of meat appears red. Light sources with an excess or deficiency of certain wavelengths of light (e.g., fluorescent light is deficient in red), will result in the meat appearing in a different colour.

**Figure 17.4.2.3** Spectral reflectance for chicken thigh meat.
17.4.3 Method for Colour Evaluations

Determining colour and expressing it in a simple way is not easy. In our daily life, we use a variety of descriptive terms for colour; e.g., the colour green can range from dark to light, bright to dull, glossy to matte, and modifiers such as grass, hunter, etc.

Colour can be evaluated and reported in different ways. Colour scales have been developed to compare the product’s colour to a reference. These colour scales are popular, for example, in home hardware stores where customers are interested in matching/selecting colours for their homes. An example of a colour fan used by the poultry industry to evaluate and report egg yolk colour and/or chicken skin is shown in Figure 17.4.3.1. Similar colour fans have been produced for meat (e.g., the Japanese pork meat colour chart). Producing chicken/pork/beef with a consistent meat/skin colour is important to consumers who have certain expectations for a wholesome product. Deviation from such a colour will raise questions and might prevent the customer from buying the product. It is also interesting to note that expectations differ regionally; in the USA light coloured chicken skin is desirable whereas in Japan darker yellow skin is praised (Note: preferences can also differ within the same country). Growers can affect skin and egg yolk colour through diet by providing feed rich in carotenoids or synthetic xanthophylls which will enhance the yellow colour.

Figure 17.4.3.1 Example of a colour fan used by the poultry industry to check for egg yolk colour and/or chicken skin
Fletcher (1999a) provided a historical review of the various methods used by the poultry/meat industry to measure and express colour. The methods can be basically divided into three categories:

a. **Visual**

b. **Chemical-spectral photometric** (e.g., direct pigment analyses)

c. **Reflectance colourimetry**

**a. Visual**

descriptions were developed in the early 1900s when colour chip standards were introduced to score colour of poultry skin and egg yolks. Originally, a series of colour standards (fairly linear scale) were created and assigned numbers. One of the most common colour standards was the Hoffman-LaRoche yolk colour fan (Fig. 17.4.3.1), which was also used for broiler skin colour evaluation. The colour fan has been used for many decades and is still used today in certain parts of the world. This applies a less subjective scoring system to evaluate skin colour and also serves as a quality control measurement. This and the Japanese pork meat chart are still used today.

**b. Chemical-spectral photometric** methods are based on spectrophotometric characterization of extraction of meat pigments. Pigments can range from the carotenoids found in feed material (e.g., corn) that are deposited in the skin and fat to the heme pigment found in meat. Several procedures for poultry are based on the extraction of skin pigments from the shank area using acetone, followed by a colourimeter evaluation. Meat pigments, including heme and cytochrome C, are also extracted and quantified (AMSA, 2012; to be further discussed below).

Several of the problems encountered with the visual and chemical methods were due to the incorrect assumption that the results were linearly related to the product’s final colour. For example, the Hoffman-LaRoche colour fan employs a linear scale to describe non-linear colour values. This problem becomes apparent when colour values do increase in a linear fashion in response to the amount of carotenoids fed in the diet. The problem can also be seen when examining the relationship between the heme content and meat colour, since the colour is often more affected by the chemical state of the heme pigment rather than its concentration in the tissue (Fletcher, 1999a). It should be mentioned that one of the major disadvantages of pigment extraction, from meat, is that the processing steps can change the chemical redox form of the pigments. Thus, extractions are best for quantification of pigments and determine spectral peaks and valleys.

**c. Reflectance colourimetry** is the most popular method used today in meat/food science colour research. It can overcome some of the previously described problems and eliminate the inherent problem of variation among panelists. It also
eliminates problems associated with changes of pigment forms when extracted and from differences due to lighting type and intensity, differences in light viewing angles of colour by panelists, and background effects (i.e., items placed on different coloured backgrounds can appear different to people). The major advantages of reflectance colourimetry, when done correctly, include its accuracy, objectivity, and reproducibility. Some of the limitations include the dependency on more expensive equipment, potential operating errors, and improper use. Overall, the three components involved in the way we perceive colour include the illumination source, the object/surface viewed, and the observer (human or instrument). When discussing instrumental colour measurements it is important to first explain the concepts of hue, lightness, and saturation (Swatland, 1989).

Hue describes a primary colour such as red, green, or blue.

Lightness or luminosity describes the brightness of the colour.

Saturation describes how vivid or dull the colour is.

As an illustration of the relationship between the three terms, consider the slow mixing of green paint into dull white paint. The colour will gradually change from the original dull white to pale green to dark green, but the hue (green in this case) remains unchanged. What changes is the saturation; the colour progressively changes from dull green to a more vivid, saturated green. The lightness or luminosity can be changed by using bright white paint instead of dull white, so the paint would be brighter.

Figure 17.4.3.2 shows a graphic description of hue, lightness, and saturation. In the example provided above, adding more green paint moves along the saturation axis toward the outside of the sphere. Using a brighter white paint (as the starting ingredient) moves upwards along the lightness line.

With scientific advancements, different numerical systems have been developed to measure colour. Established in 1931, the CIE (Commission Internationale de l’Eclairage) incorporated the spectral aspect of illumination with the three primary colours into the so-called tri-stimulus values, also known today as the X, Y, Z. The CIE X, Y, Z system defines a colour by the additive mixture of the three primary light colours, X (red), Y (green) and Z (blue) that would be required to match the colour of a mixture as viewed by a “standard observer” (human) under defined illumination and viewing conditions. This is based on the theory that the human eye possesses receptors only for these three primary colours, and that all other colours seen are a mixture of the three. Note: the system is useful for defining colours but the results are not always easily visualized.
Richard Hunter used the CIE data and established a Hunter Lab system (Mancini and Hunt, 2005). The original formulas for calculation L, a, and b, were modified in 1976 to minimize the problem that equal distances on a chromaticity diagram do not correspond to equal differences in colour perception (CIE, 1976). This system is one of the most popular systems currently used by the meat industry and is known as the CIE L*, a*, and b*, (note that the asterisk is now used to indicate the 1976 modifications).

The CIE L*, a*, and b* colour space system is presented in Figure 17.4.3.3. The L* value is an expression of the lightness of the surface ranging from 0 (black) to 100 (white). The a* spans from -60 (green) to +60 (red), and b* from -60 (blue) to +60 (yellow). Another frequently used method for food applications is the Hunter L, a, b solids scale. The relationship between the CIE and other colour scales has been discussed in the AMSA Guidelines (2012).
17.4.4 Myoglobin and Meat Colour

Meat colour is affected by various intrinsic and extrinsic factors. The main intrinsic factors include myoglobin content (also called meat pigment content), muscle fiber orientation, spacing among muscle fibers, and pH.

For many years muscle fibers have been described as “dark vs. light”, “red vs. white”, “slow vs. fast”, “aerobic vs. anaerobic”, and numerous others nomenclatures, which are based on inherent differences in myoglobin content and muscle biochemistry/physiology. When discussing meat colour, it is important to note that muscles differences in myoglobin content have a great effect on colour and colour stability when comparing different muscles (see Chapter 3 - differences between red and white muscle fibers). White chicken breast meat is predominantly
composed of white fibers, which have low myoglobin content and a light gray colour (see Pectoralis in Table 17.4.4.1; the table shows differences in total hemoglobin, and myoglobin content).

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Hemoglobin (mg/g)</th>
<th>Myoglobin (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>2.67 ± 0.65a</td>
<td>1.08 ± 0.41a</td>
</tr>
<tr>
<td>Adductor</td>
<td>0.83 ± 0.21b</td>
<td>0.56 ± 0.17b</td>
</tr>
<tr>
<td>Pectineus</td>
<td>0.09 ± 0.04d</td>
<td>0.01 ± 0.00c</td>
</tr>
<tr>
<td>Sartorius</td>
<td>0.67 ± 0.11b</td>
<td>0.12 ± 0.02d</td>
</tr>
<tr>
<td>Pectoralis</td>
<td>0.24 ± 0.04c</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Table 17.4.4.1** Myoglobin and hemoglobin content in chicken muscles.  
From Kranen et al. (1999).

The heart muscle, which has the darkest colour and more anaerobic chemistry, has the highest heme content followed by the Adductor muscle. Thigh meat is mainly composed of red fibers and appears dark. Kranen et al. (1999) used different methods to determine hemoglobin and myoglobin content including spectral photometric, size exclusion chromatography, and immunological methods. They compared their results to about half a dozen other groups and found fairly comparable data. Different poultry also vary in the inherited amount of pigment in their muscles (e.g., chicken vs. duck). Differences can also be related to muscle activity where domestic chicken breast muscle is lighter than active breast muscle of a migratory duck. The muscles’ colour is influenced by the amount of hemoglobin and myoglobin found in them. Hemoglobin is found in red blood cells and is composed of four myoglobin units (both are used to deliver oxygen to the muscle and hence can bind and release oxygen fairly easily; e.g., depending on partial pressure of the gas, pH). The structure of the heme complex is shown in Figure 17.4.4.1. Myoglobin is a complex molecule consisting of two major parts: the protein portion called globin and the non-protein portion called the heme ring. The protein component consists of a globular protein and the heme ring has an iron molecule in its center which is responsible for binding molecules such as oxygen and water. The oxidation state of the iron molecule and the compounds attached to the ring determine the shade of red colour.
In terms of extrinsic factors, myoglobin has a bright-red colour when exposed to oxygen (Fig. 17.4.4.2) and the iron molecule is in its reduced ferrous (Fe^{2+}) state.
Consumers associate this bright red colour (called oxymyoglobin) with fresh, high quality meat. This colour is sometimes also referred to as “bloom”. When
there is no oxygen, the iron molecule is in its ferric, Fe$^{3+}$, state and the pigment (called metmyoglobin) gives the meat a brown colour. This can be reversed when the meat is exposed to oxygen (i.e., the metmyoglobin first has to be converted deoxymyoglobin and this form can be converted back to oxymyoglobin; Suman et al., 2014), provided microbial count is not too high. Consumers associate brown meat with old meat because meat tends to be brown when it has been stored for long periods, and a large number of microorganisms that consume oxygen are present.

Extrinsic factors such as vacuum packaging can also result in the conversion of the myoglobin pigment into the brown colour form (Fig. 17.4.4.3). Vacuum packaging is often used to extend the shelf life of the fresh meat product (see also Chapter 11). To overcome this, a master package can be used for the small fresh meat trays. The master package is then either vacuum packed or flushed with CO$_2$. At the store, the master package is removed and time is allowed (15-30 min) for the “bloom” to develop; i.e., the packaging material of the individual tray is oxygen permeable.

Cooking results in denaturation of the meat pigment and appearance of a typical greyish/dull brown colour (Fig. 17.4.4.2). Heat usually denatures the globin portion of myoglobin and the heme ring is usually separated from myoglobin and adds to the “non-heme” pool in meat. The denaturation temperature depends on the interaction between meat pH and redox status of the myoglobin. As muscle pH increases the myoglobin is more thermally stable resulting is more pink/red colours. Thus, pH effects combined with the redox forms will have a highly significant effect on cooked colour. The relative resistance of the major redox forms to heat induced denaturation is: carboxymyoglobin > deoxymyoglobin > oxymyoglobin > metmyoglobin (AMSA, 2012).

When meat pigments are heated sufficiently, the fully denatured myoglobin becomes the “cooked pigment”, or the so-called denatured metmyoglobin. This denaturation results in the meat changing to a more opaque structure (i.e., more translucent in the raw state), and reflecting more light (i.e., appearing lighter). In the case of cooked chicken thigh meat, an almost 50% increase in both the L* (e.g., 45 to 65) and b* (e.g., 6.2 to 16.7) values and a slight decrease in a* are commonly observed. In the case of chicken breast meat, which has a much lower myoglobin content (Table 17.4.4.1), the L* value usually increases by about 60% (e.g., 52 to 82) due to cooking. During cooking, breast meat colour also becomes yellower (e.g., 6 to 14). The a* does not change much and overall the consumer sees a very light product at the end of the cooking process.
There are some other potential colour problems that can be associated with the so-called premature browning (meat appears cooked before it reaches a temperature of 65°C) and the persistent pink phenomena (meat still looks uncooked even when a temperature of 72°C has been reached). These colour problems have been investigated over the years (Seyfert et al., 2004; AMSA, 2012) and the processor should be aware of the causes and potential solutions.

During slow roasting, the surface of the meat and/or skin also develops a typical brown colour as a result of the Maillard reaction between amino acids and reducing sugars that causes brown pigment formation. Enhancing the development of the brown colour can be achieved by adding sugars such as honey to the basting media (see Chapter 13). During smoking an extra brownish/golden colour develops on the surface due to the presence of carbonyls in the smoke that also participate in the Maillard reaction (see Chapter 13). Higher than normal pH of meat (such as DFD meat) will usually decrease Maillard surface browning.

When nitrite is added to cured meat products (see Chapter 13, ham recipe), a typical pinkish-red colour will initially develop in the raw meat. Later, upon heating, it will change to the stable light pink pigment called nitrosohemochrome (Fig. 17.4.4.2). The difference between nitrite and nitrite-free meat products can easily be seen when ham or turkey leg meat is prepared at home; without nitrite cooked products have a typical brown colour whereas cured products have a pink colour. Additional discussion on unintentional nitrite contamination of fresh meat (low levels are needed) can be found at the end of the chapter.

Figure 17.4.4.3 Example of beef meat just vacuum packed (right) and same meat after 12 hr (left), showing the transformation of myoglobin to metmyoglobin. Photo by S. Barbut.
17.4.5 Animal Skin Colour

In meat producing animals sold with the skin on (e.g., broilers, turkeys, ducks, pigs), skin colour and shade are important marketing factors. In poultry, skin colour can range from light beige to yellow to even totally black. Skin pigmentation is the result of two major factors that include melanin deposition and carotenoids/xanthophyll obtained from the diet (Fletcher, 1999a). The first factor is related to the genetic ability of the bird to produce and deposit melanin in the dermal or epidermal layer of the skin (see Chapter 3). The second factor is the broiler’s ability to absorb and deposit carotenoid pigments from plant material. Studies have shown that consumers usually prefer the colour that was traditionally available in their region. For example, in the eastern US deeply pigmented poultry are most desirable, whereas in the northwestern US pale skin colour is preferred.

a. White skin colour results from little or no melanin or xanthophyll deposition in either the dermis or epidermis (Fletcher, 1999a).
b. Black skin (found in some Chinese breeds) is the result of melanin deposition in both the dermis and epidermis.
c. Yellow skin results from xanthophyll deposition in the epidermis. Breeds that have the ability to absorb and deposit carotenoids must receive this pigment in their diet.
d. Green skin is the result of the deposition of xanthophyll in the epidermis and melanin in the dermis. Greenish and bluish skin colours can be seen in some South American breeds.

In most commercial breeds, the ability to deposit melanin has been eliminated through genetic selection. Sometimes, however, consumers still return poultry showing dark spots in certain areas. The processor can quickly verify the presence of typical melanin-bodies in the skin cells by using microscope analysis and then assure the consumer that the problem is not related to microbial spoilage or a food hazard.

Various studies have been conducted to evaluate skin pigmentation in relation to natural and synthetic sources of carotenoids, and to establish the dietary levels of carotenoids required to achieve a certain colouration. Carotenoids are deposited in the epidermis. Therefore, if a yellow skin colour is to be maintained, a mild scalding procedure should be used (i.e., one that does not remove the outer skin layer during scalding and plucking. See Chapter 5).
17.4.6 Product Presentation and Light Sources

Colour is the result of light reflected back from an object. As mentioned earlier, the light source used for illumination and its intensity can affect colour. Therefore, if an unbalanced light source is used the colour will be distorted. This is mentioned because fluorescent light, which is deficient in certain wavelengths (e.g., red), is commonly used in refrigerated display cases. It should also be remembered that there are significant differences in how people perceive colours (e.g., a person who is colour blind or cannot distinguish between shades of red will have a different colour perception compared to a person with a perfect vision (AMSA, 2012).

When consumers look at a meat product in the store it is usually displayed under artificial light. The most common artificial lights include incandescent (INC), fluorescent (FL), metal halide (MH) and light emitting diodes (LED). As will be demonstrated below, these sources have different spectra (resulting from the different lighting colour temperature and colour rendering index; i.e., two terms that can be found today in the specification of light bulbs). The decision to install one over another depends on factors such as cost of the light bulb, life expectancy, energy efficiency, and heat output. For example, FL bulbs do not produce a full spectrum but radiate about 20% of the heat produced by INC bulbs of the same light output. Therefore FL bulbs are usually installed in commercial display coolers. Metal halide is the most efficient light bulb to illuminate large areas, but also does not produce a full spectrum (e.g., strong in the yellow/orange range). Barbut (2001) examined the effects of different light sources on the colours perceived by the customer and their degree of liking of certain products (whole chicken with skin on, skinless thigh meat, and breast meat). In most studies the actual colour coordinates (e.g., CIA L*, a*, and b* values) are determined using a commercial spectrophotometer. Such colour meters are equipped with a stable light source (e.g., xenon) to illuminate the surface after the instrument has been calibrated with a white plate. L*, a*, and b* colour coordinates are important in studying the effect of various test parameters (e.g., storage time, additives) but they do not reveal the actual colour the consumer will see at the store when different light sources are used. Therefore, in that particular study scanning equipment capable of utilizing the actual light source used in the store was employed.

Consumer preferences to buy the product under different light sources are shown in Table 17.4.6.1. Consumers liked the whole chicken product with skin presented under the INC (150 W, 120 V). The panelists also indicated a strong preference to buy the product when presented under INC light (data not presented here), as opposed to no specific preference when presented under FL and a significant objection when presented under MH.
Table 17.4.6.1 Preference of fresh chicken cuts under different light sources. All products presented under 70 foot candle. From Barbut (2001).

<table>
<thead>
<tr>
<th>Product</th>
<th>Incandescent</th>
<th>Fluorescent</th>
<th>Metal halide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole chicken (skin on)</td>
<td>7.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thigh meat</td>
<td>6.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breast meat</td>
<td>5.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> Means, within each row, followed by a different superscript are significantly different (P < 0.05) from each other. Twelve panelists evaluating the product on 2 successive days; 1 = dislike, 10 = like.

The reason for that can be explained by examining the luminance data (Fig. 17.4.6.1). The INC light source produces a full spectrum (balanced distribution of the different wavelengths) and imparts a full natural colour to the product (i.e., the luminance data obtained for the products under the INC source are fairly similar to data obtained by a Minolta/Hunter commercial spectrophotometer). The main descriptor colour used by 66% of the panelists to describe the whole chicken with skin on under INC light was yellow, while in the other light sources it was described as cream or white (see below). In general, observed colour is described in one to two words that indicate its main colour and shade. This is the result of our mind summarizing all the reflected wavelength data and expressing it as one colour. Since we do not have the ability to “see” the individual peaks (as measured by photo diode array equipment; Fig. 17.4.6.1), we express our overall impression.

Figure 17.4.6.1 Luminance data of whole chicken (skin on), skinless thigh meat and breast meat presented under incandescent light (70 foot candle). From Barbut (2001). With permission.
When a MH luminance light source was used, the data showed a narrow peak in the yellow region (Fig. 17.4.6.2), strong peaks in the blue and green regions, and a low red peak. The luminance data obtained and the position of the peaks were similar to published data of other commercial MH sources. The use of MH resulted in a low panel preference score (Table 17.4.6.1) and 75% of the panel to describe the colour of the whole chicken with skin on as cream white. The panelists overwhelmingly said that they would not buy the product presented under MH because of its unnatural colour.

Presenting the whole chicken under FL light produced a peak in the yellow zone and two additional, strong peaks in the blue (430 nm) and green (530 nm) regions (Fig. 17.4.6.3). The reflection curve obtained here is similar to published data for other commercial FL light bulbs that show typical strong peaks in the blue (430 nm), green (530 nm), and yellow (570 nm) regions. Seventy-five percent of panelists described the whole chicken with skin on as pale white. The reason for that was the lack of a broad enough yellow peak and the minimal red colour in this light source.

![Figure 17.4.6.2](image)

*Figure 17.4.6.2* Luminance data of whole chicken (skin on), skinless thigh meat and breast meat presented under metal halide light (70 foot candle). From Barbut (2001). With permission.

The skinless thigh meat with its typical dark poultry meat colour was also most preferred under INC light (Table 17.4.6.1). In this case, the expression of red colour could only be achieved with an adequate source of red light. The luminance curve is fairly similar to data published by Swatland (1989) for poultry leg meat measured using a fiber optic spectrophotometer. The presence of adequate red
light output in the INC source resulted in a high buying preference as opposed to a significantly lower buying preference under FL and no preference under MH. Under INC light, the leg meat was described by most panelists as pink/red, but was described as brown under FL light and brown/purple under MH light.

The skinless breast meat was liked similarly by the panel under both INC and FL light (Table 17.4.6.1). Since the colour of this raw product is actually light beige, the red and yellow components were not as important as in the other two products. The main colour used to describe the product under INC light was tan/pink. Under FL, it was described as brown/beige and beige/tan under MH. Comparing the FL and MH source showed that the product was more liked under FL light. In terms of the buying decision, there was actually a preference to buy the product under FL light (P < 0.01). Overall, skinless chicken breast meat has a neutral colour that is not much affected by the lack of red in FL light or the relatively strong blue and green peaks in the FL and MH sources (i.e., this can be related to the colour temperature of the light source, its colour rendering index and light intensity).

17.4.7 Other factors Influencing Meat Colour (PSE, DFD, White Striation)

The colour and appearance of meat are also affected by the structure and spacing of the sarcomeres (muscle building units). The physical structure of the sarcomeres (see Chapter 3) affects the way light is absorbed and reflected from the muscle’s
surface. An example is the differences between pale, soft and exudative (PSE) meat and the dark, firm, and dry (DFD) meat. In this case the PSE meat has a more open structure that reflects more light and results in a lighter appearance (Barbut et al., 2008; Swatland, 2008).

Figure 17.4.7.1 shows the lighter PSE poultry meat that is considered lower quality meat because of its poor water holding capacity (note: pork, beef, and turkey meat is also known to show PSE; see discussion below). This is important to processors as lean muscle contains about 75% water. When PSE breast muscle is used for further processing there are usually problems holding the muscle’s original water and any injected moisture (i.e., added during further processing). This can be critical when, for example, large individual turkey breast muscles or pork hams are injected, tumbled and then cooked in a bag. If there is free water in the bag it will have to be opened and the free moisture drained. This results in reduced profit and a substantially shorter shelf life.

When it comes to selling fresh meat, packaging skinless chicken breast fillets/pork chops in a tray can be a challenge if there are noticeable variations in colour (note: the human eye is very sensitive in detecting colour variations). In a survey of 1,000 packages of skinless breast fillets (four per package) conducted in the USA, Fletcher (1999b) reported an average of 7% that showed noticeable colour variation (i.e., presence of at least one fillet that was lighter or darker than the rest). Samples were evaluated at 16 different stores where packages from six brands
were marketed. It was interesting to note that the occurrence of colour variation varied among companies (0.9, 3.5, 6.1, 8.4, 12.6 and 16.9% showing one or more discoloured fillets). This clearly indicates that some companies sort their meat.

Industry and academic reports have indicated varying degrees of PSE in different species (Barbut et al., 2008). The magnitude and colour distribution (L* value – lightness) of turkey breast meat can be seen in Figure 17.4.7.2 where the occurrence of PSE meat was evaluated over one year in Ontario, Canada (4,000 samples from 40 flocks). Similar results were later published by Owens et al. (2000a) for turkey breast meat sampled in Texas. Seasonal effects can be seen in Figure 17.4.7.3 for the Ontario data, indicating that the hot summer months resulted in higher incidences of PSE meat, likely due to heat stress. Overall the mean L* value of flocks processed in the summer was significantly higher than in the spring, autumn, and winter. The occurrence of PSE in poultry meat has led various researchers to suggest that the problem is associated with genetically inherited stress susceptibility in some broilers/turkeys.

![Figure 17.4.7.2](image)

**Figure 17.4.7.2** Turkey breast meat lightness (L*) distribution; n = 4,000.

Strasburg and Chiang (2009) indicated that the mechanisms underlying the development of PSE poultry meat are poorly understood; however, it is widely
accepted that PSE meat results from postmortem hypermetabolism of skeletal muscle. Porcine stress syndrome (PSS) has historically served as the model of hypermetabolism by pigs in response to stress. Upon exposure to heat, transportation, or mating, stress-susceptible pigs can often develop malignant hyperthermia (MH). This syndrome is characterized by excessive heat and lactic acid production coupled with augmented glycogenolysis and anaerobic glycolysis, all of which are coupled with severe muscle contracture. Porcine stress syndrome-malignant hyperthermia can even result in death before slaughter whereas stress-susceptible pigs that go through the slaughter process yield a higher incidence of PSE meat than non-stress-susceptible animals (Offer, 1991). The combination of high carcass temperatures and acidic muscle pH during the early stages of postmortem conversion of muscle to meat leads to denaturation of some of the myofibrillar proteins. Malignant hyperthermia has also been recognized in humans and other animals as an inherited skeletal muscle disorder triggered by response to administration of certain anesthetics such as halothane (Gronert, 1980).

Pork breeders were able to identify two main mutations associated with the PSE condition. The mutations are related to a defect in the regulation of calcium channels (called the ryanodine receptor) a few decades ago. Later, the industry introduced a fairly successful program to remove stress susceptible animals from the herd. However, such an exact gene mutation has not yet been identified in poultry.

![Figure 17.4.7.3 Truncation values of L* measurements obtained for young turkey tom breast meat samples showing seasonal effects. From McCurdy et al. (1996).](image-url)
Although there is not a comparable malignant hyperthermia phenotype in poultry, there is ample evidence that postmortem hypermetabolism of skeletal muscle underlies the development of PSE turkey meat (Strasburg and Chiang, 2009). Pietrzak et al. (1997) observed that turkey breast muscles, segregated into groups based on high (pH > 6.2) or low (pH < 5.8) 20-min postmortem pH, displayed markedly different biochemical and meat quality characteristics. Breast muscles from the latter group displayed lower mean adenosine triphosphate concentrations, higher lactate levels, lower water-holding capacity, lower cook yield, and lighter colour. These results are consistent with the biochemical description of PSE pork and are suggestive of rapid postmortem glycolytic metabolism.

Owens et al. (2000b) showed that some live turkeys are sensitive to halothane gas, a test used to identify susceptible pigs. The turkeys were exposed to 3% of the halothane for 5 min, which caused leg muscle rigidity in 3.5% of the 4 week old turkeys. However, the susceptible turkeys did not end up with a significantly higher incidence of PSE at slaughter time, as compared to a control group. At that time the authors suggested that either the halothane response is only a limited predictor of PSE meat in turkeys or is not an appropriate stressor to induce the PSE condition in poultry. Strasburg and Chiang (2009) suggested that the ryanodine receptors play a central role in regulation of avian sarcoplasmic Ca\(^{2+}\) and changes in receptor activity may have important implications for the development of PSE meat. The authors mentioned that although numerous advances have been made in our understanding of avian ryanodine receptors, particularly with respect to the discovery of alternative splice variants, it will be important to determine whether these transcripts are translated into protein and the functional differences of these variants. As of yet, our understanding of the causes of PSE in poultry is still insufficient to start a massive selection program as the one used in pigs. More research should be conducted with a long-term goal of reducing the problem in poultry. For now, poultry processors can only monitor the problem and try to reduce stress during catching, transport, and unloading and to modify some of the processing conditions (Barbut, 2009).
As indicated above, incidences of PSE in poultry have been reported to range from 5 to 40% (Barbut, 1998b; Petracci et al., 2009; Owens et al., 2000a) depending on the season, age, and cut point used to classify PSE meat. McCurdy et al. (1996) suggested a cutoff value of L* > 50 for young turkey breast meat based on its lower water holding capacity above this point. Owens et al. (2000a) suggested L* > 53 based on the relationships they identified between colour (L* value), pH, and expressible moisture (Fig. 17.4.7.4). A cut off point of L* > 52/53 was suggested for mature turkey hens, which are known to have overall lighter breast meat colour (Barbut, 1998b). An L* > 49/50 was suggested for broiler chickens based on data shown in Figure 17.4.7.5 and the corresponding WHC plus cooking loss data for these samples. Figure 17.4.7.5 shows that the lightness of broiler breast fillets can range from L* = 41 to 56, which represents a fairly wide colour range.

**Figure 17.4.7.4** The relationship between expressible moisture (EM), pH, and L* value (1.5 h) of turkey breast fillets (EM = 14.8935 - 0.0236 * colour * colour - 2.5438 * pH * pH + 0.5435 * colour * pH; R² = 0.3714; P = 0.0001). Each point represents average pH and EM for every one increment of L* value. The surface plot represents predicted values based on raw data. From Owens et al. (2000a). With permission.
When it comes to further processing, dealing with a high percentage of PSE meat can be a challenge. Some processors use unsorted meat in large batches (combos) to produce certain products. In the case of a product made out of small pieces (ground/chopped) this would not be a large problem so long as the proportion of PSE meat is low (e.g., < 20%). Mixing is actually one of the solutions to mask the effects of PSE meat (i.e., diluted effect). However, in a product made from large whole muscle pieces (e.g., oven roasted turkey breast injected with brine; see recipe in Chapter 13), the inclusion of PSE meat will result in excessive water release during cooking. In this case, presorting can be a solution. To sort, one needs to establish cutoffs suitable for specific production needs (e.g., requirements for water holding, texture). McCurdy et al. (1996) demonstrated how this could be done. They published a table with L* cutoff values for achieving WHC of 17, 20 and 23%. The corresponding L* values for the spring season were 52.0, 50.9 and 51.3 respectively. Values were also reported for meat obtained during the other three seasons (see Fig. 17.4.7.3) as well as to achieve certain textural characteristics (i.e., maximum compression force of cylindrical cooked meat samples). The important point is that such cutoffs can be established by meat processors to accommodate for their raw meat selection criteria, product formulation, and/or specific preparation method (e.g., injection rate, tumbling time). Overall, employing a grading system based on meat quality rather than the current one that is mainly based on aesthetic
factors (e.g., skin discolouration, bruises, missing parts, confirmation) would be a useful tool to grade meat according to functional properties (e.g., water holding capacity, texture).

White striation is another phenomena that can also be seen today in young broiler’s breast meat fillets (i.e., in the past it was associated with older laying hens or mature turkeys). The consumer will see striation on the anterior part of the skinless fillet, and their intensity can vary among individual samples. The problem is related to necrosis of muscle fibers, possibly due to fast growing rate and poor blood supply to the peripheral areas, and filling the spaces with fat and connective tissue (Kuttappan et al., 2013)

17.4.8 Colour Defects and Other Issues Related to Poultry and Red Meat

Looking at a food product is the first step in the consumer decision making process. If at this initial stage customers decide that they do not like the product, there is little that can be done to change their mind. The meat industry faces several challenges when it comes to the colour of fresh meat and processed products. In fresh meat, challenges include the meat being too light (PSE; discussed before), too dark (DFD meat), blood splashes, and discolouration (e.g., greening) of the muscle due to microbial activity. In addition, uneven colouring of meat pieces/fillets placed on the same tray can be considered a problem by the consumer. In cooked products, colour problems can range from nitrate burns to pinkness in traditionally white products (e.g., poultry breast meat). Other problems can develop over storage time; for example, colours can fade or off colours can be produced by microorganisms breaking the heme ring in myoglobin and/or producing certain pigments. Several examples are presented in the section below followed by an explanation of the cause and a potential solution.

a. Hemorrhages and blood splashes – occur due to a blood vessel rupture and are related to muscle injury (e.g., bruising, bone dislocation). Large bruises are usually trimmed/removed at the processing plant. Injury can happen at different stages of the animal’s life. During the growing period, animals housed in barns can be bruised/injured by sharp objects, fighting, or even sitting down for a long period (which can create blisters). During catching and transportation there is an even greater risk of injury, including when the animals are loaded and unloaded (see Chapter 4). Susceptibility to hemorrhaging can be increased by moldy feed that contains mycotoxins at a level as low as 5 ppm (Froning, 1995), which cause a weakening of blood vessels. Determining the time of injury is not always an easy task. In general, red bruises indicate recent injuries and brownish grey
discolourations, on the surface, can indicate older bruises. However, in order to precisely determine the cause of the bruise, a histological study is needed. This process involves looking at the distribution of red and white blood cells around the bruise (see discussion in Chapter 4, including staining methods). Determining the time of the injury will help with identify and correct the problem. During injury, cellular components are released into the tissue and cause a physiological or pathological response by triggering an inflammatory response and/or a blood clot. During the process, coagulation factors (e.g., thromboplastin) can cause localized or vascular coagulation and the breakdown of cell membranes releases enzymes such as of proteases and lipases that destabilize other cells.

The hemoglobin content in muscle showing different types of hemorrhaging was reported by Kranen et al. (1999). Adductor muscles with ecchymosis (a blood spot of several square mm) contained a hemoglobin concentration between 6.5 to 9.9 mg/g of tissue. Table 17.4.4.1 indicates that the average hemoglobin level is 0.83 mg/g (i.e., tenfold lower than injured tissue). In the pectineus, blood stains (described by the authors as a small straightened hemorrhage) contained hemoglobin levels of 0.12 mg/g as compared to hemorrhage-free muscle with 0.09 mg/g hemoglobin (Table 17.4.4.1). In the Sartorius, levels of 0.75 to 4.61 mg/g of hemoglobin were seen in bruised muscle as compared to 0.67 mg/g in hemorrhage-free muscles.

Processing conditions such as electrical stunning at high voltage can also increase the rate of hemorrhages as was discussed in Chapter 8. This can be due to severe muscle contraction and physical rupture of blood vessels that later result in blood splashes or what is called a “gunshoot” pattern. Problems, such as blood splashes, that are seen in fresh meat will be exaggerated in the cooked product (e.g., dark spots in a white oven roasted chicken breast).

b. Bone darkening – sometimes seen in young animals after cooking, this phenomenon can be induced by freezing the meat. After thawing, the muscle around the bone may have a dark/bloody appearance because some bone marrow was squeezed out from the porous bone structure. Later, during cooking, the hemoglobin component of the marrow is denatured (see Fig. 17.4.4.2) and forms a dark discolouration. The problem is more commonly seen around bone ends in the knee, wing, and leg joint areas. The problem is aesthetically unpleasing but does not present a health risk.

c. Discolouring due to microbial activity (e.g., greening, yellowing) – Discolouring due to microbial activity (e.g., greening, yellowing) – can be the result of microorganisms breaking the porphyrin (heme) ring of myoglobin or
producing water soluble pigments. This problem in non cured products usually
develops over storage time. An example is the growth of *Streptococcus faecium*
subspecies *casseliflavus* in vacuum packed meat, which initially appears as little
yellow dots (areas of developing colonies) but later can cover the whole surface of
the meat and have the appearance of a layer of mustard (Fig. 17.4.8.1).

![Image](image_url)

**Figure 17.4.8.1** Yellow pigment formation on meat due to microorganisms.
Photo by S. Barbut.

Such a discolouration makes the product unappealing and potentially dangerous
to consume. In the case of the *Streptococci*, the contamination usually takes place
after cooking because the microorganism is fairly heat sensitive and is destroyed by
normal cooking procedures (Whiteley and D’Sousa, 1989). Cross-contamination
by slicing equipment, handling of the meat, and/or contaminated air can spread the
microorganism to different packages. At refrigerated temperature it takes a few
weeks for the bacteria to develop. Note that such post cooking contamination can
also represent a big safety issue when a pathogen such as *Listeria monocytogenes*
is involved as was the case of a big outbreak in the USA in the 1990s.

Green colour has been reported when microorganisms such as *Pseudomonas
fluorescence* grow and produce a shiny, transparent, greenish exudate, mainly due
to myoglobin breakdown. This is not a common problem, but is more common
than the yellow colour described above. The greenish appearance is sometimes
mistaken as an iridescence problem (Swatland, 1984). It can be distinguished from
an iridescence problem by rotating the product 90°; if the greenish colour does not
disappear, then the problem is likely microbial. If the colour does disappear, the
problem is likely related to iridescence (see discussion below).
In cooked products the appearance of a so-called “green ring” can indicate an improper cooking procedure. A green core in the middle of a sausage indicates that the target internal cooking temperature was not reached and spoilage microorganisms capable of breaking down the heme pigment are still active. This kind of discolouration is irreversible (see Fig. 17.4.4.2). The appearance of a green colour on the peripheral part of a cooked sausage might indicate the use of meat with a high microbial load. In such a case, the microorganisms might have degraded the heme pigment even before the cooking operation started.

d.  **Green discolouration of fresh meat seen during the deboning process** – also called “green muscle disease”, this problem is sometimes seen in the interior of chicken/turkey breast meat. The scientific name is deep pectoral myopathy and results from the necrosis or death of muscle fibers in the interior part of the muscle in the live animals (Sosnicki and Wilson, 1992). In the past it was more commonly seen in heavy turkeys, however, today the problem is also seen in young broilers (Petracci et al., 2009). It might be that some breeds are more susceptible to it, and that certain growing conditions (e.g., thinning towards the end of the growing period) result in higher incidences (Kijowsk et al., 2014). Some researchers suggest that selection for increased body/muscle size may have altered blood flow to the deep pectoral muscle. On the processing line, affected birds usually show a sunken area on one side of the breast that, upon cutting, reveals an initial pinkish discolouration which later (in a few days) will change to greenish discolouration. In certain areas the necrotic zone hardens after being filled with fat and connective tissue, a condition referred to as “wooden breast” syndrome.

e.  **White spots on a fresh meat cut** – can appear after a few days of refrigerated storage as a result of microbial growth on the surface and is sometimes associated with a bad smell. Since the problem arises from bacteria and yeast growth, using a microscope to look at the microflora can be a quick way to identify the microorganism. This can be done by aseptically removing a colony and spreading it on a glass slide. If the organisms show budding they are most likely yeast cells. If they are small and rod shaped, they are most likely *Lactobacilli*. A follow up Gram stain can further help in the identification (Russell, 2006). Again, good sanitation during the process is a key factor in eliminating the problem. As well, identifying where the contamination occurred will help to solve the problem.

f.  **Iridescence** – usually appears as green-orangey colour on the surfaces of meat (Figure 17.4.8.2). It can be seen in fresh or cooked meat slices and is the result of white light spitting to its components. The exact mechanism is not fully understood, but it is known that certain muscle structures can cause more optical diffraction than others (Swatland, 1984; Lawrence et al., 2002).
The processor can reduce or even eliminate the problem by using a dull knife instead of a sharp knife. However, using a sharp blade is recommended to maintain high product quality and to avoid tearing the product. The fact that using a dull knife can “eliminate” the problem indicates that a smooth surface structure must be present to cause iridescence. Some reports suggest that the use of high phosphate levels can exaggerate the problem (Wang, 1991). One can use scientific tools to evaluate the spectral emissions of iridescent samples where the greenness has an almost monochromatic purity of colour. Conversely, greening due to microbial activity will show broader spectra typical of heme pigment degradation (Swatland, 1984). As indicated above, distinguishing between green discolouration caused by microorganisms and by physical structure can also be performed by rotating the product 90° and observing if the colour disappears. If it disappears, it was caused by the unique physical structure of the cut muscle surface.

g. Nitrite burn – seen as intense pink areas surrounded by light pink areas in a cured meat product. This can be the result of uneven brine injection in a whole muscle product (e.g., blocked needles in the injector or excessive pressure that causes an uneven distribution of brine). This visual defect can be accompanied
by areas that were not cured at all or by poor distribution of nitrite in both whole muscle and ground meat products (e.g., the no/low nitrite areas showing the typical brown denatured myoglobin colour). The appearance of nitrite burn usually also indicates that other ingredients, such as salt and spices, have not been evenly distributed. However, nitrite is the most critical ingredient for even distribution as it is an anti- \textit{C. botulinum} agent.

\textbf{h. Pinkness of typically white meat products} – a phenomenon sometimes seen in cooked poultry breast meat as pink strips/patches or an overall pink colour. The problem can be formed by two different mechanisms. The first was already mentioned and is referred to as persistent pinking (AMSA, 2012) and the second is due to low nitrite contamination. These products are often rejected by consumers, who suspect that the product has not been properly cooked. Maga (1994) indicated that “one of the interesting phenomena associated with this problem is its very sporadic and random occurrence among carcasses processed in apparently the same manner. Thus, by the time the problem is detected and various changes are made in production and/or processing, the problem has usually disappeared, and one is not sure as to which variable was responsible”. However, since this problem tends to come in cycles, it is important to find the cause. Holownia et al. (2003) reviewed the topic and described a number of factors that can be involved in the pinking caused by low nitrite levels getting into the product, and result in different manifestations of the problem (e.g., pinkness throughout the product, in the seams among muscle chunks, around the product’s perimeter). In most cases one of the most common causes is nitrite contamination. Heaton et al. (2000) reported that the minimum nitrite level required to cause detectable pinking in turkey breast meat roll was 2 ppm, for chicken 1 ppm, for pork 4 ppm, and for beef 14 ppm. The nitrite can come from the water used in the plant, the spice mix, gases discharged from the truck hauling the live birds, and from gas-fired ovens. Nitrite in the water can be a problem in certain agricultural areas (i.e., where nitrogen fertilizers are used). Therefore, it is recommended that processing plants monitor the nitrite levels on a routine basis and, if needed, install special filters to remove nitrite or at least nitrite in water used to process white meat products.

In the case of persistent pinking, an important factor can be the pH of the meat system. Janky and Froning (1973) studied the effect of pH and several additives on turkey myoglobin denaturation in a model system. The type of myoglobin derivative had an effect on the amount of heat denatured pigment in the crude myoglobin extract system. Denaturation increased when the pH was lowered using sodium erythorbate (i.e., commonly used as a curing accelerator; Chapter 13). On the other hand, sodium tripolyphosphate increased the heat stability of myoglobin by increasing the pH of the model system. This was believed to be due
to the increased polarity charge on the iron of the heme group. Ahn and Maurer (1990) studied the heme-complex-forming reactions of myoglobin, hemoglobin, and cytochrome C (molecular weight of around 12,500 Da with a structure similar to myoglobin). They reported that naturally present ligands such as histidine, cysteine, methionine, or their side chains formed solubilized protein complexes with hemoglobin. They also reported that a high pH (> 6.4) was favorable for the heme-complex-forming reactions of myoglobin and hemoglobin with most naturally present ligands (histidine, cysteine, methionine, nicotinamide, and solubilized proteins).

Additives such as salt, phosphate, and non-fat dried milk can also affect pinking. It has been reported that salt added at 2.5% significantly decreased the heat stability of myoglobin and hemoglobin at 68 and 74°C, respectively, while increasing the heat stability of cytochrome C (Slesinski et al., 2000; Ahn and Maurer, 1989). Sodium tripolyphosphate salt (0.5%) added to meat heated to 68, 74, 80 and 85°C, increased the heat stability of myoglobin but decreased the heat stability of cytochrome C due to the pH increase. Dextrose increased the stability of hemoglobin at 68°C and cytochrome C at 85°C, but not myoglobin. Overall, the authors indicated that adding salt and phosphate decreased oxidation-reduction potentials and that these changes could have a strong effect on cooked turkey breast meat pinkness, particularly if the oxidation-reduction potential of the meat is around +90 mV or -50 mV. Dobson and Cornforth (1992) reported that pink discolouration in turkey rolls could be prevented by adding 3% dried milk solids. They indicated that reactive sulfhydryls or other protein side chains in the non-fat dried milk might have raised the oxidation-reduction potential, thereby preventing complexing between heme and the denatured proteins. They also indicated that casein micelles in the non-fat dried milk might mask the meat pigments.

Froning (1995) and later Holownia et al. (2003) indicated that cooking temperature and time can also play a major role in the pinking (determining the amount of undenatured pigment present in cooked meat). Research findings from both poultry and red meat indicate that the undenatured pigment in cooked meat is mainly oxymyoglobin. Cooking turkey rolls to various end-point temperatures using a rotary oven showed that pink colour problems increased when the end-point was below 71°C. Normally, the end-point temperature should exceed 71°C (e.g., the USDA requires a minimum temperature of 71.2°C for all fully cooked poultry meat products to destroy pathogens), but processing temperatures could fall below this target if not closely monitored. Froning (1995) further reported a problem with regenerated pink pigment appearing 2 hr after cooked meat samples had cooled. In that case oxymyoglobin was identified as the cause for the pink problem.
i. **Colour fading** – results in yellowish, colourless meat and can arise due to strong light exposure that oxidizes the porphyrin ring in meat pigment (see Fig. 17.4.4.2). The problem is usually more pronounced in cooked products packed in a clear package and presented in a display case. Certain light sources have a relatively high proportion of UV light (e.g., fluorescent; see earlier discussion in this chapter) and are known to be more damaging to the colour and cause faster fading than other light sources (e.g., incandescent). Overall, the meat product becomes lighter (higher L* value) and less red due to partial oxidation of the meat pigment.

In order to minimize this problem, retailers can rotate displayed packages, use a specially designed film to block some/all of the UV light, or use opaque packaging material with or without a small window. The latter might not work if the consumer is used to looking at the actual product. Protecting cured meat pigment can also be achieved by removing oxygen from the package and/or using antioxidants. Vitamin E is a common, natural antioxidant, and as such it can be incorporated into the animal’s diet without any special labeling. Vitamin E is found in different plant materials where its function is to protect the plant from oxidation. Various researchers have reported the beneficial effects of vitamin E on preserving colour and flavour, and minimizing off-flavour formation by lipid oxidation in meat (Sheldon et al., 1997).

j. **Freezer burn** – appear as whitish grey areas caused by moisture loss (e.g., freeze drying) from the surface of uncovered/unprotected meat. The dehydration causes protein denaturation and discolouration. The problem can result from the use of an inappropriate wrapping material or holes in the packaging material. Besides discolouration, the meat in this area will be dry, tasteless, and may exhibit oxidative rancidity (described by consumers as old/stale flavour). Usually the colour becomes yellowish gray with a much higher b* value compared to the raw product. The a* value can also increase by 50%, probably as a result of concentrating the meat pigment. The physical characteristics of the packaging material are very important. Overall, the film should be moisture proof and ideally stretchable so that it can come in close contact with the meat (e.g., vacuum packaging is very popular in the meat industry). Having a tight package is important because it prevents water evaporation and ice accumulation inside the bag. It also ensures faster freezing by eliminating insulating air.

k. **Slimy appearance** – can be seen on the surface of the product and is the result of microorganisms capable of producing carbohydrate polymer chains to protect themselves (see also Chapter 15). Among the microorganisms that can be isolated in this case are the lactic acid bacteria of the genera *Lactobacillus*, *Enterococcus*, *Weissella* and *B. thermosphacta*. Slime formation is favored by moist surfaces and
is usually confined to outer surfaces/casings (Jay et al., 2005). Therefore, producers of cooked products are adding ingredients, such as whey proteins, that can bind the moisture and prevent exudate in the package. This is especially important in vacuum packaged goods where there is a physical force drawing moisture out of the product.

**l. Greening of cured products** – is usually caused by H$_2$O$_2$ and H$_2$S production in cooked products. In cooked products, both chemicals can interact with the nitrosohemochrome and oxidize the porphyrin rings. By doing so, the colour is changed from pink to green. As indicated before for non cured products, the greening can appear as a green core on the surface or throughout the product. A green core can appear after an aerobically stored product is sliced and exposed to air. It usually indicates inappropriate cooking temperature where the desired internal temperature has not been achieved and microorganisms capable of producing H$_2$O$_2$ or H$_2$S have survived. An outer green ring usually indicates high contamination levels of the meat surface or the casings. Some of the most common microorganisms known to cause H$_2$O$_2$ production in processed meats are *Weissella viridescens*, *Leuconostocs*, *Enterococcus faecium* and *Enterococcus faecalis*.

Greening of vacuum packed meat stored at refrigerated temperatures is usually caused by H$_2$S production, where the H$_2$S reacts with myoglobin to form sulphmyoglobin. The main microorganism responsible for this is *Pseudomonas mephitica*. The H$_2$S is usually formed by the degradation of the cystine amino acid, which contains sulfur. The green colour usually appears when the microorganisms reach 10$^7$/cm$^2$ (Jay et al., 2005). As indicated before, internal green patches in freshly slaughtered turkey meat have also been reported. However, in this case, the problem is attributed to deep-muscle-myopathy caused by the death of muscle fibers at these regions, and not related to bacterial spoilage. The problem is associated with the fast growth of the breast muscle in the modern turkey and the inadequate development of blood supply.

**m. Off odours** – The spoilage of vacuum packaged meat products and the production of off odours, flavours, and colour is usually the result of metabolites produced by microorganisms. Long chain fatty acids cleaved into short chains can often be the byproducts of *Lactobacilli* and *B. thermosphacta* activity, and result in offensive off odours. Acetone and diacetyl have been reported to be the most significant compounds responsible for the off odour of vacuum packaged luncheon meat.
In fresh vacuum packaged products, the production of sulphide odours can be the result of *Pseudomonas* and *H. alvei* activity. The sulphide smell usually is evident when the number of microorganisms reaches $10^7-10^8/cm^2$, which usually indicates extensive proteolysis by microorganisms using amino acids as an energy source. Slime formation can also be evident as was already discussed. Jay et al. (2005) reviewed some of the volatiles produced by bacteria causing spoilage of fresh and irradiated chicken. They have identified dimethyl disulfide, methyl mercaptan, $H_2S$, methanol and ethanol as the main compounds.

Spoilage of further processed, cooked poultry products can result from high microbial loads on the fresh meat, spices and casings contamination (especially natural casings). If inappropriate production procedures (e.g., warm temperature) are used, the shelf life may be significantly shortened. Examples of bacteria that can contribute to putrid off odours include *Pseudomonas* and lactic acid bacteria (see previous paragraph).

**n. Souring** – can occur in stored, cooked meats due to the growth of *Lactobacilli, B. thermosphacta* and *Enterococci* which are capable of fermenting different sugars (usually added to the product as milk ingredients or sugars). The microorganisms use the carbohydrates as an energy source and convert them into acids that cause souring. Processed meat products commonly contain a fairly varied microflora because of the different spices and other non-meat ingredients added. Therefore, if inadequate sanitation, quality control, cooking and cooling are not employed, on a continuous basis, a variety of problems can arise.

**o. Gas production** – Gas production in vacuum packaged sliced meats is usually the result of bacteria from the Clostridium family growing in the product. The meat is then unfit for human consumption. The main gas is $CO_2$ which is a water and lipid soluble gas and has some bacteriostatic effect. In a packaged meat system, the following chemical reactions can occur:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$

The proportion of the carbonic acid which is produced in stored meat is related to factors such as temperature and pH (Jay et al., 2005).
References


