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The raw and the cooked

How changes in protein structure cause meat to change colour when it is cooked

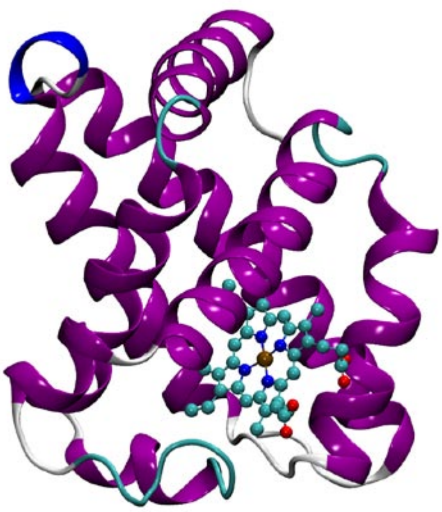
Aim

The purpose of this experiment is to show how the colour of meat changes at various temperatures. This is of importance when meat is cooked. The colour change is due to denaturation of the myoglobin: the protein in muscle fibres which gives raw meat its distinctive cherry colour. The denaturation is a structural change in proteins by heat or chemicals thereby changing some properties of the protein, like solubility and three-dimensional structure.

Introduction

Meat from different species varies in colour: beef is redder than pork, which again is redder than turkey meat. This variation is due to differences in the myoglobin content of the muscles.

Myoglobin is a small, red, oxygen-storage protein which carries oxygen from the red blood cells to the mitochondria in the muscle cells. Myoglobin is similar to hæmoglobin, the oxygen-binding protein of red blood cells. Hæmoglobin transports oxygen from the lungs to the capillary system of the various organs, including the muscles.



Myoglobin, showing the oxygen-binding hæm group. At the centre is an iron atom, bound to four (blue) nitrogen atoms and surrounded by a flat hæm group. Myoglobin was the first protein structure to be determined, by John Kendrew and his colleagues in Cambridge, in 1960.
[Data from Protein Data Bank, Protein ID: 1MBO]

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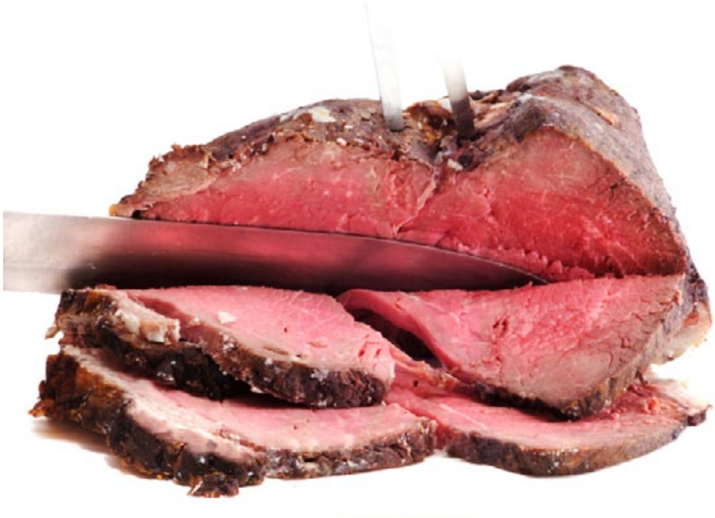
The amount of myoglobin in muscles varies considerably between species. Marine mammals have a large amount of myoglobin in their muscule tissues, which makes their meat very dark. Muscle myoglobin is an adaptation to their lifestyle and functions as a reservoir for oxygen. This reservoir allows them to stay under water for an extended time and prevents the 'bends', allowing them to dive deep into the sea.

Almost all proteins in meat are denatured by heat treatment, which has a dramatic effect on the colour of the meat. Myoglobin denatures at about 60 °C, which is apparent when one prepares roast beef — if the thermometer shows 58 °C in the middle of the meat the beef will appear red but at 68 °C the meat will appear grey. Chefs use these colours to name your piece of meat *rare*, *medium* or *well done*.



The red colour of roast beef is due to myoglobin. Denatured myoglobin, on the outside of the meat, is brown.

The muscles of vertebrates have two types of fibres: white and red. Red fibres need a lot of oxygen to function and therefore have a large myoglobin content. These fibres are in use during for example marathon running or cross-country skiing. They function under conditions where there is sufficient oxygen for cellular respiration.

The white fibres, in contrast, function without using oxygen and obtain their energy from conversion of glucose to lactic acid. They contain almost no myoglobin. White muscle cells are only efficient for short periods of time (minutes), since they depend solely on the glucose reserves in the muscle. The metabolite from anaerobic breakdown of glucose, lactic acid, is transported from these muscle fibres by the bloodstream to the liver, where it is metabolised.

The proportion of white and red muscule fibres in vertebrates varies and can be changed to some extent by exercise. You can see this in chickens. Their breast meat is white, not red, since they do not fly. Their thighs are slightly more red since they walk around — but not, of course, if they are caged. This could be a reason for the slightly different taste of organic chicken.

Chicken meat is praised by nutritionists as a healthier alternative to beef or pork because of its lower fat content. Studies have shown, however, that especially women on this diet have iron defficiency because they do not eat red meat and loose a lot of blood every month during menstruation. The problem is that hæm iron from meat is absorbed much more easily than non-hæm iron from vegetables. Furthermore, some plant metabolites in certain vegetables prevent the uptake of non-hæm iron which makes the problem worse.

Equipment and materials

Needed by each person or group

Equipment
- Small (*e.g.*, 100 mL) heat-resistant beakers, 6
- Spoons or spatulas, 6
- Test tubes, 6
- Test tube rack
- Small funnels, 6
- Heat-resistant glove
- Water-resistant marker pen
- Thermometer
- Access to a water bath, set at 90–100 °C

Materials
- Filter paper, 6 discs
- Freshly-minced beef, ~60 g
- Distilled or deionised water, 150 mL
- 1 bucket of water containing ice cubes
- Access to soap, water and towels for washing hands

Procedure

1. Wash your hands thouoghly before handling the meat. If you have any wounds to your hands, wear disposable gloves.
2. Place 10 g of meat into each of the six beakers.
3. Add 25 mL of distilled or deionised water to each beaker.
4. Place the beakers in a water bath set at about 90–100 °C.
5. Heat the samples, stirring them constantly with a spoon or spatula until they reach a final temperature of: 50 °C, 60 °C, 65 °C, 70 °C, 75 °C or 80 °C. Use the thermometer to check the temperature regularly.
6. Once each sample has reached its final temperature, remove it from the water bath and cool it down immediately in an ice bath. *Ensure that the beakers do not fall over — adjust the water level of the ice bath to the level of the content of the beakers or lower.*
7. After cooling, filter each sample and collect the filtrate from each one in a separate test tube.
8. Evaluate the colour by eye (*e.g.*, red, light brown, dark brown, brownish grey *etc*) and record your results in a table.
9. Wash your hands thoroughly after completing this work.
10. If possible, take a digital photograph as an additional record.

Safety guidelines

When you work with meat there is a risk of the presence of *Salmonella* or *Campylobacter.* Under normal conditions it is sufficient to thoroughly wash your hands and any tools that have been in contact with the meat. If you have cuts or skin damage on your hands, you should wear disposable gloves.

Prior knowledge and teaching tips

The investigation is quite simple and does not require special laboratory experience or chemical insight. It would however be an advantage if the students have been introduced to the structure and function of proteins and their denaturation. The experiment provides an opportunity to discuss protein denaturation and its importance in meat preparation procedures.

A further perspective is the discussion of oxygen transport, muscle function and the sources and function of iron in the diet.

This work also provides an opportunity to consider food hygiene and the rôle of the immune system.

Preparation and timing

The investigation takes about 45 minutes. It will increase the efficiency if each group of pupils takes responsibility for one part of the experiment, that is, a certain temperature. Once the experiment is set up each component part can be completed rapidly.

Specimen results

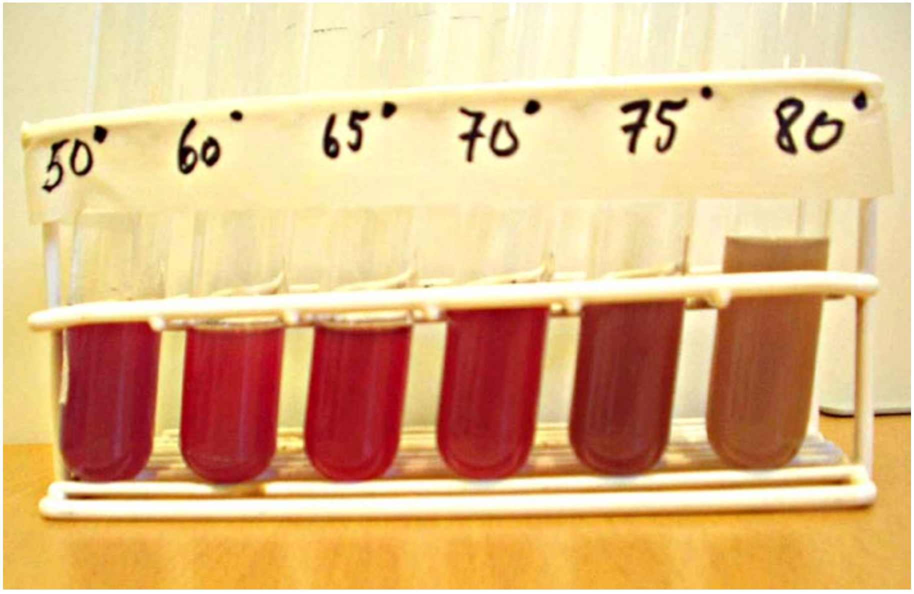


PHOTO: KAREN LUNDEN

The colours of the juice extracted from meat at different temperatures: 50 °C – purple; 60 °C – cherry red; 65 °C – cherry red; 70 °C – slightly darker red; 75 °C – dark red with a little brown; 80 °C – brown.

Additional investigations

The experiment can be tried with other types of meat *e.g.*, pork and turkey, but the colour change may not be that marked, since their myoglobin content is lower than that of beef.

You can also try fresh red salmon and compare it with fresh tuna (not tinned). While the tuna's red colour is due to myoglobin the red colour in salmon is caused by astaxantin, a pink pigment from crustations — their food source. Astaxantin is not protein-linked and

therefore does not change colour. Farmed salmon also contains astaxantin, but it may be of microbial origin. A yeast-like strain synthesizes it; the yeast or a red dye is added to the fish food (see the *SalmoFan*, below).

Troubleshooting

It is essential that once the right temperature is reached the beaker of water-meat mixture is cooled down immediately in an ice bath. Don't forget to stir to ensure a better temperature distribution from the walls of the beaker to the interior.

Disposal of waste

Pack the meat in a plastic bag, close the bag with a firm knot and put it in a dustbin.

Other sources of information

For more literature in both English and Danish, see: www.kvl.dk/forskning/oevelseshaefte.aspx

Additional information is provided by Chapter 3 of *McGee on food and cooking: An encyclopedia of kitchen science, history and culture* by Harold McGee (2004) Hodder and Stoughton Ltd, London. ISBN: 0 340 83149 9.

Burros, M. (2003) The SalmoFan: Issues of purity and pollution leave farmed salmon looking less rosy *New York Times*, 28 May

This article is available here: http://www.edwardtufte.com/files/salmofan.html

Additional discussion and photos of the *SalmoFan* are available here: http://www.edwardtufte.com/bboard/q-and-a-fetch-msg?msg_id=0000XT&topic_id=1&topic=Ask+E%2eT%2e

Godsell, D. (2000) Protein Data Bank Molecule of the month, January: Myoglobin. http://www.rcsb.org/pdbstatic/education_discussion/molecule_of_the_month/download/Myoglobin.pdf

Acknowledgements

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The myoglobin molecule was created using data from the Protein Data Bank: www.rcsb.org/pdb. The data for myoglobin was published in: Phillips, S.E. (1980) Structure and refinement of oxymyoglobin at 1.6 Å resolution. *Journal of Molecular Biology* **142** 531–554.

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