# 5-P3

## The use of electron spin resonance (ESR) to determine the mobility of solutes in meat during freezing and frozen storage

**E. Hansen, M. L. Andersen, A. Mortensen & L. H. Skibsted**
Food Chemistry, Department of Dairy and Food Science, Royal Veterinary and Agricultural University
Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

**Background:**
When the temperature of meat is decreased below the freezing point water begins to crystallise, the average mobility of solutes in the water phase decreases and ice crystals are gradually formed. Electron Spin Resonance (ESR) spectroscopy holds the potential to follow the decreasing mobility using spin probes (stable radicals). Investigation of sugar-water/water-glycerol mixtures has thus shown a good correlation between the shape of the ESR spectrum (and the $t_c$ (correlation time) of the probe) and a quantitative measure of the mobility of the probe in solution and further provided indications of glass transitions (Roozen & Hemminga, 1990). The existence of glass transitions in frozen meat is still a matter of discussion and it appears that in two widely different temperature ranges phase transitions occur, which both have been assigned as glass transitions. By using the ESR spin probe technique it is possible to get information about how the mobility of solutes in meat is affected by decreasing temperatures. This information can in principle be used to detect glass transitions. Below the glass transition temperature the viscosity is extremely high, in effect halting the diffusion of solutes and any diffusion controlled reactions. The spin probe 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethyl-1-piperidine-N-oxyl (Figure 1)) incorporated in a meat system has in the present investigation been used to explore transitions in meat that has been exposed to conditions of decreasing temperature and progressive freezing of water.

**Objectives:**
The objective is to develop a method for determination of solute mobility in pork meat during freezing and frozen storage, and to assign the glass transition temperature of pork meat, in order to reduce drip loss and increase oxidative stability as a result of improved freezing techniques.

**Methods:**
Fresh pork meat (*Longissimus Dorsi*) was cut in thin (1 mm) slices and was placed in a buffered (potassium phthalate, pH 5.6 at 5 °C and I = 0.16) solution of 2.0 mM 4-hydroxy-TEMPO (Sigma). 4-Hydroxy-TEMPO is hydrophilic and concentrate in the aqueous phase of meat. After marinating (at 5 °C) a sample (ca. 2.5 mg) was placed in a plastic cell (1.5 cm wide, 16 cm long and 1.5 mm thick) and weighed. The plastic cell was made of 115 μm thick plastic foil and was sealed using an AVC table sealer. The cell was sealed in the bottom after the meat sample was placed to avoid drying during the experiment. A plastic stick was placed in the cell to stabilise the cell during the experiment.
ESR spectra were recorded on a Bruker ECS 106 spectrometer (Bruker, Karlsruhe, Germany) with a liquid nitrogen temperature control. Measurements were made with the following spectrometer settings: microwave power 0.200 mW; modulation frequency 100 kHz, modulation amplitude 1 Gauss, time constant 41 ms, conversion time 41 ms, resolution 1024 and number of scans 2. Water content of the meat samples was determined before and after marinating with the spin probe, extracting with dried methanol. After 24 hours the water content in methanol was determined by Karl Fisher titration (Mettler Toledo AG, Analytical, Schwerzenbach, Schweiz). The water activity of the meat samples was also determined before and after marinating using an AquaLab CX-2 (Decagon Devices, Inc., Pullman, Washington, USA).

**Results and Discussion:**
The water activity in the meat samples showed no difference between marinated and raw meat samples, the average value was $a_w$ 0.988 ± 0.002 (Table 1). The water content determined by Karl Fischer titration was likewise very similar for marinated and raw meat samples (Table 1). The ESR spectra confirmed that 4-hydroxy-TEMPO had diffused into the meat sample (Figure 2). The ESR spectrum of 4-hydroxy-TEMPO changes as the temperature is lowered (from 6 °C to – 46 °C), Figure 2. At – 10 °C the spectrum changed over a narrow temperature range from a typical solution spectrum towards a typical powder spectrum (high viscosity), indicating that the spin probe becomes immobilised as the water crystallises. As the temperature was decreased further changes towards a typical powder spectrum was observed which indicated progressive freezing of water resulting in a further lowering of the rotational mobility of the spin probe. The glass transition in bigeye tuna flesh determined by DSC was found to be around – 70 °C (Inoue & Ishikawa, 1997), but in this study no change in the ESR spectrum was observed in this temperature region.

**Conclusion:**
A method for determining solute mobility in meat at subzero temperatures has been developed. The method is based on the stable hydrophilic radical 4-hydroxy-TEMPO which is incorporated in the meat sample by a marinating procedure. Line broadening of the ESR spectrum of the spin probe indicated a decrease in solute mobility in a rather narrow temperature range around –10 °C. This result indicates that solute mobility and glass transitions in meat can be explored using the ESR spin probe technique. Further progress is expected when this method is correlated with scanning calorimetric methods.

**Literature cited:**
Inoue, C. & Ishikawa, M. (1997) Glass Transition of Tuna Flesh at Low Temperature and Effects of Salt and Moisture. Journal of Food Science **62**, 496-499

Roozen, M.J.G.W. & Hemminga, M.A. (1990). Molecular Motion in Sucrose-Water Mixtures and Glassy State as Studied by Spin Probe ESR. Journal of Physical Chemistry **94**, 7326-7329

**Data:**

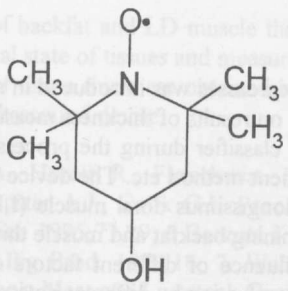


**Figure 1:** Structure of the spin probe 4-hydroxy-2,2,6,6-tetramethyl-1-piperidine-N-oxyl (4-hydroxy-TEMPO)

**Table 1:** Water content and water activity of marinated and raw meat samples used for ESR experiments.

| | Karl Fisher titration [% water in meat sample] | | Water activity at 25 °C | |
|---|---|---|---|---|
| **Raw** | 78 ± 2 | 76 ± 2 | 0.988 ± 0.003 | 0.989 ± 0.009 |
| **Marinated** | 81 ± 3 | 78 ± 3 | 0.988 ± 0.004 | 0.985 ± 0.002 |

The values are results of 3 determinations of each meat sample.

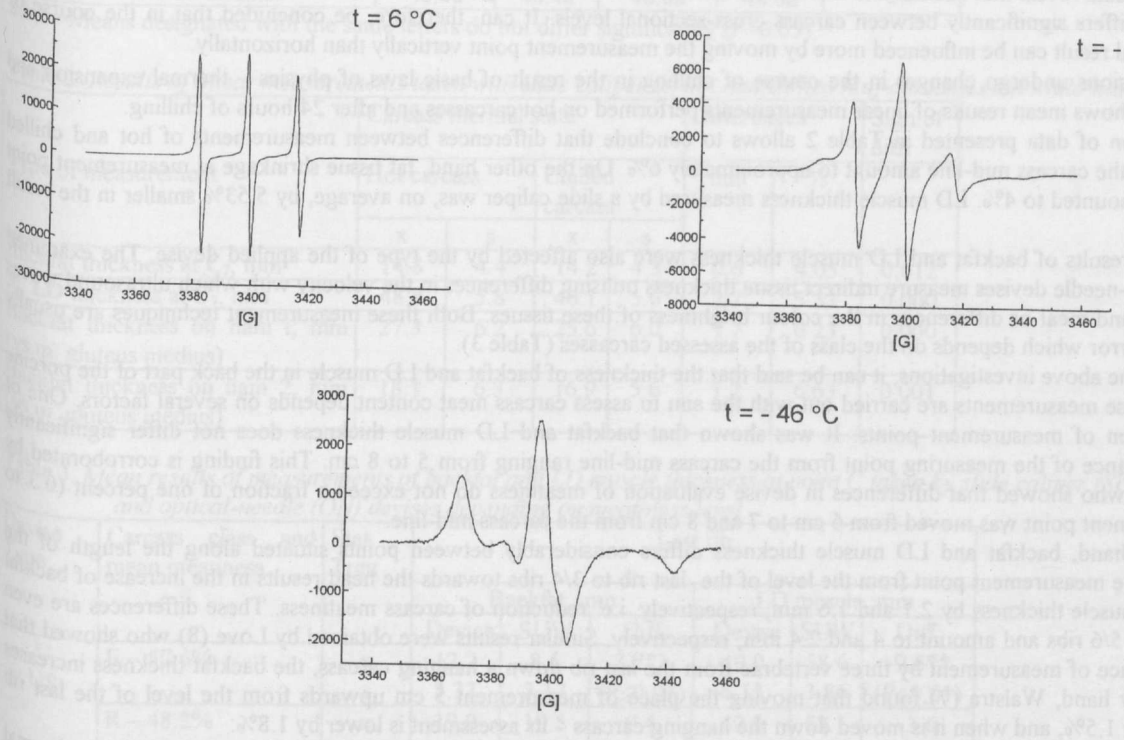


**Figure 2:** ESR spectra of spin probe 4-hydroxy-tempo (2 mM) in *Longissimus Dorsi*. Sample weight is approximately 3 mg.