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The use of dielectric properties and other physical analyses for assessing protein denaturation in beef *biceps femoris* muscle during cooking from 5 to 85 °C

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Abstract

Dielectric properties of beef *biceps femoris* muscle were recorded during heating (5–85 °C) to assess their linkage to phase changes monitored by differential scanning calorimetry (DSC) and rheology. DSC indicated endotherms between 56 and 81 °C corresponding to denaturation of actin, collagen and myosin. Matching changes in dielectric properties (dielectric constant (ε') and loss factor (ε'')) were noted at radio and/or microwave frequencies though the nature of the change differed depending upon frequency. The main observation in ε' was an increase above 65–66 °C, most likely due to fluid release on collagen denaturation. This fluid plus liquid from myosin denaturation most likely solvated ions freed during myosin denaturation which manifested as an ε'' increase. However, it must be noted that meat structural protein denaturation is compounded with physical shrinkage which can also influence dielectric properties. Rheological parameters of beef muscle heated from 5 to 85 °C also displayed marked changes relating to structural protein denaturation.

Keywords: Beef muscle; Protein denaturation; Dielectric properties; Rheology; Electrical conductivity; DSC

1. Introduction

The tenderness of meat is largely governed by two muscle components, the connective tissue which is responsible for the so-called "background toughness" while the myofibrillar proteins (including the myofibrillar cytoskeletal proteins) and cytoskeletal proteins largely govern the "myofibrillar toughness" (Geesink, 1993; Valin & Ouali, 1992). The improvement in the tenderness of meat during conditioning may be attributed to changes occurring in the myofibrillar proteins (Tarrant, 1987). Subsequent heating of meat results in the development of textural, colour and flavour properties characteristic of a cooked product. These changes are brought about mainly through the effect of heat on components such as proteins and fats found in the muscle. Myosin and actin (myofibrillar) and also collagen (major protein in connective tissue) and are the major structural proteins present in muscle foods and thus the effect of heat on these proteins has a major influence on the resulting texture of the cooked meat. Myosin which constitutes approx. 45% of total myofibrillar protein (Greaser, Wang, & Lemanski, 1981) is the least heat stable of the major structural proteins in muscle. This class of protein generally denatures in the temperature range 40–60 °C (Bendall & Restall, 1983) and this denaturation and shrinkage manifests as an increase in the shear

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values of meat (Sims & Bailey, 1992). It has been reported that denaturation of these myosin proteins is accompanied by the release of calcium and magnesium ions (Hamm, 1966) and fluid (Bendall & Restall, 1983) into the extracellular spaces but is not accompanied by shortening of the muscle fibres. Collagen is the major connective tissue protein present in meats and collagen fibres begin to shrink at around 64 °C with complete denaturation usually being complete at around 70 °C, though Rochdi, Bonnet, and Kopp (1985) reported that when collagen fibres are restrained during heating the temperature at which denaturation is complete can increase to 80–85 °C. Similar to myosin denaturation, collagen denaturation is accompanied by a further increase in shear values due to the shrinkage of collagen (although prolonged heating of meat above 70 °C will eventually produce a reduction in shear value which is believed to be due to the cleavage of peptide bonds in the collagen (Sims & Bailey, 1992)). The denaturation of collagen fibres leads to a change from an opaque inelastic fibre to a translucent swollen elastic fibre which generates a tension against the muscle fibres and also results in juice expulsion from the tissue. Given that an increase in juice expulsion has been directly related to an increase in collagen content in meats (Offer & Trinick, 1983) it has been suggested that collagen is responsible for the majority of juice expulsion from tissue during cooking (Bendall & Restall, 1983). In the majority of muscle systems actin proteins are the most heat stable and only begin to denature at temperatures from 71 °C with denaturation being complete in most instances at 83 °C (Barbut & Findlay, 1991).

As outlined above, heating brings about major changes in the water holding capacity of the constituent structural proteins, which results in the release of juice from the muscle. To date by far the most common method used for monitoring denaturation of proteins in muscle systems is differential scanning calorimetry (DSC) which measures the difference in heat flow between the sample and a reference. However, as outlined above since denaturation of proteins is accompanied by changes in some of the physical and chemical properties of the muscle it is possible to monitor changes in the denaturation state of proteins indirectly by assessing how these properties change during heating. For example rheological parameters such as the storage modulus (G') and the loss modulus (G'') have been used to monitor changes in the viscoelastic behaviour of meats and these changes have been related to the denaturation of its myofibrillar proteins (Egelandsdal, Martinsen, & Autio, 1995; Torley & Young, 1995).

A number of recent studies have attempted to relate changes in the dielectric properties (i.e. ε' and ε'') of meats to the denaturation status of its constituent structural proteins. These properties are composition dependant (Lyng, Scully, McKenna, Hunter, & Molloy, 2002; Zhang, Lyng, & Brunton, 2004) and are influenced by water (free vs. bound) and ionic (free vs. bound) content of the food among other factors (Roebuck & Goldblith, 1972). The work of Tornberg, Andersson, Goransson, and Von Seth (1993) and Hills, Manning, Ridge, and Brocklehurst (1996) has led to a better understanding of water binding in meat and it is now usually sufficient to consider three states of water, namely 'structural and bound water' (i.e. water hydrogen bonded inside the groves and cavities of globular proteins), surface water (i.e. hydration water of the macromolecule which extends only one or two molecular layers from the surface of the bio-polymer) and bulk water (i.e. the rest of the water). Li and Barringer (1996) monitored changes in the ε'' of high salt ham samples at microwave (MW) frequencies and concluded that changes in ε'' corresponded to the denaturation temperature of actomyosin. In addition Bircan and Barringer (2002) monitored ε' and ε'' (at MW frequencies) in a range of meat, fish and poultry samples over the temperature range of 20–120 °C and found that both ε' and ε'' increased at a temperature which appeared to match the DSC denaturation temperature of collagen in these foodstuffs. Researchers from this group recently published a study relating to the dielectric properties of two comminuted meat products over the temperature range 5–85 °C at both RF and MW frequencies (Zhang et al., 2004). However, in this study measurements were only taken at intervals of 20 °C and a number of non-meat ingredients were present in the meat product. Thus the effect of protein denaturation on dielectric properties may have been masked by non meat ingredients and missed because of the large temperature measurement interval. Therefore a principle objective of the present study was once again to monitor changes in the dielectric properties of whole meat across a temperature range of 5–85 °C but at 1 °C intervals (vs. 20 °C in Zhang et al. (2004)) to determine if changes in the dielectric properties when measured at a finer temperature resolution could be related to changes in the denaturation state of the constituent structural proteins as monitored by DSC. In addition, changes in the rheological properties and juice loss of the whole beef muscle were measured as a function of temperature in an attempt to correlate changes in these properties with protein denaturation.

2. Materials and methods

2.1. Meat handling

Three (4 kg) batches of beef *biceps femoris* muscle were obtained from a local supplier (Kepak Ltd., Clonee, Co. Dublin). This muscle was chosen because of its well defined fibrous structure and the fact that the muscle fibre direction was relatively uniform. Prior to trimming