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Testing meat tenderness using an in situ straining stage with variable pressure scanning electron microscopy

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Abstract

This project developed analytical protocols allowing in situ tensile testing of beef M.semitendinosus in a variable pressure scanning electron microscope (VP-SEM) fitted with a straining stage. The objective of this project was to develop an appropriate testing regime for the tensile testing of meat following various pre-treatments (marinaded, cooked, frozen). The in situ testing, at 100% relative humidity, was validated with ex situ testing using the same tensile stage. From the ex situ tensile tests the apparent elastic modulus ranged from 0.1MPa to 0.3MPa with the cooked, non-marinaded meats being stiffest. The low strain rates imposed by mechanical considerations of the straining stage resulted in lower values of fracture toughness and UTS than those reported in the literature. Fracture behaviour observed during the in situ tensile tests revealed that fracture propagated across the perimysial connective tissue. For the cooked non-marinaded steak, catastrophic rupture occurred in the crosslinks of the connective tissue. The cooked marinaded steak with more gelatinized connective tissue showed a less catastrophic rupture. Limitations of the testing geometry of an in situ straining stage are critical for their impact on image quality. The working distance for VP-SEM is necessarily short meaning that a sample needs to be close to the final lens of the SEM for high quality images. This is hard to achieve within the geometrical constraints of most sample chambers. However, it was possible to capture real time images of muscle failure during tensile testing of meat and relate that to the impact of meat processing treatments. Such information is vital for determining the structural factors affecting meat tenderness.

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Keywords: ESEM; VP-SEM; in situ microscopy, meat tenderness

1. Introduction

Meat texture, in particular the sensory property of "tenderness" is an essential parameter in meat quality and has been studied for many years [1]. Tenderness is influenced by each stage of the meat production process including slaughter, aging and cooking technique [2]. Mechanical measures of meat

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tenderness are most usually based on the Warner-Bratzler shear force (WBSF) [1,3,4] or on Texture Profile Analysis (TPA) [1,5,6]. Tensile tests are more infrequently used but have been used extensively in the past and are still vital to providing specific information on fracture behaviour [7-13]. Fracture behaviour of meat under shear, compression or tension, indicative of tenderness, is intimately related to the overall microstructure of meat including the arrangement of muscle fibre bundles and the strength of the cross links between them. These can be altered by processing routes, including aging, cooking and marinading [14,2,5,6]. .

To follow the fracture behaviour of meat at the appropriate length scale is important in understanding the failure mechanisms of the muscle fibres, vital to the breakdown of meat during oral processing (chewing). However, developing microscopy techniques that allow the investigation of any food structure, at relevant length scales, is challenging due to the hydrated and metastable state of many food materials [15]. Variable pressure scanning electron microscopy (VP-SEM), often called Environmental SEM (ESEM), can be used to study food materials in their native state [16-18]. When coupled with a suitable sample stage real time deformation can be followed [19,20].

The development of the VP-SEM, or ESEM, and details of vacuum management and electron detection are thoroughly reviewed elsewhere [21-23]. It is important to note the essentials here, however, to illustrate the particular issues of operating a straining stage, or any in situ manipulator, in the VP-SEM. VP-SEM instruments have a differentially pumped electron column, in conjunction with some method for allowing a pressure difference between the column and the specimen chamber, allowing the electron column to be maintained at high vacuum whilst the specimen chamber can be simultaneously maintained at pressures up to around 2500Pa, or about 20 torr. For ease of imaging the sample is usually imaged at low temperatures (about 5°C in many cases) and 5-10 torr. By altering the sample temperature or the chamber pressure the sample can be held in relative humidities up to 100%. A short beam gas path length is essential for image quality meaning that the ideal situation is a short working distance between the pole piece of the SEM and the sample surface. This short working distance is hard to achieve for an in situ straining stage within the geometrical constraints of most sample chambers.

This project developed analytical protocols allowing in situ tensile testing of beef M.semitendinosus in a VP-SEM fitted with a straining stage. The objective of this project was to develop an appropriate testing regime for meat following various pre-treatments (marinaded, cooked, frozen). Limitations of the straining stage geometry meant adaptors had to be used to shorten the working distance to acceptable levels, compromising the testing geometry; however, it was possible to capture real time images of muscle failure and relate that to the impact of meat processing treatments. Such information is vital for determining the structural factors affecting meat tenderness.

2. Materials and Methods

Commercially available raw beef rump steak (Hereford breed, slaughtered at approximately 30 months) was used for all tests. Samples measuring 60x40x10mm were excised from the whole steak using a sharp scalpel. Samples were examined both raw and after cooking, marinaded and unmarinaded. Marinades of varying pH were prepared from: fruit pulp (kiwifruit or lemon), vinegar and bicarbonate of soda. Beef samples were marinaded for 3 hours and quantified for weight gain using equation 1.

$$\%\,\textbf{weight gain} = \left(\frac{Initial\ weight - blotted,\ \ marinated\ weight}{Initial\ weight} \right) \qquad (1)$$

Cooked samples were prepared using a UFB500 Memmert Universal lab oven at 180°C. Samples were cooked for 20 minutes with one turn after 10 minutes. Moisture loss on cooking (cooking loss) was calculated using equation 2.