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The Use of Proteolytic Activity to Evaluate Meat Tenderising Agents

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Abstract: Tenderness is considered to be the most important meat characteristic. Currently common methods of evaluating meat tenderisation include scanning calorimetry, texture profile analysis and WBSF measurements. Here we report the use of a scientific tool based upon a colourimetric protease assay to screen natural products as tenderising agents.

Keywords: Meat tenderisation, marinades, proteolytic activity, azocasein

Introduction

Consumers and manufacturers are constantly looking for products of improved quality. The meat industry also shares the same expectation. Tenderness is rated as the most important characteristic of meat. Currently Warner-Bratzler Shear Force (WBSF) is commonly applied in order to determine meat tenderisation [1, 2]. Units of measurements are kilograms of force needed to shear a 1 cm^3 muscle sample. A tender cut requires a WBSF of 2.6 Kg whereas tough meat presents that of 5.3 Kg [1].

Meat with the fat deposited within the steak to create a 'marbled' appearance has always been regarded more tender than steaks where the fat is in a layer around the outside [3]. Additionally 'marbling' provides a dilution effect of connective tissue (collagen) and offers lubrication in the chewing process. Adequate finish and 'marbling' to meat market demands could be achieved by feeding the animals a high grain diet for a maximum of 100 days prior to slaughtering [4]. However selecting for 'marbling' to improve meat tenderisation could result in compromised meat yield and consequently customer's dissatisfaction.

Moreover tenderness is heritable due to genetic influences, such as calpain (20% variability), calpastatin (40-70% inheritability), muscle fibre thickness (40µm-80µm variability), collagen solubility (28-6% variability) and elastin fibrils (0.6-4µm) [5]. For example the USA Brahman breed of beef cattle is considered to lack reliable meat quality demonstrates a WSBF of 5.3-6.8 Kg and is considered to have a bad genetic background [6, 7]. Scientists are trying to overcome this problem by cross breeding genetic distant animals in order to achieve 'double muscled' breeds with improved tenderness [7].

The post slaughter physicochemical characteristics of the muscle tissue also affect the tenderisation process. The pH of living animals is around 7. After death the sugars in the muscles are converted to lactic acid lowering the pH to 5.5 [8, 9]. Although meat that exhibits very high or very low pH is tender, high pH meat is very dark (borderline dark cutters) and has a 'rubbery' undesirable texture [10]. High pH also indicates stress before slaughtering, which suggests that quiet handling and good transport conditions have not been employed [11].

Many studies have been carried out for numerous years regarding the disruption of meat architecture with a view to improving meat tenderness post mortem [12-15]. For example hydrodynamic pressure or shockwaves have indeed demonstrated a 25 % WBSF reduction but the technology has not been employed by the food industry [16, 17].
The use of marinades to break down connective meat tissue and improve texture and flavour has also been assessed [18]. In most cases evaluation is based on sensory evaluation by trained experts, WBSF measurements, scanning calorimetry and texture profile analysis [18-22].

The study presented here aims to investigate the use of proteolytic activity to evaluate meat tenderising agents. Proteases are found in a variety of natural products and they are enzymes that hydrolyse peptide bonds leading to the disassembly of muscle proteins and consequently meet tenderisation [23]. The effect of pH was eliminated by manually adjusting it to 4, 5 and 10.

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Materials and Methods

Azocasein Method

An aliquot of each natural product was added to 200 µl of substrate (10mg/ml of Azocasein Product A-2765 [Sigma, Lot 18H7014] in 25mM Hepes, pH 7.0.) and incubated overnight at 37^0 C. Where appropriate (liquid samples) the pH was adjusted using 1M NaOH or 1M KCl. The reaction was stopped with 750µl of stopping reagent (0.3M of Trichloroacetic acid), which precipitated the undigested azocasein [24]. The solution was mixed and centrifuged at 3000 rpm for 10 minutes in an Anderman 5414 micro centrifuge. The supernatant (1ml) was removed and optical density was spectrophotometrically determined at 450 nm (OD_{450}) using a UVIKON 930 analyser.

Results and Discussion

In an attempt to design a 'scientific' based marinade, which will significantly improve the meat tenderisation process, natural products were screened for their proteolytic activity.

Proteases found in food disrupt the peptide bonds between amino acids present in meat proteins, such as collagen. This biochemical mechanism improves meat texture and tenderness [23].

OD_{450} is a measure of enzyme activity in the products tested and it is an indication of the tenderisation potential of the material. Table I illustrates the protease activity of the products assayed by the Azocasein method. All the samples tested had demonstrable proteolytic activity. Greatest activity was seen with fresh pineapple at pH 10. Interestingly when pasteurised pineapple was examined it also contained protease activity, albeit to a lower activity (30% of that measured for the fresh extract). The significance of this is that this product appears to retain activity after heat processing suggesting that this could be a useful addition to a processed marinade.

Tinned tomato can also be employed as an ingredient in pasteurised marinades as its proteolytic activity was not significantly different to the fresh tomato. The pH of tinned tomato was not adjusted due to the high content of solids. Materials also high in protease activity include mushroom and garlic.

The objective of this study was to determine if a commonly used scientific test could be employed to screen natural products as potential tenderising agents. This would facilitate the design of a scientifically based natural marinade for meat products. Consumers perceive natural ingredients superior, healthier and of better nutritional value.

Tenderness is considered the most import trait of meat quality. Solving the problem of inconsistent meat tenderisation is a top priority of the meat industry.

There are many pre slaughter factors contributing to meat tenderisation including management, handling of the animals and supplementation of the feed with vitamins or additives, such as calcium chloride [25-28]. However these factors do not guarantee improved tenderness. The reason behind it is that direct selection for tenderness (or against toughness) can only be evaluated on dead animals. Meat marinades could be commercially valuable to the food industry by facilitating meat tenderisation post mortem and upgrading its organoleptic quality.

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Table I: OD_{450} is a measure of the protease activity in each of the samples assayed. Liquid products were tested at different pH values as shown in column two.

Natural products	pH of product adjusted as stated	Substrate	Protease activity (OD_{450})
50µl of fresh lime juice	4	200µl	0.1675
	5	200µl	0.2351
	10	200µl	0.2517
0.1g of fresh mushroom	-	200µl	0.7384
50µl of tinned tomato	4	200µl	0.3053
50µl of fresh tomato juice	4	200µl	0.3511
	5	200µl	0.4510
	10	200µl	0.3729
50µl of juice extract from fresh onion	4	200µl	0.1918
	5	200µl	0.1704
	10	200µl	0.1875
0.1 g of garlic	-	200µl	0.4507
50 µl of fresh Pineapple juice	4	200µl	0.4894
	5	200µl	0.8965
	10	200µl	0.9546
50µl of pasteurised pineapple juice	4	200µl	0.3072
	5	200µl	0.3238
	10	200µl	0.3231