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Actinidin Treatment and *Sous Vide* Cooking: Effects on Tenderness and *In Vitro* Protein Digestibility of Beef Brisket

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Abstract

Actinidin from kiwifruit can tenderise meat and help to add value to low-value meat cuts. Compared with other traditional tenderisers (e.g. papain and bromelain) it is a promising way, due to its less intensive tenderisation effects on meat. But, as with other plant proteases, over-tenderisation of meat may occur if the reaction is not controlled. Therefore, the objectives of this study were (1) finding a suitable process to control the enzyme activity after desired meat tenderisation has been achieved; (2) optimising the dual processing conditions- actinidin pre-treatment followed by *sous vide* cooking to achieve the desired tenderisation in shorter processing times. The first part of the study focused on the thermal inactivation of actinidin in freshly-prepared kiwifruit extract (KE) or a commercially available green kiwifruit enzyme extract (CEE). The second part evaluated the effects of actinidin pre-treatment on texture and *in vitro* protein digestibility of *sous vide* cooked beef brisket steaks.

The results showed that actinidin in KE and CEE was inactivated at moderate temperatures (60 and 65 °C) in less than 5 min. However, the enzyme inactivation times increased considerably (up to 24 h at these temperatures) for KE/CEE-meat mixtures, compared with KE/CEE alone. The thermal inactivation kinetics were used as a guide for optimising actinidin application parameters during the second phase of the study.

For the final experiments, beef steaks were injected with 5 % (w/w, extract/meat) of CEE solution (3 mg/mL) followed by vacuum tumbling (at 4 °C for 15 min) and cooking

(at 70 °C for 30 min) under *sous vide* conditions. This cooking time was considerably less than usual *sous vide* cooking times used in the meat industry. The actinidin-treated meat had no change in pH and colour, but showed a lower instrumental shear force; and improved sensory scores for tenderness, juiciness and flavour than the untreated meat steaks when tested by a sensory panel. Improved tenderness agreed well with the Transmission Electron Microscopy (TEM) results that showed considerable breakdown of the myofibrillar structure, particularly around the Z line. The addition of actinidin enhanced the rate of breakdown of muscle proteins, as shown by Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and led to an increase in both protein solubility and ninhydrin-reactive free amino N release, during simulated gastric digestion. These results demonstrate the positive effects of actinidin on meat tenderness and meat protein digestibility during gastric digestion *in vitro*.

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Abbreviations

ANZFSC	Australia New Zealand Food Standards Code
CA	Commercial availability
CBZ	N- α -carbobenzoxy-L-lysine P-nitrophenyl ester hydrochloride
CEE	Commercial enzyme extract
DTT	DL-Dithiothreitol
EA	Enzyme activity
FDA	Food and Drug Administration of United States
GRAS	Generally Recognised as Safe
KE	Kiwifruit extract
MPI	Ministry for Primary Industries
SGF	Simulated gastric fluid
SF	Simulated salivary fluid
SSF	Slice shear force
SDS-PAGE	Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEM	Transmission Electron Microscope
WBSF	Warner-Bratzler shear force