Kinetics of activation and deactivation of cathepsin B and L during sous vide cooking of beef pectoralis profundus

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Objectives: Meat endogenous enzymes such as cathepsin B and L have been associated with meat tenderisation during low-temperature long-time cooking (sous vide). The aim of this study was to determine the kinetics of cathepsin B and L activation and deactivation at temperatures of interest during sous vide cooking of beef *pectoralis profundus* (brisket flat).

Materials and Methods: Two beef briskets (*pectoralis profundus*) (5 days post-mortem) were bought from a local butcher. Each brisket was trimmed of external fat and connective tissue and cut across the muscle fibers direction into thin slices with 2 mm thickness to allow rapid isothermal conditions during cooking. The slices were then individually vacuum-packed and sous vide at temperatures of 46, 48, 50, 52, 54, 56, 58, and 60 °C and time range from 30 seconds to 72 h (total 52 temperature and time combi- nations). Cooking was stopped by immersing samples in iced water. Raw and cooked samples (combined meat residue and cook loss) were homogenized in an ice-cold buffer and centrifuged. The supernatant was collected and cathepsin B and L activity was measured at 37 °C using common substrate (Z-Phe-Arg-7-amido-4-methylcoumarin hydrochloride) (Koohmaraie & Kretchmar, 1990). Cathepsin B and L are lysosomal cysteine proteases and optimally active at slightly acidic environments such as found in lysosomes. High temperature has been reported to promote the disruption of lysosomal membranes and cause the release of lysosomal enzymes into meat tissue as shown by an increase in the percentage of free enzyme activity (activity in supernatant). At the same time, temperature also causes irreversible enzyme denaturation. Thus, in this study the amount of free (active) cathepsin B and L during sous vide was modelled assuming consecutive reactions in series which are activation followed by deactivation. The deactivation of cathepsin B and L was modelled by assuming that the system is composed of two isoenzymes with different ther- mal resistances, each deactivating according to a first-order reaction. All data points of cathepsin B and L activity at different temperatures were then fitted to the model by using a reference temperature (Tref) of 50°C. The solver feature in excel was used to fit the equation by minimising the sum of square residuals between predicted and experimental data. The goodness-of-fit of the model was evaluated using the adjusted coefficient of determination (Ra2). The effect of temperature on cathepsin B and L activity was also determined by incubating supernatant with the substrate at different temperatures (37, 40, 45, 50, 55, and 60 °C). The results showed that the rate of cathepsin activity increase as temperature increases before it starts to cease at temperature above 50 °C. The rate of cathepsin activity at temperatures 50 °C and below were then used to determine their activation energy (Ea) following the Arrhenius equation. The area under the curve of predicted cathepsin B and L activity after taking into account the effect of temper- ature on their activity (incorporating the activation energy) was then calculated using cumtrapz function (Matlab R2020b) to give total accumulated activity at specific temperature and time combinations during sous vide.

Results and Discussion: The amount of free cathepsin B and L activity of raw beef pectoralis profundus was found to be 1.49 nmoles of 7-amino-4-methylcoumarin released.min-1.g muscle-1 (uA). The amount is slightly lower than what has been reported in longissimus muscle of pig which is 7.24 ± 0.45 uA (Koohmaraie & Kretchmar, 1990). This might be due to different muscle and animal used in this study. The result shows that cathepsin B and L reached a maximum value of 5.73 uA after cooking for 1h and 10 min at 46 °C and 56 °C, respectively. The rate of deactivation was very high at 56°C with almost 50% of reduction after 1 hour of heating while the same reduction was observed only after 18 hours at 46 °C. The kinetics of cathepsin B and L activation and deactivation was successfully modelled (Ra2 = 0.9149). For prolonged cooking time (24 to 72 hours) such as in sous vide conditions, cooking at 46 and 48 °C will give the highest accumulated cathepsin activity. While for shorter sous vide time (8 hours) such has been prac- tised in the industry, 50 °C will give the highest accumulated activity.

Conclusions: This study will help to guide the appropriate temperature and time combination selection to maximise the use of ca- thepsin B and L during sous vide processing to enable the tenderisation of meat cuts. Further study on the effect of cooking at high- est cathepsin activity alone or combining with collagen denaturation and solubilisation such as in step-wise sous vide on meat ten- derness will provide greater opportunity to manipulate this process advantageously.

References:

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