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Kinetics of proteolysis studied by *in vitro* digestion and linear mixed effects model analysis (#57)

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Introduction

Attempts to evaluate the quality of meat proteins have been made with *in vitro* digestion models, where total nitrogen content, peptides and free amino acids (FAAs) are measured and compared to determine the overall digestibility. Pepsin and pancreatin or other types of proteolytic enzymes used for the *in vitro* digestion simulation target specific peptide linkages to release FAAs, however the rate of individual FAAs released remain relatively unexplored. The kinetics of the dietary proteins using dialysis system and peptide fractions have been studied by Vachon et al. (1987), Savoie et al. (1988) and Savoie et al. (2005). Across the different types of protein foods tested, the same kinetics of protein hydrolysis were seen with tyrosine and methionine being released faster compared to other amino acids. The current study proposes the use of linear mixed effects model analysis as a tool to study the kinetics of proteolysis in high pressure processed lamb by examining the rate of the total and individual FAAs released during the *in vitro* digestion simulation.

Methods

Flat cuts (leg) of three lamb were high pressure processed at 0, 200, 300, 400 and 500 MPa (HPP 055, Multivac, Germany) in FoodBowl Ltd., in New Zealand. Treated samples were sous vide cooked at 60 °C for two hours and ground for two minutes at room temperature. *In vitro* enzymatic digestion method by Minekus et al. (2014) was used, with pepsin from porcine gastric mucosa (P7000, Sigma Aldrich®), pancreatin (3X, U.S.P., MP Biomedicals, LLC) and bile extract porcine (B8631, Sigma-Aldrich®). The digested samples were collected at 0, 2 and 5 hr to represent the baseline before digestion, gastric and intestinal phases of digestion, respectively. The collected samples were snap-frozen with liquid nitrogen to halt further enzymatic digestion. A commercial FAAs analysis kit (EZ Faast™, Phenomenex®, Torrance, U.S.A) was used for extraction and derivatisation of FAAs in the collected samples, followed by separation and quantification by a Shimadzu GC-2010 Gas Chromatograph with an auto injector (AOC-20i, Shimadzu), a Flame Ionisation Detector (FID) and a split injector. R software (The R Foundation for Statistical Computing, version 3.3.1) was used for one-way ANOVA to separate the means at $P < 0.05$. Linear mixed effects model analysis was performed using the R-package, lme4. The results were compared using the Restricted (or Residual) Maximum Likelihood Estimate (Bates et al., 2015) with digestion time as a fixed effect and pressure as a random effect.

Results

Significant differences ($P < 0.0001$) in the quantity of total and nineteen individual FAAs among the four high pressure treated samples and the control (0 MPa) were detected, at each of the three digestion phases. Pressure treatment of 300 MPa resulted in the highest amount of total FAAs released, as well as for the individual FAAs, in the intestinal phase. Leucine, phenylalanine, lysine and tyrosine were released the most after peptic and pancreatic proteolysis when compared with those prior to digestion, in the 300 MPa treated sample. The amount of total and individual FAAs increased exponentially with respect to the digestion time. The pressure treatment did not significantly influence the release of all of the FAAs. The rate of increase in FAAs showed fixed effect for all of the pressure treatments. With the use of the Restricted (or Residual) Maximum Likelihood Estimate, we found that the linear mixed-effects model in log-scale was favored. The slope of log (total FAAs) at 300 MPa indicated that the rate of FAA release was the fastest compared to the other pressure levels (Table 1). Most of the individual FAAs showed similar pattern to that of the total FAAs. For example, log(phenylalanine) had the highest gradient at 300 MPa, indicating that the rate of phenylalanine released from the digestion was the highest with 300 MPa pressure treatment. Phenylalanine and leucine were liberated in faster rates followed by tryptophan, tyrosine and lysine, with alanine being released the slowest. Further statistical analysis has shown that six amino acids (lysine, tyrosine, leucine, tryptophan, phenylalanine and methionine) were rapidly released as FAAs from the peptic and pancreatic proteolysis. Apart from arginine which could not be detected from the current study, the six amino acids that were released fast were also seen by Vachon et al. (1987) with beef, even without the pressure treatment. Other amino acids were released more slowly during the digestion simulation, with most of these being non-essential amino acids.

Conclusion

For the first time, linear mixed-effects model was applied to analyse the relationships among the digestibility of protein measured in FAAs, digestion time and the pressure.The kinetics of proteolysis studied by measuring the rate of FAAs released from *in vitro* digestion simulation may serve as a tool to evaluate the quality of proteins. The study of kinetics may further be applied to develop protein-rich foods with maximal release of FAAs in earlier stages of digestion for those having difficulty in protein digestion.

Notes

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References

Pressure (MPa)	log (Total FAAs) = c + 0.40 Hour	log (PHE) = c + 0.58 Hour
0	c = 5.43	c = 2.73
200	c = 5.52	c = 2.79
300	c = 5.63	c = 3.00
400	c = 5.47	c = 2.81
500	c = 5.33	c = 2.71

Notes