Effects of trypsin-induced limited hydrolysis on the structural, functional, and bioactive properties of sericin

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Electronic Supplementary Information (ESI) [Detailed experimental, Fig. S1-S2]

Calculation of the degree of hydrolysis (DH)

As a quantitative characteristic, the degree of hydrolysis (DH) is used to quantify the extent of hydrolysis, which is the ratio of the number of peptide bonds dissociated to the total number of peptide bonds in the per unit weight of the substrate. (Adlernissen, 1986) It can be measured using the pH-stat method and then calculated using the formula below:

$$DH(\%) = (\mathrm{V}\times\mathrm{N_b})/(\mathrm{M_p}\times\alpha\times\mathrm{h_{tot}}) \times 100$$

V stands for the amount (mL) of NaOH used to maintain pH 8.0 during the hydrolysis reaction, N_b is the molarity of NaOH (0.1 mol/L). h_{tot} is the total number of peptide bonds in the substrate, which was determined to be 6.74 mmol/g by the amino acid composition of sericin. (Wu, Wang,

& Xu, 2008) M_p is the mass of sericin (g). α stands for average dissociation degree of the α-NH_2 groups during the enzymatic hydrolysis and can be computed using the formula below:

$$\alpha = \frac{10^{pH - pK}}{1 + 10^{pH - pK}}$$

Where pH is the value at which the enzymatic hydrolysis reaction was run.

The pK values at different temperatures are figured out using the formula below (T in Kelvin):

$$pK = 7.8 + \frac{298 - T}{298 \times T} \times 2400$$

Preparation of ANS solution

To prepare an 8 mmol/L ANS solution, 12 mg ANS was dissolved in 100 μL ethanol first, then diluted in phosphate buffer (10 mmol/L, pH 7.0) to 5 mL.

Determination of in vitro antioxidant activities

Determination of hydroxyl radical, DPPH radical, and ABTS radical scavenging activity of sericin and its hydrolysates was performed according to the method of (Zheng et al., 2015).

Hydroxyl radical scavenging activity

1 mL sample mixed with 0.5 mL $FeSO_4$ (3 mM) and 0.5 mL salicylic acid (3 mM) and the reaction was initiated by adding 0.5 mL H_2O_2 (3 mM) into the mixture followed by incubation

for 30 min in the dark. The absorbance of the mixture was measured at 536 nm. The hydroxyl radical scavenging activity of samples was computed used the formula below:

$$Hydroxyl\ radical\ scavenging\ activity(\%) = (1 - \frac{A_{sample}}{A_{control}}) \times 100$$

DPPH radical scavenging activity

1 mL sample mixed with 1 mL freshly prepared DPPH ethanol solution (0.1 mM) and incubated for 30 min in the dark. The absorbance of the mixture was measured at 517 nm. The DPPH radical scavenging activity of samples was computed used the formula below:

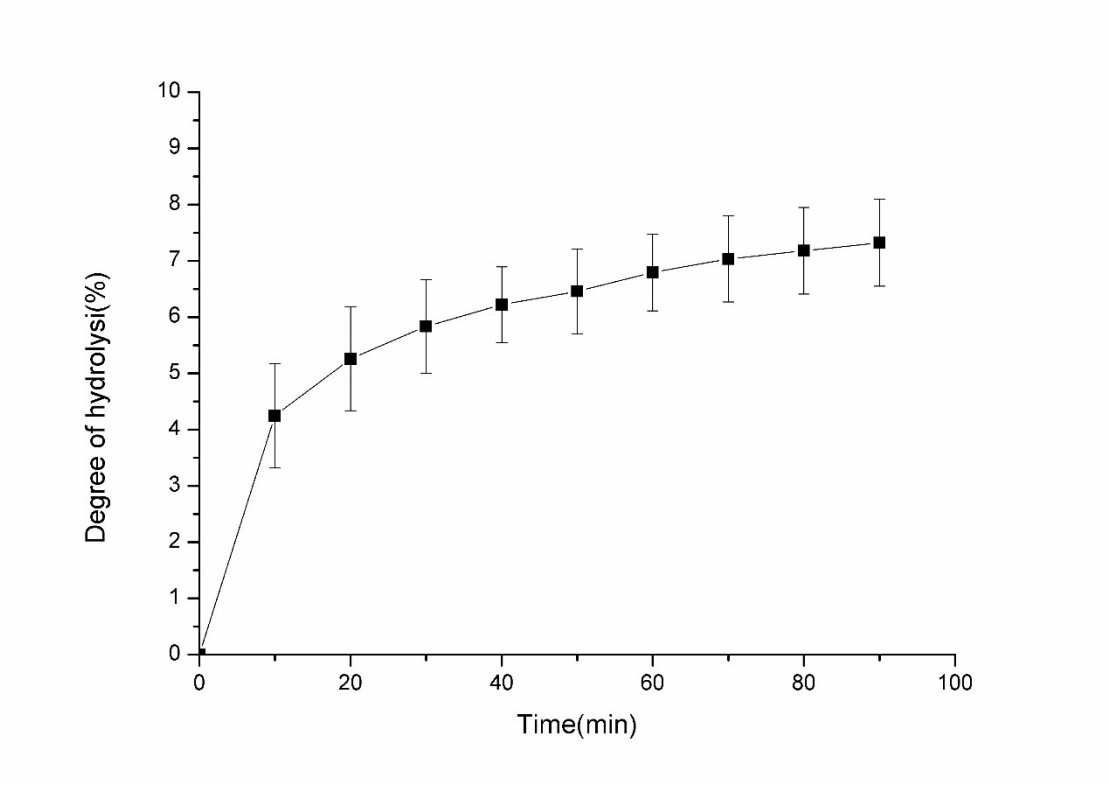
$$DPPH\ radical\ scavenging\ activity(\%) = (1 - \frac{A_{sample}}{A_{control}}) \times 100$$

ABTS radical scavenging activity

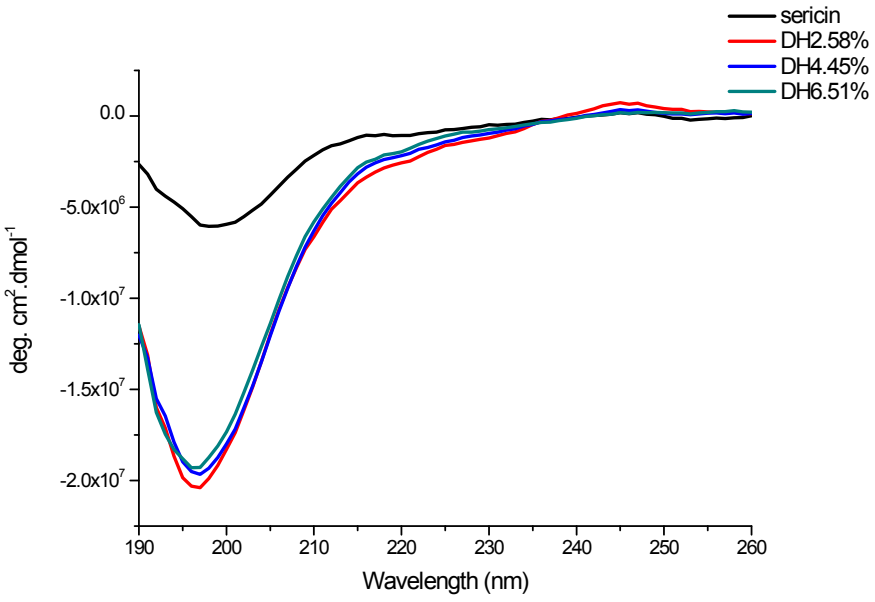
3 mL ABTS (7 mM) and 3 ml potassium persulfate (2.45 mM) solutions were mixed and allowed to stand in the dark for 12 h to prepare the ABTS stock solution. 4 mL of the ABTS stock solution diluted with 250 mL dH_2O to obtain an absorbance of 0.70±0.02 at 734 nm. Then 0.5 mL sample solution mixed with 2 mL ABTS solution and incubated 5 min in the dark. The absorbance was measured at 734 nm. ABTS radical scavenging activity was computed by the following formula:

$$ABTS\ radical\ scavenging\ activity(\%) = (1 - \frac{A_{sample}}{A_{control}}) \times 100$$

Results were expressed as mg vitamin C equivalents/g sample.



Supplementary Figure 1 Degree of hydrolysis of sericin by trypsin during 90 min, n=3.



Supplementary Figure 2 CD spectra of sericin and its hydrolysates.

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Wu, J.-H., Wang, Z., & Xu, S.-Y. (2008). Enzymatic production of bioactive peptides from sericin recovered from silk industry wastewater. *Process Biochemistry, 43*(5), 480-487. doi: https://doi.org/10.1016/j.procbio.2007.11.018
Zheng, X.-q., Wang, J.-t., Liu, X.-l., Sun, Y., Zheng, Y.-j., Wang, X.-j., & Liu, Y. (2015). Effect of hydrolysis time on the physicochemical and functional properties of corn glutelin by Protamex hydrolysis. *Food Chemistry, 172*, 407-415. doi: https://doi.org/10.1016/j.foodchem.2014.09.080