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In-situ and real-time monitoring of enzymatic process of wheat gluten by miniature fiber NIR spectrometer

Yanyan Zhang [a,b,1], Lin Luo [a,c,1], Jing Li [a], Suyun Li [a,b], Wenjuan Qu [a], Haile Ma [a,*], Ayobami Olayemi Oladejo [a], Xiaofei Ye [a]

[a] School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[b] School of Food and Biological Engineering, Zhengzhou University of light industry, 5 Dongfeng Road, Zhengzhou 450002, China
[c] School of Food Science & Technology, Nanjing Agricultural University, 210095 Nanjing, Jiangsu, China

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ABSTRACT

The degree of hydrolysis (DH), peptide content and angiotensin I-converting enzyme (ACE) inhibitory activity of hydrolysates are the three most important indexes for process control in proteins enzymatic hydrolysis and ACE inhibitory peptides preparation. In this study, a miniature fiber optical spectrometer in conjunction with multivariate analysis for in-situ and real-time monitoring of the parameters (DH, peptide content and ACE inhibitory activity) in wheat gluten (WG) hydrolysis process. Synergy interval partial least square (Si-PLS) algorithm was performed to variables selection and the regression model was calibrated. The performance of a model was evaluated by the correlation coefficient (R_p) and the root mean square error (*RMSEP*) in the prediction set. The results showed that $R_p = 0.9270$, $RMSEP = 1.73\%$ for DH; $R_p = 0.9673$, $RMSEP = 0.79$ mg/ml for peptide content; $R_p = 0.9507$, $RMSEP = 5.12\%$ for ACE inhibitory. This work demonstrated that miniature fiber optical spectrometer combined Si-PLS model can be used as an alternative method for in-situ and real-time monitoring the enzymatic process of WG.

1. Introduction

Wheat gluten (WG) is the natural water-insoluble protein portion of wheat endosperm which during wet processing of wheat flour, is separated in the form of a protein-lipid-starch complex (Mandato et al. 2013). Although WG has now become a significant ingredient in the food industry and with high protein content about 76% (w/w), its vast use is limited mainly because of its low solubility (Dadzie et al. 2013). During the past few years, numerous studies have been found that enzymatic hydrolysis is one of the methods that have been found to improve not only the solubility of wheat gluten but has also been found to release many bioactive compounds such as inhibitors of angiotensin I-converting enzyme (ACE) (Matsui et al. 2000;Yoshikawa et al. 2000).

The degree of hydrolysis (DH), which is defined as the percentage ratio of the number of peptide bonds broken (h) to the total number of peptide bonds in the substrate (h_{tot}) and measures the percentage of peptide bonds hydrolyzed during protein hydrolysis (You et al. 2009) The peptide concentration presents the extraction rate of peptide in hydrolysates. In respect to ACE inhibitory activity of WG hydrolysate, it is determined by the peptide forming process and the peptide degrading process but not DH and peptide concentration. Therefore, the DH, peptide concentration and the ACE inhibitory activity of hydrolysate were three proteolysis monitoring parameters used to indicate the enzymatic process in the preparation of the ACE peptides (Spiegelman et al. 1998). The analytical chemical method usually used in the determination of DH is PH-state method, which shows good precision, accuracy, and reliability. However, this method involves adding the NaOH to the hydrolysates throughout the enzymatic process. The adding of NaOH introduced numbers of Na^+ in the hydrolysates, which might compromise the biological activity of the peptide. The measurements of peptides concentration and ACE inhibitory activity of hydrolysates during the enzymatic process require the inactivation of enzyme by heat treatment, which is time consuming and cumbersome. Therefore, a successive, rapid and accurate method of monitoring the DH, peptides concentration and ACE inhibitory activity of hydrolysates in the enzymatic process is urgently needed for determining the endpoint of enzymolysis.

In-situ and real-time monitoring chemical and physical changes during processing of reactive biomaterials are of critical importance to scientist and engineers. A miniature fiber optical spectroscopy is a fast, easy, economical, objective, and non-destructive technique that can be a suitable substitute for the traditional analytical method. It can be used to monitor not only the final product but the various stages of

* Corresponding author. School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China.
E-mail address: mhl@ujs.edu.cn (H. Ma).
[1] The two authors contributed equally to this paper.

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the production process as well (Pereira et al. 2008). Recently, miniature fiber optical spectroscopy technique has been widely used in monitoring and predicting the food processing on-line and in-site (Chen et al. 2008; Mandatoa et al. 2013; Porep et al. 2015). However, few studies have been reported on the monitoring of enzymatic process.

NIR spectral data analysis was often made with a classical linear multivariate calibration method (Tøgersen et al. 1999). Partial least squares (PLS) model is the classical method (Lee et al. 2011). However, the full spectrum PLS model has some unrelated and collinear spectral variables that may influence the stability and prediction capability of the model. Selection of variables can significantly improve the performance of the full-spectrum calibration technique (Chen et al. 2010). Usually, a judicious selection of spectral regions would improve the performance of the PLS model (Spiegelman et al. 1998). Therefore, spectral region selection by Si-PLS was implemented to improve the performance of final model in this research.

The aim of this study was to develop a portable and low-cost NIR spectroscopy system, investigate its applicability for monitoring real-time enzymatic process parameters and choose an efficient variable selection algorithm to improve the performance of model. The specific objectives were to (1) set up a portable in-situ and real-time spectroscopy system, (2) use this system to monitor process parameters in enzymatic process, (3) select the most suitable spectral intervals by Si-PLS to build a prediction model for each process parameter, and, finally (4) real-time monitoring the enzymatic process in the enzymatic reactor.

2. Materials and method

2.1. Materials

Wheat gluten powder (protein content, 76 g/100 g) was purchased from Xu Zhou OK wheat starch Co. (Jiangsu, China). Alcalase with an activity of 2.280×10^5 U/g (by Folin Phenol method) (Zhou 1995) was purchased from Novozymes (China) Biotechnology Co., Denmark. Angiotensin-I-converting enzyme (ACE) from pig lung was extracted according to the method described by Maruyama et al.(1999). Substrate hippuryl-His-Leu (HHL) was purchased from Sigma Chemicals Co. Ltd. (St. Louis, MO., USA). All other chemicals and solvents were of analytical grade.

2.2. Enzymatic hydrolysis method of WG

An aliquot (1500 mL) of WG suspension (20.0, 30.0, 40.0 and 50.0 g/L) was placed in the water bath at a temperature of 50 °C. The enzymatic hydrolysis was referred by Zhang et al. (2015a, b) with some modifications. The above solution was adjusted and maintained at pH 9.0 using 1 M NaOH and reacted with alcalase, the enzyme-substrate ratio [E/S] was 6080 U/g. The reaction time was 80 min. For the first 20 min, the usage of NaOH was recorded every 2 min and sample taken; then every 5 min for the following 60 min. During the enzymatic hydrolysis process of WG protein, solution in the reactor is homogeneous by a magnetic stirrer. The enzyme in every sample was inactivated by boiling the hydrolysate for 10 min. The hydrolysate was then centrifuged at 5000*g* for 10 min at 4 °C and the resulting supernatant was stored at 4 °C for further subsequent analysis. The total number of samples was 88.

2.3. In-situ and real-time measurement by miniature NIR spectroscopy

The whole enzymatic process of WG was in-situ and real-time monitored by miniature NIR spectroscopy combine a fiber optic probe. In-situ and real-time recording of miniature NIR spectra was carried out directly inside the enzymolysis reactor. NIR spectra were recorded using a miniature NIR Analyzer (NIRQUEST256-2.5) (Ocean Optics, America) (Fig. 1a). The NIR instrument was connected to a fiber optic probe (TP300) (Ocean, America) with a transmission and reflection module (Fig.1b) positioned inside the reactor and allowing direct contact with the hydrolysates during enzymatic process. The optical path length was 2 mm and each spectrum was an average of 16 scanning spectra. The NIR spectrometer gathered spectra in the range 800–2500 nm, and the data were measured in every 9.5 nm, which resulted in 256 variables. For each enzymatic condition, the NIR spectra were collected in triplicate from three experiments every 2 min for the first 20 min, and then collected every 5 min for the following 60 min during enzymatic process. The background spectra of the empty cell were also obtained before each NIR measurement using distilled water at 50 ± 2 °C.

2.4. Determination of enzymatic hydrolysis process by off-line methods

2.4.1. Measurement of the degree of hydrolysis (DH)

The degree of hydrolysis (*DH*) was calculated according to the pH-stat method of Adler-Nissen (Williams 1987);

$$DH(\%) = \frac{h}{h_{tot}} \times 100\% = \frac{BN_b}{\alpha M_p h_{tot}} \times 100\% \quad (1)$$

where B (mL) is the volume of NaOH consumed, N_b is the normality of the NaOH, α is the average degree of dissociation of the α-amino groups related with the pK of the amino groups at particular pH and temperature, and α is 0.885 for alcalase, M_P (g) is the amount of the protein in the reaction mixture, and h_{tot} (mmol/g) is the total number of peptide bonds in the protein substrate used in the experiment, which is 8.33 for WG.

2.4.2. Measurement of ACE inhibitory activity

ACE-inhibitory activity was measured by the method of Zhang et al. (2015a, b), with slight modifications. An aliquot (10 uL) of sample solution (all samples diluted 5 times by 0.1 M borate buffer, pH 8.3) were mixed with 25 uL of ACE (ACE in 0.1 M borate buffers containing the 0.3 M NaCl, pH 8.3) and pre-incubated for 10 min at 37 °C. The reaction was initiated by adding 45 uL of hippuryl-His-Leu (HHL) sodium borate buffer (6.5 mM HHL in 0.1 M borate buffer containing 0.3 M NaCl, pH 8.3) and the reaction was carried out at 37 °C for 30 min. The reaction was stopped by adding 85 uL of 1 M HCl to the samples. The hippuric acid was determined in a High performance liquid chromatography (Shimadzu Inc. Japan) at 228 nm with a UV-detector. The ACE inhibition (%) was calculated with Eq. (2).

$$\text{ACE inhibition activity } (\%) = \left[1 - \frac{\Delta_{Inhibition}}{\Delta_{Control}}\right] \times 100\% \quad (2)$$

2.4.3. Measurement of peptides concentration

The determination of peptides concentration was according to Jin et al. (2015). and referenced by Lowry method (Waterborg 2009) using bovine serum albumin (BSA) as standard and with slightly modification.

2.5. Spectral data preprocessing

Before the calibration stage, the spectral data should be preprocessed for building reliable, accurate, and stable models. Four preprocessing methods, first and second derivative, standard normal variate (SNV) and multiple scattering correction (MSC) (Ouyang et al. 2013) were used comparatively in this study. The best preprocessing method was in accordance with PLS models. Finally, we find that SNV was superior to other methods in this work, SNV spectra was presented in Fig.2b.