ELSEVIER

Journal of Chromatography A, 680 (1994) 413–417

JOURNAL OF CHROMATOGRAPHY A

Rapid characterization of soy protein and hydrolysates by capillary electrophoresis

Theodore M. Wong*, Cameron M. Carey, Santa H.C. Lin

Protein Technologies International, Inc., Checkerboard Square, St. Louis, MO 63164, USA

Abstract

Rapid methods for characterizing soy proteins and their hydrolysates by free-zone capillary electrophoresis were developed. The 7S- and 11S-rich protein fractions were separated by capillary electrophoresis (CE). The relationships between degrees of hydrolysis and electropherograms were established. Protein hydrolysates were separated in less than 6 min. Data showed that the CE method can be used effectively for monitoring protein hydrolysis during processing and for fingerprinting various types of protein products.

1. Introduction

The usual techniques for qualitative and quantitative analysis of proteins and their derivatives include size-exclusion HPLC and gel electrophoresis. Both methods have their advantages and disadvantages. These two techniques for monitoring protein hydrolysis are tedious and time consuming. Therefore, as an alternative, a CE technique was explored for monitoring protein hydrolysis. Isolated soy protein (ISP) and hydrolysates were chosen for this study because of their importance in the food industry. In this study, soy protein hydrolysates treated with various enzymes were compared. A high pH buffer can be effectively used [1,2]. Since most of the protein was negatively charged above this pH, borate buffer at pH 8.5 was used.

* Corresponding author.

2. Experimental

Isolated soy protein (SUPRO 620) was obtained from Protein Technologies International (St. Louis, MO, USA). Protein was hydrolyzed with alkaline protease (Alcalase 2.4L from Novo Nordisk, Danbury, CT, USA), bromelain (2500 tyrosine units/g) and fungal protease (2500 tyrosine units/g; Enzyme Development, New York, NY, USA). Hydrolyses were carried out at the initial pH 8.5, 4% enzyme–substrate ratio, 6% protein substrate and at 50°C. The isolated soy protein was first denatured by heating up to 85°C after pH adjustment. The pH was not controlled during hydrolysis. In addition, a fungal protease hydrolysis was also conducted at pH 4.0. The degree of hydrolysis was measured by the trinitrobenzenesulfonic acid (TNBS) method [3].

Other proteins analyzed include the 7S- and 11S-rich fractions of soy protein. These 7S- and 11S-rich fractions were isolated according to

0021-9673/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)00344-9

Thanh et al. [4]. The 7S fraction accounts for about one third of the extractable protein and is designated as conglycinin. Conglycinin has been separated into three immunologically distinct fractions, α-, β- and γ-conglycinin by Catsimpoolas et al. [5]. The 11S fraction, designated as glycinin, accounts for an additional third of the total extractable protein.

The CE system used was the PHORESIS 500 (Thermo Separation Products, Freemont, CA, USA). Electrophoreses were performed in a fused-silica tube, 70 cm × 75 μm I.D., supplied by Polymicro Technologies (Phoenix, AZ, USA). Samples were first diluted at 1 mg/ml in a borate buffer at 20 m*M*, pH 8.5 and introduced into the capillary by pressure injection for 2 s. The same borate buffer was used as a running buffer. The separation of protein required the buffer pH to be either above or below isoelectric points of the sample protein [1] or under neutral conditions having high ionic strength [2]. The electrophoretic separation was maintained under constant voltage of 25 kV for up to 10 min. Proteins were monitored at 200 nm, and the temperature kept constant at 30°C. Between runs, the capillary was washed with 1.0 N sodium hydroxide solution followed by reconditioning with the running buffer.

3. Results and discussion

Protein hydrolysis curves for the enzymes tested are depicted in Fig. 1. The degree of hydrolysis in relation to time was as expected for enzyme hydrolysis. Fungal protease has a higher catalytic rate than either bromelain or Alcalase. The hydrolysis curves showed that the rate of bromelain activity was most rapid initially and then leveled off abruptly. Likewise, Alcalase hydrolysis also flattened out very quickly, and it was partly due to the pH drop to neutral. No attempt was made to keep the hydrolysis pH constant. Since fungal protease contained *exo*-peptidases, the DH measured was an expression of both the *endo*- and *exo*-peptidase activities.

The separation was accomplished very quickly, within 6 min, for all enzymes tested (Figs. 3–6). A good reproducibility of peak shape and migration time was observed. The unhydrolyzed SUPRO 620 showed a peak (Fig. 2); however, the subunits of ISP were not resolved in this buffer system. Further CE analyses were performed on the 7S- and 11S-rich fractions. Two separate peaks were observed. When these two fractions were recombined, they were very close together. The normalized graphs showed that the 7S and 11S fractions do not react. The 7S and

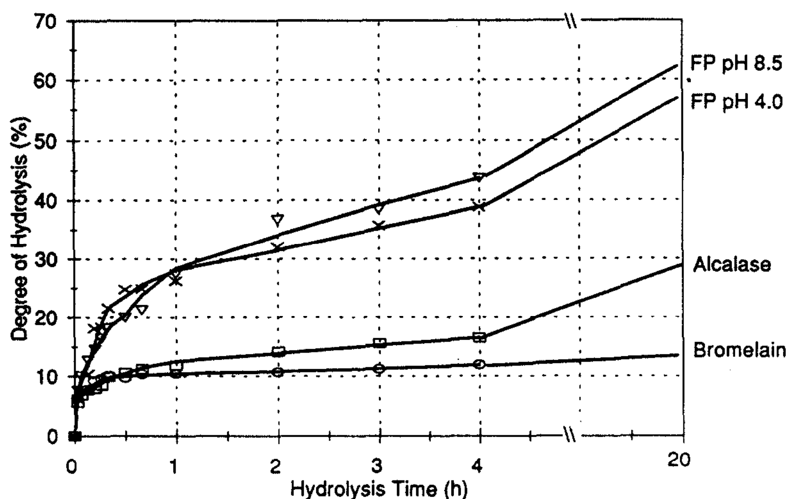

Fig. 1. Hydrolysis curves of isolated soy protein treated with Alcalase, bromelain and fungal protease (FP) at pH 8.5 and 4.0. The degree of hydrolysis (%DH) was determined by the TNBS method.

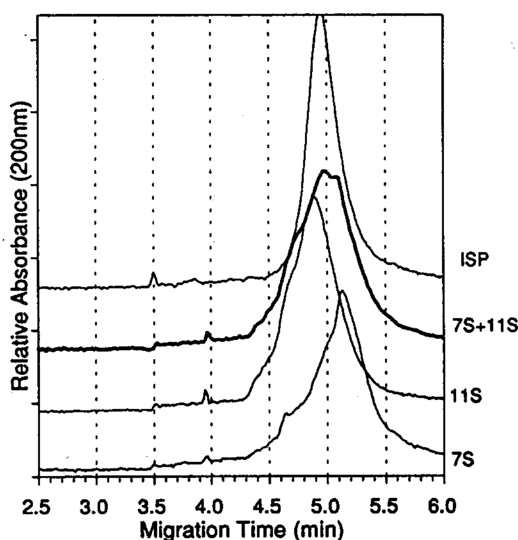

Fig. 2. Electropherograms of 7S-, 11S-rich soy protein fractions and a mixture of both fractions. Two distinct peaks were depicted for the 7S and 11S fractions. For CE conditions, see text.

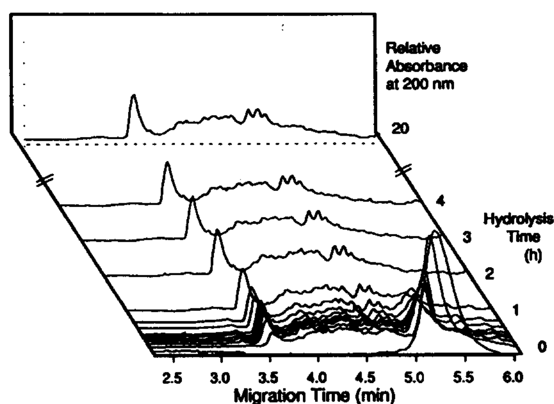

Fig. 3. Electropherograms of isolated soy protein hydrolysates treated with Alcalase from 0 to 20 h. Disappearance of intact protein is visible after 1 h. For hydrolysis and CE conditions, see text.

11S fractions are very different. The 7S fraction has a molecular mass of about 350 000. The 11S fraction has an M_r of 180 000 and 12 subunits [6]. Therefore, increased separation for these two peaks may be possible by changing the ion strength and lowering the voltage [7].

If CE is used for monitoring the progression of hydrolysis, a short retention time is preferred. Therefore, this buffer system and parameters were applied to separate the hydrolysates and will be discussed subsequently.

Protein analysis by CE under high pH can achieve fast separation without compromising resolution. Borate buffer at 20 mM allows peptide species of soy protein hydrolysates to be separated by CE with moderate success, as shown in Figs. 3–7. The CE electropherograms show a definite relationship between the extent of hydrolysis and the disappearance of the intact protein peak (4.5–5.0 min).

Alcalase hydrolysis showed a progression of breaking down the intact peak at 5.0–5.5 min migration time (Fig. 3). The intact peak began to break down as soon as the hydrolysis commenced. Some proteolysis was evident during the short time it took to inactivate the hydrolysis for the aliquot taken at 0 min. The intact protein peak lost its identity when protein was hydrolyzed to greater than 10.0% DH. The functionalities relating to this transition need to be further explored. Peaks within 3.5–5.0 min migration time continued to increase in number and magnitude.

Furthermore, a peak migration time at 3.5 min emerged and increased significantly during Alcalase hydrolysis. It is likely this peak was generated from the 7S fraction. All hydrolysates (i.e., fungal protease, bromelain and Alcalase) exhibited this peak. It was demonstrated that this peak is from the ISP (polypeptides or amino acids) and not from the enzyme preparations (data not shown).

Likewise, bromelain hydrolysis showed a progression of breaking down the intact peak at 5.0–5.5 min migration time (Fig. 4). Similarly, the intact peak began to break down as soon as the hydrolysis started. Since bromelain has a broader specificity, broader peaks were observed between 3.5–4.5 min. The CE electropherograms showed that the intact protein was broken down more rapidly. Although the intact protein continued to break down, the subsequent increase in DH was very slow. As was observed with Alcalase, some hydrolysis occurred during

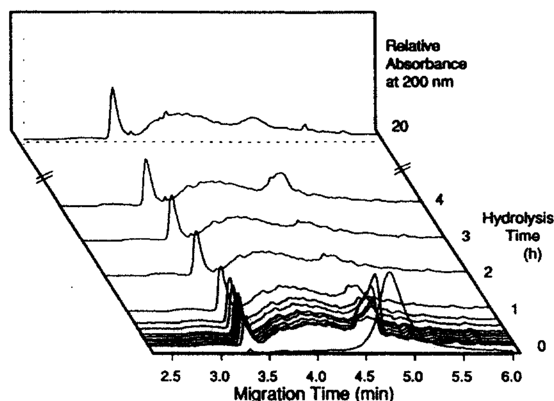

Fig. 4. Electropherograms of isolated soy protein hydrolysates treated with bromelain from 0 to 20 h. Disappearance of intact protein is visible after 1 h. For hydrolysis and CE conditions, see text.

the short time it took to inactivate the bromelain at time 0.

The CE profiles for fungal protease at pH 8.5 were unique. Similar to those from an Alcalase or bromelain hydrolysis, the CE profiles showed a progression of breaking down the intact peak at 5.0–5.5 min migration time (Fig. 5). Although the DH was higher, the intact protein disappeared much later than it did under the bromelain or Alcalase hydrolysis. In this case, the DH was a measure of both *endo*- and *exo*-peptidase activities. Additional low-molecular-mass peptides and amino acids were generated. Thus, the 4.0 min peak increased significantly during hydrolysis. This peak was unique to this particular enzyme and it was also seen in the hydrolysis conducted at pH 4.0.

The intact protein was not seen for the fungal protease hydrolysates conducted at pH 4.0. This was mainly due to the insolubility of protein substrate at this pH (Fig. 6). The solubility of the protein and enzyme specificity are both involved in providing such a unique CE electropherogram.

To distinguish the different profiles of enzyme hydrolysis, CE electropherograms at a fixed DH (10%) were shown in Fig. 7. The data confirm that CE is an effective way to elucidate the difference between hydrolysates with different enzymes. In addition, the disappearance and appearance of peptides could be quantitatively calculated to relate to the %DH for each different enzyme system.

Difficulties were also encountered with identification of the fractions due to variations of fraction mobilities. Intact protein without hydrolysis (or only slightly hydrolyzed) experienced a shift in mobilities. Therefore, spiking the hydrolysates with unhydrolyzed soy protein was employed here to verify that these intact proteins had the same mobilities (data not shown).

In conclusion, the results demonstrated that CE is an effective means for monitoring enzyme hydrolysis. The degree and types of hydrolysis

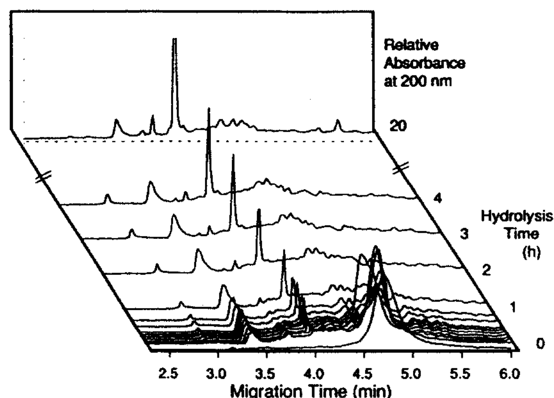

Fig. 5. Electropherograms of isolated soy protein hydrolysates treated with fungal protease from 0 to 20 h. A unique peak is visible at 4 min. For hydrolysis and CE conditions, see text.

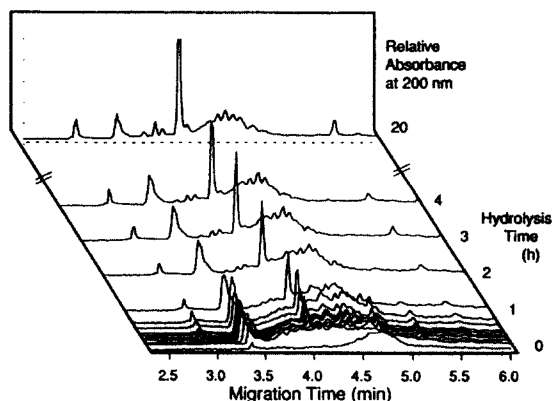

Fig. 6. Electropherograms of isolated soy protein hydrolysates treated with fungal protease at pH 4.0 from 0 to 20 h. A unique peak is visible at 4 min. For hydrolysis and CE conditions, see text.

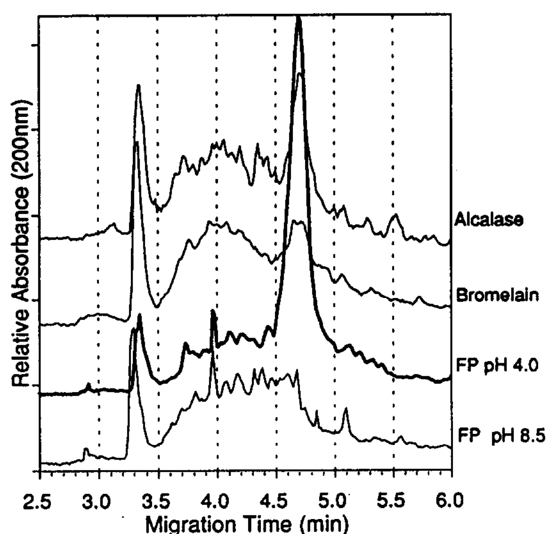

Fig. 7. Electropherograms of isolated soy protein hydrolysates at 10% DH that were treated with Alcalase, bromelain and fungal protease (FP) at pH 8.5 and 4.0. For hydrolysis and CE conditions, see text.

can readily be distinguished. The rapid CE separation is of practical value for process control and monitoring. In addition, the simplicity of sample preparation is an added advantage for ease of implementation. Therefore, the potential of the CE method to characterize protein and hydrolytic changes holds great promise. The challenge continues to be the development of an analytical method with better resolution and precision.

References

[1] H.H. Lauer and D. McManigill, *Anal. Chem.*, 58 (1986) 166.

[2] J.W. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.

[3] J. Adler-Nissen, *J. Agric. Food Chem.*, 27 (1979) 1256.

[4] V.H. Thanh, K. Okubo and K. Shibaski, *Plant Physiol.*, 56 (1975) 19.

[5] N. Catsimpoolas, T.G. Campbell and E.W. Meyer, *Arch. Biochem. Biophys.*, 131 (1969) 577.

[6] M.A. Moreira, M.A. Hermodsom, B.A. Larkins and N.C. Nielsen, *J. Biol.Chem.*, 254 (1979) 9921.

[7] L.R. Gurley, J.E. London and J.G. Valdez, *J. Chromatogr.*, 559 (1991) 431.