JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fast Characterization of Industrial Soy Protein Isolates by Direct Analysis with Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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Industrial soy protein isolates (SPIs) due to differences in their processing conditions may differ both in composition and in degree of hydrolysis. As a result, they display different performance in food production and final food properties like consistency and taste. To address this issue, a fast, cheap, and simple method for screening and characterization is required. In this article, the successful analysis of soy protein isolates, a complex mixture of proteins with glycinin and β-conglycinin as major components, by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is demonstrated. The preparation implements a fast extraction of the proteins from the raw SPI either under neutral or reducing conditions. The extracts are analyzed subsequently by MALDI-TOF-MS without further purification. Results of the two conditions are compared. Finally, different SPIs from different suppliers are analyzed and compared concerning their consistency. The method could be applied to other plant proteins and mixtures thereof. Since the composition and intactness of different subunits play important roles in functional properties of soy proteins, rapid methods for fingerprinting of different industrial soy protein sources will be valuable tools for successful product formulation.

KEYWORDS: Soy proteins; glycinin; β-conglycinin; MALDI-TOF-MS

INTRODUCTION

Proteins play an important role in determining nutritional, functional, and textural properties of modern food products. In particular, plant/seed storage proteins have become important functional ingredients in many prepared foods. The classification of these proteins as globulins, albumins, glutenins, and prolamins is widely accepted; each of these groups, in turn, represents a complex mixture of proteins. Proteins are potential health-promoting ingredients as a consequence of their biological activity or composition (owing to the presence of biologically active peptides in their primary sequences). Therefore, a research effort has been undertaken to develop techniques and methods for fast separation, purification, and characterization of food proteins (*1*). The research on structural and physicochemical characteristics of food proteins has been aimed at elucidating the link between molecular structure and functionality in food systems. Such an example are soy proteins, which became more interesting as (food) ingredients, particularly after the US Food and Drug Administration's approval of their indication in coronary prevention (*2*). On the other hand, soy proteins have found various applications in many different food products due to their high nutritional values and ability of form gels and stabilize foams and emulsions. This makes them well-suited to improve and control the texture of different food products.

A huge amount of different soy protein sources (e.g., concentrates, isolates, etc.) are currently available on the market from different suppliers. Soy proteins isolates (SPIs), commercially available in high purity >80 wt % by weight in dry product and low soy (off) flavor, in many cases are preferred soy protein sources. Due to different processing conditions (extraction, purification, and drying), soy protein isolates can display very different physicochemical properties. These isolates may differ in composition and in degree of hydrolysis. It is known that these differences can cause different behavior in further food production and final food properties like consistency and taste. Soy protein is a typical example of a plant storage protein. Its physicochemical properties related to food structuring are mainly determined by the two major proteins: glycinin and β-conglycinin (*3*). The quaternary structure of these proteins depends on pH and ionic strength. β-Conglycinin (a 7S globulin)

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10.1021/jf071321y CCC: $37.00
Published on Web 12/05/2007

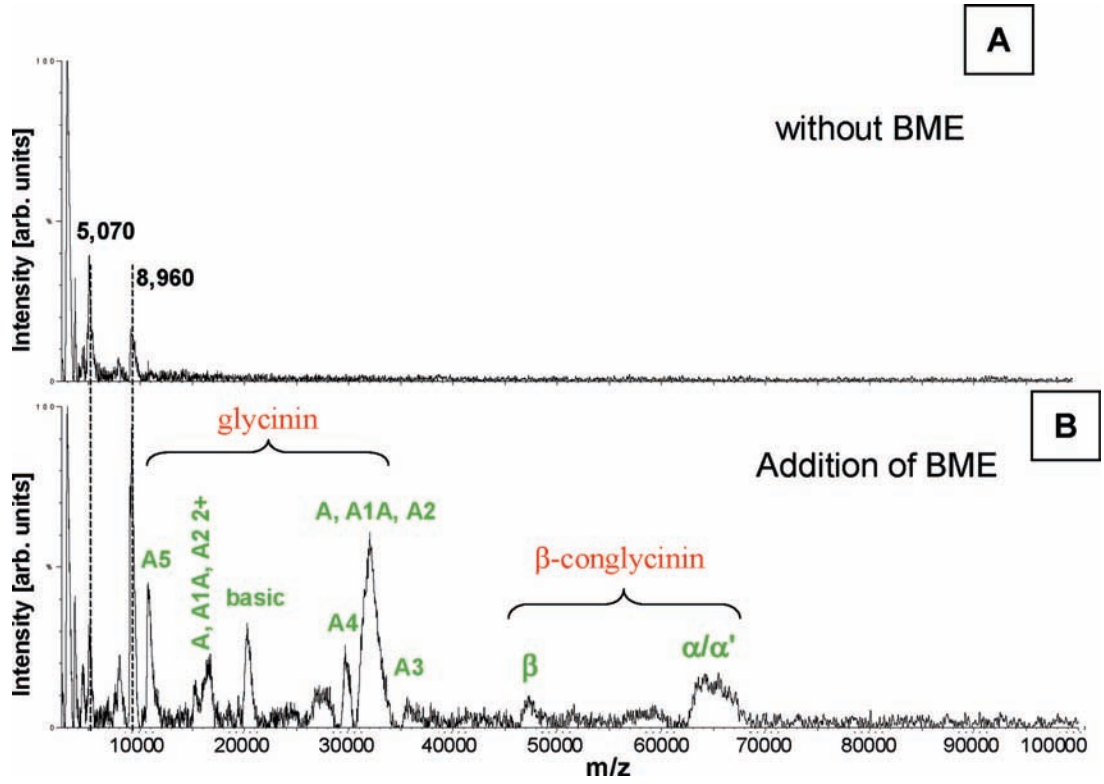

Figure 1. Comparison of MALDI-MS positive ion spectra of an SPI (Supro 651 IP) showing the influence of the reducing conditions after using BME.

consists of three subunits α′ (~65 kDa), α (~62 kDa), and β (~47 kDa) in at least six different combinations (*4*). Glycinin is built from acidic (A1–A4 ~ 30 kDa, A5 ~ 10 kDa) and basic (B, B1, ... ~20 kDa) subunits linked by a single disulfide bridge, except for the acidic polypeptide A4 (*5*).

The dominant state-of-the-art analytical approach for composition characterization applicable to protein food research is matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). It is able to measure the mass of large, labial biopolymers such as peptides, proteins, DNA, polysaccharides, etc. quite exactly as intact molecules (*6–8*). Furthermore, MALDI-TOF-MS is a tool that can determine conformational changes and molecular interaction, sequence *N*-terminally blocked protein, define N and C terminal sequence heterogeneity, locate and correct errors in DNA, identify sites of deamination and isoasparty formation, phosphorylation, oxidation, disulfide bone formation, and glycosylation (*6*).

In the past, MALDI was successfully used in determining the "fingerprint" of the dairy protein composition of various food compositions (*8*). In contrast to electrospray ionization, MALDI can be used for characterization of crude mixtures. Different applications of mass spectrometry to the analysis of food peptides and proteins have been extensively reviewed in recent years (*6*, *8*); MALDI-TOF-MS used for the structural characterization of proteins has advantages in terms of sensitivity, mass accuracy, and short analysis time. The resolution achieved with the instruments equipped with this system is high enough to separate peaks differing by a few dalton in a mass range up to 20–30 kDa. However, the use of MALDI-TOF-MS for characterizing plant storage proteins has been limited.

This paper describes the analysis of industrial SPIs by MALDI-TOF-MS. A new analytical protocol is developed because SPIs consist of a complex mixture, mainly of glycinin and β-conglycinin. The preparation implements a fast extraction of the proteins from the raw SPI either under neutral or reducing conditions. The extracts are analyzed subsequently by MALDI-TOF-MS without further purification. Results of the two conditions are compared. Finally, different SPIs from several suppliers are analyzed and compared concerning their consistency.

MATERIALS AND METHODS

Materials and Reagents. Soy protein isolates FXP H0219D IP and Supro 651 IP were purchased from PTI and Prolisse 500/510 from Cargill. TRIS buffer (pH 8), β-mercaptoethanol (BME), 2,5-dihydroxybenzoic acid (DHB), and 2-hydroxy-5-methoxybenzoic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). All water used was double deionized (Milli-Q water purification system, Millipore Corp., Bedford, MA).

Protein Extraction. The extraction of the protein from the SPI was performed with 10 mM TRIS buffer (pH 8) for 1 h and addition of 10 mM β-mercaptoethanol (BME) for reducing conditions, respectively.

MALDI-TOF-MS. MALDI-TOF-MS was performed using a TOF-Spec 2E (Micromass) in linear mode with a high mass detector for a mass range up to ~100 kDa and low mass suppression below 2500 kDa. DHBs was used as a matrix, i.e., a mixture of 2,5-dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic acid, a standard matrix for high mass range. MALDI-TOF-MS was calibrated with with a protein standard (bovine ubiquitin 8565 kDa, horse heart cytochrome C 12360 kDa, bovine trypsinogen 23981 kDa, and bovine serum albumin 66430 kDa). A single spectrum was obtained by collecting 40 laser pulses from three randomly selected spots. Thus, one spectrum represented the sum of 40 pulses per 3 positions or a total of 120 laser pulses. The sample was prepared by mixing 0.5 μL of both the sample and matrix solution on a MALDI target and air-drying.

RESULTS AND DISCUSSIONS

The molecular masses of the two major proteins glycinin and β-conglycinin in their intact form exceed the mass detection range (up to ~100 kDa). Therefore, glycinin and β-conglycinin could not be detected directly at normal (nonreducing) conditions, as shown on **Figure 1A**. Only peaks (not identified)

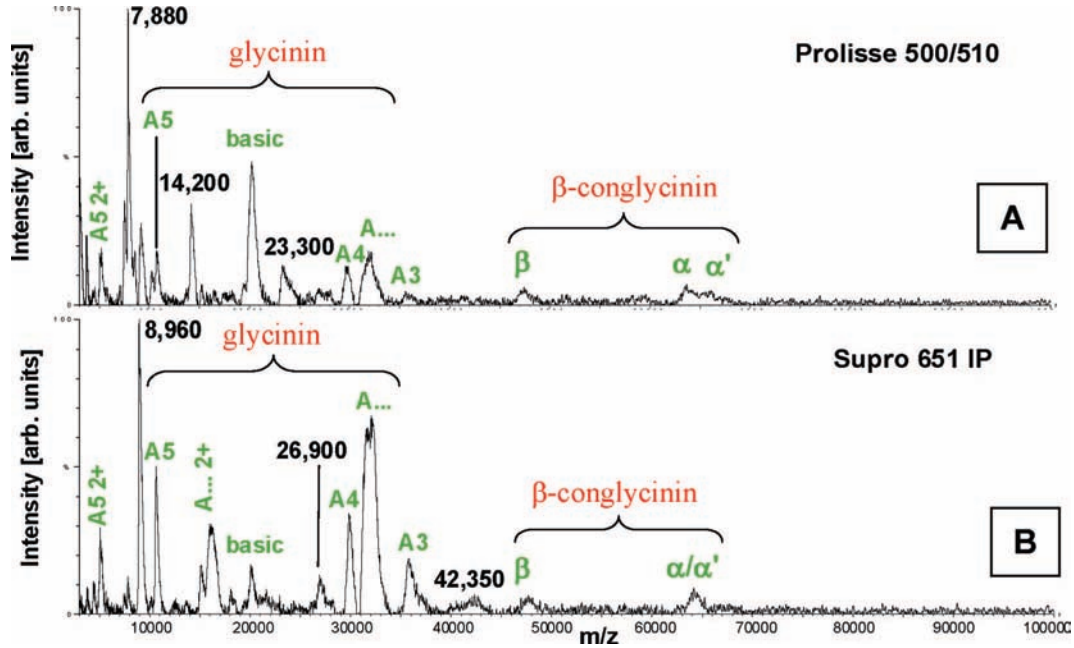

Figure 2. Comparison of the influence of reducing conditions on MALDI-MS positive ion spectra spectra: (soy protein samples after hydration and reduction) (**A**) Prolisse 500/510 and (**B**) Supro 651 IP (bottom).

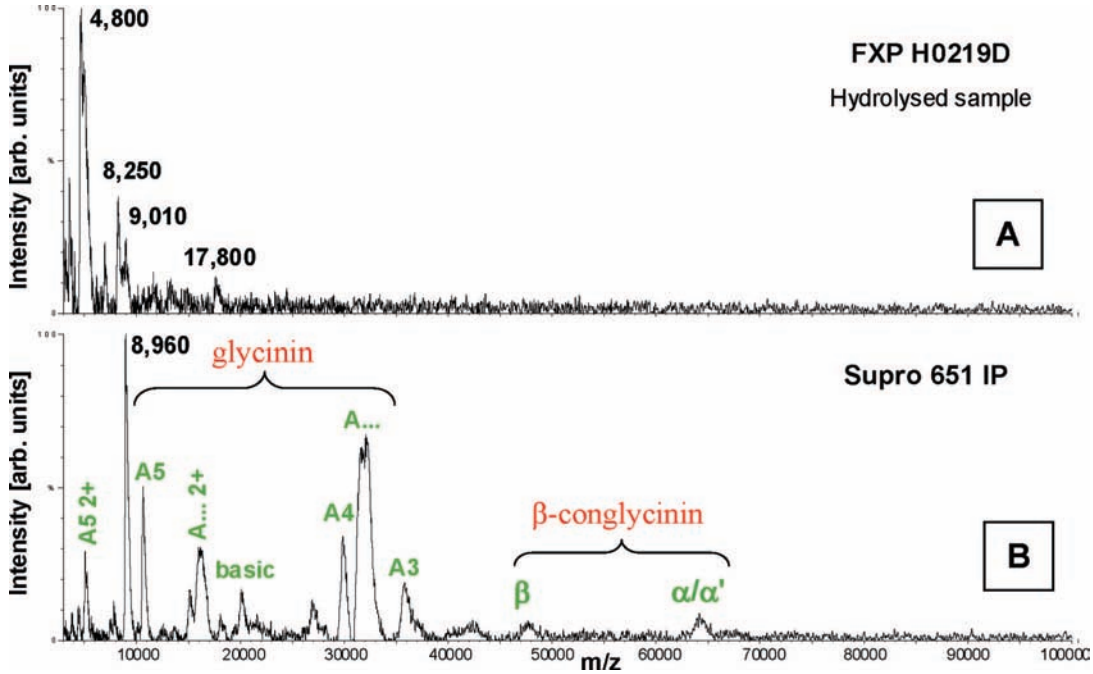

Figure 3. Comparison of MALDI-MS positive ion spectra of SPIs with (**A**) fully hydrolyzed (FXP H0219D) and (**B**) intact subunits (Supro 651 IP).

corresponding to species of mass smaller than ~9 kDa appeared without using a reduction agent. After reduction with BME, however, the different subunits building the soy protein can be detected (**Figure 1B**). Under the reducing conditions applied, the quaternary structure is destroyed and the individual subunits are detected. The three subunits of the β-conglycinin α, α', and β appear at ~64, ~67, and ~48 kDa, respectively. Typically, these peaks appear with relatively low intensity in comparison to the peaks of the glycinin subunits. The most pronounced peaks of the glycinin subunits are of A, A1A, or A2 (or a mixture of them) with mass ~32 kDa. The A4 and A5 acidic subunits appear with mass ~30 and ~10 kDa, respectively. Interestingly, the peak at ~9 kDa which is detected at nonreducing conditions appears several times larger under reducing conditions.

The comparison of SPIs from different suppliers (**Figure 2**) shows that there can be a significant difference in the type and amount of subunits present. For example, in Supro 651, the α and α' subunits appear in one peak, whereas in Prolisse 500 two peaks are well distinguished. The difference is even more pronounced for the glycinin subunits. Prolisse 500 has a strong peak for the basic subunit, whereas Supro has a strong peak for A. . .(A, A1A, or A2) subunits. In addition, there are several other differences in the in the spectra. Importantly, such differences could be used for fingerprinting of the SPI in identifying it in the crude mixture of proteins and a product. These differences could also bring insight to the different performances of the SPIs (*9–11*). Although, a direct correlation between MALDI-TOF-MS spectra and physicochemical characteristics is outside of the scope of this paper, we would like

to point out that such differences in the SPIs could provide valuable information about composition−performance correlations.

The MALDI-TOF-MS spectra also indicate differences between the SPIs from different suppliers as well as in products of the same supplier. This can be used as quality control for new samples or different batches from the same supplier. The difference in the MALDI spectra can be attributed to different processing conditions used by the suppliers. One can correlate this to the degree of "nativeness" of the protein−amount of intact subunits. **Figure 3** shows a comparison of MALDI-MS spectra of SPIs with intact subunits (Supro 651 IP) and fully hydrolyzed (FXP H0219D). The fully hydrolyzed SPI shows only one peak at ~18 kDa. Although we have chosen an extreme case, it is possible to detect a different degree of hydrolyzation. If different process of hybridization are being used, the method should in principle reveal which component and which subunit will be hydrolyzed and at what condition. Careful calibration should in principle allow quantification of the degree of hydrolyzation in an SPI for correlation with other protein functionalities and modes of processing. Finally, there is no limitation besides higher complexity and low protein content for MALDI-TOF-MS to be used for other soy protein sources like concentrates and defatted soy flavor.

The protein composition, degree of nativeness, and degree of hydrolyzation and processing conditions are very important in determining the functionality of soy protein (*3*, *11*, *12*). Soy proteins is a complex mixture of several proteins and the composition, in addition to genetic variants, can vary substantially in different sources. As the functional properties of the two main components glycinin and β-conglycinin, for which it is accepted that they are the main contributors to the overall functionality, are different (*13*–*15*). Their concentration in the protein source is important for determining the general performance. Although the ratio between glycinin and β-conglycinin can easily be determined, the exact composition could still play an important role due to the presence of other minor proteins. Therefore, commercially available SPIs could show very different performance and functionality. Fist, fingerprinting of different sources is very important for protein source selection. Second, the solubility of the two main components (glycinin and β-conglycinin) depends differently on pH (*16*). Therefore, the effective solubility will depend on their ratio/concentration. Glycinin and β-conglycinin have also different activities to Ca ions (*16*). Different compositions of their subunits may additionally influence the sensitivity to calcium ions. The viscosity of soy protein solutions can be affected by the degree of hydrolyzation. Taste is also strongly affected by the degree of hydrolyzation. Bitterness is increased (related to) the degree of hydrolysis. Composition, heat denaturation, and partial hydrolyzation can significantly improve foaming/emulsifying properties (*17*, *18*). The higher the solubility, the higher the foaming/emulsifying capacity of the soy protein (*12*, *19*–*21*). The stiffness of the gel increases with the degree of denaturation of soy protein. Glycinins are stiffer than β-conglycinin gels under the same conditions. However, there is a synergy effect between glycinin and β-conglycinin as the mixture has improved gelling properties. This synergy can be influence by their composition. Hydrolyzation should decrease the ability for gel formation, as the small polypeptides cannot form a gel network. Water or oil sorption and binding are also affected and vary with protein source and composition. All this indicates that the composition and intactness of different subunits play important roles in fictional properties of soy proteins. Therefore, rapid methods for fingerprinting of different industrial soy protein sources will be a valuable tools for successful product formulation.

Conclusions. In conclusion, MALDI-TOF-MS offers a simple, cheap, and quick way to fingerprint industrial soy protein sources. Due to its high sensitivity, the subunits of the two major proteins conglycinin and β-conglycinin can be identified. MALDI-TOF-MS results could be related to other characterization methods and correlate composition to protein performance through other physicochemical characteristics. In addition, sample purity can be detected and used for quality control for new samples or different batches from the same or new supplier. Finally, the method gives a first impression of the degree of hydrolysis. Due to its simplicity, sensitivity, and rapidness, the method can be applied to other plant proteins and mixtures of them.

ACKNOWLEDGMENT

The authors would like to thank Dr. P. Schum and Dr. R. Farr for critical reading of the manuscript and useful discussions.

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Received for review May 6, 2007. Revised manuscript received October 22, 2007. Accepted October 26, 2007.

JF071321Y