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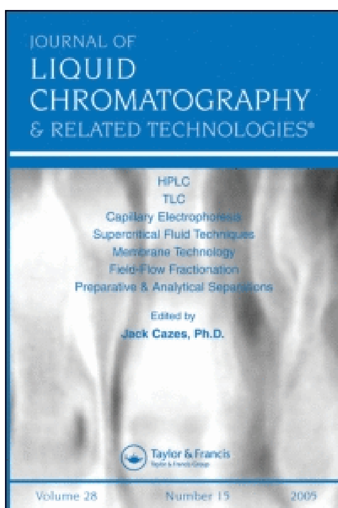


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# USE OF PHASTGEL SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS FOR RAPID CHARACTERIZATION OF SOYBEAN PROTEINS IN COMMERCIAL SOYBEAN PRODUCTS

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# USE OF PHASTGEL SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS FOR RAPID CHARACTERIZATION OF SOYBEAN PROTEINS IN COMMERCIAL SOYBEAN PRODUCTS

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## ABSTRACT

Commercial soybean products for human consumption (soybean protein isolate, soybean flour, textured soybean, and soybean powdered milks) are characterized for the first time by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) using the PhastSystem®, a semi-automatic electrophoretic system. It makes SDS-PAGE very fast and gives very sharp bands that simplify the identification. The time from loading the gel to characterization is less than 1 hour and the resolution is similar to that of large conventional gels. Proteic fractions obtained from these commercial soybean products by using a fractionation procedure described in literature are also analyzed. The electrophoretic patterns of these globulin fractions exhibited some differences which were attributed to the different technological process used for the preparation of each product investigated. Furthermore, the electrophoretic patterns obtained for commercial soybean products enabled the differentiation between



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products derived from soybean protein isolates and those prepared directly from whole soybeans.

## INTRODUCTION

The consumption of soybean products has increased in recent years, even in many Western countries, based on their low-fat and excellent vegetable protein content.[1] Furthermore, soybean derivatives are ideal substitutes for animal milk proteins, especially for individuals allergic to these proteins. Currently, there are many soybean products available in the market: dairy-like products (liquid or powdered milks, yoghourt, soybean-based infant formulas), tofu, miso, natto, tempeh, etc., in addition to the traditional whole soybeans, textured soybean, and soybean flour. These soybean derivatives are generally obtained from soybean flakes or soybean protein isolates and the industrial processes employed to obtain all these products are the following.[2,3]

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Soybean flakes are the starting material for most soybean products. They are made from whole soybeans by cleaning, heating, craking, dehulling, and flaking to 0.25-0.30 mm thickness. The oil is removed with hexane by several types of countercurrent extraction systems.

Soybean flour is prepared by grinding the flakes to pass through a US 100-325 mesh. Upon a controlled heat treatment, soybean flour provides varieties with different Nitrogen Solubility Index.

Soybean protein concentrate is prepared from soybean flakes or flour by removing most of the water-soluble non-protein constituents. This product is produced by three basic processes: acid leaching (at about pH 4.5); extraction with aqueous alcohol (70-90%), or denaturing the protein with moist heat prior to extraction with water.

Soybean protein isolate is prepared from soybean flakes or flour by removing most of the non-proteic compounds present in soybean. For this purpose, the protein is solubilized at pH 6.8-10 and separated by centrifugation and filtration. The resulting supernatant is acidified (pH 4.5) to precipitate the protein. Then, the protein is either spray-dried at its precipitation pH or neutralized to pH 6.5-7.0 as sodium or potassium proteinates to make it more soluble and functional.

Textured soybean is prepared by thermoplastic extrusion from soybean flour, protein concentrates, or protein isolates. The process consists in heating at high temperature and high pressure during a short time. During this process, quaternary structures of proteins are open, proteins polimerize and reorientate, and intermolecular bonds are produced.[4]

Powdered soybean milk-like products can be produced from soybean protein isolate or by drying soybean liquid milk-like products. Liquid milk-like products are aqueous extracts from whole soybeans.

Due to the functional properties of soybean proteins, soybean derivatives have a great potential in food manufacture. Soybean isolates, soybean concentrates, whole soybeans, and soybean flour are the most used in food preparation.[1]

The major protein components of soybean (globulins) are glycinin and β-conglycinin. Glycinin (11S) has a molecular weight of about 350 kDa[5,6] and is composed by acidic polypeptide chains (relative molecular mass ($M_r$) 42-37 kDa) and basic polypeptide chains ($M_r$, 20-17 kDa) which are paired by disulfide bonds. β-Conglycinin (7S) has a molecular weight of about 180 kDa[5,6] and exists in at least seven forms as a result of various combinations of the three subunits α, α' and β ($\beta_3$, $\beta_2\alpha'$, $\beta_2\alpha$, $\beta\alpha\alpha'$, $\beta\alpha_2$, $\alpha_2\alpha'$, $\alpha_3$).[7] Soybean proteins have been well characterized in whole soybeans by using several slab-gel electrophoretic techniques,[8-12] Size-Exclusion High Performance Liquid Chromatography (SE-HPLC),[13,14] Reversed-Phase High Performance Liquid Chromatography (RP-HPLC),[15-17] and Capillary Electrophoresis (CE).[18,19] Nevertheless, despite the great progress made in the characterization of whole soybeans and its major globulins (11S and 7S), there is very little information about the characterization of soybean globulins isolated from commercial soybean derivatives, even though it is known that the technological treatment used may affect soybean proteins. In fact, the knowledge of the different proteins of soybean products, as well as, the determination of its concentration in those products is very important to establish the quality of the product. Indeed, Yagasaki et al.[20] have postulated that decreasing glycinin/β-conglycinin ratio and structural changes caused by a lack of glycinin subunit(s) must affect the functional properties of soybean proteins.

By other ways, despite the potential of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) for the characterization of soybean proteins from whole soybeans, the high analysis times used constitute important drawbacks. Thus, in order to provide a sensitive, cost-efficient, and very rapid procedure for the characterization of soybean proteins, new systems are needed. PhastSystem® is a semi-automatic electrophoretic system, is faster and easier to operate than conventional polyacrilamide gel electrophoresis, providing reproducible and reliable results.

For all these reasons, the primary aim of this work is the rapid characterization of different commercial soybean products for human consumption and their isolated 11S and 7S globulin fractions by SDS-PAGE using the PhastSystem®.

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## EXPERIMENTAL

### Chemicals and Samples

Tris(hydroxymethyl)aminomethane (Tris) and 2-mercaptoethanol (ME) (Merck, Darmstadt, Germany) were used for the fractionation of soybean globulins and for sample preparation for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The disodium salt of ethylenediaminetetraacetic acid dihydrate (EDTA) and sodium dodecyl sulphate (SDS) (Merck, Darmstadt, Germany) were used in the preparation of the samples for the SDS-PAGE analysis.

The soybean protein isolate was obtained from ICN (Aurora, OH), and textured soybean, soybean flour, and powdered soybean milks were purchased from local markets in Madrid, Spain.

11S and 7S globulin fractions were obtained by precipitation with 0.03 mol/L Tris-HCl buffer, containing 0.01 mol/L ME at pH 6.4 (11S globulin) and pH 4.8 (7S globulin) as described by Thanh and Shibasaki.[21] Both fractions were isolated from the supernatant by centrifugation (10000 rpm, 20 min). The whey fraction remaining after precipitation corresponds to the soluble fraction at pH 4.8. The determination of the protein content in this soluble fraction was analyzed by the Kjeldahl procedure.[22]

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### Polyacrylamide Gel Electrophoresis with SDS (SDS-PAGE)

Protein fractions were characterized by means of SDS-PAGE using the PhastSystem® electrophoresis equipment (Pharmacia Biotech, Uppsala, Sweden). SDS-PAGE was performed on 20% homogeneous precast PhastGels, following the manufacturer's instructions[23] and stained with Coomassie Blue R-350[24]. Whey fractions were stained with PhastGel Silver Kit (Pharmacia).

Samples were solubilized at a final concentration of 6 mg/mL in protein solvent (0.01 M Tris/HCl buffer, pH 8.0 containing 0.001 M EDTA, 2.5% SDS, and 5% 2-mercaptoethanol (ME) (v/v)) and heated at 100ºC for 10 min. The relative molecular mass ($M_r$) of the bands was determined using a standard protein mixture consisting of phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (α-LA) (14.3 kDa) (LMW electrophoresis calibration kit, Pharmacia Biotech, Uppsala, Sweden). β-Lactoglobulin (β-LG) (Sigma, St Louis, MO) was also used as standard. Soluble fractions at pH 4.8 from soybean derived products were diluted 1:9 in the SDS-PAGE buffer.

## RESULTS AND DISCUSSION

### Characterization of Commercial Soybean Products by SDS-PAGE

SDS-PAGE using the PhastSystem® has been applied for the first time to the characterization of several products derived from soybean that are commercially available for human consumption: soybean flour, textured soybean, and powdered soybean milks. For this purpose, 7S and 11S globulin fractions obtained from the above-mentioned soybean products were firstly characterized. These fractions were obtained by means of the fractionation method proposed by Thanh and Shibasaki.[21] Results obtained showed that the resolution of the soybean proteins on PhastSystem® was similar to that obtained with conventional gels[25] and that the shorter separation distance did not impair resolution of the different soybean globulins. Band assignment was facilitated by comparison to the pattern of Honig and Wolf[26] and analysis of the 7S and 11S fractions.

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Figure 1 shows the electrophoretic pattern of the molecular weight standard and 7S and 11S globulin fractions from a soybean protein isolate, a soy-

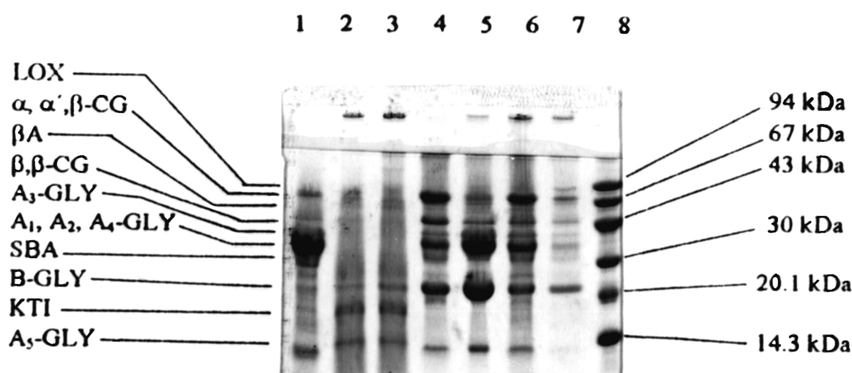


**Figure 1**. SDS-PAGE pattern using the PhastSystem® for the two globulin fractions, 7S and 11S, isolated from different soybean products. Lane 1: textured soybean fraction 7S; lane 2: powdered soybean milk fraction 7S; lane 3: powdered soybean milk fraction 11S; lane 4: soybean flour fraction 7S; lane 5: soybean flour fraction 11S; lane 6: soybean protein isolate fraction 7S; lane 7: soybean protein isolate fraction 11S; lane 8: molecular weight standards: phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.3 kDa). Bands: LOX, lipoxygenase; α-, α'-, β-CG, α and α' subunits of β-conglycinin; β-A, β-amylase; β, β-CG, β subunit of β-conglycinin; $A_3$-GLY, acidic glycinin polypeptide; $A_1$, $A_2$, $A_4$-GLY, acidic glycinin polypeptide; SBA, agglutinin; B-GLY, basic subunits of glycinin; KTI, Kunitz trypsin inhibitor; $A_5$-GLY, acidic glycinin polypeptide.

bean flour, and a powdered soybean milk. In the case of the textured soybean only the 7S fraction could be obtained when the fractionation method of Thanh and Shibasaki[21] was employed (Figure 1, lane 1). The 11S fraction of the soybean flour (Figure 1, lane 5) was enriched in acidic glycinin polypeptide chains $A_1$, $A_2$, $A_3$, and $A_4$ (37-43 kDa) and basic subunits of glycinin (22kDa) while 7S fraction (Figure 1, lane 4) showed an enrichment in the subunits of β-conglycinin, α and α' (80 kDa) and β (55 kDa).

These results agree with those obtained for whole soybeans in literature.[9] However, this behaviour is not the same for all globulin fractions obtained from the other soybean products investigated. In fact, 7S and 11S globulin fractions from the powdered soybean milk showed the same electrophoretic pattern (Figure 1, lanes 2 and 3) whereas in the case of the textured soybean for which 11S fraction could not be obtained, the electrophoretic pattern of 7S (Figure 1, lane 1) showed characteristic bands of 11S suggesting that for textured soybean the isolation of the two globulins could not be achieved by the fractionation method employed. In addition, for the soybean protein isolate a bad separation between 7S and 11S globulins was observed being the 7S fraction more enriched in proteins than the 11S fraction. Note that this last fraction presented a different electrophoretic pattern from that obtained for the 11S globulin fraction isolated from soybean flour (Figure 1, lanes 5 and 7). These differences might suggest that conformation of proteins in these commercial soybean products is different, which probably could be due to the different manufacture process used in the preparation of every commercial soybean product.

In relation with soybean products, in the soybean protein isolate (Figure 2, lane 2) the major bands can be identified as: lipoxygenase (LOX) (94 kDa), β-conglycinin subunits, α and α' (80 kDa), β-amylase (β A) (57 kDa), β-conglycinin subunit β (55 kDa), acidic glycinin polypeptide chains $A_3$ (43 kDa) and $A_1$, $A_2$, and $A_4$ (37 kDa), agglutinin subunit (SBA) (30 kDa), basic subunits of glycinin (B) (22 kDa), Kunitz trypsin inhibitor (KTI) (21.7 kDa), and acidic glycinin polypeptide chains $A_5$ (13 kDa). The electrophoretic patterns obtained for soybean flour and textured soybean (Figure 2, lanes 3 and 4 respectively) were similar to that obtained for the soybean protein isolate except for the existence of some electrophoretic bands for the soybean flour and textured soybean that could not pass through the resolution gel. These bands may be aggregates formed during the processing of these products. Two commercial powdered soybean milks (Figure 2, lanes 6 and 7) showed similar electropherograms to those obtained for the soybean protein isolate even with different concentrations. One of them (Figure 2, lane 6) also showed a band that did not pass the gel as in the case of soybean flour and textured soybean. In fact, this powdered soybean milk was the only one among the three powdered milks analyzed which was directly derived from whole soybeans, while the other two (Figure 2, lanes 5 and 7) were prepared from a soybean protein isolate.

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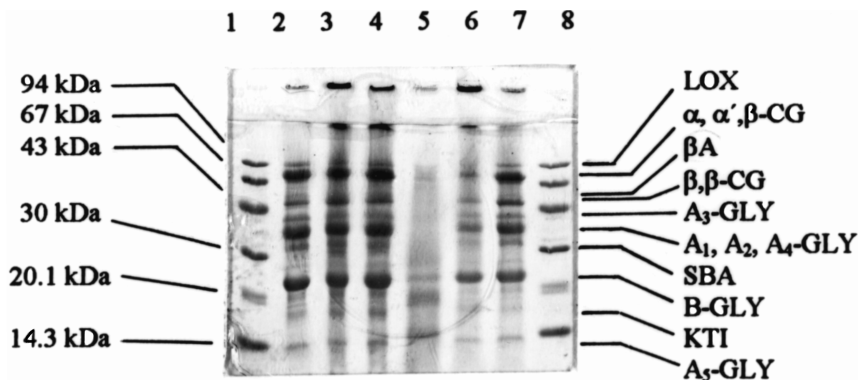


**Figure 2**. SDS-PAGE pattern using the PhastSystem® for whole extractable proteins by different soybean products. Lane 1 and 8: molecular weight standard; lane 2: soybean protein isolate; lane 3: soybean flour; lane 4: textured soybean; lanes 5 to 7: three different powdered soybean milks. Standards and band identities as in Figure 1.

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The third sample of powdered soybean milk analyzed (Figure 2, lane 5) showed a different electrophoretic pattern with only low molecular bands, between 14 and 20 kDa. These bands were not coincident with any soybean protein bands and were not found in other soybean products. These bands could be due to the presence of α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) proteins which were included in the formulation of this sample of powdered soybean milk.

## Soluble Fraction at pH 4.8

In order to achieve a better characterization of the different commercial soybean derivatives studied, the soluble fraction at pH 4.8 resulting from the fractionation of the 11S and 7S globulin fractions from the soybean protein isolate, soybean flour, textured soybean, and one powdered soybean milk (corresponding to lane 5 in Figure 2) were analyzed by SDS-PAGE using the PhastSystem® and stained with Coomassie Blue R-350. No protein band was detected (data not shown) indicating that high concentrations of proteins were not present in the soluble fraction at pH 4.8. These results were corroborated by the analysis of this soluble fraction by the Kjeldahl method which indicated a very low percentage of proteins (≤ 0.55%).

To increase sensitivity, silver stain was used. Figure 3 shows the electrophoretic pattern of the soluble fraction at pH 4.8 from the soybean protein isolate (lane 1), textured soybean (lane 2), powdered soybean milk (lane 3), and

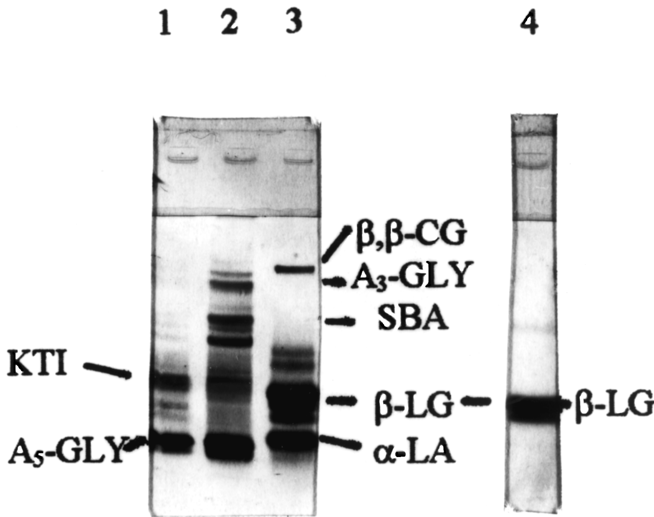


**Figure 3**. SDS-PAGE pattern using the PhastSystem® for the soluble fraction at pH 4.8 for different soybean products. Lane 1: soybean protein isolate; lane 2: textured soybean; lane 3: powdered soybean milk; lane 4: standard of bovine β-LG. Bands as in Figure 1. Silver stain was employed.

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standard β-LG (lane 4) (the electrophoretic pattern for the soluble fraction of soybean flour was similar to that obtained for the soybean protein isolate). Three major bands corresponding to lipoxygenase, β-amylase, and Kunitz trypsin inhibitor are usually described in the soluble fraction at pH 4.8 obtained in the fractionation of the 11S and 7S globulins from whole soybeans.[27] However, soluble fractions at pH 4.8 obtained from commercial soybean derivatives included in Figure 3, showed different electrophoretic patterns. The soluble fraction obtained for soybean protein isolate showed two main bands corresponding to KTI and $A_5$-GLY (Figure 3, lane 1). The soluble fraction from textured soybean showed a higher number of bands that could correspond to $A_3$-GLY, SBA, KTI, and $A_5$-GLY (Figure 3, lane 2). For the soluble fraction obtained from the powdered soybean milk, the two main bands appearing corresponded to molecular mass of 14 and 18 kDa. These bands were identified as α-LA and β-LG by comparison with bovine standards of these proteins. In fact, the addition of bovine whey to this soybean milk sample was indicated on the label of this product.

## CONCLUSIONS

SDS-PAGE using the PhastSystem® allows the rapid characterization of soybean proteins from commercial soybean products for human consumption with a high resolution, making possible the analysis of up to 16 samples within 1.5 hours. The electrophoretic patterns of 11S and 7S globulin fractions obtained from different commercial soybean derivatives exhibit some differences which could be attributed to their different technological processing. Electrophoretic patterns obtained for the commercial soybean products enables the establishment of differences between soybean products derived from soybean protein isolate and those directly prepared from whole soybeans. The main difference between these two groups is the presence of a band corresponding to a high molecular mass in the case of products prepared from whole soybeans, which, could correspond to protein aggregates originated in the processing of these products. These features, together with the short analysis times provided by the PhastSystem® in comparison with conventional SDS-PAGE, made this system suitable in routine analysis to carry out the quality control of commercial soybean products for human consumption.

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