Functional Properties of the Acidic and Basic Subunits of the Glycinin (11S) Soy Protein Fraction

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ABSTRACT: The functional properties of low- and high-M.W. (LMW and HMW, respectively) acidic subunits and the basic subunit separated from the 11S soy protein fraction were studied and compared with the functional properties of the 11S fraction. Among the functional properties investigated were solubility, emulsification, and viscosity. The results showed that the LMW acidic subunit had higher solubility than the HMW acidic subunit. Among all the samples, the LMW subunit separated by using β-mercaptoethanol (ME) was the most soluble, with a solubility of 98–100% at a pH of 6–12. The solubility profile of the HMW subunit followed a pattern similar to the solubility of 11S. The lowest solubility was observed around pH values in the range close to the isoelectric point for both the LMW and HMW subunit. The basic subunit was not soluble in the pH range 3–10; however, the solubility increased more than 50% at pH 13 compared to the solubility at pH 10. The emulsification capacity of all subunits was higher than 11S in the following descending order: LMW, basic, HMW, 11S. Emulsification activity and stability of the subunits were greater than those of the 11S samples at room temperature and 95°C. With the exception of the LMW subunit separated with ME, the subunits had a higher viscosity than 11S. The basic subunit separated with sodium bisulfite had the highest viscosity of all the samples tested.

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KEY WORDS: 11S, basic subunit, glycinin, high molecular weight fraction, low molecular weight fraction, protein functional properties.

Seventy percent of isolated soy protein is found in two protein fractions: 11S (glycinin) and 7S (β-conglycinin). The M.W. of the 11S protein fraction is 350,000 and the 7S fraction has a M.W. of 150,000 (1). Each of these fractions is composed of an acidic and a basic polypeptide linked by a single disulfide bond (2). Functional properties of proteins are related to their structures and, consequently, to their surface hydrophobicity and sulfhydryl cross-linking (3). Knowledge of the functional properties of soy proteins is fundamental to understanding their value as ingredients in food, feed, and industrial ingredients (4).

The separation of 11S into subunits may generate potential ingredients with improved functional properties that can be used in high-value industrial applications. Little is known about the role of the 11S subunits in influencing certain industrial soy protein characteristics.

The objective of this project was to study the functional properties of the individual subunits and how they compare to the original 11S fraction. The functional properties tested were solubility, emulsification capacity, emulsification activity, and viscosity.

EXPERIMENTAL PROCEDURES

The glycinin (11S) used in this study was isolated at the Center for Crops Utilization Research (Iowa State University, Ames, IA) from soybean cultivar MSB 2795 by a pilot-plant process. The subunits from this process, low- and high-M.W. (LMW and HMW) acidic subunits and a basic subunit, were prepared and donated by Dr. Patricia Murphy. One batch of subunits was isolated by using β-mercaptoethanol (ME) and another batch was isolated by using sodium bisulfite (SB). Subunit samples obtained from each batch were analyzed separately to ascertain the effect of the two methods on the functionality of the subunits. All functionality tests, except solubility of the basic subunits, were carried out at neutral pH of zero-level ionic strength. The basic subunit samples were not soluble in any solution below pH 10. Therefore, all other functionality tests were carried out at pH 12.5 to maximize the pH at which they were the most soluble. In all functionality tests, each evaluation was duplicated.

Solubility. A suspension of each sample was made at 1% wt/vol (0.05 g in 5 mL H_2O) and transferred to a centrifuge tube. Sample pH was adjusted from 1.5–12 using 0.1 N HCl or 0.1 N NaOH. The tubes were shaken (Versa-Bath® model S 224; Fisher Scientific, Fairlawn, NJ) at 120 rpm for 1 h and centrifuged (Sorvall RC 5 Plus; Sorvall, Newton, CT) at 32,500 × *g* for 30 min at 20°C. The protein content in the supernatant was determined by the biuret method (5).

Emulsification capacity (EC). In a 400-mL beaker, 25 mL of a 2% sample suspension was continuously blended with a commercial soybean oil (Flavorite® brand) at a flow rate of *ca.* 0.5 g/s using a hand-held mixer (Bamix, Mettlen, Switzerland) at high speed (*ca.* 12,000 rpm). When the inversion point of oil-in-water to water-in-oil was reached, EC was determined as the maximum amount of oil (in g) emulsified by 1 g of protein (6).

Emulsification activity (EA) and emulsification stability (ES). Five milliliters of a 2% protein suspension plus 1.6 mL (1.8 g) of soybean oil (protein suspension/oil = 3:1 vol/vol)

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were mixed for 15 s in a microcontainer (30 mL capacity) using a Waring® blender (model 31BL92; Dynamics Corp., New Hartford, CT) at low speed. The emulsion was immediately diluted 1000× with 0.1% SDS (1 mL of emulsion in 20 mL SDS and 1 mL of that mixture in 50 mL of SDS). Then 5-mL aliquots of the diluted emulsion were added to nine small glass tubes. One tube was used immediately to measure the initial absorbance at room temperature at 500 nm and was used for time 0. Four of the tubes containing the diluted emulsion were held in a 95°C water bath, and the remaining four tubes were held at room temperature. At each 15-min interval, one tube was carefully taken from each of the two holding temperatures without disturbing the cream formation on the top or the sediment on the bottom of the tube. Absorbance of the drawn sample was read immediately at 500 nm. Readings were taken at 15, 30, 45, and 60 min.

Viscosity. A 7% suspension (wt/vol) of the sample was blended in a Waring blender at low speed for about 30 s. The suspension was centrifuged at low speed (relative centrifugal force = 137) to remove air bubbles and let stand for 2 h to stabilize. The viscosity was measured using a capillary Cannon–Fenske viscometer (Cannon Instrument Company, State College, PA) at 25°C (7).

RESULTS AND DISCUSSION

Solubility. The results show (Fig. 1) that the LMW subunit separated using ME was the most soluble at all pH ranges compared with the LMW subunit separated with SB and the 11S fraction. At the extreme pH ranges, 11S had a rate of solubility similar to the LMW subunit separated with SB (Fig. 1). Both SB- and ME-separated LMW subunits had higher solubility than 11S in the pH range of 4.5–8. In contrast, HMW subunits showed a solubility similar to or lower than 11S (Fig. 2). In this case, the method used in separating the subunits had no influence on solubility. The basic subunit samples had lower solubility than 11S and exhibited no solubility at pH 3–10 (Fig. 3). The solubilities of the basic subunits from both methods of separation increased above pH 10. The increase was greater than 50% at pH 13.

The normal U-shaped solubility distribution in relation to pH for pure protein was seen for all the subunits, with the minimum solubility occurring at the isoelectric point (pI). LMW subunits had a sharp base, basic subunits had a broad base, and HMW subunits revealed a U-shaped profile with a base between pH 4 and 6. The basic subunits had a wide base at a pH range of 1.5 to 10. A protein is usually the least soluble at the pI because the electrostatic forces of the molecules are at their minimum and interaction of protein with water will yield a precipitate. This is a favorable condition for protein molecules to approach each other and aggregate (8). The sharp increase in solubility of the basic subunits above pH 10 may be the result of net negative charge of the protein. Hence, greater interaction between water and the charged protein creates greater protein solubility. On the other hand, the high solubility of LMW acidic subunits at a basic pH may be explained by extreme charge repulsion between the protein and water, which contributes to greater protein solubility.

EC. The EC of all the subunits was higher than that of the 11S fraction (Fig. 4). The EC of the LMW subunits was higher than that of the HMW subunits. The basic subunits also showed higher EC than the HMW subunits. When evaluating the effect of method of isolation (SB or ME) on the EC of the subunits, the results showed no major effect. The process of separating the

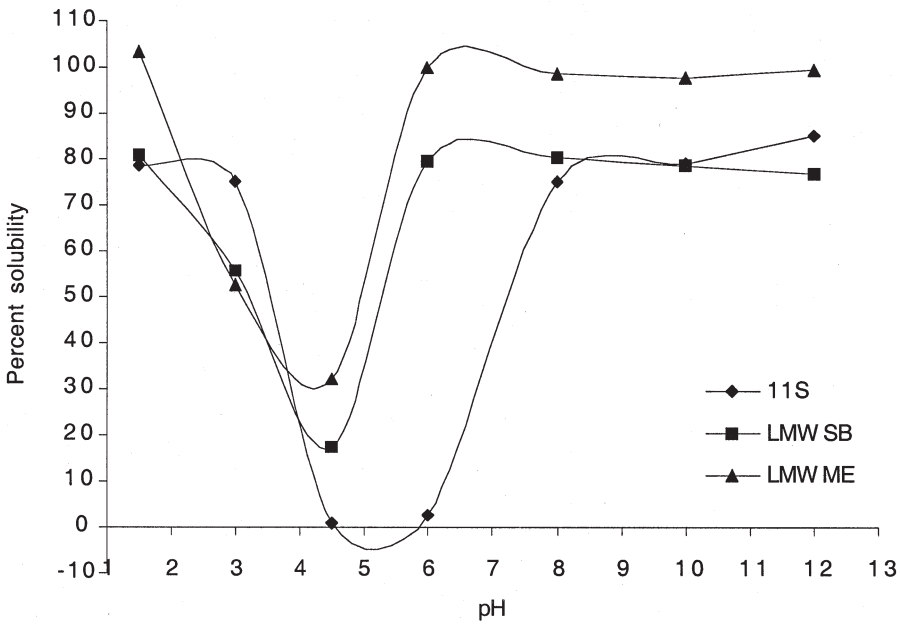

FIG. 1. Solubility of low-M.W. (LMW) acidic subunits fractionated using sodium bisulfite (SB) and β-mercaptoethanol (ME) compared with the 11S fraction. Each data point represents an average of two evaluations.

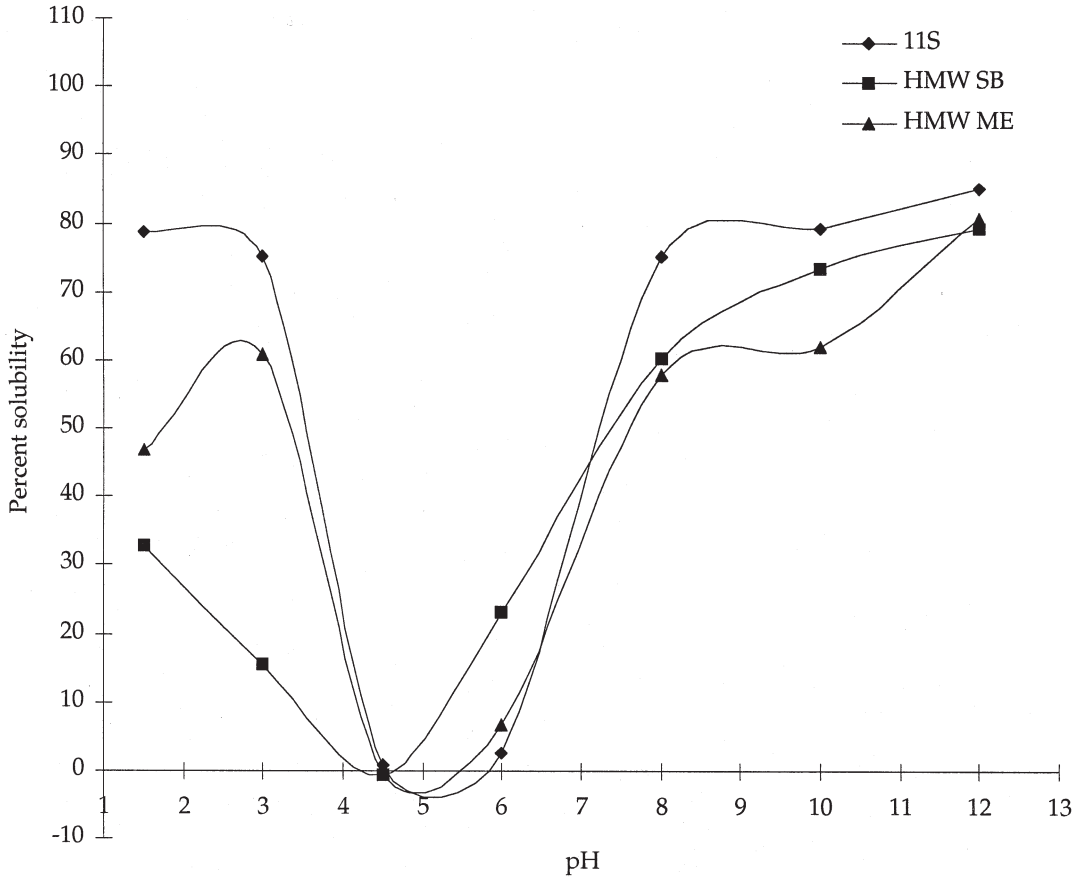

FIG. 2. Solubility of high-M.W. (HMW) acidic subunits fractionated using SB and ME compared with the 11S fraction. Each data point represents an average of two evaluations. For abbreviations see Figure 1.

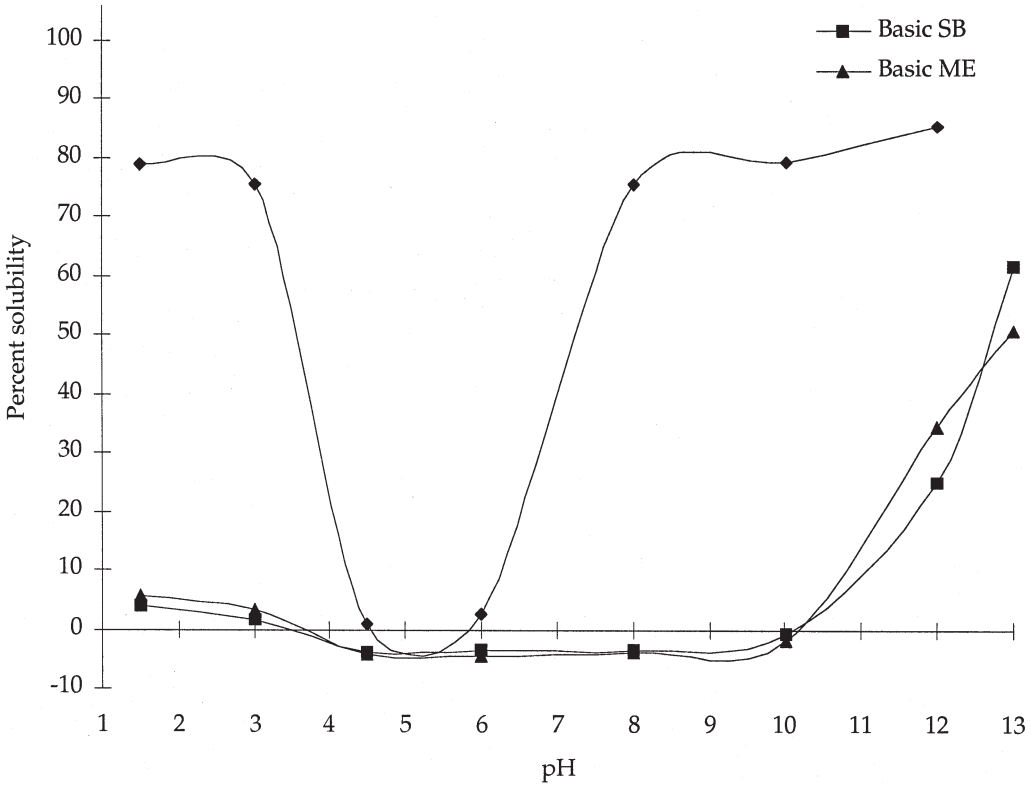

FIG. 3. Solubility of basic subunits fractionated using SB and ME compared with the 11S fraction. Each data point represents an average of two evaluations. For abbreviations see Figure 1.

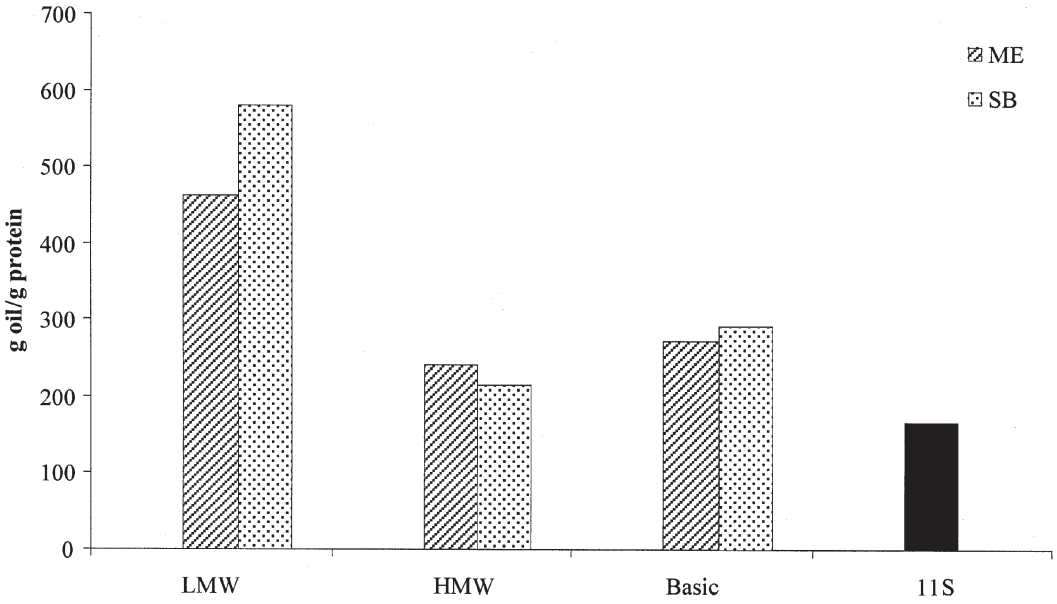

FIG. 4. Emulsification capacity of LMW and HMW acidic subunits and basic subunits compared with the 11S fraction. Data represent an average of two evaluations. For abbreviations see Figures 1 and 2.

subunits may have resulted in changes in the interfacial area of the original protein, resulting in different EC in the different subunits in comparison with the original 11S (9).

EA and ES. The results showed that the LMW and basic subunits isolated with SB demonstrated higher EA (Figs. 5A and 5C) in comparison with the ones produced using ME. There was no difference in EA between the HMW subunits (Fig. 5B). The results also showed that the ES at room temperature was similar to that at 95°C for most of the subunits. Liu *et al.* (10) reported that, of the two acidic subunits, the LMW subunit had the higher EA compared with the HMW. Furthermore, the two subunits had higher EC compared with 11S, in agreement with the findings from this study.

Viscosity. The results (Fig. 6) show that with the exception of the LMW subunit separated with ME (2.05 mm^2/s), all the subunits had higher viscosity than the 11S fraction (2.32

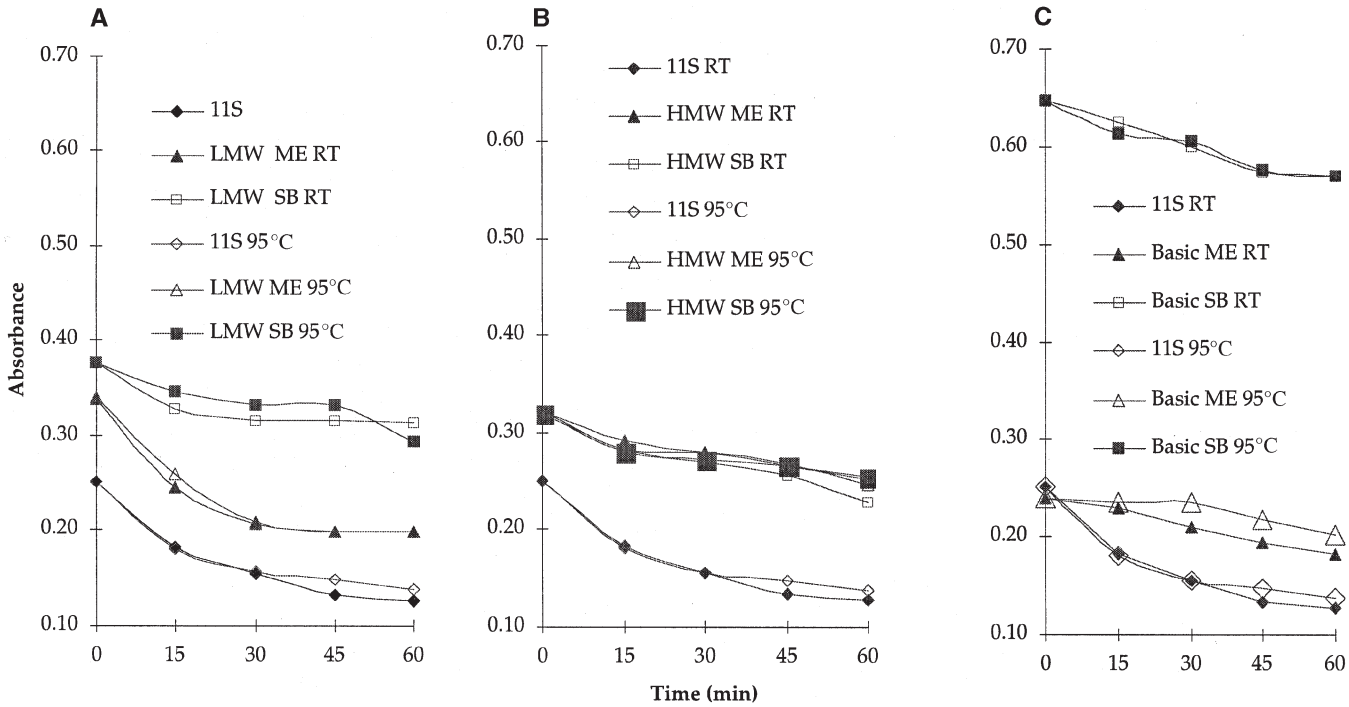

FIG. 5. Emulsification activity and stability of subunits compared with the 11S fraction. (A) LMW acidic fractions; (B) HMW acidic fractions; (C) basic fractions. Each data point represents an average of two evaluations. RT, room temperature; for other abbreviations see Figures 1 and 2.

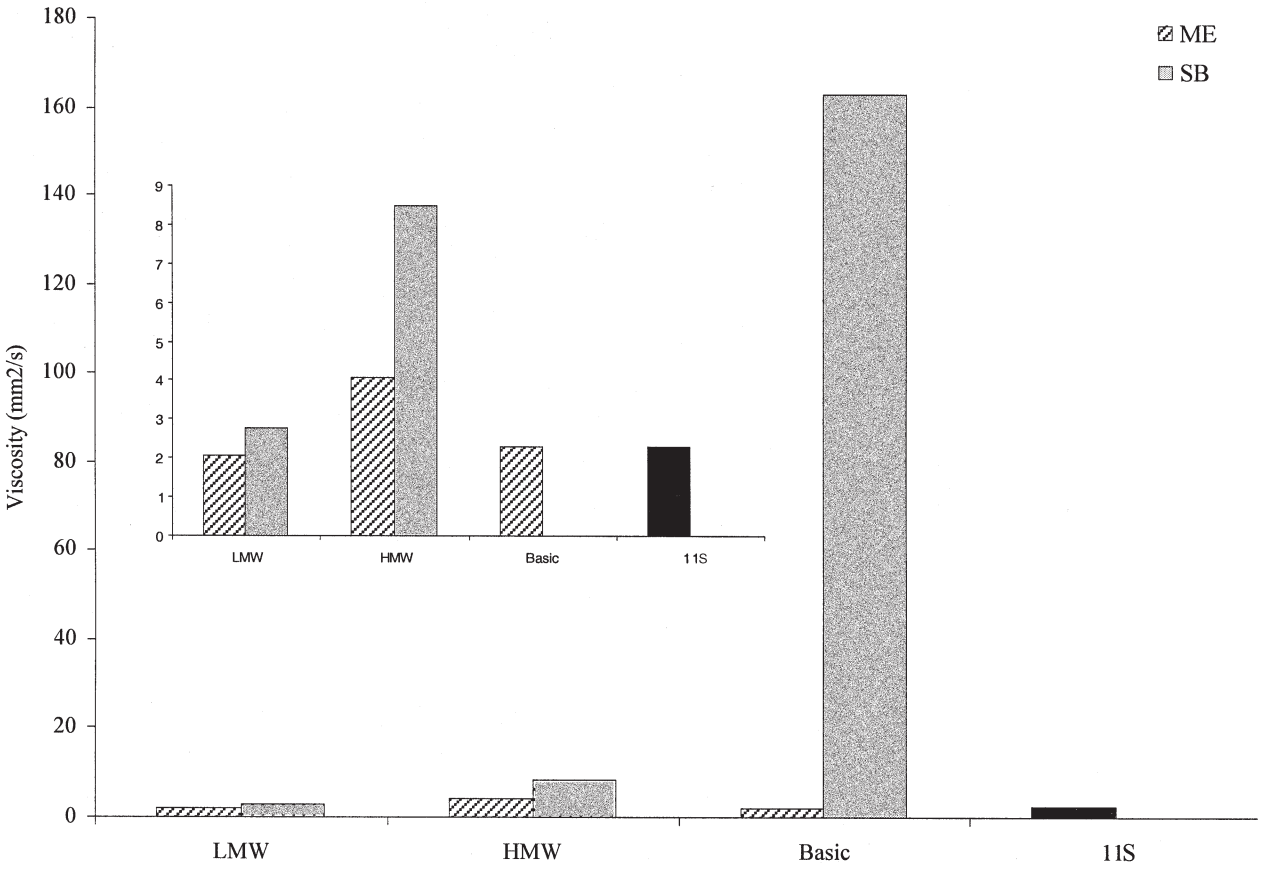

FIG. 6. Viscosity of LMW and HMW acidic subunits (SB and ME) and basic subunits (SB and ME) compared with the 11S fraction. Data represent an average of two evaluations. Inserted graph included to illustrate differences in all fractions except basic SB. For abbreviations see Figures 1 and 2.

mm^2/s). Treatment with SB produced subunits with higher viscosity than those produced with ME. Both the HMW and the basic subunits isolated with SB showed significantly higher viscosity than the other samples, and the basic subunit separated with SB had the highest viscosity (162.9 mm^2/s).

Among the subunits, the LMW acidic subunit had the highest solubility and EC compared to the other subunits and 11S; ME-separated LMW samples had higher solubilities than BS-separated LMW samples. The basic subunit separated with SB had the highest emulsification activity and viscosity. Results of this study suggest that soy protein subunits showed higher viscosities, EA, and EC than 11S. This study also shows that the functionality of the subunits can be affected by the method of isolation.

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