IRON PROTEIN COMPLEX IN SOYBEAN TEMPE

by

Mary Astuti*)

Abstract

In this study some characteristics of iron-protein complex in soybean tempe were investigated. Tempe was prepared by fermenting dehulled cooked soybeans at 30°C for 0; 24; 48; 72 and 96 hours, respectivelly. Tempe protein was extracted using Tris maleate buffer at pH 6.5 and the extract was dialyzed in cellulose tube M.W. 12,000. The residue was fractionated using Sepharose 6B/Cl-6B gel filtration. The protein and iron distribution in those fractions and their molecular weight were determined.

Results showed that fermentation increasing the number of protein fractions. The molecular weight of protein fractions was in the range of 5,000 — 70,000 daltons. Iron was distributed in all fraction measured. The precentage of protein and iron in fractions with molecular weight > 70,000 daltons decreased with longer fermentation time. Some of the small molecular weight of protein fractions failed to pass the cellulose membrane.

Introduction

Soybean is one of the most important legumes utilized as a source of protein in the world (Camacho, 1981). Soybean protein is potentially rich in iron in the diet, eventhough the iron availability of soybean products is controversial. Some researchers considered that soybean protein is a good source of available iron (Rotruck and Luhrsen, 1979; Young and Janghorbani, 1981). Others have found that the addition of soy protein to meals inhibit the absorption of iron (Halleberg and Rosander, 1982). Because of the discripencies in the literature, it is very important to understand the characteristics of iron in soybean protein.

Iron characteristics include the solubility of complex and ionic iron, the iron protein complex and also the effects of some factors which influenced on iron solubility (Smith, 1983). Solubility is often used to predict mineral bioavailability by *in vitro* method (van Dokkum, 1989). The characteristics of iron in soybean protein products are litle understood. Scnepf and saterlee (1984) reported that there are two major sites of iron binding in soy isolate. Iron bound to the surface of a large peptide is easily removed whereas iron found within the large peptide aggregates is difficult to release. Smith (1983) observed that iron binding protein in soybeans consists of two major fractions i.e. (a) large molecular weight fraction (> 600,000 daltons) which eluted at the void volume of chromatographic coloumn, and (b) the fraction with molecular weight 10,000 — 25,000 daltons. However, only limited information about the effect of processing soybeans on the characteristics of iron protein complex is available.

Food processing such as boiling, braising, frying, drying and also fermentation have an effect on the characteristics of iron. Boiling red meat increasing non heme iron (Schricker *et al.*, 1982). Cooking soybeans in autoclave increasing bioavailability or iron (Mulyopawiro *et al.*, 1987). Fermentation has been known to have a positive effect on iron bioavailability (Derman *et al.*, 1980; Mary Astuti et al., 1989). Tempe, a traditional fermented soybean is consumed in significant quantities in Indonesia and recently, widespread in other parts of the world. There are lot of chemical changes during tempe fermentation. Soluble nitrogen has been

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reported to increase with fermentation time (van der Riet *et al.*, 1987). Mary Astuti (1992) reported that soluble iron icreased during tempe fermentation.

The ability to increase both soluble nitrogen and soluble iron during fermentation makes exploration of iron protein complex of particular interest.

Material and Methods

Reagents and Materials

Glassware

Glassware was washed in ultrasonic diswasher, rinsed in distilled water, soaked overnight in 1 N HCl, and rinsed again with distilled water.

Water

Distilled, deionized, iron-free water was used in all experiments.

Protein precipitant

One-hundred g trichloracetic acid and 100 g hydroxylamine hydrochloride were dissolved in 500 ml H_2). One-hundred ml concentrated HCl was added and the mixture was brought to 1000 ml with H_2O.

Chromogen

Two-hundred fifty mg/l bathopenatroline disulfonate in 2 M sodium acetate.

Iron standard solution

Certified atomic absorption standard reference solution, 1000 ppm (Fisher Scientific Co, Pittsburg, PA).

Protein standard solution

Bovine serum albumin as a standard solution at various concentration 0—500 ppm.

Preparation of Tempe Samples

Tempe was prepared from Wilis variety soybeans grown in Yogyakarta province. Cleaned, dried whole soybeans were washed and boiled in distilled deionized water (DD water) for 30 min. Then the beans were soaked for 24 hours at room temperature, in the ratio 3 parts DD water to 1 part beans. Following soaking the DD water was discarded and the beans were dehulled by hand and then soaked again in DD water for 24 hours at room temperature. Again DD water was discarded and the beans were boiled for 60 min. The DD water was drained. As soon as the temperature reached 30°C the cooked beans were inoculated with tempe inoculum powder 0.2 g/100 g dried beans. The inoculated beans were packed in Petri dishes and incubated at 30°C. To study of the effects of fermentation time, on the soybeans frementation were stopped at intervals of 0; 24; 48; 72 and 96 hours. Following fermentation the beans were steamed for five minutes and then minced, freeze dried, ground and screened using 35 mesh.

Gel Filtration Chromatography

Tempe powder was extracted with 10 parts (w/v) of Tris maleate buffer pH 6.5 using magnetic stirrer for two hours and then centrifugated at 30,000 RPM, 0°C for 30 min. The supernatant was then dialyzed (cellulose tubing of 12,000 M.W.) in buffer pH 6.5 at 4°C for 24 hours. The residue was separated with gel chromatography using Sepharose 6B/Cl-6B, and the eluate was fractionated under standard condition. The protein content in the fractions was determined by absorbance of UV 280 nm, using standard Bovine Serum albumin. The iron in the fractions was analyzed with the colorimetric method using bathopenantroline disulfonate and measured in a spectrophotometer at 535 nm. The molecular weight of protein in

the fractions was determined by comparing the elution volume of the samples and the standards (Pharmacia Calibration Kit).

Iron Analysis by Colorimetric Method

One ml of protein precipitant was added in a two test tube each containg 2 ml of aliquot of protein fractions and standard solution. This mixture was heated in a boiling water bath for 10 min and centrifugated at 3,000 RPM. Two ml of supernatant were transferred to a clean test tube. To this supernatant, 1 ml of the chromogen solution was added. After 10 min, the absorbance at 535 nm was read in Shimadzu Spectrophotometer UV-110-02.

Molecular Weight Determination

To determine the molecular weight of protein fractions, a series of molecular weight standards of protein i.e. albumin, ovalbumin, chymotripsinogen A and ribonuclease were eluted at Sepharose 6B/Cl-68. The elution volume was measured from the start of the sample application to the center of the elution peak (fig. 1.). Blue dextran 2000 is used as a coloumn void volume. Then the elution volume was calculated using the equation:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

V_e = elution volume for protein
V_o = coloumn void volume (elution volume for Blue Dextran)
V_t = Total bed volume

Then, using semilogarithmic graph paper, the K_{av} value was plot for each protein standard against the corresponding molecular weight. Draw the straight line which best fits the points on the graph. To calculate the molecular weight of each protein fraction, locate the point on the calibration curve which corresponds to the K_{av} for protein fractions. The value on the logarithmic scale which corresponds to this point is estimated molecular weight of protein.

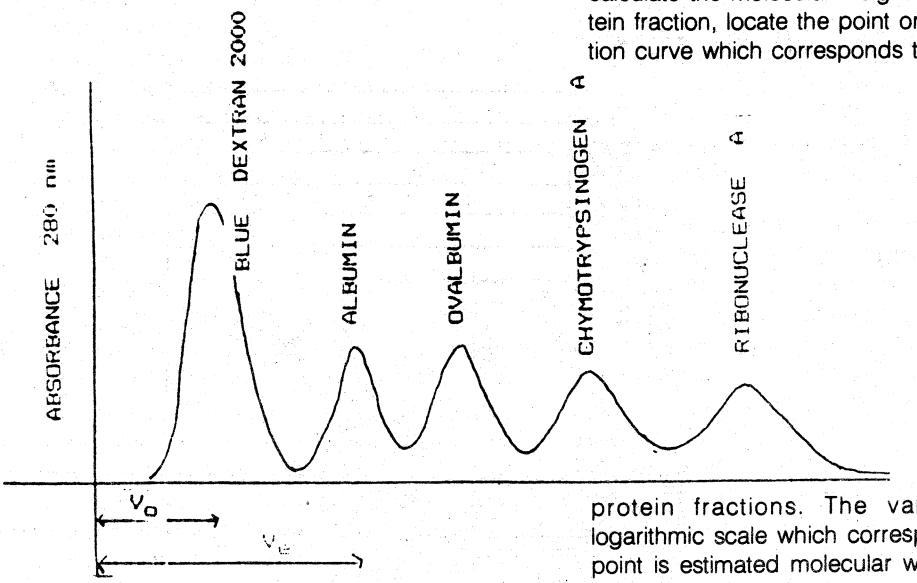

Fig 1. **Elution profiles for protein standard on Sepharose 6B/Cl-6B**
Eluent: Tris maleate buffer, pH 6.5
Sample volume for each run: 1.0 ml
Flow rate: 40 ml/hr
Total bed volume: 50 ml
Coloumn void volume: 8 ml

Fig 3. ilustrated the number of protein fractions. During fermentation of tempe, the number of protein fractions increased. Tempe fermented for 0 hour known as

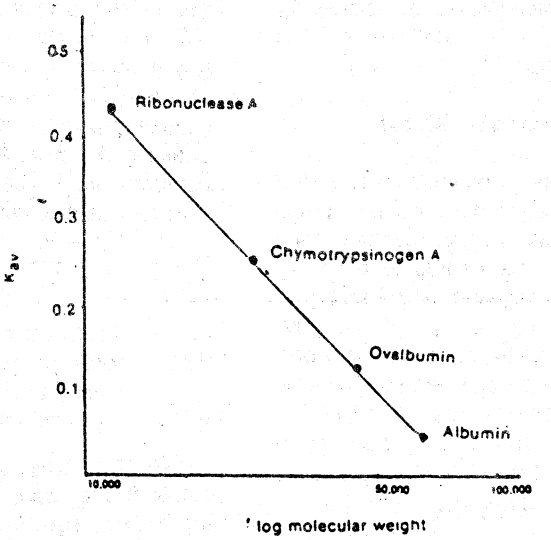

Fig 2. **Calibration curve using low molecular weight of protein standard**

RESULTS AND DISCUSSION

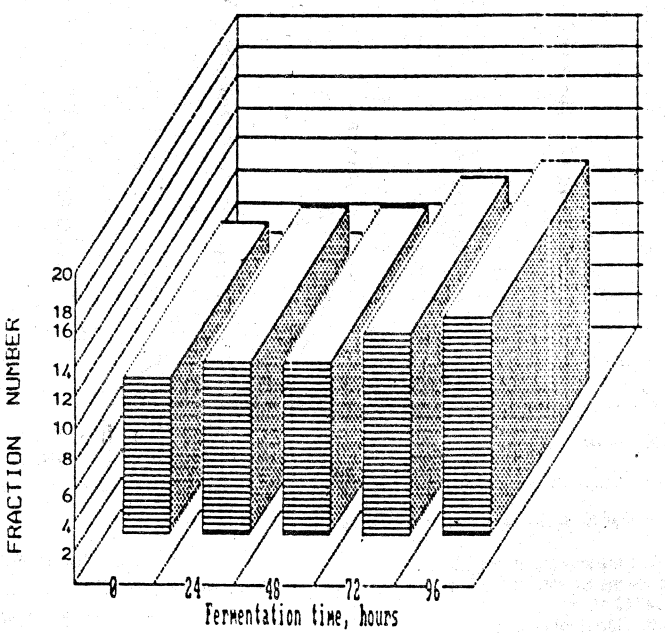

Fig 3. **Protein fractions**

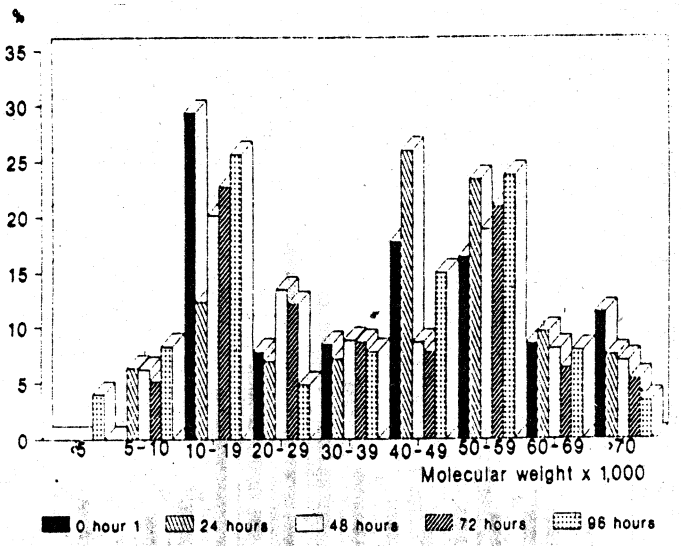

Fig 4. **Distribution and molecular weight of protein fractions**

unfermented soybeans resulted in 10 fractions compared to 11; 11; 13 and 14 fractions in tempe fermented for 24: 48; 72 and 96 hours, respectively. The increased of protein fractions proves that during fermentation proteinase enzyme is active digesting the complex protein resulting in a simple protein. This data supported the previous study by Hermana *et al*, 1992.

The molecular weight of soluble protein in soybean tempe was in the range < 5,000 — > 70,000 daltons (fig. 4). Tempe fermented for 0 hour displayed the highest percentage of protein in the fraction with molecular weight > 70,000 daltons. The lowest percentage of protein fraction of molecular weight > 70,000 daltons was found in tempe fermented for 96 hours. Protein in the fraction with molecular weight less than 10,000 daltons was not detected in unfermented soybeans, whereas protein fractions with molecular weight less than 5,000 daltons was detected in tempe fermented for 96 hours. Scnepf and Saterlee, 1984, found that hydrolized protein in soy isolate was displayed in the fraction of molecular weight between 300,000 — 5,000 daltons. The highest percentage of those protein was found in the fractions with molecular weight in the range of 30,000 — 150,000 daltons.

Most of iron in food is in the form of organic compounds. Fig. 5 ilustrated the iron distribution in protein fractions. Iron-protein complex was found in all fraction of protein in the range of molecular weight < 5,00 — > 70,000 daltons. Iron-protein complexes of molecular weight less than 5,000 daltons were only found in tempe fermented for 96 hours. The presence of iron in fractions with molecular weight less than 5,000 daltons which is smaller than the molecular weight of membrane used, indicated that some iron in the form of complex organic failed to pass through the membrane. According to Scnepf and Saterlee, 1984, in the pH neutral or slightly alkaline, iron in the form of ferric protein complex has limited solubility. Tempe

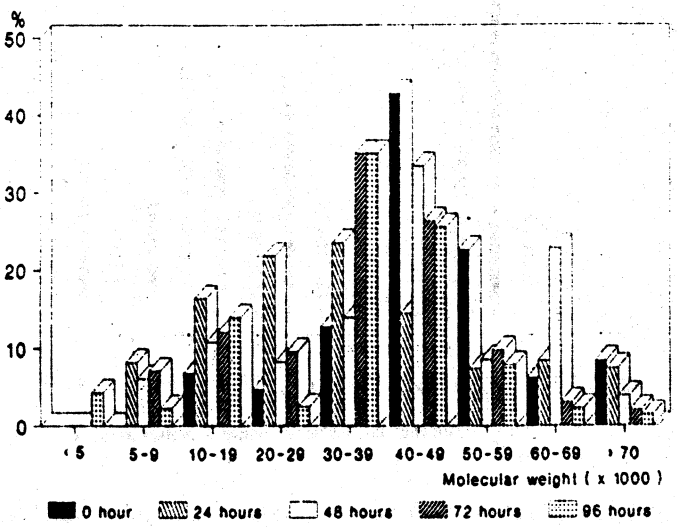

Fig 5. **Iron distribution in protein fractions**

which was fermented for 96 hours had a pH value of 7.5. This might one reason why fractions with a molecular weight less than that of the membrane remained in the dialysis bag. The other possibility is that the dialysis time was too short resulting in not all the protein fraction with molecular weight less than 12,000 passed through the membrane.

The percentage of iron in fraction with molecular weight higher than 70,000 daltons was found in unfermented soybeans. Among those fractions the highest percentage of iron was found in unfermented soybeans with molecular weight in the range 40,00 — 49,000 daltons. Whereas in fig. 4, shows that the highest percentage of protein was found in fraction with molecular weight 10,000 — 19,000 daltons. This indicated that iron is not only associated with protein but also with other organic compounds.

Conclusion

The data indicated that the number of fractions increased with fermentation time. The percentage of protein and iron in the fraction with molecular weight higher than 70,000 daltons decreased. Iron was distributed in all fraction measured. Iron-protein complex with molecular weight less than 5,000 daltons only found in tempe fermented for 96 hours Some of the low molecular weight of iron-protein complex in tempe failed to pass through the dialysis membrane of 12,000 M.W.

Acknowledment

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