Another example of the use of composite flours is the macaroni developed by the Institute for Food Technology, Campinas, Brazil. The composite flour used for the macaroni is made up of wheat, maize, and soy flour (proportions 30:40:30); its protein content is ca. 20%.

NUTRITIVE VALUE

From the standpoint of nutrition, composite flours containing soy flour compare favorably with white wheat flour in their protein characteristics.

From Table I which shows figures for the biological evaluation of some bread types and biscuits, it is evident that the nutritional protein quality is improved substantially by the addition of soy flour to the bread flour, even at levels as low as 3-5%. The protein efficiency ratio is increased from less than 1.0 to ca. 1.5.

Detection of Soy Proteins

ANDRÉ FROUIN, Olida et Caby Associes,
Levallois-Perret, France

INTRODUCTION

Many studies have been made on this up-to-date and important subject. These studies can be classified into four broad groups.

Histological Methods

They are simple and often allow strong presumptions but cannot give formal proofs, because the natural structure of the soybean is destroyed and the aspect found at the microscopic analysis essentially depends upon the transformation treatment of soy proteins.

Serology

We do not know good methods at this time, but it is possible that a quick, simple solution will be found one day, because the chemical characteristic of soy proteins is made.

Immunoelectrophoresis

Lambion and Basseman have published a precise, sensible method, but this method is complicated and combines the difficulties inherent to obtaining serum and those difficulties linked to the electrophoresis, which is not a simple chemical method.

Electrophoresis

These methods were the first to give results. They have been used more and are, in my opinion, the ones which are the best at the present time. The bibliography presented below helps to review its history, which essentially contributed to improved performances, leading to sensibility limits which are below 0.5-1% in the most difficult cases, i.e. on textured products used in products containing various ingredients in sterilized tins.

THREE METHODS

Our own works have aimed to improve these methods, mainly the Penny-Hofmann method, according to a technique similar to the one followed by Spell, the works of whom I was not aware, because they were not yet published. I think that the most interesting is to compare the Penny-Hofmann method, ours, and Spell improvements.

Spell, like ourselves, has improved the performances in preparing the sample before electrophoresis, in both cases using the known effects of high concentration of urea.

However, our methods are different. Spell makes a fat extraction with ketone, then with ether by mixing and centrifugation. We prefer a total extraction with Soxhlet with the nonpolar solvent, petroleum-ether. Spell then makes a direct test with urea 6.6M in limiting the time delay to 1 hr at 35-40 C.

We prefer first, to use NaOH with pH 9, followed by a centrifugation which eliminates all soluble proteins. Then, the dried and rinsed residual is taken over with urea 8 during one night and mercaptoethanol is added, as in the Penny-Hofmann method, to break the linked S-S, a technique which has been eliminated by Spell. Then comes the electrophoresis. As in the Spell method, we have worked on disc electrophoresis, which increases the residual quantity, i.e. the detection sensibility.

Gels are classical in most cases; ours is more concentrated than Spell's, which itself is more concentrated than Penny-Hofmann's. This is to obtain a better separation because the PM of isolated proteins is weak. Like the Penny-Hofmann method, and contrary to Spell, we have found it indispensible to maintain the dodecyl-sulfate which negatively saturates the charge of proteins. Since we cannot bring it into the gel that makes it turbid, we maintained it in a buffer electrode bath, leaving a time delay of 1/2 hr, so that it can penetrate and react in the sample gel.

We have kept the Penny-Hofmann electrode buffers and coloring systems; Spell modified them. He preferred the Tris buffer and the boric acid to tetraborate; I do not think that this is an important point. He makes the fixing, the coloring, and the decoloring in trichloracetic acid, instead of acetic acid-water-methanol. This last operation is delicate; and, since I do not have enough experience on Spell's solvent, I cannot say what is the best.

The Penny-Hofmann method allows a detection to 1% on uncooked or slightly cooked soy protein. We believe that much research effort is unnecessary if interest is for the sterilized products only. In fact, this test also allows the product to be sterilized before analysis, to eliminate the largest number of the bands. This makes the reading easier and avoids mistakes.

We have used this method many times on various products without mistakes, but we found that it is not very easy to make it quantitative because of the sugar proteins interaction during the heating treatment.

We have published two versions of our method, one, the easiest, was discussed at the European Meeting of Meat Research Workers in 1973. The other method was published in the *Annales des Falsifications et de l'Expertise Chimique;* it is more precise yet more complicated.

BIBLIOGRAPHY

Bessemans, J., and Lambion, R. 1972. Recherche des proteines etrangeres dans les produits de viande. Revue de Ferment. Ind. Alim. 27:61.

de Hogg, P.; Van Den Reek, S.; and Brouwer, F. 1970. Nachweis und Bestimmung von Aufgeschlossenem Milcheiweiss und Sojaprotein in Fleischerzeugnissen. Die Fleischwirtschaft. 12:1663.

Fleischmann, O. 1964. Nachweis und Bestimmung von Milcheiweiss in Fleischerzeugnissen. Die Fleischwirtschaft. 4:985.

Frouin, A.; Barraud, C.; and Jondeau, D. Detection des Proteines de Soja ou de Lait dans les Produits de Viande Sterilises ou Non. Reunion Europeenne des Chercheurs en Viandes, Paris, September 1973.

Guenther, F.; Burckhart, O.; and Oostinga, I. 1969. Zur Unterscheidung von Fleisch und TVA Erzeugnissen. Die Fleischwirtschaft. 4:474.
Hofmann, K., and Penny, I. F. 1971. Identifizierung von Soja und Fleischeiweiss Mittels Dodecylsulfat-Polyacrylamidgel-Elektrophorese. Die Fleischwirtschaft. 4:577.
Kruger, H., and Grossklauss. D. 1971. Untersuchungen zum serologischen Nachweis von Sojaprotein in Erhitzten Fleischerzeugnissen. Die Fleischwirtschaft. 3:315.
Linke, H. 1969. Histologischer Nachweis von TVP. Die Fleischwirtschaft. 4:469.
Olsman, W. J. Electrophoretic Detection of Non-Meat Proteins in Meat Products. Thirteenth European Meeting of Meat Research Workers, 1967.
Olsman, W.J. 1969. Electrophoretischer Nachweis von Fleischfremden Eiweissen in Fleischerzeugnissen. Zeitschrift fur Lebensmittel-Untersuchung und Forschung. 141:253.
Penny, I. F., and Hofmann, K. The Detetion of Soya Bean Protein in Meat Products. Seventeenth European Meeting of Meat Research Workers. 1971.
Spell, E. 1972. Nachweis von Milcheiweitz und Sojaweitz in Fleischerzeugnissen mit Hilfe der vertikalen Flachdisk–Electrophoreze. Die Fleischwirtschaft. 11:1451.
Talbot, D. N., and Yphantis, D. A. 1971 Fluorescent Monitoring of SDS Gel Electrophoresis. Anal. Biochem. 44:246.
Van Baal, J. P. W., and Leget, H. N. 1965. Nachweiss von Casein und Natriumcaseinat in Fleischprodukten. Zeitschrift fur Lebensmittel-Untersuchung und Forschung. 127:263.
Van Gils, W. F., and Hidskes, G. G. 1973. Detection of Casein, Soy Protein and Coagulated Egg White in Meat Products by Electrophoresis on Cellulose Acetate Membranes. Zeitschrift fur Lebensmittel Untersuchung und Forschung. 3:175.
Williams, J. G., and Gratzer, W. 1971. Limitations of the Detergents–Polyacrylamide Gel Electrophoresis Method for Molecular Weight Determination of Proteins. J. Chromatogr. 57:121.
Wittmann, J.; Kotter, L.; and Schmidt, H. 1968. Nachweis von Fremdeiweiss in Fleischerzeignissen Mittels mit serologischen Methoden. Archiv fur Lebensmittel-Hygiene. 12:272.
Wyler, O. 1971. Immunologischer Nachweis von Milchweiss und Sojaeiweiss. Die Fleischwirtschaft. 12:1743.

Soy Protein Development in Japan (Especially for Isolated Soy Protein)

SABURO KIKUCHI, Fuji Oil Company Limited, Higashi-Ku, Osaka, Japan

DEVELOPMENT OF SOY PROTEIN IN JAPAN YESTERDAY AND TODAY

In the beginning, apart from the foodstuffs, soy powder was used industrially as a material for dry paints and as an adhesive agent in the U.S. in the 1930's. After World War II, soy protein was regarded for its nutritious value.

Before the war the Japanese studied the soy protein in Manchuria. Among the studies they sampled textured soy protein, but World War II created a void in the development of soy protein for the Japanese.

Originally, the application of soy protein began with the introduction of the battery extraction system made in Germany at the end of the Taisho era. Before that, soybean oil had been obtained with the press machine only; and the cake, as residue, was used for fertilizer.

In the 1920's, with the introduction of the battery extraction system, the residue changed cake to meal. However, these meals also were used for fertilizer and were not usable for human food or meal for animals.

At the end of World War II, we Japanese were surprised to find that the country producing the most soybeans was not Manchuria, but the U.S.

The critical food situation after the war created a big demand for American soybeans and we Japanese imported them as relief material at first and concentrated our efforts upon their development.

Soybeans were used in making feedstuffs first. In our ancient history we had the custom of consuming soybeans directly. In Japan, miso, shoyu, natto, tofu, and uba are some of the soybean foods ued for direct consumption.

In May 1964, the International Symposium on Oil Seed Protein Food was held at Lake Yamanaka near Mt. Fuji, and Japanese soy protein products were impressive. To be frank, I was surprised. We came to regard our traditional soy protein products highly. Among the processed soybean products, we began to use soy meal in tofu as human food after the war. This development gave impetus to the further development of soy protein products in Japan. Before this discovery, the tofu was made of the soybean itself. But after this discovery, we use soy meal for tofu. Nowadays, much soy meal is used in shoyu also.

It is hard to grasp the whole picture of soy protein products in Japan now. This is because the development is at a turning point and each manufacturer develops his products confidentially. The potential use of soy products is certainly great. Moreover, in addition to soybean crushers, trading companies, marine companies, pharmaceutical companies, chemical companies, and confectionary companies are paying attention to this field and are beginning to study it.

DEVELOPMENT OF ISOLATED SOY PROTEIN IN JAPAN

The products made from soy protein products are as follows: soy powder and soy grits (fatted and defatted), soy meal (defatted), concentrated protein, textured protein, isolated protein, and spun protein.

However, I am going to talk about the isolated protein. The study of isolated soy protein in Japan has a long history, but it was only ca. 10 years ago that the product entered the market industrially. The first maker to market the isolated soy protein was Nikka Fats & Oils. At that time, I was associated with Nikka, and we were working toward market development.

The product in those days was inferior in quality, color, and odor. There was no education of the customers to accept the new product at all, and we often heard from the customers: "We have no interest in this product for human consumption. It is like the smell of horse's dung." We were discouraged.

The Central Soya Co. was aggressive in trying to sell isolated soy protein even in those days and was trying to penetrate the Japanese market. In 1965, Central Soya, Nikka Fats & Oils, and another pharmaceutical company concentrated their efforts to establish a joint venture to produce an isolated soy protein in Japan. However, this ended in vain.

In December 1967, Fuji Oil Company started production of isolated soy protein, though the production quantity was small. The present Fuji sales are several times larger than they were in those days. This product has viscoelasticity as one of the functional properties, as required by the Japanese meat industries, as well as improved color and flavor. At the present time, Ashai Oil Company also has produced isolated soy protein.

The growth of Fuji Oil in this field has been remarkable, and it is not too much to say that the history of the isolated soy protein in Japan is its history in Fuji Oil. However, most of its application is limited to the meat industry. Fortunately, I have been associated with Fuji Oil