# THE DEVELOPMENT OF HIGH PROTEIN NOODLE

# FROM SOY BEAN FOR THAILAND

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SUMMARY

A high protein soya noodle made from soy flour has been developed for Thailand, where there was a report of protein malnutrition. Soy bean was considered as the cheap source of protein in terms of quantity and quality and availability in the country.

The product was first made of 75% full fat soy flour, 25% wheat flour, with vitamins and minerals supplemented; and 5.5% dried egg yolk added to complete the standard identification of egg noodle. The texture of the product was not satisfactory when a consumer test was carried out among 50 Thai students.

The texture of the product was improved by changing the recipe and improving the method of processing. The new recipe was 55% full fat soy flour, 10% low fat soy flour, 25% wheat flour and 10% rice flour, with an addition of 22.5 g. fresh whole egg per 100 g. of dry mix.

The proximate chemical composition including vitamins and minerals and the amino acid composition of the noodle were analysed. A daily dietary standard for a reference Thai man was proposed for the calculation of vitamin and mineral needed to be supplemented to the noodle. Thiamine and niacin were added to the noodle in the amount that 200 g. of dry soya noodle would provide half of the daily requirement of these two vitamins. Calcium was added in the form of calcium carbonate so that the ratio of calcium to phosphorus in the noodle was 1:1.

The amino acid pattern of soya noodle was compared with the FAO 1957 pattern and it was decided to add DL methionine to balance the amino acid pattern in soya noodle.

The destruction of trypsin inhibitor in soya noodle was investigated and it was found that steaming the noodle at atmospheric pressure for 20 mins. adequately destroyed the trypsin inhibitor and the remainder was completely destroyed after cooking the final noodle.

The method of processing was investigated and a method of obtaining a uniform destribution of ingredients and a better texture of the product by controlling mixing was found. Better colour, texture and rehydration property of noodle were obtained by varying the drying condition, to give a three period drying cycle.

The noodle was packed in saran coated paper bags, with the predicted storage life of about 126-140 days. An accelerated storage test was carried out, and it was found thatat the equivalent storage condition of Thailand, the product became unacceptable due to browning in 96 days and the mould occurred in 150 days.

In the assessment of the protein quality of soya noodle, the determination of available amino acids and a chicken feeding trial were used. The availability of amino acid in dry soya noodle was very low and the weight gain of young chickens fed dry soya noodle after four weeks was about 35-50% of the weight gain of chickens fed reference protein (meat meal and butter milk powder). It would have been better if the experiment was carried out with cooked soya noodle as it was thought that the trypsin inhibitor interfered with the intake.

A preliminary cost of production was also estimated. With the batch operation of 2,000 packages of soya noodle per day, the production cost per package was 1.15 bahts. The return on investment after tax was 46%. A consumer test should be done in Thailand, before the product is going to be marketed.

# I. INTRODUCTION

## The World Problem of Protein Malnutrition

Protein malnutrition is the major nutritional problem in the world. Protein-calorie deficiency occurs at all ages but the effects of it are greater in infants and young children. It is often called "Kwashiorkor" in which protein deficiency predominates with the absence of serious calorie deficiency, whereas "marasmus" is referred to as severe malnutrition caused by both calorie and protein deficiency (Altschul, 1965). Childhood malnutrition causes both retardation of physical growth and mental development.

F.A.O. (1965) reported that apart from the effect on growth, mild or moderate protein deficiency renders infants and young children particularly susceptible to respiratory and gastro-intestinal infections. The incidence of such disease is much higher in malnourished than in well-nourished children; mortality in the age groups 1 to 4 years is 20 to 50 times higher in the developing than in the developed countries and it is probable that the difference is due in large part to malnutrition. Besides this, among people with low incomes in developing countries there is a high prevalence of weanling diarrhoea, because of the combined effect of poor hygiene and protein deficiency.

Low protein intakes, often accompanied by low calorie intakes, occuring in adults in developing countries show only a marginal deficiency, which is often seasonal or transient and does not cause obvious ill health unless it is complicated by acute or chronic disease. For pregnant women, the increase in body-weight during pregnancy observed in the women of low income groups in developing countries is often markedly less than that observed in well-nourished countries. Complications of pregnancy such as miscarriage, stillbirth or premature birth, seem to be more frequent in these situations. In the lactation period after pregnancy, although total protein content and amino acid composition of milk from malnourished mothers have not been found to be significantly different from those of milk from well-nourished mothers, there is evidence that in developing countries, the body-weights of women of low income groups may decrease progressively during the time they are feeding the baby.

The problem of protein deficiency arises from the uneven distribution of food supply among countries, within countries among families with different levels of income, and also within families, as a result of social, economic and cultural factors that limit the consumption of protein by vulnerable groups of thepopulation, even when the total supplies are adequate for the population as a whole.

A review of protein supplies for consumption in different regions of the world (Appendix I) shows the marked difference in the per capita protein supplied between the well developed and less developed regions. The amounts of protein supplied as vegetable protein are the same but, in contrast, protein supplied from animal sources is very much lower in the less developed regions where higher incidences of protein malnutrition are reported.

The three main areas in which the protein-calorie deficiencies occur are Latin America, Africa and Asia. Most of the population in Latin America suffers from protein deficiency, and it is particularly prevalent and serious in children during weaning and post-weaning period. Foods which are adequate dietary sources of protein in quantity and quality are low in availability and the price is high. Large amounts of cereals are consumed especially corn, therefore, the diets are low in lysine, methionine and tryptophane content. (Bressani, 1966).

As Sai (1966) reported, although actual production figures in terms of available protein may be high in Africa, regional distribution may be so poor that some areas may be short. Much of the protein comes from cereals, especially maize, millets and sorghum, and in the wetter areas, from root crops and starchy fruits. Because of the poor storage, processing and marketing about 10 to 30 percent of the total production is wasted. Even where enough protein enters the family menu, the distribution may be such that the children may not have their fair share. Firstly, it is not generally realised that for his size a child needs more of everything than adults. Secondly, the order of meals requires the menfolk to eat first, and by the time it is the turn for the children and women to eat, there is very little left. This results in a high mortality rate in the children aged between 6 months through to 4 years. With a death rate of 20 to 50 percent of children with protein malnutrition there will be 5,000 deaths per million population per annum and protein malnutrition will account for 15 to 20 percent of all deaths under 5 years old.

Ahmad (1966) reported that in Asia, a study of the distribution of calorie supply in relation to the requirement for calories of the population has shown that at least one-fifth and possibly more of the people in this region go hungry, and protein-calorie under-nutrition is the most important problem impeding health and economic development. From data of food consumption (Appendix II), 60-80 percent of calories are supplied by cereals. Both total protein and animal protein consumption are low in comparison to the United States and United Kingdom. When protein is poor in both quantity and quality, the children who require most protein are likely to suffer, and in Asia, children up to the age of 14 constitute 38.2 percent of the population. Mortality rate of the children aged 1 to 4 is high in Asia, 11 per 1000 in Thailand, 33 in South India, 88 in East Pakistan as compared with 1 . 1 in U.S.A.

According to the 1963 F.A.O. Third World Food Survey, it was estimated that at least 20 percent of the population of the less developed areas was undernourished and that some 60 percent received diets inadequate in nutritional quality. For the world as a whole, it was concluded that 10 to 15 percent of the people were undernourished and nearly half suffered from hunger and/or malnutrition.

If the population grows according to the United Nations medium projection, the world's total food supply would have to be trebled by the year 2000 in order to provide a reasonably adequate level of nutrition. For the less developed areas, total food supplies would have to be quadrupled and the supplies of animal products would have to be raised to nine times the present volume (F.A.O. 1967b).

But since the time of the Third World Food Survey, increase in food production has failed to keep pace with population growth. In Asia, Africa and Latin America, the population is increasing at a rate of from 2.5 to more than 3 percent per annum. The average rate of increase in food production from 1958 to 1965 was 2 percent per annum, with a reduction in the last four years of this period to about 1 percent. An increase of at least 3 percent per annum in food production will be needed to keep up with population expansion (U.S.President's Science Report, 1967).

While animal protein resources are rising in the developed countries, in the developing countries the total per capita protein supply has declined by about 6 percent since World War II, with increased dependence on protein from grain (Abbott, 1966). Therefore the other protein resources, apart from animal protein, must be developed to meet the nutritional needs of the population throughout the world.

## The Situation of Protein Deficiency and Protein Supply in Thailand

Background. Thailand, a tropical country in South East Asia, had in 1967 a population of 32,452,000 of which 16,257,000 were males and 16,195,000 females; and over 40 percent of the total population were children under 14 years of age (Bhumiratana and Nondasuta, 1968).

Thailand is geographically divided into four parts, Northern, Central, North Eastern and Southern, and has a total area of 514,000 sq.kms.

Much of the North and East, which includes about three-fifths of the total area and more than half of the population, cannot be used for agriculture because of the mountainous character of the terrain. In the East, the additional problem of scanty rainfall limits the arable areas to small valleys that can be irrigated. Most of the agricultural area is in the delta region or central part of Thailand. Rice is the most important crop, with 78 per cent of total harvested area devoted to paddy rice. The Southern part of the country, where there is plenty of rainfall, is the main source of fish supply. Because of the agricultural difficulties, people in the North and North East regions are more likely to suffer from malnutrition than the other regions.

Kwashiorkor in Thailand. In 1955, Netrasiri and Netrasiri, as reported by Thanangkul et al (1966), said that there were fifty-four cases of kwashiorkor in Bangkok over a four year period. In one half of these cases, the disease occurred in one to two year old children.

According to Valyasevi (1964), kwashiorkor was reported in Bangkok, and also from hospitals in Northern, North Eastern, and other regions of Thailand. It has been estimated that for every case of reported kwashiorkor, there will be 100 cases of protein malnutrition, so the problem is much greater than these figures suggest.

In a recent report of Thanangkul et al (1966), there were one hundred and eleven patients with protein-calorie malnutrition admitted to Chienmai Hospital (Northern region of Thailand) from January 1 to December 31, 1964. Among this number, forty-nine children suffered from third degree protein-calorie malnutrition with edema (kwashiorkor), fifty-one with third degree protein-calorie malnutrition without edema but with severe dehydration (marasmus) and only nine with second degree protein-calorie malnutrition (weight about 60-75 percent of normal weight). Those children who had third degree malnutrition with edema (kwashiorkor) were chronically and seriously ill. They showed weakness with inability to sit up, weak cry, irritability and lack of interest in surroundings and food. Muscle wasting and growth retardation were marked. Those who suffered without edema were extremely thin, weak, showed lack of appetite, were dehydrated and sometimes apathetic or irritable. There was marked muscle wasting and growth retardation. Twenty-seven deaths have been reported. Of the one hundred and eleven patients, 88 percent were between the ages of six months and four years, with the greatest number of the children between the ages of one and three years. The lack of transportation facilities and the inaccessibility of the outlying villages make it difficult to bring children to the hospital, and therefore it is likely that more children than reported by the hospital suffer from protein deficiency diseases.

Protein consumption in the past and present. There is a rather limited amount of information as there were only a few dietary surveys in Thailand between 1953 and 1957, and in 1963 as in Appendices III and IV, and one in 1960 (ICNND, 1960). The diet consists mostly of rice and glutaneous rice with some kind of vegetable and very little animal food, as summarised in Table I.

Table I. Summary of Thais Dietary Intake.

| Area | Percentage of Total Intake | | |
|---|---|---|---|
| | Animal Food | Rice and Starchy Food | Other Food |
| Bangkok | 27.2 | 48.2 | 24.6 |
| (Chandapanon, 1955) | | | |
| Uborn | 12.6 | 72.0 | 16.4 |
| Udorn | 9.2 | 80.0 | 10.8 |
| Chiengmai | 5.4 | 69.0 | 25.6 |
| Chiengrai | 4.8 | 72.0 | 23.2 |
| (Bisolyyaputra, 1957) | | | |
| Chonburi | 11.2 | 65.7 | 23.1 |
| (Bisolyyaputra, 1953) | | | |

| Area | Net Food Supply per Capita | | |
|---|---|---|---|
| | Total Calorie k.cal | Total Protein g. | Animal Protein g. |
| Bangkok | 1409 | 47.0 | 23.9 |
| (Chandapanon, 1955) | | | |
| Uborn | 2099 | 49.3 | 10.2 |
| Udorn | 1826 | 42.0 | 8.6 |
| Chiengmai | 2232 | 52.7 | 9.3 |
| Chiengrai | 2207 | 47.0 | 7.2 |
| (Bisolyyaputra, 1957) | | | |
| Chonburi | 1746 | 48.0 | 15.0 |
| (Bisolyyaputra, 1953) | | | |

The calorie intake of Bangkok and Chonburi is rather low in comparison with other parts of the country; this is due to lower consumption of rice and starchy food. Animal protein intake is high which is due to higher consumption of meat and meat products in Bangkok and fish and fish products in Chonburi.

In chiengmai and Chiengrai, almost all of the rice is glutaneous rice, which is soaked overnight in water that is discarded before steaming the next day. Large quantities of this nutritionally depleted rice are eaten with small portions of fried pork, beef, chicken and fish, primarily to give flavour to the rice. Vegetables and pulses are the main source of protein in the diet. Because of the low intake of animal protein in the diet and the traditional way of cooking, people in this area often suffer from protein malnutrition, beri beri and goitre (ICNND, 1960).

The main sources of animal protein of the North East are fresh water fish, frogs, and fermented fish products. Fruit and vegetables are rarely eaten. People in this area suffer from vitamin and mineral deficiencies as well as protein malnutrition. A high incidence of bladder stone disease is reported in this region. (Valyasevi et al, 1967).

The food consumption pattern has changed since 1957. In the North East, take Uborn as an example, the total calories decreased from 2099 (Bisolyyaputra, 1957) to 1743-1877 (Valyasevi, et al 1967). There was an increase of protein consumption, especially animal protein. The total protein intake in 1963 was about 10 percent higher than 1957 and animal protein intake was doubled.

Although there was an increase in animal protein consumption, the average daily protein intake of the North East population was

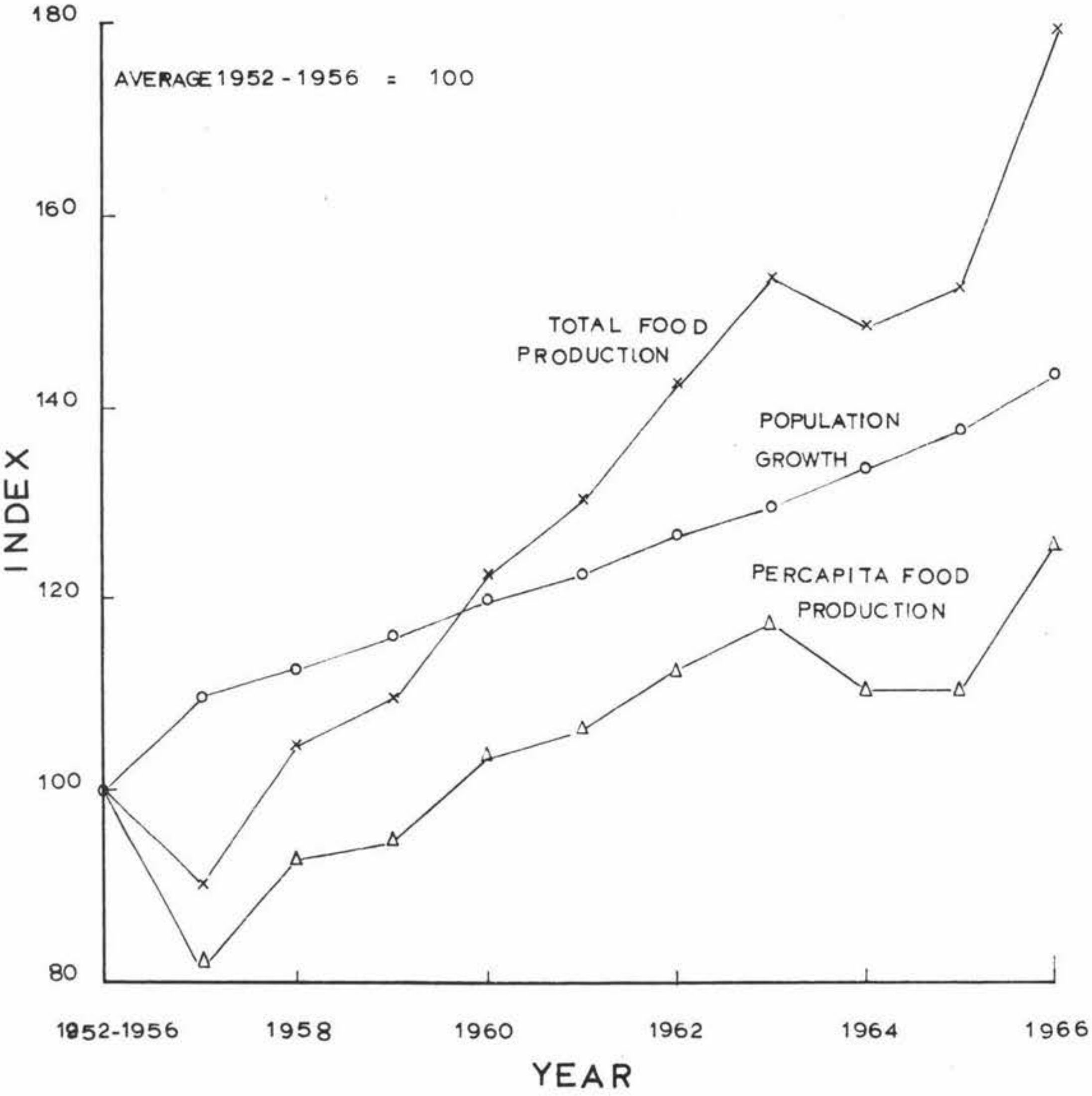



Figure 1 Trends in Food Production and Populatior in Thailand

(FAO, 1967)

still low. According to Bhumiratana and Nondasuta (1968), the protein intake from the result of the 1966 dietary surveys in the North East figures were 1.1 ± 0.2 g/kg body weight/day. If we assume that the low income families had daily protein intakes of less than one standard deviation below the mean, then they were living on 0.9 g/kg body weight, or less, per day. The protein intake per kg body weight of a pre-school child is considered to be the same as or less than that of the adult because the eating pattern of rural Thais allows no extra share of protein in favour of the children. The protein requirements of children and adults are between 0.7-0.9 g/kg body weight per day for the reference protein (NPU = 100) (F.A.O. 1965). Because of the low quality of protein intake, the daily protein requirement should be double. This i.e. 1.4-1.8 g/kg body weight per day, and therefore there is a lack of protein in the North Eastern diet.

To increase the protein intake of the population, both quantitatively and qualitatively, major high quality protein sources should be developed.

<u>Population growth and agricultural production</u>. The population in Thailand is increasing at the same rate as the average for Asian countries, 3 percent per annum. The rate of food production is increasing at a rate higher than the population growth - 4.8 per cent per annum (United Nations, 1964).

Although the country seems to have been able to keep total food production well ahead of population growth (Figure 1), the increasing of protein food supplies may not be able to keep pace with the increasing population. Therefore the problem of protein malnutrition in Thailand may arise from this difficulty in increasing total protein food production, together with the uneven distribution of protein food supplies in the country. The sources of protein food supplies should be investigated.

1. Cereal protein:

Rice and corn are the two important cereal products in Thailand. The total consumption of rice in 1963 was 3.4 million tons and projected to 1985 is 6.6 million tons. Total production in 1963 was 9 million tons and in 1967 was 11 million tons (F.A.O. 1967a). There will be plenty of rice to feed the increasing population even if the amount of consumption were doubled to increase the protein level in the diet. At the present time, there is a large amount exported. Corn production has shown an important expansion, from 68 thousand metric tons in 1952-1956 to 665 thousand metric tons in 1962, and nearly double the production of 1962 in 1966. Most of the production is exported as animal feeding stuff (F.A.O. 1967a).

2. Legume protein:

Peanuts, mungbeans and soybeans are the main sources of legume protein produced in Thailand. From the F.A.O. production year book of 1967, the production of peanuts increased from 88 thousand metric tons in 1956 to 130 thousand metric tons in 1966. Although mungbeans are not included in the government project to expand agricultural production as are corn and soybeans, the production of mungbeans has been increasing rapidly. The production in 1966 was about 130 thousand tons which was four times the production of 1953 (Bhumiratana and Nondasuta, 1968). Soybeans are used for numerous food preparations, and in particular the demand for soybean oil is now increasing. The number of vegetable oil extraction mills being established has resulted in a big requirement for soybeans. Between 1950 and 1963 acreage rose and production expanded. However, production fell off from a 1963 high of 33,000 metric tons to 19,200 metric tons in 1965 (F.A.O. 1967a). There appears to be some doubt as to the accuracy of these statistics, as according to the agricultural statistics of the Thai Ministry of Agriculture, the production of soybean in 1966 was 37,900 tons (Bhumiratana and Nondasuta, 1968) and the government intends to produce 50 thousand tons in 1971 (Polgraigriongrild, 1968).

3. Animal protein:

Only a small percentage of animal protein is consumed in the country. Cattle, swine and buffalo production have remained stable since 1962 and there was also a reduction in the number of animals exported. There was an increase in poultry production from 1950 to 1965, but since then the production remained stable (F.A.O. 1967a).

4. Fish:

In the past ten years, the total catch has risen about 10 times, from 196.3 thousand tons in 1958 to 847.2 thousand tons in 1967 and 1089 thousand tons in 1968. Fifty percent of the total catch is marketed as fresh fish and 48 percent are used for miscellaneous purposes. From 1966 to 1968 the amount of exported and imported fish remained stable, and therefore there is an increase of fish consumption in the country (F.A.O. Fishery Statistic, 1968).

Because of the shortage of animals, the only possible sources of protein for development of protein rich foods are vegetables and fish.

Although the amount of fish is increasing every year, fish is caught mainly in the Southern part of Thailand. Even if a factory is set up there, because of the cold storage and transportation costs, the price of the product will be too high for the low income group of people in the North and North East. Therefore, fish was excluded in considering a source of protein for the development of high protein products.

In cereals, although a new high protein content variety of rice and corn has been developed, the protein content is still low when comparing it with other vegetable protein sources such as legume protein. It is unlikely that the proportion contributed by cereal to the population's protein supply can be increased, as there is a limit to the amount of such relatively bulky food that a person can consume, especially children.

Only legume protein remains as a possible source. In comparing peanuts, mungbeans and soybeans, the production of peanuts and mungbeans is about four times higher than the production of soybeans. Prices per ton of peanuts, mungbeans and soybeans are 5,500, 2,500 and 2,500 bahts respectively. (Bhumiratana and Nondasuta, 1968). The protein content of peanuts and soybeans is 27 and 42 percent respectively (Milner, 1966) and the protein content of mungbeans is 21.1 percent (Bhumiratana and Nondasuta, 1968). Although the production figure for soybeans is lower than that of peanuts and mungbeans, soybeans are the cheapest souce of protein in terms of cost per protein unit.

On comparing the amino acid pattern in the proteins (g.amino acid per 16 g.N) of peanuts, mungbeans and soybeans as in Appendix V, the amino acid pattern of soybeans is better than that of peanuts in having a higher tryptophan and lysine content, but is slightly inferior to that of mungbeans in having a slightly lower proportion of all the essential amino acid content. However the protein content of soybeans is about double that of mungbeans, so the percentage of individual amino acids in soybeans will be nearly double that of mungbeans.

Soybeans have been used more extensively in food production than peanuts and mungbeans. The drawback of soybeans is that their use is limited by the bitter flavour of the mature bean, which is not easily removed. By careful searching for the proper method of processing, soybeans could be used as an excellent protein source in the future.

Moreover, the area of soybean production is found mainly in Sukhothai which is situated in the central plain and the Northern part of Thailand and in Cheingmai which is in the North. If a factory is set up there where the raw material is most abundant, it will be easy to distribute the product to other Northern areas, and Central and North East areas. The additional cost on the product due to transport will be low.

For the reasons of the high protein and amino acid content, low price, extensive uses and suitable production location soybeans were chosen as a protein source for development of high protein food.

## II. REVIEW OF POSSIBLE PRODUCT FROM SOYBEANS

### Soybean Protein Products in General

In the past few years, the use of soybean products in human food has been the subject of greatly renewed interest. The number and kinds of products continue to grow. Some are quite new and some are the result of improving traditional products. Some of the more important soybean protein products are, soymilks, fermented soy products, soyflour, soy protein concentrate and isolates, spun and textured protein and blended food products.

Soymilk. Soymilk has been used for centuries in the Orient for infant feeding and other purposes. Oriental soymilk is an emulsion of finely ground soybeans in water. The product is not stable and needs to be prepared fresh for sale. The process has been refined, Hand et al (1964a) studied the pilot plant production of soymilk, then produced a spray dried, homogenised, dehulled, whole soybean slurry. Heat sterilised, bottled, soymilk and soymilk concentrate has been developed (Lo et al, 1968a, 1968b). Mustakas et al (1964), in co-operation with the Wenger Machinery Company, developed another process in which, dehulled soybean flake, properly conditioned with moisture, was fed into an extruder and forced through an orifice under conditions of short time, high temperature, and high pressure, from which the bean mixture emerged cooled, puffed and dried. The puffed material was then finely ground and could be slurried with water to form soymilk.

As reviewed by Martin (1970), K.S. Lo of Hong Kong has been bottling soymilk as a nutritional, low-cost, soft drink and selling many millions of bottles yearly. The World Health Organisation built a $1 million soymilk plant in Indonesia in 1959, in which a spray dried soymilk powder was produced and sold in a tin as baby food.

In the Western World, soymilk is used for infants suffering malnutrition, or who are allergic to cows' milk; and also it is used by adults who, for other reasons, prefer a vegetarian milk.

Fermented soy product. In Asian countries, through many centuries, methods have been found to make soybeans alone more palatable. In almost every case soybeans are modified by fermentation, using moulds, yeast, or a mixture of micro - organisms (De and Russell, 1966).

One of the most important fermented foods made from soybeans is soysauce. Soysauce is the hydrolysate product of mould, yeast and bacterial action on soybeans and wheat flour, or sometimes rice flour instead of wheat flour. Soysauce is used in a small quantity for seasoning. It is not nutritionally significant because of the low protein content and high quantity of salt in the product. The protein and salt content of soysauce are 9.4 and 18 percent respectively (Yokatsuka, 1960).

Apart from soysauce, Miso is a very important product in Japan. Miso is prepared from crushed soybean and rice fermented with Aspergillus oryzae. Since the salt content is high, miso is used only for seasoning. Research nowadays is trying to raise the protein content and lower the salt level of miso for its better utilisation as a protein source in Japan and other Asian countries (Ito et al, 1965).

Tempeh, an Indonesian fermented soybean product, is prepared, according to Djien and Hesseltine (1961), by fermenting cooked and dehulled soybeans with tempeh mould, (Rhizopus oligosporous). The soybean and mould mixture is wrapped in banana leaves and kept for 24 hours. The final product is a solid mass in which the mycelium of the tempeh mould has grown over and through the soybean. The mass of soybean and the mould myceloum is thinly sliced. The thin slices are dipped in salt water, deep fat fried and consumed promptly.

The process for making tempeh has been modernised by the U.S.D.A's Agriculture Research Service in various ways, such as the fermentation of soybeans is carried out in plastic bags (Djien and Hesseltine, 1961), or in shallow wooden and metal trays (Martinelli and Hesseltine, 1964). Steinkraus et al (1965) have scaled up a pilot plant for small factory production of dehydrated tempeh. The equipment described can yield 54.5 kg. of fresh tempeh per 8 man hours. A cereal soybean tempeh type product also has been developed (Hesseltine, 1966). The product can be baked, deep fried as chips, and used as an ingredient in soup and also manufactured as a snack food.

Another important fermented soy product in Japan is natto, which is made by fermenting whole soybeans through the action of Bacillus subtilis (Ackoyd and Daughty, 1964).

There is no doubt that the microbiological processes considered improve the ease of digestibility of soybean (Van Veen and Schaefer, 1950) and the biological value (Gyorgy, 1961).

Soyflour. There are three types of soyflour - full fat soyflour, low fat soyflour and defatted soyflour. Full fat soyflour contains all the natural oil of the soybeans, roughly 20%. The flour has a protein content of approximately 40%. Full fat soyflour was prepared in the traditional way by screening, grading, cracking, dehulling the beans and then subjecting them to a process called debittering or improving flavour, then grinding to the desired particle size (Markley, 1951). Alternative processes for making full fat soyflour, either by simple hand procedures or by mechanical extrusion cooking, are also being developed by the Northern Laboratory U.S.D.A. (Mustakas, 1966, 1967).

Low fat soyflour contains up to 6% fat and about 40 to 50% protein. Defatted soyflour contains less than 1% fat and typically 51% to 52% protein. Both low fat and defatted soyflour are prepared the same way as full fat soyflour except that most of the oil has been removed by expelling or solvent extraction.

The present largest usage of soyflour is in baked goods. Baking takes advantage of certain chemical and physical properties it possesses to improve dough handling characteristics, in producing a white bread, and especially to lengthen shelf-life.

The second largest usage of soyflour is in the processed meat industry. Soybean flour is used as a binder and emulsifier in sausages, meat loaves, and related products. The large amount of protein in soybean flour is one of the chief attributes, since in addition to its functions as an emulsion stabiliser and excellent water absorption agent, its presence also contributes to the protein content of the meat product.

Besides these uses, soyflour is used in baby food, and as a high protein cereal product (Martin, 1970)

Soy protein concentrate. Soy protein concentrate is the product prepared from defatted soyflake by removing most of the oil and water soluble non-protein constituents. Minimum protein content is 70% on a moisture free basis (Diser, 1968).

In the preparation of soy protein concentrate, the protein is precipitated by various means and the precipitated protein is then washed or desolventised and dried. There are three different methods by which protein concentrate is made commercially. The difference between these methods is principally in the means of precipitating the protein component during separation of low molecular weight carbohydrates, mineral matter and other minor constituents. One process takes advantage of the fact that the protein components are insoluble in aqueous alcohol of about 60 to 80 percent concentration. Another method is based on the knowledge that the major soy globulins have limited solubility in aqueous acid at their isoelectric point. In the third process the protein is denatured by moist heat treatment to insolubilised protein. The commercial soy protein concentrates derived from these three processes have the same gross composition characteristic except that the concentrate prepared by the aqueous alcohol and the water extraction processes have lower nitrogen solubility indices than that of acid extraction method, which was due to protein denaturation.

Soy protein concentrates were introduced in 1960 to the United States and are used in meat products for increasing product yields, in baked goods, infant foods as well as dietary foods (Martin, 1970).

Soy protein isolate. Isolated soy protein is the major proteinaceious fraction of soybean, prepared from defatted soybean flake or soyflour. Protein content is not less than 90 percent on a moisture free basis.

The protein is extracted with an aqueous solution of neutral pH or alkaline pH. Then the pH of the clarified extract is adjusted to 4.5 with acid to precipitate the major globulin protein. The protein curd is washed, dewatered, neutralised and dried (Meyer, 1966).

Soy isolate became available for food use in the United States in 1957-1958. Like soy protein concentrate it has found a place in sausage and canned meats as a binding agent. A new food field in which the isolate is finding increased use is the so-called dairy type products such as coffee whiteners, whipped toppings, and frozen desserts, as a whipping agent, emulsifier and stabiliser (Martin, 1970).

<u>The spun protein</u>. Spun proteins are the source of the remarkable new simulated natural products that resemble such items as chicken, turkey, ham, frankfurters, and pre-fried crumbled bacon.

Basically, defatted flake or flour is extracted and purified to an isolate. This pure protein is dispersed in alkaline and then precipitated at the isoelectric point in a bath by drawing it away continuously from the face of a spinneret to form tiny monofilaments. These fibrils are combined with such other ingredients as wheat gluten, egg albumin, vegetable oil or animal fats, flavours, and colours, and are formed into simulated meat items (Martin, 1970).

<u>Textured protein</u>. Also, as reviewed by Martin (1970), textured vegetable soy proteins are made by a special extrusion type processing of soyflour to give an expanded product in which, when

treated with hot water or steam, further expansion is undergone to produce a material with the appearance and coherence of meat. It can also be used to simulate other products including vegetarian meats, coconut chips and fruit bits. Lacking the uniform fibrosity of meat they come in "chunks" or "bits" of various size. Flavour, texture and shape can be produced as desired. Supplementary nutrients can be added to overcome dietary deficiencies. Textured protein can be used by food makers to enrich existing foods without changing their colour or taste.

The textured proteins are less costly to produce than the spun fibres, which are based on the isolated protein and therefore the textured protein is much more competitive with meats in price.

Blended food products. There are many reports of using soybean protein as a supplement to a poor quality diet. Bressani and Elias (1966) developed a soybean-corn vegetable combination designed as INCAP Mixture 14. It was found that corn and toasted soybean protein complement each other when 20-40% of the protein in the diet is derived from corn and 60-80 percent from soybean.

Rao et al (1965) produced a vegetable protein mixture based on peanuts and soyflour. Growth studies on rats and feeding trials on school children showed that the protein mixture had a significant supplementary value to poor cereal-based diets.

Pre-cooked dehydrated soybean plus corn and rice was also developed by Hand et al (1964b).

In 1966, U.S.D.A. developed CSM (formula No.2 or corn-soy-milk mix) as a high protein food as part of the Food for Freedom Programme. CSM is made from processed corn meal, toasted soyflour and non-fat dry milk together with vitamin-mineral mixture. It has a minimum protein content of 20%. WSB (Formula No.3 or wheat-soy blend) is a new wheat based food supplement developed by U.S.D.A. It is supplemented with the same vitamin and mineral as CSM and has the same protein and fat content (Martin, 1970).

Soybean Protein Products in Thailand

From the data recorded by the Ministry of Industry in Bangkok, Thailand, there are about 56 small factories which produce soybean curd, fermented whole soybeans and soybean paste. Eighty percent of these factories are in Bangkok. There is no statistic reported for the factories which produce soysauce. There are two soymilk plants, one in Bangkok and the other in the Southern part of Thailand. The one in Bangkok produces a water extracted soymilk and it is sold in bottles as "Vitamilk". The other in the South uses soyflour instead of water extracted whole soy beans, to make a slurry of soymilk.

Soybean products that are marketed are, soy curds, soy sprout, fermented products, soysauce and soymilk produced by the vendor.

The Institute of Food Research and Product Development, Kasetsart University, as reported by Bhumiratana and Nondasuta, 1969, has produced a few products, utilising soybean as a main source of protein for a high protein-rich food development programme. The products that have been developed are soymilk and Kaset Noodle. Two types of soymilk have been produced by the Institute, heat sterilised bottled soymilk with a protein content of 2.7 percent and canned concentrated soymilk, protein content 6.76 percent. In the production of Kaset Noodle, soybean residue, the product remaining after separation of soymilk, was used with wheat flour. The protein content of Kaset Noodle is 14.7 percent and to produce 8.5 kg noodle, 1.5 kg of soybean residue (40% protein content) was mixed with 8.5 kg wheat flour. According to their report, another product from soybean - soy cookies is still under development.

Martin (1970) also reported an introduction of textured soy protein foods by Archer Daniels Midland Company to Thailand as a new food programme for International Development.

Evaluation of Possible Soy Protein Products to be Developed

In 1968, a possible high protein product from soybean had been searched for in the development of a high protein food for Thailand, in the Product Development Year Work Project, at the Department of Food Technology, Massey University (Thantapongee, 1968). The criteria that were used to consider suitable products for development were:

1. the product should have high protein content and could be offered to the consumer at a low price,
2. the product could be consumed in large quantities and very often,
3. the product should be easy to distribute, stable, and should also be a nation-wide product.

The products considered were:

soya noodle
soy bread
breakfastfood - dried flakes
soy biscuit
simulated meat
soy cheese
instant pudding
soymilk
soy beverage or protein drink
sweets from soyflour.

The products were rated under the market possibility, the manufacturing and technical possiblity (Appendix VII). From these aspects, the possible products that were most suitable for development were:-

soya noodle
soy biscuit
soy milk
soy cheese.

Then the four possible products were considered in detail, the method of marketing, processing, equipment needed, and nutritional value of the product. The final product suggested for development was soya noodle.

It was found that soya noodle was the most suitable product to carry on developing because:-

1. There will be more acceptance of soya noodle in the market than the other products, as noodle is a very popular food among Thai people of different ages.
2. It is a nation wide product. Noodle can be fed to various groups of people from post-weaning infants, pre-school children, school children, to adults and old people. Noodles are also eaten in every part of the country, usually at lunch time.
3. The processing method is simple and can be introduced at the village level.
4. Because noodles can be eaten in larger quantities than other products, it is the most suitable way of increasing protein intake in the diet. It can be easily supplemented with vitamins and minerals.

As far as soya noodle had been developed in the project in the preliminary development in 1968, as summary in the next chapter, it was found that a high protein noodle could be made which was generally acceptable, although the texture of this product was not as good as the texture of ordinary noodles in Thailand.

Therefore, the development of soya noodle was continued in this project, with the hope that it will be one among other various high protein products that can be a help in solving the problem of protein malnutrition in Thailand.

# III. PRELIMINARY DEVELOPMENT WORK

The purpose of the preliminary development work carried out in 1968 was to convert an idea of producing a high protein noodle from soybean into an actual product, and to study how the product which had been developed was accepted by consumers. The work reported here was concerned with the investigation of raw materials used for recipe development, the possibility of enrichment of soya noodle with vitamins and minerals, the method of processing and laboratory and consumer tests on the product.

## Examination of Raw Materials for Recipe Development

There are two types of noodles - egg noodles and plain noodles. The U.S.A. Department of Agriculture has adopted the definitions and standards for egg and plain noodles as: Egg noodles are the shaped and dried doughs prepared from wheat flour and eggs, with or without water, and with or without salt. The egg ingredient may be whole egg and/or egg yolk. In the finished product, the moisture content does not exceed 13 percent; and the egg content, on the moisture-free basis, is not less than 5.5 percent by weight of egg, calculated as whole egg solids. Plain noodles are made from plain dough of wheat flour and water, with or without salt; and also in the finished product the moisture content does not exceed 13 percent. Both egg noodles and plain noodles are commonly ribbon-shaped (Hoskins and Hoskins, 1954).

Macaroni products are often enriched by the addition of vitamins and minerals. Most vitamins incorporated in macaroni products are water-soluble vitamins of the "B" complex. In 1955, a U.S.A. standard of identity for enriched macaroni products was established, which took into account the losses to be expected in cooking and draining, and prescribed levels of thiamine, riboflavin, niacin, and iron in the uncooked product, anticipating that the cooked product would contain the same levels per pound as enriched flour (Brooke, 1968). For an enriched noodle, the allowances of vitamins and minerals which are used to supplement one pound of wheat flour are: 500-615 mg for calcium, 13 - 16.5 mg for iron, 4 - 5 mg for thiamine, 1.7 - 2.2 mg for riboflavin and 27 - 34 mg for niacin (National Academy of Science, 1956).

In the development of the new soya noodle, for raw materials untreated full fat and low fat soyflour were used for dough making together with ordinary wheat flour. To comply with the standard for egg noodles, dried egg yolk of 95% solid was used to make 5.5 per cent of egg solid in the final dried noodle.

For studying the development of dough, various levels of wheat flour were added to low fat and full fat soyflour. The results are shown in Table II. (next page).

The minimum level of wheat flour that dough developed at was 25 percent. As the percentage of wheat flour was increased, the dough became more elastic. Fat content also played an important role in development of dough. Dough was clear and smooth, and a better colour, when full fat soyflour was used rather than low fat soyflour at the same level. For higher protein content, a high ratio of soyflour to wheat flour should be used, therefore 75% full fat soyflour plus 25% wheat flour with 5.5% egg solid added were used for making soya noodle.

Table II. Dough Development at Various Levels of Soy Flour and Wheat Flour

| Trial | Ingredients | Dough Character |
|---|---|---|
| 1 | Low fat soyflour alone | Did not form dough |
| 2 | 95% low fat soyflour plus 5% wheat flour | Dough disintegrated |
| 3 | 90% low fat soyflour plus 10% wheat flour | Dough clumped together |
| 4 | 85% low fat soyflour plus 15% wheat flour | Dough clumped together |
| 5 | 75% low fat soyflour plus 25% wheat flour | Weak dough developed |
| 6 | 70% low fat soyflour plus 30% wheat flour | Dough was slightly stronger with elastic property |
| 7 | 75% low fat soyflour plus 25% wheat flour plus 5.5% egg solid | Clear dough developed but still weak |
| 8 | 75% full fat soyflour plus 25% wheat flour plus 5.5% egg solid | Smooth and clear dough developed with more elasticity |

Vitamins and minerals were decided on to supplement the soya noodle. The amount of each vitamin and mineral added in the recipe was calculated in relation to the vitamin content of soyflour and wheat flour to give the dry mix flour the amount which would meet the allowance of nutrient supplement for noodles according to the National Academy of Science (1956). The calculation is shown in Table III.

**Table III. Calculation of Vitamin and Mineral Supplement to Soya Noodle**

| Details | Calcium mg | Iron mg | Thiamine mg | Ribo-flavin mg | Niacin mg |
|---|---|---|---|---|---|
| 1. Range of Allowance to supplement 1 lb of wheat flour | 500-625 | 13-16.5 | 4-5 | 1.7-2.2 | 27-34 |
| 2. Composition of 1 lb wheat flour | 109 | 5.9 | 1.16 | 0.33 | 9.03 |
| 3. Composition of 1 lb wheat flour supplemented with vitamins and minerals (1) + (2) | 609-734 | 18.9-22.4 | 5.16-6.16 | 2.03-2.53 | 36.03-43.03 |
| 4. Composition of 0.75 lb soyflour | 675 | 28.5 | 2.8 | 1.28 | 7.20 |
| 5. Composition of 0.25 lb wheat flour | 27 | 1.5 | 0.29 | .08 | 2.26 |
| 6. Composition of 1 lb mixture, 75% soyflour plus 25% wheat flour (4) + (5) | 702 | 30 | 3.09 | 1.36 | 9.46 |
| 7. Amount of vitamin and mineral needed to add (3) - (6) | 0-32 | 0 | 2.07-3.07 | 0.67-1.17 | 26.57-33.57 |

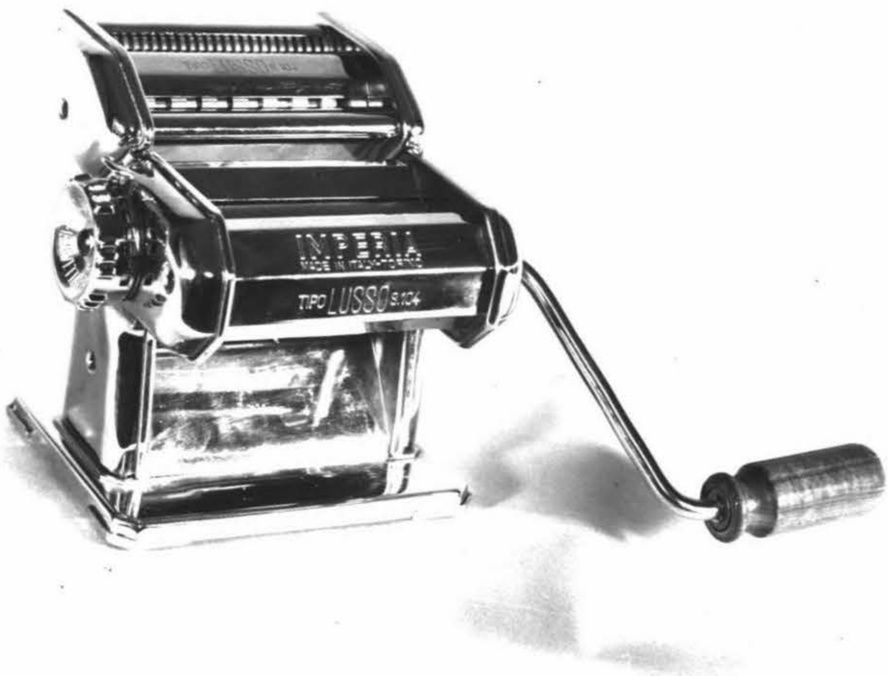



Figure 2: The Noodle Machine

The composition of the mix was:

| | |
|---|---|
| Full fat soyflour | 340 g. |
| Wheat flour | 114 g. |
| Egg (95% dried egg yolk solid) | 24 g. |
| Thiamine | 0.003 g. |
| Riboflavin | 0.001 g. |
| Niacin | 0.034 g. |
| Calcium | 0.032 g. |
| Water | 202 g. |

## Processing Method

Noodles are generally produced by mixing wheat flour with eggs and water and kneading this mixture into a homogenous dough. This dough is then extruded or rolled and cut into ribbon shaped strips and dried to about 13 percent water content.

For soya noodles, the method of processing was the same as that for ordinary noodles except that the noodles were steamed prior to drying.

Mixing. Full fat soyflour, wheat flour and dried egg yolk were mixed in a Kenwood mixer for 5 mins., the water was added and mixing continued until the dough was smooth and clear.

Rolling and cutting. The dough was rolled into thin sheets by a dough breaker, the dough breaker consisting of a pair of rollers and the gap between the rollers being adjustable. This operation was repeated a few times, setting the rollers closer together as the dough was rolled through. Then the thin sheet of about 0.035" thick dough was passed through the cutting rolls, by which the sheet of dough was cut into ribbons. The noodle machine is is shown in Figure 2.

Steaming. During dough mixing, a strong heavy smell was detected and the noodle tasted bitter. It was found from the literature that after about 10 mins. cooking in boiling water or steaming at atmospheric pressure of whole soybeans, the beany flavour was reduced (Rackis et al, 1966, and Mustakas, 1967). Therefore, the noodle was subjected to heat treatment prior to drying.

Firstly, the cut noodles were steamed for 10, 15, 20, 25 and 30 mins. It was found that steaming for 20 mins. eliminated the beany flavour in the noodles, but the straight cut noodles were curly after steaming.

Secondly, a thin sheet of dough about 6" long and 3" wide was steamed at various times, 20, 25 and 30 mins. At 25 mins. steaming there was absolutely no beany flavour and the noodle came out straight after cutting, so it was decided to steam the sheet of dough before cutting into noodles.

It was noted that, after steaming, the noodle had a sweet nutty smell.

Drying. The noodle was dried overnight in a drying cabinet at different temperatures, 180, 140, and 120°F. The colour of the noodle dried at high temperature was slightly brown, therefore, low temperature drying was preferred. The noodle was dried down to a moisture content of about 10-12 percent.

## Product Testing

Laboratory tests. The aim of the test was to see the effect of vitamin, mineral addition, and different processing methods on the characteristics of the product, as well as the general acceptability of the product.

The taste panel consisted of 6 Thai students in Palmerston North. Three products were tested.

Product A. The noodle was fortified with vitamins and minerals and was prepared by rolling and cutting prior to steaming.

Product B. The noodle was fortified with vitamins and minerals and was rolled into thin sheets, steamed and then cut.

Product C. With the same method of processing as Product B but not enriched with vitamins and minerals.

The Hedonic scale was used with the score range from 7 = like very much, to 1 - dislike very much.

The results are summarised in Table IV.

Table IV. Results of Laboratory Tests on the Preference of Three Different Products

| Product | Average Score (and one standard deviation) | | | | |
|---|---|---|---|---|---|
| | Appearance of dried product | Colour of dried noodle | Colour of cooked noodle | Texture of cooked noodle | Flavour of cooked noodle |
| A | 5.3±0.75 | 4.2±1.26 | 5.3±0.98 | 6.0±0.91 | 5.3±0.47 |
| B | 5.6±0.46 | 5.3±0.46 | 6.6±0.45 | 5.5±0.91 | 6.1±0.53 |
| C | 5.5±0.76 | 6.0±0.41 | 5.6±0.58 | 5.6±0.36 | 6.1±0.53 |

The addition of vitamins and minerals in products A and B did result in a darker colour of the dry product but gave a better colour when cooked. In comparing cutting before and after steaming, product A was scored less than product B for colour of the dry noodle and also flavour of cooked noodle. In the method of production of product A, the sheet of dough after it was rolled out, was left for the surface to dry in room temperature for a few hours. Then the sheet of noodle was cut and steamed. If the thin sheet of noodle was cut immediately after rolling, the ribbons of noodle stuck together. By leaving the noodle lying around for a few hours before steaming, an enzymatic browning reaction and oxidation could occur and these could be the possible reasons for the poor score of product A. Therefore steaming the sheet of dough prior to cutting was preferred. There was not much difference in the eating quality between the noodles with the addition of vitamins and minerals and the noodles with no addition, thus it was possible that vitamins and minerals could be added to soya noodle.

As a result of the three experiments, soya noodle was prepared from 75% full fat soyflour, 25% wheat flour and 5.5% dried egg yolk. Thiamine, riboflavin, niacin, and calcium were added to soya noodle at the quantity required by the standard allowance of the National Academy of Science for nutrient supplement to noodle. Dough was prepared from these ingredients and water, rolled into thin sheets and steamed at atmospheric pressure for 25 mins., then cut into ribbons and dried at a temperature of 120°C until the moisture content was less than 13 percent.

<u>Consumer test</u>. The purpose of the test was to find out the general acceptability of the product in comparison with ordinary Thai noodle, and also to find the approximate price the consumer would pay for the product.

50 Thai students in Wellington and Palmerston North were used

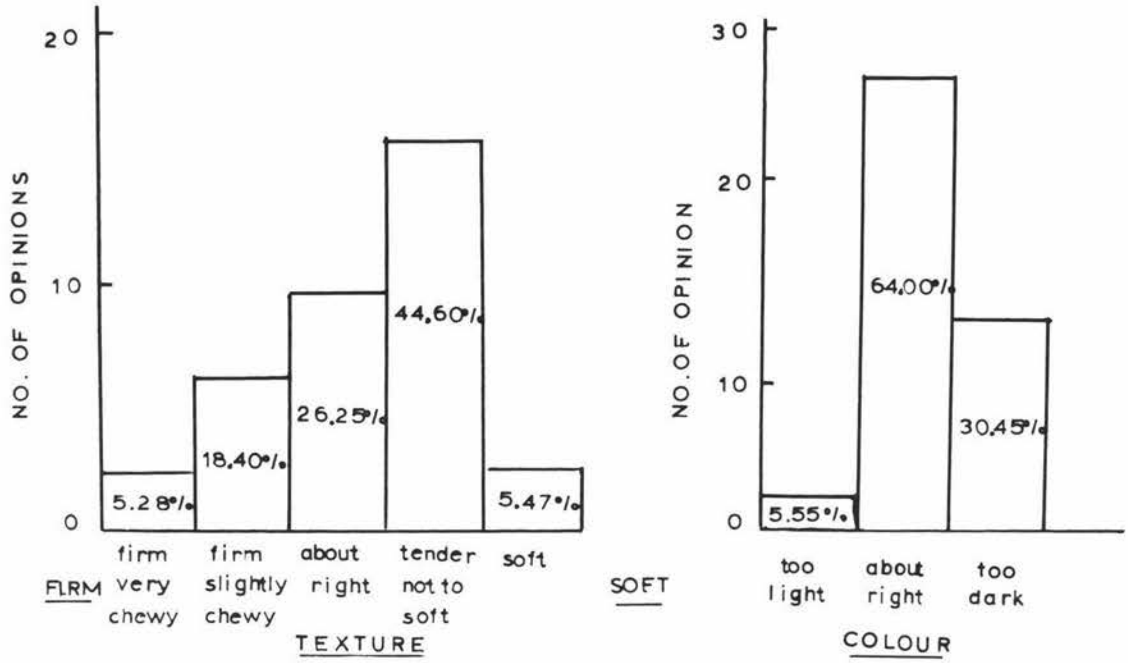



Figure 3 Histogram Showing Consumer Opinion on Texture and Colour of the Product

as consumers. 75 g. of the noodle, packed in a polythene bag 6" x 8" was presented to the consumer at home. The consumers were asked to cook the noodle, complete the form which was written in Thai, and then post back the form.

Hedonic and rating scores for all the product characteristics - colour, flavour, and texture, were used in the form.

75% of the forms were sent back and the results were analysed and are shown in Table V. and Figure 3.

Table V. Results on Consumer Preference of the Preliminary Developed Soya Noodle

| Hedonic Scale | | Appearance of Dried Noodle | | Appearance of Cooked Noodle | | Flavour | |
|---|---|---|---|---|---|---|---|
| | Score | Frequency | Score | Frequency | Score | Frequency | Score |
| like very much | 7 | 5 | 35 | 7 | 49 | 7 | 49 |
| Like moderately | 6 | 17 | 102 | 12 | 72 | 14 | 84 |
| Like slightly | 5 | 6 | 30 | 13 | 40 | 8 | 40 |
| Neither like nor dislike | 4 | 6 | 24 | 4 | 16 | 6 | 24 |
| Dislike slightyly | 3 | 0 | - | 6 | 18 | 1 | 3 |
| Dislike moderately | 2 | 1 | 2 | 0 | - | 1 | 2 |
| Dislike very much | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Total | | 36 | 194 | 38 | 196 | 38 | 203 |
| Average | | - | 5.4 | - | 5.12 | | 5.3 |
| Standard deviation | | - | 1.34 | | 1.55 | | 1.44 |

When compared with the noodles prepared in Thailand, only 23.8% of the total consumers thought it was higher quality, about 31% thought it was the same quality, and 42% thought it was lowere quality. Nearly half the consumers said that they would buy the noodle, less than a quarter would not buy it and the remainder were undecided. The average price per package of 75 g. noodle that they would pay as 2 bahts (about 10c N.Z.)

Some of the comments on the product made by the consumers were:

10% no comment on the product.

13% commented on the colour of the product. The colour was too dark before boiling and after boiling the colour was too bleached.

13% did not like the flavour. The flavour was said to be bland, and some said that it had no flavour, no taste. However, others said it had a distinct flavour.

21% like this noodle because of its high nutritional value.

63% complained about the texture. The texture before boiling was too hard and brittle, and after boiling became soft, no elasticity, not smooth. The texture disintegrated.

## Discussion

Because the product which had been developed up to this stage was reasonably acceptable, it was found worthwhile to be carried on. From the results of the consumer tests, the product was found generally acceptable in the overall appearance, flavour and colour. The main problem of the product was the texture of the dried and cooked product that could not be compared with ordinary noodle.

Further improvement of the recipe and method of processing was needed to overcome this problem.

# IV. RECIPE IMPROVEMENT

In the preliminary development work, 75 percent of full fat soyflour and 25 percent of wheat flour were used in making the soya noodle, and this resulted in a product of poor texture. It was necessary, at this stage, to investigate a new recipe that would give a better texture to the product.

## Factors Affecting Texture of the Product

The factors that were likely to affect the texture of the noodle were the quantity and quality of wheat gluten and the fact content of the dry mix.

For development of dough, a certain level of gluten is required to form a protein matrix in which the starch and the soy protein are embedded. From electron microscope and X-ray studies of the gluten in 1961 by Grosskreutz, described by Baldwin et al (1963), a structure was proposed, which visualised gluten as a sheet varying in thickeness and consisting principally of protein platelets surrounded by water and held to each other by hydrogen bonds. An essential part of the sheet-like structure is lipoprotein which acts as a slip phase. The rheological properties of dough are dependent on the quantity of disulfide and sulfhydryl groups in dough and the rate of their inter-change (Frater et al., (1960) If there are too many of the sulfhydryl group the dough is too viscous, if too many of the disulfide bond, the dough will be tough.

Recently lipids have been implicated in the mechanism of oxidising sulfhydryl groups in dough. During mixing of flour and water dough under oxygen, lipid peroxidation gradually occurred, thus exerting an improving effect (Tsen and Hlynka, 1963). Since there is no starch in soybeans or too little if it occurs, most of the proteins of soybeans are located in the so-called protein body which contains numerous spherical particles 1 - 10 $\mu$ in diameter (Wolf, 1970). In making dough from soyflour and wheat flour, because of the addition of extra disulfide and sulfhydryl groups from soy protein, lipids may possibly be needed either as a mechanical lubricant to keep the dough pliable or to oxidise sulfhydryl groups. The presence of lipids may result in a smoother and more extensible property of dough. Besides these, lipids will cause the final product to retain its firmness during cooking because there is less adsorption of water.

## Scope of the Investigation

As the aim of the project was developing a high protein noodle, the level of protein content in the noodle should be as high as possible. When using 75 percent full fat soyflour and 25 percent wheat flour, a protein content of 31 percent was obtained (from calculation). Thus, the new recipe should have about the same protein content, but offer a better textured product, which can be achieved by varying the ratio of wheat flour to full fat soyflour. Low fat soyflour can be added to maintain the protein content of the product.

It was found from previous experiments that when using low fat soyflour alone, the minimum wheat flour content from which a good dough rheology could be obtained was 30 percent and 25

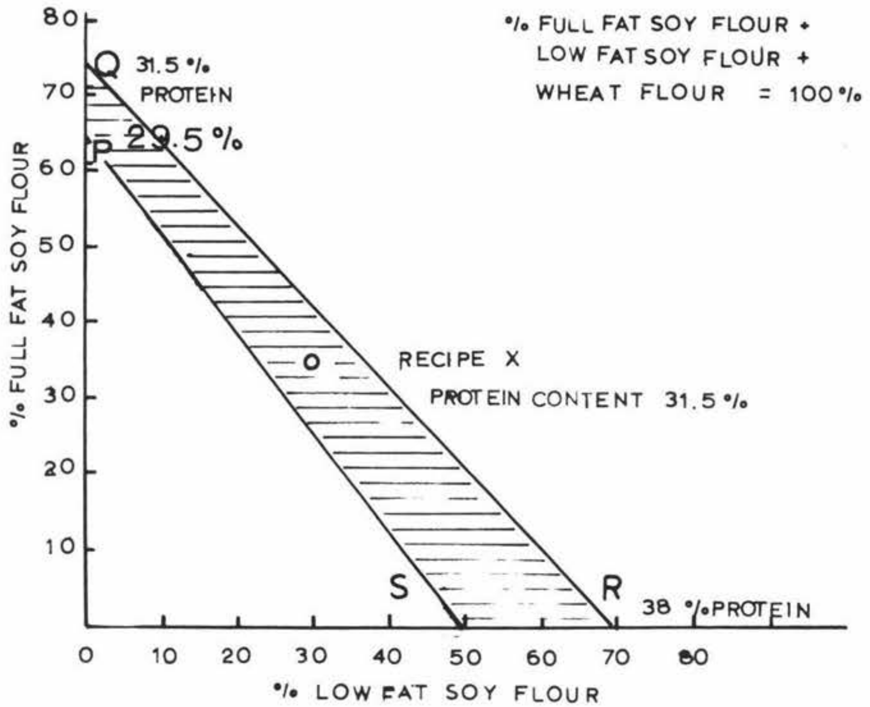



Figure 4 Diagram Shows Area in which New Recipe Should Be Investigated

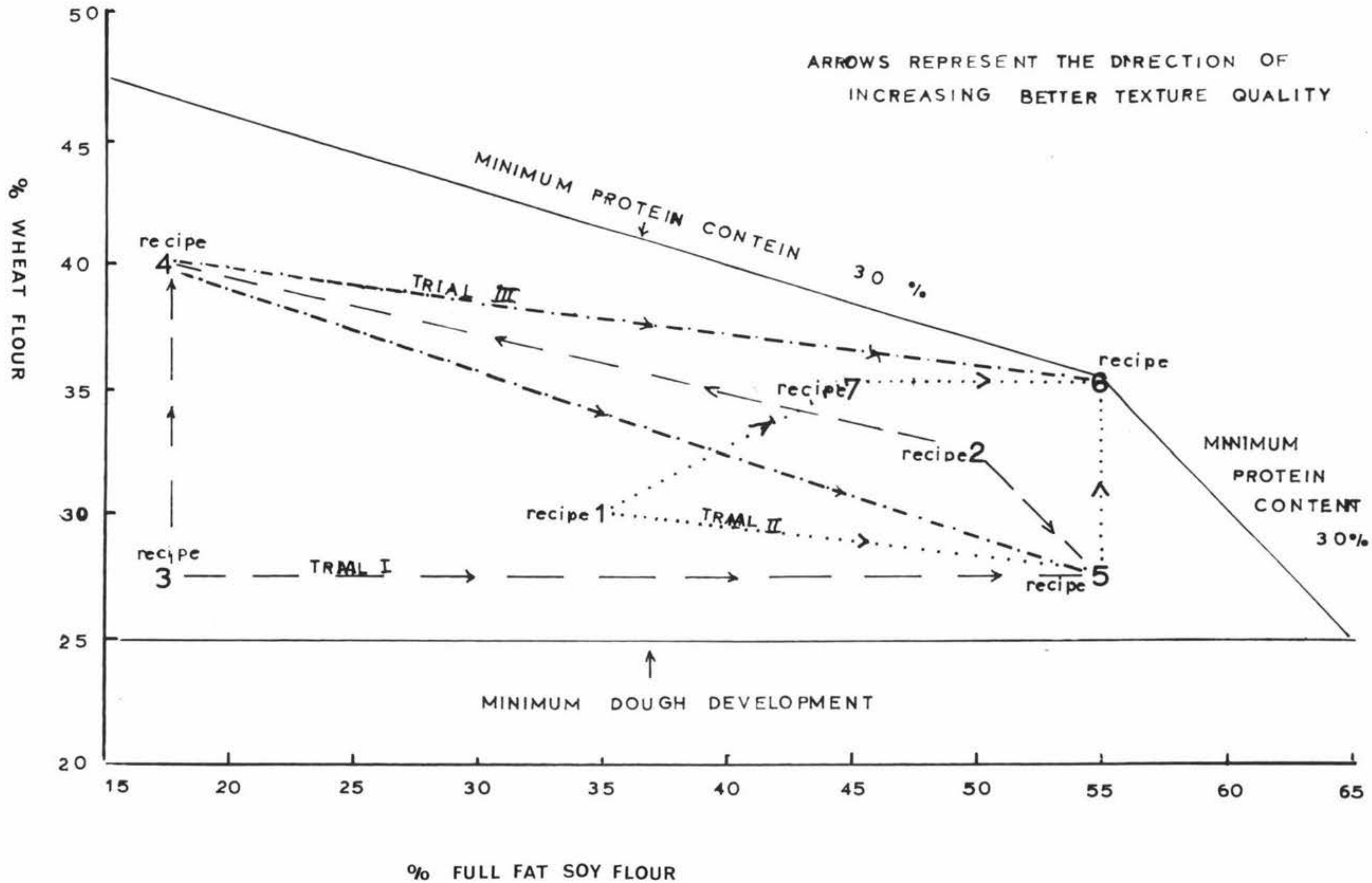
percent when full fat soyflour was used. Therefore, for the new recipe, if the low fat soy flour was used alone the amount of wheat flour should be increased or with the same amount of wheat flour (25-30%) full fat soy flour could be used together with low fat soy flour. Because the protein content of wheat flour is lower than soyflour, the amount of wheat flour that could be used in the recipe was limited. If a large quantity of wheat flour was used, the protein content of the noodle would be less than 30 percent. With all these criteria in mind, a diagram was developed to aid in the search for a new recipe. (Figure 4). It was assumed that the protein content of the ingredients are as follows:-

| | |
|---|---|
| Low fat soyflour | 50% at 8 percent moisture content |
| Full fat soyflour | 40% at 8 percent moisture content |
| Wheat flour | 12% at 15 percent moisture content |
| Whole egg | 11.9% at 73.4 percent moisture content |

The shadow area PQRS represents the area in which the new recipe should be investigated. The protein content of these recipes in this area varies from 38 to 29.5 percent and the wheat flour from 25 to 50 percent. If recipe X, for example, was chosen, the composition of the recipe is 35 percent full fat soyflour, 30 percent low fat soyflour and 35 percent wheat flour, and the protein is 31.5 percent.

Changing this to a two variable system - full fat soyflour and wheat flour, this means that they can only vary from 15 to 65% in full fat soyflour and 25 to 47.5% in wheat flour, as shown in Figure 5.

Figure 5 Diagram Shows Location of Recipes and Direction of Increasing Texture Quality



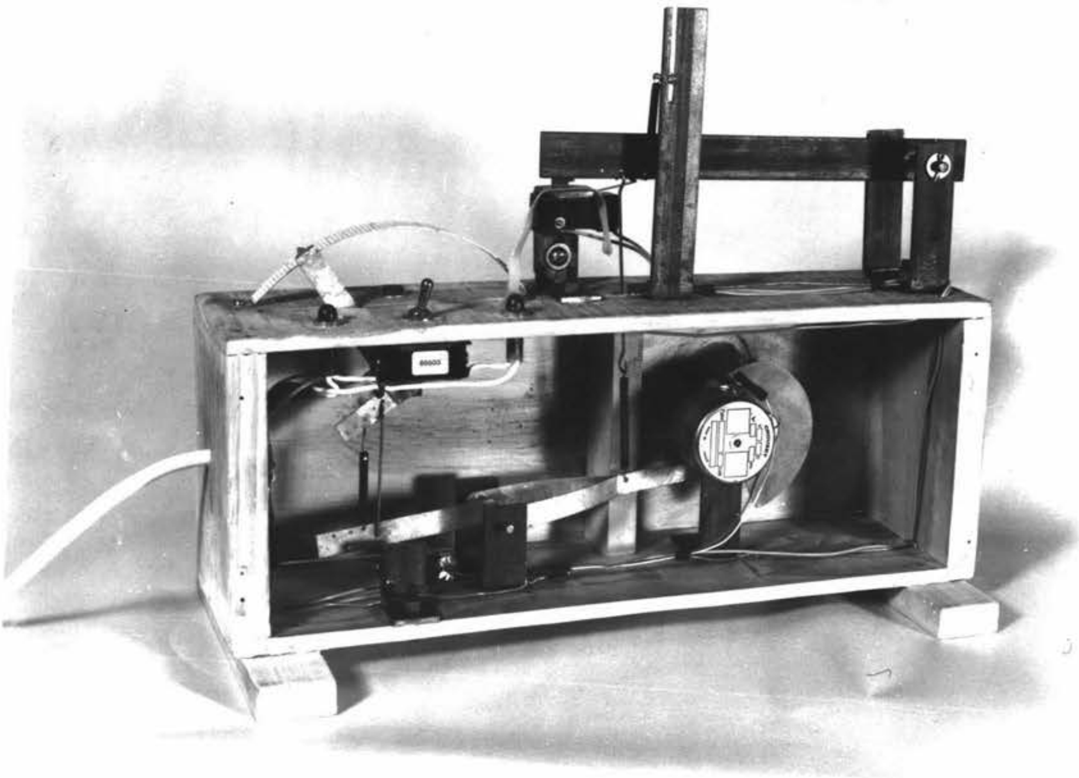


Figure 6: A Noodle Texture Testing Machine

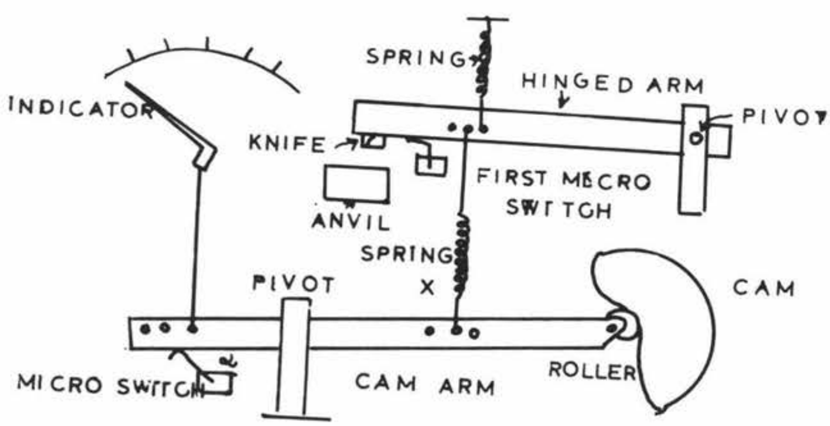



Figure 7 A Diagram Showing Different Function of the Noodle Texture Testing Machine

Experimental Method

Method of testing the texture of cooked noodle. Subjective and objective methods were used to evaluate the texture of the noodle.

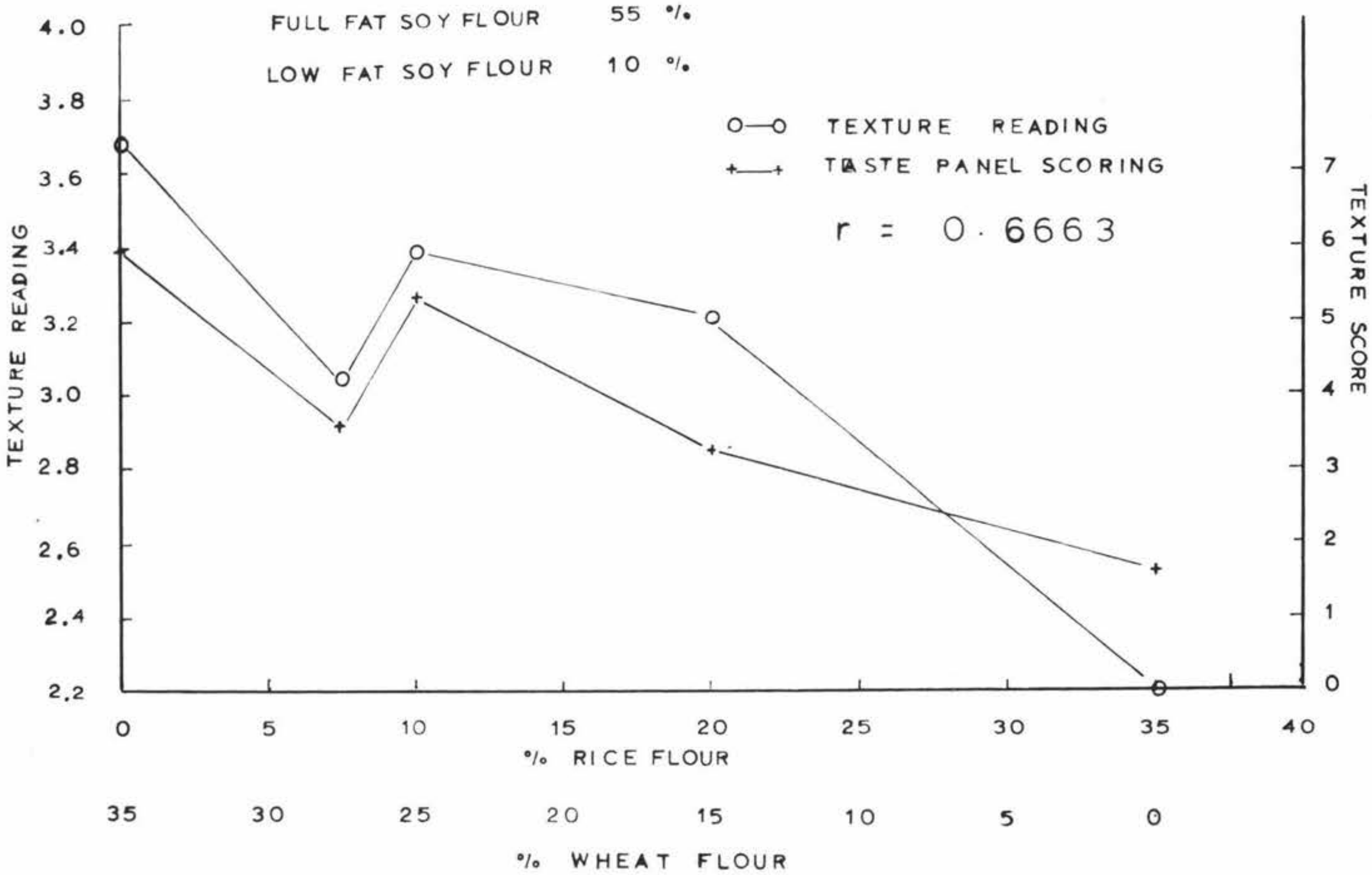
Subjective method: 6 - 7 member laboratory taste panels were used. Ranking method and hedonic preference scale were used for evaluation of the best recipe. The taste panel was asked to rank the texture of cooked noodle according to their preference, the best product was ranked first and the worst product was ranked last. For a hedonic scale, a 1 - 7 score was used, 7 = like very much, and 1 = dislike very much.

For comparison with the objective method, a scoring scale was used as shown below:-

| | |
|---|---|
| Very tough | score = 7 |
| Moderately tough | = 6 |
| Slightly tough | = 5 |
| Neither tough nor soft | = 4 |
| Slightly soft | = 3 |
| Moderately soft | = 2 |
| Very soft | = 1 |

Objective method: A noodle texture testing machine, as shown in Figure 6. was used to measure the tenderness of cooked noodle. The tenderness of the noodle sample was measured by the time taken for the knife blade to penetrate through the noodle. A strip of noodle sample was placed lengthwise on an anvil and a knife blade of a suitable size was lowered on to it. The time taken for the blade to penetrate the noodle was indicated on a graduated scale divided into 6 units. The general construction

Figure 8 Correlation between Subjective and Objective Methods on the Texture of Soya Noodle



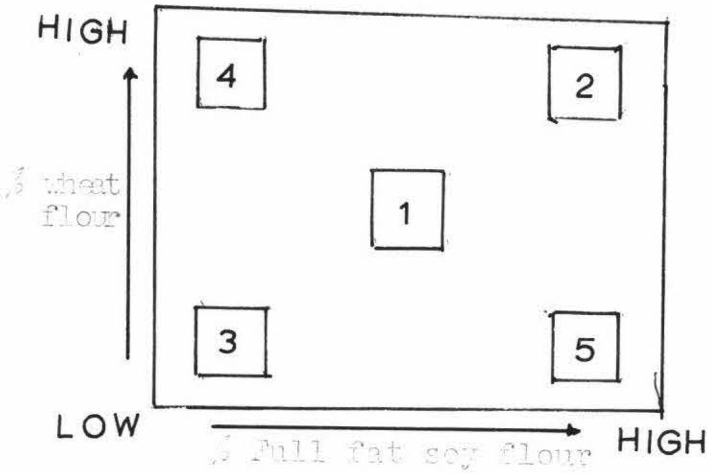
and operation of this noodle texture testing machine is described in Appendix VIII.

For testing, the noodles were boiled for 5 mins. in boiling water, drained and washed with cold water to stop further cooking. For the objective method, the cooked noodle was kept moist in a beaker prior to evaluation.

Correlation between subjective and objective methods: It was found that the results obtained from the texture reading by the noodle texture testing machine correlated quite well with the taste panel scores. (Figure 8.). When the noodle was scored low (for soft texture) the texture reading by the machine was also low and when the noodle was indicated to be tough by the taste panel, a high result of texture reading was also obtained. Therefore, this objective method was used together with the taste panel to evaluate the best recipe for soya noodle.

Design of the experiment. In searching for the best recipe, EVOP or evolutionary operation method was used. In this method, the levels of full fat soyflour and wheat flour in the recipe were varied and the effect of the variation on texture was studied. The following is the procedure based on the method described by Fox (1968):-

Firstly, starting with one level of full fat soyflour and wheat flour, for a two-level experimental design, four other recipes were needed. In one of these, (recipe 2) both full fat soyflour and wheat flour levels were higher than the centre point, (recipe 1) and in the other both full fat soyflour and wheat flour levels were lower (recipe 3). In the third, full fat soyflour was lower and wheat flour was higher, (recipe 4) and in the fourth, the recipe was reversed, (recipe 5) as in the diagram below.




If any recipe gives a significantly better texture, the recipe becomes the centre point for another five recipe trial, until a satisfactory product is obtained.

Manufacture of soya noodle. The noodles were made by the method described in the previous chapter, in which the dough was rolled to a thin sheet, steamed for 25 mins., cut and dried overnight at 120$^{o}$F.

## Results

Recipe 1, in which the content of wheat flour and full fat soyflour was 30 and 35 percent respectively was used as a centre point. Another four recipes, recipes 2, 3, 4 and 5, were developed later according to the experimental design. The composition of flour mix and results of the texture ranking score are shown in Table VI.

In trial 1, recipe 3, in which the content of both full fat soyflour and wheat flour were low, was significantly ranked poorer than the others. Recipe 5, which had the same wheat flour content as recipe 3 but a higher full fat soy flour

**Table VI.** Effect of Flour Composition on Texture by Ranking Method

| Flour mix composition and ranking score | Recipes | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Full fat soyflour % | 35 | 50 | 17.5 | 17.5 | 55 | 55 | 45 |
| Wheat flour % | 30 | 32.5 | 27.5 | 40 | 27.5 | 35 | 35 |
| Low fat soyflour % | 35 | 17.5 | 55 | 42.5 | 17.5 | 10 | 20 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Trial I<br>Ranking score of<br>7 Replication | 25 | 19 | 31* | 17 | 13 | | |
| Trial II<br>Ranking score of<br>7 Replication | 30 | 16 | - | - | 25 | 12 | 22 |
| Trial III<br>Ranking score of<br>6 Replication | | 19 | | 40 | 15 | 14 | 22 |
| Total Ranking score<br>13 Replication | | | | | | 26** | |

* Significant at 5% level ** Significant at 1% level

Ranking score 1 = 1st, score 7 = 7th

content, ranked best. And recipe 4 which had the reverse full fat soy flour content and wheat flour content of recipe 5, was ranked second. Therefore the new recipe should go for high fat content and also increasing wheat flour.

In trial II, as shown in Figure 5, recipe 2 was used as a centre point instead of recipe 5. There were two reasons that recipe 5 should not be the centre point. Firstly, the wheat flour content of recipe 5 was low, and it was not possible to lower the wheat flour content any further. Secondly, from the results of trial I, the new recipe should go for high fat content and also increasing wheat flour. For these reasons, recipe 2 was used as a centre point, and two new recipes 6 and 7 were developed; together with the old recipes 4 and 5, the texture of the noodles were evaluated. The results and composition of the mix of the recipes in trial II are also shown in Table VI. Recipe 6 ranked best and recipe 1 ranked poorest. In trial III, because there was no significant difference between recipes 1, 2, 5, 6 and 7 in trial II, the analysis was repeated again with recipe 4 used instead of recipe 1. Recipe 6 was again ranked best. When combining the results of trial II and trial III, a significant difference was obtained. Therefore the level of 55% of full fat soy flour, 35% wheat flour and 10% low fat soy flour was preferred to the other levels.

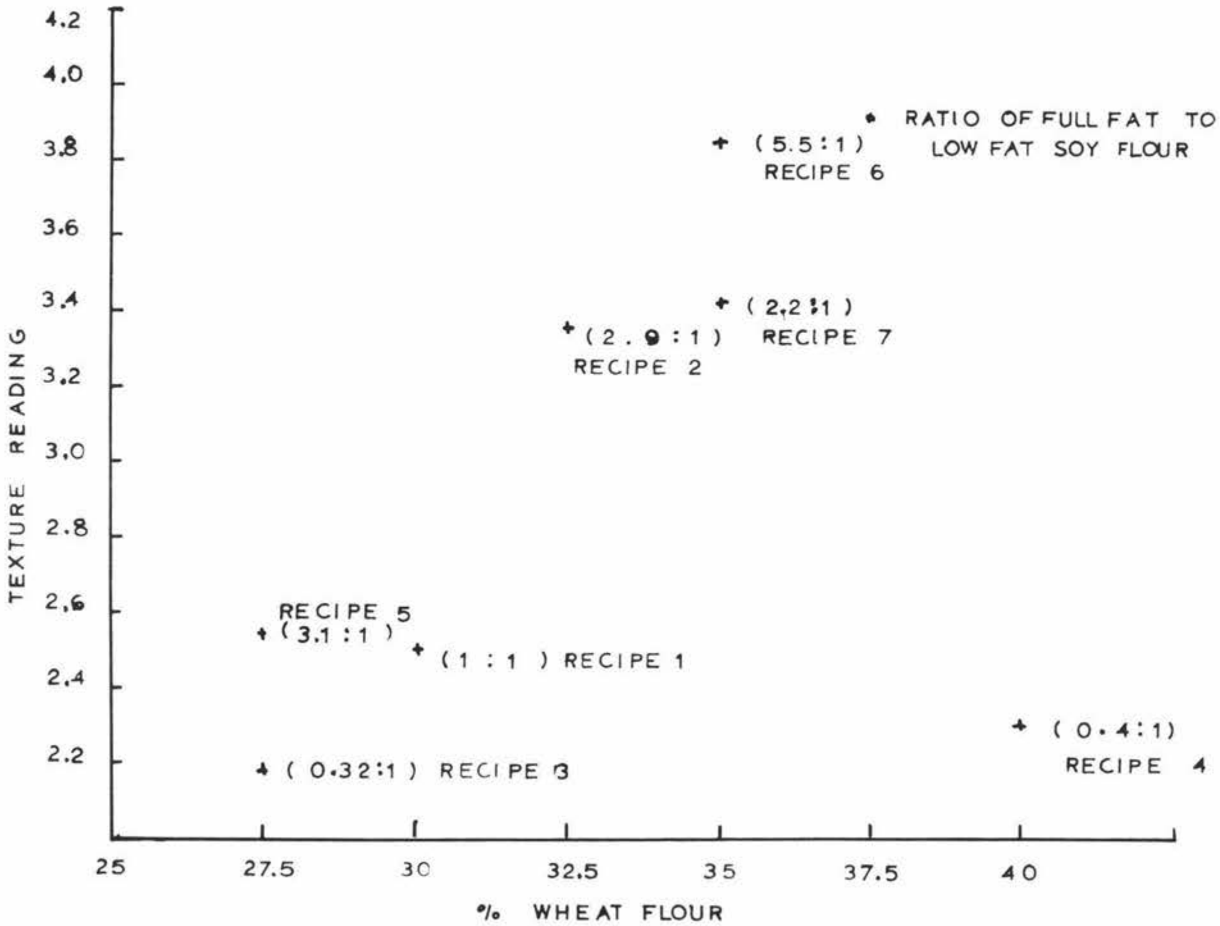



Figure 9 Effect of Variation in Recipe Composition on Texture Measurement by the Noodle Texture Testing Machine

The results obtained from the taste panel ranking agreed very well with the results from the texture machine. In Figure 9, recipe 6 which was ranked best, also obtained a high texture reading. Recipe 3 which was the poorest by ranking method was very soft as shown by the low figure of the texture reading. The higher the level of wheat flour in the recipe, the tougher the product and the higher the reading obtained. When 27.5 percent of wheat flour was used, the reading varied from 2.18 to 2.54, according to the level of full fat soyflour in the recipe. When the level of wheat flour was increased to 35 percent, the reading varied from 3.41 to 3.84. The fat content in the recipe plays an important part in the texture of the product. At the same level of wheat flour, but increasing the ratio of full fat to low fat soyflour, the texture of the product is tougher.

In the further improvement of recipe 6, different qualities of wheat flour were used. In recipe 8, standard plain wheat flour from the Timaru Milling Company was compared with Snowwhite flour from the Manawatu Milling Company, recipe 6. The net gluten content of standard plain wheat flour and Snowwhite wheat flour were 24 and 16.8 respectively. Also the use of rice flour was introduced, 7.5% of wheat flour being replaced by rice flour in recipe 9. The results and composition of each recipe are shown in Table VII.

There was a slight improvement of texture when better quality wheat flour was used. Recipe 8 was preferred by the taste panel to recipe 6 and the texture of recipe 8 was tougher than 6, from the results of the texture reading. Recipe 9 showed a highly significant softer texture than recipe 6 and 8. The texture of the product according to the taste panel gave a smooth mouth feel and it was ranked best when compared with the texture of recipes 6 and 8.

Table VII. Comparison of Recipes Using Different Qualities of Wheat Flour and Using Rice Flour on Results of Texture Measurement

| Flour mix composition, ranking, score and texture readings. | | Recipes | | |
|---|---|---|---|---|
| | | 6 | 8 | 9 |
| Full fat soyflour | % | 55 | 55 | 55 |
| Snowwhite wheat flour | % | 35 | - | - |
| Standard plain wheat flour | % | - | 35 | 27.5 |
| Rice flour | % | - | - | 7.5 |
| Low fat soyflour | % | 10 | 10 | 10 |
| Total | % | 100 | 100 | 100 |
| Ranking score with 6 replication | | 15 | 13 | 6** |
| Texture reading | | 3.84 | 3.99 | 3.05 |

** significant at 1% level.

Various levels of rice flour were investigated but the results in Table VIII did not show any significant difference. Recipe 11 showed a slightly higher hedonic score than the others. But because recipe 11 gave a slightly weak dough which could not be handled by machines in large scale production, recipe 10 was preferred. The texture reading of recipe 9 was unexpectedly lower than those of recipes 10 and 11. The reason for this is not known, but may be because of the difference in manufacturing method.

Table VIII. Comparison of the Effect of Different Levels of Rice Flour on the Texture of the Product

| Flour mix composition, hedonic score and texture reading | Recipes | | | |
|---|---|---|---|---|
| | 9 | 10 | 11 | 12 |
| Rice flour | 7.5 | 10 | 20 | 35 |
| Wheat flour | 27.5 | 25 | 15 | 0 |
| Full fat soyflour | 55 | 55 | 15 | 55 |
| Low fat soyflour | 10 | 10 | 10 | 10 |
| Total | 100 | 100 | 100 | 100 |
| Hedonic score | 4.6 | 5 | 5.5 | 4. 8 |
| Texture reading | 3.05 | 3.40 | 3.20 | 2.20 |

Discussion

In searching for the best recipe, there was a high preference for the product which had either high full fat soyflour or wheat flour, or both. Recipe 6 which contained the highest full fat soyflour and wheat flour of the recipes was ranked highest.

From Figure 5., it can be seen that recipe 6 has moved to the limit line of minimum protein content (30%), thus neither the level of wheat flour nor full fat soyflour can be increased in the recipe to obtain a product of better texture, otherwise the protein content will be lower than the limit. Therefore, the recipe 6 fulfilled the requirements of better texture of the product and optimum protein content.

Because the protein content of wheat flour and rice flour are lower than soyflour - the protein content of wheat flour and rice flour are 12 - 14 and 7 percent respectively (McCance and Widdowson, 1960) - the replacement of some of the wheat flour with rice flour will not significantly alter the protein content of the product. Rice flour was used to experiment in improving the texture, and it resulted in a product of highly significant preference rating by the taste panel. The product was described as having a smoother texture.

In the comparison of the textures of the noodles made with various different levels of rice flour, the product prepared from 20% rice flour and 15% wheat flour was given the highest score. A sheet of dough made from this mixture was however difficult to handle in mechanical rolling and cutting, thus this level of rice and wheat flour is not likely to be suitable for a large scale production. There was no significant difference between the texture of this product and the other product prepared from different levels of rice flour, therefore the recipe which used 10% of rice, 55% full fat soyflour, 10% low fat soyflour and 25% of standard plain wheat flour was taken as the most suitable for development of a high protein noodle. It fulfilled the requirement of both producing a better textured product and optimum protein content.

The results of the texture reading of the tenderness of cooked noodle correlated quite well with the taste panel scoring. Therefore this objective texture measurement method was used instead of taste panels for further development of texture by studying the method of processing. From the results of taste panel hedonic score and texture measurement of noodle when rice flour was used, the taste panel seemed to prefer the noodle of texture reading about 3.20 than the noodle of texture reading lower or higher.

# V. DESTRUCTION OF TRYPSIN INHIBITOR

## Literature Review of Anti-nutritional Factors in Soybeans

Leiner (1966) has classified the anti-nutritional substances in seed proteins into three groups: proteins and protein derivatives, glycosides and a miscellaneous group. For soybeans, the anti-nutritional substances in the first group are trypsin inhibitor and hemagglutinins, the second group are a goiterogenic substance and saponin and in the last group are metal-binding factors and anti-vitamin factors.

Trypsin inhibitor. Trypsin inhibitor is the most intensively studied of all the anti-nutritional factors. As reviewed by Pusztai (1967) and Mickelsen and Yang (1967), since Osborne and Mendel reported a beneficial effect of heat treatment on the nutritive value of soybeans in 1917, a great deal of work has been attempted to identify the factor or factors responsible for the deleterious effect of raw soybean. When a trypsin inhibitor of protein nature in untreated soybean flour was first described by Read and Haas in 1938 and rediscovered independently by Bowman and Han and Sandstedt in 1944, it seemed to offer a perfect explanation for the observed improvement in nutritional quality on heating. Further support for this concept also came from the observations of Ham and co-workers in 1945 on the growth of chicks, and of Klose and his associates in the following year on rats, that the addition of a trypsin inhibitor preparation to a heated soybean ration reduced the growth rate.

Pancreatic enlargement of chicks fed a raw soybean ration was reported by Chernick and coworker in 1948, as reviewed by Leiner

(1966). There is little doubt that pancreatic hypertrophy is one of the primary physiological effects of feeding raw soybeans. Gertler et al (1967) affirm the pancreatic hypertrophy effect, but they concluded that the inhibitor has a minor role in the growth depression of chicks and rats. Also in Leiner's (1966) review, he, himself, and other workers in 1949 found that methionine markedly improved the nutritional value of raw soybeans. He also mentioned that Booth and co-workers gave an opinion in 1960 that pancreatic hypertrophy leads to an excessive loss of endogeneous protein in the form of exocrine protein secreted by the pancreas, and since the protein is rich in cystine, this represents a net loss of cystine from the body. This increased need for cystine for protein biosynthesis during pancreatic hypertrophy is reflected by an increase in the conversion of methionine to cystine in the pancreas. This would explain why the need for methionine is particularly acute in diets containing raw soybeans. In addition De Muelenaere (1964) explained the growth depression of animals fed raw soybeans as compared with animals fed heated soybeans at the same level of intake by suggesting that there was an extra demand for protein by the animals in order to keep up with the faster epithelial cell regeneration and the sharply increased digestive enzyme secretion.

For the chemical and physical properties of soybean trypsin inhibitor, Steiner and Frattali (1969) reported that trypsin inhibitor was finally isolated and crystallised and studied by Kunitz in 1945-47. This Kunitz inhibitor has a molecular weight close to 21,500. The molecule consists of a single polypeptide chain. It contains no sulfhydryl group, but is crosslinked by two disulfide bridges. No evidence of self association has been reported. The solubility properties are those of a globulin, with a sedimentation coefficient about 2.29 and an isoelectric point of pH 4.5. The best known property of the Kunitz inhibitor is the ability to form at neutral pH a one-to-one stoichiometric complex with trypsin. The complex, which can be crystallised, is devoid of proteolytic activity.

There is another trypsin inhibitor, discovered by Bowman in 1946 and Birk in 1961. As reported by Steiner and Frattali (1969) this inhibitor is an acetone-insoluble fraction, referred to as the Bowman-Birk inhibitor. The inhibitor is a low molecular weight protein close to 8,000 which self-associates in solution. Its alpha-helical content appears to be low. It strongly inhibits trypsin and alpha-chymotrypsin, although not in a stoichiometric fashion.

Rackis and Anderson (1964) found four fractions of trypsin inhibitor. The trypsin inhibitors $A_1$ and $A_2$ when separated from the other protein constituents of the seed by chromatography on DEAE cellulose columns, both form 1:1 complexes with trypsin. $A_3$ was found to be identical with Kunitz's crystalline inhibitor. $A_1$, on the other hand, was slightly less acidic and the molecular weight was 14,300. Two more trypsin inhibitors $B_1$ and $B_2$ were isolated. They differed from A1 and $A_2$ in behaviour in ion-exchange chromatography, electrophoretic mobility, sedimentation pattern and specific activity.

Frattali and Steiner (1968) also separated commercial soybean trypsin inhibitor by DEAE column chromatography into 3 fractions, $F_1$, $F_2$ and $F_3$. Fraction $F_2$ is similar to Kunitz's inhibitor and Fractions $F_1$ and $F_3$ are not stoichiometric inhibitors of trypsin.

From a practical standpoint, trypsin inhibitors do not pose a serious problem in feeds and foods since Wolf (1966) reported that they are readily inactivated by moist heat, however, he also mentioned that Kunitz in 1947 heated trypsin inhibitor at 90°C for 2 mins., completely inactivating it but activity gradually returned on cooling. The extent of reversibility decreases as the time of heating is increased. Borchers et al (1947) found that for absolute destruction of trypsin inhibitor in soybean meal of 3 m.m. thickness, is needed 90 mins. of atmospheric steaming and 60, 30, 20 and 10 mins. at 5 lbs, 10 lbs, 15 lbs and 20 lbs autoclaving respectively.

Van Burren et al (1964) and Hackler et al (1965) reported that about 4-5% activity of trypsin inhibitor remains when soymilk is heated for 120 mins. at 200°F or 20-30 mins. at 250°F.

Rackis (1966) studied inactivation of soybean trypsin inhibitor during soybean meal processing, he found that with defatted soya bean flakes about 0.01 in. (25 mm) thick maximum inactivation was attained by atmospheric steaming for 15 mins. Steaming for 20 mins. almost completely inactivated the inhibitor in whole bean containing 25 percent water.

From the results of heat destruction of trypsin inhibitor reviewed by these three groups of workers, the rate and amount of trypsin inhibitor destruction are dependant on the type of sample, the moisture content and also the method of determination. Borchers and co-workers used Anson's method, Van Burren and associates used Learmonth's method and Rackis used Kunitz method which will be described later.

Soybean Hemagglutinins. As reviewed by Leiner (1966), hemagglutin substances, known as phytohemagglutins in many plants, were first purified in 1952. The purified soybean hemagglutin is capable of significantly inhibiting the growth of rats. In 1961, Jaffé found that soybean hemagglutinin caused impairment in the absorption of protein and fat when feeding rats with the purified form of soybean hemagglutinin. He showed that isolated intestinal loops taken from rats fed the raw soybean or the purified hemagglutinin absorbed glucose at about half the rate of loops taken from control animals. The hemagglutinin might combine with the mucosal cells lining the intestinal wall, thus interfering with the absorption of essential nutrients.

It was also found that hemagglutinin agglutinated the red blood cells in certain species of animals, as reviewed by Mickelsen and Yang (1967), but Wolf (1966) is doubtful of this reaction because firstly, hemagglutin had been shown to be inactivated by pepsin and secondly, to come into contact with red cells the undigested hemagglutinins would have to be absorbed from the intestine. Therefore the agglutinating effect seems unlikely to occur.

Weda et al (1958) studied the composition and end groups of soybean hemagglutinin. They found that it has a molecular weight 96,000 and its composition is high in glycosamine content (6 - 10%). It is composed of at least two peptide chains with N-terminal alanine residue. The C terminal could not be established with certainty but they believed it to be serine. The sedimentation constant using the ultracentrifuge is 6.4S. In contrast to crystalline trypsin inhibitor, the hemagglutinin is an albumin, being soluble in water at the isoelectric point. It also has a high isoelectric point (pH 6.1).

Like trypsin inhibitor, hemagglutinin activity of soybean meal is readily inactivated by moist heat. The maximum stability toward thermal inactivation was obtained in the region of pH 6 to 7 (Leiner, 1958). At pH 4-5, it will take about 150 mins. to completely destroy the activity at 60°C and less than 30 mins. at temperature 68°C, in comparison with 300 mins. at 60°C when the pH is increased to 7.3. It seems to be that to inactivate hemagglutinin, less heat treatment is required than for inactivation trypsin inhibitor.

Goiterogenic agents. Several workers have reported a number of cases of goitre in human infants fed soybean milk (Van Wyk et al, 1959, and Hydrowitz, 1960). An infant fed from 1 to 5 months of age on a soyabean diet had an enlarged thyroid but appeared normal. Apparently the heat treatment employed for sterilising these particular soybean preparations was not sufficient to destroy the goiterogenic agent. Iodine supplementation, however, alleviated the goitre condition in infants.

No goiterogenic agent has ever been isolated from soybeans, so it is not known what substance is actually responsible for the goiterogenicty of raw soybeans, but it has been known that some oil seed proteins such as rapeseed contain thioglycoside which releases goiterogenic isothiocyanate upon hydrolysis (Leiner, (1966).

Soybean saponins. Saponins as described by Leiner, (1966), are bitter-tasting, foam-producing glycosides in which the non-sugar residue (sapogenin) is a triterpenoid alcohol referred to as a soya sapogenol. There is evidence to indicate that at least five different saponins exhibit varying degrees of hemolytic and foam-producing activity.

Birk et al (1963) pointed out that the hemolytic property of the saponin is of little importance in determining the nutritional value of soybean meal.

Gestetner and co-workers in 1965, as mentioned by Wolf (1966) studied with rats, chicks and mice. They found that the saponins pass through the stomach and the small intestine, unhydrolysed and without being absorbed and also no growth impairment occurred.

Soybean isoflavones. Soybean isoflavones occur mainly as the glycosides, genistin and daidzein. Small amounts of genistin have been found in isolated soybean proteins and in frozen tofu. Genistin has been isolated from commercial defatted soybean meal which presumably had been heated, hence it is assumed that genistin is stable to autoclaving (Wolf, 1966).

Physiological effects of genistin and genistein isolated from soybean meal are reported, but little information is available about their effects when ingested at the levels occurring in meal and other soybean fractions. Magee (1963) fed rats with 19% casein diet containing 0.1 and 0.5% of genistin and genistein for 4 weeks. At the higher level the isoflavones decreased weight gain and decreased weight of the kidneys, and spleens, whereas the lower level had no significant effect. The genistin and genistein is usually found in soybean meal at a low level, therefore they should not have any significant physiological effect.

Although no adverse effects concerning genistin and daidzein in soybeans under practival conditions are reported in the literature, Wolf (1966) suggested more research is needed before deciding whether they are completely harmless at the level commonly ingested. A method of quantitatively determining genistin and daidzin in soybean is needed, and the possible presence of other isoflavones needs exploring. The distribution of the isoflavone in soybean products as a result of processing should also be determined.

Miscellaneous effects. Some soybean protein isolates have been shown to interfere with the availability of certain mineral constituents of the diet. The exact mechanism taken by soybean protein exerting this effect is unknown, although it may be related to the observation that a soybean protein-phytic acid complex has a special affinity for metal ions (Leiner, 1966).

Carlson et al (1964) have reported that unheated soybean protein, or the protein isolated therefrom, caused rickets in turkey poults as judged by a decrease in bone ash. This rachitogenic effect could be overcome by supplementation with vitamin D3 or by autoclaving the meal; calcium and phosphorus were ineffective in this respect.

From the literature reviews of antinutritional substances present in soybeans, trypsin inhibitor and hemagglutinin have been shown by various studies and feeding experiments to be the most important anti-nutritional factors that inhibited growth of animals feeding on raw soybeans. Fortunately, these two growth inhibitor substances can be destroyed by heat treatment. Since the rate of destruction of these inhibitors varied with the method of heat treatment, the form of the soybean material and the method of determination, it was necessary to find the optimum time and temperature for destruction of trypsin inhibitor in soya noodle. As has been said before, hemagglutinin required less heat treatment than trypsin inhibitor, therefore the time and temperature required for destruction of trypsin inhibitor would also destroy hemagglutinin.

Literature Review of Method for Determination of Trypsin Inhibitor

To measure the destruction of trypsin inhibitor by heat treatment, a quantitative measurement of the inhibitor had to be developed. There are five methods at present which have been used in determining the trypsin inhibitor activity or retention of the heat treatment, as summarised below.

Kunitz method. (Kunitz, 1946). The Trypsin inhibitor activity is measured by trypsin digestion of casein followed by optical density measurement at 280 $m\mu$. Inhibitor activity is expressed in terms of units of trypsin inhibited, and the measurement consists simply in comparing the tryptic activity of two samples of trypsin, one containing a definite amount of inhibitor and the other sample being free of inhibitor. The difference in the tryptic activity of the two samples of trypsin, provided the inhibitor is not in excess, expressed in (I.U.) or in weight of pure trypsin divided by the weight of the inhibitor used is a measure of the specific activity.

Crystalline soybean inhibitor can be used as a convenient standard for assaying samples of trypsin.

Anson method (1938). As described by Borchers et al (1947), the method was based on colour development of tyrosine which was liberated due to the action of the trypsin on the hemoglobin substrate. The colour development agent was dilute phenol of

Folin and Ciocalteu's reagent. The colour intensity was expressed as tyrosine by reference to a standard curve and the inhibitor unit can be calculated from the inhibition of liberated tyrosine when comparing with trypsin standard.

Learmonth method. (Learmonth, 1951, 1952). The method is based on the comparison of the retardation of gelatin liquefaction by trypsin in the presence of various concentrations of trypsin inhibitor. This method has the great advantage of speed and simplicity but for certainty of result it is important to obtain clear distinction between the tube in which proteolytic action had been inhibited and those in which it has not.

Titration method of Wu and Scharaga (1962). The method is based on the direct titration of trypsin with Benzoyl-arginine ethyl ester which is used as a substrate. The method is simple but the equipment is complicated. It needs a special titration apparatus.

Feigenbaum method. (Feingebaum, 1964). This method is a simple method based on direct determination of the action of trypsin on carmine-fibrin substrate. The time of appearance and intensity of the freed colour depends on the activity of the proteolytic enzyme and on the concentration of trypsin inhibitor in the soy bean extract added. Usually 4-6 hours are needed for a distinct red tinting of the carmine-dye developing in the mixture free of trypsin inhibitors or containing too small a quantity to inhibit all the trypsin activity.

For determination trypsin inhibitor in soy noodle, the method of Learmonth (1951, 1952) has been used, for the reason that it was more simple, quicker, and more suitable for using in quality control and reagents are cheaper and easier to obtain than the other mentioned methods.

## Development of Trypsin Inhibitor Determination Method

For the gelation test as described by Learmonth (1951, 1952), 0.25% papain solution was added to a buffered gelatine sol alone or with a papain inhibitor factor in soybean extract. The mixture was incubated at 40°C for 30 mins., cooled rapidly in running water and placed in a refrigerator at 1°C. Hackler et al (1965) used a modified Learmonth method in determining trypsin inhibitor in soymilk. They incubated the buffered gelatine sol with the soy extract at 98°F (36°C) for 15 mins. However these two methods could not be used directly. Therefore, it was necessary to study the variation of experimental conditions in the determination of trypsin inhibitor in soya noodle.

### Experimental procedure

(1) Effect of incubation time on gelatin digestion by trypsin enzyme: The buffered gelatine sol was prepared by dissolving 8 g. of edible quality gelatine in 100 ml of water and adding 1 g. of disodium hydrogen phosphate. 0.25% trypsin inhibitor was added in different quantities. Screwcap test tubes were used and prepared by adding ingredients shown below, in the vertical order given (top first):

| Tube No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Distilled water, ml | 10 | 9.75 | 9.5 | 9.25 | 9.0 | 8.0 | 7.0 | 6.0 |
| 8% gelatin-citrate ml | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 0.25% Trypsin, ml | - | 0.25 | 0.5 | 0.75 | 1.0 | 2.0 | 3.0 | 4.0 |
| TOTAL ml | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |

The tubes were then shaken and incubated in a water bath at 37°C for 15 and 30 mins., cooled rapidly in running water and placed in a crushed ice bath at 1°C. The setting times of the gelatin incubated for 15 mins. and 30 mins, were compared.

(2) Method of extraction of trypsin inhibitor - water extraction versus acid extraction: Unheated soy noodle was finely ground. For water extraction, 1 g. of noodle was shaken with 100 ml water for 20 mins. and left overnight at 37°F. For acid extraction this was suggested using 0.05 N HCl instead of distilled water. Then a series of tubes were prepared as:

| Tube No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Distilled water, ml | 10 | 9.0 | 8.8 | 8.6 | 8.4 | 8.2 | 8.0 | 7.8 |
| 8% gelatin-citrate, ml | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| o.25% trypsin, ml | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| soy extract, ml (acid or water) | - | - | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 |
| TOTAL | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |

The same incubation and cooling conditions were used as in the previous experiment.

(3) Effect of increasing gelatine concentration on setting time: The experiment was carried out the same as previously, except that 8, 10, 11 and 12% gelatin was used for buffer-gelatine sol, and the quantity of 0.25% trypsin was increased.

(4) Effect of variation of trypsin concentration on inhibiting power of trypsin inhibitor: For 5 sets of tubes containing 10% buffered gelatine solution and various quantities of water extract soy bean trypsin inhibitor, the volumes of 0.25% trypsin solution added to each set were 0.6, 0.8, 1.0, 1.2 and 1.5 ml.

(5) <u>Effect of mixing trypsin and the inhibitor prior to adding gelatin</u>: Tubes were set as below:

| Tube No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distilled water, ml | 8.45 | 8.4 | 8.3 | 8.2 | 8.1 | 7.9 | 7.7 | 7.5 | 7.3 | 6.9 | 6.7 | 6.5 |
| 0.25% trypsin, ml | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| soy extract (water extracted) ml | .05 | .1 | .2 | .3 | .4 | .6 | .8 | 1.0 | 1.2 | 1.6 | 1.8 | 2.0 |
| 10% gelatine-citrate, ml | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |

After trypsin and the water extract were added, the tubes were screwed and shaken vigorously before the gelatin-solution was added.

Results and Discussion

(1) <u>Effect of incubation time on gelatin digestion</u>. When the mixture of trypsin and gelatine was incubated for 15 mins. it was found that tubes 3, 4, 5, 6, 7 and 8 set at 20 mins. after they were placed in an ice bath at 1°C. When the mixture was incubated for 30 mins., tubes 3, 4 and 5 set at 21, 26 and 37 mins. respectively and tubes 6, 7 and 8 did not set at all. Tubes 1 and 2 set at 5 and 12 mins. respectively after both incubated at 15 and 30 mins. It was shown from these results that gelatin was more digested by enzyme when incubated for 30 mins. than for 15 mins. The reason why tubes 6, 7 and 8 did not set was that gelatin was completly liquefied by the enzyme action.

(2) <u>Comparison of methods of extracting trypsin inhibitor</u>. From Figure 10, it can be seen that setting time of the gelatin

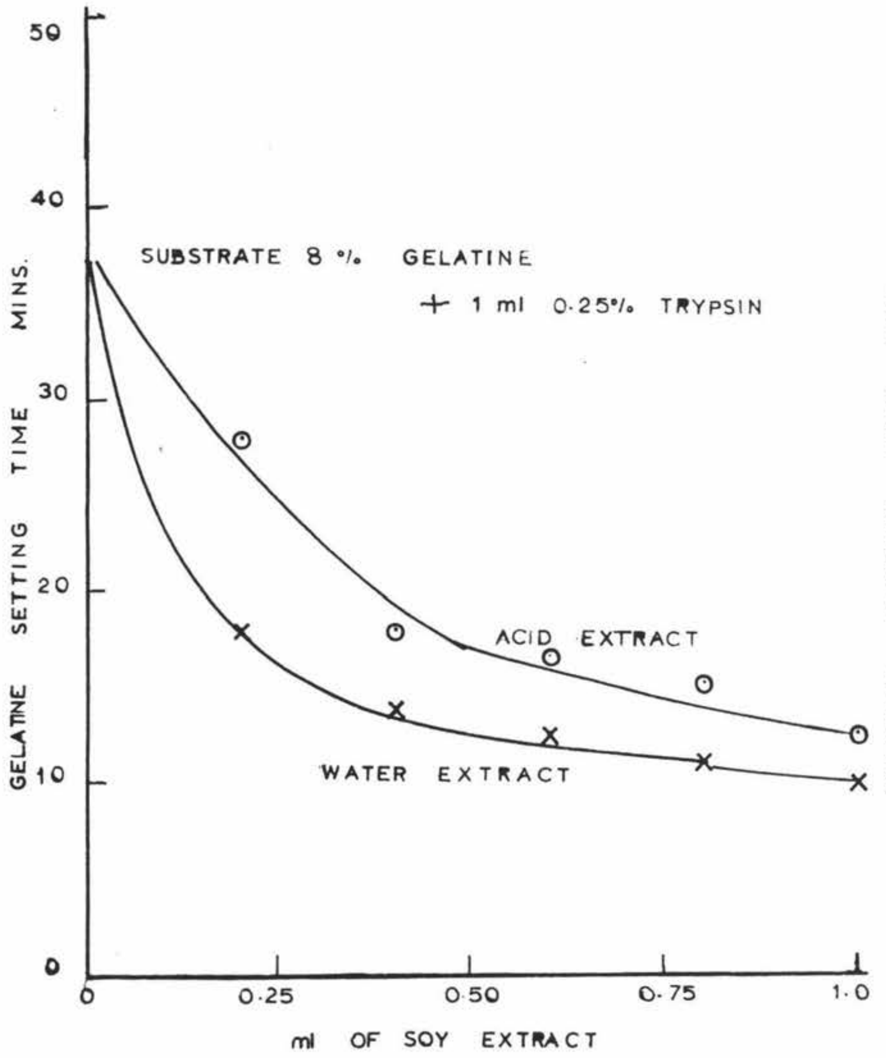



Figure 10 COMPARISION OF SETTING TIME OF WATER EXTRACTION AND ACID EXTRACTION OF TRYPSIN INHIBITOR

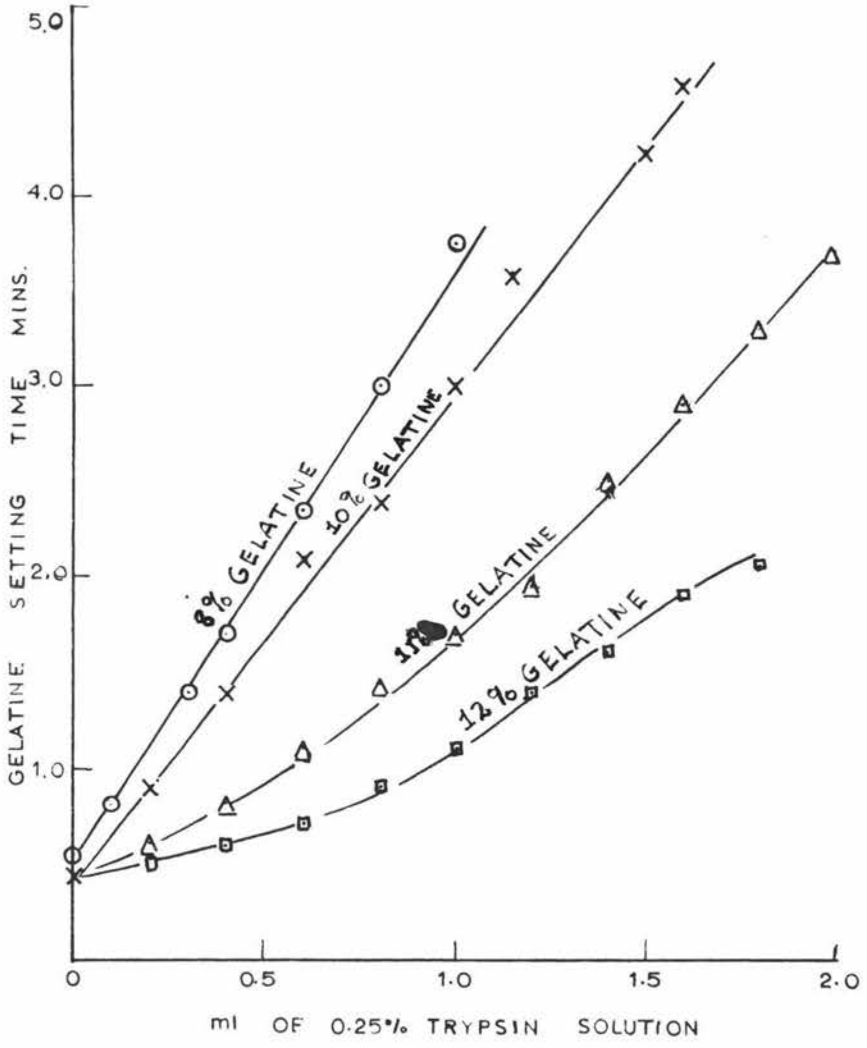



FIGURE 11 EFFECT OF INCREASING GELATINE CONCENTRATION ON GELATION TIME

containing the acid extract was longer than that containing the water extract. It was likely that less of the trypsin inhibitor was extracted by the method of acid extraction than by the water extraction method.

(3) Effect of increasing gelatine concentration on setting time. In figure 11, it was found that with the high concentration of gelatine, the setting time was quicker when the same concentration of trypsin solution was used. The gelatine at the concentration of 8% did not set when more than 1 ml of 0.25% trypsin solution was used. For the 10% gelatine concentration, the volume of 0.25% trypsin solution could be as high as 1.6 ml and the setting time was about 44 mins. When the concentration of gelatin was increased to 11% and 12% with the same concentration of trypsin, the setting times of 28 and 18 mins. were obtained. Therefore the 10% gelatine concentration was chosen. When 1.5 ml of 0.25% trypsin was added, the setting time was about 42 mins., which was the most suitable time for the test. If all the trypsin inhibitor was destroyed, the gelatine set at 42 mins. and when the amount of inhibitor was increased the setting time was shorter, until the inhibitor concentration was in excess of the trypsin present, when the setting time was equal to the control which set in about 5 mins.

(4) Effect of variation of trypsin concentration on inhibiting power of trypsin inhibitor. When volumes of soybean extract were plotted against volumes of trypsin that inhibited by the inhibitor, a linear relationship should have been obtained when trypsin inhibitor was used in low concentrations, and start to level off when the concentration of soy extract is in excess of

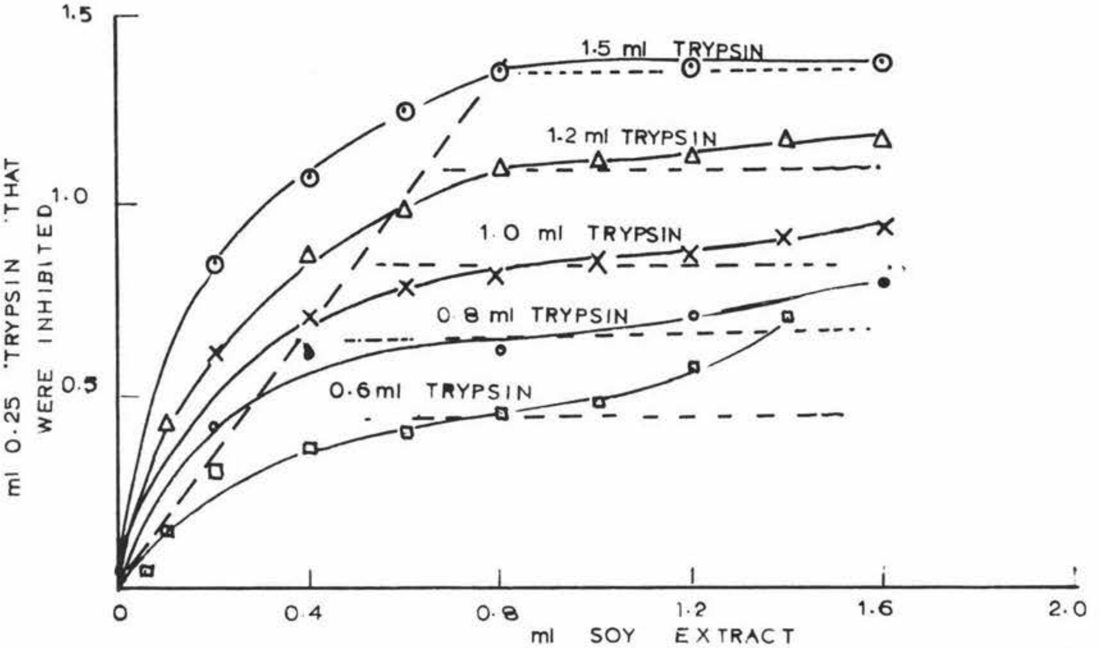



FIGURE 12 EFFECT OF VARIOUS LEVEL OF TRYPSIN CONCENTRATION ON INHIBITION OF SOY EXTRACT

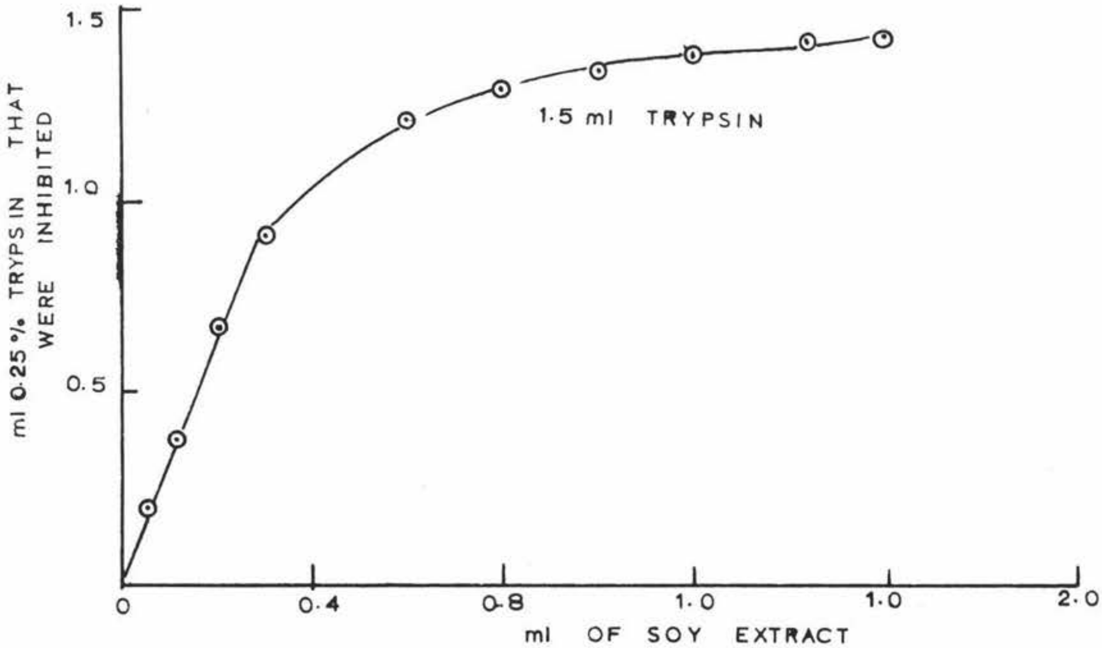



FIGURE 13 INHIBITION EFFECT OF SOYBEAN TRYPSIN INHIBITOR

enzyme as shown by dotted line in Figure 12. In the actual results, the inhibition of soy extract seemed to be increased when the level of trypsin added increased. This might be due to the fault of the experiment in which the solution of trypsin and soy extract were added to the gelatine solution at the same time. Therefore there were two reactions, digestion and inhibition, occurring simultaneously. Or it might be due to the random inhibition of the inhibitor. (Kunitz trypsin inhibitor forms a one-to-one stoichiometric complex with trypsin, Bowman-Birk trypsin inhibitor does not).

(5) Effect of mixing trypsin and inhibitor prior to adding gelatin. Because such problems as above occurred, the experimental procedure was modified so that typsin and soy extract were mixed together first, then gelatine solution was added. In this case, only the trypsin that remained after the inhibition would digest the gelatin. The results obtained from this procedure (Figure 13) showed a straight line when a low quantity of soy extract was used and the inhibition effect levelled off when higher quantities were used. Therefore in the determination of heat destruction of trypsin inhibitor 1 ml of soy extract and 1.5 ml of 0.25% trypsin solution could be used for the assay to satisfy the condition in which the enzyme was in excess of the inhibitor. In addition to this the result would be more accurate when less than half of the activity remained, because the assay would be worked on the straight line part of the graph.

Final Method for Determination Trypsin Inhibitor

The following procedure was finally adopted as the most suitable for the analysis of the trypsin inhibitor in soya noodle.

Trypsin inhibitor was extracted by water extraction method. 1 g. finely ground sample of soya noodle was extracted with 10 ml distilled water in the screw cap test tube. The mixture was shaken vigorously for 20 mins. then left overnight at 37°F. The final volume of the mixture was made to 100 ml, filtered, and 1 ml of the filtrate was used for the assay.

10 percent gelatine buffer solution was used as a substrate. The buffer gelatine solution was made by dissolving 10 g. of edible quality gelatin in 100 ml of water and adding 1 g. of disodium hydrogen citrate.

The 0.25% trypsin solution was prepared by dissolving 0.25 g. of trypsin in 100 ml distilled water, and the solution was kept in a refrigerator until required for use.

In the assay, the standard trypsin assay tubes were prepared as follows:

| Tube No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Distilled water, ml | 10 | 9.8 | 9.6 | 9.4 | 9.2 | 9.0 | 8.8 | 8.5 |
| 10% gelatine-buffer solution, ml | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 0.25% trypsin solution, ml | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.5 |
| TOTAL VOLUME | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

The soy extract assay tubes were prepared by adding 7.5 ml distilled water, 1.0 ml of 0.25% trypsin solution and 1.5 ml of

1/100 soy extract. The tubes were capped and shaken for 2 mins. before 5 ml of gelatine-buffer solution was added.

All of the tubes were shaken and incubated in a water bath at 37°C for 30 mins., cooled rapidly under tap water and placed in an ice bath at 1°C. The setting time was noted.

By comparing the setting time of soy extract with the setting time of the standard trypsin tubes the amount of uninhibited trypsin in the soy extract was estimated. By substracting the amount of uninhibited trypsin from the original amount of trypsin added in the tube, the trypsin equivalent to the trypsin inhibitor was obtained. The activity of trypsin inhibitor remaining after different head treatment was calculated from the original trypsin inhibitor of soya noodle which had not been heat treated.

Heat Treatment of Trypsin Inhibitor in Soya Noodle

Preparation of sample. Noodle from recipe 10 (previous chapter) was steamed at atmospheric pressure for 5, 10, 15, 20, 30, 40, 50, and 60 mins. and autoclaved at 10 lbs per sq.in. pressure for 2, 5, 10, 15, 20, and 30 mins. , then air dried, and the trypsin inhibitor determined as above.

For cooked noodle, the noodle was steamed at 212°F for 15 and 30 mins., then air dried before cooking. After cooking for 5 mins. with the noodle to water ratio of 1:10, the noodle was air dried and the trypsin inhibitor was determined.

Results. Results of heat destruction of trypsin inhibitor under various conditions are shown in Tables IX and X and in Figure 14.

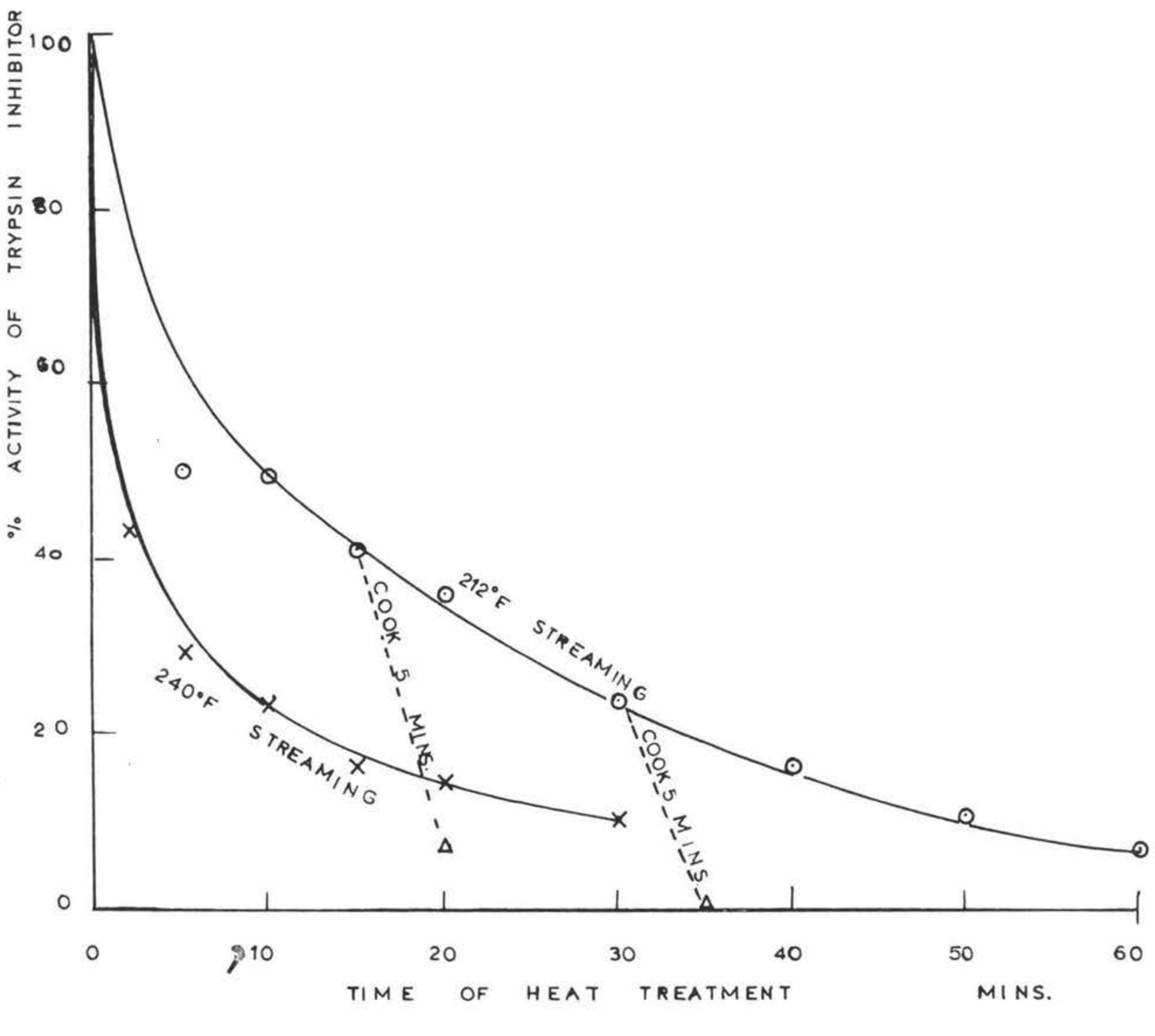



Figure 14 Heat Destruction of Trypsin Inhibitor in Soya Noodle

Table IX. Heat Destruction of Trypsin Inhibitor in Soy Noodle Steaming at 212°F.

| Time of steaming (mins.) | Setting time of gelatine* (mins.) | Trypsin (0.25%) equivalent to setting time (ml) | Trypsin equivalent to inhibitor (ml) | Activity of Inhibitor % |
|---|---|---|---|---|
| 0 | 8 | 0.15 | 1.35 | 100 |
| 5 | 25 | 0.75 | 0.75 | 55.5 |
| 10 | 27 | 0.83 | 0.67 | 50.6 |
| 15 | 30 | 0.94 | 0.56 | 41.7 |
| 20 | 32 | 1.02 | 0.48 | 36.2 |
| 30 | 36.5 | 1.18 | 0.32 | 24.0 |
| 40 | 39 | 1.28 | 0.22 | 16.65 |
| 50 | 43 | 1.35 | 0.15 | 11.10 |
| 60 | 44 | 1.40 | 0.10 | 7.40 |

* The setting time of gelatine Blank solution was 5 mins. and standard trypsin 45 mins.

From the results of heat destruction of trypsin inhibitor, it was impossible by this method to destroy all of the inhibitor. The rate of destruction was decreased when the time of heating was increased and also at severe heat treatment the noodle suffered from browning. Steaming the noodle beyond 30 mins. and autoclaving more than 5 mins. caused the noodle to become a darker colour.

Cooking the noodle for 5 mins. before eating reduced the activity of the trypsin inhibitor by about 30%, therefore, in practice, the noodle should be steamed at 212°F for 20 mins. The remaining activity will be destroyed after cooking for 5 mins. or longer.

Table X. Heat Destruction of Trypsin Inhibitor in Soy Noodle Autoclaving at 10 lbs pressure (240°F).

| Time of auto-claving (mins.) | Setting time of gelatine* (mins.) | Trypsin (0.25%) equivalent to setting time (ml) | Trypsin equivalent to inhibitor (ml) | Activity of Inhibitor % |
|---|---|---|---|---|
| 0 | 8 | 0.24 | 1.26 | 100 |
| 2 | 33 | 0.95 | 0.55 | 43.5 |
| 5 | 35 | 1.13 | 0.37 | 29.6 |
| 10 | 38 | 1.23 | 0.27 | 21.7 |
| 15 | 39.5 | 1.29 | 0.21 | 16.6 |
| 20 | 40 | 1.31 | 0.19 | 14.85 |
| 30 | 41.5 | 1.36 | 0.14 | 10.90 |
| Noodle steamed 15 mins. and cooked 5 mins. | 42 | 1.40 | 0.10 | 7.8 |
| Noodle steamed 30 mins. and cooked 5 mins. | 45 | 1.50 | 0 | 0 |

* The setting time of gelatine Blank solution was 5 minutes and standard trypsin 45 minutes.

## Discussion

The rate of inactivation of trypsin inhibitor in soya noodle when autoclaving at 10 lbs pressure was faster than when steaming at atmospheric pressure. Autoclaving for 5 mins. destroyed 70% of the activity of trypsin inhibitor but for steaming to destroy the same amount of the activity of trypsin inhibitor, 20 mins. was needed. However, the noodle autoclaved for 5 mins. was darker in colour than that from steaming for 20 mins. Therefore in this case it was preferred that the noodle was steamed for 20 mins. instead of autoclaving for 5 mins.

For the other growth impairment substances such as hemagglutinin, since the hemagglutinin activity is more readily destroyed by moist heat than is the trypsin inhibitor (Leiner, 1953), therefore steaming soya noodle for 20 mins., then cooking for 5 mins. or longer should completely destroy the hemagglutinin activity in soya noodle.

## VI. CHEMICAL COMPOSITION OF SOYA NOODLE

This study was carried out for the purpose of obtaining a general knowledge of the composition of the soya noodle so that the nutritive value could be evaluated.

### Preparation of Sample

Soya noodle, prepared according to the previous method, was ground to a fine powder until all the noodle passed through sieve B.S. 25, and the ground sample was used for analysis.

### Method of Analysis

Moisture, ash, crude fibre, calcium, phosphorous and iron were determined according to the AOAC Methods of Analysis (AOAC, 1967). Fat was determined as "ether-extract" by extracting for 16 hr. with petroleium ether (b.p. 40 - 60°C), in a Soxhlet apparatus. Nitrogen in the sample was determined by semi-micro Kjeldahl method.

Thiamine was estimated by the Thiochrome method, riboflavin by the simplified fluorescence method and niacin by cyanogen bromide method, according to AACC Cereal Laboratory Methods (AACC, 1962).

### Results

The composition of 100 g. soya noodle is shown in Table XI.

Table XI. Proximate Analysis of 100 g. Soya Noodle

| | |
|---|---|
| Moisture | 8.10 g. |
| Protein | 29.80 g. |
| Ether extract | 11.15 g. |
| Crude fibre | 1.30 g. |
| Ash | 3.70 g. |
| Carbohydrate (by difference) | 45.95 g. |
| Calcium | 89.20 mg. |
| Phosphorus | 289.00 mg. |
| Iron | 6.90 mg. |
| Thiamine | 0.12 mg. |
| Riboflavin | 0.58 mg. |
| Niacin | 1.60 mg. |

## Discussion

From the results of this analysis, the protein content of soya noodle agreed with the purpose of the project of minimum protein content 30 percent. Moisture content was lower than ordinary macaroni products which are generally dried to about 13 percent moisture content. Crude fibre, thiamine and niacin content of the soya noodle were rather low when compared with those of full fat soyflour prepared by Mustakas (1966), and the riboflavin content was higher. Ether extract and ash content were comparable with his results. Allowing for the fact that minerals content of wheat flour and rice flour are lower than soy flour, soya noodle is a good source of calcium, phosphorus an iron when compared with ordinary noodles.

# VII AMINO ACID COMPOSITION OF SOYA NOODLE

The quality of protein is greatly influenced by its content of amino acid, required for essential metabolic functions (FAO, 1965). Since soybean protein has been reported as having limiting sulfur amino acids (Milner 1966, FAO 1965, Evans and Bandermer 1967), it was necessary to determine the amino acid composition of the soya noodle to ascertain its nutritive value.

## Preparation of Sample

Ground soya noodle, prepared as in the previous chapter, was previously defatted in a soxhlet extracter with petroleum ether, b.p. 40 - 60°C, and air dried before analysis. Nitrogen content of the defatted sample was determined by the method described before.

## Methods

Acid hydrolysis. Direct hydrolysis was carried out with 6 N HCl to obtain a hydrolysate suitable for analysis of all amino acids except for cystine and cysteine and tryptophan, as Tkachuk and Irvine (1969).

Hydrolysis of a sample, containing 36 mg. protein, with 36 ml 6N HCl was carried out in a 40 ml pyrex tube. The mixture was frozen in liquid air and the tubes were evacuated with an Edwards vacuum pump to 0.1 mm of mercury and sealed under vacuum. The sample was hydrolysed in the sealed tube at 110 ± 2°C in a hot air oven for 24 hrs.

After hydrolysis, the insoluble humin was removed by filtration through Whatman No.52 filter paper. The residue was washed with a small volume of distilled water. The acid was removed from the hydrolysate by evaporating the hydrolysate under vacuum to near dryness in a rotary evaporater, followed by further evaporation with 3 small volumes of added water. The volume of the residue was made up to 25 ml. with pH 2.2 sodium citrate buffer. The sample was kept frozen until analysis.

For the determination of cystine and cysteine, the sample was first oxidised with performic acid to convert cystine and cysteine to the more stable derivative, cysteic acid, as described by Schram et al (1954), then subjected to acid hydrolysis as above.

Alkaline hydrolysis. Alkaline hydrolysis of protein is used to determine amino acids that are labile to acid, particularly tryptophan. To 36 mg protein, 2 gm of $Ba(OH)_2.8H_2O$ previously ground to a fine powder were added followed by 1 ml. water. After the mixture was frozen, the test tube was evacuated and sealed as before. Hydrolysis of the sample was carried out at 110 $\pm$ 2°C in a hot air oven for 20 hrs. After hydrolysis, the hydrolysate was prepared for analysis according to the method of Miller (1967). The cold hydrolysate was neutralised with 6 N HCl and the pH adjusted to pH 2. $Ba^{++}$ was removed as barium sulphate by precipitating with sodium sulfate and centrifuging at 2700 x g. The residue was washed with 10 ml water and recentrifuged. The supernatant was concentrated under vacuum to 25 ml volume and used for analysis.

Amino acid analysis. Analysis for amino acid content was carried out with a Beckman-Spinco model 120 C amino acid analyser, modified for 2 hrs. accelerated analysis. In the accelerated analysis, the custom spherical resin type PA 35 was used for the separation of the basic amino acids, and type UR 36 for the fractionation of the acidic and neutral amino acids. The operating conditions for the analysis are shown in Appendix IX.

A short column, 5.5 cm, was used for separation of tryptophan, lysine, histidine, ammonia and arginine in the alkaline hydrolysate. A long column 36 cm was used for separating the acid and neutral amino acid in the acid hydrolysate. The sodium citrate buffer pH 5.359 ± 0.010 was used for basic analysis and the buffer pH 5.488 ± 0.005 was used for acidic and neutral analysis.

The operational principle was based on elution chromatography from buffered columns of ion-exchange resin. The separated components were subjected to reaction with ninhydrin and the coloured complex quantitatively measured with a colorimeter. The resultant peaks were recorded by a strip chart recorder.

Computation of results. Integration by height-time-width method was used. The peak on the effluent curves were integrated by multiplying the height of the peak by the width at half the height. The height of the peak on the chart was measured, and the net height was obtained by correcting for the base line. The width of the peak at half-height was then measured by a simple method of width measurement which consisted of counting the number of dots on the chart above the half-height position.

For the final calculations, the net height H of the peak is multiplied by W, the width at half-height. The constant C, by which H x W is divided to give micro mole of a given amino acid is determined by multiplying the area of 1 $\mu$ mole norleucine, which was used as standard, with the factor F. Factor F is the area of 1 $\mu$ mole of a given amino acid to the area of 1 $\mu$ mole norleucine 0.2 ml of norleucine containing 0.2 $\mu$ mole was added together with the hydrolysate sample to the long column and the area of 1 $\mu$ mole norleucine was determined for each run of the long column.

## Results

The amino acids of soya noodle recovered from the hydrolysis methods are shown in Table XII. The amino acid analysis of duplicated samples reported are the results of analysis of 24 hr. acid hydrolysate, acid hydrolysate from a sample previously oxidised by performic acid to recover cystine and cysteine quantitatively in the form of cysteic acid, and barium hydroxide hydrolysate for quantitative recovery of tryptophan.

Lysine, histidine and ammonia content were very low when using alkaline hydrolysis and arginine was completely destroyed by this method. Performic acid oxidisation gave a higher result of aspartic acid, threonine, serine and glycine than non-oxidation method. Half cystine reported in HCOOH sample column was the recovery of cysteic acid.

Table XII. The Amino Acid Recoveries from Soya Noodle, by Acid and Alkaline Hydrolysis

g. amino acid per 16 g. N

| Amino acid | 24 hrs. 6 N HCl hydrolysis | HCOOOH sample acid hydrolysis | Alkaline hydrolysis | Maximum value |
|---|---|---|---|---|
| Tryptophan | | | 0.40 | 0.40 |
| Lysine | 6.39 | | 5.36 | 6.39 |
| Histidine | 1.67 | | 0.97 | 1.67 |
| Ammonia | 2.16 | | 1.37 | 2.16 |
| Arginine | 6.76 | | 0.00 | 6.76 |
| Aspartic acid | 8.73 | 10.28 | | 10.28 |
| Threonine | 2.73 | 2.99 | | 2.99 |
| Serine | 3.43 | 4.12 | | 4.12 |
| Glutamic acid | 19.87 | 18.93 | | 19.87 |
| Proline | 8.58 | 6.63 | | 8.58 |
| Glycine | 2.84 | 3.02 | | 3.02 |
| Alanine | 3.43 | 2.94 | | 3.43 |
| Half cystine | 1.25 | 1.41 | | 1.41 |
| Valine | 5.42 | 5.36 | | 5.42 |
| Methionine | 0.82 | | | 0.82 |
| Isoleucine | 5.60 | 3.55 | | 5.60 |
| Leucine | 6.45 | 5.90 | | 6.45 |
| Tyrosine | 2.59 | | | 2.59 |
| Phenylalanine | 3.81 | | | 3.81 |

To obtain the maximum results for the amino acid composition of soya noodle, the value of tryptophan was obtained from alkaline hydrolysis; aspartic, threonine, serine, glycine and half cystine from HCOOOH and acid hydrolysate and the remainder from 24 hr. 6 N HCl hydrolysates. The comparison of amino acid compositions of soya noodle with soybean meal and Selkirk wheat analysed by Tkachuk and Irvine (1969) and with the calculated amino acids composition of soya noodle are illustrated in Table XIII.

Table XIII. Amino Acid Composition of Soya Noodle from Analysis and Calculation, Comparing with the Amino Acid Composition of Soybean Meal and Selkirk Wheat Analysed by Tkachuk and Irvine (1969)

g. amino acid per 100 g. sample N

| | Soya noodle (from calculation)* | Soya noodle from analysis | Soybean meal | Selkirk wheat |
|---|---|---|---|---|
| Tryptophan | 10.15 | 2.50 | 11.10 | 9.55 |
| Lysine | 34.93 | 39.94 | 38.30 | 14.50 |
| Histidine | | 10.44 | 17.00 | 13.80 |
| Ammonia | | 13.50 | 10.50 | 22.30 |
| Arginine | | 42.25 | 42.20 | 24.90 |
| Aspartic acid | | 64.25 | 70.80 | 29.20 |
| Threonine | 21.70 | 18.69 | 22.10 | 17.30 |
| Serine | | 25.75 | 29.00 | 31.30 |
| Glutamic acid | | 124.19 | 113.00 | 207.00 |
| Proline | | 53.63 | 32.10 | 69.60 |
| Glycine | | 18.88 | 26.30 | 23.50 |
| Alanine | | 21.44 | 26.10 | 20.40 |
| Half cystine | 12.97 | 8.82 | 12.90 | 16.20 |
| Valine | 33.19 | 33.89 | 33.30 | 27.90 |
| Methionine | 9.57 | 5.13 | 8.08 | 10.50 |
| Isoleucine | 2[illegible].15 | 35.00 | 29.60 | 23.90 |
| Leucine | 43.83 | 40.31 | 44.00 | 42.00 |
| Tyrosine | 20.00 | 16.19 | 19.00 | 16.70 |
| Phenylalanine | 29.57 | 23.81 | 28.80 | 29.70 |
| N recovery, % | | 89.36 | 91.30 | 96.40 |

* The amino acid composition of soyflour and wheat from Tkachul Irvine (1968) and of egg from FAO (1965).

## Discussion

Generally, the amino acid compositions of soya noodle from the results of analysis agreed quite well with those of calculated results and soybean meal and wheat flour reported by Tkachuk and Irvine (1969) except for the low result of tryptophan in soya noodle. This might be due to either the destruction of tryptophan during hydrolysis in the presence of starch or absorption of tryptophan during the removal of barium sulphate as reported by Miller (1967).

The recoveries of histidine, threonine, serine, glycine, cystine, methionine, leucine, tyrosine and phenylalanine are lower than those reported by Tkachuk and Irvine (1969) and from calculation. Usually amino acids differ in the ease with which they are liberated from peptide linkage during acid hydrolysis. Valine and isoleucine required 48 and 96 hr. hydrolysis to obtain an optimum liberation (Blackburn, 1968). Sincethe volume of acid used for hydrolysis in this experiment is higher than reported in other literature, high results of valine and isoleucine were obtained. Therefore low recoveries of threonine, serine, tyrosine and methionine may be due to decomposition which may have occurred during hydrolysis. Low recoveries of other amino acids might be due to the differences in samples grown in different environments and locations.

The recovery of lysine is high probably resulting from the degradation product of tryptophan. As reported by Blackburn (1968), when hydrolysis is conducted in a sealed tube, some tryptophan and an acid decomposition product derived from it are usually present after 24 hrs. and give rise to two peaks on the short column just in front of lysine, and on several occasions, a small peak was noted in the lysine region when analysing peptides which contained tryptophan but no lysine.

# VIII. SUPPLEMENTATION OF SOYA NOODLE WITH VITAMINS, MINERALS AND AMINO ACID

During the preliminary development work, vitamins and minerals were added to soya noodle at the levels according to the standard of nutrient supplement for noodle (National Academy of Science, 1956). Now that the chemical compositions and amino acid contents of soya noodle had been analysed it was possible to look at the supplementation in more detail. The product is intended to be consumed by Thai peiple as a high quality protein product and as a source of other nutrient supplements, therefore it was important to reconsider the amount and type of vitamins and minerals needed to be added to the product and to consider the possibility of supplementation of soya noodle with the limiting amino acids to improve the protein quality.

## Vitamin and Mineral Supplementation

Literature review of nutrition surveys and deficiency diseases in Thailand. In 1960, as reported by Halstead and Valyasevi (1967), a country-wide nutrition survey by the United States Inter-departmental Committee on Nutrition for National Defence (ICNND) was carried out. They found that the most frequent nutritional deficiency syndromes recognised in Thailand are iodine-deficiency goitre and iron-deficiency anaemia. Beriberi during pregnancy and beriberi heart disease of infants are known, the latter possibly being an important cause of infant mortality. Xerophthalmia and other vitamin A deficiency syndromes appeared to be very rare. Rickets and scurvy were not reported. Angular stomatitis, a lesion seen in up to 12% of rural Thai children, may be related to riboflavin undernutrition, although this has not yet been adequately established.

Studies of adults living in three villages near Uborn city by this survey showed that by United States standard Uborn diets were deficient in riboflavin, low in vitamin A, C and thiamine and also low in calcium but adequate in niacin. Recent studies in Uborn village have implicated low phosphate intakes with the occurrence of bladder stone disease (Dhanamitta et al, 1967).

According to the report of Thanangkul and Whitaker (1966), since a nutrition survey was carried out in 1950 by the Nutrition Division of the Ministry of Public Health, Cheingmai has been considered a thiamine deficient area. Cheingvai which is 320 kilometres north-east of Chiengmai had a 24 percent incidence of peripheral neuropathy among adults and adolescents, which is the indication of thiamine deficiency. In 1964 fortyfive suspected cases of childhood beriberi were encountered in Cheingmai hospital and two of this number were shown to have beriberi. Thus, there is no question that Chiengmai Province must still be considered a thiamine deficient area.

From the above review of nutrition surveys, it seems that among the vitamin deficiency diseases, thiamine is the most serious one and this occurs mostly in the northern part of Thailand. The next important one is riboflavin which was reported as deficient in the diet of the North-eastern people. Niacin, ascorbic acid, and vitamin A deficiencies cause only a slight problem. Iron, iodine, calcium and phosphorus are the important minerals that are always lacking in the Thai diet.

Proposal of dietary standard for Thailand. In order to calculate the amounts of vitamins and minerals to be added to the soya noodle, a standard for the daily average of the quantities of certain nutrients believed to be used by an individual person was required. Since the dietary needs of a human population varies with the individual's physique, age, sex, degree of muscular activity and environmental condition, the nutrient requirement of the people in different regions of the world will not be the same. Thus each country should have a dietary standard of their own. Because of the failure to find out whether a dietary standard had been set for the Thai population, the ICNND (1960) used the United States standard and Bisolyaputra (1957) used the Philippine's standard. However, it was thought to set up a new standard for Thailand rather than using the standards of other countries.

A man of 25 years of age, 55 kg in weight, at light work was chosen as a reference man. Requirement of energy as calories per day was calculated according to calorie requirement of FAO (1957) in relation to activity and also to weight as 1400 K Cal. For fat, the British and Canadian standards hold to a minimum of 25% of total calories in order to conform to present dietary patterns. The current level of consumption in Japan is 10% (Beaton and McHenry, 1966). Therefore about 16% of calories i.e. 50 g. fat was used for Thai dietary standard. The standard for carbohydrates was based on the assumption that 75% of total calories should be supplied by carbohydrates. Thus the minimum requirement of carbohydrate for the reference man per day was 525 g.

The requirement of thiamine, riboflavin and niacin, based on the FAO (1965)* recommendation were respectively 0.40, 0.55 and 6.0 mg per 1000 calories per day.

The amount for calcium and phosphorus of 800 mg per day was used according to the Recommended Dietary Allowance, National Academy of Science (1968), and the ratio of one to one of calcium to phosphorus agreed well with the average Thai daily per capita intake data of Valyasevi et al (1967) in which the intake of phosphorus was about 775 mg per day (as in Appendix IV).

For iron, the requirement of adult females 9-12 mg/day was used, as in the Recommended Dietary Allowance, National Academy of Science, (1968) because of the need of female for higher iron.

The standard for protein was based on a requirement for ideal protein according to the National Academy of Science, in which 20 mg of ideal protein is required per basal k cal plus allowance for individual variability of 30 percent. The basal k cal requirement for 55 kg reference man was calculated from FAO (1957) which is 1400 k cal. Therefore the requirement of ideal protein plus 30% allowance is 35,4 g. Taking the utilisation value (N.P.U.) as 65 percent for actual food protein, therefore the requirement of protein became 54 g./day which was approximately 1.0 g. of protein per kg body weight per day for a 55 kg reference Thai man.

Therefore a dietary standard for a reference Thai man was proposed as in Table XIV, where it is compared with the standard for reference man of other nations. In comparison with the dietary standard of the other countries, the Thai standard is comparable with India's in the age, weight and calories needed by the reference man. For the daily requirement of protein, different

**Table XIV.** Proposed Dietary Standard for Reference Thai Man compared with the Standards of Different Countries (daily intake)

| Country | Sex | Age (years) | Weight (kg) | Energy (k cal) | Protein (g.) | Ca (g.) | Iron (g.) | Vit.A (I.U.) | B1 (mg) | Ribo-flavin (mg) | Niacin (mg) | Ascorbic acid (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Thai (Proposal) | M | 25 | 55 | 2800 | 35*<br>54** | 0.8 | 10 | - | 1.1 | 1.5 | 18.5 | - |
| (From Recommended Dietary Allowance - National Academy of Science, 1968) | | | | | | | | | | | | |
| FAO | M | 25 | 65 | 3200 | 46 | 0.4-0.5 | | | 1.3 | 1.8 | 21.1 | |
| | F | 25 | 65 | 2300 | 39 | 0.4-0.5 | | | 0.9 | 1.3 | 15.2 | |
| U.S.A. | M | 22 | 70 | 2800 | 65 | 0.8 | 10 | 5000 | 1.4 | 1.7 | 18 | 60 |
| | F | 22 | 58 | 2000 | 55 | 0.8 | 18 | 5000 | 1.0 | 1.5 | 13 | 55 |
| India | M | 25.4 | 55 | 2800 | 55 | | | | | | | |
| | F | 21.5 | 45 | 2300 | 45 | | | | | | | |
| Japan | M | 26-29 | 56 | 3000 | 70 | 0.6 | 10 | 2000 | 1.5 | 1.5 | 15 | 65 |
| | F | 26-29 | 49 | 2400 | 60 | 0.6 | 10 | 2000 | 1.2 | 1.2 | 12 | 60 |
| Phillipines | M | None | 53 | 2400 | 53 | 0.5 | | 5000 | 1.2 | 1.2 | | 70 |
| | F | Specified | 46 | 1800 | 46 | 0.5 | | 5000 | 0.9 | 0.9 | | 70 |

* ideal protein

** approximately 1.0g/kg body weight when protein came from vegetable origin (65% NPU) countries have different bases for calculation. FAO based on 0.7-0.8 g/kg body weight, India based on 1.0g/kg body weight, U.S.A. based on 0.9 g/kg body weight assuming 70% NPU of protein and a 70 kg. reference man. The protein requirement of the countries that have most of the protein supply coming from animal sources will be lower than that of the countries where the supply is based on cereal or vegetable protein. The requirements for vitamins and minerals of the proposed Thai dietary standard is comparable with the other countries, except for the calcium requirement of FAO which is too low. This proposed standard was used as a basis for calculation of nutrient supplementation of soya noodle.

Calculation of vitamin and mineral supplementation of soya noodle. It was assumed that 200 g. of dry soya noodle would be consumed per day by the reference man, instead of 200 g. cooked rice. The reason for using 200 g. of dry soya noodle instead of 200 g. of cooked noodle in comparing with 200 g. cooked rice was that, after the rice was cooked and water was allowed to evaporate the volume of cooked rice would be nearly the same as the volume of 200 g. of dry noodle after soaking in hot water. Therefore the reference man could eat 200 g. of dry noodle in place of 200 g. cooked rice.

**Table XV. Comparison of Thai Food Intake with Proposed Dietary Standard**

| | Dry Soya Noodle 200 g. (400g. cooked noodle) | Cooked Rice** (200g.) (70g. raw rice) | Average daily per capita consumption of Uborn Province (Valyasevi et al, 1967) | Proposed Dietary Standard (daily per capita consumption) | Uborn diet plus 200g. soya noodle instead of rice (daily per capita consumption) |
|---|---|---|---|---|---|
| Calories | 818* | 244 | 1853 | 2800 | 2427 |
| Fat (g.) | 22.30 | 0.6 | Not reported | 50 | not calculated |
| Protein (g.) | 59.60 | 4.2 | 54.8 | 54 | 110.2 |
| Carbo-hydrate (g.) | 91.80 | 59.2 | Not reported | 525 | not calculated |
| Calcium (mg.) | 178.40 | 2.6 | 306 | 800 | 481.8 |
| Phosphorus (mg.) | 578.00 | 68.0 | 760 | 800 | 1270.0 |
| Iron (mg.) | 13.80 | 0.32 | 10 | 10 | 23.48 |
| Thiamine (mg.) | 0.24 | 0.02 | 0.45 | 1.10 | 0.67 |
| Ribo-flavin (mg.) | 1.16 | 0.02 | 0.40 | 1.50 | 1.54 |
| Niacin (mg.) | 3.20 | 0.60 | 13.0 | 18.5 | 15.6 |

* from calculation
** from McCance and Widdowson (1960)

The figures of the last column were obtained by addition of nutrient of 200 g soya noodle that was extra to that of 200 g cooked rice to the average per capita consumption of Uborn Province. The result was the average per capita of food consumption when 200 g soya noodle was used instead of the same amount of rice.

In comparing the amount of nutrient intake (last column) with the standard requirement, it appeared that the calorie intake was about 86% of the requirement. There is no data available for fat intake of the Uborn Province. Soya noodle contributes nearly half of the requirement of fat. Calories could be increased by using fat in the cooking of soya noodle. Soya noodle contains low carbohydrate but this will be amply supplied by the other cereals and starchy food in the diet. Therefore it was decided not to add further fat to increase the calorie and fat intake.

Total calcium intake was lower than the requirement, therefore it was decided to add 400mg of calcium to 200 g soya noodle to comply with the standard requirement and make the ratio of calcium to phosphorus 1:1 in soya noodle according to the suggestion of the Recommended Dietary Allowance of the National Academy of Science, (1968). The high level of phosphorus intake when compared with the requirement will be beneficial to the problem of bladder stone disease.

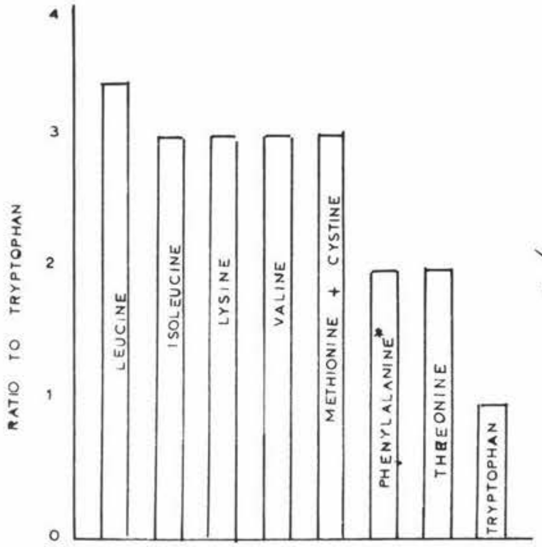
Iron obtained from only the consumption of soya noodle was enough for the requirement. There will be no problem of anaemia when this amount of soya noodle is consumed each day.

The amount of riboflavin in the soya noodle satisfied the requirement. Thiamine and niacin in both noodle and daily diet of the Uborn people were low, therefore thiamine and niacin needed to be supplemented. So that 200 g. noodle would supply half of the daily thiamine requirement, 0.40 mg of thiamine needed to be added to 200 g. noodle. When considering losses during processing and cooking of the supplemented vitamin, an allowance must be made. It was found from the literature that about 50% of thiamine in enriched macaroni was lost during cooking (Bunting, 1965) but if macaroni was cooked in a small amount of water the loss would be small. It was decided that 30% of thiamine enrichment in soya noodle whould allow for steaming and cooking loss. Therefore 0.57 mg needed to be added to 200 g soya noodle instead of 0.40 mg. With the same basis of calculation, 7.5 mg of niacin needed to be added to 200 g soya noodle allowing for 20% loss during processing and cooking.

## Amino Acid Supplementation

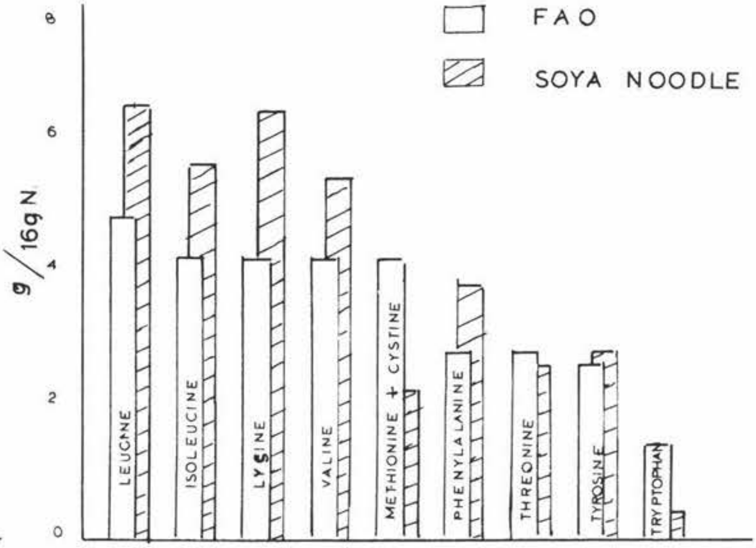
A reference amino acid pattern. In 1957, the F.A.O.Committee on protein requirement placed the emphasis on meeting the essential amino acid requirement (F.A.O. 1965). A certain amount of each essential amino acid is required for maintenance; additional nitrogen supplied by miscellaneous groups of materials is required for synthesis of the non-essential amino acids. Amino acids have many complex inter-relationships, they share certain metabolic pathways and compete with one another for the absorbtion. An unbalanced pattern may have an adverse effect on nutrition even if the quantity of essential amino acids is sufficient for a normal individual. The problem of improving protein quality by supplementation with other protein or with pure amino acids then becomes a problem of approaching as ideal amino acid pattern in which undesirable interactions, competitions, and excesses are reduced to a minimum. Thus the FAO Committee proposed a provisional pattern as a standard of reference for correcting unbalanced diets as shown in Table XVI. In the reference pattern that has been proposed, the proportion of each amino acid has been expressed in relation to tryptophan which is required in the least amount by humans. Tryptophan concentration in the diet may be set arbitrarily as one and the others listed in

Figure 15a Provisional Pattern of Requirements for Essential Amino Acid Expressed in Relation to Tryptophan (Altschul, 1965)




* ASSUME A RATIO OF TYROSINE TO TRYPTOPHAN OF ABOUT 2

Figure 15b Comparion Between Essential Amino Acid of Soya Noodle and the FAO, 1957 Pattern



terms of their proportion to tryptophan. This constitutes a proportional pattern of amounts of the essential amino acids needed for the best performance, and this is sometimes called an aminogram (Altschul, 1965). Any serious deviation from this pattern will reduce the nutritional value of the protein. The FAO provisional pattern is shown in Figure 15a.

Comparison of amino acid composition of soya noodle with FAO provisional pattern. The essential amino acid content of soya noodle expressed as g amino acid per 16 g N is shown in Table XVI together with the FAO provisional amino acid pattern.

Table XVI. Essential Amino Acids in 1957 FAO Provisional Pattern and in Soya Noodle

g of amino acid per 16 g N.

| Amino Acid | FAO 1957 Provisional Pattern | | Soya Noodle | |
|---|---|---|---|---|
| Isoleucine | | 4.2 | | 5.6 |
| Leucine | | 4.8 | | 6.5 |
| Lysine | | 4.2 | | 6.4 |
| Total "aromatic" amino acid | 5.6 | | 6.4 | |
| Phenylalanine | | 2.8 | | 3.8 |
| Tyrosine | | 2.8 | | 2.6 |
| Total sulfur-containig amino acid | 4.2 | | 2.2 | |
| Cystine | | 2.0 | | 1.4 |
| Methionine | | 2.2 | | 0.8 |
| Threonine | | 2.8 | | 3.0 |
| Tryptophan | | 1.4 | | 0.4 |
| Valine | | 4.2 | | 5.4 |
| Total essential amino acid | | 31.4 | | 35.9 |

In comparison with the FAO pattern, although the total essential amino acid of soya noodle was higher, the sulfer containing amino acids are much lower. With regard to tryptophan, the low amount of tryptophan in the noodle might be due to the error of the analysis as explained in a previous chapter; because there is no report of tryptophan as limiting in soybean protein. Therefore it was decided not to supplement with tryptophan.

Since the quantity of cystine and methionine in soya noodle was only half as much as required, and assuming that there is sufficient addition of nitrogen to satisfy the requirement for synthesis of the non-essential amino acids, then if soya noodle was eaten in sufficient quantity to satisfy the need for tryptophan, all the other requirements, but that of cystine and methionine, would be met but almost twice as much would need to be consumed to satisfy the cystine and methionine requirement. And, if only the amount of soya noodle equal to the amount of ideal protein (35 g) required was consumed, then only 50% of the other amino acids could be used as a source of amino acids to support protein growth. The remainder would serve only for energy in the same manner as carbohydrate. This is shown in Figure 15b.

In Table XVII, the amount of essential amino acids required by a reference man was compared with those supplied by soya noodle.

The essential amino acids required for a reference man per day in the first column was calculated for 35 gm of ideal protein by using the reference amino acid pattern of FAO. The total amino acid content of 200 g. soya noodle is presented in the second column. The figure in the third column was obtained from the assumption that the net protein utilisation of soya noodle was 70 percent, and the last column was obtained by comparing this figure of 70% with the requirement.

Table XVII. Comparison of the Requirement for Essential Amino Acids and the Essential Amino Acid Content of 200 g. Soya Noodle

| Essential Amino Acid | Requirement per day (g.) | Soya noodle 200 g. supplied (g.) | Soya noodle 200 g. 70% NPU* (g.) | Ratio of Soya noodle to the requirement % |
|---|---|---|---|---|
| Isoleucine | 1.47 | 3.30 | 2.30 | 156 |
| Leucine | 1.68 | 3.80 | 2.67 | 159 |
| Lysine | 1.47 | 3.78 | 2.65 | 152 |
| Phenylalanine | 0.98 | 2.26 | 1.59 | 162 |
| Tyrosine | 0.98 | 1.42 | 1.0 | 102 |
| Total aromatic amino acids | 1.96 | 3.68 | 2.59 | - |
| Cystine | 0.70 | 0.84 | 0.59 | 84 |
| Methionine | 0.70 | 0.48 | 0.33 | 47 |
| Total S-amino acids | 1.40 | 1.32 | 0.92 | 65 |
| Threonine | 0.98 | 1.76 | 1.23 | 126 |
| Tryptophan | 0.49 | 0.24 | 0.18 | 36** |
| Valine | 1.47 | 3.30 | 2.36 | 156 |

* NPU Net Protein Utilisation

** Low ratio of tryptophan in soya noodle to the requirement was due to experiment error.

By comparing the total amino acid content of 200 g. soya noodle with the amino acid requirement, it appeared that all the required amounts of the amino acids except methionine and tryptophan was met by the soya noodle. As has been mentioned before, the low content of tryptophan was due to an analysis error. Therefore only methionine was of a lower quantity than the requirement. But when it was considered that only 70% of these amino acids in soya noodle could likely be utilised, the cystine also became a limiting amino acid. Thus 200 g. soya noodle can supply only 84% of cystine and 47% of methionine in the requirement, that is

65% of the total sulfur containing amino acids. This means that about half or more of the quantity of the other amino acids was wasted. There might be enough methionine from another source of food in the diet to supplement this deficiency. But according to Altschul (1956), all of the essential amino acids must be presented at the same time for any synthesis of protein to occur. Even when only one of these is missing or supplied several hours later than the others, protein synthesis will either cease or decrease to a very low level. In addition, Van Reen et al (1967) reported low organic sulfur excretion of people in Uborn village. As the major source of urinary sulfate in man and animal has been shown to be the dietary sulfer containing amino acids, it seems likely that methionine is needed to supplement the soya noodle. Although the cystine content in 200 g. soya noodle was higher than the requirement, when 70% of soya noodle was considered to be utilised the cystine content would be lower than the requirement. Also it was reported elsewhere, that methionine can be converted to cystine. Therefore only methionine was considered as a supplement to soya noodle and it was decided that the amount of supplementation should be such that the total S-amino acid in the noodle agreed with the requirement. Thus 0.48 mg of methionine needed to be added to 200 g. soya noodle.

Possibility of supplementing soya noodle with methionine. As nowadays sythetic D-L methionine is commercially available at a very cheap cost, it is possible to use this synthetic material to supplement the imbalanced S-amino acids. However, there have been arguments on the use of methionine to supplement the diet. FAO (1965) reported the uncertain decision of the committee about supplementation of limiting amino acid with pure amino acid. If there is a need to supplement, the amino acid reference pattern should be followed closely in order to minimise any adverse effect since large amounts of single amino acids added to the diet may induce various toxic reactions including depression of growth.

Gaudin (1969) in a review stated that fortification with sulfur amino acid is generally agreed to improve the biological value of protein when the value is measured by the effect on the growth of rats and chicks. As the formation of keratin needed an adequate supply of cystine, which may perhaps be derived from methionine, hence the sulfur requirement depended partly on keratin formation and the occurrence of fur and feathers. He also pointed out that the normal animal organism can incorporate only natural L methionine into its body protein. In order that D methionine can be utilised it must first be converted into the L-isomer and this will need a special enzyme.

There is not much literature about the effect of supplementation of D-L methionine to food to be used for human consumption. Tung et al (1967) supplemented D-L methionine to rice and soybean meal for feeding babies. They reported that, administered to normal breast-fed babies aged 1-6 months on a diet based on rice and thoroughly toasted whole soya bean meal, 6 babies received a supplement of D-L methionine (160 mg/day), another group of 6 babies received no supplement. The increases in weight during 6 months 2150 and 2031g.), the coefficients of alimentary efficiency (0.41 and 0.43) and the coefficients of protein utilisation (2.18 and 2.13) were not significantly different so there is no evidence that such fortification was useful.

Clark and Woodward (1966) have proved that the amount of methionine added to the diet can be varied in a wide range without causing any adverse effect to nitrogen retention. In a study on the influence of different quantities of methionine intake on nitrogen retention of young men, they found that when methionine was added to the experimental diets at levels of 320, 470, 620 and 920 mg per day together with 770 mg cystine, the nitrogen balance of six men resulting from the different treatments did not differ significantly from each other and a wide zone of tolerance for methionine was observed under these experimental conditions. Therefore it appears that a high concentration of methionine can be consumed in the diet without any deleterious effect. Unfortunately they used L-methionine for supplementation.

In Van Reen and co-workers' (1970) sudy of bladder stone disease in Thailand, D-L methionine of 300 mg/day was given to eighteen male infants, 5-18 months of age. The methionine, 300mg/day was mixed with about 2 ml of an 80% sucrose solution and adminstered at the time of the infant's morning feeding. All infants were given breast milk and premasticated, glutinous rice. They found that before supplementation with methionine, Uborn village children excreted an average of 114 mg sulfate per day and after supplementation with 300 mg D-L methionine/day 243 mg of sulfate secretion was obtained. The experiment indicated that the low level of sulfate excretion is a reflection of a low dietary intake of sulfur containing amino acids and the excretion of most amino acids derivative was not altered markedly by the administration of methionine.

From the reviews, although there is no information exactly confirming the beneficial effect of supplementary methionine on the growth of humans, the works of Clark and Woodward and Van Reen and co-workers, showed no harm in supplementary methionine in the diet. As in Thailand most of the people are suffering from protein malnutrition, especially with sulfur amino acids as the limiting amino acids, it was thought necessary to supplement soya noodle with D-L methionine, and although full benefit might not be obtained there will be no harm and most likely some good effect on the amino acid imbalance.

Final Recipe of Soya Noodle

Calcium was added to soya noodle in the form of calcium carbonate, thiamine as thiamine hydrochloride, niacin as nicotinic acid and methionine as D-L methionine. The amount of these vitamins, minerals and amino acids added to the other ingredients is shown in Table XVIII.

Table XVIII. Final Recipe of Soya Noodle (per 400 g. of dry mix)

| | | | |
|---|---|---|---|
| Full fat soyflour | = | 220 | g. |
| Low fat soyflour | = | 40 | g. |
| Wheat flour | = | 100 | g. |
| Rice flour | = | 40 | g. |
| Thiamine hydrochloride | = | 1.40 | mg |
| Nicotinic acid | = | 16.04 | mg |
| Calcium Carbonate | = | 2.16 | g. |
| D L methionine | = | 1.03 | g. |
| Whole fresh egg | = | 90.00 | g. |
| Water | = | 130.00 | g. |

Discussion

In the calculation for vitamin and mineral supplement the average daily per capita consumption of Uborn province was used because, firstly it is the only recent dietary survey that could be obtained, and secondly because Uborn province is in the Northeast where most of the nutrition problems occur.

With the supplement of Thiamine, niacin, calcium and methionine, soya noodle if consumed alone or with other diets will solve the beriberi, anaemia, angular stomatitis, bladder stone disease and protein malnutrition problems. There is one drawback with the product in the case of goitre problems, because goiterogenic agents have been reported in soybean.

# IX. IMPROVEMENT OF PROCESSING METHOD

The quality of the soya noodle, besides depending on the raw material, also depended on the proper method of processing. To obtain a better colour, texture and appearance of the product, the processing method was studied more extensively. The general method for processing of soya noodle was described earlier in the preliminary development work. In these experiments, some parts of the processing method were studied in more detail in order to produce a better quality product.

## Blending of Dry Ingredients and Mixing of Dough

Due to the use of many ingredients - full fat and low fat soyflour, wheat flour and rice flour, together with vitamins, minerals and amino acid entichment, it was necessary to blend these ingredients thoroughly in order to obtain a uniform distribution of vitamin, mineral and amino acid and also a uniform texture in the product.

Also, it was recognised that the quality of the product depended on the rheological properties of the dough. There are many changes occurring during dough mixing; decrease in the size of the protein aggregates or protein particles through tearing and shearing action and forming of a continuous protein film and a protein network, which results in an increasing amount of protein extracted with dilute acetic acid (Tsen, 1967) and the releasing of SH groups (Mechan, 1968). On prolonged mixing in air, there is a loss of SH groups (Tsen and Bushuk, 1963) and the breaking down of S-S bonds and the protein complex caused by either physical rupture or oxidation of the S-S bonds or scission of non covalent bonds (Tsen, 1967).

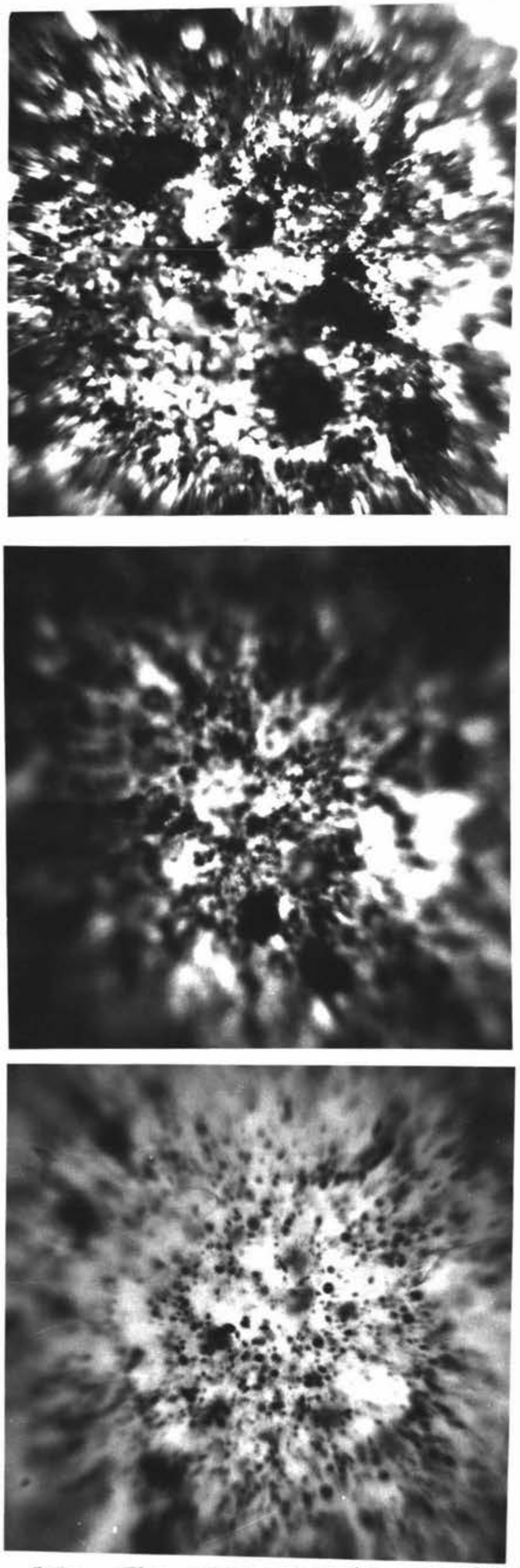
The consistency of the dough increases first in mixing, then remains at the maximum and then decreases again. Therefore, it is important to determine the time of mixing in which maximum dough development occurs.

Experimental procedure. In the experiments, the following mixing conditions were carried out:

A. Mixing dry ingredients for 2 mins. and mixing dough for 3 mins.
B. Mixing dry ingredients for 5 mins. and mixing dough for 3 mins.
C. Mixing dry ingredients for 10 mins. and mixing dough for 3 mins.
D. Mixing dry ingredients for 5 mins. and mixing dough for 5 mins.
E. Mixing dry ingredients for 5 mins. and mixing dough for 10 mins.

The results of microscopic examination of a dough section from each mixing time of dry ingredients were compared, together with the results of texture measurement on the final cooked noodle.

In the experiments, a batch operation of 400g. dry mix was used. Vitamins, minerals and amino acid were premixed with wheat flour and rice flour. Then all the ingredients were mixed in a Kenwood mixer at the speed of the driven gear of 260 rpm, with a dough hook being used instead of the mixing blade. At the end of each dry mixing time, water and egg were added and the dough was mixed for the required time. A sample of the dough was taken for microscopic study and the noodle was made according to the method described previously. For the microscopic examination, a thin section of dough was prepared and stained with iodine in potassium iodide solution. A black colour developed for the starch granules and a yellow colour for the protein matrix.



a.

b.

c.

Figure 16: The microscopic examination of the effect of mixing time of the dry ingredients on the distribution of starch granule:
a. 2 mins.mixing time. b. 5 mins.mixing time
c. 10 mins.mixing time.

## Results and Discussion

The results of the microscopic study for the mixing time of the dry ingredients are shown in Figure 16., and the results of the texture reading in the cooked noodle of different mixing times of dry ingredients and of dough are shown in Table XIX.

For the mixing of dry ingredients, at 2 mins. mixing time, a few large clumps of starch granules appeared and the size of this starch aggregation decreased as the time of mixing was lengthened, until at 10 mins. a uniform distribution of small starch particles occurred. Since vitamins, minerals and amino acid were pre-mixed with wheat flour when the uniform distribution of starch particles was obtained, it seemed likely that a uniform distribution would occur for vitamins, minerals and amino acid too. From the results of the texture reading on the cooked noodle of different mixing times of the dry ingredients, a more uniform texture of the product also occurred when the mixing time of the dry ingredients increased. In the texture readings of the noodle made by mixing for 2 mins., there was a wide range of texture readings both within a strip of noodle and also between individual strips of the noodle. The difference in these readings were reduced when the mixing time was increased to 10 mins.

In dough mixing, after water and egg were added, dough developed with 3 mins., therefore the longer mixing times of 5 and 10 mins. were used in the experiment. From the results of texture measurement of cooked noodle prepared from dough of different mixing times, when the mixing time was increased a tougher noodle was obtained. Unfortunately, a longer mixing time than 10 mins. was not tested in the experiment so it was not known whether the maximum strength of the dough had been obtained when dough was mixed for 10 mins. Upon increasing the dough mixing time to longer than 10 mins., either a higher or lower reading than 3.16 for texture measurement of cooked noodle may be obtained. But from the results of texture measurement and taste panel hedonic scores of various noodles from different recipes in Chaper IV, the preferred texture of the noodle was when the texture measurement was about 3.20. Therefore a 10 mins. mixing time for dough was preferred than 5 and 3 mins. mixing time for dough, although it was not certain that at 10 mins dough mixing time a maximum dough development occurred. In preparation of soya noodle the dry ingredients should be mixed for 10 mins., followed by 10 mins. dough mixing for a better quality of the product. This is of course only true for this one mixer and times may vary with the other mixers. However the time for the dry mix should be such that there is the uniform distribution of small starch particle for the dough maximum elasticity.

Table XIX. The Results of Texture Measurement of Noodle Made by Different Mixing Times of Dry Ingredients and of Dough

| Texture Reading | Mixing time of Dry Ingredients | | | Mixing time of dough | | |
|---|---|---|---|---|---|---|
| | 2 mins. | 5 mins. | 10mins. | 3 mins. | 5 mins. | 10 mins |
| Average reading of Noodle cooked for 5 mins.* | 2.82 | 2.70 | 2.71 | 2.70 | 2.87 | 3.16 |
| Average range within the strip of five readings | 0.33 | 0.31 | 0.25 | 0.31 | 0.37 | 0.26 |
| Average range between five strips. | 0.53 | 0.43 | 0.41 | 0.43 | 0.23 | 0.33 |

* Sample of five strips of cooked soya noodle was used, and five readings were taken from each strip.

Rolling, Cutting and Steaming Soya Noodle

As already described in the preliminary development work, after mixing the dough was rolled into a thin sheet to the required thickness. Because the wet surface of the thin sheet of dough caused the noodle to stick together during cutting, the thin sheet of dough was left at room temperature prior to cutting and steaming, so that the surface would dry. During this stage of long exposure to air, enzymic oxidation and enzymic browning reaction could occur. Therefore it was thought that the noodle should be steamed as quickly as possible after the dough was removed from the mixer. To solve this problem in the preliminary development work, thin sheets of dough were steamed quickly after rolling into thin sheets. At the end of the steaming period, the surface of the sheet was sufficiently dry for cutting without causing the noodle to stick together. Steaming the thin sheet of dough was not practical since the thin sheet of dough needed to be laid on a tray or hung on a stick in the steamer, where a large space was required for rather large batch operations.

In this experiment, the thin sheet of dough was subjected to an air blast of the velocity 600-1000 ft. per min., at room temperature, i.e $50^{o}F$ in a tray dryer for 5 mins., then cut into strips and these strips were loosely folded into a ball prior to steaming in a retort at atmospheric pressure for 20 mins. By this improved method, a large quantity of noodle could be steamed at one time and the enzymic reaction that occurred before the noodle was steamed could be reduced. The colour of the noodle obtained by this method was better than that where the sheet of dough was left for surface drying in room conditions.

Drying Method of Soya Noodle

As mentioned by Hummel (1966) in his book on macaroni products, drying is the most difficult operation in the manufacture of macaroni products. It is in fact one of the most delicate drying problems in the food industry, being more than simple dehydration as in the case of many other alimentary products. In these simpler cases, a certain amount of water has to be extracted from a food product, and the time required for the drying is only of economic importance; the drying temperature has seldom more than an upper limit, and the humidity of the drying air may be chosen by economic standards. The conditions are not so easy with macaroni products, as drying does not mean simply an extraction of a certain amount of water from the fresh goods. During drying, macaroni products develop their final colour and texture. They must not crack or warp during the drying period, and must not crack after being packed and ready for sale.

In drying soya noodle, additional problems occurred as the protein and fat contents of soya noodle were higher than ordinary macaroni products and the soya noodle was also supplemented with vitamins, minerals and amino acid. The macaroni product was usually dried down to moisture content less than 13% on a wet basis or 15% on a dry basis. Since the protein content of soya noodle is higher than wheat flour macaroni products, the soya noodle might not be stable at this moisture content. Protein foods usually have a low water holding capacity and stabilise at a lower moisture content than starchy food (Salwin, 1959).

It was found from the previous experiments that when the noodle was dried overnight at 120°C in air of low relative humidity, there was a slight haylike smell which might be caused by oxidation; and the texture of the dry noodle was brittle and easy to break. Moreover, the colour of the noodle supplemented with vitamins, minerals and amino acid was darker than the noodle without supplementation, as shown in Figure 20a. Therefore it was necessary in further experiments, to study the method of drying so as to obtain an improved product and also to find the level of moisture to which the noodle should be dried.

Moisture sorption isotherm and stability of soya noodle. It was recognised that the moisture range for the product in which maximum product stability occurred could be predicted from the moisture sorption isotherm of that product. Above or below this moisture range, the product deteriorated at a more rapid rate (Rockland, 1969).

Equilibrium moisture content of a hygroscopic material may be determined in a number of ways, the only requirement being a source of constant-temperature and constant-humidity air. Determination may be made under static or dynamic conditions, although the latter case is preferred if the data are to be used for drying calculations (Perry, 1963). In the determination of the moisture adsorption isotherm of soya noodle the static condition was used. A dried and finely ground sample was placed in ordinary laboratory desiccators containing saturated salt solutions or sulfuric acid solution which produced atmospheres of constant relative humidity. Eight relative humidities 10, 20, 30, 43.6, 64.6, 74.9, 84.5 and 92.9% were chosen for the experiment. A list of the salt solutions and sulfuric acid

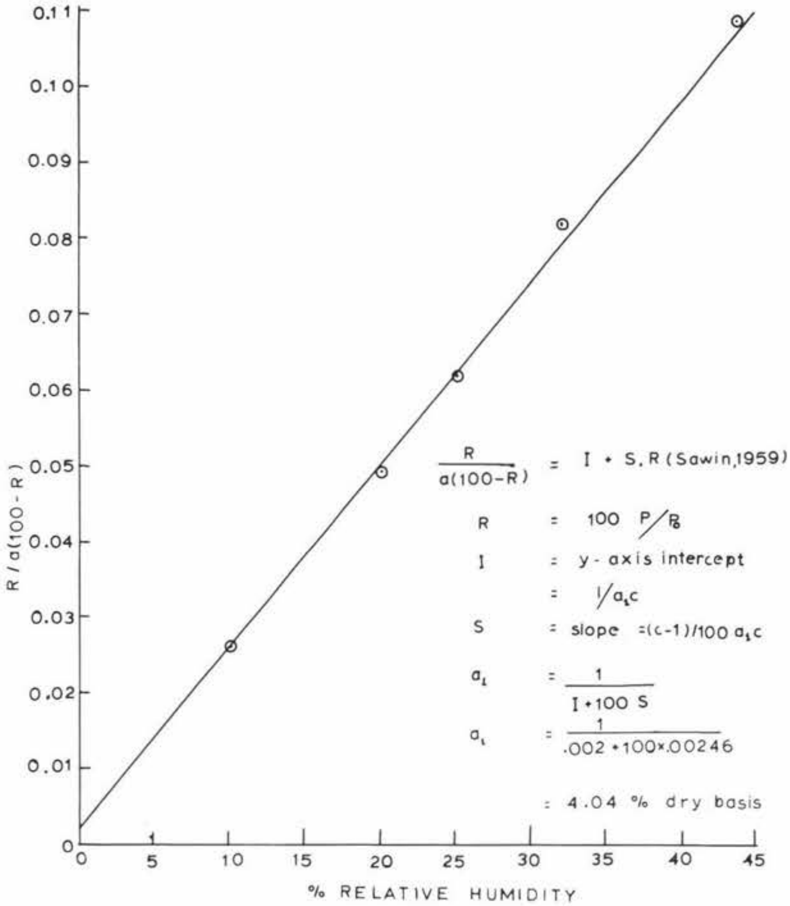



Figure 18 Modified B.E.T Moisture Sorption for Soya Noodle

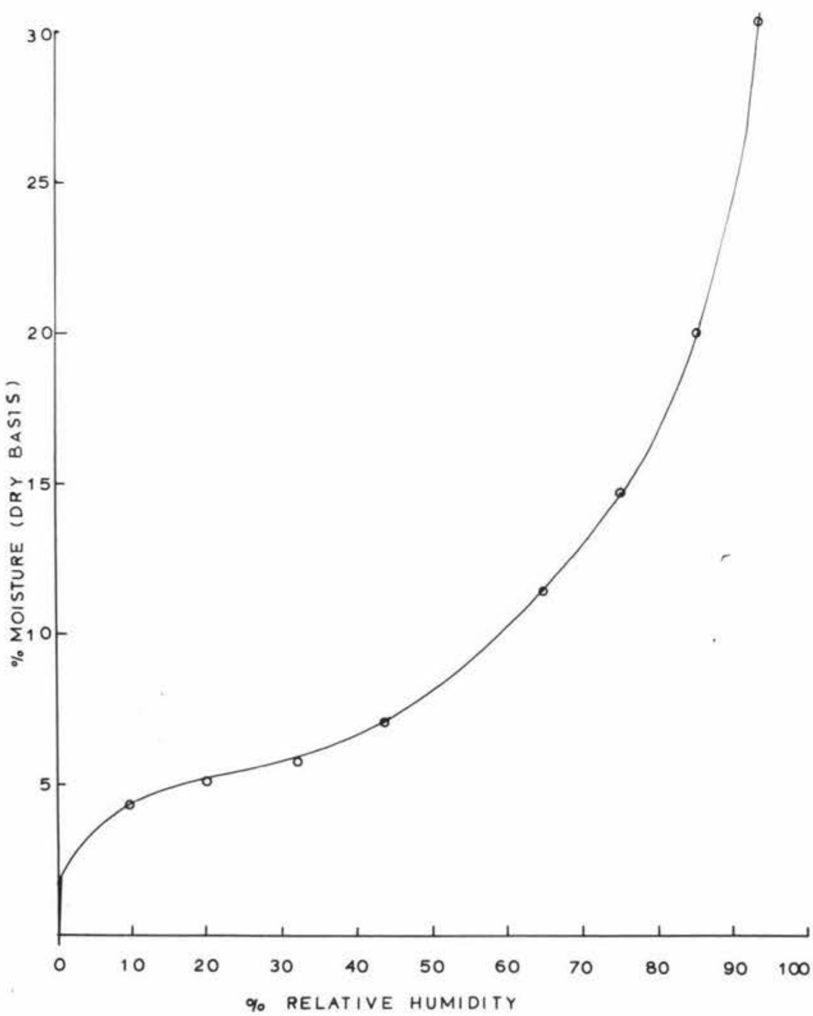



Figure 17 Moisture Sorption Isoterm of Soya Noodle

solutions which gave these relative humidity conditions is shown in Appendix X. The desiccators containing these saturated salt solutions and sulfuric acid solutions were kept in a cabinet of constant, 30°C, temperature.

The samples were allowed to equilibrate at these various relative humidities and the sample in each desiccator was weighed periodically until a constant weight was obtained. The equilibrium moisture of the samples kept at low relative humidity was obtained within two weeks and those of samples kept at 84.5 and 92.9% were obtained in 5-6 days. The moisture content of each sample was, as previously described in Chapter VI, determined by the air oven method. The moisture adsorption isotherm was obtained by plotting the results of moisture content on a dry basis against the % relative humidity as shown in Figure 17. For the calculation of monolayer moisture value the method of Sawin (1959) was used as also shown in Figure 18.

For the stability test on the product, dried soya noodle was also kept in these controlled relative humidity atmospheres for 2 weeks. As the relative humidity increased, the product became softer and darker. The noodles exposed to 74.9, 85.4 and 92.9% RH were soft and brown. There was mould growth in the noodle kept at 92.9% RH. A slightly rancid odour occurred in the noodle kept at 20 and 32% RH, but this rancidity could be detected easily at 10% RH. The noodles that were kept at 43.6 and 64.6 % RH were in good condition.

From the result of the stability tests, the optimum moisture range in which soya noodle showed maximum stability was between the relative humidities of 32% RH, and 74.9% RH. At the moisture levels lower than 32% RH, deterioration occurred in the noodle due to development of rancid flavours and odours and at the moisture levels higher than 74.9%RH, the browning reaction developed. If the moisture adsorption isotherm was divided into 3 regions of differential stability as suggested by Rockland (1969), the lower limit of region II will correspond with the so-called BET monolayer. As described by Sawin (1959) the water molecules of the monolayer might be regarded as a discontinuous phase, bound by functional groups of protein and carbohydrate, but exerting their influence over the entire surface of the food. Water attached at these sites, probably by hydrogen bonding, should protect them from reaction with oxygen, possibly by excluding adsorption of oxygen directly or on adjacent surfaces, possibly co-ordinating trace metals and reducing their catalytic effect or possibly by decomposing free radicals. The adsorbed water might also inhibit interactions between adjacent polar groups, thereby preserving their hydrophilic properties, and facilitating rehydration. On the other hand, moisture in excess of the monolayer value represents free water, which promoted browning so he suggested that this monolayer adsorbed water should represent a minimum desirable amount of water as well as a maximum permissible amount. However, Martinez et al (1968) found that freeze-dried salmon was more stable at moisture levels considerably higher than the BET monolayer. The monolayer value for soya noodle was 4.04% moisture content on a dry basis or at 10% RH. As the noodle sample kept at 20 and 32% RH showed slight rancidity, therefore it was decided to dry soya noodle to the moisture level of 45% RH or at 7.5% moisture content on a dry basis.

<u>The Theory of drying macaroni product</u>. As described by Hummel (1966),

any hygroscopic material exposed to air of given temperature and moisture will absorb or lose water according to its water content until it has reached what is called the equilibrium water content or equilibrium moisture when it will gain or lose no more water. All the water such a material can lose is therefore the difference between the total and the equilibrium water content. For macaroni products, the drying process is divided into 3 stages, pre-drying, softening period (also called sweating period) and final drying. The equilibrium water content of macaroni product at each stage can be obtained by drying a sample to constant weight in a drier with close control of temperature and humidity and adequate circulation of air to make sure that the conditions prevailing at the surface of the sample actually correspond to the temperature, and humidity, as set for each stage. It has been found that the equilibrium water content depends mainly on the wet bulb depression of the air to which the sample is exposed. When the wet bulb depression is at 2°C, the equilibrium moisture content of macaroni product is 20% on a dry basis; and the equilibrium moisture content decreases as the wet bulb depression increases. At a wet bulb depression of 4.5°C the equilibrium moisture content of the macaroni product is 15% on a dry basis or 13% on a wet basis.

As has been mentioned earlier, drying of macaroni product is more than a simple process of removing water from a fresh product. Many problems occur during the drying process. As described by Hoskins and Hoskins (1954) and Hummel (1966) checking or deformation of macaroni products often occurs unless the drying conditions are carefully controlled. In the preliminary drying, when the macaroni products are still soft, if drying proceeds too quickly the surface will dry rapidly and case harden but the interior will remain soft and plastic as the water cannot diffuse to the surface as quickly as it is evaporated. At a later period, diffusion will catch up with evaporation, the interior then shrinks more than the already case-hardened surface. This will pull the interior away from the surface resulting in a white spot or bubble in the interior. Usually these preliminary drier "checks" do not cause trouble with respect to cooking qualities.

If dried macaroni is moved into a very humid atmosphere, it will absorb moisture on the surface which will then expand. If the process is carried too far, the surface will pull away from the interior and cause serious checking.

If a product of low moisture content is moved into hot and dry air, the surface will dry and contract. This will cause a very fine network of cracks to appear on the surface. Usually these cracks do not extend very deeply into the macaroni and do not usually cause trouble in cooking. This check is called "tension check" because the surface is in tension when it occurs.

If wet macaroni is dried rapidly, the surface will be dried but no stresses will be set up because the product is plastic. Under these circumstances, the moisture content at the surface will be small and in the interior it will be large. The solids content at the surface will be large and the solids content in the interior will be small. If drying is continued with this difference in concentration down through the plastic range into the brittle range, there will be no stress set up as long as the drying rate is fast enough to keep the moisture gradient in line with the solids content. When drying is stopped, the moisture will tend to distribute itself evenly. This will cause the surface to expand because it contains too great a concentration of solids and the interior will contract because it contains too low a concentration of solids. This will cause compression check. It is the most common check encountered in macaroni drying. Sometimes it takes several days for this type of check to appear and it may appear after the product has been packaged.

For the understanding of the problems in the drying of macaroni product, a good drying diagram has been suggested by Hummel as:

(1) Preliminary drying: For an efficient predrier, a wet bulb depression should be set at the condition in which the macaroni product would stay 2-3 hours and the water content on a wet basis should be brought down to near 20%. Up to this point the warm macaroni product is soft and any stress is relieved by plastic deformation.

(2) Resting or sweating: In this stage the wet bulb depression should be decreased and the macaroni products should be allowed to rest or sweat in high humidity air and a fairly high temperature. During the rest period, drying would stop. There might even be a very small absorption of water from the air. Water inside the macaroni product is distributed evenly, aided by the fairly high temperature. At the end of the resting period which often takes about 1-2 hours, water and temperature is evenly distributed within the macaroni

product, and there is no built-in stress as the product is still sufficiently plastic for all stresses to have been relieved.

(3) Final drying: It is necessary to keep the water gradient fairly low at this stage so that differences in the water content between the interior of the macaroni product and the surface do not exceed 1%. The wet bulb depression should be adjusted to the condition that the moisture content would be brought down from 20% to 14.5% on a wet basis within 4 hours, then the wet bulb depression should be increased and drying would be finished in about 6 hours, with a final moisture content of about 13%. The temperature of drying air should be set at 113 - 123°F (45 - 50°C).

Drying of soya noodle. In the drying experiments on soya noodle, two drying methods were compared. One based on the method suggested by Hummel, in which the drying process consisted of 3 stages, pre-drying, resting or sweating, and final drying periods. In the other method, the noodle was dried to a constant weight at one constant drying condition. The suggested conditions for drying are shown in Table XX.

The wet bulb depression was calculated from the difference between the dry bulb temperature (air temperature) and the temperature at the surface of the product (wet bulb temperature). The wet bulb temperature was read from the psychometric chart at a given % relative humidity and at dry bulb temperature of 120°F.

The % relative humidity for the drying condition at a particular equilibrium moisture content was obtained from the moisture adsorption isotherm (Figure 17). It was recognised that the change in temperature results in a proportional change in equilibrium relative humidity at each moisture level. As shown by Rockland (1969) the equilibrium relative humidity at a particular moisture level increases if the temperature increases, or on the other hand, at a particular relative humidity the equilibrium moisture level will be lower when the temperature increases. The moisture adsorption isotherm of soya noodle, as shown in Figure 17, was determined at the temperature of 86°F. Therefore for the drying temperature of 120°F at the suggested relative humidity the equilibrium moisture content of the noodle would be slightly lower than that required.

Table XX. Suggested Conditions Programme for Drying of Soya Noodle

| Drying Period | Wet bulb depression | Air temperature (Dry bulb temperature) | Wet bulb temperature | Approximate Relative humidity | Equilibrium Moisture content | |
|---|---|---|---|---|---|---|
| | | | | | Wet basis | Dry basis |
| | | °F | °F | % | | |
| **3 Period Drying Condition** | | | | | | |
| Pre-drying | 3°F | 120 | 117 | 90 | 20 | 25 |
| Resting | 2°F | 120 | 118 | > 90 | 20 | 27 |
| Final drying | 7°F | 120 | 113 | 80 | 14.5 | 17 |
| **A Constant Drying Condition** | 23°F | 120 | 97 | 45 | 6.75 | 7.5 |

It was also noted that isothermal dehydration of natural products often results in a higher moisture content at a given equilibrium relative humidity than isothermal rehydration of completely dried material. This phenomenon is commonly referred to as moisture sorption hysteresis.. Rockland also explained that molecular shrinkage during desorption was believed to reduce the availability of sorptive sites on absorbent surfaces. Thus when setting the drying conditions of soya noodle at 45% relative humidity, the actual moisture content of the final noodle would be higher than 7.5% moisture content on dry basis. It was also clear that when the drying process was nearly completed, the drying rate was decreased. For an economic drying system, in the final stage, the wet bulb depression should be kept just a little bit larger than the wet bulb depression corresponding to the equilibrium moisture content of 7.5% on a dry basis for shortening the drying time. Therefore with all these considerations the wet bulb depression in the final stage of drying was set higher than 23°F and the equilibrium relative humidity was aimed for 40%.

For the second drying method, by calculation the noodle should be dried at a wet bulb depression of 23°F. Because of the hysteresis effect and the slow rate of the final stage of drying, the drying conditions were set at 40% equilibrium relative humidity, i.e. wet bulb depression of 25°F.

The results of actual drying diagram of soya noodle by these two methods are shown in Table XX. and the actual drying curve in Figure 19.

Table XXI. Actual Condition of Drying Soya Noodle by Two Different Methods

Area of sample on tray = 1 sq.ft. Air velocity 600-1000 ft/min. 600 g./sq.ft.

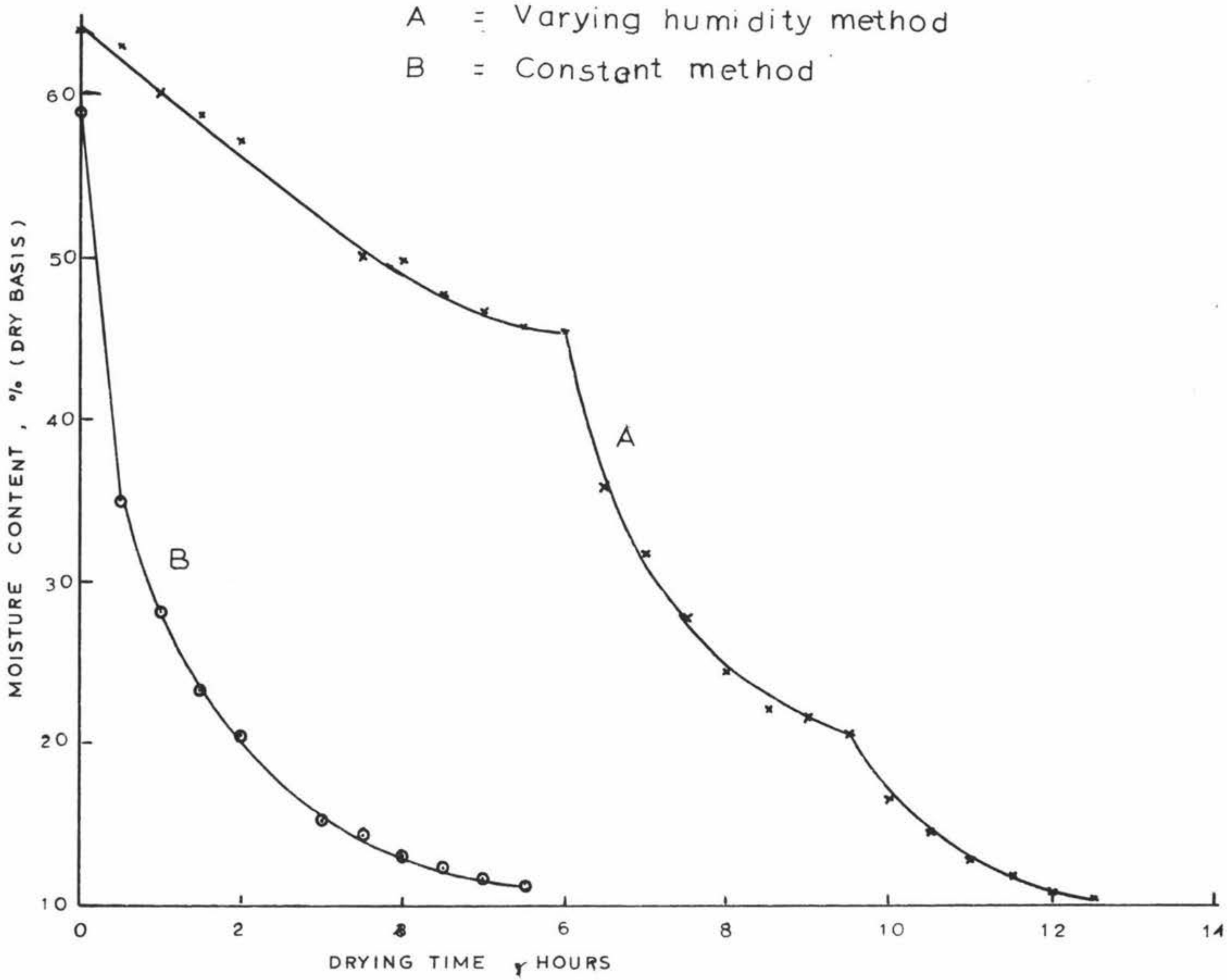
| Drying time (hr) | Wet bulb Depression °F | Wet bulb Temperature °F | Dry bulb Temperature °F | Product Temperature °F | Relative humidity % | Weight of same sample taken at intervals of time g. | Moisture Dry basis % | Content Wet basis % |
|---|---|---|---|---|---|---|---|---|
| Varying Humidity Method | | | | | | | | |
| 0 | 3 | 114-115 | 118 | 90 | 90 | 51.47 | 64.1 | 39.1 |
| ½ | 3 | 117-118 | 120 | 118 | 88-96 | 51.18 | 63.2 | 38.7 |
| 1 | 3 | 117 | 120 | 118-119 | 90 | 50.39 | 60.7 | 37.8 |
| 1½ | 3 | 117 | 120 | 118-119 | 90 | 49.75 | 58.6 | 36.9 |
| 2 | 3 | 122 | 125 | 123 | 90 | 49.25 | 57.1 | 36.4 |
| 3½ | 4 | 115 | 119 | 116 | 88 | 47.08 | 50.1 | 33.4 |
| 4 | 3 | 117 | 120 | 118-119 | 90 | 46.77 | 49.1 | 32.9 |
| 4½ | 3 | 115 | 118 | 117 | 90 | 46.33 | 47.7 | 32.3 |
| 5 | 3 | 114-115 | 118 | 117 | 90 | 45.98 | 46.6 | 31.8 |
| 5½ | 3 | 115 | 118 | 117-118 | 90 | 45.72 | 45.9 | 31.4 |
| 6 | 3 | 114 | 117 | 117 | 90 | 45.56 | 45.3 | 31.2 |
| 6½ | 9 | 108 | 118 | 113-116 | 73 | 42.93 | 36.9 | 26.9 |
| 7 | 7-15 | 110 | 117-125 | 115-117 | 71 | 41.36 | 31.9 | 24.2 |
| 7½ | 7-15 | 110 | 117-125 | 115-117 | 71 | 40.05 | 27.7 | 21.7 |
| 8 | 11 | 109 | 120 | 115 | 70 | 38.99 | 24.3 | 19.8 |
| 8½ | 9-12 | 110 | 119-122 | 116-118 | 70 | 38.28 | 22.1 | 18.1 |
| 9 | 9-12 | 110 | 119-122 | 116-118 | 70 | 38.11 | 21.3 | 17.8 |
| 9½ | 9-12 | 110 | 119-122 | 116-118 | 70 | 37.80 | 20.5 | 17.0 |
| 10 | 23-30 | 96-97 | 119-127 | 118-122 | 39-40 | 36.55 | 16.6 | 14.2 |
| 10½ | 23-30 | 96-97 | 119-127 | 118-122 | 39-40 | 35.84 | 14.3 | 12.5 |
| 11 | 25-29 | 95-97 | 120-126 | 118-122 | 37-40 | 35.39 | 12.9 | 11.4 |
| 11½ | 15-29 | 95-97 | 120-126 | 118-122 | 37-40 | 35.04 | 11.7 | 10.9 |
| 12 | 15-29 | 95-97 | 120-126 | 118-122 | 37-40 | 34.79 | 10.9 | 9.9 |
| 12½ | 15-29 | 95-97 | 120-126 | 118-122 | 37-40 | 34.59 | 10.3 | 9.3 |

continued

continued:

| Drying time (hr) | Wet bulb Depression °F | Wet bulb Temperature °F | Dry bulb Temperature °F | Product Temperature °F | Relative humidity % | Weight of same sample taken at intervals of time g. | Moisture Dry basis % | Content Wet basis % |
|---|---|---|---|---|---|---|---|---|
| **Constant Humidity Method** | | | | | | | | |
| 0 | 22-27 | 94-95 | 116-122 | 80-88 | 40-45 | 35.97 | 59.7 | 37.4 |
| ½ | 20-26 | 93 | 113-119 | 104-108 | 40-42 | 30.65 | 36.1 | 26.5 |
| 1 | 21-26 | 95-96 | 116-122 | 108-113 | 39-47 | 29.66 | 28.8 | 22.3 |
| 1½ | 21-26 | 95-96 | 116-122 | 110-113 | 39-47 | 27.76 | 23.3 | 18.9 |
| 2 | 21-26 | 95-96 | 116-122 | 110-113 | 39-47 | 27.11 | 20.4 | 16.9 |
| 3 | 20-25 | 95-96 | 115-121 | 113-114 | 40-48 | 25.98 | 15.4 | 13.3 |
| 3½ | 20-25 | 95-96 | 115-121 | 113-114 | 40-48 | 25.73 | 14.5 | 12.5 |
| 4 | 22-27 | 93-94 | 115-121 | 114.5-119 | 37-45 | 25.46 | 13.1 | 11.5 |
| 4½ | 22-27 | 93-94 | 115-121 | 114.5-119 | 37-45 | 25.31 | 12.4 | 11.0 |
| 5 | 22-27 | 93-94 | 115-121 | 114.5-119 | 37-45 | 25.15 | 11.7 | 10.5 |
| 5½ | 22-27 | 93-94 | 115-121 | 114.5-119 | 37-45 | 25.05 | 11.2 | 9.9 |

Figure 19 Actual Drying Curve of Soya Noodle by Two Methods:



In the actual drying because of the difficulty in setting the drying conditions for obtaining a desired humidity in the drier, the resting or sweating period failed to be obtained in the varying humidity method. In this method after the predrying period at 90% RH for 6 hours the drying condition was set to 70% RH instead of 80% as in the suggested drying programme. About 30% of the original moisture content of the wet noodle was removed on drying at 90% RH for 6 hours and drying at 70% RH for 3½ hours another 30% of the moisture content of wet noodle was removed. Then the rate of drying was decreased, only 10% of moisture content was removed when drying at 40% RH for another 3½ hours drying time. The moisture content of noodle at the end of each drying condition, at 90, 70 and 40% RH, (31.20, 17.0 and 9.3 respectively) were higher than the expected equilibrium moisture content as shown in Table XX. This may be due to either the hysteresis effect or the inefficiency of the drying time. In the second method of drying at 40% RH, about half of the moisture content of the wet noodle was removed in the first hour of drying, then the rate of drying was decreased and on drying for 5½ hours the moisture content was brought down to about 11% on a dry basis, or 10% on a wet basis.

To study the effect of drying method on the final quality of soya noodle, the colour, texture and rehydration property of the noodles dried by these two methods were compared. Colour of the noodle was measured by determination of pigment content and visual comparison Munsell with whirling paper discs, consisting of white, black, yellow and red according to AACC Laboratory Cereal Method (AACC, 1962). Texture was measured by texture measurement of cooked noodles with the noodle testing machine. The rehydration property was measured by soaking noodles in boiling water, for 5, 10 and 15 mins. and the weight and volume before and after soaking measured.

In the determination of pigment content, a method of AACC (1962) was modified. 2g. of sample finely ground and passed through sieve B.S.40 was extracted with 10 ml of water saturated n-butanol for 16 hours. At the end of 16 hours the contents were shaken and filtered through No.1 Whatman filter paper. The filtrate was transferred to 1 cm square cell and the optical density of the extract was read from 390 to 440 mμ wavelength. A Hitachi spectrophotometer was used. The results of colour and texture measurement and rehydration property of noodle prepared from two different drying methods are presented in Table XXII. (next page).

From the results in Table XXII, drying the noodle by varying drying conditions gave a product of better colour, texture, and rehydration property than drying at a constant condition. Browning occurred in the noodle dried by the constant condition method as shown by the higher results of optical density reading at low wavelenghth - 390, 400 and 410 mμ and a higher percentage of yellow and brown colour in the Munsell colour disc method. Oxidation of colour pigment also occurred in the noodle dried by this method, which was shown by the low result of carotinoid pigment extraction reading at 440 mμ. The higher texture reading result of texture measurement of cooked noodle dried by the constant condition method and poorer adsorption of water during rehydration than those of the noodle dried by varying the drying condition may be due to the occurring of case hardening.

Table XXII. Comparison Between Colour, Texture and Rehydration Property of Soya Noodle Dried by Two Different Methods

| Quality Test | Drying Method | |
|---|---|---|
| | Varying Humidity Method | Constant Humidity Method |
| Colour measurement by pigment extraction method | | |
| Optical density at 390 mμ | .390 | .446 |
| 400 | .317 | .350 |
| 410 | .268 | .280 |
| 420 | .247 | .238 |
| 430 | .217 | .205 |
| 440 | .203 | .175 |
| Colour measurement by disc method % yellow and brown of disc colours | 64.0 | 83.5 |
| Texture measurement of cooked noodle by noodle testing machine | 3.28 | 4.06 |
| Rehydration property | | |
| Weight before soaking | 10.0 | 10.0 |
| Volume before soaking | 8.0 | 8.0 |
| Weight after soaking for 5mins. (g.) | 18.0 | 17.2 |
| Volume after soaking for 5mins. (cc) | 17.0 | 16.0 |
| Weight after soaking for 10mins. (g.) | 20.20 | 20.0 |
| Volume after soaking for 10mins. (cc) | 20.0 | 18.5 |
| Weight after soaking for 15mins. (g.) | 22.8 | 21.5 |
| Volume after soaking for 15mins. (cc) | 22.0 | 20.0 |
| % rehydration (maximum) | | |
| Weight (g.) | 128.0 | 115.0 |
| volume (cc) | 175.0 | 150.0 |

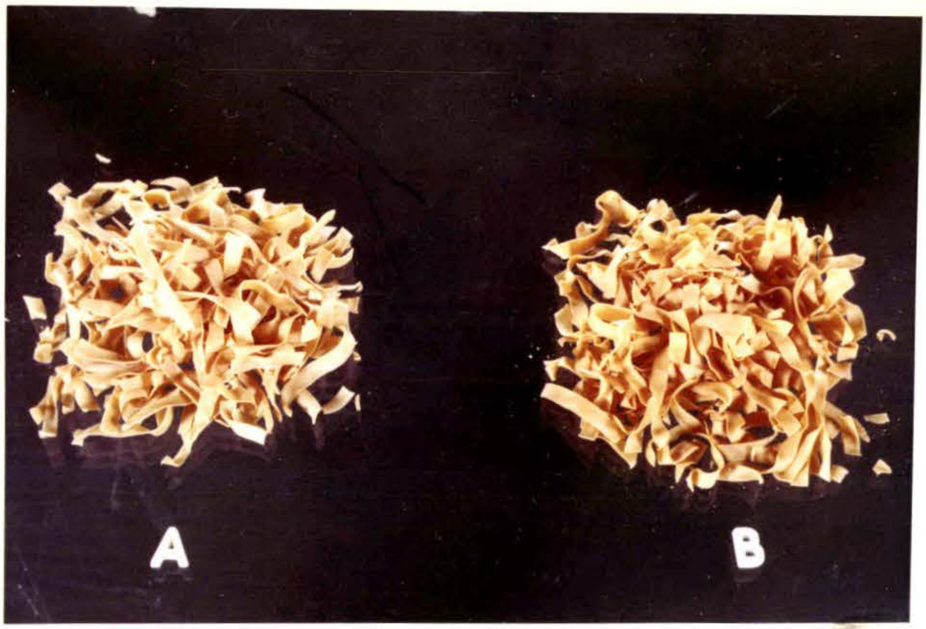



Figure 20a. Soya Noodle Dried Overnight at 120°F 20% RH.

a. Soya noodle without supplementation.
b. Soya noodle supplemented with vitamins, minerals and amino acid.

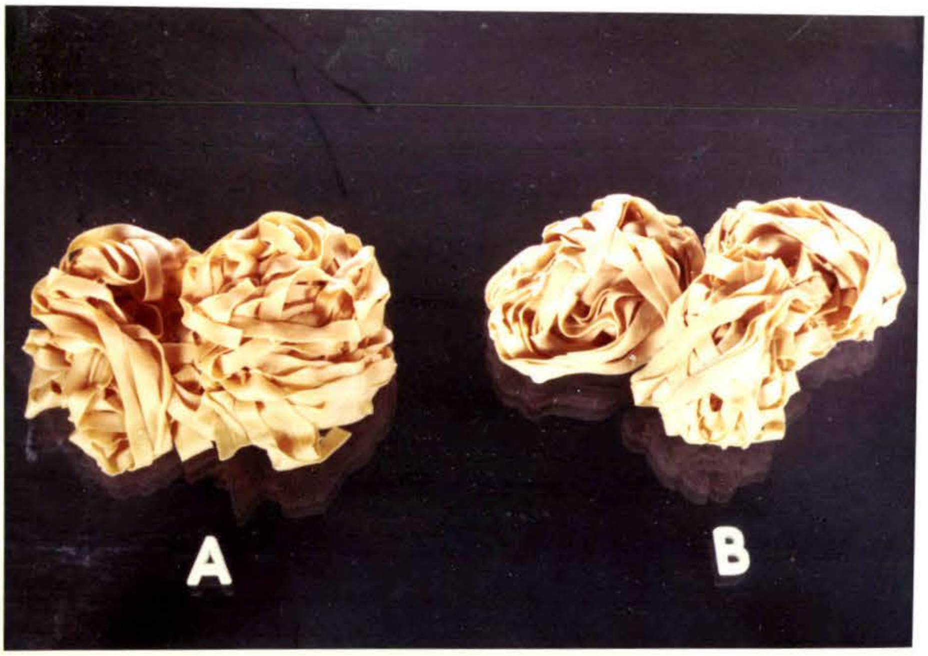


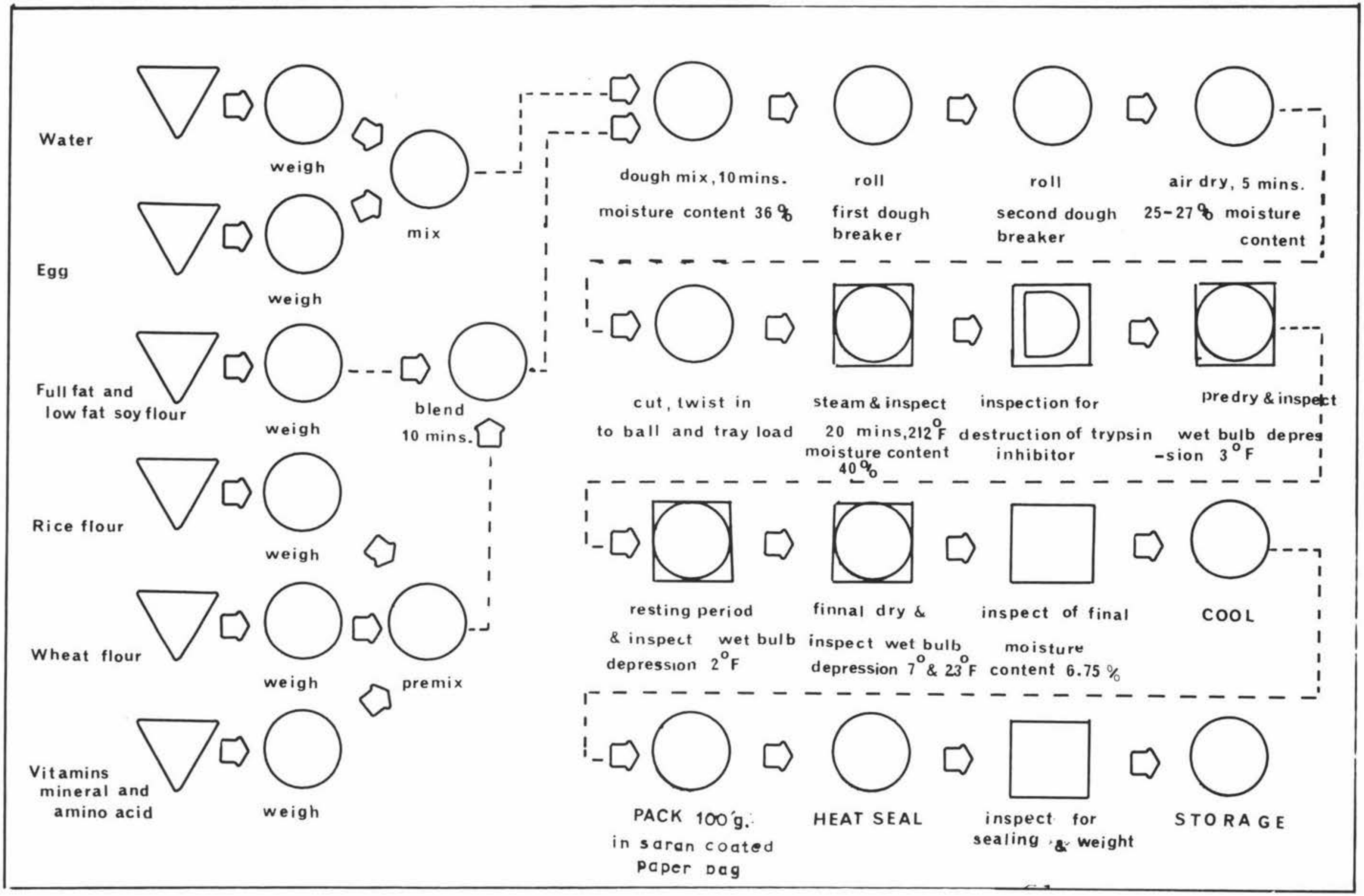

Figure 20b. Soya Noodle Supplemented with Vitamins, Minerals and Amino Acid Dried by

a. Constant Humidity
b. Varying Humidity.

For the general appearance, the noodle dried by the varying drying condition method had a smoother appearance, clearer and lighter colour than the noodle dried by the second method, as shown in Figure 20b. It was more firm and hard to break. Noodle dried by the second method showed a few small white bubbles on the surface of the noodle and the noodle was slightly brittle. However, the noodle dried by the second method was not as brittle as the noodle dried by the method described in the preliminary development work in which the relative humidity of the drying condition was about 20%. From the results of this experiment, it was suggested that the noodle should be dried by controlling the humidity in the drier and in the final stage of drying, the relative humidity should be slightly lower than 40% for obtaining the required final moisture content of 7.5% dry basis.

A summary of final processing method is shown as the flow process chart in Figure 21.

**Figure 21** **Flow Process Chart for Soya Noodle**



# X. PACKAGE DESIGN

Packaging has long been recognised for its ability to protect the product and also for its ability to communicate the product selling message. As suggested by Litchfield (1968) besides protecting the product, the other functions of packaging should be making the package easy to use, promoting the product through advertising on the package, explaining ways in which the product can be used by the consumer and providing a reasonable cost package in relation to the cost of the product. In designing a package for soya noodle, the above criteria were considered, but the main emphasis was on the protective properties of the packaging material for preserving the original characteristic of the product under the storage and distribution condition in Thailand.

## Literature Review of General Types of Deterioration in Dehydrated Food Products, The Factors Affecting Them and The Possibility of Them Occurring in Soya Noodle

Changes in quality of dehydrated foodstuffs are generally described under change in colour, flavour and rehydration texture and each product has its own characteristic change. The type of deterioration encountered, as described by Gooding (1962) and Van Arsdel and Copley (1964) are: Oxidative deterioration, enzymic deterioration, non-enzymic browning, microbial changes, nutritional loss and loss of natural colour and flavour of the product.

Oxidative deterioration. In the oxidative deterioration, oxygen plays an important part. Rancidity of fat is produced by the autoxidation of the unsaturated fatty acid probably by the addition of molecular oxygen to the double bonds of the unsaturated fatty acid with the production of labile peroxides and ultimately aldehydes, ketone and short chain fatty acid. Oxidation of carotenoid pigment has also been reported. The pigment is responsible for the colour of the product. It has been stated that beta-ionone is the major product of beta-carotene oxidation and it might be responsible for the haylike odour of dehydrated vegetables. Beta-carotene is of course provitamin A and the oxidation destruction of this pigment therefore causes the loss of vitamin A.

Oxidative deterioration of dehydrated products occurs rapidly at low moisture content. Dehydrated potato of 10% moisture content could stand in air for upwards of 6 months without developing rancidity while potato of 2 or 3% moisture content became rancid in 2 to 3 weeks. (Von Arsdel and Copley, 1964). As suggested by Sawin (1959), the moisture content of maximum resistance to oxidation is at the value corresponding to a mono-molecular layer of moisture within the internal structure of the solid. The monolayer value of potato is 6.5%. Therefore the potato of lower moisture content than 6.5% is likely to suffer from oxidative deterioration.

This oxidative deterioration may not be serious in the case of soya noodle. Since it was found from previous stability test that only the noodle kept at low relative humidity (10, 20, 32%) developed rancidity. And the moisture content in the final product is higher than the moisture at the monolayer level. The product was also planned to be sold in Thailand where the humidity is high, and the pickup of moisture from the atmosphere of the product in the package may prevent an occurrence of oxidation.

Enzymic deterioration. It is commonly known that the deterioration of a product caused by enzymes occurs when the moisture content of the product is in excess. In the dehydrated food of low moisture content, the enzymic reaction still does take place as reviewed by Acker (1962) and (1963). Dried vegetables which have not been blanched develop - obviously through the effect of oxidation - a haylike taste during storage (Joslyn, 1951). Browning reaction also occurs in freeze-dried raw meat which may be due to the enzymic liberation of glucose which is then able to take part in the Maillard reaction (Matheson, 1962). Acker also reported that oat-products spoil rapidly unless they are steamed beforehand; they develop a bitter taste through the combined action of lipases and lipoxidase. In noodle and macaroni containing egg, the lecithin can be split off to a considerable degree (in some cases to 60%) during unsuitable storage as the result of attack by a phospholipase D which separates choline from lecithin but is only slightly active in the raw material of the noodles, that is of wheat semolina. Lipase in the wheat flour also can be active after addition of fats to flour in cake processing. The changes leading to the spoiling of dried foods caused by an enzymatic process were presumed from the fact that heat-treated controls do not show these changes.

Acker (1969) found that enzymic changes generally do not take place or take place at a very slow rate when the moisture content of the food product is below the monolayer level. The lack of free water prevents the diffusion of the substrate to the enzyme.

Soya noodle had been subjected to heat treatment prior to drying. Steaming at 212°F for 20 mins. with the moisture content of the noodle about 27% wet basis should be sufficient to destroy all the enzyme activity that would be responsible for oxidation or browning deterioration.

Non-enzymic browning deterioration. Non-enzymic browning reaction which has been reviewed by several workers (Hodge, 1953, Reynolds, 1963, 1965, and Spark, 1969) is the complex chemical reaction initiated by the condensation of reducing sugar and amino acids or protein, the initial products being colourless but later products being brown. This series of reactions is commonly termed the Maillard browning. The development of a bitter flavour is also associated with the browning deterioration. Along with the browning reaction, other forms of deterioration also occur. In vegetables, this is loss of vitamin C, and structural change leading to inability to reconstitute fully and to toughness of the cooked product.

Much work has been done on the factors affecting the development of brown colour in food and in model systems containing aldose and amino acids. It was found that the rate of browning in foods and model systems increases rapidly with the increasing temperature and also depends on the moisture content of the food product or of the model system. Browning in foods and model systems also increased with increasing pH. (Reynolds, 1963).

Legault et al (1954) had obtained substantial protection against non-enzymic browning in dehydrated potato by reducing the water content to a very low level (1.4.2.4%). At 38°C a sample of 1.6% moisture content showed only a low degree of browning after 340 days compared with 63 days for a similar sample of 8% water content. The low moisture condition was obtained by in-package desiccation.

In Thailand at conditions both high in temperature and humidity, soya noodle would be likely to suffer from the non-enzymic browning. From the stability test of soya noodle placed in desiccators of various constant relative humidity as described in Chapter IX, the browning occurred in the noodle stored at 74.9, 84.5, and 92.9% RH after storage for 2 weeks at the temperature of 30°C.

Microbial deterioration. In dehydrated food products, microbial growth may occur if the moisture content of the product is at the level of the requirement for growth of a particular micro-organism. The water requirements for growth of many micro-organisms are defined in terms of the water activity of their environment. The water activity (aw) of a solution is also equal to P/PO, the ratio of its vapour pressure to the vapour pressure of pure water at the same temperature.

Christian (1963), reported that the water activity of microbial growth ranged from very close to 1.00 down to about 0.62. Most food spoils very rapidly at aw above 0.8. Between 0.75 and 0.72 the rates of mould growth are such that spoilage is delayed for some weeks, and on some food will not occur even after prolonged storage.

For soya noodle, mould growth occurred in two weeks on the noodle in the desiccator containing saturated salt solution of 92.9% relative humidity, in three weeks at 84.5% RH and in 4 weeks at 74.9% RH.

Loss of nutrition of dehydrated food product. As had been mentioned both vitamin A and carotenoid could be oxidised at high temperature storage in the presence of oxygen, when the moisture content of the product is low. The major hazard is rancidity of the fat in which the vitamin is dissolved, as the fat is oxidised the carotene and the vitamin A are destroyed. Vitamin C in fruit and vegetable products can be lost due to the browning reaction, as mentioned earlier.

The study of the loss of vitamin B in enriched flour, as mentioned by Zeleny (1960) indicated that the significant losses of thiamine occurred during storage and the extent of the loss, although quite variable, depended to a considerable extent on the time and temperature of storage and upon the moisture content of the flour. High temperature and high moisture content accelerate the rate of thiamine destruction. When thiamine hydrochloride was used as an enrichment ingredient, enriched flour was expected to lose about 10% of the thiamine in 6 months of normal storage, although losses of 20% or more might occur under severe storage conditions. No appreciable losses of riboflavin or niacin had been found to occur in enriched flour during normal storage. Riboflavin and pyridoxine are rather sensitive to light and may therefore be unstable in the product exposed to strong light.

The loss of amino nitrogen during browning of foodstuffs has been reported by several authors. As reviewed by Spark (1969) losses of amino nitrogen in stored dried fruit was noted by Tappel and Jones. They reported considerable loss of amino acids during browning of cod muscle preparation. In Spark's model study of aldohexose-glycine browning he found that free amino nitrogen did disappear during browning and in a manner which depended on the sugar involved. The loss of glycine free amino nitrogen in aldohexose-glycine browning systems was linear with time.

Soya noodle was supplemented with thiamine hydrochloride, niacin and methionine. These vitamins and amino acid are in the free form. During storage, if the moisture content of the noodle increases and browning occurs, thiamine and methionine will be lost due to the destruction of thiamine and browning reaction of free amino acid. Besides thiamine and methionine, other vitamins and amino acid content in soya noodle will be lost also if the storage conditions are unfavourable.

In conclusion, the deteriorations of soya noodle which may possibly occur during distribution and storage in Thailand where temperature is about 30°C, are mainly those resulting from increasing moisture content of the product.

<u>Requirement of Packaging Material</u>

In selecting a packaging material for soya noodle for storage and distribution in Thailand where the temperatures range from a maximum of 32°C to a minimum of 23°C and where the relative humidity may range from 62% at noon to 92% at morning and evening (Meteorological Office, London, 1966), the following criteria were considered:

(1) Protective properties of the packaging material,

(2) Cost of the packaging material and cost of production,

(3) Availability of the packaging material in Thailand,

(4) Communication between the product and consumer.

Protective properties of packaging material. As reviewed earlier the factor that causes the deterioration of soya noodle which possibly occur during distribution and storage in Thailand is the increasing moisture content of the product. The major property of the required packaging material is to stop the moisture transfer from the atmosphere to the product in the package. The dried noodle is crisp and the edge of the noodle is also sharp, so the packaging material should also be resistant to puncturing by the noodle.

The noodles are usually sold in the shops selling other dehydrated food products and it is possible that the noodle can pick up other food odours, therefore the packaging material should prevent the flavour/odour transfer too.

To prevent the transfer of moisture from the environment to the product the package should be tightly sealed, preferably heat seal, and for this purpose a heat seal able packaging material is required.

Cost of packaging material and cost of production. As the noodles are intended to be sold as a cheap source of high protein food for the low income group of people, the cost of the packaging material should be low. The material should also be made by simple methods and easily handled by the machines for a low production cost.

Availability of the packaging material in Thailand. The packaging material which gives the required properties should be common and easily obtainable in Thailand.

Communication between the product and the consumer. A consumer always wants a package that tells her quickly and truthfully what the package contains. The packaging material should be transparent for the visual characteristics of the product to be seen and/or printable so that all the information concerning the product and the method of use can be printed on the package.

The required properties of the packaging material for soya noodle are summarised in Table XXIII.

Table XXIII. Requirements for the Properties of Packaging Material for Soya Noodle

| | Properties | Requirements |
|---|---|---|
| General | | |
| | Clarity : | Transparent |
| Mechanical | | |
| | Tensile strength: | High |
| | Tear strength: | High |
| Chemical | | |
| | WVTR (water vapour transmission rate): | low |
| | Gas transmission: | reasonable |
| Converting characteristic | | |
| | Machine performance: | good to excellent |
| | Printability: | good to excellent |
| | Sealing: | heat |
| Other | | |
| | Cost: | low |
| | Availability: | in Thailand |

<u>Suggested Packaging Material for Soya Noodle</u>

With the recommendation from the Mono Division of U.E.B. Packaging Ltd., Auckland, the three possible packaging materials for soya noodle which are compatible with the requirements were:

(1) 0.003"gauge high density polythene

(2) 55 g. SBK/25 g. PVDC coating (saran coated paper)

(3) Cellophane, saran coated, .001" gauge.

All the above materials are printable, heat sealable, low cost and strong enough to resist puncture by the noodles.

The P.V.D.C. or saran coating besides being a food moisture barrier material will also give some protection from oxidation of the fat components in the product and protect it from flavour/odour migration. The high density polythene has inferior properties in these roles. All of these materials can be obtained in Thailand.

The results of chemical and mechanical tests of the three packaging materials are shown in Table XXIV.

In comparison to the properties between the three packaging materials; high density polythene, paper saran coated and cellophane saran coated, the saran coated paper was chosen for the packaging material for soya noodle. Although the cellophane saran coated was a sparkling transparency so that the consumer can see the product inside the package and has an excellent high speed performance on the packaging machine, the moisture vapour transmission rate is higher than the other two. The product packed in the package made with this packaging material would have a shorter storage life than the product in packages made from the other two materials.

Both high density polythene and paper saran coated are opague and good moisture barriers. The paper saran coated has a lower moisture vapour transmission rate, higher tensile strength than those of high density polyethylene. For the printing property, paper can be printed more easily than cellophane. With reasons such as these the paper saran coating was chosen for making a package for the soya noodle.

Table XXIV. Testing Properties of Three Different Packaging Materials: High Density Polythene, Saran Coated Paper and Cellophane Saran Coated.

| Material | H.V.T.R. (moisture vapour transmission rate) g. per sq.in/24 hr. at 100°F 100% RH | Tensile Strength (g/cm) |
|---|---|---|
| 0.003" Polythene | 2.6 | approximate 1200 |
| 55g. SBK/25 g. PVDC coating (saran coated paper) | 3.5 | Machine direction (M.D.) 3700<br>Tranverse direction (T.D.) 1300 |
| Cellophane Saran coated 0.001" gauge | 17.0 | M.D. 2410<br>T.D. 1290 |

Source: The Mono Division of U.E.B. Packaging Ltd., Auckland.

## Designing of a Pack

A preformed bag of flat type with the demensions of 6" x 9" was designed for packing 100 g. of soya noodle. The bag was heat sealed at the sides and bottom with the saran coated layer

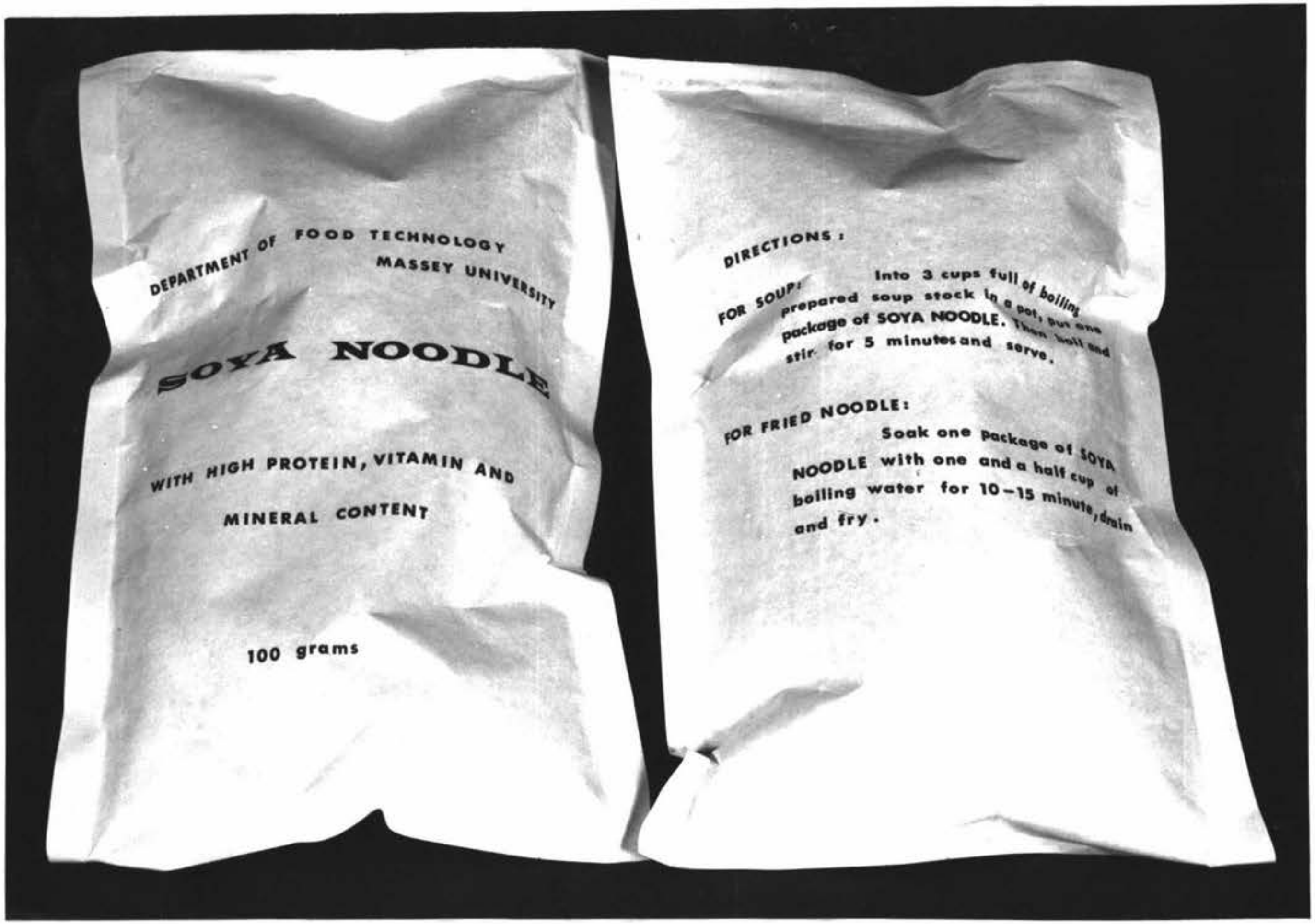



**Figure 22:** A Desigened Package for Soya Noodle

inside the bag. After packing with the noodles the top end was heat sealed.

Information, such as the name of the product, protein content, and directions for use, could be printed on both sides of the pack, together with an illustration of the noodle. A package of soya noodle with a rough graphic design is hown in Figure 22.

A carton designed for the pack was omitted due to the additional cost of the package to the price of the product. A few packages can be displayed on the shop shelf and the rest can be kept in kerosene tins.

Estimation of Shelf Life of the Product

With the knowledge of the weight of the product to be packaged, the package area, the WVTR of the packaging material at the intended storage conditions and the critical moisture content at the level of which the product was unacceptable, the approximate shelf life of the product can be estimated with the equation suggested by Davis (1970).

$$t = \frac{w}{A \times P \times \Delta P}$$

where $t$ = shelf life

$w$ = permissible moisture uptake, i.e. the weight of water absorbed by the product between its initial and critical moisture content levels.

$A$ = package area

$P$ = WVTR of the package

$\Delta p$ = mean difference in water vapour pressure between the product and the storage atmosphere over the storage period.

Soya noodle was packaged at a moisture content of 7.5% dry basis which corresponds to an ERH of 45%. It was found from storage of the noodle in the atmosphere of relative humidity as in Chapter IX that at the end of two weeks at 30°C the noodle stored in 74.9/84.5 and 92.9% RH was brown and soft. Mould growth occurred in 2 weeks at 92.9% RH, 3 weeks at 84.5% RH and 4 weeks at 74.9% RH. The noodle kept at the relative humidity lower than 74.9% RH did not change. Therefore, the critical moisture content at which the quality of the noodle starts to change is at the moisture content of 12.8% dry basis or at 74.9% RH.

Assuming that

(1) the temperature in Thailand is 30°C and the relative humidity is 77% which is the average of the maximum and minimum relative humidity reported by the Meteorological Office London, (1966).

(2) the water vapour transmission rate of 55 g. SBK/25 g. PVDC coating at 77% RH and 30°C is about the same as 100% RH 37°C (100°F).

therefore $w = 12.8 - 7.5 = 5.3$

$A = 0.07$ sq. m.

$P = 3.5$ g. per sq. m. per day

$$p = \left[77 - \left(\frac{45 + 74.9}{2}\right)\right] \times \frac{1}{100}$$

$$t = \frac{5.3}{0.07 \times 3.5 \times 0.17}$$

$$= 126 \text{ days.}$$

Thus the timefore100 g, soya noodle packaged in 6" x 9" paper saran coated bag to reach the critical moisture content is

126 days. When the moisture content of the noodle reaches the critical moisture level, the product can be kept for sometime until it goes off. Therefore the shelf life is estimated by adding together the time to reach the critical moisture content and the time the product is known to keep after the critical moisture content is reached.

In soya noodle, it was found from previous stability test that after the first two weeks the noodles kept in the desiccator containing saturated NaCl solution of 74.9% RH became soft and brown. The noodles could be kept for another two weeks before mould started to grow. For the noodles in the package if it takes 126 days for the moisture content to reach its critical moisture level, it may take another couple of weks for the mould growth. Therefore, the shelf life of soya noodle can be about 140 days.

From the results of estimation of shelf life of soya noodle, it was found satisfactory to use 55 g. SBK/25 g. PVDC coating or saran coated paper for a packaging material for soya noodle.

# XI. STORAGE TEST

In developing a new food product, it is necessary to carry out storage tests in order to study the stability of the product during distribution, transportation and storage. Then the proper method of distribution and marketing can be introduced. The shelf life of the soya noodle had been predicted from calculation in the previous chapter with the knowledge of the rate of moisture transmission of the packaging material. This prediction may be optimistic since the result of the water vapour transmission rate was obtained from a testing of a piece of packaging material rather than a pack containing the product. Therefore it is the purpose of this storage test to obtain the storage life of soya noodle in the designed package and to investigate the changes that occur during the storage of soya noodle at three different temperatures, 37°, 4[illegible]° and 50°C and at 74.9% RH.

## Storage design

An accelerated storage test was used to obtain a quicker result. The rate of a chemical reaction in a simple system normally increases when the temperature is raised and the relationship is quantified in the Arrhenius equation. Rose (1948) found that the storage life of the dehydrated potato product which may deteriorate due to discolouration is doubled by a 3.4°C (6.8°F) drop in temperature or the rate of the colour reaction increased to 8-fold for each 10°C rise of temperature.

Burton (1949) also found that the rate of oxidation in potato products increased only about 1.2 to 1.4 times by a temperature rise of 10°C.

Soya noodle, as reviewed in Chapter X, is likely to suffer most from browning during storage. Temperature is one of the effects which causes browning deterioration and the rate of browning reaction is increased about 8 times for each 10°C rise. An accelerated storage test had been used by Gooding and Duckworth for browning deterioration of dehydrated vegetable (Gooding and Duckworth, 1957). In their reviews, the accelerated storage test, by holding the product at temperatures considerably higher than required, had been first suggested by Tomkin in 1945, who considered that the brown discolouration appearing at 37° and that which appeared much more rapidly at 55° and 70° was similar in nature. Also in 1951 Legault and co-workers showed that the curve relating browning and moisture content in dehydrated potato was similar at 120°F (49°C) and 100°F (38°C) but that the rate of browning was accelerated about tenfold at the higher temperature. They found that the extent of browning deterioration of dehydrated carrot that occurred in one day at 55°C was equal to a month at 37°C. Therefore it was possible that the accelerated storage test could be used for obtaining quicker indications of the storage properties of the packaged soya noodle.

For the storage conditions, the temperature of 37°C, 43°C and 50°C were chosen for the accelerated storage test. The package of soya noodle was stored in the desiccator containing a saturated solution of sodium chloride giving 74.9% RH in the incubater at these different temperatures and in the desiccator containing saturated solution of $CaSO_4$ of 84.5% RH at 37°C. The reason for using the relative humidity of 74.9% RH for the accelerated storage test was firstly that a constant relative humidity at a variation of temperature could be obtained only from saturated solution of NaCl, secondly, at this %RH the noodle started to go brown and it was similar to the average relative humidity in Thailand. The relative humidity of 84.5% was used as a severe condition. For the control sample, the noodle was kept in a sealed tin flushed five times with nitrogen and stored at 0°F.

At intervals of 2 days for 43° and 50°C and one week for 37°C samples were taken from each type of storage and the moisture content was determined, then the package was vacuumed and resealed again and kept at 0°F for further analysis.

At the end of the storage test, the samples were examined in the laboratory for changes in colour and texture and the development of rancidity in comparing with the use of antioxidant and also by a trained taste panel who recorded their opinions of flavour and odour changes in a scoring test and gave their opinions about the acception and rejection of the colour of the dried noodle in different storage conditions.

Method of Analysis

Objective method. (1) Moisture: The moisture content of the noodle was determined by the air oven method as previously described in Chapter VI.

(2) Colour: Colour was measured by pigment extraction method as described in Chapter IX. The increase in

browning during storage was reported as the optical density reading at the wavelength 390 m$\mu$. The colour of the rejected noodle as judged by the taste panel was also measured by the colour disc comparison method as previously described.

(3) Texture: Texture changes of the noodle during storage was measured by the texture measurement of the cooked noodle with the noodle texture testing machine.

(4) Rancidity test: A thiobarbituric method was used which was based on the reaction of 2-thiobarbituric acid (TBA) with the oxidation products of unsaturated fatty acids to give a red pigment. The spectrophotometric determination of this red pigment has been used to follow rancidity in the product. Malonaldehyde, a three-carbon fragment derived from the oxidation of mono-or-polyenoic fatty acids was identified as the active colour producing compound. Therefore the distillation method for a quantitative determination of malonaldehyde in rancid foods of Tarlagis et al (1960) as described in Appendix XI was used to determine rancidity in soya noodle.

Subjective method. 6 members of a trained taste panel were asked to smell the samples which were kept in jars for the rancid odour and recorded their opinion in the form as shown below:

| Score | Description |
|---|---|
| 1 | very strong rancid odour |
| 2 | strong rancid odour |
| 3 | moderately rancid odour |
| 4 | slightly rancid odour |
| 5 | very slightly rancid odour |
| 6 | no detectable rancid odour. |

Then the taste panels were asked to indicate their opinion on the flavour of cooked noodle samples. A 1 - 7 rating score was used which gave 7 = no off-flavour, 6 = very slight off-flavour, 5 = slight off-flavour, 4 = moderate off-flavour, 3 = strong off flavour, 2 = very strong off-flavour, and 1 = objectionable.

The red lights were used in the taste panel room during these tests, to prevent the bias that could occur due to the brown colour of the sample.

The taste panels were also asked to classify the colour of the noodle samples, which were presented in a series of different browning intensity, into acceptable and objectionable colours which they would either buy or not buy.

## Preparation of Samples for Storage Test

100 g. of soya noodle prepared as the method described in Figure 21, and with a final moisture content 7.5% wet basis, were packed in the saran coated paper bag and the bag was heat sealed. For the testing of antioxidant to prevent rancidity, 0.01% BHA was incorporated in the dry ingredients and the noodle was prepared the same as above.

## Results

The results of moisture, colour, texture and flavour changes in the noodle during storage at 74.9% and 84.5% RH and at 37°, 43°, and 50°C are shown in Figure 23.

Moisture content. During storage the moistured content of the sample inside the package increased with increasing time of storage. Increase in the temperature of storage did not affect the moisture content that the sample would reach when the

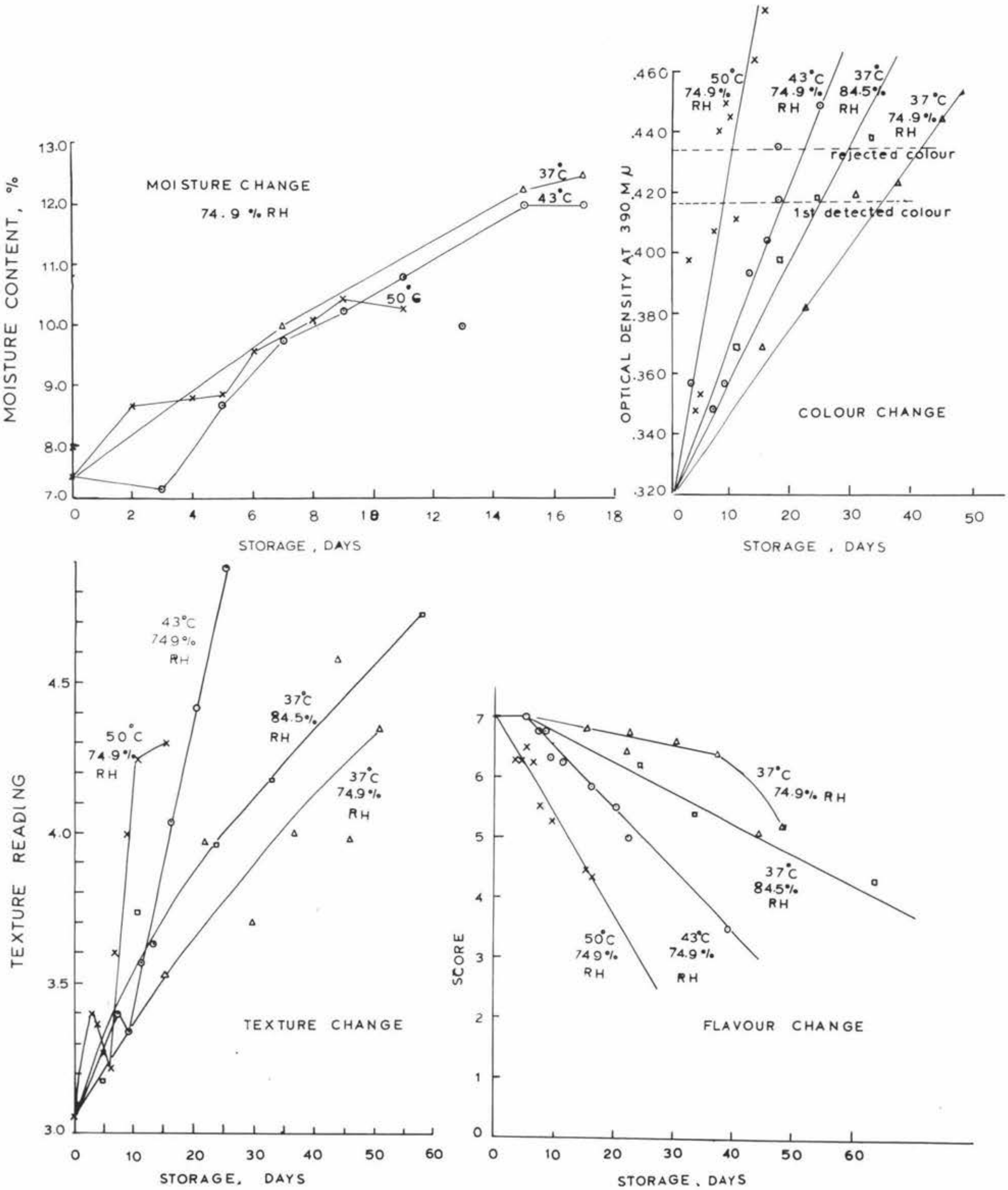



Figure 23 Moisture, Colour, Texture and Flavour Changes During Storage of Soya Noodle at 37°, 43° and 50°C, 74.9 % and 84.5 % RH

equilibrium condition occurred. The highest levels of the moisture content of the samples stored at 50°C, 43°C, and 37°C at 74.9% RH were 10.4, 12.0 and 12.5% wet basis respectively. This effect may be due to the transition of the moisture sorption isotherm when the temperature changes as illustrated by Rockland (1969). At the same ERH, when the temperature increase the moisture content of the food at this equilibrium level will decrease.

<u>Colour change</u>. The rate of browning reaction occurred rapidly, when the temperature and relative humidity increased. The rate of browning reaction increased 4.5 times and 1.5 times when the temperature and relative humidity were raised by 10°C and 10% RH respectively. From table XXV the taste panel indicated the first detectable difference in colour between the stored sample and the control sample when the reading of the butanol extraction at 390 mμ was 0.417. At this reading, about half of the taste panel members would accept the sample and the other half would reject it. When the reading was higher than 0.435 mμ the sample was rejected by all the taste panel members. The storage times at 37°, 43°, and 50° and at 74.9% and 84.5% RH for the first detectable colour to appear and for the sample rejected because of colour were read off from the graph in Figure 23, and are shown in Table XXV.

The colour of the sample was unacceptable when the sample was stored at 74.9% RH, at 37°C for 41.5 days, at 43°C for 22 days and at 50°C for 10.25 days. When the relative humidity was increased to 84.5%, the sample could be kept at 37°C for only 29.5 days. With all this information the shelf life of the

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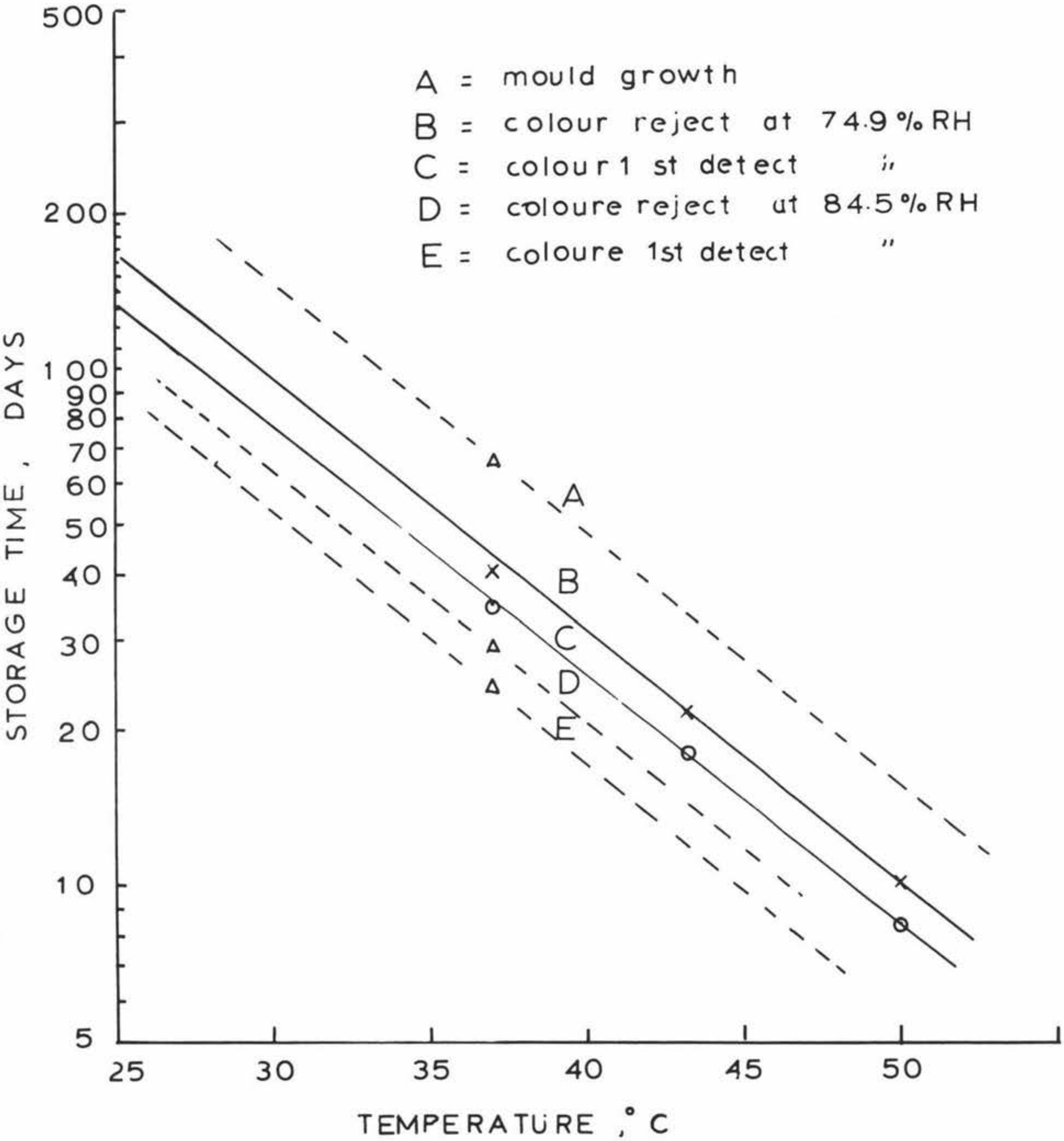


Figure 24 Time – Temperature Relationship of Storage Deterioration of Soya Noodle

product in Thailand at a temperature of 30°C could be predicted by plotting the time and temperature where colour change in product was first detected and where the product was rejected because of the colour on the semilog paper as in Figure 24 and by extrapolating the lines to 30°C. It was found that the product could be kept at 30°C, 74.9% RH for 78 days when the brown colour change was first detected and to 96 days when the product was rejected because of its colour; and similarly for 55 and 64 days at 84.5% RH.

Table XXV. Results of Measurement of the Browning Colour in the First Detectable Coloured Sample and the Rejected Sample

| Sample | Pigment reading at 390mμ (O.D.) | % yellow plus brown of Munsell disc colour | Time (days) | | | |
|---|---|---|---|---|---|---|
| | | | 37°C | | 43°C | 50°C |
| | | | 74.9% RH | 84.5% RH | 74.9%RH | 74.9% RH |
| Control sample | 0.322 | 62.0 | 0 | 0 | 0 | 0 |
| First detectable colour by taste panel | 0.417 | 84.5 | 35.0 | 24.5 | 18.5 | 8.5 |
| Rejected sample by taste panel | 0.435 | 89.5 | 41.5 | 29.5 | 22.0 | 10.25 |

Texture change. In the case of texture change, the cooked noodle toughened when the storage time increased and the toughness increased rapidly with the increase of temperature. Storage at 43°C and 50°C caused the texture of the product to become slightly tough at the beginning of the storage test, then the texture became soft when the moisture content increased, and started to become increasingly tough again when the moisture, time of storage and browning increased. It was found that the texture reading was about 4.2, that is very tough, when the product was rejected due to browning.

Flavour change. Although the product became more brown and had a tougher texture when the storage time increased, the flavour of the cooked noodle was only slightly changed. The rate of flavour change was dependant on the temperature of the storage condition. The taste panel could detect the flavour change that occurred in the sample stored at high temperature more accurately than those of low temperature as shown by the straight line of 43$^{o}$ and 50$^{o}$C and bent curve of 37$^{o}$C. The cooked noodle was indicated as slightly off-flavour by the taste panel when the colour of the dry noodle was unacceptable. The control sample and the sample stored only a few days were described as having a beany flavour and this beany flavour disappeared when browning occurred.

Rancidity. From Table XXVI, when BHA was used as an antioxidant in soya noodle at the level of 0.01% BHA incorporated in the dry mix, the oxidative deterioration was delayed. Samples with BHA had lower results on TBA readings at 538 m$\mu$, but higher results of pigment extraction reading at 440 m$\mu$, and a higher score for rancid smell.

It was noted that the sample with BHA also gave a higher pigment extraction reading at 390 m$\mu$, which represented the brown colour of the product. Therefore, it seemed likely that the antioxidant accelerated the browning reaction, thus resulting in a higher reading of pigment extraction at 390 m$\mu$.

Table XXVI. Effect of Antioxidant on the Deterioration of Stored Soya Noodle

| | T.B.A. READING AT 538mμ (O.D.) | SMELLING SCORE OF RANCIDITY+ | PIGMENT EXTRACTION READING | |
|---|---|---|---|---|
| | | | at 440mμ (O.D.) | at 390mμ (O.D.) |
| Control | 0.717 | 4.3 | 0.133 | 0.322 |
| 37°C, 44 days without BHA | 0.236 | 3.2 | 0.132 | 0.456 |
| 37°C, 46 days with BHA | 0.211 | 4.4 | 0.212 | 0.551 |
| 43°C, 18 days without BHA | 0.432 | 4.3 | 0.146 | 0.417 |
| 43°C, 18 days with BHA | 0.317 | 5.2 | 0.178 | 0.435 |
| 50°C, 3 days without BHA | 0.545 | 3.3 | 0.153 | 0.397 |
| 50°C, 11 days without BHA | 0.367 | 4.6 | 0.132 | 0.412 |
| 50°C, 8 days with BHA | 0.507 | 4.3 | 0.186 | 0.440 |

+ (Score 1 = very strong rancid odour, Score 6 = no detectable rancid odour) than the sample without BHA.

Microbiological change. It was found that on storage, the sample in 37°C 74.9% RH storage conditions after 67 days developed mould growth. By drawing a parallel line to the rejected colour line in Figure 24, it was estimated that the time for mould growth to occur at 30°C would be 150 days.

## Discussion

From the results of the accelerated storage test, it appeared that the browning deterioration was the most critical deterioration that affected the storage life of soya noodle. When the noodle became brown, the texture was tougher than the fresh noodle, but the flavour of the cooked noodle did not show much difference freshthe fresh noodle. A benefit was obtained from the occurrence of some browning, in that there was a disappearance of the beany flavour in the stored samples.

The use of antioxidant for soya noodle might not be necessary, since the rancid odour in the stored sample could not be detected by the taste panel and the antioxidant might accelerate the browning reaction.

In this experiment, two problems occurred. Firstly, in storage of the packages of soya noodles in the desiccators, the packages at the bottom layers tended to absorb more moisture than the packages in the top layer. Therefore, the results for moisture contents of samples taken at different storage times fluctuated. Secondly in the control sample, rancidity did occur as shown by the high result of TBA reading. This occurrence of rancidity in the control sample may be due to the negative temperature coefficient (McWeeny, 1968). When the temperature was decreased, the moisture that once prevented the oxidation at the high temperature could no longer prevent this oxidation when the temperature was below freezing point. Therefore in the control sample, the oxidation reaction would occur if there was some oxygen left in the tin. To solve these two problems, dynamic storage conditions should be used instead of the static conditions. A cabinet of controlled relative humidity and temperature with the air velocity about 10 ft./sec. would be preferred to the desiccator containing saturated salt solution. In the control sample, flushing with nitrogen more than five times would be preferred.

In conclusion of the storage test, a package of the noodle could be kept in Thailand during distribution and storage up to 150 days before the product has to be thrown away due to mould growth. The product will start browning after storage for 78 days at 74.9% RH or 55 days at 84.5% RH; and after 96 days at 74.9% RH, 64 days at 84.5% RH the colour of the product becomes unacceptable. If the package of soya noodle is kept in a kerosene tin during distribution and selling in the shop, the storage life of the product will be longer. If longer life is required, a more expensive package with lower water permeability would need to be used.

The results of the predicted shelf life of the product given in previous chapter is close to the actual storage test.

# XII. MEASUREMENT OF NUTRITIONAL VALUE OF SOYA NOODLE

Although the total amino acid composition of soya noodle had been analysed and the limiting amino acid in comparison with the FAO amino acid pattern was supplemented in the soya noodle, it was recognised that the nutritive value of a protein depends not only on the pattern of the component amino acids but also on their physiological availability. Availability of amino acids can be reduced by incomplete digestion and absorption; by the presence of an inhibitor of the digestive enzyme and by damage to protein and amino acids from heat treatment and other processing (FAO, 1965). Therefore it was the purpose of this study to firstly determine the availability of amino acids in soya noodle and then to determine its nutritional value by a feeding trial with young chicks. It was realised that there was still some trypsin inhibitor remaining after steaming the noodle for 20 minutes and also that the soya noodle contained a certain amount of carbohydrate which during steaming and drying in the presence of carbohydrate could reduce the availability of some of the amino acids in soya noodle, both of which might affect the nutritional value. As well, there could be destruction of some of the vitamins. Thiamine, riboflavin and niacin in soya noodle were determined before and in the supplementation an allowance for processing loss was made, therefore it is not thought necessary to determine them again.

In the experiments, the availability of the amino acids was measured by the in vitro enzymatic method; and the results of weight gain of young chicks was used for the assessment of the nutrition value of soya noodle.

Evaluation of Availability of Amino Acids in Soya Noodle

There are many methods in which availability of amino acid can be measured as reviewed by Morrison and Rao (1966) and Miller (1967). These methods are chemical method, enzymatic method, in vivo method, plasma amino acid method and microbiological method. All of these methods except the chemical method and enzymatic method are cumbersome and time consuming. In the chemical method, only the method for available lysine has been established in which the DNFB (dinitro-fluorobenzene) available lysine method of Carpenter is used widely. A suitable chemical procedure to evaluate the availability of sulfur containing amino acids has not been developed. The measurement of available amino acids by the chemical method cannot predict the protein quality of a protein in which varying levels of toxic substance such as trypsin inhibitor may occur. The enzymatic method seemed to be the only method suitable for determination of availability of amino acid in soya noodle, as there was some of the trypsin inhibitor activity left.

For the enzymatic method, the problem arises as to what should be the suitable condition for in vitro assay of availability of amino acid that can serve as a guide of the amount of amino acid which may be released by enzymatic digestion in the intestine. Sheffner et al (1956) proposed a method, referred to as the pepsin-digestion-residue index, for integration of the pattern of essential amino acid released by in vitro pepsin digestion with the amino acid pattern of the pepsin resistant residual. Since the work involved was considerable in this method - 10 amino acid had to be determined in an acid hydrolysate as well as in a pepsin digest by the use of a microbiological technique. Therefore Akeson and Stahmann (1964)devised a pepsin-pancreatin digest index based on the chromatographic determination of amino acids released by an in vitro digestion with pepsin followed by pancreatin. With the aid of an automatic amino acid analyser the method was simple and possible for the determination of availability of amino acids in soya noodle.

Recently Ford and Salter (1966) developed a method of using pepsin, trypsin, pancreatin and erepsin digestion for determination of available amino acid in fish protein, and the digested amino acids were determined by microbiological assay. They found the result of available amino acids released by these enzymes digestion was as good as that by the pronase enzyme-an enzyme from bacteria. It was recognised that a nearly complete protein hydrolysis could be obtained by using pronase digestion, (hill, 1965).

In the determination of availability of amino acids in soya noodle, the pepsin, trypsin, pancreatin and erepsin digestion as the method of Ford and Salter (1966) was used and the hydrolysate was prepared for amino acid analysis, by the use of automatic amino acid analyser, according to the method of Akeson and Stahmann (1964).

## Experimental method

Method of enzyme hydrolysis. A ground uncooked sample of soya noodle prepared by the method as described in Figure 21, was defatted prior to analysis. The nitrogen content of the defatted sample was determined. A sample containing 1 g. N (19.08 g. defatted soya noodle) was weighed into a conical flask of 500 ml capacity, to which was added 150 ml 0.05N HCl. The contents of the flask were allowed to stand for 30 mins. and their pH value adjusted to 1.8 by the addition of N HCl. 100 mg crystalline pepsin (strength 1:2500, The British Drug House Ltd., England) was added to the flask. Then the flask was placed in a water bath at 37°C and the contents stirred gently for 30 mins. The pH value was again adjusted to 1.8 and incubation at 37°C was continued for a further 23½ hrs., with frequent stirring during the first 8 hrs. of incubation. After digestion for 24 hrs. with pepsin, 1 N NaoH was added to the flask until the pH value of the digest was about 7 and then 1.5 g. $NaHCO_3$ were added. The pH value was no adjusted to 8.2, and 50 mg crystalline trypsin (40.54 Anson units per g., The British Drug House Ltd., England) and 100 mg pancreatin (conforms to B.P. 1968, The British Drug House Ltd., England) were added to the digest.

After stirring for 30 mins. at 37°C, 5 ml of sulfur-free toluene was added. The flask was stoppered tightly and incubated for a further period of 24 hrs., with frequent swirling of the contents during the first 8 hrs. of incubation. At the end of the digestion period there was added 7.5 ml of a solution, prepared by grinding 2 g. erepsin (from hog intestine) with 40 ml 0.02M phosphate of pH 7.6 for 10 mins. at room temperature, centrifuging and decanting through a plug of cotton wool in a filter funnel. Incubation was now continued for a further period of 24 hrs., again with frequent swirling of the contents of the flask during the first 8 hrs. An enzyme blank was prepared, together with the duplicate samples by incubation under the described conditions with the soya noodle sample omitted.

Preparation of sample for amino acid analysis. The volume of the digestion mixture from each flask was adjusted to 500 ml and 10 ml aliquot were added to 100 ml of one percent picric acid solution and the mixture was centrifuged for 30 mins. at $100^{\circ}$x g. to remove undigested protein and large peptides. 50 ml of supernatant were passed through a column containing anion exchange resin (Dowex 2-x 8, size 20-50 U.S. mesh in chloride form) After rinsing the column with three portions of 0.02N HCl, the samples freed from picric acid were evaporated under reduced pressure in a rotary evaporator.to near dryness. The digests were freed from toluene and the volume was adjusted to 25 ml with buffer pH 2.2 Samples were kept frozen until analysis.

Method of amino acid analysis. Amino acid analysis of the sample was made by the ion exchange method with the Beckman model 120C amino acid analyser according to the method described previously in the total amino acid analysis except that a standard 4 hr. analysis was used instead of accelerated analysis. The operational procedure of amino acid analysis by the amino acid analyser was also described in Appendix IX.

Results and discussion. The results presented in Table XXVII are the average results from the duplicate analysis, before and after substraction with the results of amino acids from Blank due to the autohydrolysis of enzymes. The result of tryptophan was not reported Tryptophan was destroyed during removal of undigested protein and large peptides with picric acid. The chromatograms of the acid hydrolysate and of the pepsin, trypsin, pancreatin and erespin hydrolysate of soya noodle are shown in Figure 25.

Table XXVII. Available Amino Acid in Soya Noodle

g. amino acid per 100 g. N

| Amino Acid | Amino Acid from Enzyme Digestion of Soya Noodle | Amino Acid from Enzyme Blank | Available amino acid in Soya Noodle | Total amino acid in Soya Noodle |
|---|---|---|---|---|
| Lysine | 16.56 | 8.82 | 12.74 (31.9)* | 39.94 |
| Histidine | 2.06 | 0.00 | 2.06 (19.7) | 10.44 |
| Ammonia | 2.82 | 0.02 | 2.80 (20.8) | 13.50 |
| Arginine | 35.09 | 0.76 | 34.33 (81.2) | 42.25 |
| Aspartic acid | 2.66 | 0.69 | 1.96 (03.1) | 64.25 |
| Threonine | 1.97 | 0.65 | 1.31 (07.0) | 18.69 |
| Serine | 6.86 | 1.10 | 5.76 (22.3) | 25.75 |
| Glutamic acid | 4.81 | 0.85 | 3.96 (03.2) | 124.19 |
| Proline | 0.00 | 0.00 | 0.00 (0.00) | 53.63 |
| Glycine | 0.75 | 0.46 | 0.30 (01.6) | 18.88 |
| Alanine | 2.88 | 0.69 | 2.20 (10.2) | 21.41 |
| Cystine | 0.87 | 0.00 | 0.87 (09.9) | 8.82 |
| Valine | 5.27 | 0.53 | 4.74 (14.0) | 33.89 |
| Methionine | 6.66 | 0.00 | 6.66 (64.9) | 10.26** |
| Isoleucine | 5.07 | 0.47 | 4.60 (13.1) | 35.00 |
| Leucine | 23.89 | 1.08 | 22.87 (56.7) | 40.31 |
| Tyrosine | 14.31 | 0.39 | 13.94 (86.1) | 16.19 |
| Phenylalanine | 16.23 | 0.35 | 15.88 (66.7) | 23.81 |
| Total | | | 136.98 | 601.21 |

* Percentage of total amino acid

** Half of total methionine was supplemented by DL methionine.

The results of amino acids obtained from enzyme digestion of soya noodle was very low except that of arginine, methionine, leucine, tyrosine and phenylalanine. About 30% of total lysine was released by the enzyme digestion. The high amount of

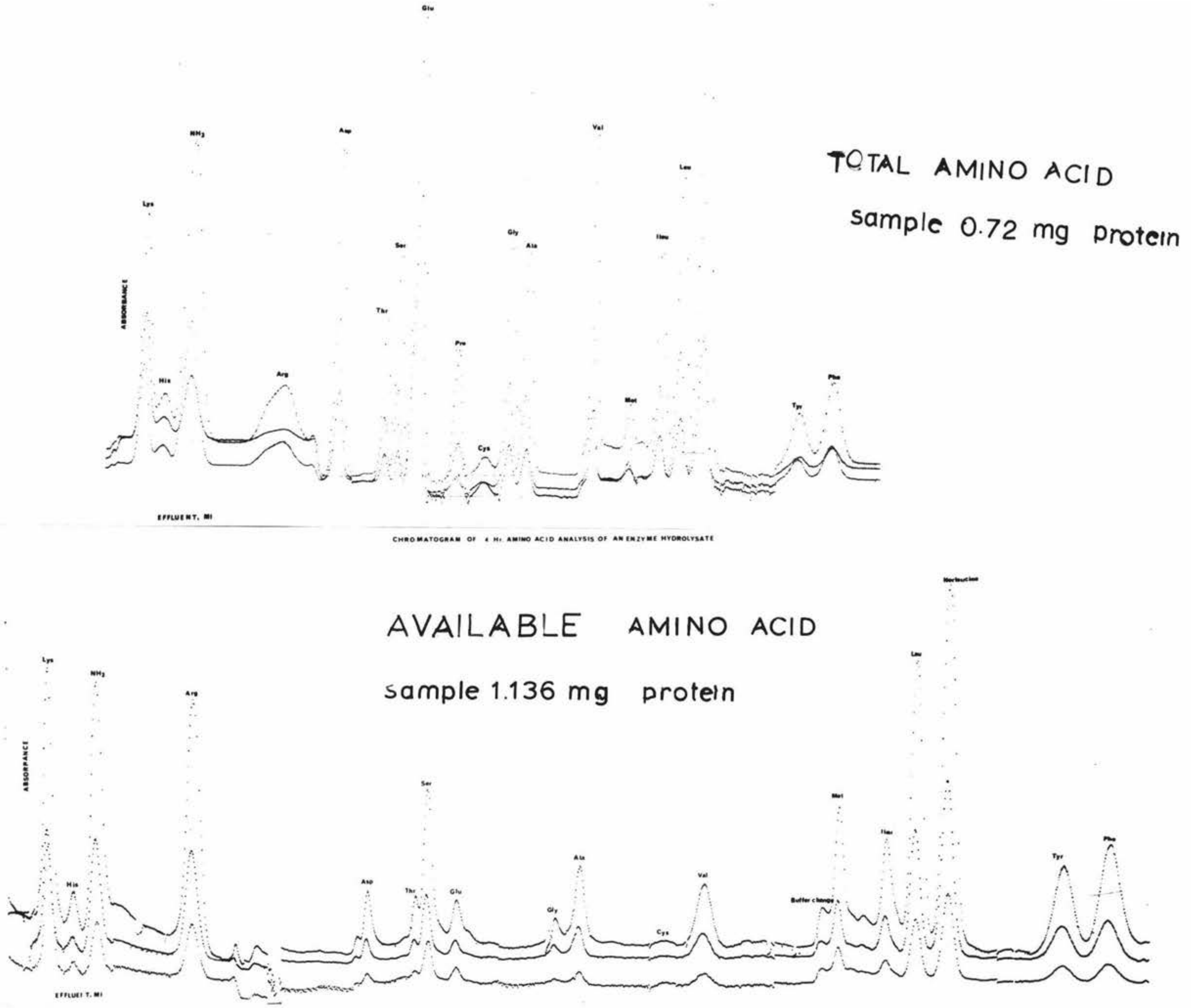


Figure 25: Comparison of Chromatogram of Total Amino Acid and Available Amino Acid.

methionine available 65% may be due to the supplementation of DL methionine in the noodle. The amino acid proline was not released by the enzyme digestion at all. Usually aspartic and glutamic contents of soya noodle are very high but from the enzyme digestion the two amino acids were not released (see chromatogram in Figure 25).

Before going to the discussion of factors affecting the low or high results of availability of some amino acids, it is worthwhile to study the specificity for hydrolysis of peptide bonds of each enzyme. As reviewed by Hill (1965), pepsin can hydrolyse bonds formed by the amino or carboxyl groups of phenylalanine, tyrosine, glutamic acid, cystine and cysteine and also the bonds formed by leucyl residues. Bonds formed by the other amino acids do not appear to be hydrolysed as readily as those of the aromatic amino acids and leucine. Trypsin can hydrolyse peptide bonds formed by the carboxyl groups of lysine and arginine. The only type of lysyl or arginyl bond which appears to be completely resistant to trypsin is that formed with proline. The specificity of chymotrypsin for hydrolysis of peptide bonds formed by the carboxyl group of tyrosine, phenylalanine and tryptophan has been recognised. From Hill and Schmidt (1962) erepsin which is the intestinal extract was shown by Cohnbeim in 1901 that it hydrolysed peptones to amino acids. With the understanding of the specific hydrolysis property of each enzyme, the results of enzyme hydrolysis can be easily discussed.

This analysis was on the dry soya noodle and it was realised that there was trypsin inhibitor in the soya noodle even though it had been subjected to steaming, therefore in the enzyme digestion a certain amount of trypsin and chymotrypsin would be lost due to the inhibition of the trypsin inhibitor. This might lead to the low results of the corresponding amino acid released by these two enzymes. Since the result of arginine availability was a high as 80% and that of lysine was 30% and the trypsin enzyme has a specificity of hydrolysis arginine and lysine bond, therefore as much as 50% of lysine availability could be lost during processing, and about 20% of arginine and lysine availability lost due to the inhibition effect of the trypsin inhibitor. Because of the work of Sealock and Laskowski (1969) this explanation could not be true. They found that the reaction site of trypsin inhibitor is the arginine (64) and isoleucine (65) peptide bond (as illustrated in Figure 26). Incubation of the inhibitor with trypsin leads to formation of modified inhibitor in which this arginine (64) isoleucine (65) bond has been hydrolysed, and the arginine (64) is easily removed from the modified inhibitor by carboxypeptidase B which is one of the pancreatin enzymes. This can explain the high result of arginine.

For the very low results of aspartic acid and glutamic acid, Hill and Schmidt (1962) in their attempt to find the method for complete enzymic hydrolysis of protein, found that the yield of these amino acids was low, as these two amino acids and serine emerged together from the chromatographic column and also a considerable quantity of glutamic acid was converted to pyrrolidone carboxylic acid which did not yield a coloured product with ninhydrin. Tower et al (1962) also employed enzymatic hydrolysis with pancreatin preparation for the liberation of

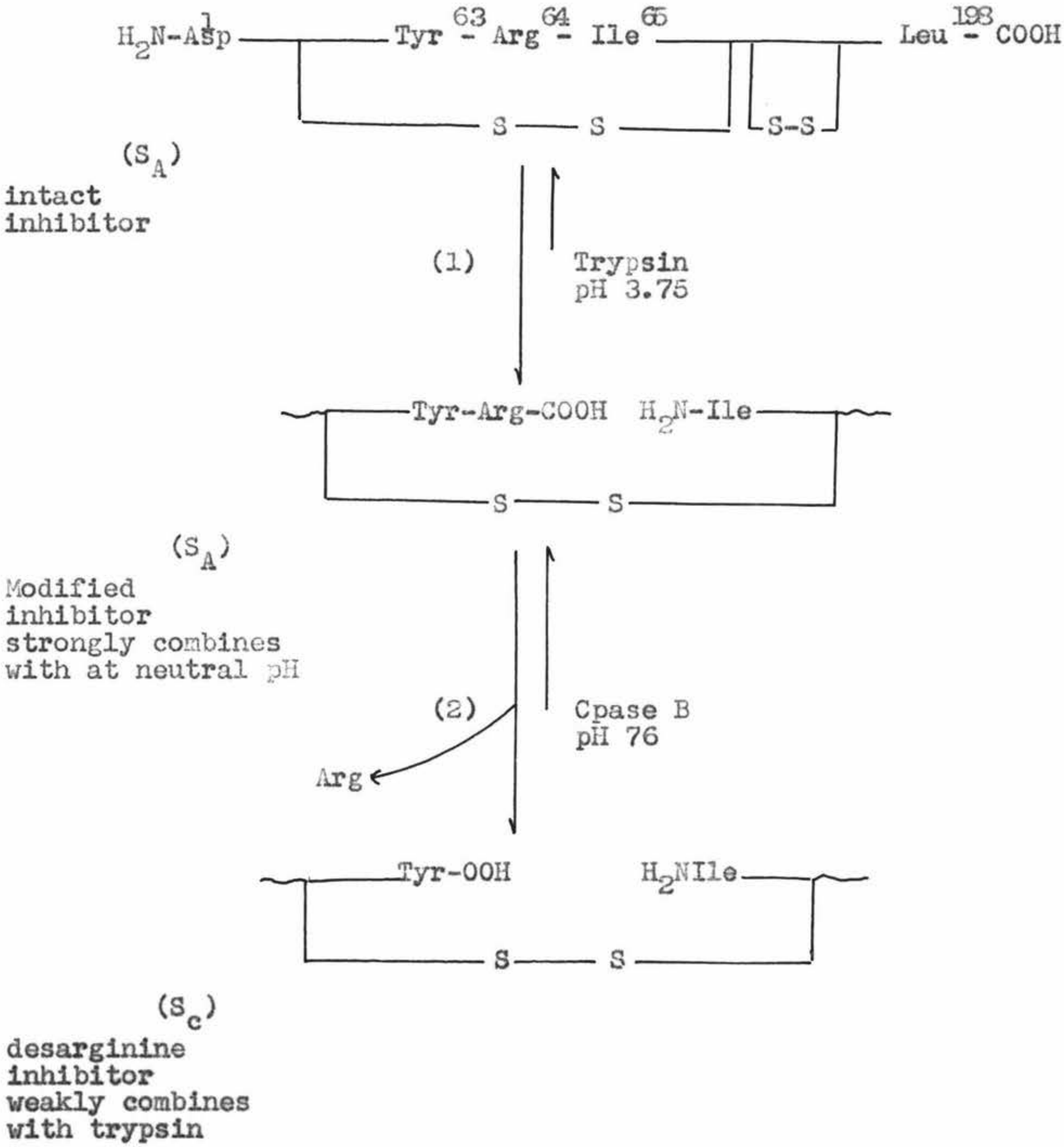


**Figure 26:** The sequence of reactions employed in the "enzymatic mutation" of soybean trypsin inhibitor. (Sealock and Laskowski, 1969)

glutamine and asparagine from proteins. Under the conditions employed, proteins were not hydrolysed to the extent of more than 50-80%.

A result of proline was not obtained in the analysis. Hill (1965) and Hill and Schmidt (1962) mentioned that for the hydrolysis of peptides containing proline a specific enzyme such as prolinase was needed.

Fukushima (1965) studied the internal structure of 7S and 11S globulin which are the major proteins of soybeans. He found that the major internal structure of both 7S and 11S globulins of soybean are not alpha-helix, but are both the antiparallel beta structure and the disordered structures. The molecules are fairly compact as a whole, even in their disordered parts. The molecules are not hydrolysed by proteinase before the internal structure is disrupted, and the initial velocity of the hydrolysis by proteinase is proportional to the degree of its disruption. Also from Wolf and Tamura's (1969) study of heat denaturation of soybean 11S protein, in which they found that heating apparently disrupts the quaternary structure of the 11S protein with subsequent separation of the subunits into two distinct fractions. One fraction consists of soluble subunits of 3-4S which are stable to heating for 30 mins. or more. The other fraction of subunits has a pronounced tendency to interact to form a soluble aggregate which, in turn rapidly converts to an insoluble state on continued heating when sulfhydryl groups are present. Therefore steaming soya noodle for 20 mins. may only disrupt the quaternary structure of the protein. The internal structure may still be compact and the enzyme cannot hydrolyse as suggested by Fukushima.

On the other hand, the high results of leucine, tyrosine and phenylalanine could be explained that in the pepsin hydrolysis at low pH (pH 1.8) the native protein was unfolded, as shown by Schlamowitz and Petterson (1959), therefore pepsin enzyme could easily hydrolyse the aromatic amino acid and leucine bonds, thus resulted higher liberation of these enzymes.

In conclusion the low results of availability of amino acid in uncooked soya noodle (only steamed for 20 mins. in processing) may be due to the presence of the trypsin inhibitors that inhibited the trypsin enzyme activity and also due to the compactness of the protein molecule which lowered the enzyme action. It may have been better to have carried out this analysis on the cooked noodle.

Measurement of Protein Value of Soya Noodle by Chick Feeding

Protein values have been extensively investigated by animal tests. Some of those frequently used are the protein efficiency ratio (PER), the biological value (BV) and the net protein utilisation (NPU) as reviewed briefly in Appendix XII.

For measuring protein quality with chick feeding, as reviewed by Calet (1967), the simplest method consists of measuring the weight gain of young chicken. The weight gain of the young chicken varies according to the protein level in the feed. Because of the inaccuracy in measuring the protein quality by relating weight gain to the amount of protein ingested, a method was introduced whereby chickens receiving a feed incorporating the protein to be tested, were compared with others receiving a reference protein. It is called the Gross Protein Value method and it expresses the value of a protein as a percentage of the value of a reference protein.

To assess the value of soya noodle for early chick growth, the growth of young chickens fed with rations incorporating dry soya noodle were compared with those fed meat meal and butter milk powder at the end of four weeks.

## Experimental Method

Sixteen pounds of soya noodle prepared by the final method and finely ground were used for feeding trials.

Three soya noodle rations were derived with protein levels of the complete ration of 12.35, 13.87 and 15.39%. Pollard, limestone and vitamin mineral premix were kept constant at the three levels. These ingredients were made up as 29% by weight of the rations and contributed 4.16% of the protein. With varying amounts by weight or of contribution of the total protein required in the rations with the soya noodles, as more or less is used, less or more of some other food ingredient has to be used to maintain the bulk ingredients equal to 100 parts. Barley meal was chosen as the other feed ingredient. The appropriate levels of barley meal and soya noodle were derived using a simultaneous equation approach.

Assuming the protein contents of soya noodles, barleymeal and pollard are 30, 11 and 15% respectively, the three rations were derived. Two control rations in which meat meal, butter milk powder and lucerne meal were used as a reference protein, were included for comparison. The percentage composition of the rations are as in Table XXVIII.

In the experiment, 14 one-day old female strain cross white leghorn (single combed) chickens were used for each replication and two replications for each trial. The chickens were wing banded and weighed individually at hatching and weighed again at four weeks. All feeds were weighed into compartment food troughs and then weighed back at the end of first two weeks to allow adjustment to food consumption for chicken,which mostly occurred on the first two weeks and weighed back again after four weeks.

TABLE XXVIII. Composition of the Rations for Chicks Feeding Trial (percentage)

| Ingredients | Control Rations | | Soya Noodle | | |
|---|---|---|---|---|---|
| | A | B | S | T | U |
| Soya noodle | - | - | 2.00 | 10.00 | 18.00 |
| Barley meal | 60.00 | 66.00 | 69.00 | 61.00 | 52.00 |
| Pollard | 20.11 | 27.75 | 27.75 | 27.75 | 27.75 |
| Limestone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Meat meal | 11.00 | 3.00 | - | - | - |
| Butter milk powder | 6.56 | 2.00 | - | - | - |
| Lucerne meal | 1.00 | - | - | - | - |
| Furone supplement* | 0.10 | - | - | - | - |
| Amprol plus** | 0.0375 | - | - | - | - |
| % Protein (from Cal.) | 15.00 | 13.80 | 12.35 | 13.85 | 15.39 |
| % Protein (N x 6.25) | 17.30 | 16.20 | 14.53 | 16.69 | 18.90 |
| % Contribution of test protein to toal protein (from calculation) | - | - | 4.85 | 21.62 | 35.08 |

## Results and Discussion

A summary of the results of the chick feeding trial of soya noodle as given in Table XXIX. It is seen from the table that the results of weight gain of chicks fed rations contributed by soya 'e were low when compared with the two controls A and B.

**Table XXIX. Results of Weight Gain and Food Consumption in 4 Weeks of Young Chicks Fed Soya Noodle**

| Results | Control | | Soya Noodle | | |
|---|---|---|---|---|---|
| | A | B | S | T | U |
| | 18% meatmeal + buttermilk & lucerne meal | 5% meatmeal + buttermilk | 2% soya noodle | 10% soya noodle | 15% soya noodle |
| **Average weight after Hatching (g)** | | | | | |
| **Replication one** | 36.6 ± 2.94 | 36.5 ± 3.33 | 36.00 ± 2.60 | 36.4 ± 4.11 | 37.1 ± 3.18 |
| **Replication two** | 38.4 ± 2.4 | 35.1 ± 3.72 | 37.5 ± 3.79 | 34.9 ± 3.89 | 38.1 ± 2.98 |
| **Average weight after 4 weeks (g)** | | | | | |
| **Replication one** | 183.6 ± 31.05 | 129.9 ± 39.82 | 78.3 ± 12.29 | 87.7 ± 15.35 | 87.6 ± 13.47 |
| **Replication two** | 189.0 ± 23.35 | 131.8 ± 40.65 | 72.2 ± 10.87 | 85.5 ± 9.36 | 88.7 ± 13.24 |
| **Average weight gain After 4 weeks (g)** | 148.8 | 95.0 | 38.5 | 51.0 | 50.6 |
| **Average feed consumption (g)** | | | | | |
| **After 2 weeks** | 159.5 | 137.3 | 119.8 | 126.2 | 130.3 |
| **After 4 weeks** | 460.0 | 367.5 | 245.7 | 284.2** | 274.5 |
| **PER (Protein Efficiency Ration)*** ** | 1.36 | 1.60 | 1.07 | 1.07 | 0.96 |
| Mortality (no) | - | - | - | 1 | - |

* Average of 13 chicks.

** Average of 13.5 chicks (one chick was dead at the third week).

*** The protein content of ration from analysis was used for calculation.

Ration S was the low protein level, therefore the chick would not gain weight very much. The protein level of ration T was the same as control B and ration U was the same as control A. The weight gain of chicks fed ration T was only half of that of control B and that of ration U was one-third of control A. The weight gain of chicks fed soya noodle did not increase when the protein level in the ration increased from 13.85 to 15.39%. The weight gain of the chick fed both control rations and soya noodles was correlated with the feeds consumed. For the mortality of chicks fed soya noodle, only one chick died when fed with ration T.

From the result of post mortem analysis of Animal Station, Wallaceville, this chicken died of rickets.

The results illustrated that the protein levels in the feeds are the factors that depressed the weight gain and feed consumption of the chicken. Comparing control A and control B, both are different only in the level of protein content. The chicken ate less in control B than in control A, therefore the weight gains were less. This effect also occurred in chicken fed ration S when comparing them with chicken fed rations T and U.

Rations T and U have the same protein content as controls A and B respectively. In rations T and U, soya noodles were incorporated in the feed instead of meat meal and butter milk powder as in controls A and B. Chicken fed rations T and U ate less and have a lower weight gain than chicken fed controls B and A. Therefore the protein quality of soya noodle cannot be compared with meatmeal and butter milk powder on the basis of weight gain.

The weight gain and feed consumption of chicken fed ration U were slightly less than chicken fed ration T, although the protein level of ration U is higher than ration T. This may be due to the higher contribution of soya noodle in ration U. The percentage contribution of soya noodle in ration T and U are 21 and 35% respectively. From this result, there may be some factors in soya noodle that prevented the chicken from eating.

Rickets caused one death in the chicken fed ration T which may be due to the indirect effect of soya noodle. There were sufficient quantities of calcium and phosphorus in the diet. Because of the low consumption, the calcium and phosphorus may not be enough for the bone formation of the growing chicken. The feed consumption of rations S and U are also low, but there were no chickens dead in these two rations. The chicken fed ration S had a very poor growth and at the end of 4 weeks, the average weight of these chickens was only double the hatched weight and the size of these chickens was very small. Therefore calcium and phosphorus may not be critical for the chickens fed that ration. In chicken fed ration U the weight gain was comparable with weight gain of the chicken fed ration T. The calcium and phosphorus contents of soya noodle are high, therefore by receiving more soya noodle the chicken fed ration U received more calcium and phosphorus than the chicken fed ration T.

Considering the factors that affect the smaller consumption by chicken fed soya noodle; size, taste and appearance of the feed may not affect the consumption by chicken in this case, since the chicken is not sensitive to size and taste but will be sensitive to the appearance of the feed. The colour of soya noodle was the same as barley and pollard, therefore the appearance of the feeds containing soya noodle did not affect the consumption by the chicken. The amount of feed consumed by the chicken fed soya noodle

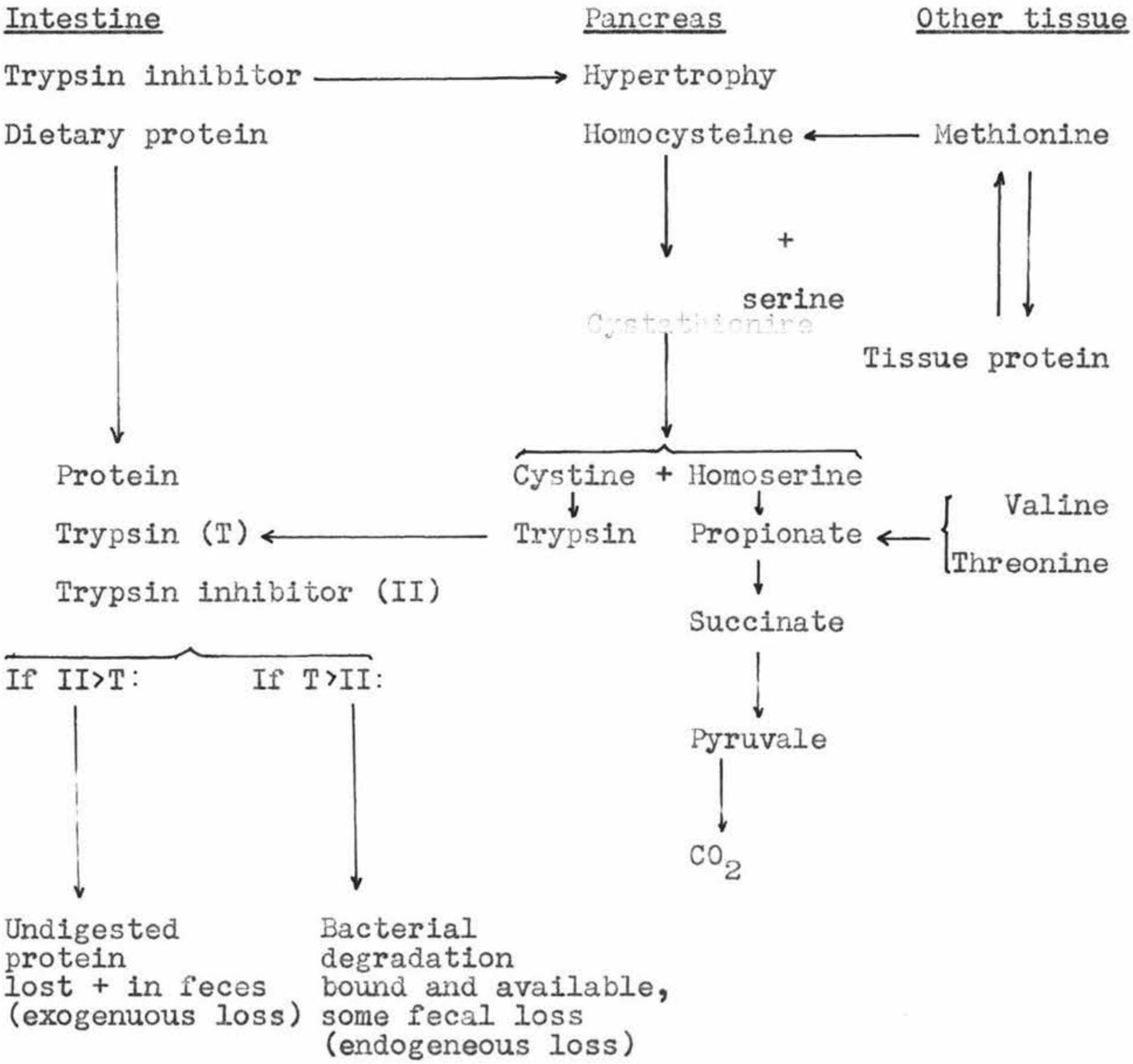


**Figure 27:** A proposed Schematic Diagram to Explain the Experimental Evidence relating to the Effect of the Soybean Trypsin Inhibitors on the Nutritive Value of Protein.

was comparable with the amount of feed eaten by the chicken fed control rations, in the first two weeks. In the last two weeks of growth this amount of feed consumption remained constant in the chicken fed soya noodle rations while the consumption of control rations rose to double the amount. Therefore the factor that caused growth depression was also responsible for the impairment of feed intake.

In feeding the chicken, soya noodle was used in the dry form, but for human food preparation using the product it will be boiled which destroys the remaining trypsin inhibitor, but this was not particularly helpful for the chicken. There is still some trypsin inhibitor left, about 20-30% of the original trypsin inhibitor activity in raw soy bean. This remaining trypsin inhibitor may be responsible for the poor growth of chicken fed soya noodle.

As reviewed by Liener and Kakade (1969), Chernik and co-worker reported that chicks fed raw soybeans developed hypertrophy of the pancreas and Lyman and Lepkovsky suggested that the growth depression caused by the trypsin inhibitor may be the result of the endogeneous loss of essential amino acids derived from an enlarged pancreas which responds in a compensatory fashion to the effects of the trypsin inhibitor. There is in fact direct evidence for the inhibition of proteolysis in the intestinal tract of chicks (Bielorai and Bondi, 1963). To explain the experimental evidence relating to the effect of soybean trypsin inhibitor on the nutritive value of protein Liener and Kakade proposed a schematic diagram in Figure 27.

In the case of the young chicken, the hypertrophic response of the pancreas is delayed so that the amount of trypsin produced by the pancreas is not sufficient to counteract the trypsin inhibitor and an inhibition of intestinal proteolysis results. Therefore, this effect could explain the depression of growth of chicken fed soya noodle during the first two weeks with the same amount of feed consumption as the control. And after that the size of the pancreas might begin to enlarge and secrete more trypsin enzyme and also increase mucosal slough-off as suggested by De Muelenaere (1964). At the same time, the slow stomach stomach emptying might have occurred. Therefore, at this stage, both endogeneous and exogeneous nitrogen was lost and the food intake was reduced, resulting in a poor growth.

It should be noted that the results of protein efficiency ratio which are reported in Table XXIX may not be accurate results. Although the chickens were weighed individually the food intake for individual chicken could not be obtained, since the chicken could not be fed alone like rats. Therefore, an average of food intake of 28 chicken was used in calculation. Also during feeding a certain amount of feed was lost due to the scattering by the chicken. The PER value of soya noodle was decreased when the level of soya noodle in the ration increased, this may be due to the effect of trypsin inhibitor.

## Conclusion

In the measurement of nutritional value of soya noodle an in vitro enzyme digestion method and chick feeding were used. The results were reported as available amino acid and weight gain after 4 weeks. Although Sheffner et al (1965) and Akeson and

Stahmann (1964) devised the pepsin-digestion-residue index and pepsin-pancreatin index which were found to be well correlated with the biological value, but these calculations were not helpful in the case of this experiment because the digestion of egg protein at the same condition was needed for comparison. The results of available amino acid from the in vitro pepsin, trypsin, pancreatin and erepsin digestion were poor, which was due to the incompleted digestion of enzyme.

In the chicks feeding trial, the weight gain of the chicken fed soya noodle at the end of four weeks was very low. This was due to the effect of the remaining trypsin inhibitor which caused incomplete digestibility of food intake and prevented the chicken from eating. For human consumption the soya noodle would need to be cooked, therefore trypsin inhibitor would be completely destroyed. In the chick feeding the wet noodle cannot be mixed with other ingredients, therefore the uncooked product was used in both feeding trials and in vitro availability amino acid analysis. It is hopeful that the protein value of soya noodle will be improved if soya noodle is cooked as in preparation for human consumption. Further experimentation will be needed to prove this.

# XIII. FINAL PRODUCT EVALUATION

## Nutrition

Soya noodle can be counted as one of the prototype products among various high protein food products developed as a cheap source of protein food for supplying the hungry populations.

The protein content of soya noodle is 30% which is comparable with the other high protein products such as INCAP vegetable mixture 14, Corn-Soy-Milk mixture and Soybean-Rice mixture for infants in Taiwan. The protein content of INCAP vegetable mixture is 27% (Bressoni and Elias, 1966), of Corn-Soy Milk mixture is about 20% (Martin, 1970) and Soybean-Rice mixture for infants in Taiwan is 23-25% (Tung et al, 1960). The pattern of amino acid in soya noodle was adjusted so that it could be compared with the FAO reference pattern in which nearly all the amino acid could be fully utilised if they are all in the available form.

The anti-nutrition factors such as trypsin inhibitor and soybean hemagglutin were nearly completely destroyed by steaming the noodle for 20 mins. and they would also be completely destroyed if the noodle was cooked at least 5 mins. in boiling water.

Soya noodle was also supplemented with the vitamins and minerals in such a way that when 200 g. of the noodle was consumed instead of the same amount of cooked rice with the ordinary diet, all the daily requirements of thiamine, riboflavin and niacin and minerals will be met.

Loss of vitamins, minerals and amino acid in cooking by draining off the cooking water was also eliminated. It was found that the noodle could be cooked in a small quantity of boiling water for 10-15 mins. by which time the noodle would absorb all of the water. The noodle can also be used for frying or preparing a soup.

Eating Quality

Upon soaking for 10-15 mins. in boiling water or cooking for 5 mins. in boiling water, the noodle absorbed only twice its weight in water and swelled only two and a half times its original valume. The rate of rehydration of soya noodle could be compared with other instant noodle, soya noodle then could be regarded as an instant noodle. The noodle retained its shape and firmness with an elastic property and smooth mouth feel. On prolonged storage, the noodle was slightly tougher but still had elasticity.

Soya noodle had a characteristic bland and nutty flavour. The freshly prepared noodles had a slightly beany flavour, but this beany flavour disappeared during storage, when there was a moisture increase and browning started to occur.

Because of the only slightly increased volume of the noodles during cooking, a large quantity of the noodles can be consumed at a time.

Cost of Production

The preliminary costing was based on the batch production of 200 kg dry ingredients of 203 kg finished product (2030 bags).

The process operation was divided into 2 periods. In the morning 4 small batches of 50 kg of dry ingredients were mixed, rolled into a thin sheet by using 2 sets of the series of thickness of the dough breaker, then air dried and again cut by the use of 2 sets of calibrated cutting rollers, then the noodle was twisted onto a tray and steamed, then pre-dried as per the flow process chart in Figure 21. 4 small batches of 50 kg each were preferred to 200 kg batch because once the dough was mixed the enzymatic oxidation and browning was likely to occur. Therefore to reduce the time from the mixing to steaming, a small batch operation and 2 sets of dough breakers were used. All the noodles prepared from these 4 batches were dried together. Therefore in the afternoon, the process workers were used for packing the noodle.

The cost of equipment, labour and raw materials as required for the process as described were considered for the production cost.

1. **Fixed Cost:** Cost of Equipment and Factory

| | U.S.$ | | |
|---|---|---|---|
| 1 Mixer, light duty capacity of 5 gallons for vitamins, minerals and amino acid premixed with wheat flour and rice flour | 400 | | |
| 1 Mixer, heavy duty, sigma blade mixer, capacity of containing 77 kg of dough (or 15 gallons) | 1,600 | | |
| 4 Dough breaker, stainless steel, $1\frac{1}{2}$ft.wide, approximately $90 each | 360 | | |
| 2 Cutter, stainless steel, calibrated cutter roller $1\frac{1}{2}$' wide, approximately $110 each | 220 | | |
| 1 Steamer including steam supply, tray area 154 sq.ft.for 77 kg of thin sheet of noodle | 700 | | |
| 1 Dryer, tray area of 154 x 4 sq.ft. stainless steel | 6,000 | | |
| 1 Recording control thermometer for steamer | 240 | | |
| 2 Weighing apparatus, small and large scale | 100 | | |
| 1 Moisture tester | 100 | | |
| Total equipment cost | | 9,720 | |
| Installation 15% equipment cost | 1,458 | | |
| Building and construction 60% equipment cost | 5,832 | 7,290 | |
| Total equipment and building cost | | 17,010 | |
| Engineering and construction 10% | | 1,701 | |
| Contingencies 5% | | 850 | |
| **Total Fixed Cost** | | $19,561 | (391,220 bahts) |
| **Working Capital 10% Fixed Cost** | | $ 1,956 | ( 39,122 bahts) |
| **Total Fixed Cost plus Working Capital** | | $21,517 | (430,342 bahts) |

II. Raw Material Cost: per annum.

| | | |
|---|---|---|
| Production Level | 200 kg dry incredients per day | = 2030 packages of soya noodle |
| | 8 hours a day | |
| | 6 days a week | |
| | 50 weeks a year (300 days) | |

Total Production 609,000 packages per annum

| Raw material | Cost | Quantity (kg) | Total Cost (baht) |
|---|---|---|---|
| Full fat soy flour | 4.50 baht/kg | 33,000.00 | 150,000 |
| Low fat soy flour | 2.25 baht/kg | 6,000.00 | 13,300 |
| Rice flour | 2.50 baht/kg | 6,000.00 | 15,000 |
| Wheat flour | 6.00 baht/kg | 15,000.00 | 90,000 |
| Egg* | 0.25 baht/egg | 13,500.00 | 67,500 |
| Methionine | 34.00 baht/100g. | 154.00 | 52,360 |
| Thiamine | 60.00 baht/10g. | 0.210 | 1,260 |
| Niacin | 50.00 baht/kg | 2.40 | 1,200 |
| Calcium Carbonate | 98.00 baht/kg | 324.00 | 31,752 |
| Package | 0.05 baht/package | 642,000 packages | 12,900 |
| Total Raw Material Cost | | | 435,272 |

* Duck egg, 50 g. per egg.

III. Labour Cost:

| | No.of workers | cost/month | | cost/annum (baht) |
|---|---|---|---|---|
| Process worker | 8 | 600 | | 57,600 |
| Night shift worker | 1 | 750 | | 9,000 |
| Total Direct Labour | | | | 66,600 |
| Supervision 20% Direct Labour | | | 13,320 | |
| Payroll Overhead 20% Direct Labour | | | 13,320 | 26,640 |
| Total Labour Cost | | | | 93,240 |

Possibility of Production in Thailand

Soya noodle can possibly be produced in Thailand with the following reasons:

1. Availability of raw materials: the production of soybean in Thailand is increasing every year and the production in 1971 is expected to be about 50 thousand metric tons. For the plant production level of 609,000 packages of soya noodle per annum, about 20 tons of soybeans are required to supply the factory yearly. Therefore even if the production of soya noodle increased ten times there would still be plenty of raw material for the supply. In the case of wheat flour, because of the small requirement - 7 tons per annum - the amount can be supplied from the local production. Rice flour presents no problem. Thailand is a rice producing country. Fresh eggs can be obtained easily. Vitamins, minerals and DL methionine are already imported into Thailand. Packaging material can also be easily obtained.

2. Investment: From the preliminary costing the requirement for the initial capital investment is low (about 430 thousand bahts for plant, equipment and working capital). It might be of interest to many private investors.

Marketing Possibility in Thailand

Soya noodle is a nation-wide product. It can be consumed by many classes of people from the children to the old people. The population in Thailand is about 35 million people and two-thirds of this number are in the North and Northeast. Assuming that the average family size is 6 people and if 1% of these families would buy soya noodle at the rate of 2 packages a month, therefore the market potential for soya noodle would be one million packages per annum, which would be about one and a half times the amount of production.

# XIV. DISCUSSION

Up to this stage of development of soya noodle, further study is still needed to be carried on particularly in the nutrition study, consumer acceptability of the improved product and the method of marketing the product in Thailand.

The determination of the availability of amino acids and a feeding trial should be carried out in the cooked noodle. If the results of these nutrition studies were not satisfactory, the method of processing would have to be improved. Steaming the whole bean prior to grinding into soy flour might be a better method for obtaining the optimum nutrition value than steaming the noodle, after being supplemented with vitamins, minerals and amino acid, as the amino acid is sensitive to heat treatment.

After the nutrition had been studied and a more satisfactory product obtained, a consumer test should be carried out to obtain the acceptability of the product. This should be done in Thailand.

The method of marketing and distribution should be investigated, together with the study of how to make the consumers learn about the product accept and adopt the product as one of their regular foods.

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Appendix I

PROTEIN SUPPLIES PER CAPITA BY MAJOR FOOD GROUPS AND REGIONS

(Abbott, 1966)

| | Vegetable Protein | | | | Animal Protein | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Grains | Starchy Roots | Pulses, Oil-seeds, and Nuts | Vege-tables and Fruit | Meat and Poultry | Eggs | Fish | Milk and Milk Products | Total |
| | Grams per Day | | | | | | | | |
| North America | 15.7 | 2.3 | 4.7 | 4.6 | 31.9 | 6.0 | 2.5 | 25.3 | 93 |
| Australia and New Zealand | 24.3 | 2.4 | 2.1 | 3.1 | 36.8 | 3.5 | 2.2 | 19.5 | 94 |
| Western Europe | 30.5 | 4.4 | 5.0 | 4.1 | 16.2 | 3.1 | 2.4 | 17.3 | 83 |
| Eastern Europe and USSR | 48.3 | 8.2 | 2.0 | 2.5 | 12.9 | 2.2 | 1.9 | 16.1 | 94 |
| Latin America | 26.5 | 2.7 | 10.7 | 2.8 | 13.8 | 1.2 | 1.5 | 7.4 | 67 |
| Far East | 32.2 | 1.8 | 12.0 | 1.7 | 3.0 | 0.4 | 2.2 | 2.2 | 56 |
| Near East | 48.5 | 0.7 | 9.5 | 3.6 | 4.6 | 0.5 | 1.1 | 7.4 | 76 |
| Africa | 32.2 | 7.1 | 9.0 | 1.7 | 5.8 | 0.4 | 1.3 | 3.5 | 61 |
| Europe, North America, Australia, New Zealand, Argentina, Paraguay, Uruguay | 33.4 | 5.2 | 3.8 | 3.6 | 19.8 | 3.3 | 2.4 | 18.5 | 90 |
| Far East, Near East, Africa, Latin America (except Argentina, Paraguay, and Uruguay | 33.2 | 2.3 | 11.6 | 1.8 | 3.8 | 0.4 | 1.9 | 2.9 | 58 |
| World | 33.4 | 3.2 | 9.0 | 2.4 | 8.8 | 1.2 | 2.3 | 7.7 | 68 |

## Appendix II

### CONSUMPTION OF CEREALS AND PROTEIN IN ASIAN COUNTRIES

(Ahmad, 1966)

| Country | Calories from Cereals (% of total calories) | Total Protein per capita in diet (grams per day | Animal Protein (% of total protein) |
|---|---|---|---|
| Indonesia | 55.8 | 38.2 | 11.8* |
| India | 67.6* | 53.0 | 11.1 |
| East Pakistan | 85.6 | 57.5 | 13.0 |
| Vietnam | - | 69.6 | 33.0 |
| Burma | 76.6 | 54.9 | 19.0 |
| Thailand | 65.7 | 49.1 | 31.0 |
| Ceylon | 61.2* | 41.0 | 22.0 |
| Philippines | 60.3 | 47.7 | 30.0 |
| Malasia | 63.3 | 75.5 | 33.0 |
| Japan | 66.7 | 69.7 | 39.0 |
| United Kingdom | 23.5* | 88.0 | 61.4 |
| United States | 20.4* | 72.0 | 91.6 |

* F.A.O. (1967a) Production Yearbook

# APPENDIX 111

## KINDS AND AMOUNTS OF FOODS CONSUMED BY FAMILIES IN RURAL THAILAND FROM VARIOUS SURVEYS

| FOODS | GRAMS CROSS WEIGHT per PERSON per DAY | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | This Study | | | | A. Chandrapanond 1955* | | | U. Bisolyaputra** | |
| | Villages of | | | | Borough of | | | | |
| | Ubon | Udorn | Chiang Mai | Chiang Rai | Bangrak, Bangkok | Sansai, Chiang Mai | Muang, Ubon | Phrae Province 1955 | Chon Buri 1953 |
| I. CEREALS | | | | | | | | | |
| Rice | 455 | 476 | 567 | 553 | 228 | 487 | 510 | 428 | 425 |
| Other cereals | *None reported* | | | | 18 | 3 | — | — | — |
| II. STARCHY ROOTS and TUBERS | *None reported* | | | | *Included with other cereals* | | | — | — |
| III. SUGARS and SYRUPS | *None reported* | | | | 6 | 1 | — | — | 1 |
| IV. PULSES, NUTS, SEEDS, and their PRODUCTS | — | — | 16.1† | 4.0† | 10.2 | 2 | — | — | 4 |
| V. VEGETABLES | | | | | | | | | |
| a. Leafy, green | 32 | 53.4 | 67.6 | 73.3 | 43 | 49 | 21 | 102 | 30 |
| b. Leguminous pods | 1.1 | 0.1 | 31.3 | 20.3 | *Included with "non-leafy"* | | | — | — |
| c. Yellow vegetables | — | — | 10.7 | 14.2 | ,, | ,, | ,, | — | — |
| d. Light green (mostly fruits) | 7.7 | — | neg. | 1.4 | ,, | ,, | ,, | — | — |
| e. Chillies dried | 0.8 | 0.6 | 5.1 | 3.2 | ,, | ,, | ,, | — | — |
| TOTAL | **41.6** | **54.1** | **114.7** | **122.4** | **43** | **49** | **21** | **102** | **30** |
| f. Other vegetables | 51.1 | 5.1 | 51.6 | 42.0 | 46 | 20 | 3 | — | 41 |
| VI. FRUITS | 0.3 | — | — | — | 15 | 20 | 3 | — | — |
| VII. MEAT and MEAT PRODUCTS | 18.7 | 11.5 | 24.6 | 20.2 | 112 | 46 | 40 | 31 | 5 |
| VIII. FISH and FROG | 23.6 | 19.2 | 13.9 | 14.6 | *Included with "meat"* | | | 6 | 69 |
| Fermented Fish | 31.6 | 23.0 | 0.4 | — | ,, | ,, | ,, | 7 | — |
| Crustaceans and Mollusks | 4.6 | — | 0.5 | — | *None reported* | | | — | — |
| IX. EGGS and INSECT EGGS | 0.7 | 0.6 | 5.3 | 1.7 | 24 | 8 | 1 | 1 | — |
| X. MILK and CHEESE | *None reported* | | | | 7 | — | — | — | — |
| XI. FATS and OILS | neg. | neg. | 0.3 | 0.4 | 28 | 7 | 1 | 1 | 5 |
| XII. CONDIMENTS and SPICES | — | — | — | — | — | — | — | — | 24 |
| XIII. SALT | 3.1 | 3.3 | 8.4 | 8.9 | — | — | — | 5 | — |

## Appendix IV

### DAILY PER CAPITA INTAKE OF CALORIES AND NUTRIENTS IN RURAL THAILAND, AVERAGE OF FAMILY LEVELS:

### Comparison of Various Surveys

| AREAS | Calories | Protein Total | Protein Animal | Fat | Calcium | Phosphorous | Iron | Vitamin A | Thiamine | Riboflavin | Niacin | Ascorbic acid |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | g. | g. | g. | g. | g. | mg. | I.U. | mg. | mg. | mg. | mg. |
| BISOLYAPUTRA, U. 1957 | | | | | | | | | | | | |
| Ubon villages | 2099 | 49.3 | 10.2 | 11.3 | 0.17 | - | 6.9 | 957 | 0.49 | 0.43 | 9.9 | 22.2 |
| Udorn Thani villages | 1826 | 42.0 | 8.6 | 7.3 | 0.17 | - | 6.1 | 1682 | 0.45 | 0.47 | 9.2 | 32.0 |
| Chiang Mai villages | 2232 | 52.7 | 9.3 | 10.8 | 0.22 | - | 7.3 | 2212 | 0.58 | 0.52 | 9.5 | 54.5 |
| Chiang Rai villages | 2207 | 47.0 | 7.2 | 11.7 | 0.19 | - | 6.7 | 3212 | 0.57 | 0.41 | 9.2 | 38.5 |
| CHANDRAPANOND, A. 1955 | | | | | | | | | | | | |
| Bangrak, Bangkok | 1409 | 47.0 | 23.9 | 42.0 | 0.18 | - | 7.3 | 1678 | 0.82 | 0.61 | 10.2 | 37.0 |
| Sansai, Chiang Mai | 1851 | 46.0 | 7.8 | 23.0 | 0.36 | - | 5.4 | 1364 | 0.8 | 1.8 | 12.0 | 39.0 |
| Muang, Ubon | 1722 | 43.0 | 6.0 | - | 0.43 | - | 0.9 | 465 | 0.56 | 0.4 | 11.0 | 12.0 |
| Sansai, Chiang Mai | 2083 | 47.0 | - | - | - | - | - | - | 0.72 | - | - | - |
| BISOLYAPUTRA, U. Minburi people, 1948 | 2637 | 65.79 | - | - | - | - | - | - | - | - | - | - |
| Phrai (families from 3 districts) 1955 | 1577 | 46.0 | - | 11.0 | 0.23 | - | 3.42 | - | 1.96 | 0.31 | 2.21 | 104.0 |
| Chon Buri 1953 | 1746 | 48.0 | 15.0 | 13.0 | 0.13 | - | 7.0 | 2006 | 0.78 | 0.46 | 11.14 | 30.0 |
| VALYASEVI ET AL, 1967 | | | | | | | | | | | | |
| Uborn 1963 | | | | | | | | | | | | |
| Hot season | 1877 | 52.9 | 21.2 | - | 0.27 | 0.76 | 10.7 | 2527 | 0.48 | 0.42 | 12.6 | 42.7 |
| Rainy season | 1929 | 56.3 | 24.5 | - | 0.26 | 0.77 | 10.3 | 1854 | 0.47 | 0.39 | 13.4 | 44.6 |
| Cool season | 1743 | 55.3 | 26.3 | - | 0.32 | 0.78 | 10.0 | 1852 | 0.42 | 0.38 | 12.3 | 43.2 |

Sources: Bisolyaputra (1957) and Valyasevi et al (1967).

Appendix V

COMPARISON OF AMINO ACID COMPOSITION OF PEANUT, MUNGBEAN, AND SOYBEAN PROTEIN WITH F.A.O. PATTERN

(g.amino acid per 16 g.N)

| | FAO (1965) FAO Provisional Pattern | Altschul (1958) | Evans and Bandermer (1967) | |
|---|---|---|---|---|
| | | Peanut Flour | Mungbean | Thailand Soybean |
| Tryptophan | 1.4 | 0.92 | 1.9 | 1.9 |
| Lysine | 4.2 | 4.53 | 8.0 | 7.0 |
| Histidine | - | 2.23 | 2.8 | 2.9 |
| Arginine | - | 12.38 | 6.6 | 6.3 |
| Threonine | 2.8 | 2.84 | 3.4 | 3.2 |
| Cystine | 2.0 | not reported | 0.8 | 0.8 |
| Methionine | 2.2 | 0.71 | 1.6 | 1.6 |
| Valine | 4.2 | 5.50 | 5.3 | 4.4 |
| Isoleucine | 4.2 | 3.89 | 4.1 | 3.8 |
| Leucine | 4.8 | 7.11 | 8.4 | 7.3 |
| Tyrosine | 2.8 | 3.28 | not reported | not reported |
| Phenylalanine | 2.8 | 5.41 | 5.6 | 5.1 |

## Appendix VI

### SOYBEAN PRODUCTION IN THAILAND

| Zone | Planted Area (rai) | Harvested Area (rai) | Production (kg) | Average Yield per Rai (kg) |
|---|---|---|---|---|
| Central Plain | 142,351 | 141,871 | 19,462,170 | 137 |
| Northern | 68,923 | 68,907 | 12,433,090 | 180 |
| North Eastern | 1,895 | 1,850 | 310,850 | 168 |
| Southern | 163 | 163 | 22,820 | 140 |
| TOTAL | 213,332 | 212,791 | 32,228,930 | 151 |
| The most productive province | | | | |
| 1st Sukhothai | 91,760 | 96,760 | 11,011,200 | 120 |
| 2nd Chiangmai | 41,622 | 41,622 | 7,491,960 | 180 |
| 3rd Lamphoon | 15,800 | 158,000 | 3,160,000 | 200 |
| 4th Nakorn Sawan | 11,552 | 11,472 | 2,064,960 | 180 |
| 5th Lopburi | 12,650 | 12,650 | 1,897,500 | 150 |

**Source:** Agricultural Statistic of the Thai Ministry of Agriculture (1965).

## Appendix VII

### SCORING SYSTEM OF SOY BEAN PROTEIN PRODUCTS

| | Maximum Score | Soya Noodle | Soy Biscuit | Breakfast Food | Simulated Meat | Soy Bread | Milk Substitute | Soy Cheese | Sweets | Soy Beverage or Protein Drink | Instant Pudding |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Home Market Possibility | 20 | 18 | 15 | 10 | 10 | 12 | 15 | 10 | 8 | 10 | 12 |
| Newness | 5 | 5 | 2 | 0 | 0 | 4 | 5 | 3 | 5 | 4 | 0 |
| Profitability | 5 | 3 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 2 |
| Nutrition | 20 | 13 | 15 | 12 | 18 | 10 | 16 | 17 | 15 | 17 | 10 |
| Raw Material Availability | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 |
| Presentation | 15 | 12 | 12 | 8 | 8 | 10 | 9 | 12 | 13 | 9 | 8 |
| Export Market | 3 | 2 | 2 | 1 | 1 | 0 | 0 | 2 | 1 | 1 | 1 |
| Raw Material Content | 8 | 6 | 5 | 6 | 7 | 2 | 4 | 7 | 2 | 6 | 6 |
| Research Cost | 10 | 8 | 6 | 6 | 3 | 5 | 6 | 5 | 7 | 6 | 6 |
| Technical Production Difficulties | 7 | 6 | 6 | 4 | 4 | 6 | 6 | 5 | 6 | 4 | 4 |
| Equipment | 5 | 3 | 4 | 2 | 3 | 4 | 4 | 4 | 4 | 2 | 2 |
| TOTAL | 100 | 81 | 73 | 54 | 58 | 58 | 71 | 70 | 67 | 67 | 54 |

Source: A report on development of soya noodle: Product Development Project 1968

# Appendix VIII

## GENERAL CONSTRUCTION AND OPERATION OF NOODLE TEXTURE TESTING MACHINE

(as in Figures 6 and 7)

The general construction of this experimental model is as follows: In the off or start position the knife which is attached to a hinged arm, as shown in the diagram in Figure 7, which is suspended above the anvil at a distance of approximately 1/16". This position of balance is achieved by the adjustment of a fine tension spring. The length of the hinged arm from pivot to blade is $8\frac{3}{4}$". At a distance of $1\frac{1}{2}$" back from the knife, a 1/16" rod is attached. To this rod is connected a fine tension spring, which at the other end is connected to a cam operated arm. This cam arm which is pivoted about halfway has a small roller bearing at one end, which engages with an accelerating cam. The accelerating cam is turned at 1 r.p.m. by a small electric motor. At the other end of the cam arm is a connecting rod joined directly to the indicating pointer. One full turn of the cam moves the pointer from 0 to 6, on the indicating scale. If there was no noodle on the anvil, the micro switch would turn off the cam motor immediately the knife came into contact with the anvil, which would be very soon after the cam started turning. With a test noodle in place, the movement of the knife towards the anvil would be slowed down to a rate depending upon whether the noodle was soft or tough. Because the downwards force applied to the knife is via the tension spring (x), further movement of the cam arm will continue to operate the indicater.

When the noodle was cut, the micro switch (triggered by the hinged arm) would stop the motor and the reading on the indicator could be noted. This operating switch is then turned to the second position and the cam continues to turn a full circle, stopping at the start position, ready for the next test. This stop position is controlled by a second micro switch (2). The operating switch has two positions, the first position to start the motor for the test and the second position to return the machine to zero scale. Two indicator lamps are used, one to show that the power is on and that the machine is in start position and the second lamp shows when the noodle has been cut and the pointed position can be noted.

Appendix IX

## OPERATING CONDITIONS OF BECKMAN MODEL 120C AMINO ACID ANALYSER

| Operating condition required | 2 hours accelerated analysis | | 4 hours analysis | |
|---|---|---|---|---|
| | Analysis basic | Analysis acidic and neutral | Analysis basic | Analysis acidic & neutral |
| **Column size** | 23 x 0.9cm | 69 x 0.9cm | 23 x 0.9dm | 69 x 0.9cm |
| **Packing** | | | | |
| Resin type | PA-35 | UR-30 | PA-35 | UR-30 |
| Height of Resin column | 5.5cm | 56cm | 5.5cm | 56cm |
| Resin Diluting buffer | pH5.3(0.35N) | pH3.5(0.2N) | pH 5.25 | pH 3.25 |
| Buffer flow rate | 70 ml/hr | 70 ml/hr | 70 ml/hr | 70 ml/hr |
| **Analysis** | | | | |
| Duration of run | 48 mins | 115 mins. | 48 mins. | 185 mins. |
| Flow rates | | | | |
| Buffer | 70 ml/hr | 70 ml/hr | 70 ml/hr | 70 ml/hr |
| Ninhydrin | 35 ml/hr | 35 ml/hr | 35 ml/hr | 35 ml/hr |
| First buffer | pH5.359±.010 | pH3.488±.005 | pH5.25±0.01 | pH3.25±0.01 |
| Second buffer | none | pH4.404±.01 | none | pH4.30±0.01 |
| Buffer change | none | 30 mins. | none | 70 mins. |
| **Operating temp.** | | | | |
| Bath tank | 53.7°C±.05°C | 53.7°C±.05°C | 55°C | 55°C |
| Column jacket outlet | 53.4°C±.05°C | 53.4°C±.05°C | 55°C | 55°C |
| Approximate column pressure | 60 psi | 160 psi | 40 psi | 130 psi |
| **Regeneration** | | | | |
| Cleaning (NaOH volume) | 3 ml | 15 ml | 3 ml | 15 ml |
| Equilibrium buffer volume) | 40 ml<br>5.359±.01 | 70 ml<br>3.438±.005 | 40 ml<br>5.25 | 70 ml<br>3.25 |

# Appendix X

## SOLUTIONS FOR MAINTAINING CONSTANT RELATIVE HUMIDITIES

| Relative Humidity % | Chemical | Conditions | Temperature °C | Reference |
|---|---|---|---|---|
| 10.0 | $H_2SO_4$ | 64.45% w/w (anhydrous) | 25 | Stokes (1949) |
| 20.0 | $H_2SO_4$ | 57.76% w/w (anhydrous) | 25 | Stokes (1949) |
| 32.0 | $CaCl_2.2H_2O$ | Saturated solution | 30 | Spencer (1926) |
| 43.6 | $K_2CO_3.2H_2O$ | " " | 30 | Spencer (1926) |
| 64.6 | $Na_2CrO_4$ | " " | 30 | Carr and Harris (1949) |
| 74.9 | NaCl | " " | 30 | Carr and Harris (1949) |
| 84.5 | KCl | " " | 30 | Carr and Harris (1949) |
| 92.9 | $NH_4H_2PO_4$ | " " | 30 | Spencer (1926) |

Appendix XI

## A DISTILLATION METHOD FOR THE DETERMINATION OF RANCIDITY IN SOYA NOODLE

(Tarlagis et al, 1960)

In the method, 10g. ground sample of soya noodle was blended with 50 ml of distilled water in a homogeniser for 2 mins. The mixture was transferred quantitatively into a digestion flask of 250 ml by washing with an additional 47.5 ml of distilled water. 2.5 ml of 4N HCl was added to bring the pH to 1.5. A small amount of Dow antifoam A and a few glass beads were used to prevent foaming and bumping. Thus, the apparatus was assembled in the same way as the macro kjeldahl distillation using the electric heating element. The highest heat was used in order to collect 50 ml of the distillate within 20 mins.

The distillate was mixed and 5 ml was pipetted into a screw cap tube, and 5 ml of TBA reagent (0.02 M 2-thiobarbituric acid in 90% glacial acid) was added. The tube was capped and the contents were mixed, then immersed in a boiling water bath for 35 mins. A distilled water-TBA reagent blank was prepared in the same way as the sample.

After heating, the tube was cooled in tap water for 10 mins., then the contents were transferred into a 1 cm cell, and the optical density of the sample read against the blank at a wavelength of 538 mμ.

## Appendix XII

### A REVIEW OF METHOD MEASURING THE PROTEIN VALUE IN FOODSTUFFS

As in the FAO Report No.37 (FAO 1965)

"The protein efficiency ration (PER) is the weight gain of a growing animal divided by the protein intake. It is a measure of protein quality when determined under specific conditions. The calorie intake must be adequate and the protein must be fed at an adequate level for a specific period of time. The PER is not a true efficiency ratio because not all the protein is used for growth, only that consumed above maintenance. When fed at surfeit levels, weight will no longer increase with protein intake and the ratio will fall. The PER has been used chiefly in feeding experiments on small animals and is also of value in studies on human infants. It is the simplest method of determining quality, requiring no chemical measurements, but it suffers from the possible error that weight gain may not be proportional to gain in body protein.

The biological value (BV) is determined by nitrogen balance and is defined by the ratio of nitrogen retained:nitrogen absorbed. This expression of protein quality measures the percentage of absorbed nitrogen retained for growth and maintenance, but it does not include a correction for incomplete absorption. The protein must be fed at or below the level needed for maintenance in order to achieve maximum efficiency of utilisation. Generally this level is 9 to 10% of the diet (w/w)."

The net protein utilisation (NPU) expresses in a single index both the digestibility and the biological value of a protein. It is the product of the coefficient of digestibility and the biological value and therefore represents the proportion of food retained (NPU = N retained/N intake), therefore a correction has to be applied for the nitrogen extracted on a protein-free diet and the protein must be fed below maintenance level. In man, the value of NPU must be obtained by nitrogen balance whereas in animals it can also be measured by direct analysis of the body. For comparison of the quality of proteins, the NPU is measured under standardised conditions, with protein supplied at or below maintenance levels in a diet providing adequate calories (NPUst). The term NPU operative (NPUop) refers to the utilisation of a protein under those conditions in which it is actually eaten.

The efficiency and concentration of protein may be combined in a single index, which has been called the net dietary protein value (NDPV). This expression is obtained by multiplying protein concentration by NPU determined at that protein level. And the term net dietary protein calories percent (NPpCal%) is used when in the latter expression protein concentration is expressed as a percentage of calories in the diet.