# EFFECT OF PROCESSING AND COOKING PROCEDURES ON THE THIAMINE CONTENT OF RICE

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# EFFECT OF PROCESSING AND COOKING PROCEDURES ON THE THIAMINE CONTENT OF RICE

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# CHAPTER I

## INTRODUCTION

Food is a fact of life. To be physically happy, to grow normally, and to be able to withstand normal pressures of a society, man must be well fed. Food is essential for human beings not only for individual survival but also for building a healthy nation.

The object of the science of nutrition is to show mankind how to eat more intelligently and live a fuller and longer life. This is possible by improving the methods of food production, selection, and preparation.

Large numbers of surveys and nutrition experiments have demonstrated a need for a definite requirement of certain food components for normal growth and health.

It has been proven that eating inadequate kinds and amounts of food causes not only specific diseases but instigates "subcritical" symptoms associated with poor health, diminished efficiency, fatigue, and lowered resistance to disease.

Vitamins, accessory factors in nutrition, promote health and regulate body processes. All vitamins are necessary for good health.

Vitamin $B_1$ deficiency resulting in beriberi has been a common disease to the Chinese since 2600 B. C. It occurs among people whose staple cereal is polished rice or refined wheat. This deficiency affects the nerves causing instability and death.

Physiological functions of vitamin $B_1$ are concerned with:

Metabolism -- Carbohydrate oxidation changes.

Alimentary system -- Controls appetite and influences the tone of the alimentary tract.

Central nervous system -- Necessary for the normal functioning of the brain and nervous system.

Circulatory system -- Essential for the maintenance of normal rate of heart beat, and also affects blood sugar level.

Reproductive system -- Required for normal reproduction and lactation.

Since vitamin $B_1$ is concerned with all these physiological functions, lack of this vitamin causes poor health and lessened mental alertness.

According to a nutritionist, H. C. Sherman (59, p. 580), "Nutrition is both a science in itself, and it is an instrument of social policy." For the health, happiness, efficiency, and for the enhanced duration and dignity of human life, the findings of scientific research in the field of vitamin $B_1$ have improved the state of physical vigor and mental alertness.

The dietary essential, vitamin $B_1$ has been found to be abundant in rice and wheat embroy. More than 75 per cent of the world's total food crop consists of rice. According to an editorial (56, p. 12), "Rice is one of the few non-allergy foods, researchers report. No one is allergic to rice. Many people who are allergic to other products have been referred to rice by physicians." It is the staple article of food for many eastern nations comprising nearly half the human race (56).

Many of the people of East Pakistan exist almost solely on rice. The climate of East Pakistan, and of some places in West Pakistan, is favorable for growing rice. The Report of the Second Five Year Plan of the Government of Pakistan Planning Commission (17) shows the planned

output in 1964-65 of rice (cleaned) in East Pakistan to be 8,752,000 tons. From the Commission's Report (17, p. 143)

> ... East Pakistan is pursuing a similar free-market policy for rice. The risk factor in the case of rice in East Pakistan is somewhat greater than in the case of wheat in West Pakistan. East Pakistan has no guaranteed supplies from external sources to protect it against any shortages resulting from natural calamities or other causes, such as floods, droughts and insect attacks.

In this situation attention should be given to the processing and cooking procedures of rice to retain the maximum of food value, especially of vitamin $B_1$.

In East Pakistan rice is processed for consumption either by the family members of the native farmer, or in the mills. When pounded in the home, more of the outer covering (pericarp) is left on the rice than when the rice is milled by machinery in modern mills. Such milling tends to remove the skins of the grain by sifting and winnowing until the product is finally polished.

Rice is sometimes prepared in the "parboiled" form. Brown parboiled rice husked by the native family is richer in nutrients, as well as more acceptable and palatable to the people for its flavor and aroma. Husking methods used by the natives are man-powered and time consuming. The best rice products in the markets are supplied by the natives. For this reason the brown, parboiled varieties of rice are more expensive than the milled white ones.

It has been found that milled rice, prepared from rice that has been parboiled, is richer in vitamin $B_1$ than ordinary milled rice; it is considered that the increase of the vitamin content is due to the diffusion of the vitamins from the germ and the pericarp into the endosperm during the steaming (34).

## Basic Assumptions

In this study it is assumed that:

1. Brown, parboiled rice, husked by the natives, is richer in vitamin $B_1$, or thiamine, than the polished, commercially milled rice.
2. Brown, parboiled rice, treated with steam before husking, contains more thiamine than the brown rice husked without being treated with the steam.
3. Cooking procedures affect the thiamine content of rice.

## Statement of the Problem

This study is concerned with the testing of the following hypotheses:

1. There is more thiamine in parboiled, brown rice than is in the commercially milled white rice of the same species when cooked in the same manner.
2. There is more thiamine in parboiled, husked, brown rice than is in the unparboiled, husked, brown rice of the same species when cooked in the same manner.
3. There is more thiamine in brown, parboiled rice cooked in just enough water to soften it than is in brown, parboiled rice cooked in a large amount of boiling water and excess water discarded.

## Purposes of the Study

The results of this experiment will help the Pakistani farmers to make decisions concerning the method of processing rice by which the

maximum thiamine content is retained. If the first hypothesis is true, then the improvement of the native husking method is to be considered in order to get the maximum amount of nutrients with the minimum expenditure of time and energy. The use of the superior cooking method identified in the third hypothesis will permit the preparation of rice with a minimum loss of thiamine.

If the Pakistani housewife is taught to apply the findings from this study in the preparation of rice for her family she can provide them with an improved intake of food nutrients which will increase their nutritional status.

# CHAPTER II

## REVIEW OF LITERATURE

### The Development of Nutritional Research

#### Pre-scientific period (460 B.C.-1830 A.D.)

Hippocrates, the first Greek physiologist of this period, became known as "The Father of Medicine" because of his interest in studying disease and trying to find a cure for it. Hippocrates decided that all food is basically of one kind but appears in many forms. It was generally believed that all of the various vegetables and animal foods contained one thing in common -- a universal "aliment" (19).

#### Chemical period (1830-1860)

This period was the beginning of modern chemistry and the beginning of modern nutritional research. It had been discovered that food contains at least four important classes of chemical substances - fats, carbohydrates, proteins, and inorganic salts. Chemical analysis was used as the "measuring stick" to find out the chemical components in food (19).

#### Caloric period (1860-1906)

During this period, use of the calorimeter was initiated in nutritional research. The calorimeter served as the "measuring stick" to find out food values, measured in terms of heat units or calories. The body was conceived as an engine which required fuel for heat and energy (19).

Biological period (1906-1935)

The beginning of this period was the birth of the vitamin hypotheses. Research in nutrition was more scientifically developed. Laboratory animals were used as the "measuring stick" in detecting the deficiency of food. Chemical analysis, calorimetric measurements, and biological assay were the various methods used inside the food research laboratory (19).

Microbiological period (1935- )

Microbiological methods in assaying food have been widely used since 1935. The short duration of microscopic organisms favors the development of a rapid test method for vitamins of the B-Complex. Industries utilize the ability of micro-organisms to cause physical and chemical changes that are necessary for manufacturing fermented food (19).

## The Story of Vitamin $B_1$

History:

The first clear evidence for the existence of dietary factors of the nature of vitamins came from the work of N. Lunin (1881). He found that proteins, sugars, fats, and mineral elements were incapable of supporting life. Lunin (19, p. 30) postulated that "A natural substance such as milk must therefore contain, besides these known principles, small quantities of unknown substances essential to life." These findings were elaborated by Pekelharing of Holland (1905), who demonstrated for the first time the existence of disease due to deficiency in diet. He pointed out that there was still an unknown substance in milk which, even in very small quantities, was of paramount importance in nutrition. About

this time, Hopkins (1906) in England concluded from similar experiments that milk contained "accessory factors," now known as vitamins (19).

Vitamins are substances that:

a. are distributed in food stuffs in relatively minute quantities.

b. are distinct from the main components of food (i.e. proteins, carbohydrates, fats, mineral salts, and water).

c. are needed for the normal nutrition of the animal organism.

d. the absence of any one of which causes a corresponding specific deficiency disease.

The discovery of vitamins began with observations made centuries ago in China, where the disease now recognized as beriberi was certainly known. This disease is now considered to result from the lack of an essential vitamin, thiamine. Beriberi prevails mainly in eastern Asiatic countries among the people living on polished rice.

That beriberi is of dietary origin was deduced in 1872 by Takaki (24), Director General of the Medical Service of the Japanese Navy. By adding more meat and vegetables to the sailors' diet and by replacing the customary rice, in part, by wheat and barley he greatly reduced the incidence of the disease in Japanese naval forces.

The tables given on the following page show the results of two experiments conducted in a lunatic asylum in a United States Army Corps, indicating positive relationship between intake of polished rice and incidence of behavior. In the United States Army Corps' experiment, it is seen that with the introduction of unpolished rice and beans, the causes of beriberi dropped from a high figure of 618 to nil.

The first step towards the isolation and synthesis of the vitamin was the discovery that a disease resembling beriberi could be produced

TABLE XIII

A DIETARY EXPERIMENT ON BERIBERI IN A LUNATIC ASYLUM, 1907 (24, p. 46)

| Diet | No. of patients | Cases of beriberi | Deaths |
|---|---|---|---|
| Polished rice | 120 | 36 | 18 |
| Steamed rice | 123 | 26 | 0 |

TABLE XIV

AN EXPERIMENT ON BERIBERI IN A U. S. ARMY CORPS (PHILIPPINE SCOUTS) (24, p. 46)

| Date | Diet | Cases of beriberi |
|---|---|---|
| 1909 | Potatoes, beef and white flour + polished rice | 618 |
| 1911 | + unpolished rice and beans | 3 |
| 1913 | + unpolished rice and beans | 0 |

in animals under laboratory conditions. In 1890, the Dutch physician Eijkman (12), working in a military hospital in Java, noticed that poultry, when fed on rough unpolished rice, remained healthy; but when fed on the polished rice which was usually reserved for the men, developed a paralytic condition in which they were unable to stand and would not hold up their heads (head retraction). Soldiers at the station were also suffering at the time from beriberi, a disease in which great weakness was accompanied by a condition resembling paralysis of the limbs and frequently causing death.

Dr. Eijkman found that he could treat his men in the same way as he treated the hens by giving them either rough, unpolished rice or rice polishings, and that this was effective in overcoming beriberi (1897). He did not, at the time, recognize that beriberi was a deficiency disease. By 1906 Eijkman (12) won the Nobel prize for showing that there was a very small amount of something in the germ and pericarp of the rice that protected fowls from a disease resembling beriberi, and for recognizing the fact that it was an unknown nutrient - neither protein, fat, carbohydrates, nor mineral. He extracted it from rice polishings with water and alcohol. In 1911, Funk (24) attempted to isolate from rice polishings the substance active in the cure of beriberi and succeeded in obtaining a crystalline fraction of material capable of curing polyneuritis in pigeons. He coined the word "vitamin" from vital amine.

It was not until 1926 that Jansen (24), working with Donath in the same laboratory where Eijkman and Jenkins worked, succeeded in isolating the factor "vitamin $B_1$" in crystalline form. Jansen and Donath used small (rice) birds instead of fowls for testing the action of the different fractions which they prepared in the course of isolating the vitamin.

The isolation of crystalline vitamin left the chemist with the difficult task of identifying its structure. In 1932, Odhake found that the substance contained sulphur. Analysis showed it to have the empirical formula $C_{12}H_{16}N_4O_2S$, united in an unknown (thiazole) ring (70). However, its structure was finally elucidated and its snythesis independently accomplished in the United States of America, Germany, and Scotland.

The merit of prior publication goes to R. R. Williams (65) who began his search for the vitamin in 1913, under the guidance of E. B. Vedder, a pioneer in the clinical study of beriberi.

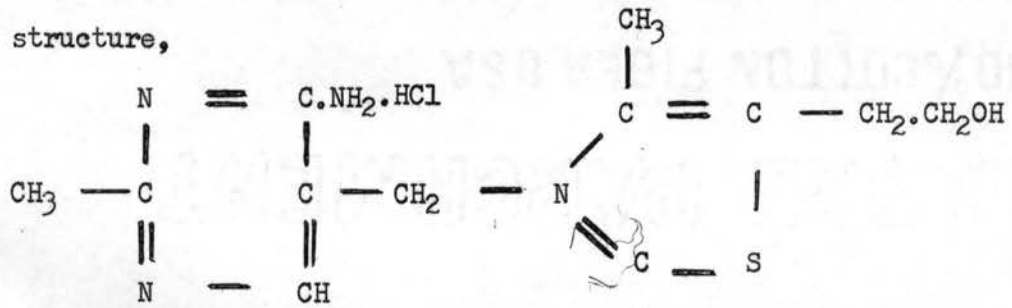
Williams (70) and his co-workers (1934), were able to devise a method for securing consistent yields of antineuritic vitamin hydrochloride of approximately 5 grams per ton of rice polishings.

Chemistry:

Thiamine was the name given to vitamin $B_1$ by Williams following its snythesis. Williams (69, p. 142) says:

> ... In an endeavor to promote the adoption of a universally accepted term based on the chemistry of the substance, I have proposed 'thiamine' (chloride, bromide, sulfate and so on) pending action of the Confederate of Vitamin Standardization.

Williams (69, p. 145) demonstrated that thiamine hydrochloride (vitamin $B_1$) is a white, crystalline subatance with the following chemical structure,



Pyrimidine ring — Thiazole ring

Thiamine hydrochloride

According to Fox and Cameron (14, p. 235), a thiamine molecule contains a primary alcohol group and an amino group. The amino group is combined with hydrochloric acid as a salt, and one of the other nitrogen atoms is also in this quadriocovalent, unielectrovalent condition. Thiamine owes its water solubility to the presence of these two electrovalencies. The alcohol group can form esters in the usual way, and before thiamine is utilized by the body it is esterified with pyrophosphoric acid.

Thiamine is readily soluble in water and alcohol but not in most fat solvents and in fats. It is rapidly destroyed by heat in neutral or alkaline solutions. In acid solutions, however, it is resistant to heat up to 120° C. Thiamine can be converted by controlled oxidation into an inactive product, thiochrome, which is strongly fluorescent in ultraviolent light. This property is used for the chemical estimation of the vitamin in biological materials (69, p. 151).

Physiological Activities:

Thiamine was the first vitamin in which precise activity in the body was stated in biochemical terms. Thiamine is important as a component of two co-enzymes, thiamine pyrophosphate (cocarboxylase), and lipothiamide pyrophosphate.

Thiamine is absorbed from both the large and small intestine. The phosphorylation of the vitamin to form the co-enzyme takes place in the liver.

The most important function of thiamine is its role in the utilization of carbohydrate. In countries where 80 per cent of the food calories may be derived from carbohydrate, the thiamine need is increased. Glucose is oxidized in the tissues to supply energy. This occurs through

a series of reactions, each requiring specific enzymes. Thiamine is a part of the co-enzyme structure. The reaction of the enzyme system brings about the oxidation of glucose. Thiamine then participates in reactions that, through the release of energy, result in the formation of carbon dioxide. This co-enzyme known as carboxylase, acts on pyruvic acid, an intermediate product in the oxidation of glucose. Carboxylase is required for the subsequent break-down of pyruvate, and then further energy is released. However, if a thiamine deficiency exists, the oxidation of glucose cannot proceed beyond this stage and pyruvic acid levels of the blood increase. One of the indications of thiamine deficiency is the accumulation of excessive pyruvic acid in the blood.

Brozek (9), in his studies about healthy young men, investigated the impact of a thiamine-free diet. Tests with a thiamine-free diet for a period of 15 to 27 days showed neurasthenia, marked emotional upset, lowering of pressure-pain threshold, general weakness, extreme anorexia, pronounced incoordination of the legs and hyperpyruvinemia. No affect was noted on intelligence.

Thiamine has been called "the morale vitamin" because of its relation to a healthy nervous system and its effect on mental attitude (8).

Chaney (10) states that thiamine functions in maintaining normal muscle tonus, especially in the digestive system and the heart. In clinical cases of antithiaminosis constipation is usually present.

Birch and Harris (7) found that thiamine is related to cardiac function. In some cases lack of thiamine causes enlargement of the heart and the heartbeat is slowed (bradycardia).

Daum, Tuttle, and Wilson (11) suggested that thiamine is necessary for the proper metabolism of tryptophan.

Keys (33) pointed out that thiamine also plays a part in promoting

appetite and better functioning of the digestive tract. It is also essential for proper growth.

According to Keys (33) the total amount of thiamine in the well-nourished human body is small, amounting in all to about 25 milligrams. The highest concentration is found in the heart, brain, liver, kidneys, and skeletal muscles. The body has no means of storing any excess so that no benefit is derived from taking large doses since the excess is lost in the urine. The amount which can be stored in the liver is small and can last for a few days only.

Both urine and blood tests are performed in the study of thiamine metabolism.

Calorie Relationship:

The amount of thiamine needed by the body is related to energy metabolism. The thiamine requirement is based on and proportional to the calorie intake. An increased need for thiamine may result from a liberal but poorly-balanced diet furnished largely by cereal grains.

Keys (33) and co-workers in their investigation on normal young men concluded that 0.23 milligrams of thiamine per 1000 calories restricted muscular, neuromuscular, cardiovascular, psychomotor, and metabolic functions.

Daum (11) and co-workers have concluded that the minimal daily thiamine need of young women is 0.25 to 0.30 milligrams per 1000 calories. They based their figures on urinary excretions.

The minimal thiamine requirement for the adult is approximated to be 0.2 to 0.3 milligrams per 1000 calories. The Food and Nutrition Board of the United States National Research Council recommends the amount of thiamine to be .5 milligrams per 1000 calories. The Board states that

the total thiamine intake of an adult should never fall below 1 milligram daily even when the calorie intake is less than 2000.

According to Davidson et al. (12, p. 235):

> The concept of a desirable thiamine calorie ratio for a diet is perhaps more useful than attempts to relate thiamine needs directly to carbohydrate intake. Whole wheat, for instance, has a ratio of about 1.2 mg./100 Cal. and is actively protective against beriberi. Raw polished rice has a value of about 0.15 mg./1000 Cal. and so is beriberi-producing.

Countries which use polished rice in large amounts will, therefore, need a liberal source of thiamine other than the rice if they are to avoid beriberi.

## Rice as a Dietary Source of Thiamine

Thiamine is found in many common foods, but in comparatively small amounts. The cereal grains are generally considered the best sources, thiamine usually occurring in the germ and outer layer.

Rice as a dietary source of nutrients has been supported by many scientists (56, p. 18).

> Rice is one of the most easily digested of all foods. Scientists report that rice may be fully and easily digested by the human body within one hour, while most other foods require two to four hours for digestion. Because of this quality, rice exerts little wear and tear on the organs of digestion and assimilation, which gives it particular significance as a food for infants, growing children and persons with digestive disturbances, as well as for healthy individuals.

The rice grain, which was grown as long ago as 2000 B. C., is made up of the hull, the seed coat (pericarp), the starchy endosperm, and the embroy or germ. The rice germ is situated at one end of the kernel and consists of five different parts -- epiblast, coleorhiza, plumule, radical, and scutellum.

The distribution of thiamine and riboflavin in rice was discussed by Simpson in 1951 (61). He confirmed that the thiamine was largely

concentrated in the scutellum.

Rice is prepared for consumption either by the natives or in commercial mills. By means of suitable milling equipment rough rice is separated into milled rice, hulls, bran, and polish. One of the initial products obtained in milling is the so-called first break bran, which is composed mainly of embroy and outer layers of the rice kernel. It is rich in members of the vitamin B complex, especially thiamine, apart from riboflavin and niacin. The inner seed coat layer, along with some starchy material, compose rice polish.

Milled rice, prepared from rice that has been parboiled, is richer in thiamine than ordinary milled rice.

Aykroyd (2) concluded that highly milled, parboiled rice was found to be rich in vitamin $B_1$, whereas roughly milled, raw rice was deficient. It is considered that the increase of the vitamin is due to the diffusion of the vitamin from the germ and the pericarp into the endosperm. Aykroyd (1940) has pointed out that machine-milled rice from raw rice contains about 1.0 microgram per gram of thiamine, whereas that from parboiled rice contains about 2.20 micrograms per gram.

Kik (37) states that rough rice or paddy has about 3.0 microgram per gram of thiamine and polished rice about 0.6 microgram per gram, whereas rice bran has as high as 21-30 microgram per gram.

## Rice Enrichment

Enrichment and fortification of food indicates progress in nutrition. Enrichment was made possible in the 1940's by the chemists' ability to prepare pure nutrients in inexpensive forms. It was stimulated by the findings that the average American diet of that period was inadequate.

By definition (29, p. 2):

> Enrichment means the improvement of staple foods through restoration or supplementation with vitamins and minerals - for better nutrition and better health. It does not in any way signify that the caloric value of the food has been increased.

Cereal foods are enriched to compensate for the nutrients lost in processing the grain to make them appeal to the consumer.

The official reasons in the United States of America for enrichment -- as stated in the National Research Council's publication (45) "The Facts About Enrichment of Flour and Bread" -- are two-fold. The average prewar American diet needed improvement because it did not contain enough thiamine, niacin, riboflavin, and iron. Flour and bread were selected for "enrichment" because they are consumed by nearly everyone, because these nutrients are natural to wheat but are lost in part in milling, because their return does not change the appearance or taste of white flour and bread, and because such return is easy for the miller and baker.

Modern enriched white bread (27) as made in the United States of America today contains the enrichment ingredients -- thiamine, riboflavin, niacin, and iron. These added nutrients are all available for easy assimilation.

The controlled enrichment of white rice consists of adding nutrients in such amounts that the final product is about the equivalent of brown rice in thiamine, niacin, and iron content.

Rice enrichment processes were first introduced in the Philippines as a result of beriberi experiments in 1913 by Vedder and Williams (65). This study helped the scientists to learn about the loss of vitamins in rice while processing, storing, and cooking. Suitable enrichment requires that the product shall not suffer any appreciable loss of the added nutrients in washing, cooking, and storing and that the cost of enrichment shall be low.

The Philippine experiments (65) with rice showed the following results -- four fowls were fed on polished rice plus a daily does of 10 grams of the polishings that had been extracted three times with alcohol. All four fowls remained well for a period of three months when the experiment was discontinued.

Furter et al (15) performed experiments regarding enrichment of rice with synthetic vitamins and iron. They assumed the two ways of improving the nutritional quality of white rice to be: (1) the older method, based on the preservation of the natural nutrients of whole grain rice and (2) newer methods for enriching polished white rice with synthetic vitamins and iron. The first category includes the processes known as undermilling, parboiling, conversion, and maleknizing[1] (35).

According to Furter et al, enriched rice is prepared by mixing premix and white rice in the ratio of 1 to 200, either in the trumboil[2] or in any other suitable device. The table below presents typical data on the vitamin and iron content of premix and enriched rice as given by Furter (15, p. 488).

TABLE III

UNIFORMITY OF ENRICHMENT

| Sampling in Trumboil | Rice Premix, Mg/lb | | | Enriched rice, Mg/lb | | |
|---|---|---|---|---|---|---|
| | Thiamine | Niacin | Iron | Thiamine | Niacin | Iron |
| Front | 480 | 2220 | 1810 | 2.9 | 19.7 | 12.5 |
| Middle | 510 | 2310 | 1940 | 3.1 | 18.6 | 13.1 |
| Rear | 500 | 2360 | 1770 | 3.1 | 19.0 | 12.3 |
| Average | 500 | 2310 | 1840 | 3.0 | 19.1 | 12.6 |

[1]Soaking rough rice for six hours at 40° C. followed by 15 minutes steaming at 15 lbs. pressure.

[2]The trumboil is a rotary drum used in the rice mill for coating or mixing rice after polishing.

A number of rice varieties, including both short-grained and long-grained, have been enriched by the Roche (29) process on a pilot plant scale in the United States of America. One of them is "Uncle Ben's" -- converted, enriched, long, and parboiled. Each cup (cooked) contains the following proportion of the minimum adult daily requirement of:

Thiamine - 22%
Niacin - 17%
Riboflavin - 1%
Iron - 14%

According to Kik and Landingham (37), the efficiency of the parboiling process, the appearance of the rice kernel after milling, and the percentage of breakage during shelling as well as the retention of thiamine in the milled product, have to be taken into consideration. These properties are of paramount economic importance in the industrial processing of rice.

While working with the methods of parboiling, Kik and Landingham (37) included boiling, boiling and steaming, steaming, soaking and steaming, and soaking. The data showed that boiling alone produced an unsatisfactory product. Of the other methods the best result was obtained from boiling for 20 minutes, followed by 15 minutes steaming at 5 pounds pressure. This method produced fairly good parboiled rice, with a thiamine content of 2.13 microgram per gram and a breakage of 4 per cent. The average thiamine content of the milled, nonparboiled samples was 0.60 microgram per gram and the average breakage 3.8 per cent.

Kik and Landingham (37) determined the thiamine, riboflavin, and niacin content of rice before and after conversion and found that conversion favors the retention of these vitamins.

Williams (69) suggested that for India and Pakistan the parboiling

process now used may be utilized by extension members in nutrition education and proved preferable to artificial enrichment. Age old custom has developed parboiling as the better way of preparing rice, but the nutritional significance was not known. Williams further said that in a great part of these countries, sun drying after parboiling is rendered practical by the long, dry season which does not require the cost of fuel.

## Rice Cultivation in East Pakistan

East Pakistan has a total area of approximately 54,000 square miles, equivalent to 34 million acres of land. Total cultivatable land is 25 million acres of which rice covers about 20 million (1). This land area indicates that rice production is 75 per cent of the total field crops in East Pakistan.

Abundant rainfall is the characteristic of all parts of East Pakistan, the district-wide annual average ranging from 60 to 140 inches. The rainy season extends from April to October. The temperature in East Pakistan varies from 40 degrees to 100 degrees Fahrenheit with an average of 75 degrees. The high temperature, monsoon rain, and fertile soil of East Pakistan are favorable for luxuriant growth of rice. Pakistan is the third largest producer of rice in the world and its production is concentrated mainly in East Pakistan (1).

Cultivated rice is known botanically as oryza sativa. Oryza sativa is divided into two geographical races - Japonica and Indica.

Japonica rices are characterized by high yield, good response to intensive manuring, and is cultivated in Japan, Korea, part of China, Italy, Spain, and Egypt. Japonica rices are less suitable than Indica types for the Indo-Pakistan Zone of cultivation in view of their photoperiodic requirements (1).

Indica rice is cultivated in Pakistan and other tropical Asian countries. This longer type of grain gives a lower yield and is not responsive to heavy manuring because of greater vegetative growth.

According to the time of harvest, the rice crops of East Pakistan are separately distinguished as spring, summer or autumn, and winter rice. They are divided into six groups according to their growing capacity in different seasons, in different levels of lands. The various groups are (26):

1. Highland Aus or early rice (summer rice)
2. Lowland Aus or autumn rice
3. Transplanted Aman or winter rice (soil)
4. Lowland Aman or floating rice (Jaliaman)
5. Boro or spring rice
6. Ryada

In the broader classification, however, there are only four classes of rice. They are:

1. Transplanted aman rice
2. Aus rice
3. Long-stemmed floating, or deep-water aman rice
4. Boro rice

The details of each class are given below:

Aman - This is the most important paddy crop in East Pakistan, grown in medium and semi lowlands. Under normal conditions, it can yield up to 3,000 pounds of paddy per acre. Most of the finest and high yielding varieties belong to this class of paddy.

Aman paddy flowers during October-November. After flowering, the crop is ready for harvest within 30 to 35 days. The crop ripens and becomes ready for harvest by the time the monsoon is completely over.

Aus - This variety is the early paddy, matures within three to four months from the date of sowing. Aus is usually grown in high and medium land. It is absolutely dependent on monsoon rain and is sown from March to May, and harvested during June-July, before Aman rice. The members of this group are short seasoned and comparatively poor yielders. The farmers grow this crop when they are greatly in want of food for themselves and for their cattle.

Floating Paddy (Jaliaman) - This is the only crop grown under deep water condition in very low land. The growing stems float on the surface of water. Sowing is done when the land is dry during the months of March and April. The crop is harvested from August to the first of November.

Boro (spring paddy) - Boro is the spring paddy grown in low lands. This rice crop is grown from November to April and harvesting has to be completed before monsoon sets in. Under favorable conditions, it can yield as high as 3,200 pounds of paddy per acre.

In regard to cooking quality, the following varieties of rice give the best results:

1. Balam rice of the district of Barisal
2. Patnai - 23 grown in saline tracts
3. Daud Khani and Katari bhog of North Bengal (Dinajpur)

All of these varieties are grown throughout East Pakistan but they grow best in the districts mentioned above.

## Processing of Rice

Rice is usually harvested when the moisture content of the grain is between 18 and 25 per cent (41).

After harvesting, the threshed rice has a thick, fibrous husk and in this state is known as "paddy" -- a rough rice.

The entire process of preparation of rice, from harvesting to milling and polishing, is known as processing.

A rice mill is an elaborate assembly of devices for handling the rough grain as it comes from the warehouse and, with a minimum of manual labor, turning it into finished white rice, sacked and ready to market. According to a group of researchers led by Stermer (64, p. 362), "Degree of milling of rice is a measure of the extent to which the germ and bran layers have been removed from the endosperm." A rice photometer is the excellent device used in determining the degree of milling of rice. Milled rice, as defined by United States standards, consists of whole or broken kernels of rice from which the hulls and practically all of the germ and bran layers have been removed.

After processing, the products obtained at home and in the commercial mill from the paddy are as described here:

Milled white rice - Rough rice (paddy) is milled by first removing the hulls. This leaves what is called brown rice. When the bran is removed from the brown rice, the grains are ready for polishing and the final product is known as white, or milled rice.

Brown rice - The brown rice is defined as the whole, unpolished grain of rice with only the outer inedible fibrous hull removed. This rice retains its natural vitamins, minerals, and oils (52). Home-pounded rice is always brown rice.

Parboiled rice - By a special steam pressure process, the paddy rice is parboiled before milling. This treatment aids in the retention of much of the natural vitamins and minerals.

In East Pakistan the parboiling of the paddy before husking is a universal practice. The variations in this treatment are many, but essentially are performed as follows. Rough rice is steeped in water for

a period of 24-36 hours, then drained and steamed (usually under pressure). After steaming, the parboiled paddy is dried under the sun for a couple of days. The properly dried-parboiled paddy can be stored for a period in suitable containers and is husked as the need arises. The parboiled rice has a better keeping quality than the unparboiled. Husking causes very little breakage, especially if steaming is done under pressure and the rice is carefully dried (1).

The native people use kahal and dheky for hulling paddy at home. Kahal is a wooden mortar with a heavy, wooden pestle of 5 to 6 feet in length. One to three people can work together, with the same number of pestles, for dehusking paddy contained in one mortar. Dheky is worked by foot. It is made with a long beam of wood resting on a fulcrum in one end of the beam. There is a pestle on the other end of the beam. The pestle, fixed under the beam, is about two feet long. There is a hole on the ground just under the pestle. The dheky is worked by giving pressure by foot on one end of the beam near the fulcrum. When the pressure is released the pestle comes to rest in the hole containing the paddy, thus putting pressure on the paddy and loosening the husk. The working capacity of dheky is 3 to 4 times more than that of a kahal.

Parboiled rice (41) has a rubbery texture and, for that reason, resists breakage when it is milled. Parboiled rice is not quick cooking, but it has certain advantages over raw rice. These are: (1) it is more nutritious, (2) it is more resistant to insect infections, (3) it can be used in canned formulations such as soups and other semi-liquids without disintegration, and (4) it can be cooked with less danger of becoming mushy than white rice. Consumption of parboiled rice in East Pakistan is 95 per cent compared to unparboiled rice consumption of 5 per cent (58).

In order that rice may be acceptable to the consumer, the product

should be free from foreign matter, uniform in grain size and color, and contain a minimum amount of broken grains. The rice should have the natural flavor and aroma characteristic of rice, both before and after cooking. Brown rice, or hulled rice, should be free from the usual shagginess and non-uniform color. White rice should be completely white and have the desired translucency which is sought by the consumer. Rice paddy of brown and white rice, should be resistant to deterioration or becoming rancid (51).

According to Durrani (53) a method of processing rice paddy comprises (1) surface drying of the paddy while heating the rice grain to a temperature of 40 to 50 degrees Centigrade, (2) heating the preheated paddy to a temperature within the range of 60 to 120 degrees Centigrade in a steam atmosphere under a pressure of 1 to 100 pounds for a time interval required to gelatinize the rice grain almost completely, (3) rapidly cooling the gelatinized rice paddy to below 60 degree Centigrade, and (4) dehydrating the gelatinized grain of the cooled paddy to a moisture content of 9 to 14 percent.

Further work of Durrani (52) relates to a method of treating rice paddy or hulled rice grains to remove oils and fats from the bran and polish coatings of rice. This method removes a small amount, up to substantially all of the fats and oils, thus producing superior rice products and conditioning the rice for storage or for processing. The removal of the hulls, bran, and polish coatings from the surface of the grain is necessary to produce a fine product.

In 1961, Mazumder et al (42) developed a new method of parboiling rice for use in India. They found quick and mechanized methods for soaking, gelatinization, and drying of paddy to produce a good quality, parboiled rice superior in appearance, flavor, and thiamine content, and

acceptable to consumers in the East.

Their preliminary studies showed that the soaking of the paddy to the desired extent could be completed in 2.25 to 3.5 hours at 65 to 80 degrees Centigrade. It was observed that there was no "white belly"[1] in the rice grains when the moisture content of paddy after soaking was approximately 50 per cent on the dry basis. It was further noted that the best result in gelatinizing could be achieved by steaming the soaked paddy for 3 to 5 minutes in open steam.

Mazumder et al (42) used two types of soaker-steamers for their studies. They are: (1) Batch-soaker-steamer and (2) continuous soaker-steamer. The soakers were designed and fabricated to obtain the paddy soaked properly (retaining 50-55 per cent moisture) at elevated temperatures. The continuous agitating type soaker-steamer gave the best performance for the amount of water imbibition in the paddy grains. The paddy, when soaked in the batch soaker-steamer, contained 45 to 80 per cent moisture, whereas in the continuous agitating type soaker-steamer it was 52 per cent, the time and temperature being 2.5 hours and 75 degrees Centigrade in all cases. They suggested that the continuous soaker-steamer method would give a larger turn-over and would permit mechanization for larger scale production of parboiled rice.

They described the conventional parboiling process used in the orient which consists of steeping paddy in cold or lukewarm water for 48 to 72 hours, steaming for 15 to 25 minutes and then drying in the sun. Both the batch and continuous soaker-steamer methods produced a rice, which retained about double the thiamine content of parboiled rice obtained in the conventional manner.

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[1]White belly is a gelatinous material inside the rice kernel.

Mazumder et al (43) carried out a second pilot plant study on parboiling of rice and the dehydration procedure. They explained that the drying of parboiled paddy is generally carried out in the orient by spreading in the sun on cement or mud plastered yards. In the rainy season drying becomes a prolonged and uncertain process and microbial action on soaked paddy often becomes very pronounced giving an unpleasant flavor. The process usually takes 3 to 5 days (from soaking to drying) but sometimes even a fortnight during rainy weather.

They observed that, dehydration of paddy, unless carried out under well regulated conditions, caused development of cracks on the surface of the paddy which resulted in excessive breakage during milling.

According to Mazumder (43, p. 441):

> Theparboiled paddy (after soaking and steaming) contained 55 to 60 percent moisture on the dry basis. The conditions of drying to be fulfilled should be:
>
> (i). Reduction of the moisture content from 55-60 percent to 15-18 percent (dry basis).
>
> (ii). The drying should be uniform.
>
> (iii). The grains should not crack during drying and subsequent milling.
>
> (iv). The dehydration of paddy should cause no appreciable change in color or nutritive value compared with sun drying.

The optimum drying conditions for parboiled paddy as observed in the experimental rotary dryer designed by Mazumder et al (43) are:

1. The steam pressure in the tubes should be about 45 pounds per square inch gauge;
2. The feed rate should be approximately 150 pounds per hour;
3. The rotation should be about 5 revolutions per minute;
4. The inclination of the shell should be nearly 2 degrees to the horizontal; and
5. Air at a rate of 100 cubic feet per minute should be blown parallel to the feed, pre-heated to a temperature of 90 degrees

Centigrade.

It has been reported by Mazumder's workers that drying can be rapid when the moisture content of the paddy exceeds 35 per cent and also when it has been reduced to 18 per cent, but between these limits drying must be slow and carefully controlled to avoid cracking of grains. For good products a rotary dryer was designed and fabricated for drying parboiled rice.

Mazumder et al (44), in their third experiment, further studied the effect of hot soaking and mechanical drying on the nutritive value of parboiled rice. They preferred the sun drying method in which the paddy was dried in open sun in half inch thick layers on a concrete floor for two consecutive days from 10 a.m. to 5 p.m. at a temperature of 30 to 35 degrees Centigrade.

The results showed that the mechanical dryer was more efficient in retaining thiamine in rice compared with the sun-drying process. Variation in the drying temperature (70 to 87 degrees Centigrade) in the mechanical dryer did not appreciably change the thiamine content if the drying time was kept within certain limits (25 to 35 minutes).

## Chemistry of Cooking Rice

Griswold states that rice is a starchy food in which the starch occurs in small particles known as granules which are insoluble in water at room temperature. No membrane surrounds the granules. The contents of the swollen granule are held together by hydrogen bonds between the branches of amylopectin (20).

Griswold (20) further pointed out that crystalline, water-soluble, yellow pigments, known as flavones and flavonols, are distributed in rice. These pigments are stable to acid but made intensely yellow by

treatment with alkali. According to Lowe (40) rice cooked with alkaline water (or softened water) usually has a yellow tinge, though sometimes it turns green. Rice from the same source cooked in distilled water, has a snow-white appearance.

Glutinous and waxy rice becomes sticky when cooked, according to Shah (58). In East Pakistan, Biran or Birori or Binni rice is classified in the waxy rice group which comprises about 5 per cent of the total rice production. These are used for special purposes only such as making cakes, firni, etc.

Shah (58) further states that other varieties of rice in East Pakistan are non-glutinous and flinty in character and have excellent cooking quality. Balam, Patnai-23, Daudkhani, and Katariphog when cooked give white, soft and juicy rice and do not show any cracks. After cooking, each grain remains separate from the other. Varieties like Badshabhog, Kalizeera, and Chiniguri have small grains and are very fragrant. These are used for cooking pilaf and other costly dishes.

The unparboiled rice, usually known as "atap" in East Pakistan, is waxy and only used to prepare sweet dishes.

Rice varieties grown in the United States are divided into short, medium, and long grain groups based on grain size and shape. Beachell and Hallick (6) state that each grain type is associated with certain specific processing and cooking characteristics, but that there are exceptions. Most long-grain varieties tend to cook dry and fluffy, and the grains do not split or stick together. Short-grain varieties are usually more firm than long-grain varieties and tend to be more cohesive. Medium-grain varieties are usually intermediate in these respects. The differences in processing and cooking behavior reported were due to inherent differences in the chemical makeup of the rice grain rather than the grain

size and shape.

Some of the inherent varietal quality differences found by these and other investigators were amylose content, gelatinization temperature of starch when cooked, and stickiness of the cooked product.

Williams et al (68) reported differences in amylose of from 12.9 to 23.5 per cent. Varieties with high amylose content were Rexoro and Texas Patna. Varieties with low amylose content were Century Patna 231 and Toro. Hallick and Keneaster (22) were able to classify rice varieties as to amylose content, using an empirical starch-iodine blue technique.

Quality differences of milled rice are based upon the yield of total milled rice, yield of whole grain rice, appearance and the processing and cooking behavior of the milled grain. Milling rice consists of removing the hulls and most of the bran layers. Most of the fats, oils, proteins, and vitamins located in the bran layers and embryo are removed in the milling operation. As a result the physical and chemical differences in the starchy component of the rice grain are generally thought to constitute the differences in processing and cooking behavior. Physical and chemical differences in the milled rice grain are used in classifying varieties.

Batcher, Dreary, and Dawson (4) classified rice varieties using cooked samples of whole-grain, milled rice. Color, cohesiveness, flavor, degree of doneness, and amount of water absorbed were recorded. Rexoro, Texas Patna, Bluebonnet 50, and Century Patna 231 all absorbed more water during cooking and were more sticky than Caloro and Colusa. They concluded these grain types appeared to be associated with water absorption but noted some overlapping. Residual cooking liquids from most of the long-grain varieties appeared to have less total solids and starch than the short and medium-grain varieties. However, Century Patna 231 and Toro

had higher amounts of total solids in residual cooking liquids than other long grain varieties.

A cooking and soaking test reported by Hallick and Keneaster (22) gave marked varietal differences in the degree of longitudinal splitting of the grains. The rice was cooked for 20 minutes and soaked overnight in petri dishes. It was concluded that varieties exhibiting the greatest degree of longitudinal splitting showed the greatest degree of stickiness when cooked.

To produce good quality, they cooked rice by several methods of cooking which could apply in different school lunch situations, long- and medium-grain white rice and a parboiled, long-grain variety were cooked in three pound quantities by three methods of heating (5). They included cooking: (1) in a covered stockpot or saucepan on direct heat, (2) in a covered baking pan in a 350 degree Fahrenheit oven, and (3) in an open steam table pan in a compartment steamer at five pounds gauge pressure. The rice-water proportions and cooking times for each method were varied. The product was then allowed to stand 5 to 10 minutes before serving. It was not rinsed after cooking. The rice cooked by the oven and steamer methods was found to be tender and slightly moist with the grains firm enough to hold their shape.

Ozai-Durrani (51) established a process for preparing quick-cooking rice. In this process hulled rice grains are steamed under certain conditions to increase their moisture content by not more than about 6 per cent. Thereafter the rice is dried to a stable moisture content. Upon subsequent cooking the rice will absorb water very rapidly and will reduce the time required for cooking.

## Effect of Washing, Cooking, and Storage On Thiamine Content of Rice

The custom of washing rice thoroughly before cooking is universally prevalent, though an appreciable loss of water-soluble nutrients lessens the nutritional value. The oriental people wash rice through four to five changes of water before cooking, to remove dust and other foreign materials.

The percentage retention of nutrients in any type of rice depends on a number of factors, including length of washing periods, volume and temperature of washing water, and vigor of agitation.

Furter et al (15) used two methods of washing in their experiment: (1) the South Carolina method (household washing practice) and (2) mechanical procedure. They defined "customary rinsing" as the procedure in which one-half pint of grits was placed in a pan containing one quart of water at 25 degrees Centigrade, stirred and rotated for one minute, allowed to settle one minute, drained off and discarded water, added a second quart of fresh water, stirred and rotated for one minute, allowed to settle one minute, drained off and discarded water.

The South Carolina method described above, shows that the portion of the loss which occurred with white rice is unavoidable and constant for any given rice variety.

Some synthetic enrichment of white rice affords protection of the added nutrients against washing. In the Roche process (29) this is accomplished by a water resistent film which protects the enrichment premix. Ordinary white rice suffers extremely high losses of vitamins and iron. Furter et al (15) concluded that the rice premix is resistent to washing, cooking, and storage losses.

In the methods used in the Roche test, seventy-five grams of rice were added with 375 milliliters of water in a screw cap jar which was rotated at a constant speed of about three rounds per minute. The washing continued for twenty minutes. As in the South Carolina washing, the losses of thiamine, niacin, and iron are extremely high for white rice, even for relatively short periods of washing.

Miller (48) determined the loss of thiamine as a result of washing and cooking brown and partially-milled rice. In preparing rice he developed the methods as:

1. Brown rice-unwashed: foreign materials were removed from the rice and 96 grams of rice (half cup) were added to 150 grams (two-thirds cup) of hot water, and the rice soaked for 5 hours. The mixture was brought to boil quickly and then gently boiled until all water was absorbed. The heat was then reduced and the product was steamed for 30 minutes.
2. Brown rice-washed: the rice was washed for 5 successive times using one cup of water each time. Hot water was added to make the weight equivalent to that for the unwashed rice. The rice was soaked and cooked as in the first method.
3. Partially parboiled rice - unwashed: 150 grams (two-thirds cup) of cold water were added to 96 grams (half cup) of rice and, without soaking, the product was cooked as for brown rice, steaming period being 20 minutes.

Miller obtained results as follows:

1. No significant quantity of thiamine was lost when brown rice was washed rapidly through 5 changes of water.
2. Partially-processed rice contained 70 per cent as much thiamine as the unprocessed, brown rice.

3. Partially-brown rice lost more thiamine than brown rice as a result of washing (about 20 per cent).

4. The use of partially milled or, better still, brown rice, is probably the best means of improving the thiamine content of the rice-eaters' diet until a highly-milled type of parboiled rice becomes generally available.

According to the studies made by Vinacke (66) the loss of thiamine in rice with excessive washings is twice or more than that with one washing.

The modern methods of boiling rice are:

Double-boiler method:

Measure 2½ cups of boiling water into the top of a double boiler. Add 1 teaspoon salt, then 1 cup rice. Cover and place over boiling water and cook without stirring until tender from 35 to 40 minutes. At the end of cooking all the water is absorbed and the rice is dry and fluffy.

The use of a double boiler is desirable to avoid high temperatures at the bottom surface of the pot which tends to accelerate vitamin losses.

Direct-heat method:

Measure 2½ cups of water and 1 teaspoon salt in a heavy 2 quart saucepan. Pour in 1 cup rice, cover pan tightly. Place over low heat and simmer without stirring about 25 minutes or until tender.

Boiling method:

Measure 2 to 3 quarts of boiling water into a 4 quart saucepan. Add 2 teaspoons salt to each quart of water used. Place over heat. Pour in cup of rice slowly so that boiling does not stop. Stir frequently and boil until soft.

When done, pour rice into a collander to drain, then rinse with hot

water.

Place the collander over a pan of hot water to make rice fluffy.

The first two methods give uniformly tender, fluffy grains of rice which stand apart. These methods conserve the food value. The boiling method is the quick method but it requires draining. This wastes food value.

The United States' Department of Agriculture, Human Nutrition Research Branch (63) included the oven method of cooking regular white rice. The method is as follows:

> Place ingredients in baking dish. Pour boiling water over rice, stir, cover with tight lid or foil. Bake at 350 degrees Fahrenheit for 25 to 30 minutes or until tender.

The cooking procedure used by Furter et al (15) was as follows:

> 750 milliliters of water containing about 10 grams of salt is brought to a boil in an aluminum pan; 200 grams of rice are added and cooked over direct heat with occasional stirring for at least 30 minutes. The cooking losses of thiamine were 7 to 10 per cent and of niacin 2 to 4 per cent, regardless of the kind of rice cooked. When the above method involved draining off any of the cooking water, the losses of thiamine in that case are roughly proportional to the volume of water drained off after cooking. The relative losses are similar for both enriched and converted rice.

According to Vinacke (66), brown and processed rice lose half of their thiamine content when cooked in large amounts of water, as compared to the loss of one-fifth and one-third when cooked in small amounts of water. White rice loses only about 10 per cent when cooked in either manner. Rice cooked in the oven has the highest retention of thiamine - 82 per

cent. In this method, twice as much water as rice was used, and the rice was baked at 400 degrees Fahrenheit for 45 minutes. The highest loss of thiamine caused by cooking occurred in rice cooked in the pressure saucepan for 11 minutes, when 52 per cent retention of thiamine was noted. In this method three-fourths as much water as rice was used and the resulting product was rather dry, but an increased amount of water tended to produce a soggy, heavy, cooked product. The pressure saucepan method is not recommended because the product is not as fluffy as boiled rice.

## Methods of Assay for Thiamine

The amount of thiamine in food is determined by several different methods. These are identified as biological assay, chemical assay, and microbiological assay.

### Biological Assay:

To measure the amount of thiamine in a food-stuff by biological test, the animals which have been used are primarily the pigeon and rat. With pigeons the protective, weight maintenance, and curative methods are the principle ones which have been used. The protective method, based on prevention of polyneuritis, was employed by early workers in this field. Eijkman maintained fowls on a polished rice diet. Funk (19) and others kept pigeons on polished rice, or synthetic diets, and Jansen and Donath (19) used the rice bird which developed polyneuritis within 2 weeks. The protective test involves an estimation of the daily ration of a particular food-stuff which is sufficient to prevent neuromuscular symptoms in birds, when added to the vitamin $B_1$ diet.

In the curative method, Vedder and Williams (65) used the fowl which developed polyneuritis within 2 weeks. Their experiment was done in

Bataan, Philippines. The fowls that developed polyneuritis when fed white rice, were cured by feeding brown rice as the entire ration. From the result they concluded that white polished rice is deficient in vitamin $B_1$, whereas brown rice contains a high percentage of thiamine. In the weight maintenance method, Harris (24) took a group of four rats and gave 1 gram of thiamine to each rat. In the same way he used more groups and fed 2 grams, 4 grams, and so on to each rat of a particular groups. The results show that the less vitamin-$B_1$ the rat received, the poorer was its growth, within limits.

In the bradycardia method Birch and Harris (7) using white rats, determined the specific cardiac action of vitamin-$B_1$. This has become the basis of a convenient, rapid and accurate test method. Young rats are placed on a vitamin-$B_1$ deficient basal diet, and after 3 weeks when the animals are beginning to decline in weight, electrocardiograms are taken. By this time the normal heart rate of 500-550 beats will have fallen to about 350 per minute. A single dose of the test substance is fed in addition to the basal diet and water as before. After 24 hours, with a sufficiently large dose of vitamin, the rate is increased. Then the gradually diminishing rate is measured every 12 hours until it reaches the original low value. The increase in rate and the period over which the increase lasts are proportional to the dose given. This method is thought to be reliable but requires expensive apparatus and a trained technician.

Chemical Assay:

The first problem in any of the chemical methods is to obtain the vitamin in solution, and this in many cases requires an extraction procedure.

For securing data on thiamine values, chemical techniques are based

either on the fluorescence of the product formed by the oxidation of the vitamin into thiochrome, or on the color intensity of thiamine when combined with a diazotized amine. Paper chromatography and paper electrophoresis permit the separate measurement of the vitamin and its phosphorylated esters.

The thiachrome method was introduced in 1936 by the Dutch chemist, Jansen, and the test has gradually been refined.

Jansen (69) oxidized thiamine in alkaline medium by ferricyanide and thus converted it into thiochrome. When irradiated by light in the near ultraviolet region of the spectrum, this substance has a strong blue-violet fluorescence, the intensity of which is proportional to the amount of thiamine from which it is formed by oxidation.

In the application of the Jansen method, the determination of vitamin $B_1$ in body tissues and fluids and analysis of urine was worked out by Westenbrink and Goudsmit (67).

Hennessy and Cerecedo (28) introduced the use of synthetic zeolites for the isolation of the vitamin from food stuffs, and later these researchers showed that materials interfering in the thiochrome method could be eliminated by the use of base exchanging zeolite.

Melnick and Field (47) developed the calorimetric method. This consists of passing the impure extract which contains the vitamin through a column of the synthetic zeolite. The vitamin is retained on the zeolite, from which it can later be removed by treatment of the zeolite column with potassium chloride solution.

According to Hennessy (27) the thiochrome method, having considerably greater sensitivity than the calorimetric method, may also be used for the assay of materials of low potency. The use of the synthetic zeolite, Decalso, as a preliminary step in both procedures effectively

eliminate interfering materials.

Microbiological Assay:

In the higher plants, Vitamin $B_1$ is formed in the green leaves. From the leaves it is transported to the roots, where it exerts its effect on root growth. This supply of vitamin may vary with the type of plant or with the stage of development and may be supplemented from the soil, either indirectly through the soil bacteria or directly from the vitamin absorbed on the clays of the soil. Since this vitamin content of the soil is derived from the debris of plants and the soil microflora, the role of the vitamin in the growth of micro-organisms, fungi, and yeasts is of importance.

The short duration of the life cycle of microscopic organisms favors the development of a rapid test method for vitamin $B_1$. The stimulation of the rate of alcoholic fermentation, the growth of fungi and cocci and the germination of seeds of higher plants have all been proposed.

The microbiological assay of foods to determine their content of certain B-vitamins is an accurate and useful method (57).

If a particular kind of microorganism is known to require a certain vitamin in its culture medium, and it cannot synthesize the substance, that microorganism can be used in vitamin assay work (57).

Knight (38) demonstrated that, under suitable conditions, the growth of Staphylococcus aureus is proportional to the amount of vitamin $B_1$ present in the medium.

Niven and Smiley (50) used the test organism Streptococcus salivarius. The culture medium of the organism required the addition of thiamine before growth could occur. Inoculum for assays are prepared by transferring the culture from the agar stab directly into the basal medium to which 10

millimicrograms of thiamine per 10 millimeter have been used. The culture is incubated at 37 degrees Centigrade for 24 hours, or until good growth occurs, before used to inoculate the assay tubes.

Nevin and Smiley in their experiment, used a thiamine-free basal medium. When sufficient thiamine is added to this medium, Streptococcus salivarius grows in intensity and rapidity equal to that in any ordinary laboratory medium. In their assaying procedure, since thiamine is partially destroyed on heating in alkaline, and even in neutral solutions, the standard vitamin solutions, as well as the test substance is necessary to add, aseptically to the medium after autoclaving. They found turbidimetric measurements with a photoelectric calorimeter to be more satisfactory for measuring growth response than titrating the developed acid. They listed that the thiamine content of the test substance may be determined by comparing the growth response to that produced by thiamine on the standard curve.

Grains and Stahly (18) carried on experiments with the organism of Leuconostoc mesenteroids for several members of the vitamin B-complex. They used thiamine chloride aqueous solutions and other B-complex vitamins.

Barnett and Lilly (3) investigated the influence of thiamine upon the formation of perithecia by the fungus C. fimbriata. It was known to them that Cerostostomella fimbriata was highly deficient for thiamine. In their experiment, the weights of all mycelia grown in media containing thiamine were remarkably uniform. From this result they concluded that the isolate C. fimbriata is completely deficient of thiamine.

To confirm that the failure of the perithecia in low concentration of thiamine was due directly to an insufficient supply of thiamine, Barnett and Lilly transferred the discs of agar and mycelium from plates having no thiamine, but containing the full amount of nutrients to tubes of each of

the following: (a) distilled water; (b) distilled water, thiamine, and purified agar. The tests showed that the mature perithecia discharging ascospores were formed within six days in all tubes containing thiamine, but no perithecia were developed in any tubes which lacked thiamine.

Further studies (3) on the effects of varying concentrations of thiamine and nutrients were made with a reduced supply of nutrients. They used replicates of 8 flasks with varying amounts of thiamine as 100, 6.25, 1.56, and .8 grams per litre. The mycelium was removed from the flasks, dried, and weighed. Estimates of the relative abundance of perithecia were made on the fifth and eleventh days. In the presence of 100 grams of thiamine per litre, the amount of growth was in direct proportion to the amount of nutrients present. In a plentiful supply of nutrients, the numbers of perithecia were influenced directly by the amount of thiamine in the medium, none being formed in the presence of 0.8 grams of thiamine.

# CHAPTER III

## METHOD OF PROCEDURE

### Choice of the Variety of Rice

In the environment of the United States of America it was necessary to choose a similar variety of rice to that usually consumed by the majority of the people in East Pakistan. It was found that a number of experiments on rice had been done in America by Dr. M. C. Kik (37), in the Department of Agricultural Chemistry, University of Arkansas. So it was reasonable to write to Dr. Kik to get information about the place where rice could be obtained. The first letter to Dr. Kik (Appendix A) was written on October 15, 1962. In reply to this letter, Dr. Kik sent two of his publications on rice. He gave the address of Mr. L. C. Carter, General Manager, Arkansas Rice Growers Association. Dr. Kik also mentioned the name of Professor Beachell, Rice Breeder, Rice Experiment Station, Beaumont, Texas, for information on varieties of rice.

Since Louisiana is one of America's largest rice producing areas, the same letter as was written to Dr. Kik, was sent to the Director of Rice Research (Appendix A), Southern Regional Laboratory, United States Department of Agriculture, New Orleans, Louisiana. Mr. E. L. Patton, Assistant Director, United States Department of Agriculture, Research Service, replied to this letter. He recommended to contact Mr. L. E. Crane, Rice Pasture Experiment Station, Beaumont, Texas.

On advise of Dr. Kik, a letter was written to Mr. Carter (Appendix A) on October 31, 1962.

The acknowledgment of the letter written to Mr. Carter was received from W. L. Knoll (Appendix A), Assistant Sales Manager, The Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas, and was dated November 7, 1962. Mr. Knoll gave the name of some varieties of available rice. They were Blue Bonnet, long-grain variety; Century Patna, long-grain, but not as long as Blue Bonnett; Blue Rose, large medium grain variety; Nato, a medium grain variety; and Pearl, a round grain variety. Mr. Knoll wanted to be advised of what variety he was to ship.

Three different sizes of rice kernel were obtained in Stillwater and were examined by Pakistani men and women students on the campus of Oklahoma State University. The majority of the students chose a long-grain variety which is similar to a medium grain rice in East Pakistan.

After the final decision, a sample of long grain variety of rice was sent to Mr. Knoll, requesting him to ship 40 pounds of paddy and 20 pounds of milled white rice as much like the sample as possible. All the rice used in the experiment was to be from the same lot and same variety.

On December 5, 1962, a letter from Mr. Knoll (Appendix A) was received in which he indicated the requested sample of rice had been shipped on December 3, 1962.

The parcel of rice ordered arrived in Stillwater on December 20, 1962. The variety which was similar to the sample chosen by the Pakistani students was Blue Bonnet, which is the best long grain variety in Stuttgart, Arkansas. The 40 pounds of paddy and 20 pounds of milled white rice were shipped packed separately in jute sacks. Upon arrival the paddy and rice were stored for later use in covered glass jars at room temperature in the Food and Nutrition Research Laboratory.

## Processing Rice from Paddy

Parboiled and unparboiled rice used in this experiment were obtained from the 40 pounds of paddy received from Stuttgart, Arkansas. The paddy was treated following the native processing procedures. Information of the home pounding method was obtained from a well known farm in East Pakistan.

Parboiled method:

The first lot of paddy was soaked on January 28, 1963. Four batches of paddy, 2 pounds each, were soaked in 8 3/4 cups of distilled water. Each batch was placed at room temperature (24° C.) in an open crockery vessel which had a glazed finish.

After 48 hours a little trace of fermentation was found on the surface water of the soaked paddy. The paddy was stirred with a wooden spoon. Each 2 pounds of this paddy, with distilled water in which it was soaked, was transferred into an aluminum vessel with tight fitting lid. The 4 vessels were placed on a gas burner, and started to heat. The paddy was heated to 70 degrees Centigrade.

The covered pans of rice paddy and water in which it was soaked were kept hot enough to barely simmer. At this temperature the paddy was steamed for 15 minutes, then the heat was turned off. The paddy in each vessel was poured into a separate colander. The small amount of water which had not been absorbed on parboiling was discarded. The water discarded was a very light brown color and was not clear.

The parboiled paddy was then spread one-half inch thick on plastic trays. The trays were kept at room temperature (24° C.). After each 24 hours the paddy was stirred.

After 5 days of drying at room temperature, the parboiled paddy was completely dry and ready to be stored in glass jars. Twelve pounds of parboiled paddy were made ready to be husked.

Soaked, unparboiled method:

Following the parboiling practice 6 batches of paddy, 2 pounds in each batch, were soaked in distilled water, the proportion of water to paddy being the same. The paddy was soaked for 48 hours, then was poured in a colander. All remaining water was discarded, the soaked paddy was spread one-half inch thick on plastic trays. It was kept at room temperature ($24^{\circ}$ C.) and was stirred at 24 hour intervals. After 5 days the paddy was completely dried. It was stored in a closed glass jar at room temperature for dehusking.

Description of the husking equipment:

The device for husking the rice used in the laboratory was similar to that practiced by the native people in East Pakistan. The only difference was the material and size of the equipment available for dehusking.

The equipment used was a hollow, conical-shaped aluminum mortar, closed at the vertex. The diameter of the open base was 6¼ inches and the height was 8 inches. The volume of the mortar permitted one-half pound of paddy to be husked at a time. There were small holes on the surface of the mortar in order to make the surface rough through which the finer husk could be passed. The mortar was held on an aluminum three-legged frame. When the mortar was placed on the frame, the total height of the whole equipment was measured to be 9 3/4 inches, the closed end of the mortar being 1 3/4 inches above the surface of the table. There was a handle attached to the mortar. While doing the pounding it

was necessary to hold this handle tightly.

A wooden pestle, 11 inches long and 6 inches in perimeter was fitted into the mortar.

Husking method:

About two-thirds of the mortar was filled with paddy. Holding the handle of the mortar with the left hand, the paddy was beaten by the pestle with the right hand. Pressure was given by each stroke of the pestle moving up and down. In response to the pressure the husks were lossened and detached from the rice kernel. The husks were separated from the rice by means of a bamboo winnowing tray known as a Kula. The outer layer (pericarp) of the rice kernel was removed up to the point at which the rice was acceptable to eat. Approximately one-third of the pericarp remained on the rice kernel. Time consumed to separate the kernel from the husk in one-half pound of paddy was 30 minutes and the quantity of rice obtained was measured to be approximately one-third pound.

It was observed that the parboiled paddy dehusked faster than the soaked unparboiled paddy. It also took less care while dehusking, because soaked unparboiled paddy broke easily during husking. Therefore it was necessary to manipulate the pestle with care so as not to put hard pressure on the paddy. In spite of all the extra care given during husking, the rice obtained from the soaked, unparboiled paddy contained 19.82 per cent broken grains. On the other hand, rice from the soaked, parboiled paddy had very few broken grains and the kernels looked solid and separate. Twelve pounds of parboiled paddy yielded 8 3/8 pounds of whole rice, whereas 12 pounds of soaked, unparboiled paddy yielded 7 pounds of whole rice kernel and 1 3/8 pounds of broken rice.

The milled white rice used in this experiment was commercially processed in the mills of Stuttgart, Arkansas.

The three qualities or rice - parboiled, unparboiled, and milled white rice - were stored in tightly covered glass jars at room temperature in the Food and Nutrition Research Laboratory for cooking and determination of thiamine content.

## Experimental Cookery

The sample of rice used for thiamine assay was prepared from cooked rice. In this study, two prevalent methods of cooking were considered. One method was cooking with a small amount of water, just long enough to tender the grains. All water was absorbed. The other method of cooking was with a large amount of water, just long enough to tender the grains, then excess water was discarded.

The purpose of experimental cookery was to determine the proportion of water to rice needed, and the length of time to tender the grains. Distilled water was used in all cooking of rice to avoid alkalinity of water which increases the destruction of thiamine in rice.

The three varieties of rice were cooked by the two methods to be used taking care to use the same amount of rice, number of rinsings before cooking, amount of water during cooking, and length of cooking time. The amount used in the recipes was sufficient to cook easily and to yield enough rice for adequate samples.

The recipes for cooking all three varieties of rice were as follows:
Rice Recipe (small amount of water)

50 grams rice

160 grams distilled water

Rinse rice through 3 waters using tap water. Add distilled water to drained rice. Place in flat-bottomed vessel with a tight-fitting lid. Bring to boil quickly, stir to prevent sticking to bottom of pan, and cover tightly. Adjust flame so that the rice continues to boil gently. Cook for 18 minutes.

Rice Recipe (large amount of water)

50 grams of rice

640 grams of distilled water

Rinse rice through 3 waters using tap water. Add distilled water to drained rice. Place in flat-bottomed vessel with a tight-fitting lid. Bring to boil quickly, stir to prevent sticking to bottom of pan, and cover tightly. Adjust flame so that rice continues to boil gently. Cook for 18 minutes. Pour rice and cooking water into a colander and allow to drain. Do not rinse.

The appearance and flavor of the cooked rice was evaluated and recorded.

Rice cooked with small amount of water:

Parboiled - Grains were distinct and chewy and the flavor was nutlike. The color was light cream.

Unparboiled - Grains were somewhat less distinct, gummy, and were not as chewy as the parboiled. The flavor was milder than the parboiled. The color was light cream.

Milled white - Grains were not distinct but fluffy. Flavor was bland. This was more sticky than unparboiled rice and the color was very white.

Rice cooked with large amount of water:

Parboiled - Grains were distinct, chewy, and waxy. The flavor was nutlike and somewhat milder than rice cooked in a small

amount of water.

Unparboiled - Grains were distinct and the color was light cream. Variation in color from white to cream was present. The grains were tender and slightly chewy with mild but pleasing flavor.

Milled white - Grains were somewhat distinct. The color was very white and the flavor was mild. The grains were tender and distinguishable.

## Selection of Method of Assay for Thiamine

This study was performed following the microbiological method of assay for thiamine.

Dr. Lynn L. Gee, Head of the Department of Microbiology, Oklahoma State University, suggested the names of two micro-organisms to be used in the assay of rice for thiamine. He recommended the methods of Niven and Smiley (21, p. 373) and Sarett and Cheldelin (21, p. 376).

The two micro-organisms chosen for assay require thiamine for their growth. When thiamine-free media was used, and known samples of cooked rice were added to them, growth could be attributed to thiamine in the rice samples.

Pure cultures of Streptococcus salivarius No. 9222 and Lactobacillus fermenti No. 9338 were obtained from the American Type Culture Collection, 2112 M. Street, Washington 7, D. C.

## Media for Thiamine Assay

Vitamin Methods edited by Gyŏrgy contains a table for the composition of media for assay of thiamine with lactic acid bacteria. It is as follows (21, p. 375):

TABLE VI

COMPOSITION OF MEDIA FOR ASSAY OF THIAMINE WITH LACTIC ACID BACTERIA

For Streptococcus salivarius

Medium No. 15, Niven and Smiley, 1943

| Constituent | Amount /100 c.c. of double strength medium. |
|---|---|
| Casein hydrolyzate[a] | 1.0 g. |
| Thiamine-free yeast extract | 0.6 g. |
| Glucose | 2.0 g. |
| Potassium phosphate buffer (0.4M, PH 7.0) | 20 cc. |
| Salt solution[b] | 2.0 cc. |
| Uracil | 1.0 cc. |
| Nicotinic acid | 100 γ |
| Riboflavin | 100 γ |
| Calcium pantothenate | 100 γ |
| Biotin (methyl ester) | 0.1 γ |

For Lactobacillus fermenti 36

Medium No. 16, Sarett and Cheldelin, 1944

| | |
|---|---|
| Alkali-treated peptone (plus sodium acetate)[c] | 2.0 g. |
| Casein hydrolyzate[a] | 0.5 g. |
| Glucose | 4.0 g. |
| Sodium acetate | 1.2 g. |
| Cystine | 20 mg. |
| Adenine sulfate | 2.0 mg. |
| Guanine hydrochloride | 2.0 mg. |
| Uracil | 2.0 mg. |

TABLE VI (Continued)

| Constituent | Amount /100 c.c. of double strength medium. |
|---|---|
| Salts A[d] | 1.0 cc. |
| Salts B[d] | 1.0 cc. |
| Riboflavin, calcium pantothenate, p-aminobenzoic acid, nicotinic acid, pyridoxine hydrochloride | 20 each |
| Biotin | 0.08 |
| Folic acid[e] | 0.015 |

a Preparation of Acid Hydrolyzed Casein. See Appendix C.
b Preparation of Salt Solution. See Appendix C.
c Preparation of Alkali Treated Peptone. See Appendix C.
d Composition of Salts A and B. See Appendix C.
e Recalculated in terms of pure folic acid (pteroylglutamic acid) Assuming this to have a potency of 137,000.

See Appendix C for: Preparation of Thiamine-free yeast extract; Vitamin dilutions in the media for Streptococcus salivarius; Vitamin dilutions in the media for Lactobacillus fermenti.

## Preparation of Rice Sample for Assay

Two-gram samples of cooked rice were chosen to assay as the estimated amount of thiamine in this size sample could be measured adquately. Three samples from each kind of processed rice, cooked by each of 2 methods, were prepared for assay by each of the 2 micro-organisms (36 sample tubes plus 1 blank). Each assay was repeated 3 times.

Two grams of the cooked rice were weighed accurately. The weighed portion was placed in a porcelain mortar. With a pestle the rice was mashed until smooth and dissolved in 15 cc of 0.1 N $H_2SO_4$. The mixture was digested on a steam bath with frequent mixing for 30 minutes. The liquid remained distinctly acid during the digestion. According to the

directions, if the pH rises above 1.5, additional dilute $H_2SO_4$ is to be added, but this was not necessary in this study. After digestion, the mixture was cooled and the pH adjusted to between 4 and 4.5 by addition of a concentrated solution of 3N sodium acetate. This is the optimum pH for digestion with phosphorolytic enzymes. A 10 per cent solution of an enzyme preparation high in diastatic and phosphorolytic enzymes was prepared in distilled water. This was done by adding 10 grams of takadiastase to 90 cubic centimeters of distilled water.

For each 10 gamma of thiamine estimated to be present in the sample used, 1 cubic centimeter of the takadiastase solution was added. Amounts of takadiastase added to samples of rice were based on thiamine content of rice as reported by Kik (35) in 1946 and were as follows:

| Kik's Raw Rice Samples | Thiamine Content |
|---|---|
| After boiling 15 minutes | 2.24 µg/gm. |
| No treatment | 0.6 µg/gm. |
| 45 hours soaking plus 10 minutes steaming | 2.20 µg/gm. |
| **Cooked Rice Samples** | **Takadiastase Added** |
| Parboiled | .46 cc. |
| Unparboiled | .40 cc. |
| Milled white | .12 cc. |

The mixture was incubated at 45 to 50 degrees Centigrade for 3 hours. It was then adjusted to pH 6.5 to 6.6, and diluted to an estimated thiamine content of 0.1 to 0.2 gamma per cubic centimeter. The sample was then filtered and preserved in the refrigerator. The total volume of the two-gram sample was determined and one-fourth of the total volume was used as a suitable aliquot for assay.

## Assay of Thiamine in Rice Samples

In the assay of prepared samples of rice for thiamine every assay tube contained 5 cubic centimeters of the double-strength medium made up for the organism to be used in the test. All tubes filled with 5 cubic centimeters of basal medium were sterilized in a pressure cooker at 15 pounds pressure for 15 minutes. All flasks, cotton plugs, pipettes, and distilled water used in measuring the rice samples and making the total volume of each assay tube to 10 cubic centimeters were sterilized in the same manner.

Samples of rice could not be pressured without possible destruction of thiamine unless they were kept at a very low pH. Such a pH is not conducive to optimal growth of the micro-organisms used in this study. Therefore each sterile assay tube containing 5 cubic centimeters of double strength-medium was made up to a total volume of exactly 10 cubic centimeters by addition of an aliquot of the rice sample (one-fourth of the total volume) plus sterile distilled water. All tubes were labeled, plugged with sterile cotton, and placed in beakers. The beakers were placed in a large aluminium vessel, and the vessel was filled with sufficient boiling water to reach the level of the liquid in the assay tubes. The water was brought to a boil, a tight-fitting lid was placed on the vessel, and the assay tubes were steamed for 15 minutes (21, p. 376). At the pH of the medium (6.5) this procedure avoids destruction of thiamine, and is adequate to prevent contamination during the short assay period used.

The sterile tubes containing 5 cubic centimeters of basal medium, an aliquot of the rice sample, and sufficient distilled water to make a total volume of 10 cubic centimeters were allowed to cool to approximately body temperature.

The procedure for making the inoculum medium and the inoculum for Streptococcus salivarius and Lactobacillus fermenti are given in Appendix B. The pure strain of these two micro-organisms needs to be in an active state in order to use the turbidity produced by their growth as a means of measuring the thiamine content of the rice samples to be assayed.

Each assay tube was inoculated with one drop of the appropriate inoculum, dependent upon the organism being used in the assay. The tubes were kept plugged and were incubated for 18 to 24 hours at 37 degrees Centigrade. Then the turbidity of each tube was matched with tubes in McFarland's Nepholometer Standards. See Appendix B for directions for making these standards.

## Standard Growth Curves for Thiamine

In order to determine the amount of thiamine necessary for various degrees of growth a standard growth curve must be made for each micro-organism used in the assays. The concentrations of thiamine and the directions for making the standard growth curves for Streptococcus salivarius and Lactobacillus fermenti are given in Appendix B.

Turbidity produced by the growth of the microorganism, when the quantity of thiamine in the basal medium is known, is used as an index against which to measure the amount of thiamine in the rice sample assay tubes.

McFarland's Nepholometer Standards were used to measure the degree of turbidity present in the tubes for the standard growth curve of thiamine. Rice sample assay tubes were also matched with McFarland's Standards and the amount of thiamine present in a given sample was calculated from this data.

## Simplification of Assay Methods for Use in Pakistan

Since the author wished to develop methods which could be duplicated in Pakistan, care was taken to choose available materials whenever possible. In lieu of a colorimeter to read the turbidity of assay tubes McFarland's Nepholometer Standards were made and were used. A pressure cooker was used whenever an autoclave was needed as a sufficiently high temperature could be reached to render materials sterile.

All solutions were mixed by the author to be certain that she could make up the basal media needed for assays with two micro-organisms. Directions for making vitamin dilutions and for making percentage, molar and normal solutions used in this study are in Appendix C. Instead of a pH meter, pH paper was used to further reduce the need for pieces of equipment which might not be available to the researcher later on.

# CHAPTER IV

## RESULTS AND DISCUSSION

Observation of the growth of the microorganism as indicated by turbidity in the assay tubes is a measure of the concentration of thiamine for standard curve. When the tubes were inoculated for making the thiamine standard curve using Streptococcus salivarius and Lactobacillus fermenti, good growth was reported from Streptococcus salivarius. No response of growth was found in the tubes of Lactobacillus fermenti. The reason for no response was not known. Therefore, it was considered that the microorganism was not virile. The incubation with Lactobaccilus fermenti was repeated using fresh culture. It could further be suspicioned that the media prepared for Lactobacillus fermenti was not effective. However, it was decided to discard the Lactobacillus fermenti microorganisms and continue the assay using the single microorganism, Streptococcus salivarius.

The inoculated tubes with known amounts of thiamine in them were read to determine the amount of growth of the microorganism. This was performed by matching the turbidity of the thiamine standard tubes against McFarland's Nepholometer Standard tubes (See Appendix B). Following is the results of turbidity of the thiamine standard tubes which has been received to obtain the standard growth curve for thiamine using Streptococcus salivarius:

| Thiamine standard tubes m /10 cc | Nepholometer standard tubes numbers |
|---|---|
| 0 | 0 |
| 0.1 | 2 |
| 0.2 | 3 |
| 0.4 | 4 |
| 0.7 | 5 |
| 1.0 | 5 |
| 1.5 | 6 |
| 2.0 | 6 |
| 2.5 | 7 |
| 3.0 | 7 |

Figure 1 presents the standard growth curve in which the concentration of thiamine is plotted against the corresponding growth of culture as indicated by Nepholometer Standard tube numbers.

The results of rice sample assays for three cookings are presented in Tables 1, 2, and 3. In these tables columns 1 and 2 list the total volume of the prepared rice samples and the corresponding aliquot used for assay. The total volume was recorded to estimate the thiamine value in the rice sample and to calculate the size of aliquot to use. A blank tube was included in the assay of each cooking. In the assay of the first cooking, the blank tube reading was 2., whereas in the second and third cooking it was 1. Columns 3, 4, and 5 indicate the amount of growth in the assay tubes. The average of the 3 tubes for each variety of rice for each cooking method is shown in the sixth column. As the blank tubes were not free of growth, it was indicated that there was a small amount of thiamine which stimulated the growth in the blank tubes without rice samples or the organisms present. So the average of the

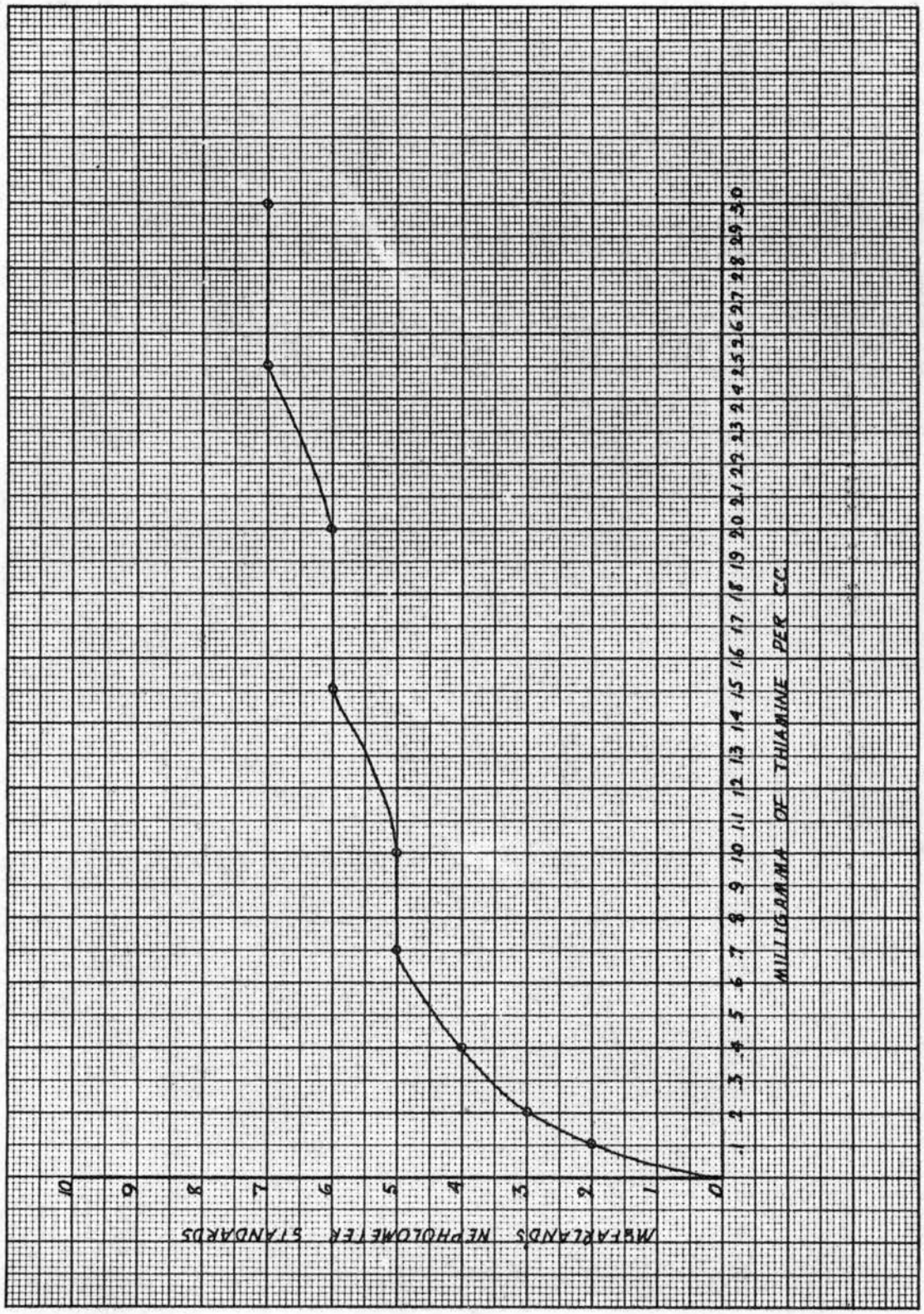


Figure 1. Concentration of Thiamine for Standard Curve for S. salivarius.

Table 1. Rice Sample Assay for First Cooking

| Cooked sample | Total Volume | Aliquot | McFarland's Nepholometer Standard Readings | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tube 1 | Tube 2 | Tube 3 | Average | Blank | Av. corrected for blank |
| | cc | cc | | | | | | |
| Lg. am't. water | | | | | | | | |
| Parboiled | 15.0 | 3.8 | 5 | 4 | 4 | 4.3 | | 2.3 |
| Unparboiled | 16.0 | 4.0 | 4 | 4 | 4 | 4.0 | | 2.0 |
| Milled | 13.2 | 3.3 | 3 | 3 | 3 | 3.0 | | 1.0 |
| Sm. am't. water | | | | | | | | |
| Parboiled | 17.5 | 4.4 | 5 | 5 | 5 | 5.0 | | 3.0 |
| Unparboiled | 14.0 | 3.5 | 4 | 3 | 3 | 3.3 | | 1.3 |
| Milled | 20.1 | 4.0 | 2 | 3 | 3 | 2.7 | | 0.7 |
| Blank | | | | | | | 2 | |

Table 2. Rice Sample Assay for Second Cooking

| Cooked sample | Total Volume | Aliquot | McFarland's Nepholometer Standard Readings | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tube 1 | Tube 2 | Tube 3 | Average | Blank | Av. corrected for blank |
| | cc | cc | | | | | | |
| Lg. am°t. water | | | | | | | | |
| Parboiled | 15.0 | 3.8 | 3 | 3 | 2 | 2.6 | | 1.6 |
| Unparboiled | 15.1 | 3.8 | 5 | 3 | 4 | 4.0 | | 3.0 |
| Milled | 16.0 | 4.0 | 4 | 4 | 4 | 4.0 | | 3.0 |
| Sm. am°t. water | | | | | | | | |
| Parboiled | 15.5 | 3.9 | 6 | 6 | 6 | 6.0 | | 5.0 |
| Unparboiled | 15.5 | 3.9 | 5 | 5 | 5 | 5.0 | | 4.0 |
| Milled | 16.8 | 4.2 | 4 | 3 | 3 | 3.3 | | 2.3 |
| Blank | | | | | | | 1 | |

Table 3. Rice Sample Assay for Third Cooking

| Cooked sample | Total Volume | Aliquot | McFarland's Nepholometer Standard Readings | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Tube 1 | Tube 2 | Tube 3 | Average | Blank | Av. corrected for blank |
| | cc | cc | | | | | | |
| Lg. am't. water | | | | | | | | |
| Parboiled | 14.0 | 3.7 | 5 | 5 | 5 | 5.0 | | 4.0 |
| Unparboiled | 12.0 | 3.0 | 4 | 4 | 4 | 4.0 | | 3.0 |
| Milled | 18.8 | 4.7 | 3 | 3 | 3 | 3.0 | | 2.0 |
| Sm. am't. water | | | | | | | | |
| Parboiled | 10.0 | 2.5 | 5 | 5 | 5 | 5.0 | | 4.0 |
| Unparboiled | 13.0 | 3.3 | 4 | 4 | 5 | 4.3 | | 3.3 |
| Milled | 10.0 | 2.5 | 4 | 3 | 3 | 3.3 | | 2.3 |
| Blank | | | | | | | 1 | |

assay tube readings was corrected by subtracting the blank tube reading. The corrected average reading for each variety of rice is presented in the last column. To determine the thiamine content of the rice samples, all the corrected averages for 3 cookings are recorded in Table 4. Column 4 of this table represents the average of each variety of rice by 2 methods of cooking and 3 repetitions of assay.

These average figures were compared with the standard growth curve for Streptococcus salivarius to determine the average amount of thiamine in each variety of rice prepared by each of 2 methods of cooking. The last column of Table 4 shows the thiamine in milligamma per gram of rice sample. The results obtained from the reading of the rice assay tubes were for one-half gram of rice since the aliquot was one-fourth of a 2 gram sample. Comparisons are easier to make when values of thiamine per gram are presented. Therefore, the thiamine values obtained from the reading of the assay tubes, and presented in Table 4, were doubled.

For the 3 varieties of rice considered, the thiamine content in milligamma per gram, presented in Table 4, was used to interpret the findings of this study.

When cooked in a large amount of distilled water the parboiled brown rice contained one-third more thiamine than was present in the milled white rice. Also, when cooked in a small amount of water, there was 5 times as much thiamine in parboiled brown than was found to be present in the milled white rice.

In respect to the unparboiled brown rice cooked in a large amount of water the thiamine content exceeded slightly the amount of thiamine present in the parboiled brown rice. This is contrary to findings of other investigators. When unparboiled brown rice is compared with parboiled brown rice cooked in a small amount of water the thiamine content of the

Table 4. Thiamine Content of Cooked Rice Samples as Calculated from McFarland's Nepholometer Standards Compared with Thiamine Growth Curve for S. salivarius

| Cooked sample | Average McFarland's Nepholometer Standard Readings | | | | Thiamine in ug per gram |
|---|---|---|---|---|---|
| | First Cooking | Second Cooking | Third Cooking | Average | |
| Large am't. water | | | | | |
| Parboiled | 2.3 | 1.6 | 4.0 | 2.6 | 0.30 |
| Unparboiled | 2.0 | 3.0 | 3.0 | 2.7 | 0.32 |
| Milled | 1.0 | 3.0 | 2.0 | 2.0 | 0.20 |
| Small am't. water | | | | | |
| Parboiled | 3.0 | 5.0 | 4.0 | 4.0 | 0.80 |
| Unparboiled | 1.3 | 4.0 | 3.3 | 2.9 | 0.36 |
| Milled | 0.7 | 2.3 | 2.3 | 1.8 | 0.16 |

unparboiled rice was 2¼ times less than the thiamine content of the parboiled rice.

When parboiled rice cooked in a large amount of water is compared with parboiled rice cooked in a small amount of water the thiamine content was found to be slightly more than 2½ times greater in the rice cooked in the small amount of water.

The thiamine content of the milled white rice cooked in a large amount of water was approximately one-fifth greater than the thiamine content of the milled white rice cooked in a small amount of water. The investigator believes that this assay should be repeated as it is contrary to findings of other researchers. The very small amount of thiamine present in milled white rice is difficult to assay and is more likely to be influenced by error than large amounts.

# CHAPTER V

## SUMMARY AND CONCLUSION

Rice, when processed and cooked by different methods shows a variability in its thiamine content. This study is concerned with the effect of three processing procedures and two methods of cooking on one variety of long-grain rice similar to that produced in East Pakistan.

Milled white rice and rice paddy of the same variety were obtained for use in this experimental work. The author processed rice paddy by two methods:

1. Soaking for 48 hours and parboiling for 15 minutes at temperature, below boiling, then drying and removing the outer husk.
2. Soaking for 48 hours, drying and then removing the outer husk.

The three varieties of rice (milled white, parboiled brown, and soaked unparboiled brown) were cooked by two methods, taking care to use the same amount of rice, number of rinsings before cooking, amount of distilled water added during cooking, and length of cooking time. The two cooking methods used were: (1) cooking in small amount of water sufficient to tender the grain, and (2) cooking in a large amount of water and discarding excess water after grain is tender.

Two microorganisms which require thiamine for growth were chosen for use in assaying thiamine content of rice samples. The microorganisms chosen for the assays were S. salivarius No. 9222 and L. fermenti No. 9338.

In order to determine the amount of thiamine necessary for various

degrees of growth an attempt was made to make a standard growth curve for each microorganism to be used. The L. fermenti failed to produce growth in the basal media used. This micro-organism was rejected and only the S. salivarius No. 9222 was chosen as the assay microorganism.

Concentrations of thiamine in the standard tubes ranged from 0 to 3.0 milligamma per 10 cubic centimeters. Turbidity produced by the growth of the microorganism, when the quantity of thiamine in the basal medium is known, was used as an index against which to measure the amount of thiamine in the rice assay tubes.

Two gram samples of cooked rice were chosen to assay as the estimated amount of thiamine in this size sample could be measured adequately. Three samples from each kind of processed rice, cooked by each of 2 methods were prepared for assay by one microorganism (36 sample tubes plus one blank). Each assay was repeated three times.

McFarland's Nepholometer Standards were used to measure the degree of turbidity present in the tubes for the standard growth curve of thiamine. Rice sample assay tubes were also matched with McFarland's Standards, and the amount of thiamine present in a given sample was calculated from this data.

In summary the findings of this study were:

1. The parboiled brown rice, when cooked in a large amount of distilled water, contained one-third more thiamine than was present in the milled white rice. When cooked in a small amount of distilled water, there was 5 times as much thiamine in parboiled brown rice as was found to be present in the milled white rice.
2. In respect to the unparboiled brown rice cooked in a large amount of water, the thiamine content exceeded slightly the amount of thiamine present in the parboiled brown rice. When

unparboiled brown rice was compared with parboiled brown rice cooked in a small amount of water, the thiamine content of the unparboiled rice was 2¼ times less than the thiamine content of the parboiled rice.

3. When parboiled rice cooked in a large amount of water was compared with that cooked in small amount of water, the thiamine content was found to be slightly more than 2½ times greater in the rice cooked in the small amount of water.
4. The thiamine content of the milled white rice cooked in a large amount of water was approximately one-fifth greater than the thiamine content of the milled white rice cooked in a small amount of water.

In conclusion, the author recommends that two parts of this study be repeated since the findings are contrary to those of other investigators. These are: (1) comparison of the thiamine content of unparboiled brown rice cooked in a large amount of water with parboiled brown rice cooked by the same methods. Parboiling usually increases the thiamine content of rice over that of unparboiled regardless of manner of cooking. (2) Milled white rice cooked in a small amount of water usually retains a higher content of thiamine than milled white rice cooked in a large amount of water. When assaying very small quantities of thiamine, as was done in this study, even a very small error can produce large differences in the results.

Upon her return to East Pakistan the author wishes to continue her assays of rice in order to determine:

1. Varieties which are rich in thiamine,
2. Methods of processing rice which conserve its thiamine content, and

3. Effect of methods of cooking upon retention of thiamine in rice.

These findings will be important to rice growers, consumers, and housewives.

This experimental study has helped the author to appreciate the following aspects of research: (1) experimental work is time consuming and tedious, (2) research procedures require great accuracy, (3) the simplified method of assay using microorganisms is not as costly as chemical assay which may require a large variety of expensive equipment, and (4) a researcher is obligated to report his exact findings and to interpret them as accurately as possible, regardless of results.

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# A P P E N D I C E S

Appendix A

September 19, 1962

Director of Rice Research
Southern Regional Laboratory U.S.D.A.
New Orleans, Louisiana

Dear Sir:

I am a graduate student in the Department of Food, Nutrition and Institution Administration of the Oklahoma State University, Stillwater, Oklahoma. I am from East Pakistan.

Now I am working on my thesis. I shall do research work with rice. The topic of my thesis I have chosen as "The effect of processing and cooking procedures on the thiamine content of rice".

For that purpose I need two or more varieties of rice as nearly like those grown in Pakistan as possible.

We want to parboil the paddy as the village people do in Pakistan. Then assay the comparative thiamine content of the parboiled and the white rice of the same variety. The amount and varieties needed are as follows:

Threshed but unmilled rice "paddy", 40 lbs.
The same varieties as obtained in paddy form after it is commercially milled (white rice), 25 lbs.

Do you know of a source of rice from which the paddy and milled white rice of the same varieties can be obtained? Any information relative to varieties available in the United States will be appreciated.

Sincerely,

Siddiqua Khatun
Graduate Student

Helen F. Barbour
Head, Dept. of FNIA

HFB:bdw

October 15, 1962

Mr. Marinus C. Kik, Ph.D.
Department of Agricultural Chemistry
University of Arkansas
Fayetteville, Arkansas

Dear Mr. Kik:

I am a graduate student in the Department of Food, Nutrition and Institution Administration of the Oklahoma State University, Stillwater, Oklahoma. I am from East Pakistan.

Now I am working on my thesis. I shall do research work with rice. The topic of my thesis I have chosen as "The effect of processing and cooking procedures on the thiamine content of rice".

For that purpose I need two or more varieties of rice as nearly like those grown in Pakistan as possible.

We want to parboil the paddy as the village people do in Pakistan. Then assay the comparative thiamine content of the parboiled and the white milled rice of the same variety. The amount and varieties needed are as follows:

Threshed but unmilled rice "paddy", 40 lbs.
The same varieties as obtained in paddy form after it is commercially milled (white rice), 25 lbs.

Do you know of a source of rice from which the paddy and milled white rice of the same varieties can be obtained? Any information relative to varieties available in the United States will be appreciated.

Sincerely,

Siddiqua Khatun
Graduate Student

Mary E. Leidigh
Acting Head, Dept. of FNIA

HFB:bw

UNIVERSITY OF ARKANSAS
COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
FAYETTEVILLE

October 18, 1962

Department of
Agricultural Chemistry

Miss Siddiqua Khatun
Graduate Student
Dept. of Food, Nutrition and Institution Administration
Oklahoma State University
Stillwater, Oklahoma

Dear Miss Khatun:

This is to acknowledge receipt of your letter dated Oct. 15. You should be able to obtain what you need to have by writing to

Mr. L. C. Carter
General Manager
Arkansas Rice Growers Association
Stuttgart, Arkansas.

State clearly to Mr. Carter that you would like to have 40 lbs of paddy and 25 lbs of milled white rice - from the same lot and same variety for your experiments. I am sure he will be glad to ship them to you.

The best thing is that Dr. Mary E. Leidigh - head of your department writes the request with you. And you can refer to my name as a recommendation.

The best information about rice varieties in U.S.A. can be obtained from Professor H. M. Beachell, Rice Breeder, Rice Experiment Station, Beaumont, Texas. Tell him that I gave you his address and suggested to write him. He is one of the authorities in USA.

I will send you one of my old reprints of your subject; in these days I used Converted Rice as my parboiled rice.

I wish you good luck in your experiments and wish your work.

Sincerely yours,

M. C. Kik, Professor

P.S. Let me know whether you receive your rice?

October 31, 1963

Mr. Marinus C. Kik
Department of Agricultural Chemistry
University of Arkansas
Fayetteville, Arkansas

Dear Mr. Kik:

Thank you very much for the reply of my letter dated October 15. I have received the two publications of your research on rice which are really helpful for my work. In the Journals of The American Dietetic Association and Cereal Chemistry I found your other researches on rice. These are also very good guides to proceed with the work.

I have written to Mr. L. C. Carter for rice and decided to write to Professor H. M. Beachell for the information about the varieties after receiving the rice. I am grateful to you for your kind direction and necessary information.

Thanking you for your co-operation.

Sincerely yours,

Siddiqua Khatun
Graduate Student

Mary E. Leidigh, Acting Head

MEL:bdw

cc: Dr. Barbour

October 31, 1962

Mr. L. C. Carter
General Manager
Arkansas Rice Grower's Association
Stuttgart, Arkansas

Dear Mr. Carter:

I got your address from Mr. Kik in the Department of Agricultural Chemistry, Fayetteville, Arkansas. He informed me to write you to get rice for my research study.

I am a graduate student in the Department of Food, Nutrition and Institution Administration of the Oklahoma State University, Stillwater, Oklahoma. I am from East Pakistan.

Now I am working on my thesis. I shall do research work with rice. The topic of my thesis I have chosen as "The effect of processing and cooking procedures on the thiamine content of rice".

For this purpose I need two or more varieties of rice as nearly like those grown in East Pakistan as possible. We want to parboil the paddy as the village people do in Pakistan. So we would like to purchase 40 pounds of paddy and 25 pounds of milled white rice from the same lot and same variety.

Any information relative to varieties available in the United States will be appreciated.

Thanking you for your co-operation.

Sincerely yours,

Siddiqua Khatun

Mary E. Leidigh, Acting Head

SK:bw

cc: Dr. Barbour

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
1100 Robert E. Lee Boulevard
NEW ORLEANS 19, LOUISIANA

REPLY TO:
P. O. Box 19687
New Orleans 19, La.

November 5, 1962

Miss Siddiqua Khatun
Department of Food, Nutrition
and Institution Administration
Oklahoma State University
Stillwater, Oklahoma

Dear Miss Khatun:

Thank you for your letter of September 19. We were most interested in reading it and learning of the research you are undertaking as your thesis requirement.

A survey of the rices from various countries has recently been completed and some Pakistanian rice samples were included in this survey. There are considerable variations however between these samples and those obtained commercially, so it is difficult to ascertain exactly the type of rice youwill need.

We would recommend that you contact Dr. L. E. Crane, Rice Pasture Experiment Station, Beaumont, Texas, or Dr. H. R. Caffey, Rice Experiment Station, Crowley, Louisiana, describing the characteristics of the rice with which you would like to work. They may be able to help you secure these.

Sincerely yours,

E. L. Patton
Assistant Director

THE ARKANSAS RICE GROWERS COOPERATIVE ASSOCIATION
STUTTGART, ARKANSAS

November 7, 1962

Oklahoma State University
Department of Food, Nutrition &
Institution Administration
Stillwater, Oklahoma

Attention: Siddiqua Khatun
c/o Mary E. Leidigh, Acting Head

Gentlemen:

We acknowledge your letter of October 31 addressed to Mr. L. C. Carter, and note that you are from East Pakistan, and are doing work on a thesis about Rice.

We are not familiar with the type of varieties grown in East Pakistan. It is our belief that they are long grain. In Arkansas this year we have the following varieties available:

Blue Bonnett, which is our best long grain variety
Century Patna, This is also a long grain variety, but not as long as Blue Bonnett.
Blue Rose, a large medium grain variety.
Nato, a medium grain variety.
Pearl, a round grain variety.

If you can tell from the above which variety you desire in your work, please advice us, and we will ship about 40 lbs. of the "paddy" and 25 lbs of the milled rice from the same lot, as you have requested.

Yours truly,

THE ARKANSAS RICE GROWERS COOPERATIVE ASSOCIATION

W. L. Knoll
Assistant Sales Manager

WLK:fr

November 20, 1962

Mr. S. A. Qadir
Assistant Chief
Planning Department
Agricultural Sector
Eden Buildings, Dacca

Dear Sir:

I am a lecturer of Mathematics, Eden Girl's College, Dacca, now studying in the Oklahoma State University for the fulfillment of the requirements for a Master's Degree in Food, Nutrition and Institution Administration. I shall submit a thesis.

The subject of my thesis is "The effect of processing and cooking procedure on the thiamine content of rice".

For the research purposes I would like to have some information from you.

1. What are the names and qualities of the varieties of rice commonly grown in East Pakistan?
2. Which parts of East Pakistan grow these varieties?
3. Is the milled rice in the market parboiled ( সিদ্ধ )?
4. Is both parboiled and unparboiled milled rice consumed by the people?
5. What are the percentages of milled and brown husked rice consumed by the people of East Pakistan?

If you have any, could you please send me reprints of papers on qualities of rice and on milling and home pounding methods of husking rice paddy?

Any information relative to rice processing methods in East Pakistan will be appreciated.

Thanking you for your cooperation.

Sincerely,

Siddiqua Khatun
Graduate Student

Helen F. Barbour
Head, FNIA Department

HFB:bdw

November 20, 1962

Director
East Pakistan Council of
Scientific and Industrial Research
Dhanmondi, Dacca

Dear Sir:

I am a lecturer of Mathematics, Eden Girl's College, Dacca, now studying in the Oklahoma State University for the fulfillment of the requirements for a Master's Degree in Food, Nutrition and Institution Administration. I shall submit a thesis.

The subject of my thesis is "The effect of processing and cooking procedure on the thiamine content of rice".

For the research purposes I would like some information from you.

1. What are the names and qualities of the varieties of rice commonly grown in East Pakistan?
2. Which parts of East Pakistan grow those varieties?
3. Is the milled rice in the market parboiled ( সিদ্ধ )?
4. Is both parboiled and unparboiled milled rice consumed by the People?
5. What are the percentages of milled and brown husked rice consumed by the people of East Pakistan?

If you have any, could you please send me reprints of papers on qualities of rice and on milling and home pounding methods of husking rice paddy?

Any information relative to rice processing methods in East Pakistan will be appreciated.

Thanking you for your cooperation.

Sincerely,

Siddiqua Khatun
Graduate Student

Helen F. Barbour
Head, FNIA Department

HFB:bdw

November 26, 1962

Mr. W. L. Knoll
Assistant Sales Manager
The Arkansas Rice Growers Cooperative Association
Stuttgart, Arkansas

Dear Sir:

Thank you for your letter dated November 7, 1962.

I am interested in buying a medium grain variety of rice. With this letter I have sent the sample of rice for your information as I need the same size of rice grain. Could you please ship 40 pounds of paddy, in a variety of rice which has a rice grain neareast the size enclosed. I also need 20 pounds of milled white rice, unparboiled. Do you have a milled parboiled rice (converted) from the same lot as the paddy and the white unparboiled milled rice? If so, I would like to have 20 pounds of this rice also.

Please ship these quantities of rice to the Food, Nutrition and Institution Administration Department, Home Economics West Building, Oklahoma State University, Stillwater, Oklahoma. Please bill the rice to Miss Siddiqua Khatun, c/o Food, Nutrition and Institution Administration Department, Oklahoma State University, Stillwater, Oklahoma.

Thank you for your assistance.

Sincerely yours,

Siddiqua Khatun
Graduate Student

Helen F. Barbour
Head, FNIA Department

HFB:bdw

EAST REGIONAL LABORATORIES
PAKISTAN COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Dated, the 3rd Dec. 1962

Dear Madams,

Your letter dated Nov. 29, 1962. Herewith I am enclosing the answers to the questions you communicated in the above reference. I hope the answers will serve your purpose.

Thanking you.

Encl: - 4 sheets

Yours sincerely,

(S. Hedayetullah)

Siddiqua Khatun
Graduate Student

Helen F. Barbour
Head, F.N.I.A. Dept. Head of the Research Divn.
Department of Food, Nutrition
and Institution Administration
Oklahoma State University
Stillwater

Dated December 5, 1962

From: Dr. H. Zaman Shah
Botanist, Govt. of East Pakistan
E. P. H. A. S.
Agric. Research Institute, Dacca-5

To : Miss Siddiqua Khatun
Graduate Student
C/O. Dr. Helen F. Barbour
Head, FNIA Dept.
Oklahoma State University
Stillwater, Oklahoma, U.S.A.

Dear Miss Khatun:

Your letter of November 20, 1962, addressed to Mr. S. A. Qadir, Asst. Chief Planning Dept. Agric. Sector, Govt. of East Pakistan was forwarded to me a few days back.

I feel very much happy that you are working on the nutritional aspects of rice. In the attached sheet, you will find some of the relevant facts that might be helpful to you.

From the title of your thesis, I believe that a more or less similar work was done by Dr. Kik of University of Arkansas, Fayetteville. In fact, Dr. Kik did a voluminous work on nutritional aspects of rice. I was in Arkansas during the year 1955-56 and got acquainted with his work. So you may write to the Publication Dept. of University of Arkansas for copies of Dr. Kik's work or you may personally go there.

For about two years, I was associated with Rice Research in Beaumont Rice Pasture Expt. Station and Dr. G.V. Helick was conducting experiments on similar lines. It will be good idea to get literature from him. I also suggest that if you find time, kindly visit the station also. They have wonderful arrangement for visiting Scientiest.

By any means, I am not a nutrition chemist but a Rice Breeder. However in the attached pages some references are supplied which may be of use to you. I hope, you will do a valuable piece of work.

May the Rahmat of Allah abide with you and crown you with success.

Please feel free to write to me if there is anything that I can do for you.

Sincerely yours,

(H. Zaman Shah)
Botanist,
Govt. of East Pakistan, Dacca-5

THE ARKANSAS RICE GROWERS COOPERATIVE ASSOCIATION

STUTTGART, ARKANSAS

December 5, 1962

Oklahoma State University
Stillwater, Oklahoma

Attention: Graduate Student Siddiqua Khatun
c/o Helen F. Barbour, Head FNIA Dept.

Gentlemen:

We acknowledge your letter of November 26, and wish to advise you that we forwarded to you the samples requested, on December 3.

Yours truly,

THE ARKANSAS RICE GROWERS COOPERATIVE ASSOCIATION

W. L. Knoll
Assistant Sales Manager

WLK:fr

January 2, 1963

Mr. S. Hedayetullah
Pakistan Council of Scientific Research
East Regional Laboratories
Dacca, East Pakistan

Dear Sir:

Thank you very much for your kind reply to my letter and the answers to my questions. The findings of your answers about the varieties of rice grown in East Bengal in different seasons will help me to write the qualities of rice consumed in East Pakistan.

Thank you for your cooperation.

Sincerely,

Siddiqua Khatun

Dr. Helen F. Barbour
Head, FNIA Department

HFB:bdw

January 2, 1963

Mr. W. L. Knoll
The Arkansas Rice Growers
Cooperative Association
Stuttgart, Arkansas

Dear Mr. Knoll:

I received 40 pounds BB Rough Rice and 20 pounds BB Milled Rice. The invoice No. 4226, dated 3rd December, 1962 listed total price of rice as $8.42. I am herewith sending a check for that amount.

Thank you.

Sincerely yours,

Siddiqua Khatun
Graduate Student

SD:BDW

Enc.

March 19, 1963

Dr. H. Zaman Shah
Botanist, Government of East Pakistan
E.P.H.A.S.
Agriculture Research Institute
Dacca - 5

Dear Mr. Shah:

I would like to thank you for the valuable literature on rice and the references on the related field, that you sent me.

I received your letter with the answer sheets attached on second week of this month. Though it is late for me to include some more about rice of East Pakistan, I shall try to add your materials if I have time. Because I got the appropriate answers from you which I needed.

I write Mr. Kik at the very beginning. He was so kind to send me his published materials. He also suggested to me the name of Mr. Halick to make correspondence. I got my rice paddy and milled white rice from Stuttgart, Arkansas.

This is the finishing stage of my thesis. Now I am ready to collect data from my research work. The thesis is due on 7 May, 1962, and lots of work is left yet to be finished.

Thank you very much for your good wish and co-operation.

Sincerely yours,

Siddiqua Khatun
(graduate student)
Department of Food, Nutrition
and Institution Administration

SK:w

## Appendix B

### Extraction of Thiamine from Rice (21, p. 372)

Thiamine occurs naturally in the free form and as the pyrophosphate, cocarboxylase. The monophosphate, derived from cocarboxylase by hydrolytic removal of one phosphate residue, probably also occurs in many samples. All of these forms may also occur bound more or less firmly to protein. For complete extraction of thiamine, hydrolysis is therefore required. The vitamin is very easily destroyed by heating in alkaline or neutral solution; but is quite stable in strongly acid solutions.

To release the bound thiamine a convenient and accurately known weight of the finely ground or homogenized sample (2 grams in case of the three varieties of cooked rice) is suspended or dissolved in at least 15 times its weight of 0.1 N $H_2SO_4$. The mixture is digested on a steam bath with frequent mixing for 30 minutes. The liquid must remain distinctly acid during the digestion: if the pH rises above 1.5 additional dilute $H_2SO_4$ is added. After heating, the mixture is cooled, and the pH adjusted to between 4 and 4.5 by addition of a concentrated solution (2N or greater) of sodium acetate. This is the optimum pH for digestion with phosphorolytic enzymes. A 10 per cent solution of takadiastase is prepared in distilled water. For each 10 gamma of thiamine estimated to be present in the sample used, 1 cubic centimeter of this solution is added. The mixture is incubated at 45-50 degrees Centigrade for 3 hours, or overnight at 37 degrees Centigrade. It is then adjusted to pH 6.5 to 6.6, diluted to an estimated thiamine content of 0.1 to 0.2 gamma per cubic centimeter, filtered if cloudy, and suitable aliquots used for assay.

Preparation of McFarland's Nepholometer Standards (39, p. 583)

(a) Arrange 10 test tubes or ampules of uniform size in a rack, and label 1 to 10.

(b) Add the following amounts of a 1 per cent acqueous solution of chemically pure barium chloride: To Tube No. 1, 0.1 ml.; tube No. 2, 0.2 ml.; and so, increasing 0.1 ml. in each.

(c) Add sufficient 1 per cent chemically pure sulfuric acid solution to make the total vol. 10 ml. in each tube.

(d) Seal the tube in a flame.

(e) When the fine white precipitate of barium sulfate which has formed in the tubes is thoroughly shaken, each tube will have a different density, increasing from No. 1 to 10. The density of the tubes corresponds approximately to bacterial suspensions as follows:

No. 1: 300,000,000

No. 2: 600,000,000

No. 3: 900,000,000

No. 4: 1,200,000,000

No. 5: 1,500,000,000

No. 6: 1,800,000,000

No. 7: 2,100,000,000

No. 8: 2,400,000,000

No. 9: 2,700,000,000

No. 10: 3,000,000,000

If assays are carried out in colored media the Nepholometer Standards should be prepared with 1 per cent sulfuric acid in media in order to convey the color of the latter.

## Inoculum Medium and Inoculum for Rice Assays

### Streptococcus salivarius (21, p. 374)

Inoculum medium - is made up of 5 cc. of basal medium plus 4 cc. of distilled water. Sterilize at 15 pounds for 15 minutes, cool, then add 1 cc. of a sterile solution containing 10 micro-gamma (.01 gamma) of thiamine hydrochloride per cc. Several tubes of medium may be made up at once but care should be taken to steam the medium for several minutes before use to insure prompt growth.

Inoculum - grown 24 hours (or until "good" growth occurs) at 37° C. Five-tenths cc. of the resulting suspension is mixed with 10 cc. of sterile 0.9% NaCl solution, and one drop of this diluted suspension used per assay tube.

### Lactobacillus fermenti (21, p. 376)

Inoculum medium - Five cc. of basal medium, 10 gamma thiamine, 5.0 mg. Difco yeast extract, with distilled water to 10 cc. Sterilized in pressure cooker at 15 pounds pressure for 15 minutes.

Inoculum - Incubated 16-24 hours, but no longer, at 37° C. Cells are centrifuged out, resuspended in 10 cc. of sterile saline, and 1 drop of this suspension further diluted with 25 cc. of sterile saline. One drop of this dilute suspension is used to inoculate each assay tube.

## Concentration of Thiamine for Standard Curves

For Streptococcus salivarius (21, p. 375)

Concentrations of thiamine for standard curve are: 0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0 milligamma per 10 cc. Samples are added to furnish thiamine at several levels within this range. Since thiamine is destroyed by autoclaving at the initial pH of the medium, sterile solutions of both standard and samples are added aseptically after the sample tubes have been autoclaved.

A sterile standard solution of thiamine hydrochloride (100 gamma/cc) is prepared by dissolving 10 mg. of the vitamin in 100 cc. of 0.1 M. acetate buffer, pH 4.5, and autoclaving for 15 minutes at 15 pounds pressure. Aliquots are diluted appropriately with sterile distilled water just before use. The sample has to be sterilized in a similar fashion before addition to the sterilized assay tubes.

In preparing the sample tubes before autoclaving, distilled water is added in volume such that addition of the sterile standard and sample after autoclaving will make the total volume to 10 cc.

Sterilization - 15 minutes at 15 pounds pressure.

Incubation time and temperature - 37° C. for 24 hours.

Measurement of response - Turbidimetric most satisfactory, although good results are reported from titration with 0.05 N NaOH.

Comment - The thiazole and pyrimidine moieties of thiamine are inactive for the test organism, both alone and in combination, at levels up to 10 gamma per cc. of medium. Cocarboxylase is approcimately 40% more active than thiamine on a molar basis, the monophosphate of thiamine is

also probably more active than thiamine itself. This makes complete hydrolysis of the cocarboxylase of natural materials to thiamine obligatory before accurate value can be obtained.

For Lactobacillus fermenti (21, p. 376)

Concentrations of thiamine for standard curve are: 0, 5, 10, 15, 20, 30, 40 and 50 milligamma thiamine hydrochloride per 10 cc. of diluted medium. Samples are usually run at four different levels ranging from 5 to 50 milligamma of thiamine per assay tube (10 cc.).

Sterilization - Plugged tubes containing standard and samples are heated in flowing steam (100° C.) for 15 min. at the pH of the medium (6.5). This procedure avoids destruction of thiamine and is adequate to avoid contamination during the short assay period used.

Incubation time and temperature - 16-18 hr. at 37° C.

Measurement of response - Turbidimetric.

The method is a recent one, and has not yet been subjected to the test of widespread use. When carried out as originally described, the test organism sometimes develops the ability to synthesize thiamine at a low rate, which is reflected in standard curves with excessively high blank values.

## Appendix C

### Preparation of Acid Hydrolyzed Casein (21, p. 364)

Where media of known salt content are desired, $H_2SO_4$ - hydrolysed casein is used. It is prepared as follows: 100 g. of purified casein are mixed with 500 cc. of 25% (by volume) $H_2SO_4$. The mixture is autoclaved for 10 hours at 15 lbs. pressure. The solution is diluted to capacity of 2 liters, and a hot concentrated solution of $Ba(OH)_2$ added until the mixture is almost neutral. The $BaSO_4$ is allowed to settle, the supernatant solution siphoned off, and the sludge filtered dry with suction and washed. Use of generous quantities of a filter aid (e.g., Super-Cel) is helpful. All liquid fractions are combined, and barium ion exactly removed with dilute $H_2SO_4$. $BaSO_4$ is removed by filtration, and the filtrate concentrated under reduced pressure to 1000 cc. Charcoal treatment, as in the procedure above, is usually necessary to remove the last traces of nicotinic acid.

Preparation of Thiamine-free Yeast Extract (21, p. 373)

Six g. of Difco Bacto-yeast extract are dissolved in 200 cc. of water and autoclaved (15 lb) for 15 min. The pH is adjusted to 3.0, 10 g. of fuller's earth added, and the mixture shaken for 30 min. The filtrate is adjusted to pH 1.0, reautoclaved for 15 min., cooled, a second 10 g. portion of fuller's earth added, and the mixture shaken over night. After filtering, 1.5 g. of $K_2HPO_4$ are added to the filtrate, which is then adjusted to pH 7.4 and autoclaved again for 15 min. The resulting precipitate is filtered out, and the volume adjusted to 200 cc. This solution is sterilized in conveniently sized lots, and stored until used.

Preparation of Salt Solution (21, p. 374)

The stock solution of salts contains 10 g. of NaCl, 0.8 g. $MgSO_4$ $7H_2O$, 40 mg. Fe $SO_4.7H_2O$, and 12 mg. $MnCl_2$ in 100 cc. of distilled water.

Vitamin Dilutions in the Media for Streptococcus salivarius

Dilution for nicotinic acid, riboflavin, calcium pantothenate, 100 gamma each.

Weigh out 1 mg. of nicotinic acid, riboflavin and calcium pantothenate. Add 10 cc. of distilled water to each. Use 1 cc. for 100 gamma quantity.

For biotin, weigh 1 mg., and dilute with 10 cc. of distilled water. Take 0.1 cc. and add 9.9 cc. of distilled water. The total volume of 10 cc. contains 1 gamma per cc. Use 0.1 cc. for 0.1 gamma quantity.

## Vitamin Dilutions for Lactobacillus fermenti Media

Weigh 1 mg. each of riboflavin, calcium pantothenate, p-amino-benzoic acid, nicotinic acid, pyrodoxine hydrochloride. Add each of them to 10 cc. of distilled water. Use 0.2 cc. from each portion.

For 0.08 gamma of biotin, add 1 mg. of biotin to 10 cc. of distilled water. Again add 9.9 cc. of water to 0.1 cc. of the first dilution. Dilute a third time taking 0.1 cc. from the second dilution, and add 9.9 cc. of water. The third dilution has 0.01 gamma per cc. Use 8 cc. of this dilution.

To obtain 0.015 gamma of folic acid, dilute 1 mg. of the vitamin with 10 cc. of distilled water. Add 9.9 cc. of water to 0.1 cc. of this dilution. Again take 0.1 cc. from the second dilution and add 9.9 cc. of water. Use 1.5 cc. from the last solution.

## Preparation of Alkali-treated Peptone (Plus Sodium Acetate) (21, p. 345)

Bacto-Peptone (Difco Laboratories, Detroit, Michigan) is suitable for use.

To 40 g. of peptone dissolved in 250 cc. of water is added 20 g. of NaOH also dissolved in 250 cc. of water. The solution is placed in the light in a crystallizing dish or plain glass bottle and allowed to stand 24 hr.

Glacial acetic acid is then added to a pH of 6.6-6.8 (27-29 cc. are usually required), 7 g. of anhydrous sodium acetate are added, and the solution diluted with water to 800 cc. This stock solution contains 50% of treated peptone and 6% sodium acetate. It may be kept indefinitely under toluene in a refrigerator.

## Composition of Salts A and B (21, p. 362)

Salts A contain 100 mg. $K_2HPO_4$ and 100 mg. $KH_2PO_4$ per cc.

Salts B contain 40 mg. $MgSO_4 \cdot 7H_2O$, 2.0 mg. NaCl, 2.0 mg. $FeSO_4 \cdot 7H_2O$, and 2.0 mg. $MnSO_4 \cdot 4\ H_2O$ per cc.

## Composition of Percentage, Molar and Normal Solutions

1. A percentage solution is a solution in which the percentage of solute is substracted from 100 and the remaining portion is the amount of water to be used as the solvent to make a total of 100 cc. of solution.

   Examples:

   a. 10% takadiastase solution

      Weigh 10 grams takadiastase and add 90 grams of distilled water to it.

   b. 25% $H_2SO_4$ solution

      Measure 75 cc. distilled water. Measure 25 cc. concentrated $H_2SO_4$. Caution - slowly add concentrated $H_2SO_4$ to water. Swirl container after each addition. Much heat will be generated as slow mixing is required to prevent solution from boiling. Never add water to concentrated $H_2SO_4$ but add $H_2SO_4$ to water.

   c. 0.9% NaCl solution

      Weigh 0.9 gram sodium chloride and add this to 99.1 grams distilled water.

2. A molar solution (M) contains one gram molecular weight of solute per liter of solution.

   Example:

   a. 0.1 M sodium acetate solution

   Calculate molecular weight of $CH_3COONa$:

| | |
|---|---|
| 2 C | = 80.160 |
| 3 H | = 3.024 |
| 2 O | = 32.000 |
| 1 Na | = 23.000 |
| Molecular Weight | =138.184 |

To make 0.1 M solution use $\frac{138.184}{10}$ = 13.82 grams. Weigh out 13.82 grams $CH_3COONa$, then add 1000 cc. distilled water. Stir until all crystalls are dissolved.

3. A normal solution (N) contains one gram equivalent weight, or the amount which reacts with one gram atom of replaceable hydrogen,

a. 0.1 N NaOH

Calculate molecular weight of NaOH: 1 Na = 23.00
1 O = 16.00
1 H = 1.008

Molecular Weight = 40.008 gms.

This chemical has one hydrogen equivalent in the OH part of the molecule. Therefore use 4.008 grams solid NaOH to 1000 cc distilled water to make 0.1 N NaOH.

b. 0.1 N $H_2SO_4$

Calculate molecular weight of $H_2SO_4$:

2 H = 2.016
1 S = 32.06
4 O = 64.00

Molecular Weight = 98.22

$H_2SO_4$ has 2 replaceable hydrogen equivalents so use $\frac{1}{2}$ the molecular weight for 1 N solutions or 0.1 of this for 0.1 N solution. Therefore weigh $\frac{98.22}{2}$ = 49.11 ÷ 10 = 4.911 grams per 1000 cc distilled water for 0.1 N $H_2SO_4$.

c. 3 N sodium acetate solution

Calculate molecular weight of $CH_3COONa$:

2 C = 24.020

3 H = 3.024

2 O = 32.000

1 Na = 23.000

Molecular Weight = 82.044 grams

Sodium acetate has one hydrogen equivalent as indicated by presence of one-OH group in which hydrogen has been replaced by sodium. Therefore use 82.044 x 3 = 246.13 grams sodium acetate per 1000 cc. distilled water for 3 N $CH_3COONa$ solution.

VITA

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Master of Science

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Personal Experience: In February 1957 she joined the Dacca Eden Girls' College as a Lecturer in Mathematics in the East Pakistan Junior Educational Service, and served there until her selection in Institutional Management at the Oklahoma State University in The United States of America. On completion of her training in this university she will be required to join, as a professor, the newly established College of Home Economics in Dacca.

Outside the academic field she has been continually connected with Radio Pakistan in program announcements since 1955, while still a student in the University.

In Pakistan she worked in several socio-economic and educational research projects of which mention may be made of the following projects connected (1-3) under the leadership of Professor A.F.A. Husain, one of the topmost social scientists of Pakistan: (1) Human and Social Impact of Technological Change in Pakistan (Sponsored by UNESCO); Employment Position of Middle Class Muslim Women of East Pakistan; (3) Marketing of Jute in East Pakistan; (4) Reading Interest of Women in Bengali in East Pakistan; conducted by the Bengali Academy under the sponsorship of UNESCO.