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POSTHARVEST MICROBIOLOGICAL STUDIES ON MANITOBA WILD RICE

by

JOHN F. LOGAN

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements for the

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TO MY PARENTS

ABSTRACT

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The characteristics and effects of the microbial population on postharvest wild rice are not fully understood at the present time. The present study is an attempt to investigate this problem. Studies were conducted to determine (a) the taxonomy of bacteria and molds on wild rice, (b) what happens to microorganisms during curing, parching, hulling and cooking of wild rice, (c) the possible health hazards associated with wild rice processing, and (d) the efficiency of microbial reduction on wild rice. From this research, an attempt was made to deduce the role of microorganisms in the processing of wild rice, especially the curing step. For the bacterial taxonomy study, the Pseudomonas spp. were the most dominant bacterial genus among all other identified bacteria while for the mold taxonomy study seven different types of molds took their turns at being dominant. The microbial analyses of wild rice indicated that microorganisms have no role to play in curing while parching, hulling and cooking were effective ways to reduce the microbial load on wild rice grains. The only possible health hazard problem associated with wild rice is due to potential mycotoxin production by molds such as Fusarium spp. The microbial reduction tests were successful but did not succeed in producing a completely sterilized wild rice.

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CHAPTER 1

INTRODUCTION

Wild rice (Zizania aquatica) is an annual aquatic grass or cereal, native to North America, especially the Upper Great Lakes' region (73,81). It has continued to be a supplementary food and money crop for Manitoba Indians for hundreds of years (2). "Manitoba is the largest producer of wild rice in Canada."(4) Today, wild rice has become a prized gourmet food of North America due to its flavor and short supply (2,26,65).

"Production of wild rice prior to 1970 was exlusively from natural stands, located on shallow banks of streams and lake edges in eastern Manitoba." Since 1970 the commercial production of paddy wild rice has been slow in Manitoba because of climate and the inability to domesticate a wild rice variety for this region. Thus the potential of paddy wild rice in Manitoba south of $54^{o}N$ has still to be realized (3,4). In the U.S., commercial paddy production of wild rice started in the mid-1960's. Since then, it has increased so much that its harvest has almost completely taken over the industry with lake wild rice having a minor role (24,65). This has caused an almost complete mechanization of the industry (2,65).

Due to the growth of this food industry, it has been necessary to improve its processing techniques (16,17). For example, in 1972 in Minnesota, 3,740,000 pounds of green paddy wild rice and 1,001,000 pounds of green lake wild rice were harvested (24). The shattering

characteristic of lake wild rice and some paddy varieties of wild rice at the present time necessitate harvesting green rice which must be matured or cured before further processing (65,80). Microorganisms and enzymes are involved in this ripening step but their mechanisms of involvement are not known, especially for the microorganisms (28, 32,34,35,80,81,82). In this thesis, the research has been directed towards the examination of the types of microorganisms involved in this processing step to determine if they have a role in ripening such as contributing to flavor, as a deteriorative factor in storage, or as an environmental contaminant because changes in the microbial population of a grain are known to affect its viability, storage quality, nutritive value, and industrial usefulness (28,35). Therefore, the main objectives of this research program were:

1. To conduct a taxonomic examination of the bacteria and mold population present on wild rice.
2. To determine the population counts of microorganisms from beginning to end of the procedure for processing wild rice, especially the curing step.
3. To assess the microbiological safety of the wild rice.
4. To determine if it is possible to reduce the microbial population on wild rice.

CHAPTER 2

LITERATURE REVIEW

2.1 Description of Wild Rice

Zizania aquatica is a single species with several varieties in North America. It is a tall aquatic grass-like plant (Figure 1) and produces its seeds at the top of the plant (2,3,4,24,57,65,73,80,81). Wild rice has a nutritive value much like other cereals. It is high in protein and vitamin B while it is low in fat, and deficient in vitamin A and some minerals. It is also high in energy (73,81).

2.2 Methods of Processing Wild Rice

2.2.1 Harvesting

Wild rice is harvested just before full maturity. The traditional method of harvesting was by canoe, with one Indian paddling and the other "ricing". The harvester reached out, first on one side and then on the other, with one stick in one hand, and bended as many stalks as possible over the canoe; while with the stick in the other hand, he beat the heads and knocked off the almost mature grains into the bottom of the canoe. When one end of the canoe was full, then the Indians would change roles to fill the whole canoe. Due to the uneven ripening of wild rice, they would cover the same field over the ricing period a number of times to gather the full crop (2,26,58,65,73,80,81).

At the present time in the United States, natural stands of wild rice are still harvested by mechanical harvester which is usually some

Figure 1. The Wild Rice Plant

(Adapted from 24)

type of converted combine machine (65). In Canada, natural stands are harvested by hand by Indians, and mechanically by white men who obtain licences from the government to do this. They use some type of pontoon boat with catcher in front, or a flat bottom boat with single blade in the front (speedhead). These machines travel at 10-12 mph., striking the rice stalks with the front apparatus and the wild rice falls into a basket, or the boat. All paddy wild rice is harvested here in the same way as it is in the States (5,6,73).

2.2.2 Old methods of processing wild rice

The old processing steps for wild rice were: 1)curing, 2)parching or drying, 3) hulling, and 4) winnowing. Curing is a complex chemical, physical and biochemical process whereby the wild rice became mature and developed its characteristic flavor and black color. There were three methods for curing wild rice: (a) prolonged sun drying, (b) smoking and heating over a slow fire, and (c) parching in a vessel. The last method was the favorite one because it combined processing steps 1 and 2, and gave the best flavored wild rice. This method involved putting the wild rice in a large kettle over a fire and heating for 15 to 30 minutes with occasional stirring until the husks became dry and brittle (2,26,58,65,73,80,81).

Hulling of wild rice was accomplished by three methods: a) treading with feet, b) pounding with a pole in a lined hole, and c) flailing. Treading was the preferred method by Indians (2,26,58,65,73,80,81).

Winnowing was the removal of the husks from the grains after hulling. One method was to pour them over a blanket when a stiff wind was blowing which would take away the chaff. If there was no wind, a

birch bark fan would be used to create a breeze. Another method was the agitation of shallow trays to remove chaff like a human anti-gravity bed separator (2,26,58,65,73,80,81).

2.2.3 Modern methods of processing wild rice

The processing steps are: 1. curing or fermentation, 2. parching, 3. dehulling, and 4. cleaning and size grading. Each processor in Manitoba has his own method of curing wild rice. He cures the wild rice in piles upon some type of flat surface such as a cement floor, or plastic sheets on the ground either outdoors or indoors but the differences come in the depth of the pile, the amount of water added or not, turning of the pile, and the length of the curing period. The depth of piles vary from 30.48 to 106.68 cm (1 to 3 1/2 feet) resulting in higher pile temperature as the bed depth is increased. Some processors add water and turn the pile every day while others add water and turn the pile only when they consider it necessary. The adding of water to the pile and the turning of it are necessary to control the temperature of the pile and to prevent mold growth. But another important reason for adding water to the pile is to keep the moisture content of wild rice above 30% to prevent aflatoxin production and the occurrence of white centers in processed wild rice. The length of the curing period is dependent on the temperature of the curing pile, maturity of the wild rice and environmental conditions. With favorable environmental conditions and reasonably mature wild rice, the higher the pile temperature is, the shorter the curing period. Thus the length of the curing period can vary from a few days to several

weeks. At the present time, the end of the curing period is determined by the proper color development in the wild rice kernels (5,6,33,52,73, 80,81).

Because of these various curing conditions, research was undertaken at the Universities of Wisconsin and Manitoba to find the best curing conditions. Wisconsin indicated the best techniques were to cure wild rice under ambient (21.1°C) and cool (10°C) conditions with daily turning and watering, and pile depth of 45.72 cm (18 in.)(28,80, 81). Manitoba found the best curing treatment was a cool one with daily turning and watering, and using a pile depth of 30.48 cm (12 in.) (20).

Parching is the drying of wild rice from an initial moisture content of 40 to 50% to a final one of 8 to 11%. It also helps to develop color (darker) and flavor (toasted). In Manitoba, the processors use the principle of a revolving cylindrical drum over burners for a parching machine, some with paddles inside and others none. The only other difference is the different forms of heat energy used for the burners such as wood, gas, or electricity. Parching temperatures are in the range of 204.4°C to 232.2°C. Length of the parching period is dependent upon the moisture content of the wild rice and the parching temperature. The end of the parching period is determined by processors when individual rice kernels cannot be very readily broken between the thumb and forefingers (5,6).

Dehulling is the removal of the husks from the rice grains by a twisting motion between two surfaces. The processors use a cylindrical

drum which is stationary with revolving rubber padded paddles or flails inside. The dehulling takes place between the paddle and inside surface of the drum. This is called a barrel huller (5,6). In the United States, some processors also use a Japanese Kyowa huller which consists of two rotating rubber padded drums moving in opposite directions at different speeds. The dehulling of the kernels takes place between the two drums (80,81).

Finally, for the cleaning and grading of the wild rice, processors use either an air screening machine, or an anti-gravity machine, or both together. These machines give three fractions: whole rice, cracked rice, and hulls and debris. The whole rice is sold and the cracked rice is disposed of in some way. The hulls and debris are burned (5,6).

2.3 Storage of Wild Rice

This term is used in the industry to define that period of time when green rice is held for the purpose of maturing the kernels.

2.3.1 Curing of wild rice

As previously stated during curing, wild rice developes its characteristic flavor and color. In other words, the green wild rice kernels become dark brown to black in color, and a dominant tea like, earthy, cereal flavor develops along with minor other flavors such as nutty and sweet (28,80,81). But the factors causing these changes are not well understood.

2.3.1.1 Color development in curing wild rice

First it was believed that these changes were due to biochemical reactions or maybe microbial activity (28,80,81). The reason for the proposed involvement of microorganisms was that certain microorganisms such as Aspergillus spp., Penicillium spp. and Xanthomonas spp. did cause color changes in seeds (13,50). Several microorganisms also excreted colored compounds into its environment, eg. Pseudomonas spp. (2). On the other hand Withycombe (82) reported that the color change in curing wild rice was probably due to biochemical changes such as oxidation of polyphenols.

2.3.1.2 Flavor development in curing wild rice

Researchers have found that certain molds (Aspergillus and Penicillium) did produce flavor compounds (47). Certain bacteria such as Pseudomonas spp. excreted chemical compounds such as pyrazine which could act as flavor compounds (12). But it was still unknown whether flavor change in curing wild rice was completely a biochemical process, or if microorganisms were involved in some way (28,32,80,81, 82). Withycombe (82) has shown in his research that certain flavor compounds appeared in the curing rice and proposed it was due to some type of biochemical process while Frank (32) indirectly tried to show that bacteria were involved in flavor production. He did this by isolating, identifying and re-inoculating the bacteria in high dosage amounts back onto green wild rice at the start of the curing period. He found that Achromobacter spp., Pseudomonas fluorescens, Flavobacterium solare and Micrococcus spp. gave acceptable wild rice flavor. But overall Frank's (32)

research did not prove that bacteria were involved in flavor production of curing wild rice. The question of the involvement still remains to be determined since an exact method to accomplish this task has not yet been developed.

2.3.1.3 Hull degradation of curing wild rice

Hull degradation took place during curing which facilitated hull removal, reduced breakage and in general, made dehulling of wild rice more efficient (32, 80, 81). This hull degradation might be due to cellulolytic activity of enzymes in the kernel or maybe from microorganisms. Rapid degradation occurred at high curing temperatures and moisture levels (80, 81). Frank (23) conducted research in this area using the bacteria, Cellulomonas spp., but he was unable to prove that bacteria are involved in celluloytic breakdown of hulls of curing wild rice. Certain molds such as Trichoderma spp., Chaetomium spp. and Mortierella nand possessed cellulolytic enzymes but again there is no definite proof of their involvement in hull degradation of grains. Therefore, at the present time, the hull degradation of wild rice must be considered to be due to only cellulolytic enzymes in the kernel.

2.3.1.4 Microorganisms in respiration and heating of grains

The role of microorganisms in respiration and heating of grain in storage is thought to be an important deteriorative factor in respiration and heating of grain in storage. The first bacteria identified with the heating of hay was Bacillus colfactor. All bacteria proposed to be associated with heating of a grain were present in high moisture content material (over 30%) which enabled the bacteria to grow. Grain containing the usual moisture content between 12 to 20%

or stored in an environment of 75% relative humidity did not allow bacterial growth but only mold growth was able to develop. Thus molds were mostly responsible for a sharp increase in respiration due to increased heat production, along with a buildup in the concentration of carbon dioxide and fatty acids. Penicillium chrysogenum I and II, Aspergillus niger, Aspergillus flavus, and Mucor racemosus were examples of molds involved in this phenomenon called spontaneous heating. In studies of thermogenic activity of microflora on various moist agricultural materials, two stages of heating were found. At first it was proposed that the first heating stage, up to 50-55°C, was due to the metabolic and respiratory activities of mesophilic nonspore formers, including molds, while the second stage, up to 70°C, was due to thermophilic bacteria. But later research proved that the first heating stage was a consequence of the metabolism primarily of the molds until the thermal death range (50-55°C) was reached, and the secondary heating stage was due to nonbiological oxidation since the temperatures of the materials were above the maximum survival levels for seeds and molds (13,56,63).

2.3.1.5 Evidence of deterioration in grain

Pomeranz (63)stated the following changes to check for in the deterioration of a grain: 1. visual observations, 2. increased fungal population, 3. weight loss, 4. decrease in germinability or viability, 5. heating, 6. production of toxins, and 7. various biochemical changes, including those that resulted in mustiness, souring, high fat acidity, or bitterness. "When grain deteriorated in storage, especially when the

deterioration is caused by spontaneous heating, the grain lost its natural luster and became rather dull and lifeless in appearance." High temperatures in grain were a positive indication of deterioration. A slight rise in temperature above what is considered normal under prevailing conditions would indicate incipient deterioration (84).

Odors, such as musty or sour, occurred in grain that was well deteriorated and heated. Musty odor was due to molds while sour ones were due to a fermentation process which could be anaerobic. This situation of bad odors usually occurred in grain in the advanced stages of deterioration (63,84).

Germ damaged grain was another distinct form of grain deterioration and was identified by the brown to black discoloration of the seed's germ. This was called "sick" wheat (63,84). But other researchers considered "sick" wheat to be any discolored or "blanched" grain (13). Heat damaged grain appeared also dark red to mahogany in color (63,84). The above discussion indicated that discoloration was due to only heating of the grain but it was believed that molds were also involved due to conditions associated with fungal infection (21,22,75). On sound wheat, Alternaria was usually isolated but on germ damaged or "sick" wheat, Aspergillus glaucus and Penicillium spp. were isolated predominately along with Fusarium, Rhizopus, Mucor, Aspergillus and Helminthosporium (13). Kim's (50) research indicated a variety of molds were involved in the discoloration of rice. High moisture contents in the grain also assisted in the discoloration process (75). The only bacterium identified with the black stained discoloration of barley was called Bacterium herbicola Burri and Duggeli (21,22).

During the curing of wild rice, deterioration was noticed by visual build up of mold on the kernels, or by excessive slime build up caused by microorganisms. This resulted in decreased yields of wild rice because the kernel itself had been degraded in some way. Also odors such as moldy, very earthy, swampy, or putrid developed in deteriorating wild rice. This was probably due to poor aeration of curing wild rice resulting in an anaerobic environment in the pile, or the curing period was too prolonged, or the bad condition of the wild rice at the start of curing. Discoloration of wild rice kernels was hard to notice because of its dark color. But this dark color had been detected to lighten upon extended curing periods which could be considered as a deterioration characteristic of wild rice. The deterioration of wild rice usually took place when the curing period became too extended, or improper curing techniques were used. This resulted in very quick deterioration of curing wild rice (20,80,81).

2.4 Microbiological Studies of Wild Rice

2.4.1 The isolation of microflora

The way in which seed flora occurred determined which method would be employed for the isolation and identification of microorganisms (61). These methods would fall into the following categories:

1. Visual methods
   (a) macro
   (b) micro
2. Washing methods and the use of the centrifuge
3. Assorted histological and staining techniques
4. Soil and blotter tests and use of a moist chamber
5. Various disinfection and plating methods (61,66)

The macro-visual methods involve continuous examination of grains in storage for visual appearance of the microorganisms, or some defect in the grain. When microorganisms are detected they are isolated by aseptic techniques. The micro-visual method involves the use of a microscope to detect microbial invasion of the seed (61).

Surface contamination of seed by microorganisms could be determined by the washing method which consisted of washing the grains in a sterile liquid and plating the washings on suitable nutrient media. Centrifugation was used to concentrate the isolates before plating (61).

Histological and other staining techniques were of great value in assessing the location and amount of microflora present in seeds, but these methods provided little or no information about the identity of the microorganisms involved (61).

The blotter method was the same one used for germination tests. Microorganisms were identified as they grew from the germinating seed. This method was inadequate for bacterial determination and slow growing molds (61).

The best procedure for identification of seed-microorganisms was the agar plating method. The technique consisted of placing seeds, either surface disinfected or not, in a suitable nutrient medium and observing the growth of microorganisms on the media and on the seeds. The purpose of disinfecting the seeds before plating was to remove post harvest surface contaminants (61).

Another study indicated two disadvantages for the plating method:

1. the method was time consuming and permitted the examination of only a small seed population, and 2. incorrect population counts could be obtained because of the selective destruction of certain species (61).

The whole kernel plating method did not provide an absolute population count. Therefore, a ground up seed mixture and serial dilution plating method was proposed (54,61). This method has been criticized because: 1. the results could not be easily correlated with the whole kernel plating method used in fungal determinations; 2. the size of the ground particles would determine the number of colonies that developed on the dilution plating; 3. fungi that produced copious numbers of condia and spores would yield population figures all out of proportion to their actual presence in terms of comparative area and weight figures (61). On the other hand, Wisconsin researchers (32,34,35) indicated this to be the best method for their microbiological studies of wild rice, for several reasons. First, the wild rice was stored with their hulls on while most other grains were stored in the dehulled state. Thus the surface disinfectant method would not work as well as on dehulled grains to assist in the appearance of the true microflora on the seed (61). But the main problem was the retention of the surface disinfectant solution in the wild rice hull which would inhibit the growth of microorganisms on plating (1). Therefore, in this research, the Wisconsin method was used with minor changes in dilution ratios and blending time (34,35). Also this grinding up or blending method was the official method of A.A.C.C. for microbiological examination of grain and grain products (7).

A serious problem in the isolation of microorganisms using the

plating method was the suppression of certain organisms by the seed flora and masking of developing colonies by other fast growing micro-organisms. Therefore, the selection and modification of the plating media was necessary in order to obtain the total representation of each type of microbe on the grain. For example, an anti-bacterial medium containing streptomycin and rosebengal was useful in the isolation of soil fungi while cycloheximide (actidione) was useful in the isolation of bacteria (61). Pepper (61) in his research tested several types of media to check their influence on the microflora of barley seed and to determine which ones were best suited for the isolation of each type of microorganism.

2.4.2 The characterization of microflora

The predominant microorganism of a grain could be determined by several methods. Hoynak (43) stated that the typical colonies were picked off a plate and inoculated onto agar slants. The number of organisms isolated would correspond to the frequency of their occurrence (43). Masood (54) used a method which crushed up the seed and inoculated the pieces into peptone broth for incubation. This was then streaked on nutrient agar to separate out the bacteria until pure. It was a very limiting isolation method because only the bacteria that could grow profusely in the growth medium were isolated, preventing the appearance of slow growing bacteria, or of bacteria present in small numbers on the seed. Ostovar (60) used a method where he selected plates with 10 to 100 colonies on them for each time period of cocoa bean curing and picked off all discernible colonies exhibiting different morphological

characteristics. Graves (36) method involved selecting plates which showed a reasonably accurate picture of species distribution from the grain. Then he selected at random a plate from this lot and picked off 25 bacteria from a sector on the plate which had been delineated with a wax pencil. Frank (32) appeared to follow the above methods. He selected a plate with an uncrowded bacterial population and then picked off at random a set number of bacteria. He did this for several samples of wild rice taken at certain time intervals in the curing period.

For all these isolation techniques above, there was no definite statistical program. Each researcher determined how many microorganisms he thought necessary to identify in order to obtain the spectrum of microorganisms on the grain. This was always an estimate due to the difficulties of obtaining a true spectrum of microorganisms on a seed because the extremely large populations of these organisms on a single seed, as well as their rapid reproductive and physiological potential, were difficult to assess. This was mostly due to a lack of a proper method to study the bacteria. Also the identification of a few bacterial isolates was time consuming, and the task would become nearly impossible when the microflora of a small sample of grain was considered (61). The isolation method could approach a statistical scheme somewhat in that the researcher used his own standardized isolation procedure throughout the experiment, along with replicates.

"The microflora of cereal grains were made up of a wide variety of fungi and bacteria, including actinomycetes (66)." The microflora of a grain consisted mainly of epiphytic microorganisms since no

internal organisms occurred in healthy seeds, only in unhealthy seeds (61, 66, 76, 77). The kind and abundance of microorganisms on grain depended on such factors as the environment under which the grains were produced, handling treatment and conditions of storage (15, 66, 76, 77). The microorganisms could be divided into three groups depending on their effect on grain: 1. saprophytic, 2. phytopathogenic and 3. pathogenic (for animals and man) (61, 76, 77).

Parasites and some saprophytes comprised the internal microflora (Table 1). Semiparasites and saprophytes such as Nigrospora oryzae, Cephalosporium acremonium, Microascus trigononsporus, Penicillium oxaliccum, Penicillium spp., Aspergillus spp., Rhizopus spp., Alternaria spp. and bacteria may be carried within grains (66, 76, 77). Trisvyatskii (77) listed a few saprophytic and phytopathogenic bacteria that infected grain. For example, Bacillus mesentericus and Bacillus subtilus caused "ropy" bread; Bacillus translucens and Bacillus atrofaciens caused a shrivelling of kernels during storage (77).

Fungi are classified the following ways. Fungi that invaded seeds could be divided into two groups, field and storage fungi. Field fungi were those that invaded seeds as they were growing on the plants in the field and on the seeds before harvesting. Common genera of field fungi are Alternaria, Cladosporium, Fusarium, and Helminthosporium plus a few species of Aspergillus and Penicillium. This fungi can discolor seeds, weaken or kill the embryos, cause prolonged dormancy, and incite various blights and root rots in the plants grown from seeds. In general, the damage caused by field fungi will be done by the time the seeds are harvested and no further injury is caused in storage. In addition they required high moisture content in equilibrium with a relative humidity of at least 90-95% to grow. This means a moisture content of 20-25% on a wet weight basis, or about 30-33% on a dry weight basis.

Table 1

Parasitic Fungi and Bacteria Carried Internally by Cereal Grain Seeds

Parasites	Wheat	Oats	Barley	Rye	Corn
Fungi					
Calonectria graminicola	+			+	
Colletotrichum graminicolum		+		+	
Diplodia macrospora					+
D. zea					+
Fusarium spp.	+	+	+	+	+
Gibberella fujikuroi					+
G. zea	+	+	+	+	+
Helminthosporium carbonum					+
H. gramineum			+		
H. sativum	+	+	+	+	
H. victoriae		+			
Pyrenophora avenae		+			
P. teres			+		
Septoria avenae		+			
S. nodorum	+				
S. secalis				+	
S. tritici	+				
Ustilago avenae		+			
U. nuda			+		
Bacteria					
Bacterium stewartii					+
Pseudomonas coronafaciens		+			
Ps. striafaciens		+			
Xanthomonas translucens	+		+	+	

(Adapted from 66)

Storage fungi are those that grow on grain in storage and grow in seeds having a moisture content between 12 to 30% on a dry weight basis. They comprise mainly several group species of Aspergillus and a few of Penicillium (21, 85).

The predominant types and total range of fungi varies for each type of grain. Flannigan (31) found that Alternaria alternata, Aureobasidium spp. Cladosporium spp. and Epicoccum nigrum were predominant on barley while on wheat he found Alternaria alternata, Cladosporium spp., Epicoccum nigrum and Fusarium avenaceum predominant. Research on microflora of cereal seeds in Finland showed that Alternaria spp., Cladosporium spp., Epicoccum spp., Fusarium spp. and Penicillium spp. were the predominant ones (83). Hyde et al.'s (44) work indicated that only Alternaria tenuis was the dominant mold on wheat kernels. In storage of rice, Aspergillus spp. and Penicillium spp. were the predominant ones plus Fusarium spp. and Curvularia spp. (27, 40). For corn, Fusarium spp. and Penicillium spp. occurred in 50% of the kernels (15). Pepper (61) found all the above mentioned molds plus Helminthosporium spp. were common on barley kernels. There were several other types of molds found on grain in minor amounts. On wild rice kernels, Mucor spp., Rhizopus spp., Aspergillus spp. and Penicillium spp. were prevalent (35).

Bacteria apparently represent 90-99% of the microflora on grain (66). Gram negative, yellow pigmented bacteria were predominant on several grains. This organism has been called the following names: Bacterium herbicola aureum Dugg, Pseudomonas trifolii Huss, Flavobacterium herbicola, Xanthomonas trifolii James and Erwinia herbicola Dye. Now it could be called Pseudomonas herbicola, Erwinia herbicola or

Xanthomonas trifolii depending on the results of taxonomic tests (36,45,59,77). The second most predominant bacteria found on grain were Pseudomonas fluorescens and several types of Pseudomonas spp. (36,45,59,77). Other major types of bacteria on grain were Bacillus spp., Aerobacter spp., Brevibacterium spp., Flavobacterium spp., Brivinia spp., Micrococcus spp., Lactobacillus spp., Xanthomonas spp., and Achromobacter spp.(36,40,43,45,59,54,59,60,61,66,72,77). Frank(32) discovered the following bacteria on wild rice: Flavobacterium spp., Achromobacter spp., Pseudomonas spp., Micrococcus spp., Enterobacter spp., Corynebacterium spp., Cellulomonas spp., Streptococcus spp., and Bacillus spp. The above was only a small list of the many types of bacteria to be found on grain. There were also several types of yeasts and actinomycetes on grain but will not be discussed here since no research was carried out on these organisms.

During the storage of grain the changes in the types of microflora were found to be dependent on storage conditions and the condition of the grain. In the storage of barley at different moisture contents and temperatures, the field fungi (Helminthosporium, Fusarium and Alternaria) decreased substantially while the storage fungi (Aspergillus and Penicillium) increased greatly (53). Initially in the storage of high moisture corn, the field fungi (Fusarium, Mucor, Cladosporium, Alternaria and Trichoderma) predominated but after 7 days, only storage fungi were predominant with the Fusarium spp. the only remaining field fungi (55). Hernandez et al (40) stated that all microorganisms were reduced during rice storage. During the storage of high moisture barley, Jorgensen (46) noticed that the original microflora of the barley

underwent few changes except for a large increase in Penicillium spp. During the fermentation of cocoa beans, the microflora changed from a mainly yeast one to a lactobacilli and bacilli one (60). Frank (32) tried to show that the bacterial population did change somewhat during the curing of wild rice but his data was too limited to draw any precise conclusions. Gram negative bacteria always predominated throughout the curing period for wild rice (28,32,64).

2.4.3 Counting microbial populations on grain

Because of the scarcity of information regarding microflora on grain and grain products, and with food safety demands increasing, greater research is required on the numbers and kinds of microorganisms present on grains. The total bacterial counts on wheat were found to range from 15,000 to 660,000 per gram while actinomycete counts ranged from 0 to 300 per gram (36). Spicker's data (70) indicated that a total mesophilic bacterial count of 3.78 million per gram, a coliform count of 99 per gram, and faecal streptococcus count of 19 per gram occurred on wheat. Microbial surveys of corn showed respective counts for bacteria, mold, actinomycetes, psychrotrophs, and aerobic spores ranged from 10^2 to 10^6, 10^2 to 10^6, 0 to 10^3, 10^2 to 10^3 and < 10 per gram of sample (14,15). For green wild rice, Goel et al. (34) discovered the following respective average counts for total bacteria, coliforms, psychrotrophs, streptococci, yeasts and molds to be 16×10^8, 36×10^6, 50×10^7, 60×10^5, 1.1×10^5 and 31×10^5 per gram. Therefore, the numbers of microorganisms on grain varies greatly.

The microbial population changed in regards to kinds and

numbers during grain storage. For the storage of white rice of different moisture contents and at different temperatures, the actinomycete, bacterial and yeast populations decreased while the mold population increased (42). But during the curing of cocoa beans, the total bacterial counts increased gradually to a certain level and remained around this level for the rest of the curing period (60). If the curing temperature was increased for cocoa beans, the bacterial population increased faster (43). During the storage of high moisture corn the counts for aerobic mesophilic bacteria increased from 10^6 per gram to 10^8 per gram while the yeast counts increased from 10^5 per gram to almost 10^8 per gram and mold counts from 10^5 per gram to between 10^7 and 10^8 per gram. These increases in numbers were gradual (55). For wild rice curing the changes in microbial population were dependent upon the curing conditions. For example, at ambient conditions (21^oC), the total bacterial counts increased to $> 10^9$ per gram and then remained at a constant level or fluctuated up and down. Psychrotrophs decreased from 10^7 per gram to 10^5 per gram, and mold counts were erratic around 10^5 per gram. In later research, the mold count increased from 10^4 per gram to 10^6 per gram in the first week of curing and remained constant at this level throughout the rest of the curing period. Also the aerobic spore type bacterial count increased in this study. Under the cool curing conditions (10^o C), only the psychrophilic count increased while the other kinds of microflora remained almost constant or decreased (28,32,35,80).

Since several types of grain are dried to a certain moisture content for better storage or processing, there has been interest in

the effect of drying on the microbial population on the grain. In the drying of barley for 1 hour, a temperature of 80°C was the most effective for bacterial reduction while the drying temperature of 60°C was the most effective for mold reduction. It reduced the bacterial load from 4.4×10^7 per gram to 200 per gram and the mold load from 1.1×10^5 per gram to 50 per gram. Lethality of drying treatments increased with increasing temperature and moisture content of the grain. Also the bacteria were more heat resistant than other types of microflora (71). The same microbial results occurred in the roasting of cocoa beans with Bacillus spp. becoming predominant (19). In the parching of wild rice, researchers have tested temperatures ranging from 51.7°C to 121.1°C but they found that the best parching temperatures ranged from 79.4°C to 121.1°C. The total bacterial count could be reduced from 10^9 per gram to 10^7 per gram under normal parching times. If the parching times were increased, the total bacterial count could be reduced another log cycle or two. Yeast counts were greatly reduced by all parching temperatures even to the point of zero per gram of rice. Mold counts varied after parching. For some cases there was very little reduction in mold counts, and in other cases, there was a substantial reduction. In general, most kinds of microorganisms were reduced by parching but the degree of microbial reduction was dependent on the parching temperature and time, the kinds and numbers of microorganisms, etc. The microbial population on wild rice kernels was reduced even further when the kernels were dehulled. For example, the total bacterial count could be around 10^3 per gram of dehulled wild rice from an initial level of 10^7 per gram of parched, not dehulled wild rice (34,35,80).

2.4.4 Food safety of grain

Current publicity on food-borne illnesses and intoxications has intensified interest in the microbial quality and safety of food products (15). This problem could have occurred in cereals naturally, or from human and animal contamination. The genera of fungi most involved in mycotoxin production in cereals were Aspergillus, Fusarium, Penicillium and Claviceps (22,41). As previously discussed, during the grain storage, the numbers of Aspergillus and Penicillium population generally increased, and thus may have caused a potential health problem if toxin-producing strains were present. Frank et al. (33) indicated that it was possible to control the production of aflatoxins from Aspergillus flavus during the curing of wild rice. This was done by maintaining the moisture content of wild rice at or above 33% during the curing period. If below this moisture content, aflatoxin may be produced. Frank et al (33) discovered that parching reduced aflatoxin B_1 content in the rice kernels significantly but detectable amounts still remained. He concluded that extensive mold growth could still occur and other mycotoxins might be produced.

In addition to the mold problem, the bacterial population on grain may cause a health hazard. For example, the following have been detected on several types of grain; staphylococci, salmonellae, anaerobic spore formers, etc. (15,34,35,70). In a microbial survey of corn, the total coliform counts and fecal streptococci counts were low, and the coagulase-positive staphylococci and salmonellae counts were negative (15). Goel et al. (34) stated that the numbers of coliforms and streptococci were high on wild rice but their numbers were

less than the total plate count. However, no coagulase-positive staphylococci were detected and one isolate of salmonella was found. During the curing of wild rice, the coliform count was erratic and their population increase was minimal (35). Also the streptococci count only increased under wet ambient conditions. Goel et al. (35) did not detect coagulase-positive staphylococci and salmonellae in their study. The presence of coliforms, streptococci, staphylococci, and salmonellae on grain kernels cannot be interpreted as indicative of direct contamination. However, the presence of large numbers of these organisms would raise a warning flag of potential health hazard and would require correction (15).

As previously mentioned the drying of a grain reduced the microbial population which included these potential health hazard organisms. Therefore grain drying helped to maintain a healthy product except for the case where mycotoxins were produced by microorganisms since these generally survived heat treatments. For the parching of wild rice the health hazard microorganisms are substantially reduced even to a point of zero (34,35). Goel et al. (34) also found under normal cooking conditions that the total bacterial plate count could be reduced to 140 gram of wild rice from an original count of approximately 108 per gram and no other organisms could be recovered.

2.4.5 Microbial reduction

The most satisfactory method of achieving sterilization or disinfectation was by physical agents such as wet or dry heat, or by ionizing radiations. There were some situations where these methods

were inconvenient, or impossible because of damage to the materials to be treated. Recourse had then to be made to chemical methods and antimicrobial gases (48). An example of a chemical method was the use of sodium hypochlorite (NaOCl) which was effective as a disinfecting and sterilizing agent against a broad range of bacteria, viruses, and fungi (1). Disinfecting seeds with strong solutions of NaOCl (5.25 available chlorine) for 1 minute to several minutes did not reduce germinability and resulted in steady decrease in microbial counts (61).

"Available chlorine" may be defined as measurement of the oxidizing capacity and is expressed in terms of the equivalent amount of elemental chlorine. In the case of hypochlorites, this term indicates the amount of chlorine initially used to prepare the hypochlorite solution in question, including the chlorine consumed to form germicidally inactive chloride ion. The mechanism of chlorine type compounds' activity on microbes has not been completely elucidated yet. It appears to be some type of bounding to activity, but the biocidal activity of chlorine compounds is affected by pH, temperature, concentration, organic content, etc. For example, an increase in pH decreases the biocidal activity of chlorine, and a decrease in pH increases this activity (29).

Two chemical sterilizing solutions consisted of a hypochlorite solution and ethyl alcohol (61), or a hypochlorite solution and water (78,79). The alcohol served as a wetting agent, dissolved lipoidal material, and was slightly germicidal. But this combination was somewhat less efficient due to chemical reactions between the two chemicals (61). Vojnovich et al. (78,79) in their research on microbial reduction

on corn and corn products, found that the microbial population could be greatly reduced by dipping in a hot solution of sodium hypochlorite and hot water. For example, an initial microbial count of 1,700,000 bacteria and 110,000 fungi per gram of corn could be reduced to about 5,000 per gram of corn or less. Also the hot sodium hypochlorite solutions gave better reduction than hot water (78,79). There was a problem with this sterilizing solution, however; seeds sterilized with it retained appreciable amounts of the compound because sodium hypochlorite had a strong oxidizing property which made it highly reactive with amino acids, nucleic acids, amines and amides (1). All the literature on liquid sterilants stated that they were only able to reduce the microbial population, and not totally sterilize the grain.

The lemma, palea and pericaps of a kernel usually contained fungi and bacteria that were difficult to kill with liquid sterilants without injury to the embryo (19). Thus to prevent this and to obtain grain totally free of microorganisms the use of antimicrobial gases (ethylene oxide, propylene oxide, etc.) have been applied since the gases penetrated relatively complex loads, were effective at low temperatures and were chemically simple substances with a broad antimicrobial spectrum (48). The mode of action upon microbes seem to be a nonspecific alkylation of such chemical groups as -OH, $-NH_2$, and -SH with the loss of a hydrogen atom and the production of an alkyl hydroxyethyl group. Their rate of kill depended on time of exposure, concentration, relative humidity and temperature of the environment, type of gas used, temperature and moisture content of the material, the

species and the numbers of microorganisms. For example, the optimal relative humidity for the most efficient sterilization by ethylene oxide was around 30%, and beyond the relative humidity range of 20 to 40% the effectiveness dropped off rapidly. Also it was common practice to dilute the antimicrobial gas with a carrier gas such as carbon dioxide or dichlorodifluoromethane up to 90% for safety reasons (18, 30, 48, 62).

Gaseous propylene oxide commonly used to sterilize plant materials, gave only partial sterilization of barley seeds (19). However, Bushnell was unable to totally or almost totally eliminate the microflora from barley kernels with 12% ethylene oxide and 88% dichlorodifluoromethane gas mixture having an ethylene oxide concentration of 1200 to 1400 mg per litre at a pressure of 3 to 3.3 atmospheres for 3 hours at room temperature and relative humidity of 42% to 60%. He discovered that a standing period after gas sterilization of the barley kernels was needed to obtain the complete sterilizing action of the ethylene oxide. Frank et al. (33) were able to sterilize wild rice, which had been dried to a moisture content of 20%, with a gas mixture of 10% ethylene oxide and 90% carbon dioxide. The wild rice was flushed with this gas over 48 hours and its sterility was checked with plate count agar. However, there were no data presented for this study (33).

CHAPTER 3

METHODS AND MATERIALS

3.1 Introduction

In the fall of 1973, the Food Science Department obtained 454.5 kg (1000 pounds) of green lake wild rice and 136.4 kg (300 pounds) of green paddy wild rice from the Indian Rice Producers Co-op Limited at Fort Alexander, Manitoba. This wild rice was frozen and stored at a temperature of -35.0°C before being processed in early 1974. Also 45.5 kg (100 pounds) of cured lake wild rice were obtained from the Northland Wild Rice Co. in Winnipeg. These lots were for the preliminary microbiological work. In the fall of 1974, the department obtained individual lots of 181.8 kg (400 pounds) green lake wild rice from Eileen Lake, Shallow Lake, and Harrop Lake in Manitoba plus 363.6 kg (800 pounds) of green paddy wild rice from Sprague, Manitoba. The rice from Eileen Lake and Sprague were processed immediately while the rest were frozen and stored at -40°C. By the late fall of 1974 45.5 kg (100 pounds) of lake wild rice was received from La Ronge, Saskatchewan which was frozen and stored for future studies. These lots of wild rice supplied the samples for the microbiological studies.

3.2 Design of Curing Studies

The wild rice processing equipment and studies have been previously described (20), and certain parts of it are repeated in this

section since they are essential to understanding the research (20).

Mixed lots of green lake (from Eileen and Harrop Lakes) and paddy (Sprague) wild rice were washed to remove insects, dirt and debris prior to curing. Next, 18 pounds or 8.2 kg. of each lot of wild rice were placed in cylindrical metal containers measuring 61 cm high and 31 cm in diameter with a perforated bottom. This gave a bed depth of 31 cm (12 inches). Then the containers were placed in controlled environmental chambers set at the desired curing conditions. The curing conditions used in this study were:

1. the curing temperature was 10°C and relative humidity of 95% and curing period of 9 weeks for lake and paddy rice.

2. the curing temperature was 15°C and relative humidity of 95% and curing periods of 3 weeks for paddy rice and 4 weeks for lake rice.

3. the curing temperature was 21°C and relative humidity of 95% and curing period of 5 weeks for lake and paddy rice.

4. the curing temperature was 32°C and relative humidity of 95% and curing periods of 15 days for lake rice and 10 days for paddy rice.

The wild rice in these containers were turned and mixed up every day. About 350 ml of fresh water was added to each container three times every week to maintain the wild rice at a moisture content above 35%.

The temperature of the rice bed was recorded daily by means of a thermometer. This thermometer was positioned in the centre of the rice pile until a constant temperature was observed. Also during the

curing period the wild rice was examined for visual changes and any detectable odors (20).

3.3 Design of Parching and Cooking Studies

Chung's (20) procedure for parching and cooking was modified as follows. Four hundred and fifty four grams of wild rice were removed from the curing containers and parched at 135°C to 141°C for 35 to 55 minutes in the coffee roaster. The final moisture content of the rice was 7 to 9% .

The wild rice was dehulled according to Chung's method (20). The AACC procedure for determining spore forming bacteria in cereals and cereal products was used for the cooking procedure (7). This was carried out for comparison with Wisconsin's method for determining spore type bacteria (32,64). The microbial results of this test indicated that the same population reduction could be obtained by boiling for 23 minutes (7) as by boiling for 45 minutes (20).

3.4 Isolation of the Microflora from Wild Rice

3.4.1 Bacteria

Samples were taken at random aseptically from all sublots of each type of wild rice and placed in sterile Whirl-Pak bags during the fall of 1973. There was no cured paddy wild rice available during this time of isolation. The cured lake wild rice was taken from an ambient (21°C) curing environment that was commonly used by wild rice processors in Manitoba. The reason for using green and cured wild rice for

bacterial isolation purposes was that the method quickly produced the predominant bacterial population at the beginning and at the end of the curing period. This enabled one to deduce if the bacterial population changed during curing, and showed the general spectrum of bacteria to be found on wild rice. All the samples from each type of sublot were mixed to give a composite sample.

The method for obtaining the total aerobic bacteria count using standard plate count medium was performed for each sublot of wild rice. (Refer to 3.7 for the detailed method.) Upon examining all the plates, it was resolved that the 10^7 dilution plates were the most ideal for the isolation of bacteria because they gave the best representative spectrum of bacteria on wild rice without being overcrowded as at low dilutions, or being screened out as at higher dilutions. From this lot of 6 plates, one plate was selected at random and colonies were delineated with a grease pencil (36). This was done for all three types of wild rice used in this study. Then 25 colonies were picked off from each plate and inoculated into test tubes of trypticase soy broth (BBL) (36). After the incubation of the test tubes at 30°C for 3 days, the cultures were streaked upon nutrient agar plates for purification (32). This streaking was continued until the bacterial culture was pure. Once purified, the cultures were maintained on TSA slants at 4° - 5°C until identification procedures were started (36). It was expected that a total of 75 bacterial isolates would appear for identification but due to mixed colonies on isolation plates there resulted in a total of 90 bacterial isolates.

Yellow colored bacteria predominated on the total count plates,

and the isolation procedures above were such that it was possible to miss some types. Therefore all yellow colored bacteria which exhibited different morphological characteristics were isolated. This totalled approximately 15 bacterial isolates. About a half dozen other bacteria, which were morphologically different from all other previous bacterial isolates, were isolated to obtain a more complete picture of total kinds of bacteria to be found on wild rice kernels.

3.4.2 Mold

Using the same wild rice samples, from which the bacterial isolates were isolated, the method for obtaining mold counts using acidified potato dextrose agar was performed. (See section 3.7 for detailed method.) Mold colonies exhibiting different morphological characteristics were picked off and streaked on acidified potato dextrose agar for purification and maintenance until identified. A total of 17 different types of mold were isolated.

3.5 Characterization of Microflora

3.5.1 Bacteria

The materials and methods based upon those described in Laboratory Methods in Microbiology, The Genera of Bacteria, BBL Manual of Products and Laboratory Procedures and Manual for the Identification of Medical Bacteria were used to identify the bacterial isolates (9,25,37,68). The following tests were performed for the identification of the bacterial isolates; motility test, gram stain and morphology, colony morphology on nutrient agar and trypticase soy agar, growth in trypticase

soy broth tube, reaction in oxidative-fermentative (O - F) medium by Hugh and Liefson, and a modified Hugh Liefson medium for weakly oxidative plant bacteria (38), action on sugars (glucose and lactose) in purple broth base media, Kovac's oxidase test, catalase test, cellulolytic test using filter paper in 0.5% peptone, spore stain, acid fast test, flagella stain on motile cultures using a modified method (69), pigment production by Pseudomonas on King's medium A and B (51), methyl red and voges-proskauer tests, production of indole, utilization of (a) nitrate, (b) urea, (c) citrate and (d) phenylalanine and reaction of TSI (8). All the above tests were not performed on every bacterial isolate. Only tests necessary for the identification of each bacterial isolate were performed according to an identification scheme (section 3.6.1).

3.5.2 Molds

The tests performed on molds for identification purposes were visual examination on plates and examination under the microscope for morphological characteristics (37,74).

3.6 Classification of Isolates

3.6.1 Bacteria

All bacterial isolates were grouped according to the schemes presented in the following tables. The non-yellow colored bacteria were grouped as presented in Tables 2 and 3 (25). The Pseudomonas spp. were further grouped according to Table 4 (67). The yellow pigmented rod cultures were classified by Tables 5 and 6 (39).

Table 2

First-stage Diagnostic Table for Gram-positive Bacteria

Shape	S	S	S	S	R	R	R	R	R	R	R	R	R
Acid-fast	–	–	–	–	–	–	–	–	–	–	–	+	–
Spores	–	–	–	–	–	–	–	–	–	+	+	–	–
Motility	–	–	–	+	+	–	–	+	–	d	d	–	–
Growth in Air	+	+	+	+	+	+	+	+	d	+	–	+	+
Catalase	+	+	=	=	+	+	+	+	=	+	=	d	+
Oxidase	–	–	–	–	–	–	–	–	–	d	–	–	–
Glucose (acid)	d	+	+	+	+	+	–	–	+	d	d	+	+
O-F test	O/–	F	F	F	F	F	–	–	F	F/O/–	F/O	O/NT	O
Micrococcus	+	–	–	–	–	–	–	–	–	–	–	–	–
Staphylococcus	–	+	–	–	–	–	–	–	–	–	–	–	–
Aerococcus	–	+	+	–	–	–	–	–	–	–	–	–	–
Streptococcus	–	–	+	+	–	–	–	–	–	–	–	–	–
Listeria	–	–	–	–	+	–	–	–	–	–	–	–	–
Corynebacterium	–	–	–	–	–	+	+	–	–	–	–	–	–
Kurthia	–	–	–	–	–	–	–	+	–	–	–	–	–
Erysipelothrix	–	–	–	–	–	–	–	–	+	–	–	–	–
Lactobacillus	–	–	–	–	–	–	–	–	+	–	–	–	–
Actinomyces	–	–	–	–	–	–	–	–	+	–	–	–	–
Bacillus	–	–	–	–	+	+	+	+	–	+	–	–	+
Clostridium	–	–	–	–	–	–	–	–	+	–	+	–	–
Mycobacterium	–	–	–	–	–	–	–	–	–	–	–	+	–
Nocardia	–	–	–	–	–	–	–	–	–	–	–	+	+

Meaning of symbols

+ = 100-80% strains positive; d = 79-21% positive; – = 20-0% strains positive;

F = fermentation; O = oxidation; (.) = delayed reaction; NT = not testable

S = sphere; R = rod.

(Adapted from 25)

Table 3

First-stage Diagnostic Table for Gram-negative Bacteria

Shape	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Motility	-	-	-	+	-	-	-	+	+	-	-	-	-	+	-	d
Growth in Air	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Catalase	+	-	+	+	-	+	+	+	+	+	+	+	+	+	NT	d
Oxidase	+	-	-	-	-	+	+	+	-	+	-	-	+	+	d	-
Glucose (acid)	d	+	+	+	+	+	+	+	+	+	+	-	-	-	NT	d
O-F test	O/-	F	F	F	F	F	F	O	O	O	O	-	-	-	NT	NT
Neisseria	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gemella	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterobacteriaceae	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Actinobacillus	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Pasteurella	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-
Aeromonas	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Vibrio	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Pseudomonas	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Chromobacterium	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Flavobacterium	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Acinetobacter	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Brucella	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Moraxella	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Bordetella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Haemophilus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Bacteroides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Meaning of symbols

+ = 100-80% strains positive; d = 79-21% strains positive;
- = 20-0% strains positive;

F = fermentation; O = oxidation; (.) = delayed reaction;
NT = not testable.

S = sphere; R = rod.

(Adapted from 25)

Identification of isolates that did not fit the schemes presented in these tables were classified according to Bergey's Manual. The identification of all bacterial isolates was checked and finalized with Bergey's Manual.

3.6.2 Molds

All mold isolates were identified according to the Diagnosis of Plant Diseases and Laboratory Methods in Microbiology (37,74).

3.7 Studies on Microbial Populations

For these studies the wild rice was analysed as follows:

1. In 1973 and 1974, each lot of wild rice was sampled aseptically. The samples were kept in sterile Whirl-Pak bags until the start of bacteriological analysis each day.

2. During the curing trials in 1974, samples from each sublot of wild rice were taken aseptically at weekly intervals for the 10°C, 15°C, and 21°C treatments, and every three days for the 32°C treatment. They were placed in the sterile Whirl-Pak bags and were examined later that day.

3. Samples were also taken aseptically from the various steps in the processing of wild rice such as parching and dehulling, besides some cooked samples.

The samples were mixed as described in 3.4.1. Ten grams were removed aseptically and placed in 90 ml of sterile phosphate buffered distilled water in a sterile Waring blendor jar (34,35). This was blended for 2 minutes (60) instead of 1 minute (34,35) because 2

Table 4

A Grouping of the Pseudomonas spp.

Behavior in the test of Hugh & Leifson (1953)			
Oxidative		Alkaline	No action
Green fluorescent + diffusible pigment	No diffusible pigment	No diffusible pigment	No diffusible pigment
Psuedomonas group I	Pseudomonas group II	Pseudomonas group III	Psuedomonas group IV

(Adapted from 67)

Table 5

Identification of Yellow-pigmented rods

	Gram	Oxidase	Phosphatase	Motility (flagellate) Polar flagella	Spreading growth	Bacteriolytic	Fruiting bodies	Microcysts	Sensitive to Polymyxin B.	Carbohydrates Oxidative	Carbohydrates Fermentative
Cytophaga	-	+	+	- -	x	-	-	-	-	x	x
Sporocytophaga	-	+	+	- -	x	-	-	+	-	x	-
Fruiting myxobacteria	-	+	+	- -	+	+	+	+	±	.	-
Psuedomonas	-	x	x	+ +	-	-	-	-	+	x	-
Xanthomonas	-	-	.	+ +	-	-	-	-	.	+	-
Cellvibrio	-	+	-	+ +	-	-	-	-	+	+	-
Vibrio/Aeromonas	-	+	.	+ +	-	-	-	-	.	-	+
Coliforms (including Erwinia lathyri Escherichia aurescens)	-	-	x	x -	-	-	-	-	.	-	+
?Flavobacterium	-	.	.	x -	-	-	-	-	.	+	-
Coryneforms	+orx	-	x	x .	-	-	-	-	.	x	x
"Pleiston A"	x	-	-	- -	x	-	-	-	-	+	-

+ = Positive; - = negative; x = variable; . = unknown; ± = weak positive

(Adapted from 39)

Table 6

Differentiation of some cellulolytic types

	Carbohydrates						
	Gram	Oxidase	Oxidative	Fermentative	Polymyxin B sensitive	Microcysts	Flagella
Cytophaga	-	+	+	-	-	-	-
Sporocytophaga	-	+	+	-	-	+	-
Cellvibrio	-	+	+	-	+	-	+
Cellulomonas	+	-	-	+	.	-	+

+ = Positive; - = negative; . = unknown

(Adapted from 39)

minutes gave a more homogenous solution. Serial dilutions were made with sterile phosphate buffered distilled water and were plated in duplicate for: a. total aerobicplate count (plate count agar (Difco), incubation at 30°C for 48 hours); b. yeast and mold count (potato dextrose agar (Difco) acidified to pH 3.5 with sterile tartaric acid, incubation at 22°C for 3-5 days); d. aerobic spore count (standard plate count agar, the sample was heat shocked at 80°C for 20 minutes, incubation at 30°C for 3 days) (32,34,35). This method for determining the aerobic spore count was not in accordance with the prescribed AACC method (7) which stated that the sample must be boiled for a certain time period, and plates must be incubated at 55°C. In order to compare data, Wisconsin's method was used.

Following incubation, the colonies were counted and average values were calculated (32,34,35). During the curing studies, the changes in the types of microbial population were also observed visually.

3.8 Possible Health Hazards of Wild Rice

The food safety of wild rice was determined by microbial counts on green, cured, dehulled and cooked samples of wild rice since high counts were usually a warning signal of a possible health problem (32,34,35). The taxonomic studies of molds on the wild rice kernels could indicate if hazardous microbes such as Aspergillus flavus and certain Fusarium spp. were present (33).

3.9 Microbial Reduction Studies on Wild Rice

The purpose for these experiments was to develop a procedure for

sterilizing green wild rice without causing any damage to the rice. The next step was to determine the possible functions of microorganisms in the curing process.

3.9.1 Microbial reduction studies using liquid sterilants

The surface disinfectant used was a 12% sodium hypochlorite solution (Diversey Divex which had a minimum available chlorine content of 10.2%. This solution was diluted to 5 1/4% available chlorine with sterilized water. The following trials describe the variables tested in this program.

Trial 1:

a. A mixture of 1 part ethyl alcohol (70%) and 2 parts sodium hypochlorite solution (5 1/4% available chlorine) was prepared. Samples of wild rice (454 g) were immersed in this mixture for one to two minutes at room temperature (61). Total aerobic plate count, and yeast and mold counts were performed on all tested samples (Section 3.7).

b. Trial 1a was modified by using 227 g of wild rice and an immersion time of 30 minutes. The rest of the procedure was not changed.

c. Trial 1b was modified by using 113.5 g samples of wild rice.

d. Trial 1b was modified by using 56.8 g samples of wild rice.

Trial 2:

a. Trial 1a was modified by immersing 56.8 g of wild rice for 30 minutes.

b. Trial 2a was modified by using one part 95% ethyl alcohol and 2 parts 12% sodium hypochlorite solution.

c. Trial 2a was modified by substituting concentrated Super Bacterole (Diamond Wax Ltd.) in place of the sodium hypochlorite. Ethyl alcohol was not used in this test.

d. Trial 2c was modified by adding one part ethyl alcohol (95%) to 2 parts Super Bacterole.

Trial 3:

a. Trial 2b was modified by using a 30 g. sample of wild rice.

b. Trial 3a was modified by excluding ethyl alcohol from the immersion solution.

Trial 4:

a. This was the same as trial 3a.

b. Trial 4a was modified by immersing 20 g of wild rice for 20 minutes.

c. Trial 4a was modified by immersing 10 g of wild rice for 10 minutes.

Trial 5:

a. Trial 3a was modified by immersing the wild rice at a temperature of $55^{o}C$.

b. Trial 5a was modified by immersing the wild rice for 20 minutes.

c. Trial 5a was modified by immersing the wild rice for 10 minutes.

Trial 6:

a. Trial 5a was modified by immersing 20 g of wild rice for 60 minutes.

b. Trial 6a was modified by having an immersion time of 30 minutes.

c. Trial 6a was modified by having an immersion time of 15 minutes.

Trial 7:

a. Trial 5a was modified by using an immersion temperature of $60^{o}C$.

b. Trial 7a was modified by using an immersion temperature of 70°C.

c. Trial 7a was modified by using an immersion temperature of 80°C.

Trial 8:

a. Trial 7c was modified by using a 20 g sample of wild rice.

b. Trial 8a was modified by using an immersion time of 20 minutes.

c. Trial 8a was modified by using an immersion time of 10 minutes.

Trial 9:

a. Trial 7c was modified by using a 25 g sample of wild rice.

b. This was the same as Trial 7c.

c. Trial 7c was modified by using a 35 g sample of wild rice.

Trial 10:

a. Trial 7c was modified by using a 10 g sample of wild rice.

b. Trial 10a was modified by reducing the immersion time to 20 minutes.

c. Trial 10a was modified by reducing the immersion time to 10 minutes.

Trial 11:

a. Trial 9a was modified by using a sodium hypochlorite solution of 5 1/4% available chlorine.

b. Trial 11a was modified by immersing 20 g of wild rice for 25 minutes.

c. Trial 11a was modified by immersing 10 g of wild rice for 12 minutes.

Trial 12:

a. Trial 11a was modified by using 20 g samples of wild rice.

b. Trial 12a was modified by using 15 g samples of wild rice.

c. Trial 12a was modified by using 10 g samples of wild rice.

Trial 13:

a. Trial 11c was modified by immersing dehulled green rice for 10 minutes at room temperature.

b. Trial 13a was modified by increasing the immersion time to 20 minutes.

c. Trial 13a was modified by increasing the immersion time to 30 minutes.

d,e,f,g. Trials 13a, b, c, and d were modified by using the 12% sodium hypochlorite solution.

Trial 14. a,b,c,d,e,f,g. Trial 13a, b, c, d, e, f and g were modified by using an immersion temperature of 55°C.

Trial 15:

a,b,c,d,e,f,g. Trial 13a, b, c, d, e, f and g were modified by using an immersion temperature of 80°C.

Trial 16:

a,b,c. Ten gram samples of wild rice were immersed in hot water (55°C) for 10, 20 and 30 minutes.

d,e,f. Trial 16a, b and c were modified by using an immersion temperature of 80°C.

3.9.2 Gas sterilization of wild rice

Wild rice was sterilized in a 1 quart glass milk bottle equipped with 2 glass tubes in a 2 holed rubber stopper and rubber caps for the open ends (11,33). The sterilizing gas mixture was 12% ethylene oxide and 88% CO_2 (11,48). The wild rice had a moisture content of 40%. The gas mixture was injected into the milk bottle with a hypodermic needle.

The bottle was flushed with the gas mixture to remove all the air, and then it was closed with rubber caps over the ends of the glass tubing to maintain a pressure inside the bottle equivalent to the outside atmospheric pressure. At the end of the exposure period, the milk bottle was flushed with inert nitrogen gas instead of evacuating with a vacuum pump to remove the sterilizing gas. This also partially served the function of a necessary standing period after gas sterilization in order to obtain the maximum sterilizing effect (19). The following sterilizing treatments were used:

1. exposure time of 4 hr. and flushing time of 2 hr.
2. exposure time of 4 hr. and flushing time of 6 hr.
3. exposure time of 24 hr. and flushing time of 2 hr.
4. exposure time of 24 hr. and flushing time of 6 hr. (11)

These were carried out at room temperature and without relative humidity control. Sample sizes of wild rice used were 200, 100 and 25 grams (11). After the sterilization trial, the wild rice was removed aseptically from the milk bottles and placed into sterile Whirl-Pak bags. Then total aerobic plate count, and yeast and mold counts were performed on the rice (Section 3.7).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

Preliminary microbiological studies on wild rice and its processing were started in October, 1973. These consisted of initial microbial counts on wild rice, isolation of bacteria and molds for taxonomic purposes, and testing of gas sterilization of wild rice. In the curing of 1973 wild rice, bed temperature and other general changes in the rice bed were monitored. The major microbiological tests were conducted during the curing studies on the 1974 crop. Bed temperatures and visual changes were also monitored. During the same time period taxonomic studies and microbial reduction studies were continued.

The main objective of this research was a taxonomic study of microbes on wild rice. A second objective was to check microbial counts on wild rice, especially during the processing studies in order to detect any potential role for microorganisms during the processing of wild rice. A third objective was a microbial reduction study on wild rice (the ultimate purpose of this test was to investigate the possible roles of microorganisms in the curing of wild rice).

4.2 General Observations and Temperature Changes of the Rice Bed during the Curing of Wild Rice

During the curing of wild rice, changes occurred in it which could be observed visually, or by means of measurements. As stated in the Literature Review the changes that occur during the storage of a grain

are affected by temperature and relative humidity of environment, moisture content of grain, maturity of the seed, etc. In these studies the curing environment and methods of handling wild rice during curing (turning and watering) were the only factors controlled while other factors affecting storage were not controlled due to the amount of work involved, or are uncontrollable at the present time in the wild rice industry.

Freshly harvested lake and paddy wild rice usually were green in color. The rate of color change in wild rice was closely associated with the conditions of the curing environment tested. During $10^{o}C$ curing, the color changed at the slowest rate and required up to 7 to 8 weeks for maximum color development. For the $15^{o}C$ curing trial, it took only 3 weeks which was very fast considering this curing temperature (The only possible reason for this occurrence was that the rice was already quite mature at the start of the curing period). Then at $21^{o}C$, the maximum color development was during the second to third week while for $32^{o}C$ it was 5 to 10 days. After this maximum color development, the dark brown color started to fade as the curing period was extended. The above information pertained only to the curing of lake wild rice. These results correlated well with Wisconsin's and Chung's data (20,28,64,80).

For paddy wild rice the normal pattern of color development did not take place. Its color changed from green to a brownish yellow in 1974 but in 1973 the color was dark brown. In some cases for the 1974 crop, the color went from green to yellow and did not develop a dark color at all. This was probably due to the fact that the paddy rice was too immature (28,80).

The development of odors in curing lake wild rice was similar for the 32°, 21° and 15°C treatments. The green wild rice had a grassy to green grain smell. The texture of the rice was firm and appeared clean. Gradually the odor became fishy or fresh marsh smell as color developed. As the color darkened, an earthy odor developed. This odor remained present for about 1/3 of the curing period. During this time, the wild rice developed its maximum dark black color and best texture. The optimum times to end the curing period are given above. After this time period, a strong earthy odor became dominant and then changed to a swampy to putrid odor indicating that the wild rice had started to deteriorate. This occurred about 2/3 of the way through the curing tests. The lake rice retained its original fresh odor throughout the whole curing period at 10°C.

The paddy wild rice had an odor development similar to lake wild rice above but it usually took place at a faster rate for some unknown reason. Also the texture of paddy wild rice was different from lake wild rice. It was firm to soft and clean to start with. But instead of becoming firm like lake rice, it became softer and started to deteriorate.

The deterioration of lake rice was usually characterized by high bed temperatures, strong swampy odor, slimy texture, dirty in appearance, color fading, and soft kernels. The higher the treatment temperature, the faster the deterioration took place. For the 32°C treatment, the wild rice started to deteriorate between 3 to 6 days and it became very moldy and putrid after this. For the 21°C treatment, deterioration started between the second and third weeks, and it was completely rotten

and moldy by the fourth week. For the 15°C, it took place between the third and fourth week along with sprouting and was completely rotten and moldy at the end of the fourth week. During the 10°C treatment, the only deterioration was the fading of color and the sprouting of the rice at the sixth week. The above results correlated well with other research (20,28,32,80).

The deterioration of paddy wild rice during curing had the same characteristics as the lake rice. But it was observed that the paddy rice deteriorated at a faster rate than the lake wild rice by a week to a few days earlier depending on the curing treatment. Paddy rice usually sprouted under all the curing treatments.

Upon examining the microbial counts (section 4.5) on wild rice it was observed that there was no substantial increase in the numbers of microorganisms during curing. Therefore the deterioration of wild rice was mainly due to biochemical reactions with microorganisms playing only a minor role.

The bed temperatures of the wild rice varied for each curing treatment. For the 10°C curing, the bed temperature of the wild rice increased only slightly and then leveled out with minor fluctuations throughout the curing period (Figures 2,3)(28). For the 15°C treatment, the bed temperature again leveled out with minor fluctuations in the temperature range of 18° to 20°C for paddy rice and lake rice remaining around 20°C (Figure 8). Finally for the 21°C and 32°C treatments, higher bed temperatures were noticed especially for 1974's wild rice (Figures 4,5,6,7)(20). The possible reason for this could be that the wild rice was frozen in 1973 for a time period before it was

cured while in 1974 the rice was not frozen before curing. The bed temperature for lake wild rice ranged from 18°C to 34°C for the 21°C curing treatment and ranged from 24°C to 44°C for the 32°C treatment. The bed temperatures for paddy wild rice were usually higher than for lake rice under the same curing treatments. Also the bed temperature for paddy rice decreased during the later stages of the curing period while for lake rice the bed temperatures increased. Wisconsin reported the same results with their curing of paddy wild rice (64). The reasons for these differences between lake and paddy wild rice could be due to the immaturity of the paddy wild rice which would cause a higher bed temperature initially and a decreasing one in later stages when the kernels' energy supply would be spent. Another reason stated in Chung's thesis (20) was that this difference was due to the difference in size of kernel for lake and paddy wild rice since the paddy rice would pack better together with less air space between the kernels resulting in more heat accumulation.

As pointed out by Milner et al(56), the bed temperature of a grain can be used to detect the onset of deterioration which was indicated by the second temperature increase. For the 21°C curing treatment, this occurred at the third week for lake rice and the second week for paddy rice while for the 32°C curing treatment for both types of wild rice, the second temperature increase occurred around the 5th day (Figures 4, 5,6,7). This second increase in temperature correlated well with the visual observations indicating the start of deterioration of the wild rice kernels. But for the 15°C curing treatment, the bed temperature did not increase when expected even though the rice deteriorated badly

Figure 2. Rice Bed Temperature of Lake Wild Rice Cured at 10°C

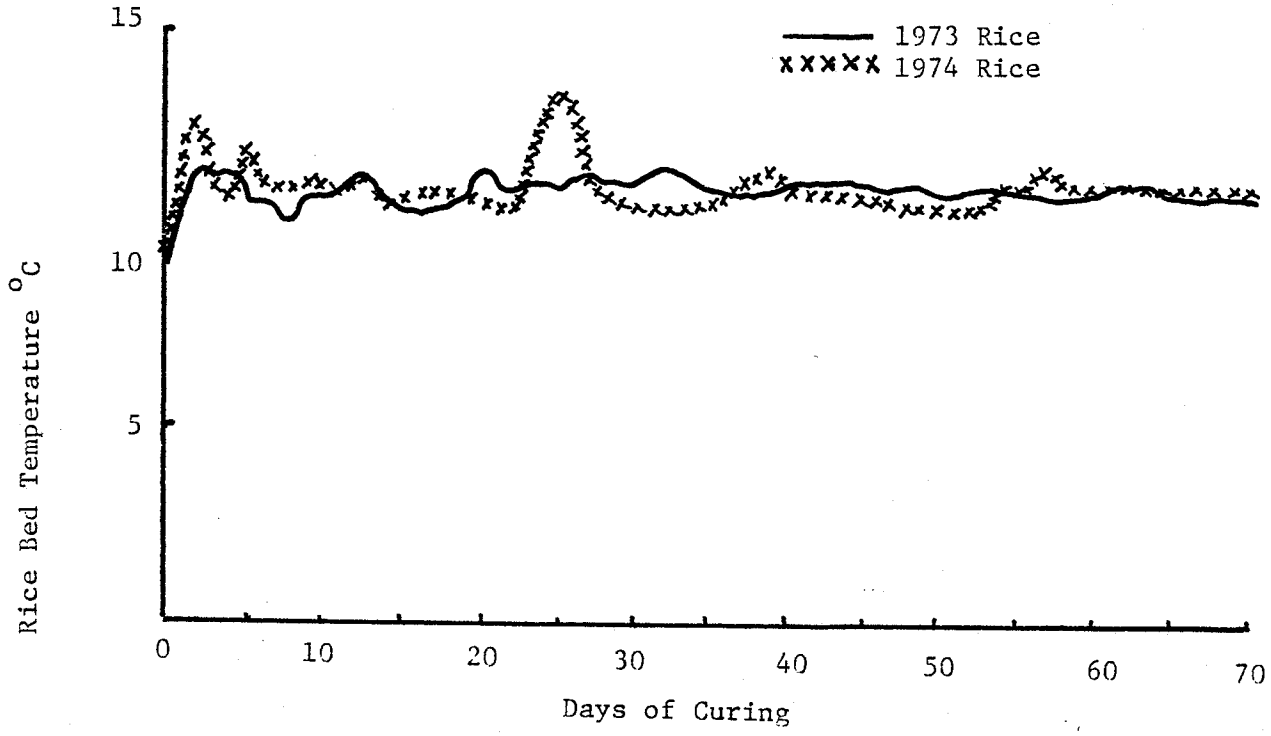


Figure 3. Rice Bed Temperature of Paddy Wild Rice Cured at 10°C

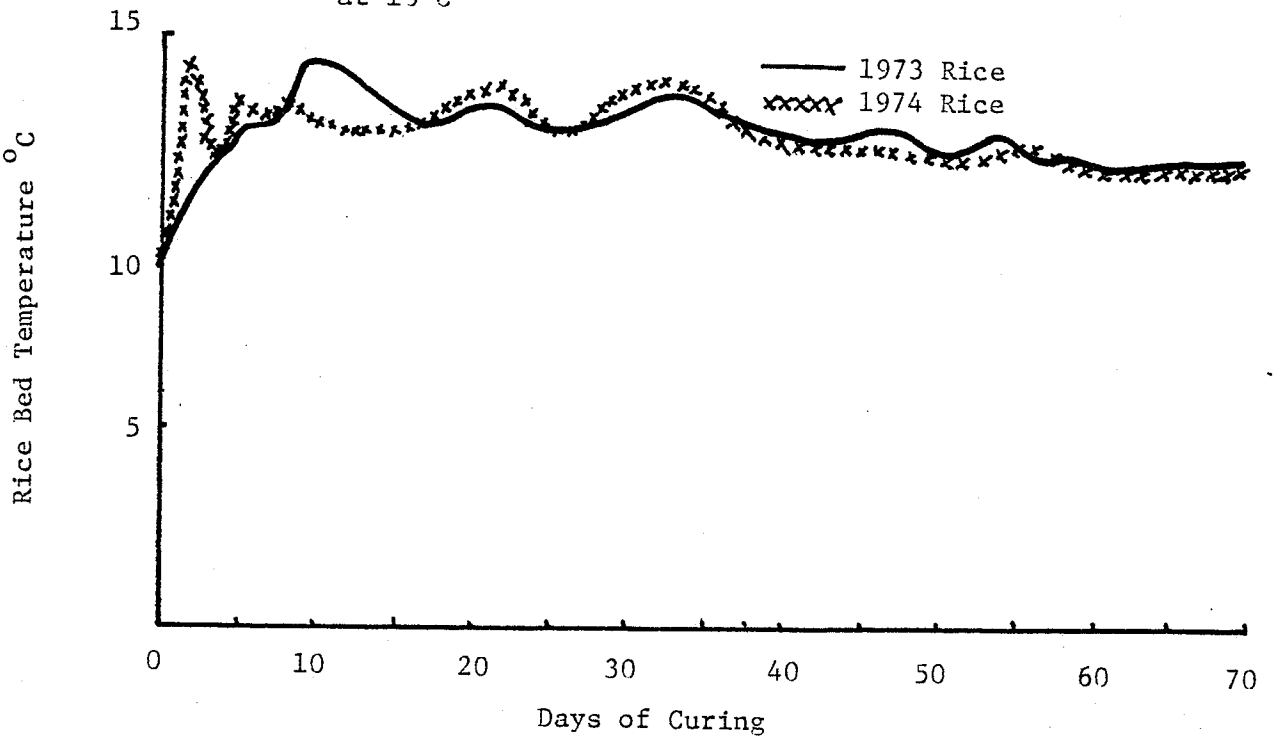


Figure 4. Rice Bed Temperature of Lake Wild Rice Cured at 21°C.

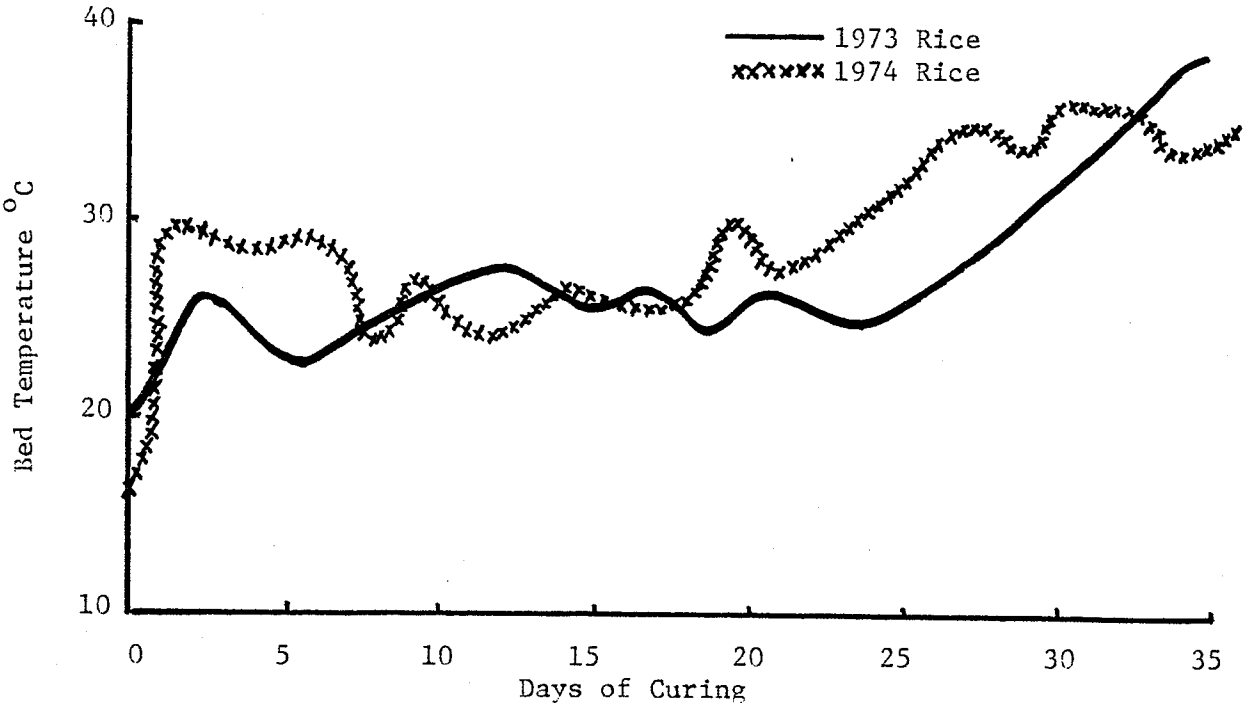


Figure 5. Rice Bed Temperature of Paddy Wild Rice Cured at 21°C.

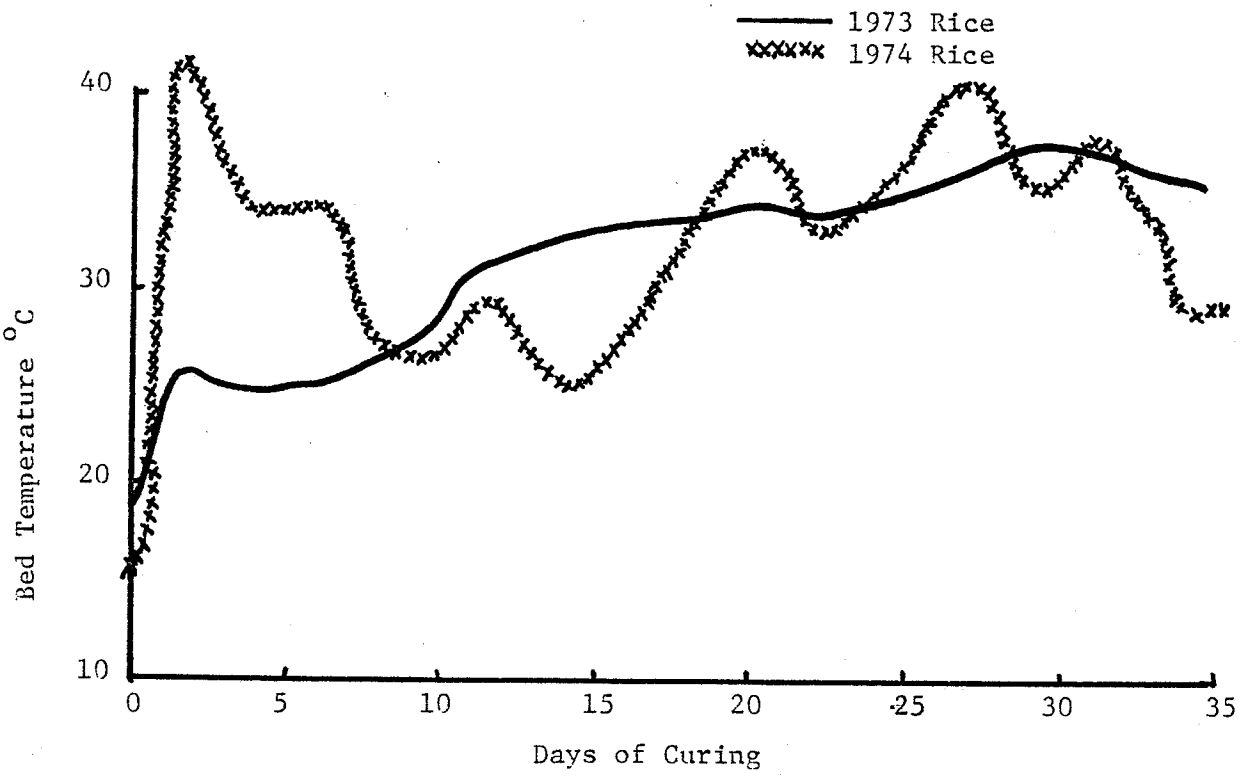


Figure 6. Rice Bed Temperature of Lake Wild Rice Cured at 32°C.

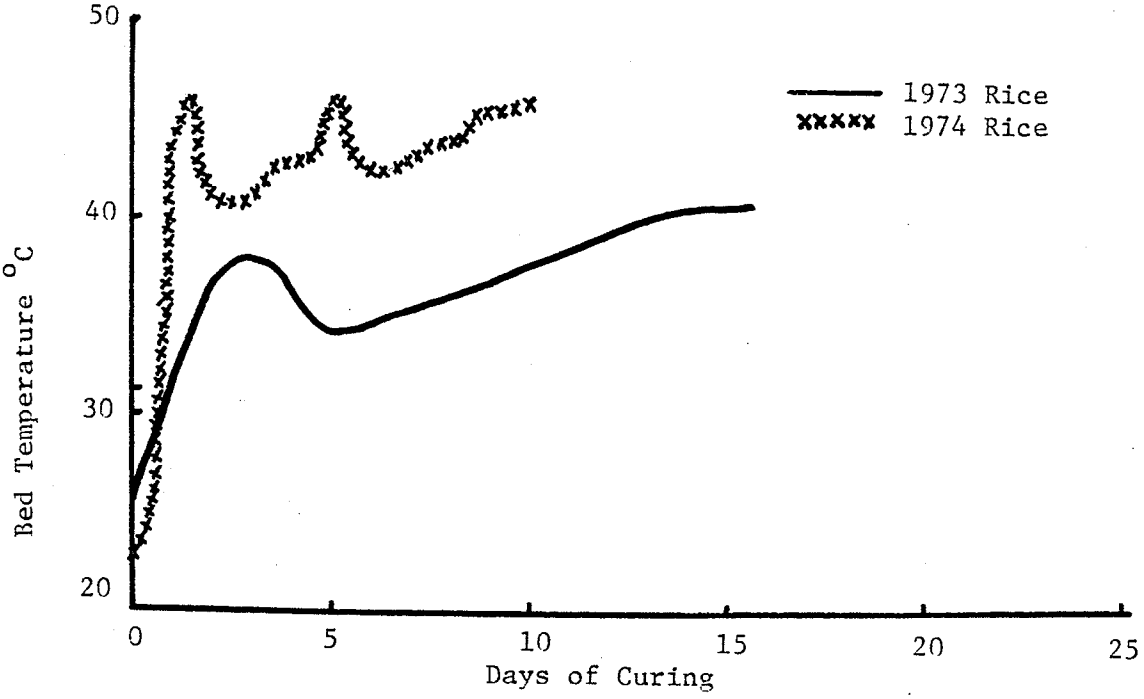


Figure 7. Rice Bed Temperature of Paddy Wild Rice Cured at 32°C.

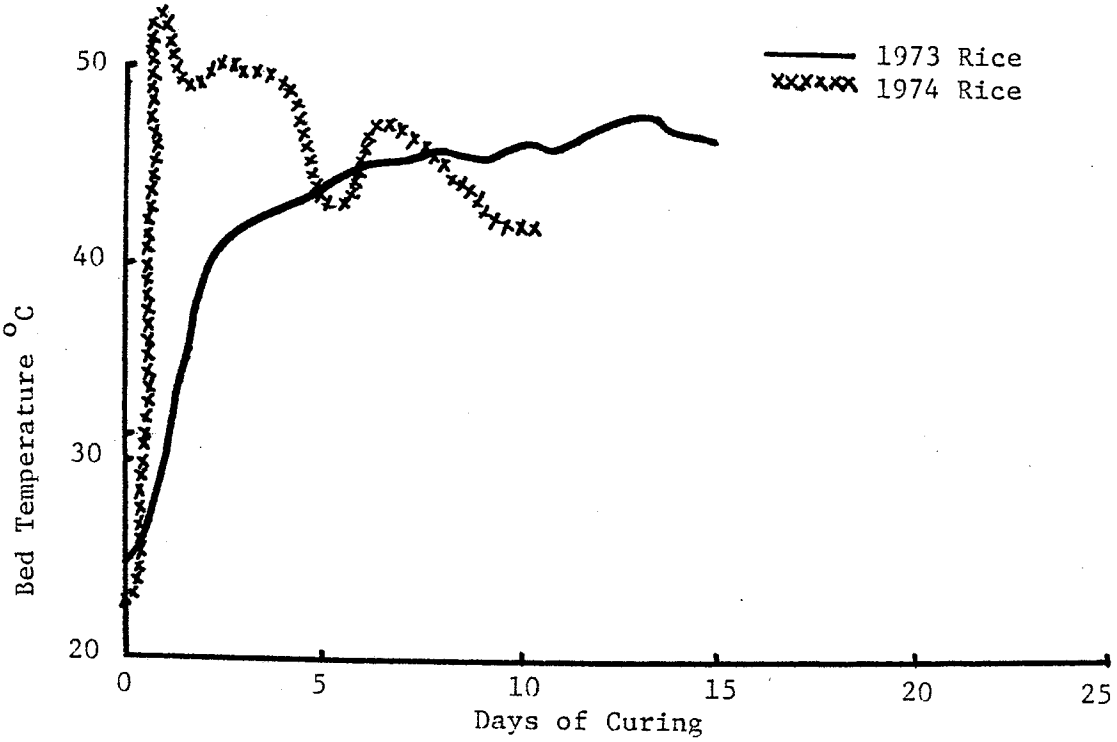
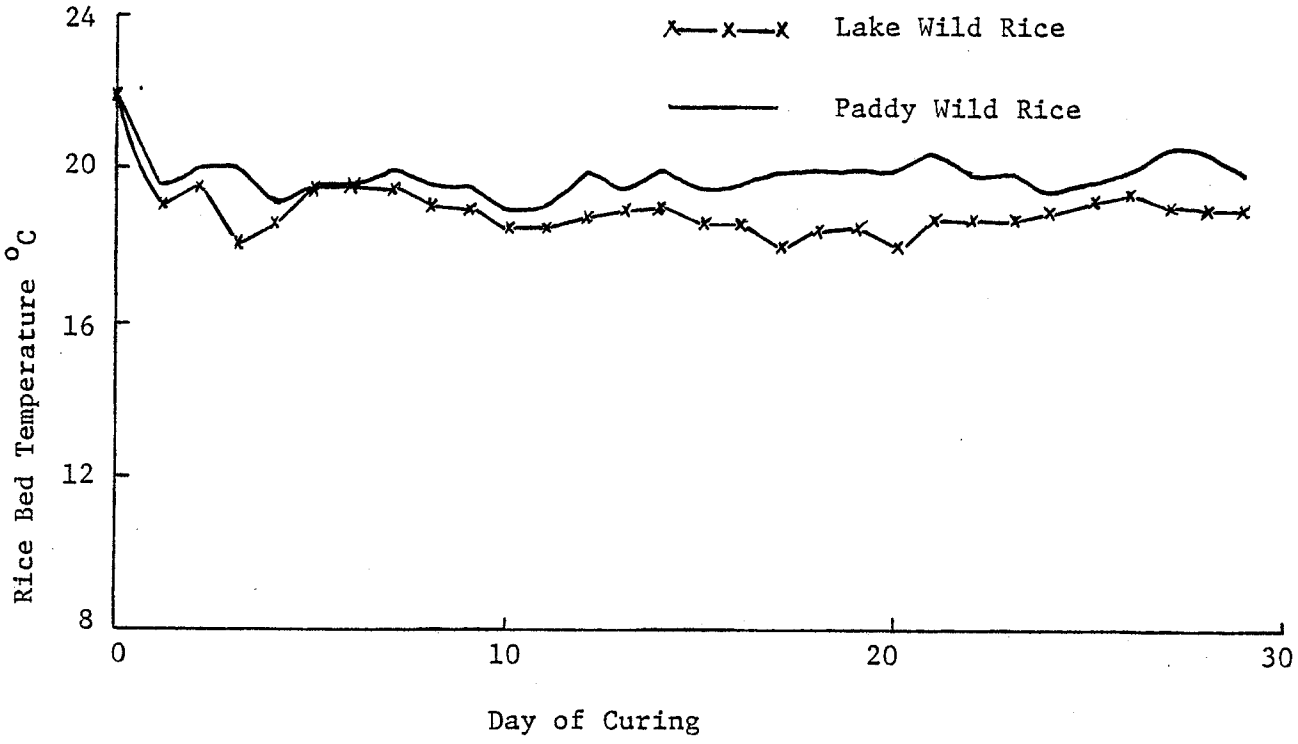


Figure 8. Rice Bed Temperature of 1974 Wild Rice Cured at 15°C.



(Figure 8). Finally in the 10°C curing treatment, the bed temperature did not increase, and the wild rice did not deteriorate in general (Figures 2,3). This also pointed out the importance of the curing environment in relation to bed temperature of the rice. From these results the higher the environmental temperature, the higher the bed temperature, and the faster deterioration occurred even though the rice was turned and watered regularly. Therefore only a low curing environmental temperature was able to control the bed temperature and the deterioration of the wild rice kernels. This again correlated well with Wisconsin's research (28,80).

Milner et al (56) also stated that the first increase in bed temperature of a grain was due to respiration and microbes (fungi) while the second temperature increase was due to biochemical causes. From the data presented in Section 4.5, it cannot be concluded that an increase in the rice bed temperature was due in part to microbial causes since there was no substantial increase in most microbial counts. Therefore it appeared to be due to mainly biochemical causes along with environmental causes, and the microorganisms apparently played only a minor role.

4.3 Taxonomy of Bacteria Isolated from Wild Rice

The types of bacteria isolated from kernels of lake and paddy wild rice harvested from different regions are listed in Tables 7,8,9 and 10. The great number of bacterial isolates obtained in these studies prevented the possibility of making specific identification to species so that only the genus is reported. In this study, it was impossible to differ-

entiate the numbers and kinds of microorganisms from environmental contamination versus the general microflora on the rice kernels.

The gram negative rods were the predominant bacteria isolated from green and cured wild rice (Tables 7 to 10) which correlated with research by Frank and Goel et al (12). For green paddy wild rice, Pseudomonas spp. were isolated most often with Flavobacterium spp. being the next predominant type of bacteria (Table 7). These two types of bacteria are followed by Acinetobacter spp., Arthrobacter spp. and Erwinia spp. Single isolates of Cytophaga spp., Bacillus spp., Citrobacter spp., Hafnia spp. and Enterobacter spp. were also identified.

For green lake wild rice, the bacterial isolates of Pseudomonas spp. and Enterobacter spp. predominated with Flavobacterium spp., Hafnia spp., Arthrobacter spp. and Erwinia spp. following (Table 8). Only single isolates of Streptococcus spp., Acinetobacter spp., Bacillus spp. and Azotobacter spp. were identified. The high number of Enterobacter spp. found on these kernels indicated that some outside contamination of the kernels had occurred.

For cured samples of lake wild rice, Pseudomonas spp. were dominant with other bacterial isolates following in order of dominance: Erwinia spp., Corynebacterium spp., Arthrobacter spp., Bacillus spp. and Escherichia spp. (Table 9). Also, there were single isolates of Flavobacterium spp., Cellulomonas spp., Proteus spp., and Alcaligenes spp. As noted at bottom of Table 9, the Alcaligenes spp. used to be called Achromobacter spp. (12).

The reason for the next set of bacterial isolates was that there was a high population of yellow-orange colored bacteria on the plates,

Table 7

Bacteria Isolated from Green Paddy Wild Rice

Type of Bacteria	No. of specific types/total isolates
Gram negative rods	34/35
Gram positive rods	1/35
Gram positive cocci	0/35
Psuedomonas spp.	17/35
Cytophaga spp.	1/35
Erwinia spp.	2/35
Flavobacterium spp.	6/35
Bacillus spp.(c)	1/35
Citrobacter spp.	1/35
Arthrobacter spp. (b)	2/35
Acinetobacter spp. (a)	3/35
Hafnia spp.	1/35
Enterobacter spp.	1/35

(a) It is now classified as Acinetobacter spp. instead of nonmotile Achromobacter spp.due to motility test in Bergey's Manual.

(b) They are pale brownish white colored and watery looking.

(c) It is a flat dirty white opaque colony with a hairy like edge.

Table 8

Bacteria Isolated from Green Lake Wild Rice

Type of bacteria	No. of specific type/total isolates
Gram negative rods	31/33
Gram positive rods	1/33
Gram positive cocci	1/33
Enterobacter spp.	7/33
Arthrobacter spp. (b)	2/33
Hafnia spp.	3/33
Pseudomonas spp.	12/33
Flavobacterium spp.	3/33
Streptococcus spp.	1/33
Erwinia spp.	2/33
Acinetobacter spp. (a)	1/33
Bacillus spp.	1/33
Azotobacter spp. (c)	1/33

(a) It is now classified as Acinetobacter spp. instead of nonmotile Achromobacter spp. due to motility test in Bergey's Manual.

(b) They are light pinkish white and watery looking.

(c) It is a very gummy dirty white colored colony but possibly it is another type of bacteria.

Table 9

Bacteria Isolated from Lake Wild Rice Cured under Ambient Conditions

Type of bacteria	No. of specific type/total isolates
Gram negative rods	24/27
Gram positive rods	2/27
Gram positive cocci	1/27
Psuedomonas spp.	9/27
Bacillus spp.	2/27
Arthrobacter spp. (c)	3/27
Escherichia spp.	2/27
Flavobacterium spp.	1/27
Erwinia spp.	4/27
Corynebacterium spp. (b)	3/27
Cellulomonas spp.	1/27
Protens spp.	1/27
Acaligenes spp. (a)	1/27

(a) It is now classified as a Acaligenes spp. instead of motile Achromobacter spp. due to motility test in Bergey's Manual.

(b) Two of them are pinkish colored and watery looking. One is golden peaked and large gram positive cocci.

(c) They are pale brownish white colored and watery looking.

Table 10

Bacteria Isolated from Green Wild Rice Having Different Morphological Characteristics

Type of bacteria	No. of specific type/total isolates
Gram negative rods	32/34
Gram positive rods	2/34
Gram positive cocci	0/34
Cytophaga spp. (e)	13/34
Erwinia spp.	1/34
Acaligenes spp. (a)	2/34
Pseudomonas spp.	8/34
Azotobacter spp. (d)	1/34
Beijerinkia spp. (b)	3/34
Cellulomonas spp. (c)	1/34
Kurthia spp.	1/34
Chromobacterium spp.	1/34
Flavobacterium spp.	3/34

(a) Appear to be Acaligenes spp. but close to Flavobacterium spp.

(b) These isolates appear to be Beijerinkia spp. but they may not be also.

(c) White colored Cellulomonas spp. and inert in Hugh-Liefson medium

(d) Brown colored

(e) Some of isolates are very close to being called Flavobacterium spp. instead of Cytophaga spp.

and in the random isolation of bacteria from the plates, they were discriminated against in sampling. In order to correct this and to obtain a better spectrum of bacteria found on wild rice kernels, the various yellow colored bacteria showing different morphological characteristics were isolated. At the same time, other bacteria showing different morphological characteristics were also isolated for the above reasons. The predominant yellow-orange colored bacteria were Cytophaga spp. and Pseudomonas spp. with Flavobacterium spp. and Alcaligenes spp. following, and there was also one isolate of Erwinia spp. (Table 10). Some of these isolates could have been called Cytophaga or Flavobacterium because the identification scheme in Bergey's Manual was very vague on differentiating the nonmotile forms of these two bacteria. Other types of bacteria isolated were Azotobacter spp., Beijerinkia spp., white Cellulomonas spp., Kurthia spp. and a purple Chromobacterium spp.

In comparison with Wisconsin's research (32,35), they found that Pseudomonas spp., Achromobacter spp. and Flavobacterium spp. were the predominant bacteria on wild rice kernels while in these studies Pseudomonas spp. were the most predominant with Flavobacterium spp., Enterobacter spp., Erwinia spp. and Cytophaga spp. alternating at being the next predominant genera (Tables 7-10). From the data presented in Table 9, if the Arthrobacter spp. had been classified as coryneforms, then the coryneform type bacteria would be the second most predominant bacteria in this isolation trial. Also, no Micrococcus spp. were found on these rice kernels as compared with other research on grain (40,45,54,60). The number of isolates of Corynebacterium spp., Enterobacter spp. and Escherichia spp. correlated well with Frank's research but he did not

detect any Arthrobacter spp. This may be because he called all his coryneforms, Corynebacterium spp. (32). Only two different species of Cellulomonas were isolated compared to six different species isolated by Frank (32). The number of isolates of Bacillus and Proteus correlated well with work by Goel et al. but they discovered more Streptococcus spp. (35). Also, no isolates of Lactobacillus, Leuconostoc and Staphylococcus were found in this research (35,60). Wisconsin's research did not find isolates of Cytophoga, Erwinia, Citrobacter, Azotobacter Hafnia, Chromobacterium and Kurthia (32,35). But other researchers have found these isolates listed above on other kinds of grain (36, 40,43,45,49,54,55,60,72,77). This difference in types of bacteria isolated by each researcher is expected since successive qualitative investigations invariably reveal the presence of previously unreported microflora on grain kernels, especially if they are from different regions.

Since Pseudomonas spp. were the most predominant bacteria on wild rice kernels, they were grouped into four separate groups (Table 11)(67). This served the partial function of classifying these bacteria down to species. Most of the Pseudomonas spp. fall into group II, closely followed by group I. Only a few isolates fell into group III or IV. Due to the identification scheme followed by Frank, no correlation for the types of Pseudomonas could be made (32).

To find out which of the yellow-orange colored bacteria predominated on wild rice, they were grouped into a separate table (Table 12). On green lake and paddy wild rice, Flavobacterium spp. were dominant while

Table 11

A Grouping of Pseudomonas spp. Isolated from Wild Rice (a)

	Type of Groups of Pseudomonas spp			
	I	II	III	IV
Source of isolates	No. of specific types/total No.			
Cured lake wild rice	3/9	4/9	0/9	2/9
Green paddy wild rice	7/17	9/17	1/17	0/17
Green lake wild rice	3/12	6/12	2/12	1/12
Different morphological run (b)	2/8	5/8	1/8	0/8

(a) See material and methods for details on this grouping scheme

(b) Bacterial isolates from the different morphological characteristics isolation.

Table 12

A Grouping of Yellow-Orange Colored Bacterial Isolates from Wild Rice

Source of isolates	Type of bacteria	No. specific type/total no.
Cured lake	Erwinia spp.	1/6
	Flavobacterium spp.	1/6
	Cytophaga spp.	0/6
	Psuedomonas spp.	3/6
	Cellulomonas spp.	1/6
Green Paddy	Flavobacterium spp.	6/9
	Cytophaga spp.	1/9
	Psuedomonas spp.	1/9
	Erwinia spp.	1/9
	Cellulomonas spp.	0/9
Green Lake	Flavobacterium spp.	3/3
	Cytophaga spp.	0/3
	Pseudomonas spp.	0/3
	Erwinia spp.	0/3
	Cellulomonas spp.	0/3
Different morphological characteristic run	Cytophaga spp.	13/21
	Flavobacterium spp.	3/21
	Erwinia spp.	1/21
	Pseudomonas spp.	4/21
	Cellulomonas spp.	0/21

on cured lake wild rice, Pseudomonas spp. were dominant. For the special isolation trial for yellow colored bacteria, Cytophaga spp. were dominant with Flavobacterium spp. and Pseudomonas spp. following. But this was a bias isolation setup so that no definite conclusions can be drawn from it. In general, by identification of isolates and visual detection on count plates, all three types of yellow colored bacteria were equally predominant and varied from one lot of wild rice to another depending on type of wild rice and source location plus the processing method used for the rice.

4.4 Taxonomy of Molds Isolated from Wild Rice

Seventeen different species of molds were isolated from wild rice kernels (Figures 9 to 28). The numbers and types of molds on each sample of wild rice varied. The following kinds of molds were identified from these isolates:

1. Fusarium spp. was a dark yellowish brown hairy colony as a young culture which became a dark reddish color for an old culture (Figures 9 and 10).
2. Alternaria spp. was dark blackish brown bottom mat with a dirty tuff-like mat on top (Figures 11 and 12).
3. Fusarium spp. was a white hairy colony for young culture and became a light pink for an old culture (Figures 11 and 13). The identification of this mold was questionable since it did not precisely fit any classification.
4. Aspergillus spp. developed black aerial condia (Figures 14 and 15).

Figure 9. Fusarium spp., on right: young culture, on left: old culture.

Figure 10. Diagram of Fusarium spp.

(Adapted from 37)

Figure 11. Left plate: *Alternaria* spp., and
Right plate: *Fusarium* spp.

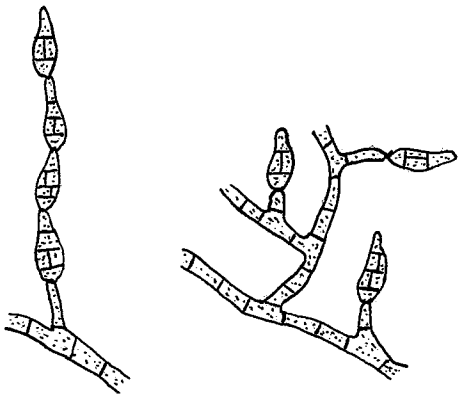
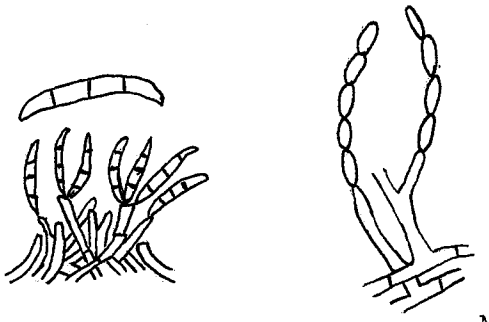


Figure 12. Diagram of Alternaria spp.
(Adapted from 37)



Moniliform

Figure 13. Diagram of Fusarium spp.
(Adapted from 74)

5. Cladosporium spp. was a dark olive green, powdery culture (Figures 14 and 16).

6. Penicillium spp. was sky blue in color and gave yellow tints to media around the colony (Figures 17 and 18).

7. Trichoderma spp. started as fluffy white colonies and became dark green in color (Figures 17 and 19).

8. Mucor spp. was a white hairy culture with a yellow base (Figures 20 and 21).

9. Rhizopus spp. was a white hairy culture with black sporangia (Figures 20 and 22).

10. Fusarium spp. started as pinkish purple culture with pink hairy hyphae and then became dark purple along with media becoming dark purple (Figure 23).

11. Fusarium spp. started as pinkish purple culture with white hairy hyphae and then became dark purple but the media was not tinted (Figure 24).

12. Aspergillus spp. had lime green aerial condia (Figure 25).

13. Mucor spp. was a short hyphae culture with black sporangia (Figure 25).

14. Aspergillus spp. had dark bluish green condia and gave greenish tint to the media which faded away as the plate aged (Figure 26).

15. Aspergillus spp. had dark tourquoise condia (Figure 26).

16. Penicillium spp. started as a velvety, white, powdery culture and became bluish grey in color (Figure 27).

17. Byssochlamys spp. was a dark gold (tawny), powdery culture (Figures 27 and 28).

Figure 14. Left plate: Aspergillus spp., and
Right plate: Cladosporium spp.

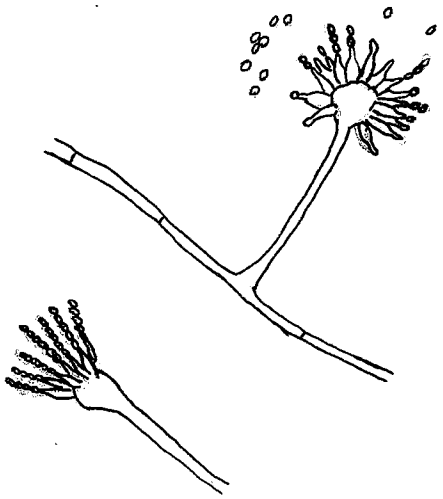


Figure 15. Diagram of Aspergillus spp.
(Adapted from 37)

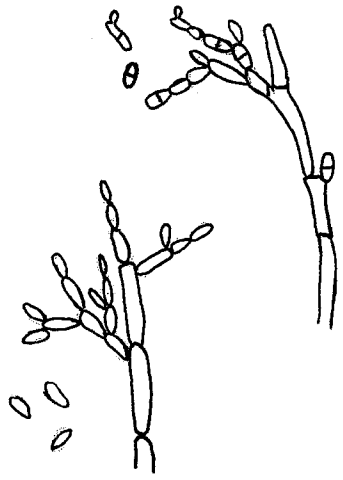


Figure 16. Diagram of Cladosporium spp.
(Adapted from 37)

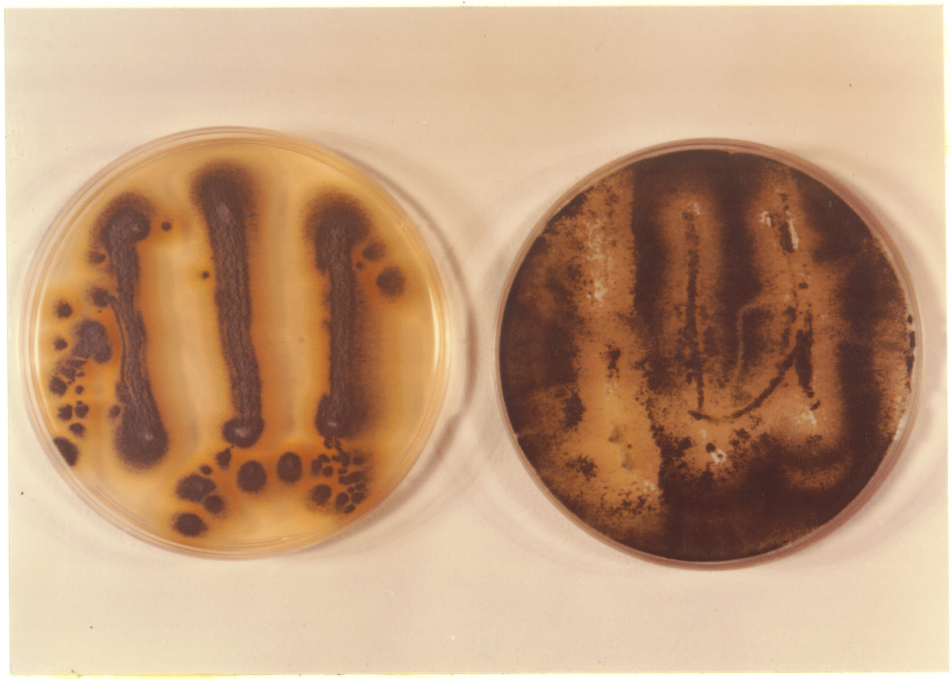


Figure 17. Left plate: *Penicillium* spp., and
Right plate: *Trichoderma* spp.

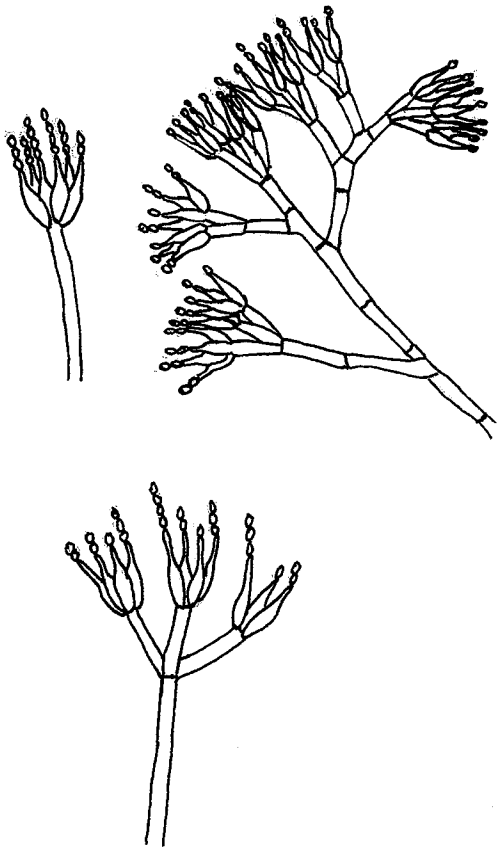


Figure 18. Diagram of Penicillium spp.

(Adapted from 37)

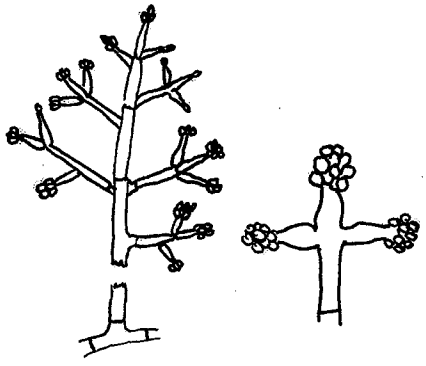


Figure 19. Diagram of Trichoderma spp.

(Adapted from 74)

Figure 20. Left plate: *Mucor* spp., and
Right plate: *Rhizopus* spp.

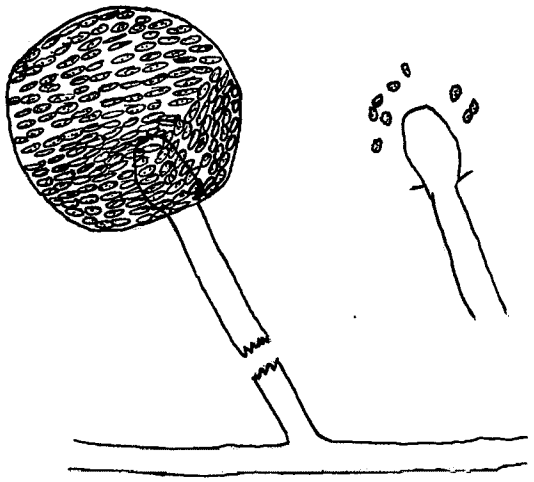


Figure 21. Diagram of Mucor spp.
(Adapted from 37)

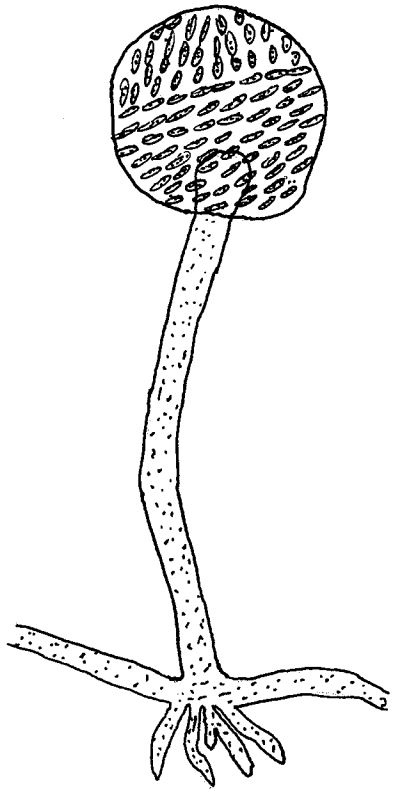


Figure 22. Diagram of Rhizopus spp.
(Adapted from 37)

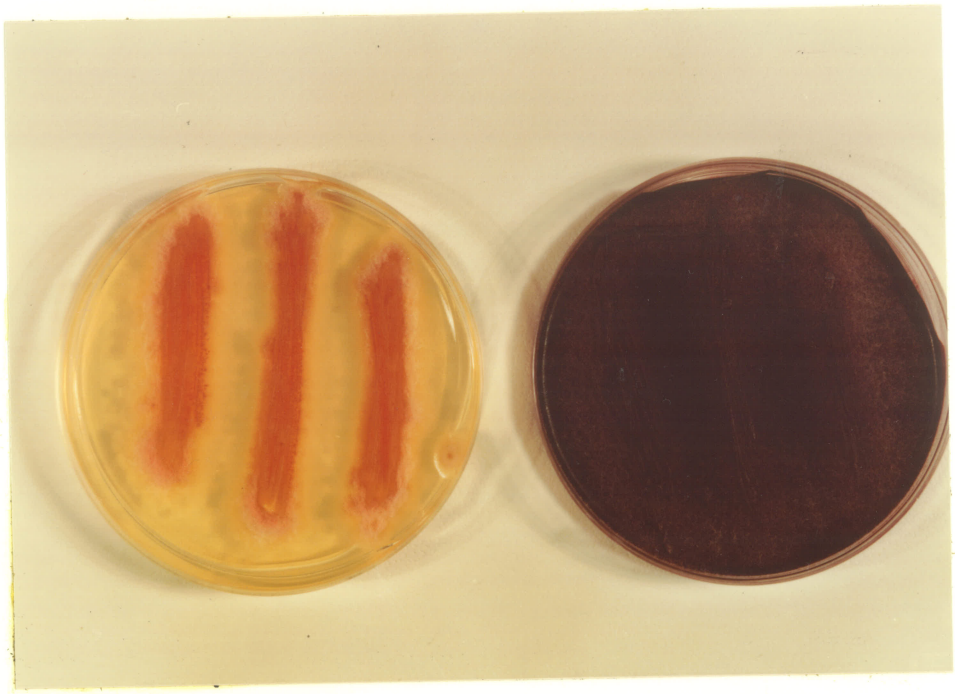


Figure 23. Fusarium spp., left plate: young culture and right plate: old culture; (see figure 10 for diagram).

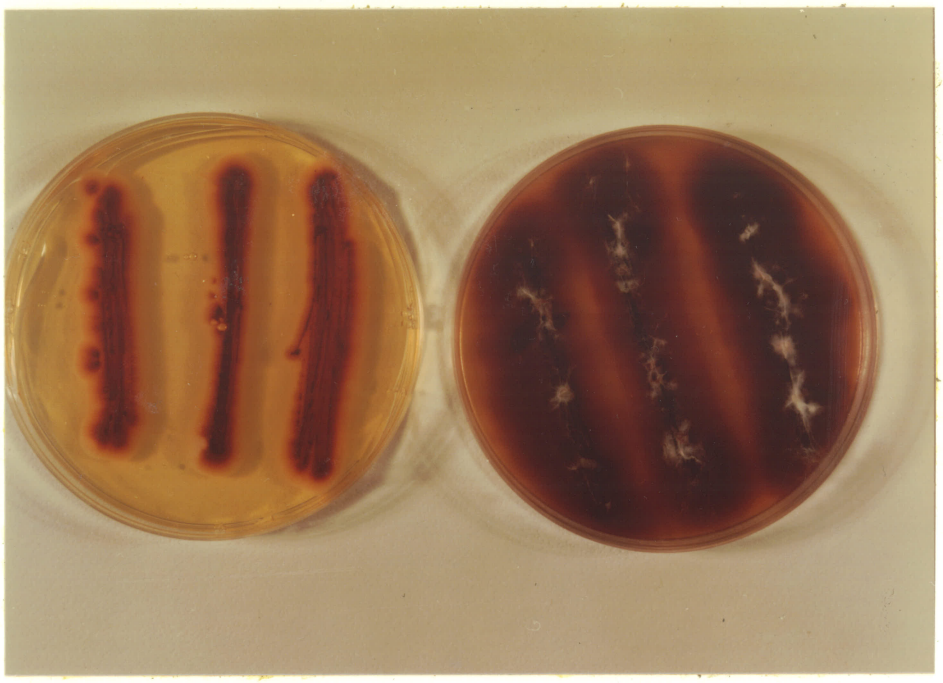


Figure 24. Fusarium spp., left plate: young culture and right plate: old culture (see figure 10 for diagram).

Figure 25. Left plate: Mucor spp., (see figure 21 for diagram) and right plate: Aspergillus spp. (see figure 15 for diagram).

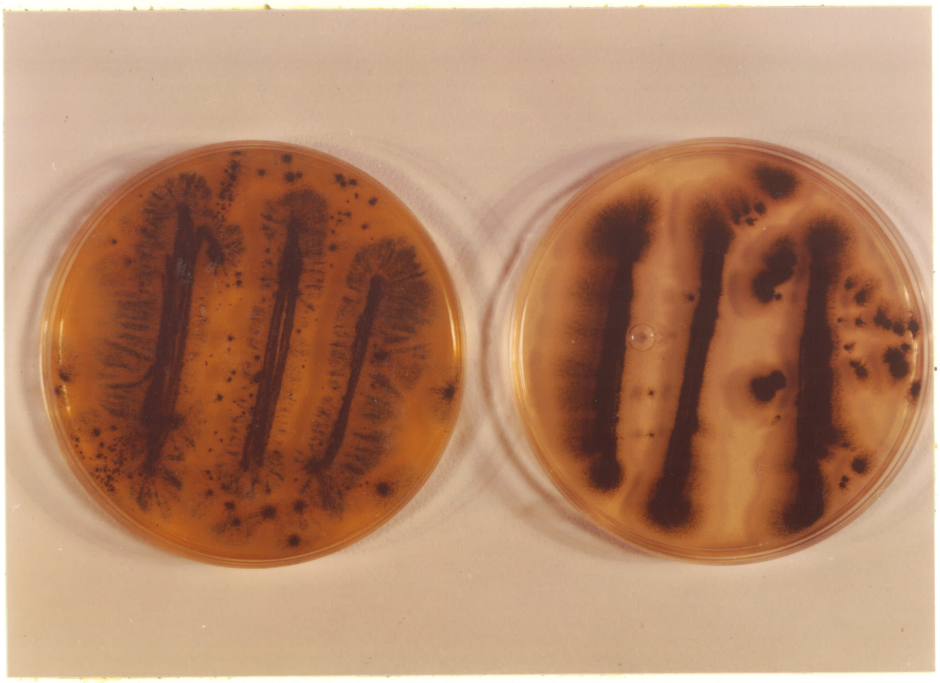


Figure 26. Left plate: blue green Aspergillus spp. and Right plate: tourquoise Aspergillus spp. (see figure 15 for diagram of both molds).

Goel et al. (35) found only Mucor spp., Aspergillus spp., Rhizopus spp. and Penicillium spp. on their wild rice kernels in comparison with the above. This difference was expected due to their source location for wild rice, and again each examination of microflora on wild rice grain would usually give a different microflora spectrum The major difference in comparing the data between Manitoba and Wisconsin was that a high population of Fusarium spp. was discovered on Manitoba wild rice. It was also possible to detect visually Fusarium infection of the wild rice kernels by the appearance of reddish colored kernels. This Fusarium infection occurred while the plant was growing and was mainly due to the Fusarium spp. illustrated in Figure 9.

On green lake wild rice obtained from Fort Alexander, Manitoba, the major types of molds isolated were Rhizopus spp., hairy yellow Mucor spp., blue green Aspergillus spp. and followed by hairy yellowish brown Fusarium spp. There was also a few blue Penicillium spp., black Aspergillus spp. and pink Fusarium spp. On green paddy wild rice from the same place, the main types of molds isolated were Rhizopus spp., hairy yellow Mucor spp., blue Penicillium spp., blue green Aspergillus spp., and followed by the Cladosporium spp. There were a few lime green Aspergillus spp. and hairy yellowish brown Fusarium spp. On cured lake wild rice kernels, the major molds isolated were blue green Aspergillus spp., followed by a few to very few blue Penicillium spp., Rhizopus spp., black Aspergillus spp., green Trichoderma spp., pink Fusarium spp. and hairy yellowish brown Fusarium spp. The above information correlated well with literature on grain storage and

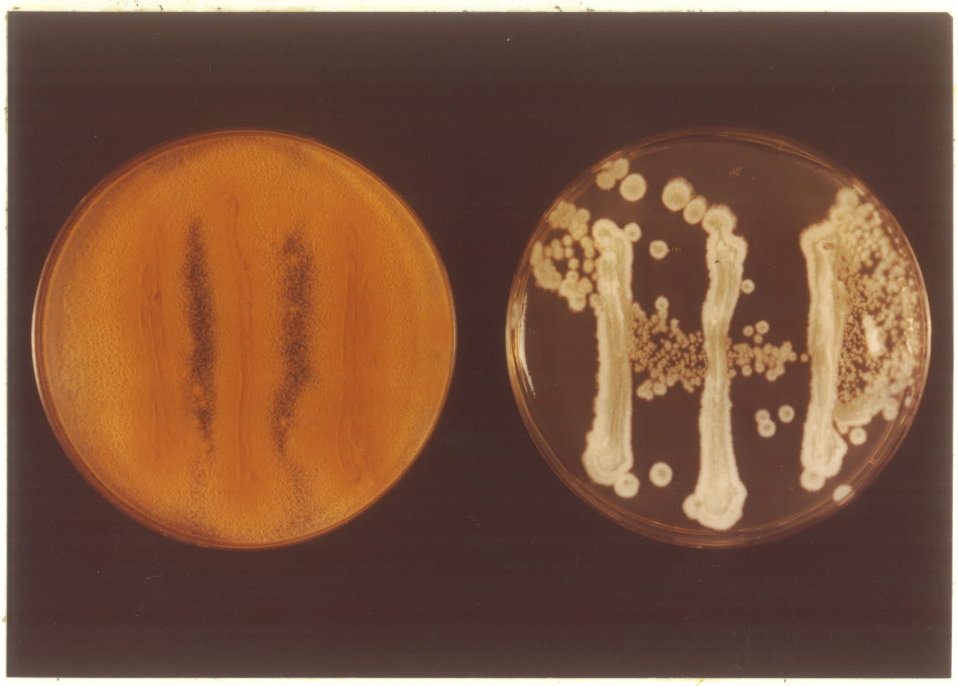


Figure 27. Left plate: Byssochlamys spp., and Right plate: Penicillium spp. (see figure 18 for diagram).

Figure 28. Diagram of Byssochlamys spp.

(Adapted from 37)

the types of molds that would appear (21,22,53,55).

The predominant molds isolated from green lake wild rice obtained from Eileen Lake, Manitoba, were low black Mucor spp. and blue green Aspergillus spp. followed in decreasing order by Rhizopus spp., blue Penicillium spp. and green Trichoderma spp. For green paddy rice obtained from Sprague, Manitoba, the major ones isolated were Alternaria spp., Cladosporium spp., hairy yellow Mucor spp. and hairy yellowish brown Fusarium spp. followed by a few Rhizopus spp., low black Mucor spp., green Trichoderma spp., blue green Aspergillus spp. and pink Fusarium spp. On Shallow Lake's wild rice, blue green Aspergillus spp., Rhizopus spp., hairy yellow Mucor spp. and Cladosporium spp. were the predominant molds while there was a few pink Fusarium spp. and hairy yellowish brown Fusarium spp. For the first lot of wild rice obtained from Harrop Lake, the low black Mucor spp. and blue green Aspergillus spp. were predominant followed by Cladosporium spp., Rhizopus spp., hairy yellowish brown Fusarium spp. and Alternaria spp. Then on the second lot of wild rice, the dominant fungi were the Alternaria spp. followed by Cladosporium spp., yellow hairy Mucor spp. and hairy yellowish brown Fusarium spp. These observations indicate the variation in types of molds that can be isolated from different lots of rice harvested from the same region. For the final lot of lake rice received from LaRonge, Saskatchewan, the hairy yellow Mucor spp. was dominant followed by low black Mucor spp. and Rhizopus spp. The above taxonomy for molds on wild rice was in accordance with other research on grain having high moisture content since harvested wild rice had a moisture content above 30 percent (15, 20, 27, 31, 40, 44, 61, 83, 85).

4.5 Analyses of Microbial Counts on Wild Rice

4.5.1 Preliminary microbial counts on wild rice

Data from the preliminary studies (Tables 13-14) indicated that the total microbial population on wild rice was not excessive but contained high numbers for each common group of microorganisms. The total mesophilic aerobic plate count for 1973 wild rice was in the range of 10^7 to 10^8 per 1 gram sample while the mold count was in the range of 10^4 to 10^6 per 1 gram sample (Table 13). For the 1974 wild rice the total mesophilic aerobic plate count ranged from 10^5 to 10^7 per 1 gram sample, the mold count from 10^3 to 10^5 per 1 gram, the yeast count from 10^4 to 10^5 per 1 gram, psychrotrophic count from 10^6 to 10^7 per 1 gram, and aerobic spore count from 10^3 to 10^5 per 1 gram (Table 14). These high numbers for a variety of microorganisms are expected when the condition of the wild rice itself, and its environmental conditions during growth, harvesting and curing are considered. This data correlated well with Goel et al.'s results within one log cycle difference (34).

4.5.2 Microbial analyses of curing wild rice

5.4.2.1 Microbial analyses of wild rice curing at 32°C

Prior to curing lake wild rice at 32°C, the types of mold present on the rice were blue green *Aspergillus* spp. and low black *Mucor* spp. along with various yellow, orange, white and grey colored bacteria. After 3 days of curing, the above types of mold were still present plus a small population of lime green *Aspergillus* spp., black *Aspergillus* spp. and grey *Penicillium* spp. Also bacilli type bacteria appeared on

Table 13

Preliminary Microorganism Counts on Wild Rice (1973)

A. Green Lake Wild Rice (Fort Alexander)

Trials	Bacteria	Mold
	Ave. count/1 g sample	Ave. count/1 g sample
1	42×10^8	79×10^4
2	32×10^8	100×10^4
3	35×10^8	95×10^4
4	46×10^8	120×10^4
5	46×10^8	25×10^5

Range 72×10^7 - 51×10^8

No yeast count due to mold overgrowth, but count is around 10^4/1 g wild rice.

B. Green Paddy Wild Rice (Fort Alexander)

Trials	Bacteria	Mold
	Ave. count/1 g sample	Ave. count/1 g sample
1	102×10^8	12×10^5
2	93×10^8	14×10^5
3	93×10^8	8×10^5
4	108×10^8	11×10^5
5	118×10^8	10×10^5

Range 129×10^7 - 156×10^8

No yeast count due to mold overgrowth, but the count is around 10^5/1 g wild rice.

Table 13 (continued)

C. Cured Lake Wild Rice (Northland)

Trials	Bacteria	Mold
	Ave. count/1 g sample	Ave. count/1 g sample
1	107×10^8	15×10^6
2	141×10^8	93×10^6
3	104×10^8	74×10^6
4	105×10^8	43×10^6
5	148×10^8	47×10^6

Range 91 - 166 x 10^8

No yeast count due to mold overgrowth but the count is around 10^5 - 10^6/1 g wild rice.

Table 14

Microbiological Counts on 1974 Wild Rice from Manitoba and Saskatchewan (Ave. count/1 g sample)

	Types and Source of Wild Rice				
	Lake Wild Rice				Paddy Wild Rice
Microbiological test	Eileen Lake	Shallow Lake	Harrop Lake	LaRonge	Sprague
Total plate count	57.5×10^7	65.5×10^7	70.3×10^6	63×10^6	30.3×10^7
Range	$43\text{-}83 \times 10^7$	$55\text{-}75 \times 10^7$	$23\text{-}102 \times 10^6$	$176 \times 10^5\text{-}138 \times 10^6$	$73 \times 10^6\text{-}56 \times 10^7$
Mold count	55×10^4	41.3×10^5	184.3×10^4	133.2×10^3	50.1×10^4
Range	$47\text{-}68 \times 10^4$	$36\text{-}48 \times 10^5$	$113\text{-}243 \times 10^4$	$118\text{-}153 \times 10^3$	$11\text{-}91 \times 10^4$
Yeast count	95×10^5	27.5×10^5	66.8×10^5	56.5×10^5	27.2×10^5
Range	$74\text{-}111 \times 10^5$	$18\text{-}34 \times 10^5$	$49\text{-}83 \times 10^5$	$43\text{-}69 \times 10^5$	$29 \times 10^4\text{-}58 \times 10^5$
Psychrotrophs	129×10^6	45×10^7	72.7×10^6	61.7×10^6	24.9×10^7
Range	$78\text{-}153 \times 10^6$	$31\text{-}61 \times 10^7$	$10\text{-}66 \times 10^6$	$21\text{-}129 \times 10^6$	$90 \times 10^6\text{-}43 \times 10^7$
Aerobic Spore count	78×10^5	214.5×10^4	65.7×10^4	33.5×10^3	18.9×10^4
Range	$50\text{-}107 \times 10^5$	$185\text{-}246 \times 10^4$	$31\text{-}98 \times 10^4$	$27\text{-}42 \times 10^3$	$5\text{-}32 \times 10^4$

the plates. After 6 days of curing, the types of mold were low black Mucor spp., blue green Aspergillus spp. and blue Penicillium spp. along with a small number of black Aspergillus spp. and lime green Aspergillus spp. There was more bacilli type bacteria present and fewer yellow colored bacteria. By the 9th day, the rice was completely moldy, and the types of mold remained the same except for the appearance of Rhizopus spp. The bacterial population had not changed during the three day interval. Therefore, there was some change in the types of mold and bacteria during curing over this short time period, but this change does not appear to have any significance.

During this curing trial the moisture content of wild rice remained above 35 percent as indicated in Chung's thesis (20). Therefore, all these types of mold would be field fungi since the literature stated that this group of molds generally appeared on high moisture grain while storage fungi did not due to competition (85). The possible reason for the apparent increase in bacilli or spore type bacteria in this trial could be due to the high temperature of the curing chamber and the rice bed.

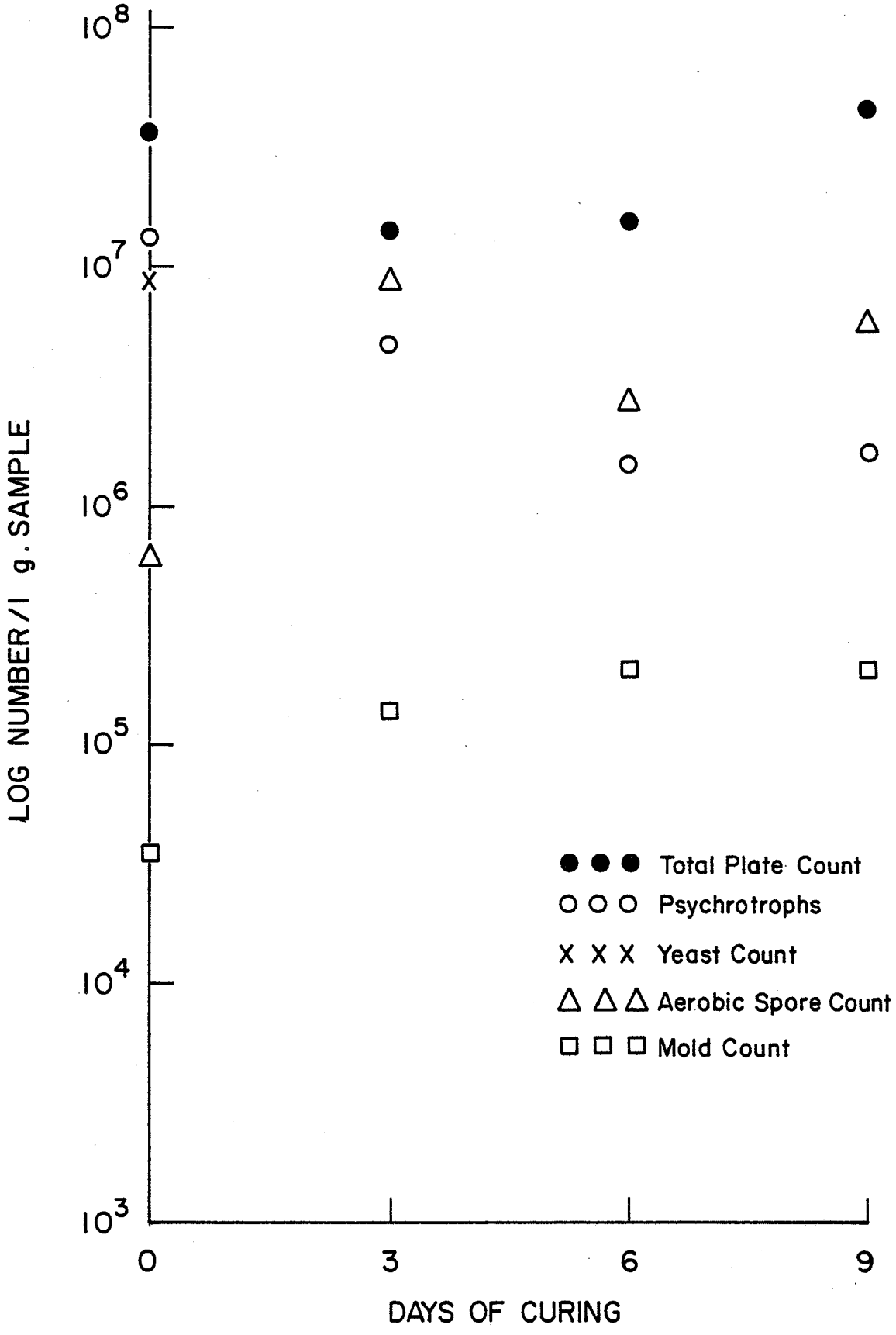
The total plate count for lake wild rice cured at 32°C remained static with a minor increase developing at the end of the curing period (Figure 29, Appendix 1). This result was not expected since a substantial increase in total plate count was anticipated under these conditions. A possible reason for this could be that aeration of the pile was insufficient producing a slightly anaerobic atmosphere in the pile preventing the growth of aerobic bacteria. Another reason was that

the high temperature of the rice pile produced a static population where growth equalled death. The psychrotrophs decreased and leveled off as expected. The yeasts were completely eliminated which was probably due to the high pile temperature. The mold count increased one log cycle and leveled off which was inconsistent with the very moldy appearance of the rice at the end of the curing period.

At the start of curing paddy wild rice at this temperature, the predominant molds were Alternaria spp., hairy yellow Mucor spp. and Cladosporium spp. There was an assortment of orange, yellow, cream, grey and white bacteria. After 4 days, the hairy yellow Mucor spp. and Alternaria spp. were still present, and blue green Aspergillus and low black Mucor spp. appeared. The bacterial population had not changed much except for the appearance of bacillus type bacteria. Ascomycetes were observed for the first time at 10^2 per 1 gram sample of rice. By the 8th day, ascomycetes had increased to 10^4 per 1 gram sample. The types of mold present were low black Mucor spp., hairy yellow Mucor spp. and a decreasing number of blue green Aspergillus spp. Therefore, there was some change in the types of molds during the curing of paddy rice since Alternaria spp. and Cladosporium spp. disappeared.

As mentioned before for curing lake wild rice under these conditions of high moisture content and relative humidity, these molds would belong to the field fungi group and not the storage fungi (85). There appeared to be little change in the types of bacteria during the curing period except for the appearance of bacilli type bacteria.

Figure 29. Microbial Counts on Lake Wild Rice Cured at 32°C and 95% R.H.

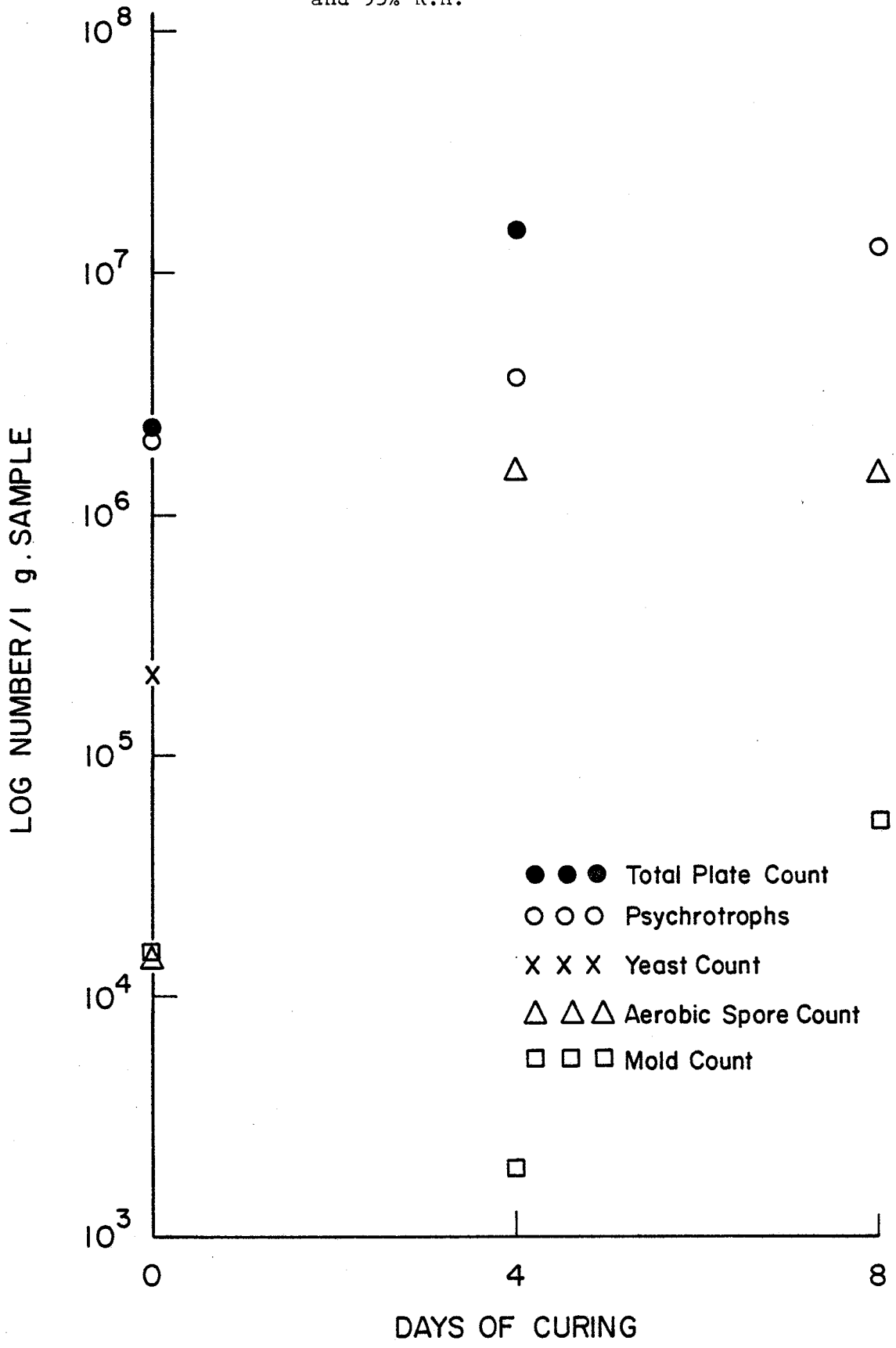


During the eight days of curing, the total plate count increased from 10^6 to 10^8 per 1 gram paddy rice which was expected (Figure 30, Appendix 2). But the psychrotrophs increased gradually when they should have decreased due to the high environmental temperatures. The yeasts were totally eliminated after the first week of curing since they are very sensitive to high temperatures. The mold count at first decreased and then increased one log cycle which was inconsistent with the moldy appearance of the rice. There should have been no decrease in the mold population, and there should have been a substantial increase. The decrease in molds could possibly be explained by their being sensitive to high temperatures initially and then becoming insensitive. The aerobic spore count increased two log cycles and leveled out which was probably due to the environmental temperatures which were high enough to cause this static condition.

4.5.2.2 Microbial analyses of wild rice curing at 21°C

With lake wild rice cured at 21°C, the following molds were detected initially: low black Mucor spp., blue green Aspergillus spp., Rhizopus spp., blue Penicillium spp. and Trichoderma spp. along with assorted types of orange, yellow, cream, grey and white colored bacteria. By the second week hairy yellow Mucor spp. and Alternaria spp. were added while the blue Penicillium spp. and Trichoderma spp. had disappeared. The bacterial population did not change. After the third week, the mold population had not changed but the yellow-orange colored bacteria had decreased. At the end of the fourth week, pink Fusarium spp. had been added to the mold population, and an ascomycete population of 10^4 per 1

Figure 30. Microbial Counts on Paddy Wild Rice Cured at 32°C and 95% R.H.



gram sample was detected for the first time. The type of bacteria had not changed much except that more bacillus type bacteria were detected visually.

During this curing trial, there were small changes in the microbial population but, in general, it was quite static. Again there was an assortment of field fungi on the rice at the start of curing. Then certain types of fungi increased slightly while others decreased slightly or remained constant during the curing period. There was no apparent reason for the mold situation, or for so little change in the bacterial population under these favorable conditions.

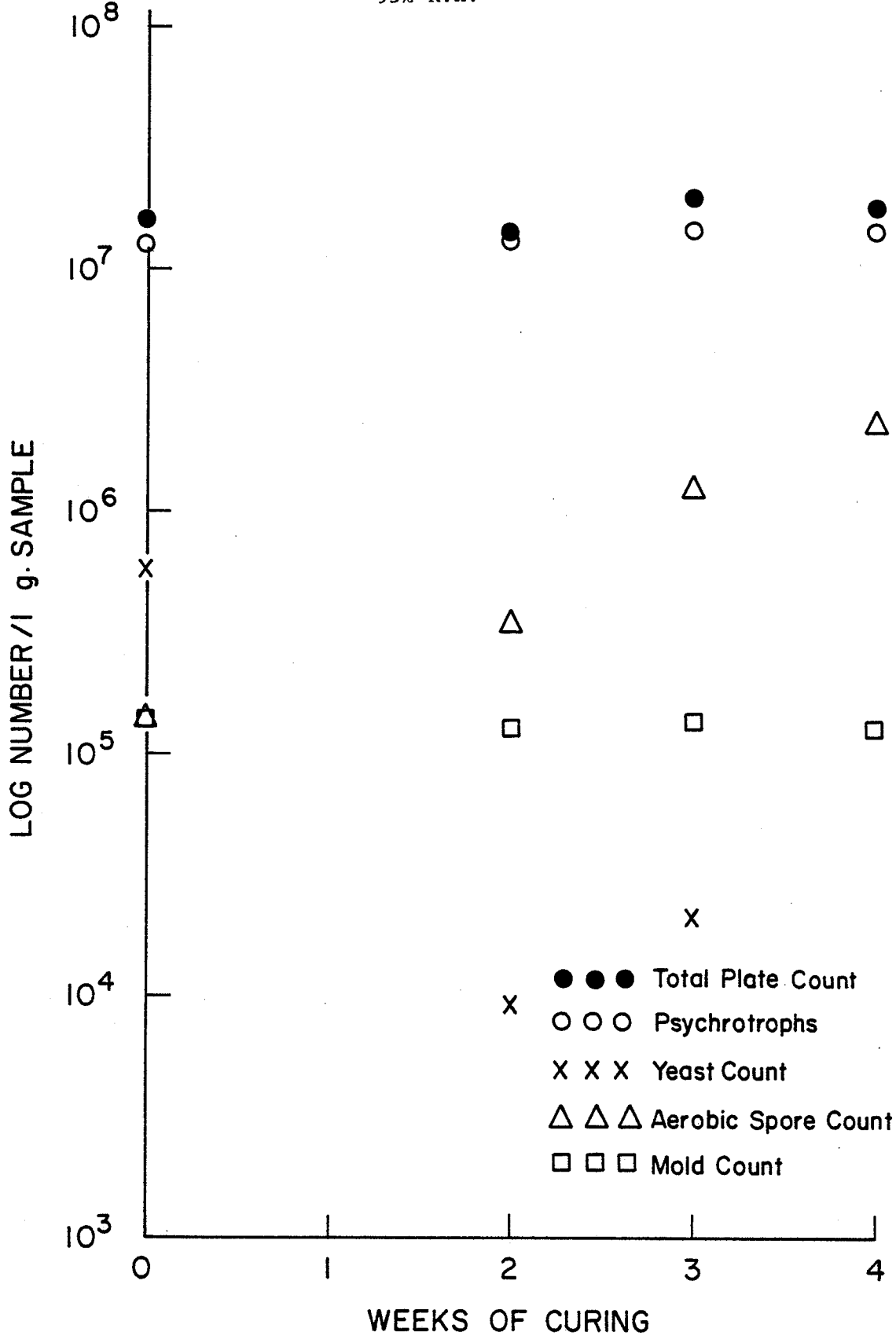
The total plate count and psychrotrophs remained static throughout the entire curing period which was strange under these conditions (Figure 31, Appendix 3). An increase in total plate count was expected by previous results in the literature (32, 35, 55, 80). Possible reasons for this could be inadequate aeration producing an anaerobic environment in the pile, or the pile temperature was high enough for a bactericidal effect. A decrease in the psychrotrophs should have occurred under these unfavorable conditions (32, 35, 80). No reasonable explanation is evident for these observations at this time. The yeast count decreased first and then started to recover. This indicated the pile temperature was not high enough to kill all the yeasts and they apparently were able to adapt to this environment. The mold count remained static during curing. This was inconsistent with the moldy appearance of the rice. Also some of the literature indicated there should be an

increase (32, 55), but other researchers found that the mold count was very erratic with great fluctuations up and down which they stated was due to variability of mold growth in different lots of rice (35, 80). There was a definite increase in aerobic spore count which was consistent with Frank's research and indicated the effect of bed temperatures (32).

The molds first detected in paddy rice cured at 21°C were Cladosporium spp., Alternaria spp., and Rhizopus spp. plus various orange-yellow, cream grey and white colored bacteria. This rice was immature (or appeared immature). After the first week, hairy yellow Mucor spp. and pink Fusarium spp. showed up while Cladosporium spp. decreased in number. The bacterial population had changed slightly; the bacillus or spore types had increased in numbers. After the second week, the pink Fusarium spp. increased while the hairy yellow Mucor spp. and Alternaria spp. decreased in numbers. The Cladosporium spp. were apparently eliminated and fewer yellow-orange colored bacteria were present. By the third week the paddy rice was sprouting and moldy. Two species of pink Fusarium spp. were the predominant molds while the rest of the molds occurred in lesser numbers. The types of bacteria did not change from the second week. Therefore there appeared to be only a slight change in the bacterial population in this curing trial which again was strange considering the environmental conditions. In the case of molds, there was no change from field to storage fungi which was anticipated due to high moisture content of the wild rice (40-50%) and high relative humidity of the curing environment as stated in Chung's thesis (20).

The reasons for little change in the microfora are not apparent.

Figure 31. Microbial Counts on Lake Wild Rice Cured at 21°C and 95% R.H.

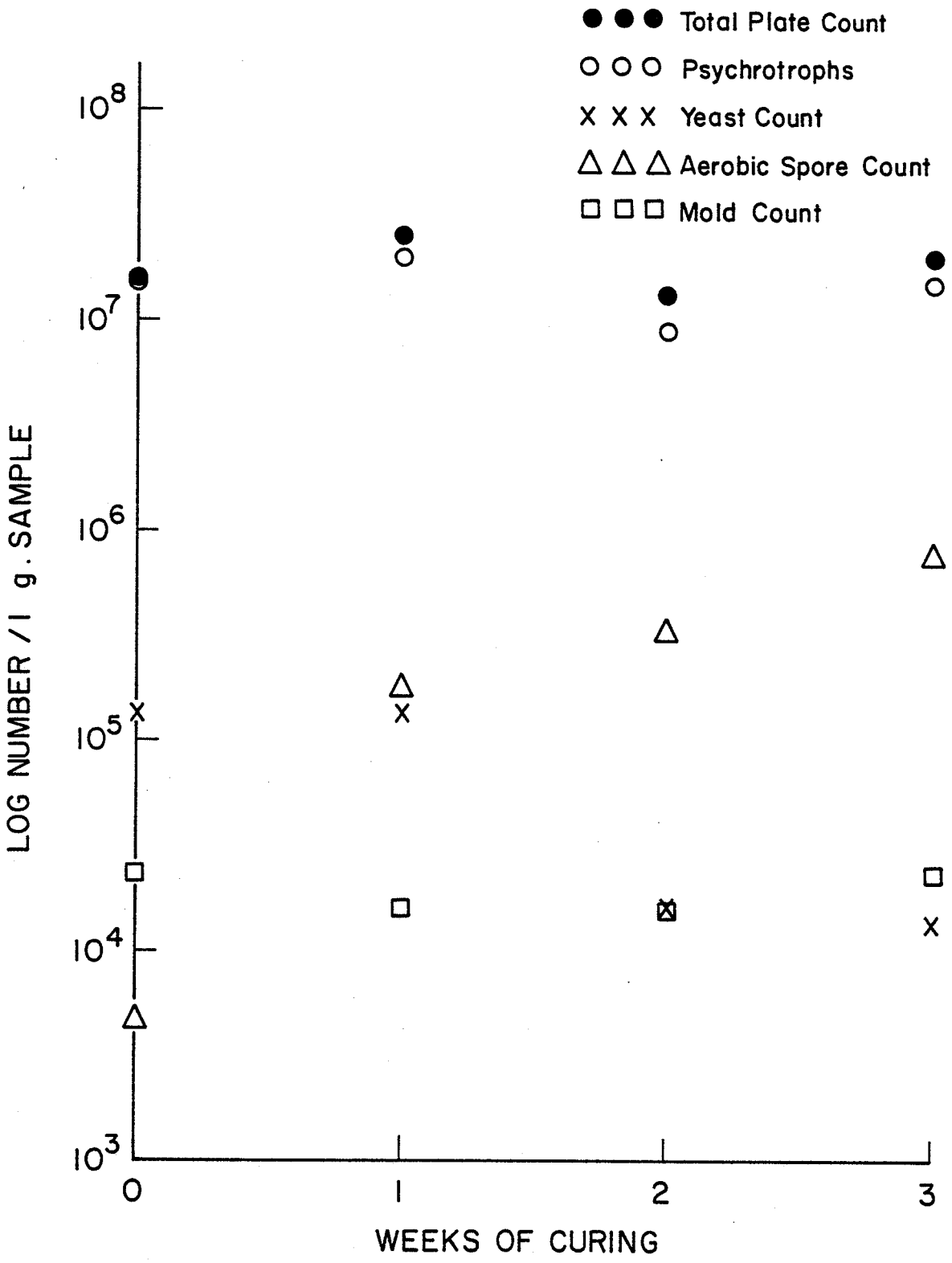


The trend of microbial counts for paddy wild rice was similar to that for the lake wild rice under the same curing conditions (Figure 32, Appendix 4). Therefore, its discussion will not be repeated since it would be the same as above.

4.5.2.3 Microbial analyses of wild rice curing at $15^{o}C$

The types of molds detected first in lake wild rice cured at $15^{o}C$ were as follows: Alternaria spp., hairy yellowish brown Fusarium spp., hairy yellow Mucor spp. and Cladosporium spp. A general assortment of bacteria (colored orange, yellow, white and grey) were observed visually. After the first week, low black Mucor spp., Rhizopus spp., blue green Aspergillus spp., light pink Fusarium spp., blue Penicillium spp. and lime green Aspergillus spp. were added to the original mold population. There was no visual change in the bacterial population. After the second week the ascomycetes became countable at 10^{5} per 1 gram sample. The types of molds remained the same and there was less yellow-orange colored bacteria. By the end of the third week, the rice had started to sprout. The mold and bacterial populations remained the same. After four weeks of curing the rice was visually moldy. The ascomycete count had increased to 10^{6} per 1 gram sample, and there appeared to be no change in the types of bacteria on the kernels. Rhizopus spp. and hairy yellow Mucor spp. were the predominant molds and to a lesser extent Alternaria spp., low black Mucor spp. and hairy yellowish brown Fusarium spp. were also present. Small numbers of lime green Aspergillus spp., blue green Aspergillus spp., pink hairy Fusarium spp. and Trichoderma spp. were also detected. In general, there were only minor changes in types of molds and bacteria during this curing trial which appeared to be consistent with the moderately cool curing environment.

Figure 32. Microbial Counts on Paddy Wild Rice Cured at 21°C and 95% R.H.



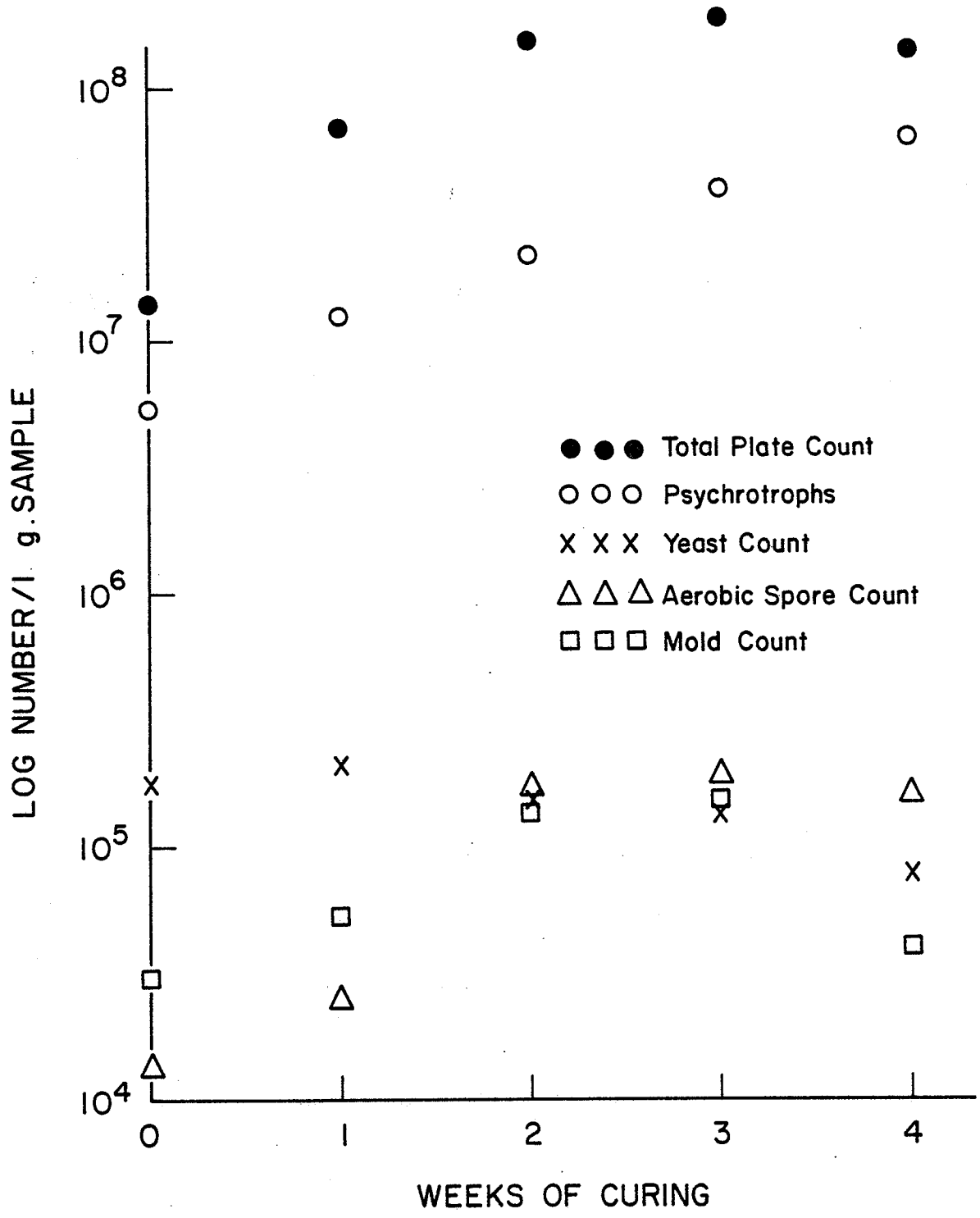
The field fungi were dominant throughout this curing period which again was consistent with literature on grain storage and high moisture grain (21, 22, 53, 55, 85).

The total plate count increased by approximately one log cycle which was expected since the moderately cool curing conditions should allow this (Figure 33, Appendix 3). The psychrotrophs also increased gradually for the same reason above. There was a slight decrease in the yeast count while the mold count increased slightly and then decreased at the end of the curing period. This decrease in mold count was inconsistent with their visual increase on the rice. The aerobic spore count increased for the first two weeks and then leveled off. In general, under these conditions a gradual increase in all counts was expected.

At the beginning of curing paddy wild rice at 15°C, the major types of molds were the Alternaria spp. and hairy yellow Mucor spp. with Cladosporium spp., blue green Aspergillus spp., light pink Fusarium spp.,being present in lesser numbers. After one week of curing, no change in the mold population was observed. At the end of two weeks, the hairy yellowish brown Fusarium spp. were more predominant, and two different species of pink Fusarium appeared for the first time. Rhizopus spp. also appeared after the third week of curing. No microbial samples were taken for the 4th week due to uncontrollable circumstances. Similar to the curing of lake rice under the same conditions, there was little change in the mold population except for change in order of dominance.

The total plate count increased one log cycle and then started to

Figure 33. Microbial Counts on Lake Wild Rice Cured at 15°C and 95% R.H.

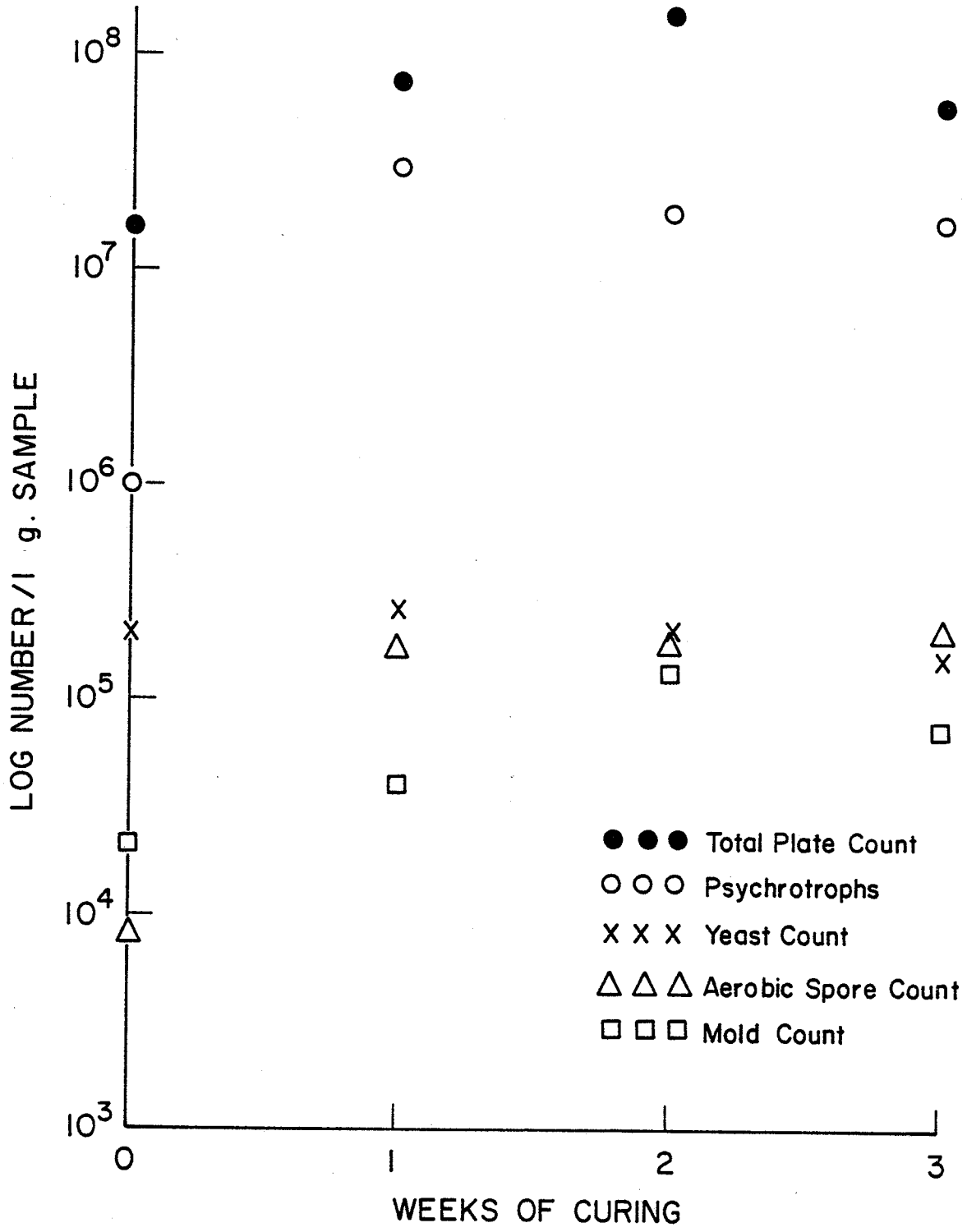


decrease at the end of the curing period (Figure 34, Appendix 4). This appeared to be consistent with the curing conditions. The psychrotrophs remained almost static or decreased slightly when an increase should have been observed throughout the curing period. If the estimated psychrotrophic count at the start of the curing trial was accurate, there would be an increase of one log cycle in the first week and then a gradual decrease for the rest of the curing period occurred. The yeast count was almost static with a minor decrease. The mold count increased one log cycle and then decreased. This did not correlate with the physical appearance of the deteriorating paddy rice. The aerobic spore count increased for the first week and then remained static. Overall, there should have been some increase in all microbial counts due to the moderate curing temperature and the high moisture levels which would allow this growth. There appeared to be no appropriate reason for the microbial counts not to increase.

4.5.2.4 Microbial analyses of wild rice curing at $10^{o}C$

The final environment used for curing lake wild rice was at $10^{o}C$. The types of mold isolated from green lake rice were blue green Aspergillus spp. and low black Mucor spp. There was a general assortment of yellow, orange, grey, white and cream colored bacteria. At the end of the first week of curing, blue Penicillium spp. and Rhizopus spp. were added to the previous molds. Also crater-looking bacteria were observed for the first time along with the rest of the bacteria. After the second week of curing, the blue Penicillium spp. disappeared and the yellowish brown Fusarium spp. and Cladosporium spp. were isolated for the first time on the plates. Hairy yellow Mucor spp. and blue Penicillium

Figure 34. Microbial Counts on Paddy Wild Rice Cured at 15°C and 95% R.H.



spp. appeared with the rest of the molds by the third week. The numbers of yellow colored bacteria decreased. By the fourth week Alternaria spp. were added and no yellow colored bacteria were observed. There was no change in the mold population during the fifth week except for the appearance of the Trichoderma spp. plus orange-yellow colored bacteria. The first pink Fusarium spp. appeared in the sixth week. For the rest of the curing period there was no more change in the types of molds. The only noticeable change in the bacterial population was the disappearance and the re-appearance of yellow and orange colored bacteria from week to week. The lake rice did not deteriorate throughout this curing trial. In general, there was little variation in the mold population except for the addition, or deletion of a few types which was expected under these cool curing conditions which should prevent many biological changes in the rice. This also applied to the bacterial population which appeared to change very little except for the fluctuating appearance of certain types of bacteria plus the appearance of the crater looking bacteria at the beginning.

The predominant molds isolated from paddy wild rice curing at 10°C were Cladosporium spp., Alternaria spp. and Rhizopus spp. initially plus hairy yellow Mucor spp. Similar colored bacteria to that on lake rice were present along with the crater looking and bacillus type bacteria. In the first three weeks of curing there appeared to be no change in the mold and bacterial populations. By the fourth week of curing, the hairy yellow Mucor spp. was the most dominant among the previous types of molds. Also yellowish brown Fusarium spp., pink Fusarium spp., lime green Aspergillus spp. and Trichoderma spp. appeared.

By now the orange colored bacteria were dominant, and there were fewer crater looking bacteria. There was no more change in the mold and bacterial populations throughout the rest of the curing period. At the end of curing period for paddy rice there was an observable ascomycete count of 10^4 per 1 gram. Therefore, under these cool curing conditions, there was very slow change over the long curing period even to the point of no change at all in the microbial population.

The total plate count for paddy rice cured at $10^{\circ}C$ was a bit erratic with up and down fluctuations throughout the curing period while for the lake rice the total plate count decreased and leveled off after the second week (Figures 35 and 36, Appendices 3 and 4). Data from the curing of lake rice correlated well with Frank's results (32) but neither correlated with research showing an increase and leveling off (35,80). The data for psychrotrophs were not similar to any previous research (32,35,80). For both types of wild rice, the psychrotrophs decreased slightly and started to level out. The yeast count was static with only a decrease to a new level half way through the curing period for the paddy wild rice but for the lake rice the yeast count was very erratic for some unknown reason. With both types of wild rice, the mold count was almost static which correlated well with all previous reports (32,35,80). Finally, the aerobic spore count was static for lake wild rice and erratic for paddy rice. The results from the curing of lake rice appeared to be somewhat similar to Frank's (32) results under the same conditions and Frank obtained erratic results only under the curing condition of forced saturated air through the rice bed. Overall, the microbial counts should have been static, or decreasing slightly over a long curing period. Only the psychrotrophic count should have

Figure 35. Microbial Counts on Lake Wild Rice Cured at 10°C and 95% R.H.

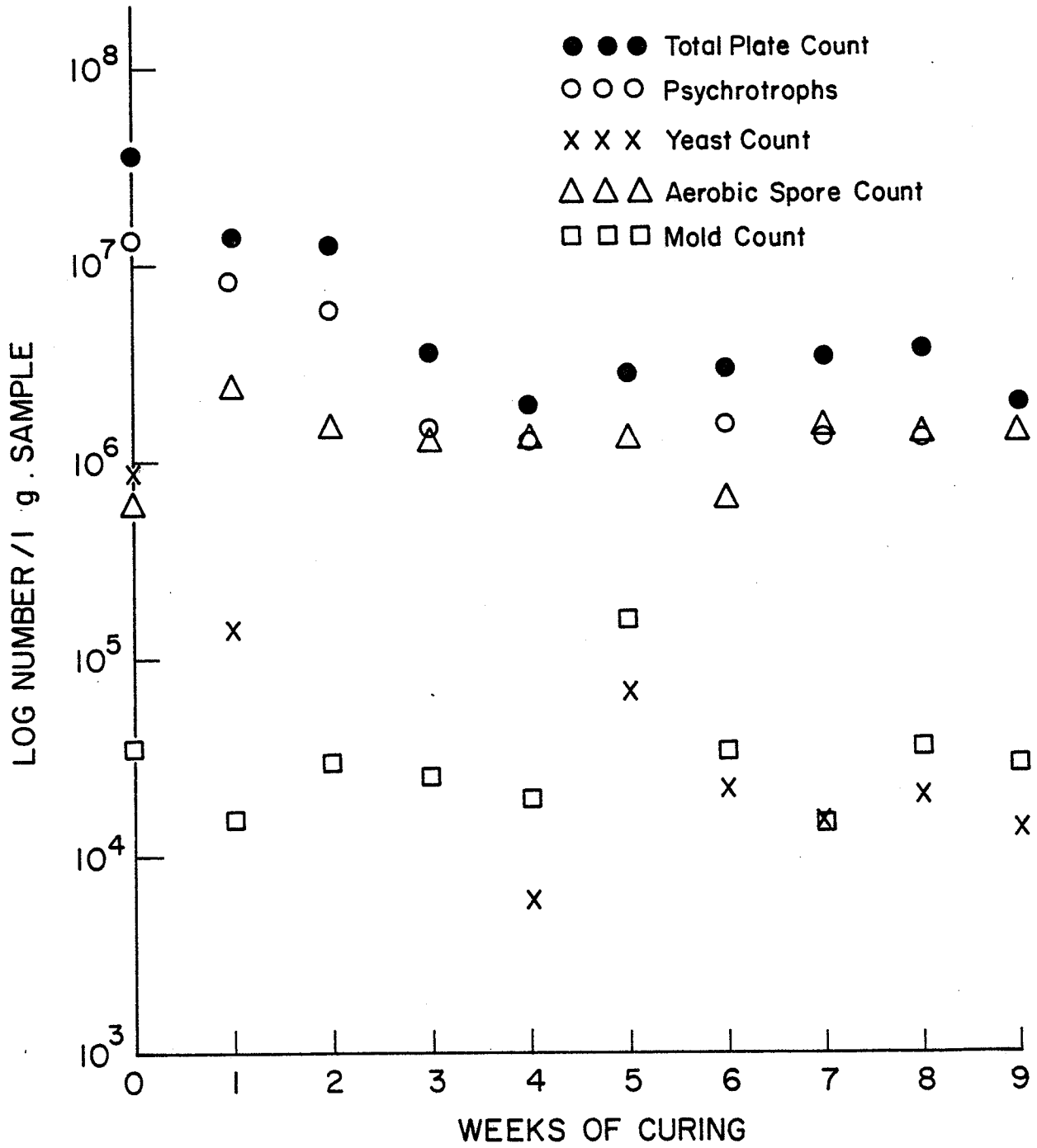
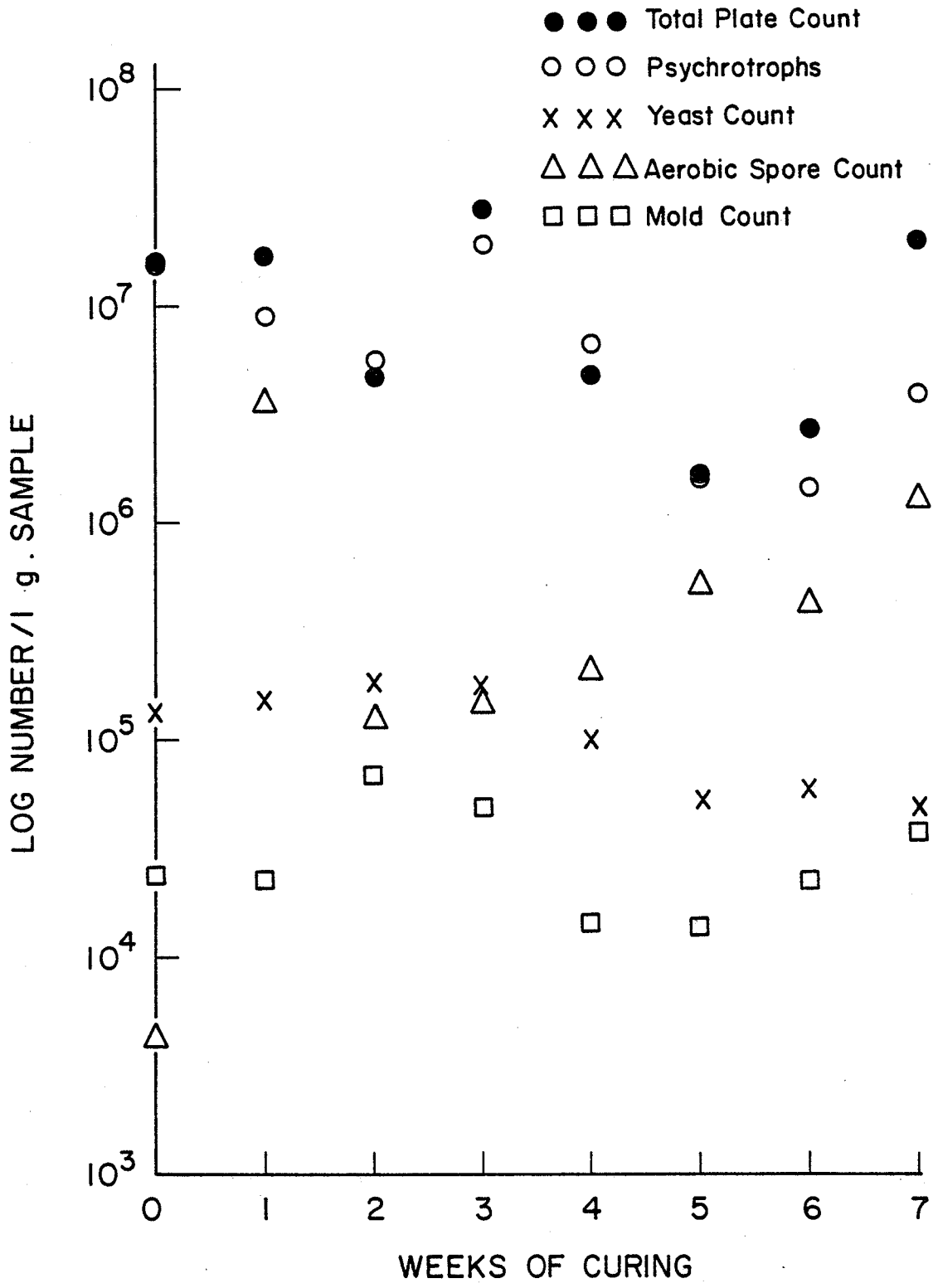


Figure 36. Microbial Counts on Paddy Wild Rice Cured at 10°C and 95% R.H.



increased gradually under these cool curing conditions.

4.5.3 The effect of parching on the microflora of wild rice

In the parching of cured wild rice the total numbers of microorganisms were in general reduced but were not eliminated except for yeast and mold (Tables 15 and 16). Parching at 135°C caused only a reduction in total plate count of one to two log cycles while yeast and mold counts were almost or totally eliminated. The aerobic spore count was reduced by one or two log cycles in some samples and very little in other samples. Psychrotrophs were reduced one to three log cycles. In comparison with Wisconsin's research, the amount of microbial reduction was the same except that their mold counts were not as greatly reduced (34,35,80). This study also correlated well with Spicker et al.'s research which indicated that bacteria were more heat resistant than other types of microflora in the drying of a grain (71).

The dehulling of parched wild rice only reduced the counts for each type of microorganisms by approximately one log cycle (Table 16). Somehow, Wisconsin researchers were able to get a substantial decrease in microbial counts by dehulling the parched wild rice (such as a three log cycle decrease)(35,80).

4.6 Possible Health Hazards of Wild Rice

The initial high microbial counts on wild rice and the usually higher counts after the curing process would cause one to expect a possible problem with the food safety of this crop. However, no Staphylococcus or Salmonella spp. were isolated during the microbial

Table 15

Microbiological Counts on Raw and Parched Wild Rice

	Parched at 135°C for 35 min.			
	Before Parching[d](a)	After Parching d	Before Parching[d](b)	After Parching d
Microbiological test				
Total plate count	69.3×10^6_4	80.5×10^4_1	48.8×10^6_4	106.3×10^5
Yeast count	100.5×10^4_3	1.3×10^1_1	33.3×10^4_4	0
Mold count	162.7×10^3_5	5.8×10^1_3	53.7×10^4_5	0
Aerobic spore count	32.5×10^5_6	79.3×10^3_3	81.3×10^5_5	47×10^5_4
Psychrotrophs	82.5×10^6	32.8×10^3	187.8×10^5	36.7×10^4

(a) used paddy wild rice cured for 4 weeks at 10°C and 95% R.H.
(b) used lake wild rice cured for 6 weeks at 10°C and 95% R.H.
(c) counts are almost 0
(d) average counts/1 g sample

Table 16

Microbiological Counts on Raw, Parched and Dehulled Wild Rice

	Parched at 121.1 - 135°C for 35-55 min.		
	Not dehulled		Dehulled
	Before Parching(a) e	After Parching e	After Parching e
Microbiological test			
Total plate count	30.2×10^{6}	52.3×10^{5}	68.7×10^{4}
Yeast count	147.7×10^{3}	0	0
Mold count	43.2×10^{4}	0	3.8×10^{1}(c)
Aerobic spore count	13.3×10^{6}	47.8×10^{5}	78.5×10^{4}
Psychrotrophs	(b)(x 10^{5})	44.2×10^{1}	(d) 41×10^{2}

(a) used lake wild rice cured for 9 weeks at 10°C and 95% R.H.
(b) unable to count
(c) must be contamination since it is zero before dehulling
(d) throughout these tests I got these very low counts for some unknown reason. It should have been around 10^{3}.

(e) average counts per 1 g sample.

analyses, and only a few streptococci and Eschericheria spp. were isolated which may be a cause for concern.

The parching of wild rice assisted in reducing the total microbial load by a factor of one to three log cycles. This process will help reduce the health hazard problem. However, the spore forming bacteria will be only slightly affected. Also any toxins produced during curing may not be affected.

The hulling operation was more effective in reducing the total microbial population than was parching (section 4.5.3). However, this process too will have little effect on spore formers and toxins depending on whether they are located on the hulls or on the kernels.

In the cooking trials, the total plate count was reduced to < 10 per gram of rice. This was lower than the total plate count of 140 per gram reported by Goel et al.(34). There was total elimination of all other types of microflora. Therefore, by the time the rice is consumed the microbial numbers have been reduced to a level where no health hazards exist except for maybe the mycotoxins if they were produced during the curing step.

From this research, molds appeared to be the only possible health hazard problem . The Aspergillus spp. isolated in this research did not have the taxonomic features of Aspergillus flavus as reported by Frank et al. (33). While this species was not isolated, its presence is not precluded on Manitoba wild rice since mycotoxins in Canadian grain has been reported by Hesseltine (41). Penicillium spp. were found in small numbers in the curing trials but their populations did not increase at

any time and therefore, the probability of these species becoming a health problem were considered remote. On the other hand, a large Fusarium population was isolated. In fact, the visual examination of green wild rice kernels enabled one to observe that a certain number of kernels had a reddish to purple discoloration which was due to these molds. Manitoba wild rice appeared to be highly infected with Fusarium spp. In some curing trials, Fusarium spp. became dominant in the rice bed. Since certain species of Fusarium produce mycotoxins (zearalenone, T-2, etc.), this could develop into food safety problems (41). Therefore, on the basis of these results further investigation on Fusarium spp. on wild rice and their mycotoxin properties should be carried out.

4.7 Microbial Reduction Studies

These studies were designed to evaluate liquid sterilants and gas sterilization for the purpose of eliminating the microflora on wild rice grains.

4.7.1 Microbial reduction studies using liquid sterilants

Five parameters were studied in these tests. They are as follows:

1. The effect of the type of sterilant used to reduce the microflora.
2. The effect of sample size used for microflora reduction tests.
3. The effect of the immersion time on microflora reduction.
4. The effect of the sterilant temperature on microflora reduction.
5. The effect of the hull on the wild rice kernel on microflora reduction.

Sterilant types were evaluated in trials 1,2,3,11,12,13,14,15 and 16. Typical data obtained from these trials are presented in Table 17.

TABLE 17. Effect of Type of Sterilant on Microbial Reduction.

Trial 3

Treatment(f)	Bacteria	Mold	Yeast
	Ave. count/1 g sample	Ave. count/1 g sample	Ave. count/1 g sample
Control	25×10^7_5	140×10^2_2	67×10^3_2
Clc (d)	156×10^3	19×10^2	11×10^2
Cla (e)	158×10^3	0	0

(d) Clc = concentrated sodium hypochlorite colution, no alcohol
(e) Cla = concentrated sodium hypochlorite solution and ethyl alcohol. Also noticed this solution produced a heat reaction with a temperature up to 55°C upon standing.
(f) for all treatments used 30 g of wild rice for 30 min. at room temperature.

Trial 11

Treatment(d)	Bacteria	Mold	Yeast
	Ave. count/1 g sample	Ave. count/1 g sample	Ave. count/1 g sample
25(a)	40×10^2_1	0	0
20(b)	4×10^1_2	0	0
10(c)	90×10^2	0	0
control	167×10^6	$-(10^3)$	8×10^4

(a) 25 = 25 g wild rice immersed for 30 min.
(b) 20 = 20 g wild rice immersed for 25 min.
(c) 10 = 10 g wild rice immersed for 12 min.
(d) used 1 part 95% ethyl alcohol and two parts sodium hypochlorite solution (5 1/4% available chlorine) at 80°C.

The combination of ethyl alcohol (95%) and sodium hypochlorite (12%) was the most effective sterilant, achieving bacterial reductions from 10^6 down to 10^3 while completely destroying yeast and mold populations. Ethyl alcohol (95%) plus sodium hypochlorite (5 1/4% available chlorine) was as effective as the above combination when used at 80°C. All other combinations used were only moderately effective in sterilizing wild rice grains.

The effect of altering the size of sample used for achieving sterilization was tested in the following trials: 1, 2, 3, 4, 9, 11 and 12. Typical data for these tests are presented in Table 18. A sample size of 30 grams or less was the optimum size for achieving good microbial reduction; yeast and mold populations being reduced to 0 and bacterial populations were reduced from 10^6 to 10^4. The volume of sterilizing solution was not controlled but the quantity was sufficient to cover the sample at all times. The data indicated that as sample size increases, the effect of the sterilizing solution decreases. This seems to indicate that liquid sterilants are not capable of sterilizing large samples of rice under the restrictions required for this project.

Trials 1, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15 and 16 tested the time of immersion required to achieve sterilization of the wild rice. The tabulated data presented in Table 18 are representative of the results that were obtained. A minimum of 30 minutes was required to achieve a maximum sterilizing effect. There was almost no difference observed in immersing the rice for time periods greater than 30 minutes on reducing the microbial population.

The temperature of sterilization was one of the more critical factors in determining the efficiency of the sterilizing solution. This factor was tested in trials 1 to 16 inclusively for the following five temperatures: 25°C (RT), 55°C, 60°C, 70°C and 80°C (Table 19). The most effective temperature was 80°C which was in agreement with Vojnovich et al. (78, 79). As the temperature

TABLE 18. Effect of Sample Size and Immersion Time on Microbial Reduction.

Trial 4

Treatment(d)	Bacteria	Mold	Yeast
	Ave. count/1 g sample	Ave. count/1 g sample	Ave. count/1 g sample
Control	94×10^{6}	31×10^{1}	128_{1}
30(a)	142×10^{4}	0	6×10^{1}
20(b)	273×10^{4}	11×10^{1}	0
10(c)	TNC $\times 10^{4}$	42×10^{o}	12×10^{1}

(a) 30 = 30 g for 30 min. immersion time.
(b) 20 = 20 g for 20 min. immersion time.
(c) 10 = 10 g for 10 min. immersion time.
(d) used one part 95% ethyl alcohol and 2 parts 12% sodium hypochlorite solution at room temperature.

TABLE 19. Effect of Temperature on Microbial Reduction.

Trial 7

Treatment(d)	Bacteria	Mold	Yeast
	Ave. count/1 g sample	Ave. count/1 g sample	Ave. count/1 g sample
60(a)	82×10^3	0	0
70(b)	38×10^2	0	0
80(c)	7×10^1	0	0
Control	145×10^7	3×10^4	250×10^4

(a) 60 = 60°C treatment
(b) 70 = 70°C treatment
(c) 80 = 80°C treatment
(d) used 30 g of wild rice for 30 min in one part 95% ethyl alcohol and two parts 12% sodium hypochlorite solution.

of the sterilizing system decreased, the sterilizing effect was reduced.

In order to determine if the hulls on wild rice kernels hindered sterilization by a liquid sterilant, the wild rice was dehulled and this factor was tested in Trials 13 to 15 inclusive From analysing all the data, hulls on wild rice kernels appeared not to hinder microbial reduction with a liquid sterilant.

The optimum conditions for sterilizing wild rice was utilizing an ethyl alcohol (95%) and sodium hypochlorite (12%) solution (1:2 ratio) at a temperature of 80^{o}C and immersing 30 grams of wild rice in this solution for 30 minutes. This system almost sterilized the wild rice but complete sterilization was never achieved. Harsher treatments would have been required to accomplish this. However, in obtaining this desired condition, the wild rice kernels would have been killed. Then they were unable to be cured as defined in this research program making it impossible to evaluate the role of microorganisms in curing. In conclusion this program did not achieve the desired state of sterility but was successful in accomplishing microbial reduction.

4.7.2 Microbial reduction studies using gas sterilization

Three parameters were evaluated in these trials;

1. The exposure time of the wild rice to the sterilizing gas.
2. The length of time of flushing with nitrogen gas.
3. The size of sample used for these sterilization studies.

The data collected from these trials are presented in Table 20. The optimum exposure time for achieving the maximum sterilizing effect

Table 20

Gas Sterilization with Ethylene Oxide and Carbon Dioxide

Trial 1: 200 g wild rice

	T.C.(ave.count)/1 g sample	Ave.Mold count/1 g sample
Control	14.5×10^7	65×10^4
Treatment 1[(a)]	165×10^6	29×10^4
Treatment 2	33×10^7	31×10^3
Treatment 3	120×10^6	151×10^3
Treatment 4	20×10^5	126×10^3

Trial 2: 100 g wild rice

	T.C.(ave.count)/1 g sample	Ave.Mold count/1 g sample
Control	124×10^7	92×10^4
Treatment 1[(a)]	130×10^6	251×10^3
Treatment 2	25×10^7	27×10^4
Treatment 3	22×10^6	37×10^3
Treatment 4	26×10^6	33×10^3

Trial 3: 25 g

	T.C.(ave.count)/1 g sample	Ave.Mold count/1 g sample
Control	84×10^6	25×10^5
Treatment 1[(a)]	116×10^5	196×10^4
Treatment 2	64×10^4	78×10^3
Treatment 3	39×10^3	28×10^2
Treatment 4	30×10^3	23×10^2

TABLE 20 (Contin)

(a) Treatment 1: exposure time of 4 hr and flushing time of 2 hr.

Treatment 2: exposure time of 4 hr and flushing time of 6 hr.

Treatment 3: exposure time of 24 hr and flushing time of 2 hr.

Treatment 4: exposure time of 24 hr and flushing time of 6 hr.

(b) No yeast counts due to unable to count due to overgrowth of molds but noticed the yeasts reduced in numbers usually one log cycle below the mold count reported for each treatment.

was found to be 24 hours. The length of the flushing period had no effect on sterilization. A sample size of 25 grams was found to be the most effective for achieving sterilization with the gas sterilizing system that was used for these trials. This did not compare well with Frank et al.'s report on gas sterilization which stated that there was complete sterilization after 48 hours exposure period of wild rice dried to a 20% moisture content (33). His technique was not tested in these studies since specialized equipment was required. In addition, the drying of the wild rice down to a certain moisture level before gas sterilization and rehydration afterwards did not appear favorable due to the difficulty of properly rehydrating such a product and the possibility of harm to the kernels. Future studies in this direction will more fully establish the role of the microflora in the curing of wild rice.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary and Conclusions

Wild rice cures faster at the higher curing temperatures; synonymously the lower the temperature the slower the curing rate. The same effect was observed for the rate of deterioration. Higher bed temperatures are warning indicators for the onset of deterioration. These high bed temperatures are normally associated with high temperature curing environments. Only the cool curing conditions prevented deterioration and high bed temperatures. The paddy wild rice deteriorated faster than lake wild rice, and its quality after curing was poor in comparison with lake rice, which was probably due to its immaturity. The deterioration of wild rice and the increase in bed temperature during curing were due mainly to biochemical reactions and not microbial activity because the microbial counts did not correlate with either. This suggests the possibility that microorganisms have very little or no role in the curing of wild rice.

The gram negative rods are the predominant group of bacteria on kernels of wild rice. On all samples of wild rice, the Pseudomonas spp. are the most predominant bacterial genus while the rest of the identified bacteria vary in numbers from a level of being the next predominant to a level of very few depending on the lot of rice tested. The numbers of yellow to orange colored bacteria vary from one sample

of wild rice to another with three types being dominant at one time or another. But there was a problem in identifying some of these yellow to orange colored bacteria because they are very similar in features, and an identification scheme has not been found to solve this problem.

Seventeen different types of molds were found in varying proportions on wild rice with the following molds being the major types: Rhizopus spp., hairy yellow Mucor spp., blue green Aspergillus spp., low black Mucor spp., Alternaria spp., Cladosporium spp., and yellowish brown Fusarium spp. The rest of the molds are present in smaller numbers. If a comparison was made between each curing condition in relation to the types of molds present from the start to the end of the curing period, this comparison would be invalid because too many variables affect the data such as the source of the rice, post harvest handling techniques prior to arrival at the department and complicated curing reactions which were noncontrollable. The same observation applies to the comparison of bacterial data under these similar circumstances.

In these microbial studies, it was impossible to differentiate the numbers and kinds of microorganisms from environmental contamination versus the natural microflora on the rice kernels since the wild rice came from different regional sources, and underwent different harvesting and handling techniques prior to arriving at the department. In spite of this situation, the microflora detected in this research are similar to that which one would expect to isolate from wild rice curing under a commercial environment in Manitoba.

The microbial population was not an important variable for curing wild rice. The trend for microbial counts should have been dependent

on the curing conditions which on occasion did occur. In other words, for a certain curing condition, some microbial counts should have increased while others should have decreased or remained static. But in several instances, this was not the case. In general, there was no apparent reason for the conflicting phenomena of changes in the microflora population under various curing conditions. During a short curing period which is typical for the wild rice industry, there was little to no change in the bacterial population. There were definite changes when the curing period was long. Therefore, bacteria appear to play no role in the curing of wild rice when the curing period is of normal length. It also appeared that the curing environment had only minimal effects on causing the bacterial population to change. The changes in the mold population during curing occurred more often than in the bacterial population and the curing conditions appeared to be more responsible for these changes. But these changes in types of molds did not follow the normal trend of field to storage fungi as was observed in grain storage. It was usually a change from one field mold to another. There appeared to be no apparent reason for this occurrence, unless it is a characteristic of curing wild rice under these experimental conditions which has not been detected before.

In examining the food safety of wild rice there was no problem with food poisoning or infective type bacteria. Due to parching and cooking, the total plate count is reduced drastically. Therefore there appears to be no health problem due to the high population of Fusarium on lots of wild rice even though certain species of Fusarium produce mycotoxins.

The using of sodium hypochlorite for microbial reduction studies was very successful but it did not completely sterilize the wild rice. The gas sterilization of wild rice was successful as far as it was tested. Further research is required to test its usefulness because it appears to be the only promising method for sterilizing the wild rice without causing harm. When this is achieved, then the role that micro-organisms play in curing wild rice can be precisely evaluated.

5.2 Recommendations

The purpose of this section is to indicate potential areas of research which requires further study and the following is a list of them:

1. Fusarium spp. was a common organism on many samples of wild rice and it is known that certain species of this genus produce mycotoxins. Thus further research should be conducted to check the infected wild rice samples for mycotoxins. Each Fusarium isolate should be investigated to see if it could produce a toxin. Since this research indicated that Fusarium infection of Manitoba wild rice is quite prevalent, a more complete study of the frequency of this infection should be done and to determine what is causing this problem, along with possible solutions.
2. The next recommendation has to do with the gas sterilization of wild rice. When the proper equipment, which can be pressurized and humidified, has been obtained, further research should be conducted on sterilization of wild rice. Once this is accomplished, any potential role of micro-organisms in curing can be finally resolved.
3. The food safety of wild rice appears to be alright at the present

time. But some type of microbial standards must be set up for wild rice due to the potential health problem from mold infection and to prevent unsanitary processing of wild rice.

4. No more microbiological work appears to be needed in the area of curing studies. However, if an indepth study of changes during the curing of wild rice is required, the biochemical changes could be examined and answers found for the anomalies that occurred in the microbial population (counts).

5. In the area of microbial taxonomy, the bacteria and molds could be identified down to species and beyond this point, no more taxonomical research is suggested.

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APPENDICES

Appendix 1. Changes in Microbiological Counts during the Curing of Lake Wild Rice [a] at 32°C and 95% R.H.

Ave. count/1 g.

Microbiological test	Days 0	3	6	9
Total plate count	57.5×10^7	14.7×10^7	19.4×10^7	66.5×10^7
Psychrotrophs	129×10^6	68.2×10^6	16.0×10^6	23.2×10^6
Yeast count	95×10^5	- [b]	-	-
Mold count	55×10^4	14.2×10^5	31.2×10^5	31.3×10^5
Aerobic spore count	78×10^5	95.5×10^6	43.3×10^6	75.8×10^6

(a) used lake wild rice from Eileen Lake, Man. and it was washed before curing.
(b) after the start of curing, no yeast count was obtainable probably due to the high temperature curing.

Appendix 2. Changes in Microbiological Counts during the Curing of Paddy [a] Wild Rice at 32°C and 95% R.H.

Ave. count/1 g

Microbiological test	Days 0	4	8
Total plate count	36.6×10^6	16.3×10^7	19.5×10^8
Psychrotrophs	30.8×10^5	57×10^6	11.4×10^7
Yeast count	34.3×10^4	- (b)	- (c)
Mold count	19.1×10^4	28×10^8	73×10^4
Aerobic spore count	18×10^4	18.1×10^6	18.3×10^6

(a) used paddy wild rice from Sprague, Man., and it has been frozen and then washed before curing.
(b) unable to count due to mold but definite decrease in yeast count to about 10^2/1 g wild rice. Ascomycete count is around 10^2/1 g wild rice
(c) same as (b) except Ascomycete count increased to 10^4/1 g wild rice

Appendix 3. Changes in Microbiological Counts during the Curing of Lake Wild Rice

Ave. count/1 g wild rice

Microbiological test	Treatments[b]	Weeks 0	1	2	3	4	5[i]	6	7	8	9
Total plate count	1	21.6×10^7	-[c]	14.6×10^8	30.3×10^8	25.8×10^8					
	2	15.3×10^7	84.7×10^7	18.6×10^8[g]	27.8×10^8[j]	15.6×10^6					
	3	57.5×10^7	15.8×10^7	11×10^7	55.9×10^8	30.8×10^6	46.3×10^6	48.8×10^6	54.8×10^6	56.8×10^6	30.2×10^6
Psychrotrophs	1	10.9×10^7	-	11.1×10^7	15.6×10^7	16.4×10^7					
	2	74.8×10^7	10.6×10^7	34.7×10^6	60.2×10^6	81.3×10^6					
	3	12.9×10^7	92.3×10^6	77.8×10^6	17.5×10^6	10.7×10^6	-	18.8×10^6	12.4×10^6	12.2×10^6	-
Yeast count	1	76.3×10^5	-	97.2×10^3	34.3×10^4	-[f]					
	2	26.1×10^5	33.2×10^5	19.8×10^5	13.7×10^5	91.5×10^3					
	3	95×10^5	15.4×10^5	-[d]	-[e]	77.8×10^3	84×10^4	33.4×10^4	20.7×10^4	30.7×10^4	14.7×10^4
Mold count	1	15.7×10^5	-	10.6×10^5	14.3×10^5	11.5×10^5					
	2	48.7×10^4	73×10^4	14.6×10^4	19.8×10^4	60.5×10^4					
	3	55×10^4	18.3×10^4	47.7×10^4	40.5×10^4	29.2×10^4	21×10^5	53.7×10^4	17×10^4	55.3×10^4	46.2×10^4
Aerobic spore count	1	15.4×10^5	-	53.8×10^5	10.2×10^6	36.3×10^6					
	2	12.9×10^4	40.3×10^4	24.2×10^5	29.5×10^5	21.9×10^5					
	3	78×10^5	39.4×10^6	17.1×10^6	10.4×10^5	11.5×10^6	11×10^6	81.3×10^5	18.4×10^6	15.1×10^6	18.3×10^6

(a) for treatments 1 & 3, used lake wild rice from Eileen Lake, Man. and it was washed before curing.
for treatments 2, used lake wild rice from Harrop Lake, Man., and it has been frozen and was washed before curing
(b) Treatment 1 - 21.3°C + 95% R.H.
2 - 15°C + 95% R.H.
3 - 10°C + 95% R.H.
(c) this run was ruined in some way
(d) unable to count because of mold but count is around 10^4/1 g
(e) unable to count because of mold but count is around 10^2/1 g
(f) unable to count due to mold and ascomycete count is around 10^4/1 g
(g) ascomycete count is around 10^5/1 g
(h) ascomycete count is around 10^6/1 g
(i) started to sprout
(j) starting to sprout

Appendix 4. Changes in Microbiological Counts during the Curing of Paddy Wild Rice

Ave. count/1 g wild rice

Microbiological test	Treatments[a]	Weeks 0	1	2	3	4	5[c]	6	7
Total plate count	1	20.5×10^7	40.3×10^7	11.8×10^7	28.4×10^7[f]				
	2	28.1×10^7	87.2×10^7	18.2×10^8	76.2×10^7				
	3	20.5×10^7	24.5×10^7	67.5×10^6	45×10^7	69.3×10^6	21.9×10^6	43.5×10^6	30×10^7
Psychrotrophs	1	18.9×10^7	30.7×10^7	94.5×10^6	16.4×10^7				
	2	-(b)	48.2×10^7	26.3×10^7	21.1×10^7				
	3	18.9×10^7	95.7×10^6	75.2×10^6	$2\cancel{5} \times 10^7$	82.5×10^6	20.1×10^6	16.3×10^6	58.7×10^6
Yeast count	1	13.8×10^5	13.4×10^5	20.2×10^4	12.7×10^4				
	2	32×10^5	42.8×10^5	32.2×10^5	18.8×10^5				
	3	13.8×10^5	18.7×10^5	26.5×10^5	25.6×10^5	100.5×10^4	72.3×10^4	77×10^4	70.7×10^4 e
Mold count	1	38.7×10^4	20.5×10^4	18.3×10^4	36.3×10^4				
	2	34.5×10^4	61.3×10^4	13.3×10^4	86.2×10^4				
	3	38.7×10^4	36.7×10^4	84.9×10^4	71.8×10^4	16.3×10^4	15.2×10^4	36.5×10^4	56.8×10^4
Aerobic spore count	1	68.3×10^3	25.8×10^5	52×10^5	89.3×10^5				
	2	91×10^3	24.6×10^5	26.4×10^5	31.5×10^5				
	3	68.3×10^3	56.5×10^6	11.5×10^5	17.4×10^5	32.5×10^5	72×10^5	63.3×10^5	11.4×10^5

(a) Treatments 1 - 21°C + 95% R.H.
2 - 15°C + 95% R.H.
3 - 10°C + 95% R.H.
(b) count estimated around 10^6/1 g
(c) starts to sprout
(d) paddy wild rice from Sprague, Man. It was washed.
(e) ascomycete count is around 10^4/1 g
(f) starts to sprout

Appendix 5. Abbreviations and Symbols Used in this Thesis

1. AACC - American Association of Cereal Chemists
2. ^{o}C - celsius
3. cm - centimeter
4. g - gram
5. hr.- hour
6. in. - inches
7. kg. - kilogram
8. mph. - miles per hour
9. ml. - millilitre
10. no. - number
11. % - percentage
12. R.H. - relative humidity
13. RT - room temperature
14. spp. - species
15. TC - total count
16. TSI - triple sugar iron media
17. TSA - trypticase soy agar
18. US - United States