The University of Manitoba

# POSTHARVEST MICROBIOLOGICAL STUDIES ON MANITOBA WILD RICE

by

JOHN F. LOGAN

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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).

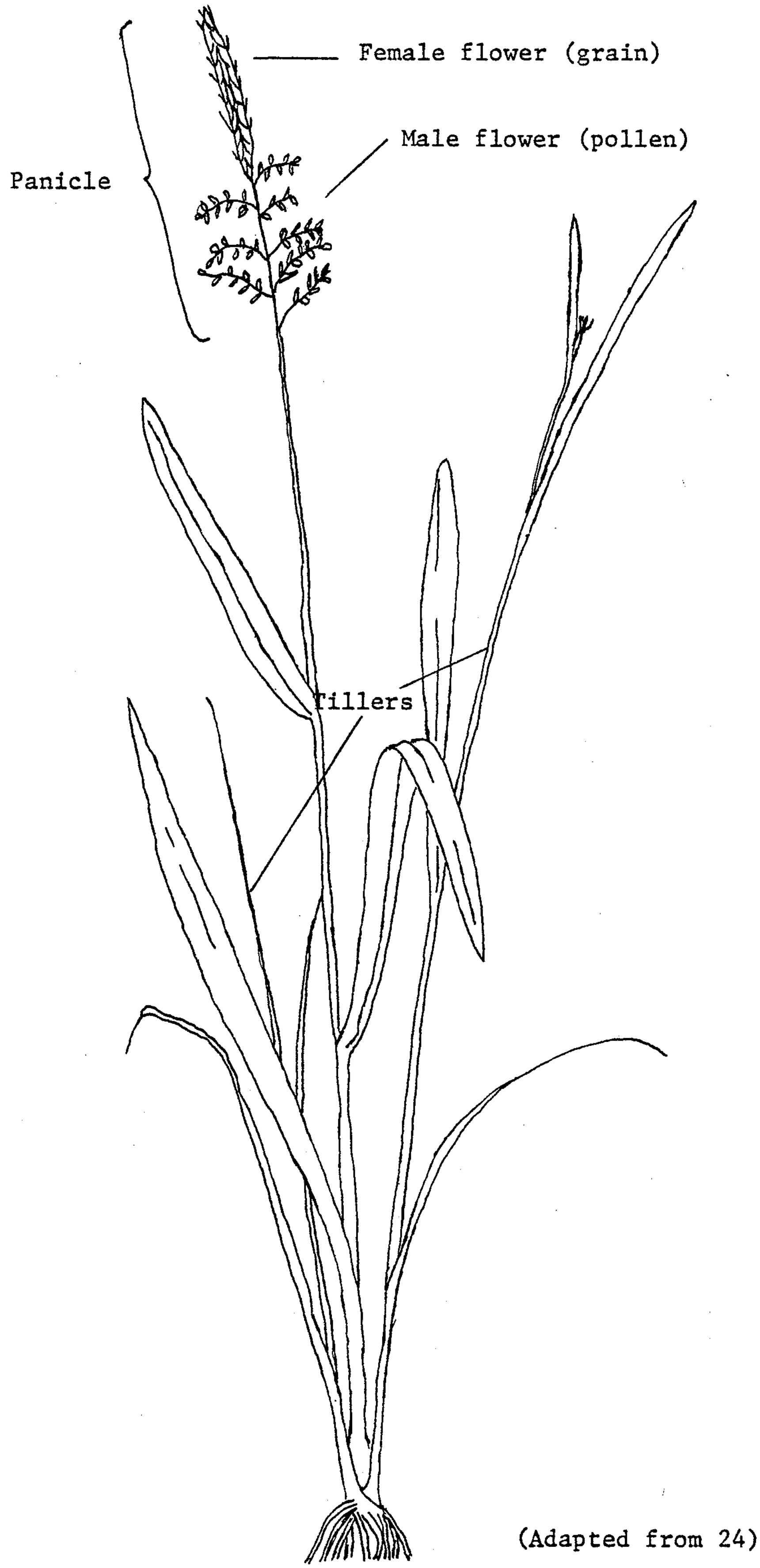
## 2.2 Methods of Processing Wild Rice

### 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$^{o}$C, was due to the metabolic and respiratory activities of mesophilic nonspore formers, including molds, while the second stage, up to 70$^{o}$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$^{o}$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