CONTROL OF MICROORGANISMS DURING RICE STORAGE

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LOAY KAREEM ALNAJI

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INTRODUCTION

Rice is one of the leading food crops of the world with more than half the population of the world consuming rice as its main food. Total world rice production from 1963 to 1967 was 259,984,000 metric tons per year. Wheat production in the same period was 277,666,000 metric tons per year (2).

Much rice in the world is harvested with a moisture content between 18% to 24% and like other grains and seeds is subject to post harvest invasion by microorganisms and cannot be stored safely. Some molds can grow on rough rice in an atmosphere having a relative humidity (R.H.) as low as 65%, and yeast can grow at 80% to 90% R.H., but it is usually considered that bacteria cannot grow unless the relative humidity is at least 95% which occurs on a visibly wet surface (3, 12, 13).

Fungi have been established as one of the primary causes of deterioration of rough rice during storage, and probably ranks second only to insects as a cause of deterioration (1, 4). The growth of mold on stored rough rice may result in the fellowing damages: discoloration of the kernels even in the absence of heating, heat damage, lowering the quality of rough and polished rice, decreased germination, deterioration in the technological characteristics of rough rice, loss of dry matter from the grain, production of toxins that if consumed may be injurious to man and domestic animals, various biochemical changes, and mustiness (3, 4, 5, 6, 7, 8, 9, 10).

Of the many factors that influence mold development in rough rice, the moisture content and relative humidity are most important. Rough rice can be safely stored with high moisture level for short periods of time but very soon mold will proliferate when the moisture and oxygen are available (10, 11).

With the development of mold, heat will be generated in ever increasing amounts.

Many methods have been introduced to prevent or reduce deterioracion of high moisture stored rough rice. These include chilling or refrigeration, preservatives, chemicals (organic acids and fungicides), and garma ray irradiation. All these methods are used to inhibit or minimize the growth of microorganisms (especially mold growth) during storage.

The object of this investigation was an attempt to sterilize damp rough rice by treatment with flash heat. The effect of flash heat treatment on the growth of mold, bacteria, yeast, and the quality of rough rice for milling was studied.

REVIEW OF LITERATURE

Under conditions of storage of cereal grains that permit growth of microorganisms, development of molds will predominate. Bacteria and yeast organisms
may grow but generally mold growth will be far greater. The main reasons for
their difference is because mold will proliferate at lower moisture levels
than yeast and bacteria. Mold will also multiply quickly at lower temperatures
(10°-20°C.) than bacteria. Molds are able to respire with the limited oxygen
supply in stored cereals and then provide the necessary enzyme system to
hydrolyze the natural energy supply of the cereals (4).

Since most common species of fungi are found on the surface of rough rice, these common species will multiply and respire when rough rice of 18-24% moisture is placed in bulk storage. These common fungi develop on the leaves, stem, and husks of the rice as it is grown in the paddy. These fungi also sporulate and thus, provide a serious source of infestation in the stored rice (11, 13).

The fungi associated with rough rice are divided into two indistinct groups, field and storage fungi. This division is based on the moisture content requirement for growth of the fungi (9, 10). Field fungi infest the kernels of seed before harvest and in general, the following genera of field fungi are found in rough rice (1, 9, 10).

Helminthosporium.

Nigrospora.

Fusarium.

Curvularia.

Cladosporium.

Alternaria.

Rhizoctonia.

Penicillium.

All these genera require a high moisture content in order to grow. A moisture content of 22-23% provides a relative humidity of 95-100% which is required for their growth (1, 9, 10). Field fungi decrease while storage fungi increase during storage. They may survive for years in dry grain, but die rapidly in grains stored at relative humidities of 70-75% or a moisture content of 13.5% to 14.8% (9, 10, 11, 14).

Storage fungi have the ability to grow in grains where moisture contents are in equilibrium with relative humidities of 70% to 90% in which no free water is present (9). The species of <u>Aspergillus</u> and <u>Penicillium</u> are the most prevalent fungi that invade rough rice during storage (1), but <u>Rizopus</u> and <u>Mucor</u> also may develop in rough rice during storage (10).

There are many conditions that influence the development of storage fungi on stored rough rice. The moisture content of the stored grains is the main factor. For each of the common species of storage fungi, there is a minimum moisture level in the grain below which the mold cannot grow (4, 9, 14).

Shibabe and Iizuka (15) stated that the only species of molds which can grow on stored rice grain containing 14% to 15% moisture is Aspergillus restrictus, but when the moisture content was 16% Aspergillus glaucus group and Aspergillus restrictus developed. At 17% moisture, growths of the molds were mainly members of Aspergillus glaucus group and Penicillium citrinum series, whereas Aspergillus restrictus and Aspergillus versicolor showed minor growth. Del-prado and Christensen (15) found that at higher moisture contents of stored rough rice, the mold population increased during storage.

The greater the moisture content of the grain, the greater will be the rate at which nutrient substances reach the mold cells and the more intensive their respiration and liberation of heat. As a result, mold reproduction rate is greatly increased (4, 11, 16, 17).

Mold population increases rapidly at about 30° to 32°C. and decreases as the temperature is lowered. Some strains of the <u>Aspergillus glaucus</u> group can grow slowly at a temperature of 1.6° to 4.5°C., and some species of <u>Penicillium</u> can grow at a temperature several degrees below freezing (14, 17).

Fungi are commonly regarded as being strictly aerobic. Under low oxygen concentration, mold growth will be greatly reduced. Schlegel, et. al. (5) pointed out that the mold population decreased gradually as the oxygen concentration was lowered and as the carbon dioxide increased.

If the rice has been invaded by storage fungi during harvest, it is already in the first stage of deterioration and if the grain is stored under conditions that permit growth to continue, greater degree of damage in a given time will occur.

Agrawal, et. al. (18) stored grain of 12% initial moisture content which was infested and non-infested with the granary weevils. After three months, the grain infested with weevils had a moisture content ranging from 17.6% to 23% while the grain not infested had a moisture content of 14.6% to 14.8%. The development of storage fungi is also affected by growth of the insects and mites. The biological activity of these organisms will localize the moisture content of grains, thus increasing the mold activity. However, these organisms may carry the fungi spores through out the stored grain (14).

The active growth of various molds on rough rice can and does cause all types of deleterious changes in stored grain (3, 4, 5, 6, 7, 8, 9, 10, 13, 14). Both field fungi and storage fungi may cause discoloration of whole seeds.

Field fungi produces dark brown, black, and red pigments (1, 9, 10, 22, 23). Storage fungi may develop orange colored stains by <u>Penicillium puberulum</u> (19) and yellowish stains by <u>Penicillium islandicum</u> (20). Discolored kernels are classified as damaged in U. S. grain standards for rough rice, brown rice, and milled rice (19). Such kernels cause a considerable loss to the rice industry because when milled, they break more easily than do sound kernels (21).

The growth of storage fungi in stored rough rice will cause a heat damage and will cause dark amber to brown coloration of kernels. Kernel discoloration (heat damage) in rough rice may develop without any detectable rise in temperature. The presence of heat damaged kernels in rough rice is considered evidence that the rice has undergone deterioration (9, 10). Schroeder and Sorenson (24) found that the percentage of heat damaged rice in experimental storage increased with number of storage fungi.

Aspergillus glaucus, if growing rapidly, can increase the temperature of the grain at least to 40°C. At moisture levels of 15% to 15.5% Aspergillus candidus can grow, and if the growth conditions are favorable, this mold can increase the temperature of the grain rapidly. When the moisture content of the grain reaches 18.5% Aspergillus flavus can grow. Aspergillus candidus and Aspergillus flavus together can increase the temperature of the grain to 55°C. and maintain this temperature for weeks. The metabolic activities of these fungi may cause accumulation of free water on the grain; therefore thermophilic bacteria may carry the temperature up to 75°C., after which the purely chemical processes may take over and carry the temperature to the point of combustion (9).

The high fungal activities will develop musty or moldy odors in rough rice.

Musty odors are associated with sporulation of some of the storage fungi. Musty
or moldy odors are not allowable in rice above Sample Grade in the U. S. Grain

Standards (9, 10). The U. S. D. A. rice inspection manual specifies that rice which is musty or sour, or which has any commercially objectionable foreign odor shall be graded U. S. Sample Grade (25, 26, 27).

All factors necessary for the development of severe aflatoxin contamination are present in rice. Rice provides an excellent substrate for aflatoxin production by Aspergillus flavus. This mold may be found on rough rice in large numbers, and harvested rice is usually at high moisture content and temperature for rapid development of this fungi (28). Christensen (29) found that the group species, Aspergillus glaucus, Aspergillus candidus, Aspergillus flavus, and Penicillium spp. increased with time in rough rice stored with moisture contents of 17% to 20% and at a temperature between 12°C. to 27°C., and these molds produce potent toxins when growing on a natural substrate such as rice. Schroeder (28) reported that Aspergillus flavus group was the principal producer of aflatoxin in the temperature range of 25-35°C. and a relative humidity of 80-100% in stored cereal crops. Schroeder (30) found certain species of Aspergillus flavus produced high concentration of aflatoxin on rice compared to peanuts. Galderwood and Schroeder (6) found that rice aerated at a rate of 0.3 C. F./M./CWT. was significantly contaminated with aflatoxin within 2 days of storage. The concentration of toxins exceeded 800 p.p.b. after 20 days storage. Schroeder et. al. (31) pointed out that most aflatoxin in contaminated rough rice was located in the bran and other layer of the kernels. Ordinary milling procedures, therefore, can be expected to remove much of the toxins. They also found that the concentration of aflatoxins in the bran functions remained more than 10 times as high as in the milled rice fractions.

Chemical changes and deterioration in the nutritive value of rough rice is also caused by the growth of storage fungi. The activaly growing fungi causes many chemical changes in stored rough rice (37). These changes occur at varying rates which depend upon the following factors: the oxygen supply, the moisture content, the temperature, and the degree of soundness of the grain. At higher levels of moisture and temperature, there are marked increases in enzymatic and respiratory activity and the various chemical changes associated with deterioration (17, 18). Fungal activity in stored rough rice will cause an increase in the free fatty acids and change the proportions of reducing and non-reducing sugars of the rice. However, the growth of fungi may actually increase the nutritive value in some cases by producing some vitamins, growth substances, essential amino acids, and other substances (10). The biological activities of fungi such as respiration results in the loss of dry matter from the grain, thus increasing the loss in total available food (10).

With a high moisture content, rough rice decreases in germination and increases in storage fungi as the storage time is extended at temperature levels between 25-31°C. (3, 16, 33, 34). Christensen and Lopez (33) found that invasion of rough rice with storage fungi caused a decrease in loss of germination which normally occurs during storage.

Yeasts are potential spoilage agents of stored rough rice. They can produce off-flavors and odors in rough rice stored in sealed bins by decomposition of the natural constituents of rice, especially the fat which is present in high concentrations in rice bran (35).

Teunisson (12) storing rough rice with 18% to 20% moisture in sealed glass-lined bins 34 and 210 days found that both samples became sour. Some

molds survived but did not increase while aerobic bacteria survived or decreased in number and the yeast number increased tremendously. The number of yeast cells in these samples were millions per gram, compared to less than 1000 per gram in the control sample.

The types of yeasts isolated from sour rough rice by Teunisson (35) were as follows:

Endomycopsis chodati.

Hansenula anomala.

Pichia farinosa.

Candida krusei.

Oospora lactis.

Candida tropicalis.

Bacteria normally do not appear to be involved in the deterioration of stored seeds. None of the bacteria can grow at moisture levels below those where free water is not available. This means bacteria grow in a relative humidity of about 100% (7, 9, 12). If fungi has caused the temperature of spoiling grain to be about 55°C., and if the grain has a moisture content to promote free water, thermophilic bacteria may become involved in the heating process (9). There are many genera of bacteria which are present on the stored grains (36). In general, these genera are as follows:

Lactobacillus.

Micrococcus.

Pediococcus.

Pseudomonas.

Serretia.

Streptococcus.

Acetobacter.

Achromobacter.

Bacillus.

Bacterium.

Clostridium.

Escherishia.

Flavobacterium.

Graves, et. al. (37) studied samples of wheat taken from 11 flour mills located in different places in the U. S. A. The most frequently encountered and widely distributed bacteria in these samples were those belonging to the genus <u>Flavobacterium</u>. Members of this genus accounted for more than 53% of the total number isolated from the wheat.

Several methods and combinations of methods have been developed to maintain the quality during storage of the grains which had a high moisture content. Fungicides were used to inhibit the storage fungi on high moisture content grains. All the fungicides may have one or more of the following difficulties: toxicity to man and animals, excessive cost, difficulty of application, undesirable effects on processing quality of the grains, and lack of toxicity to storage fungi (7, 9).

Organic acids such as propionic, acetic, formic, and a combination of these has been used to protect grains in storage at a high moisture level from deterioration by mold and bacteria (1, 38, 39).

Schroeder (1) stored sodium propionate treated, damp, 14% and 16.5% rough rice for 4 and 6 months. The rate of infection by storage fungi was significantly reduced in the 14% moisture rough rice, while the other sample of 16.5% moisture content, sodium-propionate suppressed infection

by Aspergillus candidus but did not control Aspergillus glaucus.

Airtight storage has been developed to prevent mold growth in high moisture grain storage. In this type of storage, oxygen is eliminated in the airtight structure to a level which kills or inactivates the harmful organisms. Neither insects or molds become numerous enough to cause serious damage to the grain. Some fungi can grow at a very low oxygen concentration. At oxygen concentrations between 0.5% to 1%, many microorganisms such as molds and yeasts can grow rapidly and if the temperature is suitable these microorganisms will cause deterioration of the grain. It is therefore important with damp grain that the storage structure be completely airtight. A high moisture grain can be safely stored in this type of storage for feeding to livestock without the development of molds (40).

Another method was introduced to prevent the growth of mold in a high moisture grain. Aeration with cold air greatly reduced the need for drying (41). Air with temperatures of 5°C. to 10°C. retard the growth of storage fungi and maintained uniform temperature throughout the grain (18). Aeration is only possible in cool climates or by mechanical refrigeration (chilling). There is a balance between moisture content and safe storage temperature, the higher the moisture content, the lower must be the temperature for the safe storage (42). Some fungi, when sufficient moisture is present, will grow at a temperature well below freezing.

Houston, et. al. (43) pointed out that when rough rice samples with an average moisture content of 12%, 13.1%, and 15.9% were stored for three years at -29°C., -7°C., and +1°C., the yeast counts fluctuated markedly during storage. They varied between seven thousand and forty-nine million per gram. However, molds showed no significant change but did survive, and viability

of the higher moisture rices at +1°C. decreased significantly. Kondo, et. al. (44, 45) found that rough rice stored at low temperatures for 11 and 15 years retained many good quality characteristics but failed to germinate.

Gamma ray irradiation treatment was used in Japan for treatment of a high moisture rough rice. Shibaba, et. al. (15) treated rough rice (14% moisture and a temperature of 30°C.) with gamma ray irradiation at a dosage of 0.1 mrad per hour. They found that rough rice so treated could be safely stored for 2 to 3 months. By use of 0.2 mrad/hour, rough rice (16% moisture) storage could be safely extended to 3 to 4 months. Irradiation dosage of 0.3 mrad/hour extended the storage still longer. They found it possible to safely store 17% moisture rough rice if it had been irradiated with 0.4 mrad/hour.

From the review of the literature it is evident that molds are one of the main causes of rough rice deterioration during storage. This investigation was an attempt to develop a new economical method to destroy mold which invades damp grain, thus reducing damp grain deterioration during storage.

MATERIALS AND METHODS

Medium grain, rough rice (not parboiled) with a moisture content of 1.0.2%, a protein content of 8.2%, a fat content of 2.1%, and ash content of 5.4% was used in these studies. Rough rice was divided into two parts. One part was tempered to 18% moisture by addition of water and subdivided into seven 5 pound samples and treated as follows:

Control.

Flash heat at 600° ± 10°C, once.

Flash heat at 600° ± 10°C, twice.

Flash heat at 700° ± 10°C, once.

Flash heat at 700° ± 10°C, twice.

Flash heat at 800° ± 10°C, once.

Flash heat at 800° ± 10°C, twice.

The second part of the rough rice was subdivided into ten 5 pound samples with various moisture contents (M.C.) as follows:

Control 14% M.C.

14% M.C. flash heat treatment at 800 ± 10°C, twice.

Control 16% M.C.

16% M.C. flash heat treatment at 800 ± 10°C, twice.

Control 18% M.C.

18% M.C. flash heat treatment at 800 ± 10°C, twice.

Control 20% M.C.

20% M.C. flash heat treatment at 800 ± 10°C, twice.

Control 22% M.C.

22% M.C. flash heat treatment at 800 ± 10°C, twice.

An instrument was designed and built to treat grain samples with flash heat at different temperatures. This instrument consisted of a 60" X 5.0" steel tube. Six baffle plates were located alternately inside the tube to regulate flow of grains. Two gas burners were installed inside the tube at 40" and 45" from its bottom. These burners were connected to natural gasair mixing valves. A 9" long hopper with slide and ventilation gate were attached to the top of the tube, while a metallic 10" X 8" X 6" receiving box was attached at the bottom of the tube to receive the grain after flash treatment. A pyrometer was inserted just above the burners to measure the temperature of the flash heat. The instrument was held vertically by a triangular iron stand. Treated rough rice samples were passed quickly through flash heat into the receiving box. The average time for kernels to pass through the intense heat (600°-800°C.) was 0.1 second, so that the rate of passing of grain was regulated at 6 pounds per minute. This instrument is illustrated in Plate 1.

All 17 samples of rough rice were stored in 5 pound capacity metal cans, which were insulated with glass wool. The storage time was 15 days at room temperature (about 28°C.). Compressed air was passed to the samples through sterilized glass-wool periodically (every 48 hours of the storage time).

Total count of microorganisms (mold, bacteria, yeast) was performed on the sample before treatment, after treatment, and after 15 days storage. Milling and physical tests were performed on the polished rice. Samples were milled on a McGill experimental sheller and polished with Satake experimental pearler. Samples from all the treated samples and the control samples were milled and polished before and after storage. Plate count method was used to determine number of mold, bacteria, and yeast colonies.

Potato dextrose agar (PDA) was used as a medium for mold, nutrient agar (NA) was used for bacteria, and orange agar (OA) was used for yeast. Three different dilutions and three plates for each dilution were made for each sample. The temperature of incubation was 30°C., incubation time was 3 days for bacteria and yeast, and 5 days for mold.

U. S. grade requirement and grade designations for medium grain milled rice (illustrated in Table 11) were used for grading the samples after milling and polishing, and to evaluate the color and other physical characteristics. The percentage of broken kernels was determined by the hand separation of the broken kernels in 100 grams of each polished rice sample.

The other part of this study was made as an attempt to store damp rough rice in large quantities. Two 3 bushel lots of rough rice samples were tempered to 18% moisture. One was treated with flash heat at 800°C. ± 10°C. twice, and the other was the control. These samples were stored for 8 weeks in an insulated barrel. Total count of microorganisms was made of these samples after every week of storage time. The storage conditions were the same as in the small 5 pound samples, the same method as used for the small samples was used to count the number of microorganisms in the large sample.

During the period of storage, temperature of grain inside the barrels (large samples) was recorded every 84 hours of storage time by a thermometer inserted into each barrel. All treated samples were brought to the same temperature as the untreated samples before storage.

Germination of seeds was performed for all samples to test viability of grains. One hundred kernels were placed on moist toweling and incubated at 28°C., and the seedlings counted each day for 5 to 7 days. Any seed which produced a root or coleoptile was considered to have germinated.

Illustration of Flash Heater Used for Sterilizing Grains (Plate 1).

To the left of tube is a thermostat leading to pyrometer for reading the temperature of flash heat. Below the thermostat are two gas burners joined to gas mixing valves. At the top of the tube is the hopper and regulating valve, while the receiving box is attached to the bottom of the tube. This box was located to receive the treated grain.

Inside the tube are baffle plates to reduce the flow rate of the grain.

The entire assembly is supported by a tripod.

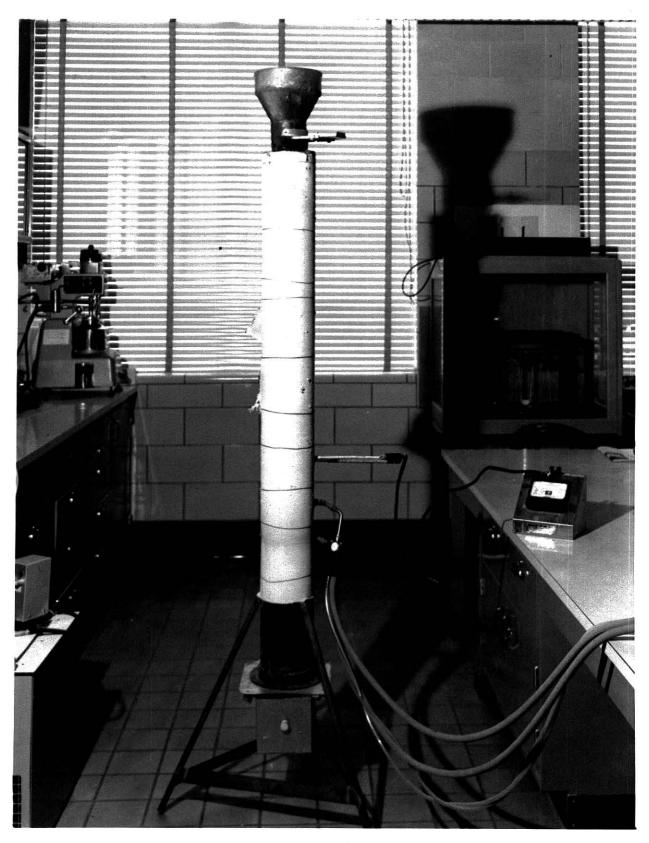


Plate 1. Flash heater .

RESULTS AND DISCUSSION

1. Total Count of Microorganisms.

A. Different flash heat temperature treatments.

Total count (mold, bacteria, and yeast) for all the samples were determined and are summarized in Tables 1 and 2. The 18% moisture content rough rice was used to test the ability of flash heat treatment to control the development of microorganisms during storage. The microbial counts of rough rice decreased as the temperature of flash heat increased. The same trend was found when the number of passes through the flash heater increased (Tables 1 and 2). However, after 15 days of storage, the number of microorganisms increased in all treatments except flash-heated treatment at 800°C., twice.

Analysis of interaction between treatments and storage time is summarized in Table 3. There were significant differences between treatments with flash heat at 800°C. once or twice and the following treatments: flash heat at 600°C. once, or twice, flash heat at 700°C. once, or twice. There were no significant differences between the following treatments: flash heat at 600°C. once, or twice, flash heat at 700°C. once, or twice. Table 3 also shows that there were no significant differences between flash heat treatments at 800°C. once or twice, before and after storage. There were significant differences in mold populations between flash heat treatments at 600°C. once or twice,

Total count of microorganisms (mold, bacteria, and yeast) and temperatures, of 18% moisture content rough rice samples after treatment.

TABLE 1

Treatment		Temperature of Rough Rice After Treatment	Mold Colonies Per Gm Rough Rice	Bacteria and Yeast Colonies Per Gm Rough Rice
Control.		27°C.	5600	1,800,000
Treated with flash heat	600°C. once.	43°C.	210	1,050,000
Treated with flash heat	600°C. twice	. 44°C.	49	850,000
Treated with flash heat	700°C. once.	45°C.	180	890,000
Treated with flash heat	700°C. twice	. 48°C.	60	21,500
Treated with flash heat	800°C. once.	49°C.	50	620,000
Treated with flash heat	800°C. twice	. 52°C.	0	2,550

TABLE 2

Total count of microorganisms (mold, bacteria, and yeast) of 18% moisture content rough rice samples after 15 days of storage.

Treatment	Mold Colonie Per Gm Rough Rice	Bacteria s Yeast Colonies Per Gm Rough Rice
Control	10,800,000	2,000,000
Treated with flash heat at 600°C. once.	1,750,000	3,000,000
Treated with flash heat at 600°C. twice	830,000	1,150,000
Treated with flash heat at 700°C. once.	1,030,000	1,300,000
Treated with flash heat at 700°C. twice	590,000	26,000
Treated with flash heat at 800°C. once.	40,000	680,000
Treated with flash heat at 800°C, twice	. 133	3,000

TABLE 3

Effect of storage on numbers of mold per gram of rough rice 18% moisture content treated with different flash heat temperature.

	Storage	Mold Colonies
Treatment	Before and After	Per Gm Rough Rice
Flash heat at 600°C.	Before	133.997
Flash heat at 600°C.	After	1,292,999.99 **
Flash heat at 700°C.	Before	120.0
Flash heat at 700°C.	After	828,300.0 *
Flash heat at 800°C.	Before	25.0
Flash heat at 800°C.	After	20,066.5

Significant range of mold = 526,274.81

Protection level = 0.05

^{*} Significantly different

^{**} Highly significantly different

From the analysis of variance it was found that there were no significant differences in the means of numbers of bacteria between samples treated with flash heat and non-treated (control) samples, before and after 15 days storage for rough rice of 18% moisture content.

B. Different moisture content samples.

Five different moisture levels including 14%, 16%, 18%, 20%, and 22% moisture of rough rice were used in these studies. Total microbial count (mold, bacteria, and yeast) of all the samples were determined before and after 15 days of storage. The results are given in Tables 4 and 5.

The 14%, 16%, 18%, 20%, and 22% moisture content rough rice were used to test the effectiveness of treatment with flash heat at 800°C., twice, to retard development of microorganisms during storage. Data in Table 6 shows the mean number of mold spores. There were no significant differences before and after 15 days of storage between the following treatments: 14% moisture control and 14% moisture samples treated with flash heat at 800°C. twice, 16% moisture control and 16% moisture treated with flash heat at 800°C., twice. There was a significant difference between before and after 15 days of storage for control and flash heat treated rice at 800°C. twice, for the following samples: 18% moisture, 20% moisture, and 22% moisture.

Numbers of mold, Table 7, indicated no significant differences between 14% moisture control and 14% moisture treated with flash heat at 800°C., twice, before and after storage, 16% moisture control and 16% moisture treated with flash heat at 800°C., twice, before and after storage. There were significant differences between the control samples and those treated with flash heat at 800°C., twice, among the following: 18, 20, and 22% moisture, respectively,

TABLE 4

Total count of microorganisms (mold, bacteria, and yeast) of 14%, 16%, 18%, 20%, and 22% moisture content rough rice samples. Control and treated before storage.

% Moisture Content	Treatment	Mold Colonies Per Gm Rough Rice	Bacteria and Yeast Colonies Per Gm Rough Rice
14	Control.	4200	1,600,000
14	Treated with 800°C., twice.	0	110,000
16	Control.	7000	2,730,000
16	Treated with 800°C., twice.	0	32,000
18	Control.	5600	1,800,000
18	Treated with 800°C., twice.	0	2,550
20	Control.	5500	2,360,000
20	Treated with 800°C., twice.	0	10,500
22	Control.	78000	3,400,000
22	Treated with 800°C., twice.	0	1,000

TABLE 5

Total count of microorganisms (mold, bacteria, and yeast) of 14%, 16%, 18%, 20%, and 22% moisture content rough rice control and treated samples after 15 days storage.

			Deserved and
% Moisture Content	Treatment	Mold Colonies Per Gm Rough Rice	Bacteria and Yeast Colonies Per Gm Rough Rice
14	Control.	4,400	2,550,000
14	Treated with 800°C., twice.	0	115,000
16	Control.	3,000,000	3,000,000
16	Treated with 800°C., twice.	82	32,000
18	Control.	10,800,000	2,000,000
18	Treated with 800°C., twice.	133	3,000
20	Control.	12,500,000	25,000,000
20	Treated with 800°C., twice.	29,000	31,000
22	Control.	19,000,000	4,400,000
22	Treated with 800°C., twice.	540,000	5,800

TABLE 6

Effecting initial moisture and flash heat treatment on mold count in rough rice.

% Moisture Content	Before or After Storage	Mold Colonies Per Gm Rough Rice
14	Before	2,101
14	After	2,201.5
16	Before	3,533.0
16	After	1,666,540.7
18	Before	2,800
18	After	5,465,065 *
20	Before	2,766.5
20	After	6,364,749.9 *
22	Before	38,330.0
22	After	9,876,647.9 **

Significant range of mold = 4,622,940

Protection level = 0.05

^{*} Significantly different

^{**} Highly significantly different

 $\label{table 7} \mbox{Numbers of mold per gram of rough rice after 15 days storage. }$

% Moisture Content	Treatment	Mold Colonies Per Gm Rough Rice
14	Control.	4,303.0
14	Treated 800°C., twice.	0.0
16	Control.	670,032.9
16	Treated 800°C., twice.	41.329
18	Control.	5,867,799.9
18	Treated 800°C., twice.	66.5 *
20	Control.	6,352,765.9
20	Treated 800°C., twice.	14,750.0 *
22	Control.	9,638,327.9
22	Treated 800°C., twice.	276,650.0 **

Significant range of mold = 3,629,308

Protection level = 0.05

^{*} Significantly different

^{**} Highly significantly different

before and after 15 days storage.

Table 8 shows that for the five levels of moisture there were significant differences in the numbers of mold between the control before and after storage to 15 days, but there were no significant differences between the treated samples before and after 15 days storage.

Data in Table 9 indicates that there were significant differences in the number of bacteria between the control samples and all samples treated with flash heat at 800°C., twice. This appeared true for both before and after 15 days of storage at all five levels of moisture.

C. Large (3-bushel) sample studies.

Flash heat temperature of 800°C. was selected to treat large quantities of rough rice. This temperature was used because the preliminary evidence indicated that this temperature would destroy the maximum number of microorganisms on the grain surface, and this temperature has no significant change on the milling quality and grade designation of the rice.

Two 3-bushel samples of 18% moisture, one control and the other treated with flash heat at 800°C., twice, were used in these studies. Numbers of microorganisms of both samples were determined before storage and after each week of the total eight weeks storage period at room temperature (about 78°F.). The barrels were insulated to maintain temperature generated during storage. The data are summarized in Table 10. The number of microorganisms in the control sample increased very rapidly after storage, compared to the treated sample.

The number of microorganisms in these studies were less when samples of rough rice were subjected to flash heat. This indicated that most of the

TABLE 8

Effect of moisture level and flash heat treatment on number of mold per gram of rough rice.

Treatment	Mold Colonies Per Gm Rough Rice
Control before storage.	19,812.39
Control after 15 days storage.	9,233,477.9 **
Treated before storage.	0.0
Treated after 15 days storage.	116,603.125

Significant range of mold = 5,132,621.0

Protection level = 0.05

The significant difference is shown by the number of stars.

TABLE 9

Effect of moisture content and flash heat treatment on number of bacteria per gram of rough rice before and after storage.

% Moisture Content	Treatment	Mold Colonies Per Gm Rough Rice
14	Control.	2,081,000
14	Treated with 800°C., twice.	111,950 *
16	Control.	2,941,500
16	Treated with 800°C., twice.	33,650 *
18	Control.	1,913,000
18	Treated with 800°C., twice.	2,795 *
20	Control.	2,398,000
20	Treated with 800°C., twice.	21,680 *
22	Control.	3,933,000
22	Treated with 800°C., twice.	3,514 **

Significant means range for bacteria = 675,085.625

Protection level = 0.05

^{*} Significantly different

^{**} Highly significantly different

microorganisms on the surface of grains had been destroyed by the intense heat of the instrument. Sharp decreases in the number of microorganisms occurred as the temperature of flash heat increased.

In the samples of rough rice, where the moisture contents increased, the number of microorganisms increased very rapidly. Many investigators have studied the relationship between the moisture content of rough rice and the growth of microorganisms during storage. They found a positive relationship between moisture content and mold population (3, 7, 9, 10, 11, 12, 15, 16, 17, 35). Deterioration occurs in high moisture rough rice during storage because of the rapid growth of mold and other microorganisms.

Control samples in this study showed sharp increases in mold and other microorganisms during storage while the microorganism population in treated samples increased only slightly. In some treatments such as using flash heat at 800°C., once and twice, the number of microorganisms remained constant or decreased during storage. This was because of the effect of flash heat which destroyed the microorganisms and prevented their growth during storage.

Both the control and treated samples had the temperature adjusted to the same level before storage (about 78°F.). The treated samples were cooled until they reached the temperature of the control samples. The temperature of both the control sample and treated sample was recorded twice a week during storage. The barrels were insulated with 3" fiberglass batting from the outside. Figure 1 shows that there was a sharp increase in the temperature of the control sample while the sample treated with flash heat at 800°C., twice, showed almost a constant temperature for five weeks of storage. After that the temperature started to increase slowly until the end of the storage period.

TABLE 10

Total count of mold, bacteria and yeast of 18% moisture content rough rice (3-bushel samples) during storage time.

	Mold Colonies Per Gm Rough Rice		Bacteria Colo Gm Rough Rice	
Storage Time	Control	Treated	Control	Treated
1st Week	8,000	0	93,000	1,330
2nd Week	2,700,000	0	110,000	1,500
3rd Week	2,880,000	610	150,000	1,680
4th Week	27,700,000	6,500	180,000	1,800
5th Week	137,500,000	20,000	185,000	1,850
6th Week	179,000,000	22,200	750,000	2,300
7th Week	380,000,000	600,000	1,800,000	2,865
8th Week	420,000,000	1,650,000	4,200,000	3,050

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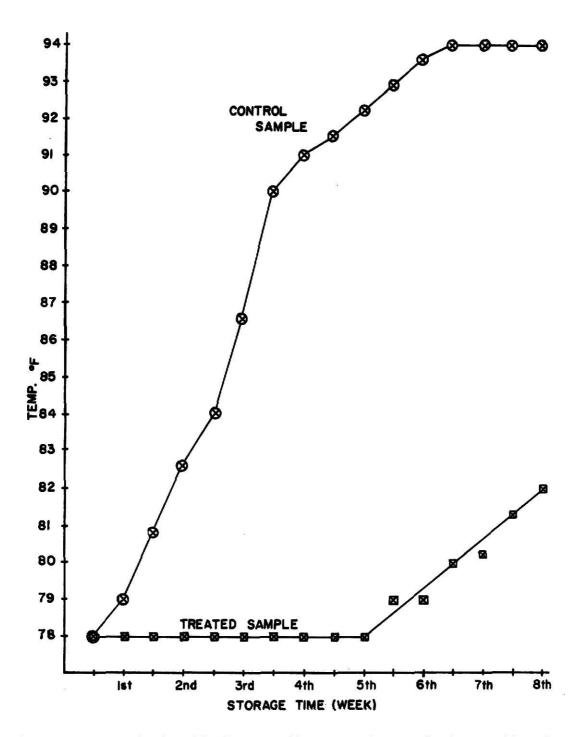


Figure 1. The relationship between the temprature and storage time in 3-bushels of control and treated samples (rough rice).

The large number of microorganisms which invaded the control sample caused the temperature of the stored grain to increase sharply, because of their respiration and other biological activities. Gilman and Barron (46) observed that there was no marked rise in temperature in storage grains except where microorganisms were present. Christensen and Gordon (47) also noticed that at moisture levels favorable to the growth of mold in stored grains, molds caused the temperature to rise within a few degrees of the maximum temperature that the molds could endure.

Figures 2-8 show a heavy growth of mold, bacteria, and yeast in all the control samples, but slight growth in the samples given a flash heat treatment before storage. Microorganisms in the control samples increased sharply in number after 15 days in small samples and in 8 weeks in the 3-bushel samples. Samples which were treated with flash heat at 800°C. once and 800°C. twice (800°C. twice for 3-bushel samples) showed a lower number of mold, bacteria, and yeast after storage.

Figures 9-12 show a heavy growth of microorganisms in the control samples of all five levels of moisture before and after 15 days of storage, but in samples which were treated with flash heat at 800°C. twice there was a slight growth of mold, bacteria, and yeast. After 15 days storage of the same samples, there was a lower number of mold, bacteria, and yeast at the moisture levels of 14%, 16%, and 18% and sharp increases in the number of the same organisms at moisture levels of 20% and 22% after the same period of storage.

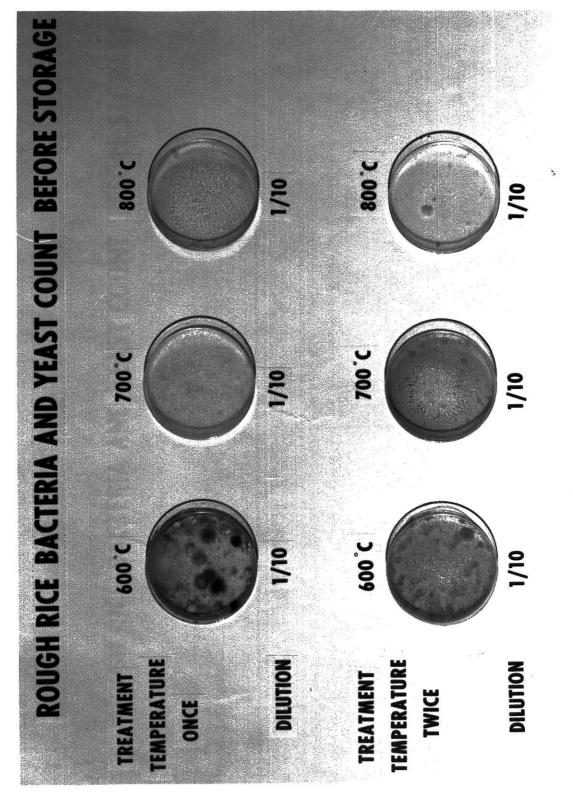


Figure 2 . Effect of flash heat temperature and time of passage on bacteria and yeast count before storage .

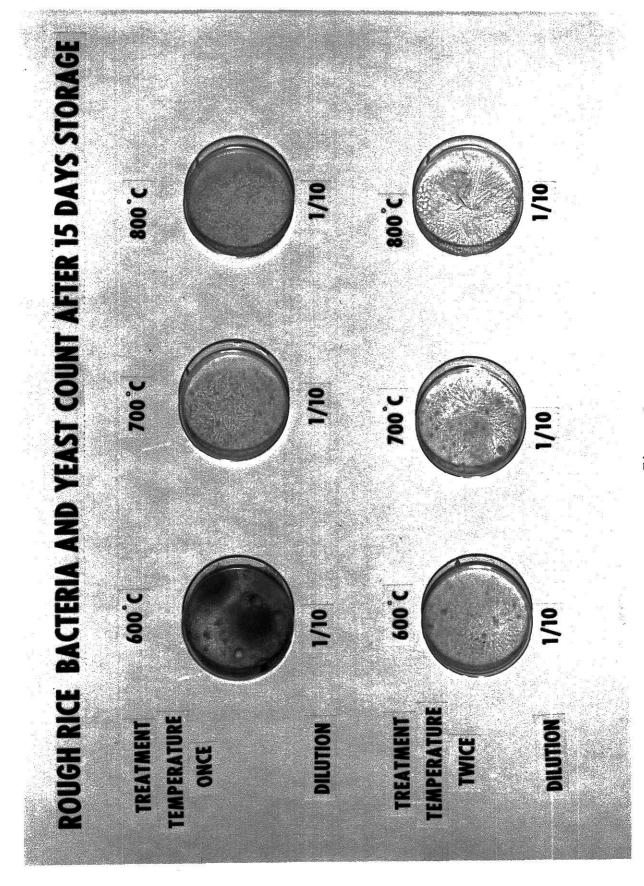


Figure 3. Effect of flash heat temperature and time of passage on bacteria and yeast count after 15 days of storage.

ROUGH RICE MOLD COUNT BEFORE STORAGE J. 008 S00°C 700°C J. 00/ J. 009 TEMPERATURE TEMPERATURE TREATMENT TVICE

Figure μ . Effect of flash heat temperature and time of passage on mold count before storage.

DILUTION

ROUGH RICE MOLD COUNT AFTER 15 DAYS STORAGE 300g 780°C 700°C ر 909 2 **TEMPERATURE** TEMPERATURE TREATMENT ONCE

Figure 5. Effect of flash heat temperature and time of passage on mold count after 15 days of storage.

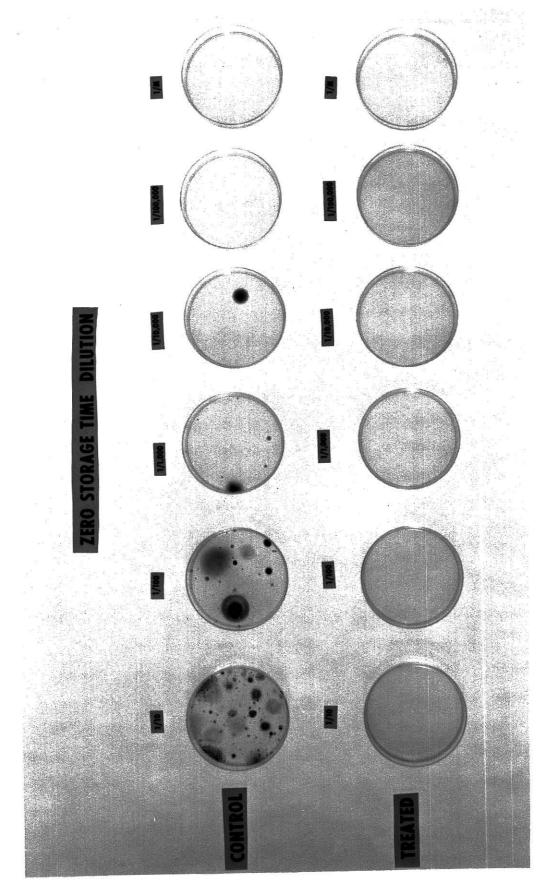


Figure 6. Effect of flash heat at 800C, twice on mold growth before storage (3- bushel sample control and treated).

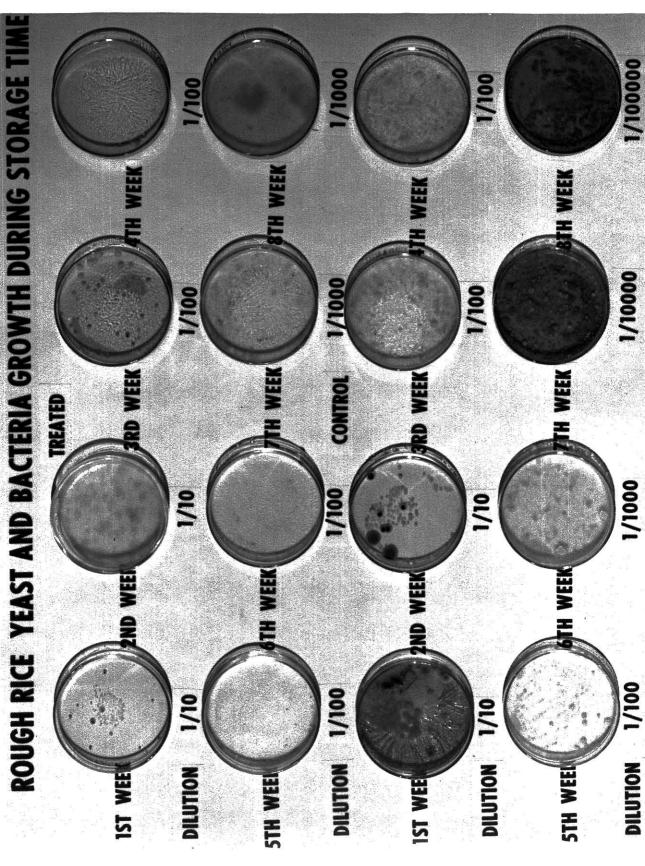


Figure 7. Effect of storage time on development of yeast and bacteria growth in both control and treated with flash heat at 800C, twice samples.

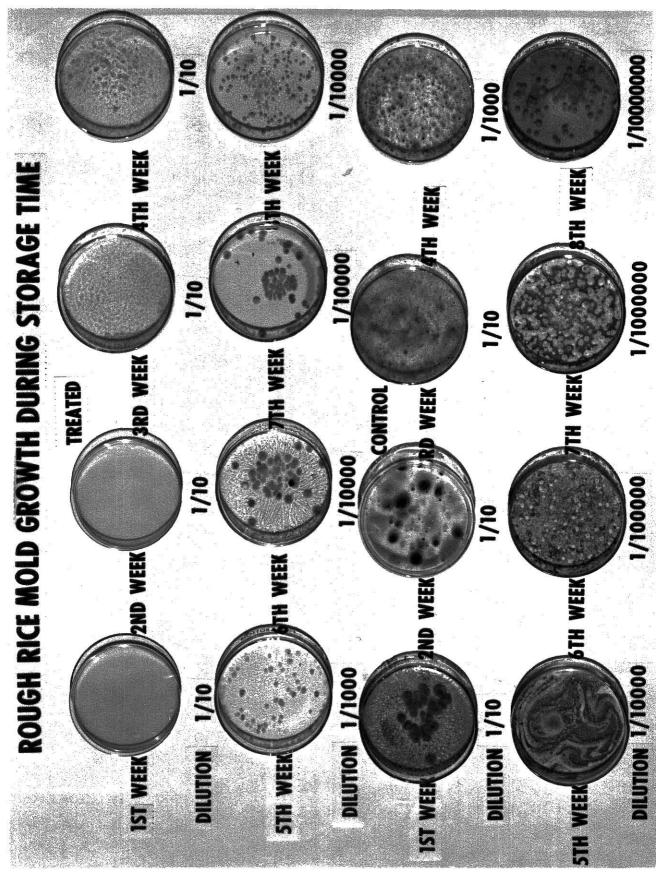


Figure 8. Effect of storage time on development of mold in both control and treated with flash heat at 800C, twice samples .

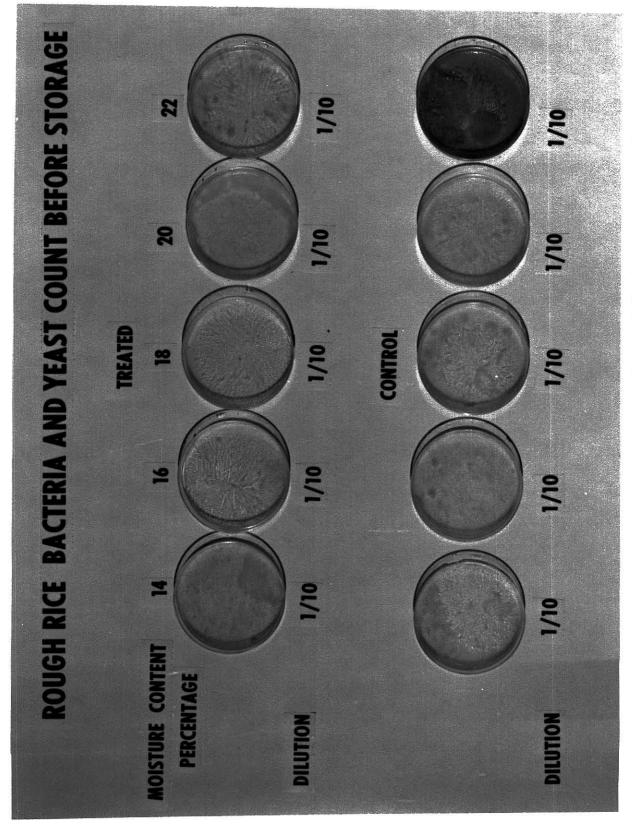


Figure 9. Effect of moisture content and flash heat treatment at 800C, twice on bacteria and yeast count before storage.

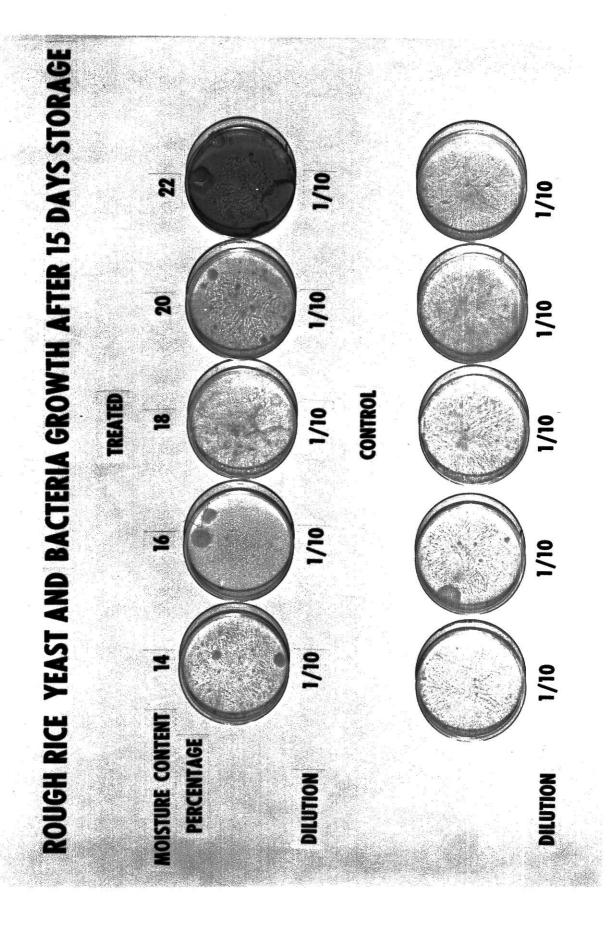


Figure 10. Effect of moisture content and flash heat treatment at 800C, twice on bacteria and yeast count after 15 days of storage.

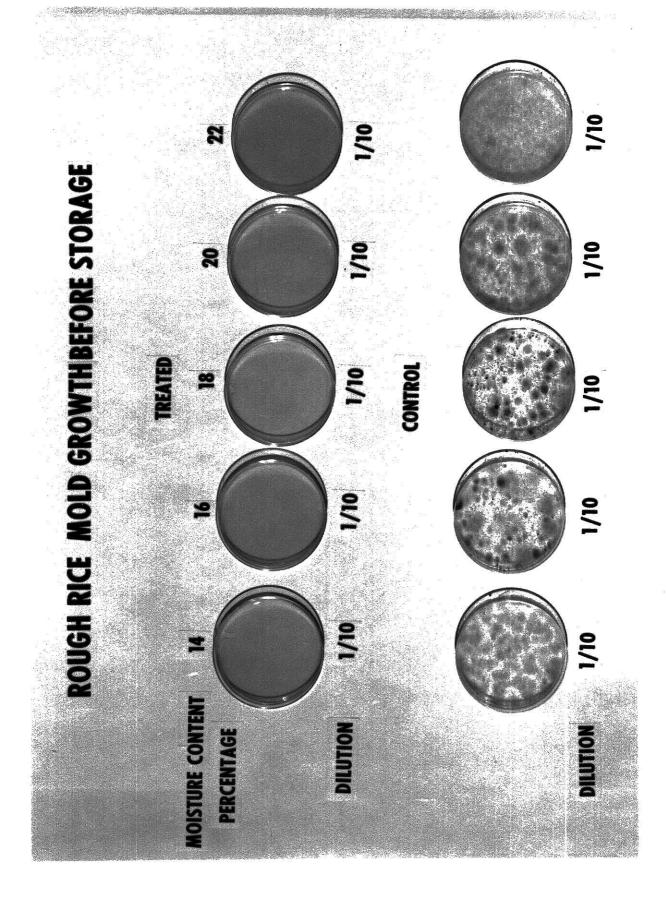


Figure 11. Effect of moisture content and flash heat treatment at 800C, twice on mold count before storage.

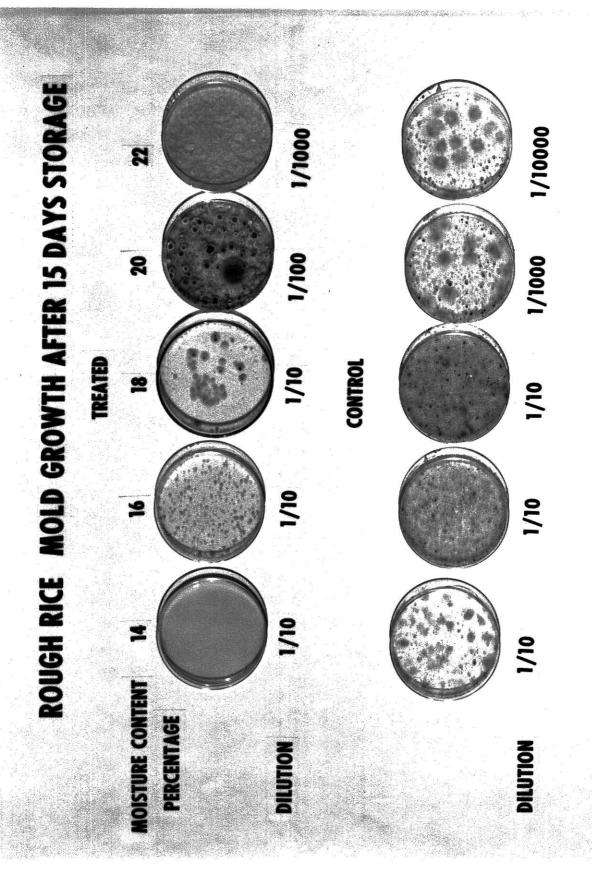


Figure 12.* Effect of moisture content and flash heat treatment at 800C, twice on mold count after 15 days of storage.

From the above data and figures, it may be observed that treatment with flash heat at 800°C. twice may be used as a successful method to prevent or reduce deterioration caused by the development of microorganisms on damp rough rice with a moisture content at a maximum level of 18%. But, when the moisture content exceeds this level, this method has a limited effect on preservation of rough rice.

2. Milling and Germination Studies.

All the samples of rough rice were milled to determine whether there was any change in the milling quality and grade of milled rice due to treatment.

Table 11 was used as a guide to determine the quality and grade of milled samples.

Tables 12 and 13 show no significant differences between flash heattreated samples and control samples either before and after 15 days of storage. Table 13 shows that as the moisture content of rough rice increased, the percentage of head rice decreased in all the samples, before or after storage, and in all treated or non-treated rough rice.

Data in Table 14 shows that there were no significant changes in the quality and grade of rice milled from treated rough rice during the 8 weeks of storage time (3-bushel sample) while in the control sample, the quality and grade of milled rice reduced after each week of storage. This included the percentage of head rice, color, grade, and odor. Schroeder (21) observed that the growth of mold on rough rice will cause discolored kernels. Such kernels cause a considerable loss to the rice industry because when milled, they break more easily than the sound kernels thus lowering the percentage of head rice and increasing the percentage of broken kernels.

TABLE 11

Grade, grade requirements, and grade designations for the medium grain milled rice. st

Maximum Limits of

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singly
kernels
paddy

Color and milling	Requirements	Shall be white or creamy and shall be well milled.	May be slightly gray and shall be well milled.	May be light gray and shall be at least reasonably well milled.	May be gray or slightly rosy and shall be at least reasonably well milled.	May be dark gray or rosy and shall be at least lightly milled.	May be dark gray or rosy and shall be at least lightly milled.
Broken Kernels	% %	4.0	7.0	15.0	25.0	35.0	50.0
Chalky Kernels	84	2.0	4.0	0.9	0.8	10.0	15.0
Red rice and damaged kernels (singly or combined)	%	0.5	1.5	2.5	4.0	6.0	15.0
	Number in 500 gm		2	'n	15	25	75
Total Number	in 500 gm	2	4	7	20	30	75
		- !	. 2	е	7	٠.	9
	e	No	No	No	No	No	No
2-	Grade	U.S. No.	U.S. No. 2	U.S. No.	U.S. No. 4	U.S. No.	U.S. No. 6

TABLE 11 (continued)

U. S. Sample grade for medium grain milled rice shall be milled rice which (a) does not meet the 15% moisture, (c) is musty, or sour, or heating, (d) has any commercially objectionable foreign odor, requirements for any of the grades from U. S. No. 1 to U. S. No. 6 inclusive, (b) contains more than (e) contains more than 0.1% foreign material, (f) contains live or dead weevils or other insects, insect webbing, or insect refuse, or (g) is otherwise of distinctly low quality.

Broken Kernels - Kernels of rice which are less than three-fourths of whole kernels.

U. S. Dept. Agr. * United States Standards for Rough Rice, Brown Rice for Processing Milled Rice. Consumer and Marketing Service (Rev. 1972).

The effect of flash heat treatment on the milling quality of rice before and after 15 days storage (18% M.C.).

TABLE 12

Treatment	Storage Before and After	% of Hulls	% of Polished Rice	% of Broken <u>Kernels</u>
Flash heat at 600°C., once.	Before	21	61	25
Flash heat at 600°C., once.	After	21	60	28
Flash heat at 600°C., twice.	Before	21	61	25
Flash heat at 600°C., twice.	After	22	59	27.5
Flash heat at 700°C., once.	Before	21	62	24.8
Flash heat at 700°C., once.	After	20	61	25
Flash heat at 700°C., twice.	Before	21	62	28
Flash heat at 700°C., twice.	After	20	62	24.5
Flash heat at 800°C., once.	Before	21	62	25
Flash heat at 800°C., once.	After	22	60	26
Flash heat at 800°C., twice.	Before	21	64	24.9
Flash heat at 800°C., twice.	After	20	64	24.8

TABLE 13

The effect of moisture content and flash heat treatment on the milling quality of rice before and after 15 days storage.

% Moistur Content	e <u>Treatment</u>	Storage Before and After	% of Hulls	% of Polished <u>Rice</u>	% of Broken Kernels
14	Control.	Before	21	65	15
14	Control.	After	21	65	15
14	800°C., twice.	Before	21	65	14.5
14	800°C., twice.	After	21	65	15
16	Control.	Before	21	65	18
16	Control.	After	21.5	66	18
16	800°C., twice.	Before	20	64	17.8
16	800°C., twice.	After	21	65	17
18	Control.	Before	21	62	25.5
18	Control.	After	23	60	24.5
18	800°C., twice.	Before	21	63	25
18	800°C., twice.	After	22	60	26
20	Control.	Before	22	60	54
20	Control.	After	24	55	61
20	800°C., twice.	Before	21	62	52.5
20	800°C., twice.	After	22.5	61	58
22	Control.	Before	26	54	85
22	Control.	After	28	51	89.5
22	800°C., twice.	Before	23	57	84
22	800°C., twice.	After	25	54	88.8

TABLE 14

The effect of storage time on the milling quality and rice grade of treated and non-treated rough rice (3-bushels 18% M. C. rough rice).

	Treated with 800°C. Flash Heat or Control	Storage Time	% of Hulls	% of Pol- ished Rice	% of Broken Ker- nels	Color	<u>Odor</u>	Grade U. S. No.
	Treated	1st Week	21	63.2	27	White	Good	5
-1	Control	1st Week	20	63.2	27.8	White	Good	5
	Treated	2nd Week	20	64	27	White	Good	5
- <i>p</i>	Control	2nd Week	20.5	63	30	Cream	Musty	Sample grade
	Treated	3rd Week	20	63.5	27.3	White	Good	5
	Control	3rd Week	21	62.8	37.5	Cream	Musty	Sample grade
	Treated	4th Week	20.5	63.2	27.7	White	Good	5
	Control	4th Week	22.8	57.2	33	Dark cream	Musty	Sample grade
	Treated	5th Week	20	63.1	28	White	Good	5
	Control	5th Week	26.7	53.5	33.6	Dark cream	Musty	Sample grade
	Treated	6th Week	20	64	28.8	White	Good	5 ,
	Control	6th Week	27.7	50.3	37.5	Dark cream	Musty	Sample grade
	Treated	7th Week	20	63.5	27	Cream	Good	5
	Control	7th Week	34.3	39.7	48	Gray	Musty	Sample grade
	Treated	8th Week	20.5	63.5	26.8	Cream	Good	5
	Control	8th Week	35.0	39.0	50.5	Yellow brown	Musty	Sample grade

^{*} Fit all the requirements of U. S. Grade except the moisture content. (In all the samples the moisture content was higher than 15%.)

	<u>Ge</u>	% of rmination			
Control.				Ē	85
Treated with	flash heat	at	600°C.	once.	68
Treated with	flash heat	at	600°C.	twice.	32
Treated with	flash heat	at	700°C.	once.	41
Treated with	flash heat	at	700°C.	twice.	4
Treated with	flash heat	at	800°C.	once.	23
Treated with	flash heat	at	800°C.	twice.	0

TABLE 16

The effect of mold growth on the seed germination of 18% moisture content rough rice (3 bushels non-treated sample) during storage time.

Storage Time	Mold Count	% of Germination
1st Week	8,000	
2nd Week	2,700,000	78
3rd Week	2,880,000	75
4th Week	27,700,000	67
5th Week	137,500,000	59
6th Week	179,000,000	57
7th Week	380,000,000	49
8th Week	420,000,000	43

Germination of the rice seeds are shown in Tables 15 and 16. Data in Table 15 indicates a decrease in germination with increasing flash heat temperature or number of times grain was passed through instrument. Table 16 shows that in the 3-bushel control samples, as the mold population increased, the percentage of germination decreased during storage. Christensen and Lopez (33) found that invasion of rice seed by fungi will cause loss of germination of the seed as storage time and moisture content of the seed are increased.

SUMMARY

Rough rice with 18% moisture content was divided into seven 5-pound samples. One sample was used as a control and others were treated with flash heat at different temperatures through an instrument called a flash sterilizer. Total count of mold, bacteria, and yeast showed high numbers of microorganisms on the control sample and fewer microorganisms on the samples treated with flash heat at 700°C. twice, 800°C. once, and 800°C. twice. All the samples (the control and treated samples) were stored at room temperature (about 78°F.) for 15 days with air being supplied at intervals. Numbers of microorganisms increased very rapidly in the control sample and treated samples with flash heat at 600°C. once and twice, and 700°C. once and twice, while treated samples with flash heat at 800°C. once and twice showed slight development of microorganisms.

Another sample of rough rice was divided into ten 5-pound samples. They were tempered to the following moisture content: 2 samples at 14%, 2 samples at 16%, 2 samples at 18%, 2 samples at 20%, and 2 samples at 22%. One sample from each moisture level was used as a control and the others treated with flash heat at 800°C. twice. Total count of mold, bacteria, and yeast showed a high number of microorganisms on the control samples. There was no mold growth and a low number of bacteria and yeast showed on the treated samples. All the control and treated samples were stored at room temperature for 15 days with air being supplied at regular intervals. Numbers of microorganisms increased greatly in control samples except in the 14% moisture sample, 20% moisture treated, and 22% moisture treated, while treated samples of 14% moisture, 16% moisture, and 18% moisture and 14% moisture control showed a

little development of microorganisms during storage.

A temperature of 800°C. with two passes was used to treat large quantities of damp rough rice with flash heat as a procedure to safely store damp, rough rice. Total count of microorganisms after each week of storage indicated a significant difference in the number of microorganisms between control sample and treated sample. Temperature of grain was checked during storage period; control sample showed a sharp increase in temperature while treated sample temperature increased very slowly.

Milling quality and grade of rice milled from control and treated samples before and after storage were studied. There were no significant differences in the quality between treated samples and control samples, before and after 15 days storage (for the 18% moisture content rice). Rice milled from the control sample of 18% M. C. after more than two weeks storage, and rough rice with more than 18% M. C. treated or control gives very low quality milled rice. Germination of seeds decreased as the temperature of flash heat increased or number of times grains passed through instrument and as the mold population increased.

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CONTROL OF MICROORGANISMS DURING RICE STORAGE

by

LOAY KAREEM ALNAJI

B. S., University of Baghdad 1968

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ABSTRACT

Rice is one of the major food crops of the world. Much rice is harvested with a moisture content between 18% to 24% and like other grains and seeds, is subject to post harvest invasion by microorganisms. Fungi have been established by many investigators as one of the primary causes of deterioration of rough rice during storage, and probably ranks second only to insects as a cause of deterioration.

In this investigation an attempt was made to sterilize damp rough rice by using flash heat. The treated and control samples of rough rice were tested for the mold, bacteria, and yeast population before and after storage. All the samples were also examined for any changes in milling quality and grade of milled rice.

Rough rice with 18% moisture content was divided into seven 5-pound samples. The first sample was used as the control and others were treated with flash heat at different temperatures. Treated samples and control sample were examined for microbiological population before and after 15 days of storage at 78°F. Samples were supplied with air during storage.

There were significant differences between samples treated with flash heat at 800°C. once, twice, and the following treatments: flash heat at 600°C. once and twice, and 700°C. once and twice. Storage of the same samples for 15 days showed a high number of microorganisms in the control sample and samples treated with flash heat at 600°C. once, 600°C. twice, 700°C. once, and 700°C. twice while the number was very low in samples treated with flash heat at 800°C. once

or twice. The sample treated with flash heat at 800°C. twice gives the lowest number.

Different moisture contents were used to test the ability of flash heat at 800°C. twice to kill maximum numbers of microorganisms and to increase the storage period. Rough rice was divided into ten 5-pound samples. They were tempered to the following moisture content: 14, 16, 18, 20, and 22% respectively. One sample from each moisture level was used as control and the others were treated with flash heat at 800°C. twice. All the samples were examined for microbiological count before and after 15 days storage at 78°F. All samples were supplied with air during storage.

There were significant differences between control samples and treated samples. Storage of the same samples for 15 days showed a high number of microorganisms in control samples, treated samples of 20% moisture and 22% moisture, while the number was very low in the following treated samples: 14% moisture, 16% moisture, and 18% moisture, respectively.

A temperature of 800°C. twice was used to treat a larger quantity of damp rough rice with flash heat. Two 3-bushel samples of 18% moisture content rough rice, one control and the other treated with flash heat at 800°C. twice, were stored in insulated barrels for 8 weeks at 78°F. with air supplied at regular intervals. Microbiological count of the control sample before storage showed 50,200 colonies and 212,100,000 colonies after storage, while treated sample was invaded by 650 colonies before storage and 826,025 after storage. Temperature of grains was measured during storage by using thermometers which were inserted into storage barrels. There was a marked increase in the temperature of the control sample and an almost imperceptible change in the treated sample temperature.

Milling quality and grade of rice milled from all the samples were performed. There were no significant differences between treated samples and control samples before and after 15 days storage, while rice milled from control sample of 18% moisture after more than 2 weeks of storage and rough rice with more than 18% moisture gives a low quality and grade of milled rice.

Germination of seeds decreased rapidly as the flash heat temperature increased. It was reduced to 27% when rough rice was treated with flash heat at 800°C. once, and 51% after 8 weeks storage in untreated rough rice samples.