FUNGUS DETERIORATION OF RICE: EFFECTS OF FUNGUS INFECTION ON FREE AMINO ACIDS AND REDUCING SUGARS IN WHITE AND PARBOILED RICE[1]

HARRY W. SCHROEDER

ABSTRACT

Growth of *Fusarium chlamydosporium* in Bluebonnet 50 and Belle Patna rice greatly increased the numbers and concentrations of free amino acids as determined by paper chromatography. Those detected in noninfected rice were aspartic acid, glutamic acid, serine, glycine, threonine, alanine, valine, leucine(s), tyrosine, asparagine, proline, methionine, gamma-amino-n-butyric acid, and glutamine. In infected rice, histidine, lysine, arginine, phenylalanine, tryptophan, cysteine, and cystine were also detected. A marked decrease in concentration of glutamic acid, histidine, asparagine, proline, and glutamine, with corresponding increases in valine, leucine(s), phenylalanine, tryptophan, and methionine, resulted from parboiling infected rice. The bulk of the reducing sugars detected was glucose. Glucose was not found in the infected rice but reappeared when infected rice was parboiled. Studies with rice infected and discolored by *F. chlamydosporium* indicated that the change in color associated with parboiling was not solely a result of the browning phenomenon (Maillard reaction).

Fungus infections of rice kernels are known to cause various types of endosperm discolorations. The parboiling process usually increases the intensity and/or darkens the discoloration and increases the portion of the kernel that is affected. Schroeder (1) reported that *Fusarium chlamydosporium* Wollenweber and Reinking was the cause of a red discoloration of rice which turned dark brown to black when infected kernels were parboiled.

The present investigation of changes in free amino acids in rice, resulting from the metabolic activities of the fungus, *F. chlamydosporium,* is part of a comprehensive program to determine the physical and chemical aspects of microbiological deterioration of rough rice. Indications were sought of a relation between gross changes with respect to these compounds and the development of pigments in infected rice, particularly the changes in pigmentation occurring during parboiling.

This report shows how the free amino acids and reducing sugars in rice are affected by the metabolic activities of *F. chlamydosporium* and the parboiling process.

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Materials and Methods

Bluebonnet 50 and Belle Patna rough rice from commercial lots were used in this experiment. A portion of each variety was retained without treatment. The remainder was divided into 12 equal samples per variety and sterilized in an autoclave for 15 min. with steam pressure of 15 lb./sq. in. on each of three successive days (the rice was in sealed containers to prevent contact with steam). Six samples of each variety were inoculated with *F. chlamydosporium*; inoculated and noninoculated samples were adjusted to a moisture content of 22% and incubated at 30°C. for 2 weeks (1). Half of the samples (three inoculated and three noninoculated per variety) were then parboiled in the laboratory, using a 3-hr. steeping period at 65°C. followed by autoclaving 5 min. with a steam pressure of 15 lb./sq. in. All samples were dried at room temperature to approximately 12% moisture content and shelled. The discolored kernels were then hand-picked from the inoculated samples and used in the determinations.

Five-gram portions from each treatment were ground to pass a 40-mesh screen and extracted with 70% (w/v) ethanol. The sugars were separated from the amino acids with a Dowex 50 resin column following the methods described by Linko (2). The solutions were evaporated under vacuum; the amino acid solutions were adjusted to 1 ml. and the sugar solutions to 2 ml. The concentrated solutions were used in chromatographic studies.

The techniques described by Smith (3) were followed in the detection of amino acid and sugars by paper chromatography. One- and two-dimensional ascending chromatography on sheets of Whatman No. 1 paper 9 × 9 in. were used to detect amino acids. The sugars were also chromatographed on Whatman No. 1 paper but in only one direction.

The following solvent systems were used: (BuA) n-butanol-acetic acid-water (12/3/5); (Ph) phenol-water (160 g./40 ml.); (PhAm) phenol-water solvent-ammonia (200/1); and (BuP) n-butanol-pyridine-water (1/1/1). Proportions are by volume for solvent systems BuA, PhAm, and BuP. The solvent pairs employed were: BuA-Ph; BuA-PhAm; BuP-Ph.

Amino acids were located by dipping in 0.2% ninhydrin, 0.2% isatin, sulfanilic acid reagent, and Ehrlich's reagent. These reagents and the aniline reagent, utilized for the location of reducing sugars, were prepared as described by Smith (3).

The individual amino acids were identified by comparing their R_F values with those published by Smith (3) and with those obtained

by concurrent chromatography of a mixture of 22 pure amino acids. The amino acid standards were prepared by mixing equal volumes of a 10-millimolar solution of each amino acid. For confirmation, the more specific reagents were utilized on one-dimensional chromatograms. Cochromatography with knowns was used to verify doubtful identifications. The concentrations of the various amino acids were visually estimated on two-dimensional chromatograms on the basis of the intensity and size of the spots after reaction with ninhydrin, with two exceptions: histidine and proline concentrations were estimated on one-dimensional chromatograms after reaction with sulfanilic acid and isatin, respectively (Table I).

Identifications of the sugars are based only on their position relative to glucose (R_G value) on the chromatogram developed with the PhAm solvent.

TABLE I

CONCENTRATIONS[a] OF AMINO ACIDS IN BLUEBONNET 50 BROWN RICE AS AFFECTED BY INFECTION WITH *Fusarium chlamydosporium* AND PARBOILING

AMINO ACIDS	STANDARD	INITIAL CONTROL	NONINFECTED		INFECTED	
			Nonparboiled	Parboiled	Nonparboiled	Parboiled
Aspartic acid	++	+	tr	tr	+	+
Glutamic acid	++	+	tr	+	+++	+
Serine	++	+	+	+	++	++
Glycine	++	+	+	+	+++	++
Threonine	++	+	+	tr	+++	++
Alanine	++	+++	+++	+++	+++	+++
Valine	++	tr	tr	tr	++	+++
Leucine(s)	++	tr	tr	tr	++	+++
Histidine[b]	+++				+++	+
Lysine	++				+	+
Arginine	++				++	+
Phenylalanine	++				++	+++
Tyrosine	++		tr		+	++
Tryptophan	++				++	+++
Proline[c]	++	tr	tr		+++	+
Cysteine	+				+	tr
Cystine	tr				tr	
Methionine	++	tr	tr	tr	++	+++
Asparagine	++			tr	++	tr
Gamma-amino-n-butyric acid	++	tr	tr	tr	tr	tr
Glutamine	++	tr			+++	tr

[a] Concentrations are expressed as a visual estimate on the basis of intensity and size of the spot after reaction with ninhydrin on two-dimensional chromatograms, except as noted. Legend: tr, trace; +, weak; ++, moderate; +++, strong.
[b] Reaction with sulfanilic acid reagent on one-dimensional chromatograms.
[c] Reaction with isatin reagent on one-dimensional chromatograms.

Results and Discussion

Twenty-one amino acids were identified by one- and two-dimensional chromatography in the samples of Bluebonnet 50 and Belle

Patna rice (Table I). Although leucine and isoleucine were not adequately separated for identification by the solvent systems employed in this study, the presence of both was indicated by the size and shape of the spot. The differences between varieties were minor and quantitative only, probably well within the range of the error of estimation; therefore, the tabulated results (Table I) also apply to Belle Patna.

Hunter *et al.* (4) identified 18 amino acids in fresh and aged parboiled rice; however, their extracts represented a 20-fold increase in concentration of amino acids on a rice weight basis. In the present study, only 11 of the amino acids reported by Hunter were detected in noninoculated rice; however, gamma-amino-n-butyric acid and glutamine were detected (although they were not reported by Hunter). Cysteine (also not reported by Hunter) was detected in the inoculated samples, and there was a marked quantitative increase in glutamine in the inoculated nonparboiled rice. The differences between the results of the two studies may be caused by differences in microfloral infestations of the rice prior to the respective investigations.

Growth of *F. chlamydosporium* in rice greatly increased the number and concentration of detectable free amino acids (Table I). Con-

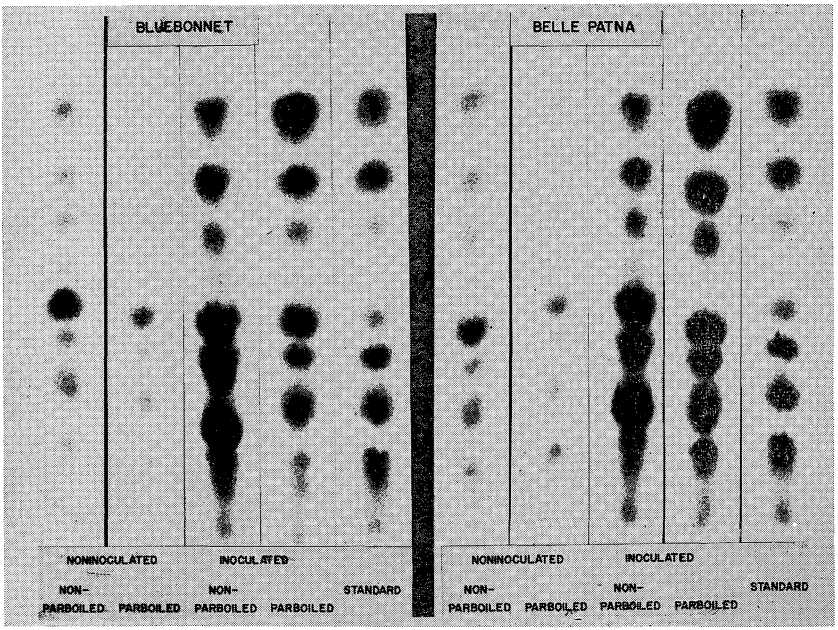

Fig. 1. Comparison of free amino acids in Bluebonnet 50 and Belle Patna brown rice as affected by infection with *F. chlamydosporium* and parboiling. Separation with BuA solvent. The standard contains 22 pure amino acids in equimolar concentrations.

centrations of glutamic acid, glycine, threonine, valine, histidine, arginine, tryptophan, proline, asparagine, and glutamine were apparently increased. Synthesis of some of these compounds by the fungus, but primarily enzymatic hydrolysis of rice proteins, could account for their increase. Most infected kernels break when milled without parboiling, indicating a weakening of kernel structure through hydrolysis of the proteins in a matrix binding the starch granules in the endosperm.

When the noninoculated samples were parboiled (Fig. 1), total amino acids decreased. This reduction may have resulted from a chemical reaction with reducing sugars, resulting in the formation of brown pigments as suggested by Hunter *et al.* (4); however, the highly water-soluble amino acids may have leached during the steeping process. Although total amino acid concentrations were not measured quantitatively, large changes in concentrations of individual amino acids were observed after parboiling (Table I). Parboiling infected rice apparently resulted in decreases in concentrations of glutamic acid, histidine, asparagine, proline, and glutamine and increases in valine, leucine(s), phenylalanine, tryptophan, and methionine.

Kretovich and Kasperek (5) reported the synthesis of alanine from pyruvate, in growing rice, to be accompanied by consumption of gamma-amino butyric acid, glutamine, aspartic acid, and asparagine. Glutamine was a particularly important source of combined nitrogen. In the present study, because of a high concentration of alanine in all the rice extracts, similar observations were not possible. The apparent decrease of some amino acids and increase of others during parboiling may have resulted from leaching of the highly water-soluble amino acids and of more efficient extraction of the more apolar amino acids from the gelatinized infected portions of the endosperm.

Cysteine and methionine were also detected, in the extracts of inoculated samples, in their oxidized forms: cysteic acid, methionine sulfone, and methionine sulfoxide.

Glucose, fructose, and raffinose were detected by the aniline reagent (Fig. 2). These sugars were also found by Williams and Bevenue (6) to be present in parboiled and nonparboiled rice. In the present study, the reducing-sugar portion was almost entirely glucose as reported by these authors. The initial control sample contained approximately twice the concentration of reducing sugars as was detected in the sterilized noninoculated samples (Fig. 2). There was no detectable change induced by parboiling the noninoculated samples;

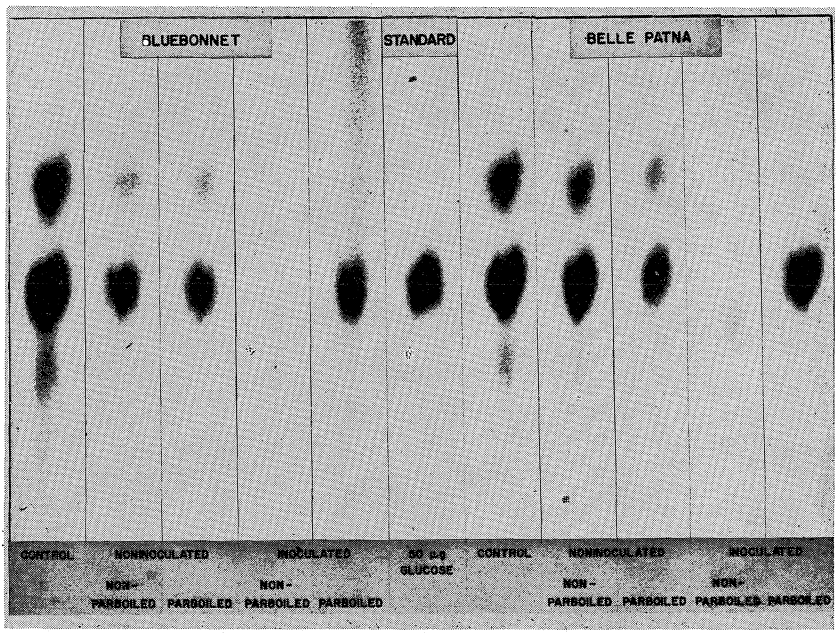

Fig. 2. Comparison of reducing sugars extracted from Bluebonnet 50 and Belle Patna rice as affected by infection with *F. chlamydosporium* and parboiling. The initial controls are equivalent to one-half of the other rice extracts. Fifty γ of glucose was applied to the standard.

however, reducing sugars were not detected in the nonparboiled infected rice, but glucose appeared again when infected samples were parboiled.

When these data are considered in respect to the browning phenomenon (Maillard reaction), steam sterilization of the rice can be expected to result in a decrease in reducing sugars and free amino acids and browning of the rice. Slight browning of the sterilized noninoculated rice was observed, accompanied by a decrease in reducing sugars (Fig. 2); but there was no marked decrease in free amino acids in comparison to those detected in the initial control (Table I).

Parboiling of noninfected sterile rice reduced free amino acid concentrations and increased the browning of the kernels, but caused no detectable change in reducing sugars.

Parboiling infected rice caused a shift in the relative concentrations of individual amino acids but no major change in total amino acids, and was accompanied by an increase in reducing sugars and a noticeable increase in brown to black pigmentation.

It is difficult to equate these data with the assumption that the change of color resulting from parboiling rice originally discolored

by the growth of *F. chlamydosporium* occurs solely as a result of the Maillard reaction. Attempts to separate pigmented compounds extracted from discolored rice have failed, as most of the color disappears when extracts are chromatographed. However, some colored compounds usually remain at the origin and these react with ninhydrin, indicating an association with amino nitrogen. Further investigation of the phenomena of changes in the concentrations of individual amino acids which was accelerated by the parboiling process may produce an explanation for the change in color and intensity of pigmentation. It is suggested that the initial red color developed in rice infected with *F. chlamydosporium* results from a combination of products of the fungus' metabolic processes.

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