

## **Fermented rice, a food from Ecuador**

A. G. VAN VEEN\*, D. C. W. GRAHAM\*, K. H. STEINKRAUS\*\*

### **SUMMARY**

"Fermented Rice" (arroz fermentado or requemado) is a food consumed in the Ecuadorean Andes, about which little information is available. One paper published ten years ago (1) describes its manufacture, distribution, consumption and its economical aspects. The unhusked rice is fermented by the microorganisms with which it is contaminated. During the fermentation the grain acquires a yellowish or brownish color. After drying and milling the resulting product is still colored, after cooking it develops a faint, specific flavor.

We isolated from samples of the fermented product a mixture of fungi and bacilli, in which *Aspergillus flavus* (2 strains), *Asp. candidus*, and *B. subtilis* were preponderant. To judge from the increase in "soluble N" a part (a few percent) of the protein is broken down by the microorganisms and as a result of this and other microbiological effects the cooking properties of the rice (and the consistency of the cooked product) have been changed. In the rat-experiment the nutritive value of the protein had not improved; the riboflavin content increased considerably.

The fermented rice samples did not contain aflatoxin, and the two *Aspergillus flavus* strains were not able to form aflatoxin on the usual wheat medium.

### **INTRODUCTION**

Fermented foods play a less important role in the diet in Latin America than in Southeast Asia and in part of Africa. As a part of our research program in nutritional and health aspects of fermented foods, we undertook the study of a little

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\* Graduate School of Nutrition, Cornell University, Ithaca, New York.

\*\* New York State Agriculture Experiment Station, Cornell University.

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known fermented product from Ecuador, *arroz fermentado* also called *arroz amarillo* or *requemado*, or *Sierra rice*. The adjectives *amarillo* and *requemado* are used because of the brownish yellow color of the product reminding one of yellow toasted rice. So far, to our knowledge only one paper devoted to this interesting product has been published (1), which mentions the following:

"This 'Sierra rice' is used exclusively in the preparation of 'dry rice', which is considered indispensable to all meals. 'Dry rice' is prepared by cooking the kernels until they separate evenly. Inasmuch as fermentation already precooks the rice by subjecting it to temperatures of 50 to 80 degrees C. 'Sierra rice' requires less cooking time in the Andean altitudes where water boils below 100 degrees C.\* 'Dry rice' is distinguished from white 'soup rice' and 'wet rice' in that the latter are cooked to a mushier consistency than the former.

"Inasmuch as varieties characterized by slender ligneous integuments ferment more easily, so-called 'Fortuna' and 'Carolina' varieties are preferred. Occasionally, shortage of supply of these varieties will cause 'Chato-Canilla' (with a thicker integument) to be used. In the milling process, fermented rice is more susceptible to breakage; while 'Sierra rice' is not highly polished, yields of between 32 and 40% brokens (kernels smaller than three-fourths of the whole grain) considerably exceed those of hardmilled white rice. This does not present a serious marketing problem, however, because the trade accepts rice with up to 35% brokens. In fact, the greatest demand is for low-priced rice — hence for rice containing a large percentage of brokens."

The authors were not able to verify all this and the situation may have changed since this article was written.

The authors were interested not only in the nutritive value but also in the possibility of the occurrence of mycotoxins which might present health hazards to the consumer of fermented rice (2).

Studies on fermented corn products have been made repeatedly (3,4,5) and recently an interesting study has been made on fermented wheat (wheat tempeh) (6), but to our knowledge studies on fermented rice have not been published.

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\* At 3000 m. altitude water boils at about 87°C.

In general, fermentation may increase the acceptability as in the case with tempeh (7), ontjom (2) and bongkrek (8). Protein quality (at least of the total protein mixture) is in our experience, usually not improved in the animal experiment. Digestibility for human beings (not necessarily for rats) may be increased, as is the case with tempeh and ontjom prepared from, for human beings rather undigestible, basic materials, soybeans and peanut presscake.

## MATERIALS AND METHODS

*Manufacture in Ecuador (according to Hertzfeld) (1).* "Fermentation is induced by dumping moist rice on large cement or cane floors out in the open, and covering it with tarpaulins. The rice normally used for this process is grain which already has a relatively high moisture content. Rice with a 22% moisture content begins to ferment in three days, while it takes up to ten days for rice with a moisture content of 17% to ferment. Once fermentation sets in, the rice is left under tarpaulins for four or five days. Then laborers remove the tarpaulins, replacing them after turning the rice with shovels. At a decreasing rate, the rice continues to ferment; six to fifteen days elapse, depending on relative humidity and temperature, before the rice is turned once more and left to dry in the open. The hulls turn a cinnamon color, the shade becoming darker the longer fermentation proceeds. Individual kernels of fermented milled rice, on the other hand, range in color from golden to deep cinnamon brown. The most acceptable color to the trade is golden or light cinnamon."

Some samples from Ecuador were investigated (Exp. I).<sup>\*</sup> The husked and unhusked samples were labelled *Arroz medio quemado*, *Arroz fermentado o quemado*, *Arroz amarillo Fortuna*, and *Arroz quemado Canilla*.

### *Preparation of Fermented Rice in the Laboratory*

For comparative purposes one batch of fermented rice was prepared with the fungus, *Aspergillus candidus* isolated from our Ecuadorian samples (Exp. II) and one batch with *Bacillus subtilis*, another isolate (Exp. III). Because the authors were

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interested in the action of these microorganisms on the protein, milled rice (Riceland Rice) was used and not rice in the husk as is done in Ecuador.

a) Suspensions were made of the *Aspergillus* spores and small quantities of rice (60 g) were inoculated with about 10 ml of the spore suspension. The rice was incubated for four days at 30°C and portions of about 1800 g rice were, after soaking with 300 ml of distilled water, mixed with the small batches of inoculated rice mentioned before; the batches were incubated at 30°C for three days and then autoclaved at 105°C to kill the mold.

b) For the experiment of rice fermented by *Bacillus subtilis*, two tubes of 10 ml of a brain-heart-infusion broth were inoculated with *Bacillus subtilis* and incubated for 48 h at 37°C. The tubes were centrifuged and the washed cells were diluted with 200 ml saline solution. A batch of 3400 g of dry rice plus 400 ml of distilled water was, after soaking for a few hours inoculated with this bacterial suspension and then incubated for seven days, at 37°C; the rice was stirred two times during this period. The product was autoclaved for 15 minutes at 120°C.

c) Both batches were freeze-dried, ground and sieved (20 mesh). The unfermented rice controls were also autoclaved and ground. The final products were incorporated into the diet formula for the animal experiments (Tables 1, 3, and 5).

#### *Microbiological Analysis of samples from Ecuador*

All husked samples had the same yellowish-brown (uneven) color and gave practically the same microbiological results. A number of husked grains of the samples were either soaked in A.P.T. broth, or in distilled water and inoculated on A.P.T. agar, Nutrient agar, or B.H.I. agar. A great number of isolates were obtained which were transferred to either slants or plates. The isolated bacteria were determined with the help of Bergey's Manual of Determinative Bacteriology (7th Ed., 1957). For the determination of the fungi we received the cooperation of specialists in this field.\*

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Moisture, crude protein, fat, ash, and riboflavin were determined according to the "Methods of Analysis of the A.O. A.C." (9).

True protein was determined by precipitation with 5% trichloroacetic acid (10). The rice samples were cooked in ten times their weight of boiling water and the cooking water, and also the rice plus cooking water, diluted with 20 times the volume of 5% trichloroacetic acid.

Water absorption of rice in cooking was determined by heating the rice samples at 90, and 100°C in the tenfold volume of water during 30, 40 and 60 minutes, respectively, centrifuging at 3500 RPM and measuring the volume of the supernatant.

#### *Protein Efficiency Ratio and Apparent Digestibility*

Both were determined by the method described by Barnes and Fiala (11).

The fermented and unfermented rice products after freeze-drying were analyzed and compared in the rat experiment (6 rats, initial weight 50-60 g, in each group) with standard casein. The composition of the diets is given in Tables 1, 3, and 5.

#### *Aflatoxin*

Aflatoxin was determined by the method of the Tropical Product Institute, London, England, by Broadbent *et al.* (12).

## RESULTS AND DISCUSSION

We isolated several different fungi and bacteria from the different Ecuadorian fermented rice samples (husked) and it is difficult if not impossible, to state which microorganisms are the most important ones for the fermentation process. However, in general, all samples showed more or less the same population of microorganisms.

All bacteria were gram-positive spore-formers, which is not surprising because the fermenting rice heats itself up considerably. The most prominent bacteria appeared to be *Bacillus subtilis* with a strong proteolytic and amylolytic activity; two other less important bacilli resembled *Bacillus pumilus* and *Bacillus cereus*, though both were not quite identical with

these strains (in connection with some of the biochemical properties), as described in Bergey's Manual.

Amongst the fungi, the Aspergilli were prominent especially *Aspergillus flavus*, L. K. var. *flavus* and *Aspergillus flavus* var. *columnaris* Raper and Fennel, *Aspergillus candidus* L. K., and *Aspergillus fumigatus*. Furthermore, we isolated *Rhizopus rhizopodiformis*, *Absidia corymbifera* and an *Actinomyces* species.

With the help of the usual techniques we tried to investigate the possibility that one or more of the microorganisms isolated by us, would be predominant in the fermentation process because of the production of substances with antibiotic action on the others, but in spite of using different techniques we only found very weak or no antibiotic activity.

From the cooking experiments with two unfermented and two fermented Ecuadorian rice samples, under carefully controlled conditions, it appeared that after treatment with trichloroacetic acid the two unfermented samples contained 20 and 40 mg soluble nitrogen (calc. per 100 g of raw rice), after fermentation 40 and 90 mg. The total N-content of the raw rice was 1400 mg.

From our cooking experiments it appeared moreover that the fermented grains retained their consistency much better than those of unfermented rice which is in accordance with Herzfeld's observations (1.).

From Table 2 it is clear that on the 6% protein level the P.E.R. of the Ecuadorian fermented rice (and the apparent digestibility) are certainly not higher than that of the unfermented product. The P.E.R.'s and apparent digestibility of the rice samples fermented with *Aspergillus candidus* and *B. subtilis* (Tables 4 and 6) were inferior to those of unfermented rice. This is not surprising, because during the rather lengthy fermentation (4 days) some amino acids are decomposed by the fungi, and the fungus mycelium is rather indigestible. We did not perform any crude fiber determinations, because this fact is well known. Therefore, fermentation has not increased the protein quality, but only broken down some of the protein.

More interesting are the riboflavin figures in Table 7 which show that the Ecuadorian fermented rice has about double the riboflavin content of the non-fermented rice and

that the riboflavin content of ordinary milled rice fermented with *Bacillus subtilis* has increased nearly fourfold. An increase in riboflavin content through microbial action has been noted in many fermented foods such as tempeh (7), ontjom (2), and idli (2).

Aflatoxin could not be detected in any of the Ecuadorian rice samples, and both *Aspergillus flavus* strains did not produce aflatoxin on the whole-wheat medium on which other *Asp. flavus* strains produced much aflatoxin.

Table 1 - Analytical Data  
EXPERIMENT I  
ECUADORIAN RICE, FERMENTED AND UNFERMENTED

	Crude Protein (N×6.25) %	Fat %	Moisture %	Calories per 100 g
Unfermented Rice	7.00	0.84	12.9	—
Fermented Rice	8.81	1.10	11.3	—
Casein Diet	6.00	6.32	16.5	356.1
Unfermented Rice Diet	5.75	6.54	15.0	360.5
Fermented Rice Diet	5.75	6.86	15.3	360.9

Table 2 - Protein Intake, Weight Gain and Apparent Digestibility  
in Rats on Three Diets (Mean values for 6 male rats per group)

	Casein	Unfermented Rice	Fermented Rice
Average Feed Intake g/rat/28 days	317.5	334.0	322.2
Average Protein Intake g/rat/28 days	19.0	19.2	18.5
Average Weight Gain g/rat/28 days	46.5	36.5	30.2
Protein Efficiency Ratio	2.45	1.90	1.63
Apparent Digestibility	88.6	88.5	84.2

Table 3 - Analytical Data

## EXPERIMENT II

## RICE FERMENTED WITH ASPERGILLUS CANDIDUS

	Crude Protein (N×6.25) %	Fat %	Moisture %	Calories per 100 g
Unfermented Rice	6.1	—	—	—
Fermented Rice	8.5	—	—	—
Casein Diet	7.8	6.27	16.3	353.9
Unfermented Rice Diet	5.3	6.42	16.5	353.6
Fermented Rice Diet	6.7	6.09	15.9	366.9

Table 4 - Protein Intake, Weight Gain, and Apparent Digestibility in Rats on Three Diets (Mean values for 6 male rats per group)

	Casein	Unfermented Rice	Fermented Rice
Average Feed Intake g/rat/28 days	348.2	363.4	319.7
Average Protein Intake g/rat/28 days	27.2	19.3	21.4
Average Weight Gain g/rat/28 days	70.0	42.8	38.0
Protein Efficiency Ratio	2.57	2.22	1.77
Apparent Digestibility	91.6	86.8	72.6

Table 5 - Analytical Data

## EXPERIMENT III

## RICE FERMENTED WITH BACILLUS SUBTILIS

	Crude Protein (N×6.25) %	Fat %	Moisture %	Calories per 100 g
Unfermented Rice	8.56	0.8	5.3	—
Fermented Rice	8.75	0.1	4.2	—
Casein Diet	7.12	6.50	18.1	347.7
Unfermented Rice Diet	6.69	6.77	15.1	361.2
Fermented Rice Diet	7.12	5.90	15.0	357.1

Table 6 - Protein Intake, Weight Gain, and Apparent Digestibility in Rats on Three Diets (Mean values for 6 male rats per group)

	Casein	Unfermented Rice	Fermented Rice
Average Feed Intake g/rat/28 days	413.2	319.2	290.0
Average Protein Intake g/rat/28 days	29.4	21.4	20.6
Average Weight Gain g/rat/28 days	68.6	35.8	25.6
Protein Efficiency Ratio	2.33	1.67	1.24
Apparent Digestibility	91.2	82.6	78.9

Table 7 - Riboflavin Content in mcg/gr

	Samples from Ecuador	Riceland Rice Inoculated with Bacillus subtilis
Before fermentation	0.15	0.28
After fermentation	0.32	1.04

## RESUMEN

## Arroz fermentado, alimento del Ecuador

Arroz fermentado o requemado es un alimento de consumo popular en los Andes Ecuatorianos sobre el cual se ha publicado poca información científica. En un trabajo publicado hace diez años (1) se describe su preparación, distribución, consumo y aspectos económicos. El arroz entero es fermentado por los microorganismos que lo contaminan espontáneamente. Durante la fermentación adquiere un color amarillo o parduzco. Después del desecado y beneficio, el producto resultante es todavía coloreado y al cocinarlo se le percibe un débil sabor específico.

De muestras del producto fermentado se han aislado varios hongos y bacili, entre los cuales prevalecen 2 cepas de *Aspergillus flavus* y otros *Asp. candidus* y *Bacillus subtilis*. Durante la fermentación se hidroliza una pequeña parte de las proteínas por acción de los microorganismos, resultando en un aumento en el nitrógeno soluble. Igualmente, se observa un cambio en las características de cocción y en la consistencia del producto cocido como consecuencia de la acción microbiana. En experimentos con ratas sobre el valor biológico no se destacó una mejora del valor proteico. Se observó, sin embargo, un aumento significativo del contenido en riboflavina.

No se encontró aflatoxina en las muestras de arroz fermentado y las dos cepas de *Aspergillus flavus* no eran capaces de producir este producto cuando se cultivaron en el medio de trigo, usado generalmente para tal fin.

## BIBLIOGRAPHY

- (1) Hertzfeld, H.C.—Rice fermentation in Ecuador. *Economic Botany*, 11 (3): 267-270 (1957); see also short reference in R. C. Mickus, *Cereal Science Today*, 4 (5): 138-147 (1958).
- (2) Van Veen, A. G., Graham, D. C. W., Steinkraus, K. H.—Fermented peanut presscake. *Cereal Science Today*, 13 (3): 96-99 (1968).
- (3) Platt, B. S.—Some Traditional Alcoholic Beverages and their importance in Indigenous African communities. *Proc. Nutr. Soc.*, 14: 115 (1955).
- (4) Platt, B. S.—Biological Ennoblement: Improvement of the Nutritive value of Foods and Dietary Regiments by Biological Agencies. *Food Technology*, 18 (5): 662 (1964).
- (5) Novelle, L.—Biological Ennoblement and Kaffir Beer. *Food Technology*, 20 (12): 1607 (1966).
- (6) Wang, Hwa L. and Hesseltine, C. W.—Wheat Tempeh. *Cereal Chemistry*, 43 (5): 563 (1966).
- (7) Van Veen, A. G. & Schaefer, G.—The influence of the tempeh fungus on the soybean. *Doc. Neerl. et Indon. de Morbis Tropicis*, 2 (1): 18 (1958).
- (8) Van Veen, A. G.—In: Toxicants occurring naturally in foods. Publ.

- No. 1354, Nat. Ac. Sc. - Nat. Res. Council, Washington, D. C., p. 174 (1966).
- (9) Methods of Analysis, Published by the Association of Official Agricultural Chemists, 1960 edition.
  - (10) B. L. Oser.—Hawk's Physiological Chemistry, 14th ed., McGraw-Hill (1965).
  - (11) Barnes, R. H. & G. Fiala.—Effects of the Prevention of coprophagy in the rat. I. Growth Studies. *J. Nutrition*, 64: 533 (1958).
  - (12) Broadbent, J. H., Cornelius, J. A. & Shone, G.—The detection and estimation of aflatoxin in groundnuts and groundnut materials. *Analyst*, 88: 142 (1963).