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Parakari, an indigenous fermented beverage using amylolytic *Rhizopus* in Guyana

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Abstract: The alcoholic beverage parakari is a product of cassava (*Manihot esculenta* Crantz) fermentation by Amerindians of Guyana. While fermented beverage production is nearly universal among indigenous Amazonians, parakari is unique among New World beverages because it involves the use of an amylolytic mold (*Rhizopus* sp., Mucoraceae, Zygomycota) followed by a solid substratum ethanol fermentation. The mycological significance of this dual fermentation process previously was unrecognized. A detailed study of parakari fermentation was made in the Wapisiana Amerindian village of Aishalton, South Rupununi, Guyana. Thirty steps were involved in parakari manufacture, and these exhibited a high degree of sophistication, including the use of specific cassava varieties, control of culture temperature and boosting of *Rhizopus* inoculum potential with purified starch additives. During the fermentation process, changes in glucose content, pH, flavor, odor and culture characteristics were concomitant with a desirable finished product. Parakari is the only known example of an indigenous New World fermentation that uses an amylolytic mold, likely resulting from domestication of a wild *Rhizopus* species in the distant past. Parakari production is remarkably similar to dual fermentations of Asia, yet it was independently derived.

Key words: cassava, ethnomycology, fermentation, neotropics, *Rhizopus,* Zygomycota

INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) forms the principal carbohydrate food source for most indigenous peoples of tropical South America. Many indigenous groups regularly manufacture fermented cassava beverages as well as consuming solid cassava in the form of flatbread and toasted granules (farine) (Im Thurn 1883, Forte 1992, Salick et al 1997). Most indigenous cassava fermentations involve prolonged cooking and exposure to salivary enzymes as the method of initially breaking down the starch to sugars (amylolysis), which subsequently are fermented to ethanol by ambient yeasts (Steinkraus 1996). Parakari is a more complex cassava fermentation using an amylolytic mold in place of salivary amylolysis. Production of parakari was noted anecdotally by ethnologists among the Wai Wai and Wapisiana Amerindians of Guyana (Farabee 1924, Forte 1992). However, the details of this highly sophisticated fermentation technology have remained undocumented. This paper reports the first detailed investigation of the process by which the Wapisiana of Guyana use an amylolytic mold (*Rhizopus* sp., Mucoraceae, Zygomycota) in combination with ambient yeasts to produce parakari, a unique fermented beverage. The origin of parakari and the remarkable similarity of its production to indigenous fermentations of Asia are discussed.

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MATERIALS AND METHODS

Ethnogeographic backround.—Guyana is a country of 83 000 square miles located north of Brazil, east of Venezuela and west of Suriname (Fig. 1). Nine indigenous Amerindian groups inhabit Guyana, each of which produce or formerly produced fermented cassava beverages. Only three of the tribes currently manufacture parakari; these are the Wapisiana, Macusi and Patamona. These tribes have overlapping territories that lie in or adjacent to the Rupununi savanna region in the country's southwest. Production of parakari among the Wai Wai, who live to the south of the Wapisiana in extreme southern Guyana, was noted by Farabee early in the 20th century but now has ceased due to suppression by missionaries of the New Tribes Mission (Farabee 1924, Marawanaru pers comm).

The indigenous group featured in this study, the Wapisiana, lives in the savanna south of the Kanuku Mountains along the upper Rupununi River and its tributaries, at 1–4°N and 59–61°W. Villages are scattered on the savanna periphery near forests converted to farmlands. Population of the Wapisiana in Guyana is 4000–6000, with a lesser number living in adjacent Brazil (Forte 1992). Traditional subsistence economy of the Wapisiana consists of shifting cassava cultivation, hunting and fishing. Introduction of cattle rearing, mining and limbering over the past century have led many Wapisiana to seek seasonal employment in these enterprises.

The Wapisiana of Aishalton cultivate cassava as their main

FIG. 1. Map of northeastern South America with Guyana and the Wapisiana village of Aishalton indicated.

source of carbohydrate. Farming technique is one of shifting plots, from one to several hectares, in forested areas adjacent to the savannas. Forests are cleared and burned, and cassava is planted along with maize, sweet potato, yam, sugarcane, watermelon, pineapple, tobacco, bananas, papaya and medicinal plants in a polycultural system in which crops are harvested at various times according to their maturation over a 1–5 y period. Cassava, being the primary crop, is harvested as needed for the day-to-day making of parakari, farine and cassava bread.

The Wapisiana manufacture two cassava beverages, sarawau and parakari, with different fermentation techniques. Fully fermented parakari is consumed throughout the day by adults, and the sweet, weakly fermented form is relished by children.

The origins of parakari fermentations are lost in antiquity. The overwhelming prevalence of fermented beverages throughout Meso- and South American indigenous cultures indicates a long-term, pre-Columbian practice (LeBarre 1938). Parakari production, with its exceptional degree of microbial manipulation, certainly has a long history. The Wapisiana noted that the beverage has been around since the ''beginning of time''; they have no cultural memory of a time without it.

Study area and objectives.—In May 1994 an expedition was undertaken to the Wapisiana village of Aishalton on the southern Rupununi savanna (2°23′N, 59°18′W; FIG. 1). Experience lead the author to think that parakari-making was widespread in this village. Interviews with villagers indicated that numerous women were expert at brewing. From this pool of artisans two women, Bernadette James and Grace Smith, were engaged to make batches of parakari for detailed field study. Objectives were to (i) document all stages of parakari manufacture from farm to consumption and (ii) record cultural characteristics of the parakari fermentation process.

Cultivation and harvest of cassava.—Farming techniques and cassava cultivation by the Wapisiana of Aishalton were examined during three trips to local farms. The farms belonged to the brewers who participated in the study. These women, along with Regis James, served as informants concerning cultivation and harvest of cassava and other plants used in brewing. Access to cassava farms was not subject to gender restrictions as reported in other studies (e.g., Salick et al 1997).

Cassava varieties.—Informants identified varieties of cassava using leaf morphology, plant stature and tuber characteristics, such as skin and flesh color and size. Wapisiana names were applied and were reported here in italics. Use of each variety in parakari, farine and cassava bread was noted. Varieties preferred for parakari making were identified and their qualities noted.

Steps in the manufacture of parakari.—Preparation of cassava, preparation of fungal inoculum, and Stage I and Stage II fermentations were examined for parakari Batch A (Bernadette) and Batch B (Grace). The women prepared these batches from start to finish as they normally would.

Glucose concentration and pH.—Changes in glucose concentration and pH of both batches of parakari were made throughout Stage I and II fermentation. Parameters were measured at approximately 12 h intervals from 0 to 60 h for both batches and included a measurement at 43 h. Subsequent measurements were made at 84, 112, 135, 188 and 205 h. For glucose measurements, four 1 g samples of semisolid parakari were chosen randomly at each interval from each of the cakes, mixed together and diluted to 10× or 100× in distilled water in a 100 mL Erlenmeyer flask. The glucose concentration of the dilutions were determined colorimetrically using glucose test strips (Chemstrip bG, ''Glucose in Whole Blood for Diabetes'') and calculated to mg/mL in the original undiluted sample. At each interval pH was determined by applying a pH strip to predilution, semisolid samples. Ethanol concentration could not be measured accurately under these field conditions and was assessed subjectively by taste. Ambient and culture temperatures were recorded at 43, 48 and 60 h for Batch A; culture temperatures were obtained for Batch B at 43, 48 and 60 h with a thermometer inserted into the fermenting parakari.

Flavor and odor.—Parakari flavor and odor were assessed and described subjectively for both batches at each interval.

Culture characteristics.—Appearance and extent of mycelial growth and sporulation during Stage I fermentation were noted at each interval up to 39 h (Batch B) or 48 h (Batch A), after which the respective batches were transferred to vessels for Stage II fermentation. At each interval during this stage gas production, bubbling and consistency of the mashes were assessed.

RESULTS

Harvest of cassava.—Informants traveled nearly 10 km one way to their farms, using bullock carts for transport. Areas with mature cassava suitable for parakari were determined, and weeds were cleared from beneath the plants. Digging began. The shallow, ripe tubers had spread horizontally and were extracted easily (FIG. 2a).

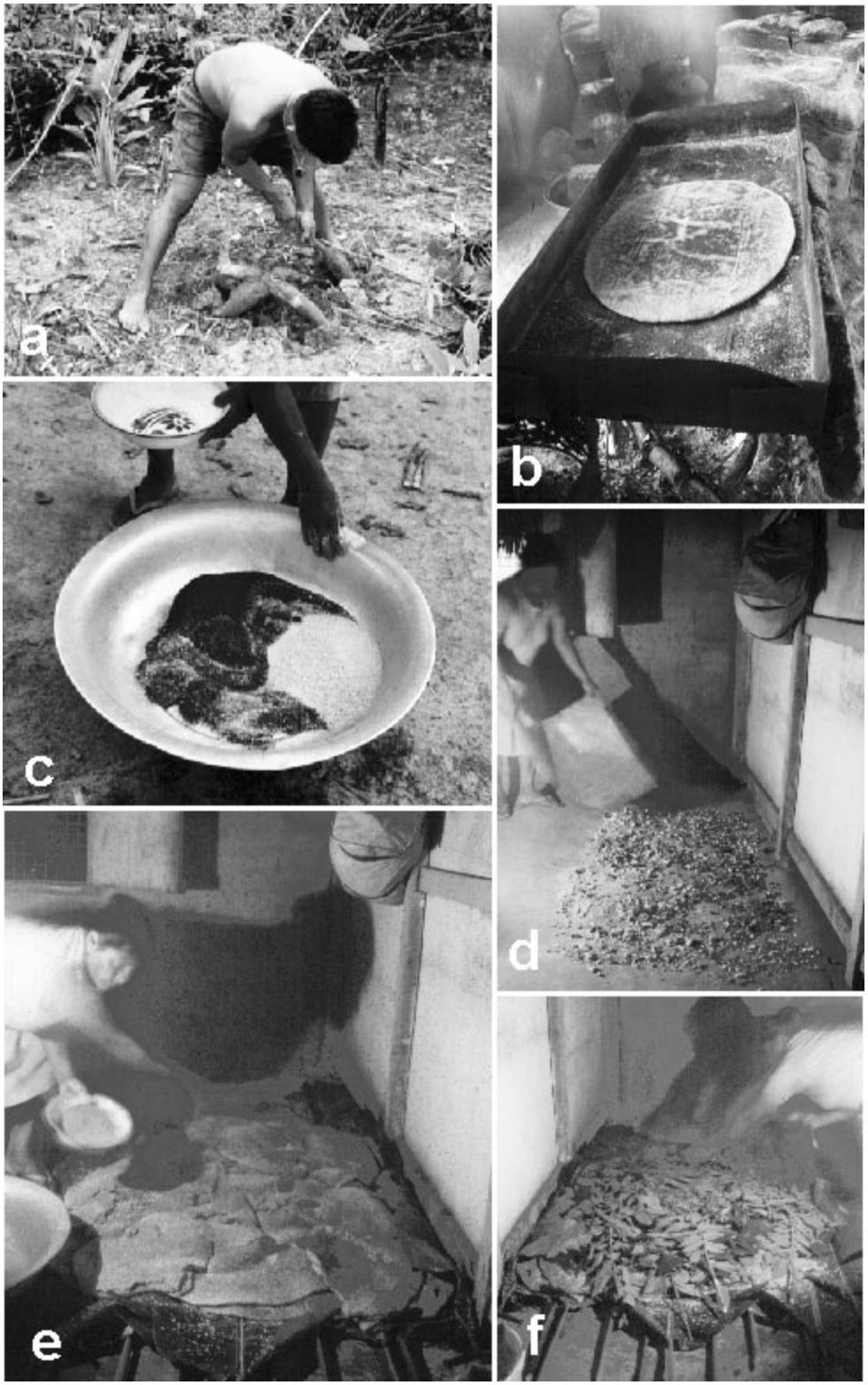

FIG. 2. Selected steps in the manufacture of Wapisiana parakari. a. Harvest of cassava tubers. b. Baking cassava bread. c. Pulverized *Trema micrantha* leaves, containing *Rhizopus* mycelium and spores from previous parakari batch, mixed with purified cassava starch to produce inoculum. d. Fanning coals. e. Layers of wet cassava bread, placed on fresh *Heliconia* leaves, are sprinkled with *Rhizopus* inoculum. f. Layer of fresh *Trema* leaves are placed on top of cassava mass.

TABLE I. Varieties of cassava (*Manihot esculenta* Crantz) grown by the Wapisiana of southern Guyana, with notes on color characteristics, products in which varieties are used, and fermentation qualities for some varieties used in making the beverage parakari

Variety	Tuber flesh color	Parakari	Farine	Bread	Parakari qualities
romiedar	yellow	+	−	−	ferments slowly
oradowniro	yellow	+	−	−	ferments slowly
simant A	yellow	+	−	−	ferments slowly
maidanair	cream	+	−	−	ferments rapidly
powatra	yellow	+	+	−	ferments slowly; ages poorly
anarwaip A	white	+	−	+	ferments and sours rapidly
tunip	white	+	−	+	consumed sweet
bouirain	white	+	−	+	minimal sweetness
kodoiwine	yellow	+	+	−	minimal sweetness
mapuid	cream	+	+	−	minimal sweetness
warizwine	cream	+	+	−	minimal sweetness
boioro	yellow	+	+	−	minimal sweetness
koiari	yellow	+	+	−	minimal sweetness
kamp	yellow	+	+	−	minimal sweetness
romie	yellow	+	+	−	minimal sweetness
bechai	yellow	+	+	−	minimal sweetness
karimatau	yellow	+	+	−	minimal sweetness
daraudan	yellow	+	+	−	minimal sweetness
simant B	white	+	−	+	minimal sweetness
madornai	white	+	−	+	minimal sweetness
madidikmad	cream	−	+	−	na
madakainok	yellow	−	+	−	na
boimawakin	yellow	−	+	−	na
kirikdan	yellow	−	+	−	na
kamichap	yellow	−	+	−	na
kowararaip	yellow	−	+	−	na
bakarai	yellow	−	+	−	na
anarwaip B	white	−	−	+	na
San Pedro	white	−	−	+	na
wirawin	white	−	−	+	na
zak	white	−	−	+	na
kiripi	white	−	−	+	na
karbaiyash	white	−	−	+	na
irachab	white	−	−	+	na
kiripinaun	cream	−	+	+	na
makshir A	yellow	sweet cassava; tubers consumed directly			
makshir B	white	sweet cassava; tubers consumed directly			

In the two farms 37 varieties of cassava were recognized as distinct by the informants (TABLE I). All but two of the varieties are ''bitter'' cassava, with tubers containing cyanogenic compounds that must be removed by grating and squeezing out the toxic juices. Cassava varieties differed according to desired characteristics such as color and suitability in making parakari, farine or cassava bread. Twenty of the varieties were reported as being used in making parakari; eight of these 20 were considered most desirable for making parakari, based on their fermentation qualities. Four varieties were used exclusively in parakari. Three of these, *romiedar, oradowniro* and *simant A* ferment slowly; one variety, *maidanair,* ferments quickly and is used in ''fast parakari''. Overall yellow, cream or white varieties can be used in parakari, while only yellow or cream varieties are used in making farine and only white-fleshed varieties are used in making cassava bread. Two varieties were noncyanogenic (''sweet'') cassavas that are consumed directly as boiled tubers.

Approximately 200 lb of tubers were harvested from each farm to prepare the batches (10–15 gallons undiluted product per batch). Equal quantities of slow-fermenting cassava varieties *romiedar* and *oradowniro* were harvested for Batch A. The fast-fermenting variety *maidanair* was harvested for Batch B. Tubers carefully were packed in *warishis* (carrying

baskets), carried out of the farm to waiting bullocks and taken to the village. Because the Wapisiana work their farms on a strictly subsistence basis, monetary value could not be assigned to the labor and end products of parakari manufacture.

Several fresh, leafy branches of the early successional tree *Trema micrantha* (L.) Blume (Ulmaceae; *bishawad*) were collected along with 10–15 leaves of a large species of *Heliconia* L. (Heliconiaceae). *T. micrantha* leaves were used as a *Rhizopus* inoculum carrier between parakari batches, and *Heliconia* leaves were used to enclose cassava layers during primary fermentation.

Steps.—Manufacture of Wapisiana parakari involved 30 steps from harvesting to the consumption of the beverage (FIG. 2). The manufacturing process was divided into six phases: I. preparation of cassava; II. preparation of fungal inoculum; III. inoculation of cassava; IV. Stage I fermentation; V. Stage II fermentation; VI. consumption.

Phase I. Preparation of cassava. (i) Harvest cassava tubers (200 lb fresh tubers = 15 gallons unstrained parakari ''mash''; FIG. 2a). (ii) Scrape skin from tubers. (iii) Wash tubers with water. (iv) Grate tubers on stone-point graters, requiring 2 h labor for 200 lb (or more quickly with bicycle graters). (v) Squeeze poisonous juice from meal with woven *matape*. Leave meal overnight in large tub, where it goes sour, further reducing or eliminating toxins. (vi) Let starch settle out; pour off juice; collect starch into three grapefruit-size balls and sun-dry for use in inoculum. (vii) Squeeze meal with *matape* a second time. (viii) Sift squeezed meal through woven screen. (ix) On metal pan over fire bake meal into cassava bread ''rounds'' or ''ovals'', 2 cm thick and ~75 cm diam; bake brown (well done) to a greater degree than is normal for cassava bread (FIG. 2b). It takes 3.5 h to bake ca 200 lb of meal. (Overbaking produces bitter parakari.) Spread rounds on thatched roof to sun-dry.

Phase II. Preparation of fungal inoculum. (x) Take sun-dried inoculum leaves (*T. micrantha*) from previous batch, dry further in pan over low heat; when warm and crumbly grind with wooden mortar and pestle to make 1 lb of powdered leaf inoculum. (xi) Sift and parch powdered starch granules (from step vi) over low heat until lightly brown. (xii) Mix warm starch (step xi) with leaf powder (step x) in tub (FIG. 2c).

Phase III. Inoculation of cassava. (xiii) Spread thin layer of red-hot coals in 1.5–2 m^2 area on clean earthen floor (FIG. 2d). Cover coals with scraped cassava skins in a layer 2 cm thick to prevent *Heliconia* leaves from burning. (xiv) Cover coals with two layers of fresh *Heliconia* leaves. (xv) Soak one cassava round ca. 6 s in a large basin of water. (xvi) Sprinkle freshwater atop *Heliconia* leaves. (xvii) Dust *Heliconia* leaves with inoculum powder (i.e., starch/leaf mixture from step xii). (xviii) Place soaked cassava round (broken into large pieces) atop inoculum, sprinkle with water, dust with inoculum, add next cassava round. Neatly fit broken cassava pieces together (FIG. 2e). (xix) Repeat previous step to produce three layers, 8 cm thick, forming a rectangular cassava cake ca. 2 m^2 in area. (xx) Cover cassava cake with 5–10 fresh *T. micrantha* branches; this allows the leaves to be covered with *Rhizopus* mycelium during culturing so that it can be used as inoculum in a subsequent batch (FIG. 2f). (xxi) Cover mass with two layers of *Heliconia* leaves, secure corners with wood (FIG. 3a).

Phase IV. Stage I fermentation. (xxii) Leave undisturbed 40–48 h, allowing extensive growth of *Rhizopus*; culturing may be extended to 72 h if slower fermenting varieties of cassava are used. (xxiii) Remove *Heliconia* leaves; remove inoculum leaves (with adhering mycelium and spores) and sun-dry for further use (FIG. 3b, c). (xxiv) Smell cultured cake; odor indicates if culturing has proceeded properly. (xxv) Taste cake to determine sweetness (indicative of hydrolysis); if judged sufficiently sweet peel up cake by hand and transfer to large ''mother'' vessels (natural rubber *gobies* or buckets) for Stage II fermentation (FIG. 3d, e). A large portion of the sweet cake may be consumed at this time, particularly by children.

Phase V. Stage II fermentation. (xxvi) Cover vessel tightly to block air flow; let sit while fermentation proceeds. Duration depends on desired taste and alcohol content. Periodically relieve pressure by loosening, then tightening, lid. (xxvii) Mash may be strained and drunk (see step xxix) at 24 h; the drink will be sweet, with little or no alcohol content. (xxviii) For higher ethanol content, mash is left in covered vessel up to 5 wk; strain and drink when desired. Mash continues to ferment, ethanol content rises, eventually turning bitter and sour.

Phase VI. Consumption. (xxix) When ready to drink mix 3 gallons parakari mash with 6 gallons water and hand-strain through finely woven sifter (leftover mash can be mixed and strained again but produces weaker flavor). (xxx) Strained parakari can be drunk immediately (FIG. 3f); if stored 1½ d ethanol content is reported to increase significantly; after 1½ d taste and texture degenerate.

Culture characteristics.—Glucose concentration and pH. Glucose in Batches A and B was nil 0–12 h after inoculation (FIG. 4). Glucose was low in both batches (7 mg/mL) by 24 h and rose dramatically at 24–48 h. At 48 h each batch had reached its highest glucose

FIG. 3. Selected steps in the manufacture of Wapisiana parakari. a. Mass is covered with *Heliconia* leaves for ≈48 h Stage I fermentation. b. Parakari mass, uncovered after 48 h, showing dense white *Rhizopus* mycelium around *Trema* leaves. c. Close-up of mycelium and leaves. d. Cross section of Stage I parakari, showing dense surface mycelium and gelatinized interior layers. e. Solid-state parakari being transferred to balata vessels for Stage II fermentation. f. Final product in glass bottle.

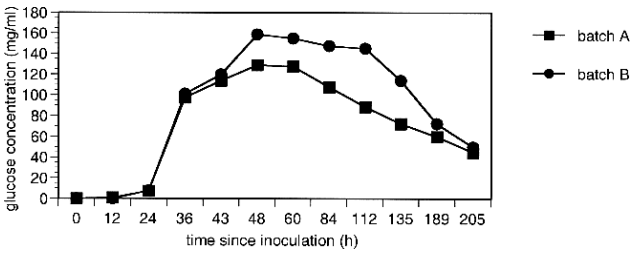

FIG. 4. Glucose concentrations of parakari Batches A and B during fermentation at 0–205 h.

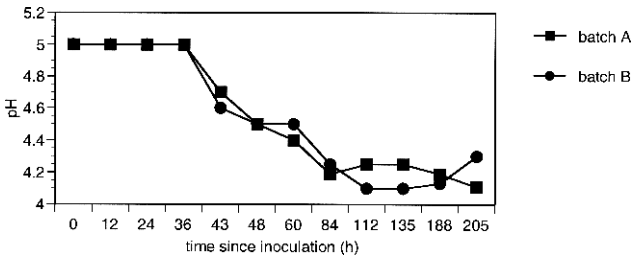

FIG. 5. pH of parakari Batches A and B during fermentation at 0–205 h.

concentration, 129 mg/mL for A and 159 mg/mL for B. From 48 to 60 h glucose concentrations of the respective batches remained at these peaks. From 60 to 205 h Batch A glucose dropped steadily to 45 mg/mL before consumption. Batch B, however, maintained a high glucose concentration (ca 150 mg/mL) at 48–112 h, after which the concentration dropped rapidly to 50 mg/mL at 205 h.

Changes in pH were similar in both batches throughout brewing. At 0–48 h, pH was 5, after which it dropped to 4.1–4.2 by 84–112 h and remained there (FIG. 5).

Flavor and odor. The first detectable fermentation flavor and odor was that of the tempeh-like *Rhizopus* mold, appearing in both batches by 24 h (TABLE II). The *Rhizopus* taste was strong during Stage I fermentation (<36–48 h) but declined during Stage II. Both batches were sweet by 36 h. Batch A tasted very sweet at 43 h and 48 h, after which sourness increased. Batch B was very sweet by 48 h and remained so

TABLE II. Parakari odor, taste, and culture characteristics derived from observations of batch A and B from 0–205 h

Fermentation	Time since inoculation (h)	Odor	Taste	Culture characteristics
Stage I	0	toasted cassava	wet cassava bread; not sweet	no mycelial growth detected
	12	toasted cassava	slight *Rhizopus*; not sweet	no mycelial growth detected
	24	*Rhizopus*-like (tempeh)	*Rhizopus* (like tempeh); not sweet	surficial growth over cake; aerial hyphae minimal
	36	strong *Rhizopus*	*Rhizopus*; slightly sweet; no ethanol	luxuriant mycelial growth on surface and deeper layers; no sporulation
	~40 (transfer to vessels)	dense, heady *Rhizopus*	*Rhizopus*; very sweet; slight ethanol	luxuriant mycelial growth in aerial interstices and on *Trema* leaves; sporulation along edges
Stage II	48	slight *Rhizopus*; slight ethanol	*Rhizopus* declining; very sweet, slightly sour; slight ethanol	mash bubbling rapidly; emission of clear juice; texture gelatinous
	60	sour; ethanol	sweet yet strongly sour; moderate ethanol	mash bubbling rapidly; heavy juice production, gelatinization
	84	sour; strong ethanol	sweetness declining; sour; ethanol pronounced	as above
	112	sour; strong ethanol	slightly sweet; sour; strong ethanol (burns); bitter overtones	as above; consistency of bread pudding
	135	sour; strong ethanol	little detectable sweetness; very sour, strong ethanol	as above; mash disintegrating to semi-liquid
	188	sour; strong ethanol	very sour; strong ethanol	bubbling reduced; mash semi-liquid
	205	sour; strong ethanol	very sour; strong ethanol	bubbling minimal; mash semi-liquid; clear juice abundant on top of mash

through 112 h; sourness also was detected at 84 h. By 188 and 205 h both batches were very sour. Odor and taste of ethanol closely tracked the development of sourness, appearing at 43–48 h and becoming dominant by 112 h in both batches.

Culture characteristics. No mycelial growth of *Rhizopus* was detected in either batch at 0–12 h (TABLE II). At 24 h mycelium had covered the surface of both parakari cakes. Luxuriant mycelium was present until Stage 2 (43 h Batch A; 39 h Batch B), with brilliant white, aerial hyphae covering the cakes and growing vertically into downy masses among the overlying *T. micrantha* leaves. Black sporangia appeared along the culture edges by 43 h in Batch A and 36 h in Batch B. Mycelial mats were disrupted when the cakes were broken and transferred to vessels.

Both mashes fermented rapidly in the vessels, with vigorous gas production and violent bubbling at 48–135 h (TABLE II). After 135 h bubbling was reduced. The mashes grew increasingly gelatinous, disintegrating to a semiliquid after 135 h. Metabolic byproducts high in ethanol pooled as a clear liquid along the edges of the mash at 48–60 h and continued to build on the surface of the mash through 205 h.

Temperature. Stage I culture temperatures for Batch A were: 43 h = 40 C, 48 h = 36.5 C and 60 h = 35 C.

Laboratory isolation of mold. The filamentous mold that dominated Stage I was isolated on potato-dextrose agar (PDA) in the laboratory from samples of dried parakari. Cultures were grown at 30 C until they had covered the agar surface and began to sporulate. They subsequently were examined under light microscopy for micromorphological features. The mold was confirmed as a species of *Rhizopus* (Mucoraceae, Zygomycota) and was deposited as *Rhizopus* sp. NRRL 22796, Northern Regional Research Laboratory, Peoria, Illinois 61614. Preliminary examinations by Dr Clifford Hesseltine indicated that the isolate morphologically was similar to the tempeh mold *Rhizopus oligosporus* Saito but differed in sporangiophore morphology; spore size also differed, and striations of the sporangiospores, present on *R. oligosporus,* were absent in the parakari isolate. NRRL 22796 is likely new to science and awaits formal description (Hesseltine pers comm).

DISCUSSION

The use of the mucoraceous mold *Rhizopus* for the initial breakdown of starch during fermentation is unique in the production of New World indigenous fermented beverages. Most indigenous fermented beverages of South America involve starch breakdown via mastication of maize or cassava or germination (malting) of maize in the case of Andean chicha (LeBarre 1938, Cutler and Cardenas 1947, Steinkraus 1996). The use of *Rhizopus* for amylolysis in parakari contrasted sharply with these other techniques. Although the use of ''a white mold'' in parakari manufacture was noted previously by ethnologists among the Wai Wai and Wapisiana of Guyana (Farabee 1924, Yde 1965, Forte 1992), the mycological significance of the process has gone unrecognized until now.

The use of *Rhizopus* by the Wapisiana constituted a more sophisticated control of useful micro-organisms than that present in other indigenous South American beverages. Although production is detailed and subject to great artisanship, chicha, along with cassiri, sarawau and other cassava fermentations, require no complex manipulation of fermenting microorganisms (Cutler and Cardenas 1947). The transfer of *Rhizopus* from batch to batch via *T. micrantha,* the addition of purified cassava starch to boost the inoculum's potential and the maintenance of favorable culture temperature by addition of hot coals demonstrated a high degree of sophistication among the Wapisiana women in the care and maintenance of this useful fungus. The complexity of this technology is similar to those in Asia, when compared to the other, relatively simple, native beers of South America (see Steinkraus 1996).

Rhizopus was effective at starch hydrolysis. The luxuriant Stage I growth of *Rhizopus* mycelium was concomitant with a sharp increase in glucose concentration and sweetness, characteristics resulting from the depolymerization of starch. Similar patterns in mycelial growth and sugar production by *Amylomyces rouxii* Calmette, a species closely related to *Rhizopus,* were found in Indonesian *tape ketan* (rice wine) fermentation (Ellis et al 1976, Cronk et al 1977). Species of *Rhizopus* are known to be effective starch hydrolyzers in a variety of Asian fermentations (Hesseltine et al 1988). Given the rapid domination of the parakari cakes by *Rhizopus,* it was probable that this fungus, and not ambient yeasts that likely were present, performed the bulk of the amylolysis. Yeasts in other mixed culture fermentation studies have been found to be ineffective starch hydrolyzers (Cronk et al 1977, Nout 1992).

Although the Stage I integrity of the *Rhizopus* mycelium was broken during transfer, it was likely that the *Rhizopus* continued hydrolyzing starch in Stage II. This was especially evident in Batch B, where glucose concentration (and thus production) remained high through 112 h, although ethanol concentration rose after 48 h as indicated by taste. While the compact, gelatinous consistency of the parakari mash in

Stage II could restrict oxygen in the interior, such conditions would not necessarily have inhibited further *Rhizopus* hydrolysis. Other amylolytic *Rhizopus* spp. are known to grow with little oxygen in solid substrate fermentations (Hesseltine et al 1985, Nout et al 1987).

The drop in pH from 5.0 to nearly 4.0 within 0–205 h likely resulted from the production of organic acids as primary metabolites of the rapidly growing *Rhizopus* (Nout 1992). This drop could function in a feedback loop to enhance successful parakari production. Reduced pH might have enhanced the growth of *Rhizopus,* because growth of the tempeh mold *R. oligosporus* is optimal near pH 4.0 (Medwid and Grant 1984). At pH less than 5.0 growth of competitive spoilage bacteria in solid substrata normally is inhibited (Nout 1992). With parakari such mechanisms could have enhanced the success of *Rhizopus* at dominating the substratum. While yeasts were not isolated in this study, it is likely that they were present and responsible in part for the Stage II production of ethanol, as they are in most dual fermentation beverages known from Asia (Steinkraus 1979, pers comm). The growth of yeasts would be favored by the lower pH during later stages of fermentation because optimum pH for growth of most yeasts is between 3.5 and 5.0 (Rose 1987). A pH range of 4–5.5 also has been shown to be optimal for the function of fungal glucoamylase enzymes (Buettner et al 1987); this positive effect could help explain the consistently effective hydrolysis seen at 24–100 h in parakari. An effect of organic acid production in parakari could be the development of attractive flavors and aromas (Ko 1972, Steinkraus 1979), although prolonged fermentation led to sourness.

Purified cassava starch added to powdered *T. micrantha* leaves might have served as a booster for the initial growth of *Rhizopus*. The purified starch, in intimate contact with fungus-containing leaf, might be a more immediately digestible carbohydrate for the germinating spores and mycelium than the coarse, fiber-rich cassava bread. After proliferation on the starch, *Rhizopus* was free to spread into the adjacent, sodden bread. The use of purified starch in parakari inoculum is analogous to a variety of amylolytic fermentation starters from Asia, in which mucoraceous fungi, yeasts and bacteria are prepared as a dry product, and added, along with one or more pulverized plant materials, to a base of starch-rich rice flour that makes up the bulk of the starter (Hesseltine et al 1988).

The addition of coals under the cake in Stage I might have provided a favorable temperature for rapid initial growth of *Rhizopus*. Under controlled laboratory conditions parakari *Rhizopus* isolate NRRL 22796 grew faster on PDA at 31 and 38 C than at the ambient temperature of 22 C (FIG. 6); a similar temperature optimum has been shown for *R. oligosporus* (Nout 1992). Coals, while cooling after application, nonetheless might sufficiently raise culture temperature for rapid *Rhizopus* growth, letting the mold dominate the culture. Cronk et al (1977) found that amylolysis and ethanol production by *A. rouxii* and *Endomycopsis burtonii* Boidin et al in *tape ketan* fermentation was increased at t = 36 h when incubation temperature was raised from 25 to 35 C. At 48 h past inoculation in the study reported here, the 40 C culture temperature recorded for Batch A was likely a result of metabolic heat generated by *Rhizopus*. In addition to growth stimulation an added benefit of the rising temperature provided by coals and *Rhizopus* metabolism itself might have been an inhibition of bacterial contaminants, which usually have a low tolerance for high temperatures (Nout 1992). Modest temperature increases from exogenous heat sources have been shown to eliminate bacterial contamination in grain mashes undergoing *Rhizopus* amylolysis (Matsumoto et al 1982).

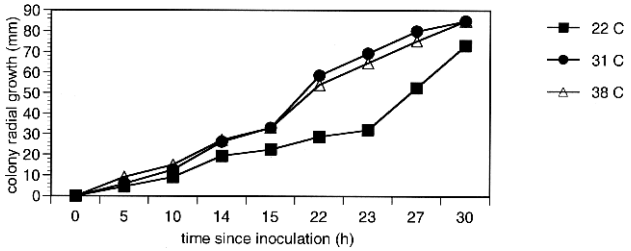

FIG. 6. Colony radial growth of parakari *Rhizopus* isolate NRRL 22796 on potato-dextrose agar at three temperatures.

The use of *Rhizopus* for parakari fermentation is more akin to certain Asian practices than to those of South America. In particular Indonesian *tape ketan* fermentation involves the hydrolysis of rice starch by the mucoraceous mold *A. rouxii,* followed by ethanol production by yeasts including *E. burtonii*. In Indonesian *tape ketella,* the same micro-organisms are used, with cassava as the substratum (Steinkraus 1996). Specific use of amylolytic *Rhizopus* species occurs in Chinese *lao-chao* (Wang and Hesseltine 1970) and the Indonesian rice beer *pachwai* (Steinkraus 1979). The well known culturing of tempeh from soybeans uses *R. oligosporus* to destroy undesirable digestive factors in the soybeans and to enhance their nutritional value through proteolytic activity (Whitaker 1978, Hesseltine 1985).

One possible scenario for the capture and control of *Rhizopus* from the wild could have involved the following events. Mucoraceous fungi such as *Rhizopus* are common saprotrophs in nature, frequently oc-

curring on carbohydrate-rich substrata (e.g., ripe fruit). In the warm, humid neotropics one can envision cassava bread being inadvertently moistened and colonized by a wild *Rhizopus*. The indigenous people could have tasted the molding cassava bread and noticed the sweetness. This sweetness might have been associated with the flavor derived from mastication in pre-existing cassava beverages such as *sarawau* (considered the more ancient form of amylolysis; Steinkraus 1979). A period of controlling the amylolytic *Rhizopus* could have followed to produce a sugar-rich substrate for subsequent fermentation to ethanol in concentrations higher than those achieved in *sarawau*. Such a sequence of events is corroborated by patterns in Asia, where indigenous fermentations emphasize mucoraceous fungi such as *Rhizopus* in tropical latitudes, whereas *Aspergillus* is used more in temperate countries such as China and Japan; relative humidity and warmth might be a selective factor (Hesseltine 1983). While boosts in ethanol content in native beverages might have been desirable (Im Thurn 1883, Yde 1965, Holmberg 1969), nutritional improvements in vitamin and protein contents of the fermented cassava, as well as production of beneficial antibiotics, might not have gone unnoticed by the indigenous people (Wang et al 1969, Keuth and Bisping 1993). The domestication of *Rhizopus* in South America appears independent from, yet analogous to, ancient *Rhizopus* domestications in Asia.

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