Microbiological Studies on Corn Dough Fermentation

K. OBIRI-DANSO[1]

ABSTRACT

Yeasts involved in the fermentation of corn grist into dough, a raw material for *kenkey* , a major staple for the people of southern Ghana, were isolated by dilution and pour-plate techniques from a number of dough samples prepared by local *kenkey* producers. The morphological characteristics and biochemical properties of the isolates were studied in pure cultures. The dough's yeast microflora consisted of mainly *Saccharomyces* spp and *Candida* spp. The isolates utilized different carbohydrates as carbon sources. Glucose, sucrose, and galactose gave the best growth for *S. cerevisiae*, *C. tropicalis*, and *C. kefyr*, while *C. krusei's* multiplication was supported only by glucose. Isolates and combinations of isolates were inoculated into fresh corn grist. Fermentation was monitored daily for 96 hr; moisture, pH, viable yeast count, temperature, and dough extension were monitored. These parameters increased significantly ($\alpha \leq 0.05$) during the initial 24 hr of fermentation; however, pH decreased significantly ($\alpha \leq 0.05$) due to yeast activity from 6.55 to 3.70–4.00 at the end of 96 hr.

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Ghana presently requires a low-cost method of providing nutritious protein-rich foods for its population; however, it lacks a sound, scientific basis for quality production of such foods. Corn (*Zea mays*) constitutes an important food item in many African diets. In Ghana, it is the most widely eaten cereal. Corn is used mainly in the form of fermented dough for the preparation of a wide variety of dishes: porridge, *kenkey*, *banku*, *abolo*, *agidi*, etc.

Even though *kenkey* is the staple of the majority of the people in southern Ghana, very little is known about the traditional fermentation process. Corn dough fermentation is done in villages and low-income settlements in an uncontrolled manner and under poor hygienic conditions. To prepare the dough, the corn is cleaned and steeped for 12–72 hr, depending on the type of food to be prepared. The steeped corn is drained of water and wet-milled into corn grist. The grist is added to water in a 5:1 ratio and mixed well with wooden ladles or by hand to form a stiff paste. The paste is placed in wooden, plastic, or enamel vessels to ferment for 24–72 hr (Nyako 1977). Corn dough fermentation is caused by a mixture of microbial inocula. This often results in a product with considerable flavors and taints. Consequently, the quality of the derived products is variable (not uniform from one producer to another), and the shelf life varies between 24–240 hr, depending on the product.

This study was conducted to isolate and identify the yeasts involved in the fermentation of corn grist into dough and determine conditions favoring the growth of the isolates. This would enable researchers to develop a pure culture to ferment corn in a manner similar to that used in some oriental fermented foods.

[1]Department of Biological Sciences, University of Science and Technology, Kumasi, Ghana.

MATERIAL AND METHODS

Source of Isolate

Isolates were obtained from 10 local producers of corn dough who were within a 10-km radius of the University of Science and Technology campus, Kumasi, Ghana.

Isolation Procedure

To obtain representative isolates of the yeasts involved in the fermentation, the corn dough from the local producers was sampled daily for 72 hr. Samples were taken in duplicate.

Corn dough (10 g) was weighed and diluted 10-fold. Samples were serially diluted to obtain concentrations of 10^{-2} to 10^{-7}. Aliquots (1 ml) of each of the six dilutions were inoculated onto malt extract agar and incubated at 28°C. Representative colonies of the various isolates were streaked on fresh isolation media. Pure cultures resulting from the isolation were subcultured and prepared on malt agar slants.

Identification of Isolates

Identification of the yeasts was based on standard morphological and biochemical characteristics useful for yeast identifications (Lodder 1971).

Fermentation

The effects of different carbon sources (glucose, sucrose, maltose, galactose, lactose, and raffinose) on the growth of the isolates were tested in 3.5-cm^3 durham tubes (Diddens and Lodder 1942). In making the dough, fermentation was initiated by mixing corn grist (*Zea mays* var. Aburotia) (Tuxpeno Planta Baja Cycle 16) with water in a 5:1 ratio. The water for mixing contained an appropriate yeast inoculum or yeast combinations at a cell count of 1×10^6 cells per milliliter as a starter to bring the yeast to approximately 2×10^5 cells per 1 g of dough. Fermentation was allowed to proceed for 96 hr at room temperature (25–28°C). Determinations were made at 0, 24, 48, 72, and 96 hr for pH, temperature, moisture, viable yeast numbers, and dough extension.

The moisture content was determined by the vacuum oven method (AACC 1983). Dough extension was measured by attaching calibrated graph sheets on three different sides of the deep petri dish used for fermentation. Malt extract agar was used to enumerate viable yeast numbers.

RESULTS AND DISCUSSION

The yeast microflora isolated in the study were *Saccharomyces cerevisiae, Candida tropicalis, C. kefyr,* and *C. krusei.* Some variation existed in the numbers and types of yeasts isolated from doughs collected from different localities. *C. krusei* could possibly have come from the corn grain itself, as it was present in all the products sampled (data not shown). Thus, the main yeasts in corn dough were species of *Saccharomyces* and *Candida.* Akinrele (1966) isolated *Saccharomyces* and *Candida* species during the fermentation of a Nigerian maize-fermented food (*ogi*).

Corn from the local producers in Ghana is deficient in lysine and tryptophan. However, these amino acids are contained in yeasts (Latham 1965). Hence, the use of yeasts in the preparation of a starter culture for fermented cereal foods would naturally result in improved protein content (Nyako and Obiri-Danso 1991).

All the isolates, with the exception of *C. krusei,* had a wide range of sugar utilization abilities, as determined by a fermentability test (Table I). Glucose, sucrose, and galactose were the best carbon sources for the growth of *S. cerevisiae, C. tropicalis,* and *C. kefyr.* This is significant because sugars are constituents of corn, and sucrose is the principal sugar (Mbugua 1981).

Moisture content for all isolates and combinations of isolates increased significantly ($P > 0.05$) within the first 24 hr of fermentation (Fig. 1). This could be attributed to the corn dough retaining

TABLE I
Acid and Quantities (cm^3) of Gas Produced[a] from Sugar Fermentation of *Saccharomyces cerevisiae* (P), *Candida tropicalis* (S), *C. kefyr* (T), and *C. krusei* (N)

	Fermentation time, hr							
Substrate/Isolate	**0–24**	**24–28**	**48–72**	**72–97**	**96–120**	**120–144**	**144–168**	**168–192**
Glucose								
P	3.00	3.50						
S	1.10	3.50						
T	A[b]	A	0.40	3.50				
N	A	A	1.85	3.50				
Sucrose								
P	0.85	3.00	3.50					
S	A	2.35	3.50					
T	A	A	0.70	3.50				
N	0[c]	0	0	0				
Maltose								
P	0	A	2.35	3.50				
S	0	A	A	3.15	3.10	3.50		
T	0	0	0	0	0	0		
N	0	0	0	0	0	0		
Galactose								
P	0	23.5	3.50					
S	0	0	A	A	A	A	0.30	1.50
T	A	A	A	A	A	0.40	1.20	3.50
N	0	0	0	0	0	0	0	0
Lactose								
P	0	0	0	A	A	A	A	A
S	0	0	A	A	A	A	A	A
T	0	0	0	0	0	0	0	0
N	0	0	0	0	0	0	0	0
Raffinose								
P	0	A	0.20	0.80	1.35	1.80	1.80	1.80
S	0	0	A	A	A	A	A	A
T	0	A	A	A	A	A	1.10	1.15
N	0	0	0	0	0	0	0	0

[a] Volume of fermentation tube: 3.5 cm^3. Values are average of three replicates.
[b] Acid but no gas produced.
[c] No acid or gas produced.

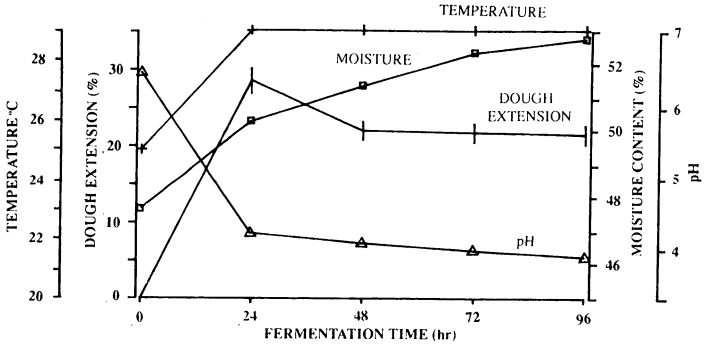

Fig. 1. Physical changes during corn grist fermentation by *Candida tropicalis*. Vertical lines represent standard errors of mean ($n = 3$).

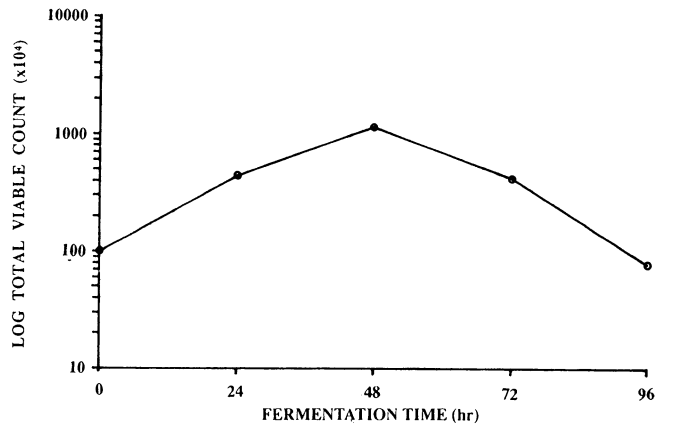

Fig. 2. Total viable yeast count during corn grist fermentation by *Candida tropicalis*.

some metabolic water formed during fermentation (Odunfa and Oyeyiola 1985). However, moisture content was not influenced by differences with respect to isolates or combinations of isolates within a range of 47.0–48.0% initially to 50.0–50.7% at 24 hr.

After the initial increase in temperature from 25 to 29°C, the temperature remained constant for all isolates and combinations of isolates; no significant ($P > 0.05$) differences in temperature were observed. This is indicative of the normal atmospheric temperature. Yeasts are known to be active at a temperature of 22–29°C (Hesseltine 1965)

The pH for all the isolates and combinations of isolates decreased significantly ($P > 0.05$) during the initial 24 hr of fermentation from pH 6.55–6.41 to pH 4.40–3.97 (Fig. 1). No significant ($P > 0.05$) changes in pH were observed between isolates and combinations of isolates. The low pH not only limits the growth of bacteria that might be pathogenic, but it also creates an environment favorable for yeast growth. The yeasts ferment the hexoses produced during the saccharification process to give alcohols and organic acids (Hesseltine and Haynes 1973). Yeast activity is optimum between pH 4 and 5 (Presott and Dunn 1987). Thus, the yeast multiplication rate was slower (Fig. 2) during the initial 24 hr, when the pH was decreasing from pH 6.55–6.41 to pH 4.40–3.97. A rapid increase in yeast number was observed during the next 24 hr, when a desired pH of 4.40–3.97 was attained.

Initially, the fermenting dough increased rapidly in volume; during the first 24 hr, it increased by 10–33%, depending on the isolates or combination of isolates (Fig. 1). *C. krusei* caused the least increase in volume. Subsequently, the volume stabilized at a 9–25% increase. The coarse nature of corn grist contributes to the escape of gas. A significant drop ($\alpha \leq 0.05$) in the dough volume was observed with *C. krusei* because of the organism's inability to metabolize any sugar other than glucose (Table I). The production and evolution of carbon dioxide by yeasts accounted for the leavening action of the dough. Spicher et al (1979) reported that the leavening of sour dough rye bread was due to the activities of *S. cerevisiae* and *C.* spp.

The rate of increase in cell numbers during the first 24 hr of fermentation was relatively low (Fig. 2). A 10–61% increase in cell numbers occurred every 2 hr during the initial 48 hr, depending upon the isolate or combination of isolates. For the subsequent 48-hr period, a 13–62% decrease in yeast population occurred. Limited nutrients (sugars) and space within the fermenting dough were inhibitory factors contributing to the decreasing yeast numbers (Nyako and Obiri-Danso 1991). The rate at which *C. krusei* multiplied was very slow, while the rate for *C. tropicalis* was fastest. This is one of the reasons why *C. tropicalis* is widely used in the production of single-cell protein for food and fodder (Smith 1980).

CONCLUSION

The results of this study showed that acceptable maize dough could be prepared with *S. cerevisiae*, *C. tropicalis*, and *C. kefyr*, as well as combinations of them. *C. tropicalis* alone, however, seemed more promising as starter inoculum. Additions of glucose, sucrose, and galactose to the fermenting mash would also be effective in accelerating fermentation.

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