ISOLATION AND IDENTIFICATION OF MICROORGANISMS DURING SPONTANEOUS FERMENTATION OF MAIZE

[Isolasi dan Identifikasi Mikroorganisme pada Fermentasi Spontan Jagung]

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ABSTRACT

Maize was traditionally the second most common staple food in Indonesia. Conversion to maize flour has been accomplished to improve its convenience. Traditionally, maize flour is produced by soaking the kernels in water followed by grinding. It was reported that final physicochemical characteristics of the maize flour were influenced by spontaneous fermentation which occurred during soaking. This research aimed to isolate and identify important microorganisms that grew during fermentation thus a standardized starter culture can be developed for a more controlled fermentation process. Soaking of maize grits was conducted in sterile water (grits:water=1:2, w/v) in a closed container at room temperature (±28ºC) for 72 hours. After 0, 4, 12, 24, 36, 48, 72 hours, water and maize grits were sampled and tested for the presence of mold, yeast, and lactic acid bacteria (LAB). Isolates obtained from the spontaneous fermentation were reinoculated into the appropriate media containing starch to observe their amylolytic activity. Individual isolate was then identified; mold by slide culture method, while yeast and LAB by biochemical rapid kits, i.e. API 20C AUX and API CH50, respectively. The number of each microorganism was plotted against time to obtain the growth curve of the microorganisms during spontaneous fermentation. The microorganisms were identified as *Penicillium chrysogenum, P. citrinum, A. flavus, A. niger, Rhizopus stolonifer, R.oryzae, Fusarium oxysporum, Acremonium strictum, Candida famata*, *Kodamaea ohmeri, Candida krusei/incospicua*, *Lactobacillus plantarum 1a, Pediococcus pentosaceus, L. brevis 1, L. plantarum 1b,* and *L. paracasei ssp paracasei 3*. Four molds and one yeast were amylolytic while none of the LAB was capable of starch hydrolysis. The growth curve suggested that the amylolitic mold and yeast grew to hydrolyze starch during the course of fermentation, while the LABs benefited from the hydrolyzed products and dominated the later stage of the fermentation.

Keywords: amylolytic, LAB, maize, mold, spontaneous fermentation, yeast

ABSTRAK

Jagung merupakan makanan pokok kedua terpenting di Indonesia dan berbagai upaya telah dilakukan untuk memproduksi tepung jagung guna meningkatkan kemudahan penggunaannya. Secara tradisional, tepung jagung dibuat dengan merendam biji jagung dalam air, diikuti dengan proses penggilingan. Selama perendaman terjadi fermentasi spontan yang telah dilaporkan dapat mempengaruhi karakteristik fisiko kimia tepung jagung yang dihasilkannya. Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi mikroba penting yang tumbuh selama fermentasi spontan jagung sehingga dapat dikembangkan kultur starter standar untuk proses fermentasi terkendali. Grits jagung direndam dalam air steril (grits : air = 1:2, b/v) dalam wadah tertutup pada suhu kamar (±28ºC) selama 72 jam untuk mensimulasi proses fermentasi spontan. Setelah 0, 4, 12, 24, 36, 48, 72 jam, sampel air dan grits jagung diambil untuk dianalisis jenis kapang, kamir, dan bakteri asam laktat (BAL) yang tumbuh. Isolat yang diperoleh dari fermentasi spontan diinokulasi kembali pada media yang sama dengan menambahkan pati untuk mengamati aktivitas amilolitiknya. Selanjutnya, masing-masing isolat diidentifikasi: kapang dengan metode slide culture, sementara kamir dan BAL dengan perangkat cepat biokimiawi berturut-turut API 20C AUX dan API CH50. Jumlah masing-masing mikroba diplotkan terhadap waktu untuk mendapatkan kurva pertumbuhan mikroba selama fermentasi spontan. Hasil penelitian menunjukkan bahwa mikroba yang tumbuh selama fermentasi spontan terdiri dari kapang Penicillium citrinum, P. chrysogenum, A. flavus, A. niger, Rhizopus stolonifer, R. oryzae, Fusarium oxysporum, Acremonium strictum, khamir Candida famata, Kodamaea ohmeri, Candida krusei/incospicua, serta bakteri asam laktat Lactobacillus plantarum 1a, Pediococcus pentosaceus, L. brevis 1, L. plantarum 1b, dan L. paracasei ssp paracasei 3. Empat kapang dan satu kamir bersifat amilolitik sementara tidak ada BAL yang bersifat amilolitik. Kurva pertumbuhan menunjukkan bahwa kapang dan kamir amilolitik tumbuh dan menghidrolisis pati selama fermentasi awal sementara BAL memanfaatkan produk hasil hidrolisis dan mendominasi tahap selanjutnya selama fermentasi.

Kata kunci: amilolitik, BAL, fermentasi spontan, jagung, kamir, kapang

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INTRODUCTION

Maize used to be an important staple food in Indonesia but has became less popular because of the inconvenience in its preparation. Traditionally, maize flour is made by soaking of the maize kernel in water followed by draining, grinding and drying. The length of the spontaneous fermentation occurred during soaking was reported to have influence in the physicochemical characteristics of the maize flour (Aini et al. 2010). Several studies on spontaneous fermentation of maize have been reported, such as in ogi production (Nago et al. 1998) and pozol (ben Omar and Ampe, 2000). During the fermentation, amylolytic, lipolytic, and proteolytic bacteria, molds as well as yeasts were isolated.

Presence of various microorganisms during fermentation were thought to affect the physicochemical properties of white maize flour produced (Aini et al. 2010). The spontaneous fermentation decreased the protein, fat, crude fiber, ash, starch, reducing sugar, pH, bulk density, and fat absorption capacity of the flour produced, while increased the bulk angle, whiteness, and water absorption capacity. Aini et al. (2010) reported that fermentation for 24 hours reduced the gelatinization temperature from 82 to 76.2°C. Meanwhile fermentation for 72 hours increased the gelatinization temperature of the maize flour to 85.2°C. Fermentation for 48 hours increased the flour peak viscosity (648 BU), while 72 hours fermentation decreased its peak viscosity to 550 BU which was similar to the unfermented flour. Fermentation for 12 to 60 hours increased the heat stability of the maize flour. Meanwhile, fermentation for up to 36 hours reduced the retrogradation tendency of the maize flour and soaking 48 hours increased the gel strength as compared to unfermented flour. The above results suggest that it is possible to control the fermentation process to achieve desired physicochemical properties of the maize flour. At the present, maize fermentation commonly rely on the naturally occuring microorganisme in the raw materials. Therefore, a consistent quality of the product may be difficult to achieve. The use of known microorganisms originated from the spontaneous fermentation is expected to better control the quality of the product. The study aims to isolate and identify microorganisms naturally growing during spontaneous fermentation of maize. The microorganisms isolated can be further used to design a starter culture for a controlled fermentation to produce maize flour with desired physicochemical characteristics.

MATERIALS AND METHODS

Materials

The maize used in this study was of Anoman type1 local variety obtained from the Cereal Crops Research Center, Maros, Sulawesi. Maize grits was obtained by grinding using a pin disc mill followed by sievering with a shaker-siever.

Spontaneous fermentation of maize grits

Maize kernels were washed with sterile distilled water (kernel:water=1:4, w/v, 5 mins) and made into grits (±4 mm in diameter) by using pin disc mill. The resulting grits were then sieved by mesh siever 10 and the grits retained in the siever were collected. After that, grits were washed in sterile water (grits:water =1:4, w/v, 5 mins), floating and unused part were discharged, and drained for 30 mins. Fermentation of the maize grits was carried out by soaking the grits with sterile water (grits:water = 1:2 w/v) in a covered container at room temperature (±28°C) for up to 72 hours (modified from Aini et al. 2010). Sampling was done at 0, 4, 12, 24, 36, 48, and 72 hours of fermentation.

Enumeration of microorganisms (Nago et al. 1998)

Samples of maize grits and water (10 g) were homogenized with 90 ml of sterile peptone physiological saline solution. One ml of the appropriate dilution were plated onto media (a) Plate Count Agar (PCA, Oxoid) for total plate count (TPC), (b) de Man Rogosa and Sharpe Agar (MRSA, Oxoid) added with 0.5% $CaCO_3$ for LAB; (c) Yeast Extract Glucose Agar (YEGA, Oxoid) and 0.01% oxytetracyclin for yeasts and (d) Acidified Potato Dextrose Agar (APDA, Oxoid) for molds. Plates were incubated at 30°C for 24 hours for TPC and LAB, 48 hours for yeast and 5 days for mold. Enumeration for each visually distinct colony was quantified at all sampling times.

Microbial isolation and identification (Nago et al. 1998)

Any visually distinct bacterial, yeast or mold colony appearing on the plates was isolated and streaked onto the appropriate media until single colonies were obtained. The isolated bacterial colony was Gram stained, microscopically observed and tested for catalase activity. LAB isolates were further identified using API 50CH. Yeast isolates were identified using API 20C AUX rapid kits. Individual mold isolate was streaked onto media APDA and Czapek Yeast Extract Agar (CYA) and incubated for 7 x 24 hours at 5, 28 dan 37°C. The color and diameter of the colonies were observed and recorded. The mold growth on the above media made into slide culture preparates on CYA and observed under microscope and the conidiophore types, metulae, phialide, and conidia forms were compared to these described in Pitt and Hocking (2009) and Samson et al. (1981). The amylolytic activity of the isolates was observed on MRS containing-2% starch + 1% anyline blue for LAB; or YEGA containing-2% starch for molds and yeasts.

Determination of pH (AOAC, 1995)

The pH of the media at various fermentation time was measured according to AOAC (1995).

Enzyme activity (EC.3.2.1.1)

The mixture of maize and water after 0, 4, 12, 24, 36, 48, and 72 hours of fermentation was centrifuged at 7000 rpm at 4°C for 10 min and the substrate-free supernatant was used for estimation of enzyme activity. The amylase activity was determined by measuring the reducing sugar formed by the enzymatic hydrolysis of starch (EC.3.2.1.1).

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RESULTS AND DISCUSSION

Growth of naturally occuring microorganisms during spontaneous fermentation

During spontaneous fermentation for 72 hours, the total microorganisms grew from 4.3 to 8.7 log CFU/mL. In the first two hours the number of microorganisms remained constant suggesting an adaptation period (lag phase). This phase was followed by slow growth during 2-4 h and a rapid growth (log phase) during 4-12 hours of fermentation. After 12 hours the population reached stationary phase as shown by a plateau curve, during which a subset of the population underwent death phase, and concomitantly another group grew. Overall, there is an increase of microbial population of 4 log cycles throughout the 72 hours of fermentation process (Figure 1).

The pH value

During the first four hours of the fermentation, the pH of medium was relatively stable (pH 5.8-5.9). However, the pH experienced a sharp decline during 4-12 hours of fermentation, reaching a pH of 4.4 at 12 hours. The pH was relatively stable afterwards. During lag and early log phase fermentation (0-4 hours), pH did not change much due to lack of microbial metabolisms (pH 5.8-5.9). The significant decrease in pH corresponds to the rapid growth of the microorganisms during fermentation (Figure 1).

Microbial isolation and identification

Mold isolates

During enumeration of the colonies, five visually distinct molds were observed. Initial identification suggested that molds growing during fermentation consisted of *Penicillium sp, A. flavus, A. niger, Rhizopus sp,* and *Fusarium sp.* Further identification based on slide culture suggested that the *Penicillium* consisted of 2 species i.e. amylolytic *P. citrinum* and non-amylolytic *Penicillium chrysogenum,* the *Rhizopus* consisted of non-amylolytic *Rhizopus stolonifer* and *R.oryzae,* while the *Fusarium* can be differentiated into amylolytic *Acremonium strictum* and non-amylolytic *Fusarium oxysporum*. Since the species of the three genuses can not be differentiated visually, the number of these species was reported as the corresponding genus (Figure 2). Description of all molds based on slide culture observation is presented in Table 1. An example of such observation can be seen in Figure 3.
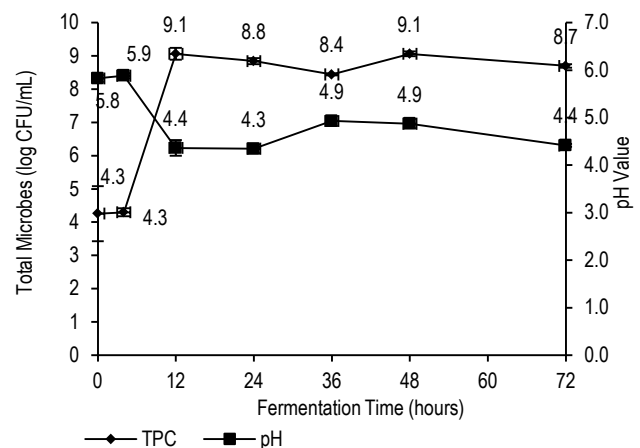

Figure 1. Total plate counts (TPC) of microorganisms and the pH changes during spontaneous fermentation of maize

Table 1. Description of molds growing during spontaneous fermentation maize grits

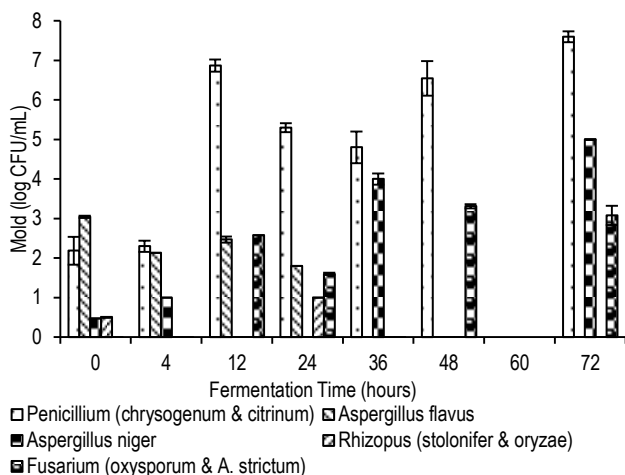| Visual Observation of Colonies	Slide Culture Observation	Identification	Amylolytic Activity
White hypae, blue-greenish spores	Colonies are yellow-gray with diameter of 27.7 mm after 7 days incubation at room temperature. When incubated at 37°C colonies are tosca green with 25-32 mm diameter. Conidiophores have three-stage branch pattern. Metulae somewhat cylindrical with an average number phialide 5. Phialide looks like a flask. Conidia roundish shaped and translucent.	*Penicillium chrysogenum*	Non-amylolytic
	Conidiophores have one-stage branch pattern which grow from the surface of hyphae. At the top there are metulae with an average of 3, growing spread. Phialides looks like a flask with an average number 6. Conidia are spherical and translucent.	*Penicillium citrinum*	Amylolytic
White hypae, blue-greenish yellow spores	Conidiophores are translucent. Vesicles are roundish in shape. Phialides grow directly on the vesicle. Conidia are spherical and conidial heads have radiate type.	*Aspergillus flavus*	Amylolytic
White hypae, short, gray to black spores	Conidiophores are translucent. Vesicles are roundish. Phialides grow on the metulae. Metulae are translucent. Conidia are spherical and conidial heads have radiate type.	*Aspergillus niger*	Amylolytic
White hypae, long, grays and black spore	Sporangiophore are tall, mostly grown on their own. Sporangium are rounded, rounded-oval columella, and elliptical sporangiospore. There are branched rhizoid and no clamydospora. There are long stolon that connected sporangiospore.	*Rhizopus stolonifer.*	Non-amylolytic
	Sporangiophore mostly grown on their own. The sporangiospore grows directly from the stolon without rhizoid. Sporangium are roundish shape. Clamydospora are oval and columella are rounded shape.	*Rhizopus oryzae*	Non-amylolytic
White hypae, pink and orange	Mycelium grow sparsely and pink colored after 24 hours incubation at room temperature. After 48 hours incubation, mycelium are purple. Conidia have septat. Phialids are oval-shaped. Conidiophores have a short branch and there are chlamydospora.	*Fusarium oxysporum*	Non-amylolytic
	Colonies are orange with a diameter of 13-17 mm after 7 days incubation at 37°C. Conidiophores are simple and not branched. Phialides are slender and unbranched which grow at slightly fasciculate aerial hyphae. Slimy head are rounded-ellips and translucent which contain conidia.	*Acremonium strictum*	Amylolytic

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Figure 2. Mold growth during spontaneous fermentation of maize

Penicillium (chrysogenum & citrinum) □ Aspergillus flavus
Aspergillus niger ■ Rhizopus (stolonifer & oryzae)
Fusarium (oxysporum & A. strictum)

Figure 3. An example of microscopic observation of mold slide culture identified as *Acremonium strictum*

Figure 2 shows that *Penicillium* and *Fusarium* were isolated throughout the fermentation process. For *Penicillium* and *Fusarium*, each had two peaks of growth. Very likely the first peaks were amylolytic *P. citrinum* and *Acremonium strictum* while the following growth curves belongs to the non-amylolytic *P. chrysogenum* and *F. oxysporum*.

Penicillium citrinum is mesophillic and grows at 5-40°C with optimum temperatures of 26-30°C. It grows at pH 2-10 with optimum pH between 5-7. This mold is also xerophillic and grows at aw 0.80-0.84. *Penicillium citrinum* can produce a toxin, citrinin, at 15-37°C with the optimum temperature of 30°C (Sweeney and Dobson, 1998). Citrinin, also known as a yellow rice toxin (Pitt and Hocking, 2009) is found in rice (Kumar, 2008). *Penicillium chrysogenum* is commonly found in maize. The mold is an endophyte which is widespread in nature and is often found living on food and indoor environment. This mold can produce antibiotics such as penicillin, chrysogine, xanthocillins, secalonic acids, sorrentanone, and PR-toxin (Meng, 2011; van den Berg, 2010).

The main source of *Acremonium strictum* is maize, but it can be found in black bean seeds, raw cork, wheat, barley, rice, bananas with crown rot, fresh vegetables, peanuts, pecans, hazelnuts and walnuts, soybeans, frozen meat, salami and biltong. *A. strictum* and other *Acremonium* species encountered in foods are not known to produce mycotoxins (Pitt and Hocking, 2009). *Acremonium strictum* as an endophytic fungi

benefits from the host plant, i.e. nourishment, water, and physical protection against biotic and abiotic adversities. Meanwhile, the host plant may be protected by the endophyte that produce secondary metabolites, e.g. alkaloids, antibiotics, or toxins that may be toxic to pathogenic fungi (D'amico *et al.* 2008). Wicklow *et al.* (2005) reported that *A. strictum,* also known as *Acremonium zeae* is antagonistic to kernel rotting and mycotoxin producing fungi *A. flavus* and *Fusarium verticillioides* in cultural tests for antagonism, and interferes with *A. flavus* infection and aflatoxin contamination of preharvest maize kernels.

Fusarium oxysporum is known to produce trichothecenes which have been linked to alimentary toxic aleukia, fusario-toxicoses and are cytotoxic to mammalian cells. These toxins are produced at the optimal growth conditions for *Fusarium* (Sweeney and Dobson, 1998). The average concentration of trichotheces found in maize is 226.2 µg/kg and ranging from 9.6-745.1 µg/kg (Adejumo *et al.* 2007).

Both Aspergilli were amylolytic but *A. niger* seemed to grow throughout the fermentation process while *A. flavus* grew well in the first 24 hours and decline afterward. This observation suggested that *A. flavus* was not a good competitor and *A. niger* as well as other microorganisms may have over grown *A. flavus*. This phenomenon is good with regard to food safety because inhibition of *A. flavus* is expected to decrease the aflatoxin production during fermentation. Purwijantiningsih *et al.* (2005) found that *Rhizopus oligosporus* dan *Candida* can inhibit the growth of *A. flavus* (37.21%) and aflatoxin production (99.96%). Recent study in East Java, Indonesia, showed that aflatoxins were found in 30% of maize at farmer level at the concentrations of more than 20 ppb and 10% of maize contains aflatoxin more than 100 ppb. At the trader level, the frequency of finding the toxin in maize was even higher, i.e. 45% of maize contain aflatoxin more than 20 ppb with 18% contain more than 100 ppb of aflatoxins (Rahayu, 2008). Unfortunately, *A. niger* is also known to produce other mycotoxin, i.e. ochratoxin A (OTA) at 15-40°C which could lead to nephrotoxin. OTA's production is low at aw 0.92 and temperature 25-40°C (Alborch *et al.* 2011).

According to Sweeney and Dobson (1998), *Fusarium, Aspergillus* and *Penicillium* are commonly found as contaminants in cereals and legumes during drying and storage. Kaaya and Kyamuhangire (2006) also isolated *Aspergillus, Fusarium, Penicillium,* and *Rhizopus* in dried and stored maize. Amusa *et al.* (2005) also reported that during spontaneous fermentation of maize and soybeans for the making of ogi, *A. niger, A. flavus, Fusarium oxysporum, and Rhizopus stolonifer* were found. Pitt and Hocking (2009) stated that *Fusarium* sp, and *Penicillium* sp were commonly found in preharvest maize, while *A. flavus* can be found in preharvest maize and during storage.

Maize is a good substrate for mold growth because it contains high protein and carbohydrate needed for mold growth (Alborch *et al.* 2011). Generally maize has moisture contents of 10.4–15.1% at 12-40°C. When stored at ambient temperature, maize will support *A. flavus* and *A. niger* that grow well at 32-33°C and 25-40°C, *Penicillium* sp at 26-30°C, *Fusarium* sp at 24-26°C (Sweeney and Dobson, 1998). Meanwhile *R. stolonifer*

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and *R. oryzae*'s optimum growth are at 25°C (Ramos-Gracia *et al.* 2012) and 37°C (Pitt and Hocking, 2009), respectively.

Both Rhizopus were non amylolytic and grew in low number during fermentation. Amusa *et al.* (2005) reported the presence of *Rhizopus sp* in white maize (10.5%) and yellow maize (9.5%). *R. oryzae* has cellulase, xylanase, pectinase, tannase, protease, phytase and lypase activities (Ghosh and Ray, 2011) which probably explains the low number of the mold when competing with various amylolytic mold presence. *R. stolonifer* is not pathogenic (Pitt and Hocking, 2009), but causes rot in fruits (Hahn *et al.* 2004).

Growth of amylolytic Fusarium, Aspergilli and Penicillium resulted in carbohydrate hydolysis to produce simpler sugar. This will trigger growth of the successive molds of *P. chrysogenum, F. oxysporum*, and *Rhizopus.* This overall process is expected to influence the quality of the maize, especially with regard to its digestibility.

Yeast isolates

Isolation of yeast in YEGA resulted in four visually distinct colonies (Table 2). However, biochemical identification suggested that the four isolates belong to three species, i.e. *Candida famata, Kodamaea ohmeri,* and *Candida krusei* or *C. incospicua* (Figure 4).

Table 2. Description of yeasts isolated during spontaneous fermentation of maize grits

| Morphology Characteristics	Amylolytic Activity	API Identification	% ID
Round-shaped, white, mucous, surface is dull	Amylolytic	*Candida famata*	92.8
Round-shaped, serrated at the edges, cream, mucous, surface is dull	Non- amylolytic	*Kodamaea ohmeri*	94.7
Round-shape with growing spread, creamy-white, mucous, surface is dull	Non- amylolytic	*Candida krusei/incospicua*	98.9
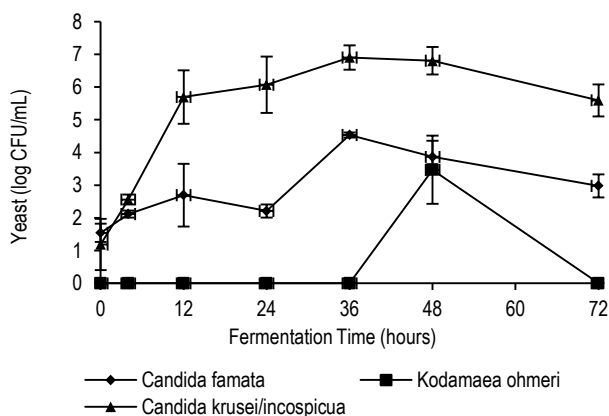
Figure 4. Yeast growth during spontaneous fermentation of maize

Growth of yeast and LABs are commonly observed in naturally fermented maize, such as those during the production of ogi, kenkey, mawe, and mahewu, traditional foods of of West Africa. These microorganisms are reported to play important role in aroma and flavor production as well as the stability of the final products. LABs and yeast affect the taste and structure of the dough in kenkey making (Omemu *et al.* 2007).

C. krusei is commonly isolated during maize fermentation. Nago *et al.* (1998) reported that during the fermentation of ogi, yeasts (7 log CFU/g) consisting of *Candida krusei* and *C. humicola* (41%) as well as *Geotricum* spp. (26%) were found. Omemu *et al.* (2007) isolated *Candida krusei, Saccharomyces cerevisiae, Candida tropicalis, Geotrichum candidum, G. fermentans, Rhodotorula graminis* on maize fermentation for making ogi. *Candida krusei* can live in the presence of lactic acid because it has a high tolerance to lactic acid (Halm *et al.* 2004*).* In addition *C. krusei* can stimulate the growth of *L. plantarum* and found in fermented maize for ogi production that has lipase and esterase activity that may contribute the final flavor of food (Omemu *et al.* 2007). In this study of *C. krusei* grew well and the number was higher than the other two yeasts.

Candida famata is usually found in plant and soil (Chaturvedi, 2003), dairy product especially cheese (Jacques and Casaregola, 2008) and Moroccan sourdough (Mohamed *et al.* 2007). *Candida famata* isolated from Moroccan sourdough shows high glucoamylase activity and produces biomass. This amylolytic yeast however did not grow as well as *C. krusei*. It has been reported that the yeast also exhibited lipolytic and proteolytic activities (Wojtatowicz *et al.* 2001).

Kodamaea ohmeri, previously known as *Pichia ohmeri* or *Yamadazyma ohmeri,* is a yeast within the family of *Saccharomy cetaceae. K. ohmeri* is the teleomorphic form of *Candida guilliermondii* and is widely used for the fermentation of fruit, pickles, and rinds (Yang *et al.* 2009).

Lactic acid bacteria

Identification of LAB isolates using API 50CH rapid kit suggest that the the isolates were *Lactobacillus plantarum1a, Pediococcus pentosaceus, Lactobacillus brevis 1, Lactobacillus plantarum 1b,* and *Lactobacillus paracasei ssp paracasei 3.* All LABs were not capable of degradating starch. Description of the characteristics of LAB isolated during spontaneous fermentation of maize is presented in Table 3 and Figure 5.

Table 3. Identification and characteristics of LABs isolated during spontaneous maize fermentation

Morphological Characteristics	API Identification	% ID
Round-shaped, creamy white, mucous, embossed on the surface of the media, shiny	*Lactobacillus plantarum1a*	99.9
Oval-shaped, white, mucous	*Pediococcus pentosaceus*	96.9
Round shaped, white mucus, growing in the center of the media	*Lactobacillus brevis1*	73.4
Half-round shaped, white, mucous	*Lactobacillus plantarum1b*	54.2
A small oval-shaped, white, mucous	*Lactobacillus paracasei ssp paracasei3*	99.3

Lactic acid bacteria are the predominant bacteria found in the fermentation of maize products. Nago *et al.* (1998) reported that during the fermentation of ogi, maize flour is immersed in water for 1-3 days at 25-35°C and lactic acid bacteria consisting of *Lactobacillus fermentum cellobiosus*, *L. brevis* and *L. fermentum* spp grow up to 9 log CFU/g. Other researcher isolated *L. plantarum, Saccaharomyces cerevisiae, Candida crusei, C. tropicalis, Geotrichum candidum, G. fermentans, and Rhodotorula graminis* during ogi fermentatin (Omemu *et al.* 2007).

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LAB is also found in Pozol and reach 7-8 logs CFU/g during the first day of fermentation, 9 logs CFU/g the second day and then decline to 8 logs CFU/g on the third day. During fermentation of ogi, *Streptococus* sp (25-75%) and *L. fermentum* were isolated in the first 2 days and followed by *L. plantarum, L casei, L. delbruekii* (ben Omar and Ampe, 2000).

This study shows that all LABs are non amylolytic, however they grew well throughout the fermentation process and maintained viability until 72 hours of fermentation. Presence of LABs may also contribute to the decrease in *A. flavus*. Edema and Sanni (2008) reported that LABs (*L plantarum, L brevis, L. fermentum, L. acidophilus*) and yeasts (*Pediococcus acidilactici, Leuconostoc mesenteroides, Leuconostoc dextranicum* and *Saccharomyces cerevisiae*) isolated from spontaneously fermented maize can inhibit the growth of *A. flavus.*
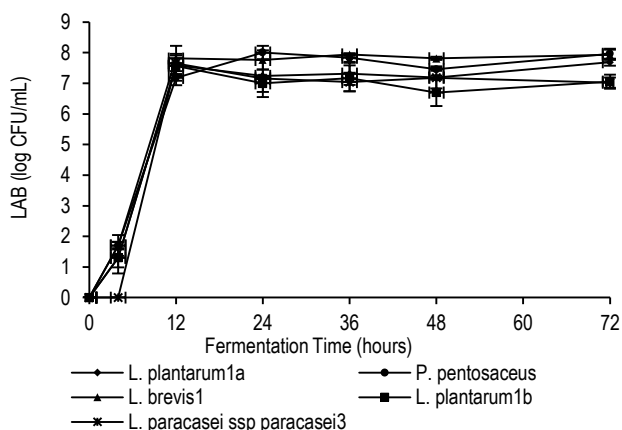

Figure 5. Growth of LABs (log CFU/mL) during spontaneous fermentation of maize

Amylase activity

The amylase activity observed during the spontaneous fermentation of maize grits shows similar pattern with the growth of amylolytic microorganisms. The amylolytic molds and yeast grew at the beginning of fermentation and hydrolyzed starch into simple sugars, which was used by the non-amylolytic organisms. The highest amylase activity was found at 12 hours fermentation (1.7 unit/mL.min), and the activity declined afterward (0.7 unit/mL.min) at 72 hours fermentation (Figure 6). The dominance of the lactic acid generated by LAB possibly led to the decrease in mold growth, thus decrease the amylolytic activity.
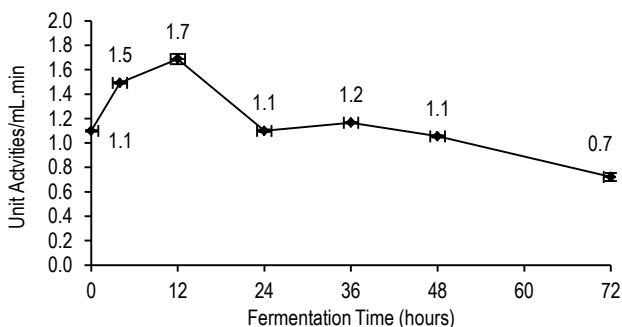

Figure 6. Changes in amylase activity during spontaneous fermentation of maize

CONCLUSION

Eight species of molds, three species of yeasts, and five species of LAB grew sequentially during spontaneous fermentation of maize grits. Four of the eight mold isolates and one of three yeast isolates were amylolytic, i.e. *P. citrinum, A. flavus, A. niger, Acremonium strictum,* and *Candida famata*. Non amylolytic molds and yeasts that grew during the spontaneous fermentation of maize were *P. chrysogenum, R. oryzae, R. stolonifer, F. oxysporum*, *Kodamaea ohmeri* and *Candida krusei* or *C. incospicua*. Five non amylolytic species of LAB were *Lactobacillus plantarum 1a, Pediococcus pentosaceus, Lactobacillus brevis 1, Lactobacillus plantarum 1b, Lactobacillus paracasei ssp paracasei3.*

Based on the growth curves, it was very likely that amylolytic mold such as *P. citrinum, A. flavus, A. niger, Acremonium strictum,* and *Candida famata* grew and hydrolized starch during the course of fermentation. Non amylolytic microorganisms grew later on the fermentation benefiting from the hydrolyzed products. The amylolytic activity in the early fermentation was high and decreases with longer fermentation time.

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REFERENCES

Adejumo TO, Hettwer U, Karlovsky P. 2007. Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. Int J Food Microbiol 116: 350–357. DOI: 10.1016/j.ijfoodmicro. 2007.02.009.

Aini N, Hariyadi P, Muchtadi TR, Andarwulan N. 2010. Hubungan antara waktu fermentasi *grits* jagung dengan sifat gelatinisasi tepung jagung putih yang dipengaruhi ukuran partikel. J Teknol dan Industri Pangan 21: 18-24.

Alborch L, Bragulat MR, Abarca ML, Cabañes FJ. 2011. Effect of water activity, temperature and incubation time on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus carbonarius* on maize kernels. Int J Food Microbiol 147: 53–57. DOI: 10.1016/j.ijfoodmicro.2011.03. 005.

Amusa NA, Ashya OA, Oladapo MO. 2005. Microbiological quality of ogi and soy-ogi (a Nigerian fermented cereal porridge) widely consumed and notable weaning food in southern Nigeria. J Food Agric Environ 3: 81-83.

[AOAC] Association of Official Analytical Chemists. 1995. Official Methods of Analysis of the Association Analytical Chemistry Inc, Washington D.C.

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ben Omar N, Ampe F. 2000. Microbial community dynamics during production of the Mexican fermented maize dough pozol. Appl Environ Microb 66: 3664-3673.

Chaturvedi V. 2003. Mycology Proficiency Testing Program January 2003. Wadsworth Center New York State Department of Health.

D'amico M, Frisullo S, Cirulli M. 2008. Endophytic fungi occurring in fennel, lettuce, chicory, and celery-commercial crops in southern Italy. Mycol Res 112: 100-107. DOI: 10.1016/j.mycres.2007.11.007.

Edema MO, Sanni AI. 2008. Functional properties of selected starter cultures for sour maize bread. Food Microbiol 25: 616–625. DOI: 10.1016/j.fm.2007.12.006.

Ghosh B, Ray RR. 2011. Current commercial perspective of *Rhizopus oryzae*: A Review. J Appl Sci 11: 2470-2486. DOI: 10.3923/jas.2011.2470.2486.

Hahn F, Lopez I, Hernandez G. 2004. Spectral detection and neural network Discrimination of *Rhizopus stolonifer* spores on red tomatoes. Biosyst Eng 89: 93–99 DOI: 10.1016/j.biosystemseng.2004.02.012.

Halm M, Hornbæk T, Arneborg N, Sefa-Dedeh S, Jespersen L. 2004. Lactic acid tolerance determined by measurement of intracellular pH of single cells of *Candida krusei* and *Saccharomyces cerevisiae* isolated from fermented maize dough. Int J Food Microbiol 94: 97–103. DOI: 10.1016/j.ijfoodmicro.2003.12.019.

Jacques N, Casaregola S. 2008. Safety assessment of dairy microorganisms: The hemiascomycetous yeasts. Int J Food Microbiol 126: 321–326. DOI: 10.1016/j.ijfoodmicro.2007.08.020.

Kaaya AN, Kyamuhangire W. 2006. The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. Int J Food Microbiol 110: 217–223. DOI: 10.1016/j.ijfoodmicro.2006.04.004.

Kumar M, Dwivedi P, Sharma AK, Telang AG, Patil RD, Singh ND. 2008. Immunotoxicity of ochratoxin A and citrinin in New Zealand white rabbits. World Rabbit Sci 16: 7-12. DOI: 10.4995/wrs.2008.641.

Meng L, Sun P, Tang H, Li L, Draeger S, Schulz B, Krohn K, Hussain H, Zhang W, Yi Y. 2011. Endophytic fungus *Penicillium chrysogenum*, a new source of hypocrellins. Biochem Syst Ecol 39: 163–165. DOI: 10.1016/j.bse.2011.02.003.

Mohamed L, Zakaria M, Ali A, Senhaji W, Mohamed O, Mohamed E, Berny EL Hassan, Mohamed J. 2007. Optimization of growth and extracellular glucoamylase production by *Candida famata* isolate. Afr J Biotechnol 6: 2590-2595.

Nago MC, Hounhouigan JD, Akissoe N, Zanou E, Mestres C. 1998. Characterization of the Beninese traditional Ogi, a fermented maize slurry: Phisicochemical and Microbiological aspects. Int J Food Sci Tech 33: 307-315. DOI: 10.1046/j.1365-2621.1998.00169.x.

Omemu AM, Oyewole OB, Bankole MO. 2007. Significance of yeasts in the fermentation of maize for *ogi* production. Food Microbiol 24: 571-576. DOI: 10.1016/j.fm.2007.01.006.

Pitt JI, Hocking AD. 2009. Fungi and Food Spoilage. Springer.

Purwijantiningsih E, Dewanti-Hariyadi R, Nurwitri CC, and Istiana. 2005. Penghambatan produksi aflatoksin dari *Aspergillus flavus* oleh kapang dan kamir yang diisolasi dari ragi tempe. Biota 10: 146-153.

Rahayu ES. 2008. Mengantisipasi kontaminasi mikotoksin. Food Rev Indonesia 3: 28-30.

Ramos-García M, Bosquez-Molina E, Hernández-Romano J, Zavala-Padilla G, Terrés-Rojas E, Alia-Tejacal I, Barrera-Necha L, Hernández-López M, Bautista-Baños S. 2012. Use of chitosan-based edible coatings in combination with other natural compounds, to control *Rhizopus stolonifer* and *Escherichia coli* DH5a in fresh tomatoes. Crop Prot 38: 1-6. DOI: 10.1016/j.cropro.2012.02.016.

Samson RA, Hoekstra ES, van Oorschot CAN. 1981. Introduction to Food-Borne Fungi. Institute of The Royal Netherlands Academy of Arts and Sciences.

Sweeney MJ, Dobson ADW. 1998. Mycotoxin production by *Aspergillus*, *Fusarium* and *Penicillium* Species. Int J Food Microbiol 43: 141–158. DOI: 10.1016/S0168-1605(98)00112-3.

van den Berg MA, 2010. Functional characterisation of penicillin production strains. Fungal Biology reviews 24: 73-78. DOI: 10.1016/j.fbr.2010.03.006.

Wicklow DT, Roth S, Deyrup ST, Gloer JB. 2005. A protective endophyte of maize: Acremonium zeae antibiotics inhibitory to Aspergillus flavus and Fusarium verticillioides. Mycol Res 109: 610–618. DOI: 10.1017/S0953756205002820.

Wojtatowicz M, Chrzanowska J, Juszczyk P, Skiba A, Gdula A. 2001. Identification and biochemical characteristics of yeast microflora of Rokpol cheese. Int J Food Microbiol 69: 135–140. DOI: 10.1016/S0168-1605(01)00582-7.

Yang BH, Peng MY, Hou SJ, Sun JR, Lee SY, Lu JJ. 2009. Fluconazole-resistant *Kodamaea ohmeri* fungemia associated with cellulitis: Case report and review of the literature. Int J Infect Dis 13: e493-e497. DOI: 10.1016/j.ijid.2009.02.003.