2014 2nd International Conference on Food and Agricultural Sciences
IPCBEE vol.77 (2014) © (2014) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2014. V77. 13

Profile of Microorganisms and Amylose Content of White Corn Flours of Two Local Varieties as Affected by Fermentation Process

Rahmawati Arifin [1, 2+], Ratih Dewanti-Hariyadi [2, 3], Purwiyatno Hariyadi [2, 3] and Dedi Fardiaz [2, 3]

[1] Department of Food Technology, Sahid University, Jakarta, Indonesia

[2] Department of Food Science and Technology, Bogor Agricultural University, Kampus IPB Darmaga, Bogor, Indonesia

[3] Southeast Asia Food Agricultural Science and Technology (SEAFAST) Center, Bogor Agricultural University, Kampus IPB Darmaga, Bogor, Indonesia

Abstract. This research was aimed to evaluate the profile of microorganisms and amylose content of white corn flour made from two local (Anoman 1 and Pulut Harapan) varieties as affected by fermentation process of the corn grits. The fermentation were carried out by soaking corn grits in tap water (i) without addition of starter culture (spontaneous, SF), (ii) with the addition of a complete starter culture at 0 hour of incubation (CC) and (iii) CC followed with additional inoculation of starter culture containing amylolytic microorganisms at 16 hours of incubation (AC). The profile of microorganisms evaluation was done on the soaking water of corn grits fermented for 0, 36, 48, and 72 hours and the amylose content evaluation was done on the flour made from corn grits fermented as the same time. Our results showed that during spontaneous, the number of molds and yeast increased to 36 hours of fermentation and decreased afterward up to 72 hours of fermentation for both of corn varieties, while the number of lactic acid bacteria (LAB) tended to increase. CC and AC fermentation resulted in increased number of molds and yeasts up to 72 hours of fermentation, but only increased the number of LAB at the beginning of fermentation. CC fermentation could increase the amylose content in flour of Pulut Harapan variety at 36 and 48 hours fermentation.

Keywords: profile of microorganisms, amylose content, white corn flour, Anoman 1 & Pulut Harapan, fermentation

1. Introduction

Corn is an important carbohydrate source after rice in Indonesia. However, the utilization of white corn especially Anoman 1 and Pulut Harapan varieties as a local varieties are being developed by Indonesian Center for Agricultural Post Harvest Research & Development (ICAPRD) as a national seed varieties because they contain a high starch, attractive white color, and higher productivity than the yellow one and more resistant to drought [1]. White corn Anoman 1 variety contains high amylose (29.92%), whereas Pulut Harapan as a glutinous corn (waxy corn) contain 95.75% of amylopectin and amylose 4.25% [2]. The differences in amylose and amylopectin content affect the profile of microorganisms and amylose content of flour produced.

Traditionally, corn flour is made by soaking corn kernels in water followed by the process of draining, drying and milling. Aini *et al.* (2010) [3] showed that changes in the physicochemical properties of white corn flour produced was attributable to the spontaneous fermentation occurring during soaking. Several other studies of spontaneous fermentation of corn have been published, such as in the production of ogi [4] and pozol [5] which are African traditional foods.

+ Corresponding author. Tel: +628161390211; fax: +62218354763.
E-mail address: rahmafarasara@gmail.com.

Previously we have identified that microorganisms responsible for the spontaneous fermentation of white corn were *Penicillium chrysogenum, Penicillium citrinum, Aspergillus flavus, A. niger, Rhizopus stolonifer, R.oryzae, Fusarium oxysporum, Acremonium strictum, Candida famata, Kodamaea ohmeri, Candida krusei/incospicua, Lactobacillus plantarum1a, Pediococcus pentosaceus, Lactobacillus brevis1, Lactobacillus plantarum1b,* and *Lactobacillus paracasei ssp paracasei3* [6]. Of all microorganisms identified; four molds (*Penicillium citrinum, Aspergillus flavus, Aspergillus niger, Acremonium strictum*) and one yeast (*Candida famata*) were found to be amylolytic, while none of the LAB was capable of starch hydrolysis. The amylolytic activity is thought to be important for physicochemical changes of flour due to its high carbohydrate content.

This study aimed to use microorganisms involved in spontaneous fermentation of corn as a starter culture for corn fermentation process and examined the profile microorganisms as well as the amylose content of the resultig corn flour. As *Aspergillus flavus* is known to produce aflatoxin in corn, the mold was not used for the starter culture.

2. Materials and Methods

2.1. Corn

Corn used in this research were local white maize Anoman 1 and waxy maize Pulut Harapan varieties obtained from the Cereal Crops Research Institute, Maros, Sulawesi, Indonesia. Corn was made into grits for a more standardized fermentation process. Kernels of corn were washed with potable water (corn : water = 1 : 4 w/v) and drained on a siever. Drained corn kernels were then ground using pin disc mill and sieved to produce grits with diameter of $\geq$ 4 mm. The grits were washed with potable water (corn grits : water = 1: 4 w/v) for 30 minutes and then drained and ready for fermentation.

2.2. Microorganisms

Microorganisms used for starter culture preparation were amylolytic *Penicillium citrinum, Aspergillus niger, Acremonium strictum,* and *Candida famata,* as well as non amylolytic *Penicillium chrysogenum, Rhizopus stolonifer, Rhizopus oryzae, Fusarium oxysporum, Kodamaea ohmeri* and *Candida krusei / incospicua, Lactobacillus plantarum1a, Pediococcus pentosaceus, Lactobacillus brevis1,* and *Lactobacillus paracasei ssp paracasei3.* The microorganisms used were previously isolated and identified from a spontaneous fermentation of corn grits [5].

2.3. Culture preparation and enumeration

One loop of each mold was streaked onto fresh Potato Dextrose Agar (PDA) slant and then incubated at 30 ℃ for 5 days. After 5 days molds were harvested by scrapping, suspended in 10 mL sterile water and appropriately diluted for enumeration using hemacytometer. Yeast culture was prepared as above but incubation was carried out at 30 ℃ for 2 days. Yeast enumeration was also carried out using hemacytometer. Meanwhile Lactic Acid Bacteria (LAB) cultures were prepared by transferring one loop of each LAB growth into de Man Rogosa Sharpe (MRS) Broth for 24 hours at 30 ℃ using shaking incubator. After 24 hours, the culture was centrifuged aseptically for 15 mins, 3500 rpm at 4 ℃ and the cell pellets were resuspended in phosphate buffer. The 24 h culture was also enumerated by plating on MRS agar.

2.4. Fermentation with added starter culture

Five days old molds and two days old yeast in sterile water as well as 24 hours LAB in phosphate buffer made up the complete starter culture. For amylolytic starter culture, only *Penicillium citrinum, Aspergillus niger, Acremonium strictum,* and *Candida famata* were used. Each microorganism was inoculated aseptically into container (15 L) containing of maize grits and potable water (1: 2 w/v) such that each microorganism has an initial load of ca. 10^6 CFU/mL.

The fermentation studied included spontaneous fermentation (SF), i.e. soaking of corn grits in water as a control and fermentation with added starter cultures. Two types of fermentation with added starter were: that with addition of a complete starter culture at 0 hours (CC), and CC with additional inoculation of amylolytic

starter culture at 16 hours of fermentation (AC). Observations were done on flour made from corn grits after 0, 36, 48, and 72 hours fermentation.

2.5. Enumeration of microorganisms [4]

2.6. Amylose content by spektrofotometry [7]

3. Results and Discussion

3.1. Profile of Microorganisms

The profile of microorganisms grown in SF increased to 36 hours and then decreased at 72 hours of fermentation (Fig. 1). In general, the number of mold (Fig. 1A and 1B) was higher when culture starter was added and the number of mold in AC fermentation was more than that of CC. The addition of a starter culture significantly increases the number of molds in 36-72 hours of fermentation, both on corn grits Anoman 1 and Pulut Harapan. While the mold growth in SF decreased sharply after 36-72 hours of fermentation. Addition of high number of microorganisms could control the fermentation process.

The number of mold in this study was relatively lower than [6]. It was suspected that this was related to the use of different types of water. Rahmawati *et al.* (2013) [6] used sterile water while this study used potable water that may contain bacteria that suppress the growth of the added mold. The number of mold decreased after 36 hours of fermentation due to the growth of LAB. LAB produce lactic acid and other organic acids as well as antimicrobial compounds [8], [9], which could inhibit the growth of mold. In general, addition of starter cultures in AC or CC fermentation resulted in increased number of molds until the end of fermentation as compared to the SF treatment.

The number of yeasts (Fig. 1C and 1D) up to 72 hours increased during all fermentation processes. In general, the number of yeast in SF was lower than CC which was lower than AC. The addition of starter cultures either in AC or CC could increase the number of yeast until the end of fermentation as compared to SF. There was a difference in the number of yeast growing for 72 hours of fermentation as compared to the mold, yeast in the SF treatment increased up to 36 hours and then is relatively stable. Therefore at 72 hours of fermentation the yeast number was relatively higher than the mold. This pattern was observed in both corn types. Higher number of yeasts at 36-72 hours of fermentation showed that the yeast were more resistant to acidic conditions than mold. Halm *et al.* (2004) [10] reported that yeast has high tolerance to lactic acid. In fact, *Candida krusei* found on the fermentation of corn during ogi production may stimulate the growth of *Lactobacillus plantarum*.

The number of LAB increased during 72 hour fermentation for both corn types. The addition of the starter culture in CC and AC increased the number of LAB initially. However, after 36 hours, the number of LAB was relatively the same throughout the treatment. It seems that LAB addition does not affect the number of LAB at the end of fermentation. It is suspected that since the LAB used have no amylolytic activities, LAB naturally exist in corn could grow and develop after the amylolytic mold breaking down amylose into simple sugars. Therfore the number LAB in SF was similar to that in CC or AC. Presence of yeast also could stimulate the growth of LAB. Nago *et al.* (1998) [4] reported that during Ogi fermentation for 1-3 days at 25-35 °C, *Lactobacillus fermentum cellobiosus, L. brevis* and *L. fermentum* spp were isolated up to 9 log CFU / g.

3.2. Amylose content

The amylose content of white corn flour Anoman 1 variety tends to decrease for all treatments during fermentation up to 72 hours, with the number between 37.17% at 0 h fermentation and 30.24 - 35.97% at 72 h of fermentation (Table 1). Claver *et al.* (2010) [11] reported that during fermentation, the amylolytic microorganisms produces amylase that attacks the 1,4-α-D-glycosidic bonds of starch granules. Besides, glucose contains many hydroxyl groups, thus amylose is hydrophilic and soluble in water during soaking. These has caused the amylose content to decrease. AC fermentation resulted in corn flour with the same

amount of amylose that of CC. It is thought that increasing of the number of microorganisms caused the competition between them so that the activity does not get higher.

The amylose content of white corn flour Pulut Harapan variety tends to decrease during fermentation up to 72 hours, which is 13.70% at 0 h to 11.95 - 12.90% at 72 hours of fermentation. The decrease in amylose content caused by the hydrolisis of amylose by enzymes. However, the CC treatment up to 48 hours of fermentation relative increase the amylose content. The increasing of amylose content presumably due to the activity of glucoamylase enzyme which can cut the outer amylopectin branch chain [12]. This will increase the amount of straight-chain, thus increasing amylose content. After that the amount of amylose decreased until 72 hours fermentation. The decreasing was caused by the hydrolyzed of the straight line into simple sugars that can reduce the levels of amylose. The amylose content of flour Pulut Harapan variety with the AC treatment tends to decrease during the 72 hours of fermentation, although it is higher than the SF treatment. Its suspected that the addition of amylolytic microorganisms after 16 hours fermentation, make the competition bigger so the activity becomes not optimal, especially amylolytic activity.

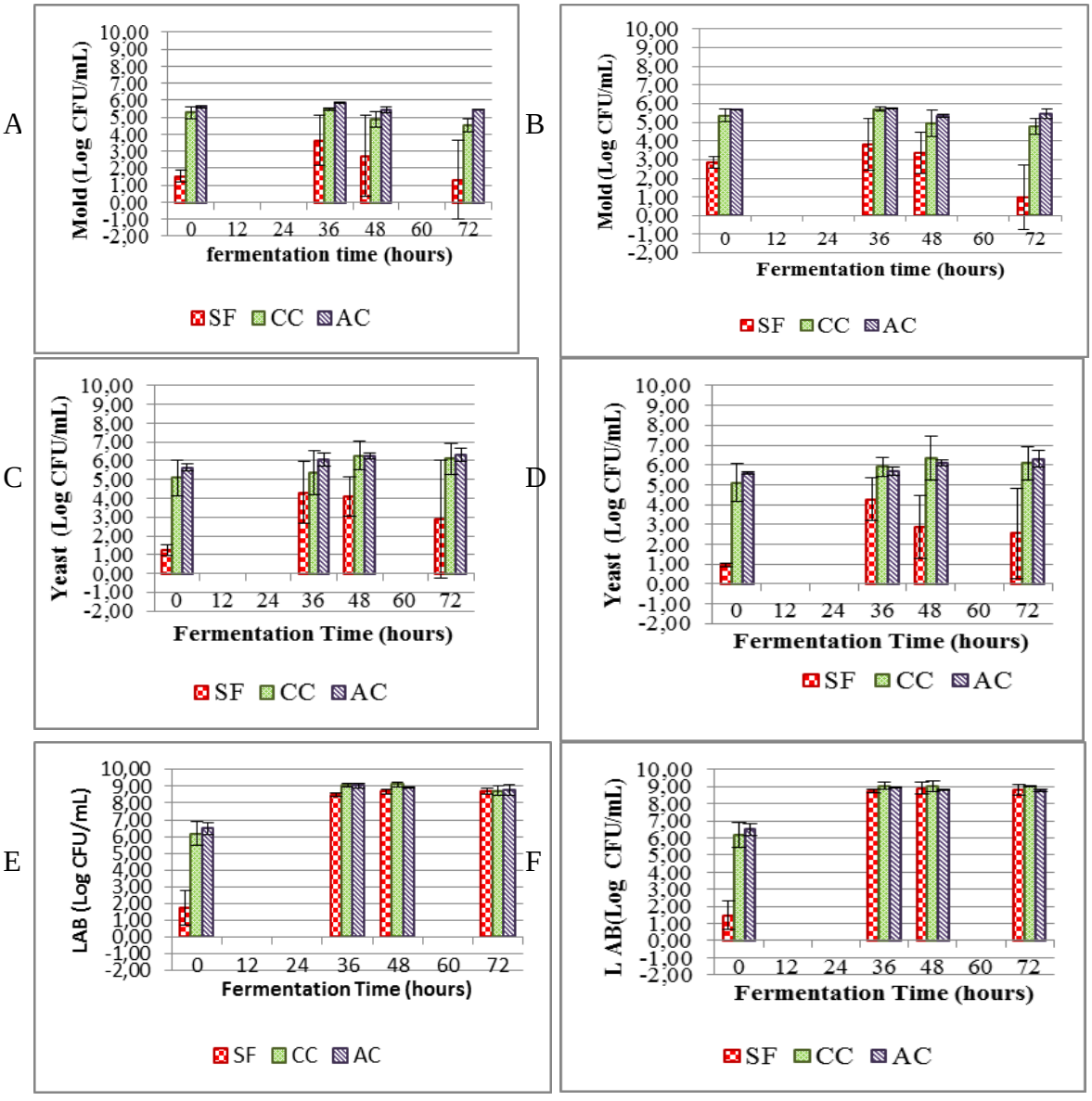

Fig. 1: The profile of growth of mold, yeast, LAB at corn grits on Anoman 1 (A, C, E) and Pulut Harapan varieties (B, D, F) during fermentation. SF: corn grits with spontaneous fermentation; CC: corn grits after fermentation with the addition of a starter culture complete; AC: CC grits corn after fermentation with the addition of amylolytic cultures after 16 hours of fermentation

3.3. Conclusion

During fermentation the number of mold tended to increase up to 36 hours of fermentation and then decreased up to 72 hours of fermentation either in white corn grits Anoman 1 and Pulut Harapan varieties. While the number of yeasts and LAB tended to increase for all treatment. The addition of a starter culture (CC and AC treatment) can increase the number of molds and yeasts in the beginning until the end of the 72 hours of fermentation compared to the SF treatment, but it seems the addition of starter culture only increases the number of LAB at the beginning of fermentation and does not affect the number of LAB at the end of fermentation.

Table 1: The amylose content (% dw) of white corn flour of Anoman 1 and Pulut Harapan varieties during 72 hours fermentation

Treatment (hours)		Anoman 1 corn flour	Pulut harapan corn flour
SF	0	37.17 ±2.39	13.70 ±1.39
	36	37.37 ±4.19	11.14 ±3.30
	48	37.26 ±3.87	10.88 ±2.46
	72	35.97 ±5.20	10.19 ±2.09
CC	0	37.17 ±2.39	13.70 ±1.39
	36	35.05 ±2.65	13.79 ±2.28
	48	34.87 ±2.63	13.81 ±1.66
	72	30.24 ±2.22	12.90 ±1.17
AC	0	37.17 ±2.39	13.70 ±1.39
	36	36.89 ±1.21	13.09 ±1.12
	48	35.25 ±1.16	12.47 ±1.15
	72	31.14 ±1.13	11.95 ±1.10

Enzyme activity during the fermentation can decrease amylose content in white corn flour Anoman 1 variety, but 36CC and 48CC treatments tend to increase the amylose content in white corn flour of Pulut Harapan variety.

3.4. Suggestion

It is recommended to not add the LAB when will ferment with the starter culture because it seems that there is naturally the material can grow well during fermentation.

4. References

[1] Qanytah dan TR. Prastuti. Penerapan teknologi pascapanen jagung di Desa Kedawung Kecamatan Bojong, Kabupaten Tegal. Prosiding Seminar Nasional Teknik Pertanian 2008, Jogjakarta.

[2] U. Suarni. Karakteristik sifat fisikokimia dan amilograf tepung jagung sebagai bahan pangan. Prosiding seminar dan lokakarya nasional Makasar 2005. Pusat penelitian dan pengembangan tanaman pangan. Badan penelitian dan pengembangan pertanian. Departemen Pertanian.

[3] N. Aini, P. Hariyadi. Gelatinization properties of white maize starch from three varieties of corn subject to oxidized and acetylated-oxidized modification. International Food Research Journal 2010, 17, 961-968.

[4] MC. Nago, JD. Hounhouigan, N. Akissoe, E. Zanou, C. Mestres. Characterization of the Beninese traditional Ogi, a fermented maize slurry: Phisicochemical and Microbiological aspects. Int J Food Sci Tech 1998. 33. 307-315.

[5] N. Ben Omar, F. Ampe. Microbial community dynamics durin production of the Mexican fermentee maize dough pozol. Applied & environmental microbiology 2000, 66, 3664-3673.

[6] Rahmawati, R. Dewanti-Hariyadi, P. Hariyadi, D. Fardiaz, N. Richana. Isolation and Identification Of Microorganisms During Spontaneous Fermentation Of Maize. J.Teknol. dan Industri Pangan 2013, 24, 38-44.

[7] BO. Juliano. A simplified assay for milled rice amylosa. Cereal Science today 1971, 16:334-360.

[8] AM. Omemu, OB Oyewole, MO Bankole. Significance of yeasts in the fermentation of maize for ogi production.

Food Microbiology 2007 (24): 571-576.

[9] AK. Rai, JP. Tamang, U. Palni. Microbiological studies of ethnic meat products of the Eastern Himalayas. Meat Science 2010, 85: 560–567.

[10] M. Halm, A. Osei-Yaw, A. Hayford, KA. Kpodo, and WKA. Amoa-Awua. Experiences with the use of a starter culture in the fermentation of maize for 'kenkey' production in Ghana. World Journal of Microbiology & Biotechnology 1996. 12: 591-536.

[11] IP. Claver, H. Zang, Q. Li, K. Zhu, H. Zhou. Impact of the soak and the malt on the physicochemical properties of the sorghum starches. *Int J Mol Sci.* 2010, 11, 3002-3015. Doi:10.3390/ijms11083002.

[12] SX. Xie, Q. Liu, and SW. Cui, in Cui, S.W. (Eds). *Food Carbohydrates: Chemistry, Physical Properties, and Applications.* Taylor & Francis Group, 2005.