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Modeling and Optimising the Growth of *Lasiodiplodia theobromae* During Gathotan Fermentation

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Gathotan is fungal fermented cassava, and a raw material for a Javanese snack called 'gathot'. This type of food is now hardly to find, and the process of making gathotan is relatively lack of process control, leads to failure in process. To make gathotan, peeled cassava tubers are left on the ground or roof for several weeks or months until they become black inside an important characteristic of gathotan. This work aims to improve gathotan fermentation by optimizing fermentation process. The effect of incubation temperature and time, inoculum level, soaking time, and drying, on the growth of *Lasiodiplodia theobromae*, the main gathotan fungus, in cassava tubers was studied. Experimental design was set up according to response surface methodology. Five parameters measured were pH, titratable acidity, and fungal growth. Results showed that incubation temperature affected pH in linear ($P<0.01$) and quadratic functions ($P<0.05$). Titratable acidity was not affected by any treatment. Fungal growth was significantly affected by incubation time ($P<0.01$) or inoculum level ($P<0.05$), and interaction of several factors: incubation time and incubation temperature ($P<0.05$) or drying time ($P<0.01$). Optimization model indicated that incubation temperature at 34.5°C for 2.4 days, soaking for 26.4 hours, drying time of 3.7 hours at 40°C, and inoculum level of 2% resulted in maximum growth of *L. theobromae* in gathotan.

Key words: gathotan, *Lasiodiplodia theobromae*, fermentation

Gathotan adalah singkong yang terfermentasi oleh jamur, dan merupakan bahan mentah jajan tradisional Jawa yang bernama 'gathot'. Makanan ini saat ini sulit ditemukan di pasaran, dan proses pembuatan gathotan umumnya tidak memiliki pengendalian proses yang cukup baik, sehingga kegagalan proses sering terjadi. Gathotan dibuat dengan cara mengupas singkong, lalu membiarkannya di permukaan tanah atau di atas atap rumah beberapa minggu atau beberapa bulan sehingga menjadi berwarna hitam di dalamnya. Warna hitam merupakan salah satu ciri penting gathotan. Penelitian ini bertujuan mengoptimasi proses fermentasi gathotan. Dikaji pengaruh suhu dan waktu inkubasi, kadar inokulum, dan waktu perendaman terhadap pertumbuhan *Lasiodiplodia theobromae*, jamur utama pada gathotan, di singkong. Rancangan percobaan disusun mengikuti rancangan pada metodologi permukaan tanggap. Ada lima parameter yang diukur, yaitu pH, keasaman tertitrasi, dan pertumbuhan jamur. Hasil menunjukkan bahwa suhu inkubasi mempengaruhi pH sesuai fungsi linear ($P<0.01$) dan kuadratik ($P<0.05$). Keasaman tertitrasi tidak dipengaruhi oleh perlakuan. Pertumbuhan jamur dipengaruhi oleh waktu inkubasi ($P<0.01$) atau kadar inokulum ($P<0.05$), atau interaksi beberapa faktor berikut: waktu dan suhu inkubasi ($P<0.05$) atau waktu inkubasi dan waktu pengeringan ($P<0.01$). Model optimasi menunjukkan bahwa suhu inkubasi pada 34,5°C selama 2,4 hari, perendaman selama 26,4 jam, pengeringan selama 3,7 jam pada suhu 40°C, dan kadar inokulum 2% memberikan hasil pertumbuhan jamur *L. theobromae* maksimum.

Kata kunci : gathotan, *Lasiodiplodia theobromae*, fermentasi

Gathotan is dried fermented cassava tuber, seemingly mostly found in Central and East Java, Indonesia. Fungal types and distribution in gathotan has been reported previously (Purwandari 2000), consisting of spore forming hypomycetes on surface, and *Lasiodiplodia theobromae* -a dematiaceous fungus- the dominant fungus grew inside the tuber. Dominant spore-forming hypomycetes are *Rhizopus oryzae* and *Aspergillus flavus*. Gathotan is characterized by black colour which comes mostly from mycelia of *L. theobromae*, since it is the only fungus having dark colour of hyphae and the fungus isolated mostly from the inside part of gathotan (Purwandari 2000). Pairing *L. theobromae* with either *R. oryzae* or *A. flavus* on three types of plate agar (DG18/dichloran glycerol agar, AFPA/A. flavus-parasiticus agar, and MEA/malt extract agar) consistently shows dominant growth of *L. theobromae* and restricted growth of the other fungus (Purwandari 2000). In the beginning of growth stage, *R. oryzae* is dominating over *L. theobromae*, but on the later stage of growth, *L. theobromae* grows overcompeted *R. oryzae*.

Gathot, the cooked gathotan, is characterised by its chewy texture. The chewy nature may relate to the

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change in starch nature. Such change maybe due to enzyme(s) or acid produced by fungus/fungi. *L. theobromae* is potentially a producer of a starch hydrolizing enzyme called glucoamylase (Navaratman *et al*. 1996) to result in D-glucose and degraded starch fraction. One possibility of starch fraction is dextrin, a hydrocolloid.

The chewy nature of gathot enables it to be used as raw material of gluten-free noodle (Purwandari *et al*. 2014b), since elasticity is a very essential characteristic of noodle. Moreover, gathotan flour has also been used as texturing agent of various gluten-free noodle made from some types of tubers or starchy fruit such as breadfruit (Purwandari *et al*. 2014a). Gathotan noodle has been identified for several health-relating benefits including hypoglycemic and antioxidant properties (Purwandari *et al*. 2014c). The hypoglycemic effect of gathotan noodle may come from fermentation product of starch during gathotan making, while antioxidant activity may come from melanin produced by *L. theobromae* (Purwandari *et al*. 2014c).

The growth of *L. theobromae* in gathotan can raise health concern, since the fungus is a plant pathogen. It is an important plant pathogen, and highly prevalent in the tropical areas. Factors affecting growth of the fungus have been reported with reference to tropical tuber (Kihurani *et al*. 2008) or fruits (Mortuza and Ilag 1999; Muniz *et al*. 2011; Sivakumar *et al*. 2011; Pawar 2012), such as temperature, water activity, and UV exposure (Uduebo and Madelin 1974). Antagonistic studies of several microorganisms against *L. theobromae* has also been reported, which involves fungi (Mortuza and Ilag 1999; Haggag and Nofal 2006; Fadahunsi *et al*. 2013), yeast (Hashem and Alamri 2009), or bacteria (Fadahunsi *et al.* 2013; Sajitha *et al*. 2014). So far, there is no study in the area of safety of gathot in relation to *L. theobromae* growth. However, there seems to be no report on incidence of gathot toxicity caused by the fungus. Instead, one may start thinking of potential health benefits can be generated by the growth of *L. theobromae* in gathot, such as anti-cancer (Pandi *et al*. 2010), anti-thrombotic (Vasconcelos *et al*. 2013), and antioxidant (Giese *et al.* 2015). The fungus also produces β-D-glucan called lasiodiplodin with several functional properties (Vasconcelos *et al.* 2013).

Although growth of *L. theobromae* as plant pathogen has been extensively studied, its growth during gathotan fermentation does not seem to attract much attention. Traditionally, gathotan making process is conducted without any addition of inoculum, thus allowing different types of soil fungi including those antagonistic to *L. theobromae*, grow during the process (Purwandari 2000). This can lead to failure in the fermentation, or very little *L. theobromae* grows in the tuber, a common problem faced by gathotan makers. Therefore, this work is dedicated to construct a model and to optimise the growth of *L. theobromae* during gathotan fermentation, using gathotan powder as inoculum.

MATERIALS AND METHODS

Materials. Raw material for gathotan making is fresh cassava tuber. The tuber was purchased commercially from local market in Kamal, Madura. The tubers were intact without any sign of cut. It had white flesh, and diameter of base of tuber varied between 3 to 5 cm. Typical commercial cassava is characterised by relatively low water content and optimum starch content, without fibrous part in the flesh. Tuber was free from any defect caused either by biochemical reaction so called 'vascular streak', or signs of visible microbial growth. The second material was gathotan powder. Gathotan chunk taken from previous batch was characterised by black colour covering more than 50% of inside part of tuber. The chunks were washed with running tap water, until washing water became clean, did not show discolouration of tuber. Washed gathotan chunk was then dried in a cabinet dryer at 40 °C for one hour, after which it was ground with a mechanical grinder. The flour resulted from grinding was passed through 60 mesh sieve, and this flour was used as inoculum in the experiment. Black part inside gathotan is the place for the growth of *L. theobromae* (Purwandari 2000).

Cassava Tubers Preparation. Tubers were peeled, and washed thoroughly with tap water three times to ensure they were free from dirt, and cut into about 10 cm long chunks. The chunks were drained well, and then treated according to experimental design.

Fermentation of Gathotan. The next step was soaking the tubers in tap water, according to experimental design: 0, 12, 24, 36, and 48 hours. After soaking, tubers were again washed to remove soaking water from tubers. Tubers were then dried in a cabinet dryer (40°C) for several hours according to experimental design: 1, 2, 4, and 6 hours. After that, tubers were mixed thoroughly with gathotan suspension (gathotan powder : distilled water, 1:2, w/w). Level of inoculum used in the experiment was 2,

3, 4, 5 and 6 % (w/w) of fresh cassava tubers. Then 1 kg of tubers of each treatment combination were placed in a sterile 5 L plastic container. The plastic container was then incubated at different temperatures: 27, 30, 35, 37, and 40 °C, according to experimental design. Incubation time was 2, 3, 4, 5, and 6 days.

Response Surface Methodology Observation. Experimental design was constructed according to Response Surface Methodology, using five factors (soaking time, drying time, inoculum level, incubation time, and incubation temperature), with five levels for each factor. During fermentation, samples were taken for examination on pH, total acidity, and growth of *L. theobromae*. Determination of pH of gathotan chunk was carried out using electronic pH-meter. Gathotan chunk was first washed by spraying chunk with distilled water thoroughly to remove excess of soaking water. Then, chunk was put in a blender, and was added with distilled water 5 times of weight of sample. It was then blended for around 3 minutes, until slurry was formed. The slurry was then passed through a number 40 Whatman filter paper to collect filtrate. Filtrate was examined for pH, using pH meter. The same slurry was examined for titratable acidity, by first added with 1 mL of 1% phenolphtalein, and then titrated with 1N NaOH. Titratable acidity was expressed as percentage of lactic acid. Fungal growth examination was carried out by examining the presence of hyphae of *L. theobromae* inside tubers, using a light microscope connected with a camera (Olympus CX31 LED, Japan) and a PC. Mycelium of *L. theobromae* is septate and grows parallel to longitudinal rays (Muniz *et al*. 2011). Tuber chunk was dissected using a manual microtome without freezing the sample. The dissected piece was divided into three sections towards center.

Zone Division. First section (zone A) was the outer part one-third near the surface of tuber. The middle section (zone B) was between one-third area from surface and one-third was from the center of tuber. Zone C was one-third area from the center of tuber.

Statistical Calculation. Statistical analysis was performed on Minitab® 14 (Minitab Inc., Pensylvania, USA) for response surface methodology, employing a quadratic equation. Response was expressed as following equation:

$$Y = \beta_0 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{55} X_5^2 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{12} X_1X_2 + \beta_{13} X_1X_3 + \beta_{14} X_1X_4 + \beta_{15} X_1X_5 + \beta_{23} X_2X_3 + \beta_{24} X_2X_4 + \beta_{25} X_2X_5 + \beta_{34}X_3X_4 + \beta_{35} X_3X_5 + \beta_{45} X_4X_5 + \varepsilon \ldots . (1)$$

where, Y was response (pH, total acidity, or fungal mycelia), X_1 was incubation temperature (°C), X_2 was drying time (hour), X_3 was inoculum level (% w/w), X_4 was soaking time (hours), and X_5 was incubation time (days).

RESULTS

pH. Result on pH of gathotan chunk throughout fermentation showed that pH was influenced significantly ($P<0.01$) only by linear and quadratic function of incubation temperature (Table 1), and pH was not affected significantly ($P\geq0.05$) by other factors such as drying time nor inoculum level, following the equation:

$$pH = 31.3043 + 0.0160 X_1^2 - 0.0396 X_2^2 - 0.0509 X_3^2 - 0.0002 X_4^2 - 0.0071 X_5^2 + 1.3165 X_1 - 0.0075 X_2 - 0.9866 X_3 + 0.0177 X_4 - 1.0465 X_5 + 0.0047 X_1X_2 + 0.0352 X_1X_3 + 0.0003 X_1X_4 + 0.0185 X_1X_5 -- 0.0644 X_2X_3 + 0.0002 X_2X_4 + 0.0625 X_2X_5 + 0.0004 X_3X_4 + 0.0625 X_3X_5 - 0.0625 X_4X_5 \ldots . (2)$$

where, X_1 was incubation temperature (°C), X_2 was drying time (hour), X_3 was inoculum level (% w/w), X_4 was soaking time (hours), and X_5 was incubation time (days).

High regression coefficient of incubation temperature (1.3165), fermentation time (-1.0465), and inoculum level (-0.9866) showed that the three factors affected pH of gathotan in a relatively considerable extent. The correlation between pH and incubation temperature was negative, indicating that pH was lowered by increasing temperature during fermentation. Contrary, longer fermentation time or higher inoculum level reduced pH. The coefficient of determination for pH was 72.24% (data not shown), indicating relatively high sufficiency of model.

Incubation temperature between 35 to 40 °C resulted in final pH of 4.5 (Fig 1). Incubation at room temperature to 34 °C resulted in pH between 7 to 5. Similarly, at high level of inoculum (6 %), low pH at 4.8 could be reached at incubation temperature of 32 °C (data not shown). Although statistically not significant ($P\geq0.05$), lower drying time seemed to reduce pH at high incubation temperature near 40 °C (data not shown).

Titratable Acidity. In contrast to pH, titratable acidity was not affected significantly ($P\geq0.05$) by any factor examined. Regression model for titratable acidity was as follows:

$$\text{Titratable acidity} = -1.3199 - 0.0009 X_1^2 + 0.0006 X_2^2 + 0.0032 X_3^2 - 0.00004 X_4^2 + 0.0012 X_5^2 \; 0.0776 \; X_1 -$$

Table 1 pH of cassava chunk during fermentation of gathotan

Source	pH		Titratable acidity		Fungal growth in zone A	
	Coefficient	P	Coefficient	P	Coefficient	P
Constant	31.3043	0.004	-1.31990	0.152	-60.0810	0.141
Incubation temperature	-1.3165*	0.010	0.07765	0.092	1.5516	0.426
Drying time	0.0075	0.994	0.02336	0.805	-6.4361	0.145
Inoculum level	-0.9866	0.335	-0.02380	0.811	9.8713*	0.043
Soaking time	0.0177	0.824	-0.00020	0.979	-0.6726	0.075
Incubation time	-1,0465	0.308	0.08526	0.399	14.0059*	0.008
Incubation temperature * Incubation temperature	0.0160*	0.022	-0.00098	0.130	-0.0083	0.762
Drying time * Drying time	-0.0396	0.518	0.00061	0.919	-0.3822	0.173
Inoculum level * Inoculum level	-0.0509	0.410	0.00324	0.594	-0.1322	0.624
Soaking time * Soaking time	-0.0002	0.635	-0.00004	0.349	0.0078*	0.001
Incubation time * Incubation time	-0.0071	0.907	0.06124	0.838	-0.5072	0.079
Incubation temperature * Drying time	0.0047	0.836	-0.00091	0.689	0.4279*	0.001
Incubation temperature * Inoculum level	0.0352	0.142	-0.00023	0.920	-0.2163*	0.050
Incubation temperature *Soaking time	0.0003	0.888	-0.00001	0.964	0.0084	0.326
Incubation temperature *Incubation time	0.0185	0.424	-0.00264	0.257	-0.2644*	0.021
Drying time*Inoculum level	-0.0644	0.439	0.00087	0.914	-0.3125	0.397
Drying time*Soaking time	0.0002	0.976	0.00019	0.783	-0.0156	0.607
Drying time*Incubation time	0.0550	0.507	0.00038	0.963	-1.1875*	0.006
Inoculum level*Soaking time	-0.0004	0.951	0.00021	0.759	-0.0156	0.607
Inoculum level*Incubation time	0.0625	0.453	0.00113	0.890	0.0625	0.863
Soaking time*Incubation time	-0.0042	0.541	0.00048	0.485	0.0365	0.243

*indicated significance level of 0.05

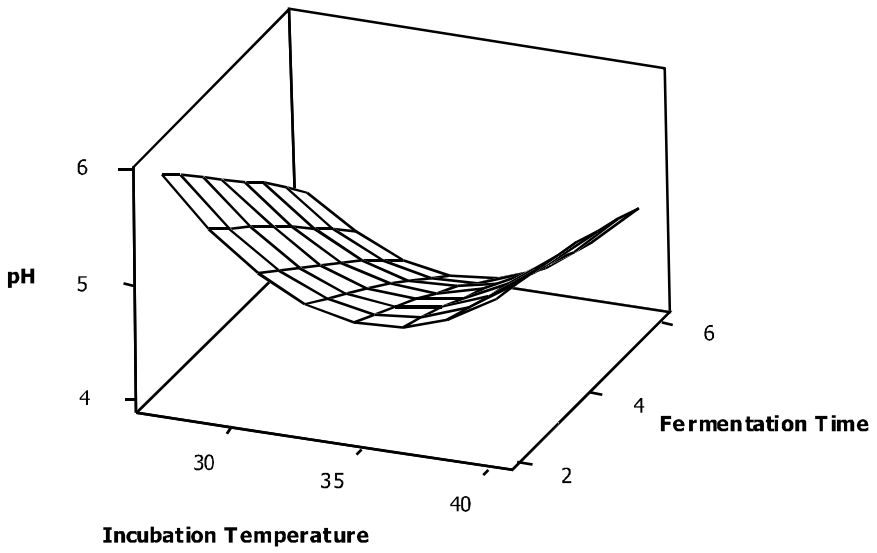

Fig 1 The effect of incubation temperature and incubation time on pH of cassava chunk during gathotan fermentation

$0.0233\ X_2 - 0.0238\ X_3 - 0.0002\ X_4 + 0.0852\ X_5 - 0.0009\ X_1X_2 - 0.0002\ X_1X_3\ \ 0.00001\ X_1X_4 - 0.0026\ X_1X_5 + 0.0009\ X_2X_3 + 0.0002\ X_2X_4 + 0.0004\ X_2X_5 + \ 0.0002\ X_3X_4 + 0.0011\ X_3X_5\ \ 0.0005\ X_4X_5 \ldots . (3)$

where, X_1 was incubation temperature (°C), X_2 was drying time (hour), X_3 was inoculum level (% w/w), X_4 was soaking time (hours), and X_5 was incubation time (days).

Although all regression coefficients in Equation 3 were small, those of incubation temperature (-0.0776) and fermentation time (0.0852) were relatively greater then the others. While higher incubation temperature

resulted in reducing titratable acidity, longer incubation time led to the increase in titratable acidity. Coefficient of determination for titratable acidity was 65.97% (data not shown), indicating that data fitted model relatively well.

Surface plot of incubation temperature against fermentation time indicated that high temperature from 36 to 40°C resulted in high titratable acidity (Fig 2). Similarly, high temperature (30 to 36°C) and longer incubation time also caused high concentration of titratable acidity. Inoculum level at 3% also gave high titratable acidity (data not shown).

Fungal Growth. Fungal growth was expressed as the number of *L. theobromae* hyphae inside gathotan. Hyphae of *L. theobromae* looked as relatively large septate hyaline hyphae that grew parallel to vascular tissue. Every parallel hyphae of the fungus was counted as one hyphae. Young hyphae of *L. theobromae* is hyaline, and becomes dark, grey, or black in later stage of growth (Alasoadura 1970). Data of the number of hyphae of *L. theobromae* growth inside cassava chunk during fermentation of gathotan was only meaningful when in zone A. The model for fungal colonization in zone B and C showed negative number of hyphae, thus possibly indicated unreliable model correlatively absence of fungus in the area. Therefore, data on the number of hyphae in zone B and C were not analysed further. Regression equation for mycelial growth in zone A was:

Number of hyphae = $-60.0810 - 0.0083\ X_1^2 - 0.3822\ X_2^2 - 0.1322\ X_3^2 + 0.0078\ X_4^2 - 0.5072\ X_5^2 - 1.5516\ X_1 - 6.4361\ X_2 + 9.8713\ X_3 - 0.6726\ X_4 + 14.0059\ X_5 + 0.4279\ X_1X_2 - 0.2163\ X_1X_3 + 0.0084\ X_1X_4 - 0.2644\ X_1X_5 - 0.3125\ X_2X_3 - 0.0156\ X_2X_4 - 1.1875\ X_2X_5\ 0.0156\ X_3X_4 + 0.0625\ X_3X_5 - 0.0365\ X_4X_5 \ldots$. (4)

where, X_1 was incubation temperature (°C), X_2 was drying time (hour), X_3 was inoculum level (% w/w), X_4 was soaking time (hours), and X_5 was incubation time (days).

High regression coefficients were shown by fermentation time (14.0059) and inoculum level (9.8713), indicating significant role of the two factors in determining fungal growth, with positive correlation. Coefficient of determination for the equation was 88.62%, reflecting good fitness of data to the regression.

The number of hyphae in zone A was affected positively and significantly by inoculum level ($P<0.05$) and incubation time ($P<0.01$) (Table 1). Several interactions between factors showed significant effect were: quadratic function of soaking time ($P<0.005$), interaction between incubation temperature and drying time ($P<0.005$), incubation temperature and incubation time ($P<0.05$), and incubation time and drying time ($P<0.01$). No mycelium was detected during the first three days,

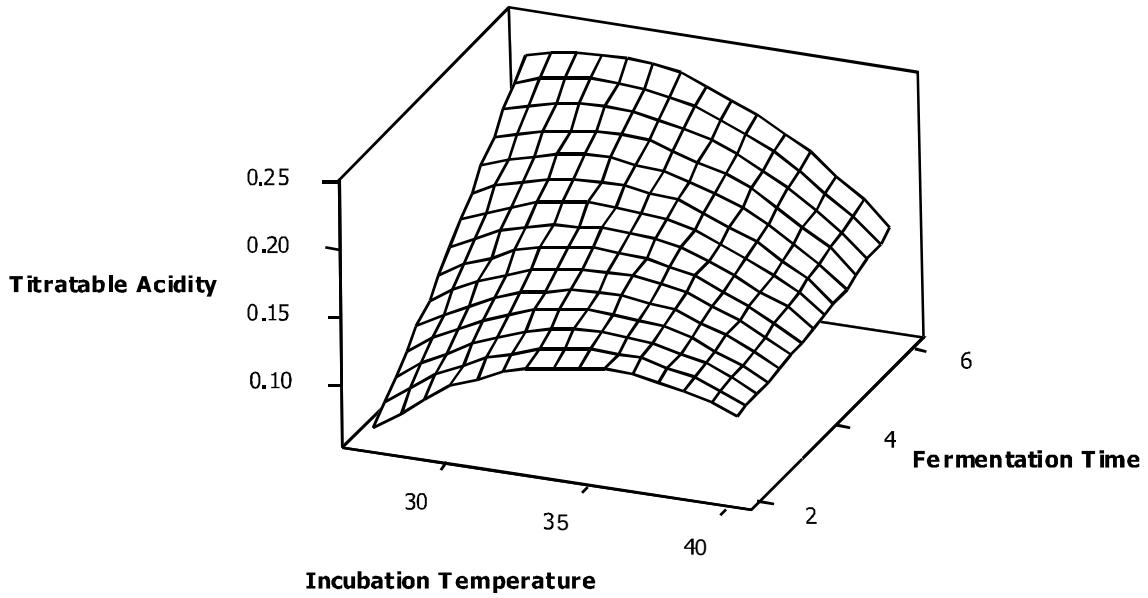

Fig 2 The effect of incubation temperature and fermentation time on titratable acidity of cassava chunk during gathotan fermentation

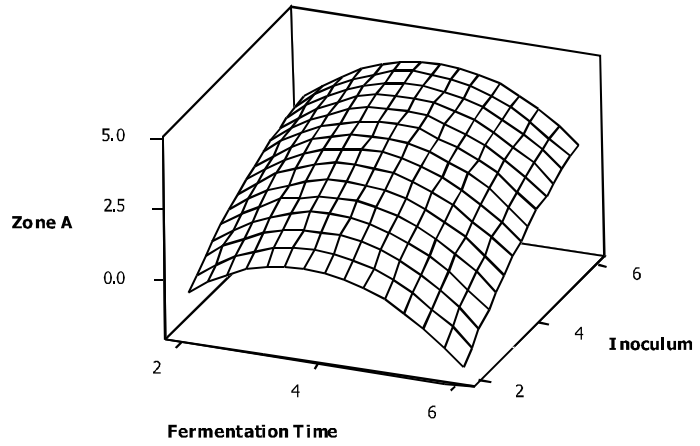

Fig 3 Growth of *Lasiodiplodia theobromae* during gathotan fermentation as affected by incubation time and inoculum level

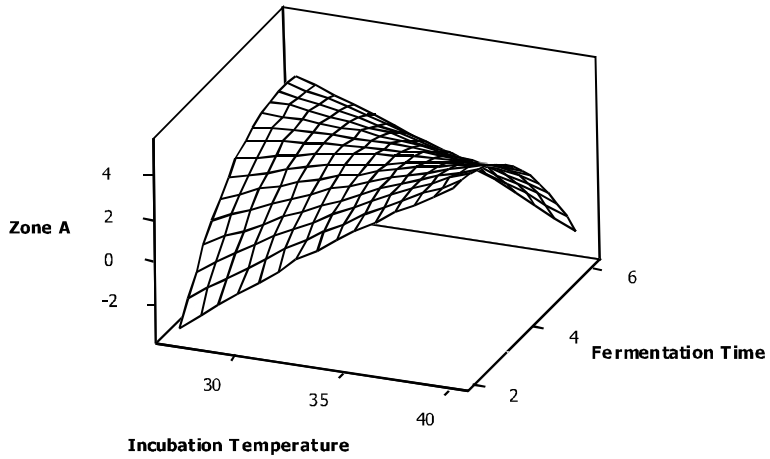

Fig 4 Growth of *Lasiodiplodia theobromae* during gathotan fermentation as affected by incubation time and incubation temperature

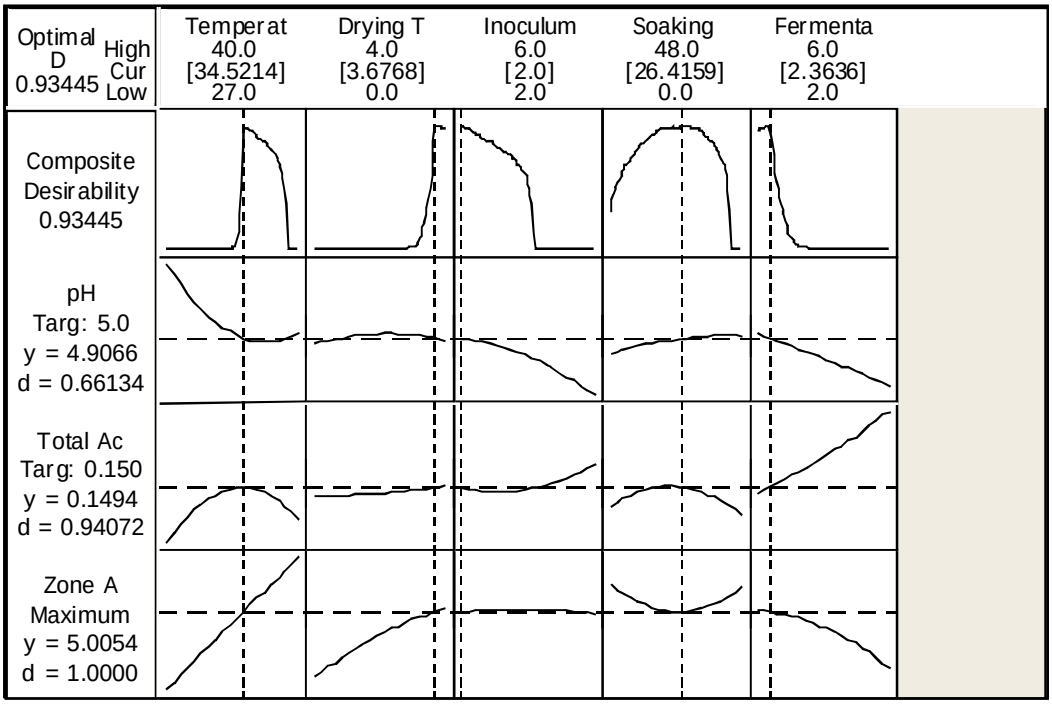

Fig 4 Optimization of conditions for *Lasiodiplodia theobromae* growth during gathotan fermentation

when inoculum level was below 4 %. However, when inoculum level was high enough (over 5 %), hyphae started colonizing cassava as soon as two days incubation (Fig 3). Maximum fungal growth was reached after four days at 6 % inoculum level, or six days at 4 % inoculum level. Incubation temperature around 30 °C and fermentation time at 4 d or longer resulted in highest mycelium number (Fig. 4).

DISCUSSION

The changes of pH during gathotan fermentation was not as fast as when it was grown in liquid cassava medium with added nitrogen source (Navaratnam *et al.* 1996). In this liquid cassava medium, pH was lowered to reach pH 1.8 during 50 hours of fermentation, showing a significant acid production by *L. theobromae* when nitrogen source was available (Navaratnam *et al.* 1996). Cassava is low in nitrogen content, so that pH reduction in gathotan fermentation was not as rapid as that in the liquid medium. Relatively slow pH reduction in gathotan fermentation seemed to be of benefit to the growth of *L. theobromae*, since the fungus grow well at relatively high pH (Eng *et al*. 1998). High pH reduction rate would not allow sufficient colonization of cassava tuber by the fungus, resulting in failure of fermentation. Previous report mentioned that mycelial production of *L. theobromae* was maximum at pH 8 to 10 (Eng *et al.* 1998). The pH during gathotan fermentation did not seem to go lower than 4, thus allowed sufficient fungal growth inside tuber. Assuming that chewy texture of gathot, the cooked gathotan, is caused by glucoamylase activity, pH during gathotan fermentation (4-6) seems to support activity of the enzyme (Navaratnam *et al.* 1996), resulted in good and chewy gathot.

Titratable acidity indicates free proton neutralised during titration using a strong base solution. Titratable acidity also includes buffering capacity of sample (Rajkovic *et al*. 2007). Not all of hydrogen ions can be detected during titration, due to probable influence of monovalent cation exchange (Boulton 1980) and dissociation strength of acids. There was no significant difference of titratable acidity among our samples, although pH was different among gathotan samples. Measurement of pH was reported to be more reliable method to determine acidity in wine (Rajkovic *et al.* 2007). This may also apply to gathotan, where measurement of pH gives more meaningful information than titratable acidity.

Incubation temperature at around 30°C seemed to support growth of *L. theobromae* not only in gathotan fermentation, but also in various media. For example, in a liquid medium (Eng *et al*. 1998), cassava-containing medium without any addition of nitrogen source (Navaratnam *et al*. 1996), potato dextrose agar medium (Kausar *et al*. 2009), and mango during postharvest (Pawar 2012). It was also reported that light was important to support growth of the fungus (Kausar *et al*. 2009). In our work, fermentation was conducted inside building with minimum light, so that growth of *L. theobromae* may not be at optimum level.

Gathotan fermentation was prone to contamination, as it is usually carried out in an open and unsterilized container. Some fungi are antagonistic to *L. theobromae*, such as some species of *Trichoderma* (Mortuza and Ilag 1999, Haggag and Nofal 2006, Fadahundi *et al.* 2013). One of antagonistic bacteria is *B. subtilis* which grows during soaking of cassava (Anyogu *et al.* 2014). A species of yeast, *Pichia anomala* restricts growth of *L. theobromae* (Hashem and Alamri 2009). Thus, reducing contamination of those antagonistic microorganisms is essential, and it can be carried out by improving sanitation of process. Lowering pH can inhibit bacterial contamination, but pH may not be allowed to be too low as it can inhibit growth of *L. theobromae*. Therefore, it is important to ensure fermentation conditions to support dominant growth of *L. theobromae* over antagonistic microorganisms.

We tried to determine optimum condition for growth of *L. theobromae* during gathotan fermentation. Using optimisation procedure, and by putting relative importance and weight of fungal colonization at 10, titratable acidity at 2 and pH at 2, we found that maximum fungal growth was achieved at incubation temperature of 34.5°C for 2.4 days, 3.7 hours drying at 40°C, inoculum level of 2%, and 26.4 hours soaking (Fig 5). Relative importance and weight is determined approximately to reflect distribution of desirability between lower or upper bound and the target value. The setting used in this optimization is evaluated by determining composite desirability value which is between 0 and 1. Our setting resulted in composite desirability of 0.934, indicating that the setting gave satisfactory results for all response as a whole. We noticed that during duration of fermentation in this work, there was no black fungal coloration of tubers, suggesting that additional time to allow maturation is needed in order to achieve final products with black colour characteristics of gathotan.

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