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Enhanced Solid State Fermentation of Gibberellins From Selected Fungi

By
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Enhanced solid state fermentation of gibberellins from selected fungi

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Abstrak Penyelidikan

Pertumbuhan tumbuhan boleh dikawal dengan menambahkan hormon tumbuhan seperti auksin, sitokinin, gibberellins, asid abscisic dan etilena. Hormon tumbuhan ini boleh mengawal pembangunan dan pertumbuhan tumbuhan dengan memberi kesan kepada pembahagian, pemanjangan, dan pembezaan sel-sel, dan juga mampu untuk menjadi pengantara tindak balas jangka pendek fisiologi terhadap rangsangan persekitaran. Gibberellins (GAs) adalah kumpulan asid diterpenoid yang berfungsi sebagai pengawal selia pertumbuhan tumbuhan dengan mempengaruhi pelbagai proses pembangunan dalam tumbuh-tumbuhan yang lebih tinggi. Antara gibberellins, yang paling penting dari perspektif industri adalah asid gibberellic (GA_3), yang boleh dihasilkan oleh proses penapaian dengan kepekatan yang tinggi, dan juga boleh digunakan dalam aplikasi yang lebih luas ke arah penggalak pertumbuhan tumbuhan. Biasanya, hormon tumbuhan yang dihasilkan adalah dalam jumlah yang sangat kecil tetapi boleh memberi kesan kepada pertumbuhan dan pembangunan organ tumbuhan. Walaubagaimanapun, terdapat kekurangan maklumat dalam literatur pada pengeluaran oleh spesis dan keluarga Trichocomaceae.

Hasil kajian menunjukkan bahawa pengeluaran GA_3 yang paling tinggi (31.9 mg/kg substrate) telah dicapai pada 7 hari tempoh pengeraman, 21% (v/w) saiz inokulum dan 2% (v/w) kepekatan bahan perangsang (minyak zaitun). Ini menunjukkan GA_3 boleh dihasilkan oleh sejenis microfungus, *Penicillium variable* yang telah diisolasikan daripada sumber semulajadi kita, dan kulit pisang, iaitu sisa dari industri makanan telah digunakan sebagai substrat. Ini mungkin merupakan satu langkah pertama penggunaan sumber asli kita untuk aplikasi yang berguna dalam usaha untuk menangani masalah pencemaran alam sekitar. Pengurusan produktif diversiti atau pemuliharaan sumber asli kita dan pemeliharaan alam sekitar boleh membawa kepada pembangunan lestari.

Abstract of Research

Growth of plant can be controlled by adding plant hormones which are auxin, cytokinins, gibberellins, abscisic acid and ethylene. These plant hormones controlled the development and plant growth by affecting the division, elongation, and differentiation of the cells and also able to mediate short term physiological responses to the environment stimuli. Gibberellins (GAs) were a group of diterpenoid acids that function as plant growth regulators influencing a range of developmental processes in higher plants. Among the gibberellins, the most important from an industrial perspective is gibberellic acid (GA_3), which can be produced by fermentation at relatively higher concentration and also applicable in wider application toward plants growth acceleration. Normally, plant hormones are produced in very small amounts but can affected the growth and development of plant organ. However, there is lack of information in the literature on its production by the species of a trichocomaceae family. In this study, solid state fermentation of GA_3 production by *P. variable* using banana peel as a substrate was carried out in flask culture. The parameters were optimized using statistical tool via response surface methodology (RSM) couple with Box-Behnken Design.

Results showed that higher GA_3 production (31.9 mg GA_3/kg substrate), was attained at 7 days incubation period, 21% (v/w) inoculum size and 2% (v/w) precursor (olive oil) concentration. Thus showing that GA_3 can be produced by a microfungus, *Penicillium variable* that was isolated from our natural resources, and using banana peel, waste from food industry as a substrate. This could be the first step utilization of our natural resources into useful application in order to address the problem of environmental pollution. The productive management of the diversity or conservation of our natural resources and preservation of the environment lead to sustainable development.

RESEARCH OBJECTIVES

This project addresses an alternative method for gibberellic acid production by selected fungi using selected agro residues as a substrate.

The measurable objectives are:

(i) To select the best food processing residues that can be used as a substrate

(ii) To screen the presence of gibberellic acid by selected fungi.

(iii) To optimize the media components and process parameters for the gibberellic acid production in flask reactor using a statistical tool

Objective 1: To select the best food processing residues that can be used as a substrate

Table 1 showed the residues used as substrates for GA_3 production. These residues corn bran (CB), banana peel (BP), mango wastes (MW), soy wastes (SW), papaya peel (PP), and mango peel (MP) which were obtained from Soon Soon Oil Mill Prai, local fruits stall, Frutania Industry Perlis, local small scale of soy milk store and Pak Ali's pickles, respectively.

The residues were dried in an oven at 60 °C in order to reduce the moisture content to less than 10%. They were then blended into smaller sizes and sieved into 45 μm mesh size using a (Retsch AS200, Germany). However for PP and MP, they were initially washed thoroughly to remove the salt which was normally used during processing in the pickles industry.

Table 1: Different types of agro-residues used for substrates in GA3 production

Substrates	Substrates Preparation
Corn Bran	Dried at 60°C and kept in cool room until further used
Banana Peel and Mango waste	Washed, dried at 60 °C and ground into 45 μm mesh size and kept in cool room until further used
Papaya Peel and Mango peel	Washed thoroughly with tap water, dried at 60 °C, ground into 45 μm mesh and kept in cool room until further used

Selected food processing residues were analyzed for its capabilities to support growth of selected fungi for the production of GA_3. Almost all of these selected residues were readily available throughout the year in Malaysia and they can be used as value added substrates instead of polluting the environment. Besides, these residues were chosen as they were rich in organic matters that made them suitable for the bioconversion process in solid state fermentation (Pandey et al., 2000; Machado et al., 2004). The proximate compositions of the nutrients present in each substrate are listed in Table 2. According to Escamilla et al. (2000) and Machado et al. (2004), production of GA_3 began when the amount of nitrogen is depleted, and continued when sufficient carbon content was available.

Table 2: Proximate analysis of different types of substrates

Proximate analysis	Substrates				
	Corn Bran	Banana Peel	Mango Peel	Papaya Peel	Mango Waste
C (%)	42.42 ± 0.15	44.85 ± 0.15	44.24 ± 0.15	34.97 ± 0.15	40.38 ± 0.15
N (%)	13.40 ± 0.16	12.16 ± 0.16	15.32 ± 0.16	19.27 ± 0.16	14.17 ± 0.16
H (%)	6.86 ± 3.75	7.68 ± 3.75	8.12 ± 3.75	7.87 ± 3.75	7.94 ± 3.75
S (%)	0.51 ± 0.15	0.70 ± 0.15	0.76 ± 0.15	0.81 ± 0.15	0.70 ± 0.15
Glucose (g/kg substrate)	40.53 ± 1.37	311.23 ± 0.41	71.45 ± 1.30	253.90 ± 1.29	323.76 ± 0.78
Sucrose (g/kg substrate)	105.48 ± 1.55	22.40 ± 1.01	81.42 ± 1.02	27.06 ± 1.29	37.63 ± 0.84
Fructose (g/kg substrate)	71.37 ± 0.51	516.68 ± 1.12	193.72 ± 1.82	1154.01 ± 1.27	1008.73 ± 1.71
Reducing Sugar (mg/kg substrate) (Before Sterilized)	263.26 ± 1.62	635.28 ± 1.08	503.25 ± 0.54	616.61 ± 0.95	341.72 ± 0.41
Reducing Sugar (mg/kg substrate) (After Sterilized)	205.09 ± 1.08	636.63 ± 0.41	418.02 ± 1.35	489.18 ± 1.08	297.08 ± 1.10

Results showed that among all, the banana peel (BP) has smaller amount of nitrogen (12.16 % ± 0.16) but higher in the carbon content (44.85 % ± 0.15). According to a few researchers, higher carbon to nitrogen ratio of the substrates used could provide positive effect towards GA_3 production (Brückner and Blechschmidt, 1991; Tudzynski, 1999; Machado et al., 2004; Machado et al., 2008; Rodrigues et al., 2009). In addition, BP also contained basic sugars such as glucose and sucrose, of which both were common carbon sources for GA_3 production. These sugars can be used as carbon sources as long as the concentration are less than 20% at the beginning of the fermentation process, as it causes catabolic repression (Machado et al., 2008). Whereas, mango peel (MW) and papaya peel (PP) contain high amount of fructose which might causes catabolic repression during fermentation process that inhibit microbial growth and GA_3 production. Conversely, both corn bran (CB) and mango peel (MP) have low content of glucose which is only at 40 g/kg substrate and 71 g/kg substrate respectively. Small amount of glucose can contribute insufficient nutrient for microbial growth thus low down the production of GA_3.

In the present study, all solid substrates used for the fermentation process were sterilized at 121 °C (1.3 bar) for 15 mins. The amount of reducing sugar of BP before and after sterilized was found to be almost similar at 635 – 636 mg/kg substrate. Results of reducing sugar for others substrate (CB, MP, PP and MW) were decreased after sterilized which might be due to high temperature effect. Thus these substrates were not suitable utilized as solid substrates for the production of GA_3 due to fluctuation of reducing sugar concentration and tend to decreased after sterilized.

On the other hand, banana is one of the most abundantly available fruits in tropical countries and its peels are available at cheap price or it is simply discarded as garbage (Karthikeyan and Sivakumar, 2010). Due to that, BP was chosen as a substrate in subsequent studies for the production of GA_3 via SSF.

Objective 2: To screen the presence of gibberellic acid by selected fungi.

16 species of selected macro- and micro-fungi has been screened in order to select the best gibberellic acid producer (Table 3). The tested fungi were obtained from the Wood Decay Fungi Laboratory, Forest Research Institute of Malaysia, Kepong and Plant Pathology Laboratory, Institute of Biological Science, University of Malaya, respectively.

Micro-fungi were cultured on PDA (Potato Dextrose Agar) while macro-fungi were cultured on MEA (Malt Extract Agar) which were incubated at 30 °C for 5 to 7 days. The plates full of mycelial mat were then kept at 4 °C until further used. Subculture of all species was carried out every 2 months in order to maintain and ensure sufficient stock for subsequent studies.

Table 3: Different micro- and macro-fungi used for screening of gibberellic acid production

Microorganism	**Family**
Micro Fungi	
Aspergillus niger	Trichocomaceae
Aspergillus terreus	Trichocomaceae
Trichoderma viridae	Hypocreaceae
Penicillium rubrum	Trichocomaceae
Penicillium islandicum	Moniliaceae
Penicillium simplicissimum	Trichocomaceae
Penicillium spinulosum	Trichocomaceae
Penicillium expansum	Trichocomaceae
Penicillium variable	Trichocomaceae
Penicillium clarifame	Trichocomaceae
Macro Fungi	
Pycnoporous sanguines	Polyporaceae
Trametes lactinea	Coriolaceae
Trametes feei	Coriolaceae
Trametes pocas	Polyporaceae
Lentinus sajor caju	Lentinaceae
Scyzhophyllum commune	Schizophyllaceae

Cell suspension was prepared by scraping and adding 10 ml Tween 80 (0.01% (v/v)) in sterile distilled water onto the agar plate containing the tested strain. The cell suspension is then transferred into a sampling bottle that contains sterile distilled water. Sterile method had been used for preparation of cell suspension to avoid any contamination.

BP used as substrate has been dried at 60°C, and then kept in cool room until further used. The media was autoclaved at 121°C for 15 min prior using it as a substrate for further fermentation process.

Solid state fermentation of GA_3 productions by the tested fungi were carried out in static flask culture. 35% (w/w) of banana peel and 65% (v/w) of sterile distilled water and inoculums were added into 250 ml flask so that total substrate used for fermentation becomes 20 g in wet basis. The initial pH of the substrate was adjusted to 3.5 with sterile acidic distilled water. Each flask was inoculated with 15% (v/w) of spore suspension and incubated at room temperature (27 °C) for 7 days in a dark condition. Prior to inoculation, the media used was autoclaved at 121 °C for 15 minutes.

The flasks were then harvested by extracting the fermented material with distilled water and then analyzed for GA_3 and reducing sugar concentration. Prior to extraction, approximately 1 g of the fermented material were dried for biomass estimation.

Analytical Methods

a) Extraction of gibberellic acid

The concentration of gibberellic acid was determined using a spectrophotometric method as described by (Berríos et al., 2004). Gibberellic acid was extracted from the supernatant using ethyl acetate as a solvent via double liquid-liquid extraction method. In this study, gibberellic acid was extracted from ethyl acetate by adding phosphate buffer pH 7.4 followed by 10 ml of sample (1 ml extract sample mix with 1 ml ethanol absolute and balance HCl 3.75 M). The absorbance of the mixture was then analyzed using a UV-Vis spectrophotometer at 254 nm.

b) Biomass estimation

Estimation of biomass was carried out using modified glucosamine method as suggested by (Zheng and Shetty, 1998). The dried sample will be mixed with 5 ml HCl (2M) which was then boiled for two hours (except for blank). Then 1 ml hydrolysate was taken and neutralized with NaOH to pH 7 and diluted to 5 ml from which 0.5 ml will be mixed with 0.5 ml of $NaNO_2$ (5% w/v) and 0.5 ml of $KHSO_4$ (5% w/v) in a centrifuge tube. After shaking for 15 min, it will be centrifuged at 1500 rpm for 2 min. Then 0.6 ml supernatant is taken and mixed with 0.2 ml 0f $NH_2SO_3NH_2$ (12.5% w/v) and shake for 3 min. The reaction mixture was mixed with 0.2 ml of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH, 0.5% w/v need to be prepared daily) which then boiled for 3 min. The reaction mixture was immediately cooled to room temperature and added with 0.2 ml $FeCl_3$ (0.5% w/v, prepared within 3 days). After standing for 30 min, the absorbance at 650 nm was measured using a spectrophotometer. Table 2 and Figure 4 summarized the presence of gibberellic acid for the tested fungi.

Table 4: Gibberellic acid after screening with different species of micro and macro – fungi

Microorganism	Family	Presence of gibberellic acid
Micro Fungi		
Aspergillus niger	Trichocomaceae	Yes
Aspergillus terreus	Trichocomaceae	No
Trichoderma viridae	Hypocreaceae	No
Penicillium rubrum	Trichocomaceae	No
Penicillium islandicum	Moniliaceae	No
Penicillium simplicissimum	Trichocomaceae	Yes
Penicillium spinulosum	Trichocomaceae	Yes
Penicillium expansum	Trichocomaceae	No
Penicillium variable	Trichocomaceae	Yes
Penicillium clarifame	Trichocomaceae	No
Macro Fungi		
Pycnoporous sanguines	Polyporaceae	No
Trametes lactinea	Coriolaceae	No
Trametes feei	Coriolaceae	No
Trametes pocas	Polyporaceae	No
Lentinus sajor caju	Lentinaceae	No
Scyzhophyllum commune	Schizophyllaceae	No

The anamorphic genera of the *Trichocomaceae* family were widely used in biotechnology and some produced mycotoxins and other various secondary metabolites (Markovina et al., 2005; Pitt and Hocking, 2009). In fact, *Aspergillus niger* also has also reported to be capable in producing GA_3 (Brückner and Blechschmidt, 1991; Bomke and Tudzynski, 2009).

In this present study, *P. variable* was found of producing the highest GA_3 followed by *A. niger*, P. *simplicissimum* and *P. spinulosum* at 16.25 mg/kg substrate, 12.45 mg/kg substrate, 5.56 mg/kg substrate and 3.90 mg/kg substrate respectively. Likewise, *P. variable* showed the highest biomass estimation followed by *A. niger*, P. *simplicissimum* and *P. spinulosum* but reducing sugar trend were opposite (Figure 1) which indicated that a lot of glucose had been consumed for growth and also product formation. Besides GA_3, *P. variable* also produced tannase at pH 6 and 60 °C which inoculated on tannic acid agar plate. Due to that, this tested strain was used in subsequent optimization studies.

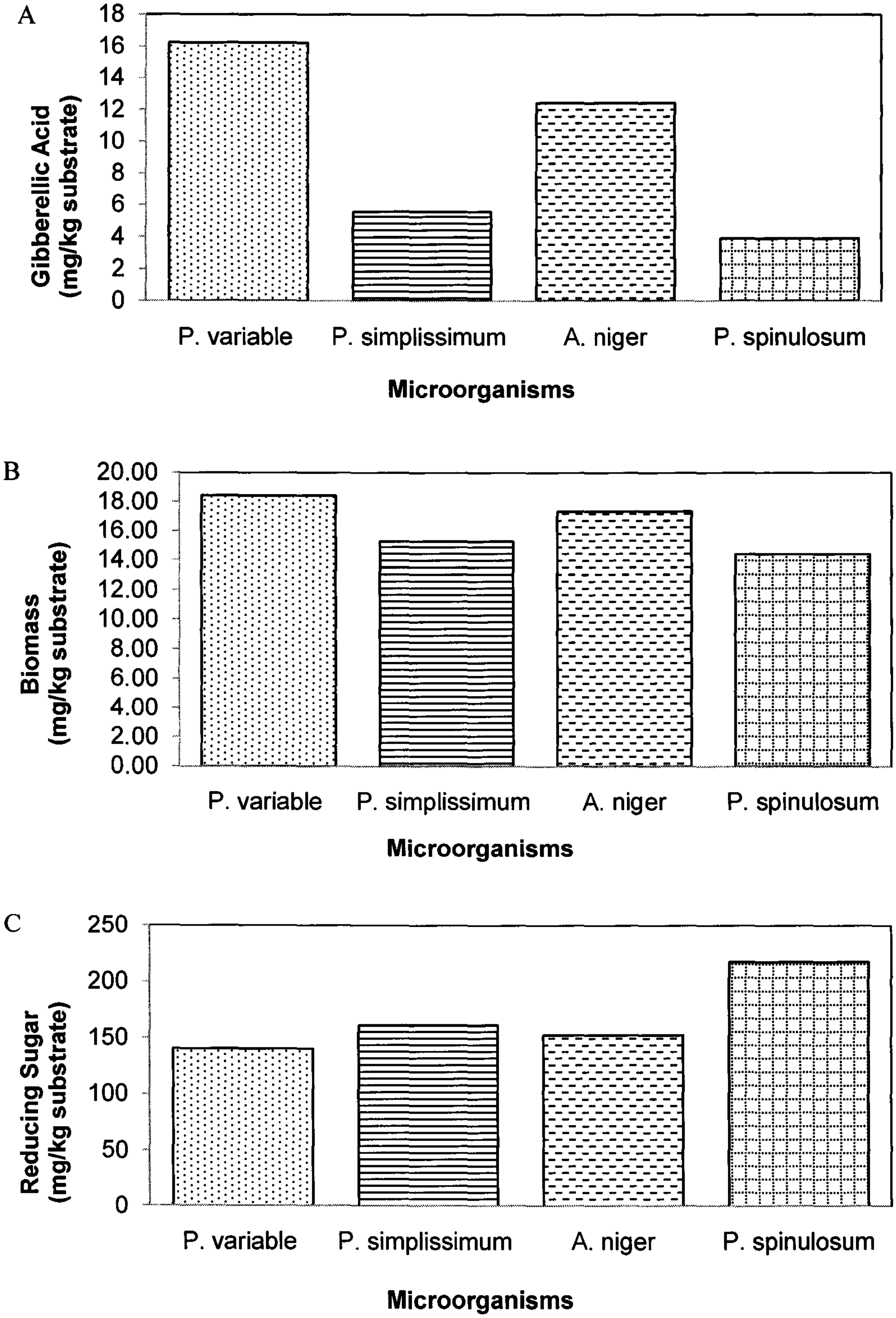

Figure 1: Profile of GA_3 production, biomass and reducing sugar during static flask cultivation by selected fungi of the family Trichocomaceae (Condition: 35% (w/w) total solid (Banana peel), 50% (v/w) liquid (sterile distilled water pH 3.5), 15% (v/w) inocubated at 27 °C)

Objective 3: To optimize the media components and process parameters for the gibberellic acid (GA_3) production in flask reactor using a statistical tool

Critical media components

To find the media component that stimulates the growth of *P. variable*, a statistical tool namely Plackett Burman design was chosen. In this present study, experiments were carried in static flask reactor, using chosen substrate (banana peel), supplemented with glucose and raw sugar (sucrose) as the carbon source, soy waste and yeast extract as nitrogen source, olive oil as a natural precursor, KH_2PO_4 as K-P source, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeSO_4.7H_2O$, $MnSO_4.7H_2O$, NH_4SO_4 as Zn, Cu, Mg, Ca, Fe, Mn and S trace elements, respectively (Table 4). These supplemented nutrients were selected and screened its potential as the best media component for GA_3 production in Placket Burman experimental design (Table 5). The variables denote as A – N represented the critical media components and O – T represented the dummy variables (unassigned variable). All flasks were incubated in room temperature under dark condition for 7 days.

Glucose and sucrose which were used as carbon source, soy waste and yeast extract as nitrogen source, olive oil as precursor and four components as trace elements (Zn, Fe, Mg and Mn) have showed positive effect (Figure 2) with higher concentration influenced the GA_3 production. And in this study, lower level indicated that the concentration of media components were zero whereas higher level indicated that supplements of media components were highly needed. Selected amount of each media with high and low concentration was showed in Table 6.

Glucose and sucrose (raw sugar) were employed as extra carbon sources or co-substrate of banana peel, and both of these extra carbon sources gave positive effect on the GA_3 production. In view of an economical aspect, the raw sugar which cost only RM 2.00/kg and also showed higher positive effect was selected for further study. Darken et al. (1959) in their research have showed that different types of carbon sources such as sucrose, glycerol, glucose and lactose also influenced GA_3 production. Sucrose was found as a favourable carbon source for this effective GA_3 production. Similar results were also reported by Rodrigues et al. (2009) who also used sucrose as the most significant component as co-substrate in their kinetic studies of GA_3 production using citric pulp (CP) as a substrate in solid state fermentation by *F. moniliforme*.

Table 5: Plackett Burman experimental design of 20 trials with 13 independent variables (A - N)

Run	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T
1	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+
2	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-
3	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-
4	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+
5	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	+
6	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+	+	+
7	-	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-
8	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+
9	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-	+	+
10	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	+	+	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-
13	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-
14	+	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+
15	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	+
16	-	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-
17	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-
18	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+
19	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+
20	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	+	+	-	-

Nitrogen sources from soy wastes and yeast extract also gave positive effect on GA_3 production, but they also contain other elements such as vitamins, amino acid and others. The yeast extract gave greater positive effect than soy wastes with *P*-value less than 0.05. GA_3 started producing when the nitrogen source was depleted, but with the presence of sufficient amount of carbon source (Kumar and Lonsane, 1989). Thus sufficient amount of nitrogen with appropriate concentration was important for GA_3 formation.

Table 6: Variables showing medium components used and concentration in Plackett Burman design

Designation	Components	Low level (-)	High level (+)
A	Glucose	0	2% w/w
B	Sucrose (Raw sugar)	0	2% w/w
C	Soy waste	0	1% w/w
D	Yeast Extract	0	1% w/w
E	Olive Oil	0	1% v/w
F	K-P (KH2PO4)	0	0.05% v/w
G	Zn (ZnSO4.7H2O)	0	0.05% v/w
H	Cu (CuSO4.5H2O)	0	0.05% v/w
J	Mg (MgSO4.7H2O)	0	0.05% v/w
K	Ca (CaCl2.2H2O)	0	0.05% v/w
L	Fe (FeSO4.7H2O)	0	0.05% v/w
M	Mn (MnSO4.7H2O)	0	0.05% v/w
N	S (NH4SO4)	0	0.05% v/w

Olive oil was also found to show positive effect on GA_3 production with *P*-value 0.0471. According to Rios-Iribe et al. (2011) and Escamilla et al. (Escamilla et al., 2000), the mixture of substrate with corn oil has increased GA_3 production higher in which it act as an inert material for carbon catabolite repression and additionally might contained natural precursor for GA_3 biosynthesis. In fact oil plant extracts such as sunflower oil might contain precursor of the GA_3 pathway (Shukla et al., 2003).

Naturally, microorganism required nutrients in order to maintain its growth which can be classified as macronutrients that are needed in larger amount and micronutrients in lower concentration. Carbon, nitrogen and magnesium are classified as macronutrients whereas iron (Fe), and manganese (Mn) are classified as micronutrients. Magnesium, iron and manganese were important cofactors for some enzymes, and played a regulatory role in some fermentation process. In fact, they also contributed in the regulation of secondary metabolism and excretion of primary metabolites, respectively (Shuler and Kargi, 2002). However among all trace elements employed in this study (Table 7), only four components

(Zn, Fe, Mg and Mn) showed positive effect towards effective GA_3 production. But Zn was considered insignificant with *P*-value more than 0.05. The source of phosphate from KH_2PO_4 showed negative effect on GA_3 production with significant *P*-value (Table 7). This showed that banana peel might contain an adequate amount of phosphate for the growth of *P. variable* since GA_3 production was influenced with the presence of KH_2PO_4 at lower concentration.

In accordance with the results, sucrose (raw sugar) was selected as carbon source, yeast extract as nitrogen source and Mn, Fe and Mg as trace elements since they give positive and significant effect towards gibberellic acid production yield. Therefore, optimization of GA_3 production is further carried out by using one factor at a time (OFAT) method for the influence of nutritional and physical factors, respectively.

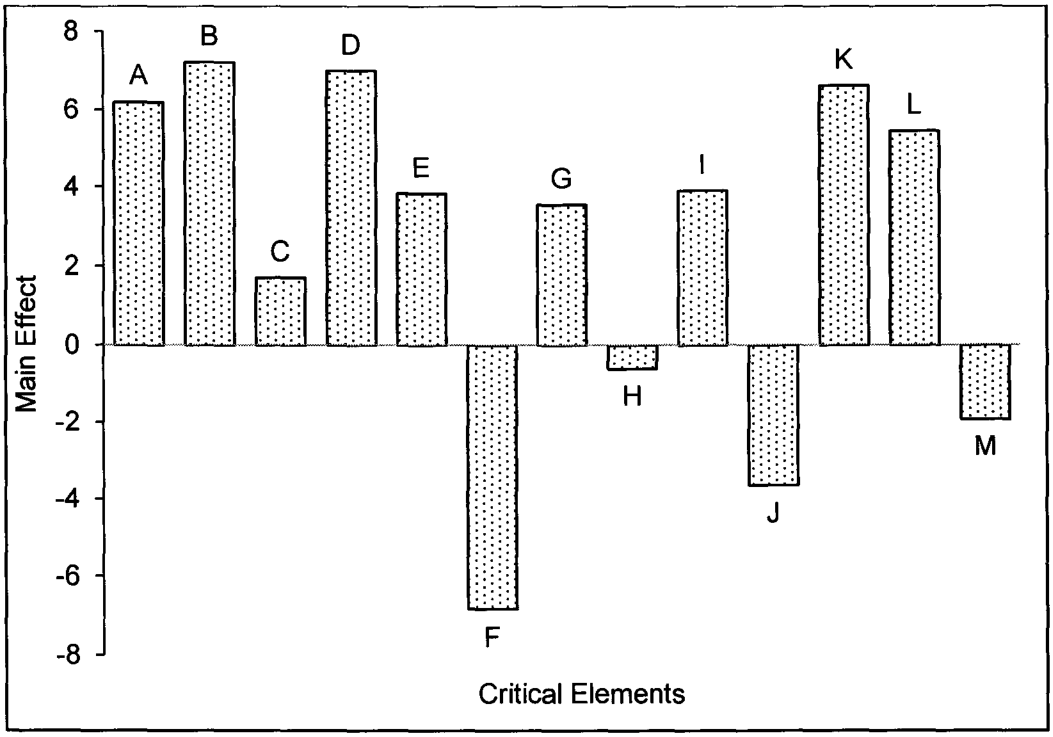

Figure 2: Effect of media components on the production of GA_3. (A – Glucose, B – Sucrose (raw sugar), C – Soy waste, D – Yeast extract, E – Olive oil, F – K-P (KH_2PO_4), G – Zn ($ZnSO_4.7H_2O$), H – Cu ($CuSO_4.5H_2O$), I – Mg ($MgSO_4.7H_2O$), J – Ca ($CaCl_2.2H_2O$), K – Fe ($FeSO_4.7H_2O$), L – Mn ($MnSO_4.7H_2O$), M – S (NH_4SO_4))

Table 7: Effect and probability of different media components on GA_3 production

Critical elements	Term	Effect	Sum Square	*P*-value
Glucose	A	6.19342	191.7923	0.0069*
Sucrose (Raw sugar)	B	7.20378	259.4722	0.0034*
Soywaste	C	1.70078	14.4633	0.3104
Yeast Extract	D	6.98242	243.7709	0.0039*
Olive Oil	E	3.82362	73.1003	0.0471*
K-P (KH2PO4)	F	-6.81882	232.4815	0.0044*
Zn (ZnSO4.7H2O)	G	3.54182	62.7224	0.0605
Cu (CuSO4.5H2O)	H	-0.61538	1.8935	0.7025
Mg (MgSO4.7H2O)	J	3.90238	76.1428	0.0440*
Ca (CaCl2.2H2O)	K	-3.64922	66.5840	0.0550
Fe (FeSO4.7H2O)	L	6.58678	216.9284	0.0051*
Mn (MnSO4.7H2O)	M	5.43698	147.8038	0.0122*
S (NH4SO4)	N	-1.93178	18.6589	0.2551

* Significant value

Media optimization by one factor at a time (OFAT) method

OFAT is used in order to observe the possible optimum level of parameters for the production of GA_3. In this experiment, the concentration of selected nitrogen source and trace elements are maintained. Four main parameters that would affect GA_3 production were chosen as sucrose concentration (1 – 15 % w/w), incubation time (1-13 days), inoculum sizes (10-50% v/w) and natural precursor olive oil (1-5% v/w) which are the physical and nutritional parameters, respectively. These experiments were carried out using static flask reactor with 65% (v/w) of liquid volume, 35% (w/w) of solid content, initial substrate pH of 5 and incubated at room temperature (27°C).

The carbon source like glucose, sucrose, flour or others were normally used as carbon source. Rangaswamy and Balu (2007) also reported that glucose or sucrose was used for the optimization of carbon source in submerged fermentation. Conventionally, Darken et al. (1959) had investigated different type of carbon source like glycerol, glucose and lactose for GA_3 production. Results showed that glucose was favorable carbon source for effective GA_3. However, Rodrigues et al. (2009) recently has successfully used citric pulp supplement with sucrose as carbon source of solid state fermentation medium gave highest production of GA_3.

As in present study, Figure 3 plotted the effect of sucrose concentration on the production of GA_3 and growth of *P. variable*. Result showed that the highest GA_3 concentration was attained at 14.5 mg/kg substrate when the substrate banana peel was supplemented with 2% (w/w) of sucrose (raw sugar). Beyond this concentration, a reversed trend was observed. In addition, the growth of *P. variable* with 2% (w/w) of sucrose also at the higher value compared to others concentration of sucrose. According to Rios-Iribe et al. (2011) this condition could be due to "catabolite repression that generated intermediate from rapid catabolism of glucose thus interfering with the enzyme in the secondary metabolism process.

Previously, Shukla et al. (2003) in their research also stated that higher concentration of glucose (> 20%) inhibited the growth of *G. fujikuroi*. However at low (< 4%) glucose concentration became stimulated the production of GA_3 and maintenance of biomass in the production phase. Moreover, Darken et al. (1959) had identified that slowly utilization of carbon source could permit more effective production of GA_3 after the maximum rate of growth has been obtained with rapidly utilized glucose, sucrose or corn starch. Thus indicates that sucrose and glucose which act as valuable carbon sources, at lower concentration could support growth of microorganisms for better product formation.

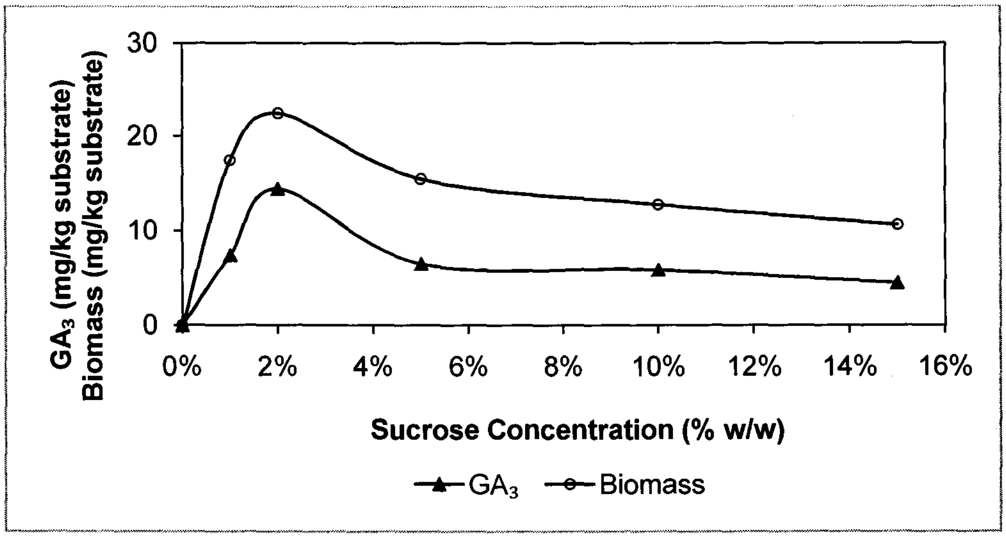

Figure 3: Effect of sucrose concentration on growth and GA_3 production. [Condition: 7 days incubation, 35% (w/w) total solid (varies sucrose concentration + BP), 50% (v/w) liquid (2% v/w mineral solution + sterile distilled water pH 5), 15% (v/w) inoculums, 27 °C]

Figure 4 summarized the effect of incubation time on the production of GA_3, growth (biomass) and reducing sugar within 14 days of the incubation period. It is found that the concentration of reducing sugar reduced gradually throughout the fermentation period. More sugar was consumed by the tested fungus for the first 5 days and then remained constant till the 13 days. According to a few researchers, glucose was used as a good carbon source for growth and in a respiratory chain (Rios-Iribe et al., 2011). The maximum biomass concentration of 22.4 mg/kg substrate was obtained in 7 days of incubation period. During this period, more than half of initial glucose was consumed for metabolism growth and product formation GA_3 was steadily increased. The GA_3 production achieved the highest concentration after 9 days of fermentation period, and then it reduced as the amount of the carbon source in the fermentation media tends to reduce. Moreover, GA_3 production was the highest at the stationary phase and the profiles seemed to be non-growth product related trend. However at this phase, the fungus was metabolically active and produced secondary metabolites which are non-growth related.

In fact, many secondary metabolites such as antibiotics and others were non-growth associated products (Shuler and Kargi, 2002). In contrast, Machado et al. (2002) reported that the highest GA_3 production was achieved only at 7 days incubation period using *G. fujikuroi*. This opposition might be due to different fungus used and the nutritional and physical factors involved. As of Figure 4, it can be seen that growth of *P. variable* started to increase from day 1 to day 7 but beyond that its growth slowly decreased.

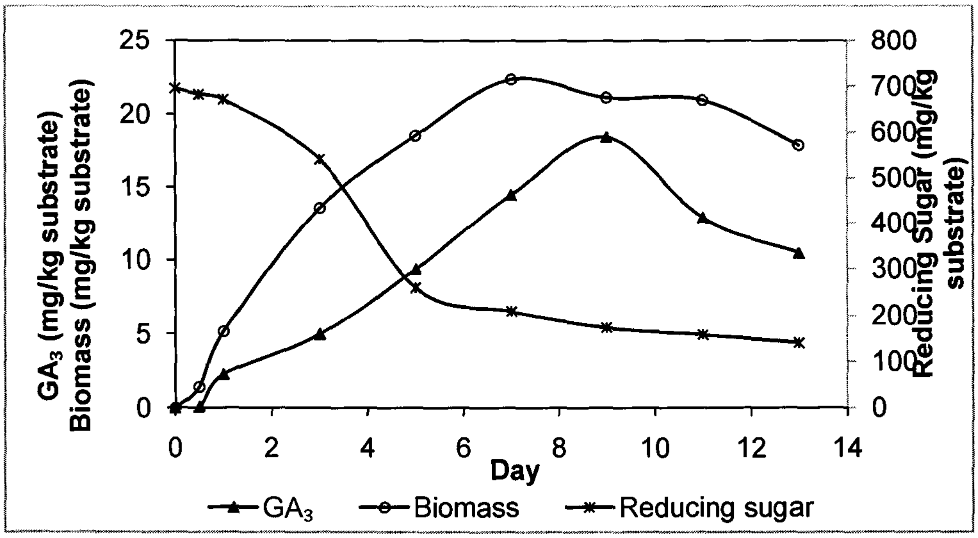

Figure 4: Effect on incubation time on GA_3 production, growth and glucose consumption by *P. variable* in SSF. (Condition: 35% (w/w) total solid (2% w/w sucrose + BP), 50% (v/w) liquid (2% v/w mineral solution + sterile distilled water pH 5), 15% (v/w) inoculums, 27 °C)

Inoculum sizes are another important physical factor that influenced GA_3 production instead of incubation time. Different inoculums size (10, 15, and 20%) with different production medium which are Czapek medium, PDA medium and citrus pulp (CP) extract were used for the production of GA_3 (Rodrigues et al., 2009). Their observation showed that the highest yield was achieved by using 15% (v/w) inoculums grown in Czapek medium and medium of citrus pulp was chosen with 20% (v/w) inoculums size. Due to that, the inoculums sizes used in the present study were varied from 10% to 50% (v/w).

Results showed that inoculums size of 20% (v/w) produced the highest GA_3 yield at 28.23 mg GA_3/kg substrate (Figure 5). This is in agreement with the result of Kumar & Lonsane (1990) whose also studied the effect of inoculums ratio and found that the maximum production of GA_3 was obtained when 20% (w/v) spore suspension was employed. In another study, Rodrigues et al. (2009) also showed that the highest yield was attained using 15% (v/w) of *Fusarium moniliforme* inoculums when grown in Czapek medium. In can be seen that maximum biomass was achieved at 28 mg/kg substrate when 30% (v/w) of inoculums size was used and tend to decrease as the inoculums size increased. Therefore inoculums size 20% (v/w) of *P. variable* was adequate for growth and as well GA_3 production. Thus, for optimizing GA_3 production an inoculums size 20% (v/w) of *P. variable* were used for subsequent study.

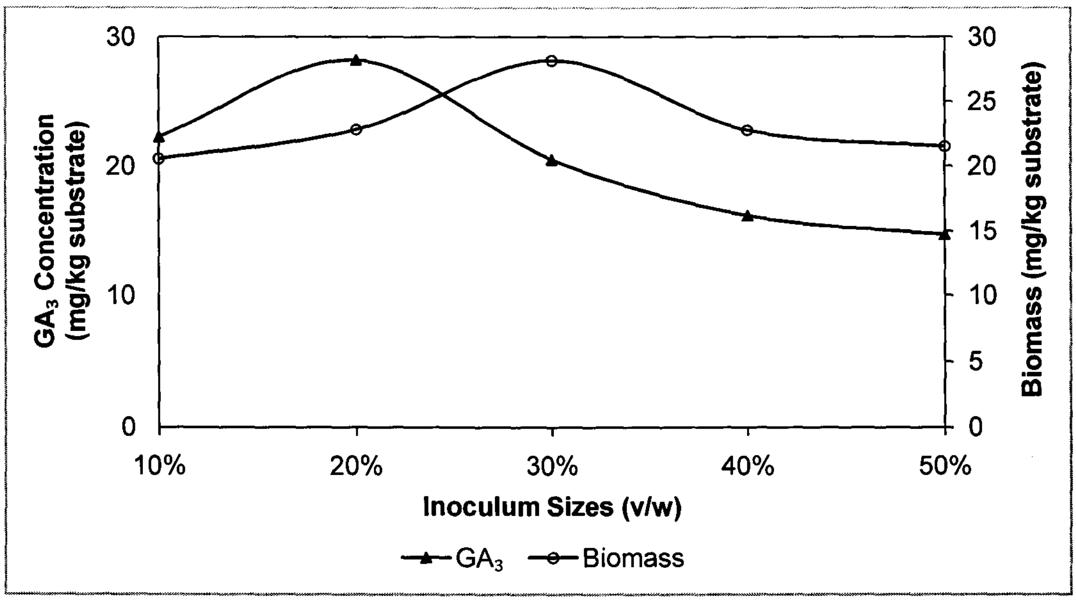

Figure 5: Effect of inoculums sizes on the gibberellic acid yield and growth(Condition: 9 days incubation, 35% (w/w) total solid (2% w/w sucrose + BP), 50% (v/w) liquid (2% v/w mineral solution + sterile distilled water pH 5), 27 °C, varies inoculums of varying sizes (10% - 50 % v/w))

Furthermore, a natural precursor, the olive oil was chosen as nutritional parameters in order to observe its influence towards GA_3 production. Plant oils also is a carbon source that is not only inert for carbon catabolite repression but also make available as a pool of acetyl-CoA and as well as might contain natural precursors for gibberellin biosynthesis (Tudzynski, 1999). Rios-Iribe et al. (2011) in their study has proved that using glucose-corn oil mixture as the carbon source minimized the use of acetyl-CoA in the biosynthesis of fatty acids and thus increasing the flux of acetyl-CoA to GA_3. A metabolic analysis indicated that the acetyl-CoA for the biosynthesis of fatty acids is switched to the synthesis of GA_3 which consequently GAs and lipids are synthesized from a common precursor, acetyl-CoA (Rios-Iribe et al., 2011). Figure 6 showed that 2% (v/w) of olive oil introduced to the production medium is proficient enough for producing highest GA_3 at 30.9 mg/kg substrate as compare to others. During this 2% (v/w) of olive oil concentration, the biomass concentration of 24 mg/kg substrate was achieved. Thus, addition of olive oil that contain natural precursor for GA_3 biosynthesis has increased in the production of GA_3 than when using sucrose only. The addition of the olive oil, acted as a precursor to increase the yield of GA_3 in present studies was further optimized using Box Benhken Design (BBD) couple with response surface methodology (RSM).

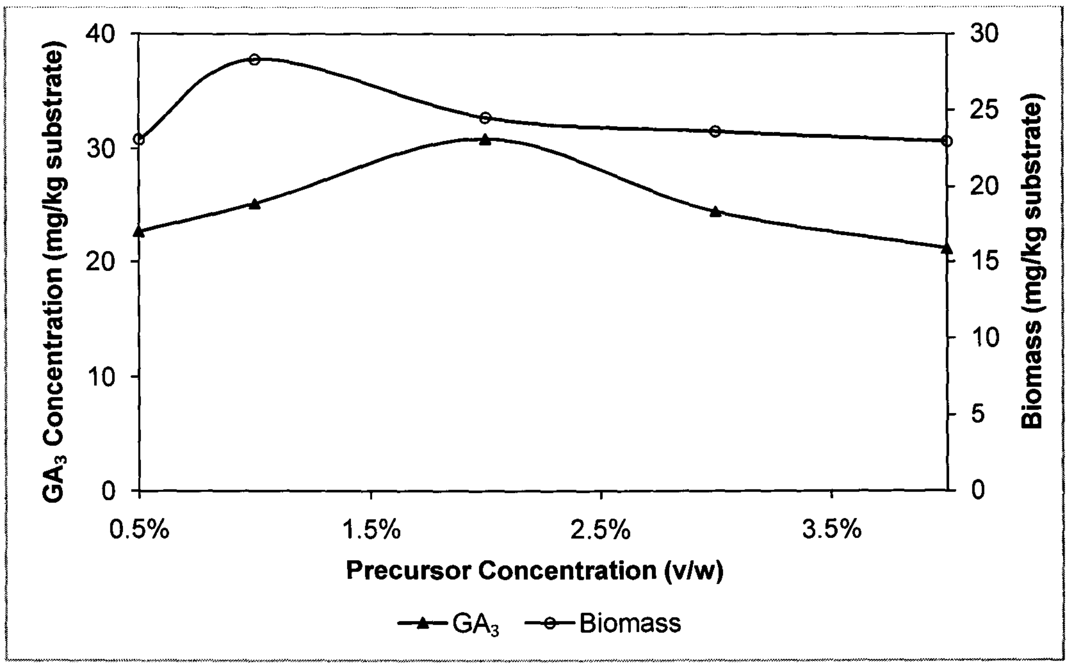

Figure 6: Effect of olive oil concentration on gibberellic acid production and growth (Condition: 9 days incubation, 35% (w/w) total solid (2% w/w sucrose + BP), 50% (v/w) liquid (2% v/w mineral solution + varies precursor concentration + sterile distilled water pH 5), 27 °C, 20% v/w inoculums sizes)

Product optimization using Box-Behnken Design (PBD) couple with Response Surface Methodology (RSM)

Optimization of product formation can be identified by using various statistical experimental designs such as response surface methodology (RSM) using Design Expert software version 6.0.6 (STATE-EASE Inc., Minneapolis, USA). In addition this statistical analysis method was one of the simplest ways to determine optimal product formation that influenced by independent variables and also interaction between selected variables studied.

In this study, three independent variables had been chosen base on the previous experiment and subjected to Box-Behnken design coupled with RSM. These variables chosen represent the physical and nutritional factors namely incubation time, inoculums size and precursor (olive oil) concentration. For each variables studied, the lower and upper limit set point were selected base on the result obtained from one factor at a time (OFAT) method experiment (Table 8). In this design, seventeen run of the experiments were conducted in which five of them are at the centre point and the production formation, GA_3 concentration as a response.

A regression analysis was designed by the software in order to fit the response and process variables with a suitable model. A fit model with significant value of P must less than 5% using the analysis of variance (ANOVA) that also described the interaction between each factor. From this analysis, three-dimensional surface plots were assembled that showed the effect of variables interaction and independent variables toward GA_3 production. The models were then validated by comparing experimental and predicted results in order to ensure its precision.

Table 8: Independent variables for GA_3 production and their coded values

Variables	Unit	Factor code	Experimental region	
			Lower limit	Upper limit
Incubation time	Day	A	5	13
Inoculum sizes	% (v/w)	B	10	30
Precursor concentration (Olive oil)	% (v/w)	C	0.5	3.5

(a) Statistical Analysis and Development of Regression Model Equation

By means of the 17 runs of experiments, regression analysis of these three independent variables and response of GA_3 concentration (Table 9) were found to be fitted well with the quadratic model which *P*-value less than 0.05. The quadratic regression model that described the GA_3 formation in terms of coded factor was shown in equation 1:

$$GA3 = +29.79 - 6.1 * A - 1.49 * B - 1.29 * C - 5.34 * A^2 - 7.40 * B^2 - 7.97 * C^2 - 3.6 * A * B - 0.90 * A * C - 2.46 * B * C \quad (1)$$

where A, B, C and GA_3 were the coded values of incubation time, inoculums size, precursor concentration and GA_3 concentration, respectively. From Table 9, experimental GA_3 production were in the range of 6.3 mg/kg substrate to 31.9 mg/kg substrate.

The statistical analysis of the regression model was taken from the analysis of variance (ANOVA) as presented in Table 9. The model F value of 59.19 implies the model significant and also showed that there was only 0.01% possibility that the model could occur due to noise (Gangadharan et al., 2008). In order to ensure the significant of each coefficient, value of *P* must be small which this value indicated the pattern of the interaction between the coefficients (Wang and Lu, 2005). It can be seen that all regression coefficients are significant except interaction of incubation time with precursor. Although it is not significant but still need to be considered in the model equation because it is a hierarchical model. This insignificant interaction might be due to physical factor of incubation time cannot showed the relationship with chemical factor of olive oil concentration which in agreement with result reported by Gangadharan et al. (2008) that also showed interaction between physical factor and chemical factor was insignificant . Although interaction of incubation time with olive oil concentration was insignificant, the presence of both parameters alone was highly significant towards GA_3 optimization with *P* value less than 0.05.

Table 9: Experimental design matrix with experimental and predicted values of GA_3 concentration

Run	Variable[a]			GA_3 yield (mg/kg substrate)	
	A	B	C	Actual value	Predicted value
1	13.00	20.00	0.50	13.365	12.57
2	13.00	20.00	3.50	7.862	8.2
3	9.00	20.00	2.00	29.16	29.79
4	5.00	10.00	2.00	20.44	20.95
5	5.00	20.00	3.50	21.393	22.19
6	9.00	30.00	0.50	15.152	16.12
7	13.00	30.00	2.00	14.913	14.74
8	5.00	30.00	2.00	26.301	25.33
9	9.00	30.00	0.50	15.366	16.67
10	13.00	30.00	2.00	6.289	5.78
11	9.00	20.00	2.00	30.017	29.79
12	9.00	20.00	2.00	28.302	29.79
13	9.00	20.00	2.00	29.588	29.79
14	9.00	30.00	3.50	9.005	9.18
15	9.00	10.00	3.50	18.391	17.08
16	5.00	20.00	0.50	23.299	22.96
17	9.00	20.00	2.00	31.875	29.79

All variables are in uncoded units

[a] A: Incubation time; B: Inoculums size; C: Precursor concentration (olive oil)

The correlation coefficient (R^2) value is closed to unity which signified that the quadratic model satisfactory adjusted to the experimental data. In addition, the adjusted R^2 and predicted R^2 (Table 10) values also lied within a realistic value. The value of lack of fit for regression is not significant which indicated that the model equation was adequate for predicting optimal GA_3 concentration with a combination of three variables (Wang and Lu, 2005).

Table 10: Analysis of variance (ANOVA) for the regression model of GA_3 yield

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob > F	
Model	1098.05	9	122.01	59.19	< 0.0001	Significant[a]
A	297.25	1	297.25	144.21	< 0.0001	
B	17.81	1	17.81	8.64	0.0217	
C	13.24	1	13.24	6.42	0.039	
A2	120.11	1	120.11	58.27	0.0001	
B2	230.69	1	230.69	111.91	< 0.0001	
C2	267.3	1	267.3	129.68	< 0.0001	
AB	54.2	1	54.2	26.29	0.0014	
AC	3.23	1	3.23	1.57	0.2505	
BC	24.2	1	24.2	11.74	0.011	
Residual	14.43	7	2.06			
Lack of Fit	7.38	3	2.46	1.4	0.3663	not significant[b]
Pure Error	7.05	4	1.76			
Cor Total	1112.48	16				

$R^2 = 0.9870$; Adjusted $R^2 = 0.9704$; Predicted $R^2 = 0.8840$

[a] significant under 95% confidence level

[b] not significant relative to the pure error due to noises

The coefficient of variance (CV) indicates the degree of precision with which the experiments are evaluated. High value of CV usually indicates that the experimental value was low in reliability (Gangadharan et al., 2008). In this present study, with CV value of 7.16 indicate that the experimental value is reliable. In addition, adequate precision was used to measure the signal to noise with desirable value if the ratio greater than 4. In this study, ratio of 21.806 indicates an adequate signal. With that, tested quadratic model is ample to represent the actual relationship between the variables and response.

(b) Process Parameters Studies

The three dimensional surface plot were constructed due to clearly visualize the relationship between the responses of various variables tested for determination of optimal GA_3 production (Figure 7 and 8). Optimal region lies in the middle of the curve which indicates the interaction between two variables while the other variable is constant.

The 3D surface plot of Figure 7 showed the interaction of incubation time with inoculums size at optimal level. Higher GA_3 concentration was detected when the substrate was inoculated with inoculums size 20% (v/w) on the 9 days of fermentation period. Shorter and longer incubation time seem to lower the product formation. This could be due to the GA_3 as secondary metabolite was normally produce at the beginning of stationary phase where the nitrogen source was depleted and the carbon source is still available in the medium (Escamilla et al., 2000). This is in agreement with the research done by Kumar & Lonsane (1989) where the optimal production of GA_3 used inoculums size of 15% to 20% v/v. However, most researchers reported prefer used 15% of inoculums size in their study for optimization (Kumar and Lonsane, 1987; Bandelier et al., 1997; Machado et al., 2002). Furthermore, inoculums size might be depend on the nutritional factor supplied to the microorganism growth and might also affected the day of optimum production. Therefore in this study, optimum production of GA_3 was achieved after 9 days of fermentation which shorter than (Rios-Iribe et al., 2011) which also used plant oil for improvement of GA_3 production. Since the optimum production fall within the selected range and showed positive contour plot thus, there was interaction and influenced of both variables towards optimum GA_3 production.

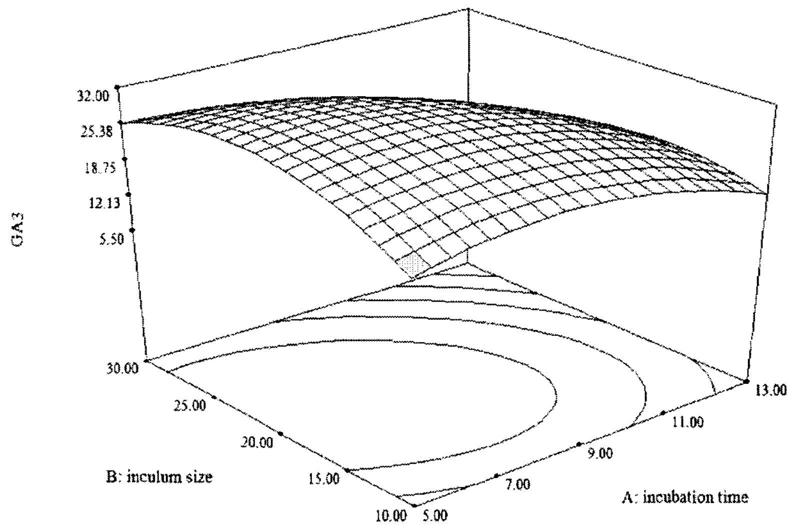

Figure 7: Response surface plot for the effect of incubation time and inoculums sizes on GA_3 production

Figure 8 indicated that the presence of small amount of olive oil (2% v/w) effected the production of GA_3. This positive interaction showed that olive oil can act as a precursor towards optimization of product formation since a higher concentration of such oil restrained the product yield. Formerly, Kumar and Lonsane (1989) had reported that addition of linseed oil to the fermentation medium successfully increased the product yield of GA_3 by 16.7%. In another studies, Tudzynski (1999) and Rios-Iribe et al. (2011) also reported that the presence of natural plant oil supplied extra carbon source as an inert for carbon catabolite and also make an available pool of acetyl-CoA, and thus might contain natural precursors for gibberellin biosynthesis. Besides, formerly known that biosynthesis of gibberellins was initiate by present of acetyl-CoA as initial stage of melvalonate pathway that provides isopentenyl diphosphate (IPP) for synthesis of all terpenoids including GA_3 (Sponsel et al., 2010).

It can be showed from the result obtained that lower and higher of inoculums size and olive oil inhibits the product formation. Hence with 20% of inoculums size ample to synthesis GA_3 from presence pool of acetyl-CoA in olive oil. According to Rios-Iribe et al. (2011) presence of acetyl-CoA from plant oil is used for synthesizing GA_3 in melvalonate pathway instead fatty acid which also make available the lipid requirements of cell function. Thus results indicated that the optimum condition was within selected range with optimum concentration of GA_3 and there is positive interaction between inoculums size with olive oil concentration.

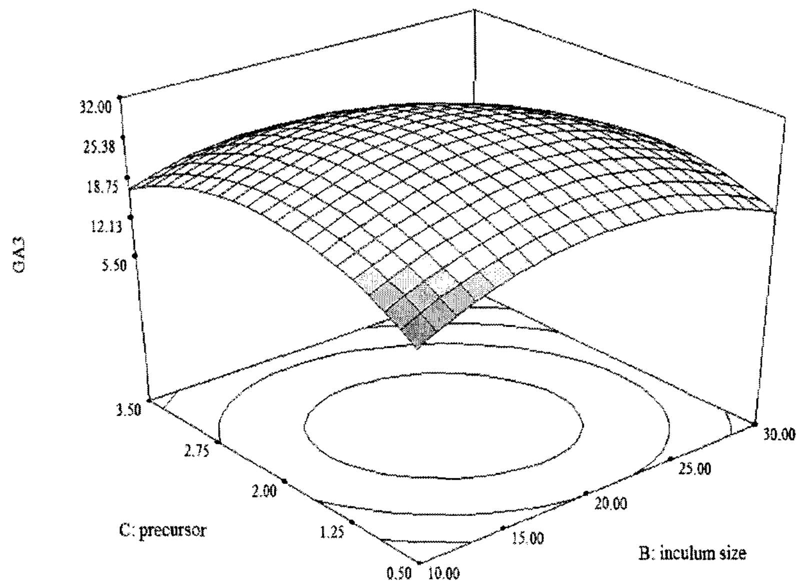

Figure 8: Response surface plot for the effect of inoculums size and precursor (olive oil) on GA_3 production

(c) Optimization Range of Parameters Studies

This study showed that the three independent variables chosen contributed to optimum GA_3 production. The optimum conditions with higher responses were chosen from predicted condition proposed by Box-Behnken design with real model presented in Equation 2.

$$GA_3 = -48.43010 + 6.62491 * \text{incubation time} + 3.96778 * \text{inculum size} + 17.93568 * \text{precursor} - 0.33381 * \text{incubation time}^2 - 0.074020 * \text{inculum size}^2 - 3.54120 * \text{precursor}^2 - 0.092025 * \text{incubation time} * \text{inculum size} - 0.14987 * \text{incubation time} * \text{precursor} - 0.16398 * \text{inculum size} * \text{precursor} \quad (2)$$

The optimum condition for GA_3 production (31.57 mg/kg substrates) by *P. variable* in static flask culture was at 2% v/w olive oil (precursor), inoculums size 20% v/w and 7 days of the incubation period. With this predicted condition proposed by Box-Benhken design, the validation of experimental run was conducted.

(d) Validation of The Model

Validation of the proposed model by Box Behnken design was done by carried out fermentation processes using a predicted optimum condition. Numerical optimization was used that predicted by box Benhken the optimum level of each independent variable with incubation time at 6.66 day, inoculums size was 20.54% and olive oil concentration at 1.92%. Therefore experiments were conducted with zero decimal points in term of realistic value with 7 days incubation time, 21% inoculums size, and 2% olive oil concentration (Table 10).

In order to validate the proposed model by Box Behnken design, the fermentation processes were carried out using a predicted optimum condition. Numerical optimization was predicted by box Benhken and the optimum level of each independent variable we at incubation time 6.66 day, inoculum size 20.54% v/w and olive oil concentration at 1.92% v/w. Therefore, the experiments were conducted with zero decimal points in term of realistic value with 7 days incubation time, 21% v/w inoculums size, and 2% v/w olive oil concentration as shown in Table 11.

Under the above predicted optimum condition, the maximum production of GA_3 was estimate as 31.57 mg/kg substrate. Experimental and predicted response values were in closed agreement with the error less than 1. Since the error is small, thus the predicted conditions level of variables and response were verified for optimizing GA_3 production. Thus, the model developed was considered to be accurate and reliable for predicting GA_3 production.

Table 11: Experimental and predicted values for GA_3 production at optimized conditions

Run	Incubation time (Days)	Inoculums size (% v/w)	Precursor (olive oil) (% v/w)	GA_3 yield (mg/kg substrate)		Error (ε)
				Experimental	Predicted	
1	7	21	2	31.947	31.5657	0.012
2	7	21	2	31.304	31.5657	-0.008
3	7	21	2	31.089	31.5657	-0.015
4	7	21	2	31.518	31.5657	-0.002
5	7	21	2	30.660	31.5657	-0.029

Conclusion

The present study had enhanced the production of gibberellic acid via solid state fermentation using *P. variable*. Banana peel (BP) was found as the most suitable solid substrate for solid state fermentation of GA_3 in static flask culture. It consist high amount of carbon to nitrogen ratio and sugar (glucose, sucrose and fructose) at 44.9 C/12.2 N, 311.2 g/kg substrate, 22.4 g/kg substrate and 516.7 g/kg substrate respectively.

Among 16 selected micro and macro-fungi, 4 species from *Trichocomaceace* family showed positive effect towards GA_3 production by solid state fermentation in static flask reactor and among all *P. variable* produce the highest GA_3 concentration. Placket Burman design was employed to select the critical media components that effected GA_3 production in static flask culture. Among 13 independent critical media components, 7 variables namely glucose and sucrose as carbon source, yeast extract as nitrogen source, olive oil as precursor, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ and $MnSO_4.7H_2O$ as trace elements showed positive and significant effect towards GA_3 production.

Response surface methodology couple with Box-Behnken design was then used to optimize the cultivation condition (physical and nutritional factors) of *P. variables* for the enhancement of GA_3 production in static flask culture. The statistical model predicted maximum GA_3 concentration at 31.57 mg/kg substrate at 7 days of incubation period, 21% (v/w) of inoculums size and 2% (v/w) of precursor concentration. Thus GA_3 production was enhanced by using *P. variable* and banana peel, waste from food industry as a substrate in solid state fermentation process.

Research out-put

List of conference and publication

1. Nur Kamilah, M. I. & Mashitah, M. D. (2011), Solid state fermentation of gibberellic acid by *Pencillium variable* using selected food processing wastes as a substrate. In International Conference on Biotechnology Engineering (ICBioE'11), Legend Hotel, Kuala Lumpur, Malaysia, May 17 - 19, ICBioE'11-105.
2. Nur Kamilah, M. I. & Mashitah, M. D. (2011), Solid state fermentation of gibberellic acid by *Pencillium variable* using selected food processing wastes as a substrate. *Journal of Revelation and Science*. (Accepted)
3. Nur Kamilah, M. I. & Mashitah, M. D. (2012), Statistical screening of media components for the production of gibberellic acid. In International Conference on Environment (ICENV 2012), Penang, Malaysia, December 11 – 13, ICENV 2012 - 38

Statement of Project Expenditure [60310020]

Vot No.	Budget (RM)	Expended Amount (RM)	Balance (RM)
111	16,800.00	15,414.06	1385.94
221	2,050.00	2,285.00	(235.00)
224	400.00	0.00	400.00
227	7,000.00	10,733.57	(3,733.57)
228	8,000.00	0.00	8,000.00
229	1,200.00	5,763.25	(4,563.25)
335	2,550.00	0.00	2,550.00
TOTAL	**38,000.00**	**34,208.38**	**3,791.62**

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OUTPUT

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Our Reference : SCE/ICENV2012/UF145

Date : 02 November 2012

Ms/Mr/Dr./Ir/Assoc. Prof./Prof.:
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ACCEPTANCE OF FULL PAPER FOR THE INTERNATIONAL CONFERENCE ON ENVIRONMENT 2012 (ICENV 2012), PENANG, MALAYSIA, 11-13 DECEMBER 2012

We would like to take this opportunity to thank you for your full paper submission and your interest to participate in ICENV 2012. We are pleased to inform you that upon evaluation by the conference's Paper Evaluation Committee, your full paper entitled **"STATISTICAL SCREENING OF MEDIA COMPONENTS FOR THE PRODUCTION OF GIBBERELLIC ACID"** has been accepted for **ORAL** presentation at the above mentioned conference. The detail program for the conference will be posted in the website around mid-November.

Participants that have yet to make payment for the conference registration fees, kindly be reminded that the payment should be made by **10 NOVEMBER 2012.** We regret to inform that if the payment is not received by **10 NOVEMBER 2012**, the paper may be withdrawn from the proceedings. We have now made the online-payment method available on the conference webpage. You could make the payment using your credit card on the web by following the given instructions.

For your kind information all food served during the conference is halal. If you need a vegetarian diet kindly notify the conference secretariat as soon as possible.

Please do not hesitate to contact us for any further enquiry. Looking forward to seeing you in Penang, Malaysia in December 2012.

Thank you.

Yours faithfully,

PROF. AZLINA HARUN @ KAMARUDDIN
Dean/Chairman ICENV 2012

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STATISTICAL SCREENING OF MEDIA COMPONENTS FOR THE PRODUCTION OF GIBBERELLIC ACID

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ABSTRACT

Gibberellic acids (GA_3) is high value plant growth regulators have received great demand in agriculture sector, and can be produced at higher concentration using fungi in solid state fermentation. In this study, *Penicillium variable* (*P. variable*) a wild species from the family of *Trichocomaceae* family was tested, and showed its ability to produce GA_3 at 16.25 mg /kg substrate in static flask culture using banana peel as a carbon source. Screening of the media components that influenced GA_3 production by *P. variable* were also carried out using Plackett Burman design. Results showed that banana peel supplemented with sucrose, yeast extract, olive oil and trace elements (Fe, Mg and Mn) were found as significant media components that influenced GA_3 production at 31.7 mg/kg substrate compared to that of banana peel alone as a fermentation medium.

Keywords: *Penicillium variable*; Plackett Burman Design; Banana Peel; Solid State Culture; *Tricocomaceace*

BIOTECHNOLOGY ENGINEERING
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Date: 12/01/2011

Author(s): **NUR KAMILAH MD ISA**

Dear Prof./Dr.

Paper ID 91: "Solid state fermentation of gibberellic acid by Penicillium simplicissimum in corn bran"

I am pleased to inform you that your extended abstract mentioned above has been accepted for oral presentation at the Second International Conference on Biotechnology Engineering [ICBioE'11] to be held during 17-19 May, 2011 at Kuala Lumpur, Malaysia.

The extended abstract had been reviewed and comments are sent via email (New results have been presented. Submit extended abstract using appropriate format for consideration.). Kindly prepare the full paper according to the comments suggested by the reviewer. The full paper should be prepared strictly as per the ICBioE'11 paper format (posted in website) and submitted by 28 February 2011.
Early bird registration fees are applicable to the papers registered by 1 April 2011.

All accepted contributions will be published in the conference proceeding. The selected high-quality papers presented at the conference will be considered for publication in the Journal of Applied Sciences and Biotechnology (ISI indexed).
I would like to remind you that the last date for the registration fee is 15 April 2011. Peer reviewed papers will be published only if the registration fee is paid by 30 April 2011.
Thank you for your interest in ICBioE'11 conference. We look forward to seeing you at ICBioE'11, Kuala Lumpur, Malaysia.
Yours Sincerely,

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ID-91:

Solid State Fermentation of Gibberellic Acid by *Penicillium* variable Using Selected Food Processing Wastes as a Substrate

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ABSTRACT

Gibberellic acid (GA_3) is one of the high valued plant growth regulators which have received great demand for boosting up agriculture sector, and can be produced by fungal growth via solid state fermentation. The usage of gibberellic acid is limited to high-premium crops due to the higher cost. In view of that, this study involved identifying the presence of GA_3 by cultivation of *Penicillium* variable in flask reactor with different substrates. Three types of food processing wastes namely corn bran; papaya peel and mango peel were selected for the production of GA_3 by the tested fungus. Results showed that the highest GA_3 production was detected at 18 mg GA_3/kg substrate after 9 days of fermentation period on corn bran.

Keywords: Gibberellic acid, *Penicillium* variable, solid state fermentation.

Subject: Selection of ICBioE paper's to Revelation and Science Journal

From: International Conference BioTech Engineering (icbioe@iium.edu.my)

To: nur_camiel@yahoo.co.uk;

Date: Tuesday, 28 June 2011, 9:48

Greetings from ICBIoE'11
Please be informed that your paper (ID 91) has been selected for publication in Revelation and Science Journal.
Kindly confirm with us within three days from the date of this e-mail whether you agree (or disagree) that the paper (ID 91) to be published in the above-mentioned Journal.

NOTE: Your paper (Solid state fermentation of gibberellic acid by Penicillium simplicissimum in corn bran) will be automatically withdrawn if we do not receive a positive reply within three working days.

All the best
Wassalam

Associate Professor Dr. Nassereldeen Ahmed Kabbashi
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SOLID STATE FERMENTATION OF GIBBERELLIC ACID BY *PENICILLIUM VARIABLE*

by

NUR KAMILAH BINTI MD ISA

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

MARCH 2013

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Nur Kamilah Binti Md Isa

School of Chemical Engineering, USM (2013)

OPTIMIZATION OF GIBBERELLIC ACID-3 PRODUCTION BY *PENICILLIUM* VARIABLE IN SOLID STATE FERMENTATION

By

(52) **ROSYANTI BINTI RAMLI**

Thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Chemical Engineering

April 2011

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