PRODUCTION OF ANIMAL FEED SUPPLEMENT BY EDIBLE FUNGI USING FRUIT WASTES AS CARBON SOURCE

BY

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ABSTRACT

Fruit wastes constitute high percentage of biodegradable residues emanating from fruit processing industries where they cause environmental challenges. These fruit wastes contained sufficient carbon source that can support fungi growth for conversion to animal feed supplement through biotechnological approach. Banana peel (Bp), pineapple peel (PAp) and papaya peel (Pp) were selected as substrates and the proximate analysis of the high solid content (HSC) type and low solid content (LSC) were performed. All samples contained simple and complex sugars that support fungi growth and development. Three white rot fungi –*Phanerochaete chrysosporium* (*P. chrysosporium*), *Panus tigrinus* (*P. tigrinus*) and *Schizophyllum commune* (*S. commune*), demonstrated profound growth and protein enrichment of the substrate with high enzyme secretion and elevated substrate consumption. Composite substrate from the three peels supported growth and protein enrichment by the fungi compared with individual substrates. All three fungi cells grew together on commercial media and formulated media. *P. chrysosporium*/*P. tigrinus* interaction and *P. chrysosporium*/*S. commune* interaction were deadlocked at contact, *P. tigrinus*/ *S. commune* interaction gave mutual intermingling while cultivation of the three together gave both deadlocked at contact and mutual intermingling. All microbial mixed cultures improved the protein secretion compared with their monocultures. Combination of *P. chrysosporium* and *S. commune* synthesized highest protein, enzymes and improved substrate consumption. Product synthesis in submerged phase bioconversion (SmB) was lower than solid state bioconversion (SSB); SSB was adopted after microbial interaction study. Substrate reformulation increased metabolizable sugar from 251 mg/g to 500 mg/g consisting 0.35g Bp, 5.5g PAp and 0.15g Pp; protein content increased from 104.22 mg/g to 160.68 mg/g. Media screening with Plackett-Burman design and optimization with face-centered-central composite design (FCCCD), gave KH_2PO_4 (1.2g/L), $CaCl_2$ (0.8g/L) and peptone (0.8g/L) as media components. Protein synthesis increased to 175.23 mg/g. Optimum pH (5.4), inoculum size (6.1%) and moisture content (70.2%) was achieved by FCCCD and protein synthesis increased to 198.77 mg/g. Kinetic study of biomass growth best fit with Monod equation (R^2 = 0.936), μ_{max} of 0.641 (day^{-1}) and K_s of 23.35 mg/g. Haldane equation had R^2 of 0.931, μ_{max} of 0.644 (day^{-1}) and K_i of 233.37 mg/g. Luedeking-Piret equation for substrate consumption gave R^2 of 0.9384; growth associated co-efficient (γ) of -49.08 mg/g and non-growth associated parameter (λ) of 48.862 mg/g/day. Product formation gave R^2 of 0.9888, growth associated co-efficient (α) of 0.0148 mg/g and non-growth associated parameter (β) of 0.0517 mg/g/day. Hanes-Woolf model fitted α-amylase (R^2 = 0.9108) and cellulase enzyme (R^2 = 0.9882) production. K_m and V_{max} of both were 11.55 Units/ml and 25.19 units/ml/day and 57.47 Units/ml, 3.05 units/ml/day respectively. Validation of parameters (media, process and kinetics) in 7 kg capacity reactor increased protein synthesis (228 mg/g), enzyme production, substrate consumption and improved productivity. Optimization of substrate depth and bioconversion period gave 2.5 cm as optimum depth and six days as optimum bioconversion period. Kinetics of in-vitro digestibility of flask optimized product and reactor, fitted into zero order model while nutritional analysis of final product showed great improvement in protein, amino acids and sugars.

ملخص البحث

تشكّل نفايات الفاكهة نسبة عالية من المخلفات القابلة للتحلل الناجمة عن صناعات معالجة الفاكهة، تحدياً للبيئة. تعدّ هذه النفايات مصدراً كافياً من الكربون يمكن أن يدعم نمو الفطريات لتحويلها إلى علف حيواني من خلال التكنولوجيا الحيوية. تم اختيار قشر الموز (BP)، وقشر الأناناس (PAP) وقشر البابايا (Pp) بمثابة ركائز للتحليل المباشر للمحتوى الصلب العالي (HSC) والمحتوى الصلب المنخفض (LSC). احتوت جميع العينات على السكريات البسيطة والمعقدة التي تدعم نمو الفطريات وتكاثرها. أظهرت ثلاثة فطريات نمواً عميقاً، خصوبة في البروتين إضافة إلى إفراز مرتفع للإنزيمات واستهلاك مرتفع للمادة: العفن الأبيض ذهبية الأبواغ *Phanerochaete chrysosporium* (*P. chrysosporium*)، *Panus tigrinus* (*P. tiginus*) وَ *Schizophyllum commune* (*S. commune*). دعم مخلوط القشور الثلاث نمو الفطريات وخصوبة البروتين مقارنة بكل واحد على حدة. نمت جميع الفطريات معاً في بيئة تجارية وكذلك في بيئة مصاغة. أظهر التفاعل بين *P. tigrinu* *P. chrysosporium*/ والتفاعل بين *P. chrysosporium*/*S. commune* إخفاقاً في النمو عند الاتحاد بينما أظهر التفاعل بين *P. tigrinus*/ *S. commune* اختلاطاً متبادلاً بينما زراعة الثلاث معاً في آن واحدٍ نتج عنه إخفاقاً في النمو وكذلك اختلاط متبادل. حسن مخلوط الفطريات معاً من إفراز البروتين مقارنةً بفطر مستقل بذاته في حين أن مزيجاً من *P. chrysosporium* وَ *S. commune* أنتج تركيزاً أعلى من البروتين، الإنزيمات وكذلك من حيث استهلاك المادة. من ناحيةٍ أخرى فإن مرحلة التحول الحيوي المغمور (SmB) أعطت تركيزاً أقل للناتج مقارنةً بالتحول الحيوي الصلب (SSB) ولذلك تم اعتماد (SSB) بعد دراسة التفاعل الميكروبي. ارتفعت صياغة السكر التمثيلي من ٢٥١ ملجرام/ جرام إلى ٥٠٠ ملجرام / جرام تتألف من ٠.٣٥ جرام Bp ، و ٥.٥ جرام PAp وَ 0.15 جرام Pp. كما ارتفع محتوى البروتين من ١٠٤.٢٢ ملجرام / جرام إلى ١٦٠.٦٨ ملجرام / جرام. فحص بيئة النمة مع تصميم Plackett-Burman والتحسين باستخدام التصميم المركب (FCCCD) أعطى النقاط المثلى كالتالي: KH_2PO_4 (١.٢ جرام /لتر)، $CaCl_2$ (٠.٨ جرام/ لتر)، وبيتون(٠.٨ جرام/ لتر) كمكونات لبيئة النمو.ارتفع كذلك تخليق البروتين إلى ١٧٥.٢٣ ملجرام/جرام وعند درجة الحموضة المثلى (٥.٤)، وحجم اللقاح (٦.١%) ونسبة الرطوبة (٧٠.٢%) من خلال FCCCD ، زاد تخليق البروتين إلى ١٩٨.٧٧ ملجرام/ جرام. دراسة حركية النمو للكتلة الحيوية تلاءمت مع معادلة Monod (R^2 = 0.936)، μ_{max} (٠.٦٤١) يوم$^{-١}$ وK_s (٢٣.٣٥ ملجرام/جرام). أما معادلة Haldane فأعطت (R^2= 0.931) ، μ_{max} ٠.٦٤٤،(٠.٦٤٤ يوم$^{-١}$) ، و K_i (٢٣٣.٣٧) ملجرام / جرام. أما معادلة Luedeking-Piret لاستهلاك الركيزة فأعطت (R^2= 0.9108) ، المعامل المرافق للنمو (γ) -٤٩.٠٨ ملجرام / جرام والمعامل غير المرافق (λ) ٤٨.٨٦٢ ملجرام/جرام/يوم. أعطى تشكيل المنتج R^2 بقيمة ٠.٩٨٨٨، والمعامل المرافق للنمو (α) ٠.٠١٤٨ ملجرام / جرام والمعامل غير المرافق (β) ٠.٠٥١٧ ملجرام / جرام / يوم. لاءم نموذج هنس وولف Hanes-Woolf إنزيم ألفا-أميليز بقيمة (R^2 = 0.9108) وإنزيم سليولين (R^2 = 0.9882). كانت K_m وV_{max} لكل من الإنزيمين ١١.٥٥ وحدة / ملل و ٢٥.١٩ وحدة / ملل / يوم، ٥٧.٤٧ وحدة/ ملل، ٣.٠٥ وحدة / ملل / يوم على التوالي. التحقق من العوامل (بيئة النمو، والحركية) في مفاعل بحجم ٧ كحجم أثبت إمكانية زيادة البروتين (٢٢٨ ملجرام / جرام)، إنتاج الإنزيم، واستهلاك الركيزة وتحسين الإنتاجية. أعطى الاستفادة من القيم المثلى من عمق الركيزة وفترة التحويل البيولوجي ٢.٥ سم كأفضل عمق وستة أيام للتحويل البيولوجي الأمثل. حركية الهضم في المختبر لاءمت معادلة الدرجة صفر في حين أظهر تحليل التغذوية للمنتج النهائي تحسن كبير في البروتين والأحماض الأمينية والسكريات.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degree at International Islamic University Malaysia or other institutions.

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Signature ……. Date ……

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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LIST OF SYMBOLS

μ	micro
^{o}C	degree Celsius
~	approximate
%	percentage
+	addition
×	multiplication
±	plus or minus
γ	Growth associated constant for substrate consumption
λ	Non-growth associated constant for substrate consumption
β	Non-growth associated constant for product formation
α	Growth associated constant for product formation
ln	Natural logarithm
t	Time

LIST OF ABBREVIATIONS

mm	millimetre
nm	nanometre
ml	milligrams
cm	centi metre
g	gram
Bp	Banana peel
PAp	Pineapple peel
Pp	Papaya peel
HSC	High solid content
LSC	Low solid content
HPLC	High Pressure Liquid Chromatography
FCCCD	Faced Centered Central Composite Design
RSM	Response Surface Methods
rpm	revolution per minutes
SSF	Solid state fermentation
TC	Total carbohydrate (mg/g)
TOS	Total Soluble Sugar (mg/g)
TRS	Total reducing sugar (mg/g)
w/v	Weight/Volume
WHO	World Health Organization
FAO	Food and Agricultural Organization
Y	Yield (mg/g/day)
$Y_{P/S}$	Yield of product based on substrate (mg/g/day)
$Y_{P/X}$	Yield of product based on growth (mg/g/day)
$Y_{X/S}$	Yield of microbial growth based on substrate (mg/g/day)
dx	Change in biomass (mg/g/day)
x	Biomass (mg/g)
V_{max}	Maximum velocity (Units/ml/day)
K_i	Substrate inhibition constant (mg/g)

K_s	Substrate consumption constant (mg/g)
μ_{max}	Maximum growth rate (mg/g/day)
μ	Specific growth rate (mg/g/day)
dP	Change in product formation (mg/g/day)
dt	Change in time (day)
K_m	Michealis menten constant
APHA	American public health association
ANOVA	Analysis of variance
et al.	(*et alia*): and others
etc.	(*et cetera*): and so forth
FCCCD	Free centered central composite design
CCD	Central composite design
SmB	Submerged state bioconversion
OPF	Oil palm fronds
POME	Palm oil mill effluent
EFB	Empty fruit bunches
PKC	Palm kernel cake
CP	Crude protein
ADF	Acid detergent fibre
NDF	Neutral detergent fibre
DNS	Dinitro salicylic acid
OM	Organic matter
UV	Ultra violet
UV-VIS	Ultra violet visible light
FTIR	Fourier transformation infra-red
AOAC	American organization of analytical chemists
BSA	Bovine serum albumen
PITC	Phenylisothiocyanate
mg/g	Milligram per gram
hrs	Hours
IIUM	International Islamic University Malaysia
OFAT	One factor at-a-time
PDA	Potato dextrose agar

MEA	Malt extract agar
ATCC	American Type Culture Collection
SEM	Scanning Electron Microscope
SDS	Sodium-dodecyl sulphate
NPN	Non-protein Nitrogen
SCP	Single cell protein
g/kg	Gram per Kilogram
U/g	One micro mole of substrate per minute per gram
U/mL	One micro mole of substrate per minute per millilitres
FPU/mL	Filter paper unit of cellulase per minute per millilitres
Min	Minutes