APPENDICES

APPENDIX A

Food waste analysis

Appendix A–1 Determination of moisture content according method of AOAC 1990 (Helrich, 1990)

Method

1. Dry the moisture can and lid in the oven at 100±2°C for 30 min and transfer to desiccator until cool for 30 min. Weigh the empty moisture can and lid (W_1).
2. Weigh about 5 g of sample to moisture can. Spread the sample to the uniformity. Then, weigh the moisture can and sample (W_2).
3. Place the moisture can with sample in the oven. Dry for 3 h at 100±2°C.
4. After drying, transfer the moisture can with partially covered lid to the desiccator until cool for 30 h. Weigh the moisture can and dried sample (W_3).

Calculation

$$\text{Moisture content (\%)} = \frac{(W_2 - W_3)\text{x } 100}{W_2 - W_1}$$

W_1 = Weight of moisture can (g)

W_2 = Weight of moisture can and sample before drying in an oven (g)

W_3 = Weight of moisture can and sample after drying in an oven (g)

Appendix A–2 Determination of ash content according method of AOAC 1990 (Helrich, 1990)

Method

1. Dry the crucible and lid in the furnace at 550°C for 30 min and transfer to desiccator until cool for 30 min. Then, weigh the crucible and lid (W_1).
2. Weigh the sample about 2–3 g into the crucible (W_2). Heat the crucible on a steam bath with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
3. Heat at 550°C overnight until the sample turns to gray (2–3 h). If not, return the crucible and lid to the furnace for the further ashing.
4. Cool down in the desiccator and weight the ash with crucible and lid (W_3).

Calculation

$$\text{Ash content (\%)} = \frac{(W_3 - W_1) \times 100}{W_2 - W_1}$$

W_1 = Weight of crucible and lid (g)

W_2 = Weight of crucible, lid and sample before drying in an oven (g)

W_3 = Weight of crucible, lid and sample after drying in an oven (g)

Appendix A–3 Determination of crude fat with Soxhlet extracts method according method of AOAC 1990 (Helrich, 1990)

Reagents

1. Petroleum ether

Method

1. Place the bottle and lid in the oven at 100±2°C overnight to ensure that weight of bottle is stable. Transfer to desiccator for 30 min and weigh the crucible and lid (W_2).
2. Weigh the sample about 0.5–1.0 g into paper filter and wrap (W_1).
3. Take the sample into extraction thimble and transfer into soxhlet.
4. Add the petroleum ether about 150 mL into the bottle and take it on the heating mantle.
5. Connect the soxhlet apparatus and turn on the water to cool them for 30 min at 10°C and then, switch on the heating mantle at 4–5 level about 14 h.
6. After that, switch off the heating mantle and evaporate the petroleum ether by using the vacuum condenser.
7. Dry the bottle in oven at 80–90°C until solvent completely evaporation and bottle is completely dry and then, transfer the bottle with partially covered lid to the desiccator until cool. Weigh the bottle and its dried content (W_3).

Calculation

$$\text{Crude fat content (\%)} = \frac{(W_3 - W_2) \times 100}{W_1}$$

W_1 = Weight of sample (g)

W_2 = Weight of bottle (g)

W_3 = Weight of bottle with crude fat (g)

Appendix A–4 Determination of crude protein with Kjeldahl method according method of AOAC 1990 (Helrich, 1990)

Reagents

1. conc. H_2SO_4
2. 0.1 M H_2SO_4
3. 50% NaOH solution
4. 4% Boric acid (H_3BO_3)
5. Indicator solution: Mix 100 mL of 0.1% methyl red in 95% ethanol with 200 mL of 0.2% bromocresol green in 95% ethanol.
6. Kjeldahl catalyst

Method

1. Weigh about 0.5–2.0 g of sample (W) and transfer into digestion tube.
2. Add 8.0 g of Kjeldahl catalyst and 20 mL of conc. H_2SO_4.
3. Prepare a tube containing the only chemical as blank. Place digestion tubes in inclined position and heat gently unit frothing ceases. Boil briskly until solution clears about 1 h.
4. Place the digested samples in digestion tubes to the distilling unit and add 70–90 mL of 50% (w/v) NaOH.
5. The sample is distilled until 100 mL of distillate collected in 50 mL of 4.0% (w/v) boric acid.
6. Add 2–3 drops indicator to the Erlenmeyer flask and titrate it with 0.1 M H_2SO_4 until the end point of the titration (Pink color).

Calculation

$$\text{Crude protein content (\%)} = \frac{(V_a - V_b) \times H_2SO_4 \times 1.4007}{W}$$

V_a = Volume (mL) of 0.1 M H_2SO_4 used sample titration

V_b = Volume (mL) of 0.1 M H_2SO_4 used in blank titration

H_2SO_4 = Concentration of sulphuric acid (M)

W = Weight of sample (g)

Appendix A–5 Determination of carbohydrate content with phenol-sulphuric method (Dubois et al., 1956)

Reagent

1. conc. H_2SO_4
2. 5% Phenol solution

Method

1. Pipette 1.0 mL of sample to test tubes and then, add 1.0 mL of 5% (w/v) phenol and 5.0 mL conc. H_2SO_4 in test tubes and mix well thoroughly.
2. Stand the mixture at room temperature for 30 min and measurement the absorbance at 490 nm
3. Prepare the standard glucose in the rage of 0–100 mg concentration from 100 mg/L stock solution. Plot the standard graph with absorbance at 490 nm (Y) and concentration of glucose (X).

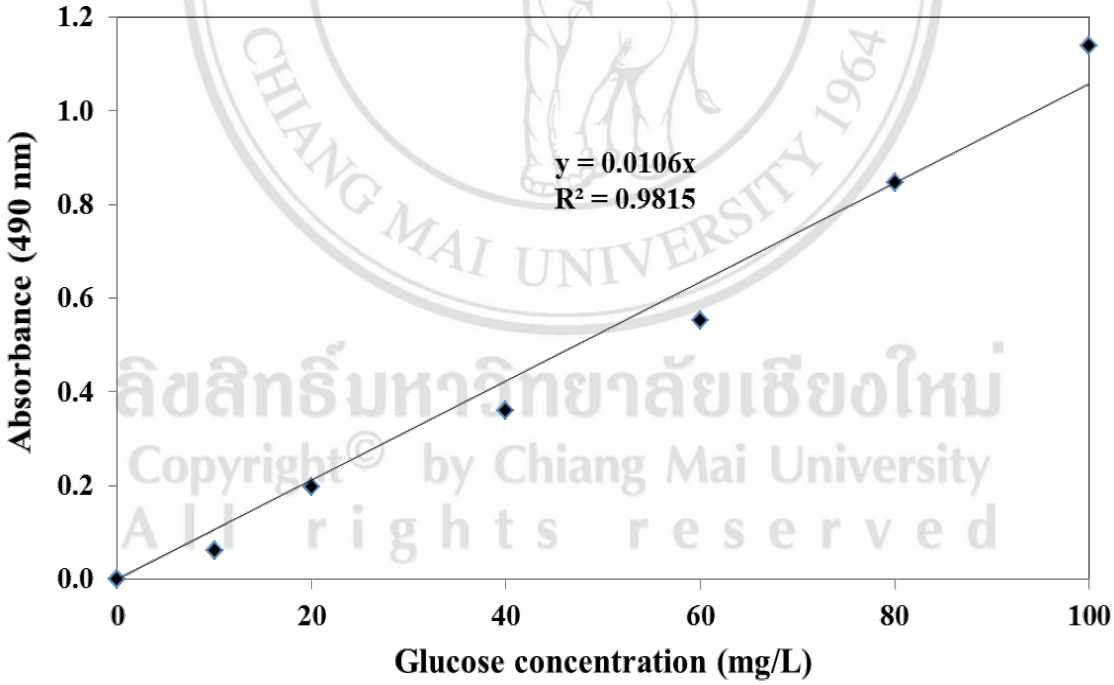


Figure A–1 Calibration curve of glucose

Appendix A–6 Determination of total carbon (Watanabe et al., 2011)

Energy dispersive X-ray spectrometry (EDXS) is the standard method for elemental analysis using an electron microscope. The total carbon content of rice residue was analyzed with EDXS on TM1000 Tabletop electron microscope. The point analyses of 10–20 nm in diameter at an accelerating voltage of 80 keV. The time of registration of the signals forming the spectrum is 400 s for each point analysis.

Appendix A–7 C:N ratio determination (Braunwald et al., 2013)

The C:N ratio was calculated based on molar basis of the carbon content of rice residue from food waste and the nitrogen content of rice residue and nitrogen source in the production medium according to the method of Braunwald et al. (2013). The nitrogen content of rice residue, yeast extract and $(NH_4)_2SO_4$ were analyzed with Kjeldahl method (Helrich, 1990) (Appendix A–4). The carbon content of rice residue was analyzed with Energy dispersive X-ray spectrometry (EDXS) (Watanabe et al., 2011) (Appendix A–6).

Calculation

$$\text{C:N ratio} = \frac{\text{Carbon source (g)} \times \%\text{C}_{\text{Carbon source}}}{\text{Nitrogen source (g)} \times \%\text{N}_{\text{Nitrogen source}}}$$

Rice residue from food waste contains 41.40% C (w/w) and 1.30% N (w/w).

Yeast extract contains 11.16% N (w/w) and $(NH_4)_2SO_4$ includes 21.20% N (w/w)

APPENDIX B

Media

Appendix B–1 Yeast malt-extract medium (YM) (per Liter)

Yeast extract	4.0	g
Malt extract	10.0	g
Glucose	4.0	g

YM preparation

Mix all components of yeast-malt extract medium, adjust to a final volume of 1,000 mL with distilled water and then, autoclave at 121°C for 15 min.

Appendix B–2 Basal medium supplemented with glucose

Yeast extract	1.0	g
Glucose	10.0	g
di-Potassium hydrogen phosphate (K_2HPO_4)	3.7	g
Potassium di-hydrogen phosphate (KH_2PO_4)	5.5	g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.5	g
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	0.2	g
Ammonium sulphate ($(NH_4)_2SO_4$)	5.3	g
Sodium chloride (NaCl)	0.5	g

Mix all components of basal medium, adjust to a final volume of 1,000 mL with distilled water. Adjust the pH to 6.0 with H_3PO_4 or 0.1 M KOH and then autoclave at 121°C for 15 min.

Appendix B–3 Basal medium supplemented with rice residue hydrolysate from food waste

Yeast extract	1.0	g
Rice residue hydrolysate from food waste	10.0	g
di-Potassium hydrogen phosphate (K_2HPO_4)	3.7	g
Potassium di-hydrogen phosphate (KH_2PO_4)	5.5	g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.5	g
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	0.2	g
Ammonium sulphate ($(NH_4)_2SO_4$)	5.3	g
Sodium chloride (NaCl)	0.5	g

Mix all components of basal medium, adjust to a final volume of 1,000 mL with distilled water. Adjust the pH to 6.0 with H_3PO_4 or 0.1 M KOH and then autoclave at 121°C for 15 min.

Appendix B–4 Basal medium supplemented with soluble starch

Yeast extract	1.0	g
Soluble starch	10.0	g
di-Potassium hydrogen phosphate (K_2HPO_4)	3.7	g
Potassium di-hydrogen phosphate (KH_2PO_4)	5.5	g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.5	g
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	0.2	g
Ammonium sulphate ($(NH_4)_2SO_4$)	5.3	g
Sodium chloride (NaCl)	0.5	g

Mix all components of basal medium, adjust to a final volume of 1,000 mL with distilled water. Adjust the pH to 6.0 with H_3PO_4 or 0.1 M KOH and then autoclave at 121°C for 15 min.

Appendix B–5 Basal medium supplemented with rice residue from food waste

Yeast extract	1.0	g
Rice residue from food waste	11.1	g
di-Potassium hydrogen phosphate (K_2HPO_4)	3.7	g
Potassium di-hydrogen phosphate (KH_2PO_4)	5.5	g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.5	g
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	0.2	g
Ammonium sulphate ($(NH_4)_2SO_4$)	5.3	g
Sodium chloride (NaCl)	0.5	g

Mix all components of basal medium, adjust to a final volume of 1,000 mL with distilled water. Adjust the pH to 6.0 with H_3PO_4 or 0.1 M KOH and then autoclave at 121°C for 15 min.

Appendix B–6 The optimized basal medium supplemented with rice residue from food waste (CCD optimization model)

Yeast extract	0.5	g
Rice residue from food waste	73.0	g
di-Potassium hydrogen phosphate (K_2HPO_4)	0.5	g
Potassium di-hydrogen phosphate (KH_2PO_4)	10.0	g
Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$)	0.2	g
Manganese sulphate monohydrate ($MnSO_4.H_2O$)	0.1	g
Ammonium sulphate ($(NH_4)_2SO_4$)	1.0	g
Sodium chloride (NaCl)	1.0	g

Mix all components of basal medium, adjust to a final volume of 1,000 mL with distilled water. Adjust the pH to 5.44 with H_3PO_4 or 0.1 M KOH and then autoclave at 121°C for 15 min.

APPENDIX C

Analytical methods

Appendix C–1 Sudan black B technique (Kitcha and Cheirsilp, 2011)

Reagent

Sudan black B solution: 0.3 g of Sudan black B powder in 100 mL of methanol

Method

1. Smear yeast strain on a slide.
2. Let it dry thoroughly in the air and heat fix.
3. Flood the entire slide with Sudan black B solution and allow the slide to remain undisturbed for 10 min.
4. Wash with water and air dry.
5. Observe stained yeasts under a phase contrast microscope on oil immersion for the presence of blue or greyish colored fat globules within the cell.

Appendix C–2 Quantitative analysis of carotenoids by high performance liquid chromatography (HPLC) (Manowattana et al., 2015)

Conditions

System controller	SCL-10Avp
Column	C18 (4.6 mm × 250 mm, 5μm)
Detector	UV detector at 454 nm
Flow rate	1.00 mL/min
Oven temperature	30°C
Mobile phase	Acetonitrie: Dichloromethane: Methanol (80:10:10, v/v/v)
Injection volume	20 μL
Total run time	40 min

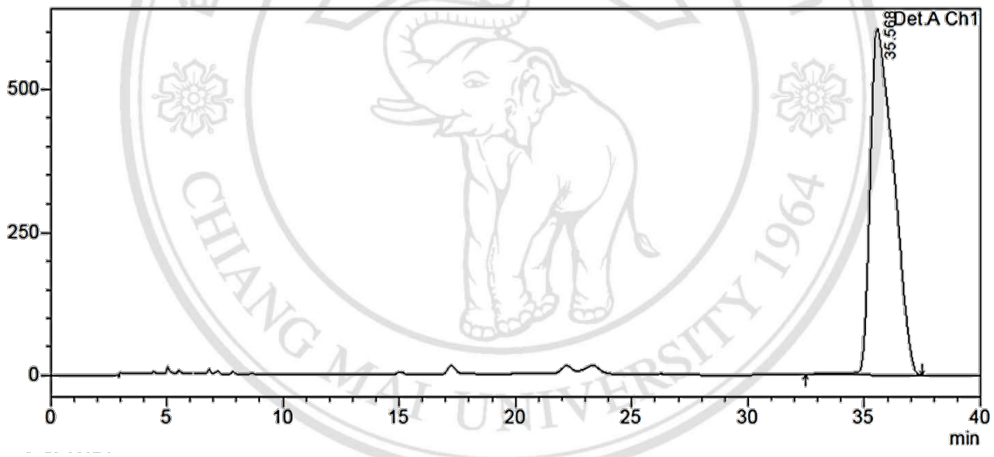
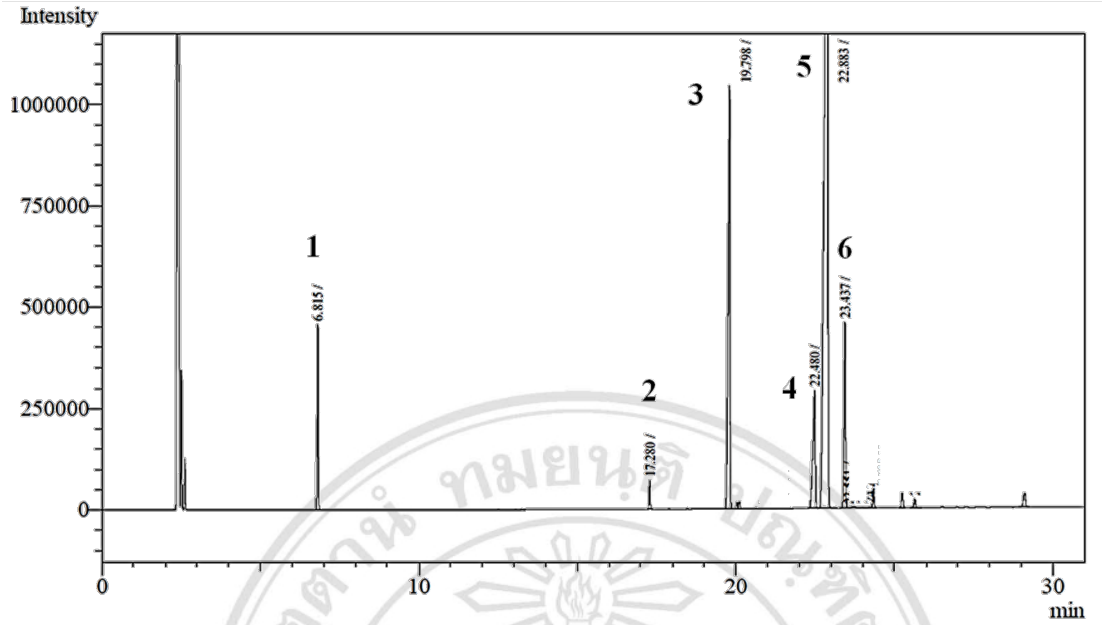


Figure C–1 Chromatogram of β-carotene analyzed by HPLC. The retention time was 35.568 min for β-carotene

Appendix C-3 Quantitative analysis of fatty acid composition by gas chromatography with flame-ionization detector (GC-FID) (Chaiyaso et al., 2012)

Conditions

System controller	GC-2010 Autosystem Gas Chromatography
Column	HP-INNOWax column (30 m × 0.25 mm, 0.25 μm)
Split ratio	100:1
Detector	Flame ionization
Temperature program	60°C (2 min), 200°C (10°C/min), 240°C (5°C/min), hold 240°C (7 min)
Injection volume	1.0 μL
Injector temperature	250°C
Detector temperature	250°C



Peak	Name	Retention time (min)	Height (mV)	Area	Area (%)
1	Methyl hexanoate (C6:0)	6.815	451,524	962,863	Internal standard
2	Myristic acid (C14:0)	17.280	70,204	155,915	0.76
3	Palmitic acid (C16:0)	19.798	1,040,646	4,249,602	20.69
4	Stearic acid (C18:0)	22.480	289,346	1,516,636	7.38
5	Oleic acid (C18:1)	22.883	1,908,490	13,142,138	63.99
6	Linoleic acid (C18:2)	23.437	453,411	1,473,914	7.18
			3,762,097	20,538,205	100

Figure C–2 Chromatogram of fatty acid analyzed by gas chromatography with flame-ionization detector.

Appendix C–4 Quantitative analysis of fatty acid composition by gas chromatograph mass spectrometer (GC-MS) (McCurry, 2011)

Conditions

Gas Chromatography (GC)

System controller	GC-7890A Gas Chromatography
Column	DB5-MS column (30 m × 0.25 mm × 0.25 μm)
Split ratio	100:1
Temperature program	60°C (2 min, 10°C/min), 200°C (5°C/min), 240°C (7 min)
Injection volume	1.0 μL
Inlet temperature	250°C
Detector temperature	280°C

Mass Spectrometer (MS)

System controller	MSD 5975C (EI) Mass Spectrometer
Scan parameter	30-500 amu
MS Quadrupole	150°C
MS Source	230°C

Appendix C–5 Reducing sugar analysis by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959)

Reagent

1. 3,5-Dinitrosalicylic acid
2. Glucose (AR grade)
3. Potassium sodium tartrate solution (300 g of potassium sodium tartrate in 500 mL deionized water)
4. 2 M NaOH solution

Method

Preparation of DNS solution

1. Dissolve 10.0 g of 3,5-dinitrosalicylic acid in 200 mL of 2 M NaOH solution.
2. Heat and stir until well dissolve but did not boil.
3. Add 500 mL of potassium sodium tartrate solution.
4. Adjust the solution to 1,000 mL with deionized water.

Preparation of calibration curve

1. Prepare the standard glucose in the rage of 0.0–1.0 mg/mL concentration from 1.0 mg/mL stock solution.
2. Pipette 100 µL of each concentration and 300 µL of DNS solution in microtube.
3. Mix well by vortex and boiling for 5 min.
4. Stand the microtube in ice bath for 20 min.
5. Add 600 µL of distilled water.
6. Mix well by vortex and measure at absorbance 540 nm.

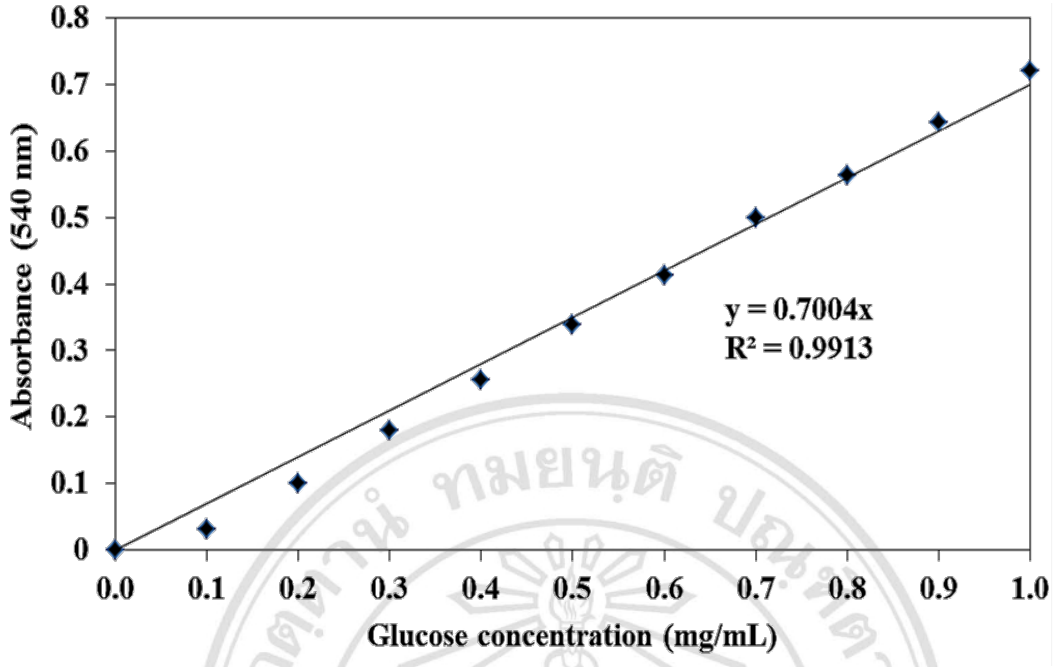


Figure C–3 Standard curve of glucose

Appendix C–6 α-Amylase activity assay (Xie et al., 2014)

Substrate: 0.5% (w/v) soluble starch in 0.05M citrate pH 5.5

Buffer: 0.05M citrate pH 5.5

Table C–1 Reaction mixture of α-amylase activity assay

Reaction mixture	Enzyme (µL)	Substrate (µL)	Buffer (µL)
Control	-	-	100
Enzyme + Substrate (ES)	50	50	-
Enzyme + Buffer (EB)	50	-	50
Substrate + Buffer (SB)	-	50	50

Method

1. Pipette the reaction mixture in microtube (Table C–1) and incubate at 30°C on water bath for 10 min.
2. Stop the reaction immediately by adding 300 µL of DNS solution in microtube.
3. Mix well by vortex and boiling for 5 min.
4. Stand the reaction immediately by stand the microtube in cool bath.
5. Add 600 µL of distilled water in the reaction mixture and mix well by vortex.
6. Measure at absorbance 540 nm.

Calculation

$$\text{Net absorbance (Abs)} = \text{ES-EB-SB}$$

$$\text{α-Amylase activity (U/mL)} = \frac{\text{Net Abs} \times \text{substrate (mL)} \times 1000 \left(\frac{\mu L}{mL}\right)}{\text{Slope} \times 180 \left(\frac{g}{mole}\right) \times 10 \text{ (min)} \times \text{enzyme (µL)}}$$

One unit (U) of enzyme activity was defined as the amount of enzyme that liberated 1.0 µmol of reducing sugar as glucose per minute under the assay conditions.

Appendix C–7 Amyloglucosidase (AMG) activity assay (Teste et al., 2010)

Substrate: 5 mM *p*-Nitrophenyl-α-D-glucopyranoside in 0.1 M citrate buffer, pH 4.0

Buffer: 0.1 M Citrate buffer, pH 4.0

Method

1. Pipette 50 µL of enzyme and 400 µL of substrate in microtube.
2. Mix well by vortex and incubate at 30°C for 5 min.
3. Then, stop the reaction by add 1000 µL of sodium carbonate (Na_2CO_3).
4. Mix well by vortex and measure the releasing of *p*-Nitrophenyl at absorbance 400 nm.

Calculation

$$\text{Activity (U/mL)} = \frac{\Delta E}{\varepsilon \times d \times C}$$

△E = Absorbance at 400 nm

ε = Molar extinction coefficient at pH 4.0 (L/mol/cm)

d = Cuvette width (cm)

C = Amount of enzyme (mL)

The molar extinction coefficient of *p*-Nitrophenol at 400 nm at pH 4.0 = 18 L/mol/cm (Bessey et al., 1946)

APPENDIX D

Nucleotide sequence

Appendix D–1 Nucleotide sequence of 26S rDNA gene of *Rhodotorula glutinis* (Isolate C7)

Sample name: Isolate C7

Identify: *Rhodotorula glutinis*

Accession number: KM281508

GCGAGTTCGGGCNCCAAGNTAAATNGACACCCTNGATNCACNAANGANCCTTCCGTAGG
TGAACCTGCGGAAGGATCATTAGTGAATCTAGGGTGTCCAATTTAACTTGGAGCCCGAA
CTCTCACTTTCTAACCCTGTGCATCTGTTATTGGTTAGTAACTCTTCGGAGTGAACTCC
ATTCACTTACAAACACAAAGTCTATGAATGTATACAAATTATAACAAAACAAAACTTTC
AACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAAT
GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTA
TTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACCCACCTCTTTCTTAGTG
AATCTGGTGGTGCTTGGTTTCTGAGCGCTGCTCTGCTTCGGCTTAGCTCGTTCGTAATG
CATTAGCATCCGCAATCGAACTTCGGATTGACTTGGCGTAATAGACTATTCGCTGAGGA
TTCTAGTCTCTGACTAGAGCCGGGTTTGGTTAAAGGAAGCTTCTAATCCTAAAGTCTAT
TTTTTGATTAGATCTCAAATC

Appendix D–2 Nucleotide sequence of 26S rDNA gene of *Rhodosporidium* sp. (Isolate C10)

Sample name: Isolate C10

Identify: *Rhodosporidium* sp.

Accession number: KX281510

TCAAGAACATGATCACAGGAACTTCACAAGCTGATTGCGCCGTATTGATTGTTGCTGCC
GGTACTGGTGAGTTCGAAGCTGGTATCTCAAAGAACGGACAGACCCGTGAGCACGCCCT
TCTCGCCTTTACCCTTGGTGTCAAACAACTCATTGTTGGAGTCAACAAGATGGACTCAA
CTGAACCACCATACAGCGAATCACGTTTCGAGGAAATCAAAAAAGAAGTTTCATCTTAC
ATCAAGAAAATTGGTTACAACCCAGCTGCTGTTGCATTCGTACCAATCTCAGGATGGCA
CGGAGACAACATGTTGGAGCCATCCACCAAAATGCCCTGGTTCAAGGGATGGGCTGTTG
AGCGCAAAGAAGGTAAAGCTGATGGCAAGTGCCTCATTGAAGCTTTGGATGCCATCCTT
CCTCCAAGCCGTCCCACAGAGAAACCACTCCGTCTTCCCCTTCAGGATGTCTACAAAAT
TGGAGGTATT

Appendix D–3 Nucleotide sequence of 26S rDNA gene of *Sporidiobolus pararoseus* (Isolate TC32)

Sample name: Isolate TC32

Identify: *Sporidiobolus pararoseus*

Accession number: KX709872

GCGGCGAGCGAAGCGGGAAAAGCTCAAATTTGTAATCTGGCGTCTTCGACGTCCGAGTT
GTAATCTCGAGAAGTGTTTTCCGTGATAGACCGCATACAAGTCTCTTGGAACAGAGCGT
CATAGTGGTGAGAACCCAGTACACGATGCGGATGCCTATTACTTTGTGATACACTTTCG
AAGAGTCGAGTTGTTTGGGAATGCAGCTCAAATTGGGTGGTAAATTCCATCTAAAGCTA
AATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTT
GGAAAGAGAGTTAACAGTACGTGAAATTGTTGGAAGGGAAACACATGCAGTGATACTTG
CTATTCGGGGCAACTCGATTGGCAGGCCCGCATCAGTTTTTCGGGGCGGAAAATCGTAG
AGAGAAGGTAGCAGTTTCGGCTGTGTTATAGCTCTTTACTGGATTCGCCCTGGGGGACT
GAGGAACGCAGCGTGCTTTTAGCATGAGCTTCGGCTTATCCACGCTTAGGATGCGGGTT
TATGGCTGTATATGACCCGTCTTGAAAACACGGACCAA

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24th July–3rd August 2016	Japan-Asia Youth Exchange Program in Science, Japan Science and Technology Agency, Japan
2016–2017	The National Research Council of Thailand (NRCT), Thailand

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Proceeding Srisuwan S., Techapun, C., Seesuriyachan, P., Watanabe, M. and Chaiyaso, T. 2016. Screening of oleaginous yeast for lipid production using rice residue from food waste as a carbon source. The 6th International Conference on Fermentation Technology for Value Added Agriculture Products (FerVAAP2015).

Presentation Srisuwan S., Techapun, C., Seesuriyachan, P., Watanabe, M. and Chaiyaso, T. 2015. Screening of oleaginous yeast for lipid production using rice residue from food waste as a carbon source. The 6th International Conference on Fermentation Technology for Value Added Agriculture Products (FerVAAP2013). July 29th–31st, 2015. Centara Hotel & Convention Centre Khon Kaen, KhonKaen, Thailand. [Poster Presentation]