Characteristics of used palm olein and its bioconversion into polyhydroxybutyrate by *Cupriavidus necator* H16

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ABSTRACT

Aims: The accumulation of synthetic plastic waste that pollutes the environment has raised awareness of the advantages of biodegradable polymers such as polyhydroxybutyrate [P(3HB)]. Nevertheless, high production costs of such environmental-friendly plastics have held back its more widespread use. One major cost component is the carbon source used in its production. Hence, this study was undertaken to evaluate used palm olein (UPO) as a potential carbon source for the production of P(3HB).

Methodology and results: Both UPO and fresh palm olein (FPO) were analysed for physicochemical and proximate characteristics and thermal properties. Compared with FPO, the composition of palmitic acid (C16:0) in UPO was increased from 40.3% to 43.7%, while the composition of linoleic acid (C18:2) was reduced from 11.3% to 7.6%. The iodine value declined from 55.8 g I_2/100g in fresh palm olein to 39.5 g I_2/100g in UPO, this being an indication of increased saturated bonds that contribute to a high efficiency of P(3HB) synthesis. Production of P(3HB) by *Cupriavidus necator* H16 using UPO as the carbon source reached 83% of dry cell weight, with the highest P(3HB) content attained after 48 h.

Conclusion, significance and impact study: We conclude that UPO has the potential to be used as a low cost carbon source for the efficient biosynthesis of P(3HB).

Keywords: Cupriavidus necator H16*,* palm olein, polyhydroxybutyrate, vegetable oil

INTRODUCTION

The widespread use of synthetic polymers for disposable consumer products has resulted in an accumulation of non-degradable petroleum-based plastics in the environment. The presence of such wastes is especially harmful in rivers, lakes and the sea where they pose as a hazard to aquatic inhabitants. While the stability of petroleum-based plastics is useful in many applications that demand durability, its resistance towards degradation remains a severe pollution to the environment (Park and Kim, 2011).

Polyhydroxyalkanoates (PHA), a potential substitute material for some conventional plastics, is an environmental-friendly plastic that undergoes total biodegradation while possessing close similarities in physical characteristics with some petrochemical plastics. This biocompatible and biodegradable polymer is produced when nutrients such as nitrogen, phosphorus, or magnesium are depleted while a suitable carbon source is present in excess (Sudesh *et al.*, 2000; Rao *et al.*, 2010).

The entire process of PHA production has been evaluated in the aspects of substrate selection, bacterial strain, fermentation process strategy and recovery dynamics (Park and Kim, 2011). Nevertheless, the commercialization of this bioplastic was restrained due to high production costs as compared to the lower costs in producing petrochemical-synthetic polymers (Lee *et al.*, 1999). A major component of the production costs is the carbon source used in the fermentation substrate, which can account for one half of the capital outlay (Song *et al.*, 2008).

Among the carbon substrates for fermentation, vegetable oils have been found favourable owing to its relatively low cost and its efficiency in PHA production (Song *et al.*, 2008). In this regard, the biosynthesis of PHA from vegetable oils is worthy of consideration as they have a higher number of carbon atoms per unit weight in comparison to sugars that are commonly used as the carbon source. In studies that have been carried out,

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vegetable oils produced 1.0 g-PHA per g of vegetable oil as compared to 0.32-0.48 g-PHA per g of sugar used (Yamane, 1992).

However, the consumption of food-grade oils for non-food purposes, such as fuel for energy needs, has received heavy criticism since such a move could hasten the depletion of world food supplies. Hence, a diversion of palm olein from the human food-chain for the production of bioplastics could meet with similar resistance. On the other hand, the use of waste vegetable oils such as used palm olein (UPO) as the carbon source of PHA would not invoke this criticism. The disposal of waste vegetable oils is increasing from year to year, and in the European Union, approximately 700,000−1,000,000 tonnes are discarded every year (Kulkarni and Dalai, 2006). The disposal or spillage of UPO into the waterways is potentially dangerous as it causes serious physical damage to the aquatic environment. The resulting increases in the chemical and biological oxygen demand impose severe stress on fresh water and marine life (Akaraonye *et al.*, 2010). Hence, collection of UPO from local community provides a solution to the disposal problems created by improper treatment of waste cooking oil. From the health perspective, re-using UPO for cooking can be harmful. Its constituents such as peroxide, aldehyde, *trans*-fatty acid and other by-products may stimulate various kinds of diseases and disorders over the long-term (Kulkarni and Dalai, 2006). Thus, the use of UPO as carbon source in PHA production could mitigate this environmental issue and at the same time address the problem pertaining to the cost of PHA production (Akaraonye *et al.*, 2010).

In this study, we used *Cupriavidus necator* H16 to produce the most common type of PHA, poly(3-hydroxybutyrate) [P(3HB)] utilizing UPO as the sole carbon source. It is suspected that the composition in the waste cooking oil due to the reactions during the frying process assist the efficient production of PHA. Thus, we carried out comparative characterisation of fresh palm olein (FPO) and UPO, about which little has been reported.

MATERIALS AND METHODS

Palm olein characterisation

Physicochemical analyses

AOAC (Association of Official Analytical Chemists) 2000 methods were used for the determination of free fatty acids (FFA), acid value (AV), peroxide value (PV) and iodine value (IV). FFA was analysed according to AOAC 940.28. PV and IV were determined according to AOAC 965.33 and AOAC 993.20 respectively (Horwitz, 2000). The K-value and *p*-anisidine value (PAV) were determined according to IUPAC (International Union of Pure and Applied Chemistry) (1992). To determine the K-value, 25 mL of 2, 2, 4-trimethylpentane was added to 0.25 g of the oil sample and then shaken well to obtain a clear solution. The absorbance of the mixture was measured by using a

Hitachi U-1900 spectrophotometer at 232 and 270 nm. A further reading was taken at K_{350} before 1 mL *p*-anisidine was added to 5 mL of the mixture. The mixture was incubated in the dark for 10 minutes prior to absorbance measurement at 350 nm. The PAV was calculated according to Eq. (1) (Dieffenbacher and Pocklington, 1992):

$$PAV = \left[25 \times \left(1,2A_s - A_b\right)\right]/\left(g\,sample\right)$$

(1)

Where, A_s and A_b is the absorbance of the mixture before and after addition of *p*-anisidine solution respectively, while g refers to the mass of oil sample.

Viscosity

Viscosity of the oil sample was analysed at 25 °C using a sine-wave Vibro-Viscometer SV-10 (A&D Company, Limited, Japan) from 0.3−10.000 mPa·s, with resonance frequency of 30 Hz in sample container of 35 mL. The viscometer was calibrated with JS20 standard calibration solution.

Proximate analysis

Fatty acid composition and moisture content

Fatty acid composition was determined according to AOCS (American Oil Chemists' Society) Ce 1e-91, and moisture content was measured by Karl Fischer titration. Both procedures were undertaken by SGS Laboratory Services (M) Sdn. Bhd.

Pigment content

Pigment content such as chlorophylls and carotenoids in oil sample was analysed using Hitachi U-1900 spectrophotometer. Approximately 7.5 g oil sample was dissolved in 25 mL cyclohexane (C_6H_{12}). It was then transferred into a cuvette, and measured at 670 nm and 470 nm wavelengths, which originates from the photometric absorbance of chlorophylls and carotenoids, respectively. The formulae to determine these contents are as follow (Moyano *et al.*, 2008):

[Chlorophylls] = [(A_{670} × 10^6)/ (613 × 100 × oil density)] mg/kg   (2)

[Carotenoids] = [(A_{470} × 10^6)/ (2000 × 100 × oil density)] mg/kg   (3)

Elemental analysis

Analysis of carbon, hydrogen and nitrogen content in the oil sample was performed on a Perkin Elmer Series II CHNS/O analyser 2400 by the Environmental Laboratory, School of Industrial Technology.

Attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR)

ATR-FTIR spectra of oil samples were collected in the range of $4000 - 650$ cm^{-1} with a resolution of 2 cm^{-1} using Nicolet iS10 spectrophotometer. Number of background and sample scans was 64 times.

Thermal analyses

Thermal properties of oil sample were investigated using the techniques of thermogravimetric analysis (TGA) and differential scanning calorimeter (DSC). In TGA analysis, approximately $10-20$ mg oil sample was weighed in porcelain crucible, and heated under dry nitrogen (flow rate: 50 mL min^{-1}, purity 99.9997%) from 25 °C to 700 °C at a heating rate of 20 °C min^{-1} using a Perkin Elmer TGA6 thermogravimetric Analyser equipped with C6 chiller. The coolant used was a mixture of alcohol and water in ratio 1:1. Melting characteristics of oil sample were determined by differential scanning calorimeter (DSC Q200, TA Instruments, New Castle, DE, USA) equipped with refrigerated cooling system (RCS-90, TA Instruments, New Castle, DE, USA). An exact amount of oil sample ($5-10$ mg) was heated under dry nitrogen gas (flow rate: 50 mL min^{-1}, purity 99.9997%) from 30 °C to 80 °C at a heating rate of 20 °C min^{-1} and hold for 5 min. Soon after, the sample was cooled to -80 °C at a cooling rate of 5 °C min^{-1} and hold for 5 min. The sample was then re-heated to 80 °C.

Poly(3-hydroxybutyrate), [P(3HB)] biosynthesis

Bacterial strain and culture conditions

Cupriavidus necator H16 was used for the production of PHA. The strain was maintained on mineral salts medium (MM) agar containing (per litre): 0.5 g NH_4Cl, 2.8 g KH_2PO_4, 3.32 g Na_2HPO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 14 g agar, 5 g fructose and 1 mL of trace elements. The bacterium was cultured in nutrient rich medium containing (per litre): 2 g yeast extract, 10 g meat extract and 10 g peptone. Trace elements were added based on Kahar *et al.* (2004).

Carbon source

UPO collected from households in the Teluk Bahang community (Penang, Malaysia) was used as the sole carbon source for P(3HB) biosynthesis. UPO and FPO (Vesawit palm olein cooking oil manufactured by Yee Lee Corp., Malaysia) were sterilized separately at 121 °C for 15 min prior to adding into MM broth for P(3HB) biosynthesis.

Biosynthesis of P(3HB)

Two loopsful of bacterial cells were inoculated into 50 mL nutrient rich medium in 250 mL shake flasks and incubated at 30 °C on a rotary shaker set at 200 rpm. The culture was transferred into 50 mL MM medium using of 3% (v/v) as the starter inoculum when the optical density of 4.5 to 5.0 at 600 nm (OD_{600}) was reached after approximately 7 h. Incubation was carried out at 30 °C, 200 rpm, for 72 h. Various nitrogen sources, adjusted to 5 mM, were evaluated for their use in the culture medium. The carbon source, UPO, was added to a concentration of 5 g/L. The dry cell weight (DCW) and the PHA produced were collected for further experiments.

Different concentrations of UPO were evaluated with selected nitrogen source adjusted to a concentration of 0.54 g/L. Cells from the culture were harvested by centrifugation at 4 °C (15,816 *g*) for 10 min. Cell pellets were re-suspended with 5 mL hexane to remove residual oil and centrifuged at 4 °C (15,816 *g*) for 5 min. Prior to freeze-drying, the pellets were washed with 50 mL of distilled water and centrifuged at 4 °C (15,816 *g*) for 10 min before freezing at –20 °C for 24 h.

Residual oil analysis

The residual oil in the culture media was measured by adding 2 mL culture medium into 5 mL hexane. After 1 min of vortex mixing, the mixture was allowed to stand for phase separation. The upper layer of hexane phase was sampled and dried on a pre-weighed plastic plate to constant weight.

Analytical procedures

P(3HB) content in freeze-dried cells was determined by gas chromatography (Shimadzu GC-2010, Japan). Freeze-dried cells were subjected to methanolysis by heating in a mixture of 2 mL chloroform and 2 mL methanolysis solution (15% v/v of sulphuric acid:85% v/v of methanol) for 140 min at 100 °C. Samples of the converted hydroxyacyl methyl ester solutions (50 µL) were injected at an injector temperature of 270 °C (Braunegg *et al.*, 1978). SPSS *version 17.0* was used in the statistical analyses.

RESULTS AND DISCUSSIONS

In this study, UPO was selected as the carbon source for P(3HB) synthesis by *C. necator* owing to its low price and the fact that it is a renewable resource and a waste product. Various nitrogen sources and UPO concentration suited for PHA production by *C. necator* was standardised at 5 g/L as initial concentration in order to evaluate the P(3HB) production. Table 1 shows the effect of various nitrogen sources to dry cell weight (DCW) of PHA production as well as the yield. A DCW of 5.8 g/L and P(3HB) accumulation of 51 wt% was achieved when 0.54 g/L urea was fed to *C. necator* utilising UPO after 72 h. Similar results were also obtained when ammonium sulphate and sodium nitrate were used. Since urea is currently cheaper than ammonium sulphate and sodium nitrate, the concentration of urea is chosen and studied. According to Ng *et al.* (2010), urea gave a satisfactory bacterial growth rate, and it is also an economical nitrogen

source for potential large scale production of PHA. Figure 1 shows P(3HB) biosynthesis as a function of urea concentrations. It is clear that urea at a concentration of 1 g/L supported the maximum DCW of 14.6 g/L and P(3HB) content at 78 wt%. The residual oil remained constant and DCW increased as the urea concentration increased due to the effect of low C/N ratio. This result is supported by the elemental analysis done on UPO and compared with

Table 1: Effects of different nitrogen sources on the biosynthesis of P(3HB) from UPO.

Nitrogen source	Total P(3HB) (g/L)	Cell dry weight (g/L)	P(3HB) content (wt%)
NH_4Cl	$2.7^{ab} \pm 0.2$	$5.3^{b} \pm 0.3$	$51^{a} \pm 1$
$(NH_2)_2CO$	$3.0^{ab} \pm 0.5$	$5.8^{b} \pm 0.4$	$51^{a} \pm 6$
$(NH_4)_2SO_4$	$3.3^{b} \pm 0.5$	$5.6^{b} \pm 0.4$	$59^{a} \pm 5$
$NH_4H_2PO_4$	$2.6^{ab} \pm 0.4$	$5.0^{b} \pm 0.8$	$51^{a} \pm 2$
NH_4NO_3	$2.8^{ab} \pm 0.2$	$5.4^{b} \pm 0.1$	$51^{a} \pm 3$
$NaNO_3$	$3.2^{b} \pm 0.6$	$5.4^{b} \pm 0.7$	$58^{a} \pm 4$

[1]3HB, 3-hydroxybutyrate.
[2]Data shown are the means of triplicate readings ± standard deviations.
[3]Means in the same column with different superscripts are significantly different ($p<0.05$).
[4]The total nitrogen concentration in each nitrogen source was standardized at 5 mM, and UPO (5 g/L) was used as the carbon source. Samples were incubated for 72 h, at 30 °C, initial pH 7.0 and shaken at 200 rpm.
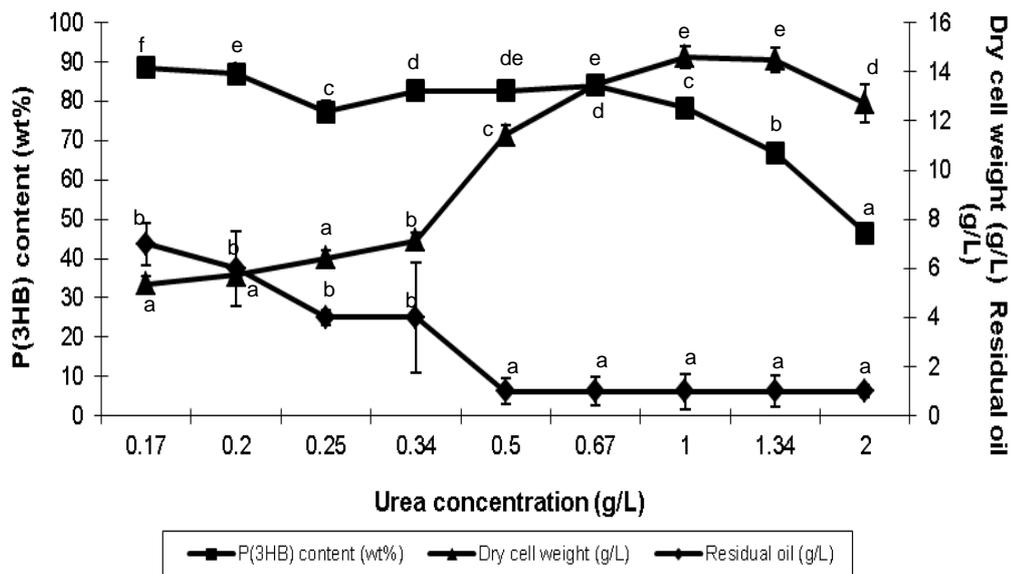

Figure 1: Effects of urea concentration on the production of P(3HB) and *C. necator* growth.
[1]Data shown are the means of triplicate tests. Standard deviations are represented by vertical bars.
[2]Means with different superscripts are significantly different ($p<0.05$).
[3]UPO concentration was fixed at 12.5 g/L and urea in different concentrations was used as the nitrogen source. Samples were incubated for 72 h, at 30 °C, at an initial pH 7.0 and shaken at 200 rpm.

Table 2: Effects of different concentrations of UPO on the biosynthesis of P(3HB).

(g/L)	Total P(3HB) (g/L)	Dry cell weight (g/L)	P(3HB) content (wt%)	Residual oil (g/L)
2.5	$0.9^a \pm 0.1$	$3.1^a \pm 0.1$	$28^a \pm 1$	$0^a \pm 0.3$
5	$2.4^b \pm 0.1$	$5.9^b \pm 0.1$	$40^b \pm 1$	$0^a \pm 0.1$
7.5	$5.9^c \pm 0.3$	$8.6^c \pm 0.2$	$68^c \pm 2$	$0^a \pm 0.1$
10	$8.9^d \pm 0.8$	$11.4^d \pm 0.1$	$78^e \pm 6$	$0^a \pm 0.3$
12.5	$10^{de} \pm 0.3$	$13.2^e \pm 0.5$	$76^{de} \pm 1$	$1^a \pm 0.1$
15	$10^e \pm 0.5$	$13.3^e \pm 0.7$	$77^{de} \pm 1$	$1^a \pm 0.3$
17.5	$11^e \pm 0.5$	$13.9^{ef} \pm 0.3$	$78^e \pm 3$	$1^a \pm 0.3$
20	$10^e \pm 0.5$	$14.5^f \pm 0.2$	$70^{cd} \pm 4$	$1^a \pm 0.1$

[1]3HB, 3-hydroxybutyrate.
[2]Data shown are the means of triplicate test readings ± standard deviations.
[3]Means in the same column with different superscripts are significantly different ($p<0.05$).
[4]$(NH_2)_2CO$ concentration was fixed at 0.54 g/L. Different concentrations of UPO were used as the carbon source. Samples were incubated for 72 h, at 30 °C, initial pH 7.0 and shaken at 200 rpm.
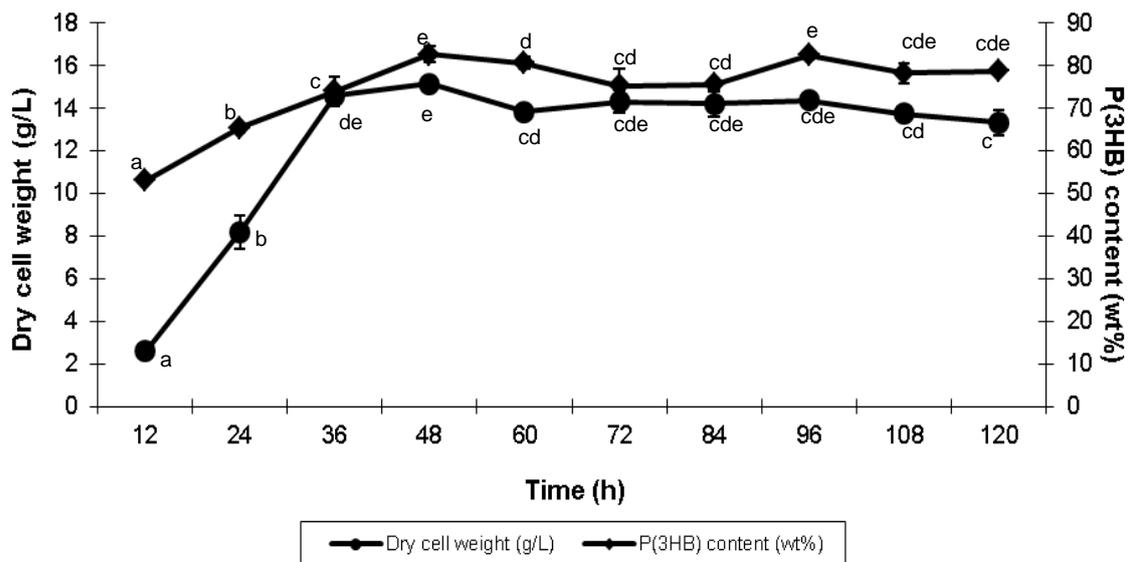

Figure 2: Time profile of *C. necator* culture with UPO used as the carbon source.
[1]Data shown are the means of triplicate readings. Standard deviations are represented by vertical bars.
[2]Means with different superscripts are significantly different ($p<0.05$).
[3]The total nitrogen concentration was fixed at 1 g/L and 12.5 g/L of UPO was used as carbon source. Samples were incubated for 72 h, at 30 °C, initial pH 7.0 and shaken at 200 rpm.
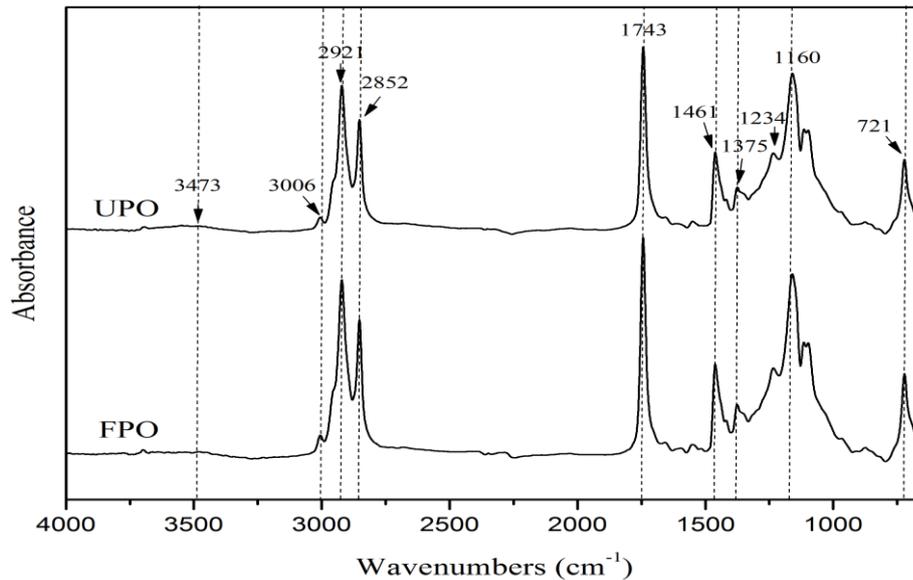
Figure 3: FTIR characterisation of UPO and FPO.

FPO. The carbon contents in FPO and UPO were similar which approximately 77%. The nitrogen content was not traceable for FPO and 1% for UPO, while the hydrogen content for FPO and UPO were not much different which are 12.4% and 12.2%, respectively.

Table 2 shows the effect of UPO concentration with respect to the synthesis of P(3HB) by *C. necator*. Here, the concentration of UPO at 12.5 g/L gave the best PHA yield [DCW=13.2 ± 0.5 g/L and P(3HB) content=76 ± 1 wt%] with minimal amount of residual oil. Further increase of UPO concentration did not give significant advantage. The low concentration of UPO that produced these satisfactory results indicated that UPO could be a viable low priced carbon source for PHA production, and hence it would keep PHA production costs low. The accumulation of P(3HB) and growth rate trend of *C. necator* H16 over time is shown in Figure 2. With UPO and urea concentrations fixed at 12.5 g/L and 1 g/L, respectively, a remarkable bacterial growth (DCW=16 g/L) and P(3HB) production (83 wt%) were achieved in just 48 h. P(3HB) production is influenced by the composition of UPO that, in turn, is influenced by how the oil is used in cooking during which time essential compounds that support the cell growth and P(3HB) accumulation are added (Verlinden *et al.*, 2011). Therefore, characterisation of UPO becomes crucial to understand the higher production of P(3HB) as compared to FPO (Kamilah *et al.*, 2013).

Figure 3 shows the FTIR spectra of UPO and FPO. In general, there were no changes or shifting of wavenumbers as it would have been expected when the oil was used. The most prominent peak at 1743 cm^{-1} represents the ester carbonyl functional group (Mirghani *et al.*, 2002). The wavenumber at 2921 cm^{-1} and 2852 cm^{-1} corresponds to asymmetric and symmetric stretching vibrations of the CH_2 functional groups respectively (Guillen and Cabo, 1997). The methyl asymmetric bending and methyl symmetric bending are observed in the wavenumber regions of 1461 cm^{-1} and 1375 cm^{-1} respectively (Man *et al.*, 2005). The wavenumber regions of 1250-1100 cm^{-1} correspond to the -C-O- functional groups, while the wavenumbers 1234 cm^{-1} and 1160 cm^{-1} signify the stretching vibration of the C-O ester groups (Mirghani *et al.*, 2002; Vlachos *et al.*, 2006). The wavenumber of 3006 cm^{-1} is due to the *cis* C=CH stretching which can be related to the iodine value (Manaf *et al.*, 2007). The wavenumber region of 721 cm^{-1} is linked to the overlapping of the CH_2 and bending-rocking vibration while wavenumber 3473 cm^{-1} represents the OH functional group (Guillen and Cabo, 1997; Man *et al.*, 2005; Vlachos *et al.*, 2006).

The thermal properties of FPO and UPO were also analysed to examine the differences between UPO and FPO. There were not much variation in terms of thermal decomposition between UPO and FPO analysed by TGA. Both UPO and FPO thermally decompose at approximately 430 °C (results not shown). The melting temperature (T_m) and crystallisation temperature (T_c) of UPO and FPO, in contrast, were different in terms of the peak numbers and the transition temperatures, as depicted in Figure 4. FPO displayed a maximum peak at 4.7 °C and two shoulder peaks of high-temperature endotherm. On the other hand, UPO showed multiple peaks with the T_m peak at 2.2 °C and two shoulder peaks of a high-temperature endotherm and two shoulder peaks of a low-temperature endotherm. Heating of the palm olein during cooking affected the melting crystallisation temperature of the samples and, accordingly, the T_m of UPO shifted to a lower temperature compared to FPO. The presence of extra by-products that shift the peaks of T_m and T_c of the cooking oils could be related to changes in IV, PAV, FFA and the C18:2/C16:0 ratio (Tan *et al.*, 2002). A similar phenomenon was observed for T_c, for

which the exothermic peak of UPO was shifted from 2 °C to −0.1 °C.

Results presented in Table 3 shows that the physiochemical characteristics of FPO and UPO. The FFA content in UPO (FFA=1%) is indeed higher than that of FPO (FFA=0.28%). Presence of higher FFA may enhance the uptake of fatty acid into the β-oxidation cycle by microorganism, which led to and contributed to higher production of P(3HB). FFA in UPO is generated by hydrolytic reactions from the steam produced during food preparation when triglycerides were hydrolysed to glycerol, monoglycerides and diglycerides (Mittelbach and Enzelsberger, 1999). The acid value (AV) which is related to FFA also follows a similar trend. The higher peroxide value (PV) of UPO at 3.9 meq/kg as compared with 2 meq/kg for FPO suggested that oxidative reactions occurred during the frying process. Trace amounts of heavy metals in the palm olein could also elevate the PV reading (Siddique et al., 2010). The p-anisidine value (PAV) of FPO (5.6 Abs/g) was a more favourable reading than that of UPO (23 Abs/g), which reflects the production of secondary products derived from peroxides (Tan et al., 2002). Similar trends were observed for the K-values of FPO and UPO, measured at K_{232} as 1.9 Abs and 3.6 Abs, respectively, and at K_{270} as 0.4 Abs and 2.4 Abs, respectively. Formation of diene and triene from the reaction of oxidation can be detected from the result of K-value (Nzikou et al., 2009).

The composition of fatty acid methyl esters (FAME) in palm olein (Figure 5) is in general agreement with that reported by Sundram et al. (2003) where the aliphatic acids, palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids are the major fatty acids. Palm oil has approximately equal parts of saturated and unsaturated fatty acids, as can be seen in Figure 5. In FPO, the ratio of unsaturated bond to saturated bond is 54:46, while in UPO it is 51:49, respectively. In present study, the saturated bond increased after palm olein had been used in frying, with changes observed in the 16:0 and 18:2 fatty acids. The amount of the 18:2 fatty acids decreased from 11.3% to 7.6% after exposure to heat while the 16:0 fatty acids increased from 40.3% to 43.7%. On the other hand, the 18:1 fatty acids did not appear to have been affected by heating. Similar changes were reported by Verlinden et al. (2011) where the 16:0 fatty acid content increased and the 18:2 fatty acids decreased after heating in its use for frying (Verlinden et al., 2011). The production of more saturated fatty acids contributes to a higher rate of P(3HB) formation in the bacteria since saturated fatty acids are more readily to be converted to acetyl-CoA through β-oxidation cycles (Verlinden et al., 2011). The formation and presence of saturated and unsaturated bonds can be observed from the iodine value (IV), which the trend in the iodine value (IV) was different, with FPO showing higher values (Table 3). The readings of 55.78 g I_2/100g oil for FPO and 39.54 g I_2/100g oil for UPO indicated that FPO contained more double bonds from unsaturated compounds (Siddique et al., 2010). The reduction in IV of UPO may occur due to thermolytic reactions during the frying process that break the double bonds in the oil (Nawar, 1984). Such changes in the
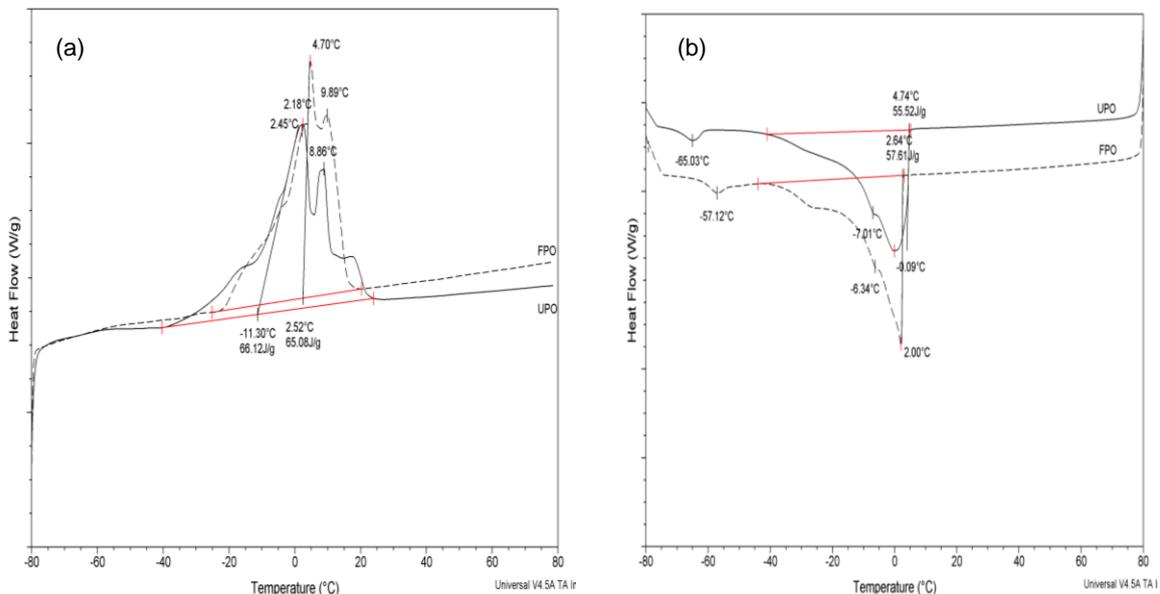

Figure 4: Thermal properties of FPO and UPO. a) T_m (Melting temperature) of FPO and UPO b) T_c (Crystalisation temperature).

Table 3: Physicochemical characteristic of FPO and UPO.

Physicochemical characteristics	Fresh Palm Olein (FPO)	Used Palm Olein (UPO)
Free Fatty Acid (as oleic %)	0.28 ± 0.14^a	1.00 ± 0.08^b
Acid Value (as oleic %)	0.56 ± 0.27^a	2.00 ± 0.15^b
Peroxide Value (meq/kg)	2.00 ± 0.40^a	3.93 ± 0.31^b
P- Anisidine (Abs/g)	5.57 ± 3.54^a	23.27 ± 3.94^b
K_{232} (Abs)	1.90 ± 0.02^a	3.61 ± 0.06^b
K_{270} (Abs)	0.41 ± 0.02^a	2.42 ± 0.45^b
Iodine value (g I/100g oil)	55.78 ± 2.80^a	39.54 ± 7.44^b
Viscosity (mPaS)	64.03 ± 1.38^a	80.73 ± 1.20^b
Density (kg/m^3)	909.57 ± 0.06^a	913.27 ± 0.12^b
Moisture content (%)	0.04 ± 0.01^a	0.12 ± 0.01^b

[1]Data shown are the means of triplicate test readings ± standard deviations.
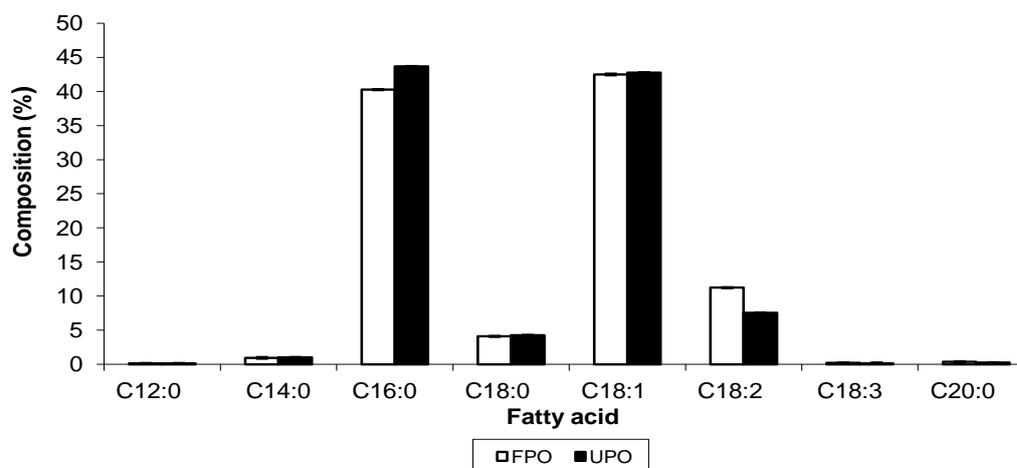[2]Means in the same column with different superscripts are significantly different ($p<0.05$).

Figure 5: Fatty acid methyl esters in FPO and UPO.

physicochemical characteristics of UPO are expected to be the cause of higher P(3HB) production by *C. necator* H16.

In addition, an evaluation of the composition such as vitamins and nutrients could be taken into consideration as the contributor of P(3HB) production. Hence, chlorophylls and carotenoids, which are suspected as nutrients contributor, are two natural pigments that are present as minor components of palm olein. Carotenoids that include β-carotene are the precursor of vitamin A, and

they provide oxidative stability to a variety of vegetable oils (Sundram *et al.*, 2003; Giuffrida *et al.*, 2007). Pigments in UPO showed distinct differences as compared with those in FPO (results not shown). Surprisingly, the chlorophylls and carotenoids composition in UPO gave results of 3.09 ± 0.27 mg/kg and 5.04 ± 0.11 mg/kg, respectively, which were considerably higher than corresponding values in FPO, i.e. 0.94 ± 0.08 mg/kg and 0.56 ± 0.02 mg/kg, respectively. The chlorophylls to carotenoids ratio in UPO indicated that carotenoids had a

larger presence as compared to chlorophylls. However, the opposite was true in FPO. Theoretically, the pigments composition should be reduced due to the exposure of heat during the cooking process (Turkmen *et al.*, 2006; Adebooye *et al.*, 2008). However, the increased value of chlorophyll and carotenoids pigment in this study might have occurred due to the leaching of these pigments from the fried food into the oil and the softening process of the food that occurred during the frying process (Miglio *et al.*, 2008; Yuan *et al.*, 2009). This probably accounted for the increased ratio of pigments composition in the cooking oil.

CONCLUSION

In conclusion, UPO collected from the community has the potential to synthesise P(3HB) efficiently. It is hypothesized that short carbon chains, saturated carbon chains, and polar compounds that are formed contribute to cell growth and P(3HB) accumulation. Other constituents in UPO, such as pigment compounds, polymers, free fatty acid, *p*-anisidine, fatty acid dimers and trimers that are higher in their contents than in FPO, might also contribute to the production of P(3HB) and cell growth. A more thorough analysis of FPO and UPO might identify more compositional differences in these oils and help in managing strategies to maximise PHA production by micro-organisms in the future.

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