Research Article

Characterisation studies of cocoa butter β(V) polymorphs from Sabah, Malaysia

Nur Fadzilah Basri and Fauziah Abdul Aziz

Physics with Electronics Program, School of Science and Technology, Universiti Malaysia Sabah, Kota Kinabalu, 88502, Sabah, Malaysia.

Email: afauziah@ums.edu.my

Abstract

Characterization of cocoa butter β(V) polymorphs from Sabah were studied in this research using GC-MS, pNMR, DSC and XRD techniques. CB_{Tawau} had the lowest melting point followed by CB_{Kunak} and CB_{LD} (highest melting point). The results from GC-MS analysis showed the same results where CB_{Tawau} had the highest composition of oleic acid followed by CB_{Kunak} and CB_{LD}. The crystallinity for all β(V) polymorphs CB increased from CB_{LD} (lowest crystallinity) followed by CB_{Kunak} and CB_{Tawau} (highest crystallinity).

Keywords: GC-MS, pNMR, DSC, XRD, confectionary, chocolate, crystallinity.

Introduction

Polymorphism of cocoa butter is a key research area in chocolate and confectionery studies. There are 6 polymorphs in CB with all types of nomenclature but mainly used are, roman numeral, I-VI, with increasing stability [1] and Greek nomenclature, γ, α, β', and two β phase: β(V) and β(VI)[2]. The main composition of cocoa butter (CB) comprises triglycerides (POP, POS and SOS) which are monounsaturated triglycerides that give stability to β crystal in chocolate manufacturing [3, 4].

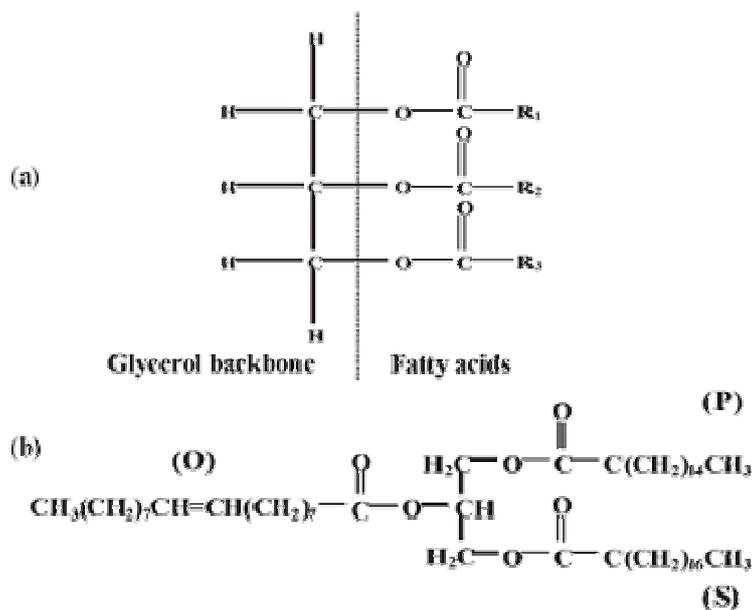**Figure 1: (a) Basic structure of triglycerides molecules with glycerol backbone attach to the three fatty acids. (b) POS triglycerides structure [4].**

X-ray powder diffraction (XRD) is a non-destructive technique widely applied for the characterization of crystalline material [5, 6, 7]. For most applications, the amount of information which is possible to extract depends on the nature of the sample microstructure (crystallinity, structure imperfections, crystallite size, texture), the complexity of the crystal structure (number of atoms in the asymmetric unit cell, unit cell volume) and the quality of the experimental data (instrument performance, counting statistics) [7].

Crystallite size studies have been conducted by several workers [8, 9, 10, 11]. The crystallite size (*D*) is calculated using the Scherrer equation which was published in 1918 [5]:-

$$B(2\theta) = \frac{k\lambda}{D\cos\theta} \tag{1.0}$$

Where *B* is the full width at half maximum (FWHM), *k* is the Scherrer constant, λ is the wavelength of X-radiation, *D* is crystallite size and θ is half the diffraction angle.

The widths of the peaks in a particular phase pattern provide an indication of the average crystallite size. Large crystallites give rise to sharp peaks, while the peak width increases as crystallite size reduces. Peak width due to crystallite size varies inversely with crystallite size.

Methodology

All samples were extracted using soxhlet apparatus according to AOAC methods [12]. Approximately 15.0 g of cocoa samples wrapped in 15.0 cm diameter filter paper and inserted in a thimble, then put into the soxhlet apparatus and the extraction process begun. Petroleum ether was used as a solvent.

The fatty acid composition of each fat was determined as fatty acid methyl esters (FAME) analysis using Perkin Elmer Gas Chromatography Mass Spectrometer (GC-MS) with DB5 MS Column (5% Phenyl Methyl crosslink siloxane, 30.0 m x 250 μm). The oven temperature program was set at 60°C and increased until 230°C at 4°C/min and held at 230°C for 17.5 minutes. Helium was used as carrier gas. All the fatty acids in samples were determined using spectrum database in Perkin Elmer software [13].

Solid fat content (SFC) was measured with a pulsed nuclear magnetic resonance (pNMR) Minispec pc 20 analyzer (Bruker, Karlsruhe, Germany) at Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia. The samples in the NMR tube were first melted at 80°C and held for 20 minutes at 60°C, then cooled at 0°C for 90 minutes, stabilized for 35 minutes at each measurement temperature. Temperature of the samples were decreased exponentially with the initial rate of $25°C \ min^{-1}$ and the SFC contents in the samples were taken at a given range of time.

The crystallization process was performed using static crystallization method [14]. The melting point analyses for each CB sample was done using differential scanning calorimeter (DSC). Samples were sealed in an aluminium hermetic pan with sample press. Approximately 5 mg samples were used for the investigations. The instrument used was a Perkin Elmer DSC6 and heated up from 18°C to 50°C: this treatment was directly monitored recording the relevant DSC signal that attained peaks, corresponding to the melting points of the crystal thus making it possible to identify which forms were present. The polymorphic form of the fat crystals in all samples were then analyzed and determined using XRD model X'Pert Pro PW3040/60 (XRD MPD) located at School of Science and Technology, UMS, Sabah, Malaysia.

Results and Discussion

GC-MS Analysis
Table 1 shows the fatty acid composition for each CB. From the results, it was shown that the 3 main constituents in all CB are palmitic, stearic and oleic acid. The stearic acid compositions in all three samples are almost the same, which is 36%. The palmitic acid for each CB are different, with the composition increased from CB_{Tawau} (24.990%) followed by CB_{Kunak} (25.753%) and CB_{LD} as the highest (26.814%).

Table 1: Fatty acid compositions for CB_{Tawau}, CB_{Kunak} dan CB_{LD}

Fatty acids	CB Tawau (%)	CB Kunak (%)	CB LD (%)
Palmitic	24.990	25.753	26.814
Stearic	36.649	36.817	36.026
Oleic	33.285	32.714	32.104
Arachidic	1.099	0.984	1.160
Palmitoleic	0.157	0.114	0.157
Others	3.820	3.618	3.739

DSC and SFC Analysis

Melting point analysis for each CB polymorph showed that CB_{Tawau} had the lowest melting point, 34.0°C, followed by CB_{Kunak}, 36.0°C and CB_{LD} with 37.0°C (highest melting point). DSC analysis thermograms are shown in Figure 2. It can be seen from the melting point that β(V) polymorphs for all CB from Sabah are higher than that found in the literature. As stated by Roberts [15], β(V) polymorph for hard cocoa butter can have higher melting points than β(VI) polymorph of soft cocoa butter. Malaysian cocoa butter is categorized as hard cocoa butter and is the hardest cocoa butter compared to other countries [16], thus the crystallization behaviour for CB Sabah is different according to its thermodynamic stability. The SFC curves for the three CB samples are shown in Figure 3. From the curves, it can be seen that the hardness of the three CB increased from CB_{Tawau} (softest), followed by CB_{Kunak} and CB_{LD} (hardest).
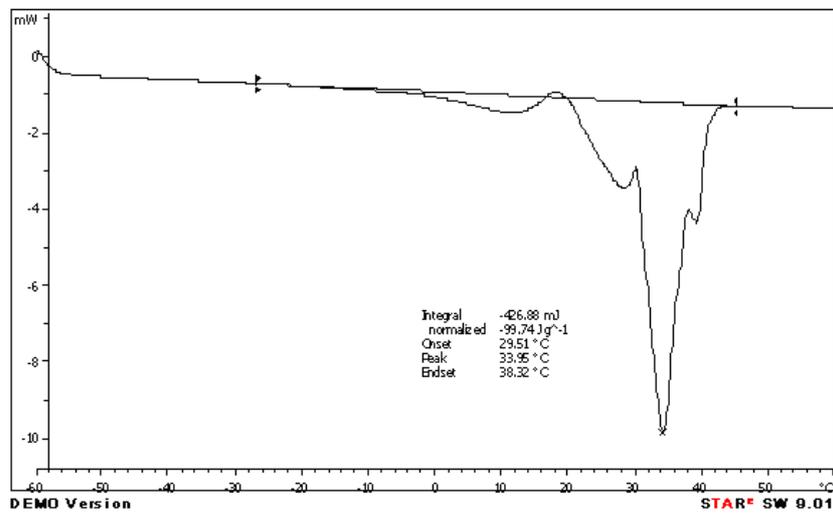

Integral	-426.88 mJ
normalized	-99.74 Jg^-1
Onset	29.51 °C
Peak	33.95 °C
Endset	38.32 °C

Figure 2a: DSC thermogram for CB$_{Tawau}$

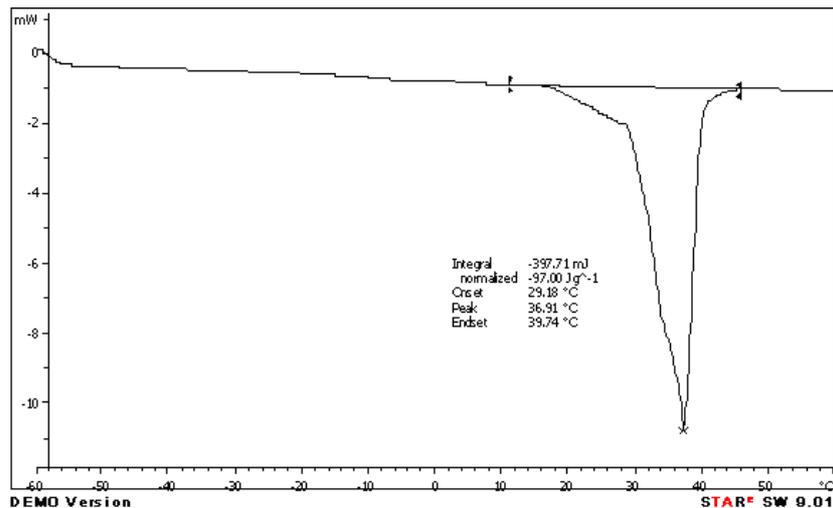| Integral | -397.71 mJ |
normalized	-97.00 Jg^-1
Onset	29.18 °C
Peak	36.91 °C
Endset	39.74 °C

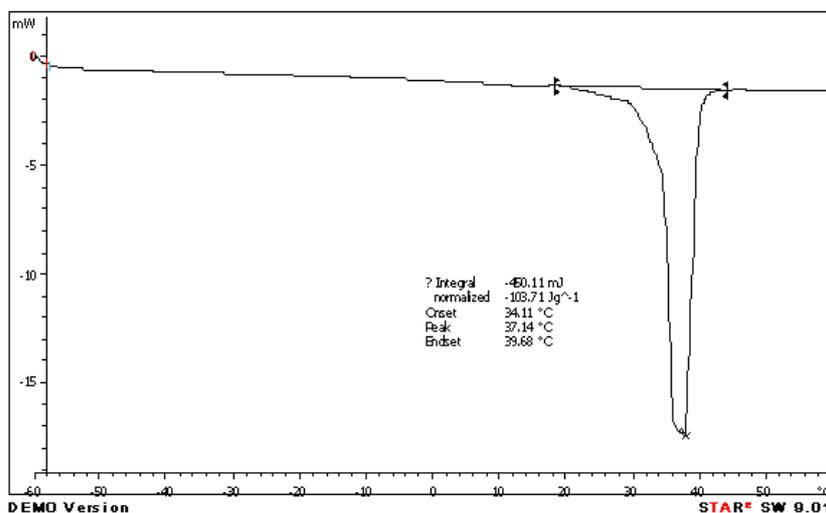**Figure 2b: DSC thermogram for CB$_{Kunak}$**

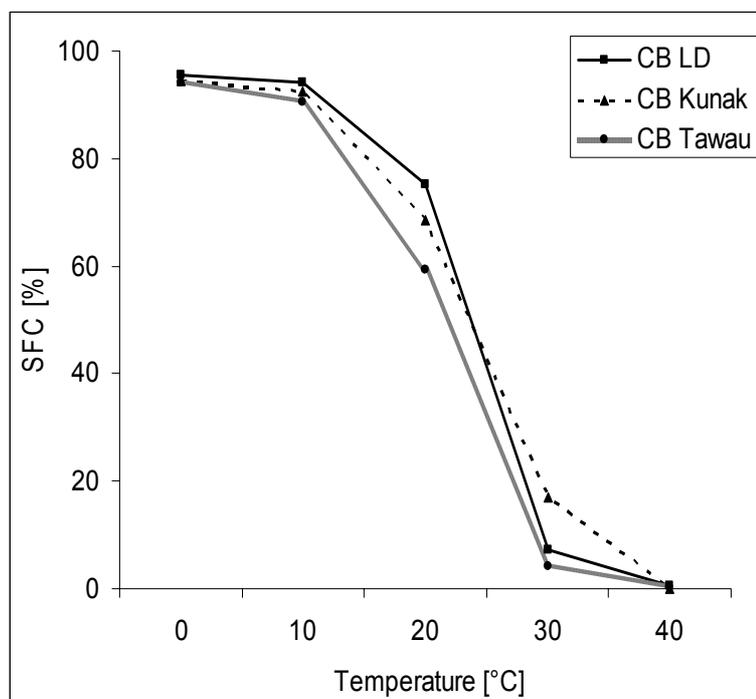**Figure 2c: DSC thermogram for CB$_{LD}$**

Figure 3: SFC plot versus temperature for CB$_{Tawau}$, CB$_{Kunak}$ and CB$_{LD}$

XRD Analysis

Figure 4 shows the XRD diffractograms for CB$_{Tawau}$, CB$_{Kunak}$ and CB$_{LD}$ β(V) polymorphs with the short spacing value at the highest peak. According to plot of FWHM average versus CB samples in Figure 5, it can be seen that the crystallinity of CB$_{Tawau}$ β(V) polymorph is the highest followed by CB$_{Kunak}$ β(V) polymorph and CB$_{LD}$ β(V) polymorph (lowest crystallinity). The crystallite sizes for each β(V) polymorph were calculated using the Scherrer equation and the value for each crystallite size is shown in Table 2. CB$_{Tawau}$ β(V) polymorphs had the largest crystallite size,

295.6130 Å, followed by CB_{Kunak} β(V) polymorphs, 292.5601 Å and CB_{LD} β(V) polymorphs, 199.7098 Å (smallest crystallites size).
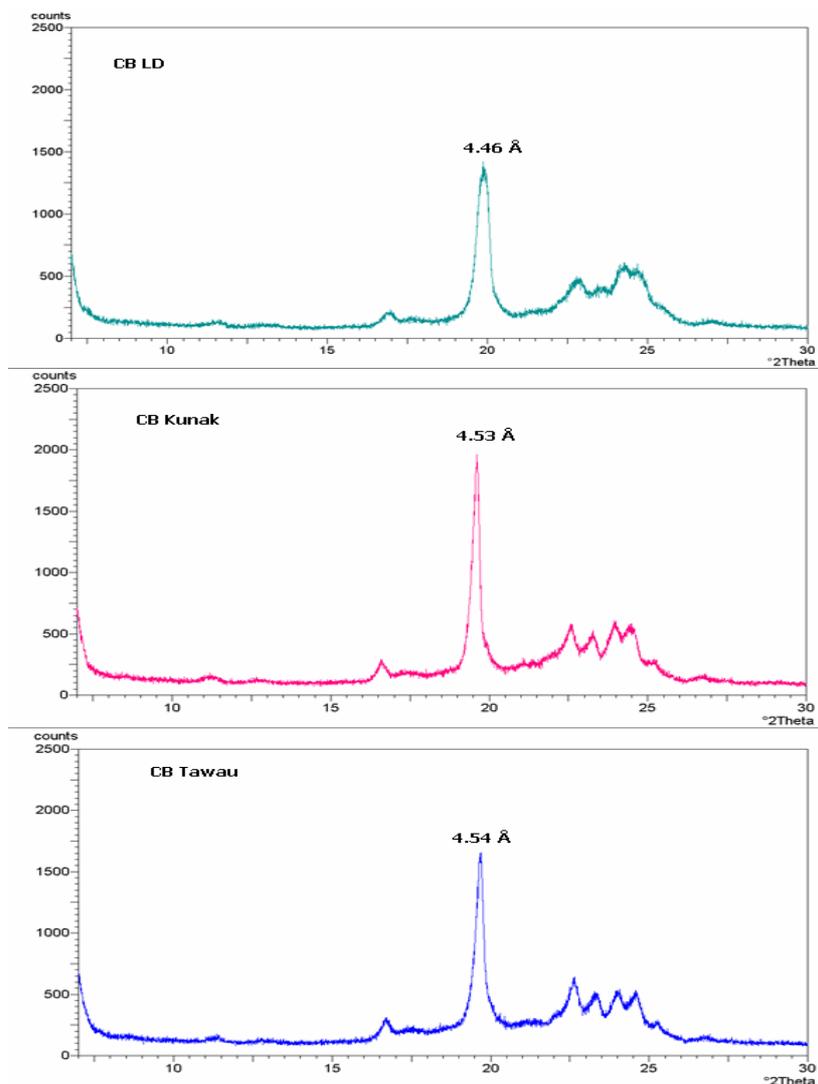

Figure 4: XRD diffractogram for β(V) polymorphs of CB_{Tawau}, CB_{Kunak} and CB_{LD}
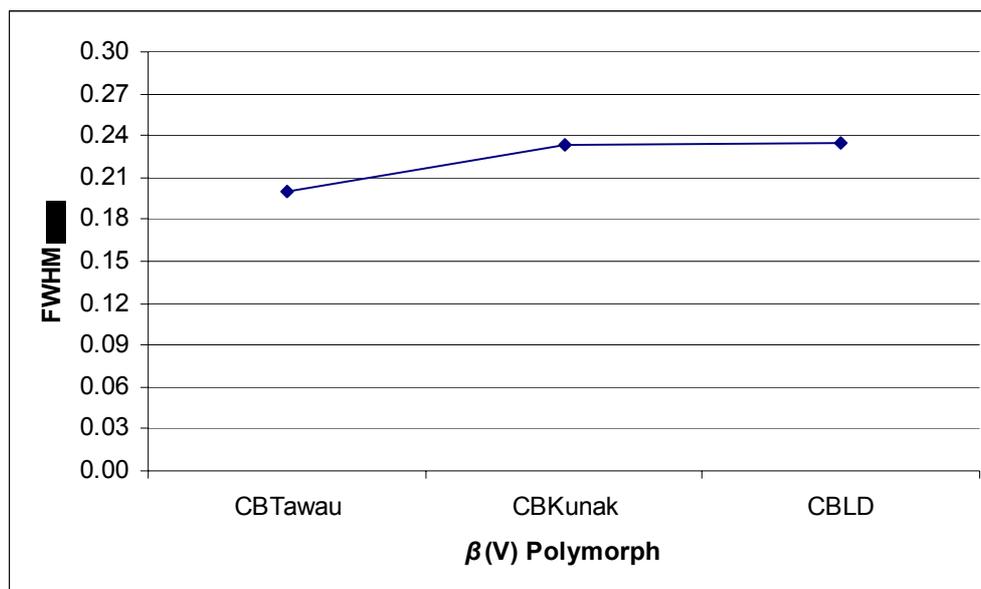
Figure 5: Plot of FWHM average versus samples.

Table 2: Crystallite sizes for each β(V) polymorph CB at the highest peak on the 2θ peak profile.

Polymorph β(V) CB	2θ (°)	d-spacing (Å)	FWHM (°)	Crystallite size, D (Å)
CB_{Tawau}	19.52409	4.5429	0.2848	295.6130
CB_{Kunak}	19.58911	4.5280	0.2878	292.5601
CB_{LD}	19.89251	4.4596	0.4218	199.7098

Conclusion

From the results and analysis conducted, it was shown that the quality of CB_{Tawau} β(V) polymorph is the highest, followed by the CB_{Kunak} β(V) polymorph and CB_{LD} β(V) polymorph (lowest).

Acknowledgement

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