CHAPTER IV

EFFECT OF HEATING ON LARD ADULTERATION IN RBD PALM OIL USING GAS CHROMATOGRAPHY AND CHEMOMETRICS

4.0 INTRODUCTION

Refined Bleached Deodorized (RBD) Palm oil was widely used and highly appreciated by Asian's people due to its excellence properties for frying and cooking besides its reasonable price. However, adulteration was reported in certain cases including cooking oil because of economical reason to obtain maximum profits. (Rohman & Che Man, 2011b). Authentication of palm oil covers many different areas such as adulteration, mislabeling, and confusing source (Aparicio et al, 2000).

Chromatography technique is most widely used to analyse the composition of the natural constituents of the oil and adulterants. (Webster et al, 2000). Gas Chromatography Mass Spectrometry Headspace (GC-MS-HS) is able to analyse the complex mixtures of volatiles and respond to all volatile compounds (Zubritsky, 2000). It is cheaper and faster as no prior sample preparation steps are required.

Generally, the compounds injected in the gas chromatography (GC) were volatile at the temperature of analysis and they do not degraded during the analysis. Mass Spectrometry (MS) technique provides molecular mass data, structural information and identification of compounds using the build in library (Cert et al, 2000).

Triacylglycerol constitutes about 95-98 % of vegetable oils and 2-5 % of complex mixtures of minor compounds. The minor constituents in vegetable oil

consist of fatty alcohols, wax esters, hydrocarbons, tocopherols and tocotrienols, phenolics compounds, volatiles, pigments, compound of minor glyceridic, phospholipids and triterpenic acids (Cert et al, 2000). Meanwhile, the flavor and aroma of the oils are due to the number of volatiles constituents that are present but at low concentration. In south East Asia, the amount of fatty acids (FA) such as lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) were higher compared to palm oil from South American and Africa (Tres et al., 2012).

Lard has been a sensitive issue for the Muslims especially involving food, pharmaceutical and cosmetics. According to Che Man and Rohman (2011), the presence of lard may be viewed on two aspects which are from the religious and economics perspectives. In terms of economic perspectives, manufacturer would blend some vegetable oils with lard to produce cost-effective margarines, shortenings and other oil based food. While, in terms of religious perspective, religion such as Islam ad well as Judaism forbid their followers from consuming products or food that contain lards.

Previous report mentioned that the most abundant volatile compound detected in oxidized lard of ENL (Enshi No 1 native breed) and TML (three way crossbred) were short and medium chain aldehyde. Among the detected alcohols, 1-octen-3-ol generated from the oxidation of linoleic acid and 1-octanol were the most abundant. Meanwhile 2-pentyl furan was the only furan compound identified in the two lard sources (Xu et al., 2012).

Using GC-MS, Dirinck et al (2006), were able to examine the volatile trace constituents isolated from palm oil. The result showed that trans-2-octenal, n-nonanal,

truns-2-decenal, trans-2-undcenal, b-ionone, cis-2,4-decadienal and trans 2,4-decadienal as important contributors to palm oil.

Therefore, the objectives of this research was to study the profiling of different percentage of lard (0 %, 15 %, 30 %) when heated at three different temperatures (120 °C (A), 180 °C (B), 240 °C (C)) for 1 hour, 2 hours and 3 hours heating time in fresh RBD palm oil.

4.1 MATERIALS AND METHODS

4.1.1 Materials

Fresh Refined Bleach Deodorized (RBD) Palm oil and sample of pig's adipose tissue were purchased from a local supermarket at Nilai, Negeri Sembilan. Adipose tissues were stored at -20 ^{0}C prior to the analysis.

4.1.2 Sample Preparation

Lard samples were extracted by rendering adipose tissue in a conventional oven (Pensonic AE-11N) at 100 ^{0}C for 2 hours according to Rohman and Che Man (2009a) with slight modification. The melted fat was filtered through Whatman filter paper and dried by the addition of anhydrous Na_2SO_4 to remove the water content. The filtered samples were stored in a tightly closed container in a refrigerator until further analysis. The RBD Palm oil was adulterated with lard at various percentages (0 %, 15 % and 30 %) in w/w. In this method, lards were spiked into the fresh RBD palm oil as a way to act as preliminary study to emulate RBD palm oil recycle oil.

4.1.3 Heating procedure

All adulterated oils were heated at 3 different temperatures (120 °C, 180 °C and 240 °C) using a digital hotplate (Daihan, Korea) with a controlled temperature probes for 3 hours. Each sample were taken at 1, 2 and 3 hour of heating time and cooled to a room temperature before kept in a tightly closed and sealed universal bottles in a refrigerator until further analysis.

4.1.4 Headspace GC-MS analysis

The method for determining the volatile compounds was done according to Lorenzo et al. (2002). For the analysis of volatile compounds, 4.0 ml of each oil samples were placed into 10ml vials sealed hermetically with a cap. The experimental conditions of the head space sampler were as follows; oven temperature: 120 °C; loop temperature: 130 °C. Transfer line temperature: 135 °C, headspace generation time 30 min. The mass range measured between in the mass spectrometer was 35-100. The carrier gas was helium, at an approximate flow rate of 20 ml/min. the column used was non polar column (Agilent Technologies). The mass spectra obtained were compared to the NIST Mass Spectral Search Program for compound identification. The peak areas were selected for further data analysis using chemometrics technique.

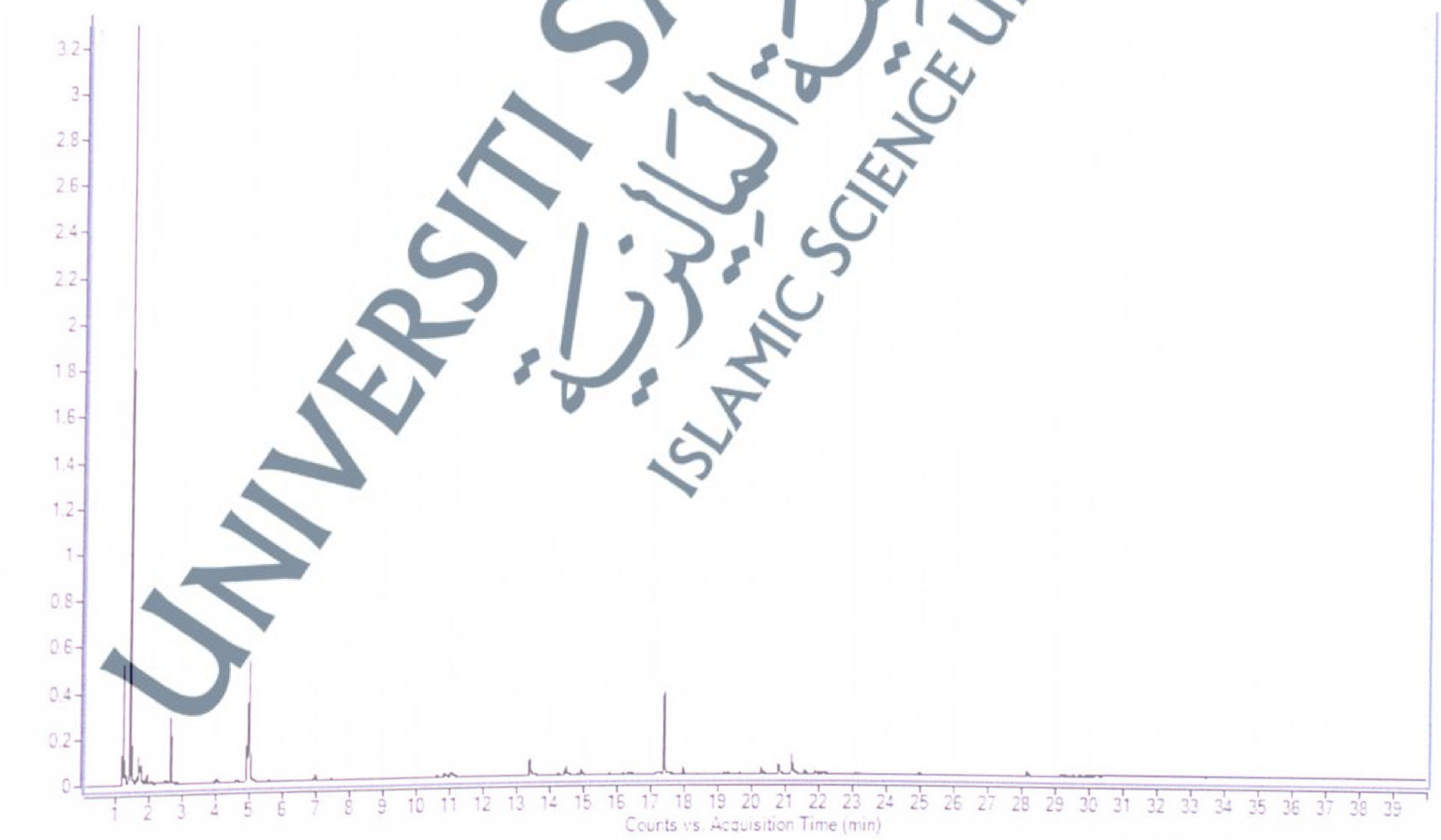
4.1.5 Statistical analysis

Unsupervised multivariate analysis, principal components analysis (PCA) was performed by mean centered on data of GC-MS-headspace using Unscrambler Software (X10.3) version.

4.2 RESULTS AND DISCUSSION

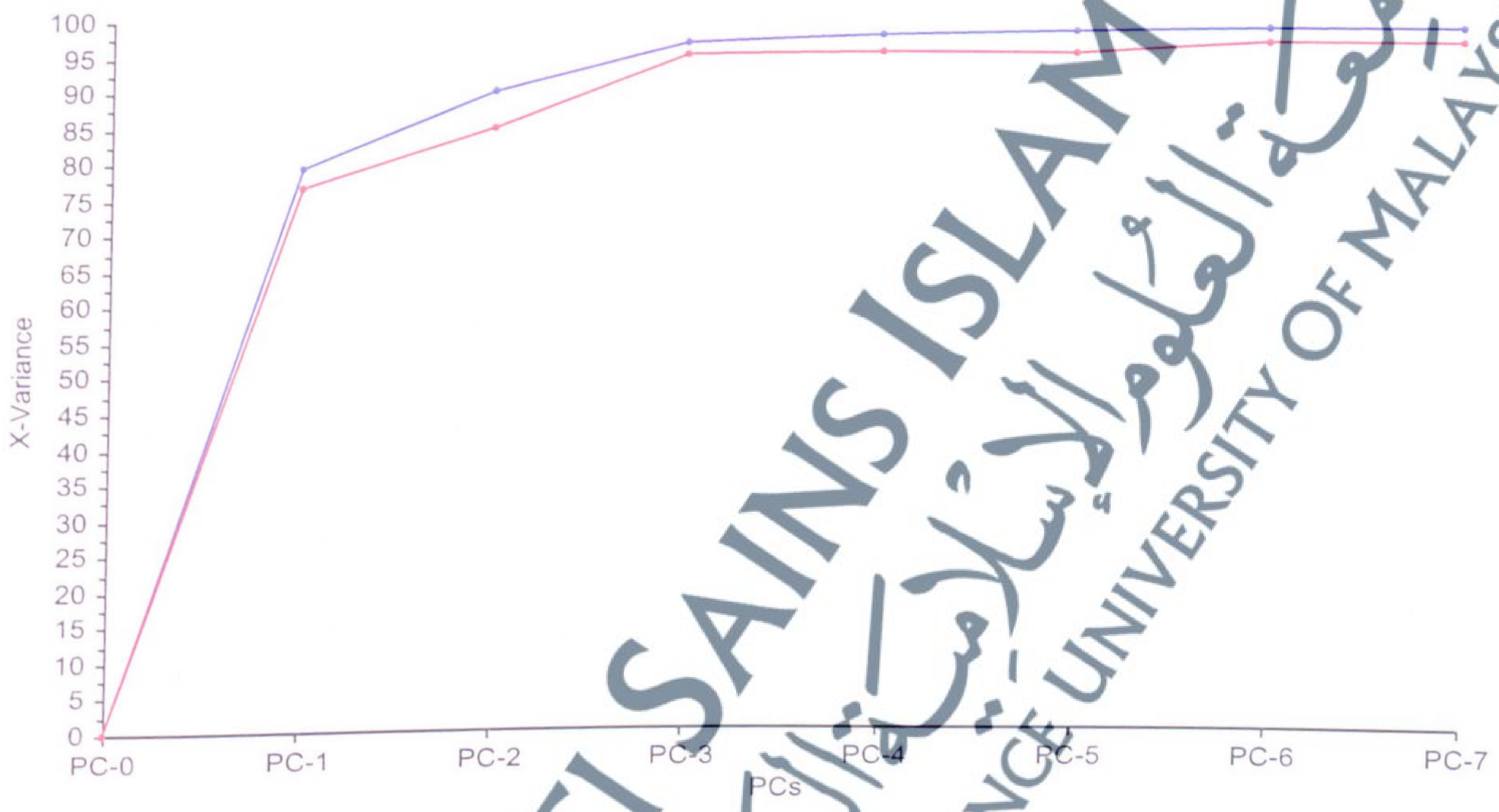
Gas chromatography mass spectrometry headspace (GC-MS-HS) was used to analyse the volatile compounds released in sample **a** (0 % lard in RBD palm oil), **b** (15 % lard in RBD palm oil) and **c** (30 % lard in RBD palm oil) after heating at 120 ^{o}C (A), 180 ^{o}C (B) and 240 ^{o}C (C) for 1, 2 and 3 hours. Raw data before the data was mean centred was shown in **Appendix A**. The chromatogram for GC-MS-HS taken from one selected sample was shown in **FIGURE 12**. A total of 16 compounds were identified and separation of the individual compound was observed to select the peak before further analysed statistically using PCA. PCA has the ability to reduce the dimensionality of data set that contain a huge number of interrelated variables, at the same time retaining the variation that present in that data set (Jolliffe, 1986).

FIGURE 12: volatile compound detected using GC-MS-HS for sample 0 % lard heated at 120 ^{o}C for 3 hours heating time



The scree plot of explained variance was shown in **FIGURE 13** as it showed the most significant PCs generated from samples treatments. Total 7 PCs were generated but only PC1 and PC2 were taken into account. PC1 described 80 % and PC2 described 11 % of the total variance in data. Results of PCA were summarized from Scores table (**Appendix C**) and Loadings table **(Appendix D).**

FIGURE 13: Scree plot of explained variance from 0 % and 15 % lard in 120 °C, 180 °C, 240 °C for 3 hours heating time



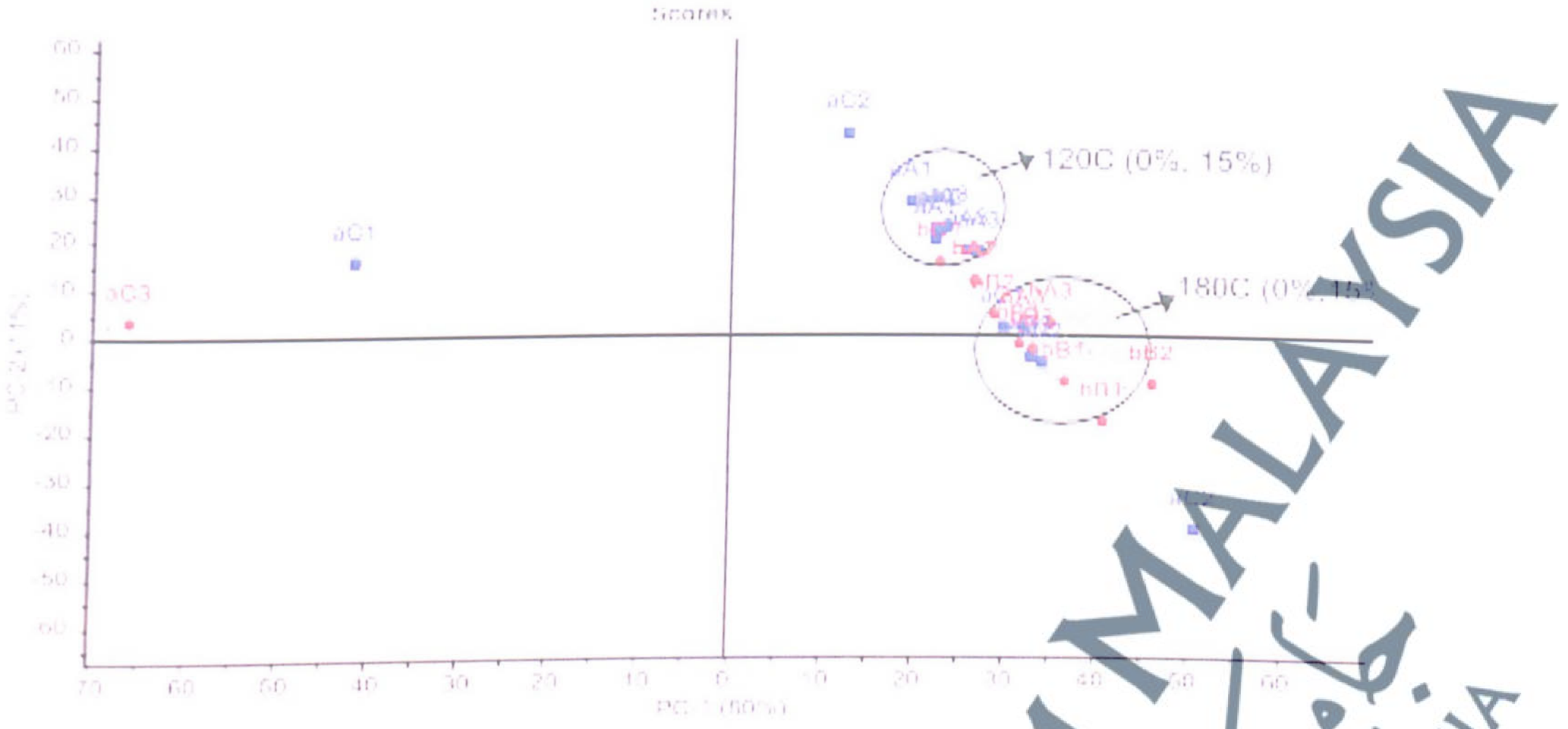
The scores plot for 15 % lard spiked in RBD palm oil when compared with control sample a (0 % lard in RBD palm oil) was shown in **FIGURE 14**. Samples were found to be clustered based on their difference in their heating temperature at 120 °C and 180 °C. In each cluster, it was found that control (0 % lard in RBD palm oil) and samples (15 % lard in RBD palm oil) were located closed together. The same

pattern was observed in **FIGURE 17** when 30 % lard were spiked into RBD palm oil where samples were clustered according to their heating temperature.

Loadings plot show the importance of the different variables for the two components specified. Based from loadings plot in **FIGURE 15** and **FIGURE 18**, only 5 variables were differed significantly from other variables. Those 5 variables were pentane (marked as X), hexanal (marked as G), nonanal (marked as B), octane (marked as U) and heptane (marked as Y). Variables that are close to each other in the loadings plot will have a high positive correlation if the two components explain a large portion of the variance X. All variables lying closed to the centre except for variables X, G, B, U and Y. Since PC1 in **FIGURE 15** showed the highest variance compared to other PCs (refer **Appendix D**), variables X (0.897818), G (0.05656885), B (0.150071), U (0.125145) and Y (0.3822328) showed the highest x- loading value compared to other variables. While in **FIGURE 18** the value for x-loading also showed the highest where X was (0.923411), G (0.0302783), B (0.1286729), U (0.1029063) and Y (0.3320893) (refer **Appendix F**).

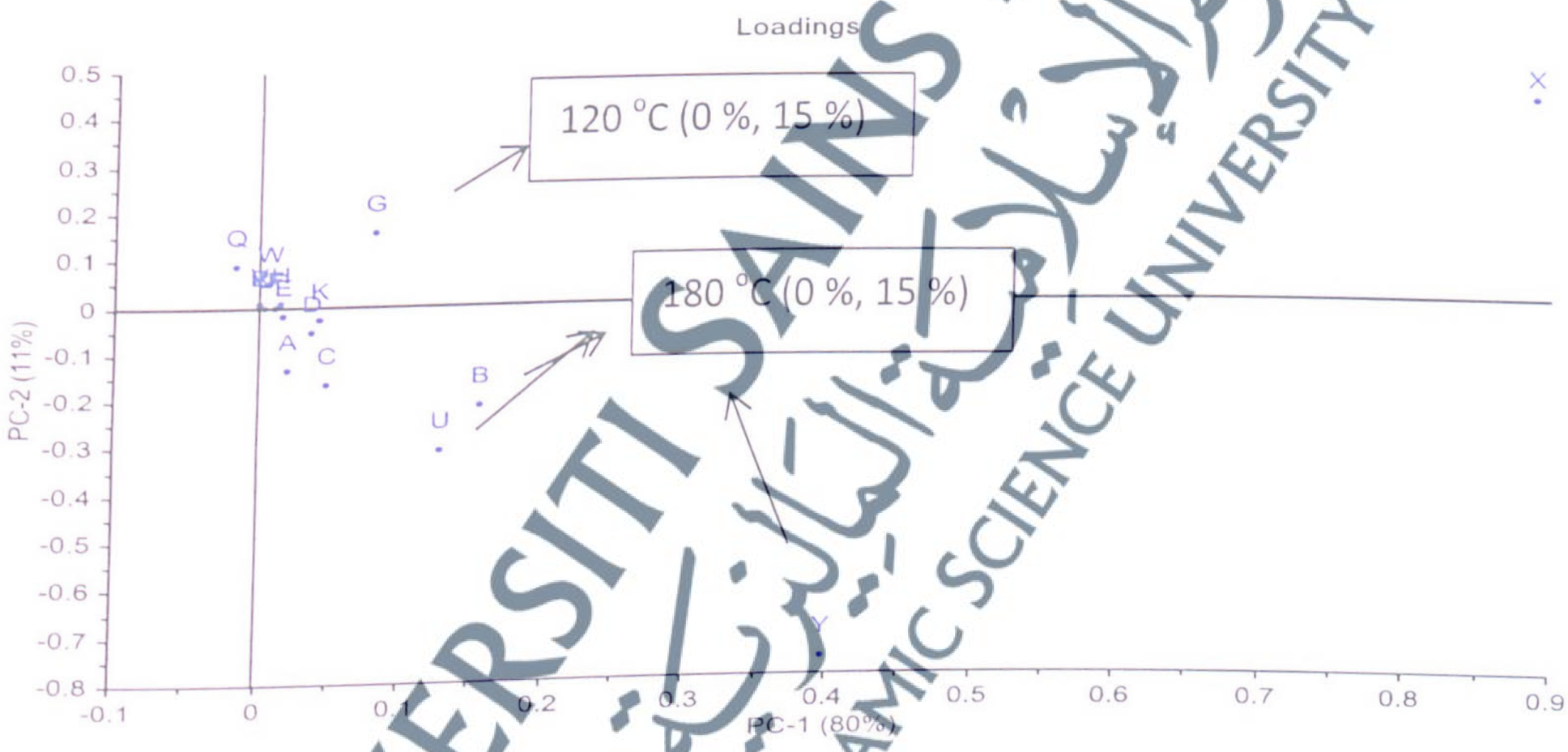
In **FIGURE 15** and **FIGURE 18**, variables G was correlated with heating temperature 120 °C while variables B,U and Y were correlated with heating temperature 180 °C. No variables were correlated with heating time 240 °C. Hassan and Salleh (2015) analyzed the fatty acids in lard by gas chromatography flame ionization (GC- FID) method. They found that fatty acids linolelaidic acid methyl ester (C18:2n6t), oleic acid methyl ester (C18:1n9c) and palmitic acid (C16:0) were observed in all loading plots. However, as the heating time increased, other fatty acids were centred in the middle and located closed to each other.

FIGURE 14: The scores plot of GC-MS-HS for 0 % and 15 % lard spiked in RBD palm oil heating at 120 °C and 180 °C.



* a = 0 % lard in 100 % % RBD palm oil, b = 15 % lard in 85 % RBD palm oil, A= 120 °C, B= 180 °C, C= 240 °C, 1= 1 hr heating time, 2= 2 hrs heating time, 3= 3hrs heatin g time

FIGURE 15: The loadings plot of GC-MS-HS for 0 % and 15 % lard spiked in RBD palm oil heating at 120 °C and 180 °C.

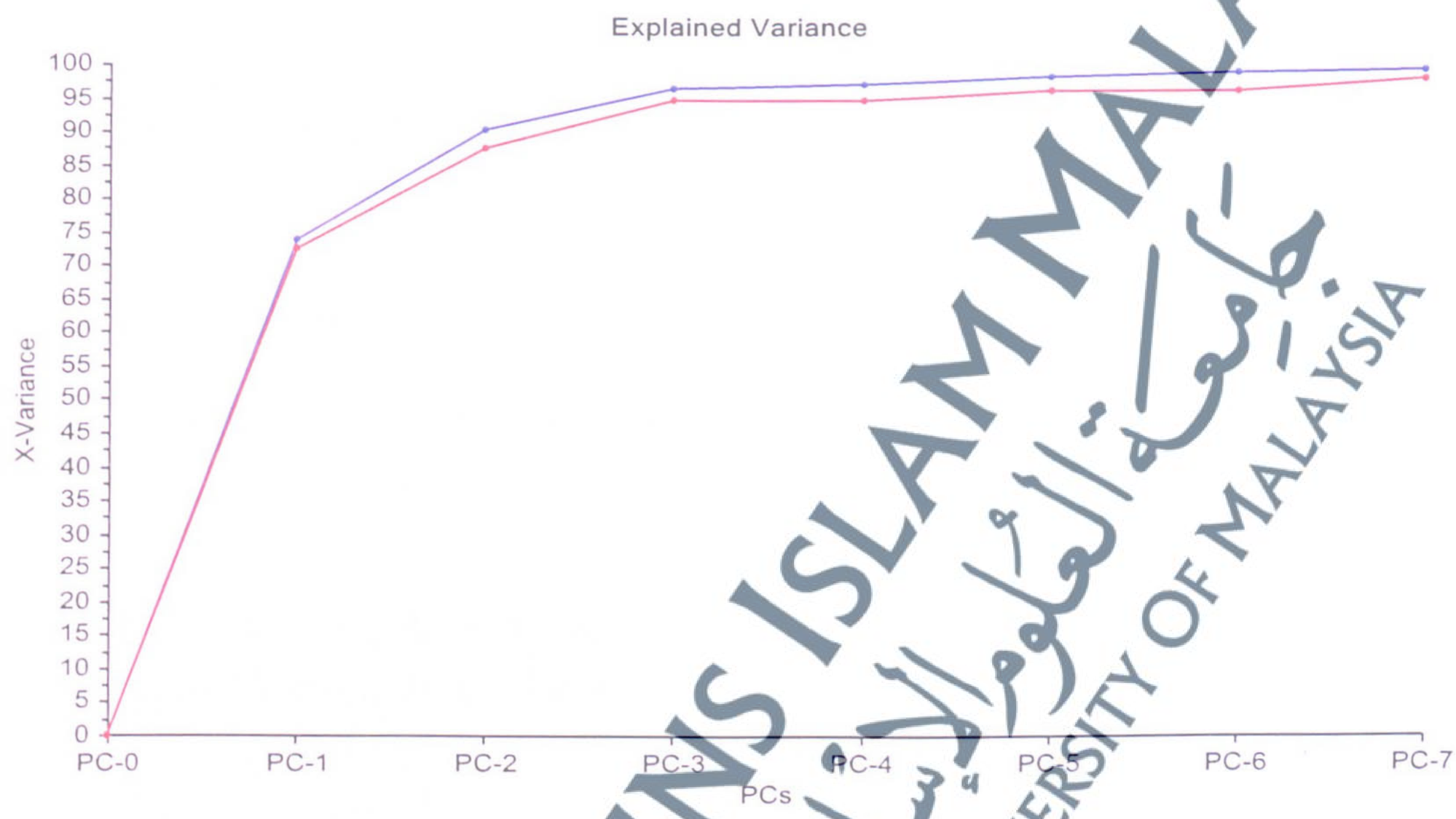


* A= heptanal, B=nonanal, C= octanal, D= E-2-decenal, E=2-undecenal, F= 2-pentylfuran,G= hexanal, H= 2,4-decadienal, K= E-2-heptenal, I= 1-pentanol, P= 2-heptanal, Q= butanal, 2-methyl, U= octane, X= pentane, Y= heptane, Z= butane.

The scree plot of explained variance for samples treatments for GCMS-HS for 0 % and 30 % lard was shown in **FIGURE 16**. Eventhough 7 PCs were generated from the scree plot, only 2 PCS were taken into account which were PC1 and PC2.

PC1 described 74 % while PC2 described 17 % of the total variance in data. Results of PCA were summarized from Scores table (refer **Appendix E**) and Loadings table (refer **Appendix F).**

FIGURE 16: Scree plot of explained variance from 0 % and 30 % lard in 120 °C, 180 °C, 240 °C for 3 hours heating.

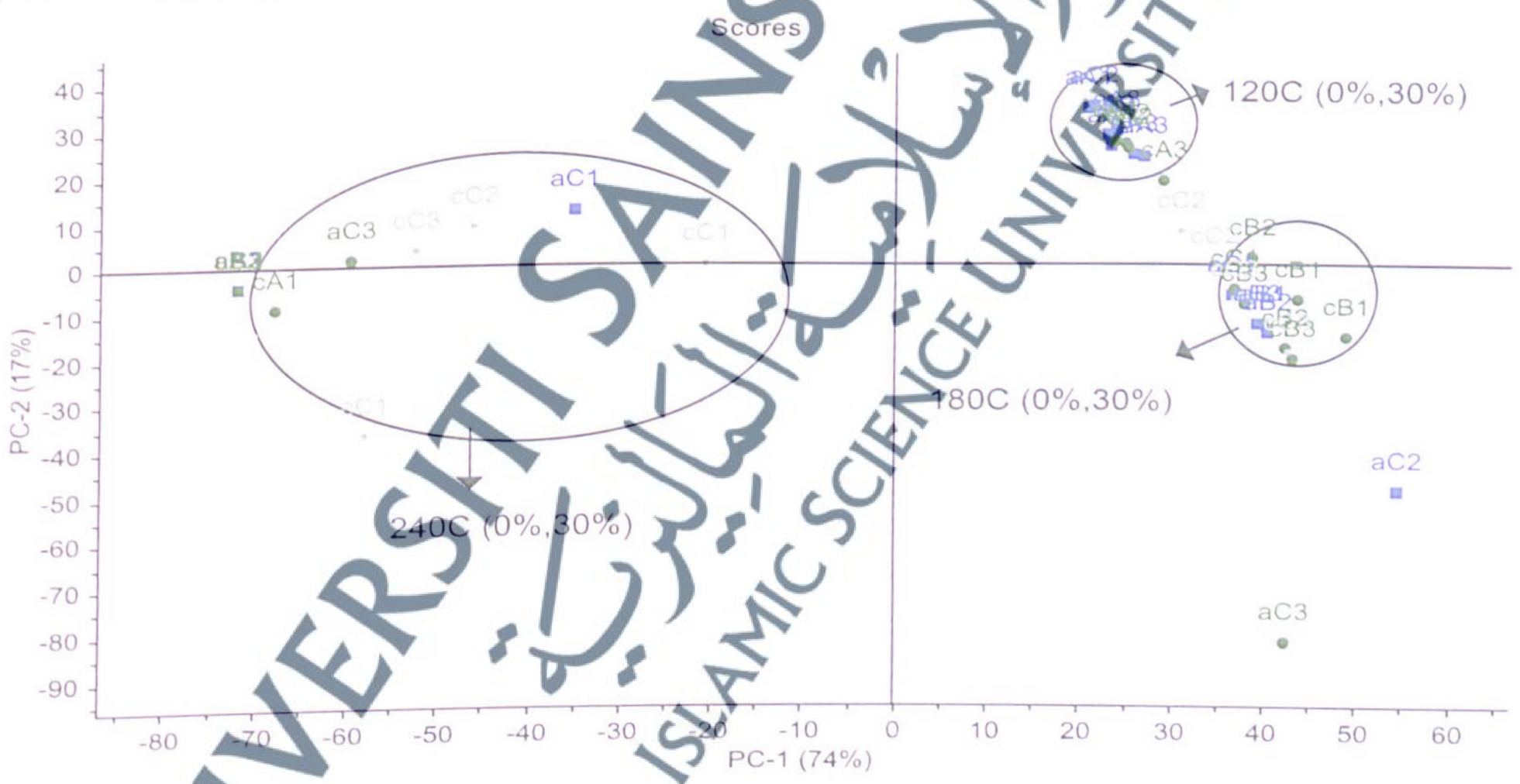


Unlike **FIGURE 14**, some volatile compounds were detected by GC-MS-HS when the lard was increased for 30 % and thus were observed at the scores plot (**FIGURE 17**). Since the amount of lard was doubled (30 % lard in RBD palm oil), therefore some volatile might present and detected by GC-MS-HS but they were scattered and did not located closed to each other in their group. Most of the targeted volatile could not be detected by GC-MS-HS as compounds already lost during high heating temperature (240 °C). Chen et al. (2004) also claimed that there was a

significant difference between volatile compounds from pork jerky when roasted at 150 °C and 200 °C for 2 minutes.

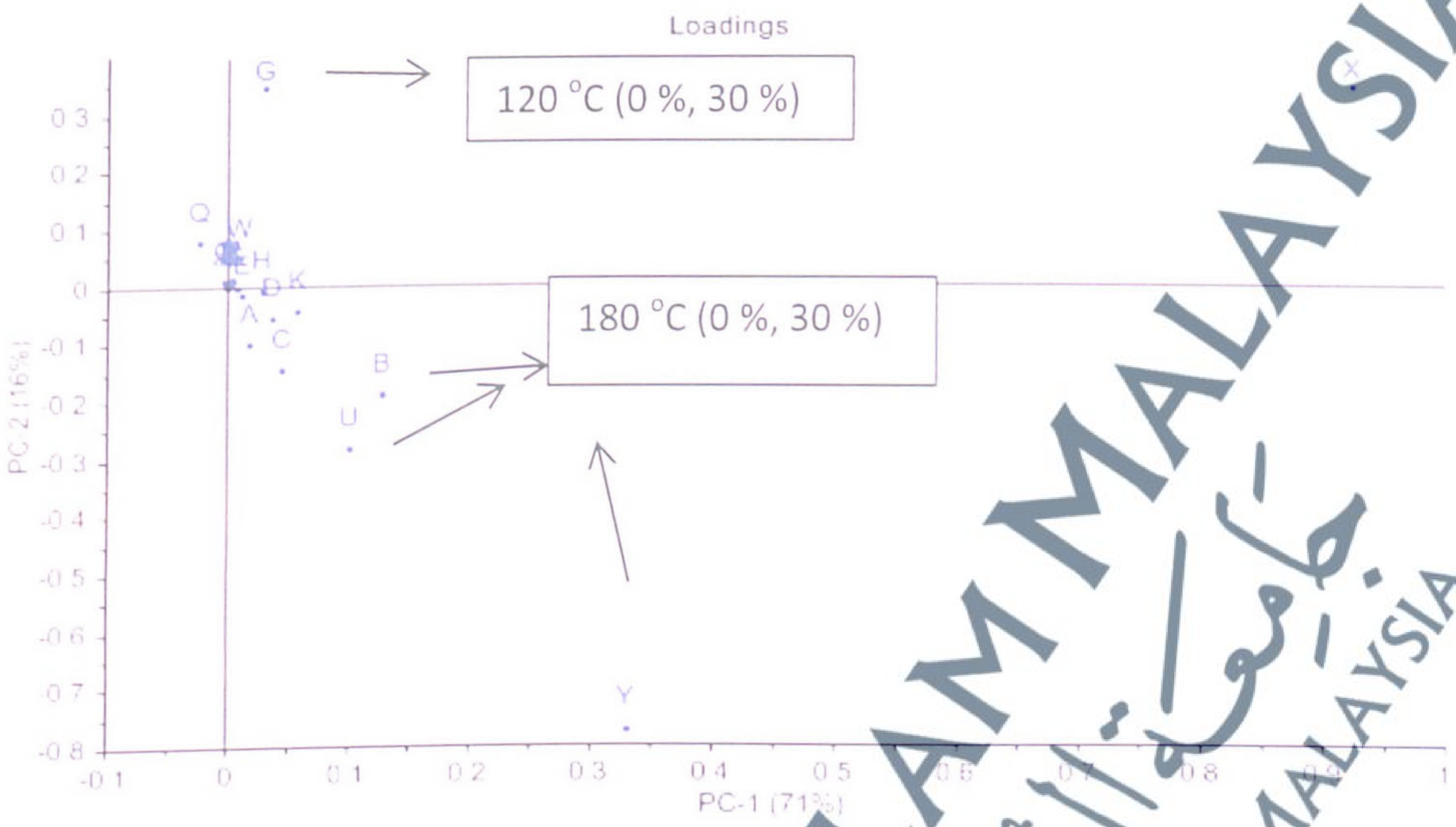
In PCA, the closer the sample in scores plot, the more similar they are with respect to the two components concerned. This pattern showed that samples containing lard (15 % and 30 %) could not be distinguished with control (0 % lard) at 120 °C and 180 °C. Meanwhile at 240 °C for 30 % lard, it was observed that samples were not clustered closely in the scores plot (**FIGURE 18**). Since volatile compounds were influenced by fatty acids, this findings was also observed by Hassan (2015) that found that at 240 °C (maximum heating time was 3 hours), there were no difference in fatty acids between sample (contain lard) in palm oil and control.

FIGURE 17: The scores plot of GC-MS-HS for 0 % and 30 % lard spiked in RBD palm oil heating at 120 °C, 180 °C and 240 °C.



*a = 0 % lard in 100 % RBD palm oil, c= 30 % lard in 70 % RBD palm oil, A= 120 °C, B= 180 °C, C= 240 °C, 1= 1 hr heating time, 2= 2 hrs heating time, 3= 3hrs heating time

FIGURE 18: The loadings plot of GC-MS-HS for 0 % and 30 % lard spiked in RBD palm oil heating at 120 °C and 180 °C.



* A= heptanal, B=nonanal, C= octanal, D= E-2-decenal, E=2-undecenal, F= 2-pentylfuran,G= hexanal, H= 2,4-decadienal, K= E-2-heptenal, I= 1-pentanol, P= 2-heptanal, Q= butanal, 2-methyl, U= octane, X= pentane, Y= heptane, Z= butane.

Production of volatile oxidation compounds were greatly influenced by fatty acid composition of oil. According to Rohman et al.(2012), the main fatty acid compositions for lard are from palmitic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2). Meanwhile in the palm oil, the main fatty acids are myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2) (Koushki et al., 2015). Pentane and hexanal were suggested and has been an indicator in determining the extent of the oxidation of oil (Choe, 1997). Aldehydes compounds such as nonanal, E-2-decenal and 2-undecenal are being correlated with oleic acids while hexanal and 2,4-decadienal are being correlated with linoleic acids. It was reported that hexanal is also a source of fatty aroma (Stahnke, 1994). Meanwhile

hydrocarbon such as heptane and octane was from oleic acids and pentane was from linoleic acids (Frankel, 1985).

4.3 CONCLUSION

GC-MS-HS could not distinguish the control and sample containing lard. However, the score plot showed that volatiles compounds were clustered according to the heating temperature. The presence of lard did not affect the overall volatile compounds when determined by GC-MS-HS. Some volatile compounds were observed to be related with the heating temperature. In the loading plot, hexanal was related with heating temperature 120 °C, while nonanal, octane and heptane was related with heating temperature 180 °C.