CHAPTER III

PHYSICOCHEMICAL CHARACTERISTIC OF LARD ADULTERATION IN RBD PALM OIL

3.0 INTRODUCTION

Malaysia is one of the exporters of palm oil and it is consumed in more than 150 countries (Lai, 2005). The special characteristic of palm oil has encouraged it to be used in a wide range of products. An oil palm tree consists of 10 % oil and 90 % biomass. In order to obtain the final products, palm oil needs to undergo many processing steps such as milling, crushing or extraction, refining, pressing, bleached and deodorization. After the milling steps, the fresh fruit bunches will produce CPO (crude palm oil), PKO (palm kernel oil), palm kernel cake, shell as well as fiber.

In Malaysia, the oil palm products consists the following categories which are palm oil and palm kernel oil products, oleochemicals, bio-fuels, and biomass (Lai, et al., 2012). CPO is further processed into refined products such as refined bleached deodorized (RBD) palm oil, RBD palm olein and RBD palm stearin. Towards the end, different products are being produced from this process. For example, RBD palm olein is normally used to produce frying and cooking oils, shortenings and margarines. While RBD palm stearin is used for fatty acids, soaps, fuel and emulsifiers. Palm olein is the liquid fraction from the fractionation process of palm oil after crystallization step at controlled temperature. The physical characteristics of palm olein are fully liquid in warm climate and it also has a narrow range of glycerides.

Double- fractionated palm olein or superolein has an iodine value between 60 and 67. It comprise of high content of oleic acid (C18: 1), palmitic (C16: 0) as well as linoleic acids (C18: 2). Super olein will becomes cloudy and tend to crystallizes when the temperature is lower than its melting point. Since palm olein can offer several technical characteristics that are desirable in food applications, such as resistance to oxidation, thus may increase the shelf life of end products. Moreover, palm olein also does not emit undesirable odors, does not contain linolenic acid and has a favourable nutritional composition for being free of trans fatty acids and contain tocopherols in its composition.

Deep fat frying will result in desirable and undesirable flavour compounds, changes the flavour stability, quality, colour and texture of fried food and nutritional quality of foods. Common chemical reactions in frying oil include hydrolysis, oxidations and polymerization. The non volatile compounds will affect the flavour, stability, quality as well as texture of the fried foods during storage. During frying, factors such as frying temperature and time, frying oil, antioxidants and the type of fryer will affect the hydrolysis, oxidation and polymerization of the oil.

Therefore, the objective of this research was to measure the physichochemical (Iodine Value, Peroxide Value, and colour intensity) changes of the RBD palm oil containing 0 % (**a**), 15 % (**b**) and 30 % (**c**) of lard after heating each sample for 1 hour, 2 hours and 3 hours at 120 °C (**A**), 180 °C (**B**) and 240 °C (**C**).

3.1 MATERIALS AND METHODS

3.1.1 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.

3.1.2 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 volume. The purpose of spiking lard in the fresh RBD palm oil was to act as preliminary study to emulate recycle RBD palm oil that might contain lard.

3.1.3 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 hours of heating time and cooled to room temperature before kept in a tightly closed and seal universal bottles in a refrigerator until further analysis.

3.1.4 Measurement of Iodine value (IV)

0.04 g samples were weight to the nearest 0.0001 g in the glass weighing scoop. The scoop was placed in a 500 ml flask. 20 ml of cyclohexane was added to dissolve the fat. Exactly 25 ml of Wijs solution was added. The flask was shakes gently before being placed in dark for 1 hour. After standing, 20 ml of potassium iodide solution and 100 ml of water were added into the flask. The solution was titrated with sodium thiosulphate solution until the yellow colour due to iodine has disappeared. 1 to 2 ml of starch indicator solution was added and the titration was continued until the blue colour just disappeared after very vigorous shaking. Two determinations on the same test sample were carried out. Blank test was also simultaneously carried out under the same conditions (PORIM Test Method, 1995).

The Iodine Value (IV) is given by:

$$\text{Iodine value} = \frac{12.69N\,(V_2 - V_1)}{W}$$

Where; N is the exact normality of the sodium thiosulphate solution used

V_2 is the volume, in millilitres of the sodium thiosuphate solution used for blanks

V_1 is the volume, in millilitres of the sodium thiosuphate solution used for the determinations.

W is the weight in grams of the test portion.

3.1.5 Measurement of Peroxide Value (PV)

2 to 5 grams of sample was weight into 250 ml flask. Then, 30 ml of the acetic acid-chloroform was added. The flask was swirled until the sample dissolved in the solution. 0.5 ml of saturated potassium iodide was added using graduated pipette. The solution was later swirled for 1 minute before being added with 30 ml of distilled water. Later, it was titrated with 0.01 N sodium thiosulphate solution. The titration was continued until near the end point to liberate all the iodine from the chloroform layer. The thiosulphate solution was added dropwise until the blue colour just disappear. The blank test was carried out parallel with the determinations (PORIM Test Method, 1995).

The peroxide value (PV) is given by:

$$PV = \frac{(V_s - V_b)N \times 1000}{W}$$

Where V_s is the volume of millilitres, of the sodium thiosulphate solution of normality N, used for the determination

V_b is the volume in milliliters of the sodium thiosulphate solution used for the blank test

W is the weight, in grams of the test portion

N is the normality of the sodium thiosulphate solution

3.1.6 Colour Intensity

Colour was measured using the Lovibond tintometer. (Labscan XE Hunter lab). Oil samples were taken in a cuvette and were placed in the provided space in tintometer . The hunter colour scale parameters which are redness, yellowness and lightness were used to estimate colour changes during frying as a function of the process variables (oil temperature, heating time, percentage of lard). The dominant factor in this measurement was the red colour. Then, the result was expressed in Lovibond colour units.

3.1.7 Statistical analysis

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

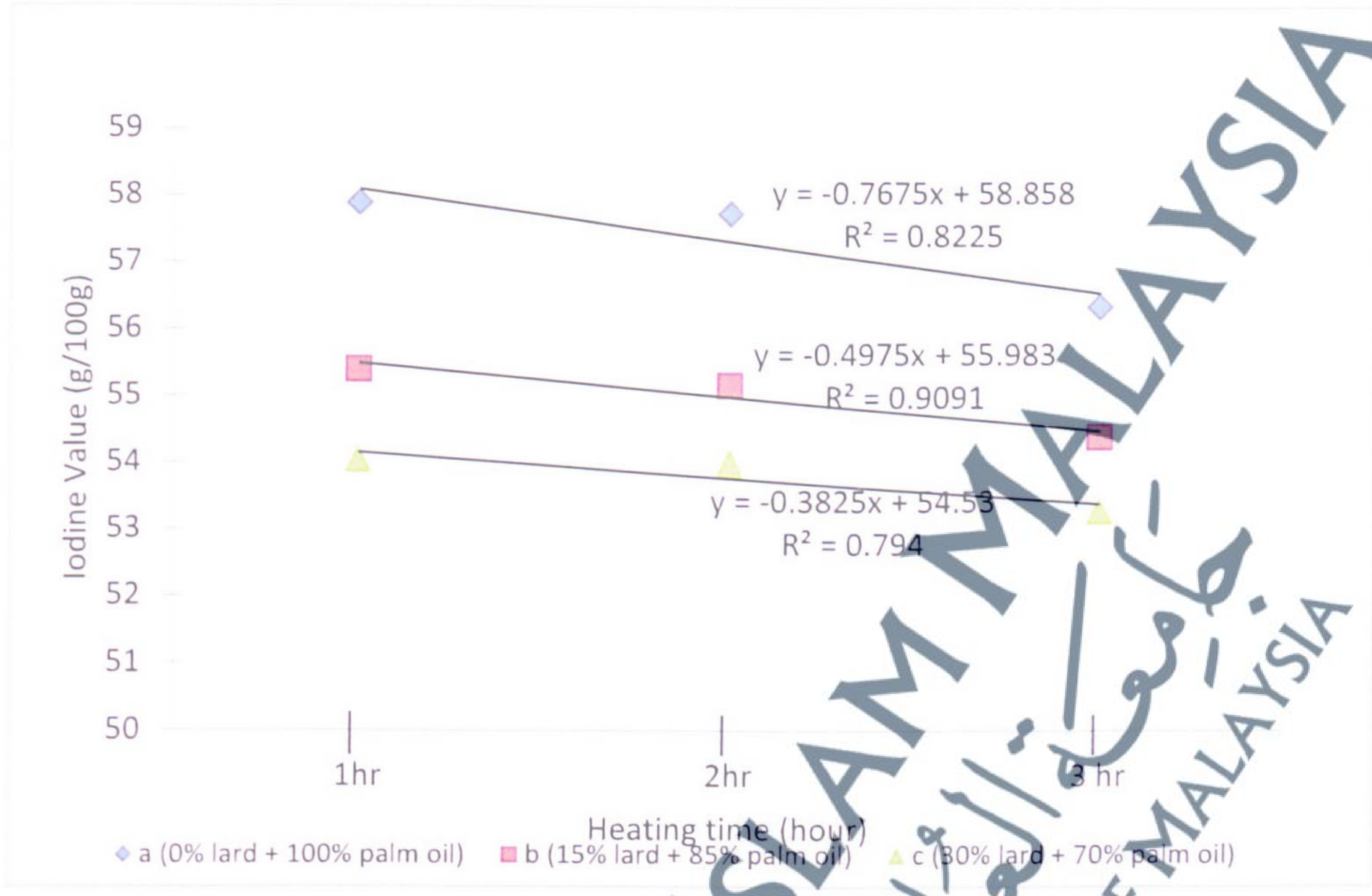
3.2 RESULTS AND DISCUSSION

When subjected to repeat heating, many properties of oil's characteristic features were exhibited with respect to different range of heating temperatures and time of heating. Since vegetable oils consist of mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA), these compounds begin to degrade and produce various oxidation products. Raw data for sample **a** (0 % lard), **b** (15 % lard) and **c** (30 % lard) for IV, PV and colour intensity was shown in **Appendix G.**

3.2.1 Iodine Value (IV)

During Wijs reaction, iodine will react with the unsaturated fatty acids which are in the form of double bonds. The high value of iodine number indicates that high amount of carbon double bonds in the fats and oil. Therefore, iodine value can be used to directly measure the amount of unsaturated fatty acid present (Gopinath et al., 2009). As heating time was prolonged, all samples showed a decreased in the amount of iodine value as shown in **FIGURE 1**. According to Cuesta et al. (1991), the decreased of iodine value can be attributed to the destruction of double bonds by oxidation, scission and polymerization. During this heating temperature, it was observed that all samples did not show much change in terms of iodine value. This might be because at low temperature (120 ^{o}C), the heat is not high enough to break most of the double bonds.

FIGURE 1: Relationship between iodine values heated at 120 °C and 3 hours heating time



The iodine values for sample **a**, **b** and **c** at more immense temperature (180 °C and 240 °C) were shown in **FIGURE 2** and **FIGURE 3** respectively. All samples showed a decreased in terms of iodine value with sample **c** showed the highest changes at both 180 °C and 240 °C. According to Marikkar and Yanty (2013), in lard, oleic acid are the most dominant fatty acid (24 % to 51 %) followed by palmitic, linoleic and stearic acid consecutively. The major triacylglycerol (TAG) in lard are PLL, OOL, LPO, OPO, PPO and SPO (O = oleic acid, P = palmitic acid, S = stearic acid, L = linoleic acid) where OPO are the most dominant TAG molecular species while LPO and SPO are the second and third most abundant. In palm oil, the major species are PPO, POO, POL, and PPL (Andrikopoulos, 2002). Since lard contains more unsaturated fatty acid, thus the high temperature might cause the unsaturated fatty acid to breakdown into other intermediate compounds. Therefore it has

undergone the highest changes in terms of iodine value when subjected to higher temperature. Alireza et al. (2010) obtain the same pattern in the reduction of iodine value when subjected the RBD palm oil, sesame oil, canola oil and their blending to 5 days of heating at 180 °C. The highest significant changes ($p < 0.05$) was observed in canola oil due to the presence of high amounts of polyunsaturated fatty acids (30 g/100 g).

FIGURE 2: Relationship between iodine values heated at 180 °C and 3 hours heating time

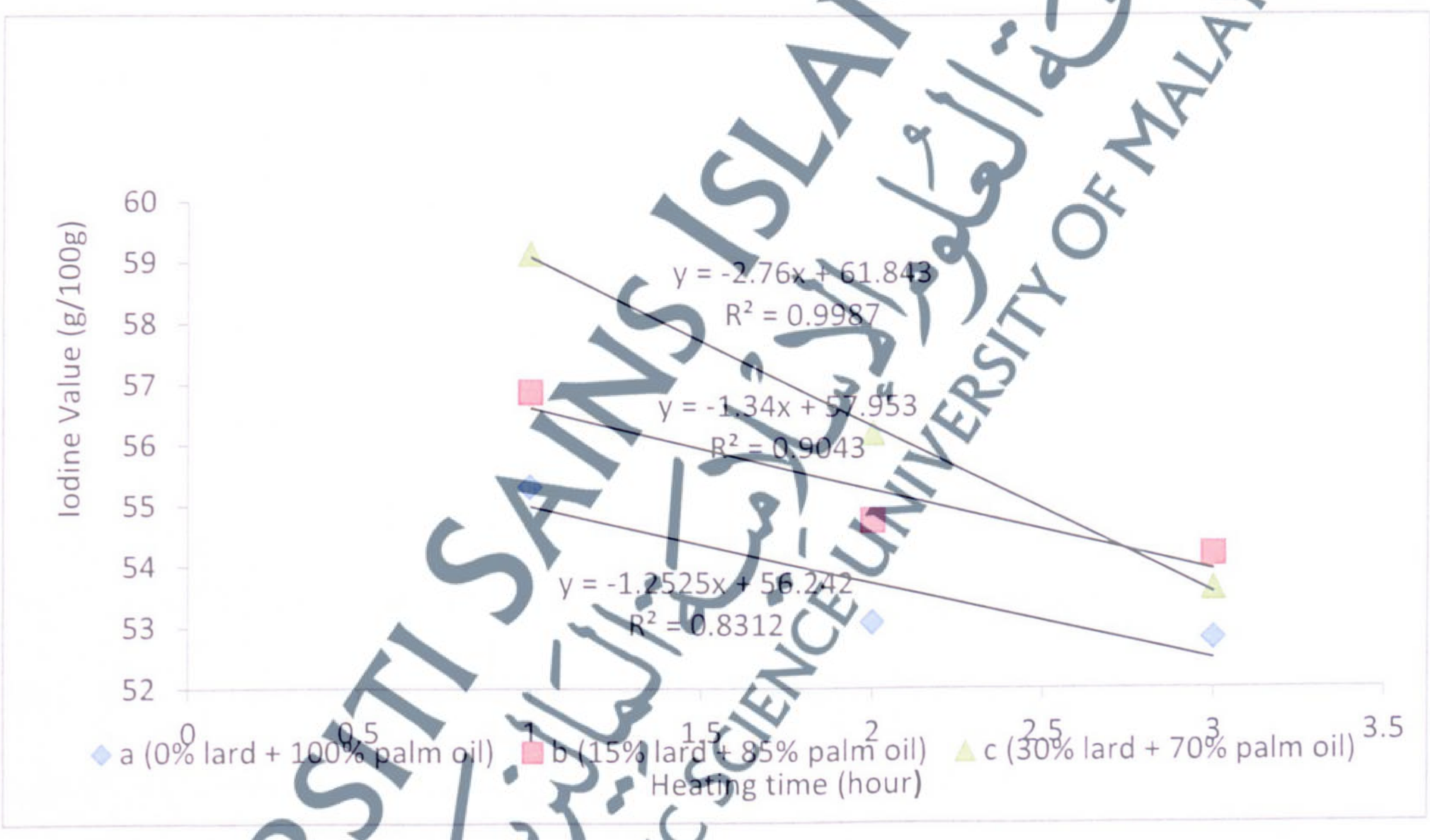
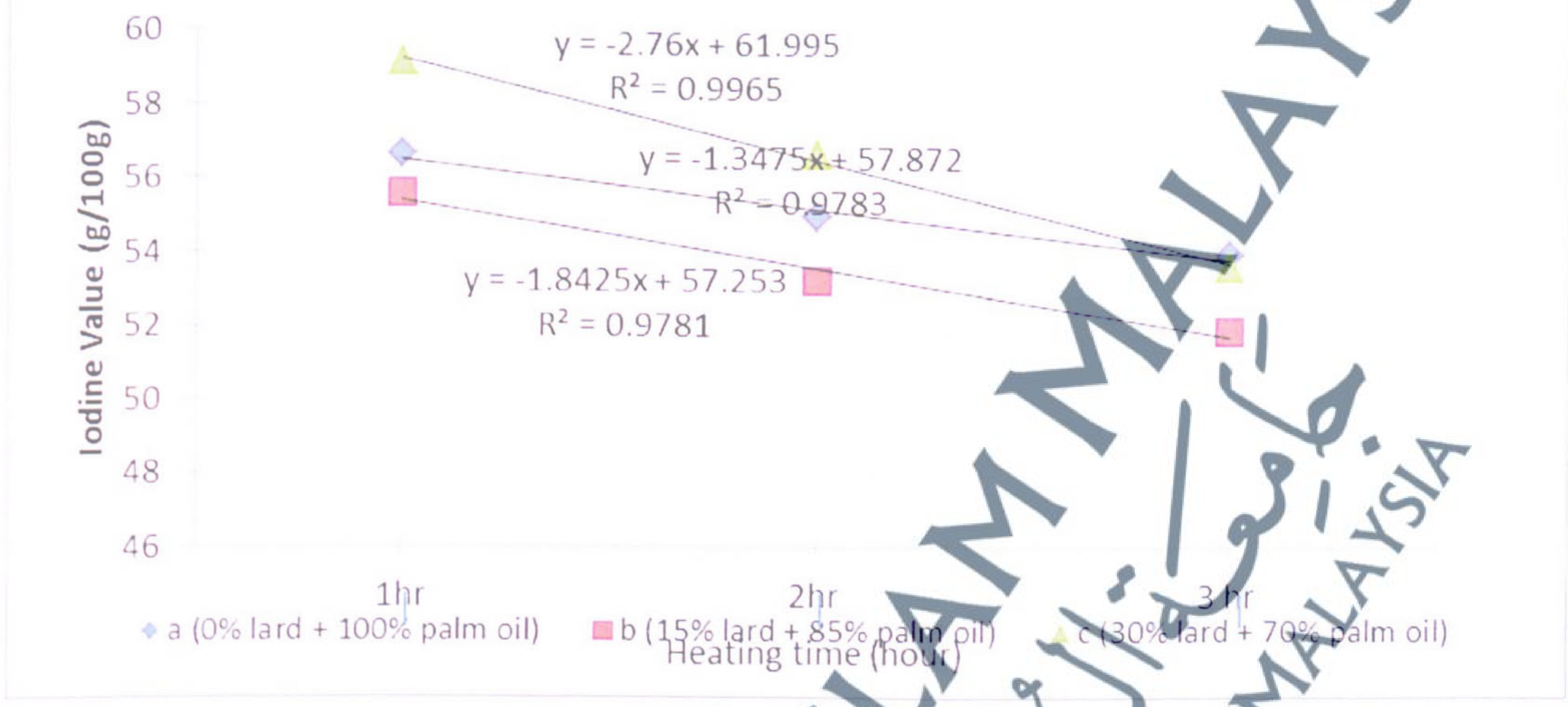


FIGURE 3: Relationship between iodine values heated at 240 °C and 3 hours heating time



3.2.2 Peroxide Value (PV)

Peroxide value measures the extent to which oil has undergo primary oxidation. It is also a measure of rancidity of the oil. Peroxide value is not a good indicator to evaluate the oil quality changes because of the degradation of hydroperoxide in high temperature. However, it can be the indicator of oil instability. According to Olaniyan (2010), an increase in heating time and temperature will increase the acid value, peroxide value and the colour intensity of the oil. Initially in **FIGURE 4**, all samples showed a low peroxide value, however, the increased in time of heating has increased the peroxide value for all samples. Sample **c** showed the highest changes in peroxide value as the heating time was prolonged. This might due to the high amount of unsaturated fatty acid contributed by 30 % lard. Bolourian et al.

When the heating temperature was increased to 180 °C (**FIGURE 5**), at 1 hour heating time, all samples showed a higher peroxide value compared to 1 hour heating time at 120 °C. As the heating time was prolonged, sample **b** and sample **c** showed a decreased pattern in peroxide value, while peroxide value for sample **a** was still increased even though not as rapid as in temperature 120 °C. When a higher temperature was subjected to all samples as in **FIGURE 6**, they showed a decreased in peroxide value. This pattern might occur because at 180 °C, the secondary oxidation products started to form and accumulate. Since peroxide value measure the primary oxidation products, thus the value decreases as the secondary oxidation products formed.

FIGURE 5: Relationship between peroxide values heated at 180 °C and 3 hours heating time

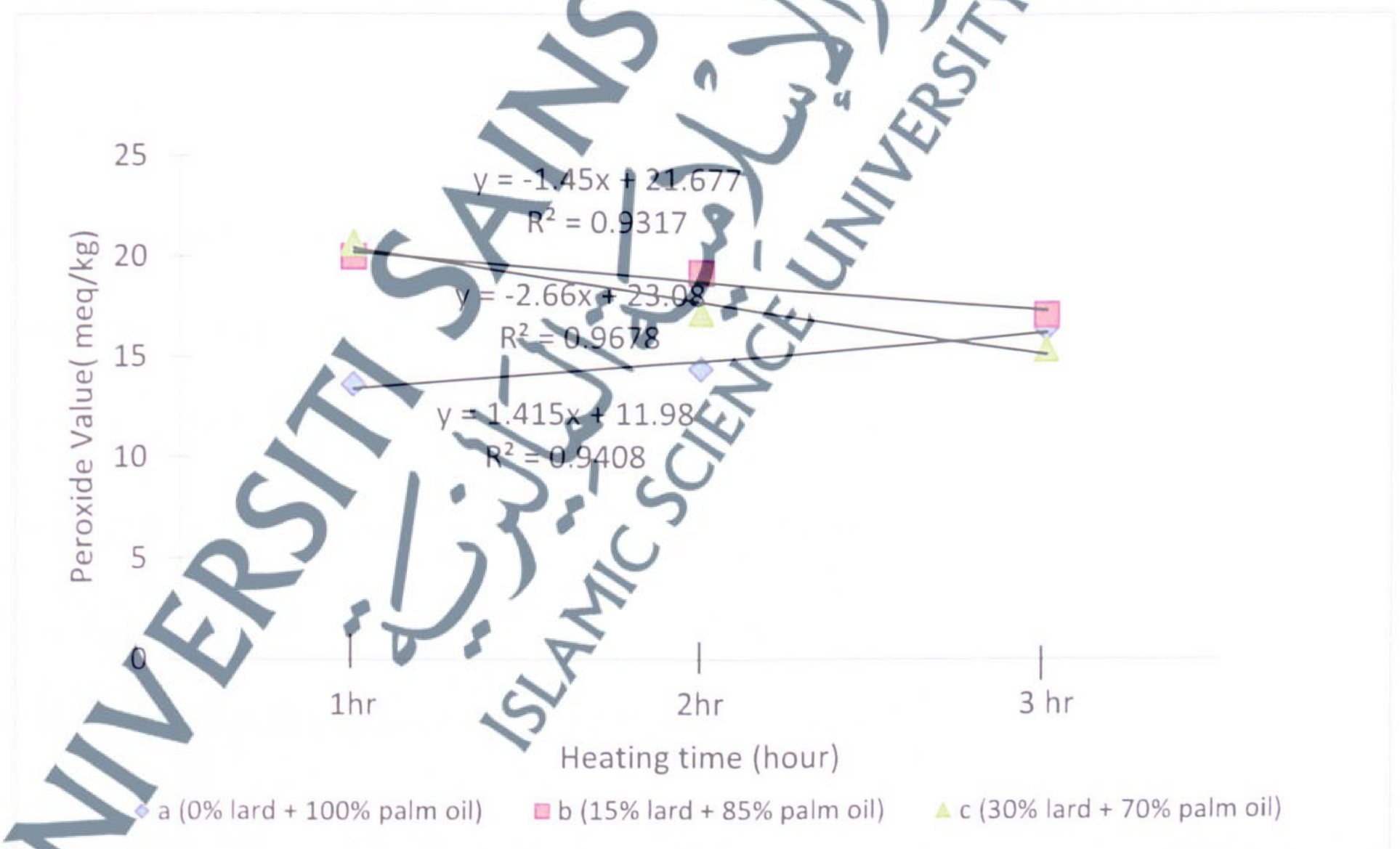
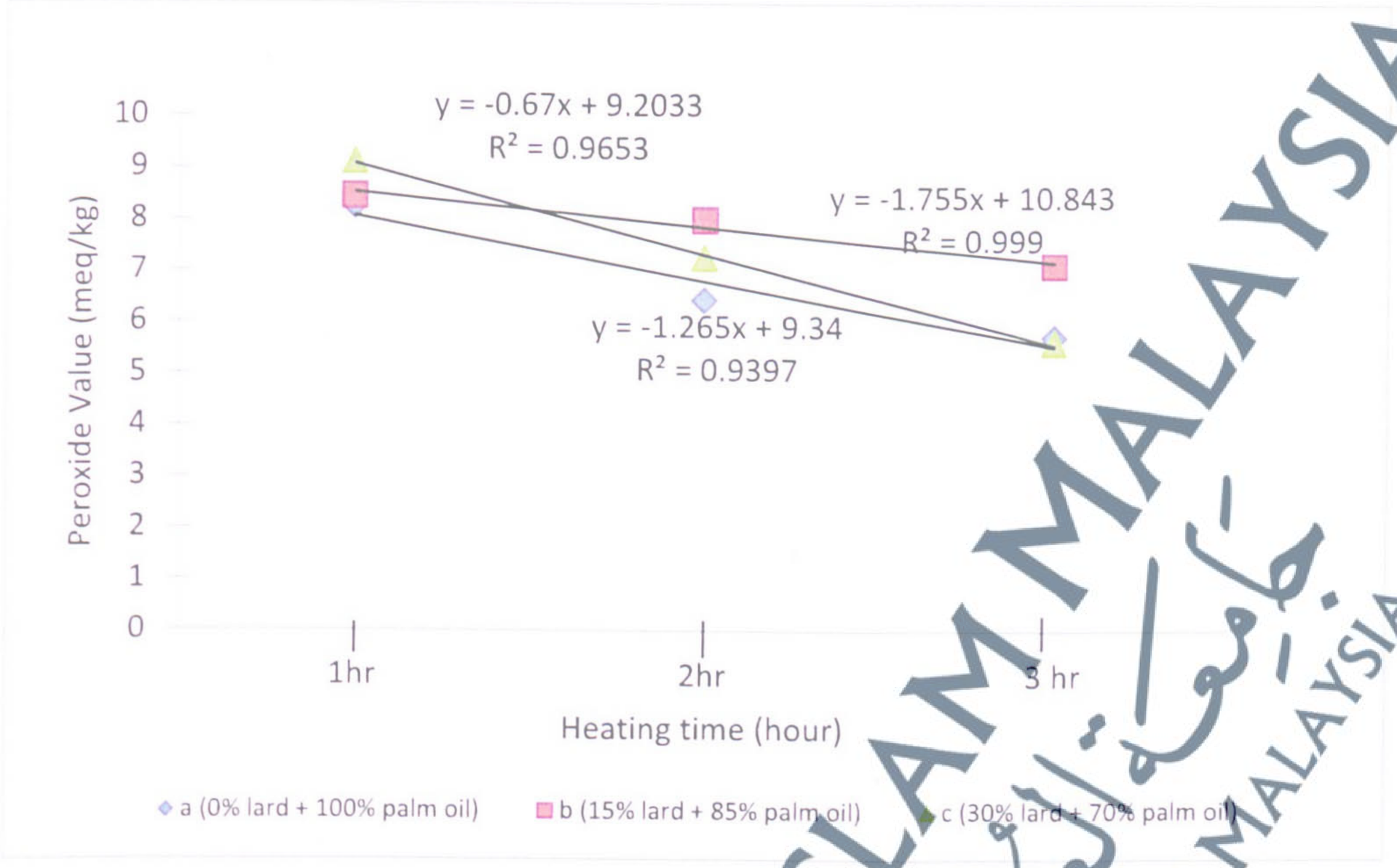


FIGURE 6: Relationship between peroxide values heated at 240 °C and 3 hours heating time



3.3 Colour Intensity

As heating or frying proceeds, oil colour become darker. Many factors may influent the changes in oil colour such as type and amount of food being fried. Food components will interact with oil and form coloured constituents like Maillard browning products. Since changes in oil colour may results from more than one chemical process, therefore the use of oil colour to monitor the quality level is not valid when evaluating a wide range of frying operations (Takeoka et al., 1997).

The value for L*, a* and b* for samples **a**, **b** and **c** at 120 °C, 180 °C and 240 °C heating at 3 hours were shown in **Table 1**. L defined lightness, +a depicts a shift towards red and –a towards green, while +b represent a shift towards yellow and –b towards blue. As the heating time increased from 1 hour to 3 hours, all samples shows

a trend in reduction of L* value indicating dark oil formed. On the contrary, all samples showed an increased in terms of a* value (Refer **TABLE 1**). This trend showed that the samples become darker as the time of heating was prolonged.

TABLE 1: Hunter Lab value for 0 %, 15 %, 30 % lard in RBD palm oil at 120 °C, 180 °C and 240 °C for 3 hours of heating

	L*	a*	b*
aA1	34.26 ± 0.03	-1.97 ± 0.02	7.13 ± 0.01
aA2	34.05 ± 0.01	-1.62 ± 0.01	7.15 ± 0.03
aA3	33.25 ± 0.02	-1.58 ± 0.01	7.29 ± 0.03
aB1	39.29 ± 0.01	-1.72 ± 0.03	7.42 ± 0.02
aB2	31.06 ± 0.04	-1.31 ± 0.04	7.95 ± 0.01
aB3	30.6 ± 0.02	-1.29 ± 0.02	7.84 ± 0.02
aC1	39.76 ± 0.03	-1.76 ± 0.02	8.11 ± 0.03
aC2	36.88 ± 0.02	-1.33 ± 0.01	8.73 ± 0.02
aC3	35.84 ± 0.01	-1.23 ± 0.03	8.81 ± 0.02
bA1	38.00 ± 0.04	-1.79 ± 0.01	7.6 ± 0.03
bA2	37.86 ± 0.02	-1.54 ± 0.01	7.67 ± 0.02
bA3	32.76 ± 0.03	-1.26 ± 0.03	7.61 ± 0.01
bB1	36.78 ± 0.03	-1.83 ± 0.04	8.2 ± 0.01
bB2	36.35 ± 0.01	-1.81 ± 0.02	8.44 ± 0.05
bB3	36.38 ± 0.04	-1.44 ± 0.01	8.47 ± 0.03
bC1	36.75 ± 0.04	-1.56 ± 0.01	8.52 ± 0.02
bC2	36.54 ± 0.01	-1.33 ± 0.04	8.44 ± 0.02
bC3	33.9 ± 0.02	-1.18 ± 0.01	9.08 ± 0.04
cA1	35.44 ± 0.03	-1.9 ± 0.02	8.27 ± 0.02
cA2	33.87 ± 0.04	-1.6 ± 0.02	8.22 ± 0.04
cA3	34.09 ± 0.03	-1.36 ± 0.03	8.07 ± 0.02
cB1	33.83 ± 0.02	-1.6 ± 0.01	9.30 ± 0.03
cB2	33.9 ± 0.05	-1.55 ± 0.02	9.15 ± 0.02
cB3	33.58 ± 0.02	-1.17 ± 0.01	9.16 ± 0.03
cC1	34.23 ± 0.01	-2.38 ± 0.01	8.23 ± 0.01
cC2	34.74 ± 0.04	-1.31 ± 0.04	8.49 ± 0.02
cC3	35.38 ± 0.05	-1.07 ± 0.02	8.36 ± 0.03

Note: mean value ± standard deviation

* +a = red, -a = green, +b = yellow, -b = blue, L, 0 = black, 100 = white

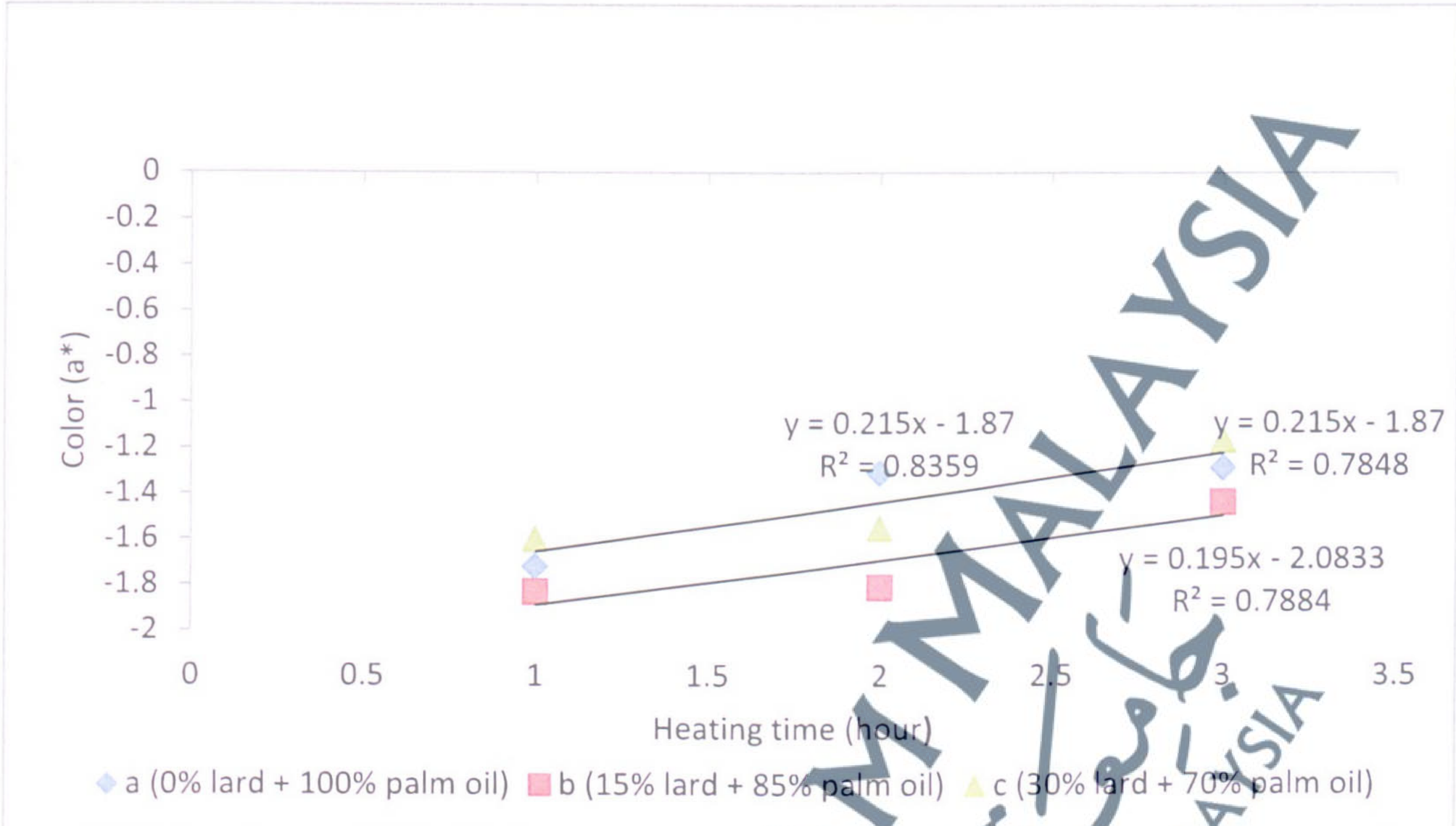
*a = 0 % lard, b = 15 % lard, A= 120 °C, B= 180 °C, C= 240 °C, 1= 1 hr, 2= 2 hrs, 3= 3hrs

At low heating temperature (120 °C), even though the a* value increased, however not much changes was observed in a* value for all samples as in **FIGURE 7**. It was observed that the lowest a* value among 3 samples were 0 % lard while the 15 % and 30 % lard showed almost the same values for 3 hours heating time. The formation of non volatile decomposition which contained carbonyl group caused the darkening of palm oil (Tsaknis, 2002). During frying, the carbonyl will absorb the energy of the visible light magnitude.

FIGURE 7: Colour changes a* for all samples heated at 120 °C for 3 hours heating time

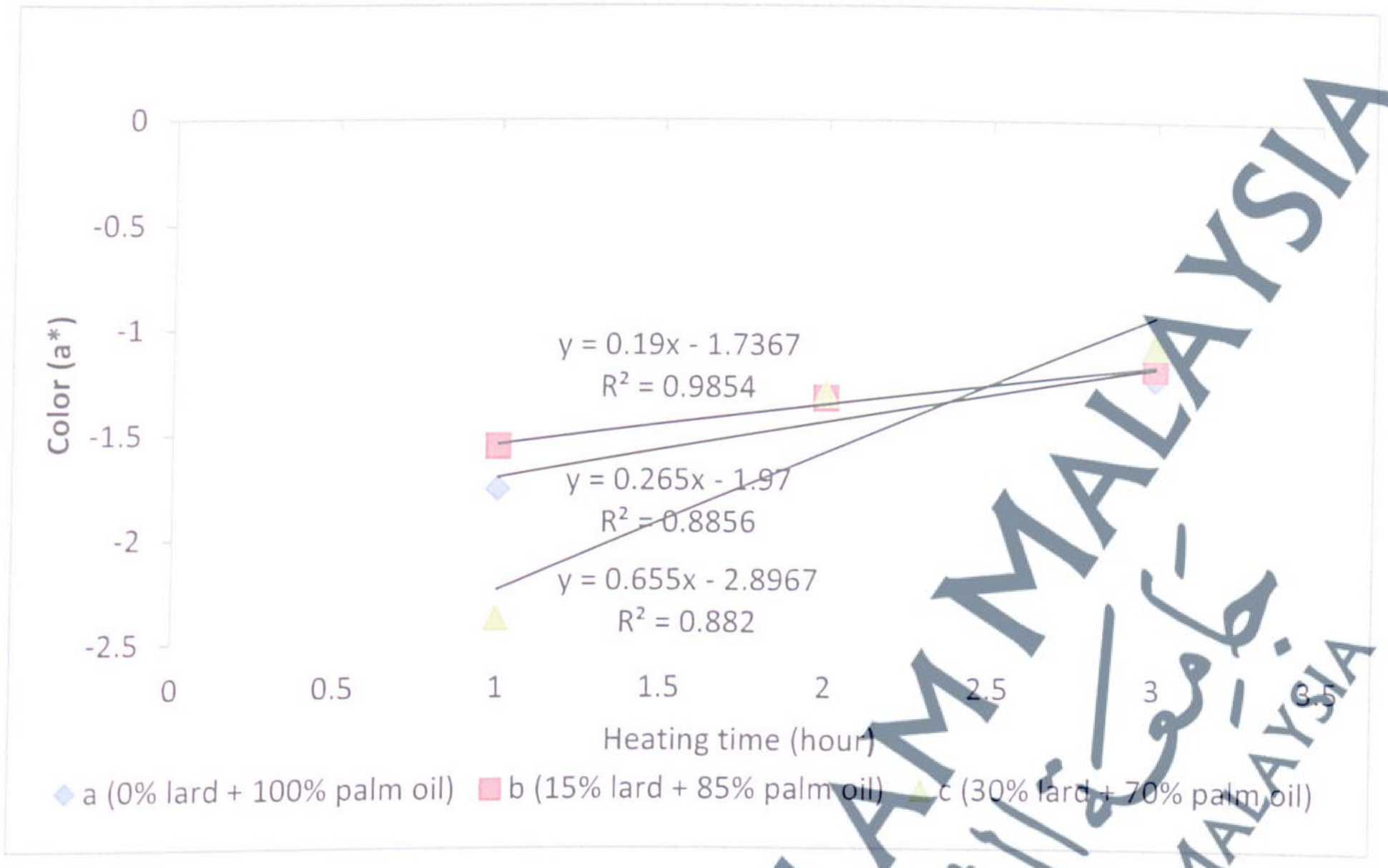
When the temperature was increased to 180 °C for 1 hour heating time, not much differences were observed when compare to 3 hours heating time at 120 °C. However, as the heating time was prolonged, the a* value increased (**FIGURE 8**).

FIGURE 8: Colour changes (a*) for all samples heated at 180 °C for 3 hours heating time



The changes in colour was the biggest when all samples were heated at highest heating temperature (240 °C) as shown in **FIGURE 9.** As the heating time was increased, the a* values for all samples also increased with sample **c** showed the highest changes during 3 hours heating time at 240 °C.

FIGURE 9: Colour changes (a*) for all samples heated at 240 °C for 3 hours heating time



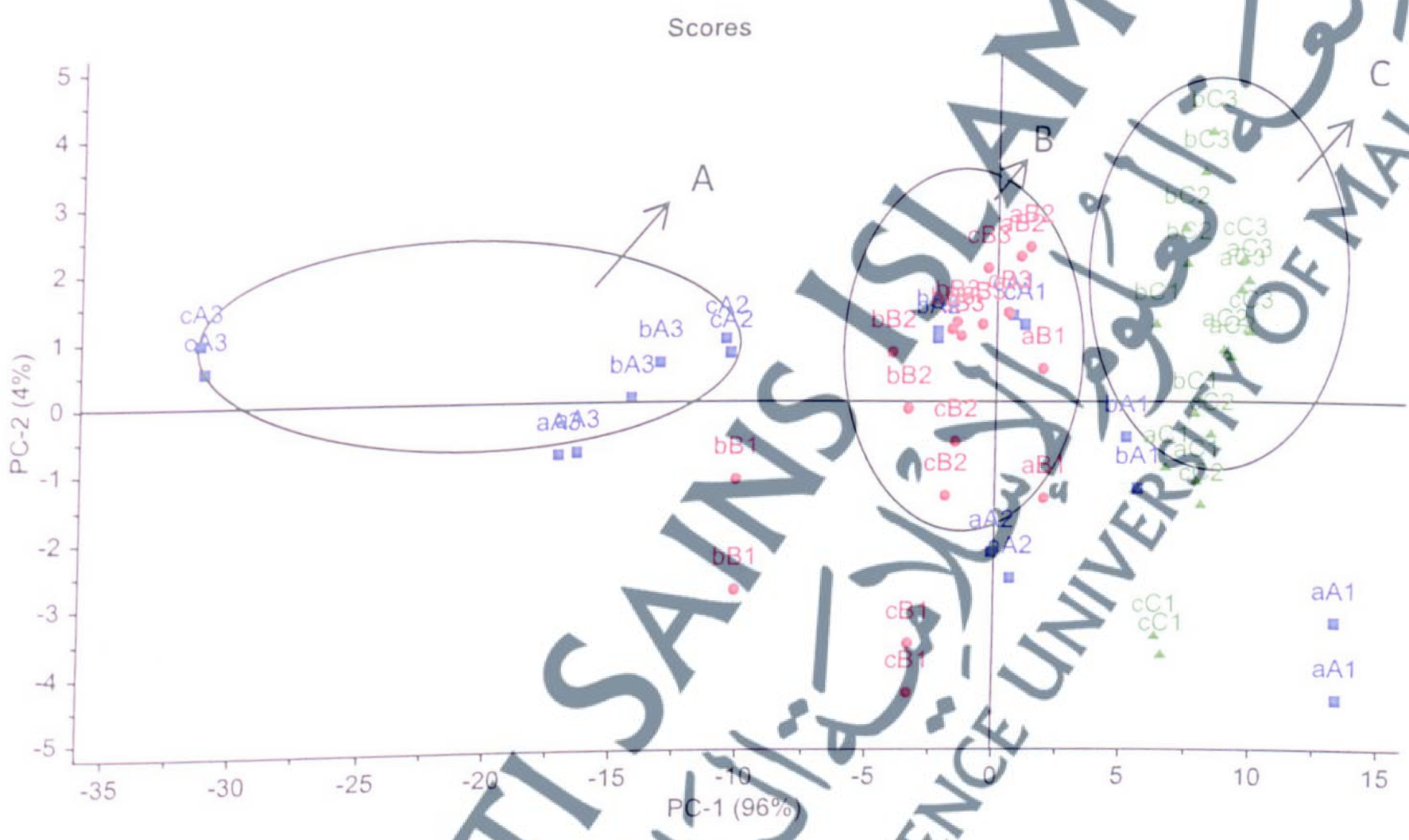
3.2.4 Principal Components Analysis

Principal components analysis is a tool to reduce a large set of variables to a small scale but still contains most of the information from the large set. Results of PCA were summarized from scores plot (**Appendix H**) and loadings plot (**Appendix I**).

The scores plot for sample **a**, **b** and **c** at 120 °C, 180 °c and 240 °C was shown in **FIGURE 10**. From the plot, it was observed that samples are clustered according to their heated temperatures despite contain a lard or no lard. Scores plot may be used to interpret the similarities or differences among samples. The closer the sample in the score plots, the more similar they are with respect to the two components concerned.

Conversely, samples that are far away from each others are different from each other. In **FIGURE 10**, each group was different from each other since a significant clustered was observed. **A** was denoted for samples heated at 120 °C, **B** for samples heated at 180°C, while **C** for samples heated at 240 °C. However, some samples that were heated at 120 °C were seemed to be presented at group **B**. This might be because of the high heating temperature as they were heated at 2 or 3 hours of heating at 120 °C.

FIGURE 10: Scores plot of physicochemical tests for all samples heating at 120 °C, 180 °C and 240 °C for 1, 2 and 3 hours heating time

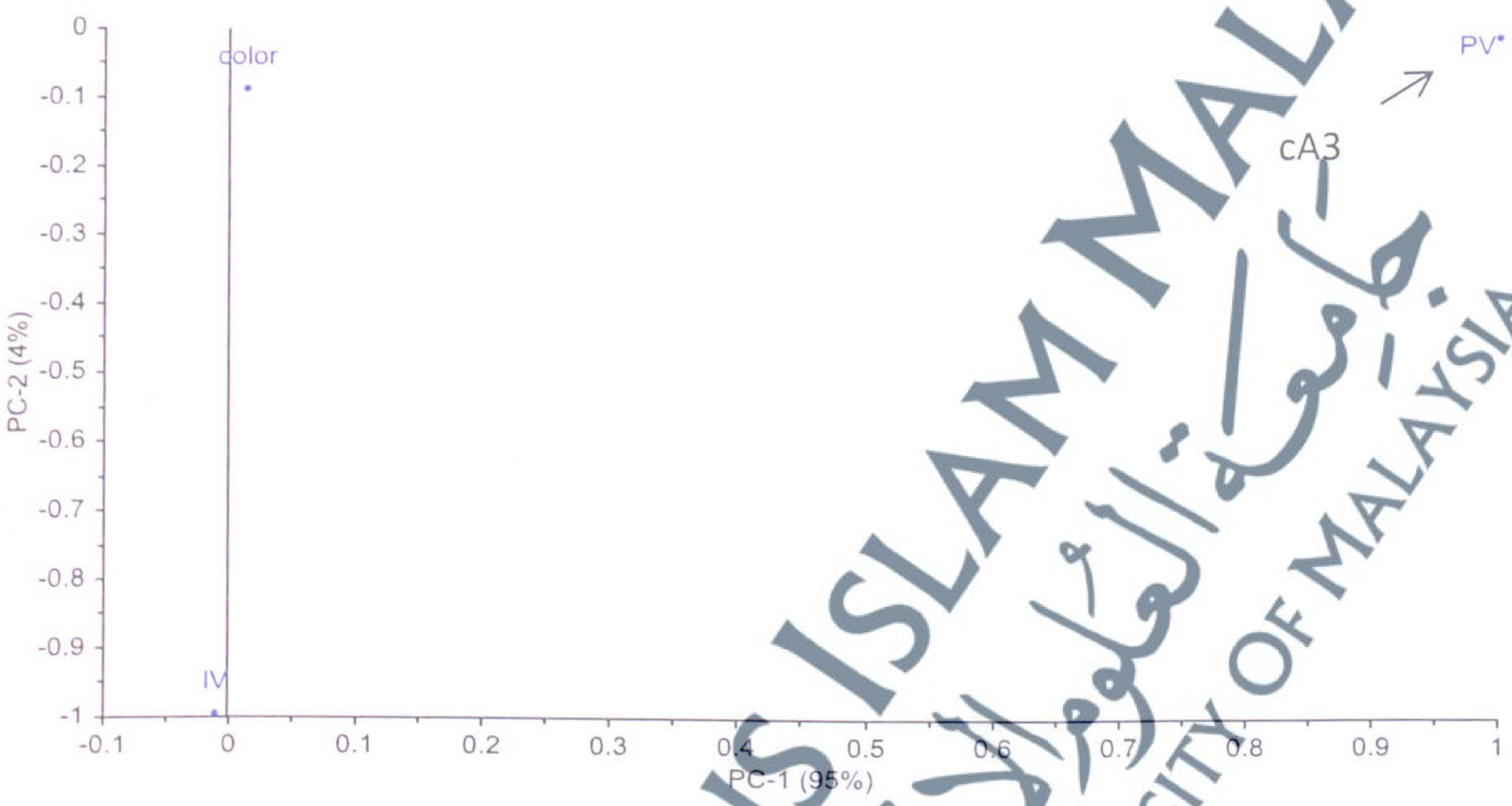


*a = 0 % lard in 100 % RBD palm oil, b = 15 % lard in 85 % RBD palm oil, c= 30 % lard on 70 % RBD palm oil, A= 120 °C, B= 180 °C, C= 240 °C, 1= 1 hour heating, 2 = 2 hours heating, 3= 3 hours heating

The importance of the different variables for the components specified was shown in **FIGURE 11**. PV was correlated with cA3 (samples **c** heated at 120 °C for 3 hours). Among all samples, it showed the highest PV which were 46.67 ± 0.01. This might be because it contains high amount of unsaturated fatty acid compared to other

sample and the heating time was longer. When the heating temperature was increased, the PV value was low due to the formation of secondary oxidation products.

FIGURE 11: Loadings plot of physicochemical tests (PV, IV and color intensity)



3.3 CONCLUSION

Heating affects the physicochemical characteristic of oil. For iodine value, at low heating temperature (120 °C), all samples did not showed much changes. However, when the heating temperature was increased to 180 °C and 240 °C, all samples showed a decrease in iodine value with sample **c** showed the highest changes. Heating destructs the double bonds in the unsaturated fatty acid. As for peroxide value, at prolong heating time at 120 °C, all samples showed an increased in peroxide value with samples **c** with the highest changes due to the formation of primary oxidation product. However, as the heating temperature was increased to 180 °C, a different pattern was observed as there was a decreased in PV for sample **b** and **c** due

to the formation and accumulation of the secondary oxidation products. While sample a still showed an increase in PV. At 240 °C, all samples showed a decreased in PV. In terms of colour, at temperature 120 °C, not much changes was observed in a* values for all samples. It was observed that as heating time increased, there was a reduction in the L* value and increment in the a* value. Changes in colour was the highest when all samples were heated at 240 °C. The scores plot showed an accumulation of samples based on the heating temperature and there was no difference observed between samples that contain lard and no lard. Therefore, it was concluded that the physicochemical tests in terms of colour intensity, PV and IV could not differentiate between 0 %, 15 % and 30 % lard heating at 120 °C, 180 °C and 240 °C for 3 hours heating time.