OCCURRENCE OF LIPID PEROXIDATION IN REPEATEDLY USED COOKING OIL IN KELANTAN

by

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of the requirements for the
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CERTIFICATE

This is to certify that the dissertation entitled
"Occurrence of Lipid Oxidation in Repeatedly Used Cooking Oil in Kelantan"
is the bona fide record of research work done by Nurul Syahmi Binti Mohd. Ramli during the period from August 2004 to March 2005 under my supervision.

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At the end there is only my name showing on the cover of the thesis, claiming all faults of this work as mine. As for the contributions, research is not done in thin air and so there are many people I would like to thank here.

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ABBREVIATION

1.	LPO	Lipid peroxidation
2.	$OH^{\bullet}$	Hydroxyl radical
3.	LH	Lipid (polyunsaturated fatty acid)
4.	$L^{\bullet}$	Lipid radical
5.	O_2	Oxygen
6.	$LOO^{\bullet}$	Lipid peroxyl radical
7.	LOOH	Lipid hydroperoxide
8.	MDA	Malondialdehyde
9.	DNA	Deoxyribonucleic acid
10.	RNA	Ribonucleic acid
11.	ROS	Reactive oxygen species
12.	LDL	Low density lipoprotein
13.	PCB	Polychlorinated biphenyls
14.	PAHs	Polycyclic aromatic hydrocarbons
15.	TBARS	Thiobarbituric acid reactive substance
16.	TBA	Thiobarbituric acid
17.	TEP	Tetra – ethoxy propane
18.	HCl	Hydrochloric acid
19.	TCA	Trichloroacetic acid
20.	NaOH	Sodium hydroxide
21.	USM	Universiti Sains Malaysia

ABSTRACT

Lipid peroxidation (LPO) is the oxidative deterioration of lipids containing any number of carbon – carbon double bonds. It is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation.

The purpose of this study is to standardize a simple and cheap method for estimating oxidation of lipids in the cooking oil samples. The other objective of this study is to compare the occurrence of oxidation of lipids in fresh cooking oil and repeatedly used cooking oil samples.

The reused oil samples are obtained from four stalls who were selling fried bananas. They were supplied with EKONOMI palm oil packs or pouches to be used on daily basis. The oil has been used solely for deep – frying the bananas. The banana hawkers were instructed to keep using the oil left at the end of the day for the next day business and they were allowed to refill the oil with the fresh one when needed. The collection of the oil was on daily basis for 7 consecutive days followed by the 14^{th} day, 21^{st} day and 30^{th} day following the reusage.

Our study has shown that the malondialdehyde concentration in the reused oil at all four banana stalls was increased during the period (30 days) of our study. The results have shown that the increased malondialdehyde concentration in the reused oil following rancidity were due to many factors. The factors which caused an increased amount of malondialdehyde concentration in the reused oil were air pollution, dirty utensils, length of

time period that the oil has been used, an infrequent replacement of reused oil with fresh one and the locality of the stall.

The pH of the reused oil samples was also been measured. Most of the reused oil samples from all stalls showed a decrease of pH throughout the study period. The reduced in pH showed that the reused oil samples had become more acidic in comparison to the fresh oil samples. This might have enhanced malondialdehyde formation.

Future studies, in animal models following feeding of oil samples might open new horizons in the ill – health effects of such oil in human population.

ABSTRAK

Pengoksidaan lemak adalah suatu proses yang melibatkan lemak yang mempunyai sebarang bilangan ganda dua karbon – karbon. Ia merupakan suatu reaksi rantaian yang membekalkan radikal bebas yang berterusan dan ia menjadikan proses pengoksidaan yang seterusnya akan terus berlaku.

Tujuan utama kajian ini dilakukan adalah untuk mempiawaikan satu kaedah yang mudah dan murah untuk menganggar pengoksidaan lemak di dalam sampel minyak masak yang telah digunakan berulang kali. Selain itu, kajian ini turut membuat perbandingan pengoksidaan lemak di antara minyak masak yang belum digunakan dan yang telah digunakan berulang kali.

Sampel minyak yang telah digunakan berulang kali diperolehi daripada empat gerai yang menjual pisang goreng. Peniaga – peniaga pisang goreng tersebut telah dibekalkan dengan pek – pek minyak EKONOMI untuk digunakan pada setiap hari. Minyak tersebut hanya digunakan untuk menggoreng pisang tersebut. Peniaga – peniaga pisang goreng tersebut telah diarahkan untuk terus menggunakan minyak yang berlebihan dan terus menggunakan minyak tersebut untuk hari – hari seterusnya. Mereka dibenarkan untuk menambah minyak baru apabila diperlukan. Sampel minyak telah dikumpulkan pada setiap hari selama 7 hari berturut – turut diikuti sampel minyak pada hari ke – 14, hari ke – 21 dan hari ke – 30.

Kajian ini telah menunjukkan kandungan kepekatan malondialdehida di dalam minyak masak yang telah digunakan berulang kali daripada keempat – empat gerai pisang goreng

telah meningkat sepanjang tempoh kajian (30 hari) ini dijalankan. Keputusan kajian menunjukkan peningkatan kepekatan malodialdehida di dalam minyak masak tersebut adalah disebabkan oleh beberapa faktor. Faktor – faktor yang telah menyebabkan peningkatan kepekatan malondialdehida di dalam minyak yang telah digunakan berulang kali adalah pencemaran udara, penggunaan alatan memasak/ menggoreng yang tidak bersih, tempoh penggunaan minyak masak itu digunakan, kekerapan menggantikan minyak masak yang telah digunakan dengan minyak masak yang baru dan lokasi gerai tersebut.

Tahap pH minyak masak yang telah digunakan berulang kali juga telah ditentukan. Kebanyakan minyak masak tersebut dari keempat – empat gerai telah menunjukkan penurunan pH sepanjang tempoh kajian dijalankan. Penurunan pH telah menunjukkan minyak masak yang telah digunakan berulang kali itu menjadi semakin berasid jika dibandingkan minyak masak yang belum digunakan. Faktor ini juga mungkin menyumbang kepada pembentukan malondialdehida di dalam minyak masak tersebut.

Kajian selanjutnya yang melibatkan model haiwan dengan memberikan sampel minyak masak yang telah digunakan berulang kali kepada haiwan tersebut dapat membuka lembaran baru kepada kesan buruk minyak masak tersebut ke atas kesihatan populasi manusia.

1.0 INTRODUCTION

1.1 Lipid peroxidation

Lipid peroxidation (LPO) is the oxidative deterioration of lipids containing any number of carbon – carbon double bonds. It is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation.

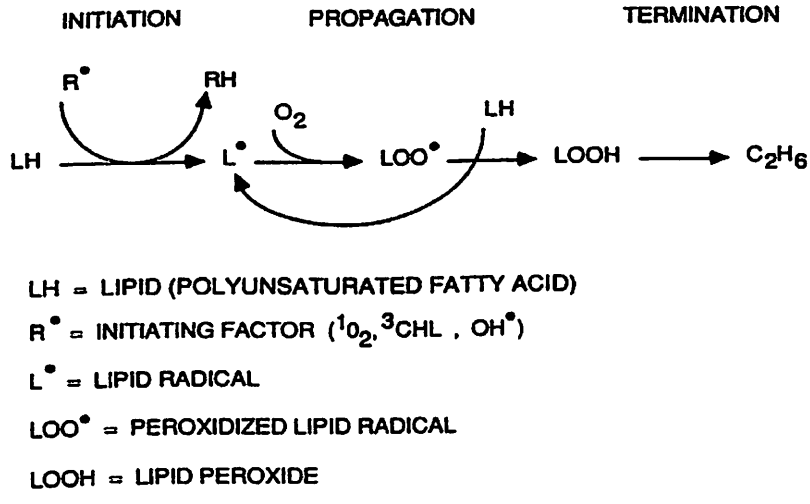

Figure 1: The lipid peroxidation chain (http://billie.btny.purdue.edu/btny504/lipidperox.html)

Some of common unsaturated fatty acids are palmitoleic acid (ω7, 16:1), oleic acid (ω9, 18:1), linoleic acid (ω6, 18:2), linolenic acid (ω3, 18:3) and arachidonic acid (ω6, 20:4).

LPO is a classic free radical chain reaction that consists of chain initiation, propagation, and termination steps. Figure 1 illustrates the free radical process of LPO. Initiation of LPO occurs when a free radical compound, such as the hydroxyl radical ($OH^{\bullet}$), removes hydrogen from a polyunsaturated fatty acid (LH). The removal of the hydrogen from the LH forms a lipid radical ($L^{\bullet}$). In an aerobic environment this radical reacts with oxygen (O_2), giving rise to a lipid peroxyl radical ($LOO^{\bullet}$). Propagation reactions will continue the process by the addition of O_2, resulting in lipid hydroperoxide (LOOH) and a second lipid radical ($LOO^{\bullet}$). Lipid radical ($LOO^{\bullet}$) can undergo through the same reactions as the LOOH, generation additional lipid LOOH. The propagation step goes through approximately eight rounds of peroxide generation on an average, before a termination event occurs.

1.2 Lipid peroxidation and its ill – health effects

As the result of single electron rearrangement, the lipid undergoes degradation and malondialdehyde (MDA) is formed. MDA is a soluble compound and can be found in most biological samples including foodstuffs, serum, plasma, tissues and urine. The termination event can occur as the result of any reaction with another radical, protein, or compound that acts as a free radical trap to form a stable oxidized compound.

Escaped or released reactive oxygen metabolites into the microvascular environment can cause injury to tissues and connective tissue matrix. Reactive oxygen metabolites may lead to the peroxidation of lipids in the cell membranes resulting in the generation of fatty acid radicals that can react with other lipids, proteins, or free radicals in tissues. The overall effect may result in biochemical changes in the liver during the infection, including depletion of glycogen, lipid infiltration, and decrease in nucleic acid content of both DNA and RNA. (Al – Omar et. al. 2004).

Oxidative damage to nucleic acids will occur in the presence of reactive oxygen species (ROS), radical – mediated damage to DNA is complex and proceeds via peroxy radicals of the DNA bases and sugar, deoxyribose. Base peroxidation occurs most readily intensively with thymine and guanine whereas adenine and cytosine appear to be more stable toward oxidation. Alternatively, ROS also can disrupt pathways critical for the maintenance of normal, adenine and cytosine nucleotide status, such as the nucleases that repair strand breaks via base excision repair. DNA damage by ROS can also lead to chromosome abnormalities. In proteins and amino acids, oxidative damage caused by superoxide anions may prevent collagen gelation. Collagen gelation involves the interaction of single collagen peptide chains by hydrogen bonding to form triple peptide chain helices. Carbohydrates are also susceptible to oxidative damage by ROS. Hyaluronic acid is one of the main components of synovial fluid in joints that can be degraded by ROS. (Al – Omar et. al. 2004).

Oxidative damage by ROS can also be seen on lipids. Specific enzymatic oxidation of polyunsaturated fatty acids leads to the formation of extremely potent and biologically important compounds, e.g. prostaglandins and leukotrienes. In contrast, unspecific oxidation of polyunsaturated fatty acids can lead to lipid peroxidation, a radical mediated pathway. Furthermore, cholesterol is oxidized during lipid peroxidation, yielding 5, 6 – epoxide in addition to a 5 – hydroperoxide. The epoxide occurs in high concentratios in human breast milk and has been identified as a directly acting mutagen. (Al – Omar et. al. 2004).

Macrophages possess receptors that recognize and bind to modified low – density lipoprotein (LDL), called scavenger receptors. Modified LDL bound to these scavenger receptors are rapidly engulfed by macrophages, so the intracellular cholesterol accumulates and may convert the macrophage into a foam cell, which in turn is involved in atherosclerosis development. ROS involvement in LDL peroxidation has been shown recently. It was also found that modified forms of human LDL initiate the accumulation of cholesterol esters in macrophages as a result of oxidative stress. Vascular endothelial cells can also uptake and destroy oxidized LDL. However, take up of oxidized LDL by macrophages is more rapid and might be regarded as a defense mechanism to protect the vascular wall, but excess oxidized LDL can kill macrophages, either by initiating necrosis or by apoptosis. Macrophage death can release proteolytic enzymes and transition – metal ions, causing more oxidative stress to the surrounding cells that may leads to atherosclerosis. (Al – Omar et. al. 2004).

Some of the diseases which are associated to free radical injury are ageing, acute renal failure, cervical cancer, cerebrovascular disorders, diabetes, Down's syndrome, ischemia, reperfusion injury and Parkinson's disease. (Marks et. al. 1996).

The products of lipid peroxidation are malondialdehyde, ethane and pentane. Malondialdehyde level which appears in blood and urine can be estimated. This estimation is used as a biomarker of free radical damage and of lipid peroxidation. (Marks et. al. 1996).

1.3 Cooking oils

Cooking oil is a purified fat of plant or animal origin, which is liquid at room temperature (http://en.wikipedia.org/wiki/Cooking_oil). Common usage of cooking oil is for frying or deep – frying the bananas, meat and vegetables. Common types of cooking oils available in the market are palm oil, corn oil, soyabean oil, coconut oil, sunflower oil, olive oil, peanut oil, margarine and butter. In this study, palm oil has been chosen to evaluate the occurrence of oxidation of lipids. In Malaysia, palm oil is widely used for cooking by the majority of the population because of its availability, great economic value, exceptional resistance to rancidity, owing to the high levels of Vitamin A and Vitamin E contents, very low concentration of linolenic acid and only a moderate proportion of linoleic acid. Furthermore, palm oil

does not produce unpleasant odours making it one of the best frying oil available. (http://www.tropicaltraditions.com/red_palm_oil.htm and http://www.fedepalma.org/oil_uses.htm).

A recent study by researchers in Spain has unraveled a correlation between re - using a cooking oil and high blood pressure. They have suggested that repeated reuse of cooking oil for a number of times may also affect the health. When the same pot of oil is repeatedly reheated, the oil begins to degrade and results in release of substances known as polymers and polar compounds. These substances are absorbed with the food. The authors have concluded that the more polar compounds and polymers present in oil samples, the more likely it was that participant had hypertension. (http://www.hvlib.integris-health.com).

Heart disease and stroke are associated with cholesterol level in the plasma and free radicals in the body. Hydrogenated plant oil, contained in nearly all margarines and foods, may form cholesterol in the body. When low density lipoprotein (LDL) undergoes oxidation, free radicals will stick to the blood vessel walls. Hence, this will lead to less blood flow and higher blood pressure following blockage of blood vessel by cholesterol.

Our study seems to be singular attempt in Kelantan in unraveling oxidation of lipids in reused oils. There are harmful substances that may be present in cooking oils.

These substances may be classified into two types:

a) compounds originating from the degradation of oil
b) liposoluble contaminants, such as polychlorinated biphenyls (PCB) and dioxin
c) polycyclic aromatic hydrocarbons (PAHs) (Riera et. al. 2000)

The data on oxidation of lipids in cooking oils are essential for finding out whether reused cooking oils constitute a significant carrier of thiobarbituric acid reactive substance (TBARS) that may accumulate in human and produce oxygen toxicity.

2.0 OBJECTIVES

The main objectives of this study were:

1. To standardize a simple and cheap method for estimating oxidation of lipids in the cooking oil samples.
2. To compare the occurrence of oxidation of lipids in fresh cooking oil and repeatedly reused cooking oil samples.

3.0 MATERIALS AND METHODS

3.1 Samples

For the present study, four stalls, who were selling fried bananas, were randomly chosen within the Kelantan area because these stalls are found at the roadside everywhere in Malaysia. Furthermore, the reused oil is mostly used for deep – frying the bananas. Each stall was supplied with EKONOMI palm oil packs or pouches (Fig. 2) to be used on daily basis. They were instructed to use the oil for frying the bananas only and to keep using the oil left at the end of the day of their business. They were allowed to refill the used oil with the fresh one but not to discard the used ones away. The reused oil samples were collected on a daily basis for 7 consecutive days. This was followed by collection on the 14^{th} day, 21^{st} day and 30^{th} day following the reusage. All the reused oil samples, from different stalls, were collected in screw capped glass vials (as shown by Figs. 3, 4, 5 and 6) The fresh EKONOMI oil was used as the control sample.

3.2 Qualitative tests performed in the oil samples

3.2.1 Measurement of pH

The pH was measured by dipping the pH (litmus) paper into the bottle containing the oil. The pH of the oil was recorded within 1 to 10 minutes by the colour change on litmus paper.

FIGURE 2: THE POUCH OF EKONOMI (FRESH) OIL USED AS CONTROL

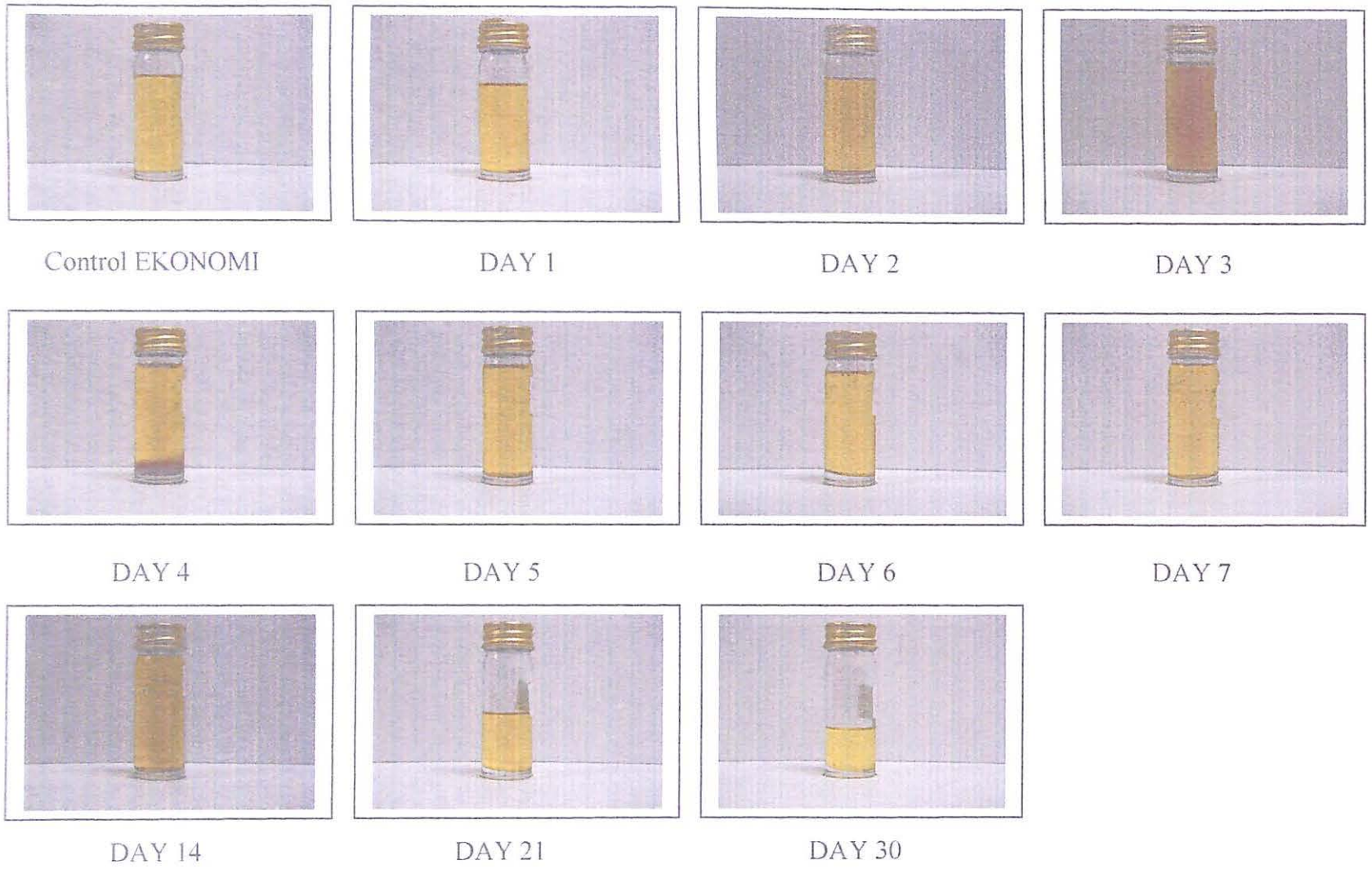

FIGURE 3: THE FRESH AND REUSED OIL SAMPLES FROM STALL 1

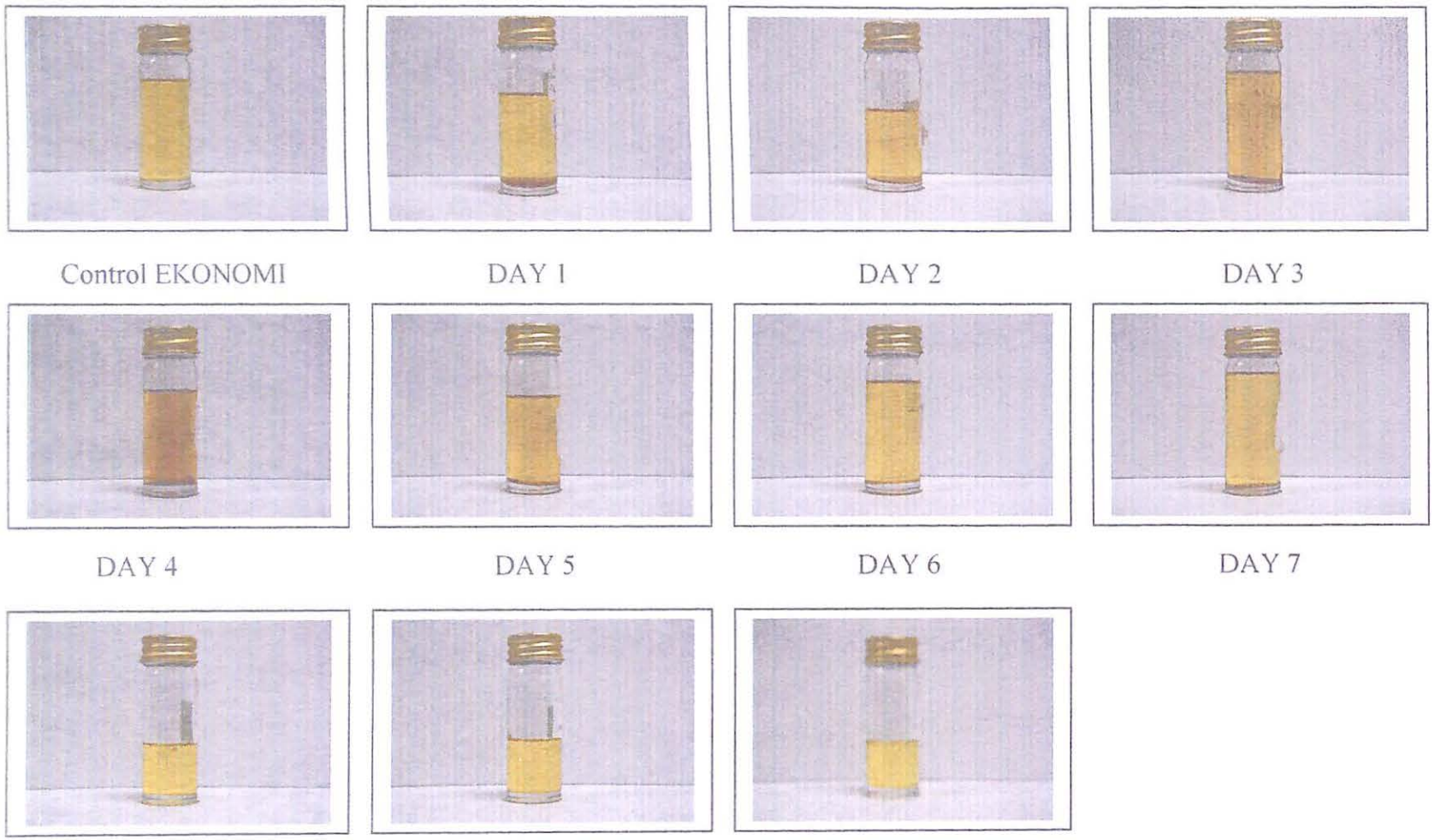

FIGURE 4: THE FRESH AND REUSED OIL SAMPLES FROM STALL 2

FIGURE 5: THE FRESH AND REUSED OIL SAMPLES FROM STALL 3

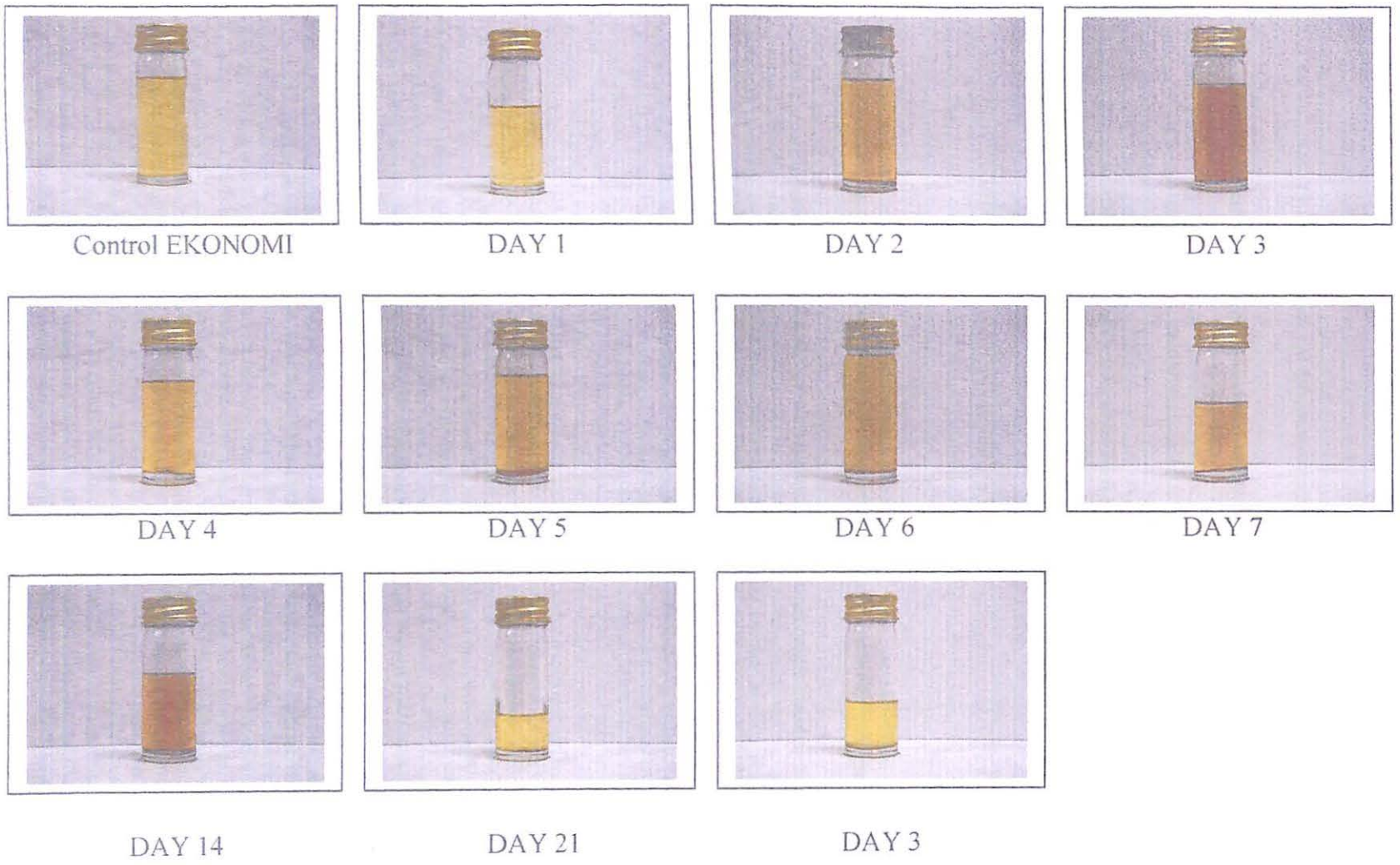

FIGURE 6: THE FRESH AND REUSED OIL SAMPLES FROM STALL 4

3.2.2 Estimation of thiobarbituric acid reactive substance (TBARS) as biomarker of lipid peroxidation

Thirteen corning glass test tubes (16.5 x 1.5 in cm) were taken. They were washed with distilled water and were labeled. The test tubes were labeled as B (for blank), 1 (for day 1 used oil), 2 (for day 2 used oil), 3 (for day 3 used oil), 4 (for day 4 used oil), 5 (for day 5 used oil), 6 (for day 6 used oil), 7 (for day 7 used oil), 8 (for day 14 used oil), 9 (for day 21 used oil) and 10 (for day 30 used oil). The test tubes for control oil samples were labeled as C (fresh EKONOMI oil).

The assay for TBARS in the oil samples was performed by thiobarbituric acid reaction method as described by Fatum and Haider (2002). The principle of the method was based on the reaction of one molecule of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA) and the pink chromogen formed was measured at 532 nm.

3.2.3 Reagents

A. Standard Stock Solution

This solution was prepared by dissolving 24.6 mg of tetra – ethoxy propane (TEP) in 100 ml of distilled water.

B. Working Standard Solution

This solution was prepared by diluting one (1) ml of TEP stock solution to 100 ml with 0.01N HCl, which releases malondialdehyde instantly. The working solution must be prepared fresh daily. A (1 : 100 v/v) dilution of stock solution contained 15 nanomoles (2.45 μg) of malondialdehyde per ml.

C. Tricholoracetic Acid (TCA) (20% w/v)

This was prepared by dissolving 20 gram of TCA in 100 ml of distilled water.

D. Thiobarbituric Acid (TBA) (0.8% w/v)

This was prepared by dissolving 0.8 gm of TBA in 100 ml distilled water with two pellets of sodium hydroxide (NaOH).

E. n – butanol and pyridine (15 : 1 v/v)

This extraction solvent mixture was prepared by mixing 15.0 ml of n – butanol with 1.0 ml of pyridine.

3.2.4 Procedure

The method consisted of the addition of the following reagents to tubes: one (1) ml of oil sample, one (1) ml of distilled water, one (1) ml of 20% TCA solution and one (1) ml of 0.8% solution of TBA were pipetted into each tube by using automated pipette (1000 µl). The blank was prepared by pipetting one (1) ml of distilled water instead of oil sample. The total volume of the reaction mixture in the tubes was 4.0 ml. The tubes were covered with parafin film and were mixed on vortex mixer. The tubes were heated in a water bath at 100°C for 30 minutes. The tubes were next cooled with tap water, one (1) ml of distilled water and 5.0 ml of mixture of n – butanol and pyridine (15 : 1 v/v) were added and shaken vigorously. The material in the tubes was centrifuged for 15 minutes at 4000 rpm. After centrifugation, the upper layer (organic layer) was transferred into the cuvette and absorbance was read against the blank at 532 nm. A standard curve (as shown by Fig. 7), with different concentrations of TEP, was prepared by treating similarly and the level of TBARS was calculated from the standard curve and was expressed as picomoles of malondialdehyde formed per 100 ml of oil by using the following formula.

3.2.5 Calculations

Picomoles of malondialdehyde per 100 ml of oil

$$= \frac{\text{concentration in nanomoles from standard curve} \times \text{total reaction mixture volume (5 ml)} \times \text{1000 (factor to convert the unit from nanomoles to picomoles)}}{\text{volume of oil samples taken into estimation (1 ml)} \times \text{100 (factor to express the values as per deciliter)}}$$

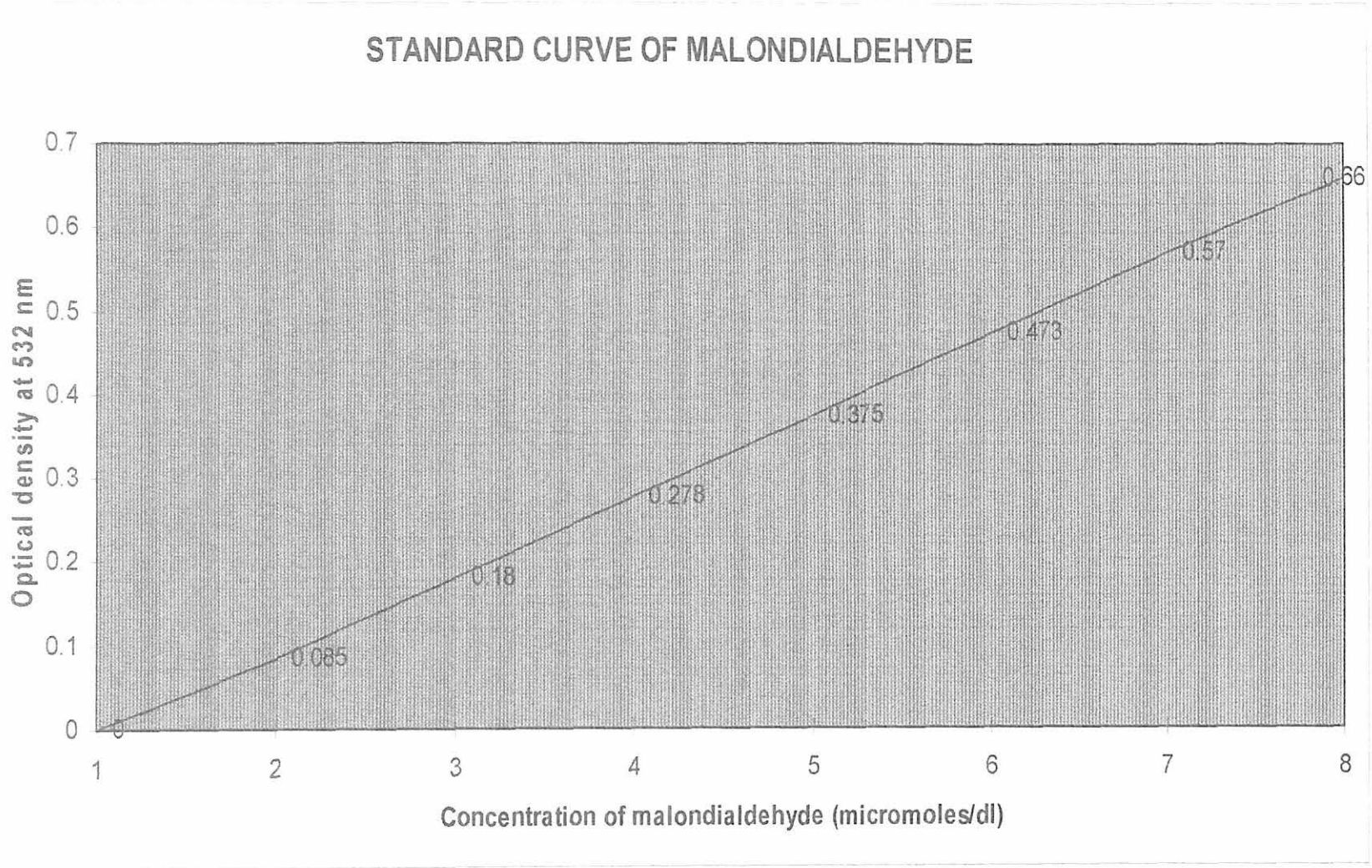

FIGURE 7: STANDARD CURVE OF MALONDIALDEHYDE

3.3 Statistical analysis

The results were analyzed by using SPSS 11.0 and Excel program. In the SPSS 11.0 program, repeated calculations of analysis of variance (ANOVA), were made to determine an association and relative correlation with respect to malondialdehyde concentration amongst the different stalls and within the stalls.

4.0 RESULTS

4.1 Comparison of pH in fresh oil and reused oil samples on different days between different stalls in Kota Bharu

Table 1 shows the comparison of pH value in fresh oil and reused oil samples on different days between different stalls in Kota Bharu. The pH on day 0 at all the stalls was 6.0. Eventually as time went by, the pH was reduced and made the oil more acidic. This pattern was seen in all the reused oil samples that were used by all four stalls. A plateau of pH was noted after the first week of this study. Our study showed that the pH remained constant (pH 5.0) throughout this study.

4.2 Comparison of malondialdehyde concentration in fresh oil and reused oil samples on different days at various stalls

Table 2.1 shows the comparison of malondialdehyde concentration in fresh oil and reused oil samples on different days at Stall 1. The results showed that there was a significant increase in the occurrence of oxidation of lipids in Pair 2 (day 0 and day 14), Pair 3 (day 0 and day 21) and Pair 4 (day 0 and day 30). The p values for Pair 2, Pair 3 and Pair 4 were less than 0.005 in different pairs. However, the rest of the pairs exhibited non – significant increase in oxidation of lipids.

Table 2.2 shows the comparison of malondialdehyde concentration in fresh oil and reused oil samples on different days at Stall 2. Our results have shown that there was a non – significant increase in oxidation of lipids in different pairs.

Table 2.3 shows the comparison of malondialdehyde concentration in fresh oil and reused oil samples on different days at Stall 3. The results have shown that there was a significant increase in the occurrence of oxidation of lipids in Pair 2 (day 0 and day 14), Pair 4 (day 0 and day 30) and Pair 7 (day 7 and day 30). The p values for different pairs were less than 0.005. However, the rest of the pairs showed insignificant increase in oxidation of lipids.

Table 2.4 shows the comparison of malondialdehyde concentration in fresh oil and reused oil samples on different days at Stall 4. The results exhibited that there was a significant increase in the occurrence of oxidation of lipids in Pair 2 (day 0 and day 14), Pair 3 (day 0 and day 21), Pair 5 (day 7 and day 14), Pair 6 (day 7 and day 21), Pair 8 (day 14 and day 21), Pair 9 (day 14 and day 30) and Pair 10 (day 21 and day 30). The p values for different pairs were less than 0.005. However, the rest of the pairs showed insignificant increase in oxidation of lipids.

4.3 Comparison of malondialdehyde concentration in fresh oil and reused oil samples between different stalls in Kota Bharu

Table 3 shows comparison of malondialdehyde concentration in fresh oil and reused oil samples between different stalls. Our study has revealed that there was a significant difference in the occurrence of oxidation of lipids among different stalls from day 1 to day 30 in the following sequence: Stall 4 (+ 185%) > Stall 3 (+ 123%) > Stall 2 (+ 84%) followed by Stall 1 (+ 71%).

4.4 The concentration of malondialdehyde (picomoles/dl) on different days at various stalls.

Figure 8 shows the concentration of malondialdehyde (picomoles/dl) from day 0 to day 30 at Stall 1. A trend of increase in the malondialdehyde concentration was seen as the oil was reused for several days. Day 6 recorded the highest malondialdehyde concentration with the value of 123.33. A plateau pattern was noted on day 7, day 14, day 21 and day 30 respectively.

Figure 9 shows the concentration of malondialdehyde (picomoles/dl) from day 0 to day 30 at Stall 2. A trend of increase in the malondialdehyde concentration was noted as the oil was reused for several days. Day 30 exhibited the highest malondialdehyde concentration with a value of 101.67. A plateau pattern was seen on day 6, day 7, day 14 and day 21 respectively.

Figure 10 shows the concentration of malondialdehyde (picomoles/dl) from day 0 to day 30 at Stall 3. A significant increase in the malondialdehyde concentration was noted as the oil has been reused for several days. Day 30 recorded the highest malondialdehyde concentration with a value of 123.33.

Figure 11 shows the concentration of malondialdehyde (picomoles/dl) from day 0 to day 30 at Stall 4. A trend of increase in the malondialdehyde concentration was seen as the oil has been reused for several days. Day 21 recorded the highest malondialdehyde concentration with a value of 207.50.

4.5 Concentration of malondialdehyde (picomoles/dl) in different weeks at various stalls

Figure 12 shows the concentration of malondialdehyde (picomoles/dl) in different weeks at Stall 1. The comparison is made between 1st, 2nd, 3rd and 4th week. Concentration of malondialdehyde exhibited at Stall 1 showed a plateau between the 4 weeks.

Figure 13 shows the concentration of malondialdehyde (picomoles/dl) in different weeks at Stall 2. Concentration of malondialdehyde recorded at Stall 2 showed a plateau between the 4 weeks. The highest value (101.67) was recorded in the 4th week.