Journal of Science and Technology

Storage Stability of α-tocopherol Extracted from Heated and Un-heated Palm Oil Mesocarp

Noor Akhmazillah Mohd Fauzi[a], Mohamad Roji Sarmidi[b]

[a] Chemical and Bioprocess Engineering Department, Faculty of Civil and Environmental Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat,Johor.

[b] Chemical Engineering Pilot Plant, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor.

**Corresponding email: akhma@uthm.edu.my, mroji@cepp.utm.my*

Abstract

α-tocopherol is one of the eight vitamers in palm oil which known as powerful biological antioxidants. It is known as phytonutrient which is natural occurring bioactives. However, it has high affinity towards heat and light. An investigation was carried out on the effect of different heating time on the α-tocopherol concentration of the extracted palm oil mesocarp. The work was also aimed to determine the stability of α-tocopherol after stored for three months period. A set of samples were stored at room temperature range between 28˚C - 32˚C. Another set of samples were refrigerated at a temperature between -14˚C to – 18˚C. The result showed that the highest yield was obtained at 40 minutes of heating with 19.9 ± 0.21% (w/w). Statistical analysis showed that there was a significant difference in α-tocopherol content ($p < 0.05$) between room and freezer temperature storage. The results also showed that unheated sample records the highest losses of α-tocopherol with 65.42 ± 3.06% compared to heated sample which is 39.10 ± 1.78%.

Keywords: Storage Stability; α-tocopherol; Heated; Unheated Palm Oil Mesocarp

INTRODUCTION

In recent years, the production and consumption of plant edible oils has been increasing and a number of these oils are found to be good source of natural occurring phytonutrients. Currently, constituents of plant, phytochemicals are attracting a lot of attention as they are associated with the capacity to provide health benefits, both in foods and isolated form (Dillard and German, 2000). Vitamin E is a fat soluble vitamin and it is identified as minor components in palm oil and important nutritionally, comprising of tocopherols and tocotrienols which is known as amphipilic lipid antioxidant. The major forms of tocopherols and tocotrienols present in palm oil are α-tocopherol and γ-tocotrienols (Sambanthamurthi et al., 2000). In fact, no other vegetable oil has as much Vitamin E compared to palm oil.

One of the issues in plant material processing is the effect of processing methods on the quality of the products. Sterilization is an important heating process because it determines the efficiency and effectiveness of the downstream and the refining processes in producing high grade palm oil. Sterilization is known to influence the stability of α-tocopherol in the extracted palm oil mesocarp since this phytonutrient is biologically active and extremely sensitive towards heat and light (Chun et al., 2006).

The impacts of sterilization process on yield and extraction of palm oil were well established. However, the loss and stability of α-tocopherol as affected by sterilization process and storage condition has not been investigated to similar extent. Consequently, the investigation on sterilization time and storage temperature for α-tocopherol concentration in palm oil is of high interest.

In the present paper, the comparison of heated and unheated palm fruits on the palm oil yield and concentrations loss of α-tocopherol were investigated. Kinetics degradation of α-tocopherol after stored at two different temperatures for three months storage were also determined. Stability of α-tocopherol is the important step for further research in developing health application of phytonutrients from palm oil.

2 MATERIALS AND METHODS

2.1 Materials

Fresh fruit bunches (FFB) was obtained from Universiti Teknologi Malaysia's plantation, Skudai, Johor. The oil palm fruits were freshly harvested, reddish

in color and of full maturity. The fruitlets have an average dimension of 4 cm in length and 2.5 cm in diameter. All chemicals used were of analytical high performance liquid chromatography (HPLC) grade.

2.2 Sample preparation

2.2.1 Stripping of Fresh Fruit Bunches

The method was adapted from Owolarafe et al. (2007) with some modification. Palm fruitlets were removed from the bunch manually. The fruit-laden spikelets were cut from the bunch with a machete. Then, the fruits were separated manually from the spikelets before washing. Washing was necessary in order to remove dirt and foreign matter present in the fruitlets.

2.2.2 Heating of Palm Fruitlets

The method was adapted from Owolarafe et al. (2007) with some modification. Three batches of cleaned fruitlets were divided equally into two fractions. For each batch, a set of oil palm fruitlets was heated while another set was not heated. Heat treatment was operated at constant temperature and pressure with 121˚C and 4MPa, respectively for 20, 40 and 60 minutes using a sterilizer (HV-110, Pyrometro Services (M), Hirayama, Japan).

2.3 Extraction of Palm Oil Mesocarp

2.3.1 Soxhlet method

The Soxhlet method was carried out according to PORIM Test Method, 1995. Soxhlet extraction was carried out using Soxhlet apparatus (Model EAM 9204, MTOPS) to determine the total extractable oil content in mesocarp extract. A 20g of mesocarp was weighed and transferred into a filter paper extraction thimble and then inserted into a 500 ml reflux flask. 300 ml of hexane was used as solvent at its boiling point. Extraction was terminated after six hours. The extract was then concentrated by removing the hexane using rotary evaporator and left in the oven at 60˚C. The extraction was done in triplicates using the same amount of the sample and within the same duration. The oil yield obtained was expressed in terms of mass percentage of the samples and calculated as:

$$Total\ yield\ (\%\ (w/w)) = \frac{Mass\ of\ oil\ extract(g)}{Mass\ of\ fruit\ pulps(g)}\ x\ 100 \quad (1)$$

The relative recovery was then determined as Equation 2:

$$\text{Relative recovery}\ (\%) = \frac{\text{Expellable yield from expeller}\ (\%\,(w/w))}{\text{Total yield from Soxhlet extraction}\ (\%\,(w/w))} \times 100 \quad (2)$$

2.3.2 Laboratory Scale Expeller

The method was adapted from Owolarafe and Faborode (2008) with some modification. The heated and unheated mesocarp of palm fruitlets were pressed using stainless steel laboratory scale expeller, The Baker (Premium Quality, Malaysia). Pressing was done by loading the inlet hopper with weighed mesocarp and the mesocarp starts to be pressed. The puree containing oil was then released through a filter and collected. Pressing process was done for several times until no oil secreted. The resulting puree was then centrifuged using KUBOTA KR- 20000T centrifuge (Kubota Medical Appliance Supply, Japan) to separate the oil and the impurities. The process was operated at 4000 rpm for 30 min. The oil yield was calculated as:

$$\text{Expellable yield}\ (\%\,(w/w)) = \frac{\text{Mass of oil obtained}\ (g)}{\text{Mass of puree}\ (g)} \times 100 \quad (3)$$

2.4 Analysis of α-tocopherol

The determination of α-tocopherol in palm oil mesocarp extract was adapted from Elham et al. (2008) works with some modification. 40 mg – 50 mg of samples were weighed and dissolve in 1 mL 2-propanol. Then, 0.2 µm nylon filter is used to filter the solution for HPLC analysis. The Waters HPLC system (Massachusetts, USA) used was equipped with pump 515 HPLC Pump Waters, Column Heater Control Model 7971. The column used was a Synergy 4u hydro – RP80A. The mobile phase was a mixture of methanol/ acetonitrile (1:1 v/v) and the flow rate was 1 mL/min with a temperature of 40˚C. Detection used was fluorescence detector Varian 9070 (Walnut Creek, CA, USA) at the excitation wavelength of 294 nm and emission wavelength of 320 nm. The compounds were identified by chromatographic comparisons with α-tocopherol standard from Merck (Darmstadt, Germany). The results were obtained from triplicate measurements.

2.5 Storage of Extracted Oil

The effect of storage on extracted oil was investigated using method developed by Monday et al. (2000) works. One set of samples containing four different

degree of heat treatment were stored at a temperature range of 28˚C to 32˚C (room temperature). The other set was refrigerated at a temperature between -14˚C to – 18˚C (freezer temperature). Each sample was monitored for α-tocopherol content twice a month for 3 consecutive months. A mean value of triplicate samples was calculated.

2.6 Degradation Kinetics Analysis

The determination of degradation kinetic was adapted from Chanderasekaram et al. (2009). The degradation reaction order α-tocopherol was determined by hypothesizing the zero and first-order kinetics using the general rate expression –dC / dt = kCn, where C is the β-carotenes concentration (ppm), k is the reaction rate constant (days-1), t is the reaction time (days) and n is the order of the reaction. The representative of the current degradation reaction was selected with the order which gave the best regression (R2) and the best correspondence among the experimental values and the theoretical half – life (t1/2); in the formula, t1/2 = C0/ 2k for zero order (C0 is the initial concentration), while t1/2 = ln 2/ k for first order. Regression analysis of Microsoft® Excel 2000 was used to analyze the kinetics data.

3 RESULTS AND DISCUSSIONS

3.1 Yield of Palm Oil Mesocarp Extract

The result obtained in the form of relative extraction recoveries is shown in Figure 1. The highest recovery was noted at 40 minutes of sterilization. Statistical evaluation shows that the yield was significantly difference between unheated and heated mesocarp, $p < 0.05$. The percentage of yield obtained increased slightly with increasing sterilization time. Higher oil yield for heated compared to unheated mesocarp is expected since sterilization is a heat rendering and moisture adsorption process which achieves the objectives of lowering the viscosity of oil as well as coagulation of protein (Monday et al., 2000; Abbas et al., 2006; Owolarafe and Faborode, 2008). Little amount of yield obtained in the unheated mesocarp were due to fibrous and loose pounded mass fruit which are not able to squeeze out all the oil from the voids in the fibre since there was no heat applied to soften the tissues of oil-bearing material. Of all, 40 minutes of sterilization gives maximum oil yield compared to others at constant temperature of 121˚C and constant pressure of 4MPa.

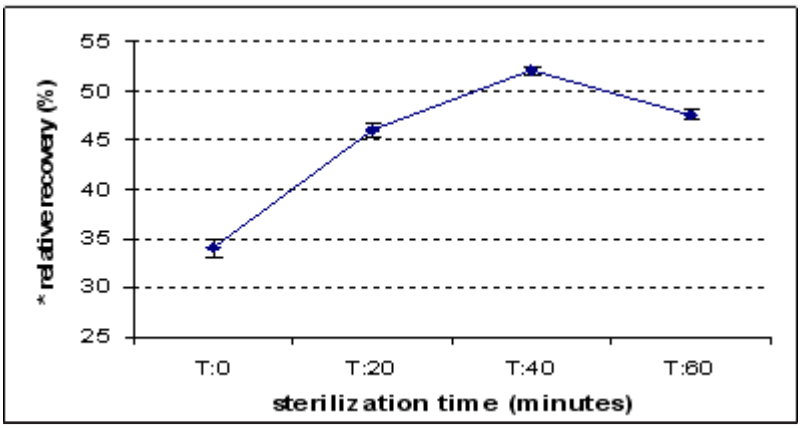

Figure 1 : Effect of sterilization time on small scale expeller efficiency (error bars represent SEM of results, n = 3); *(weight of whole palm oil extract using small scale expeller/ weight of whole palm oil extract using Soxhlet) x 100.

3.2 Stability of α-tocopherol Upon Storage

The change of α-tocopherol concentration in the samples stored at room temperature and refrigerated was shown in Figure 2. The concentration was gradually lost by the month. The result shows that all treatments with different heating time suffer a loss in α-tocopherol concentration.

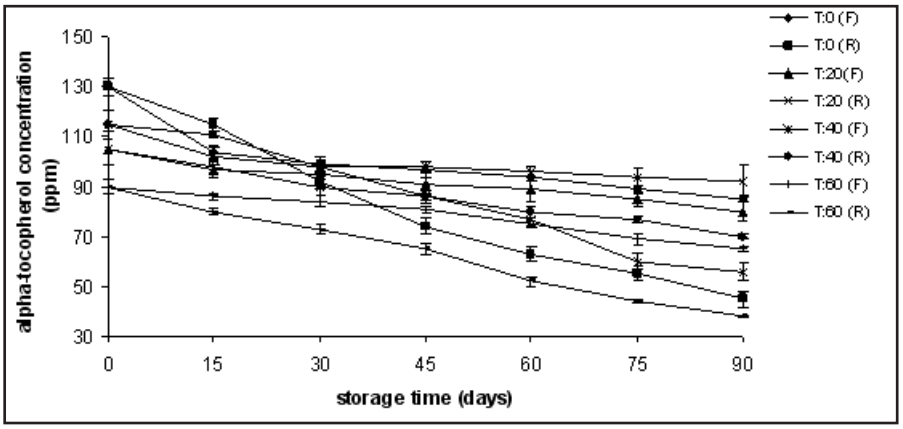

Figure 2 : Declination of α-tocopherol content in palm oil mesocarp extract during three months storage. T denoted was heating time (min), R for room temperature and F for refrigerated. Values are mean ± SEM for triplicate determination.

The result also shows that α-tocopherol concentration in the sample stored at room temperature disintegrated considerably compared to their concentration at the start of the study which is more than 40% loss as presented in Figure 3. This finding was support to the theory that at normal room temperature, phytonutrients would be able to disintegrate (Alyssa and Andrea, 2008). On contrary, the refrigerated samples recorded only a minimal amount of losses that do not exceeding 30% of the starting α-tocopherol concentration. This result showed that refrigerated sample was far more stable than the sample stored at room temperature.

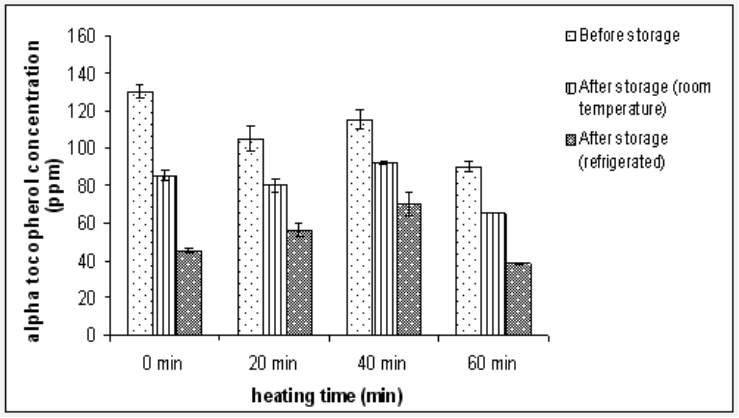

Figure 3 : The concentration of α-tocopherol after three months storage as compared to before storage for room temperature and refrigerated. Values are mean ± SEM for triplicate determination.

3.3 Kinetic Degradation of α-tocopherol

The degradation kinetics of α-tocopherol heated and unheated mesocarp extract was shown in Figure 4. The kinetics was hypothesized by applying the general reaction rate expression – dC/ dt = kCn (where C is the α-tocopherol concentration (ppm), k is the reaction rate constant, t is the reaction time (in days) and n is the order of the reaction. From the result, the order with the best coefficient of correlation, R2 and the half life, t1/2 suggest a first order equation for the best modeling of α-tocopherol degradation. The result shows that that first order reaction has better correlation coefficient and half life with R2 > 0.94 and t1/2 > 17 day, respectively.

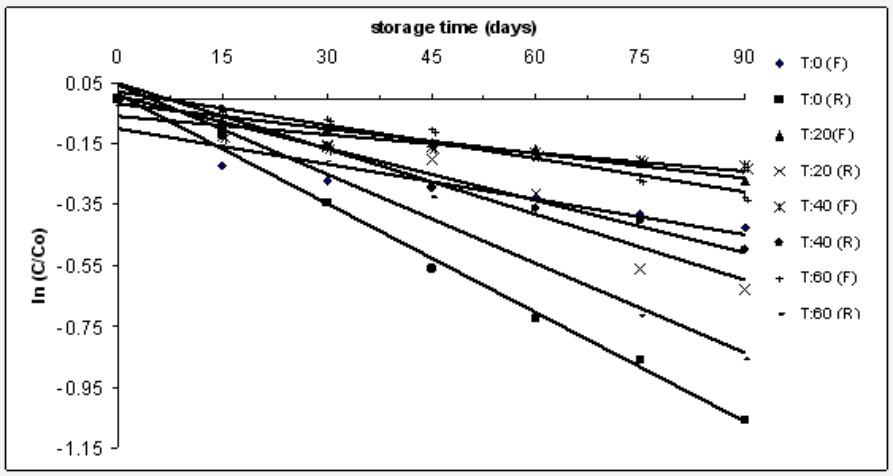

Figure 4 : Degradation kinetics of α-tocopherol of mesocarp extract for different heating time stored at room temperature and freezer temperature. T was denoted for heating time, R was for room temperature and F for freezer temperature. C was concentration of α-tocopherol at respective time; Co was its initial concentration.

The result indicates that the degradation of α-tocopherol in the extract was well described with increasing storage time. α-tocopherol decreased as a function of temperature and time upon storage. It was observed that unheated sample which stored at room temperature was found to have the quickest degradation with highest k of 1.793 x10-1% day-1. For heated sample, longer heating time (60 minutes) showed the highest k with 1.45 x10-1% day-1 and 0.55 x10-1% day-1 for room and freezer temperature, respectively. Meanwhile, 40 minutes of heating and stored at freezer temperature was found to have the lowest k value with only 0.204 x10-1% day-1.

With regards to storage temperature, α-tocopherol was found to be more stable when stored at freezer temperature than stored at room temperature. The result shows that k value decreased as the temperature decreased, indicating a slower degradation of the α-tocopherol. Possible explanation might be due to the superior content of other antioxidants compounds such as β-carotene in the refrigerated samples. The result indicated that they may have protective or synergistics effects (Chanderasekaram et al., 2009).

4 CONCLUSION

Sterilization process had been proved to provide a higher oil yield. Lower temperature storage contributes lower concentration loss of phytonutrient in

the sample. 40 minutes of heating time used in this experiment shows the appropriate time which was applicable in the palm oil processing, not only because of the highest yield obtained (with 19.9 ± 0.2 % (w/w)) but also the least concentration lost of α-tocopherol with degradation rate of 0.204 x10-1% day-1 towards the end of three months storage. The determination of minimal heating time in this study compared to normal heating time of industrial would be able to reduce the operating cost; which is one of the primary aims of palm oil processing.

ACKNOWLEDGEMENT

The authors would like to thank to The Ministry of Science, Technology and Environment of Malaysia and Tun Hussein Onn University of Malaysia (UTHM) for financial support and Chemical Engineering Pilot Plant, UTM for laboratory facilities.

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