

# Extraction of Heat Treated Palm Oil and Their Stability on $\beta$ -carotene During Storage

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## Abstract

An investigation was carried out on the effect of different sterilization time on the  $\beta$ -carotene concentration of whole palm oil extract after stored for three months period. Palm fruits were collected, cleaned and sterilized for 0, 20, 40 and 60 minutes. The kernels were then stripped from the sterilized fruits to get the pulp and later the pulp was pressed using small scale expeller. The resulting puree was centrifuge at 4000 rpm for 20 minutes. The whole palm oil extract were then collected and stored at two different temperatures. 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 sterilization with  $19.9 \pm 0.21\%$  (w/w). There was a significantly difference between the degree of sterilization time in total concentration loss of  $\beta$ -carotenes after three months storage. 20 minutes of sterilization gave the lowest total concentration loss with  $10.42 \pm 1.07\%$  towards the end of storage. Samples that stored at room temperature were observed to suffer a huge amount of loss compared to the refrigerated sample.

Keywords:  $\beta$ - Carotenes, Heat Treatment, Stability, Storage

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## 1 INTRODUCTION

Palm oil is derived from the fleshy mesocarp of the oil palm fruits, *Elaeis guineensis*. About 80% of palm oil production is destined for human consumption with the balance going to animal feed and to various industries. Harvesting, handling and processing methods used are known to influence the quality of the extracted palm oil. Fruit sterilization is one of the basic operations to obtain palm oil besides of fruit loosening, fruit digestion, oil extraction and oil clarification. Sterilization is a heat rendering operation involves steaming of fruits and reported as an important process because it determines the efficiency and effectiveness of the downstream and the refining processes in producing high grade palm oil. Increased in sterilization time has been found to increase yield of palm oil (Monday et al., 2000; Abbas et al., 2006; Owolarafe et al., 2008).

Carotenes are minor components which are responsible for the characteristic orange-red color of crude palm oil. They are organic pigments that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria; and they have an important role in living organism. In plants and algae, they absorb light energy for use in photosynthesis, and they protect chlorophyll from photo damage. While in humans, carotene such as  $\beta$ -carotene is a precursor to vitamin A, a pigment essential for good vision and eye health besides of normal cell division.  $\beta$ -carotene is known as a powerful antioxidant because it destroys toxic free radicals (Bonnie et al., 1999). Therefore, it is widely used for vitamin enrichment of margarine, nutrient preparation and pharmaceuticals. Besides, it was found that palm oil has 15 times more retinol equivalent than carrot and 300 times more than tomato. However, carotenes are susceptible to degradation by oxidation and thermal process, especially under severe processing and storage condition due their highly unsaturated nature (Bonnie et al., 1999; Chandrasekaram, 2009). It has been reported that normal room temperature was found to decrease carotenes concentration due to naturally occurring bioactive compounds which has high affinity towards heat and light. This research studies on the effect of different sterilization time on the palm oil yield and  $\beta$ -carotene concentrations. Also, to study the stability of  $\beta$ -carotene after stored at two different temperatures for three months period.

## 2 MATERIALS AND METHOD

### 2.1 Materials

The raw material used for the study is tenera species of fresh palm fruit bunches 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 or high performance liquid chromatography (HPLC) grade.

### 2.2 Extraction of Whole Palm Oil using Soxhlet method

Soxhlet extraction was carried out to determine the total extractable oil content in palm oil whole extract. The yield represents a standard for oil yield from small scale expeller and was taken as the maximum extractable oil from the samples. It was assumed that Soxhlet method could achieve 100% oil extraction. The determination of oil was according to PORIM Test Method. A 20g of mesocarp was weighed and transferred into a filter paper extraction thimble and then inserted into a 500 ml reflux flask. Extraction was carried out using 300 ml of hexane as a solvent at its boiling point. Extraction was terminated after six hours or when the orange color of the sample was faded. The extract was concentrated by removing hexane using rotary evaporator and left in the oven at 60°C. Soxhlet extraction was done with triplicates using the same amount of the sample and within the same duration.

The oil yield was expressed in terms of mass percentage of the samples:

$$\text{Percentage of oil} = \left( \frac{m_1}{m_0} \right) \times 100 \quad (1)$$

Where  $m_1$  = mass of extracted oil in gram  
 $m_0$  = mass of sample in gram

### 2.3 Extraction of Whole Palm Oil using Small Scale Expeller

Palm fruitlets were removed from the bunch. The fruit-laden spikelets were cut from the bunch with a machete. Then, the fruits were separated manually from the spikelets before cleaning. The cleaned fruitlets were sterilized for 0, 20, 40 and 60 minutes (Owolarafe et al., 2007) at constant temperature and pressure, 121°C and 4 MPa respectively. The fruitlets were then stripped from the sterilized fruits to get the pulp and later the pulp was pressed using small scale stainless steel expeller. The resulting puree was centrifuge, operated at 4000 rpm for 20 minutes to obtain the palm oil whole extract.

The yield of palm oil whole extract was determined using Equation (2)

$$\text{Yield (\%)} = \frac{\text{Mass of oil xtracted (g)}}{\text{Mass of the mash (g)}} \times 100\% \quad (2)$$

#### 2.4 Determination of $\beta$ - Carotene

The determination of  $\beta$ -carotenes was according to PORIM Test Method, 1995, PORIM p2.6. The sample was melted at 60°C - 70°C and homogenized thoroughly before filtered through a fast filter paper Whatman No.1. 0.1  $\pm$  0.0001 g of sample was dissolved in iso-octane in a 25 mL volumetric flask and dilute to the mark. The solution was then transferred to a 1 mm cuvette and absorbance at 446 was measured using Lambda 25 UV/VIS Spectrometer, PerkinElmer Precisely (Massachusetts, USA) that was calibrated previously. The carotene content is expressed as ppm  $\beta$  - carotene and is calculated as Equation 3.

$$\beta\text{-Carotene (ppm)} = 25 \times \frac{383}{100W} (a_s - a_b) \quad (3)$$

Where  $a_s$  = absorbance of the sample

$a_b$  = cuvette error

$W$  = weight of the sample in gram

#### 2.5 Storage of Whole Palm Oil Extract

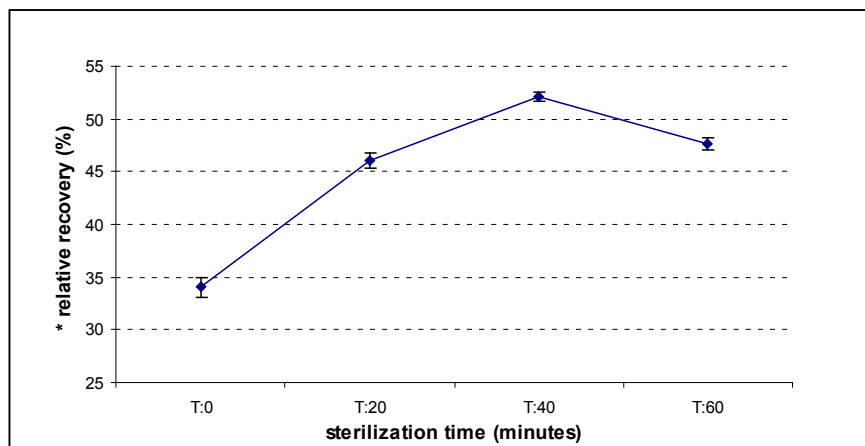
One set of samples containing four different time of sterilization were stored at room temperature with a temperature range of 28°C to 32°C. The other set was refrigerated at a temperature between -14°C to - 18°C. Each sample was monitored for its quality twice a month for 3 consecutive months. A mean value of triplicate samples was calculated .

### 3 RESULTS AND DISCUSSION

#### 3.1 Yield of Whole Palm Oil Extract at Different Sterilization Time

In this study, four different sterilization durations were performed and the results on the percentage yield of palm oil whole extract were recorded. The highest oil yield was obtained when the fruits were sterilized at 40 minutes with 19.9  $\pm$ 0.21% (w/w) yield. It was observed that, increase in sterilization time beyond 20 minutes does not increase the yield significantly. However, 0 minute of sterilization gave a mean difference about 6.9% to 40 minutes of sterilization. The result obtained in the form of relative extraction recoveries (for Soxhlet recoveries considered as equal to 100%) was illustrated in Figure1.

The highest recovery was obtained at 40 minutes of sterilization. However, a decrease of recovery was noted for 60 minutes of sterilization due to the coagulation of protein which consequently reduces the viscosity of the oil to be expelled. The percentage of yield obtained increased slightly with increasing sterilization time. Higher oil yield for sterilized fruit compared to nonsterilized fruit 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 (Owolarafe et al., 2007). Little amount of yield obtained in the nonsterilized fruits 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 $\beta$ - Carotene Contents in Heat Treated Whole Palm Oil Extract

The content of  $\beta$ -carotene as valuable minor component in palm oil whole extract was analyzed and compared with four different times of sterilization. The initial concentration of  $\beta$ -carotene at different time of sterilization is shown in Table 1. It shows that the initial content of  $\beta$ -carotene in all treatments were in the range of 1000 ppm – 1600 ppm, indicates that the samples presence higher  $\beta$ -carotene with 6.7% difference compared to  $\beta$ -carotene obtained by other researchers (Tan et al., 2009). Although the production of palm oil whole extract in this research involved a sterilization temperature at 121°C

which is about two times higher than processing temperature done by previous researchers (Tan et al., 2009), the amount of natural antioxidants of carotenes that were retained is still higher than other conventional crude palm oil. This finding was in good agreement with the study reported by Siew et al. (1992). The highest initial  $\beta$ -carotene content in the palm oil whole extract was found in the treatment 4 with  $1585.35 \pm 40.98$  ppm. However, it suffers the maximum concentration loss towards the end of storage. Meanwhile, 20 minutes of sterilization shows the lowest initial  $\beta$ -carotene content ( $1045.46 \pm 38.98$  ppm) and the lowest total loss concentration after three month storage ( $108.06 \pm 49.38$  ppm). This is the most appropriate treatment compared to others where the total loss in  $\beta$ -carotene concentration showed the lowest value with only  $10.42 \pm 1.07\%$  for refrigerated samples and  $28.59 \pm 1.02\%$  for the samples stored at room temperature.

**Table 1** : Initial concentration of  $\beta$ -carotene (ppm) in whole palm oil extract for different sterilization time.

Storage temperature	Sterilization time			
	0 minute (Treatment 1)	20 minutes (Treatment 2)	40 minutes (Treatment 3)	60 minutes (Treatment 4)
Room ( $30 \pm 2$ )°C	$1320.34 \pm 38.97^a$	$1285.27 \pm 68.78^a$	$1265.36 \pm 25.87^a$	$1585.35 \pm 40.98^a$
Refrigerated ( $-16 \pm 2$ )°C	$1060.45 \pm 25.56^a$	$1045.46 \pm 38.98^a$	$1305.42 \pm 15.76^a$	$1270.08 \pm 36.80^a$

<sup>a</sup> Mean  $\pm$  SEM (n = triplicate determinants)

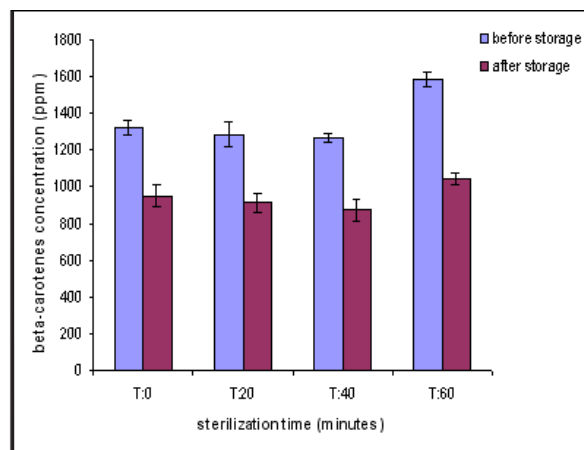
### 3.3 Stability of $\beta$ - Carotene Contents

A set of samples was stored at room temperature range between  $28^\circ\text{C}$  -  $32^\circ\text{C}$ . Another set of samples was refrigerated at a temperature between  $-14^\circ\text{C}$  to  $-18^\circ\text{C}$ . Based on the theory that normal room temperature is enough to cause the compounds of phytonutrients to disintegrate, Table 2 showing of the overall loss and percentage of deterioration in the concentration of  $\beta$ -carotene in palm oil whole extract after three months of storage at room temperature and refrigeration. The results show that the loss of concentration in  $\beta$ -carotene phytonutrient occurred in both samples which stored at room temperature and refrigerated. However, samples that stored at room temperature suffered a huge amount of loss compared to the refrigerated sample. This is in line with the finding by Seiza et al. (2006) where 59% of  $\beta$ -carotene was recorded to be

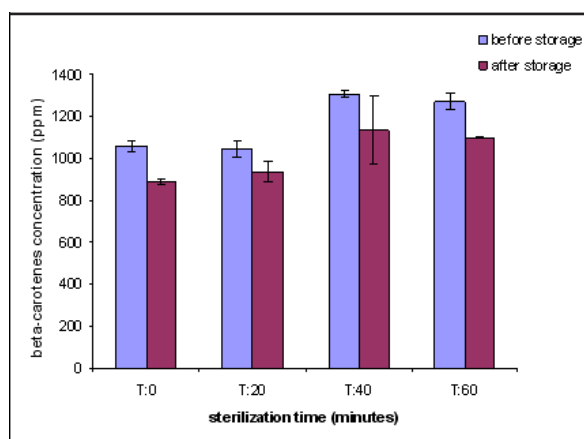
reduced at higher storage temperature. The losses of  $\beta$ -carotene concentration in palm oil whole extract within the three months period were clearly pictured in Figure 2 and Figure 3. Both sets of samples show the decrease in  $\beta$ -carotene content after stored at room temperature and freezing temperature for all sterilization time.

The change in the concentration of this phytonutrient in the samples stored at room temperature is shown in Figure 2. All treatments with different time of sterilization suffer a loss in  $\beta$ -carotene concentration. However, this finding suffers higher losses with about 10% more than the finding reported by Chandrasekaram et al. (2009). It was observed that the percentage of losses in the  $\beta$ -carotene concentration was highest in treatment 4 ( $33.85 \pm 2.46$  % loss). The slightly decreased in  $\beta$ -carotene after three months storage provide further support to the previous study (Siew et al., 1992).

On the other hand, the changes of  $\beta$ -carotene in the refrigerated samples were also denoted. However, by comparison with Figure 2 it could be seen to be minimal. Refrigerated samples present lower total loss in  $\beta$ -carotene concentration than at room temperature towards the end of storage. It was found that the carotene which stored in the freezer is stable for at least three months (Baharin et al., 2001). It was observed that treatment 2 with 20 minutes of sterilization gives the lowest total concentration loss with  $10.42 \pm 1.07$ % followed by treatment 3 ( $13.03 \pm 0.84$ %), treatment 4 ( $14.04 \pm 0.26$ %) and treatment 1 ( $16.34 \pm 2.45$ % loss).



**Figure 2** : Concentration of  $\beta$ -carotene stored at room temperature approximately at  $(30 \pm 2)^\circ\text{C}$ . T was denoted for sterilization time.



**Figure 3 :** Concentration of  $\beta$ -carotene stored at freezing temperature approximately  $(-16 \pm 2)^\circ\text{C}$ . T was denoted for sterilization time.

Overall, it showed that  $\beta$ -carotene concentration in the sample stored at room temperature disintegrated considerably compared to their concentration at the start of the study. This finding was support to the theory that at normal room temperature, phytonutrients would be able to disintegrate. On contrary, the refrigerated samples recorded only a minimal amount of losses that do not exceeding 18% of the starting  $\beta$ -carotene concentration. This result showed that refrigerated sample was far more stable than the sample stored at room temperature. It also was recorded that the loss concentration of  $\beta$ -carotene recorded in this study was found to be  $10 \pm 1\%$  higher (for room temperature and refrigerated sample) than in the loss concentration of  $\beta$ -carotene found by Chandrasekaram et al. (2009) after three months of storage.



**Table 2** : The percentage and total loss in the concentration of  $\beta$ -carotene in whole palm oil extract after three months storage.

Sterilization time (minutes)	Storage temperature ( $^{\circ}\text{C}$ )	Concentration of $\beta$ -carotene after three months storage	
		Loss of concentration <sup>a</sup> (ppm)	Percentage of losses <sup>a</sup> (%)
0 (treatment 1)	30 $\pm$ 2	370.34 $\pm$ 59.56	27.68 $\pm$ 0.21
	-16 $\pm$ 2	170.25 $\pm$ 14.99	16.34 $\pm$ 1.45
20 (treatment 2)	30 $\pm$ 2	373.48 $\pm$ 52.03	28.59 $\pm$ 1.02
	-16 $\pm$ 2	108.06 $\pm$ 49.38	10.42 $\pm$ 1.07
40 (treatment 3)	30 $\pm$ 2	392.35 $\pm$ 58.71	31.09 $\pm$ 1.86
	-16 $\pm$ 2	169.18 $\pm$ 159.94	13.03 $\pm$ 0.84
60 (treatment 4)	30 $\pm$ 2	541.18 $\pm$ 29.79	33.85 $\pm$ 2.01
	-16 $\pm$ 2	171.36 $\pm$ 3.47	14.04 $\pm$ 0.26

<sup>a</sup> Mean  $\pm$  SEM (n = triplicate determinants)

#### 4 CONCLUSION

Sterilization process had been proved to provide a higher oil yield. In this study, 40 minutes of sterilization gives the most appropriate treatment with 19.9  $\pm$  0.21% (w/w) yield. 20 minutes of sterilization gives the lowest total  $\beta$ -carotene concentration loss with 10.42  $\pm$  1.07% towards the end of three months storage. The different in sterilization time contributes to the different in the total loss concentration in the palm oil whole extract. Samples that stored at room temperature were observed to suffer a huge amount of loss compared to the refrigerated sample.

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