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Heat Degradation of α-Tocopherol in Sri Lankan Commercially available Palm Olein

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ABSTRACT

Objectives: *Palm olein, which is the fractionated form of palm oil, is commonly used as cooking oil in Sri Lanka. Processing of crude oil degrades α-tocopherol as does exposure to high heat during cooking. This study was aimed to determine the content and heat degradation of α-tocopherol in Sri Lankan commercially available palm olein.* ***Method:*** *Four different brands of Sri Lankan commercially available palm olein (100 mL) were heated to 180°C for 10 minutes. The oil was subsequently cooled and re-heated five times and assayed in duplicate. Between each cycle of re-heating oil was left in room temperature to cool for 5 hours. Fresh palm olein and the heated samples were analyzed for α-tocopherol content using reversed-phase High-pressure liquid chromatography (HPLC) (Shimadzu, Japan).* ***Results:*** *The mean α-tocopherol content in unheated palm olein was 3.02 ± 0.35 ppm, with no significant differences between brands. Heating Palm olein for 10 minutes resulted in 56.6% reduction in α-tocopherol compared to unheated oil. Re-heating resulted in further reduction with a 100% loss by the fourth time.* ***Conclusion:*** *The Sri Lankan commercially available palm olein did not provide expected α-tocopherol content, and is not a good source of dietary α-tocopherol. Further analysis is required to quantify α-tocopherol content. Since re-heating further reduced α-tocopherol levels, repeated frying during cooking is likely to result in a minimal level of α-tocopherol being provided through palm olein to the diet.*

Keywords: Crude palm oil, Palm olein, α-Tocopherol, Re-heating, HPLC

Abbreviations: HPLC: High-pressure Liquid Chromatography; RBD: Refined, Bleached and Deodorized; CPO: Crude Palm Oil; VLDL: Very Low-density Lipoprotein; USDA: United States Department of Agriculture; PUFA: Polyunsaturated Fatty Acids

INTRODUCTION

Vitamin E, a fat-soluble vitamin is reported as an excellent antioxidant. It has two subfamilies. The subfamilies are tocopherols and tocotrienols. Each subfamily has alpha (α), beta (β), gamma (γ) and delta (δ) isomers [1,2]. Tocols (tocopherols and tocotrienols) are essential antioxidants in the human body and most of their functions are related to antioxidant properties [3,4]. α-Tocopherol is the predominant form of vitamin E in the human body. Hepatic α-tocopherol transfer protein selectively transfers α-tocopherol into nascent Very-low-density lipoprotein (VLDL) and further to other tissues. Other tocols cannot be taken up by this process and they are excreted via bile, this explains the reason why α-tocopherol is the main focus on vitamin E research [5].

Vitamin E cannot be synthesized in the human body and should be taken from food [5]. There are several natural sources of vitamin E available from both animals and plants. The animal sources of vitamin E are fatty tissues. The amount of vitamin E found in animal sources is much less than in plant sources. Tocopherols and tocotrienols are synthesized in the chloroplast of the plants [6]. Plant sources are oils (oily seeds) and nuts [7]. Oily seeds, fruits such as avocado, mango and nuts accumulate vitamin E to prevent lipid oxidation [5,8]. Edible oils are considered the richest sources of vitamin E and are included in the United States Department of Agriculture (USDA) food composition tables [2,9]. Palm oil is one of the most widely consumed edible oil, globally [10]. Crude palm oil (CPO) contains 600-1000 ppm of tocols as 70% tocotrienols and 30% tocopherols [1,11,12]. CPO undergoes many processes before it reaches the customer [13]. CPO is refined to remove undesirable impurities, producing refined bleached and deodorized (RBD) palm oil. Refined palm oil is expected to contain about 350-450 ppm of vitamin E, present as tocopherol (30%) and tocotrienol (70%) [12]. Fractionation of semi-solid palm oil separates liquid olein from solid stearin. As a result of fractionation, unsaturated fatty acids are preferentially concentrated in the palm olein (liquid phase), whereas saturated fatty acids are preferentially concentrated in the palm stearin (solid phase) [13]. Diacylglycerols, squalene, carotenoids, tocopherol, and tocotrienols are also concentrated in palm olein compared to palm stearin [14]. Palm olein contains 42.1% of oleic acid and 38.3% of palmitic acid and it is less saturated than Palm oil.

Palm olein is widely used as vegetable oil for food preparation due to economic reasons [15,16]. Palm olein has excellent frying properties with a high resistance to oxidation, which results in a long shelf life compared to other cooking oil [17]. Fried products using palm olein do not leave an after taste due to its low melting point (22-24°C) [16].

Cooking oils are usually subjected to high temperatures during food preparation. In deep-frying, oil is continuously exposed to air and light for extended periods at temperatures approaching 180°C which leads to material transfer from oil to food [16,18]. Frying not only changes the texture of food but also the nutritional value of the food being fried. Oil undergoes complex chemical changes which include oxidation, polymerization, and hydrolysis of unsaturated fats forming trans-fat [10,19,20]. In many parts of the world including Asia, oil is repeatedly used for food preparation to minimize the cost [10,21]. This practice is not only limited to homes but also observed in restaurants and other large-scale food preparing industries as well as the informal food sector [22,23]. When cooling the oil from the high-temperature solubility of the oxygen in oil increases. In addition, the oil degradation products too increase with the repeated number of heating cycles [23]. The multiple reasons for the unhealthy nature of repeatedly heated cooking oil include lipid peroxidation products that are harmful to human health and trans-fat generation.

Palm olein is the most commonly used cooking oil in Sri Lanka. α-Tocopherol content in Sri Lankan commercially available palm olein is not known and heat degradation of α-tocopherol in Sri Lankan commercially available palm olein has not been studied so far. The objective of this study was to determine the content and heat degradation of α-tocopherol in Sri Lankan commercially available palm olein.

MATERIALS AND METHODS

Materials

High-pressure liquid chromatography (HPLC) grade methanol was purchased from Sigma/Aldrich, Germany. α-tocopherol synthetic 96% (HPLC), hexane and dichloromethane were purchased from Sigma, Germany. Retinyl acetate was purchased from Fluka Analytical USA, Purified deionized water was prepared using Milli Q50 water purification system (Millipore). All the reagents and chemicals used were of analytical and HPLC grade as relevant. Four most common brands (A, B, C, D) of commercially available palm olein were purchased from one major retail outlet.

Preparation of Standard Solutions

Ten milligrams α-tocopherol standard was weighed and transferred to a 10 mL volumetric flask, making up the volume (10 mL) with methanol. The same procedure was repeated for retinyl acetate for use as the internal standard (1 mg/mL). Stock solutions were stored in amber-colored glass bottles at -20°C until analysis. 100 μg/mL and 10 μg/mL for α-tocopherol and retinyl acetate working solutions were made in volumetric flasks (10 mL) using methanol. The concentration of the internal standard in standard solutions was kept constant. HPLC chromatogram of α-tocopherol standard prepared in methanol is shown in Figure 1.

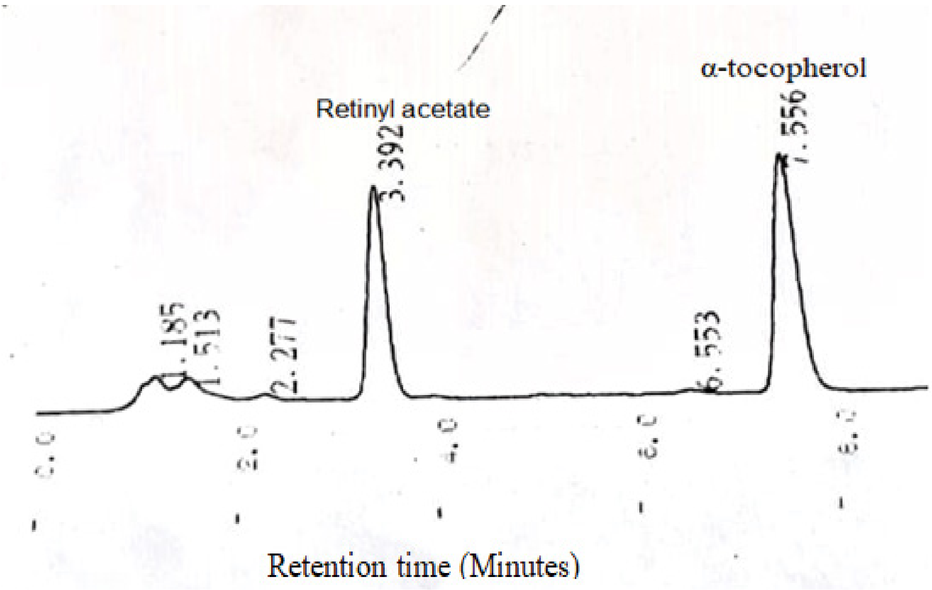

Figure 1 Chromatogram of α-tocopherol standard (5 µg/mL) with the internal standard

Method Validation and Quality Control Procedure

The analytical method for the determination of α-tocopherol using HPLC was found to be specific as no endogenous sources of interference (Figure 1) were observed at the retention time of the analytes (α-tocopherol and internal standard retinyl acetate).

A set of standards at different concentrations (1 µg/mL-13 µg/mL) was prepared and injected. Linearity was evaluated according to the relationship between the peak area ratios (peak area of α-tocopherol/peak area of retinyl acetate (IS)) at each concentration of standard. Microsoft Office, Excel (2010) was used to plot the calibration curve. From the regression line, the coefficient of determination (R^2) was obtained to statistically assess the linear relationship.

Internal standard (retinyl acetate) was used to improve precision. Since retinyl acetate has a similar chemical property (the degree of fat-solubility and polarity (nonpolar)) to α-tocopherol, they can be detected in the same column with slightly different retention times. Retinyl acetate is not found in palm olein naturally. Retinyl acetate was used to correct for the loss of α-tocopherol during sample processing and handling by using the α-tocopherol peak area/retinyl acetate peak area ratio to find the concentration of α-tocopherol in the sample.

The precision was assessed by repeated injections of at least ten replicate injections of α-tocopherol standard spiked processed palm olein. The value for tocopherol was calculated for each sample from the peak area ratio (peak area of α-tocopherol/peak area of retinyl acetate (IS)) and by applying it to the standard curve. The precision of the obtained values was calculated using the relative standard deviation (RSD% or the coefficient of variation) as follows:

$RSD\% = (SD/M) \times 100$

Where SD is the standard deviation of α-tocopherol concentration and M is the mean of α-tocopherol concentration.

Intra-assay and inter-assay precision of the extraction method was determined. Repeatability or intra-assay precision was 5.8% and inter-assay was 5.9%.

Reheating Process

Heating of the oil was done according to the method of Adam, et al. [18] with modifications detailed below; heating involved 100 mL of the palm olein in a metal wok and heated to 180°C (deep-frying temperature) and the heating process lasted for 10 minutes. A smaller volume of oil (100 mL) was used in the current study compared to Adam, et al., (2500 mL). In a previous study 25, fish-flavored chips were fried in the oil, but in the current study, oil was heated without food [18]. This was done to avoid the potential addition of α-tocopherol, moisture and any other component of food as well as the uptake of oil from frying material.

After heating, oil was cooled for 5 hours to make sure that it had reached room temperature. Oil (10 mL) was taken into a falcon tube and the tube was covered with aluminum foil and stored at -20°C until analysis. Then the heating process was repeated with the remaining oil. This process was repeated five times.

α-Tocopherol Extraction and HPLC Analysis

Palm olein samples (24 samples in duplicates) were prepared according to the standard method of Khan, et al., with modifications as stated below [24]. A 1.5 mL Eppendorf tube was used for sample preparation and volumes of the reagents were halved compared to Khan, et al., 112.5 µL of oil was transferred into an Eppendorf tube and 12.5 µL of internal standard solution (10 µg/mL) was added to the oil sample. 325 µL of ethanol:methanol (95:5) mixture was added and centrifuge for 5 minutes at 1600 xg. The clear supernatant was transferred into a new Eppendorf tube and diluted with 325 mL of hexane: dichloromethane (80:20) and was vortexed for 5 minutes. A clear solution was obtained and the solvent was evaporated under vacuum until a pale-yellow color residue alone remained with no solvent. The residue was dissolved in methanol (250 µL) and vortexed for 1 minute. 100 µL of this extracted oil solution was injected into HPLC for analysis. Since α-tocopherol and retinyl acetate are light and heat sensitive sample processing and handling were carried out in a dark room at room temperature (25°C).

A revered phase 150 × 3.0 mm, ultra C-18 column (RESTEK) was used as the stationary phase for analysis of α-tocopherol in palm olein. The mobile phase was 99% methanol prepared by mixing 990 mL of methanol and 10 mL of filtered deionized water in a 1000 ml measuring cylinder. The mobile phase was sonicated for 30 minutes before use to remove dissolved gasses (degassing). A flow rate of 0.7 mL/minute and a wavelength of 292 nm were used to detect α-tocopherol and internal standard (retinyl acetate) in palm olein.

RESULTS

The mean ± SD α-tocopherol content in unheated palm olein was 3.02 ± 0.35 ppm. α-Tocopherol content in commercially available unheated (room temperature, 25°C) palm olein (A, B, C, and D) are given in Table 1.

Table 1 α-Tocopherol content in Sri Lankan commercially available palm olein

Brands	α-tocopherol content (ppm)
A	3.01 ± 0.00
B	3.28 ± 0.46
C	2.53 ± 0.05
D	3.25 ± 0.39

*Results are presented as mean ± standard deviation (SD). Values presented are the average of two determinations.

Heat degradation pattern of palm olein A, B, C and D with the number of heating cycles are shown in Figure 2. Each sample was processed and injected in duplicates and mean and SD are presented in bar charts. Figure 2 illustrates that heating palm olein once resulted in a significant reduction in α-tocopherol and heating four times resulted in further reduction up to 100%.

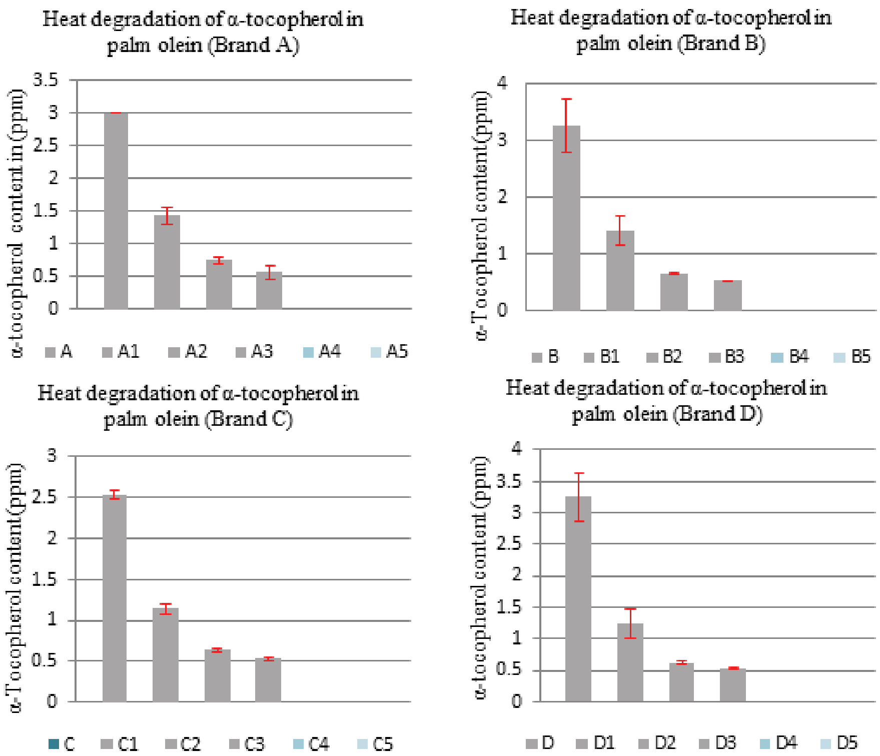

Figure 2 Plot of the content of α-tocopherol in ppm (mean and SD of the duplicate sample) against the number of heating cycle. (A-unheated, A1-once heated, A2- twice heated, A3- thrice heated, A4-heated four times and A5- five times heated)

DISCUSSION

We used HPLC to quantify the α-tocopherol in unheated and heated palm olein as HPLC is a robust method to determine vitamin E content [9,11]. α-Tocopherol content in unheated palm olein was lower than previously reported values in Palm oil. Adam et al reported 178 ppm α-tocopherol in fresh palm oil, with samples collected at the point of production. In another study, Azlan, et al., reported 51.91 ppm of α-tocopherol in Palm olein [25]. RBD palm olein was used in our study as that is the most widely used form for retail purchases. Although it is expected that RBD oil would not contain levels as high as the unrefined oil, it is important that this is documented. Production of RBD palm olein is through adsorption on activated bleaching earth, followed by high-temperature steam deodorization which results in an approximate reduction of 50% of the tocols along with free fatty acids [12]. Osawa and Goncalves reported 26 ppm α-tocopherol in Fresh palm olein [26].

The present study used an imported RBD palm olein product with potential additional loss of α-tocopherol due to heat exposure during longtime transport, improper handling, packing (packed in transparent plastic bottles) and storage. Mohd-Fauzi and Sarmidi reported the loss of α-tocopherol in Palm olein when stored at room temperature [27].

In the present study, heating palm olein once resulted in a significant reduction in α-tocopherol content and heating four or five times resulted in a further reduction of up to 100% reduction. This finding is comparable to the results of previous studies. Al-attar, observed the complete degradation of α-tocopherol in Palm olein with the pan-frying method [28]. Adam, et al., showed that heating Palm olein repeatedly five times for 10 minutes at 180°C resulted in 98.13% degradation in α-tocopherol [18]. Similar results were reported by Schroeder, et al., and Bansal, et al., [29,30]. Schroeder, et al., studied the effect of repeated heat treatment at 160°C on yellow and red Palm olein and reported a significant reduction of α-tocopherol with repeated heating [29].

Chiou, et al., reported the loss of antioxidants during frying especially during pan-frying compared to deep-frying. This is because the surface-to-volume ratio with atmospheric oxygen is higher in pan-frying [31]. Vitamin E protects unsaturated fatty acids in vegetable oil from oxidation. When the oil is heated, vitamin E is consumed by reacting with lipid-free radicals. Vitamin E can interrupt the propagation of oxidative chain reactions [18]. Some previously published studies reported that inclusion of α-tocopherol to frying oil was found to protect Polyunsaturated fatty acids (PUFA) from oxidation while of α-tocopherol degrade after each frying cycle. Therefore, repeated heating of frying oils destroys the vitamin E content while protecting the fatty acids from oxidation. However, when the content of α-tocopherol is completely lost as was observed in this study following repeated heating, the fatty acids are likely to be exposed to peroxidation and would yield the oil unhealthy for consumption.

CONCLUSION

Sri Lankan commercially available palm olein did not provide the expected α-tocopherol level when compared to USDA food tables. Heating caused a further reduction of up to 100%. Repeated use of palm olein for food preparation is not an acceptable practice, as regards the loss of vitamin E. Further analysis is required to establish content of α-tocopherol in Sri Lankan commercially available palm olein.

This was the first study to determine the content of α-tocopherol in Sri Lankan commercially available palm olein and their heat degradation. The other isomers of tocopherols and tocotrienols in palm olein were not analyzed and, are a limitation of the study. Future studies can be designed to determine the other isomers of vitamin E and their heat degradation and antioxidant property in commercially available palm olein in Sri Lanka.

DECLARATIONS

Authors' Contribution

The authors are solely responsible for the design and conduct of this study. PF, IW, MDL, and PL participated in the design of the study. PF conducted the study, collected the data, and wrote the manuscript. PF, PL contributed to the analysis. IW, MDL, and PL reviewed and edited the manuscript. All authors read and approved the final manuscript.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing Interests

The authors have disclosed no potential conflicts of interest, financial or otherwise.

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