Introduction
Refined, bleached and deodorised palm olein oil (palm olein) and soya bean oil are used as cooking oils. The oils differ in their degree of saturation and composition of fatty acids. Consumption of edible oils with different fatty acid composition results in these fatty acids being incorporated into cell membranes and organelles of cells and unsaturated fatty acids can undergo lipid peroxidation. Recently, it has been reported that the consumption of palm olein results in the production of less lipid peroxidation products than does the consumption of soya bean oil. It is generally agreed that the more unsaturated the oil, the greater the amount of lipid peroxidation products formed when the oil is consumed. Factors that contribute to the formation of lipid peroxidation products when oils and fats are consumed include the degree of unsaturation of fatty acids and other components of the oil, such as the cholesterol oxides found in ghee.

On the other hand, compounds such as carotenoids, tocopherols and tocotrienols that may be present in edible oils inhibit the formation of lipid peroxidation products. It has been reported that in hyperthyroidism there is an increase in the level of lipid peroxidation products in the soleus muscles of the hyperthyroid rats. It is generally agreed that the more unsaturated the oil, the greater the amount of lipid peroxidation products formed when the oil is consumed. Factors that contribute to the formation of lipid peroxidation products when oils and fats are consumed include the degree of unsaturation of fatty acids and other components of the oil, such as the cholesterol oxides found in ghee.

Materials and methods
Eight-weeks-old male Wistar rats weighing between 250 and 300 g were divided into four groups of 10 rats each. The groups were weight-matched and treated for a period of 8 weeks. Group 1 rats (euthyroid control) were injected intraperitoneally with alkalinized saline (pH 9–9.5) thrice weekly and were fed ground rat chow. Group 2 rats (hyperthyroid control) were injected intraperitoneally thrice weekly with 500 µg/kg L-thyroxine dissolved in 0.9% sodium chloride made alkaline with sodium hydroxide and adjusted to pH 9–9.5 with hydrochloric acid, and were fed ground rat chow (H group). Group 3 rats were treated similarly to group 2 rats but, in addition, were fed 20% w/w of palm olein mixed with ground rat chow (HPO group). Group 4 rats were treated similar...
Preparation of soleus muscle extract for assay of vitamin E

The vitamin E extraction method adopted was that of Lang et al. with a slight modification. Prior to sacrificing the rat, the soleus muscle was isolated, excised and dipped into liquid nitrogen and then stored at −70°C for no longer than 2 weeks. Subsequently it was allowed to thaw out on ice and cut into very small pieces. Exactly 100 mg of the soleus muscle was weighed placed in a 10 mL test tube and 1.0 mL of distilled water added. To this was added 50 µL of ethanolic 2,6-di-tert-butyl-p-cresol (BHT; 10 mg BHT in 1 mL ethanol) to prevent lipid autoxidation. The mixture was then homogenised for 20 s before 1.0 mL of 0.1 mol/L sodium dodecyl sulphate was added and the mixture homogenised for a further 10 s.

The homogeniser was rinsed with 2 mL of 95% ethanol which was combined with the homogenate. The mixture was vortexed for 1 min. Vitamin E was then extracted from this homogenate by adding 2 mL hexane and the tightly screwed test tube vortexed for 5 min. This mixture was then centrifuged at 1000 g for 7 min at a temperature of 20°C in order to separate the organic phase from the aqueous phase. The hexane layer containing vitamin E was pipetted out and placed in a test tube and the hexane was dried under nitrogen. The dried test tube containing vitamin E was stored at −20°C for no longer than 2 weeks before assay. Throughout this process the test tubes were covered with aluminium foil to prevent degradation of vitamin E in the sample.

The soleus muscle extracts of the four different groups (seven rats per group) were made on two different days. On the first day, extractions of the soleus muscles of three rats per group of the four different groups were made. On the second day, extractions were done on the remaining 16 rats (i.e. the remaining four rats per group for the four different groups of rats). A similar schedule was adopted for the assay of vitamin E to ensure that the storage time of the soleus extract of the four different groups of rats was similar. The assay was done within 2 weeks of extraction. Unpublished observations in our laboratory show that extracts of vitamin E can be kept out of seven extracts in the H group, which account for their large standard errors.

High pressure liquid chromatographic analysis of vitamin E

A total of 100 µL of mobile phase was added to the dried sample of vitamin E and the sample was vortexed for 20 s. The solution was then filtered using a filter of porosity 0.45 µm before 20 µL of it was injected into the column. The mobile phase was made up by mixing 99 volume of hexane and 1 volume of isopropanol and degassed before using. The equipment used was a Gilson single pump, normal phase liquid chromatography with a fluorescence detector (EX: 294 nm; EM: 330 nm). The chromatographic analysis was carried out at room temperature using a silica column (250 mm × 4.6 mm, silica spherisorb 5 µm). The flow rate of the mobile phase was 1.5 mL per min throughout. Solvents used were of high pressure liquid chromatographic (HPLC) grade.

Determination of standard curve

Serial dilutions of the various isomers of vitamin E were made with hexane to the limits of their detection. A graph of area against concentration of each isomer was plotted. The concentration of each isomer of vitamin E in the extract was determined by means of this standard curve.

Statistical analysis

The data are presented as mean ± SEM. Statistical significance was by unpaired Student’s t-test after a one-way analysis of variance.

Results

The α-tocopherol level in the soleus of the HPO group of rats was significantly higher than that in the HSBO group of rats; the α-tocopherol level in the soleus of the H group of rats was significantly higher than that in the control group of rats; the α-tocopherol levels in the soleus of HPO group of rats or the HSBO group of rats were significantly higher than that in the H group of rats (Table 1). α-Tocopherol levels were detectable in all 7 of the extracts in each group of rats. The α-tocotrienol level in the soleus HPO group of rats was significantly higher than that in the HSBO group of rats (Table 1). The α-tocotrienol level in the soleus of the H group of rats was significantly lower than that in the control group (Table 1). The α-tocotrienol level in the soleus of the H group was not significantly different from that of the HSBO group but significantly lower than that of the HPO group (Table 1). α-Tocotrienol levels were detectable in each of the seven extracts in the control group and in the HPO group but were detectable at much lower levels in only one out of seven extracts in the HSBO group and in two out of seven extracts in the H group, which account for their large standard errors.

The γ-tocotrienol level in the soleus of HPO group of rats was significantly higher than that in the HSBO group of rats.

Table 1. Levels of α-tocopherol (α-TF), α-tocotrienol (α-TT), γ-tocotrienol (γ-TT) and δ-tocotrienol (δ-TT) in the soleus muscle of the four groups of rats

<table>
<thead>
<tr>
<th>Groups of rats</th>
<th>α-TF</th>
<th>α-TT</th>
<th>γ-TT</th>
<th>δ-TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.473 ± 0.370</td>
<td>0.840 ± 0.287</td>
<td>0.642 ± 0.220</td>
<td>0.104 ± 0.104</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>9.079 ± 0.252b</td>
<td>0.150 ± 0.093a</td>
<td>0.179 ± 0.116c</td>
<td>0.240 ± 0.162</td>
</tr>
<tr>
<td>Hyperthyroid + palm olein</td>
<td>23.682 ± 0.363a</td>
<td>1.974 ± 0.040b</td>
<td>1.418 ± 0.054a</td>
<td>0.403 ± 0.226</td>
</tr>
<tr>
<td>Hyperthyroid + soya bean oil</td>
<td>14.299 ± 0.378b</td>
<td>0.053 ± 0.053</td>
<td>0.184 ± 0.120</td>
<td>0.176 ± 0.113</td>
</tr>
</tbody>
</table>

Values indicate mean ± SEM. Superscripts indicate significant difference from: hyperthyroid + soya bean oil α-c (P < 0.0001); control b (P < 0.003), a (P < 0.005), t (P < 0.05); hyperthyroid b-c-k (P < 0.0001). n = 7 in each group.
The γ-tocotrienol level in the soleus of the H group is significantly lower than that of the control group (Table 1). The γ-tocotrienol level in the soleus of the H group was not significantly different from that of the HSBO group but was significantly lower than that of the HPO group. The γ-tocotrienol was detectable in all seven samples in the HPO group, in five out of seven samples in the control group and, at much lower levels, in two out of seven samples each in the H group and in the HSBO group.

The δ-tocotrienol level in the soleus was not significantly different among all four groups (Table 1). Out of the seven samples in each group, the δ-tocotrienol level was detectable in only one sample in the control group, two samples in the H group, three samples in the HPO group and two samples in HSBO group.

**Discussion**

It has been shown by Zaiton et al. that MDA and CD levels were lower in the soleus of hyperthyroid rats on the palm olein diet compared with their levels in the soleus of hyperthyroid rats on the soya bean oil diet. One obvious reason for this finding is that palm olein, being more saturated than soya bean oil, is less prone to lipid peroxidation attack by reactive species, resulting in less production of lipid peroxidation products. The authors, in that paper, however, also made the assumption that the decrease in the level of lipid peroxidation products was due to a higher content of vitamin E in the soleus of palm olein-fed hyperthyroid rats compared with the soleus of soya bean oil-fed hyperthyroid rats. The results of this paper show that α-tocopherol, α-tocotrienol and γ-tocotrienol levels are indeed higher in the soleus of hyperthyroid rats fed palm olein compared with the level in the soleus of hyperthyroid rats fed soya bean oil, thus providing strong evidence in favour of their assumption.

The evidence would be more complete if γ- and δ-tocopherol levels were also measured. However, it has been shown that although γ-tocopherol is found in higher quantities in the diet (including in soya bean oil and absent in palm olein), plasma and tissue levels of α-tocopherol are higher. The reason for the higher level of α-tocopherol in the muscles may be a result of a tocopherol transfer protein activity. Furthermore, several studies indicate that the level of γ-tocopherol in adult human plasma averages only 10–15% of the concentration of α-tocopherol. Furthermore, α-tocopherol is the most potent isomer of vitamin E in terms of antioxidant activity. Thus the contribution of γ-tocopherol to the antioxidant activity of soya bean oil would be small. The average total isomers of vitamin E measured in this study in the soleus of HPO rats (27.50 µg/g) is approximately double the total isomers of vitamin E measured in this study in the soleus of HSBO rats (14.7 µg/g). Transfer of soya bean oil-fed hyperthyroid rats fed palm olein compared with the soleus of hyperthyroid rats fed soya bean oil is, at least partly, due to the higher level of the antioxidant, vitamin E, in these muscles.

The study shows further that, as expected, the soleus muscles of HPO rats have significantly higher levels of α-tocopherol, α-tocotrienol and γ-tocotrienol compared with those of H rats; however, the δ-tocotrienol level is not significantly different, perhaps because of its low concentration compared with the other isomers in palm olein. As expected, the soleus muscles of HSBO rats also have significantly higher levels of α-tocopherol but not tocotrienols compared with H rats, given that soya bean oil contains tocopherols but does not contain the tocotrienols. In addition, the results showed that α-tocopherol and γ-tocotrienol levels in the soleus of H rats were significantly lower but that the α-tocotrienol level is significantly higher than its level in the soleus of control rats (eutryhoid rats fed rat chow only). It has been reported that thyroid hormones influence the metabolism of vitamin E in humans and animals; from this result it would appear that the effects on tocopherols and tocotrienols are not similar.

**References**