Serum cholesterol and triglyceride levels of rats fed with consumer selected coconut oil blends

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Abstract: Double blends were prepared by mixing copra oil (CO) with seed oils of Brassica juncea (BO), Madhuca nerifolia (MO) and Sessamum indicum (SO) in different volume proportions. The consumer-acceptable oil blends were selected based on the opinion of a panel of consumers about the palatability and other physical properties of French fries prepared using the oil blends. The effect of the selected oil blends on serum lipid parameters of Wistar rats was examined. The serum levels of total cholesterol, LDL and triglycerides decreased and the serum levels of HDL increased significantly in rats fed with selected oil blends, when compared to those fed with coconut oil. The beneficial effects on lipid parameters of the rats fed with the oil blend containing the seed oil of BO (40%) and CO (60%) were closely comparable with those of the rats fed with soybean oil. The results recommended that it could be helpful to prepare essential fatty acid-rich nutritional oil blends based on CO in industrial scale.

Keywords: Coconut oil, soybean oil, oil blends, total cholesterol, HDL/LDL, triglycerides

Introduction

Coconut oil is the main fat of choice in many Asian countries.Apparently, the common method of preparation of coconut oil is by pressing dried coconut kernel. As a result, the oil prepared by this method is known as copra oil. It is well-known that the composition of the saponifiable fraction of edible oil has a profound effect on serum lipoprotein cholesterol and triglyceride levels (Harris, 1989; Grundy and Denke, 1990; Kris-Etherton and Shaomei, 1997). The results of these studies indicate that saturated fatty acids increase the plasma total cholesterol and LDL-cholesterol concentrations, whereas polyunsaturated fatty acids lower these parameters. Coconut oil contains up to 93% of saturated fatty acids. However, the important feature of coconut oil is that it is responsible for increasing serum HDL cholesterol concentrations more profoundly than other sources of saturated fat (Quig and Zilversmit, 1989; Carlson and Kottke, 1990). One noticeable drawback of coconut oil is due to its low level of essential fatty acids, with the percentage weight of linoleic acid ranging from 1.0 to 2.6 (Dale and Meara, 1955). Therefore, blending coconut oil with other edible oils containing higher percentages of polyunsaturated fatty acids is suggested as a convenient way of improving the essential fatty acid content and associated nutritional and health properties of coconut oil. Blended oils containing coconut oil with groundnut oil or olive oil are proven to be responsible in reducing of LDL oxidation and enhancing hepatic antioxidant enzymes activity in rats (Nagaraju and Belur, 2008). Recent reports also indicate that blending of coconut oil with soybean or sunflower oil improves hypolipidemic effects (Chandrashekar et al., 2010). On the other effort, improvement of the polyunsaturated fatty acid levels by blending soybean oil, sunflower oil and flaxseed oil also results in beneficial effects on the serum lipid profiles of rats fed with a hypercholesterolemic diet (Ramadan et al., 2009).

When the oil blends are prepared, it is important to check the consumer acceptation about such blends formulation. Even though the blending effort of coconut oil with other edible oils has been reported extensively, these studies do not indicate the significant information about the consumer feedback especially on the sensory evaluation. In addition to the nutritional properties, the ultimate consumer acceptability of oil blends depends on the organoleptic properties and the physico-chemical properties of food prepared using such oil blends. In order to reduce the cost of production of coconut oil blends, it is also important to use polyunsaturated oils that are easily available in the coconut growing countries. In the present study, sixteen oil blends were prepared with different proportions of coconut oil and three other easily and cheaply available edible oils. The hypolipidemic effects of the consumer selected oil blends among the prepared oil blends were investigated using Wistar rats.
Materials and Methods

Materials

The reagents for the determination of cholesterols and triglycerides were purchased from Biolabo (Maizy, France). The authentic standards for GC analysis were purchased from Fluka (Buchs, Switzerland).

Preparation of CO

Coconuts of same maturity (12 - 14 months) of “Ordinary Tall” coconut cultivars were used to prepare copra by drying coconut kernels under the sunlight for three weeks. Coconut oil was extracted by pressing copra in a small-scale expeller.

Preparation of seed oils

BO, MO and SO were prepared by pressing dried seed kernels of the relevant plants in a small-scale pressing machine. Anhydrous sodium sulphate was added to oil samples and allowed to stand for 30 min to remove excessive residual moisture. The resultant dry oil was centrifuged at 1080 g and filtered through Whatman No1 filter papers under mild vacuum using a Buchner funnel.

Determination of quality parameters

Acid value (AV), iodine value (IV), peroxide value (PV) and saponification value (SV) of the oils were determined by the reported methods (Kirk and Sawyer, 1991).

Preparation of oil blends

After preparation, the seed oils were used immediately to prepare blends with CO. The blends were prepared by mixing each three seed oils (BO, MO and SO) with CO in different volume proportions.

Quality evaluation by consumers

The panelists of 20 consumers which included 10 males and 10 females of the age 20-50 were selected. All the panelists had sensory evaluation experience and were trained in the evaluation of tested properties. French fries of 0.4 cm x 0.4 cm x 6 cm dimensions were prepared by frying them in the prepared oil blends and coconut oil. Fries were left on blotting papers for 3-5 min and were offered to the panelists. The panel members were requested to judge sensory attributes such as taste, color, texture, appearance and overall acceptability of the fried French fries. A 1-5 scale was used for each tested property meaning that 1 (dislike very much), 2 (dislike moderately), 3 (neither like nor dislike), 4 (like moderately and 5 (like very much). The French fries prepared by frying potato in coconut oil was selected as reference sample and arbitrarily given the scale of 5 for each tested property and the panel was asked to evaluate the other samples of French fries with respect to this reference sample. The values obtained from the panelists were converted to percentages and the statistical significance was analyzed.

Determination of fatty acid composition

Methylation of fatty acids was carried out by a proposed method (Garcés and Mancha, 1993). The fatty acids were identified by comparing the retention times of the signals with those of authentic fatty acid methyl ester standards. A gas chromatograph (Thermo Finnigan Trace K01332734500000, Milan, Italy) equipped with capillary column RtxR- WAX (Crossbond with PEG, 30 m x 0.32 mm x 0.25 µm) and flame ionization detector (FID) was used for this analysis. The injector and detector temperatures were set at 230°C and 250°C respectively. The carrier gas used was helium at 30 kpa in constant pressure mode and the analyses were performed on split mode (split ratio 100: 1). The sample (0.4 µl) was injected into the GC system. A temperature programme of 130°C (3 min), 130°C to 210°C at 45 ºC/min, and 210°C (12 min) was used throughout the study.

Animal study

Six week old male weaning Wistar rats (weighing 125–150 g) were randomly collected from the Medical Research Institute, Colombo. They were then placed individually in cages and housed in a room with temperature range of 25 ± 2°C with 12 h light-darkness cycle. Prior to the initiation of the experiment, the rats were acclimatized to the basal diet for six days. Then the rats were randomly assigned to treatment groups (5 rats/group) and fed with a semi synthetic diet containing tested oil blends recommended by WHO (Sabourdy, 1988). Briefly, the diet was prepared by mixing maize (2005 g), brown rice (500 g), rice bran (125 g), wheat bran (100 g), wheat flour (675 g), fish meal (400 g), soya meal (400 g), milk powder (300 g), molasses or brown sugar (125 g), tested oil (100 g), grass powder (9150 g), bone meal (75 g), mineral mix (20 g), vitamin mix (12 g), sodium chloride (10 g), Betamix E 50 (1 g), d,l-methionine (2.5 g) Vit B Complex (30 standard tablets) and 1.4 L of water for total weight of 5 kg. Free access to the diet and water were provided to the rats for a period of 84 days. The food intake was monitored daily and the growth of animals was monitored weekly. The experimental protocol was approved by the ethical committee of University of Sri Jayawardenapura, Sri Lanka (Approval number 359/7, 2007). The rats were fasted...
Determination of cholesterol and triglyceride levels

The blood samples were centrifuged promptly (3000 g, 15 min) at room temperature. Separated top layer of serum from each sample was used for the analysis. The test kit method provided by Biolabo was used for the analysis. Biolabo reagent (REF 80106) was used to determine the total cholesterol based on a reported enzymatic method (Allain et al., 1974). Biolabo reagent (REF 90416) was used to determine the LDL-cholesterol (Bachorik and Ross, 1995). HDL cholesterol was determined by the difference between total cholesterol and LDL-cholesterol. Biolabo reagent (REF 80019) was used to determine triglyceride levels based on a reported enzymatic method (Fossati and Prencipe, 1982). The procedures given by the supplier was followed without any modifications for the determination of cholesterol and triglyceride levels. For example, for the determination of total cholesterol, three mixtures were prepared according to the method given by the supplier as follows. Test sample was prepared by mixing serum (10 µl) with assay reagent (1.0 ml). Standard sample was prepared by mixing a 200 mg/dl cholesterol solution (10 µl) with assay reagent (1.0 ml). Blank sample was prepared by mixing distilled water (20 µl) with assay reagent (1.0 ml). The mixtures were incubated at 37°C for 5 min and the absorbance was read at 500 nm using a UV-visible spectrophotometer (Optima SP-3000 plus, Tokyo, Japan). Similarly, the procedures given by the supplier was used to determine and calculate LDL-cholesterol and triglyceride levels.

Statistical analysis

All analyses were run in triplicate unless otherwise indicated. Kruskal – Wallis and Mann Whitney test (Minitab, 13.3) was used to convert the data of consumer opinion about oil blends to percentages and evaluate statistical significance. Turkey’s pairwise test was carried out after ANOVA for the determination of significant differences (P < 0.05) between the means of the values obtained for cholesterol and triglyceride levels.

Results and Discussions

Consumer acceptance

Quality parameters of the individual oils used in the present study are given in Table 1. These properties were well within the range of reported values (Zajcew, 1956; Andrews, 1933; Menezes et al., 1950; Awasthi et al., 1975). Table 2 summarizes the consumer acceptance on the oil blends with respect to CO based on the quality of French fries. In BO blends, the highest score was obtained by the composition of the blend in entry 2. The result shows that level of BO was inversely proportional with the consumer acceptability. It is suggested that the blends with the amount of BO above 60% were not considered in this study. Among the blends of MO, the consumer acceptability abruptly dropped for the blend composition with MO above 60%. For SO blends, the highest score was obtained by the composition in entry 14. Further increment of the amount of MO abruptly reduced the consumer acceptability. Therefore, the amount of SO was not increased beyond 70%. The blends in the entries 2, 8 and 14 were selected for the studies of health effects because their overall acceptability was above 75 points. The oiliness is a major factor that affects the consumer overall acceptability of the final fried products. In the panel tests, oiliness of the French fries was taken into account by the panel considering the effect of oiliness on taste texture and appearance.

Fatty acid composition

Composition of the individual fatty acids of the three selected oil blends used for the animal study is given in Table 3. Fatty acid composition of the three selected oil blends and individual oils based on the degree of saturation is shown in Table 4. The results indicate that the saturated fatty acid (SFA) content of the three oil blends are 20-34 % lower than that of CO. Monounsaturated fatty acid (MUSF) contents are 2–7 times higher while polyunsaturated fatty acid (PUFA) contents are 5–16 times higher in the three oil blends compared to CO. The three oil blends which were selected for the in vivo studies contained lower percentages of MUSF and PUFA than the recommended values by experts (FAO/WHO 1994). However, the three oil blends were selected based on the consumer acceptability. Any blend with higher PUFA contents prepared by mixing BO, SO or MO with coconut oil did not score above 75 points in the consumer panel test according to the Table 2.

Table 1. Quality parameters of the individual oils used for the preparation of blends

<table>
<thead>
<tr>
<th>Type of oil</th>
<th>AV (mg of KOH/g)</th>
<th>PV (g of I$_2$/100 g)</th>
<th>IV (absorbance at 500 nm)</th>
<th>SV (mg of KOH/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>1.1 ± 0.2</td>
<td>62 ± 6</td>
<td>0.95 ± 0.1</td>
<td>241 ± 4</td>
</tr>
<tr>
<td>BO</td>
<td>0.9 ± 0.1</td>
<td>117 ± 6</td>
<td>0.52 ± 0.10</td>
<td>204 ± 9</td>
</tr>
<tr>
<td>MO</td>
<td>1.2 ± 0.4</td>
<td>62 ± 3</td>
<td>0.63 ± 0.10</td>
<td>170 ± 3</td>
</tr>
<tr>
<td>SO</td>
<td>0.9 ± 0.3</td>
<td>128 ± 5</td>
<td>0.70 ± 0.10</td>
<td>197 ± 6</td>
</tr>
</tbody>
</table>

Each data point represents the mean ± standard deviation of five replicates.

Table 2 also shows that the SFA/MUFA/PUFA ratios of the blends are not equal to the values that can be calculated by considering these ratios of...
individual oils used to make the oil blends. This can be understood by considering the fact that the volume ratios of individual fatty acids were used in the preparation of oil blends while the molar ratios of the fatty acids were obtained by gas chromatography. The densities of oils and the molecular weight distribution of individual fatty acids in the oils used for making blends are not the same for all the oils.

Table 3. Individual fatty acid compositions of oil blends

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>BO(40%) + CO(60%)</th>
<th>SO(50%) + CO(50%)</th>
<th>MO(60%) + CO(40%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 8:0</td>
<td>6.8 ± 0.3</td>
<td>5.9 ± 0.4</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>C 10:0</td>
<td>5.7 ± 0.3</td>
<td>4.4 ± 0.3</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>C 12:0</td>
<td>12.3 ± 0.7</td>
<td>10.0 ± 0.5</td>
<td>16.9 ± 0.9</td>
</tr>
<tr>
<td>C 14:0</td>
<td>19.2 ± 0.5</td>
<td>16.9 ± 0.5</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>C 16:0</td>
<td>16.7 ± 0.6</td>
<td>15.6 ± 0.6</td>
<td>16.5 ± 0.3</td>
</tr>
<tr>
<td>C 18:0</td>
<td>15.3 ± 0.1</td>
<td>14.0 ± 0.3</td>
<td>14.0 ± 0.3</td>
</tr>
<tr>
<td>C 18:1</td>
<td>12.9 ± 0.9</td>
<td>11.2 ± 0.4</td>
<td>7.3 ± 0.5</td>
</tr>
</tbody>
</table>

Each data point represents the mean ± standard deviation of three replicates.

Table 4. Fatty acid composition of oil blends and individual oils based on the degree of saturation

<table>
<thead>
<tr>
<th>Oil blend/oil</th>
<th>SFA</th>
<th>MUFA</th>
<th>PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BO (40%) + CO (60%)</td>
<td>71.8 ± 0.5</td>
<td>11.3 ± 0.6</td>
<td>16.5 ± 0.5</td>
</tr>
<tr>
<td>SO (50%) + CO (50%)</td>
<td>72.3 ± 0.7</td>
<td>16.5 ± 0.5</td>
<td>11.2 ± 0.4</td>
</tr>
<tr>
<td>MO (60%) + CO (40%)</td>
<td>60.1 ± 0.9</td>
<td>32.5 ± 0.7</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>CO</td>
<td>90.0 ± 0.5</td>
<td>5.0 ± 0.2</td>
<td>4.3 ± 0.06</td>
</tr>
<tr>
<td>BO</td>
<td>9.6 ± 0.02</td>
<td>24.8 ± 0.1</td>
<td>65.6 ± 0.2</td>
</tr>
<tr>
<td>SO</td>
<td>152.0 ± 0.3</td>
<td>42.4 ± 0.1</td>
<td>42.4 ± 0.01</td>
</tr>
</tbody>
</table>

Each data point represents the mean ± standard deviation of three replicates.

Animal study

Table 5 shows the serum total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels in rats fed with the oil blends. The results in Tables 4 and 5 also show the same variation of serum total cholesterol and LDL cholesterol levels of rats with the fatty acid content in the diet. However, the variation of serum lipid parameters is not linearly proportional to the MUFA or PUFA for all the edible oils. For example, the total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels in rats fed with the BO (40%) + CO (60%) oil blend is closely comparable with those levels in rats fed with soybean oil. However, the SFA/MUFA/PUFA ratio of this oil blend is 72:12:16 while that of soybean oil is roughly 15:27:58. These results suggest that even though the serum cholesterol levels vary beneficially with the PUFA contents in dietary fats, such variations cannot be fully explained by considering the ratio of SFA/MUFA/PUFA.

Table 5. Serum total cholesterol, LDL, HDL and triglyceride levels of the Wistar rats fed with oil blends

<table>
<thead>
<tr>
<th>Type of oil</th>
<th>BO (40%) + CO (60%)</th>
<th>SO (50%) + CO (50%)</th>
<th>MO (60%) + CO (40%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 days</td>
<td>18.0 ± 0.5</td>
<td>20.0 ± 0.7</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>56 days</td>
<td>20.0 ± 0.7</td>
<td>22.0 ± 0.7</td>
<td>22.0 ± 0.7</td>
</tr>
<tr>
<td>84 days</td>
<td>22.0 ± 0.7</td>
<td>24.0 ± 0.7</td>
<td>24.0 ± 0.7</td>
</tr>
</tbody>
</table>

Each data point represents the mean ± standard deviation of three replicates. Letters a, b, c and d were used to compare statistical significance in columns (p < 0.05). Letters q, r, and s were used to compare statistical significance in rows (p < 0.05). 

Statistically significant compared to 28 days reading.

Statistically significant compared to 56 days reading.

Statistically significant compared to initial level.

Statistically significant compared to 84 days reading.

(Zanni et al., 1987; Katan et al., 1994). The results in Tables 4 and 5 also show the same variation of serum total cholesterol and LDL cholesterol levels of rats with the fatty acid content in the diet. However, the variation of serum lipid parameters is not linearly proportional to the MUFA or PUFA for all the edible oils. For example, the total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride levels in rats fed with the BO (40%) + CO (60%) oil blend is closely comparable with those levels in rats fed with soybean oil. However, the SFA/MUFA/PUFA ratio of this oil blend is 72:12:16 while that of soybean oil is roughly 15:27:58. These results suggest that even though the serum cholesterol levels vary beneficially with the PUFA contents in dietary fats, such variations cannot be fully explained by considering the ratio of SFA/MUFA/PUFA. Studies have shown that the total serum cholesterol levels of the rats fed with groundnut oil and virgin coconut oil after 45 days were 91.58 mg/l and 75.72 mg/l respectively (Nevin and Rajamohan, 2004) even though the SFA/MUFA/PUFA ratio of these oils was 40:20:40.
PUFA ratio of groundnut oil and virgin coconut oil are 27:40:33 and 93:5:2 respectively. Despite the higher SFA content and lower PUFA contents, the effect of lowering serum cholesterol levels in rats is higher for virgin coconut oil than for groundnut oil.

The contribution of fatty acids to the cholesterol levels varies with the nature of fatty acids (Williams, 1998). Therefore, the composition of fatty acids in triglycerides, especially the chain length of fatty acids is an important factor which may influence the serum cholesterol levels (Lim-Sylianco et al., 1992; Nevin and Rajamohan, 2008). The content of fatty acids with chain lengths C16 and shorter in soybean oil is 8% while that of the oil blend BO (40%) + CO (60%) is 70%. Among the fatty acids detected in this oil blend, 46% of them is saturated fatty acids with chain lengths of C16 and shorter. The short chain fatty acids can be directly absorbed and converted to energy so that their influence on lipid parameters may be minor.

In addition to lipid fraction, unsaponifiable materials in the oils are also responsible for the beneficial antioxidant properties and health effects. Antioxidant properties of coconut oil improve with the phenolic content (Seneviratne et al., 2009). Even though coconut oil with high phenolic contents can be prepared by different methods, phenolic antioxidant content of CO prepared by pressing dry coconut kernel is very small (Seneviratne and Disanayake, 2008). However, CO is the cheaply available coconut oil affordable to most consumers and therefore it was used in the present study. BO is known to improve oxidative stability of vegetable oils due to the presence of natural antioxidants (Susheelamma et al., 2002). Unsaponifiable matter present in the oils used to blend with CO may also play an important role in controlling the serum cholesterol levels. The beneficial effects on lipid parameters resulting from unsaponifiable compounds may be due to the relative rates of synthesis and catabolism of these lipids which are influenced by unsaponifiable compounds as suggested (Nevin and Rajamohan, 2004).

**Conclusion**

Serum lipid parameters resulting from the consumption of CO can be beneficially improved by blending CO with BO, MO and SO. The oil blend containing BO (40%) and CO (60%) confers the best beneficial effect on lipid parameters. Consumer acceptability suggests that the given oil blends in present study can be used as alternatives to coconut oil while retaining most of the sensory characteristics of CO. Even though the improvement of the serum lipid quality is associated with the increase of the amount of mono-and polyunsaturated fatty acids in oils, such variations seem to be not strictly linear.

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