Determination of dietary fibre in selected fruits and vegetables by Prosky method
(Penentuan serabut diet dalam buah-buahan dan sayur-sayuran terpilih dengan kaedah Prosky)

B. T. Lim* and I. Khatijah*

Key words: dietary fibre, Prosky enzymatic-gravimetric method, fruits, vegetables

Abstract
The total dietary fibre (TDF), soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) of selected fruits and vegetables were determined. The Prosky enzymatic-gravimetric methods were used for the determination of dietary fibres. Guava had the highest TDF content among the fruit samples while petai had the highest TDF content among the vegetable samples. Fractionation of TDF into SDF and IDF showed that in the majority of the samples, IDF values were higher than those of SDF. Crude fibre content of the samples were found to be 2.23–6.25 times lower than their corresponding TDF values.

Introduction
In recent years, there has been much interest in the total dietary fibre (TDF) content of foods and the physiological effect of its intake on man. Some diseases and disorders frequently related to inadequate intake of TDF are constipation, colon cancer, atherosclerosis, hypercholesterolemia, diabetes, diverticulosis, hypertension, obesity and gallstones (Cummings et al. 1976; Van Itallie 1978; Anderson and Ward 1979; Heaton 1981; Gordon 1989).

Food composition tables generally give the crude fibre (CF) content of food which actually differs from its dietary fibre content. CF is defined analytically as the residue remaining after solvent extraction, digestion with acid and alkali, and the subtraction of mineral ash. According to Cashel and Lewis (1990), CF values are more appropriate to animal feeds and are not useful for human nutrition. The conditions of analysis for CF generally result in the destruction of the soluble fibre fraction as well as a variable amount of the insoluble fibre fraction.

There is no universally accepted definition of dietary fibre. Analytically, TDF is defined by the method used. TDF was defined by Jenkins et al. (1985) as ‘the..."
endogenous components of plant material in the diet which are resistant to digestion by enzymes produced by man. These are predominantly non-starch polysaccharides and lignin, and may include, in addition, associated substances. This definition was a modification of that suggested by Trowell et al. (1976). Further, TDF can be subdivided into water-insoluble dietary fibre (IDF) and water-soluble dietary fibre (SDF). SDF comprises mainly pectin, gums and some hemicelluloses while IDF comprises mainly lignin, cellulose and some hemicelluloses. However, there were also arguments as to whether indigestible polymers such as the Maillard reaction products and resistant starch should be considered as part of TDF (Hall 1989).

Methods for dietary fibre determination have been rapidly evolving since 1976. Among the more established methods are the Prosky enzymatic-gravimetric method (Prosky et al. 1985; Prosky et al. 1988), neutral detergent method (Schaller 1977, 1981), Southgate fractionation method (Southgate 1969), Englyst method (Englyst and Cummings 1984, 1988), Mongeau and Brassard method (Mongeau and Brassard 1986), and NIR (near infra-red reflectance) spectroscopy method (Baker 1983). A review of TDF method has been written by Hall (1989). The Prosky enzymatic-gravimetric method has been accepted by both the Association of Official Analytical Chemists (Prosky et al. 1988) and the American Association of Cereal Chemists (AACC 1988) as their official methods. This method is also used by the commercially available Fibertec system E developed by Tecator (Halvarson and Alstin 1984). The Prosky method is used in this study for the determination of TDF, IDF and SDF as it is now the most acceptable and widely used method.

Good sources of dietary fibre include legumes, tubers, vegetables, whole grain cereals and fruits. New high dietary fibre food ingredients and products are also available commercially. In this study, TDF and its related fractions were determined for some selected locally available fruits and vegetables. This information is useful to individuals and nutritionists interested in the dietary fibre contents of fruits and vegetables. A comparison was also made between the TDF values and the CF values of these samples.

**Materials and methods**

**Samples and reagents**

Fresh samples of the fruits and vegetables were purchased randomly from markets in Kuala Lumpur. The samples were first cleaned and the edible portion taken. Three replicates (from different sources) for each sample were analysed. The water content of the samples were determined by drying at 105 °C overnight in an air oven with ventilation. The dried samples were then ground into powder by using an IKA micromill and stored in air-tight glass containers until analysed.

All chemicals used were of analytical grade. The enzymes used were protease from *Bacillus licheniformis* (Sigma P5380), amylloglucosidase from *Aspergillus niger* (Sigma A3042) and termamyl (type 120L, heat-stable α-amylase) from *B. licheniformis* (Novo Laboratories Inc.).

**Determination of dietary fibre**

The enzymatic-gravimetric methods for the determination of TDF, SDF and IDF as described by Prosky et al. (1985) and Prosky et al. (1988) with a dietary fibre total assay control kit were followed (*Figure 1*). Since protease sticks to spatula, it is desirable to prepare the enzyme solution just before use. Thus, instead of adding protease directly, it was first dissolved in phosphate buffer (pH 6.0) at a concentration of 0.05 g/mL, and 100 µL was then added to the enzyme extract.

**Determination of TDF**

Ground sample (1 g) was suspended in 50 mL of phosphate buffer (0.08 M, pH 6.0) with 0.1 mL termamyl and left for 30 min at
100 °C in a shaking water bath. The extract was allowed to cool at room temperature followed by adjustment to pH 7.5 with 0.275 M sodium hydroxide (10 mL). Protease (100 µL, equivalent to 5 mg) was added and the extract left in the shaking water bath for 30 min at 60 °C. It was adjusted to pH 4.5 with 0.325 M hydrochloric acid (10 mL) and 0.3 mL amyloglucosidase added. The extract was left at 60 °C in the shaking water bath for another 30 min. SDF was then precipitated by adding 280 mL of 95% ethanol (preheated to 60 °C) and left at room temperature for 60 min. The insoluble material was recovered by filtration on a pre-weighed sintered glass crucible (porosity 2) containing a layer (about 0.5 g) of celite 545. The residue was washed successively with three 20-mL portions of 78% ethanol, two 10-mL portions of 95% ethanol, and two 10-mL portions of acetone. The crucible was dried overnight at 105 °C in an air oven. It was then cooled in a desiccator and weighed to obtain the residue weight. The residue from one of the duplicate determinations was analysed for ash, and the other for protein. Ash was determined by incineration at 525 °C in a muffle furnace for 5 h. Protein (N x 6.25) was determined by the Kjeldahl method. Blank determinations were also carried out. TDF was determined as the weight of residue less the weight of residual protein and ash. All determinations were done in duplicate.
Determination of dietary fibre by Prosky method

**Determination of SDF and IDF**
After similar enzymatic treatment as that for the determination of TDF, the enzyme digest was filtered through a pre-weighed sintered glass crucible (porosity 2) containing a layer (about 0.5 g) of celite 545. The residue was washed with two 10-mL portions of water. The combined filtrate and water washings were kept for the determination of SDF. The residue was further washed with two 10-mL portions of 95% ethanol and two 10-mL portions of acetone. The crucible containing the residue was dried overnight at 105 °C in an air oven and the weight of residue determined. The residue on the crucible from one of the duplicate determinations was determined for protein, and the other one for ash content. The IDF is the weight of the residue less the weight of residual protein and ash.

To determine the SDF, four 100-mL portions of 95% ethanol (preheated to 60 °C) were added to the combined filtrate and water washings (adjusted to 100 mL) from the IDF determination. The precipitate was allowed to form at room temperature for 60 min. The suspension was then filtered through a pre-weighed sintered glass crucible (porosity 2) containing a layer (about 0.5 g) of celite 545. The residue was washed successively with three 20-mL portions of 78% ethanol, two 10-mL portions of 95% ethanol, and two 10-mL portions of acetone. The crucible containing the residue was dried overnight at 105 °C in an air oven and the weight of residue determined. Similarly, the residue from one determination of the set of duplicates was determined for protein, and the other one for ash content. The SDF is taken as the weight of residue less the weight of residual protein and ash.

**Determination of crude fibre**
CF is the insoluble residue which remains after the carbohydrate, fat and protein have been removed. The fat is removed by ether, followed by successive treatments with acid and alkali to remove the carbohydrate and proteins. The method used is described by Tee et al. (1986).

**Discussion**

**Dietary fibre content**
Even though the Prosky method is tedious, and the corrections for residual protein and ash contents require extra analysis, the analysis time can be reduced by using the semi-automated Fibertec system E from Tecator. This method of analysis depends upon an efficient removal of starch, as any starch residue will add to the dietary fibre content and cannot be differentiated from other dietary fibre components. Therefore, foods have to be heated to swell and disintegrate the starch granules (gelatinization) to make starch completely available for degradation by amylases. Analysts have to ensure efficient starch removal, especially if foods containing large amounts of starch are to be analysed.

The TDF and CF values of six fruits and six vegetables were determined (Table 1). Among the fruit samples, guava had the highest TDF content while rambutan the lowest. The high dietary fibre content of guava has also been reported by Anuradha and Prakash (1989) to be 5.16% as neutral detergent fibre on a fresh weight basis. The TDF values of the vegetable samples ranged from 1.43% to 7.94%, of which petai had the highest and watercress the lowest. Petai, a tree native to Malaysia, produces edible seed pods which are very popular among many Malaysians.

The CF contents of fruit and vegetable samples are lower than their TDF contents. Among the samples analysed, the TDF contents were higher than the CF values by as much as 2.23–6.25 times. Since CF measures primarily cellulose and not other components, it cannot be used to estimate the dietary fibre contents of foods.

The IDF and SDF contents of six fruits and four vegetables were also determined (Table 2). Samples with high IDF contents were guava, chilli and oyster mushroom. With the exception of rambutan, the SDF
Table 1. Total dietary fibre (TDF) and crude fibre (CF) contents of selected fruits and vegetables

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water content (%)</th>
<th>TDF (%)</th>
<th>CF (%)</th>
<th>TDF/CF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava (<em>Psidium guajava</em>)</td>
<td>86.07</td>
<td>4.63 ± 0.03</td>
<td>1.72 ± 0.01</td>
<td>2.69</td>
</tr>
<tr>
<td>Rambutan (<em>Nephelium lappaceum</em>)</td>
<td>80.93</td>
<td>2.20 ± 0.12</td>
<td>0.67 ± 0.02</td>
<td>3.28</td>
</tr>
<tr>
<td>Jackfruit (<em>Artocarpus heterophylla</em>)</td>
<td>75.82</td>
<td>2.66 ± 0.22</td>
<td>1.11 ± 0.02</td>
<td>2.39</td>
</tr>
<tr>
<td>Banana (<em>Musa acuminata</em>)</td>
<td>70.10</td>
<td>2.74 ± 0.04</td>
<td>0.66 ± 0.06</td>
<td>4.15</td>
</tr>
<tr>
<td>Starfruit (<em>Averrhoa carambola</em>)</td>
<td>87.57</td>
<td>2.52 ± 0.13</td>
<td>0.71 ± 0.01</td>
<td>3.07</td>
</tr>
<tr>
<td>Papaya (<em>Carica papaya</em>)</td>
<td>90.74</td>
<td>2.79 ± 0.16</td>
<td>0.87 ± 0.07</td>
<td>3.21</td>
</tr>
<tr>
<td>Watercress (<em>Nasturtium officinale</em>)</td>
<td>94.83</td>
<td>1.43 ± 0.03</td>
<td>0.64 ± 0.01</td>
<td>2.23</td>
</tr>
<tr>
<td>Lotus root (<em>Nelumbo nucifera</em>)</td>
<td>84.57</td>
<td>2.21 ± 0.28</td>
<td>0.84 ± 0.03</td>
<td>2.63</td>
</tr>
<tr>
<td>Chilli (<em>Capsicum annuum</em>)</td>
<td>84.65</td>
<td>4.78 ± 0.15</td>
<td>1.79 ± 0.05</td>
<td>2.67</td>
</tr>
<tr>
<td>Fresh oyster mushroom</td>
<td>91.54</td>
<td>3.50 ± 0.38</td>
<td>0.56 ± 0.01</td>
<td>6.25</td>
</tr>
<tr>
<td>Mungbean sprouts (<em>Phaseolus aureus</em>)</td>
<td>94.61</td>
<td>1.57 ± 0.08</td>
<td>0.48 ± 0.02</td>
<td>3.27</td>
</tr>
<tr>
<td>Petai (<em>Parkia speciosa</em>)</td>
<td>74.88</td>
<td>7.94 ± 0.18</td>
<td>1.43 ± 0.08</td>
<td>5.55</td>
</tr>
</tbody>
</table>

Values of TDF and CF reported are mean ± SD and are on fresh weight basis.

Table 2. Insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents of selected fruits and vegetables

<table>
<thead>
<tr>
<th>Sample</th>
<th>IDF (%)</th>
<th>SDF (%)</th>
<th>IDF/TDF</th>
<th>SDF/TDF</th>
<th>(IDF + SDF)/TDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava (<em>Psidium guajava</em>)</td>
<td>3.28 ± 0.05</td>
<td>1.13 ± 0.02</td>
<td>0.71</td>
<td>0.24</td>
<td>0.95</td>
</tr>
<tr>
<td>Rambutan (<em>Nephelium lappaceum</em>)</td>
<td>1.00 ± 0.08</td>
<td>1.25 ± 0.13</td>
<td>0.45</td>
<td>0.57</td>
<td>1.02</td>
</tr>
<tr>
<td>Jackfruit (<em>Artocarpus heterophylla</em>)</td>
<td>1.55 ± 0.15</td>
<td>1.24 ± 0.20</td>
<td>0.58</td>
<td>0.47</td>
<td>1.04</td>
</tr>
<tr>
<td>Banana (<em>Musa acuminata</em>)</td>
<td>1.50 ± 0.05</td>
<td>1.04 ± 0.02</td>
<td>0.55</td>
<td>0.38</td>
<td>0.92</td>
</tr>
<tr>
<td>Starfruit (<em>Averrhoa carambola</em>)</td>
<td>1.61 ± 0.02</td>
<td>0.72 ± 0.04</td>
<td>0.64</td>
<td>0.28</td>
<td>0.92</td>
</tr>
<tr>
<td>Papaya (<em>Carica papaya</em>)</td>
<td>1.72 ± 0.06</td>
<td>1.13 ± 0.02</td>
<td>0.62</td>
<td>0.40</td>
<td>1.02</td>
</tr>
<tr>
<td>Lotus root (<em>Nelumbo nucifera</em>)</td>
<td>1.58 ± 0.07</td>
<td>0.33 ± 0.02</td>
<td>0.71</td>
<td>0.15</td>
<td>0.86</td>
</tr>
<tr>
<td>Chilli (<em>Capsicum annuum</em>)</td>
<td>3.30 ± 0.22</td>
<td>1.70 ± 0.09</td>
<td>0.69</td>
<td>0.35</td>
<td>1.04</td>
</tr>
<tr>
<td>Fresh oyster mushroom</td>
<td>3.11 ± 0.09</td>
<td>0.26 ± 0.01</td>
<td>0.89</td>
<td>0.07</td>
<td>0.96</td>
</tr>
<tr>
<td>Mungbean sprouts (<em>Phaseolus aureus</em>)</td>
<td>1.35 ± 0.04</td>
<td>0.26 ± 0.02</td>
<td>0.86</td>
<td>0.16</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Values of IDF and SDF reported are mean ± SD and are on fresh weight basis.

The analyses of SDF, IDF and TDF. Within acceptable limits of laboratory errors, the values of IDF and SDF can be summed up to give an estimate of the TDF contents of the samples.

Many food labels still include crude fibre values as required by out-of-date food laws and regulations. There are also food...
labels including dietary fibre values but without naming the analytical method used. It is important that the analytical method used for determining dietary fibre be included in the labelling as different analytical methods give different dietary fibre values. In the U.S.A., nutritional labelling that includes dietary fibre values, needs to use the AOAC method (Prosky method) for the analysis. For the benefit of the consumers, fibre in foods should be reported as TDF, divided into SDF and IDF fractions.

As TDF values can be used as an indicator for the amount of physiologically unavailable non-starch polysaccharides and lignin in the diet, the values reported in this study can be used by nutritionists for planning of high or low fibre diets by incorporating fruits and vegetables.

Conclusion
The Prosky enzymatic-gravimetric method used in this study is suitable for routine analysis of dietary fibre where the details of its composition are not required. It closely resembles the action of the upper digestive tract and yields results that are in line with the physiological definition of TDF. It is robust and easy to handle, and does not require sophisticated equipment.

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References


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