Trypsin Inhibitor Content in Some Varieties of Soya Bean
(*Glycine max* L.) and Sunflower Seeds (*Helianthus annuus* L.)

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Some varieties of soya beans and sunflower seeds were analysed for protein, fat, moisture and trypsin inhibitory activities. The soya beans were high in protein content and all of them were equally high in trypsin inhibitory activity. Some varieties of sunflower seeds were adequate in fat and proteins, some were very low and others were reasonably free from trypsin inhibitors. Extensive cultivation of the sunflower seeds having adequate amount of fat and protein, but free from trypsin inhibitor should be encouraged.

1. Introduction

The presence of trypsin inhibitors in soya beans and navy beans has been reported. The existence of trypsin inhibitor in Indian pulses and vegetables in sweet potato and in field beans has also been reported by different workers. The presence and biological activity of trypsin inhibitors in *Lathyrus sativus* has also been described.

Kakade et al. have reported that there is a wide variation of trypsin inhibitor content in different varieties of soya beans. There are also controversial reports about the existence of trypsin inhibitors in sunflower seeds. Sunflower appears to be one of the few species whose seeds are comparatively free from anti-tryptic factors. However, Agren and Lieden and Agren and Eklund reported that sunflower seeds contain a weak type of trypsin inhibitor. Biological tests on rats with sunflower seed meal or aqueous extracts of sunflower seed meal have indicated the presence of some trypsin inhibitor(s) in the seeds, but the biological effects of the inhibitor seem to be comparatively weak.

Soya beans and sunflower seeds can play an important role in human nutrition. Attempts have been made to evolve new varieties of soya beans and sunflower seeds with high protein content. Increasing attention has been given in recent years for large-scale cultivation of soya beans in North India and sunflower in South India.

In this connection, attempts have been made to estimate some major nutrients and trypsin-inhibitory activity in some newly introduced varieties of soya beans and sunflower seeds.
2. Experimental

2.1. Materials

2.1.1. Soya bean seeds

Five varieties of soya beans such as Lee, Bragg, Seemes, Punjab-1 and JS-2 were obtained from the Production Units of the Agricultural Universities at Pantnagar (Uttar Pradesh) and Jabalpur (Madhya Pradesh).

2.1.2. Sunflower seeds

Five varieties of sunflower seeds such as E.C. 68413, E.C. 68414, E.C. 68415, E.C. 69874 and Sunrise Selection were obtained from an oil seed specialist, Andhra Pradesh Agricultural University.

2.1.3. Extraction of the materials

Four g of the powdered defatted soya bean samples were treated with 40 ml of 0.05 M-sodium phosphate buffer, pH 7.0, and 40 ml of distilled water while 2 g of powdered, deoiled and defatted sunflower seeds were extracted with 20 ml of 0.05 M-sodium phosphate buffer, pH 7.0, and 20 ml of distilled water. All the above samples were shaken for 3 h and then centrifuged at 700 g for 30 min at 15 °C. The supernatants were filtered to get clear solutions. All the extracts were diluted 10 times (i.e. 0.5 ml to 5 ml) with the appropriate fluids and thus made ready for the analysis of protein and trypsin inhibitory activity (T.I.A.).

2.2. Analytical methods

2.2.1. Trypsin inhibitory activity assay

The method used earlier by Roy and Rao\(^7\) was employed here for determining the trypsin inhibitory activity in the crude preparations of both the phosphate buffer and water extracts. A 2% casein solution in phosphate buffer (0.1 M, pH 7.6) was used as substrate, while the enzyme used was trypsin (E. Merck) (5 mg/ml). The incubation mixture consisted of 0.5 ml of trypsin solution, 2 ml of 2% casein, 1.0 ml of phosphate buffer (pH 7.6, 0.1 M), 0.4 ml of HCl solution (0.001 M) and extracts, 0.1 ml. In all cases the total volume of incubation mixture was kept at 4 ml. Incubations were carried out at 37 °C for 20 min after which 6.0 ml of 5% TCA solution was added to stop the reaction and corresponding blanks were run concurrently. In this method, one trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance unit at 280 nm in 20 min for 10 ml of reaction mixture under the conditions described, and the trypsin inhibitory activity as the number of trypsin units inhibited (TUI).

2.2.2. Total proteins

Total proteins were given by the values calculated from the total nitrogen estimated by the microKjeldahl method.\(^16\)

2.2.3. Protein analysis of the extracts

Protein analysis for the extracted samples was carried out by the method of Lowry et al.\(^17\) where bovine serum albumin (Sigma, U.S.A.) was used as standard.
2.2.4. **Total fat analysis**
Fat analyses were carried out by the method described.\(^1\)

2.2.5. **Moisture content**
Moisture was determined by heating the samples at 100 °C until the successive weights were constant and calculated by difference.

3. Results and discussion
Table 1 shows the variation of trypsin inhibitory activities, fat, protein and moisture content in soya bean varieties. The protein and fat percentages are uniform. It also shows that the trypsin inhibitory activities in both water and buffer extracts were quite high and maintained a mean value of 37.7 and 38.8, respectively. Some variation in values could also be observed between the two extracts of the same sample. This may indicate the variation in the extractability of the proteins having trypsin inhibitory activity. This was also observed while working with *L. sativus*.\(^7\)

<table>
<thead>
<tr>
<th>Soya bean varieties</th>
<th>Total moisture content (%)</th>
<th>Total fat content (%)</th>
<th>Total protein content (N x 6.25) (%)</th>
<th>Water extract (0.1 ml)</th>
<th>Buffer extract (0.1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Punjab-1</td>
<td>6.95</td>
<td>19.42</td>
<td>42.70</td>
<td>0.230</td>
<td>0.239</td>
</tr>
<tr>
<td>Bragg</td>
<td>7.13</td>
<td>17.64</td>
<td>37.52</td>
<td>0.225</td>
<td>0.267</td>
</tr>
<tr>
<td>Seemes</td>
<td>6.66</td>
<td>20.94</td>
<td>36.57</td>
<td>0.267</td>
<td>0.317</td>
</tr>
<tr>
<td>Lee</td>
<td>7.11</td>
<td>19.77</td>
<td>37.70</td>
<td>0.276</td>
<td>0.239</td>
</tr>
<tr>
<td>JS-2</td>
<td>7.45</td>
<td>17.29</td>
<td>39.37</td>
<td>0.216</td>
<td>0.248</td>
</tr>
<tr>
<td>Mean values</td>
<td>7.06</td>
<td>19.01</td>
<td>38.77</td>
<td>0.243</td>
<td>0.262</td>
</tr>
<tr>
<td>Control value (TU)</td>
<td></td>
<td></td>
<td></td>
<td>44.3</td>
<td>44.3</td>
</tr>
</tbody>
</table>

T.I.A., trypsin inhibitory activity; TUI, trypsin units inhibited.
Protein in the extracts analysed by the method of Lowry *et al.* (1951).
For T.I.A. assay: incubation mixture consists of 0.5 ml trypsin solution, 1.0 ml phosphate buffer, 0.4 ml HCl solution (0.001 M), 2 ml 2% casein solution, 0.1 ml of the extract, to make the total volume of 4.0 ml.

Table 2 shows the protein, fat, moisture content and trypsin inhibitory activities in the sunflower seed samples. The variation of protein was from 15.75 to 19.42% with a mean of 18.07. On a dry weight basis the mean value can be raised to 18.9%. Similarly, the fat content varied from 37.65 to 46.59 with a mean of 43.23%, which can again be raised to 46.45% on a dry weight basis. In the case of sunflower seeds, there are variations in the trypsin inhibitory activity and between the extracts of buffer and water in the same samples. The samples such as Sunrise Selection, E.C. 68413 and E.C. 69874 showed very low trypsin inhibitory activity, while the samples E.C. 68414 and E.C.
TABLE 2. Variation in fat and protein content and trypsin inhibitory activity in different Indian varieties of Sunflower seeds (Helianthus annuus)

<table>
<thead>
<tr>
<th>Sunflower varieties</th>
<th>Total moisture content (%)</th>
<th>Total fat content (%)</th>
<th>Total protein content (N × 6.25 %)</th>
<th>Water extract (0.1 ml) Protein (mg)</th>
<th>T.I.A. (TU)</th>
<th>Buffer extract (0.1 ml) Protein (mg)</th>
<th>T.I.A. (TU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunrise selection</td>
<td>4.4</td>
<td>37.65</td>
<td>15.75</td>
<td>0.138</td>
<td>0.8</td>
<td>0.152</td>
<td>0.0</td>
</tr>
<tr>
<td>E.C. 68413</td>
<td>5.1</td>
<td>45.71</td>
<td>19.33</td>
<td>0.133</td>
<td>0.8</td>
<td>0.216</td>
<td>2.3</td>
</tr>
<tr>
<td>E.C. 68414</td>
<td>5.2</td>
<td>44.84</td>
<td>19.42</td>
<td>0.115</td>
<td>0.0</td>
<td>0.202</td>
<td>0.0</td>
</tr>
<tr>
<td>E.C. 68415</td>
<td>4.5</td>
<td>46.59</td>
<td>19.42</td>
<td>0.150</td>
<td>0.0</td>
<td>0.235</td>
<td>0.0</td>
</tr>
<tr>
<td>E.C. 69874</td>
<td>4.6</td>
<td>41.37</td>
<td>16.45</td>
<td>0.110</td>
<td>0.3</td>
<td>0.175</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean values</td>
<td>4.76</td>
<td>43.23</td>
<td>18.07</td>
<td>0.129</td>
<td>0.38</td>
<td>0.196</td>
<td>0.52</td>
</tr>
<tr>
<td>Control value (TU)</td>
<td></td>
<td></td>
<td>44.3</td>
<td></td>
<td></td>
<td>44.3</td>
<td></td>
</tr>
</tbody>
</table>

T.I.A., trypsin inhibitory activity; TUI, trypsin units inhibited.
Protein in the extracts analysed by the method of Lowry et al. (1951).
For T.I.A. assay: incubation mixture consists of 0.5 ml trypsin solution, 1.0 ml of phosphate buffer, 0.4 ml HCl solution (0.001 M), 2 ml 2% casein solution, 0.1 ml of the extract, to make the total volume 4.0 ml.

68415 did not show any trypsin inhibitory activity under the conditions described in either of the two extracts used.

From the observations shown in the Tables, the following conclusions may be drawn.

The total protein content in both soya bean and sunflower seeds analysed do not correlate with the magnitude of the trypsin inhibitory activity. The trypsin inhibitory activities in all the soya bean samples were uniform and generally on the higher side of the scale.

Two varieties of sunflower seeds such as E.C. 68414 and E.C. 68415 did not show any trypsin inhibitory activity, but the samples did show fairly good amount of protein and fat.

The low trypsin inhibitory activities in some of the varieties of sunflower seeds and the absence of it in some varieties may possibly explain why the two earlier groups of workers got contradictory results. As the content of trypsin inhibitors in some varieties of sunflower seeds examined was nil, and in others was negligibly low, it was thought that animal experiments for growth performance was not necessary.

It is well known that the majority of the trypsin inhibitors in soya beans are heat labile. According to Agren and Lieden heating improves the quality of protein in sunflower seeds, thus indicating indirectly that the trypsin inhibitor is heat labile.

The sunflower seed varieties E.C. 68414 and E.C. 68415 are high yielding when compared to the other three varieties. Variety E.C. 68415 records highest oil content among the varieties studied. All of the five varieties proved to be equally susceptible to various diseases such as sunflower rust. In this context, it may be suggested that introduction of the two varieties of sunflower seeds like E.C. 68414 and E.C. 68415 may be profitable as these are reasonably free from trypsin inhibitors, at the same time...
they may be considered to be a fairly good source of protein and fat, and they are also high yielding varieties.

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References