Fatty acid composition of grass pea (Lathyrus sativus L.) seeds

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Introduction
Grass pea or chickling vetch (Lathyrus sativus L.) is a well-established, commercially available, tropical semi-arid crop. Pods of this crop are flat, dorsally broad with two ridges, short and 3-5 cm in length. Each pod contains 2-7 seeds. Mature seeds are rhomboid or triangular in shape, dull whitish grey brown and variously mottled (4). Grass pea is a relatively productive crop compared to other pulses in regions characterized by poor soil (7). It is very well adapted to adverse climatic conditions and requires very little management for crop production. Moreover, its deep taproot and nitrogen-fixing ability make this crop an ideal choice for sustainable agriculture. Grass pea seeds are a major source of protein for large sections of the population in Bangladesh, China, Ethiopia and India (13, 15). It is also grown to a lesser extent in the Middle East, southern Europe and some parts of South America. In India grass pea is considered one of the most economical pulses for fodder and green manure in rice fields during the cool winter period (1).

Lathyrus species contain very high protein, but a neurotoxin, 3-(N-oxalyl)-L-2,3-diamino propionic acid (ODAP), is present in wild and most cultivated forms that if consumed in sufficient amounts can cause the irreversible crippling disease known as lathyrism (10, 17). This toxin to a considerable extent has been bred out of some cultivars although lathyrism in Asia from consuming grass pea is common. Because of its drought tolerance, grass pea has been judged to have good potential as a future new pulse crop for low rainfall areas of the Canadian prairies (12). It acts as a ground cover alternative to summer fallow, helping to prevent wind and water erosion, as well as adding nitrogen to the soil (11).

The nutritional health and well being of humans are entirely dependent on plant foods. Plants are critical components of the dietary food chain in that they provide almost all essential minerals and organic nutrients to humans either directly, or indirectly when plants are consumed by animals, which are then consumed by humans (9). Grass pea seeds may represent a potential source of several important nutrients for human and animal nutrition. Therefore, it is necessary to analyze grass pea seeds for their nutrient composition. The present investigation proposes to determine the fatty acid composition of different lipid classes in mature seeds of Indian grass pea.

Material and Methods
Seed materials
Mature seeds of grass pea were procured from a local market in Kolkatta (Calcutta), West Bengal, India.

Extraction and estimation of total lipids
Total lipids were determined by the gravimetric method (2). One gram of dried grass pea seeds in each of three replicates was powdered using a 700S Waring blender (Waring Products Co., USA) and homogenized in 10 ml of 50 mM Tris-HCl buffer containing 0.5 M NaCl at pH 7.2. The homogenate was combined with a mixture of chloroform and methanol in a ratio of 1.25:2.25 (v/v) to extract lipids. The chloroform layer (supernatant) was separated by centrifugation at 5000 g for 20 min and allowed to stand overnight after combining with a mixture of chloroform and distilled water in a ratio of 1:1 (v/v). The chloroform layer was collected in a pre-weighed vial for evaporation under nitrogen gas. The vial with the lipid residue was weighed again to estimate the amount of total lipids.

Separation of lipids and analysis of fatty acid composition
Total lipids were fractionated into 5 lipid classes [phospholipids (PL), monoglycerides (MG), diglycerides (DG), free fatty acids (FFA) and triglycerides (TG)] by thin layer chromatography and the constituent fatty acids in each lipid class were separated and estimated using gas chromatography as described in Chinnasamy et al. (6). The measurement of each fatty acid was calculated as a relative weight percentage to 10 selected fatty acids [C14:0 (myristic acid), C14:1 (myristoleic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (linolenic acid)].
DBI = \Sigma (\% \text{ of fatty acid content} \times \text{no. of double bonds}) / 100

Among 10 major fatty acids, sum of all saturated fatty acids, unsaturated fatty acids with one double bond and unsaturated fatty acids with more than one double bond gave total saturated fatty acids (TSFA), total monounsaturated fatty acids (TMUFA) and total polyunsaturated fatty acids (TPUFA) respectively. Unsaturated to saturated ratio (USR) was calculated by dividing total unsaturated fatty acids by total saturated fatty acids.

**Statistical analysis**

For all sets of data, one way analysis of variance was performed using the SPSS computer package (16). Means were compared by Duncan’s multiple comparison test at P = 0.05. For the purpose of statistical analysis, data in percentage were transformed to arcsine values (10).

**Results and Discussion**

Total lipid content of mature grass pea seeds was 20.93 ± 0.27 mg/g dry weight. The nutritive value of seeds is determined by not only quantity but also by quality of lipids they contain. Thus, fatty acids present in lipids are playing important role in deciding shelf life, nutrition and flavor of food products (8). Chavan et al. (3) reported the presence of 20 fatty acids varying from C8 to C22 in total lipids of Canadian grass pea seeds. The relative weight percentages of 10 major fatty acids in 5 lipid classes isolated from mature seeds of Indian grass pea are summarized in Table 1.

C18:2, C18:1, C18:0 and C16:0 were the major fatty acids present in PL, MG, FFA and TG separated from grass pea seeds. DG contained higher quantities of C18:0, C16:0 and C18:2 compared to other fatty acids. PL and TG showed higher overall DBI than FFA, MG and DG. The content of TSFA was higher in DG than in other lipid classes. High amounts of TMUFA were observed in FFA and TG. PL registered high quantity of TPUFA. MG, PL and FFA showed higher USR compared to MG and DG. Although lipids constitute a minor portion of many leguminous seeds, their profiles indicate the desirable nature of fatty acid constituents present (3). In the present study, grass pea seeds exhibited a high amount of total unsaturated fatty acids (56.37% – 59.98%) and a low amount of total saturated fatty acids (40.01 – 43.65%) in all lipid classes except MG and DG, which contained 31.49 – 47.29 % total unsaturated fatty acids and 52.72 – 68.53% total saturated fatty acids. Overall, in the present work, Indian grass pea seeds showed higher total unsaturated fatty acids than total saturated fatty acids that are in agreement with fatty acid composition of beach pea and Canadian grass pea seeds (3,5). Therefore, grass pea seeds may be important for nutritional health and may serve as a valuable nutritional source. Further in depth study is necessary to elucidate the nutritional quality and importance of grass pea.

### Table 1. Composition of major fatty acids (relative weight percentage) in phospholipids (PL), monoglycerides (MG), diglycerides (DG), free fatty acids (FFA) and triglycerides (TG) isolated from total lipids of mature seeds of Indian grass pea. Values are means (± SE) of three replications.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>PL</th>
<th>MG</th>
<th>DG</th>
<th>FFA</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.65 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.51 ± 2.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.09 ± 1.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.06 ± 0.85&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.66 ± 0.53&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.77 ± 1.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ± 1.63&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.49 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.82 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>24.88 ± 1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.62 ± 2.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.21 ± 0.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.07 ± 3.60&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.57 ± 5.28&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.57 ± 0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.77 ± 2.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 ± 0.48&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2.66 ± 2.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.26 ± 1.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:0</td>
<td>16.24 ± 4.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.59 ± 1.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.31 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.49 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.38 ± 1.96&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1</td>
<td>26.20 ± 6.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.44 ± 2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.30 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.99 ± 8.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.91 ± 6.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:2</td>
<td>28.65 ± 11.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.84 ± 8.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.55 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.31 ± 2.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.17 ± 3.73&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>C18:3</td>
<td>1.16 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.57 ± 2.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75 ± 0.69&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.81 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.55 ± 1.90&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:4</td>
<td>0.64 ± 0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.93 ± 1.61&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75 ± 1.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.36 ± 1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.89&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.18 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.52 ± 1.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DBI&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.92 ± 0.18</td>
<td>0.71 ± 0.20</td>
<td>0.56 ± 0.08</td>
<td>0.79 ± 0.02</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>TSFA&lt;sup&gt;j&lt;/sup&gt;</td>
<td>41.77</td>
<td>52.72</td>
<td>68.53</td>
<td>43.65</td>
<td>40.01</td>
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<tr>
<td>TMUFA&lt;sup&gt;j&lt;/sup&gt;</td>
<td>27.43</td>
<td>26.98</td>
<td>11.31</td>
<td>39.14</td>
<td>36.99</td>
</tr>
<tr>
<td>TPUFA&lt;sup&gt;j&lt;/sup&gt;</td>
<td>30.81</td>
<td>20.31</td>
<td>20.18</td>
<td>17.23</td>
<td>22.99</td>
</tr>
<tr>
<td>USR&lt;sup&gt;j&lt;/sup&gt;</td>
<td>1.39</td>
<td>0.90</td>
<td>0.46</td>
<td>1.29</td>
<td>1.50</td>
</tr>
</tbody>
</table>

<sup>a-e</sup> Means in the same column followed by different letters are significantly different using Duncan’s multiple comparison test at P = 0.05.

<sup>f</sup> DBI, or double bond index, is calculated as the sum of all fatty acids in a sample, multiplied by the number of double bonds in each fatty acid.

<sup>j</sup> TSFA, TMUFA and TPUFA are calculated as the sum of all fatty acids in each lipid class, multiplied by the number of fatty acids in each class.
Acknowledgements
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16. SPSS Inc. 1990. SPSS/PC + Statistics TM 4.0 for the IBM PC/XT/AT and PS/2. SPSS Inc., Chicago, IL.