**In vitro** binding of bile acid by apple pomace fibre and isabgol (*Plantago ovata*) fibre

P C Sharma*, K Issar and A Gupta
Department of Food Science and Technology
Dr Y S Parmar, University of Horticulture and Forestry, Nauni Solan HP – 173230

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In vitro binding studies of apple pomace fibre and isabgol (*Plantago ovata*) fibre were studied. Apple fibre exhibited higher (19.63 to 30.24%) **in vitro** binding properties to cholic and deoxycholic acid than that of isabgol fibre (17.20 to 22.20%) thereby indicating the superiority of apple fibre for its use in the development of hypocholesterolemic diets. The rate of adsorption increased with the increase in fibre contents, thus a linear relationship was observed for rate of adsorption. In vitro adsorption of cholic and deoxycholic acid to apple fibre found ranging between 10.86 to 22.19% as compared to 5.91 to 13.14% in isabgol fibre. The adsorption of bile acids to apple fibre was recorded to be the highest after 48 hrs of incubation. Besides these two bile acids, the adsorption of fibre to phosphate buffer was recorded to be the highest in isabgol fibre (9.83%) as compared to apple pomace fibre (8.39%). Thus, apple pomace fibre has better binding properties with deoxycholic acid against cholic acid than that of isabgol fibre and can be used in the formulation of diet with cholesterol lowering properties.

**Keywords:** In vitro, binding, apple fibre, isabgol fibre, hypocholesterolemic, cholesterol.

**Introduction**

Apple pomace is a heterogeneous mixture consisting of peel, core, seed, calyx, stem and soft tissue. It has 2.2-3.3% seed, 0.4-0.9% stem, 70.0-75.7% apple flesh. It contains 36.8% dietary fibre and 54.4% carbohydrates with an average pH of 4.3 and water holding capacity of 3.7 ml/g. Apple pomace accounts for about 25% of the original fruit mass having 85% moisture content (wet basis). It is highly biodegradable in nature because of high Biological Oxygen Demand (BOD) and abundant saccharide content. There are numerous ways of utilizing apple pomace and the utilization can be categorized either as a waste reduction strategy or obtaining a high value product or both. The value added products from pomace include enzymes, organic acids, protein-enriched feeds, aroma compounds and edible fibres. Though, the commercial production of apple fibre from the pomace after juice extraction has been practiced by certain companies in abroad, yet; the information on utilization of apple pomace for preparation of fibre enriched products is lacking. Further, the apple products have been known for their cholesterol lowering property. The dietary fibre content in apples is reported to lower down cholesterol with renal dysfunction and enhanced fecal steroid excretion. Dietary fibres bind and remove the extra bile acids from the system and thereby cause cholesterol to be converted into bile acids. The mechanism of cholesterol lowering action of dietary fibre is actually a result of binding of bile acids in the intestine. Apple fibre is among top five high fibre diets rich in cellulose, hemicellulose, lignin and pectin contents with hypocholesterolemic effect. The fibre content of apples is not particularly high (2-3%) and soluble fibres especially pectin represents less than 50% of the fibre in apples. The apple fibre is also reported to contain 31% cellulose, 15% lignin, 12% water-insoluble hemicellulose as well as 9% water-insoluble pectin. In addition, apple fibre also contains 18% water-soluble hemicellulose. Fibres are known to act as hypocholesterolemic agent. Binding prevents re-absorption of bile salts in the intestine, which in turn reduces cholesterol levels in the blood. Maximum binding occurred at alkaline pH and was reduced at acidic pH. Casterline and Yuoh Ku reported the *in-vitro* binding of zinc to apple fibre, wheat bran and other fibre components.

*Author for Correspondence
E-mail: pesharmasolan@gmail.com
In-vitro bile adsorption of deoxycholic acid was more than that of cholic acid\textsuperscript{19}. This could be due to the differences in the polarity of bile acids\textsuperscript{20}. Even Story and Kritschevsky\textsuperscript{21} reported the cholesterol lowering action of diet rich in fibres, yet very little information is available on the effect of fibres to act as hypocholesterolemic agent. Therefore, present study on in-vitro binding and adsorption of bile to apple pomace fibre and isabgol \textit{(Plantago ovata)} fibre was carried out in order to study the cholesterol lowering properties of apple pomace fibre.

**Experimental Section**

Apple fibre from pomace was obtained by washing, coring, chopping and separation of juice by pressing. Apple pomace (Fig. 1) was washed and blanched in hot water followed by drying either in polytunnel solar drier (45±8°C) or in mechanical drier (55±2°C) to a constant weight. Dietary fibre (Fig. 2) from dried apple pomace was extracted by acid-alkali digestion method\textsuperscript{22} with slight modification. Dried apple pomace (40 g) was taken in a digestion flask and boiling sulphuric acid (200 ml) was added to it. The digestion flask was immediately connected to the condenser and heated for an hour. It was washed with boiling water till the washings became free of acid. Then, boiling sodium hydroxide solution (200 ml) was added to the digestion flask and again connected to the condenser. It was boiled for an hour and later on kept for washing. After thorough washing of the residue, it was washed twice with 70% alcohol. Further, in vitro binding properties of apple dietary fibre were estimated by evaluating the extent of bile acid adsorption according to Eastwood\textsuperscript{23}. Solutions of cholic and deoxycholic acid (2.5mM concentration) were prepared in 35 ml of phosphate buffer at pH 8.0. Known quantity (1.0, 2.0 and 3.0 g) of apple and isabgol \textit{(Plantago ovata)} fibre was added to 35 ml of buffered bile acid solution containing cholic or deoxycholic acid. The conical flasks were shaken in an orbital shaker at 37°C for 16 hours at 400 rpm speed. At the end of 16 hours, the supernatant was removed by filtration through Whatman No. 2 filter paper and the volume of the filtrate was recorded. The fibre was returned to the flasks and was shaken for 16 hours at 37°C. Triplicate determinations were made for each attribute and the data was analyzed by using CRD design\textsuperscript{24}.

**Results and Discussion**

The evaluation of \textit{In vitro} binding properties of apple pomace fibre was studied and compared with the \textit{In vitro} binding properties of isabgol \textit{(Plantago ovata)} fibre (commercially used as a source of fibre). \textit{In vitro} adsorption of cholic acid and deoxycholic acid to apple fibre ranged between 19.63 to 30.24% as compared to 17.20 to 22.20% in isabgol fibre (Table 1). The percent adsorption of cholic and deoxycholic acid was recorded to be 10.86 to 22.19% for apple fibre and 5.91 to 13.14% for isabgol fibre respectively. This might be due to the differences in the polarity of bile acids\textsuperscript{20}. Further, Oakenfull and Fenwick\textsuperscript{25} reported that certain minor dietary components like polyphenols and saponins are known to be associated with these fibres, which may

![Fig 1—Apple pomace](image1)

![Fig 2—Apple pomace fibre](image2)
contribute to their bile acid adsorption and hypocholesterolemic properties. The adsorption of bile acids to apple pomace fibre and isabgol fibre has been observed to be higher in apple fibre as compared to isabgol fibre, thereby indicating better binding properties of apple fibre in comparison to isabgol fibre. This might be due to the ability of apple fibres to adsorb bile acids to lower blood cholesterol/lipid levels. Similar conclusion has been drawn by Park and Cho in cereals and pulses. In vitro adsorption of cholic and deoxycholic acid to apple fibre ranged between 19.63% to 30.24% as compared to 17.20% to 22.20% in isabgol fibre. Further, the adsorption of cholic and deoxycholic acid was found to be increased with the increase in the quantity of fibre. The adsorption of cholic and deoxycholic acid was recorded to be 16.95% and 27.90% respectively in 1 g of apple fibre and found to be increased in 3 g of apple fibre as 22.24% and 32.17% respectively. Similarly, the bile acid adsorption was observed to be 14.47% and 19.99% in cholic and deoxycholic acid respectively in 1 g of isabgol fibre. The adsorption further increased to 19.71% and 23.80% respectively in 3 g of isabgol fibre (Fig. 3). Similar findings have been reported by Vadhera who observed adsorption of cholic and deoxycholic acid in spinach and mustard fibre. Both apple and isabgol fibre recorded the least adsorption of phosphate buffer as compared to cholic and deoxycholic acid. However, it was observed that the adsorption to phosphate buffer by fibre was found to be the highest in isabgol fibre (9.83%) as compared to apple fibre (8.39%) (Table 1). The adsorption was recorded to be 8.56% in 1 g and 10.66% in 3 g of isabgol fibre whereas in case of apple fibre, the adsorption was recorded as 5.71% and 10.63% respectively. With the increase in quantity of fibre the rate of adsorption increased, thus, a linear relationship was observed for rate of adsorption. However, it was observed that the bile acids adsorption to fibre increased with the incubation time at 37°C. The adsorption of bile acids to fibre was recorded to be maximum after 48 hrs of incubation in apple fibre (32.17%) as against 16 hrs (27.90%) in our study. Similar to these results, Uberoi also observed increased adsorption of bile acid with increased incubation time. Thus, apple pomace fibre has better binding properties than that of isabgol fibre and can be used in formulation of diet for cholesterol lowering purposes. Further, the binding of bile acids to apple fibre was higher with deoxycholic acid as against cholic acid. Hence, deoxycholic acid has the highest bile acid binding capacity as compared to cholic acid, thereby resulting into lowering of high amount of cholesterol.

References
3 Boas SG & Esposito E, Bioconversão do bagaço de maça: enriquecimento nutricional utilizando fungos para

Table 1—In-vitro binding properties of cholic acid and deoxycholic acid (% adsorption) by apple pomace fibre and isabgol (Plantago ovata) fibre

<table>
<thead>
<tr>
<th>Fibre concentrations (g)</th>
<th>Phosphate buffer (B)</th>
<th>Cholic acid</th>
<th>Deoxycholic acid</th>
<th>Phosphate buffer (B)</th>
<th>Cholic acid</th>
<th>Deoxycholic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.71</td>
<td>16.95 *(11.24)</td>
<td>27.90 (22.19)</td>
<td>8.56</td>
<td>14.47 (5.91)</td>
<td>19.99 (11.43)</td>
</tr>
<tr>
<td>2</td>
<td>8.85</td>
<td>19.71 (10.86)</td>
<td>30.65 (21.80)</td>
<td>10.28</td>
<td>17.42 (7.14)</td>
<td>22.82 (12.54)</td>
</tr>
<tr>
<td>3</td>
<td>10.63</td>
<td>22.24 (11.61)</td>
<td>32.17 (21.54)</td>
<td>10.66</td>
<td>19.71 (9.05)</td>
<td>23.80 (13.14)</td>
</tr>
<tr>
<td>Mean</td>
<td>8.39</td>
<td>19.63</td>
<td>30.24</td>
<td>9.83</td>
<td>17.20</td>
<td>22.20</td>
</tr>
</tbody>
</table>
CD_{0.05} (F)             | 0.02                 | 0.01        | 0.01             | 0.02                 |            |                  |

*Figures in parenthesis indicate % adsorption of cholic acid and deoxycholic acid excluding relative adsorption by phosphate buffer.


