Note

Relationship between Molecular Weights of Pectin and Hypocholesterolemic Effects in Rats

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Hypocholesterolemic activities and other properties of three different molecular weight pectins were examined. The low-molecular-weight pectin (M_r ~ 66,000) obtained by decomposition of original pectin (M_r ~ 750,000) had the properties of low viscosity and high solubility, but it lost hypocholesterolemic activities in rats. On the other hand, the medium-molecular-weight pectin (M_r ~ 185,000) had characteristics of both low viscosity and hypocholesterolemic activities.

Pectin generally has the properties of low solubility, high viscosity, and strong gelatinization that are caused by its high molecular weight. For these reasons, it is difficult to add sufficient pectin to food and drink to produce the physiological effects of a dietary fiber. Mokady has reported that highly viscous pectins are very effective in repressing the level of the blood cholesterol content, but the close relationship between the molecular weight of pectin and the activity has not been examined. Previously we reported that molecular weight of citrus pectin was rapidly reduced at the beginning, and finally to about 66,000 by a Kluyveromyces fragilis (K. fragilis) crude enzyme solution, and the pectin (66,000), which had low viscosity and high solubility, did not have the effect of repressing the serum cholesterol level.

In this report, we obtained low-molecular-weight pectin (LP) and medium-molecular-weight pectin (MP) by changing the time of the enzyme reaction, and compared the hypocholesterolemic activities of LP, MP, and original pectin.

A commercial citrus pectin (Wako Pure Chemicals Co., Osaka, Japan) was used as the pectin source in this experiment. The K. fragilis JTF-1 culture fluid (0.17 unit/ml) was prepared as described previously. The other chemicals were reagent grade from Wako Pure Chemicals Co.

Citrus pectin (100 g) was suspended in 4 liters of a 0.025 M acetic acid buffer solution (pH 4.8), and 1 liter of the K. fragilis culture fluid was added to the solution. The solution was incubated at 40°C for 2 h and 24 h to obtain MP and LP, respectively. The enzyme reaction was stopped by putting it in boiling water for 10 min. The resultant reaction mixture was concentrated to about 500 ml by a rotary evaporator at 60°C and then was dialyzed against deionized water. The pectins were obtained by freeze-drying the dialyzed solution.

Molecular weight distribution, viscosity, and the ratio of galacturonate to neutral sugar were measured as described previously.

Male Sprague-Dawley (SD) rats (3 weeks of age, Charles River Japan, Kanagawa, Japan) were fed a commercial non-purified diet (Type MF, Oriental Yeast Co., Tokyo, Japan) for 4 days. The rats were then divided into five groups of six or nine animals and fed on a purified diet (Basal diet) or an atherogenic diet containing 1% cholesterol and 0.25% sodium cholate (Cholesterol diet) for 27 days. The composition of the basal diet was in weight percent: casein 22, lard 9, salt mixture 3.5, vitamin mixture 1, choline chloride 0.2, and sucrose to 100. Salt and vitamin mixtures were AIN-76 preparations (Oriental Yeast Co., Tokyo, Japan). The cholesterol diet was replaced with 5% each of pectin (Pectin diet), MP (MP diet), or LP (LP diet). Rats were individually housed, and the food intake and body weight were measured every day during the experimental period. Small volumes (less than 0.1 ml) of blood were withdrawn from the tail vein periodically (at 4, 11, 19, and 25 days) during the feeding period for the analysis of serum cholesterol. At the end of the experimental period, the rats were lightly anesthetized with diethyl ether, and blood was withdrawn from the vena cava. The liver and cecum were quickly excised and weighted. Cecums were divided into content and wall and weighted. Cecal contents' pH was measured after it was suspended with equal volumes of water.

Serum cholesterol levels were enzymatically measured by using a commercial assay kit (determiner TC5, Kyowa Hakko Kogyo Co., Tokyo, Japan). After extracting the lipid from livers by the method of Folch et al., the cholesterol levels were assayed by the enzymatic method as described before.

Values were analyzed by one-way analysis of variance (ANOVA), and differences of means were inspected using the Tukey-Kramer method.

The reaction procedure of citrus pectin solution and culture fluid produced a 63.9% yield of medium-molecular-weight pectin (a preparation of 2 h incubation) and 58.3% of the low-molecular-weight pectin (24 h incubation). The molecular weights of the original citrus pectin, MP, and LP were about 750,000, 185,000, and 66,000, respectively. As the reaction proceeded, the galacturonic content of pectin, 87.6%, was slightly lowered to 85.6% for MP and 80.3% for LP. The viscosities of MP and LP in 5% solution were lower than that of the original pectin (pectin 319.2 mPa-s, MP 47.5 mPa-s, and LP 16.0 mPa-s, respectively). With the original pectin, it is not possible to make a highly concentrated solution, but the MP and LP can be soluble up to 15% or more in water.

The differences in the body weight, food intake, liver weight and cecum weight among the groups are shown in the attached table. The value of the body weight gain and feed efficiency were approximately equal among the groups. The increase of liver weight to body weight ratio was repressed by the addition of original pectin.

Figure shows the changes of cholesterol concentrations in serum. MP repressed the increase of serum cholesterol the same level as original pectin, but LP did not. Significant differences of cholesterol concentration between the cholesterol diet group and LP diet group were detected only after 19 days of feeding on the experimental diets. These results indicated that the high molecular weight such
Hypcholesterolemic Effects of Pectin

<table>
<thead>
<tr>
<th></th>
<th>Basal diet</th>
<th>Cholesterol diet</th>
<th>Pectin diet</th>
<th>MP diet</th>
<th>LP diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Initial body wt. (g)</td>
<td>89 ± 2</td>
<td>88 ± 2</td>
<td>89 ± 2</td>
<td>88 ± 2</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>Body wt. gain (g)</td>
<td>191 ± 7</td>
<td>185 ± 6</td>
<td>186 ± 8</td>
<td>177 ± 5</td>
<td>188 ± 6</td>
</tr>
<tr>
<td>Food intake (g)</td>
<td>404 ± 14</td>
<td>387 ± 13</td>
<td>406 ± 10</td>
<td>380 ± 13</td>
<td>412 ± 11</td>
</tr>
<tr>
<td>Body wt. gain/food intake</td>
<td>0.47 ± 0.01</td>
<td>0.46 ± 0.02</td>
<td>0.46 ± 0.01</td>
<td>0.45 ± 0.03</td>
<td>0.46 ± 0.01</td>
</tr>
</tbody>
</table>

**Liver**

- Liver wt./100 g of body wt.: 6.0 ± 0.2* 7.7 ± 0.2* 6.6 ± 0.2* 7.5 ± 0.1* 7.2 ± 0.2*
- Liver chol. (mg/liver): 71 ± 9* 1341 ± 52* 710 ± 68* 1140 ± 100* 1256 ± 59*

**Cecum**

- Total wt. (g): 1.88 ± 0.13* 1.93 ± 0.12* 3.78 ± 0.71* 5.03 ± 1.13* 4.97 ± 0.58*
- Content wt. (g): 1.33 ± 0.17* 1.47 ± 0.10* 2.87 ± 0.75* 4.00 ± 1.06* 4.01 ± 0.52*
- Wall wt. (g): 0.58 ± 0.03* 0.48 ± 0.03* 0.84 ± 0.03* 0.91 ± 0.08* 0.50 ± 0.06*
- Content pH: 7.37 ± 0.09* 7.00 ± 0.05* 6.51 ± 0.06* 6.46 ± 0.06* 6.51 ± 0.08*

Each value is mean ± SEM. Values in the same line without common superscript letters denote significant difference (p < 0.05).

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as MP is necessary to repress the increase of serum cholesterol.

After 27 days of feeding, the liver cholesterol level was slightly lowered for LP and MP, but it wasn’t a significant decrease compared with the cholesterol diet (Table). In our previous report, the liver cholesterol level was decreased to some extent by the addition of LP or pectin for 9 days of feeding. A possible explanation for the different results may be feeding time.

It has been reported that hypocholesterolemic effects resulted from the inhibition of cholesterol synthesis by the volatile fatty acids produced by microorganisms. So we measured cecum weight and cecum content’s pH (Table) to surmise the mechanism of the effects. Weights of cecum, cecal wall, and its content were increased by adding MP and LP, the same as adding pectin. Furthermore, the level of lowering cecal content’s pH by MP or LP was superior to that by pectin, although reduction of the serum cholesterol level was smaller than that of pectin. This indicated that a mechanism of the hypocholesterolemic effect of pectin cannot be explained only by inhibition of cholesterol synthesis by the volatile fatty acids.

The results that LP lost the activity of repressing the serum cholesterol level and MP preserved the activity suggested that there were some relationship between molecular weight and hypocholesterolemic activity of pectin. Citrus pectin such as MP, which preserve both the character of low viscosity and hypocholesterolemic activity will be useful to add to food and drink.

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**References**