Adsorption of Purine Compounds in Beer with Activated Carbon Prepared from Beer Lees

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Abstract
Six hundred thousand tons of beer lees are discharged annually in Japanese breweries. It is well known that purine compounds are one of substances which cause the gout and beer especially contains a lot of purine compounds such as adenosine, adenosine 5'-phosphate and so on, compared with the other alcoholic drinks. The application of activated carbon prepared from beer lees was investigated in order to remove purine compounds in beer. The reuse and recycling of beer lees to activated carbon for removing purine compounds in beer could make a contribution to the zero emission in breweries. In this study, the effect of physical properties of activated carbon derived from beer lees on the adsorption behavior of purine compounds was researched. Adenine, adenosine and adenosine 5'-phosphate, which are one of purine compounds, are selected as an example of model compounds in beer. The size of purine compounds and the pore structure of activated carbon affect the adsorption characteristic of purine compounds. It is found that beer lees can be reused and recycled in breweries by transforming to beer lees activated carbon for removing purine compounds in beer.

Key words: Beer Lees, Activated Carbon, Purine Compound, Adsorption

1. Introduction
In breweries, it is necessary to reuse and recycle various wastes discharged in the brewing process. Since a lot of malt is used as an ingredient of beer, the amount of beer lees occupies about 80% in all wastes in breweries. The amount is estimated to be about 620,000 tons (with 80% moisture content) per year in 20081-4. Recently, the commercial products of beer have shifted to the third beer, which is produced in a different way from beer and low-malt beer. The discharged amount of beer lees is decreasing year by year, but remains a lot still now5. The components of beer lees are mainly cellulose, hemi cellulose and lignin, and they also contain proteins and minerals such as Si, P, Mg and Ca6. Usually, the beer lees are immediately dried to prevent them from putrefying because the percentage of moisture content is about 80 wt% after a filtration process5. The dried beer lees are reused as a feed, but the usage as a feed is not so good because of high costs for transportation. That is the reason why the development of new recycling method is required for beer lees.

We have already reported that the activated carbon is prepared from beer lees by carbonation and activation8. It was found that the beer lees activated carbon could be prepared to give a high specific surface area of 1600 m²/g by KOH activation. The beer lees activated carbon could adsorb various organic materials such as phenol, methylene blue, 1,2-dichloroethane and 1,2-dichloropropane9.

The preparation of activated carbon from beer lees was investigated in this study. Carbon dioxide recovered in the fermentation process of beer production may be used in the activation process. The removal experiments of purine compounds in beer were carried out as one of the effective uses of beer lees activated carbon. The purine compounds in beer are one of substances which cause the gout10. The “low purine beer” is now on sale in Japan. In the production process of the low purine beer, the special activated carbon with high adsorption ability of purine compounds is used to remove purine compounds in beer. The usage of
Adsorption of Purine Compounds in Beer with Activated Carbon Prepared from Beer Lees

2. Experimental

2.1 Preparation of activated carbon from beer lees

In order to clarify the effects of demineralization from beer lees and ash removal from activated carbon with acid mixture, three kinds of activated carbons were prepared from beer lees with or without ash removal treatment. Dried beer lees were carbonized in an electric furnace (EP-K-1200, Isuzu Seisakusho Co., Ltd.) under the condition of N₂ atmosphere, 700°C and 2 hr. The dried beer lees contain moisture of about 3.0 wt%. The beer lees charcoal of 1.0 g sieved to −106 µm was put into a ceramic board, and the board was set in the electric furnace. The temperature in the activation reactor was increased to an activation temperature of 940 and 960°C under N₂ atmosphere. At the activation temperature, CO₂ gas of purity 99% was introduced into the reactor. The flow rate of CO₂ was 100 cm³/min. After the beer lees charcoal was activated by CO₂ for 1.5 hr, the reactor was cooled down to room temperature under N₂ atmosphere. The expressions of “C940H1.5(1)” and “C960H1.5(1)” mean the activated carbons obtained under the condition of 940 and 960°C reaction temperatures, 1.5 hr activation time without ash removal treatment.

Dried beer lees of 120 g were added to HF-HCl acid mixture (2.0 mol/dm³ HF and 1.0 mol/dm³ HCl) of 4 dm³. The activated carbon and HF-HCl acid mixture were contacted by stirring for 1.5 hr. The beer lees after leaching were dried at 80°C for 24 hr. The beer lees without mineral contents were carbonized by the electric furnace in the same condition mentioned above. The obtained beer lees charcoal of 1.0 g sieved to −106 µm was set in the electric furnace and the beer lees activated carbon was prepared in the same activation condition. For example, the expression of “C950H1(2)” shows the activated carbon obtained under the condition of 950°C reaction temperature, 1.0 hr activation time with the ash removal treatment to beer lees.

On the other hand, the ash contents in the beer lees activated carbon were removed by the same method with HF-HCl acid mixture. The beer lees activated carbon of 3.0 g and HF-HCl acid mixture of 30 cm³ were mixed for 90 min. The obtained beer lees activated carbon was washed by pure water. The terms of “C940H1.5(3) and C960H1.5(3)” mean the activated carbons obtained by the ash removal treatment.

The specific surface area and the pore size distribution of the obtained beer lees activated carbon were analyzed by a surface area analyzer (AS1MP-LP 2, Quantachrome Instruments). The pore size distribution was calculated by DFT model. The amount of ash in beer lees activated carbon was measured by a thermogravimetry equipment (TGA-50/50H, SHIMADZU Corporation). For comparison, the above physical properties of “LPN36” and “LPN37” which are one of commercial activated carbons (Japan Enviro Chemicals, Ltd.) having high purine compounds absorption ability recommended in the patent, were investigated in this study.

2.2 Adsorption of purine compounds with various activated carbons

Adenine, adenosine and AMP were used as purine compounds. These purine compounds are supplied by Wako Pure Chemical Industries, Ltd. Three kinds of aqueous solutions containing a single component of purine compounds at 100 mg/dm³ were prepared. LPN37, C950H1(2), C940H1.5(3) and C960H1.5(3) were used as activated carbons for adsorption of purine...
compounds. C950H1(2), C940H1.5(3) and C960H1.5(3) are the beer lees activated carbon, respectively. The activated carbon of 10 mg was put in the aqueous solution of 50 cm$^3$. The solution was stirred for various time intervals with a magnetic stirrer at room temperature, and then filtration was carried out with a membrane filter of 0.20 µm. The concentration of purine compounds was analyzed by a high-performance liquid chromatograph. The effect of physical properties of the activated carbon on adsorption of purine compounds was studied.

2.3 Removal of purine compounds in beer with various activated carbons

LPN37, C950H1(2), C940H1.5(3) and C960H1.5(3) were used for the removal of purine compounds in beer. Various amounts of activated carbon were added to beer of 50 cm$^3$. After stirring for 90 min with a magnetic stirrer, the filtration was conducted with the membrane filter of 0.20 µm. The concentration of purine compounds in mother liquor was measured according to the Fujimori’s method$^{13}$. The mother liquor of 10 cm$^3$ was freeze-dried with a freeze dryer (FD-1000, Tokyo RIKAKIKAI Co.) in the condition of 20 Pa and $−50^\circ$C. The freeze-dried substance was put in 70% HClO$_4$ of 15 cm$^3$. Then, all purine compounds were hydrolyzed to purine bases (adenine, guanine, hypoxanthine and xanthine) by stirring for 1 hr at 100$^\circ$C. After the neutralization with KOH and the centrifugation, the concentration of purine bases in the solution was analyzed by HPLC.

3. Results and discussion

3.1 Preparation of activated carbon from beer lees with low ash contents

The property of activated carbon is shown in Table 1. The description “C960H1.5” expresses the beer lees activated carbon prepared in the activation condition of 960$^\circ$C and 1.5 hr. LPN37 has 1580 m$^2$/g specific surface area and the amount of ash is very low (0.28 wt%). The specific surface area of C960H1.5(1) is 1390 m$^2$/g and the amount of ash is 30.8 wt%. The specific surface area of C950H1(2) is 2240 m$^2$/g and the amount of ash is 1.56 wt%. The use of LPN37 and C950H1(2) in the brewing may not debase the quality of beer, because the ash contents are less than 2.0 wt%, for standard value of ash contents. C940H1.5(3) and C960H1.5(3) have low ash content due to removal of ash. Then, the specific surface area does not change, and the total pore volume increases by removal of ash. The removal of ash affects the total pore volume. There is no problem caused by the dissolution of ash contents in C940H1.5(3) and C960H1.5(3), because the ash contents derive from beer lees themselves in the same beer production process. The use of C940H1.5(3) and C960H1.5(3) in the brewing may not debase the quality of beer although the ash contents are more than 2.0 wt%.

3.2 Adsorption property of purine compounds with various activated carbons

The pore size distribution of activated carbon is shown in Figure 1. For C960H1.5(3), the pore volume of the size 10–20 Å and 23–60 Å is well developed than the other activated carbons. The pore size of LPN37 and C950H1(2) is from 4 Å to 15 Å, but C950H1(2) has larger pore size from 15 Å to 40 Å compared with LPN37.

Table 2 indicates the property of adenine, adenosine and AMP. The size of purine compounds were analyzed by Facio$^{14}$ which is 3D-graphics program for molecular modeling. The size of molecule is larger in the order of molecular weight. It is considered that three purine compounds are adsorbed on the activated carbons shown in Table 1, because activated carbons have suitable pore

<table>
<thead>
<tr>
<th>Table 1 Property of activated carbon.</th>
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<tbody>
<tr>
<td>Specific surface area [m$^2$/g]</td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>LPN36</td>
</tr>
<tr>
<td>LPN37</td>
</tr>
<tr>
<td>C940H1.5(1)</td>
</tr>
<tr>
<td>C960H1.5(1)</td>
</tr>
<tr>
<td>C950H1(2)</td>
</tr>
<tr>
<td>C940H1.5(3)</td>
</tr>
<tr>
<td>C960H1.5(3)</td>
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Fig. 1 Pore size distribution of various activated carbons.
Adsorption of Purine Compounds in Beer with Activated Carbon Prepared from Beer Lees

Table 2 Property of purine compounds.

<table>
<thead>
<tr>
<th>Compositional formula</th>
<th>Molecular weight</th>
<th>Chemical structural formula</th>
</tr>
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<tbody>
<tr>
<td>Adenine</td>
<td>C₅H₅N₅</td>
<td>135.1</td>
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<tr>
<td>Adenosine</td>
<td>C₁₀H₁₃N₅O₄</td>
<td>267.2</td>
</tr>
<tr>
<td>Adenosine 5'-phosphate (AMP)</td>
<td>C₁₀H₁₄N₅O₇P</td>
<td>347.2</td>
</tr>
</tbody>
</table>

which can absorb these purine compounds.

The residual concentration of adenine after adsorption with various activated carbons is shown in Figure 2. The concentration of adenine reaches an equilibrium state by stirring time of 20 min for every activated carbon. The equilibrium adsorption amount for adenine is large, and the order is as follows; LPN 37 > C950H1(2) > C960H1.5(3) > C940H1.5(3). The beer lees activated carbons such as C950H1(2) have good adsorption ability for adenine, which is nearly equal to the commercial activated carbon. The activated carbon with large pore volume from 4 Å to 15 Å tends to adsorb adenine molecules. The pore structure of activated carbon may affect the adsorption amount of adenine.

Figure 3 shows the residual concentration of adenosine after adsorption with various activated carbons. The concentration of adenosine attains an equilibrium state for 60 min stirring with every activated carbon. The equilibrium adsorption amount for adenosine becomes large in the following order; C950H1(2) = LPN37 > C960H1.5(3) > C940H1.5(3). The increase in adsorption amount of adenosine may be responsible for the size of adenosine. The pore structure of activated carbon may affect the adsorption amount of adenosine.

Figure 4 shows the residual concentration of AMP with various activated carbons. The concentration of AMP becomes constant, the equilibrium state being obtained for 90 min stirring. The equilibrium adsorption amount for AMP becomes large in the following order; C960H1.5(3) > C950H1(2) > LPN37 > C940H1.5(3). It is considered that the adsorption order of AMP with C950H1(2) and LPN37 becomes nearly equal to adenosine because of the similar molecular structure and size between adenosine and AMP.

The time for adsorption equilibrium increases
Junji SHIBATA, Norihiro MURAYAMA and Masato TATEYAMA

with increasing the molecular size of purine compounds as shown in Figures 2, 3 and 4. As one of the reasons, the geometric nature of purine compounds may affect adsorption rate. The adsorption rate also decreases due to the decline in driving force for adsorption caused by the concentration decrease of purine compounds. C940H1.5(3) and C960H1.5(3) have the behavior like a molecular sieve in the viewpoint that the adsorption ability decreases for relatively small size purine compounds such as adenine and increases for relatively large size purine compounds such as AMP. Therefore, it is considered that C940H1.5(3) and C960H1.5(3) are superior in the adsorption ability of purine compounds in beer. The actual beer contains purine compounds such as adenosine and AMP which are the relatively large size molecules and does not have the relatively small size purine bases such as adenine, guanine, hypoxanthine and xanthine. It is considered that beer lees can be reused and recycled in breweries by transforming into the beer lees activated carbon, to remove purine compounds in beer.

3.3 Application of beer lees activated carbon to brewing of low purine beer

The adsorption of purine compounds in beer with various activated carbons is shown in Figure 5. The total concentration of purine bases decreases with an increase in the additional amount of activated carbon. C940H1.5(3) and C960H1.5(3) are superior in adsorption ability of purine compounds to LPN37, which is the commercial activated carbon. It is found from the results that C940H1.5(3) and C960H1.5(3) tend to adsorb relatively large size purine compounds, and beer also does not contain small size purine compounds a lot. All beer lees activated carbons have almost the same adsorption ability for purine compounds as the commercial activated carbons. It is found that the activated carbon derived from beer lees enables to remove purine compound in beer.

The recycling process of beer lees is shown in Figure 6. The beer lees of 49,000 tons per year are discharged in one brewing plant, though it depends on the beer production scale. C940H1.5(3) of 810 tons per year can be produced from beer lees. The $1.53 \times 10^5$ m$^3$ of low purine beer can be...
Adsorption of Purine Compounds in Beer with Activated Carbon Prepared from Beer Lees

produced annually by applying the 3-stage batch operation with the activated carbon to removal of purine compounds, and this amount of low purine beer may be enough to cover the usual shipment of low purine beer.

4. Conclusion

The application of activated carbon prepared from beer lees to the removal of purine compounds in beer was investigated in this study. The beer lees activated carbon prepared from the beer lees has about 30 wt% of ash contents, because the beer lees has a lot of minerals such as Si, P, Ca and Mg. The treatment of the beer lees with HF-HCl acid mixture enables to prepare the beer lees activated carbon with low ash contents and high specific surface area.

The effect of physical properties of activated carbon derived from beer lees on the adsorption behavior of purine compounds was researched. The size of purine compounds and the pore structure of activated carbon may be one of the important factors determining the adsorption amount of adenine, adenosine and AMP. The beer lees activated carbon has an ability to remove purine compounds and the removal performance of beer lees activated carbon almost equals to the commercial activated carbon. It is concluded that beer lees can be reused and recycled in breweries by transforming to the activated carbon to remove purine compounds in beer.

References

9. H. Lee, Y. Hirano, N. Murayama, J. Shibata:


