Surface Microstructure of Various Calcium Alginate Xero-gels and Its Fractal Analysis

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Air-dried, freeze-dried and alcohol-replaced calcium alginate xero-gels were compared with by the specific surface area, pore-size distribution and fractal dimension. The specific surface areas of air-dried and −20°C freeze-dried xero-gels were less than 0.5 m²/g, while those of ethanol- or 1-propanol-replaced xero-gels were about 400 m²/g. The macro pore size distribution of −196°C freeze-dried xero-gel had a maximum at about 1 μm of pore radius, while that ethanol-replaced xero-gel had broad distribution in the range less than 100 nm in pore radius. The micro-pore size distribution was very much affected by the contents of mannuronic acid and guluronic acid of alginate. The micro-pore was most effectively developed by use of 1-propanol. Air-dried, −20°C freeze-dried and ethanol-replaced xero-gels had fractal structures with the fractal dimension of 2.4-3.0. The specific surface area of ethanol-replaced xero-gel was independent of the particle diameter, showing that the surface was the fractal with the fractal dimension of 3.0.

Introduction

The various physical properties of food reflect not only the chemical constituents of the food but also the surface microstructure of the food. Since heat transport, mass transport, equilibrium and kinetic properties are closely related to the surface microstructure, transformation of the surface microstructure is of special importance in improving the physical properties of food.

The surface microstructure of catalysts was transformed by adding a solvent12-30. For example, INOGUCHI et al. reported that the specific surface area of the alumina was not affected significantly by the transformation, although larger pores were developed when hydrophilic alcohols with high molecular weight were used30. The specific surface area of casein micelles, when it was dehydrated by solvents serially and dried under vacuum, became ten times larger than that of the directly freeze-dried casein micelles31. The porous structure of spores dried in air and by freeze-drying collapsed completely, but, if the spores were dried after the replacement of water by a solvent, the specific surface area of spores increased by about ten-fold30. In these works, only the changes in the specific surface area and the pore-size distribution were measured, but the changes in the structure was not analyzed quantitatively.

Recently the fractal concept, first proposed by MANDELBROT in 1975, has been used to study quantitatively the irregular structure of various objects and even the irregular physical phenomena40. By using this concept the degree of irregularity may be evaluated by the non-integer fractal dimension $D$, the value of $D$ being $1.0 \leq D \leq 2.0$ for irregular lines and $2.0 \leq D \leq 3.0$ for irregular surfaces. The higher the value of $D$, the more wiggly the object. The geometric surface irregularities were already determined by applying the fractal concept to a variety of particulate materials37-13).

In a previous report, we transformed potato
starch, rice flour and wheat flour into porous materials by adding hydrophilic alcohols (e.g. methanol, ethanol and 1-propanol) after gelatinization\textsuperscript{13}. The transformed surface microstructure was evaluated quantitatively using the fractal concept\textsuperscript{13,14}.

In this study, various calcium alginate xero-gels, of which antitumour effect\textsuperscript{15}, ion exchange property\textsuperscript{16}, and adsorption of mutagens\textsuperscript{17} have been known, were prepared by different drying procedures, and the fractal analysis is attempted for their surface microstructure.

**Materials and Methods**

**Preparation of calcium alginate xero-gels**

One liter of 2% (w/w) sodium alginate (Kanto Chemical Co., Inc.) solution in distilled water was dropped into one liter of 1% (w/w) CaCl\textsubscript{2} solution to make calcium alginate beads (about 4 mm in diameter). The beads were hardened in the CaCl\textsubscript{2} solution for at least 2 days and rinsed with distilled water.

**Air-dried xero-gel**

The gel beads were dried by hot air at 80°C for 24 hours or more.

**Freeze-dried xero-gel**

The gel beads were frozen at −20°C, or with liquid nitrogen (−196°C), and freeze-dried under a reduced pressure of about 20 Pa.

**Alcohol-replaced xero-gel**

The gel beads were dehydrated by mixing with an equal weight of reagent-grade alcohols, stirring for about 12 hours at room temperature. Then the gel beads were filtered from alcohol solution and mixed again with an equal weight of alcohols, stirring for about 12 hours. After this treatment was repeated 1 ~ 8 times, the gel beads were dried under a reduced pressure of about 20 Pa at room temperature.

**Specific surface area**

The nitrogen adsorption isotherm of sample at −196°C was measured with an ACCUSORB 2100 E-02 (Micromeritics Instrument Co., Georgia, USA.), after the sample was completely dried at a temperature below 40°C under vacuum in the apparatus. From the adsorption isotherm, the moles of N\textsubscript{2} to cover the surface by monolayer was calculated using the BET plot. The specific surface area was calculated, taking the effective cross-sectional area of N\textsubscript{2} as 0.162 nm\textsuperscript{2}. Adsorption equilibrium was assumed when the pressure change became less than 0.025% of the selected span of pressure transducer for at least 1 minute.

**Pore-size distribution**

The micro-pore size distribution was calculated for pores of less than 50 nm in diameter from the N\textsubscript{2} adsorption isotherm at −196°C, using the method of CRANSTON and INKLEY\textsuperscript{18}.

The macro-pore size distribution was measured for pores of larger than 10 nm in diameter with a Poresizer 9130 (Micromeritics Instrument Co., Georgia, USA.), using the mercury intrusion method.

**Mean particle diameter**

The xero-gels, air-dried or freeze-dried at −20°C, were ground and sifted with vibrating screen. The different size of ethanol-replaced xero-gels were prepared from different size of calcium alginate gel beads and sifted with vibrating screen after drying. The mean particle diameter of a sifted fraction was taken to be the arithmetic mean of the aperture of the two sifting screens from which the fraction was obtained.

**Fractal dimension**

Fractal dimension, \( D_s \), is defined by Eq. (1).

\[
S_w \propto d_p^{D_s-3}
\]

where \( S_w \) [m\textsuperscript{2}/g] is the specific surface area and \( d_p \) [\( \mu \text{m} \)] is the mean particle diameter of a sample. The fractal dimension \( D_s \) was calculated from the log-log plots of Eq. (1).

**Scanning electron micrographs (SEM)**

SEM S-510 (Hitachi, Ltd., Tokyo, Japan) was used at an accelerating voltage of 15 kV, the representative images being recorded on FUJI NEOPAN SS FILM.

**Results**

Table 1 shows the specific surface areas of 80°C air-dried, −20°C freeze-dried, −196°C freeze-dried and alcohol-replaced xero-gel, prepared from calcium alginate gel beads (about 4 mm in diameter). For the alcohol replacement, methanol, ethanol and 1-propanol
Table 1 Specific surface area of various calcium alginate xero-gels

<table>
<thead>
<tr>
<th>calcium alginate xero-gel</th>
<th>$S_w$ [m$^2$/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>80°C Air-dried</td>
<td>0.132</td>
</tr>
<tr>
<td>-20°C Freeze-dried</td>
<td>0.354</td>
</tr>
<tr>
<td>-196°C Freeze-dried</td>
<td>227</td>
</tr>
<tr>
<td>Dried after mixed with</td>
<td>(1) (3) (4) (5) (6) (7) (8)</td>
</tr>
<tr>
<td>Methanol</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5.76 34.2 262 370 409 417 437</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>111 209 — 430 — —</td>
</tr>
</tbody>
</table>

Figures in ( ) shows the number of replacement.

Fig. 1 Macro-pore size distribution of dried alginates
(by mercury intrusion method)

Fig. 2 Effect of number of EtOH replacement on the micro-pore size distribution (by Cranston-Inkley method)

were used. The specific surface areas of 80°C air-dried and -20°C freeze-dried xero-gels were less than 0.5 m$^2$/g, while those of -196°C freeze-dried and of ethanol and 1-propanol-replaced xero-gels were more than 200 m$^2$/g. The specific surface areas of ethanol and 1-propanol-replaced xero-gels increased over 400 m$^2$/g with increase of number of replacement. Methanol replacement was not effective for increment of the specific surface area.

Fig. 1 shows the macro-pore size distribution of -196°C freeze-dried xero-gel and 6 times ethanol-replaced xero-gel. The size distribution curve of -196°C freeze-dried xero-gel had a peak at about one micron which was approximately equal to the size of ice crystals, while the size distribution of ethanol-replaced xero-gel was broad with pore diameter less than 100 nm.

Fig. 2 shows effect of ethanol replacement on the pore size distribution. By increasing the number of replacement, pore volume at about 7 ~ 8 nm in radius increased. The size distribution curve of 6 times-replaced sample and that of 8 times-replaced sample were almost identical.
Fig. 3 shows the scanning electron micrographs of the 80°C air-dried, -20°C freeze-dried (prepared from 2% (w/w) sodium alginate solution), and the 6 times ethanol-replaced (prepared from 0.5% (w/w) sodium alginate solution) xero-gels. The micrograph of ethanol-replaced xero-gel shows more convoluted porous structure as compared with the smooth, spherical micrographs of the other two.

Fig. 4 compares the micro-pore size distribution of 6 times ethanol-replaced xero-gels of sodium alginate (prepared from 1% (w/w) solution), of which the ratio of D-mannuronic acid (M) to L-gluronic acid (G) was known to be M/G = 2.4 and M/G = 0.53 (Kimitsu Chemical Industries Co., Ltd.). The specific surface areas of the two xero-gels were 359 and 341 m²/g, respectively. The gluronic acid-rich alginate (M/G = 0.53) developed much smaller pores than the mannuronic acid-rich sample (M/G = 2.4). The two peaks observed in Fig. 2 for 6 or 8 times ethanol-replaced xero-gels might be due to the mixture of gluronic acid-rich and mannuronic acid-rich alginate.

Fig. 5 shows effect of alcohols on the development of micro-pores. The alginate samples were replaced by each alcohol by 5 times.
1-Propanol was most effective for developing micro-pores with a peak at about 7–8 nm in radius, ethanol was also effective for developing smaller micro-pores, and methanol was not effective at all.

Fig. 6 shows the log-log plots of the specific surface area versus the mean particle diameter of samples. For spheres, cylinders and cubes, the plot of Fig. 6 must give straight lines with the slope of -1. For the alginate xero-gels, however, the slopes of the straight lines were not -1, showing that the surface microstructures of xero-gels were fractal with the fractal dimension $D_s$ of between 2.4 and 3.0 (see Eq. (1)).

**Discussion**

Microstructures of catalysts were transformed by adding solvents to improve their selectivity and activity\(^1\)-\(^3\). In a sol-gel method of synthesizing glasses, the glasses were dried with addition of a surface tension lowering substance\(^18\). In these cases, the addition of a solvent may increase porosity by evaporation of the solvent and at the same time prevent the sample from shrinking and cracking by lowering the surface tension.

In this study, ethanol or 1-propanol played the same role as the solvents for catalysts and glasses mentioned above, to increase the porosity, preventing the alginate gels from shrinking and cracking when the gels were dried under vacuum. The surface tensions of pure methanol, ethanol and 1-propanol are almost same and lower than that of pure water (methanol: $22.5 \times 10^{-3}$ N/m; ethanol: $22.4 \times 10^{-3}$ N/m; 1-propanol: $23.7 \times 10^{-3}$ N/m; water: $72.8 \times 10^{-3}$ N/m; at $20^\circ C^20\) However, the surface tension of 1-propanol-water solution was as low as that of pure 1-propanol at a dilute concentration of 15 mol%, while the surface tension of methanol-water and of ethanol-water solutions was as low as that of pure alcohol at a concentration of 90 mol% and 60 mol%, respectively\(^21\). This may be the reason why the surface microstructure was developed effectively by the addition of 1-propanol.

The difference in M/G ratio of sodium alginate greatly affected the micro-pore size distributions of ethanol-replaced xero-gels as shown in Fig. 4. This may be a reflection of the structure of $\alpha_1-4$ linked-gluronic acid block and that of $\beta_1-4$ linked-mannuronic acid block. This micro-pore distribution was closely similar to that of calcium alginate gel beads measured by solute exclusion method in wet condition\(^22\). So, by ethanol or 1-propanol replacement, calcium alginate gel beads could be transformed into the xero-gel. The microstructure of the xero-gel was similar to that of calcium alginate gel in wet condition.

A quantitative analysis of the irregular microstructure of food materials using a fractal concept, especially knowing the change in the fractal dimension $D_s$ as shown in Fig. 6, is important not only for the prediction of the specific surface area of a powder of known particle-size distribution, but also for the evaluation of the transformation methods. As Fig. 6 shows, by 6 times ethanol-replacement, not only the specific surface area of the xero-gel became more than 100 times larger than
that of the air-dried and −20°C freeze-dried xero-gels, but also the fractal dimension $D_s$ increased to 3.0, that is supposed to be the maximum. By this transformation, the self-similar porous structure was developed three-dimensionally within the ethanol-replaced xero-gel. Assuming that the microstructure of particle is fractal with a fractal dimension of between 2.0 and 3.0, three well-known different empirical equations for the energy requirement for soil grinding could be interpreted by a single so-called fractal–Rittinger equation. So the estimation of the fractal dimension $D_s$ will be effective for the evaluation of the physical properties such as surface area, mechanical strength, pressure drop etc.

Through transformation of the surface microstructure of calcium alginate xero-gels as described, substantial change in their sorption behavior might be possible, which will be treated in our following paper.

References

乾燥ゲルの構造とフロクタル解析

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熱風乾燥、凍結乾燥、アルコール置換乾燥によるアルギン酸カルシウムセロゲルの微細構造を比較し、細孔分布、フロクタル次元によって比較した。80°C 熱風乾燥試料と-20°C 凍結乾燥試料の比表面積は 0.5 m²/g 以下であるのに対し、エタノールや 1-プロパノール置換乾燥試料の比表面積は 400 m²/g 程度であった。

-196°C 凍結乾燥試料のマクロポアの細孔分布は細孔半径約 1 μm にピークを持つが、エタノール置換乾燥試料の細孔分布は細孔半径 100 nm 以下の広い分布を示した。一方、アルギン酸の構成糖であるマンノロン酸とグルロン酸の含有量はミクロポアの細孔分布に大きな影響を与えた。また、ミクロポアは 1-プロパノールによって最も効果的に発達することがわかった。熱風乾燥試料、-20°C 凍結乾燥試料、エタノール置換乾燥試料はいずれもフロクタル構造をしており、その次元は 2.4から 3.0 となった。エタノール置換乾燥試料のフロクタル次元は 3.0 で、その比表面積は粒径に依存せず一定となっ