

Original Research Article

# Aragonite type of shell powder prepared from the fired *Corbicula japonica* shell prevented liver deterioration and lowering lipid level in alcohol-fed mice model

Jin-Ichi Sasaki<sup>1\*</sup>, Yoshinori Minegishi<sup>2</sup>, Mana Yasui<sup>3</sup>, Atsushi Yamazaki<sup>3</sup> and Changlong Lu<sup>4</sup>

## Abstract

Aragonite type of shell powder created by firing bivalve *Corbicula japonica* shell at 200°C for 40min was provided for the bio-activity studies using the alcohol-fed C57BL/6 mice model. Solubility of the aragonite shell powder in 1/10 N acetic acid was 85% and that of non-fired shell powder was 70%. On the test in the alcohol-fed mice model, the aragonite type of shell powder effectively worked to lower AST and ALT values in serum to compare with the values of the saline-fed or just alcohol-fed group. Aragonite shell powder-fed mice lowered level of neutral lipid and cholesterol against control groups in the alcohol-fed mice sera. Aragonite type of shell powder was also functional to protect the alcohol-caused liver deterioration.

**Keywords:** Alcohol-fed mice model, Aragonite, *Corbicula japonica*, Lipid value, Liver function

<sup>1</sup>Hirosaki University of Health and Welfare, 2-5 Ohgi-machi, Hirosaki 036-8102, Japan

<sup>2</sup>Aomori Clean-Tech Ltd.2-9-21 Tomita, Aomori 038-0004, Japan

<sup>3</sup>Department of Resources and Environmental Engineering, Waseda University, 3-4-1 Ohkubo Shinjyuku 169-8555 Tokyo, Japan

<sup>4</sup>Institute of Immunology, China Medical University, 92 North Second Road, Heping District, Shenyang 110001, China

\*Corresponding Author's Email: [sasakij@jyoto-gakuen.ac.jp](mailto:sasakij@jyoto-gakuen.ac.jp)

## INTRODUCTION

Fired shell powder of the bivalve *Corbicula japonica* has been used for a long period of time as a traditional folk medicine to improve disorder of liver functions in Japan without any scientific evidences until recently. Lately our laboratory had attested the anti-hepatitis efficiency of the calcite type of shell powder prepared from the *Corbicula japonica* shell fired at 400-500°C (calcite) in the LEC rat hepatitis model (Sasaki et al., 2011). The LEC rat is a special hepatitis model, which spontaneously develops hepatitis around at age 4-6 months, and the survived animals shift to cirrhosis stage, followed by development of liver cancer at one and half years of age (Masuda et al., 1988). In the above experiments it became clear that the aragonite type of shell powder fired at 200-300°C was less in activity to compare with these of the calcite type shell powder fired at 400-500°C in the LEC rat model.

Whereas our prefecture Aomori is a well-known place to culture the high quality bivalve *Corbicula japonica* in brackish water lakes and the meat-extracts drink is one of the hot-selling products as a liver protective healthy drink. Additionally the calcite type of shell powder product has been also commercialized as a health supplement.

However the aragonite type of shell powder was at the moment remaining as a waste without any way of concrete solution.

The present study was then focused on whether the aragonite type of *Corbicula japonica* shell powder demonstrated any possible bio-action(s), which eventually contribute to recycling the shell waste and development of health improve products.

From the biological studies of the aragonite it became obvious that the aragonite type of shell powder also possessed the restorable potency likely to be the calcite against the alcohol-induced liver deterioration, accompanying with improvement of the hyper neutral-lipemia and cholesterolemia in the alcohol-fed mice studies.

## MATERIAL AND METHODS

### Bivalve *Corbicula japonica*

Bivalve *Corbicula japonica* is widespread in Japan Sea



**Figure 1.** Bivalve *Corbicula japonica*

**Table 1.** Test samples used for the bio-functional experiments were;

1.	Non-fired naïve shell powder	Aragonite (100%)
2.	Fired at 200°C, 40 min	Aragonite (100%)
3.	Fired at 360°C, 120 min	Aragonite + Calcite
4.	Fired at 500°C, 120 min	Calcite (100%)

from East Korea Bay to western coast of Sakhalin, and distributed in brackish water lakes and tidal flats of river from South of Japan to South of Sakhalin (Yamamuro and Koike, 1993). Shell of *Corbicula japonica* was extensively washed in clean water to remove debris of meat et al. and dried for next stage firing (Figure 1).

### Crystal structure analysis and scanning microscope observation of the fired shell powder

Three types of shell powder fired at 200, 360 and 500°C were provided for analysis. Analysis of crystal structure was done by both X-ray diffraction apparatus XD-610 Shimadzu Ltd., Japan, and scanning electron microscope (JEOL Ltd. Japan) for observation of granule after firing (Table 1).

1 Non-processed naïve shell powder (aragonite type): Non-fired shell powder was 100% aragonite in crystal structure.

2 Fired at 200°C for 40 min (aragonite type): Extensively washed shell in water was fired at 200°C for 40 min with the electronic firing equipment (AT-E 58, Isuzu Ltd., Japan) and cooled down to the room temperature, followed by milling to create fine powder (granule) at 10 $\mu$ m in diameter. This powder was 100% aragonite in crystal structure.

3 Fired at 360°C for 120 min (mixture of aragonite and calcite): Powder formed 10 $\mu$ m granule in diameter showing mixture of aragonite and calcite by X-ray diffraction analysis.

4 Fired at 500°C for 120 min (calcite type): Pure calcite was formed by above described conditions.

Samples 1 (non-fired shell powder) and 2 (fired at 200°C for 40 min) were used for biological tests. Sample 3 (fired at 360°C for 120 min) and 4 (fired at 500°C for 120 min) were used as reference for crystal structure analysis to compare with sample 1 and 2.

### Solubility test of the fired shell powder in 1/10 N acetic acid

Above listed samples were subjected to solubility test in acetic acid. Briefly each test sample at 0.5 gr was stirred in 40 mL acetic acid (1/10 N) for 30 min. Insoluble residues were weighted to evaluate solubility (% w/w).

### Amino acid analysis of *Conchiolin* on the fired shell powder

Amino acid analyses were carried out by JLC-500V2 JEOL Japan whether there existed the heat-resistant *Conchiolin* in shell powder after fired at 200°C for 40 min.

### Liver function improvement potency and lipid lowering efficacy in the alcohol-fed C57BL/6 mice model

Two-month-old female mice (18-22 gr.) were obtained from the Animal Laboratory Center, Shanghai China to

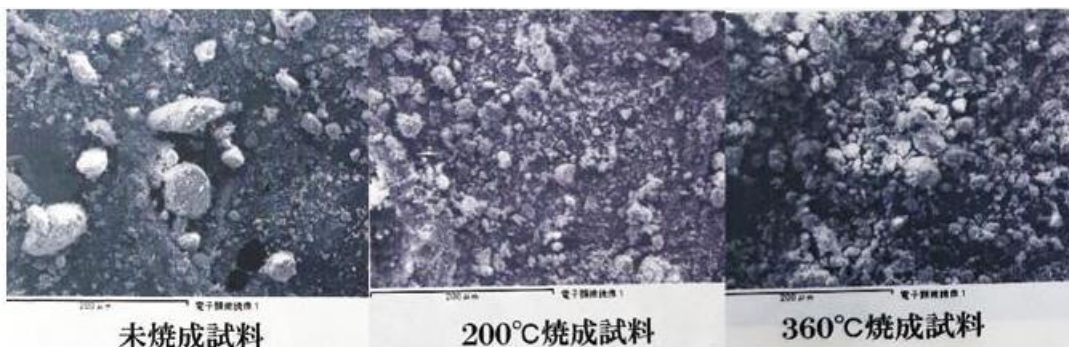
**Table 2.** Following four groups (5 mice per each group) were formed for the biological test

G 1; (Physiological saline/1.0mL, *ip group)
G 2; (10% ethanol/1.0 mL, ip group)
G 3; (10% ethanol/1.0 mL, ip + Non-fired shell powder, oral administration group)
G 4; (10% ethanol/1.0 mL, ip + Shell powder fired at 200°C for 40 min, oral administration group)

\*ip: intra-peritoneal injection

**Table 3.** Solubility of the fired shell powder in 1/10 N acetic acid (% w/w)

Non-fired shell powder; aragonite type	70 %
Fired powder at 200°C for 40 min; aragonite type	85
Fired powder at 360°C for 120 min; aragonite + calcite type	95

**Figure 2.** Scanning electron micrographs of fired shell powder

examine the bioactivity potency of fired *Corbicula japonica* aragonite. Following four groups (5 mice per each group) were formed for the biological test (Table 2)

For evaluation of bio-activity of aragonite, test sample of the shell powder was serially administered for 18 days to mouse by below manner.

Alcohol (1.0 ml at 10% alcohol/mouse/day) or distilled physiological saline (1.0 ml/mouse/day) was injected into peritoneal cavity in the morning, and the non-fired or fired shell powder at 50 mg/0.5 ml in distilled water was orally administered using catheter in the afternoon.

Animal experiments were conducted with the Approvals of the Animal Ethics Committee of University, stating to avoid an excess distress burden to the animals through the experiments.

### Statistical analysis

Student's t-test (SPSS for Windows 16.0) was employed for the evaluation of the statistical significance.

## RESULTS

Solubility of *Corbicula japonica* shell powder increased in acetic acid in proportion to the shell firing temperature

rise (Table 3). Shell powder fired at 360°C, being a mixture of aragonite and calcite, showed the highest solubility among the samples tested.

### Scanning electron microscopy of the fired test samples

Scanning electron microscopy showed that the high temperature fired shell powder became fine in granule size (Figure 2) accompanying with high solubility in acetic acid (Table 3).

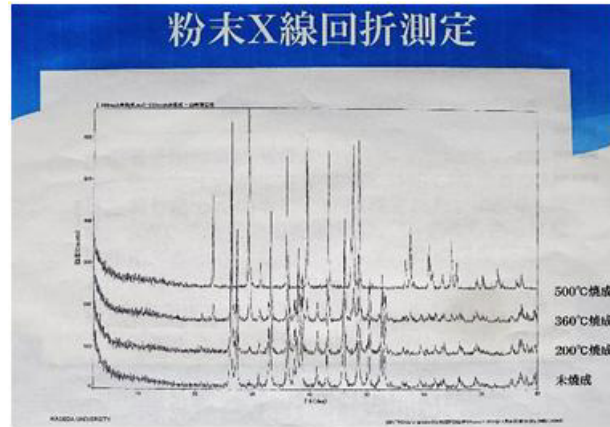
### X ray diffraction analysis of the fired shell powder

X ray diffraction pattern for determination of crystal structure of fired shell powder was shown in Figure 3.

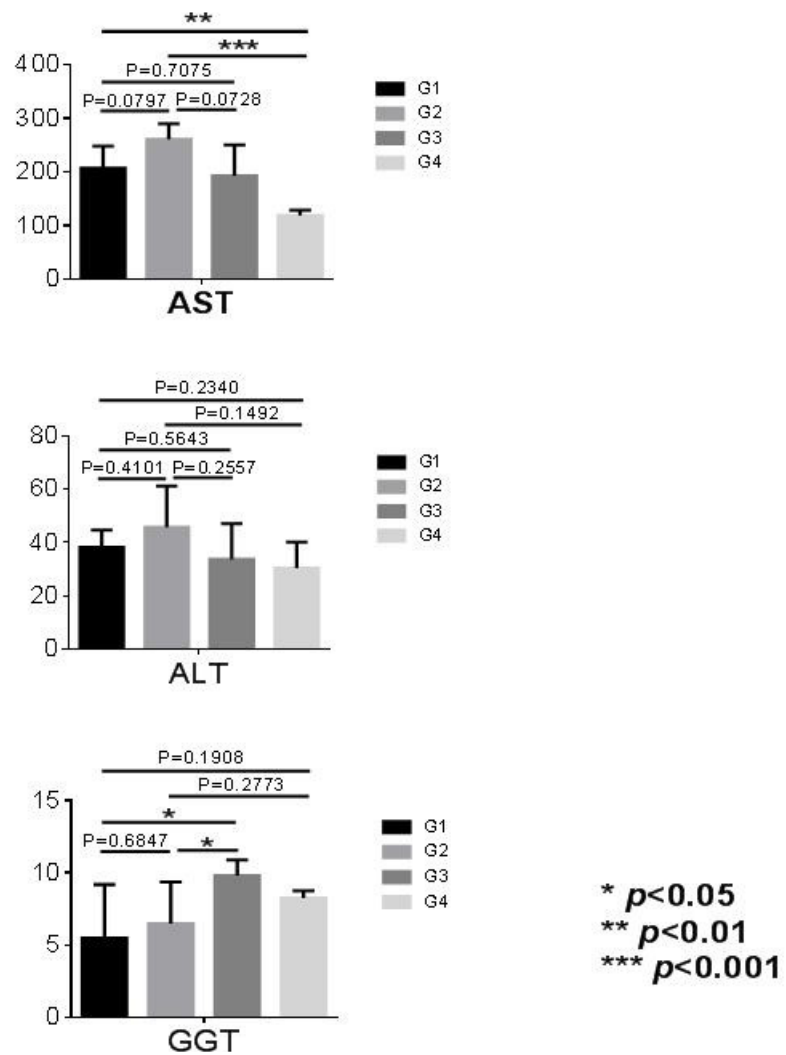
Tested samples of the aragonite, aragonite + calcite, calcite were different in their profiles.

### Amino acid analysis of the shell powder fired at 200°C for 40 min (Aragonite)

Amino acids were detectable at 0.85 g/100 g in the above shell powder by JLC-500V2 analysis (JEOL, Japan).



**Figure 3.** X ray diffraction feature of test sample (bottom; non-fired powder)



**G1;** Control (Physiological saline, ip)  
**G2;** Control (Alcohol, ip)  
**G3;** 10% ethanol/1.0 mLip + Non-fired shell powder, oral administration  
**G4;** 10% ethanol/1.0 mLip + Aragonite shell powder, oral administration  
 \*ip: intra-peritoneal injection

**Figure 4.** Liver protective potency of the aragonite shell powder in the alcohol-fed mice model.

**Table 4.** Aragonite shell powder lowered lipid value in the alcohol-fed C57BL/6 mice model

	Neutral lipid (mil mol. /L)	Total cholesterol (mil mol. /L)
Physiological saline	Median; 1.2 (1.0~1.3)	2.4 (2.0~2.8)
Alcohol alone	Median; 1.7 (1.5~1.9)	4.1 (3.5~4.8)
Alcohol + Non fired Sample	Median; 1.7 (1.6~1.8)	3.8 (3.0~4.5)
Alcohol + Fired A Sample	Median; 0.5 (0.3~0.6)	2.6 (2.3~2.9)

*Conchiolin* secreted by a mollusc's outer epithelium4) was not burnt out even after 200°C for 40 min heat treatment. Amino acids detected were aspartic acid 16.5% (w/w), glycine 36.5%, tyrosine 8.2%, phenylalanine 5.9%, glutamic acid 4.7% and proline 4.7%. No amino acid was detected in the 500°C fired sample. *Conchiolin* was a part of matrix of organic macromolecules, mainly proteins and polysaccharides to hold and bind the crystals of aragonite to give shells their stiffness. Its role was known to facilitate calcification during shell formation (Cariolou and Morse, 1988), but bio-logical function remains unclear up to now. No amino acid was undetectable in the sample fired at 500°C for 120 min.

#### Improvement of liver dis-function by the fired shell powder (Aragonite) in the alcohol-fed mice model

Shell powder fired at 200°C for 40 min (G4 group; aragonite group) effectively worked by lowering of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) values in the alcohol-fed mice, and  $\gamma$ -GT ( $\gamma$ -glutamyl transferase) value was at low level against that of non-fired shell powder treated group (G3). Non-fired aragonite shell powder also demonstrated activity but it was faint (Figure 4).

#### Lipid lowering potency of the aragonite fired at 200°C for 40 min in the alcohol-fed mice model

Aragonite shell powder was orally given to the alcohol-fed mice for 18 serial days to assess lipid lowering efficacy, and outcomes were summarized in Table 4. Fired aragonite-fed mice lowered lipid level of neutral lipid and total cholesterol against those of saline-fed, alcohol-fed, and non-fired shell powder-fed mice.

## DISCUSSION

In the previous experiments we had demonstrated that the calcite type of shell powder prepared from the bivalve *Corbicula japonica* by firing at 500°C was much effective to improve mal-function of liver in the LEC rat hepatitis mode (Sasaki et al., 2011). Our present great concern

was to know whether there exists some effectiveness on the aragonite type of shell powder, because the aragonite type of shell powder was discarded as waste.

Aomori prefecture is a well-known site to produce the high quality *Corbicula japonica*, and the meat or meat-extracts are also a trade mark product in Japan. While the fishermen around the lake have a long period known that the fired shell powder of *Corbicula japonica* has beneficial potency to improve mal-function of liver, and they are using it as a folk medicine without any scientific evidences until now.

Present studies clarified that the aragonite type of shell powder, regardless of fired or non-fired, had similar potency to lower AST (aspartate aminotransferase) and ALT (alanine aminotransferase) value in alcohol-fed mice (Figure 4), even though its activity of the non-fired (naïve) was somewhat feeble in anti-deterioration activity.

Additional prominent activity of the aragonite was to lower serum lipid levels. Neutral lipid and total cholesterol values in the aragonite-fed mice were reduced their values from 1.7→0.46 mil mol (neutral lipid) and 4.1→2.6 mil mol (cholesterol) against the values of alcohol-fed control mice as seen in Table 4.

Persuasive considerations are not clearly presented on why the aragonite or calcite shell powder works for liver protection by lowered the hepatocellular enzyme and serum lipid level in the alcohol-fed animal model. We think now that the shell powder solubility may affect to bio-activity elevation *in vivo* (Figure 4, Table 4). By the cell culture analysis of human adipocytes, increasing calcitriol (a form of vitamin D that is used to treat and prevent low levels of calcium in the blood) can increase lipogenesis, decrease lipolysis, and increase messenger RNA expression of a number of fat-metabolism-related genes by stimulating calcium influx (Boon et al., 2005), however animal level experiments will be required to establish acceptable standardized explanation in near future.

The *Corbicula japonica* calcite worked protectively against the alcohol-led liver degeneration by enhancement of the liver cell proliferation (Sasaki et al., 2011), and the aragonite type of shell powder appears working similarly to restore the alcohol-damaged liver cells (by enhancement of cell proliferation?).

The aragonite or the calcite shell powder of *Corbicula japonica* is a very fascinating calcium material armed with

the important bio-functions, even though unsolved problems still remained.

## CONCLUSION

Aragonite type of *Corbicula japonica* shell powder prepared by description above demonstrated such bio-activities as lowering of AST and ALT values, along with decrease of levels of neutral lipid and cholesterol in the alcohol-fed mice model. This newly-created material, the aragonite type of shell powder, will be expected to contribute as applicant for the health control material likely to be the calcite type of *Corbicula japonica* shell powder.

## Conflict of Interest

All authors explicitly state no conflicts of interest including financial and non-financial.

## ACKNOWLEDGEMENT

The authors gratefully acknowledged the Aomori Clean Tech. Ltd. for the financial supports to conduct the

present studies, and state the equally sharing of experimental findings and information obtained in this study.

## REFERENCES

- Boon N, L.L.J. Koppes, W.H.M. Saris, W. Van Mechelen (2005). The relation between calcium intake and body composition in a Dutch population *Am J Epidemiol* 162 (1): 27-32
- Cariolou MA, DE. Morse (1988). Purification and characterization of calcium-binding conchiolin shell peptides from the mollusk, *Haliotis rufescens*, as a function of development. *J. Comp Physiol. B* 157; 717-729
- Masuda R., MC. Yoshida, M. Sasaki, K. Dempo, M. Mori (1988). High susceptibility to hepatocellular carcinoma development in LEC rats with hereditary hepatitis *Jpn J Cancer Res* 79; 828-835
- Sasaki J, M. Wang, L. Liu, J. Wang, H.Uchisawa, C. Lu (2011). Fired shell powder of bivalve *Corbicula japonica* improves mal-function of liver – Possible development of multi-functional calcium – *J. US-China Medical Science*, 8(6); 449-457
- Yamamuro K, I. Koike (1993). Nitrogen metabolism of the filter-feeding bivalve *Corbicula japonica* and its significance in primary production of brackish lake in Japan *Limnology and Oceanography* 38; 997-1007.