

The Effect of High Hydrostatic Pressure Treatment on the Preservability and the Immunological Activity of Bovine Colostrum

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ABSTRACT : Bovine colostrum, which contains a large quantity of immunoglobulins, is indispensable for newborn animals. The establishment of a new procedure for preserving colostrum without losing the immunological activity is significant. We examined the effect of high hydrostatic pressure treatment (100-500 MPa) on the preservability and the immunochemical activity of bovine colostrum. When high hydrostatic pressure treatment was 300 MPa or more, the increase of the total viable count, coliforms and psychrotrophic gram-negative bacteria was suppressed effectively. In particular, the number of coliforms in colostrum treated at 300 MPa or more hardly increased for 35 days at 4°C. At 400 MPa or more, both gelling of the colostrum and denaturation of immunoglobulins were observed. However, if the pressure was 300 MPa, immunoglobulins were scarcely influenced and the neutralizing titers against the bovine coronavirus did not decrease. Therefore, it was suggested that 300 MPa was the best pressure for good preservability of colostrum without reducing the immunochemical response. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 9 : 1323-1328)

Key Words : Bovine Colostrum, High Hydrostatic Pressure, Preservability

INTRODUCTION

Bovine colostrum, which contains a large quantity of immunoglobulins, is indispensable for newborn animals. Sakai (1994) suggested supplying preserved surplus colostrum for newborn calves instead of bad quality colostrum secreted by the mother, and for calves infected with the rotavirus or coronavirus it might also be effective. To preserve colostrum without losing the immunological activity, which is likely to become inactivated by heating, several treatments such as freeze-drying and biopreservation by addition of lactic starter culture have been tried (Takahashi et al., 1979; Hashiguchi and Hatta, 1981). The purpose of this study was to kill bacteria in colostrum without a great loss in the immunological activity. We examined the effect of high hydrostatic pressure treatment on the preservability of colostrum and obtained some promising results.

MATERIALS AND METHODS

Colostrum

The tested colostrums were obtained within 24 hours after parturition from the dairy farm at the College of Bioresource Sciences, Nihon University, Japan.

High hydrostatic pressure treatment

A Cold Isostatic Press (CIP: internal volume 280 ml, 7,000 kgf/cm² pressurizing type) manufactured by

Nikkiso Co., Ltd. was used. Each 100 ml of the tested colostrum was poured into five polyethylene bags and sealed tightly by a heat sealer. The sealed bags were then inserted in the CIP vessel. Test pressures were 100, 200, 300, 400 or 500 MPa. The holding time was 10 minutes, and the pressure vessel was maintained at room temperature.

Bacterial counts

We determined bacterial counts of the treated colostrums stored at 4°C by an agar plate count method. A standard plate count agar (Nissui Seiyaku Co., Ltd.) was used to estimate the total viable cell count. The agar plates were incubated at 30°C for 48 hours. Desoxycholate agar (Nissui Seiyaku Co., Ltd.) was used to estimate the coliform count. The culture condition of the plates was 37°C for 24 hours. CVT agar (Nissui Seiyaku Co., Ltd.) was used to determine the psychrotrophic gram-negative bacterial count. A number of red colonies were counted after 7 days of incubation at 20°C.

Whey fraction and its fractionation

The treated colostrums were diluted to a 1:9 volume of distilled water. After adjusting the pH to 4.6, they were warmed at 40°C for 20 minutes and cooled at 4°C for one hour. The whey fraction was obtained by filtration (No. 5C filter paper: Advantec MFC).

The non-casein N fraction and the globulin N fraction of the treated colostrums were fractionated using the Rowland method (Rowland, 1938).

SDS-polyacrylamide gel electrophoresis

Polypeptide patterns of the whey fractions obtained from the treated colostrums were compared with a 3

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to 15% gradient slab, SDS-PAGE (Lambin et al., 1976). The whey fractions were heated at 100°C for 5 minutes after adding the same volume of 0.05 M Tris-HCl buffer containing 2.5% SDS (pH 6.8) and β -mercaptoethanol (5% of final volume).

Immunoblotting procedure

After the SDS-PAGE, the polypeptides of the whey fractions were transferred to a PVDF membrane using a Western blotting procedure (Towbin et al., 1979). After being treated with Block Ace solution (Dainippon Pharmaceutical Co., Ltd.) and Avidin Biotin Blocking reagent (Vector Laboratories, INC.), the PVDF membrane was immersed in a biotin-labeled anti-bovine IgG solution (EY Laboratories, INC., 1.25 mg/ml). After washing with TTBS (0.01 M - Tris, 0.15 M - NaCl, 0.1% Tween 20, pH 7.5), the PVDF membrane was immersed in HRP streptavidin solution (Vector Laboratories, INC.). The H-chain of the IgG on the PVDF membrane was colored using a POD immunostaining set (Wako Pure Chemical Industries, Co. Ltd.).

Neutralization test against bovine coronavirus

The treated colostrums were examined for immunochemical effectiveness against the bovine coronavirus. Bovine coronavirus, Kakegawa strain and MA 104 (embryonic kidney cells of rhesus monkey [*Macaca mulatta*]) as the object cells were used in this experiment. The result of the neutralization test was expressed as the reciprocal of the maximum dilution of the treated colostrums showing suppression of the cytotoxic activity of the bovine coronavirus.

RESULTS AND DISCUSSION

Bacteriological examination

As shown in figure 1, there was little or no effect of the high hydrostatic pressure treatment at 100 MPa on the total viable cell count of the test colostrum. Although the bacterial count decreased immediately after the treatment at 200 MPa, the surviving bacteria began rapid multiplication during refrigeration. The treatment at 300 MPa or 400 MPa was found effective to suppress bacterial growth for 9 days at 4°C.

Psychrotrophic gram-negative bacteria are considered mainly responsible for the microbiological deterioration of colostrum stored in a refrigerator. The day after treatment, the count of these bacteria in the colostrum treated at 200 MPa was almost the same as that at 300 MPa. However, at 200 MPa, multiplication was observed after three days of refrigeration (figure 2). Therefore, the high hydrostatic pressure treatments at 300 MPa or more were considered necessary to repress the growth of psychrotrophic gram-negative

bacteria during refrigeration.

Figure 3 shows that most coliforms in the test

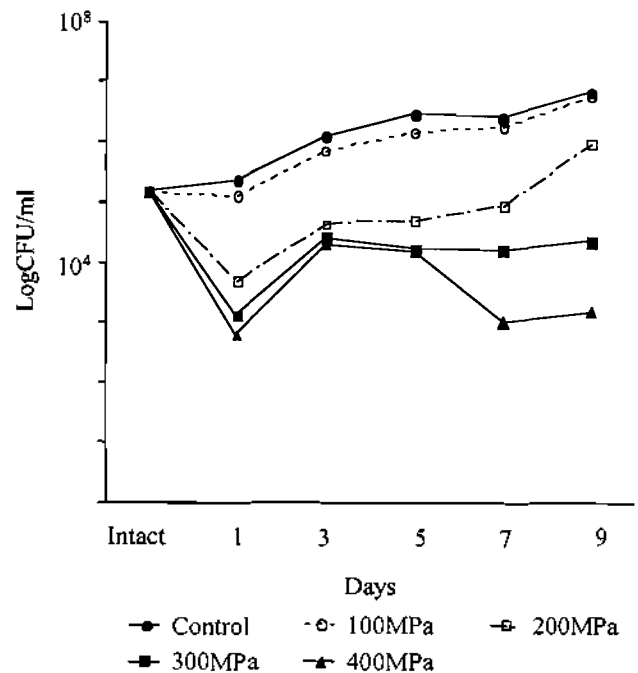


Figure 1. The effect of high hydrostatic pressure treatment on the total viable count in the treated colostrums stored at 4°C

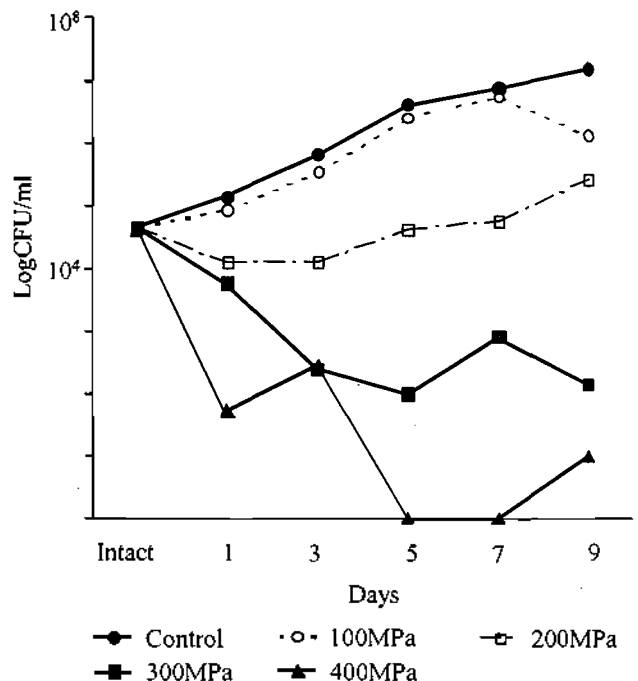


Figure 2. The effect of high hydrostatic pressure treatment on the count of psychrotrophic gram-negative bacteria in the treated colostrums stored at 4°C

colostrums died after high hydrostatic pressure treatment at 300 MPa or more, and it was confirmed that the count of coliforms did not increase afterwards.

The bacterial counts of the treated colostrums preserved in the refrigerator for 35 days are shown in figure 4. There were no coliforms in the colostrum treated at 300 MPa, and there were only a few psychrotrophic gram-negative bacteria in the colostrum treated at 400 MPa. Meanwhile, the total viable cell count of the colostrum treated at 300 MPa or 400 MPa was confirmed to increase considerably. There might be a possibility that psychrotrophic spore-forming bacteria participate in this increase because it was said that they have a remarkable tolerance for high hydrostatic pressure treatment (Raabe and Knorr, 1996; Roberts and Hoover, 1996; Mills et al., 1998; Raso et al., 1998).

Effect of high hydrostatic pressure on whey proteins

It was reported that a conformational change occurred when protein was treated with high hydrostatic pressure (several hundred MPa), and solidification or gelling of protein was promoted (Lambier et al., 1983). Colostrum contains a large amount of pH 4.6 soluble whey proteins. We examined the influence of high hydrostatic pressure treatment on the content of the non-casein N fraction. Figure 5 shows the contents of the non-casein N fraction in the treated colostrums in comparison with that in intact colostrum as a control. The content of the non-casein N fraction hardly changed within the

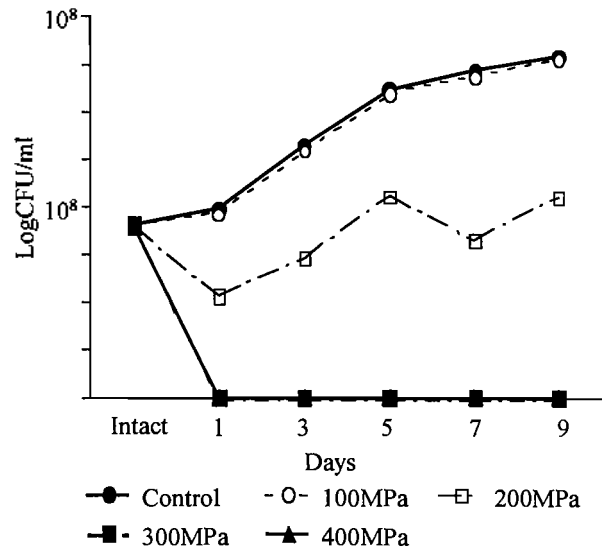


Figure 3. The effect of high hydrostatic pressure treatment on the count of coliforms in the treated colostrums stored at 4°C

range of 100 to 300 MPa, but it became about 80% and 50% of the control at 400 MPa and 500 MPa, respectively. The gel formation in colostrum treated at 500 Mpa was observed macroscopically. Therefore, it was shown that whey proteins, which were important components in colostrum, were found to denature at a high hydrostatic pressure treatment of 400 MPa or more, and suggested that a part of whey protein became insoluble at pH 4.6.

Figure 6 shows the influence of the high

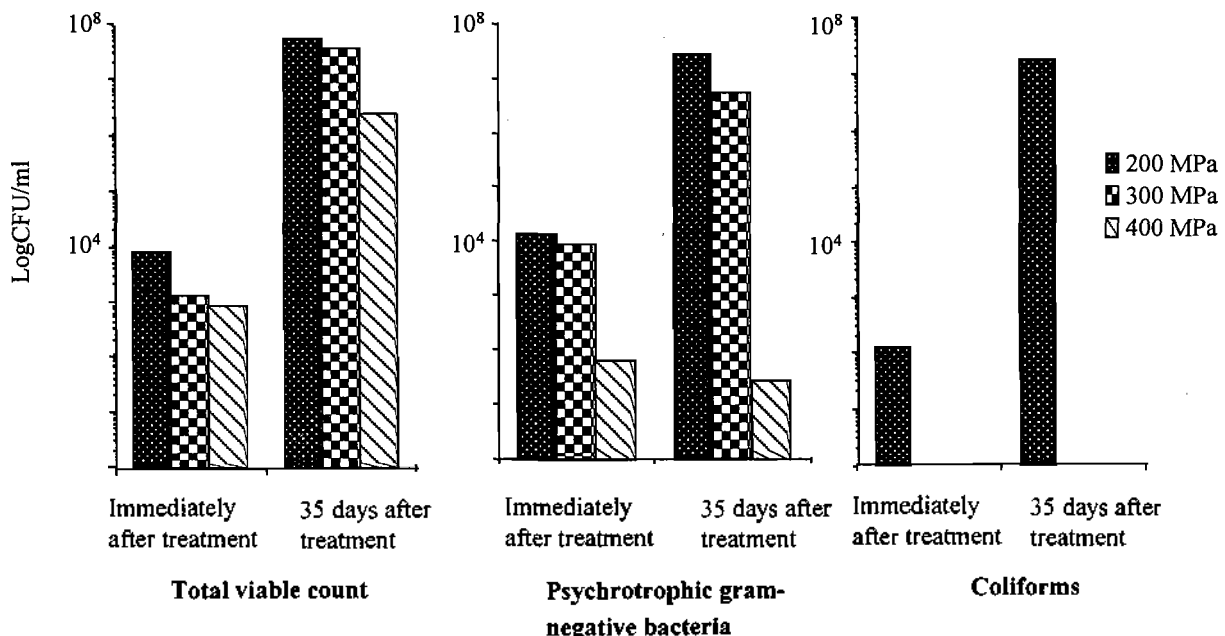


Figure 4. The bacterial counts of high hydrostatic pressure treated colostrums preserved in the refrigerator for 35 days

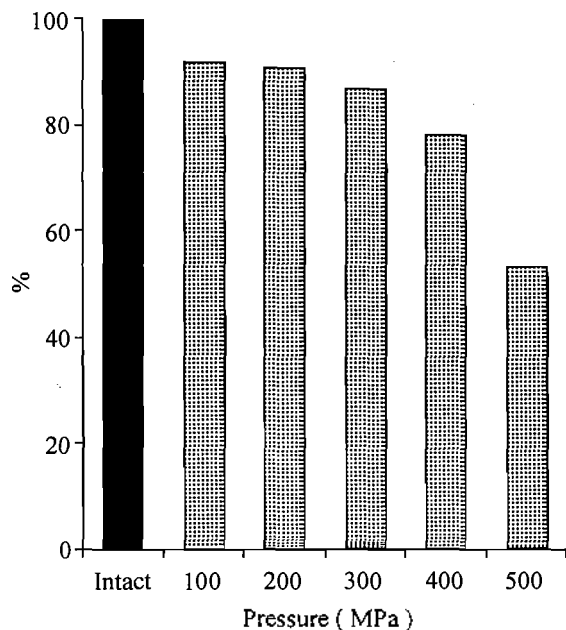


Figure 5. The influence of high hydrostatic pressure treatment on the content of the non-casein N fraction in the treated colostrums. The contents are given as a percent of the content of intact colostrum.

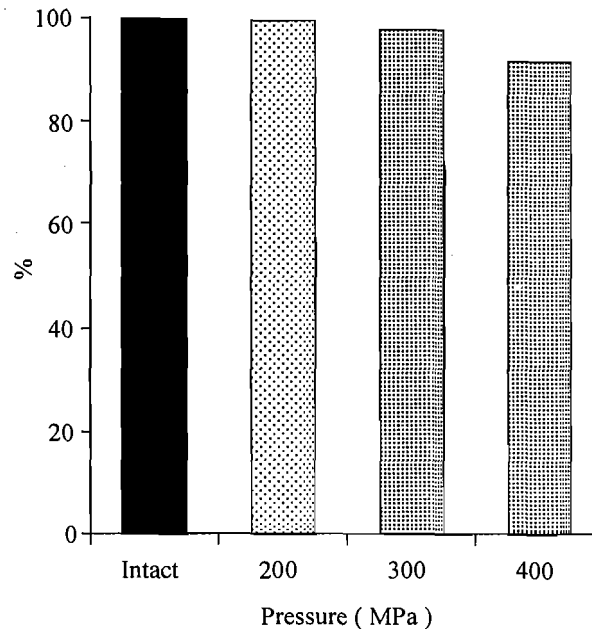


Figure 6. The influence of high hydrostatic pressure treatment on the content of the globulin N fraction in the treated colostrums. The contents are given as a percent of the content of intact colostrum.

hydrostatic pressure treatment on the content of the globulin N fraction. The content of the globulin N fraction was slightly decreased of a high hydrostatic pressure treatment of 200 to 400 MPa.

SDS-PAGE

Comparing the polypeptide pattern of the colostrum treated at 300 MPa or more with that of an untreated one, it was confirmed that little difference existed even if the colostrum was treated by high hydrostatic pressure (figure 7). When observed in detail, the band of β -lactoglobulin became narrow on the SDS-PAGE pattern for the colostrum treated at 300 MPa or more. Although we speculated that immunoglobulins were easily influenced by the high hydrostatic pressure treatment because it was said that immunoglobulins are most susceptible to heat treatment in whey proteins (De Wit and Klarenbeek, 1984), and the effect of high hydrostatic pressure treatment on whey proteins resembles that of heat treatment (Kadharmestan et al., 1998), it was shown that immunoglobulins are not as affected by high hydrostatic pressure treatment as β -lactoglobulin, which was described to be affected easily by high hydrostatic pressure treatment (Hayashi, 1987).

Immunochemical examination

Newborn calves do not have the antibody transferred through the placenta and cannot produce strong neutralizing antibodies in the body until about

four weeks after birth. If an antibody in colostrum and normal milk covers the mucous membrane of the intestinal tract at any time, it can defend against infection by various kinds of virus and bacteria. Therefore, the immune system, so-called milk immunity (Porter et al., 1970), has valuable effects and newborn calves must consume high quality colostrum immediately after birth. However, there are all kinds of colostrums (i.e., the content of immunoglobulin varies from 3.0 to 39.5 mg per ml of colostrum). There are many reports about utilizing various types of treated colostrums (e.g., heated colostrum, frozen colostrum, freeze-dried colostrum and colostrum containing propionic acid as preservative) as substitutes for fresh colostrum. However, the heated colostrum can not be expected to have enough milk immunity due to the deterioration of immunoglobulin and inactivation of the antibodies. Frozen colostrum and freeze-dried colostrum need pretreatment before use. In this experiment with colostrum treated by high hydrostatic pressure treatment, the response to anti-bovine IgG changed with the increase of pressure, but the reaction site for anti-bovine IgG was clearly confirmed even when the pressure was 400 MPa (figure 8).

Bovine coronavirus is a common virus detected in the diarrhea of calves as is the bovine rotavirus. It is known that the diarrhea caused by the bovine coronavirus develops in calves as early as 6 months of age, and it is said that the bovine coronavirus

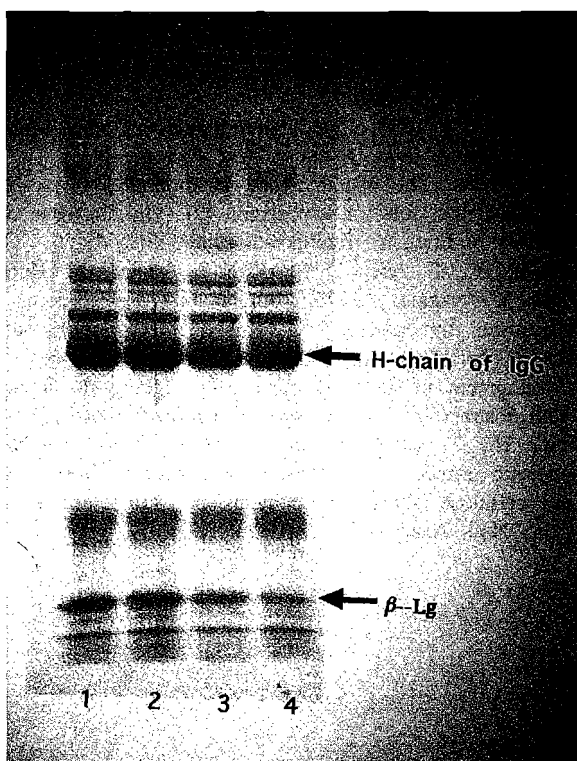


Figure 7. SDS-gel electrophoresis of the whey fraction obtained from colostrums treated with high hydrostatic pressure on a 3 to 15% polyacrylamide gradient slab gel. Line 1, intact whey protein; line 2, treated at 200 Mpa; line 3, treated at 300 Mpa; line 4, treated at 400 Mpa. All samples were denatured by SDS and β -mercaptoethanol.

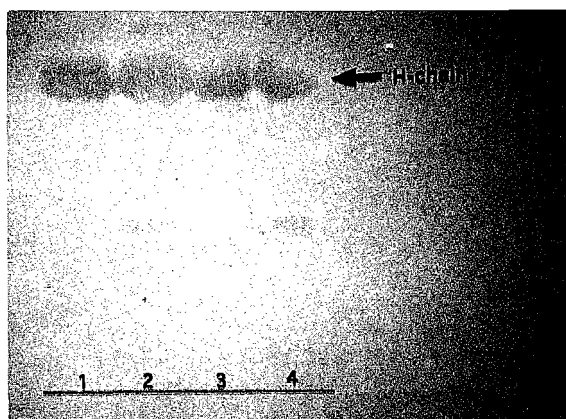


Figure 8. Immunochemical detection of IgG in colostrum treated with high hydrostatic pressure. Line 1, intact colostrum whey; line 2, treated at 200 MPa; line 3, treated at 300 MPa; line 4, treated at 400 MPa.

shows more serious diarrhea conditions than the bovine rotavirus. As presented in figure 9, the titer of neutralizing antibody against the bovine coronavirus

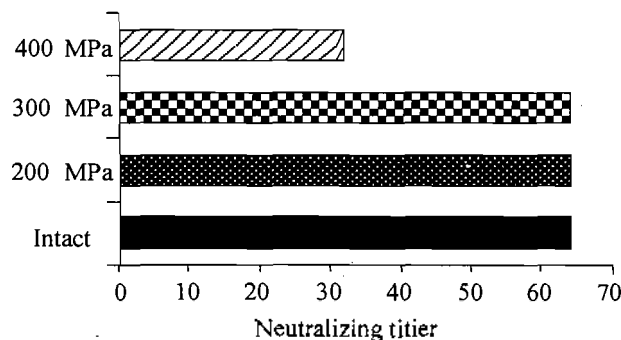


Figure 9. The influence of high hydrostatic pressure treatment on colostrum for the neutralizing ability against the bovine coronavirus. The neutralizing titer showed the reciprocal of the maximum dilution of the colostrums showing a suppression of the cytotoxic activity of the bovine coronavirus.

did not change even when colostrum was treated at 300 Mpa.

When the high hydrostatic pressure treatment was 300 MPa or more, an increase in the total viable cell count, coliforms and psychrotrophic gram-negative bacteria was suppressed effectively. In particular, the number of coliforms in the colostrum treated at 300 MPa or more hardly increased for 35 days at 4°C. At 400 MPa or more, both gelling of the colostrum and denaturation of immunoglobulins were observed. However, if the pressure was 300 MPa, immunoglobulins were scarcely influenced and the neutralizing antibody against the bovine coronavirus did not decrease. Therefore, it was suggested that 300 MPa was the best pressure for good preservability of colostrum without reducing the immunochemical response.

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