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Comparison of Effects of High-Pressure Processing and Heat Treatment on Immunoactivity of Bovine Milk Immunoglobulin G in Enriched Soymilk under Equivalent Microbial Inactivation Levels

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Immunoglobulin-rich foods may provide health benefits to consumers. To extend the refrigerated shelf life of functional foods enriched with bovine immunoglobulin G (IgG), nonthermal alternatives such as high-pressure processing (HPP) may offer advantages to thermal processing for microbial reduction. To evaluate the effects of HPP on the immunoactivity of bovine IgG, a soymilk product enriched with milk protein concentrates, derived from dairy cows that were hyperimmunized with 26 human pathogens, was subjected to HPP or heat treatment. To achieve a 5 log reduction in inoculated *Escherichia coli* 8739, the HPP or heat treatment requirements were 345 MPa for 4 min at 30 °C or for 20 s at 70 °C, respectively. To achieve a 5 log reduction in natural flora in the enriched soymilk, the HPP or heat treatments needed were 552 MPa for 4 min at 30 °C or for 120 s at 78.2 °C, respectively. At equivalent levels for a 5 log reduction in *E. coli*, HPP and heat treatment caused 25% and no detectable loss in bovine IgG activity, respectively. However, at equivalent levels for a 5 log reduction in natural flora, HPP and heat resulted in 65 and 85% loss of bovine IgG activity, respectively. Results of combined pressure−thermal kinetic studies of bovine milk IgG activity were provided to determine the optimal process conditions to preserve product function.

KEYWORDS: High-pressure processing; bovine milk IgG; immunoactivity; *E. coli* 8739; natural flora

INTRODUCTION

Infections by rotavirus (*1*, *2*), enterotoxigenic *Escherichia coli* (*1*, *3*), *Shigella flexneri* (*4*), and *Cryptospordia* (*5*, *6*) were reported to be clinically preventable by oral administration of cow colostrums. Enrichment of bovine immunoglobulins in infant formula and other foods may help reduce viral and microbial infections (*7*, *8*). Foods enriched with bovine immunoglobulins may provide consumers with improved immune activity. However, bovine immunoglobulins are sensitive to high-temperature processing (*9*−*11*). The *D* values (decimal reduction time) of bovine immunoglobulin G (IgG) were reported to be 90, 170, and 200 s at 80 °C in phosphate-buffered saline, ultrahigh temperature (UHT) sterilized milk, and boiled milk (*9*), respectively. Meanwhile, in the range of 72−80 °C, the *z* values (temperature coefficient) were reported as 6.7, 8.5, and 8.9 °C in phosphate-buffered saline, UHT milk, and boiled milk, respectively. No detectable antibody level survived the retort and UHT sterilization processes (*9*, *12*). Temperatures higher than 73 °C significantly reduced bovine IgG activity (*9*, *10*, *13*, *14*). In addition, the antigen-binding sites in IgG molecules are more sensitive to thermal processing than the rest of the IgG molecule (*15*, *16*). Yolken et al. (*17*) reported that heat treatment caused a significant decrease in anti-rotavirus capacity of antibodies in commercially pasteurized milk. Nevertheless, thermal processing at 62.5 °C for 30 min did not cause a significant loss in IgG activity in human milk (*18*). The addition of thermal protectants, such as glycerol (20%), glutamic

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acid (0.2%), and maltose (20%), may help stabilize bovine IgG in simulated systems (*19*). However, the addition of the thermal protectants may alter the main attributes (such as viscosity and flavor) of the product and also needs to be listed on the product label. Exposure to high temperature has been considered the primary cause of loss in IgG activity. Chen et al. (*19*) suggested that the severity of heat treatments is reduced to retain IgG activity.

Application of nonthermal processing technologies, such as pulsed electric field (PEF) processing (*10*, *11*, *20*−*22*) and high-pressure processing (HPP) (*23*−*25*), to extend the shelf life of foods with immunoglobulins may result in minimal loss in IgG activity. The major benefit of applying HPP in food processing is the fact that HPP can be effective in inactivating microorganisms and certain enzymes while leaving small molecules, such as flavors and vitamins, intact (*26*, *27*). Since the early report of Hite (*28*) on the effects of pressure in milk preservation, efficacy of HPP has been demonstrated in inactivating microorganisms and extending the shelf life of foods (*22*−*24*, *29*). The sensitivity of proteins to HPP varies along with the changes in the molecular structures. Different enzymes showed significantly different sensitivities to HPP. HPP was reported to be effective in inactivating some enzymes such as pectin methyl esterase (*30*, *31*) and lipoxygenase (*27*). HPP at 600 MPa for 1 min at 25 °C reduced lipoxygenase activity from 180 to 0 units/g in diced tomato, while 800 MPa for 5 min at 25 °C showed no significant change in pectinesterase activity (*27*). Nevertheless, there is a lack of literature regarding the effects of HPP on the activity of bovine milk IgG.

The purposes of this study were to investigate the effect of HPP on the activity of bovine milk IgG and to compare the efficacy of HPP and heat treatments. Pressure inactivation of *Escherichia coli* 8739, a potential surrogate of the human pathogen *E. coli* O157:H7 (*32*), and natural flora was determined and compared with heat treatment to define the equivalent HPP process conditions.

MATERIALS AND METHODS

Preparation of Immunized Milk Protein Concentrate Powder. An inactive vaccine was made by heating the media-free bacterial suspension containing 26 potentially disease-causing microorganisms, including *Staphylococcus aureas* (ATCC 11631), *Salmonella enteritidis* (ATCC 13076), *E. coli* (ATCC 26), and *Shigella dysenteriae* (ATCC 11835), in a glass flask at 80 °C overnight. A 5 day 37 °C incubation test was performed to evaluate the viability of the 26 bacteria. The mixed culture was then injected intramuscularly into cows using microencapsulated slow-release vaccine supra 3−4 times weekly at Stolle Milk and Biologics, Inc. The dairy milk was collected from hyperimmunized cows, ultrafiltered, and low-temperature thermally processed under vacuum to concentrate IgG up to 30-fold (*33*). The milk protein concentrate was spray dried at a temperature lower than 60 °C to prevent any impact on IgG activity. The Stolle milk protein concentrate (SMPC) powder was packed and sealed in plastic bags and stored at 4−6 °C before use.

Preparation of Enriched Soymilk Powder (SMBI Soymilk Powder). SMPC powder, MicroLactin (Stolle Milk and Biologics, Inc.), was mixed into soymilk base powder that was purchased from Devansoy Farms (Carroll, IA). The enriched soymilk powder contained 50.8% Devansoy soymilk base powder, 20.4% MicroLactin, 27.1% sucrose, and 0.7% table salt. The enriched soymilk powder was sealed in polyethylene bags and stored at 4 °C until use within 2 h.

Preparation of the Enriched Soymilk. Enriched soymilk powder (29.5 g) was added into 240 mL of preautoclaved water at room temperature and stirred with a magnetic stirring bar. Utensils and water used for hydration were autoclaved at 121.1 °C for 30 min in 1 L glass bottles to ensure sterility. The enriched soymilk was prepared prior to use or stored at 4 °C if it was prepared 2 h or earlier before use.

Table 1. Process Temperatures of the Enriched Soymilk Samples at Different Applied Pressures

target pressure (MPa)	initial temp (T_0) (°C)	maximum temp (T_{max}) (°C)	process temp at the end of the pressure holding time (T_{end}) (°C)
0.01	30.0	30.0	30.0
137.9	25.2	31.1	30.5
158.6	24.4	30.5	30.2
231.0	21.9	29.7	28.7
324.2	18.7	30.2	29.3
413.8	15.5	30.4	29.4
503.5	12.4	29.8	28.9
600.0	9.0	29.6	28.6
689.7	6.0	30.2	29.4

Preparation of *E. coli* 8739 Culture and Inoculation of the Samples. The *E. coli* 8739 storage culture was obtained from Silliker Laboratories (Columbus, OH) and stored at −80 °C in a deep freezer until use. The frozen culture was first reactivated by transferring one loop into three tryptic soy broth (TSB) tubes (10 mL each) and incubated at 35 ± 2 °C for 24 h. The procedure was repeated twice to ensure the recovery of *E. coli* cells. The incubated culture was transferred into 100 mL of TSB broth at an inoculation rate of 2% (v/v) and incubated at 35 ± 2 °C for 18 h to make the starter culture. The starter culture was inoculated into 500 mL of TSB at a ratio of 5% and incubated at 37 °C for 10 h to make the working culture (10^9−10^{10} cfu/mL). The working culture was centrifuged at 2500*g* and 23 °C with a refrigerated high-speed tabletop centrifuge (MR18-12, Long Island Scientific, Port Jefferson, NY) to harvest the cells for soymilk inoculation. *E. coli* cells were harvested right before inoculation of the enriched soymilk. The enriched soymilk samples inoculated with *E. coli* 8739 work culture at an initial count of approximately 10^9−10^{10} cfu/mL were subjected to HPP at a processing pressure up to 600 MPa with a holding time up to 8 min or thermal treatments.

Incubation of Natural Flora at Room Temperature in the Enriched Soymilk. The prepared enriched soymilk was stored in loosely capped 1 L glass bottles at room temperature as described previously (*10*) within an Airclean work station. The workstation was sterilized using a surface swab with 70% alcohol and exposure to UV light for 30 min. Sterilization of the work station was maintained by blowing clean air vertically (from top to bottom). The air was filtered through a 0.23 μm HEPA filter. The total plate count of the samples was monitored along the incubation time to ensure a natural flora population ranging from 10^6 to 10^8 cfu/mL.

HPP of the Enriched Soymilk. The incubated *E. coli* 8739 culture of 100 mL was transferred into two 50 mL centrifuge tubes, and the cells were harvested. The cells were inoculated into 500 mL of soymilk and stirred with a sterile magnetic bar. A 200 mL plastic bag was filled with 50 mL of inoculated soymilk and heat sealed with minimal headspace in the package. The sealed package was then double packed in another 200 mL plastic bag vacuum packed at −97 kPa using a MC-30 sealer (Sipromac Inc., St. Germain Grantham, Quebec, Canada).

The processing temperature was maintained at 30 ± 2 °C by adjusting the initial temperatures of sample and pressure transmitting fluid (glycol/water ratio 1/1) to T_1 using a water bath and refrigerator

$$T_1 = 30\ ^\circ\mathrm{C} - \Delta T \quad (1)$$

where T_1 is the initial temperature of samples and pressure transmitting fluid and ΔT is the adiabatic temperature increase due to the heat of compression as described by Balasubramanium et al. (*34*) as in eq 2.

$$\Delta T = a \times \Delta P \quad (2)$$

where a is the adiabatic compression heating factor, which is 3.5 °C per 100 MPa for 50% glycol, and ΔP is the pressure applied above atmosphere pressure in MPa. HPP treatment was provided by a QFP-6 unit (ABB Autoclave Systems Inc., Columbus, OH) with a 2 L pressure chamber.

The HPP of the enriched soymilk sample for the purposes of investigation of IgG immunoactivity was also provided by the same QFP-6 HPP unit under the same conditions as those for *E. coli* inactivation studies. These noninoculated samples were vacuum packed at −97 kPa in a single layer plastic bag of 200 mL and then double packed. The effect of HPP on bovine IgG immunoactivity was investigated using a three-factor three-level orthogonal experimental design, L_9 (3^3). The factors and their levels were pressure (413.8, 551.8, and 689.7 MPa), holding time (2, 4, and 6 min) and holding temperature (30, 45, and 60 °C). Each treatment was duplicated on a different date. After HPP, the samples were aseptically transferred into sterile 15 mL VacuTainer blood collection tubes (Becton Dickinson and Company, Franklin Lakes, NJ) and shipped to Stolle Milk and Biologics Inc. under refrigeration for immunoactivity and antigen-binding activity analyses.

Heat Treatment of the Samples. Heat treatment of the enriched soymilk samples, either inoculated or noninoculated, was provided by a set of thermal death time (TDT) tubes made by Washington State University (Pullman, Washington) from aluminum with a capacity of 1.5 mL (*35*). The soymilk sample formed a thin layer between the inner and the outer cylinders of the TDT tube. The sample temperature was monitored in two control tubes with *k* type thermocouples running through the lid and connected to a thermocouple reader. All TDT tubes were immersed in the same water bath at the designated temperature simultaneously. Treatment time was measured from the time when samples reached the target temperature. Samples were removed from the water bath at selected intervals and immediately immersed on ice to stop further heating. Samples for IgG activity studies, 50 mL packed in a 200 mL plastic pouch, were immersed into a 75.5 or 78 °C water bath for a designated time and immediately cooled in an ice water bath after treatment. Each treatment was repeated three times on the same date.

Enumeration of *E. coli* 8739. *E. coli* 8739-inoculated soymilk samples were diluted to a suitable level with 0.1% peptone water and plated on the surface of violet red bile agar (VRBA). The inoculated plates were incubated at 35 ± 2 °C for 48 h. The typical pink colonies of *E. coli* 8739 on VRBA plates were counted. It is worth noting that the injured cells may not recover on the VRBA plates and the plate counts may be lower than the actual number of total viable *E. coli* 8739 cells in a sample.

Determination of Natural Flora. The aerobic total plate count in the samples was determined using standard plate count agar (PCA) by spreading the samples at suitable dilution levels on the surface of the agar and incubating at 37 °C for 48 h; the number of colonies was counted.

Measurement of IgG Immunoactivity. Immunoactivity is the capacity of bovine milk IgG molecules to be identified and captured by anti-bovine IgG antibodies to form an antigen−antibody complex when measured using an enzyme-linked immunosorbent assay (ELISA) method as described previously (*10*). The term "immunoactivity" was used instead of "antigen-binding activity" to specify the activity of bovine IgG molecules functioning as antigens for a known anti-bovine IgG, rat monoclonal anti-bovine IgG_{1and2} heavy chain IgG in this study, from the activity functioning as an antibody to capture other antigens, such as *S. enteritidis* in this work. Peroxidase-conjugated AffiniPure Rabbit anti-bovine IgG, F(ab′)2 was used as the detecting antibody. A standard curve was constructed using linear regression (Minitab, version 13.32, State College, PA), showing a maximum OD reading of 2.0−2.5 at 450 nm, a minimum of 0.01−0.5, and a clearly defined linear portion.

Assay of Antigen-Binding Activity of Bovine Milk IgG against *S. enteritidis*. The measurement of specific antigen-binding activity of bovine IgG after treatments in the SMBI soymilk samples was conducted with modified ELISA techniques as described by Dominguez et al. (*16*) and others (*16*, *10*). *S. enteritidis* culture was used as the coating agent to coat the microtiter plates and incubated at 5 °C for at least 18 h overnight. Binding titers for the samples and controls were calculated from the standard curve using sample data points that fall within the linear portions of the standard curve.

Statistical Analysis. Results were analyzed using Minitab version 13.32 (Minitab, Inc.) with $\alpha = 0.05$. One-way and multiway analysis of variance (ANOVA) and Tukey's multicomparison test were performed to identify the significance of influences from each operation parameter and the combinational effects of the variables.

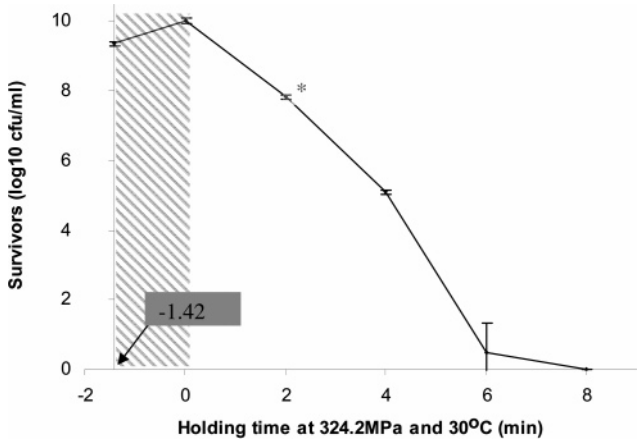

Figure 1. Log survivor of *E. coli* 8739 in the enriched soymilk during HPP at 324.2MPa and 30 °C. The shadowed area in the graph represents the pressure come-up time (1.42 min) for the QFP-6 pilot scale high-pressure unit to bring the pressure up to 324.MPa. "*" indicates the point where inactivation became significant ($p < 0.05$).

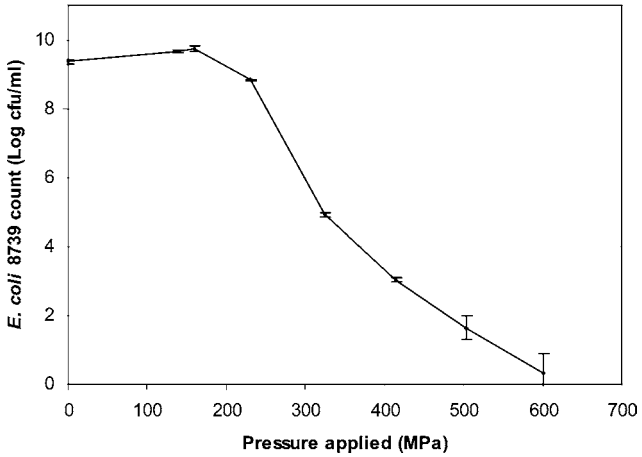

Figure 2. Log survivor of *E. coli* 8739 in the enriched soymilk processed at various pressures at 30 °C for 4 min. Inactivation became significant ($p < 0.05$) when the pressure was higher than 300 MPa.

RESULTS AND DISCUSSIONS

Inactivation of *E. coli* 8739 by HPP at 30 °C or Heat Treatment. The effects of holding time and pressure on *E. coli* 8739 at 30 °C are illustrated in **Figures 1** and **2**, respectively. The *E. coli* 8739 work culture at an initial count of approximately 10^9−10^{10} cfu/mL was subjected to a pressure of 324.2 MPa with a designated holding time. The inactivation effects of HPP against *E. coli* 8739 increased with an increase in holding time (**Figure 1**) or pressure (**Figure 2**) at 30 °C. There was a slight increase in *E. coli* 8739 population (9.2−10.0 logs) at the end of the process come-up time (**Figure 1**), shown as in the samples with a holding time of 0.1 s at a designated pressure. This might be attributed to the breakdown of aggregates formed by living vegetative cells during the process (*36*). The *E. coli* plate counts decreased steadily during a holding time from 0.1 s up to 6 min. However, the rate of reduction of *E. coli* plate count decreased when further increasing the holding time from 6 to 8 min. After treatment at 324.2 MPa and 30 °C holding for 8 min, soymilk samples showed zero plate count on VRBA after incubation at 37 °C for 48 h. As compared to the 0.1 s processing time control

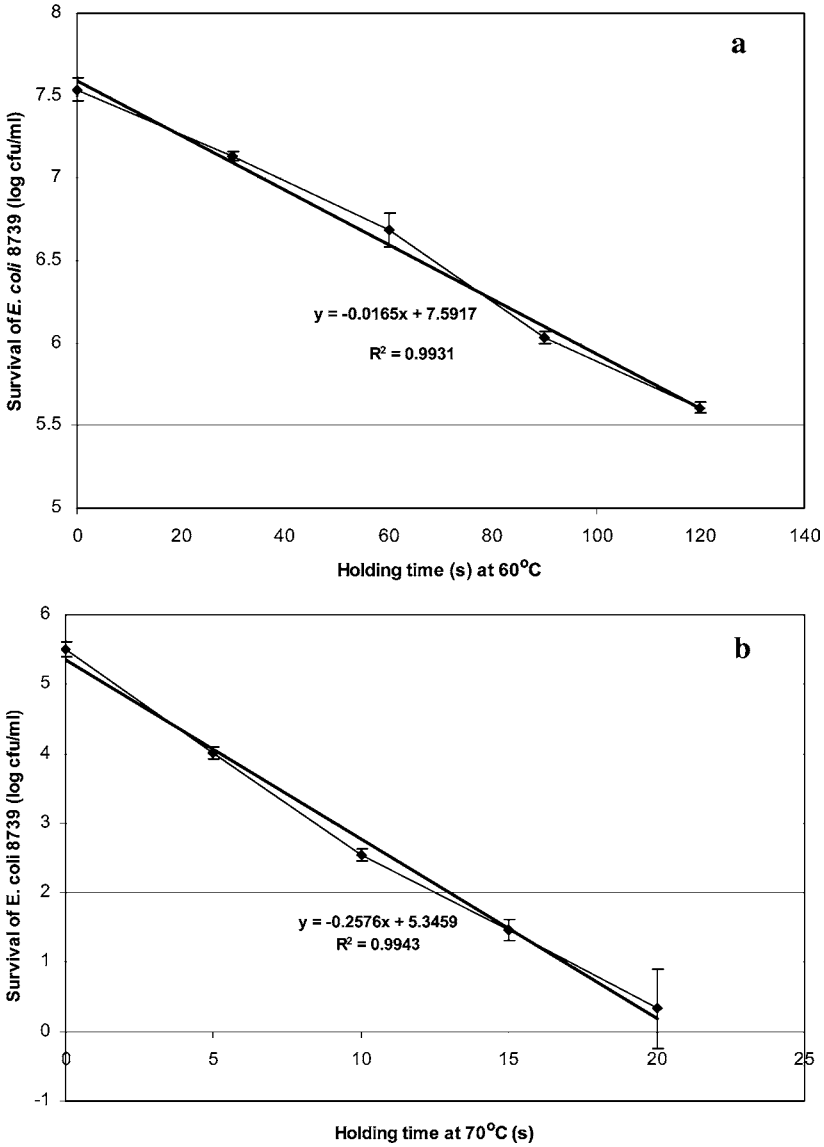

Figure 3. Thermal inactivation of *E. coli* 8739 in the enriched soymilk at (**a**) 60 and (**b**) 70 °C.

samples, a 5 log reduction in *E. coli* 8739 plate counts was achieved when HPP at 324.2 MPa and 30 °C for 4 min (**Figure 2**).

As illustrated in **Figure 2**, pressure had a significant influence on the inactivation of *E. coli* 8739 in the enriched soymilk ($p < 0.01$). There appears to be a critical pressure below which there is no significant inactivation of *E. coli* cells during treatment. For example, for a holding time of 4 min at 30 °C, when pressure was below 206.9 MPa, no significant inactivation of *E. coli* 8739 ($p > 0.05$) was observed. However, when the pressure was greater than 206.9 MPa, significant inactivation of *E. coli* 8739 was observed and the inactivation effects increased along with the increase in the applied pressure. At a holding time of 4 min at 30 °C, the applied pressure required to achieve a 5 log reduction in *E. coli* 8739 was 345. This condition was defined as the equivalent HPP condition for a 5 log inactivation of *E. coli* 8739 to compare with heat processing.

Inactivation of *E. coli* 8739 in the enriched soymilk by heat using the previously described aluminum TDT tubes is illustrated in **Figure 3a**,**b** at 60 and 70 °C, respectively. The log reduction of *E. coli* 8739 against the heating time at a fixed temperature can be described by a first-order model, and the *D* values in the enriched soymilk were 60.6 and 3.9 s at 60 and 70 °C, respectively. Heat treatment was effective in inactivation of *E. coli* 8739, to enhance the safety of the enriched soymilk. To achieve a 5 log reduction in *E. coli* 8739 population, a heat treatment of 60 °C for 303 s or 70 °C for 19.5 s was required.

Inactivation of Natural Flora in the Enriched Soymilk. Effects of HPP on the natural flora developed at room temperature in the enriched soymilk are presented in **Figure 4**. Significant inactivation was observed when the applied pressure was higher than 150 MPa ($p < 0.01$). Samples processed at a designated pressure for a very short holding time of 0.1 s were used as controls to identify the microbial inactivation effect by holding for 4 min. A 5 log reduction of natural flora count (as the aerobic total plate count on PCA) was achieved when the samples were processed at 552 MPa for 4 min at 30 °C. An 8 log inactivation of *E. coli* 8739 was observed under this condition. Inactivation of natural flora increased along with the increase in applied pressure beyond the threshold pressure of 150 MPa ($p < 0.01$). Increasing the pressure in the range of 0.1 to 690 MPa at 30 °C while holding only for 0.1 s did not cause significant inactivation of natural flora ($p > 0.05$). On the other hand, for a 4 min holding time, a 5 log reduction was achieved with 552 MPa at 30 °C. More than an 8 log reduction was observed when the samples were processed at 690 MPa

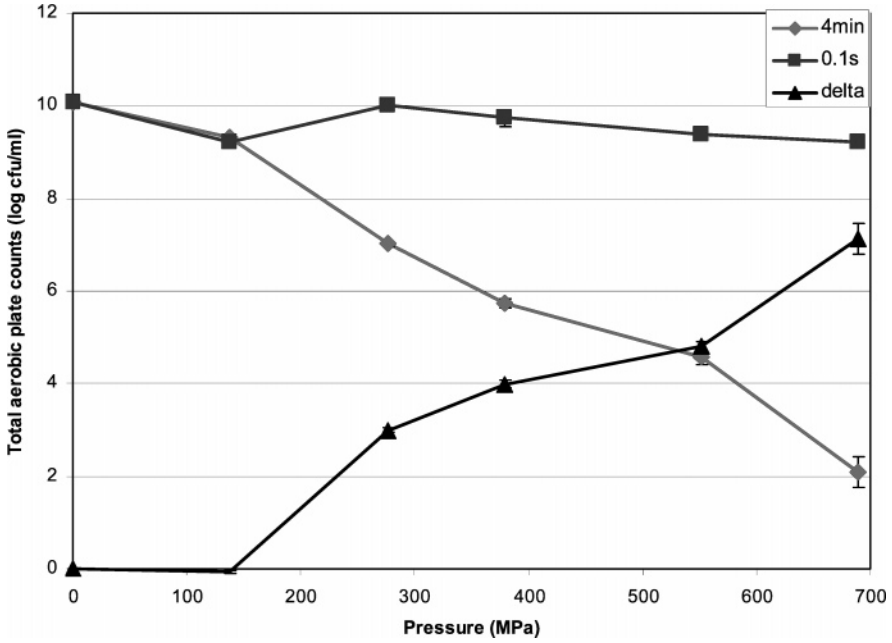

Figure 4. Inactivation of natural flora under different pressures at 30 °C. "Delta" refers to the difference in the aerobic counts between a sample treated for 0.1 or 240 s at the designed pressure.

and held for 4 min at 30 °C. A holding time of <5 min is generally recommended for an economically viable HPP operation (*34*).

Natural flora developed in the enriched soymilk was heat resistant and required a heat treatment at 78 °C for 120 s to achieve a 5 log inactivation during a continuous flow process (*10*). Inactivating natural flora in the enriched soymilk required a much higher processing dosage than inhibiting *E. coli* 8739 (**Figure 3a**,**b**). A target of 5 log reduction to natural flora in soymilk is a conservative goal for food processing. Effects of processing on bovine IgG immunoactivity were evaluated under conditions to achieve 5 log reduction of both *E. coli* 8739 and natural flora.

Effect of HPP on Bovine IgG Immunoactivity in the Enriched Soymilk. The effect of applied pressure for 4 min at 30 °C on the immunoactivity of bovine IgG is illustrated in **Figure 5a**. With an increase in the applied pressure from 0.1 to 689.7 MPa, bovine IgG immunoactivity decreased significantly ($p < 0.001$). However, there was a critical pressure point (275.9 MPa), which was higher than that bovine IgG significantly reduced its immunoactivity (**Figure 5a**). At 689.7 MPa for 4 min, 94% of bovine IgG immunoactivity was destroyed. At 414 MPa, increasing the holding time from 2 to 6 min significantly ($p < 0.05$) reduced the immunoactivity of bovine IgG (**Figure 5b**). Nevertheless, no further significant reduction in IgG immunoactivity was observed when increasing the holding time from 4 to 6 min at 414 MPa (**Figure 5b**). The reduction in bovine IgG immunoactivity took place in the first 4 min of holding time during HPP process. Further extending the holding time did not result in an additional decrease in IgG activity. As illustrated in **Figure 5b**, reduction in immunoactivity of bovine milk IgG along with the increase of holding time revealed a tailing effect.

Combined effects of pressure, holding time, and processing temperature on the immunoactivity of bovine IgG in the enriched soymilk are illustrated in **Figure 6a**,**b**. Two-way ANOVA revealed that applied pressure had a significant influence on the survival of IgG activity ($p < 0.001$), while the processing temperature ($p = 0.196$) and holding time from 2 to 6 min ($p = 0.324$) showed no significant influence at the 95% confidence

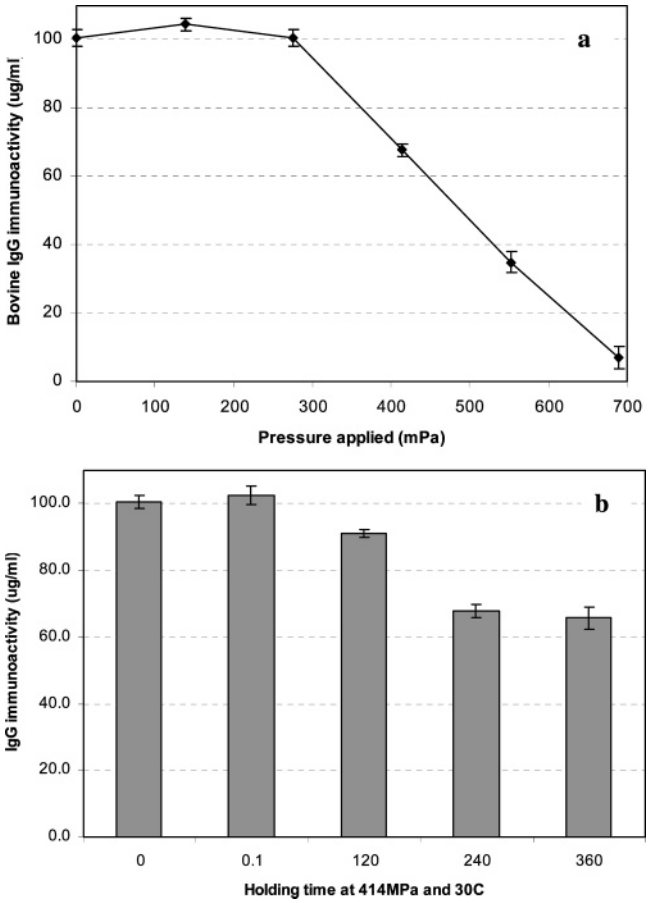

Figure 5. Effect of HPP on bovine IgG immunoactivity at 30 °C: (**a**) different pressure for 4 min of pressure-holding time; (**b**) different holding time at 414 MPa of pressure.

level. A temperature increase from 30 to 60 °C did not show a significant influence. This may be due primarily to the fact that applied pressure was high enough to achieve significant inactivation in 2 min and the kinetics reached its tailing phase after 2 min.

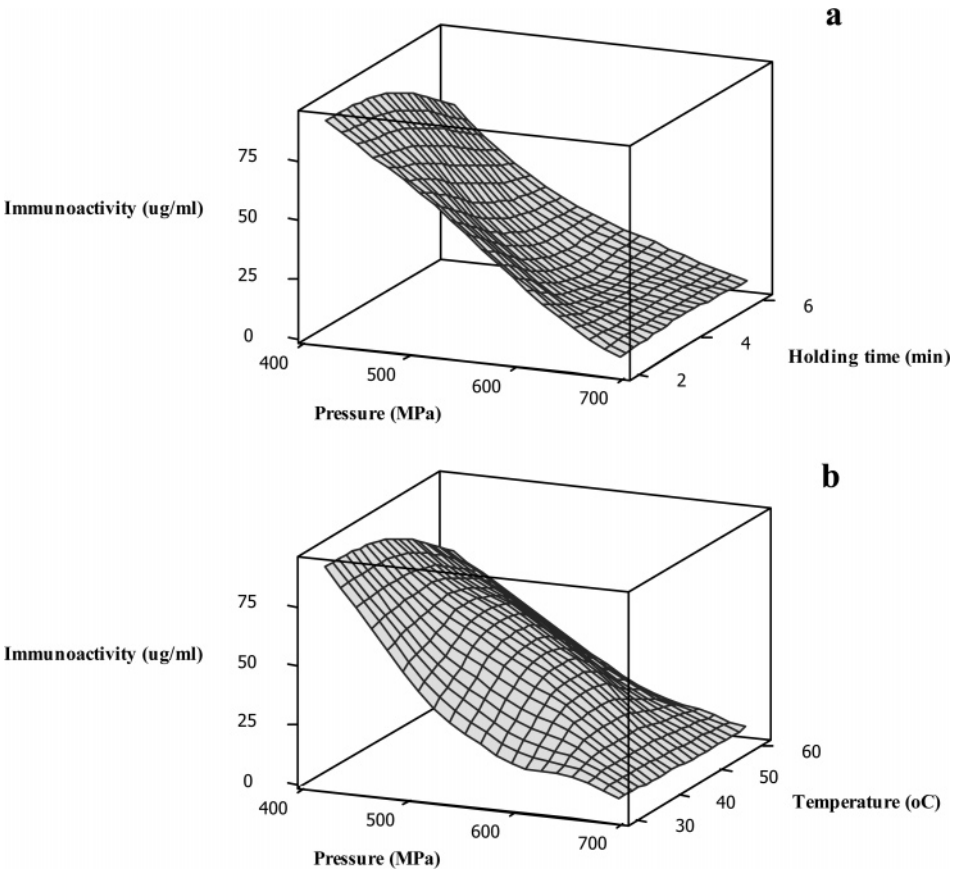

Figure 6. Surface plots (interpolated meshes) for the orthogonal experiment to investigate the combined pressure–temperature effect on immunoactivity of bovine IgG in the enriched soymilk: (**a**) effect of pressure and holding time and (**b**) effect of pressure and processing temperature.

The influence of applied pressure, treatment temperature, and holding time on the IgG immunoactivity in the enriched soymilk can be correlated by a regression equation (eq 3) derived from the data of the L_9 (3^3) orthogonal experiment.

$$\text{IgG } (\mu\text{g/mL}) = 219 - 0.274P - 0.282T - 3.93t \quad (3)$$

where P is the applied pressure (MPa), T is the holding temperature (°C), and t is the holding time (min); the regression p value = 0.001.

Heat treatment showed a significant influence on IgG activity ($p < 0.05$). **Figure 7** illustrates the effects of a batch heat treatment at 75.5 and 78 °C for various time periods. An 85% loss of immunoactivity of bovine (decreasing from 100.6 to 14.9 μg/mL) in the enriched soymilk was observed when treated at 78 °C for 120 s, which was the condition for heat treatment to achieve a 5 log reduction in natural flora (*10*). HPP at 552 MPa for 4 min at 30 °C resulted in a 5 log inactivation of natural flora in the enriched soymilk and reduced 65% of bovine IgG immunoactivity. As compared to heat treatment, HPP resulted in 20% less loss in immunoactivity of bovine IgG when processed to achieve a 5 log reduction in natural flora.

Correlation between Immunoactivity and Specific Antigen-binding Activity against *S. enteritidis* under HPP. The specific antigen-binding activity of bovine IgG against *S. enteritidis* was proportionally correlated with immunoactivity and antigenocity and measured with ELISA (**Figure 8**). The high correlation coefficient ($R^2 = 0.98$) suggests that the ELISA-measured immunoactivity is closely related to the antigen-binding activity of bovine IgG. However, because the immunoactivity measured using ELISA is the ability of bovine IgG to be identified and bound by anti-bovine IgG rat monoclonal IgG, the immunoactivity is determined by the active sites on the two heavy chains in an IgG molecule. It is different, conceptually, from the specific antigen-binding activity of the IgG molecule. The two antigen-binding sites of an IgG molecule are located at the ends of the two variable regions, consisting of both heavy chains and two light chains. The correlation between the immunoactivity and the specific antigen-binding activity suggests that both function sites against rat monoclonal anti-bovine IgG and the pathogen-binding sites at the ends of the variable regions of a bovine IgG molecule are structurally dependent. Measurement of immunoactivity of bovine IgG can be used as an indicator

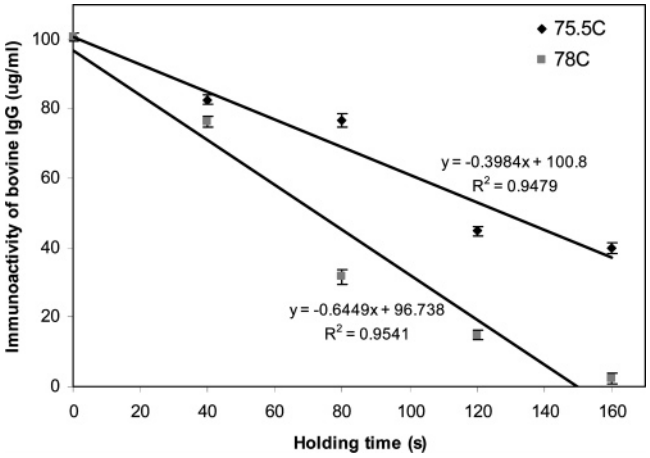

Figure 7. Effect of thermal processing on the immunoactivity of bovine IgG enriched in soymilk (sample size of 50 mL). The samples were vacuum packed in 200 mL plastic bags and immersed in a water bath that stabilized at 75.5 or 78 °C.

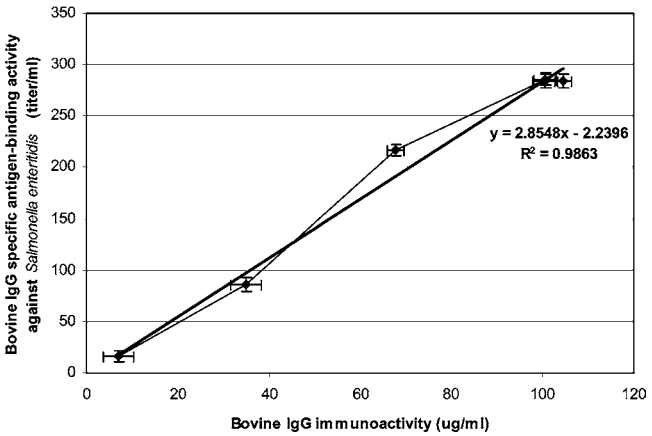

Figure 8. Correlation between the ELISA measured immunoactivity and specific antigen-binding activity against *S. enteritidis* of bovine IgG.

for specific antigen-binding activity. Nevertheless, measurement of immunoactivity of bovine IgG may overestimate the capacity of a bovine IgG molecule to bind its antigens. For instance, when the enriched soymilk was processed under 689.7 MPa at 60 °C holding for 2 min, no detectable specific antigen-binding activity against *S. enteritidis* was observed ($p < 0.01$). However, on the other hand, measurement of immunoactivity of sample indicated that there was 4.98 μg/mL (4.95%) IgG immunoactivity that survived the process.

Conclusions. Both HPP and heat treatment were effective in inactivating *E. coli* 8739 in the enriched soymilk. A 5 log reduction in inoculated *E. coli* 8739 was achieved by HPP at 345 MPa for 4 min at 30 °C or heat treatment at 70 °C for 20 s. Under these conditions, HPP resulted in a 25% loss in immunoactivity of bovine IgG while heat resulted in an insignificant change in immunoactivity. Soymilk natural flora was more resistant to both HPP and heat treatment than *E. coli* 8739 cells. To achieve a 5 log reduction in natural flora in the enriched soymilk, HPP resulted in 65% loss in immunoactivity of bovine IgG while heat resulted in an 85% loss. HPP retained 20% more bovine IgG than heat treatment. Bovine IgG was sensitive to HPP at a pressure greater than 276 MPa or a heat treatment at a temperature higher than 72 °C. The specific antigen-binding activity of bovine IgG in the enriched soymilk was proportionally correlated with its immunoactivity measured against anti-bovine IgG rat monoclonal IgG in the range of 16−105 μg/mL. The measurement of IgG immunoactivity can be used as an indicator of its specific antigen-binding activity.

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