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Investigation of the effects of food constituents on *Bacillus subtilis* reduction during high pressure and moderate temperature

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Abstract

Our published results and our studies for optimization of process conditions to inactivate *Bacillus subtilis* by high hydrostatic pressure and mild heat using response surface methodology indicated that the optimum process parameters for a six-log-cycle reduction of *B. subtilis* were obtained as temperature, 46 °C; pressure, 479 MPa; and pressure holding time, 14 min. Based on the results, response surface methodology (RSM) was employed in the present investigation, the effects of food constituents like soybean protein, soybean oil, sucrose, and pH of food matrix on the *B. subtilis* reduction during high pressure and moderate heat was studied, and a quadratic polynomial predictive model for the effects of food constituents and pH of food matrix on *B. subtilis* reduction during high pressure and moderate heat was built with RSM accurately. The experimental results showed that the efficiencies of *B. subtilis* reduction in milk buffer and food matrix designed in the present work, under the condition of high pressure treatment process parameters described above, had some differences. The soybean protein ($P < 0.0001$), sucrose ($P < 0.0001$), and pH ($P = 00006$) significantly affected reduction of *B. subtilis*. The effect of soybean oil on reduction of *B. subtilis* was not significant ($P = 0.8363$). The adequacy of the predictive model equation for predicting *B. subtilis* reduction in food matrix by high pressure and moderate heat was verified effectively using experimental test date that was not used in the development of the model.

Keywords: High pressure processing (HPP); Food constituents; Response surface methodology (RSM)

1. Introduction

In recent years, consumer demands are more and more directed towards high-quality, minimally processed, nutritious and fresh-like foods. Traditional thermal processing methods cause loss of desirable properties related to texture, flavor, color, and nutrient components. Food scientists and the food industry are therefore searching for innovative and emerging methods that may destroy undesired microorganisms with less adverse effects on food quality. Thanks to technological progress in the engineering aspects, physical alternative such as high pressure processing (HPP) is becoming more attractive. HPP offers an alternative potential non-thermal preservation method for processing food. The major benefit of pressure is its immediate and uniform effect throughout different media, avoiding complications such as non-stationary conditions typical for convection-type and conduction-type processes (Crawford, Murano, Olsen, & Shenoy, 1996; Knorr, 1993; O'Brien & Marshal, 1995; Patterson, Quinn, Simpson, & Gilmour, 1995; Raso, Gongora-nieto, Barbosa-Cánovas, & Swanson, 1998; Yuste, Mor-Mur, Capellas, Guamis, & Pla, 1998). Compared with the classical heat treatment, HPP treatment causes less deterioration of essential vitamins, phytochemicals, aroma compounds but microorganisms can be inactivated if the applied pressures are high enough (Cheftel, 1995; Hite, 1899).

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Our investigation of optimization of process conditions to inactivate *Bacillus subtilis* by high hydrostatic pressure and mild heat using response surface methodology showed that the optimum process parameters for a 6-log-cycles reduction of *B. subtilis* were obtained as temperature, 46 °C; pressure, 479 MPa; and pressure holding time, 14 min (Gao & Jiang, 2005). Based on the results, in this study, the influence of nutrient composition in food matrix on *B. subtilis* inactivation was evaluated. Cells stressed by high pressure can recover in a nutrient rich medium (Metrick, Hoover, & Farkas, 1989). A low pH-value and a high a_w-value make vegetative microbial cells more sensitive to a high pressure treatment (Arroyo, Sanz, & Préstamo, 1997; Carlez, Rosec, Richard, & Cheftel, 1993). Nutrient rich medium tend to exert more of a protective effect than an aqueous buffer (Kalchayanand, Sikes, Dunne, & Ray, 1998). Milk and cream protect microorganism against pressure (Gervilla, Sendra, Ferragut, & Guamis, 1999; Patterson et al., 1995). Therefore, *B. subtilis* reduction by high pressure and mild heat in buffer solutions are not applicable to food system. It is a fact that results of studies in buffers or laboratory media cannot be directly extrapolated to real food situations (Smelt, 1998).

Despite the fact that there are many foods currently on the Japanese market, including fruit preparations, fruit juices, rice cakes and raw squid (Watanable, Arai, Kumeno, & Honma, 1991), as yet, a accredited predictive model of HPP against food spoilage microorganism has not been established theoretically in food matrices and in real food situations. For this study, we therefore evaluated the effects of food constituents like the soybean protein, soybean oil, sucrose, and pH of the food matrix on the *B. subtilis* inactivation by high pressure and moderate heat, and develop a response surface model using a Central Composite Design (Ambati & Ayyanna, 2001; Murthy, Rakshit, & Kosugi, 2000) for predicting the magnitude of *B. subtilis* cell reduction. This would result in an accurate model and accelerate the introduction of HPP technology. The development of response surface predictive model to predict the HPP inactivation level of *B. subtilis* should be beneficial to the application in food preservation and construct HACCP program to maintain food safely.

2. Materials and methods

2.1. Preparation of B. subtilis As 1.1731 culture

Stock cultures of *B. subtilis* As 1.1731, obtained from China General Microbiological Culture Collection Center China, were maintained on nutrient agar (Oxoid CM3, Basingstoke, UK) during 48 h at 37 °C and stored at 4 °C and subcultured every month. The purity of the cultures was evaluated microscopically. For growth, a loop of *B. subtilis* was transferred from a nutrient agar plate to nutrient broth tubes.

2.2. Preparation of food constituents understudy

Soybean protein and soybean oil were purchased from Shangdong Yuwang Industrial and Commercial Co., Ltd. (Yucheng, Shandong Province, China). Sucrose used was of highest available purity and purchased from Sigma. The food matrix samples were prepared in sterile physiological solution (0.85% NaCl solution). According to the proportion of food constituents in Tables 1 and 2, the food matrix groups were prepared by adding the weighed food

Table 1
Code and level of variables chosen for the trials

Factor	Symbols		Level[a]				
	Coded	Uncoded	−2	−1	0	1	2
Soybean protein (%)	x_1	X_1	0.00	1.25	2.50	3.75	5.00
Soybean oil (%)	x_2	X_2	0.00	1.25	5.00	7.50	10.00
Sucrose (%)	x_3	X_3	0.25	3.50	6.75	10.00	13.25
pH	x_4	X_4	4.00	5.50	7.00	8.50	10.00

[a] $x_1 = (X_1 - 2.50)/1.25$; $x_2 = (X_2 - 5.00)/2.5$; $x_3 = (X_3 - 6.75)/3.25$; $x_4 = (X_4 - 7.00)/1.50$.

Table 2
Central composite design arrangement and responses

Trail no.	Variable				Y[a]	
	x_1	x_2	x_3	x_4	Observed	Predicted
1	−1	−1	−1	−1	6.46(0.03)	645
2	1	−1	−1	−1	5.92(0.01)	5.92
3	−1	1	−1	−1	6.72(0.04)	658
4	1	1	−1	−1	5.87(0.05)	5.89
5	−1	−1	1	−1	5.92(0.02)	5.95
6	1	−1	1	−1	5.85(0.05)	5.88
7	−1	1	1	−1	6.01(0.07)	615
8	1	1	1	−1	5.89(0.09)	5.92
9	−1	−1	−1	1	6.86(0.05)	684
10	1	−1	−1	1	6.63(0.06)	651
11	−1	1	−1	1	6.81(0.05)	679
12	1	1	−1	1	6.30(0.02)	629
13	−1	−1	1	1	5.92(0.04)	5.91
14	1	−1	1	1	5.89(0.07)	604
15	−1	1	1	1	5.90(0.01)	5.92
16	1	1	1	1	5.86(0.03)	5.88
17	−2	0	0	0	6.14(0.06)	616
18	2	0	0	0	5.65(0.06)	5.60
19	0	−2	0	0	5.91(0.04)	5.90
20	0	2	0	0	5.90(0.07)	5.88
21	0	0	−2	0	6.12(0.02)	628
22	0	0	2	0	5.56(0.01)	5.37
23	0	0	0	−2	6.99(0.03)	695
24	0	0	0	2	7.30(0.04)	7.32
25	0	0	0	0	6.03(0.09)	5.92
26	0	0	0	0	5.89(0.03)	5.92
27	0	0	0	0	5.89(0.06)	5.92
28	0	0	0	0	5.91(0.05)	5.92
29	0	0	0	0	5.87(0.02)	5.92
30	0	0	0	0	5.90(0.07)	5.92

[a] Values in parentheses are coefficients of variation of measures; $Y = \log(N_0/N_1)$ (N_0 = the initial number of cells (CFU/ml), N_t = the number of survivals after an exposure time t (CFU/ml)).