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Influence of High Hydrostatic Pressure on Microbial Growth and Shelf-life of Vacuum-Packed Sliced Ham

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In order to evaluate the influence of HPP on microbial growth and shelf-life, HPP of 200MPa, 400MPa and 600MPa for 10min or 20min were applied to vacuum-packed sliced ham, respectively. Modified Gompertz equation was used to model the growth of specific spoilage organisms: mesophilic aerobic bacteria, lactic acid bacteria and psychrophile during refrigerated storage (4°C, 90days). Besides, chemical and sensory properties were analyzed to confirm the end of the shelf-life. According to the results, 10^5cfu/g of mesophilic aerobic bacteria was suggested as shelf-life criterion for vacuum-packed sliced ham, which differed from previous researches. The analysis of the model showed that HPP could significantly extend the lag phase, slow the growth rate of microorganisms, and thus prolong the shelf-life of vacuum-packed sliced ham ($P<0.05$). The shelf-life was extended to about 40days by 200MPa, 2months by 400MPa and more than 3months by 600MPa. Furthermore, the main factor affected the inhibition on microbial growth is the pressure level. However, the inhibition efficiency between 10min group and 20min group showed no significant difference ($P>0.05$).

Key words: High hydrostatic pressure processing; Vacuum-packed sliced ham; Microbial growth; Shelf-life; Predictive microbiology.

Sliced ham is a popular low-temperature meat product, its superior organoleptic quality merit the consumer's particular attention and favorite. However it is highly perishable and the shelf-life of sliced ham was normally limited to 21-42 days at 1-8°C (tekelenburg *et al*. 2001; Garriga *et al*., 2004; Vermeiren *et al*., 2005; Hu *et al*., 2009; Liu *et al*., 2011). To extent the shelf-life, normal practice such as adding chemical additives or heating is obviously effective. But it would fail to satisfy consumers' demands for minimally processed, additive-free and high organoleptic quality ham (Vercammen *et al*., 2011). Hence alternative process such as high hydrostatic pressure processing (HPP) has been proposed and investigated. And it is no doubt that HPP is a very promising repasteurization technology for vacuum-packed sliced cooked ham after packaging.

Predictive microbiology method was efficient in modeling the SSOs growth and the shelf-life of meat products (Baranyi *et al*., 1995; Gospavic *et al*., 2008), and the possibility of the method in sliced ham has been confirmed (Mataragas *et al*., 2006; Kreyenschmidt *et al*., 2010). In terms of the influence of HPP on microorganisms, the reduction and inhibition on microorganisms by HPP has been confirmed (Aymerich *et al*., 2005; Garriga *et al*.,

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2004; Jofré *et al*., 2009). Pressure and the pressurized time are the two main factors contribute to the efficiency of HPP (López-Caballero *et al*., 1999; Briones *et al*., 2010; Rubio *et al*., 2007). However, few researches had mathematically modeled the growth of microorganisms and product shelf-life after HPP during the subsequent storage.

Therefore, our study aimed to confirm the viability of predictive microbiology methods in evaluation of microbial growth and shelf-life extension after HPP. New combinations of pressure level and pressurized time: 200MPa for 10min or 20min, 400 MPa for 10min or 20min, 600 MPa for 10min or 20min were applied to vacuumed-packed sliced ham. A re-parameterized version of the modified Gompertz equation was applied to model the growth of specific spoilage organisms: mesophilic aerobic bacteria, LAB and psychrophile. And the kinetic parameters including the lag phase duration, the maximum growth rate, and the shelf-life were evaluated. Meanwhile, physical, chemical (pH, TVB-N values) and sensory properties were analyzed to confirm the end of the shelf-life.

MATERIALS AND METHODS

Sliced cooked ham

Vacuum-packed sliced hams were prepared in a local slaughtering and meat processing plant. Pork ham were stripped of fat and injected with brine containing (in g/kg): water 400, salt 20, sodium nitrite 0.30 and spice 0.30. After injection hams were molded, pressed and cooked by steam until the internal temperature reached 72°C. Afterwards, cooled down to 4°C, sliced in 0.5 mm thick slices and vacuum-packaged in plastic pressure resistant bags in portions of 30±5g (3 slices each bag). The oxygen permeability of the plastic bags was 3 cm^3/m^2 24 h l atm.

High hydrostatic pressure treatment

Combinations of pressure and pressurized time were: 200MPa, 10min; 200MPa, 20min; 400MPa, 10min; 400MPa, 20min; 600MPa, 10min; 600MPa, 20min respectively. The pressurization was carried out in a research hydrostatic pressurization unit (Baotou Kefa High Pressure Technology Co., Ltd, Inner Mongolia, China), which was capable of operating up to 600MPa. The temperature of process water was 20°C before HPP and below 30°C during the pressurized processing. The required time to reach 200MPa, 400MPa and 600MPa are 40s, 70s and 100s respectively, and the pressure was released within 15s.

Storage of the sample

According to The Food Safety and Inspection Service, ready to eat foods are recommended to be stored at 4.4 °C or below to avoid temperature-abuse situation. In the present study, all samples including the high-pressure treated samples and non-treated samples (NT) were stored at 4°C for 90 days. Growth of the specific spoilage organisms: mesophilic aerobic bacteria, LAB, psychrophile were sampled in duplicate at selected time: immediately after treatment (0 day), and during storage (10, 20, 30, 40, 50, 60, 70, 80 and 90 days).

Microbiological analysis

25g sample were taken aseptically and diluted to 10-fold in sterile saline (0.85% NaCl), homogenized sufficiently (200rpm, 20min, 4°C), then made into decimal serial dilutions. The appropriate dilutions were chosen and plated onto culture media to determine mesophilic aerobic bacteria in Plate Count Agar (PCA, Beijing Land Bridge technology company, Beijing) at 36±1°C for 48±2 h; LAB in MRS agar (MRS agar, Beijing Land Bridge technology company, Beijing) at 36±1°C for 48±2 h; psychrophile in Plate Count Agar (PCA, Beijing Land Bridge technology company, Beijing) at 6.5±0.5°C for 10 d. Microbiological counts were expressed as $\log_{10}$CFU/g. The lowest detection limits for measuring of the analysis was 10CFU/g (except 2.0 $\log_{10}$ CFU/g for mesophilic aerobic bacteria). The microbial growth at each level was measured by replicating the experiments twice.

Growth curve modelling

Experimental data was fitted into the re-parameterized version of modified Gompertz equation (Zwietering *et al*., 1990).

$$\text{Log } (N(t))=K+A\cdot\exp\{-\exp\{[(\mu_{max}\cdot 2.7182\cdot(\lambda-t)/A]+1\}\}$$

Where: K is the initial bacteria load ($\log_{10}$ CFU/g), A is the increase in microbial concentration ($\Delta\log_{10}$ CFU/g) between time =0 and the maximum microbial concentration achieved at the stationary phase, μ_{max} is the maximal growth rate (Ä$\log_{10}$ CFU/g/day), λ is the lag phase (days) and t is the storage time (days). Mean square error (MSE), correlation coefficients (r^2) and bias factor

(BF) were employed to analyze the accuracy of primary model. The lower the MSE value means the better goodness-of-fit of the model. The bias factor index the agreement between predictions and observations, and it is perfect to be 1.0.

Measurements of pH

The pH values were determined by homogenizing 10 g of ham sample in 10 mL distilled water (pH 7.00) with a PB-10 microcomputer pH meter (Sartorius Group). Analyses were performed in duplicate for all samples.

Total volatile basic nitrogen (TVB-N) measurements

The sample was pretreated by using Conway's diffusion method (Conway, 1950). And the TVB-N values were determined using a Kjeltec 2300 Analyzer Unit (Foss Tecator AB, Sweden) following the process described by Liu *et al.* (2012).

Sensory analysis

Sensory analysis was carried out according to the method of Rubio *et al*. (2007) with some modifications. Color, odor, taste, hardness, juiciness and overall acceptability were scored on a 5-point hedonic scale as follows: 5=excellent, 4=good, 3=acceptable, 2=fair and 1=unacceptable. The end of the sensory shelf-life was confirmed when any of the parameters was less than 3.

Statistical analysis

The obtained results were subjected to analysis of variance (ANOVA) and Student-Newman-Keul (SNK) test (5% of significance), using the Statistical Product and Service Solutions (SPSS 12).

RESULTS AND DISCUSSION

Reduce of initial microbial load by HPP

All HPP treatments reduced the initial microbial load of vacuum-packed sliced ham (Fig. 1). Based on NT samples, HPP had an 18-75% inhibition rate on initial microbial load depending on the pressure level (200MPa to 600MPa) and type of SSOs. In contrast to mesophilic aerobic bacteria and LAB , the psychrophile got a higher inhibition rate under the same pressure level. The discrepancy of suppression between different kinds of microorganisms may due to their different sensitiveness to HPP (Garriga *et al*., 2002). As shown in Fig.1, pressure level had a positive correlation to inhibition rate of mesophilic aerobic bacteria (R^2=0.975) and LAB (R^2=0.967) between 200-600MPa. However, pressurized time between 10 min and 20 min shown no significant difference (P>0.05) on the initial inhibition (data not show).

Effects on microbial growth parameters of HPP

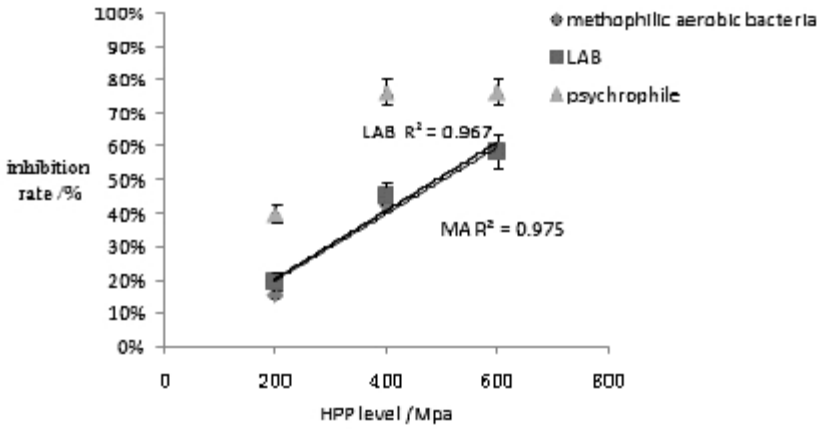

Fig. 1. Reduction of microbial load in vacuum-packed sliced ham stored at 4°C after HPP. Bars denote standard deviation of the mean. Different letters between bars are significantly different (α=0.05, SNK)

The microbial growth with the storage time are displayed in Figure 2. As expected, microorganisms grew quickly in non-treated (NT) samples. In contrast, the growth of microorganisms in pressurized samples showed a significant delay.

In order to evaluated the influence of HPP

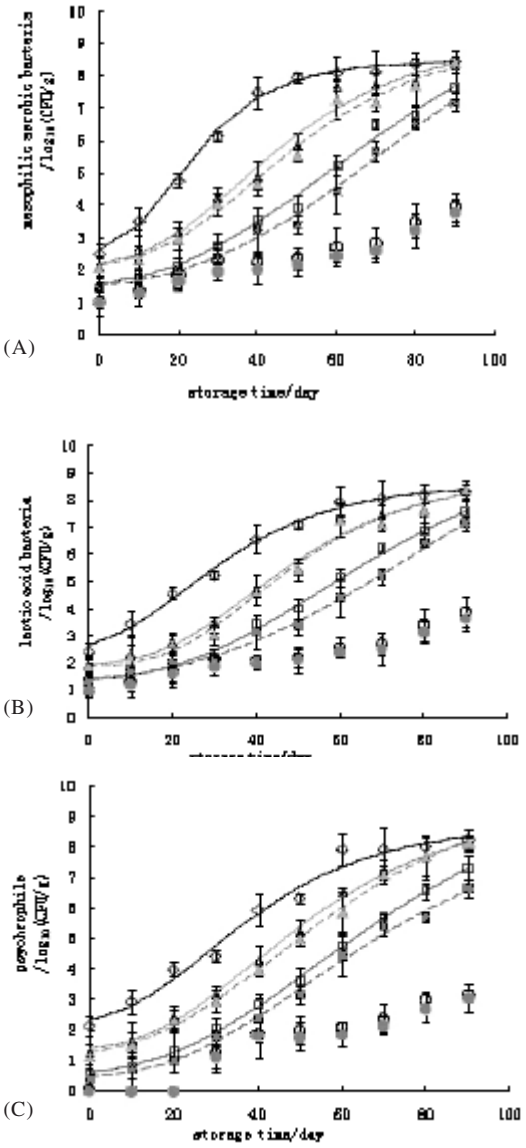

Fig. 2. Growth curves of methophilic aerobic bacteria (a), LAB (b), and psychrophile (c) in sliced ham ◇ Non-treated; △ 200MPa 10min; ▲ 200MPa 20min; □ 400MPa 10min; ■ 400MPa 20min; ○ 600 10min; ● 600MPa 20min with the storage time at 4°C. Data were fitted by modified Gompertz model

on microbial growth in pressurized samples. A modified Gompertz model was used to describe the growth of mesophilic aerobic bacteria, LAB and psychrophile. The kinetic growth parameters for maximum growth rate (μ_{max}), lag phase (λ) of SSOs and shelf-life of vacuum-packed sliced ham under different HPP conditions are shown in Table 1. And the statistical indexes of the model are displayed in Table 2, which suggest the applicability of the modified Gompertz model. 5, 6, 7 $\log_{10}$ CFU/g of mesophilic aerobic bacteria, LAB and psychrophile were used respectively as criterion for shelf-life. Compared to NT samples, HPP significantly extended the shelf-life (P<0.05). As to the growth parameters, HPP extended the lag phase of all the spoilage microorganisms detected. The magnitude of the lag phase extension varied depending on pressure level and types of SSOs. At the same time, a slower maximum growth rate was observed in HPP samples compared to the NT ones. What is interesting that, the maximal growth rate of LAB and psychrophile were not affected by HPP. The extension of lag phase and similar maximal growth rate suggested that, there were some microorganisms not been detected right after HPP, but survived then recovered. Wu (2008) has reported that there were three kinds of microbial cells after HPP stress, i) killed; ii) sublethally injured; iii) non-injured. Sublethally injured cells could not be detected immediately on an agar plate (Bozoglu *et al*., 2004). They endured a longer lag phase preparing for the necessary proteins and nucleic acids for growth (Shintani, 2006). And finally survived the HPP stress, proliferate in the following storage by given a suitable environment, leading to spoilage same as the non-injured ones(Garriga *et al*., 2002; Bozoglu *et al*., 2004; Bull *et al*., 2005; Koseki *et al*., 2006; Koseki *et al*., 2007; Jofré *et al*., 2009). Table 1 Growth parameters (μ_{max}, λ and *shelf-life*) of mesophilic aerobic bacteria, lactic acid bacteria and psychrophile obtained in sliced vacuum-packed sliced ham (HPP treated and non-treated) during refrigerated storage by fitting modified Gompertz model .

Effects on Chemical parameters and sensory by HPP

As shown in Fig.3, HPP has significant impact on the changes of pH and TVB-N with the storage time. The pH values of all the samples decreased, which agrees with previous research e.g. Li *et al.* (2012). Compared with the NT samples, HPP treatments slowed down the descent of pH value. Furthermore, the pH value decreased slower in the higher pressure treated samples, however no significant difference (P<0.05) were observed between 10min and 20min groups under the same

Table 1.

	Pressure level (MPa)	Pressurized time (min)	t_{sl}-M (day)			μ_{max} ($dias^{-1}$)	λ (day)	t_{sl}-L (day)			μ_{max} ($dias^{-1}$)	λ (day)	t_{sl}-P (day)			μ_{max} ($dias^{-1}$)	λ (day)
			SL_5	SL_6	L			SL_5	SL_6	SL_7			SL_5	SL_6	SL_7		
NT	-	-	21 [a]	28[a]	37 [a]	0.16[a]	6[a]	26 [a]	35 [a]	46 [a]	0.12[a]	3[a]	31 [a]	41 [a]	54 [a]	0.11[a]	6[a]
1	200	10	39 [b]	48 [b]	60 [b]	0.11[b]	7[a]	43 [b]	52[b]	64[b]	0.12[a]	16[b]	46[b]	56 [b]	68 [b]	0.11[a]	13[b]
2	200	20	41 [b]	51 [b]	63 [b]	0.11[b]	7[a]	44 [b]	53[b]	64 [b]	0.12[a]	18[b]	50 [b]	60[b]	72 [b]	0.11[a]	13[b]
3	400	10	58 [c]	69[c]	81 [c]	0.09[c]	10[b]	58 [c]	70 [c]	83[c]	0.10[b]	20[b]	64 [c]	74[c]	86 [c]	0.10[b]	18[c]
4	400	20	65 [d]	76 [d]	87[c]	0.09[c]	12[b]	67[d]	77 [d]	89[d]	0.10[b]	29[c]	68 [c]	80 [d]	95 [d]	0.09[b]	19[c]

NT=non-treated; M=mesophilic aereobic bacteria; L=lactic acid bacteria; P=psychrophile; SL_5, SL_6, SL_7 =The time taken for microorganisms to reach $5\log_{10}$CFU/g, $6\log_{10}$CFU/g and $7\log_{10}$CFU/g respectively-end of the product shelf-life; Mean value with different letters (a-d) in the same column with the same product and spoilage organisms are significant different ($p<0.05$, SNK).

Table 2. Statistical indexes obtained from fit Modified Gompertz model to growth curves of mesophilic aerobic bacteria, lactic acid bacteria and psychrophile in pressurized and non-pressurized (control) sliced vacuum-packaged ham stored at 4 °C

Trials	M			L			P		
	r^2	MSE	Bias factor	r^2	MSE	Bias factor	r^2	MSE	Bias factor
NT	0.997	0.034	1.009	0.994	0.051	1.006	0.984	0.152	1.004
200/10	0.983	0.168	0.998	0.986	0.152	0.993	0.998	0.025	1.004
200/20	0.988	0.123	1.005	0.986	0.158	0.990	0.998	0.027	1.009
400/10	0.991	0.077	1.001	0.995	0.079	0.994	0.999	0.008	1.023
400/20	0.993	0.053	1.003	0.993	0.053	1.005	0.996	0.040	1.009

M=mesophilic aereobic bacteria; L=lactic acid bacteria; P=psychrophile;

pressure level. Similarly, the increase of TVB-N was affected by HPP as well. As shown in Fig.3 (b), the increase rate is low in higher pressure treated samples. Sensory analysis of samples under different pressure levels at 1, 30, 60 and 90 days were shown in Fig.4. At day 1, there were no significant ($P<0.05$) difference between the HPP treated samples with the NT ones. However, sensory parameters including hardness, color, odor, juiciness and overall acceptability of all the samples decreased with the storage time. Shelf-life based on the limitation level of TVBN in China (must be <20 mg/100 g of ham sample), and the sensory analysis (higher than 3), is similar to that predicted by 10^5cfu/g for mesophilic aerobic bacteria or LAB. This criterion for microorganism were much lower

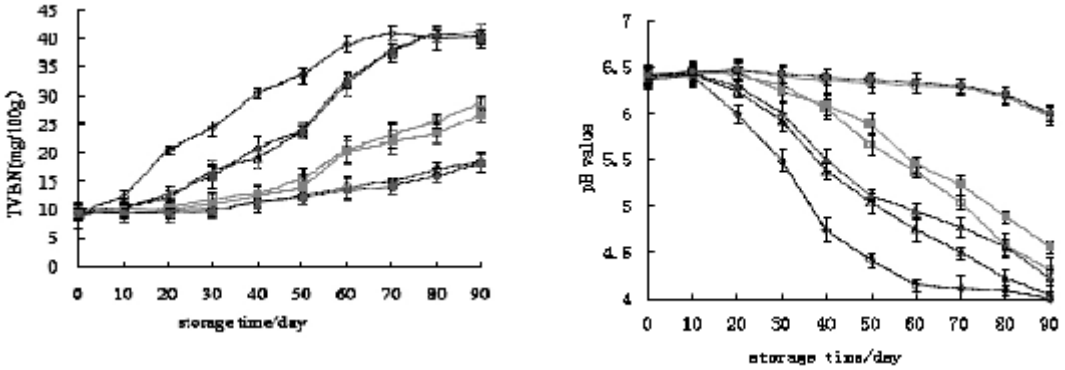

Fig. 3. Changes of pH (a) and TVBN (b) of sliced ham Non-treated; 200MPa 10min; 200MPa 20min; 400MPa 10min; 400MPa 20min; 600 10min; 600MPa 20min with the storage time at 4°C

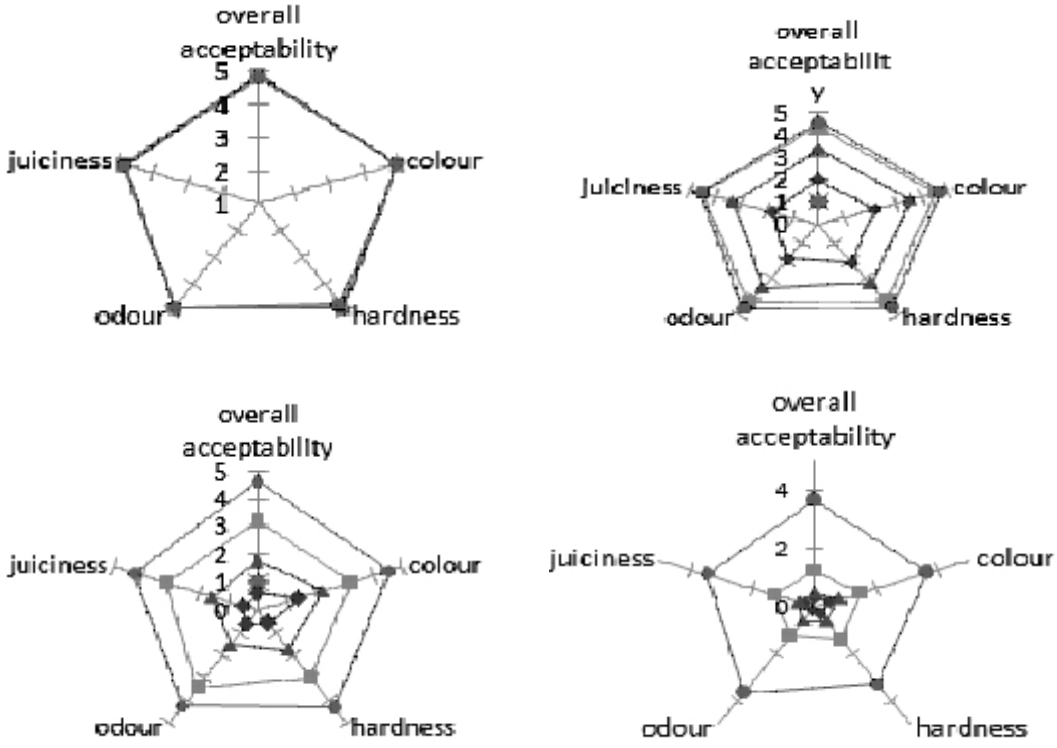

Fig. 4. Sensory analysis of sliced cooked ham Non-treated, with 200MPa (10min, 20min), 400MPa (10min, 20min), 600MPa (10min, 20min) at days 1(a), 30(b), 60(c), 90(d)

than 10^6-10^8CFU/g in previous reports (Ruiz-Capillas *et al*., 2007; Slongo *et al*., 2009; Kreyenschmidt *et al*., 2010).

CONCLUSIONS

In conclusion, predictive microbiology methods were proved and applied to evaluate the efficiency of HPP on shelf-life extension of vacuumed packed sliced-ham. Combinations of pressure level and pressurized time were analyzed: 200MPa (10min/20min), 400 (10min/20min), 600 (10min/20min) during refrigerated storage. We proved that HPP could significantly reduce the initial microbial load, extend the lag phase, and lower the maximum growth rate of microorganisms, which are the main contributors to shelf-life extension. And, 400MPa 10min treatment with a 2 months shelf-life was recommended for producer and consumers. Differ from the previous research, where LAB of 10^6-10^8 CFU/g was applied, we suggested 10^5cfu/g of mesophilic aerobic bacteria as a new criterion for shelf-life of sliced ham.

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