Article

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Predicting bacterial growth in raw, salted, and cooked chicken breast fillets during storage

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Abstract

Growth curves were evaluated for aerobic mesophilic and psychrotrophic bacteria, *Pseudomonas* spp. and *Staphylococcus* spp., grown in raw, salted, and cooked chicken breast at 2, 4, 7, 10, 15, and 20 °C, respectively, using the modified Gompertz and modified logistic models. Shelf life was determined based on microbiological counts and sensory analysis. Temperature increase reduced the shelf life, which varied from 10 to 26 days at 2 °C, from nine to 21 days at 4 °C, from six to 12 days at 7 °C, from four to eight days at 10 °C, from two to four days at 15 °C, and from one to two days at 20 °C. In most cases, cooked chicken breast showed the highest microbial count, followed by raw breast and lastly salted breast. The data obtained here were useful for the generation of mathematical models and parameters. The models presented high correlation and can be used for predictive purposes in the poultry meat supply chain.

Keywords

Chicken, bacteria, modeling, predictive, shelf life

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INTRODUCTION

The presence of pathogenic and spoilage microorganisms in poultry meat and its by-products is still of significant concern to suppliers, consumers, and public health officials worldwide and to international trade (Okolocha and Ellerbroek, 2005). Bacterial contamination of these foods, which is undesirable but unavoidable, depends on the bacterial level of the poultry carcasses used as the raw product, the hygiene practices employed during handling, and storage time and temperature (Bruckner et al., 2013). Unless appropriate actions are taken, e.g. packaging, transportation, and storage at refrigerator temperatures, the product can spoil in a relatively short time. Spoilage is affected by intrinsic (e.g. pH, aw, composition, type, and extent of initial contamination) and extrinsic factors (e.g. packaging temperature and atmosphere) (Koutsoumanis et al., 2006). The combination and interaction of intrinsic and extrinsic factors determines the microbiology of meat. Among these factors, temperature is considered the most important one responsible for microbial spoilage, influencing microbial growth during the shelf life (SL) of a food product (Allen et al., 1997; Koutsoumanis et al., 2006).

Because most chicken is sold fresh, it is essential to preserve the product's SL for as long as possible (Allen et al., 1997). The need for fresh food supplies sent to distant markets has increased the interest in procedures for extending product SL (Galarz et al., 2010). Obviously, the overall SL of a product should include not only the time spent in reaching the market but an

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additional period for its sale and for its subsequent storage by the consumer, who may decide to use it several days after its purchase. Therefore, the issue of SL is a major challenge for producers (Jiménez et al., 1999).

Chicken and other types of poultry have higher pathogenic and spoilage bacteria counts than almost any other food (Jiménez et al., 1999). Spoilage floras are usually dominated by the bacteria that grow the fastest under the conditions in which a product is stored. Under aerobic conditions, the dominant spoilage organisms are the strictly aerobic *Pseudomonas* spp. (Kanatt et al., 2005), which are considered the primary cause of spoilage of chilled poultry meat.

The spoilage of poultry products represents an economic loss to both producer and consumer. Therefore, the development of methods to increase the SL of food products is of major research interest to the food processing industry (Balamatsia et al., 2006). Modeling the growth and survival of food poisoning and spoilage bacteria is a basic tool for predicting the SL and safety of food products during processing and storage. Accurate predictions provide valuable data on food deterioration rates during shipping and storage, and enable manufacturers to reduce the amount of experimental work that is required to test and ensure the quality and safety of products and to assign an adequate SL. In addition, more realistic SLs can be assigned to products, with higher profits for the manufacturer, less wastage, and lower costs for the consumer (Bovill et al., 2000). Although theoretical models are available, data for modeling the interaction of microbial populations in poultry are still scanty (Coleman et al., 2003).

To determine the SL of chicken meat products and predict microbial growth on them, aerobic mesophilic and psychrotrophic bacteria *Pseudomonas* spp. and *Staphylococcus* spp. were analyzed, since these bacteria are the main causes of chicken meat deterioration and spoilage. The present study focused on modeling the growth of these microorganisms on raw, salted, and cooked chicken breasts stored aerobically at 2, 4, 7, 10, 15, and 20 °C, respectively, using the modified Gompertz and modified logistic models (Zwietering et al., 1990), and determining the SLs of these products based on microbiological counts and sensory analysis.

MATERIALS AND METHODS

Preslaughter and slaughter treatments

Preslaughter and slaughter treatments of poultry were carried out under hygienic conditions according to Regulations (EC) 1099/2009, (EC) 854/2004, and (EC) 853/2004. Cobb 500^{TM} broilers at the age of 40–45 days were mechanically catched and transported to a local slaughterhouse in trucks using container system. In the slaughter plant, birds were received in an air-conditioned room with light intensity control (7–8 lux) where they waited for 2 h before slaughter. The healthy birds were electrically stunned by partial immersion in an electrified water tank for 10 s. The unconscious birds were hanged and then bled by cutting the jugular veins, without severing the head and the trachea. After bleeding, birds were washed by high-pressure showers and immediately immersed in two sequential scalding tanks (29 s per tank) with water heated at least at 56 °C by direct steam injection. Then, birds passed through a defeatherer to completely remove all feathers. After plucking, broilers were washed with abundant water by high-pressure nozzles, eviscerated by automatic eviscerating machines. After viscera removing, and before final washing, all carcasses were visually inspected for bile and feces contamination. Carcasses were unloaded from the aerial transporter by an automatic de-hanger in prechiller and chiller systems, from 16 to 4 °C, with continuous iced water renewing and a bubbling system by air-injection hoses. After chilling, carcasses were sent to the cuts room, where chicken breasts were obtained in a semiautomated deboning line.

Raw materials

From the chicken breasts, the three different raw materials (raw chicken breast (RB), salted chicken breast (SB), and cooked chicken breast (CB)) were obtained frozen and transported to the laboratory at −18 °C. RB contained 74.8 ± 1.7% moisture, 23 ± 1% protein, 1.2 ± 0.9% fat, 1.5 ± 0.5% ash, pH 6.3 ± 0.5, and water activity (aw) of 0.98. SB contained 74 ± 1.5% moisture, 23 ± 1% protein, 1.2 ± 0.9% fat, 2.5 ± 1% ash, 1.5 ± 0.3% salt (NaCl), pH 6.3 ± 0.5, and aw of 0.98. CB contained 68 ± 2% moisture, 29 ± 2% protein, 2.4 ± 0.6% fat, ash 2.5 ± 1%, pH 6.3 ± 0.3, and aw 0.98. All products were boneless and skinless. Upon arrival, the products were divided into individual portions of chicken breasts, stored in sterile low-density polyethylene bags, and immediately refrozen at −18 °C until their utilization.

Experimental design

Portions of chicken breast fillets were defrosted overnight at 4 °C. The portions were stored under controlled temperature conditions in high-precision low-temperature incubators (Biopar SI50BA) at 2, 4, 7, 10, 15, and 20 °C. Samples were removed from the fillets at appropriate time intervals according to the evaluated temperature, varying from a few hours (20 °C) to an interval of 2–3 days between two consecutive samplings.

Sample preparation and microbiological analysis

For each sampling, parts of raw or salted breast were obtained by removing 25 g of the meat from the surface of a unique breast. For cooked breast, it was weighted 25 g from three different representative places of the breast. Each sample was placed in a Stomacher bag and homogenized for 60 s with 225 g of chilled saline peptone diluent (0.85% NaCl with 0.1% peptone). Another 10-fold dilution of the homogenate was prepared with peptone saline diluent. Two replicas were prepared for each dilution blank. A total of 0.1 ml of each dilution was spread on the surface of solid media in Petri dishes. The plate count was analyzed according to the classical methodology. Mesophilic bacteria in agar were determined from deep plate counts (35 °C, 48 h) and psychrotrophic bacteria in agar from surface plate counts (20 °C, 120 h). *Pseudomonas* spp. were determined using a Pseudomonas agar base plus Pseudomonas CFC selective agar (25 °C, 48 h) and *Staphylococcus* spp. using Baird-Parker agar with sterile 1% potassium tellurite solution and sterile egg yolk emulsion (35 °C, 48 h).

Modeling

The modified Gompertz model (equation (1)) and modified logistic model (equation (2)) were used to describe the curves of bacterial growth in chicken breasts

$$\ln\frac{N}{N_0} = A.\exp\left\{-\exp\left[\frac{\mu_{\max}.2.718}{A}.(\lambda - t) + 1\right]\right\} \quad (1)$$

$$\ln\frac{N}{N_0} = \frac{A}{\left\{1 + \exp\left[\frac{4.\mu_{\max}}{A}.(\lambda - t) + 2\right]\right\}} \quad (2)$$

where N is the microbial population (CFU/g) at time (h); N_0 is the initial microbial population (CFU/g); A is the asymptote (ln N_{max}/N_0); μ_{max} is the maximum specific growth rate during the exponential growth phase, defined as the tangent at the inflection point per hour; and λ is the lag time (h). The three parameters (A, μ_{max}, and λ) were optimized by nonlinear regression.

Sensory analysis

The sensory analysis of RB was performed by a trained sensory panel of at least eight people. Color, odor, and texture were evaluated using a simple 6-point scoring system (1–6), with 6 representing the highest quality score and 2 the limit of acceptance. Each criterion presented a different score: color: C = 2, odor: O = 2, and texture: T = 1, according to equation (3)

$$S = \frac{2.C + 2.S + 1.T}{5} \quad (3)$$

For that, chicken breasts stored at 2, 4, 7, 10, and 15 °C were collected after one, four, seven, nine, and 11 days. Samples were then cut into 2 cm cubes, immersed in water containing 3% NaCl, and kept in this solution for 5 min. After soaking, the samples were cooked in a microwave oven and kept under heating (50 °C) until sensory evaluation. Samples were served in disposable containers, coded with three-digit random numbers. Three samples were analyzed each day to avoid sensory fatigue, and one sample was identical to the reference sample and two samples from different storage times. The statistical analysis was performed by ANOVA using the Statistica v.8.0 software, and means were compared by the Tukey test (5% probability) using Microsoft Excel (Fonseca et al., 2013).

Determination of count parameters and SL

The initial (X_0) and maximum (X_{max}) counts were determined, respectively, by the logarithm of the lowest and highest values for the microbial counts. The products' SL was determined as the time (days) required for each microorganism to reach the microbial count established as safe, combined with the results of the sensory analysis (Galarz et al., 2010).

RESULTS AND DISCUSSION

Aerobic mesophilic and psychrotrophic microorganisms

Figures 1 to 4, respectively, depict the growth curves (log (CFU/g)) of the aerobic mesophilic and psychrotrophic microorganisms *Pseudomonas* spp. and *Staphylococcus* spp. in RB, SB, and CB samples at each studied temperature. Note the inverse relationship between storage temperature and time, i.e. the higher the temperature the shorter the time required to complete the microbial growth curve and reach the asymptote (a). The maximum specific growth rate (μ_{max}) also increases with increasing temperature, and the lag phase (λ) generally decreases over time (Tables 1 and 2). Increasing the temperature accelerates microbial growth, thus reducing the SL (Table 3). The effect of temperature on specific growth rates is amply demonstrated in the literature (Dominguez and Schaffner, 2007). A close fit was found for both the Gompertz (Table 1) and logistic (Table 2) models due to the strong correlation between the experimental and predicted data.

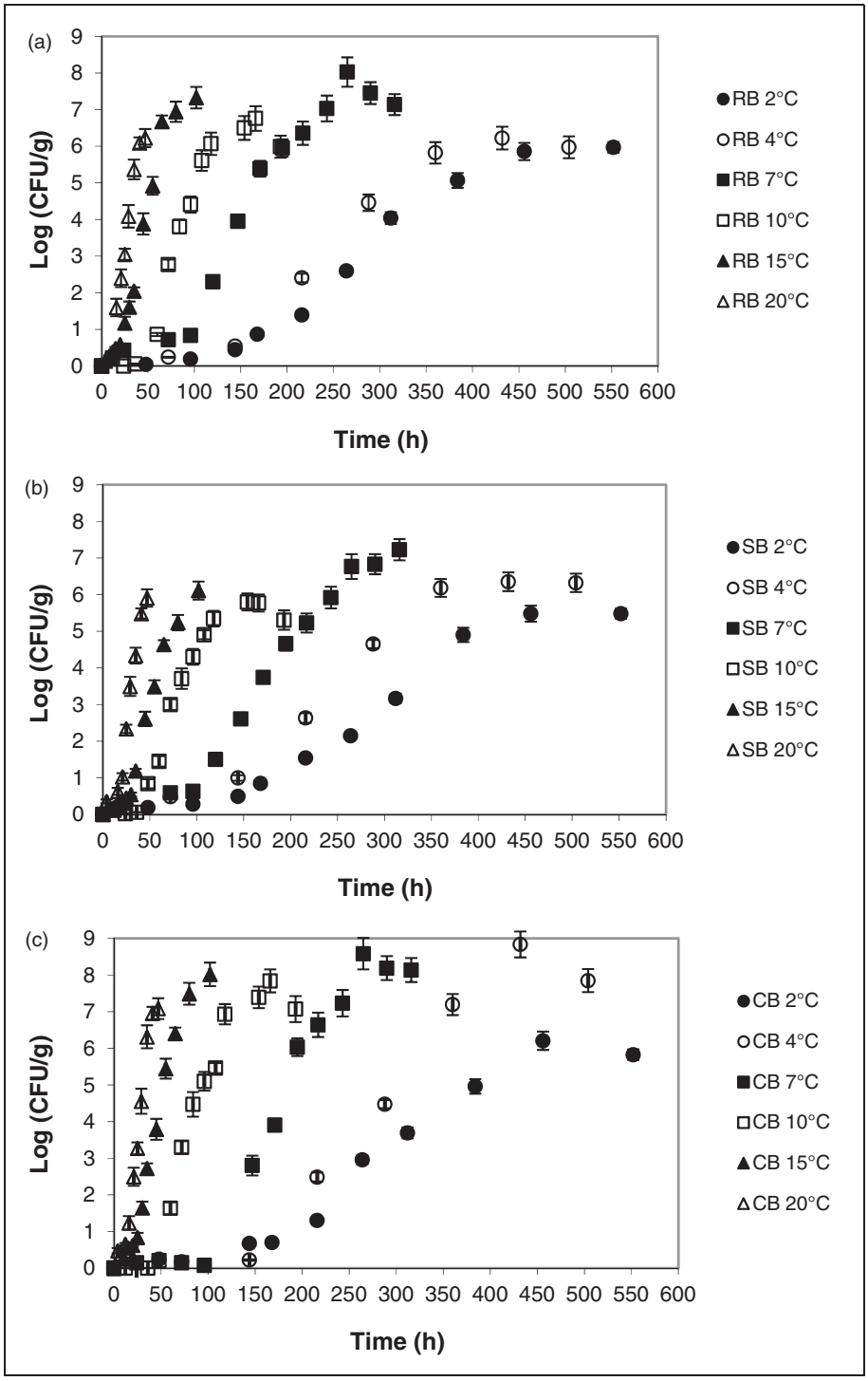

Figure 1. Growth curves for aerobic mesophilic bacteria at the temperatures of 2, 4, 7, 10, 15, and 20 °C. (a) Raw breast (RB), (b) salted breast (SB), and (c) cooked breast (CB).

Microbiological counts serve to assess the degree of deterioration of chilled foods (Franco and Landgraf, 2007). The ICMSF (1978) recommended the range of 10^6–10^7 CFU/g as the standard to determine the end of SL of meat products. Later, it established the aerobic bacteria count of 10^7 log (CFU/g) as an indicator of the end of SL (ICMSF, 1986). Other authors have reported values ranging from 10^6 to 10^8 CFU/g (Davies and Board, 1998; Franco and Landgraf, 2007; Senter et al., 2000; Smolander et al., 2004). Poultry products are considered outside the ideal sanitary conditions when

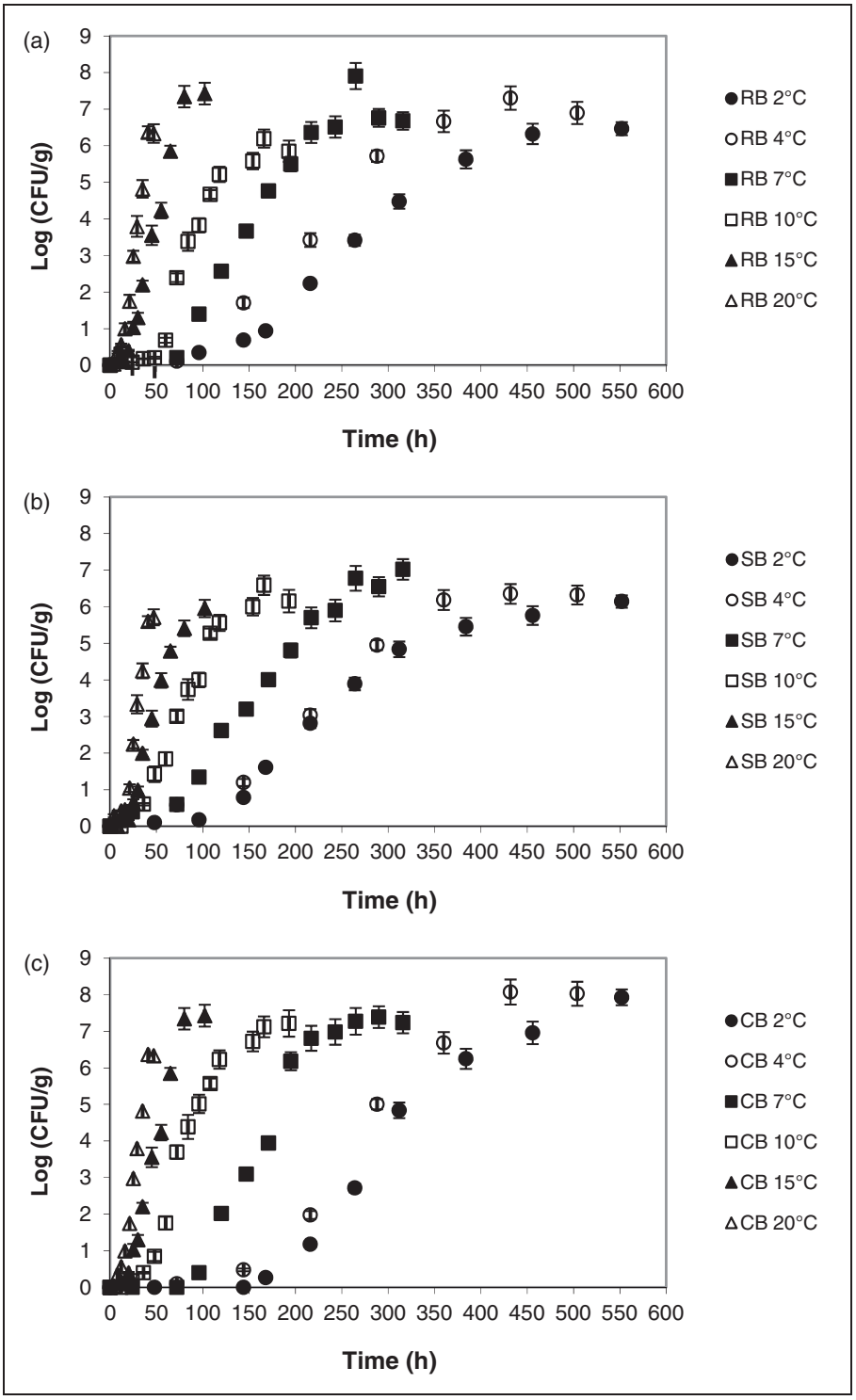

Figure 2. Growth curves for aerobic psychotropic bacteria at the temperatures of 2, 4, 7, 10, 15, and 20 °C. (a) Raw breast (RB), (b) salted breast (SB), and (c) cooked breast (CB).

mesophilic aerobic bacteria counts exceed 10^6 CFU/g (Ritter and Bergmann, 2003). Based on these values, the range of 10^6–10^7 CFU/g was considered the limit for determining SL in this study.

Among the particularities of the different microorganisms studied here, products containing aerobic mesophilic bacteria showed a SL of 12–19 days at 2 and 4 °C (Table 3), which is much higher than that

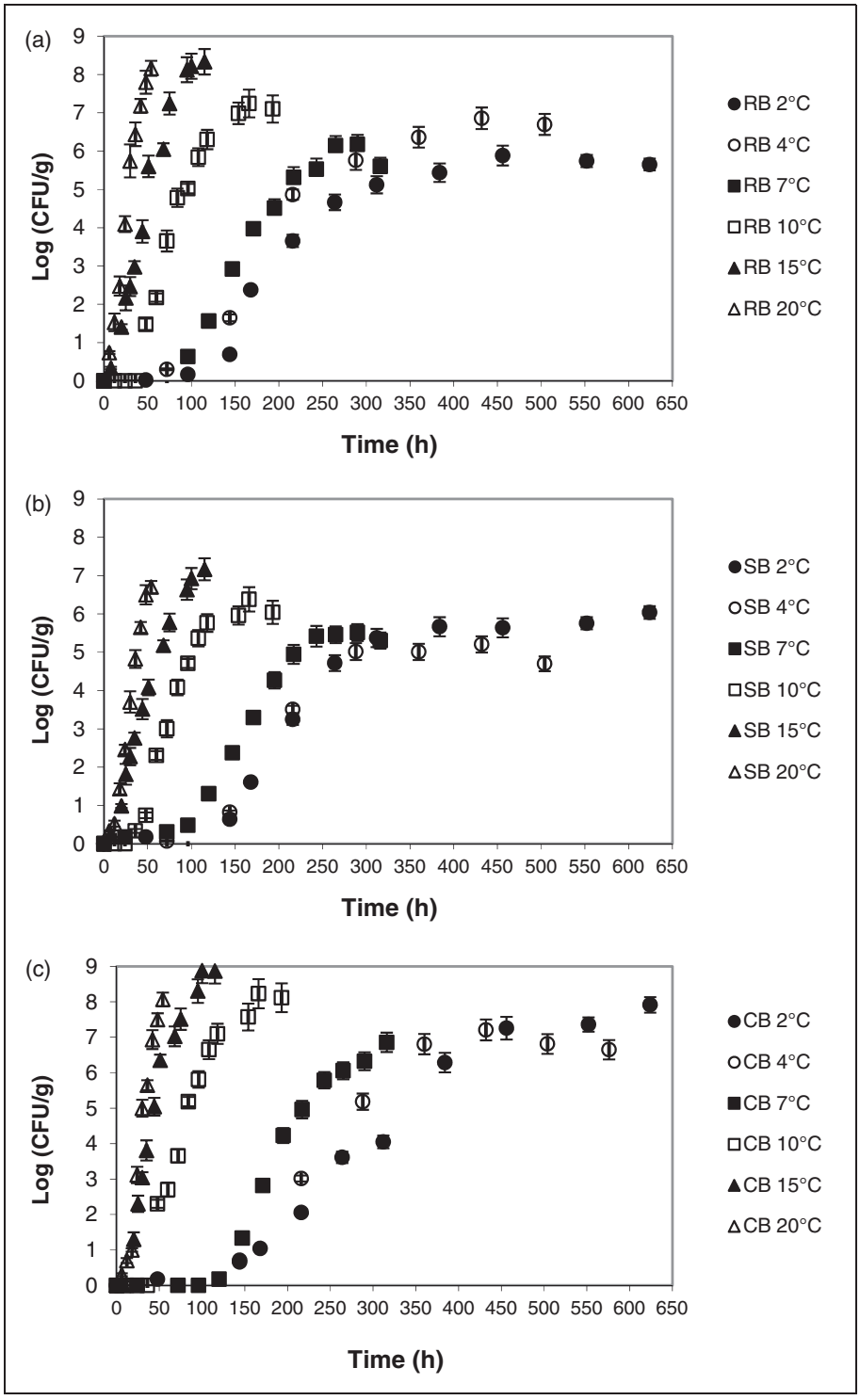

Figure 3. Growth curves for *Pseudomonas* spp. at the temperatures of 2, 4, 7, 10, 15, and 20 °C. (a) Raw breast (RB), (b) salted breast (SB), and (c) cooked breast (CB).

estimated for psychrotrophic bacteria. The reason for this is that those temperatures are far below the ideal one for mesophilic bacteria, which thrive at 20–45 °C (Madigan et al., 2004). Studies have demonstrated that at chilling temperatures, the tolerable limits for bacteria in RB can be reached between four and 17 days (Balamatsia et al., 2006, 2007; Chouliara et al., 2007; Dominguez and Schaffner, 2007; Göksoy et al., 2000;

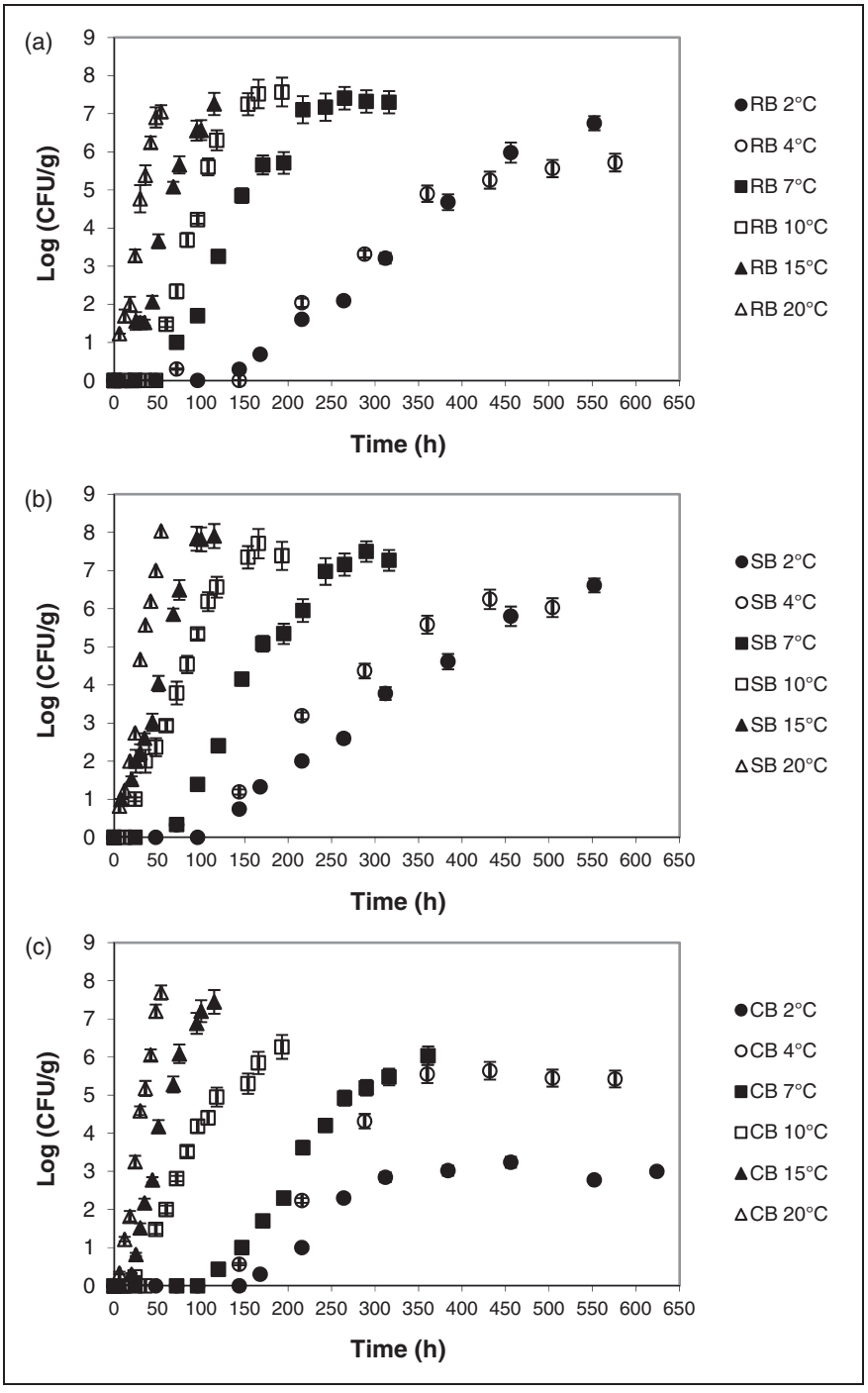

Figure 4. Growth curves for *Staphylococcus* spp. at the temperatures of 2, 4, 7, 10, 15, and 20 °C. (a) Raw breast (RB), (b) salted breast (SB), and (c) cooked breast (CB).

Ismail et al., 2000; Miyagusku et al., 2003; Patsias et al., 2006). Such variations may be due to different values of the initial count, type of processing, accompanying microbiota, etc. (Davies and Board, 1998; Mano et al., 2002). When the temperature rose from low values (2 and 4 °C) to 10 °C, the product's SL was reduced by 50% and at 15 °C it was four to fivefold shorter. At temperatures above 10 °C, each increment of 5 °C was found to represent a decrease in SL of about 50% (Jay, 2005). Psychrotrophic bacteria and *Pseudomonas* spp. showed the same behavior.

In the conditions of this study, the maximum mesophilic bacteria count was around log 7.5–10 (Table 3). Studies of chicken meat cuts stored at temperatures

Table 1. Values of parameters asymptote (A), maximum specific growth rate (μ_{max}), and lag phase (λ) obtained from the modified Gombertz model for aerobic mesophilic and aerobic psychotropic bacteria, *Pseudomonas* spp. and *Staphylococcus* spp. present on raw, salted, and cooked chicken breasts at different temperatures

		Raw chicken breast				Salted chicken breast				Cooked chicken breast			
	T(°C)	A (ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	r	A ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	r	A ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	R
Aerobic mesophilic bacteria	2	6.4	0.025	151.5	0.998	6.2	0.021	143.5	0.995	6.4	0.025	153.0	0.993
	4	6.3	0.034	143.5	0.998	6.7	0.030	120.6	0.996	8.4	0.039	149.1	0.998
	7	7.7	0.061	81.4	0.994	8.0	0.042	84.5	0.998	8.6	0.073	111.7	0.997
	10	6.4	0.126	53.1	0.995	5.7	0.095	42.6	0.997	7.5	0.116	46.6	0.995
	15	7.8	0.153	19.7	0.996	6.3	0.122	25.1	0.998	8.5	0.155	19.2	0.999
	20	7.2	0.232	10.8	0.997	6.6	0.250	15.9	0.996	8.3	0.274	11.9	0.996
Aerobic psychotropic bacteria	2	6.9	0.026	131.6	0.999	6.1	0.027	114.2	0.999	7.9	0.037	187.6	0.998
	4	7.4	0.033	100.5	0.997	6.7	0.029	104.9	0.996	8.5	0.040	164.1	0.999
	7	7.0	0.054	74.4	0.998	7.9	0.036	58.9	0.995	8.5	0.061	95.5	0.994
	10	6.0	0.096	50.4	0.996	6.6	0.072	31.4	0.996	7.5	0.098	39.2	0.998
	15	7.2	0.137	20.2	0.998	6.1	0.123	20.9	0.998	8.1	0.141	20.7	0.996
	20	7.3	0.195	9.2	0.995	6.5	0.252	16.2	0.996	7.8	0.231	12.5	0.997
Pseudomonas spp.	2	5.7	0.039	120.8	0.997	5.8	0.040	131.2	0.999	8.2	0.027	139.9	0.997
	4	6.7	0.043	103.1	0.998	5.0	0.044	128.8	0.997	7.0	0.041	141.7	0.997
	7	6.1	0.051	90.3	0.997	6.4	0.046	94.0	0.997	6.8	0.061	125.5	0.999
	10	7.2	0.100	36.9	0.998	6.2	0.095	39.1	0.998	8.2	0.103	33.8	0.995
	15	8.9	0.125	10.2	0.996	7.6	0.099	8.9	0.996	8.8	0.160	11.6	0.997
	20	8.9	0.238	6.5	0.999	7.9	0.201	11.4	0.999	8.1	0.330	15.5	0.997
Staphylococcus spp.	2	8.0	0.021	157.9	0.999	7.6	0.019	117.9	0.998	3.0	0.029	177.6	0.996
	4	5.7	0.027	150.6	0.996	6.5	0.025	95.2	0.998	5.6	0.035	147.4	0.997
	7	7.6	0.056	62.3	0.997	7.7	0.051	71.2	0.997	6.4	0.037	125.8	0.998
	10	7.7	0.103	49.9	0.998	8.0	0.071	15.9	0.996	6.2	0.061	26.4	0.998
	15	7.8	0.108	20.4	0.997	9.5	0.098	7.8	0.995	7.7	0.123	19.4	0.998
	20	8.2	0.189	5.9	0.997	10.3	0.190	7.4	0.996	9.3	0.196	8.0	0.998

varying from 3.4 to 8.3 °C showed that, in most cases, both aerobic mesophilic and psychrotrophic bacteria counts reached the maximum log 8 after nine days of storage (Smolander et al., 2004). After this time, the level of microorganisms remained stable. Values of 7–9.3 are also reported in the literature (Balamatsia et al., 2006, 2007). The maximum count depends mainly on the presence of microbial flora, because their competition and metabolites interfere in each other's growth, i.e. one of them can inhibit or even facilitate the development of the others.

At low temperatures (2 and 4 °C), the growth of aerobic microorganisms presented a difference in the SL of the products. In these conditions, the mesophilic bacteria took longer to reach the maximum count established as safe, i.e. 12–19 days, while the psychrotrophic bacteria took from nine to 13 days (Table 3). This result was expected because these temperatures are far from optimal for mesophilic bacteria growth. On the other hand, psychrotrophic bacteria have a good growth rate at these temperatures because they are more efficient in the absorption of solutes at low temperatures, since their membrane mobility is greater than that of mesophilic bacteria (Davies and Board, 1998; Dominguez and Schaffner, 2007; Jay, 2005). In their experiments, Smolander et al. (2004) found no difference between mesophilic and psychrotrophic bacteria counts in chickens cuts stored at temperatures varying from 3.4 to 8.3 °C. At higher temperatures, the mesophilic and psychrotrophic bacteria led to the same SL times, which were 6–9 days (7 °C), 4–5 days (10 °C), 2–3 days (15 °C), and 1–2 days (20 °C). The literature reports SLs similar to those obtained here, i.e. 7.2 days (5 °C), 2–3.5 days (10 °C), 2.3 days (15 °C), and 15 h (25 °C) (Davies and Board, 1998; Dominguez and Schaffner, 2007).

Table 2. Values of parameters asymptote (A), maximum specific growth rate (μ_{max}), and lag phase (λ) obtained from the modified logistic model for aerobic mesophilic and aerobic psychotropic bacteria, *Pseudomonas* spp. and *Staphylococcus* spp. present on raw, salted, and cooked chicken breasts at different temperatures

		Raw chicken breast				Salted chicken breast				Cooked chicken breast			
	T(°C)	A (ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	r	A ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	r	A ln N_{max}/N_0)	μ_{max} (1/h)	λ (h)	r
Aerobic mesophilic bacteria	2	6.0	0.027	167.2	0.999	5.8	0.023	162.6	0.998	6.0	0.027	167.7	0.993
	4	6.2	0.034	148.8	0.999	6.5	0.031	129.5	0.999	8.1	0.040	158.6	0.999
	7	7.5	0.060	83.8	0.995	7.3	0.045	92.0	0.998	8.3	0.073	114.6	0.996
	10	6.4	0.123	54.5	0.993	5.6	0.095	44.5	0.996	7.3	0.116	48.7	0.991
	15	7.4	0.166	22.7	0.998	5.9	0.133	28.1	0.997	7.9	0.168	22.2	0.998
	20	6.5	0.256	12.5	0.997	6.0	0.269	17.0	0.996	7.5	0.305	13.6	0.998
Aerobic psychotropic bacteria	2	6.4	0.028	146.0	0.998	5.9	0.028	123.7	0.997	7.4	0.042	201.5	0.996
	4	7.1	0.034	112.6	0.997	6.5	0.030	114.2	0.998	8.1	0.042	172.1	0.999
	7	7.1	0.054	79.9	0.991	7.2	0.039	67.8	0.995	8.1	0.065	103.1	0.993
	10	5.8	0.094	51.7	0.994	6.3	0.076	35.9	0.996	6.9	0.100	41.6	0.995
	15	6.7	0.151	23.5	0.995	5.8	0.132	23.6	0.996	7.6	0.153	24.3	0.996
	20	6.4	0.217	11.2	0.994	5.9	0.270	17.3	0.997	6.8	0.260	14.4	0.997
Pseudomonas spp.	2	5.6	0.037	119.2	0.994	5.7	0.041	137.4	0.998	7.7	0.029	157.0	0.996
	4	6.6	0.043	106.5	0.997	5.0	0.045	135.2	0.998	6.9	0.041	147.4	0.997
	7	5.9	0.052	95.5	0.995	5.5	0.047	98.6	0.999	6.5	0.062	128.8	0.996
	10	7.0	0.102	39.7	0.994	6.1	0.096	41.5	0.997	7.9	0.107	37.5	0.992
	15	8.3	0.132	12.9	0.993	7.1	0.103	11.4	0.992	8.5	0.167	13.6	0.992
	20	8.1	0.259	8.5	0.998	7.0	0.220	13.4	0.994	7.7	0.340	16.4	0.993
Staphylococcus spp.	2	7.0	0.024	180.5	0.998	6.7	0.021	139.4	0.994	3.0	0.029	181.3	0.997
	4	5.5	0.027	157.2	0.994	6.2	0.026	105.1	0.997	5.7	0.034	153.2	0.999
	7	7.3	0.056	65.6	0.996	7.3	0.051	75.4	0.994	5.9	0.041	134.8	0.998
	10	7.5	0.108	53.7	0.996	7.6	0.075	21.4	0.994	6.1	0.065	31.3	0.994
	15	7.1	0.118	23.5	0.991	8.4	0.107	12.5	0.996	7.3	0.130	22.3	0.995
	20	7.5	0.193	6.5	0.995	8.4	0.220	13.4	0.995	8.0	0.214	10.1	0.995

Aerobic psychrotrophic bacteria are among the microorganisms that grow well at chilling temperatures of 0–7 °C (Jay, 2005). This group includes species responsible for the product's deterioration, which indicates their significance in reducing the SL of chilled foods (Miyagusku et al., 2003). The RB, SB, and CB samples presented similar SLs at each evaluated temperature, as shown in Table 3. This indicates that the different substrates did not influence the behavior of aerobic psychrotrophic microorganisms, as was also observed in the growth curves. A comparison of the SLs of the samples stored at 2 and 4 °C showed no apparent difference, especially between SB and CB. The analyzed chicken samples showed SLs of 9–13 days at 4 °C, which is a long period when compared to other data reported in the literature: five days (Miyagusku et al., 2003) and seven days (Jiménez et al., 1999). Longer SLs of 14 days (Barbut, 2002) and 15 days (Miyagusku et al., 2003) have been reported, although, in the latter case the authors used irradiated samples, which extends the SL of meats (Farkas, 1998). The longer SLs presented here are probably due to the lower initial microbial counts, i.e. consistently below log 3 CFU/g, when compared to those of other studies that usually range from log 4.0 to 5.0 CFU/g (Jiménez et al., 1999; Miyagusku et al., 2003).

Comparing the treatments at each temperature, it seems that in most of the situations CB presented higher maximum count and μ_{max} than RB and SB. Cooked meats provide ideal conditions for microbial growth due to their high concentrations of nutrients, neutral pH, and high moisture content, which allow microorganisms to grow rapidly (Jay, 2005). It has been shown that denatured poultry meat can be a better substrate for microbial spoilage,

Table 3. Initial (X_0) and maximum (X_{max}) counts of aerobic mesophilic and aerobic psychotropic bacteria, *Pseudomonas* spp. and *Staphylococcus* spp. and shelf-lives (SL) for raw, salted, and cooked chicken breasts at different temperatures

		Raw chicken breast			Salted chicken breast			Cooked chicken breast		
	T(°C)	X_0 (log CFU/g)	X_{max} (log CFU/g)	SL (days)	X_0 (log CFU/g)	X_{max} (log CFU/g)	SL (days)	X_0 (log CFU/g)	X_{max} (log CFU/g)	SL (days)
Aerobic mesophilic bacteria	2	2.0 ± 0.5	7.9 ± 0.0	16–19	2.2 ± 0.1	7.7 ± 0.0	15–17	1.4 ± 0.0	8.6 ± 0.2	15–18
	4	2.5 ± 0.0	8.8 ± 0.1	12–13	2.5 ± 0.0	8.4 ± 0.0	13–14	1.0 ± 0.0	9.0 ± 0.1	13–14
	7	1.6 ± 0.1	10.7 ± 0.2	6–7	1.8 ± 0.1	10.1 ± 0.3	8–9	1.4 ± 0.1	10.0 ± 0.4	8
	10	1.5 ± 0.1	8.3 ± 0.2	4–5	1.6 ± 0.2	7.5 ± 0.0	4–5	1.4 ± 0.1	9.2 ± 0.1	4–5
	15	1.7 ± 0.5	8.6 ± 0.4	2–3	2.2 ± 0.1	8.3 ± 0.0	3	1.4 ± 0.0	9.4 ± 0.3	2–3
	20	1.7 ± 0.5	8.4 ± 0.1	1	2.2 ± 0.1	8.1 ± 0.2	1–2	1.4 ± 0.0	8.4 ± 0.1	1
Aerobic psychotropic bacteria	2	2.5 ± 0.2	9.0 ± 0.1	11–13	2.5 ± 0.1	8.9 ± 0.0	10–12	2.9 ± 0.2	10.4 ± 0.1	11–13
	4	2.4 ± 0.0	9.7 ± 0.0	9–11	3.1 ± 0.2	9.3 ± 0.0	11–13	2.0 ± 0.0	10.1 ± 0.0	11–13
	7	2.3 ± 0.2	10.2 ± 0.1	6–7	2.5 ± 0.2	10.0 ± 0.2	8	2.0 ± 0.0	10.3 ± 0.0	7–8
	10	2.4 ± 0.1	8.6 ± 0.0	4–5	2.5 ± 0.4	8.6 ± 0.1	4–5	2.0 ± 0.0	9.2 ± 0.2	4
	15	2.5 ± 0.2	9.0 ± 0.6	2	2.5 ± 0.0	8.5 ± 0.1	2–3	2.9 ± 0.2	9.4 ± 0.0	2
	20	2.5 ± 0.2	8.4 ± 0.0	1	2.5 ± 0.0	8.4 ± 0.0	1–2	2.9 ± 0.2	8.8 ± 0.0	1
Pseudomonas spp.	2	2.2 ± 0.0	8.1 ± 0.0	10–12	2.0 ± 0.0	8.0 ± 1.2	11–14	2.0 ± 0.0	9.9 ± 0.4	13 to14
	4	2.0 ± 0.0	8.8 ± 0.0	11 to13	< 2.0 ± 0.0	7.0 ± 0.0	13–14	<2.0 ± 0.0	9.3 ± 0.0	11 to13
	7	2.4 ± 0.0	9.3 ± 0.1	7–8	2.0 ± 0.0	8.8 ± 0.6	10–11	2.0 ± 0.0	8.9 ± 0.3	8–9
	10	<2.0 ± 0.0	7.8 ± 0.4	5–6	<2.0 ± 0.0	6.9 ± 0.2	8	<2.0 ± 0.0	8.8 ± 0.3	4–5
	15	<2.0 ± 0.0	8.3 ± 0.1	3	<2.0 ± 0.0	8.2 ± 0.0	3–4	<2.0 ± 0.0	8.9 ± 0.0	2–3
	20	<2.0 ± 0.0	8.2 ± 0.0	1–2	<2.0 ± 0.0	7.0 ± 0.0	2	<2.0 ± 0.0	8.1 ± 0.0	2
Staphylococcus spp.	2	<2.0 ± 0.0	8.0 ± 0.1	14–17	<2.0 ± 0.0	7.4 ± 0.0	14–16	<2.0 ± 0.0	4.7 ± 0.9	>26
	4	<2.0 ± 0.0	7.7 ± 0.0	12–13	<2.0 ± 0.0	7.2 ± 0.0	13–14	<2.0 ± 0.0	7.6 ± 0.0	14–16
	7	<2.0 ± 0.0	9.4 ± 0.0	7–8	<2.0 ± 0.0	8.1 ± 0.0	6–9	<2.0 ± 0.0	8.1 ± 0.0	11–12
	10	<2.0 ± 0.0	7.6 ± 0.1	4–5	<2.0 ± 0.0	7.7 ± 0.1	4–5	<2.0 ± 0.0	6.7 ± 0.3	5–7
	15	<2.0 ± 0.0	7.2 ± 0.2	3–4	<2.0 ± 0.0	7.9 ± 0.1	3	<2.0 ± 0.0	7.4 ± 0.0	3
	20	<2.0 ± 0.0	7.0 ± 0.0	1–2	<2.0 ± 0.0	8.0 ± 0.0	1–2	<2.0 ± 0.0	7.7 ± 0.1	1–2

i.e. microorganisms grow better in heat-treated meat than in raw meat (Göksoy et al., 2000). Cooking causes meat proteins to coagulate and become denatured due to the unfolding of these protein molecules or the decrease in their conformation traits (Pearson and Gillett, 1999). Microorganisms can take advantage only of the smaller protein molecules, and peptides, but not intact protein, because it cannot cross the cell membrane. Therefore, some microorganisms secrete extracellular enzymes that rapidly hydrolyze protein molecules into soluble peptides and amino acids. Some bacteria secrete enzymes that hydrolyze a wide variety of important proteins in meat products (Franco and Landgraf, 2007). Thus, cooking seems to be a facilitator for microorganisms.

The initial pH values were: CB = 6.1, RB = 6.0, and SB = 5.9. It has been shown that there is a correlation between psychrotrophic counts and pH (Allen et al., 1997), and that a high pH (>6.0) does not accelerate the growth of spoilage microorganisms, but reduces the lag phase (Newton and Gill, 1981). However, this behavior could not be observed here due to the negligible difference in the pH of the different treatments. When food is kept under aerobic conditions, it is normal for the pH to increase, possibly due to the formation of substances derived from the growth of *Pseudomonas* and other related microorganisms (Mano et al., 2002). However, monitoring the pH during storage at different temperatures did not reveal an increase in pH in any of the cases (data not shown).

At the temperatures of 2, 4, 7, and 10 °C, the lag phases of psychrotrophic bacteria were longer in CB. This was attributed to the injury the cells sustained during the heat treatment, which required time to recover before starting their exponential growth phase. Another possibility is that cooking modifies the substrate, requiring a readaptation of the surviving cells (Madigan et al., 2004). The remaining bacteria from the original raw product would be heat resistant, multiplying more easily at higher temperatures (15 and 20 °C).

All the RB, SB, and CB samples showed very different curves of microbial growth bacteria at temperatures of 7, 10, 15, and 20 °C. However, the difference was not so evident at 2 and 4 °C (Figure 2). The μ_{max} parameter was also helpful to visualize the effect of temperature on the microbial growth, and the values of μ_{max} at 2 and 4 °C were found to be very similar, while the differences at the other temperatures were greater (Tables 1 and 2).

The initial levels of aerobic microorganisms were consistently below 2.5 log CFU/g (Table 3). These counts reflect the good handling of meat during industrial processing, differing significantly from the usual food handling practices in commercial establishments (Mano et al., 2002). Other studies have reported initial counts ranging from 3 to 6.4 CFU/g (Balamatsia et al., 2006; Chouliara et al., 2007; Dominguez and Schaffner, 2007; Ismail et al., 2000; Miyagusku et al., 2003; Patsias et al., 2006).

Pseudomonas spp. and *Staphylococcus* spp.

Pseudomonas spp. and *Staphylococcus* spp. are used by poultry processing plants as general indicators of product hygiene during processing, storage quality, and SL (Del Río et al., 2007). The growth curves of *Pseudomonas* spp. indicate that increasing the temperature hastened the development of these microorganisms (Figure 3). The higher the temperature of incubation the steeper the growth curve. This tendency was confirmed by the increase in μ_{max} values in response to rising temperature. However, at 2 and 4 °C, the three products (RB, SB, and CB) showed very similar curves; moreover, the curves of SB at 2, 4, and 7 °C almost overlapped, indicating that, in this temperature range, *Pseudomonas* spp. behave similarly in all the products. Before each growth curve stabilized, which was indicated by the maximum count (X_{max}), the products had already exhibited characteristics unsuitable for human consumption (data not shown).

Indicators of poultry spoilage, such as putrid and ammonia-like odors, occur when spoilage bacteria populations reach 10^6–10^7 cells/cm^2 (Allen et al., 1997). Chicken spoilage off-odors can be attributed to the growth of psychrotrophic bacteria that degrade the amino acids in the muscle. Among these psychrotrophic bacteria, *Pseudomonas* spp. have been identified as the primary bacterium producing sulfurous off-odors associated with spoiled poultry (Russell, 2002). Thus, in this study, the end of SL was considered to be when the *Pseudomonas* spp. count reached the reported range.

pH plays an important role in bacterial spoilage rates. In chicken meat, *Pseudomonas* spp. grow well at pH 6.2–6.4 (Franco and Landgraf, 2007). The samples presented pH values lower than that range (CB = 6.1, RB = 6.0, SB = 5.9). The value closest to the reported ones found in CB may indicate its slight tendency to higher proliferation of this bacterium and its shorter SL at most of the tested temperatures (Table 1).

Pseudomonas spp. are the main psychotrophic bacteria that are present at temperatures of 0–7 °C (Jay, 2005; Kraft, 1992). In this study, the *Pseudomonas* spp. count demonstrated high levels of the bacteria, particularly at 2 and 4 °C, with μ_{max} values close to those observed for psychrotrophic bacteria (Tables 1 and 2). In eviscerated chicken stored at 10 °C or lower, spoilage occurs mainly by *Pseudomonas* spp. (Franco and Landgraf, 2007).

Even at higher temperatures (15 and 20 °C), *Pseudomonas* spp. growth was quite significant considering the short SL and high values of μ_{max} and maximum count (X_{max}) observed (Figure 4, Tables 1 to 3). However, *Pseudomonas* spp. reportedly do not predominate in chicken meat stored at high temperatures, such as 10 and 20 °C (Kraft, 1992).

A comparison of the products at each temperature (Table 3) indicated a slight tendency for the SB samples to present a longer SL, as well as a lower maximum microbial count. However, it is not possible to conclude that *Pseudomonas* spp. showed a lower μ_{max} in SB (Tables 2 and 3). The differences in relation to SL and μ_{max} appeared to be too minor to be considered relevant, especially between CB and RB. Our results are in agreement with a previous study, in which growth curves presented similar behavior, without evident differentiation between cooked and raw products (Kraft, 1992). Furthermore, low *Pseudomonas* spp. counts have been reported in precooked chicken stored for more than 20 days (Patsias et al., 2006).

The products under study presented SLs exceeding seven days when chilled (up to 7 °C), and at 2 and 4 °C the products exhibited acceptable conditions of conservation for up to 10–14 days. These results are better than the 5–11 days for spoilage by *Pseudomonas* spp. to begin in chilled chicken, according to the reports of other authors (Balamatsia et al., 2007; Chouliara et al., 2007; Dominguez and Schaffner, 2007). In anaerobic conditions, the chilled product can last for more than 17 days (Balamatsia et al., 2007). In this work, CB presented a spoilage time by *Pseudomonas* spp. similar to that observed in RB. The *Pseudomonas* spp. lag phases of CB and RB did not differ, indicating that the different substrates did not influence the adaptation of these bacteria. However, studies on chicken stored aerobically at 4 °C revealed that *Pseudomonas* spp. contributed to a lesser degree to the spoilage of the product, which could exceed 20 days of storage (Patsias et al., 2006).

In this study, up to 15 °C, aerobic mesophilic organisms exhibited a longer lag phase than *Pseudomonas* spp.,

indicating that the latter bacteria predominated during spoilage. It has been demonstrated that these bacteria predominate when poultry is already spoiled, and that they serve as a good spoilage indicator for poultry meat stored aerobically (Dominguez and Schaffner, 2007; Koutsoumanis et al., 2006). However, the lag phases for aerobic mesophilic organisms were shorter at 20 °C, indicating that, at this temperature, these bacteria are relevant competitors of *Pseudomonas* spp.

The effect of temperature was clearly visible in this study (Figures 1 to 4), particularly at temperatures above 2–4 °C. The SL of chicken meat at 10 °C was around 4–8 days. At 15 °C, the SL was reduced by half and at 20 °C it was reduced fourfold.

Staphylococcus spp. are potentially dangerous to public health due to the presence of enterotoxins. Moreover, their occurrence can impair hygiene, particularly when the process involves food handling (Franco and Landgraf, 2007). An analysis of the curves indicated a long phase lag for *Staphylococcus* spp., especially in CB. The CB samples also clearly showed low bacterial growth at 2 °C (Figure 4, Tables 1 and 2). Cooking temperatures destroy most pathogens and usually only spores survive, although heat-resistant vegetative bacteria may also survive. Moreover, recontamination may occur at low levels (10^1 or 10^2), mainly through handling, which makes *Staphylococcus* spp. a suitable indicator of hygiene in poultry processing (Barbut, 2002; Jay, 2005).

An analysis of the μ_{max} values (Tables 1 and 2) confirms that increasing the temperature hastens the growth of *Staphylococcus* spp. Up to 15 °C, the μ_{max} seems to increase only slightly with temperature, but it increases sharply at 20 °C. This may have been due to the fact that these bacteria are mesophilic, like most pathogenic bacteria.

The initial level of contamination of the products was quite low, i.e. consistently log <2.0. The maximum counts were also mostly lower than log 8 (Table 3). However, earlier studies have found that about 10^5–10^6 CFU of *Staphylococcus aureus* per gram of food are necessary to form sufficient toxins to cause food poisoning (Barbut, 2002; Franco and Landgraf, 2007). Thus, to determine the SL of the products analyzed in this study, the maximum limit of log 5–6 of *Staphylococcus* spp. was considered.

Despite the low initial count and lower μ_{max} than that of the other microorganisms under study, the tolerance to *Staphylococcus* spp. was lower. This explains why the SL of the products at temperatures above 7 °C did not differ much from the values found for the other microorganisms. Thus, at 2 and 4 °C, the bacterial development was low enough to allow for a longer SL. This demonstrates the significant difference that just a few degrees of temperature can make in product preservation and consumer safety.

Finally, a sensory index (SI) was determined by linear regression and applied to determine the SL of RB (Figure 5). The product was considered unacceptable if the SI exceeded 1.8. According to this limit, the SLs for the total counts of *Pseudomonas* spp. were determined as 275 h at 2 °C, 254.5 h at 4 °C, 198.6 h at 7 °C, 142.8 h at 10 °C, and 122 h at 15 °C. These values are in accordance with the SL interval established only by the microbial counts (Table 3).

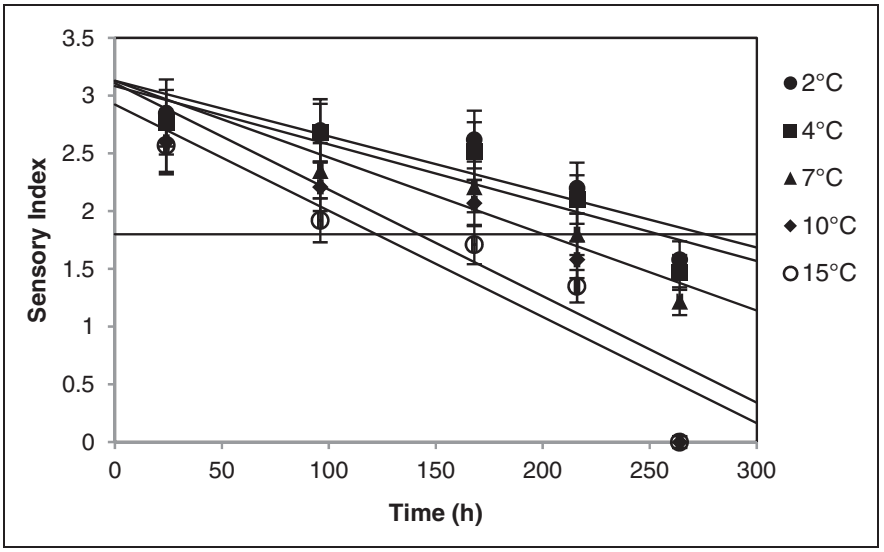

Figure 5. Sensory index for raw breast (RB) determined after one, four, seven, nine, and 11 days of storage at the temperatures of 2, 4, 7, 10, and 15 °C.

CONCLUSIONS

Increasing the temperature reduced the SL of the three products due to the growth of microorganisms. In general, the products presented SLs of 10–26 days at 2 °C, of 9–21 days at 4 °C, of 6–12 days at 7 °C, of 4–8 days at 10 °C, of 2–4 days at 15 °C, and of 1–2 days at 20 °C. The maximum microorganism count in the different products remained relatively stable at the various tested temperatures. In most cases, CB showed the highest microbial count, followed by RB and lastly SB. The data obtained in this study served to create mathematical models and parameters for different microorganisms growing at several temperatures on raw, cooked, and SB fillets. These models showed high correlations and can be used for predictive purposes in the poultry meat supply chain.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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