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Kinetic behaviour of *Staphylococcus aureus* on cheese as a function of water activity and temperature

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This study developed mathematical models in order to evaluate the effect of A_w (Water activity) and growth temperature on *Staphylococcus aureus* kinetic behaviour. The A_w levels (0·970, 0·975, 0·983, and 0·991) of cheese were adjusted by NaCl; then, *Staph. aureus* was inoculated on the cheese, followed by storage at 7–30 °C for 72–720 h. Total bacterial and *Staph. aureus* cell counts were enumerated on tryptic soy agar and mannitol salt agar, respectively. The Baranyi model was fitted to the *Staph. aureus* growth data in order to calculate the maximum specific growth rate (μ_{max}; log CFU/g/h), lag phase duration (λ; h), lower asymptote (N_0; log CFU/g) and upper asymptote (N_{max}; log CFU/g). The effects of storage temperature and A_w on the kinetic parameters (μ_{max} and λ) were then further analysed with the Ratkowsky-type model and a polynomial equation, respectively. The root mean square error (*RMSE*) and relative error (*RE*) were calculated in order to estimate the model performance. No significant effect of A_w on *Staph. aureus* growth was observed at 7 °C; thus, the Baranyi model was fitted to the growth data from 15, 25 and 30 °C. The μ_{max} values (0·011–0·303 log CFU/g/h) increased ($P<0·05$) as the storage temperature and A_w increased. In addition, λ values (2·42–63·48 h) decreased ($P<0·05$) as storage temperature and A_w increased; yet, the effect of A_w on λ was observed only at 15 °C. The theoretical minimum storage temperature and A_w were 10·15 °C and 0·882, respectively. *RMSE* (0·010–1·544) and *RE* values (−0·131 to 0·187) from validation indicated that model performance was appropriate. Hence, these results suggest that the developed models in this study should be useful in describing the effect of temperature and A_w on the growth kinetic behaviour of *Staph. aureus* in cheese along with the exposure assessment of *Staph. aureus* in cheese as well.

Keywords: *Staphylococcus aureus*, water activity, cheese, mathematical model.

Staphylococcus aureus is a facultative anaerobic and gram-positive foodborne pathogen, which is able to grow at 7–48·5 °C, pH 4·2–9·3 and 0–15% NaCl concentration (Bergdoll, 1989; Schmitt et al. 1990; Le Loir et al. 2003). Due to the wide range of these factors, *Staph. aureus* has been easily isolated from a variety of foods, such as milk and dairy products, meat, and chicken (Jablonski & Bohach, 2001; Tamarapu et al. 2001; Jorgensen et al. 2005; Colombari et al. 2007).

In the past decade, *Staph. aureus* has been one of the major foodborne pathogens (MFDS, 2014). In the U.K., although 95·8% of pasteurised milk cheese samples were satisfactory ($<10^2$ CFU) for microbial quality among 4437 cheese samples, 2·2% of the cheese samples were borderline (10^2–10^3 CFU), and 2·0% had unsatisfactory quality ($>10^3$ CFU), according to the recommendation of 2005/175/EC (EC, 2005; Little et al. 2008). In addition, 0·4% of all foodborne outbreaks in 2006 were caused by contaminated cheeses in the EU (Kousta et al. 2010). Thus, the control of *Staph. aureus* in various cheeses needs to be studied.

The A_w of cheeses varies due to various NaCl concentrations formulated in cheeses. NaCl has been added in order to improve the flavour as well as to inhibit foodborne pathogens in cheeses. Recently, the concentration of NaCl in cheese has been lowered as a result of consumers' demand. However, the decreased NaCl concentration, resulting in increased A_w, may increase the microbial risk of cheese. Therefore, the effect of A_w on the microbial growth in cheese should be studied.

Predictive models have been developed by many researchers in order to predict microbial survival under various conditions, such as storage temperature, pH and A_w. Moreover, predictive models have been used to predict the fates of foodborne pathogens. Primary models calculate

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the maximum specific growth rate (μ_{max}), lag phase duration (λ), lower asymptote (N_0) and upper asymptote (N_{max}) of bacteria using mathematical models (Whiting & Buchanan, 1993; Baranyi & Roberts, 1994). Secondary models describe the effect of various factors on the parameters, which were calculated by the primary models (Whiting, 1995).

Therefore, the objective of this study was to develop predictive models in order to evaluate the effect of storage temperatures and A_w on the kinetic behaviour of *Staph. aureus* in cheese.

Materials and methods

Inoculum preparation

Five strains of *Staph. aureus* (ATCC13565, ATCC14458, ATCC23235, ATCC27664 and NCCP10826) were cultured in 10 ml tryptic soy broth (TSB; Bacto™, Becton Dickinson and Company, Sparks, MD, USA) at 35 °C for 24 h. The 0·1 ml proportions of the cultures were transferred into 10 ml fresh TSB at 35 °C for 24 h. These subcultures were then mixed and centrifuged at 1912 ***g*** and 4 °C for 15 min. The supernatant was discarded, and the cell pellet was washed twice with phosphate buffered saline (PBS, pH 7·4; 0·2 g KH_2PO_4, 1·5 g Na_2HPO_4, 8·0 g NaCl, and 0·2 g KCl in 1 l of sterile distilled water). The mixture was serially diluted with PBS in order to obtain 6 log CFU/g.

Sample preparation and inoculation

Cheddar slice cheese (no antimicrobial added) containing 0·3% NaCl was ground with a grinder (HR 1372, Philips, Amsterdam, Netherlands); subsequently, NaCl was added to the ground cheese in order to obtain A_w of 0·970 (1·2%), 0·975 (1·0%), 0·983 (0·6%) and 0·991 (0·3%). These cheese samples (8 g/well) were then restructured in a 6-well microtiter plate by pressing; then, the 0·1 ml inoculum were inoculated on the surface of restructured cheeses, followed by spreading with a sterile spreader. Next, the 6-well microtiter plate was placed in polyethylene bag (Food Saver®, Rollpack, Pyeongtaek, Gyeonggi-do, Korea) and sealed by a packager (Food Guard®). The samples were then aerobically stored at 7, 15, 25 and 30 °C for 72–720 h.

Microbial analyses

The cheese samples in the 6-well microtiter plates were analysed at appropriate time intervals in order to enumerate bacterial populations. The cheese samples were aseptically transferred into a filter bag (3M™, Seoul, Korea). Then, 16 ml 0·1% buffered peptone water (BPW; Difco™, Becton Dickinson and Company) was added into the filter bag and pummelled for 120 s by a pummeler (BagMixer®, Interscience, St. Nom, France). The homogenates were then serially diluted with BPW, and 0·1 ml portions of the diluents were plated on tryptic soy agar (TSA; Difco™, Becton Dickinson and Company) and mannitol salt agar (MSA; BBL™, Becton Dickinson and Company) in order to enumerate total bacterial and *Staph. aureus* cell counts, respectively. The colonies were counted manually after incubation at 35 °C for 48 h.

Primary model development

The growth data of *Staph. aureus* on cheese, according to A_w, was fitted to the Baranyi model (Baranyi & Roberts, 1994) using the DMFit (Institute of Food Research, Norwich, UK), which is an MS Excel add-in; then, the maximum specific growth rate (μ_{max}; log CFU/g/h), lag phase duration (λ; h), lower asymptote (N_0; log CFU/g) and upper asymptote (N_{max}; log CFU/g) were calculated. The Baranyi model is:

$$N_t = N_0 + \mu_{max} \times A_t - \ln\left[1 + \frac{\exp(\mu_{max} \times A_t) - 1}{\exp(N_{max} \times N_0)}\right] \quad (1)$$

where N_t is the *Staph. aureus* cell counts at time t, and N_0 and N_{max} are the initial and maximum *Staph. aureus* cell counts, respectively. A_t is the adjustment function, which denotes the physiological status of *Staph. aureus* cell in order to define the λ (Baranyi & Roberts, 1994).

Secondary model development

The secondary model was developed to evaluate the effects of A_w and storage temperature on the kinetic parameters. The values of μ_{max} were fitted to the secondary models as a function of A_w and storage temperatures, as follows (Ratkowsky et al. 1982; Adams et al. 1991; Wijtzes et al. 1993);

$$\sqrt{\mu_{max}} = b\,(T - T_{min})\sqrt{(A_w - A_{w_{min}})} \quad (2)$$

where b is the coefficient value, T is storage temperature and A_w is water activity. T_{min} and A_{wmin} are the theoretical minimum temperature and A_w of *Staph. aureus* growth in cheese, respectively.

The values of λ were fitted to the secondary models with the polynomial equation as a function of A_w and storage temperatures;

$$\mathrm{Ln}\,\lambda = a_0 + a_1 \times T + a_2 \times A_w + a_3 \times T \times A_w + a_4 \times T^2 + a_5 \times A_w^2 \quad (3)$$

where a_i (where 'i' represents any number from 0 to 5) are coefficients, T is storage temperature (°C), and A_w is water activity. To fit the values of Ln λ to the polynomial equation, the regression analysis function in Excel 2007 (Microsoft®, USA) was used to select significant variables ($P<0{\cdot}05$).

Validation

Commercial Brie (A_w=0·987), Camembert (A_w=0·983), Cheddar (A_w=0·973), Mozzarella (A_w=0·975) and Gouda (A_w=0·958) cheeses were used to validate the performance of the developed model in this study. *Staph. aureus* was

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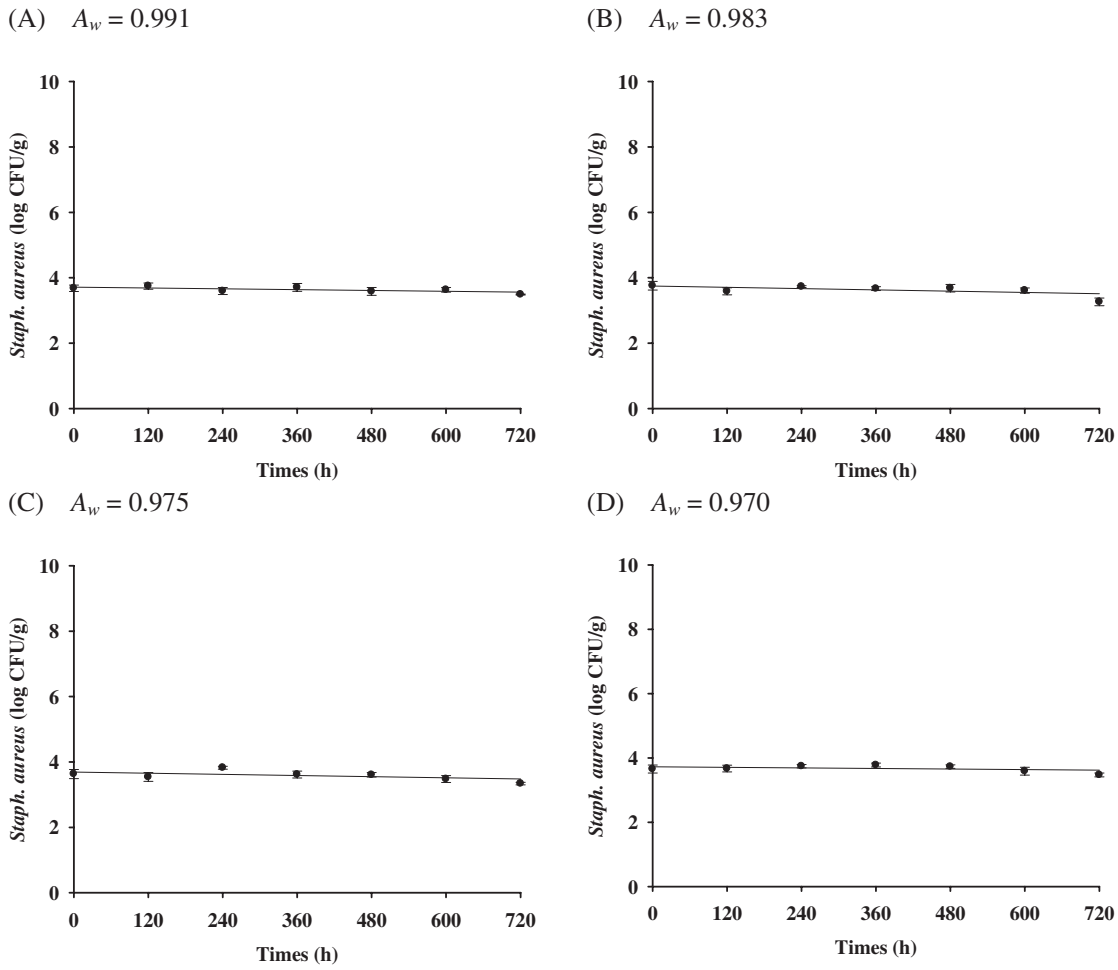

Fig. 1. Bacterial populations of *Staphylococcus aureus* in cheeses adjusted at different water activities (A_w=0·991, 0·983, 0·975, 0·970) during storage at 7 °C for 720 h; ●: observed value.

inoculated on the cheeses, and bacterial cell counts were enumerated in order to collect the observed values during storage at 4–30 °C. The observed values were then compared with the predicted values, which were estimated by the developed models. Subsequently, the model performance was evaluated by the root mean square error (*RMSE*) and relative error (*RE*) with the following equation:

$$\text{RMSE} = \sqrt{\frac{\sum (\text{predicted value} - \text{observed value})^2}{n}} \quad (4)$$

$$\text{RE} = (\text{predicted value} - \text{observed value})/\text{predicted value} \quad (5)$$

where n represents the number of data points.

Statistical analysis

This study was repeated twice using two samples per repeat (n=4). The kinetic parameters (μ_{max}, λ, N_0, and N_{max}) were analysed using the general linear model procedure of SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA), and all mean comparisons were performed with the pairwise *t*-test at α=0·05.

Results and discussion

No significant *Staph. aureus* growth was observed on the cheeses at 7 °C for all A_w (Fig. 1); however, there was a significant growth of *Staph. aureus* at 15–30 °C, regardless of A_w (Fig. 2). Only growth data (15, 25, and 30 °C) were used to develop the primary models with the Baranyi model. The R^2 values (0·900–0·995) of the developed primary model were high (Table 1), indicating that the Baranyi model was appropriate for calculating the growth parameters of *Staph. aureus* in cheeses. Although the μ_{max} values (0·011–0·303 log CFU/g/h) were increased (P<0·05) as storage temperature increased, the μ_{max} values were decreased (P<0·05) as A_w decreased. In addition, λ values (2·42–63·48 h) were decreased as storage temperature increased; yet, the effect of A_w on λ values was observed only at 15 °C (Table 1). This result indicates that A_w may not influence *Staph. aureus* growth in cheese during storage at 25 and 30 °C. The N_0 values ranged from 3·5 to 3·8 log CFU/g; the significant difference in N_{max} values (7·5–9·2 log CFU/g) was shown at 15 °C, which may have been caused by A_w.

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Table 1. Growth parameters (mean ± SD) of *Staphylococcus aureus* in cheese according to storage temperature and water activity, calculated by the Baranyi equation (Baranyi & Roberts, 1994)

Storage temperature (°C)	A_w	λ (h)	μ_{max} (log CFU/g/h)	N_0 (log CFU/g)	N_{max} (log CFU/g)	R^2
15	0·991	$29·18 \pm 0·00^{C}$	$0·022 \pm 0·01^{E}$	$3·5 \pm 0·2^{AB}$	$9·2 \pm 0·2^{A}$	0·985
	0·983	$14·19 \pm 9·40^{C}$	$0·015 \pm 0·00^{E}$	$3·6 \pm 0·0^{AB}$	$8·2 \pm 0·3^{AB}$	0·980
	0·975	$39·67 \pm 17·09^{B}$	$0·017 \pm 0·05^{E}$	$3·5 \pm 0·1^{B}$	$7·5 \pm 1·8^{B}$	0·963
	0·970	$63·48 \pm 24·62^{A}$	$0·011 \pm 0·05^{E}$	$3·6 \pm 0·2^{AB}$	$8·4 \pm 0·1^{AB}$	0·900
25	0·991	$6·86 \pm 5·45^{C}$	$0·240 \pm 0·01^{B}$	$3·6 \pm 0·2^{AB}$	$9·1 \pm 0·2^{A}$	0·989
	0·983	$8·65 \pm 6·06^{C}$	$0·230 \pm 0·02^{BC}$	$3·6 \pm 0·2^{AB}$	$9·0 \pm 0·2^{A}$	0·978
	0·975	$10·29 \pm 3·71^{C}$	$0·203 \pm 0·00^{C}$	$3·9 \pm 0·2^{A}$	$9·0 \pm 0·1^{A}$	0·990
	0·970	$5·74 \pm 4·87^{C}$	$0·150 \pm 0·01^{D}$	$3·8 \pm 0·0^{AB}$	$9·0 \pm 0·1^{A}$	0·995
30	0·991	$2·55 \pm 0·01^{C}$	$0·303 \pm 0·03^{A}$	$3·8 \pm 0·0^{A}$	$9·1 \pm 0·1^{A}$	0·981
	0·983	$2·42 \pm 1·05^{C}$	$0·301 \pm 0·01^{A}$	$3·7 \pm 0·4^{AB}$	$9·1 \pm 0·2^{A}$	0·993
	0·975	$3·64 \pm 0·31^{C}$	$0·298 \pm 0·04^{A}$	$3·8 \pm 0·2^{AB}$	$9·1 \pm 0·2^{A}$	0·995
	0·970	$3·35 \pm 1·22^{C}$	$0·281 \pm 0·01^{A}$	$3·8 \pm 0·2^{AB}$	$9·1 \pm 0·1^{A}$	0·990

μ_{max}, maximum specific growth rate; λ, lag phase duration; N_0, lower asymptote; N_{max}, upper asymptote
$^{A–E}$Different letters in the same column mean significantly different $P < 0·05$

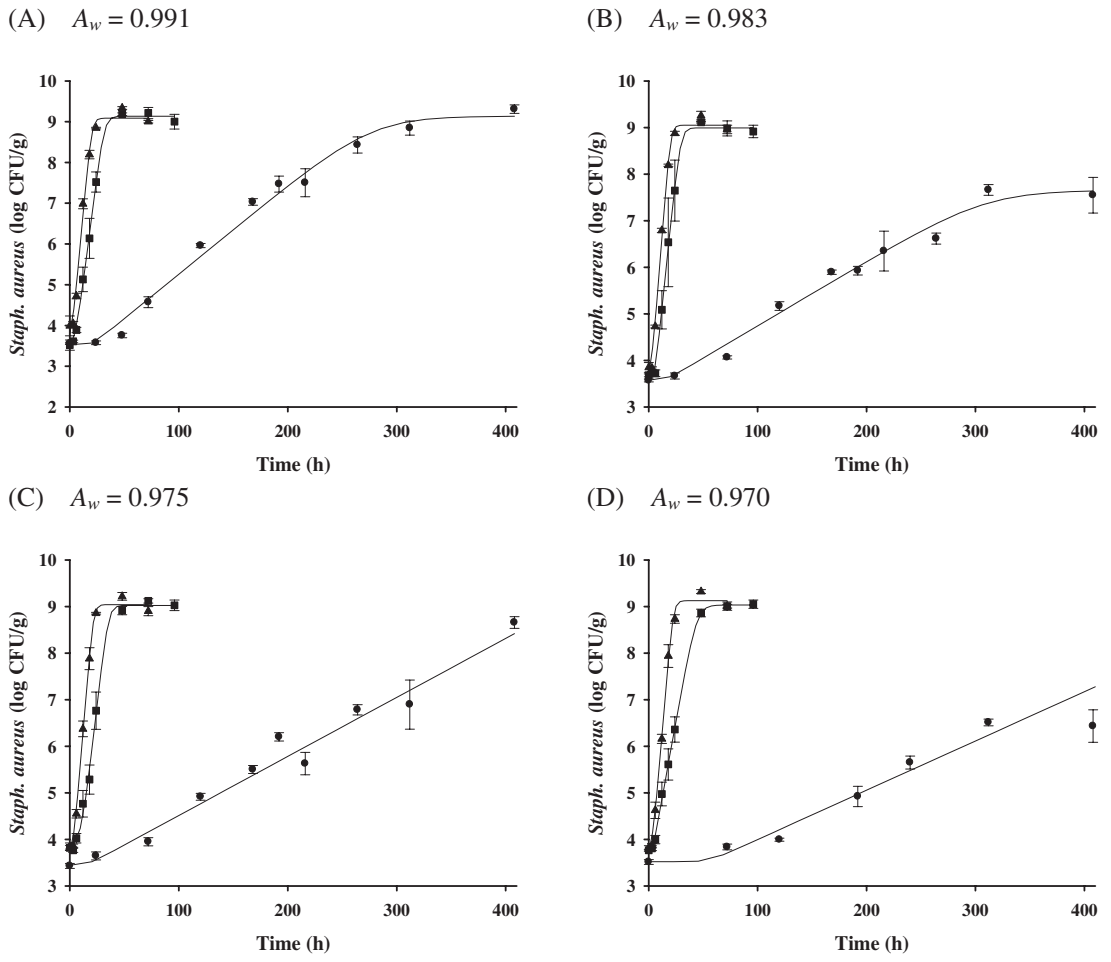

Fig. 2. Bacterial populations of *Staphylococcus aureus* in cheeses according to storage temperature and water activity; ● (15 °C), ■ (25 °C), ▲ (30 °C): observed data, —: fitted line with the Baranyi model.

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Table 2. Estimates of parameters, calculated by the Ratkowsky-type model for μ_{max} of *Staphylococcus aureus* in cheese

Coefficient	Estimate	SE	R^2
b	0·0912	0·0035	0·967
T_{min}	10·1944	0·0519	
A_{wmin}	0·8821	0·0392	

Table 3. Estimates of parameters, calculated by a polynomial equation for λ of *Staphylococcus aureus* in cheese

Coefficient	Estimate	SE	P-value
Intercept	26·7418	15·15	0·093
T	−0·1541	0·02	<0·001
A_w	−21·4674	15·50	0·181

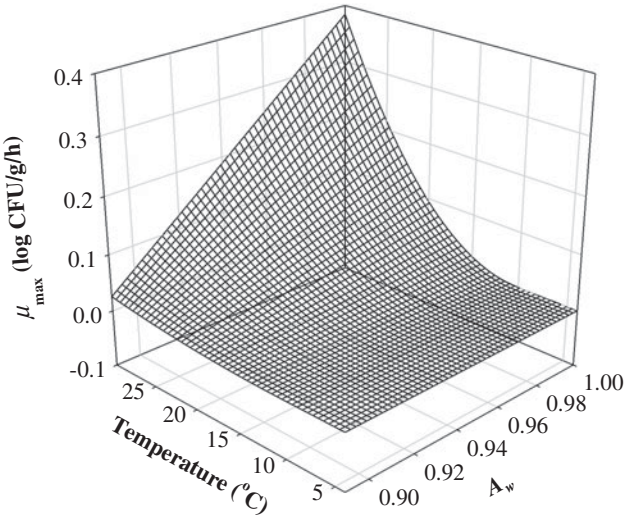
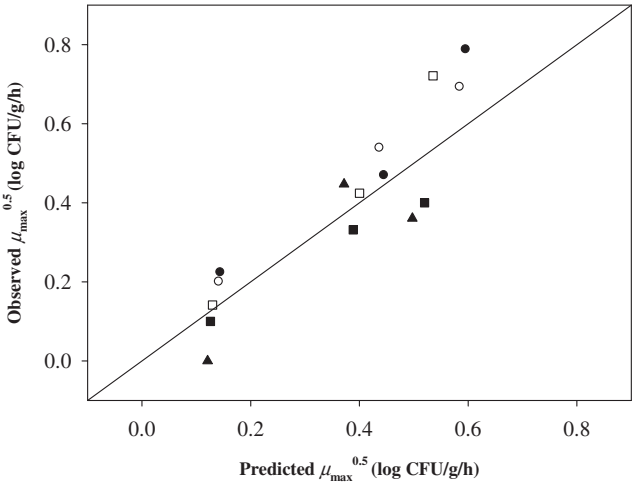


Fig. 3. Secondary model for the μ_{max} of *Staphylococcus aureus* in cheeses as a function of storage temperature and water activity.



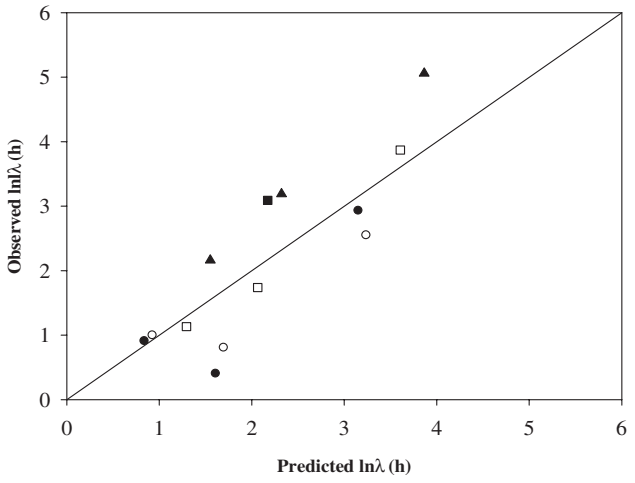


Fig. 4. Comparison of observed and predicted μ_{max} and λ values for validation.

To describe the storage temperature and A_w effect on μ_{max}, the Ratkowsky-type model was fitted to the μ_{max} values (Ratkowsky et al. 1982; Adams et al. 1991; Wijtzes et al. 1993). The R^2 value was 0·967, indicating that the Ratkowsky-type model was appropriate to describe the effect of storage temperature and A_w on μ_{max} (Table 2 and Fig. 3). T_{min} and A_{wmin} were then calculated to be 10·19 °C, and 0·882, respectively (Table 2). Although the value of T_{min} was slightly high, a survey by Jol et al. (2006) indicated that 20% of domestic and commercial refrigerators in the U.S. were kept at more than 10 °C temperature. Thus, *Staph. aureus* in cheese may possibly be exposed to the T_{min}. For Mozzarella (A_w=0·970) and Cheddar cheeses (A_w=0·965), T_{min} values were 6·35, and 5·72, respectively (Kim et al. 2013). Because the T_{min} values from the study by Kim et al. (2013) were estimated only at high A_w, the T_{min} values were lower than that of our study. These results were impacted by the kinetic behaviour of pathogens, which depends on the food matrix (Yoon, 2010). In addition, in research by Valik & Gorner (1993), the minimum A_w for *Staph. aureus* growth was estimated to be between 0·86–0·93; the results of Baird-Parker's research (2000) suggested that the minimum A_w in BHI was 0·86 at 30 °C. To describe the effect of storage temperature and A_w on λ, the λ values were transformed to a natural logarithm because λ showed an exponential relationship as a function of storage temperature and A_w, and a polynomial equation was fitted to the ln(λ) values. The estimates of parameters are shown in Table 3. R^2 was 0·766, indicating that the polynomial equation was acceptable for describing the effects of storage temperature and A_w on λ. Although, the P-value of the estimated coefficients for A_w was not significant, A_w was involved in the model because the linear relationship between λ and A_w was obviously observed.

To validate the developed models, a mixture of *Staph. aureus* was inoculated on commercial Brie (A_w=0·987), Camembert (A_w=0·983), Cheddar slice (A_w=0·973),

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Mozzarella slice (A_w=0·975) and Gouda slice cheeses (A_w=0·958). Then, the *Staph. aureus* cell counts were enumerated during storage at 15, 25 and 30 °C, as described above. Subsequently, the observed μ_{max} and λ values were also calculated using the Baranyi model (Baranyi & Roberts, 1994), and the observed data were then compared with the predicted values calculated by the developed models (Fig. 4). In our study, bias (*B*) and accuracy (*A*) factors were not used to validate the model performance because Oscar (2005b) suggested that *B* and *A* factors had limitations for evaluation of the developed model performance. Thus, *RMSE* and *RE* were calculated for the evaluation of model performance. The *RMSE* value of μ_{max} and λ values were 0·010, and 1·544, respectively. If the *RMSE* value is close to '0', indicating a lower difference of value between the observed data and the predicted data, the performance of the developed model is appropriate. In addition, the *RE* was calculated to be −0·131 for μ_{max} and 0·187 for λ. When the *RE* value is less than zero, it indicates that the model is 'fail-safe,' and when the *RE* value is greater than zero, it represents a 'fail-dangerous' prediction (Oscar, 2005a). A study by Oscar (2005a) suggested that an *RE* range from −0·6 to 0·3 is appropriate. Taken together, these values indicate that the performance of the developed model is appropriate for describing the kinetic behaviour of *Staph. aureus* in cheese as a function of A_w and storage temperature.

In conclusion, the developed model in this study should be useful in describing the kinetic behaviour of *Staph. aureus* as a function of storage temperature and A_w. In addition, the developed model in this study can be used for the exposure assessment, as a part of microbial risk assessment, to predict *Staph. aureus* in cheeses.

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