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# The effect of food processing factors on the growth kinetics of *Aeromonas* strains isolated from ready-to-eat seafood

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ABSTRACT

*Aeromonas* are ubiquitous aquatic bacteria and frequently isolated from seafood. There is growing awareness of *Aeromonas* as foodborne pathogens, particularly in connection with consumption of ready-to-eat (RTE) seafood. The aim of this study was to investigate the effect of food processing factors on the growth kinetics of eight *Aeromonas* strains (representing seven species) isolated from RTE seafood. The effect of low temperature (4 and 8 °C) in combination with different NaCl concentrations (0.5–6.5 %) or with two purified condensate smokes (PCSs; Red Arrow SmokEz VTABB and JJT01) at different concentrations (0–0.26 %) was studied in Trypton Soy Broth (TSB). In food processing, application of PCS is considered healthier than traditional smoking. Growth kinetics parameters of each strain were estimated by using a primary predictive model. Our result showed that the addition of 3.5 % NaCl at refrigeration temperature (4 °C) was not sufficient to inhibit the growth of *A. media*, *A. bestiarum*, *A. piscicola*, and *A. salmonicida*, while higher NaCl concentration (≥5.0 %) at 8 °C suppressed their growth. On the other hand, our result demonstrated the antimicrobial potential of using PCS at maximal allowed concentration (0.26 %) against *Aeromonas*. PCS concentration and phenol content were important factors influencing the growth kinetics parameters of *Aeromonas*. Moreover, the growth kinetics of three *Aeromonas* strains were further studied in commercially produced vacuum-packed fresh and cold-smoked salmon stored at 4 °C for 14 and 21 days, respectively. Our results demonstrate that vacuum packing combined with cold storage at 4 °C was insufficient to inhibit the growth of *Aeromonas* in fresh salmon, while the growth was inhibited in a minimally salted cold-smoked salmon (salt content of 1.8 %). Our study implies that mild food processing factors applied in the production of RTE seafood might not guarantee the total inhibition of *Aeromonas*. Even though further studies on evaluating the antimicrobial potential of PCSs in actual seafood matrixes are necessary, the present study suggests that PCS technology might be a promising approach to prevent the potential growth of *Aeromonas*.

## 1. Introduction

With the recent trend of consuming food perceived as healthier and more natural, consumption of ready-to-eat (RTE) seafood such as sushi, sashimi, and cold-smoked fish products has increased (Menozzi et al., 2020). In the production of RTE seafood, the growth of microorganisms is controlled by establishing a series of hurdles that challenge microbial growth to prolong the product's shelf life, an approach known as hurdle technology (Leistner, 2000). The hurdles applied are combinations of two or more mild processing technologies to retain high nutritional and sensory value. Mild processing technologies include a variety of processes e.g., chilling, salting, and smoking (Abel et al., 2022). RTE seafood are consumed without further cooking process, and proper refrigerated storage is thus needed to inhibit the growth of microorganisms (Herrera, 2016). However, mild processing technology combined with cold storage have shown to not completely prevent the growth of potential pathogenic microorganisms, such as *Listeria monocytogenes*, or *Aeromonas* spp. (EFSA BIOHAZ Panel et al., 2018; Hoel et al., 2019). Consequently, consumption of RTE seafood is often one of the major causes of foodborne diseases (EFSA and ECDC, 2021).

*Aeromonas* are ubiquitous aquatic bacteria, and some species are known as fish pathogens (Janda and Abbott, 2010). Additionally, there

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is a growing awareness of *Aeromonas* as foodborne pathogens (Hoel et al., 2019). This is due to their widespread occurrence in different types of water and food (Stratev et al., 2012), and their pathogenic potential by producing various virulence factors such as hemolysin, aerolysin, and cytotoxic enterotoxins. (Hoel et al., 2017; Nagar et al., 2011). The occurrence of potentially pathogenic *Aeromonas* possessing virulence-associated toxin genes has been reported in various RTE seafood (Hoel et al., 2015; Lee et al., 2021) as well as drinking water (Pablos et al., 2011). Several virulence-associated toxins of *Aeromonas* spp. are likely linked to clinical symptoms of *Aeromonas* infections such as gastrointestinal and extraintestinal infections to humans (Tomás, 2012). The consumption of oysters, salads and drinking water contaminated with *A. hydrophila* has been reported as the source of foodborne outbreaks (Abeyta et al., 1986; Ventura et al., 2015; Zhang et al., 2012). In addition, *Aeromonas* are commonly recognized as spoilage organisms in seafood (Jakobsen et al., 2020; Parlapani et al., 2013). The spoilage potential of *A. salmonicida* in vacuum-packed salmon has been previously reported (Jakobsen et al., 2020).

Microbial growth is influenced by several intrinsic and extrinsic factors, such as temperature, water activity, and pH. Refrigeration is one of the most common practices of controlling microbial growth in order to maintain the quality of food (Mercier et al., 2017). *Aeromonas* is known to grow at a wide range of temperatures from 0 to 45 °C, and the ability of some *Aeromonas* to grow under refrigeration temperature was highlighted in previous studies (Hoel et al., 2018; Vivekanandhan et al., 2003). Salt is a widely used preservative as well as a flavor enhancer. However, reducing salt intake to <5 g/day is recommended due to unhealthy effects of high sodium intake (WHO, 2013). The combination of low temperature and high salt contents can be a limiting factor for *Aeromonas* growth (Gram, 1991), while some *Aeromonas* can grow at the concentration of 4.0 % NaCl at 5 °C (Vivekanandhan et al., 2003). In addition, smoking is one of the oldest methods of preserving fish, and a traditional cold-smoking process includes salting, dehydration, and smoking, which establish combined hurdles (Leroi et al., 2000). To produce a cold-smoked salmon, filets with salt (<6.0 % in the water phase) are subject to wood smoking at 25–30 °C or subject to artificial smoke flavoring by applying atomized purified condensed smokes (PCS) (Løvdal, 2015). However, due to the potential health risk by polycyclic aromatic hydrocarbons (PAHs) from wood smoking, the use of atomized PCS has gained increased attention to produce smoked fish products with healthier and better sensory perspectives (Lingbeck et al., 2014; Waldenstrøm et al., 2021). PCS consist of several compounds including phenols, organic acids, and carbonyls, which are responsible for the antimicrobial effects in smoked products (Lingbeck et al., 2014). Previous studies suggested the antimicrobial potential of PCS against foodborne pathogens including *Aeromonas* (Lingbeck et al., 2014; Sofos et al., 1988; Suñen et al., 2003). Moreover, RTE seafood are usually packed under vacuum (VP) or modified atmosphere (MAP) and subjected to refrigeration to extend the shelf life (Aberoumand and Baesi, 2020). Previous studies showed that VP in combination with refrigeration at 4 °C is insufficient to inhibit *Aeromonas* spp. in RTE salmon and trout products (Jakobsen et al., 2020; Suñen et al., 2003). Although a high $CO_2$ concentration in MAP has shown better antimicrobial activity against *Aeromonas* than VP, MAP (60–67 % $CO_2$, initial headspace concentration) could not completely inhibit their growth in salmon nor cod stored at 4 °C (Hoel et al., 2022; Jakobsen et al., 2020).

The prevalence of potentially pathogenic *Aeromonas* was highlighted in different types of RTE seafood on the Norwegian market, which implies a potential risk of consuming perishable RTE seafood without heat treatment (Hoel et al., 2015; Lee et al., 2021). In order to prevent and control the growth of potentially pathogenic *Aeromonas* in RTE seafood, it is critical to understand the growth behaviors under different growth conditions. However, there is limited information about growth characteristics of *Aeromonas* under various processing factors applied in production of RTE seafood. In addition, available information on growth kinetics of *Aeromonas* is limited to some species such as *A. hydrophila*, *A. caviae*, and *A. sobria*. More knowledge on growth characteristics of *Aeromonas* would be beneficial for the practical development of advanced hurdle technology to produce the RTE seafood with higher quality and safety. Thus, we aimed to assess the growth kinetics of eight *Aeromonas* strains representing seven species isolated from RTE seafood subjected to various processing conditions. In the first part of the study, we investigated the effects of different food processing factors such as temperature, NaCl, and PCS. In the second part, the growth of three *Aeromonas* strains were studied in commercially available vacuum-packed fresh and cold-smoked salmon fillets stored at 4 °C for 14 and 21 days, respectively.

## 2. Materials and methods

### 2.1. Bacterial strains

Eight *Aeromonas* strains were isolated from retail sushi (*A. caviae* SU4-2, *A. dhakensis* A536, *A. hydrophila* A538, *A. media* SU10, *A. piscicola* SU58-3, and *A. salmonicida* SU2) and Atlantic salmon (*Salmo salar*) loins (*A. salmonicida* SL21 and *A. bestiarum* SL22) in previous studies (Hoel et al., 2015; Lee et al., 2021).

### 2.2. Salmon juice preparation

As a model system to mimic the nutrient composition of salmon, enriched salmon juice (SJ) was used as a growth medium. The SJ was prepared from fresh salmon loins obtained from a local retailer, according to the method described by Wiernasz et al. (2017) and modified by Stupar et al. (2021). In brief, 500 g of salmon loin was blended with 1 l of distilled water, boiled for 2 min, filtered through a 185 mm folding filter (Schleicher & Schuell, Dassel, Germany), and sterilized at 100 °C for 30 min. Sterile SJ was stored at −45 °C. Before use, 90 ml of SJ was supplemented with 10 ml of 1 M $K_2HPO_4/KH_2PO_4$ buffer solution (Merck, Darmstadt, Germany) at pH 6.7, 1 g of D-glucose (Merck, Oslo, Norway), and 1.5 g of NaCl (VWR, Oslo, Norway). After enrichment, the medium was filtrated through a 0.45 μm syringe filter (VWR).

### 2.3. Growth kinetics study in liquid culture systems

Growth kinetics parameters of the *Aeromonas* strains were investigated in two liquid culture systems (TSB and SJ) under different food processing factors; temperature (4 and 8 °C), NaCl (0.5–6.5 %) and purified condensed smoke (PCS, 0–0.26 %).

#### 2.3.1. Temperature

*Aeromonas* were grown on a tryptone soya agar (TSA) (Oxoid, Oslo, Norway) at 30 °C for 48 h, and one colony was precultured in 10 ml tryptone soya broth (TSB (Oxoid)) at 15 °C for 24 h. From the preculture, 300 μl was inoculated into 30 ml TSB and SJ, respectively, to achieve the initial bacterial concentration of approximately 7 log CFU/ml. The pH of TSB and SJ was measured as 7.4 ± 0.02 and 6.7 ± 0.02, respectively, by using a pH meter (Testo, Germany). Optical density (OD) was measured at 600 nm with a UV spectrophotometer (UV-1800, Shimadzu Crop., Japan) immediately after inoculation. Three parallel cultures were grown for each strain, and was incubated at three different temperatures (4, 8, and 15 °C) over a certain period (69–220 h) and OD was measured until it reached the stationary phase to estimate growth rates. Each TSB and SJ without bacterial inoculum were used as a media control during the OD measurement, respectively.

#### 2.3.2. Sodium chloride (NaCl)

NaCl (Merck) was dissolved into TSB (containing 0.5 % NaCl) in Scott bottles (1000 ml) to obtain the final NaCl concentration (2.0, 3.5, 5.0, and 6.5 %), and subjected to sterilization at 121 °C for 15 min. TSB without supplements (0.5 % NaCl) was used as a control group. The pH of TSB containing 2.0, 3.5, 5.0, and 6.5 % NaCl was measured as 7.28 ±

0.02, 7.18 ± 0.02, 7.18 ± 0.02, and 7.11 ± 0.02, respectively. Each strain was precultured in TSB as described in Section 2.3.1. Each bacterial inoculum (approximately 7 log CFU/ml) was prepared in TSB containing different concentrations of NaCl and stored at two different temperatures (4 and 8 °C). Three parallel cultures were grown for each strain and OD was measured until it reached the stationary phase. TSB containing different concentrations of NaCl without bacterial inoculum was used as a media control during the measurement.

#### 2.3.3. Purified condensed smoke (PCS)

Two purified condensed smokes (PCS) (SmokEz VTABB RA12012 and JJT01 30764575) were purchased from Red Arrow™ (Manitowoc, WI, USA). The abbreviation VTABB and JJT01 are used in the later context. Each PCS concentrate was dissolved in TSB to obtain the final concentration (0.026, 0.13, and 0.26 %), where 0.26 % is the recommended maximum level (2.6 g/1 kg processed fish product) according to the manufacturer. The pH of TSB containing 0.026, 0.13 and 0.26 % VTABB was 7.38 ± 0.02, 7.21 ± 0.02 and 6.30 ± 0.02, respectively, and the pH of TSB containing 0.026, 0.13 and 0.26 % JJT01 was 7.38 ± 0.02, 7.21 ± 0.02 and 6.67 ± 0.02, respectively. TSB without supplements was used as a control group. Each strain was precultured in TSB as described in Section 2.3.1. Each bacterial inoculum (approximately 7 log CFU/ml) was prepared in TSB containing different concentrations of PCS and stored at two different temperatures (4 and 8 °C). Three parallel cultures were grown for each strain and OD was measured until it reached the stationary phase. TSB containing different concentrations of each PCS without bacterial inoculum was used as a media control during the measurement.

### 2.4. Growth kinetics study in fresh and cold-smoked salmon

Growth kinetics parameters of three *Aeromonas* strains (*A. bestiarum* SL22, *A. piscicola* SU58-3, and *A. salmonicida* SU2) were investigated in commercially produced vacuum-packed fresh salmon and cold-smoked salmon fillets stored at 4 °C for 14 and 21 days, respectively.

#### 2.4.1. Sample and bacterial inoculum preparation

Fresh and cold-smoked salmon fillets (SALMA, Oslo, Norway) from farmed Atlantic salmon (*Salmo salar*) were purchased from a local retail. The cold-smoked salmon (SALMA) was lightly smoked and containing 1.8 g salt/100 g product. The salmon fillets were cut in 20 ± 2 g at five days post-harvest for the fresh salmon and six days post-harvest for the cold-smoked salmon. Each bacterial inoculum was prepared by growing the strain overnight on TSA, and one colony was transferred to a 250 ml conical flask containing 100 ml TSB. The culture was then grown at 8 °C at 230 rpm for 48 h to allow the bacteria to adapt to cold storage. Further, the culture was diluted with TSB to an OD value corresponding to a bacterial concentration of approximately log 3 CFU/ml. Each salmon piece was inoculated with 1 ml of its respective bacterial culture (strain SL22, SU58-3 or SU2), which was spread evenly on the surface with a sterile spreader. Uninoculated salmon (fresh and cold-smoked) was used as a control, resulting in eight experimental groups. Both inoculated and uninoculated salmon samples were air-dried on the bench for 30 min before vacuum packaging (VP). Each sample was packaged in 20 μm polyamide (PA)/70 μm polyethylene (PE) bag (120 × 80 mm, Star-Pack Productive, Boissy-l'Aillerie, France) with a Webomatic Supermax-C vacuum machine (Webomatic, Bochum, Germany). Air was evacuated to an end pressure of 10 mbar before sealing. The vacuum-packed fresh salmon and cold-smoked salmon were stored at 4 °C for 14 and 21 days, respectively.

#### 2.4.2. Microbiological analysis

A representative sample (10 g) from each product was aseptically cut and transferred to a sterile stomacher bag and diluted 1:10 with sterile peptone water (1.0 g of bacteriological peptone and 8.5 g of NaCl/l) and homogenized for 60 s in a Stomacher 400 lab blender (Seward Medical, Worthington, UK). Serial dilutions of the homogenate were prepared for further analysis. For quantification of *Aeromonas* spp., the homogenate was streaked on starch ampicillin agar (SAA) supplemented with ampicillin (10 mg/l) (Sigma-Aldrich, Oslo, Norway) and incubated at 37 °C for 24 h according to Nordic Committee on Food Analysis (NMKL) method No. 150 (NMKL, 2004). Total aerobic plate count (APC) including black colonies of $H_2S$-producing bacteria, were quantified on Lyngby's iron agar (IA) (Oxoid) supplemented with 0.04 % L-cysteine (Sigma-Aldrich), and the plates were incubated at 22 °C for 72 h according to NMKL method No. 184 (NMKL, 2006). Sampling was performed at day 0, 2, 4, 6, 8, 10, 12 and 14 for fresh salmon, and at day 0, 3, 7, 11, 15, 17, 19 and 21 for cold-smoked salmon.

### 2.5. Total phenolic content (TPC)

#### 2.5.1. TPC of purified condensed smoke (PCS)

Total phenolic contents (TPC) of the two PCSs (VTABB and JJT01) were analyzed in three replicates according to the method described by Singleton et al. (1999). For analysis, 1 ml of each PCS (0.26 % VTABB and 0.26 % JJT01) was added 1 ml Folin-Ciocalteu reagents (VWR International, France) and diluted with 5 ml deionized water. After 7 min at room temperature, 10 ml $Na_2CO_3$ (7 %, Alfa Aesar, Germany) was added before the mixture was diluted to 100 ml in a volumetric flask. Both sample solutions and standard solutions made from a serially diluted gallic acid (GA) monohydrate solution (CAS: 5995-86-8, Sigma-Aldrich, USA) were stored dark at room temperature for 2 h before measurement at 750 nm on the UV spectrophotometer. The results of standard solutions were plotted into a standard curve to calculate the total phenolic content of sample solutions. TPC was expressed as mg GA equivalents/100 ml or g sample.

#### 2.5.2. TPC of cold-smoked salmon

The TPC of cold-smoked salmon (5 g) was also analyzed in three replicates according to the method described by Singleton et al. (1999). Before analysis, all samples were added to 20 ml of methanol:water (100:80) and homogenized for 5 min (11,000 rpm) with a IKA®T25 digital ULTRA TURRAX® homogenizer (GmBH & Co, Germany). Then the mixture was centrifuged for 15 min at 4800 rpm before the supernatant was filtrated through a 185 mm folded filter (Schleicher & Schuell, Dassel, Germany) into a conical flask. One milliliter of the filtrated supernatant was analyzed as described in Section 2.5.1.

### 2.6. Growth kinetic parameters and statistical analysis

Growth kinetic parameters were estimated from log-transformed bacterial concentrations. OD values were used to convert bacterial concentrations in the liquid culture systems to log CFU/ml, with a conversion factor of $OD_{600}$ of $0.1 = 8.0 \times 10^7$ cells/ml. Bacterial concentrations deriving from salmon samples were calculated as log CFU/g. The primary growth model of Baranyi and Roberts (1994) (available at combase.cc) was fitted to the log-transformed data to estimate maximum growth rate ($\mu_{max}$, (log CFU/h)), lag-phase duration ($h$), and maximum population density ($Y_{max}$, (log CFU/g)). Each value was calculated and expressed as mean values ± standard deviation (SD), together with the coefficient of determination ($R^2$) and standard error of the fit (SE). In addition, the maximum growth rates ($\mu_{max}$, (log CFU/h)) obtained from the primary model of Baranyi and Roberts were modelled as a function of temperature, using a Ratkowsky square root model (Ratkowsky et al., 1982) (Eq. (1)).

$$\mu = (b(T - T_{min}))^2 \quad (1)$$

where $b$ is the slope of the regression line, $T$ is the storage temperature and $T_{min}$ is the theoretical minimum temperature for growth. The software program SPSS Statistics (ver. 28, IBM) was used for all statistical analysis (including a one-way ANOVA, a two-way ANOVA, Tukey post

hoc, and *t*-test ($P = 0.05$)).

## 3. Results

### 3.1. Effect of temperature, NaCl, and PCSs on Aeromonas growth

#### 3.1.1. Temperature

The effects of different temperatures (4, 8, and 15 °C) on growth kinetics parameters of eight *Aeromonas* strains were studied in TSB and SJ. In TSB, all eight strains were able to grow at 8 and 15 °C (Fig. 1a, c). At 4 °C, five strains were able to grow, whereas three strains of *A. hydrophila*, *A. dhakensis* and *A. caviae* did not grow (Fig. 1e). A significant decrease in maximum growth rates ($\mu_{max}$) was observed as lower temperature was applied (a one-way ANOVA, $P < 0.001$), whereas lag phases ($h$) were not significantly affected by the temperature ($P = 0.078$). In salmon juice (SJ), the growth pattern of each strain was similar to the TSB at all temperatures, excluding *A. caviae* which did not grow at 8 °C (Fig. 1b, d, f).

When growth kinetics parameters of all strains were compared in the two liquid culture systems, $\mu_{max}$ were significantly higher in TSB than SJ (*t*-test, $P < 0.001$). For example, the $\mu_{max}$ of *A salmonicida* SU2 was two times higher in TSB ($\mu_{max} = 0.096 \pm 0.003$ log CFU/h) than in SJ ($\mu_{max} = 0.043 \pm 0.002$ log CFU/h) at 15 °C. However, there were no significant differences in lag phases and maximum population densities ($Y_{max}$) between the two media ($P = 0.188$ and $P = 0.235$, respectively). When the effect of temperature was further examined, significantly higher $\mu_{max}$ in TSB were observed at 8 and 15 °C for all strains ($P < 0.001$), but not at 4 °C ($P = 0.165$) (a two-way ANOVA, Tukey post hoc) (Fig. 2a). Significantly longer lag phases were observed in SJ compared to TSB at 4 °C ($P < 0.001$), whereas storage at 15 °C resulted in shorter lag phases in SJ (but not significant $P = 0.103$) as several strains had no detectable lag phase in SJ (Fig. 2b). In addition, $Y_{max}$ was not significantly affected by the temperature.

For each strain, a Ratkowsky square root model was used to describe maximum growth rate ($\mu_{max}$) as a function of storage temperatures (4, 8, and 15 °C) in TSB and SJ media (Table 1). In general, the slope of the regression line (b) was higher in TSB than SJ, indicating a stronger temperature response in TSB than in SJ. Particularly, *A. piscicola* and two strains of *A. salmonicida* showed a stronger temperature response in TSB than SJ. In TSB, and the strongest response to increased temperature was observed for *A. piscicola* compared to the other strains but the interspecies variation was relatively low in TSB. In SJ, a stronger temperature response was observed for *A. media* and *A. bestiarum* compared to the other strains. Three strains of *A. hydrophila*, *A. dhakensis* and *A. caviae* were excluded from the analysis due to no detectable growth at 4 °C.

#### 3.1.2. Sodium chloride (NaCl)

The effects of NaCl at different concentrations (0.5–6.5 %) were studied in TSB at 4 and 8 °C. At 8 °C, all strains were able to grow at concentration ≤3.5 % NaCl (Fig. 3a, b), while none of them were able to grow at higher concentrations (≥5.0 %). *A. dhakensis* showed the longest lag phase (about 50 h) at 3.5 % NaCl (8 °C) compared to the other strains. At 4 °C, all tested strains were able to grow at concentration ≤3.5 % NaCl (Fig. 3c, d). At both temperatures, the concentration of 2.0 % NaCl did not significantly affect any of the growth parameters compared to the TSB (containing 0.5 % NaCl). On the other hand, an increase in NaCl concentration to 3.5 % resulted in significantly lower $\mu_{max}$, longer lag phases and lower $Y_{max}$ (a one-way ANOVA, $P < 0.001$). For instance, the $\mu_{max}$ of *A. bestiarum* ($\mu_{max} = 0.026 \pm 0.002$ log CFU/h) was two times higher in TSB (0.5 % NaCl) than at 3.5 % NaCl ($\mu_{max} = 0.009 \pm 0.001$ log CFU/h). In addition, significantly higher $\mu_{max}$ were observed at 8 °C compared to 4 °C at each NaCl concentration for all strains (a two-way ANOVA and *t*-test, $P < 0.001$) (Fig. 4a). For example, at 3.5 % NaCl, the $\mu_{max}$ of *A. media* at 8 °C ($\mu_{max} = 0.036 \pm 0.001$ log CFU/h) was four times higher than at 4 °C ($\mu_{max} = 0.009 \pm 0.001$ log CFU/h). A significantly shorter lag phase was observed at 8 °C than 4 °C at the concentration of 2.0 % and 3.5 % ($P < 0.01$), but not at 0.5 % ($P = 0.392$) (Fig. 4b). Moreover, significantly higher $Y_{max}$ was observed at 8 °C than 4 °C at 3.5 % ($P < 0.001$) but not at 2.0 % ($P = 0.175$) and 0.5 % ($P = 0.171$).

#### 3.1.3. Purified condensed smoke (PCS)

The effects of two PCSs (VTABB and JJT01) at different concentrations (0–0.26 %) were tested in TSB at 4 and 8 °C. The TPC of 0.26 % VTABB and JJT01 were measured as 10.9 ± 0.003 mg/100 ml and 4.7 ± 0.002 mg/100 ml, respectively. At 8 °C, the addition of VTABB at the maximal concentration (0.26 %) completely inhibited the growth of all *Aeromonas* strains. In case of JJT01, the growth of most strains was inhibited, except for two strains of *A. salmonicida* SU2 and *A. piscicola* with an extended lag phase (245 h and 351 h, respectively). When the concentration of both PCSs was reduced to 0.13 % at 8 °C, all strains were able to grow, except for *A dhakensis* which did not grow with VTABB (Fig. 5b). At lower concentration of 0.026 % VTABB, all strains could grow after 6 h of lag phase (Fig. 5a), whereas significantly longer lag phases (approximately 14 h) were observed with JJT01 ($P < 0.001$) (Supplementary Fig. A). At 4 °C, all tested strains were able to grow at lower concentrations (0.13 % and 0.026 %), excluding *A. media* and *A. bestiarum* which did not grow with VTABB at 0.13 % (Fig. 5c, d).

When the growth kinetics parameters for all strains were compared to growth in TSB (without supplements), the addition of each PCS at the lowest concentration (0.026 %) did not significantly affect any of the parameters. At the concentration of 0.13 %, significantly lower $\mu_{max}$ and longer duration of lag phase were observed for both PCSs (a one-way ANOVA, $P < 0.001$). For example, the $\mu_{max}$ of *A. salmonicida* SU2 in TSB ($\mu_{max} = 0.046 \pm 0.003$ log CFU/h) was two times higher than the rate in 0.13 % VTABB ($\mu_{max} = 0.024 \pm 0.003$ log CFU/h) at 8 °C. For both of PCSs, significantly higher $\mu_{max}$ was observed at 8 °C than 4 °C at each concentration (Fig. 6a, a two-way ANOVA and *t*-test, $P < 0.001$). For example, at 0.13 % VTABB, the $\mu_{max}$ of *A. salmonicida SL21* at 8 °C ($\mu_{max} = 0.026 \pm 0.005$ log CFU/h) was three times higher than the rate at 4 °C ($\mu_{max} = 0.008 \pm 0.002$ log CFU/h). On the other hand, the duration of lag phase was not significantly influenced by the temperature (Fig. 6b). Significant higher $Y_{max}$ was observed at 8 °C than 4 °C at each concentration of VTABB ($P < 0.01$), whereas $Y_{max}$ was not affected by the temperature in case of JJT01.

### 3.2. The growth of Aeromonas in salmon

#### 3.2.1. Vacuum-packed fresh salmon loin

Three strains; *A. bestiarum* SL22, *A. piscicola* SU58-3 and *A. salmonicida* SU2 were inoculated in fresh salmon fillets respectively, and vacuum-packed and stored at 4 °C for 14 days. Uninoculated pieces of salmon fillet were packed and stored under the same conditions as a control group. The mean concentration of *Aeromonas* in the samples inoculated with *A. salmonicida* SU2 was 2.56 ± 0.41 log CFU/g at day 0. The number increased proportionally as a function of storage time and a mean concentration of 7.81 ± 0.37 log CFU/g was reached at 14 days (Fig. 7, dotted line). Even though all strains of *Aeromonas* were inoculated by the same procedure, *Aeromonas* were not detected in the samples inoculated with *A. bestiarum* and *A. piscicola* at day 0. Probably, these two strains were temporarily non-culturable on the starch ampicillin agar (SAA) due to the selective condition of the media. The growth of *A. bestiarum* and *A. piscicola* were detected from day 2 and 4, respectively and the highest concentrations (4.95 ± 0.13 and 4.58 ± 0.10 log CFU/g, respectively) were observed at day 8 and flattening out. No *Aeromonas* were detected in the control samples, except for day 10, where a concentration of 2.95 log CFU/g was detected in one of three parallels.

The mean concentration of APC in the samples inoculated with the *Aeromonas* strains ranged from 2.57 ± 0.21 to 3.22 ± 0.30 log CFU/g at day 0. The APC increased as a function of storage time, and the highest

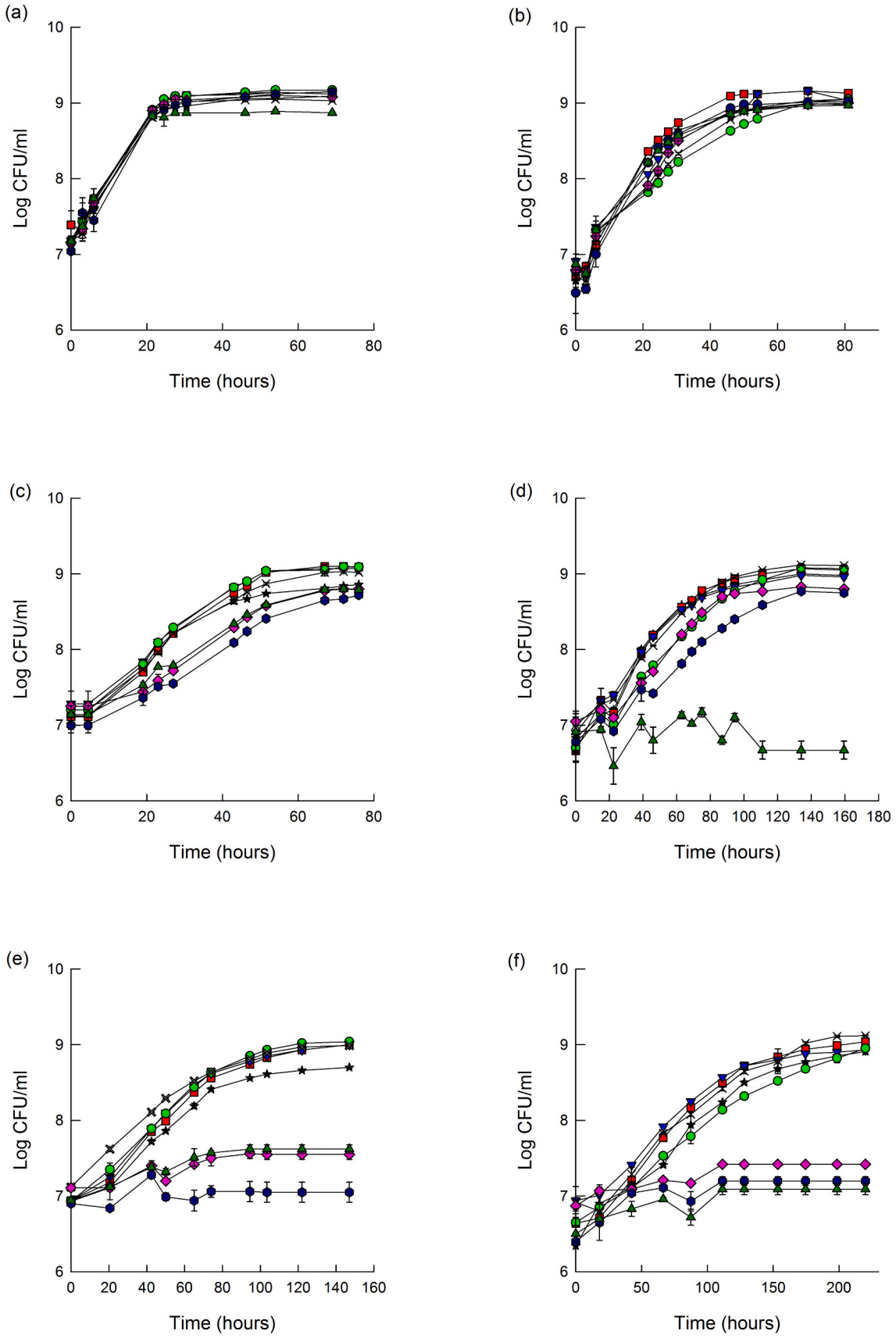


**Fig. 1.** Effect of temperature on the growth of eight *Aeromonas* strains in TSB and SJ model system. (a) TSB at 15 °C, (b) SJ at 15 °C, (c) TSB at 8 °C, (d) SJ at 8 °C, (e) TSB at 4 °C, (f) SJ at 4 °C. *A. media* (★ black), *A. bestiarum* (■ red), *A. piscicola* (▼ blue), *A. salmonicida* SU2 (● green), *A. salmonicida* SL21 (× gray), *A. hydrophila* (◆ pink), *A. dhakensis* (⬢ dark blue) and *A. caviae* (▲ dark green). Each point represents the mean bacterial count ($n = 3$), and vertical bars indicate ±SD.

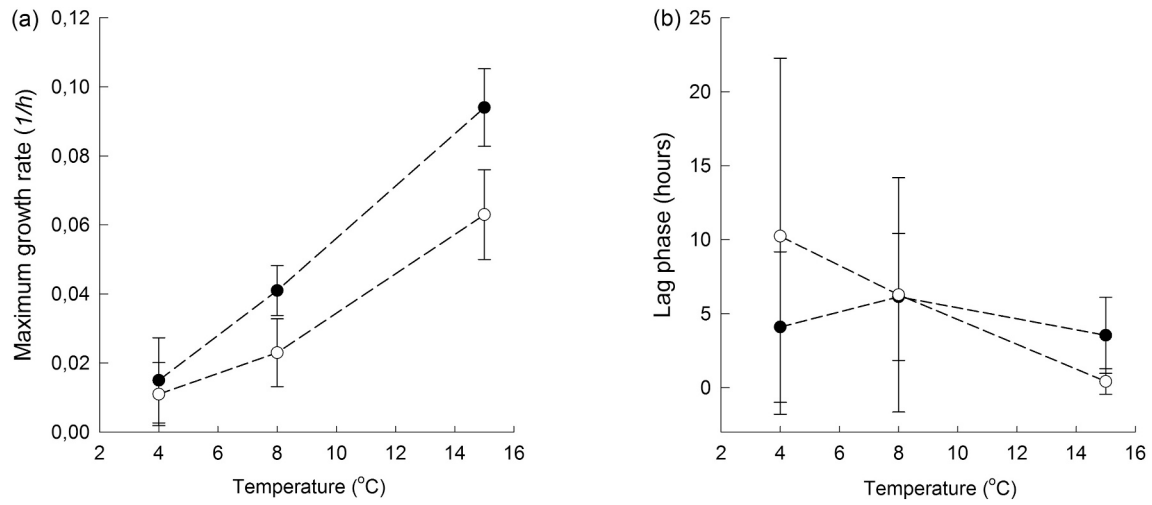


**Fig. 2.** Estimated marginal means of (a) maximum growth rate ($\mu_{max}$) and (b) lag phase (h) of all eight strains in TSB (●) and SJ media (○) at three temperatures (4, 8 and 15 °C). Each point represents the mean ($n = 24$) of eight strains ($n = 3$ for each strain) and vertical bars indicate ±SE, calculated by a two-way ANOVA.

**Table 1**
Parameters of the square root model for the effect of temperature on the maximum growth rate of all eight strains in TSB and SJ media, where $b$ (±SE) is the slope of the regression line, $T_{min}$ (±SE) is the theoretical minimum temperature for growth, and $R^2$ is the fit of the model.

| Media | Strain | $b$ | $T_{min}$ (°C) | $R^2$ |
|---|---|---|---|---|
| TSB | *A. media* | 0.013 ± 0.002 | −8.8 ± 1.4 | 0.98 |
| | *A. bestiarum* | 0.011 ± 0.001 | −10.6 ± 1.0 | 0.99 |
| | *A. piscicola* | 0.015 ± 0.001 | −6.7 ± 0.5 | 0.99 |
| | *A. salmonicida* SU2 | 0.014 ± 0.000 | −7.1 ± 0.0 | 1 |
| | *A. salmonicida* SL21 | 0.014 ± 0.000 | −6.4 ± 0.0 | 0.99 |
| SJ | *A. media* | 0.013 ± 0.001 | −6.1 ± 0.5 | 0.99 |
| | *A. bestiarum* | 0.012 ± 0.002 | −7.3 ± 1.2 | 0.98 |
| | *A. piscicola* | 0.009 ± 0.001 | −10.0 ± 1.1 | 0.98 |
| | *A. salmonicida* SU2 | 0.008 ± 0.000 | −11.4 ± 0.0 | 0.99 |
| | *A. salmonicida* SL21 | 0.009 ± 0.000 | −9.2 ± 0.0 | 0.99 |

concentrations (ranging from 6.52 ± 0.39 to 7.80 ± 0.30 log CFU/g) were observed after 14 days of storage. The quantification of APC in the inoculated samples resulted mainly from black colonies, representing $H_2S$-producing bacteria (Fig. 7, solid line). $H_2S$-producting bacteria had a similar growth pattern to the inoculated *A. salmonicida* SU2 (quantified on SAA), implying that observed black colonies on IA were *A. salmonicida* SU2. Moreover, a significant correlation was found between the concentration of *A. salmonicida* SU2 on SAA and the $H_2S$-producting bacteria on IA ($R^2 = 0.93$, $P < 0.001$). The correlation was weaker but also significant for *A. bestiarum* and *A. piscicola* ($R^2 = 0.56$, and $R^2 = 0.52$, $P < 0.01$, respectively). Thus, the quantification data of $H_2S$-producing bacteria was used to estimate the growth kinetic parameters of the *Aeromonas* strains (Table 2). The mean concentration of APC in the control group was 1.52 ± 0.52 log CFU/g at day 2 and developed to the level of 5.53 ± 0.19 log CFU/g at 14 days, but $H_2S$-producing bacteria were not detected, except for day 11, where a concentration of 3.04 log CFU/g was detected in one of three parallels.

### 3.2.2. Vacuum-packed cold-smoked salmon

The same strains (*A. bestiarum* SL22, *A. piscicola* SU58-3, and *A. salmonicida* SU2) were inoculated in cold-smoked salmon, and vacuum-packed and stored at 4 °C for 21 days. Uninoculated cold-smoked salmon was packed and stored in the same conditions as a control group. The mean concentration of TPC in the cold-smoked salmon was 1.49 ± 0.01 mg/100 g product. The mean concentration of *Aeromonas* in the samples inoculated with *A. salmonicida* SU2 was 2.73 ± 0.08 log CFU/g at day 0. *Aeromonas* concentration fluctuated over the storage period of 21 days, and no *Aeromonas* was detected at day 15 and 17 except for one of three parallels (2.95 log CFU/g). After 21 days, the highest concentration of 4.43 log CFU/g was detected in one of three parallels, indicating an increase of approximately 1.5 log compared to time zero. No *A. piscicola* and *A. bestiarum* were detected on the SAA throughout storage, except for some sporadic colonies of *A. piscicola* detected in one of three parallels at day 11 (3.73 log CFU/g), and 21 (3.04 log CFU/g) and *A. bestiarum* at day 7 (2.56 log CFU/g). This is most likely due to the non-culturable state of these two strains on the SAA, as previously noted for the fresh salmon samples. No *Aeromonas* were detected in the control samples during the storage.

Throughout the storage time, there were no significant changes in the mean concentration of APC in the cold-smoked salmon inoculated with each *Aeromonas* strain, even if there was a tendency toward a slight increase in the APC from day 0 to day 21. Similar to the fresh salmon sample, APC was represented by a significant portion of $H_2S$-producing bacteria, and $H_2S$-producting bacteria had a similar growth pattern to the inoculated *A. salmonicida* SU2. A significant correlation was observed between the concentration of *A salmonicida* SU2 on SAA and the $H_2S$-producting bacteria on IA ($R^2 = 0.58$, $P < 0.01$). However, no significant relation was found between $H_2S$-producing bacteria and storage time ($P = 0.455$). This might indicate poor growth of *A. salmonicida* SU2 on SAA over the storage time. The mean concentration of APC in the control sample was 1.2 ± 0.25 log CFU/g at day 3 and developed to 4.6 ± 1.47 log CFU/g after 15 days, and no $H_2S$-production bacteria was detected during the storage.

## 4. Discussion

In this study, the growth kinetics parameters of eight *Aeromonas* strains (*A. media*, *A. bestiarum*, *A. piscicola*, *A. hydrophila*, *A. dhakensis*, *A. caviae* and two strains of *A. salmonicida*) were studied under different growth conditions. First, the effect of different temperatures (4, 8 and 15 °C) was tested in liquid culture (TSB) and in a salmon juice (SJ) model. Then, the effect of NaCl concentrations (0.5–6.5 %), or two types of PCSs (VTABB and JJT01) in combination with low temperatures (4 and 8 °C) was investigated in liquid broth (TSB). Moreover, the growth kinetics parameters of three *Aeromonas* strains were investigated in inoculated fresh and minimally processed cold-smoked salmon fillets under vacuum packaging at 4 °C for 14 and 21 days, respectively.

The effect of temperatures (4, 8 and 15 °C) on the growth of *Aeromonas* was studied, as we hypothesized that moderate (8 °C) to severe (15 °C) temperature abuse of refrigerated food is likely to happen during transport and retail display. Our study presented that refrigeration could

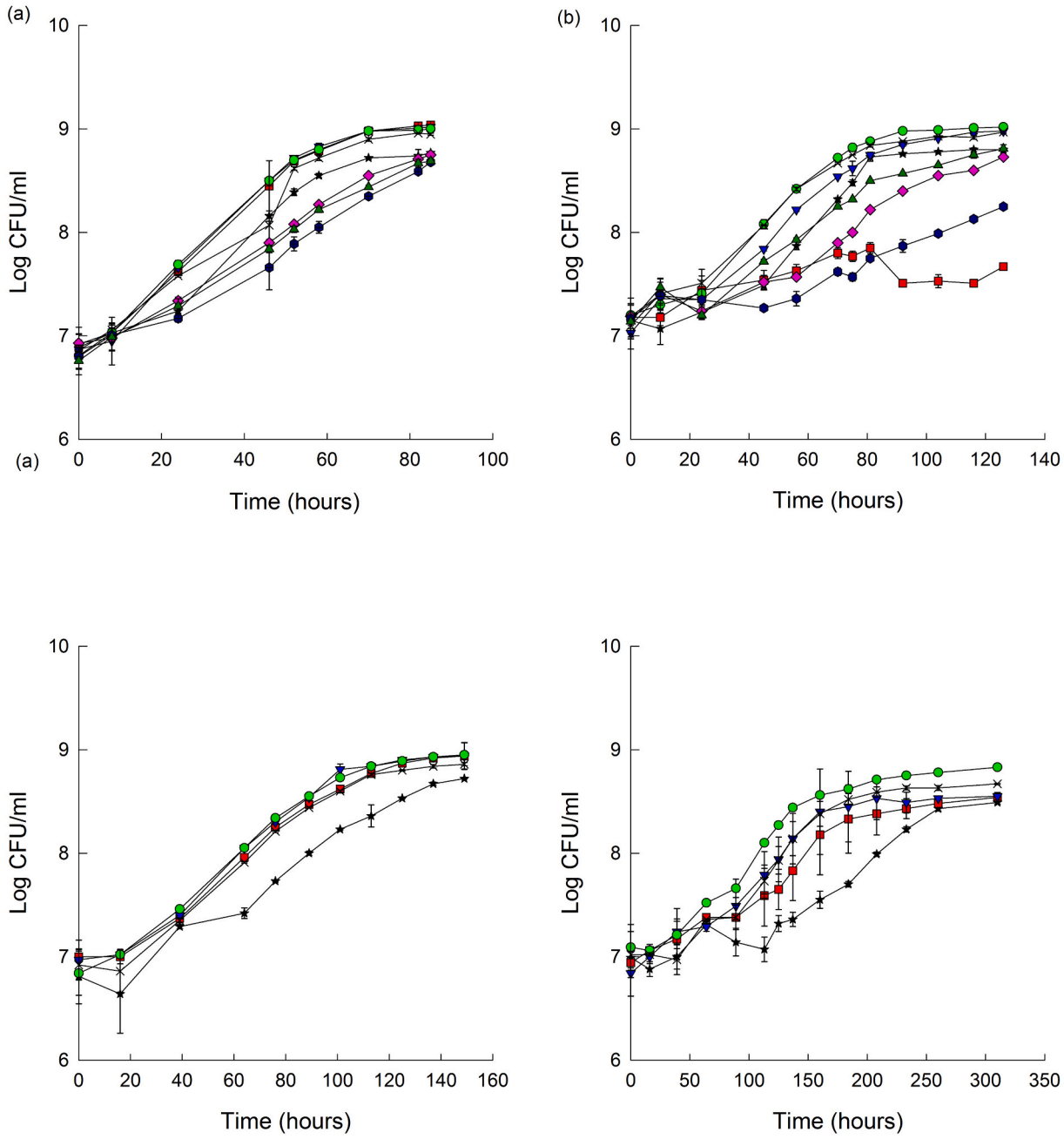


**Fig. 3.** Effect of NaCl concentration on the growth of eight *Aeromonas* strains in two different temperatures (4 and 8 °C). (a) 2.0 % NaCl at 8 °C, (b) 3.5 % NaCl at 8 °C, (c) 2.0 % NaCl at 4 °C, (d) 3.5 % NaCl at 4 °C. *A. media* (★ black), *A. bestiarum* (■ red), *A. piscicola* (▼ blue), *A. salmonicida* SU2 (● green), *A. salmonicida* SL21 (× gray), *A. hydrophila* (◆ pink), *A. dhakensis* (⬢ dark blue) and *A. caviae* (▲ dark green). Each point represents the mean bacterial count ($n = 3$), and vertical bars indicate ±SD.

not sufficiently inhibit the growth of tested *Aeromonas* strains, except for *A. hydrophila, A. dhakensis* and *A. caviae*. Our data showed that a temperature abuse scenario at 8 and 15 °C could enhance the growth of all tested *Aeromonas* strains, as an increase in temperatures resulted in significantly higher maximum growth rates with shorter lag phases for all strains, in accordance with previous studies (Kim et al., 2022; Zuccolotto et al., 2006). In addition, the *Aeromonas* strains in the present study displayed diversity in tolerance to temperature, particularly at refrigeration temperature (4 °C). *A. media*, *A. bestiarum*, *A. piscicola* and *A. salmonicida* showed ability to grow at 4 °C, whereas no growth of *A. hydrophila*, *A. dhakensis* and *A. caviae* was observed. The growth of *A. media*, *A. piscicola* and *A. salmonicida* at 4 °C was previously reported (Allen et al., 1983; Beaz-Hidalgo et al., 2009; Hoel et al., 2018), while the growth characteristics of *A. bestiarum*, *A. caviae*, and *A. dhakensis* at low temperature is poorly described. The presence of *A. hydrophila* in refrigerated food is frequently reported and their growth at refrigeration temperature has previously been highlighted (Palumbo et al., 1985; Vivekanandhan et al., 2003). On the other hand, a previous study demonstrated that most *A. hydrophila* isolated from either environment or clinical background could not grow or weakly grow at 4 °C (Zuccolotto et al., 2006). The ability of *A. hydrophila* at low temperature might be dependent on isolation source. *A. hydrophila*, *A. dhakensis*, and *A. caviae* are more often associated with clinical background; however, all *Aeromonas* strains in this study were environmentally isolated from seafood. Environmental isolates are known to be well adapted to low temperatures as low as 4 °C, where the growth of clinical isolates could

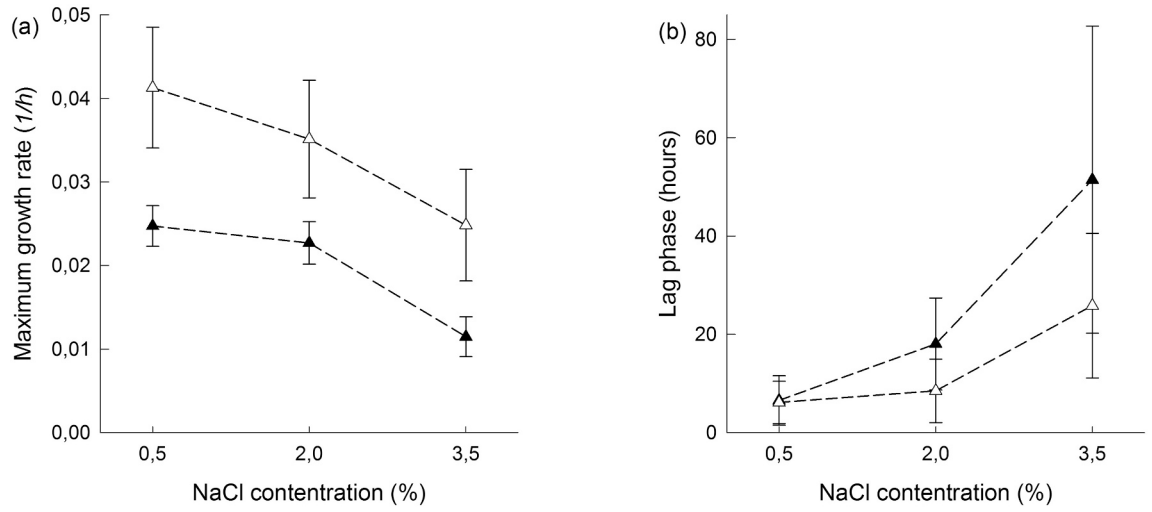


**Fig. 4.** Estimated marginal means of (a) maximum growth rate ($\mu_{max}$) and (b) lag phase (h) of eight strains in different NaCl concentrations (0.5 %, 2.0 % and 3.5 %) at 4 °C (▲) and 8 °C (△). Each point represents the mean ($n = 24$ for 8 °C and $n = 15$ for 4 °C) of eight strains ($n = 3$ for each strain) and vertical bars indicate ±SE, calculated by a two-way ANOVA.

be inhibited (Mateos et al., 1993).

The SJ model was introduced to imitate the growth condition of salmon. In this study, most tested *Aeromonas* strains were able to grow in the SJ model at all tested temperatures, implying the potential growth of *Aeromonas* in salmon. However, the overall maximum growth rate was significantly lower in the SJ than in the TSB ($P < 0.001$), suggesting that the growth of *Aeromonas* in SJ was somewhat inhibited. The low composition of protein, peptides, and free amino acids were suggested to be possible factors limiting the antimicrobial activity of lactic acid bacteria in shrimp juice (Fall et al., 2010). Further study of defining physicochemical characteristics of salmon juice seems necessary to find the limiting factors to the growth of *Aeromonas*. As the growth kinetic parameters were significantly affected by the media, further studies were carried out in TSB media. Nevertheless, in both TSB and SJ, temperature was the most important factor influencing the growth kinetics of *Aeromonas*, particularly maximum growth rates. In addition, the inter-species diversity was observed in their growth kinetic parameters and their temperature responses in both medium.

Different NaCl concentrations (0.5, 2.0, 3.5, 5.0, and 6.5 %) were chosen to simulate the salt tolerance of *Aeromonas* at two different temperatures in liquid culture (TSB). Our data showed that all tested *Aeromonas* strains were able to grow at concentrations ≤3.5 % NaCl at 8 °C, but not at the higher concentrations (≥5.0 % NaCl). Higher concentrations of NaCl resulted in significant reduction in maximum growth rates and prolonged lag phases ($P < 0.001$), which is in accordance with a previous study (Palumbo et al., 1985). Previous studies also showed salt content-dependent growth of *Aeromonas,* where mesophilic *Aeromonas* including *A. media*, *A. bestiarum*, *A. piscicola* and *A. hydrophila* could grow in concentration up to 3.0 % NaCl, while only a few strains could grow in media with >5.0 % NaCl (Ali et al., 1996; Allen et al., 1983; Beaz-Hidalgo et al., 2009; Gram, 1991).

Our results demonstrated that the combination of 3.5 % NaCl and refrigeration temperature (4 °C) was insufficient to inhibit the growth of *A. media*, *A. bestiarum*, *A. piscicola* and *A. salmonicida*. Growth kinetic parameters of all tested strains were significantly influenced by salt concentration as well as temperature, as higher salt concentration with lower temperature resulted in significant reduction of maximum growth rates and longer lag phases ($P < 0.001$). Temperature-dependent salt tolerance of *Aeromonas* was described in the previous studies, where *Aeromonas* could grow uninhibitedly at the concentration of 5.0 % NaCl at 37 °C (Gram, 1991) and even at 6.0 % NaCl at 28 °C (Delamare et al., 2000), while no growth was observed at the concentration of 5.0 % NaCl at lower temperature (5–10 °C) (Gram, 1991; Vivekanandhan et al., 2003). In practice, to inhibit unwanted microorganisms, adequate amount of salt (<6.0 % in the water phase) together with preservatives such as sorbate, benzoate or smoke are needed to produce lightly preserved fish products such as cold-smoked salmon (Løvdal, 2015). Lower salt content is however desirable due to the unhealthy effects of high sodium intake, and for example, the final salt content of cold-smoked salmon is recommended to be <3 g NaCl/100 g product in Norway (Ministry of Health and Care Services, 2015). Considering the current trend of lowering salt content, establishing additional hurdles to the salt-tolerant bacteria is critical to control their growth in the processing of lightly salted product. Investigating the viability of *Aeromonas* between 3.5 and 5.0 % NaCl concentrations would be still needed to define a minimum inhibitory NaCl concentration for these strain at low temperature.

Moreover, our study demonstrated the antimicrobial potential of PCS (at maximal concentration) against *Aeromonas* in TSB, in accordance with previous studies (Sofos et al., 1988; Suñen et al., 2003). Phenol concentration and pH of the smoke extract are considered important factors, and previous studies demonstrated that higher level of phenols in smoke extracts and pH drop in smoked products were the reasons for the better antimicrobial activity (Suñen et al., 2003; Valø et al., 2020). The two times higher TPC content of VTABB might explain better inhibitory potential than JJT01. On the other hand, no inhibitory effect on *Aeromonas* growth was observed with lower TPC levels of both PCSs. Measured pH in liquid culture varied between 6.3 and 7.4; however, *Aeromonas* are usually sensitive to lower pH (<6.0) (Daskalov, 2006). Moreover, the growth of *Aeromonas* in PCS enriched medium was also temperature-dependent, as lower temperature resulted in significant reduction of maximum growth rates for all strains ($P < 0.001$).

Overall, our study demonstrated the inter-species variation in the ability to grow at different temperatures in combination with salt content or PCSs in liquid culture systems. The liquid culture systems such as TSB or SJ could be reproducible and useful to study inter-species diversity and screen the strains for further analysis. However, such system might be not directly comparable to the cold-smoking process used in a real product. For example, the smoking process using PCS are usually performed by atomization process, where compressed air is used to vaporize PCS in a closed chamber to produce the smoked products (Valø et al., 2020). In addition, a previous study reported that antimicrobial activity of smoke extracts in microbiological culture media was not comparable to a real food matrix (Hao et al., 1998). Evaluating the

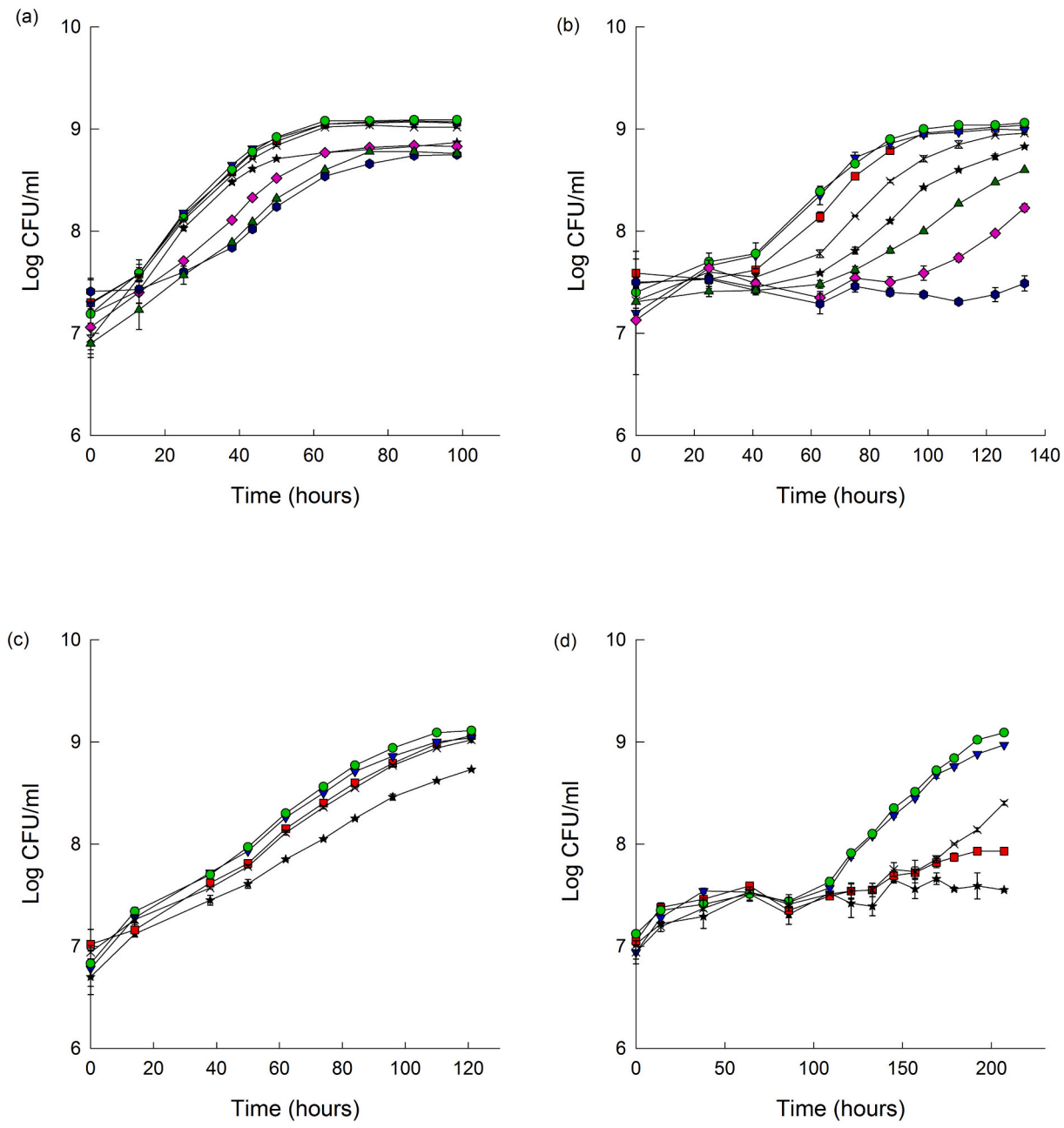


**Fig. 5.** Effect of adding VTABB at different concentrations on the growth of eight *Aeromonas* strains in two different temperatures (4 and 8 °C). (a) 0.026 % VTABB at 8 °C, (b) 0.13 % VTABB at 8 °C, (c) 0.026 % VTABB at 4 °C, (d) 0.13 % VTABB at 4 °C. *A. media* (★ black), *A. bestiarum* (■ red), *A. piscicola* (▼ blue), *A. salmonicida* SU2 (● green), *A. salmonicida* SL21 (× gray), *A. hydrophila* (◆ pink), *A. dhakensis* (⬢ dark blue) and *A. caviae* (▲ dark green). Each point represents the mean bacterial count ($n = 3$), and vertical bars indicate ±SD.

antimicrobial activity of PCS in a real product was suggested to be more relevant since a food environment is different from microbiological media and several compounds in smoke extracts could interfere with antimicrobial activity (Suñen et al., 2003). Further experiments in actual food matrixes such as PCS-treated salmon products would be required to confirm the antimicrobial potential of PCS against *Aeromonas*. In addition, as PCS is often applied in combination with salting in the cold smoking process, a follow-up study would be needed to investigate the combined effect of PCS and salt contents on *Aeromonas* growth.

Among eight *Aeromonas* strains, *A. bestiarum* SL22, *A. piscicola* SU58-3 and *A. salmonicida* SU2 which exhibited higher growth rates compared to the other strains in the liquid culture system were selected for growth kinetic studies in fresh and cold-smoked salmon products. SAA media is specifically recommended for *Aeromonas* isolation from food samples (NMKL, 2004); however, the selectivity and sensitivity of this media could be dependent on the species and isolation sources (Latif-Eugenín et al., 2016). It is worth noting that the growth of *Aeromonas* might have been somehow underestimated due to the temporarily non-culturable state of *Aeromonas* strains in the selective media (SAA). Nevertheless, our study demonstrated that vacuum packaging with cold storage (4 °C) could not sufficiently inhibit the growth of *Aeromonas* in VP salmon, in accordance with previous studies where they could detect the growth of *Aeromonas* in VP products stored at low temperature (Hudson et al., 1994; Jakobsen et al., 2020; Suñen et al., 2003). On the other hand, our data showed that the growth of the three *Aeromonas* strains was inhibited in cold-smoked salmon. Cold-smoked salmon is generally characterized by a NaCl concentration ranging from 2.5 to 3.5 % (*w*/w), and smoke treatment corresponding to 0.6 mg of phenol/100 g of

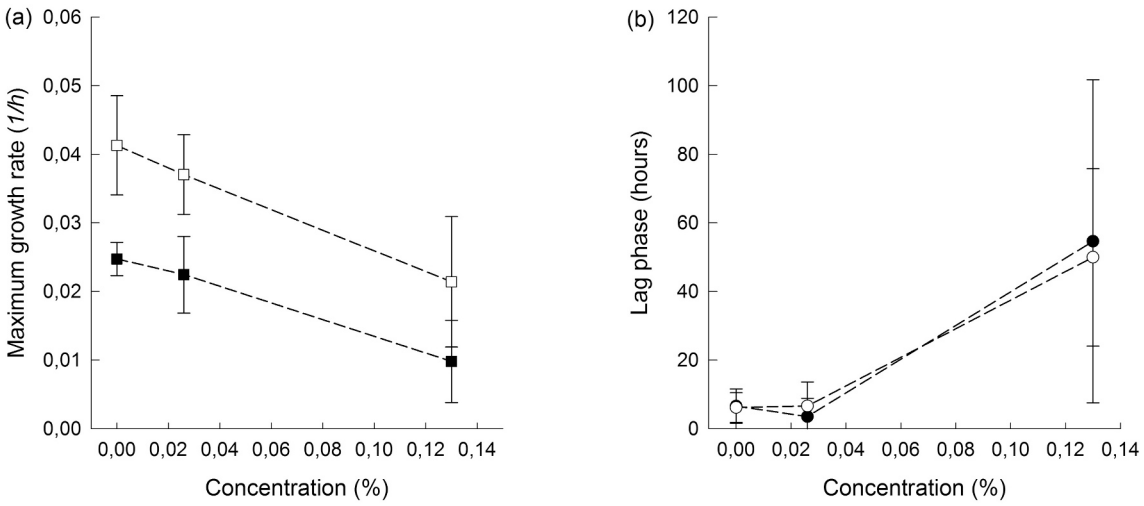


**Fig. 6.** Estimated marginal means of (a) maximum growth rate ($\mu_{max}$) and (b) lag phase (h) of all eight strains in different concentrations (0 %, 0.026 % and 0.13 %) of VTABB at 4 °C (■) and 8 °C (□). Each point represents the mean ($n = 24$ for 8 °C and $n = 15$ for 4 °C) of eight strains ($n = 3$ for each strain) and vertical bars indicate ±SE, calculated by a two-way ANOVA.

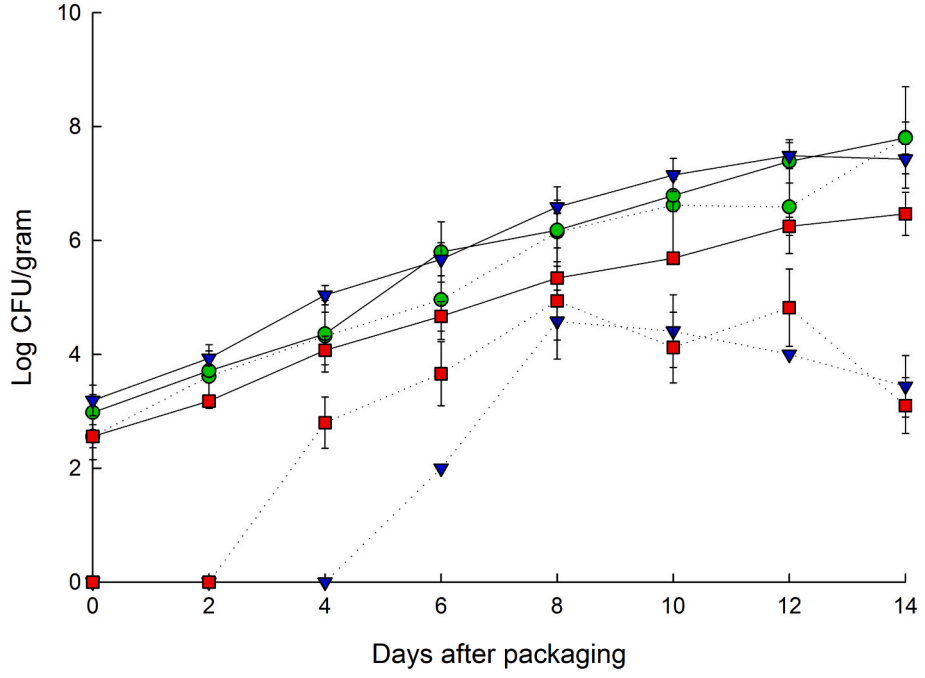


**Fig. 7.** Growth of $H_2S$-producing bacteria inoculated with *A. bestiarum* SL21 (■ red), *A. salmonicida* SU2 (● green), and *A. piscicola* SU58-3 (▼ blue) (solid line) quantified on iron agar (IA), and growth of *A. bestiarum* (■ red), *A. salmonicida SU2* (● green), and *A. piscicola* (▼ blue) (dotted line) quantified on starch ampicillin agar (SAA) in vacuum-packed salmon product stored at 4 °C for 14 days. Each point represents the mean bacterial count (n = 3), and vertical bars indicate ±SD.

product according to the French Standard NF V45-065. pH in cold-smoked salmon products is >5.0 and usually varies between 6 and 6.3 (Løvdal, 2015). Previous studies suggested the antimicrobial effect of salting and smoking in a cold-smoking process was proportional to the contents of salt and smoke (Leroi et al., 2000) and their effects were synergistic (Neunlist et al., 2005). In our study, the cold-smoked salmon contained a relatively low level of salt (1.8 g/100 g product) but contained a higher level of phenol (1.49 mg/100 g product) than the French Standard. Among the *Aeromonas* strains in the present study, *A. salmonicida* SU2 and *A. piscicola* SU58-3 were the most tolerant strains toward PCS treatment in liquid culture (containing 4.7 mg/100 ml), which is three times higher than the TPC level in the cold smoked salmon. Both strains were also able to grow in the concentration of 3.5 % NaCl at low temperature (4 °C) in liquid culture. Nevertheless, observed inhibition of these strains in the cold-smoked salmon might imply that phenol content alone was not the limiting factor influencing growth of these strains. It is most likely that the combined effect of high phenol, low salt contents and other processing factors such as low pH resulted in effective hurdles to inhibit growth of three *Aeromonas* strains in the cold-smoked salmon product.

## 5. Conclusion

In conclusion, we demonstrated that the combined effect of low temperature and 3.5 % NaCl was insufficient to inhibit the growth of *A. media*, *A. bestiarum*, *A. piscicola* and *A. salmonicida*, implying that mild

**Table 2**
Growth kinetic parameters for *Aeromonas* strains inoculated in vacuum-packed fresh salmon, estimated from the primary model of Baranyi and Roberts (1994). The quantification data of $H_2S$-producing bacteria on iron agar (IA) was used for growth prediction. Each sample point is represented as mean ($n = 3 \pm$ SD). Maximum growth rate ($\mu_{max}$, log CFU/d), lag phase duration (d), maximum population density ($Y_{max}$, log CFU/g), $R^2$ (fit of model), SE (standard error of $R^2$) and NL: no lag.

| Inoculated strain | $\mu_{max}$ (log CFU/d) | Lag phase (d) | $Y_{max}$ (log CFU/g) | $R^2$ | SE |
|---|---|---|---|---|---|
| *A. salmonicida* SU2 | 0.40 ± 0.03 | NL | 7.82 ± 0.28 | 0.98 | 0.225 |
| *A. piscicola* SU58-3 | 0.43 ± 0.03 | 0.062 ± 0.045 | 7.48 ± 0.08 | 0.99 | 0.107 |
| *A. bestiarum* SL22 | 0.33 ± 0.02 | NL | 6.48 ± 0.13 | 0.99 | 0.117 |

processing as applied in the production of RTE seafood may not guarantee the total inhibition of *Aeromonas*. On the other hand, cold-smoking process including the application of PCS could be an alternative approach to control the growth of *Aeromonas*. Our data could be used as a basis in the decision making for the practical development of cold-smoked salmon products with PCS technology Follow-up studies in a food matrix are still necessary to evaluate the actual antimicrobial activity of PCS against these bacteria. Moreover, our study presented that eight *Aeromonas* strains representing seven species displayed a variability in tolerance toward various processing factors, which might be useful for growth prediction of *Aeromonas* in RTE seafood. Our study highlighted that mild processing technology should be optimized to control the growth of potentially pathogenic *Aeromonas*, and further studies are required to expand our knowledge for growth prediction under mild processing factors in RTE seafood.

## CRediT authorship contribution statement

Hye-Jeong Lee: Formal analysis, Writing-Original Draft & Editing, Visualization, Ingebjørg Fagerheim Tokle: Investigation, Formal analysis, Bjørn-Tore Lunestad: Supervision, Writing-Review & Editing, Jørgen Lerfall: Supervision, Writing-Review & Editing, Sunniva Hoel: Supervision, Conceptualization, Writing-Review & Editing, Anita Nordeng Jakobsen: Conceptualization, Supervision, Writing-Review & Editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2022.109985.

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