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This is a "Post-Print" accepted manuscript, which has been published in "Food Packaging and Shelf Life"

Please cite this publication as follows:

Heising, J. K., van Boekel, M. A. J. S., & Dekker, M. (2015). Simulations on the prediction of cod (Gadus morhua) freshness from an intelligent packaging sensor concept. Food Packaging and Shelf Life, 3, 47-55. https://doi.org/10.1016/j.fpsl.2014.10.002

Simulations on the prediction of cod (*Gadus morhua*) freshness from an intelligent packaging sensor concept.

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Abstract

A non-destructive method that monitors changes in the freshness status of packed cod fillets has potential for the development of an intelligent packaging concept. The method is based on monitoring volatile compounds that dissolve and dissociate in the sensing aqueous phase. A mathematical model was developed to predict the freshness of the packed fish from the sensor signal (based on trimethylamine (TMA)). The model is based on physical and (bio)chemical principles of biological formation, mass transport, partitioning, and dissociation of TMA. The parameters in the model are derived partly from physical chemical properties, partly estimated from fitting the non-destructive sensor measurements in the aqueous phase and destructive TMA measurements in cod fillets. The model predicts a TMA increase in the aqueous phase comparable with sensor measurements from experimental storage trials. The initial freshness of fish is variable and taken into account in the model in the predictions of the freshness status of the packed fish.

The model was used to test different scenarios for sensor design. This showed clearly that minimizing the aqueous phase will strongly improve the sensitivity of the sensor. Reducing the package headspace can further improve the sensitivity.

In conclusion, the model can make accurate freshness predictions at a constant temperature of 0 °C and also in case of temporally temperature abuse, but needs a temperature-dependent correction for higher temperatures. Therefore combining the conductivity-sensor with a temperature sensor enables this model to be used in the development of an intelligent packaging to monitor the freshness of fish.

Keywords

Mathematical modelling, trimethylamine (TMA), fish freshness, dynamic models, temperature effect, intelligent packaging sensor

1. Introduction

Dynamic information about the quality status of foods supplied by intelligent packaging can contribute substantially to the optimization of supply chain management (Realini & Marcos, 2014). Intelligent packaging for foods requires the development of sensors that monitor and communicate freshness from the moment of packaging until the day the fish is spoiled (Kuswandi et al., 2011). Foods like fresh fish, with a highly variable quality on the moment of packaging, require sensors monitoring compounds directly correlated with food quality (Heising, Dekker, Bartels, & Van Boekel, 2014b). Freshness is a very important factor determining the quality of fish and freshness can be evaluated by different approaches, e.g. from analysis of volatiles (Ólafsdóttir et al., 1997).

An intelligent packaging sensor concept that consists of a non-destructive method to monitor changes in the freshness of packed cod fillets has been introduced in a previous study (Heising, Dekker, Bartels, & Van Boekel, 2012). The principle of this method is the introduction of an aqueous phase in the headspace of the fish package. In this aqueous phase, changes in the electrical properties can be monitored by electrodes, e.g. by using a conductivity electrode (Heising, Bartels, Van Boekel, & Dekker, 2014a). The changes in the electrical properties of the aqueous phase were related to the total volatile basic nitrogen content (TVB-N) of the fish itself, which has proven to be a good indicator for the freshness of many marine fish (Botta, Lauder, & Jewer, 1984).

The increase in the TVB-N content is mainly caused by the formation of trimethylamine (TMA) in fish, the compound that is one of the dominant components of spoiling fish and that has a typical fishy odour (Huss, 1995; Howgate, 2010). The TMA content is strongly correlated to the sensory quality of cod (Burt, Gibson, Jason, & Sanders, 1976; Gill, 1990).

In this article, we describe the framework for a mathematical model to predict the sensor response of the intelligent packaging concept from the TMA content in the aqueous phase inside a fish package. The model was fitted on data of the electrode response measured during a trial when fish was stored at 15 °C. Furthermore, simulations were conducted using the model with changes in the parameters in order to predict the sensor response on miniaturization, a necessary step in the further development of the intelligent packaging concept (Vanderroost, Ragaert, Devlieghere, & De Meulenaer, 2014).

In a previous publication models for TMA formation were developed, based on microbial growth models (Heising, Van Boekel, & Dekker, 2014c). The aim of this research is to develop a mathematical model, based on physical and biochemical principles of mass transport, to translate the sensor signal of an intelligent packaging concept into a prediction of fish freshness and to simulate the miniaturized intelligent packaging concept.

2. Materials and Methods

2.1 Data collection

2.1.1 Storage trial of cod fillets

Data for parameter estimation were collected in the experimental trials with cod fillets stored at 0-15 °C as described by Heising et al. (2014a),.

Cod (*Gadus morhua*) was bought at a wholesale in IJmuiden (NL) in May 2008. The cod was caught in the North Sea off the Netherlands, gutted on board the fishing vessels, stored on ice and brought to IJmuiden. After the auction, the wholesaler prepared skinned fillets from the cod and the fillets were transported on ice to the laboratory in ~3 hours. Purchase, fillet preparation and transport all took place the same morning. Immediately after arriving in Wageningen, the fillets were prepared for analysis and storage, and from this moment the storage trial started. The batch of fish was used for both the non-destructive and destructive analysis during the trial.

2.1.2 Non-destructive method

The non-destructive measurement setup consisted of a glass-cell with holes in the lid for air tight fitting of the electrodes to analyze an aqueous phase in a beaker separate from the fish (Figure 1) (Heising et al., 2012).

Cod fillets (~375 g) sliced into pieces of approximately 30 g, were put in the glass cell. Each experiment contained randomly mixed pieces from different cod fillets. The glass cell contained a conductivity electrode (TetraCon 325 conductivity electrode with inoLab Cond 730 precision conductivity meter, WTW) with the electrode-tip in 65 ml Milli-Q (deionized) water in the beaker. The conductivity electrode was logged automatically at time-intervals of 15 minutes.

The glass cells were placed in a cryostat set at temperatures from 0 till 15 °C, filled with water and antifreeze, located in a room that was temperature controlled.

2.1.3 Destructive TMA analysis

The fish samples for the destructive TMA analysis were packed separately in aluminum boxes, one box for each measurement day. After arrival in Wageningen, the fillets were sliced into pieces of approximately 30 g, the pieces were mixed and 120 g fish was put into each box with a lid for storage. The boxes were stored in a refrigerator at temperatures from 0 till 15 °C. The temperature of the fillets and the storage rooms was monitored with Automatic wireless temperature loggers as described in Heising et al. (2012).

The TMA content was determined in duplicate in an extract of the cod fillet according to the steam distillation method from Malle & Tao (1987) as described in Heising et al. (2012). 20 ml of ~36% aqueous formaldehyde-solution (Fluka 47630) (formaldehyde complexes with primary and secondary amines, but not with tertiary amine TMA) was added to 25 ml of filtrate, followed by 5 ml of 10% (w/v) NaOH. Steam distillation (Gerhard Vadopest 12-Kjedahl type distillatory) was carried out for 7.3 minutes on the TCA extract. A beaker containing 10 ml of a 4% aqueous boric acid solution (Merck 1.00165) and 0.04 ml of Mixed indicator 5 for ammonia titrations (Merck 1.06130) was placed at the end of the condenser. The boric acid solution turned green when alkalinized by the distilled TMA. The green alkaline distillate was titrated using a digital burette (Schott type T80 /20) containing an aqueous 0.1N hydrochloric acid solution (Merck 1.09973). Complete neutralization was obtained when the colour turned pink on the addition of a further drop of hydrochloric acid. This procedure was repeated for duplicate analysis.

2.2 Parameter estimation and simulations

149 The mass transfer of TMA in packed cod fillets was modelled using sets of algebraic
150 and differential equations. Simulations and parameter estimation from numerical
151 integration of the differential equations, including the statistical evaluation of the
152 parameters and performance of the complete model, were obtained by least squares
153 regression with the help of the software package Athena Visual Workbench (Stewart
154 et al., 1992; www.athenavisual.com).
155
156
157 ## 3. Results and Discussion
158
159 ### 3.1 Model development
160
161 The modelling approach is based on the formation of volatile compounds. In freshly
162 caught cod the volatile NH_3 and some other volatile compounds are present. During
163 subsequent storage the content of volatiles increases, mainly due to the formation of
164 TMA. In a later stage, when the fish is already spoiled, NH_3 is increasing further. This
165 formation of volatiles can be linked to the freshness and quality of fish (Ólafsdóttir et
166 al., 1997). We realize that quality is a broad concept and the combined analysis of
167 several quality attributes (e.g. protein and fat degradation, microbial growth and
168 sensory aspects) could lead to more accurate description of the quality status.
169 However for the development of a sensor a non-destructive approach is required.
170 Volatiles can be measured non-destructively. The TMA content is a good indicator
171 since it is correlated to the freshness status and also other quality attributes (Burt et
172 al., 1976).
173

The non-destructive method consists of an aqueous phase in which electrodes measure the changes in the electrical properties of the aqueous phase (Figure 1). These changes are caused by volatiles produced by the packed fish fillet, that will partition in the headspace and dissolve in the aqueous phase.

3.1.1 Formation of TMA

TMA is produced on fresh cod fillets stored at chilled and higher temperatures (in the range 0-15 °C) by the micro-organisms *Shewanella putrefaciens* and *Photobacterium phosphoreum* and the formation can be described by a dynamic model (Heising et al., 2014c):

$$\frac{dC_{TMA}}{dt} = \mu_{max} C_{TMA}\left(1-\left(\frac{C_{TMA}}{C_{max}}\right)\right) \quad (1)$$

With:

C_{TMA} concentration of TMA at time t (mg TMA-N per 100 g fish)

t time (hours)

C_{max} upper asymptote concentration (mg TMA-N per 100 g fish)

μ_{max} maximum specific formation rate coefficient ($hours^{-1}$)

With parameter for initial value in the numerical integration:

C_0 initial concentration at time t=0 (mg TMA-N per 100 g fish)

The parameter C_0 incorporates the initial freshness status and the effect of natural variation in the quality that influences the freshness of fish and C_{max} was estimated to be 62.2 mg N/100 g cod (Heising et al., 2014c). Since TMA is a metabolite formed by

198 microbial growth, microbiological models and parameter estimations were used to
199 describe the effect of temperature on the formation of TMA on fish. The effect of
200 temperature on the maximum formation rate μ_{max} of the formation of TMA could be
201 described by a model that is analogous to the microbiological extended square root
202 model of Ratkowsky (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983)
203 (equation 2):

204 $$\mu_{max} = (b(T - T_{\min})(1 - exp^{c(T-T_{max})}))^2 \quad (2)$$

205

206 With:

207 T_{min} minimum temperature at which the rate of TMA formation is zero (°C)

208 T_{max} maximum temperature at which the rate of TMA formation is zero (°C)

209 b regression coefficient (°C h^{-1})

210 c additonal parameter for fit (°C h^{-1})

211

212 The parameter estimate for T_{max} was 25 °C (taken from Dalgaard (1993)) and was
213 based on the maximum growth temperature of the bacteria *Photobacterium*
214 *phosphoreum*. The parameter estimates for the parameters T_{min}, b and c were -4 °C,
215 0.029 °C h^{-1} and 0.12 °C h^{-1}, respectively (Heising et al., 2014c).

216

217 *3.1.2 Dissociation of TMA in fish*

218 The TMA that is formed by the micro-organisms will partly dissolve and dissociate in
219 the fish tissue and a part will be present in the free form being able to partition to the
220 headspace of the package. The dissociation reaction of TMA is:

221

222 $$(CH_3)_3N + H_2O \leftrightarrow (CH_3)_3NH^+ + OH^- \quad (1)$$

The fraction of the total TMA that is formed (from equation 1, formation model) that remains in the undissociated form can be expressed according to equation 3. The density ρ_f of cod (1.0541 g/ml (Lowndes, 1955)) was used for converting the unit of mg N/100 g fish from equation 1 to the unit of mg/l.

$$F = \frac{[TMA]}{[\sum TMA]} = \frac{[TMA]}{[TMA]+[TMAH^+]} \quad (3)$$

With:

F	fraction of TMA in ΣTMA of the fish (-)
$[TMA]$	concentration of undissociated TMA (mg l^{-1})
$[TMAH^+]$	concentration of dissociated TMA (mg l^{-1})
$[\Sigma TMA]$	concentration of total TMA from equation X (mg l^{-1})

The dissociation equilibrium is described by the dissociation constant, which is expressed as (equation 4):

$$K_a = \frac{[TMA][H^+]}{[TMAH^+]} \quad (4)$$

With:

K_a	dissociation constant
$[H^+]$	concentration of hydrogen ion (mg l^{-1})

The dissociation constant pK_a (=-log K_a) for TMA at 25 °C is 9.81. The dissociation constant depends on the temperature of the fish. Equation 5 describes the temperature dependence of the pK_a of TMA. This equation was adapted from the temperature

248 dependence of the pK_a of NH_3 (Emerson, Russo, Lund, & Thurston, 1975), assuming
249 the same temperature coefficient for TMA as was reported for NH_3 (Howgate, 2010).

250 $$pK_a = 0.6516 + 2729.2T^{-1} \quad (5)$$

251

252 With:

253 pK_a dissociation constant of TMA

254 T temperature (K)

255

256 The pH of the fish changes during storage, e.g. due to autolytic reactions or dissolving
257 gases. For the modelling and simulations a pH of 6.9 for raw cod fillets was used
258 (Sivertsvik, Rosnes, & Jeksrud, 2004).

259

260 *3.1.3 Partitioning of TMA between the fish and the headspace*

261 When TMA is formed by micro-organisms on the surface of the cod fillets, part of the
262 TMA will be released to the headspace of the fish package. The partitioning between
263 the fish and the headspace is based on the total TMA content that is formed.

264

265 The ratio of volatiles between the fish and the headspace can be described by K_{hf}:

266 $$K_{hf} = \frac{c_f}{c_h} = k_H * RT \quad (6)$$

267 or rewritten to equation 7 (Sander, 1999):

268 $$T * k_H = 12.2 * K_{hf} \quad (7)$$

269

270 With:

271 K_{hf} ratio of concentrations in headspace and fish (-)

272 c_h concentration of TMA in headspace (mg l^{-1})

273 c_f concentration of TMA in fish (mg l^{-1})

274 k_H Henry's Law constant (mol l^{-1} atm^{-1})

275 R gas constant (8.314 J K^{-1} mol^{-1})

276 T temperature (K)

277

278 The Henry's Law constant for TMA between water and gas phase at 25 °C is 9.6 (mol
279 L^{-1}) atm^{-1} (Sander, 1999), it was assumed that the effect of dissolved salts in the fish
280 on this constant can be neglected. This value needs to be calculated for the
281 temperature at which the packed fish is stored. The temperature dependence of the
282 Henry constant can be described by the van 't Hoff equation (Equation 8):

283 $$\frac{d \ln k}{d\frac{1}{T}} = -\frac{\Delta H^{\ominus}}{R} \quad (8)$$

284

285 In integrated form (Equation 9):

286 $$\ln\left(\frac{k_2}{k_1}\right) = \frac{\Delta H^{\ominus}}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (9)$$

287

288 With:

289 R gas constant (8.314 J K^{-1} mol^{-1})

290 T temperature (K)

291 k Henry constant (mol l^{-1} atm^{-1})

292 $\Delta H^{\ominus}$ standard enthalpy change (J mol^{-1})

293

294 The value taken for the slope $\frac{d \ln k}{d\frac{1}{T}}$ was 4100 M atm^{-1} (Sander, 1999), assuming the
295 temperature dependence of TMA to be similar to that of NH_3. After the Henry's law

296 constant has been adjusted for the storage temperature of the fish, equation 6 is used
297 to calculate the ratio K_{hf} of concentration of TMA in the fish and in the headspace.
298
299 *3.1.4 Mass transfer coefficient*
300 On the surface of the fish that is exposed to the headspace, the release of TMA will
301 take place when there is a driving force if the concentrations of undissociated TMA in
302 the fish and in the headspace are not in equilibrium. The rate of change of TMA
303 concentration in the fish per unit of time (h^{-1}) is described by equation 10:
304 $$V\frac{dc_{TMA}}{dt} = K_L A_f\left(c_f - c_h\right) \quad (10)$$
305
306 With:
307 c_{TMA} concentration of dissolved TMA in fish (mg l^{-1})
308 V volume of fish (=M_f/ρ_f) (=0.36 l)
309 K_L mass transfer coefficient (mm h^{-1})
310 A_f surface of fish exposed to headspace (= 0.02 m^2)
311
312 The mass transfer coefficients of TMA were assumed to be similar to the mass
313 transfer coefficient for NH_3. Since there is no airflow inside the package, the overall
314 mass transfer rate is mainly dependent on the diffusion coefficient. The diffusion
315 coefficient changes proportionally with the temperature change (according to the
316 Stokes-Einstein equation, the other parameters of the Stokes-Einstein equation were
317 assumed to remain constant in the sensor for the temperature range of 0-15 °C). In the
318 model, the the mass transfer coefficient K_L at temperature T (K) was estimated from
319 K_{Lref}, using the experimental data (Heising et al., 2014a) at a reference temperature of
320 15 °C (Equation 11).

$$K_L = K_{Lref} * \frac{T}{T_{ref}} \quad (11)$$

With:

K_L mass transfer coefficient (mm h^{-1})

K_{Lref} reference mass transfer coefficient at 15 °C (mm h^{-1})

T temperature (K)

T_{ref} reference temperature (=288 K)

3.1.5 Mass transfer of TMA from the headspace to the sensor aqueous phase

The same equations as described above for the mass transfer between the fish and the headspace can be established for the mass transport of TMA from the headspace to the sensing aqueous phase as well. These equations need to be based on the dissociation and partitioning of TMA between the headspace and the sensor aqueous phase, but the dissociation constants and Henry constants are assumed to be similar for the fish and the aqueous phase (but the pH of the sensing aqueous phase is assumed to be 6.0).

The mass transfer of TMA in the fish package is schematically shown in figure 2.

3.1.6 Sensor measurement

According to the reaction 1 ions are formed when TMA dissolves in the aqueous phase. These ions cause an increase in the conductivity (molar conductivity of TMA is 47.2 S-cm^2/mol and that of OH^- is 199.1 S-cm^2/mol) (Coury, 1999). The molecular weight of TMA of 59.11 g mol^{-1} was used for converting the unit of mg TMA to the unit moles. The conductivity in the aqueous phase is monitored by the conductivity

345 electrode, from which the $TMAH^+$ concentration in the aqueous phase is calculated.
346 From this signal the freshness stage of the fish is predicted.
347
348 *3.1.7 Model equations*
349 The formation and mass transfer of TMA from the fish to the aqueous phase of the
350 sensor is described by differential equations, based on the equations described above.
351 The model equations are based on mass balances, for example the TMA content in the
352 fish is based on the formation of TMA from microbial growth minus the release of
353 TMA from the fish to the headspace. The mass balances are described separately for
354 the TMA in the fish, the headspace and the sensing aqueous phase. Finally, a
355 dissociated $TMAH^+$- concentration in the sensing aqueous phase can be calculated at
356 each time *t* resulting in a conductivity value. This conductivity value represents the
357 freshness status of the fish.
358
359 The differential equations are numerically integrated with the software in order to
360 simulate the TMA content in the fish, the headspace and the sensing aqueous phase.
361 The initial conditions for numerical integration of the differential equations are:
362 U(1) initial concentration of TMA in fish at time 0 = $C_{0*}\rho_f$ (mg l^{-1})
363 U(2) initial concentration of TMA in headspace at time 0 = 0 (mg l^{-1})
364 U(3) initial concentration of TMA in aqueous phase at time 0 = 0 (mg l^{-1})
365 U(4) initial concentration of $TMAH^+$ in aqueous phase at time 0 = 0 (mg l^{-1})
366
367 **3.2 Model application**
368
369 *3.2.1 Fit of the mathematical model on measurements of a fish storage trial*

The model was fitted on the measurements of conductivity during a storage trial (Figure 3). The fits of the model and the measurements are quite similar, therefore the general trend of the measurements is confirmed by the model.

The value for the parameter mass transfer coefficient from the release of TMA from the fish to the headspace was $3.81*10^{-3} \pm 2.0*10^{-4}$ mm*h^{-1} and from the uptake of TMA from the headspace into the sensing aqueous phase was $6.93*10^{-3} \pm 2.7*10^{-4}$ mm*h^{-1} (both values were estimated from least squares regression of the model on the measured data). It was not expected that the release from the fish proceeds slower than the uptake in the sensing aqueous phase, but the parameters are strongly correlated (-0.998) and perhaps the matrix of the fish tissue plays a role in the release of TMA.

The mass transfer coefficient is dependent on the dimensions of the system. Since convection does not play a role in the transport of TMA in the package, molecular diffusion is expected to influence the mass transfer coefficient the most (Equation 12):

$$K_L = \frac{D}{\delta} \qquad (12)$$

With

D diffusion coefficient (m^2/s)

δ distance across diffusion occurs (m)

TMA-H$^+$ that is dissolved in the fish fillet and is released to the headspace is expected to diffuse over a small distance, since it was assumed that spoilage changes are normally present and most active on the surface of the fish, therefore most TMA will be accumulated at the surface zone (Dyer, Sigurdsson, & Wood, 1944). The estimated

mass transfer coefficients for the release of TMA from the fish is in the order of 10^{-9} m/s, which is in the same order as diffusion coefficients for NH_3 reported by Frank, Kuipers, & Van Swaaij (1996), but is expected to be higher because of the low δ.

The conductivity in the aqueous phase is measured by the sensor, but the conductivity electrode is non-specific and can measure all volatile compounds that dissolve and dissociate in the aqueous phase. Therefore, also other volatile compounds, e.g. NH_3, CO_2, and H_2S that can be formed by the fish can influence the signal that is measured by the sensor. Furthermore, from reaction 1 it can be seen that OH^- ions are formed together with the $TMAH^+$. Besides, the compounds can interact with each other, e.g. the carbonic acid from dissolved CO_2 can react with the hydrogen ions formed from the dissociation of trimethylamine in the aqueous phase.

Furthermore, the parameter μ_{max} for the formation of TMA at different temperature is estimated from equation 2. Small deviations in this parameter will influence the rate of TMA formation and TMA concentrations in the fish, headspace and aqueous phase strongly. This might influence the predictions for the mass transfer coefficient as well. Despite these drawbacks very characteristic profiles of the electrode signals were observed during various storage trials at temperatures between 0 and 15 °C, which proofs the reliability of this method.

3.2.2 Simulations with geometry

Simulations at 0 °C were conducted with the model to study the effect of the geometric parameters. The parameters sensor volume and surface, and headspace volume were varied and compared with the standard laboratory experimental setup (Figure 1), except when other values for geometry parameters are mentioned. The

simulation results need to be validated during the future experimental design of the sensors.

3.2.2.1 Effect of sensor volume and surface

In the standard experimental setup the sensor had a large volume of 65 ml with a surface of sensor exposed to headspace of $3.85*10^{-3}$ m^2. To convert this laboratory setup into an intelligent packaging sensor the sensing aqueous phase and electrodes need to be minimized (Vanderroost et al., 2014). Simulations at 0 °C were conducted with the model to study the effect of the geometric parameters. When the volume of the aqueous phase decreases, the surface of the aqueous phase decreases as well. The surface exposed to the headspace depends on the shape of the aqueous phase, however for the simulations we used equation 13 to calculate the surface belonging to the reduced volume.

$$A_2 = A_1 \left(\frac{V_2}{V_1}\right)^{2/3} \quad (13)$$

In the sensor in the laboratory setup, dissolved TMA needs to diffuse over ~10 mm before being measured. When the geometry of the sensor changes, this diffusion distance will change as well. To take this effect on the mass transfer coefficient of the sensor uptake into account in the simulations, the mass transfer coefficient was corrected according to equation 14:

$$K_{L2} = K_{L1} \left(\frac{V_2}{V_1}\right)^{1/3} \quad (14)$$

When the volume of the aqueous phase is reduced, the concentration of TMA in the aqueous phase increases (Figure 4). This increased $TMAH^+$ concentration in the

aqueous phase will increase the sensitivity of the sensor response to different stages of freshness. So when minimizing the sensor, the signal will be optimized as well.

3.2.2.2 Effect of headspace volume

In the non-destructive setup in the laboratory, a glass cell with a large volume (1.6 L) compared to the mass of the packed fish (0.375 kg) was used. A ratio between the volume of a gas and volume of food product (G/P ratio) in a modified atmosphere packaging for cod is usually 2:1 or 3:1 (Sivertsvik, Jeksrud, & Rosnes, 2002). In the simulations the volume of the headspace was varied from a ratio of 1:1 until 3:1 and compared with the laboratory experimental setup (4.3:1). In the simulations a volume of 0.1 ml and surface of $5.13*10^{-5}$ were taken as values to simulate the parameters of a minimized sensor. From figure 5 it can be seen that the signal of the electrode will increase when the headspace volume is decreased, therefore the sensor sensitivity will improve when the concept is applied on a package with a regular volume, but it will only be a small effect.

3.2.3 Simulations with variation in initial freshness on the prediction of freshness in the supply chain

TMA is produced on fresh cod fillets stored at chilled temperatures by micro-organisms. The species and number of microorganisms on fish on the moment of catch varies greatly; A normal range of 10^2-10^7 cfu/cm^2 on the skin surface and between 10^3 and 10^9 cfu/g on both the gills and the intestines have been reported (Huss, 1995). This variability is influenced by (partially) uncontrollable factors, like season and environmental conditions (e.g. pollution, temperature) of place of catch

(Gram & Huss, 1996). Besides, the time and temperature between catch and moment of packaging varies, resulting in differences in the initial freshness status of the fish fillets. The initial freshness is incorporated in the model of the formation of TMA in the value of parameter C_0, which is the initial TMA concentration (mg l^{-1}) in the packed fish. The effect of natural variation in the initial freshness status was simulated using different values for the parameter C_0 (Figure 6), the range of the values for C_0 taken from parameter estimations from real trials from Heising et al., 2014c. To simulate minimized sensor conditions a volume of 0.1 ml and surface of $5.13*10^{-5}$ were taken and the headspace volume was set on 750 ml (G/P ratio 2:1). A higher C_0 will lead to a faster increase in the sensing aqueous phase. But the simulations also show that the initial freshness status does have a large impact on the freshness predictions at advanced storage times since the concentrations still increase exponentially.

3.2.4 Simulations with dynamic temperatures on the prediction of freshness in the supply chain

In the simulations above the temperature was set at 0 °C. Figure 7 shows that according to simulations with other temperatures (with other parameters set for a miniaturized sensor), the dissociated TMA in the sensing aqueous phase increases strongly with increasing storage temperature.

However, the temperature fluctuates in the cod supply chain (Hafliðason, Ólafsdóttir, Bogason, & Stefánsson, 2012). A chain with temperature abuse was simulated: In a simulation (with a sensor with miniaturized conditions) fish was stored at 0 °C, but

after 100 hours, the temperature increased to 15 °C for 10 hours, and then returned to 0 °C. The temperature abuse is clearly seen in a sudden fast increase in the TMA concentration in the packed fish (Figure 8A). This sudden increase is not seen directly in the aqueous phase, but after the temperature abuse the concentration of dissociated TMA in the sensing aqueous phase is considerably higher compared to the simulation at constant 0 °C (Figure 8B).

3.2.5 Practical considerations to translate the predicted sensor outcome to a freshness signal

The non-destructive method has potential to be developed into an intelligent packaging. Taken this in perspective, the predicted sensor signal needs to be translated into a freshness signal that can be communicated as freshness status of the packed fish.

Although a level of 30 mg TMA 100 g^{-1} has been found at rejection level for packed cod (Dalgaard, 1995), the spoilage level was set to the acceptability limit for chilled cod of 15 mg TMA 100 g^{-1} reported by Venugopal, 2002 to calculate the moment of spoilage according to the sensor predictions (this acceptability limit is taken to illustrate the principle, every other TMA value can be taken as well). However, different TMA acceptability limits have been reported in literature, since this depends on the definition of the rejection point that is regarded as unacceptable (e.g. Dalgaard, Gram, & Huss (1993) found a level of >30 mg TMA 100 g^{-1} as rejection point, but the rejection point was defined as the point when 50% of the panelists rejected the fillets, which might not be realistic in commercial practice).

Simulations where performed with the miniaturized parameter conditions, the temperature and initial TMA concentration in the fish were varied for the simulations of different scenarios. The freshness predictions based on the TMA content of the fish were compared to the model prediction of the content of $TMAH^+$ in the aqueous phase.

At a constant temperature of 0 °C, the spoilage limit of 15 mg N TMA 100 g^{-1} fish was reached after 387 h. At this time, the $TMAH^+$ in the sensing aqueous phase was 0.0552 mg l^{-1} (Table 1). In the temperature abuse simulation (fish stored at 0 °C, the temperature increases to 15 °C for 10 hours after 100 hours, then returns back to 0 °C for remaining time) the fish reached the spoilage limit after 278 hours, which is more than 100 hours earlier compared to the simulation at a constant temperature of 0 °C. At 278 hours the $TMAH^+$ concentration in the sensing aqueous phase is 0.0503 mg l^{-1}, the $TMAH^+$ concentration of 0.0552 mg l^{-1} (comparable to $TMAH^+$ concentration in aqueous phase at spoilage moment at 0 °C constant) is reached after 286 hours. If one would base the spoilage limit on 0.055 mg l^{-1} in the aqueous phase, this would give a difference in the remaining shelf life of 8 hours.

The sensor should also give accurate predictions with different initial TMA concentrations C_0. A higher initial TMA concentration will lead to a shorter remaining shelf life. When the initial TMA concentration was increased in the simulation from 1.53 mg l^{-1} to 3 mg l^{-1} the fish reached the spoilage limit of 0.055 mg l^{-1} in the aqueous phase after 334 hours. At this time also the spoilage limit of 15 mg N TMA 100 g^{-1} fish in the packed fish was reached. This shows that the sensor is able to give accurate freshness predictions with a variable initial freshness status.

When a simulation was conducted at a constant 4 °C storage temperature, the fish would reach the spoilage limit after 142.3 hours. But the $TMAH^+$ concentration in the aqueous phase is only 0.015 mg l^{-1}. The $TMAH^+$ concentration of 0.0552 mg l^{-1} is reached after 269 h when the fish is far beyond spoilage. This implies that the freshness of the fish cannot be estimated solely from the sensor signal in the aqueous phase. Also information on the storage temperature is necessary to determine the cut-off point.

So the sensor signal at higher temperatures can still be translated into a freshness status of the fish, but the sensor needs to be combined with a temperature sensor. When the sensor signal is combined with the temperature history the model can be used to calculate the initial freshness C_0 and from here a remaining shelf life can be predicted.

The simulation results can be used in the future experimental design of the sensors, during this development the results need to be validated.

4. Conclusions

This manuscript presents the framework for a mathematical model that describes the mass transport of TMA that is formed on packed fish, released in the headspace and dissolves and dissociates in the sensing aqueous phase. This model is necessary to predict the freshness of the packed fish from the data produced by a non-destructive sensor that monitors TMA in the sensing aqueous phase.

The model predicts an TMA increase in the sensing aqueous phase comparable with sensor measurements from a storage trial at 15 °C. Model outcomes from simulations with variation of the sensor geometry show that minimizing the sensing aqueous

phase and the package headspace will improve the sensitivity of the sensor to different freshness stages.

The model can make accurate freshness predictions at a constant temperature of 0 °C and also in case of temporarily temperature abuse. The initial freshness of fish is variable, the model can be used to estimate it based on the data and use this parameter in the predictions of the freshness status of the packed fish. At 4 °C and higher, the freshness of the packed fish can be estimated when the temperature history is also measured. For variable storage temperatures, the conductivity-sensor has to be combined with a temperature sensor in order to use this model for the development of an intelligent packaging to monitor the freshness of fish.

Acknowledgement

This research is financially supported by the Wageningen University Graduate School VLAG and the Dutch Ministry of Agriculture, Nature and Food Quality (KB 030013121).

References

Botta, J.R., Lauder, J.T., & Jewer, M.A. (1984). Effects of methodology on total volatile basic nitrogen (TVB-N) determination as an index of quality of fresh Atlantic cod (*Gadus morhua*). *Journal of Food Science, 49*, 734-736, 750.

Burt, J.R., Gibson, D.M., Jason, A.C., & Sanders, H.R. (1976). Comparison of methods of freshness assessment of wet fish II. Instrumental and chemical assessments of boxed experimental fish. *Journal of Food Technology, 11*, 73-89.

591 Coury, L. (1999). Conductance measurements part 1: Theory. *Current Separations*,
592 *18*, 91-96.
593 Dalgaard, P. (1993). *Evaluation and prediction of microbial fish spoilage.* Ph.D.
594 Thesis. The Technological Laboratory of the Danish Ministry of Fisheries and
595 the Royal Veterinary and Agricultural University, Copenhagen, Denmark.
596 pp.143.
597 Dalgaard, P., Gram, L., Huss, H.H. (1993). Spoilage and shelf-life of cod fillets
598 packed in vacuum or modified atmospheres. *International Journal of Food*
599 *Microbiology, 19,* 283-294.
600 Dalgaard, P. (1995). Qualitative and quantitative characterization of spoilage bacteria
601 from packed fish. *International Journal of Food Microbiology, 26,* 319-333.
602 Dyer, W.J., Sigurdsson, G.J., & Wood, A.J. (1944). A rapid test for detection of
603 spoilage in sea fish. *Journal of Food Science, 9,* 183-187.
604 Emerson, K., Russo, R.C., Lund, R.E., & Thurston, R.V. (1975). Aqueous ammonia
605 equilibrium calculations: Effect of pH and temperature. *Journal of the Fisheries*
606 *Research Board of Canada, 32,* 2379-2383.
607 Frank, M.J.W., Kuipers, J.A.M., & Van Swaaij, W.P.M. (1996). Diffusion
608 coefficients and viscosities of CO_2 + H_2O, CO_2 + CH_3OH, NH_3 + H_2O, and
609 NH_3 + CH_3OH liquid mixtures. *Journal of Chemical and Engineering Data,*
610 *41,* 297-302.
611 Gill, T.A. (1990). Objective analysis of seafood quality. *Food Reviews International,*
612 *6,* 681-714.
613 Gram, L. & Huss, H. H. (1996). Microbiological spoilage of fish and fish products.
614 *International Journal of Food Microbiology, 33,* 121-137.

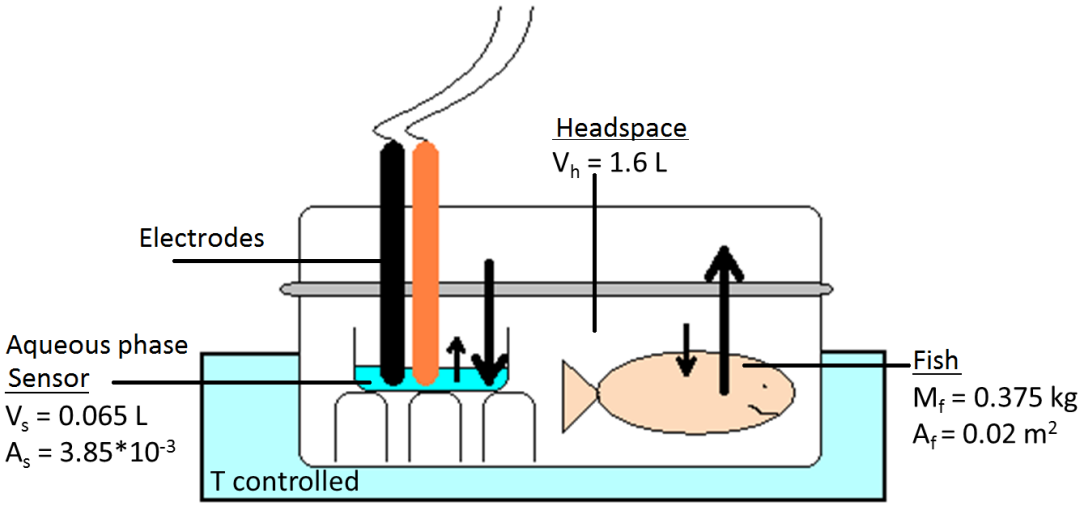
615 Hafliðason, T., Ólafsdóttir, G. Bogason, S., & Stefánsson, G. (2012). Criteria for
616 temperature alerts in cod supply chains. *International Journal of Physical*
617 *Distribution & Logistics Management, 42,* 355-371.
618 Heising, J.K., Dekker, M., Bartels, P.V., & Van Boekel, M.A.J.S. (2012). A non-
619 destructive ammonium detection method as indicator for freshness for packed
620 fish: Application on cod. *Journal of Food Engineering, 110,* 254-261.
621 Heising, J.K., Bartels, P.V., Van Boekel, M.A.J.S., & Dekker, M. (2014a). Non-
622 destructive sensing of the freshness of packed cod fish using conductivity and
623 pH electrodes. *Journal of Food Engineering, 124,* 80-85.
624 Heising, J.K., Dekker, M., Bartels, P.V., & Van Boekel M.A.J.S. (2014b). Monitoring
625 the quality of perishable foods: opportunities for intelligent packaging.
626 *Critical Reviews in Food Science and Nutrition, 54,* 645-654.
627 Heising, J.K., Van Boekel, M.A.J.S., & Dekker, M. (2014c). Mathematical models for
628 the trimethylamine (TMA) formation on packed cod fish fillets at different
629 temperatures. *Food Research International, 56,* 272-278.
630 Howgate, P. (2010). A critical review of total volatile bases and trimethylamine as
631 indices of freshness of fish. Part 1. Determination. *Electronic Journal of*
632 *Environmental, Agricultural and Food Chemistry, 9,* 29-57.
633 Huss, H. H. (1995). Quality and quality changes in fresh fish. FAO Fisheries
634 Technical paper no. 348. FAO, Rome, Italy.
635 Kuswandi, B., Wicaksono, Y., Jayus, Abdullah, A., Heng, L.Y., & Ahmad, M. (2011).
636 Smart packaging: sensors for monitoring of food quality and safety. *Sensing*
637 *and Instrumentation for Food Quality and Safety, 5,* 137-146.

638 Lowndes, A.G. (1955). Density of fishes – some notes on the swimming of fish to be
639 correlated with density, sinking factor and load carried. *The Annuals and*
640 *Magazine of Natural History, 8,* 241-256.
641 Malle, P. & Tao, S.H. (1987). Rapid quantitative determination of trimethylamine
642 using steam distillation. *Journal of Food Protection, 50,* 756-760.
643 Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B.,
644 Undeland, I., Mackie, I. M., Henehan, G., Nielsen, J., & Nilsen, H. (1997).
645 Methods to evaluate fish freshness in research and industry. *Trends in Food*
646 *Science and Technology, 8,* 258-265.
647 Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., & Chandler, R.E.
648 (1983). Model for bacterial culture growth rate throughout the entire biokinetic
649 temperature range. *Journal of Bacteriology, 154,* 1222-1226.
650 Realini, C.E. & Marcos, B. (2014). Active and intelligent packaging systems for a
651 modern society. *Meat Science, 98,* 404-419.
652 Sander, R. (1999). Compilation of Henry's law constants for inorganic and organic
653 species of potential importance in environmental chemistry. Version 3.
654 Unpublished manuscript. 107 pp. http://www.henrys-law.org (accessed on 2
655 October, 2013).
656 Sivertsvik, M., Jeksrud, W.K., & Rosnes, J.T. (2002). A review of modified
657 atmosphere packaging of fish and fishery products – significance of microbial
658 growth, activities and safety. *International Journal of Food Science and*
659 *Technology, 37,* 107-127.
660 Sivertsvik, M., Rosnes, J.T., & Jeksrud, W.K. (2004). Solubility and absorption rate
661 of carbon dioxide into non-respiring foods. Part 2: Raw fish fillets. *Journal of*
662 *Food Engineering, 63,* 451-458.

Stewart, W. E., Caracotsios, M., & Sorensen, J. P. (1992). Parameter-estimation from multiresponse data. *AIChE Journal*, 38(5), 641-650.

Vanderroost, M., Ragaert,P., Devlieghere, F., & De Meulenaer, B. (2014). Intelligent food packaging: The next generation. *Trends in Food Science & Technology, 39,* 47-62.

Venugopal, V. (2002). Biosensors in fish production and quality control. *Biosensors and Bioelectronics, 17,* 147-157.



671

672 *Figure 1: Systematic picture of the measurement set-up with geometric parameter*

673 *values (M = mass, V = Volume, A = Surface, T=Temperature) of fish (subscript f),*

674 *headspace (subscript h) and sensor (subscript s).*

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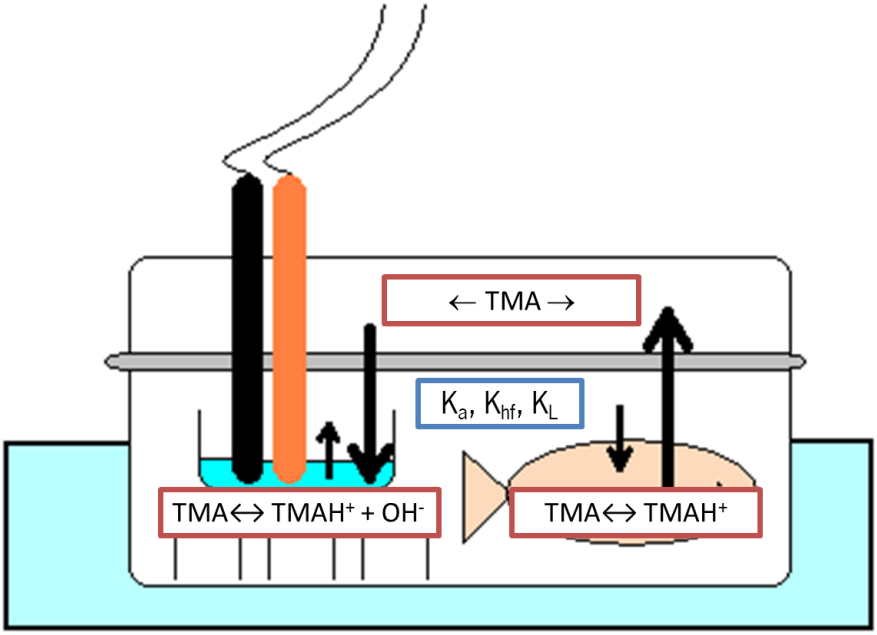

676

677 *Figure 2: Schematic picture of mass transfer of TMA in the fish package*

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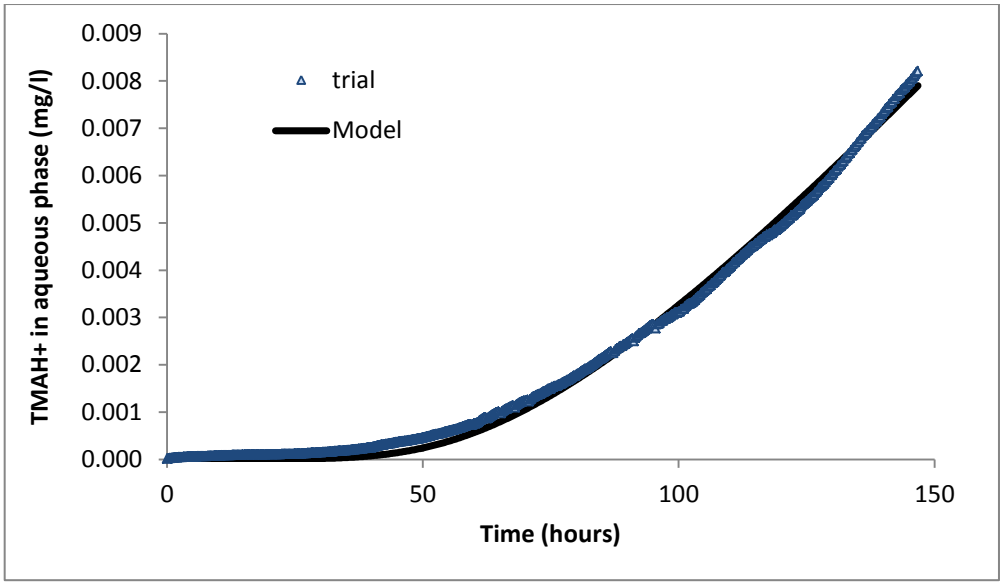

679

680 *Figure 3: Fit of the model on sensor measurements of $TMAH^+$-concentration from a*

681 *storage trial with cod stored at 15 °C.*

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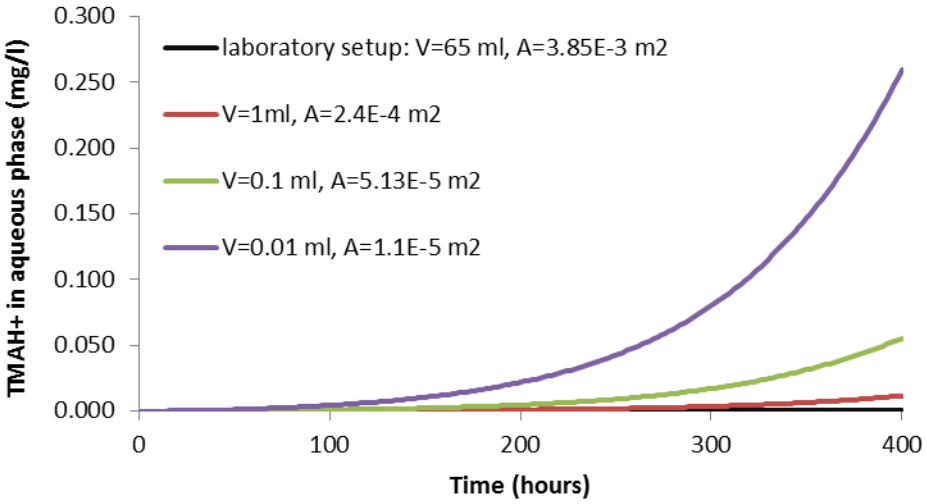

683

684 *Figure 4: Effect of volume and surface of sensor exposed to headspace (with*
685 *corrected K_L) on the concentration of TMA (mg/l) in the sensing aqueous phase from*
686 *simulations at 0 °C.*

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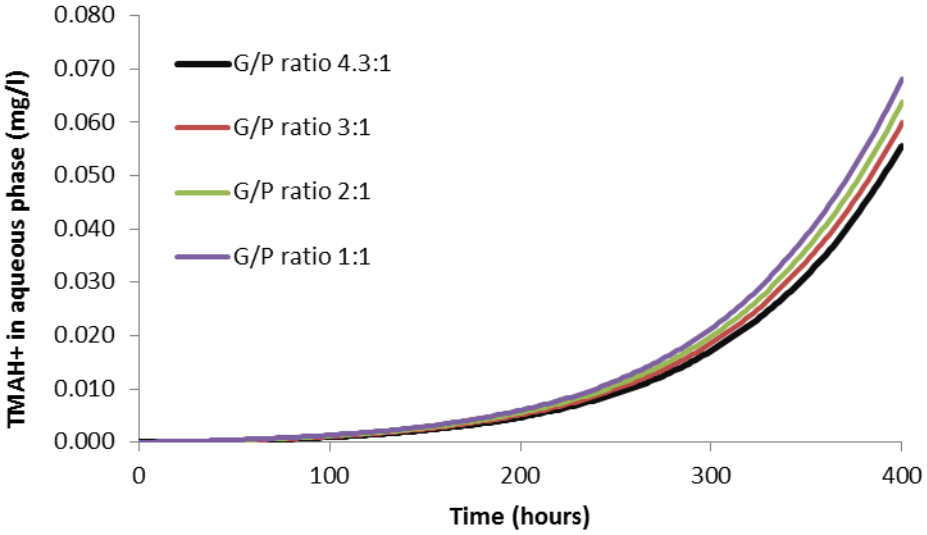

688

689 *Figure 5: Effect of headspace volume on the concentration of $TMAH^+$ in the sensing*

690 *aqueous phase from simulations at 0 °C (V_s=0.1 ml; A_s=5.13*10^{-5}).*

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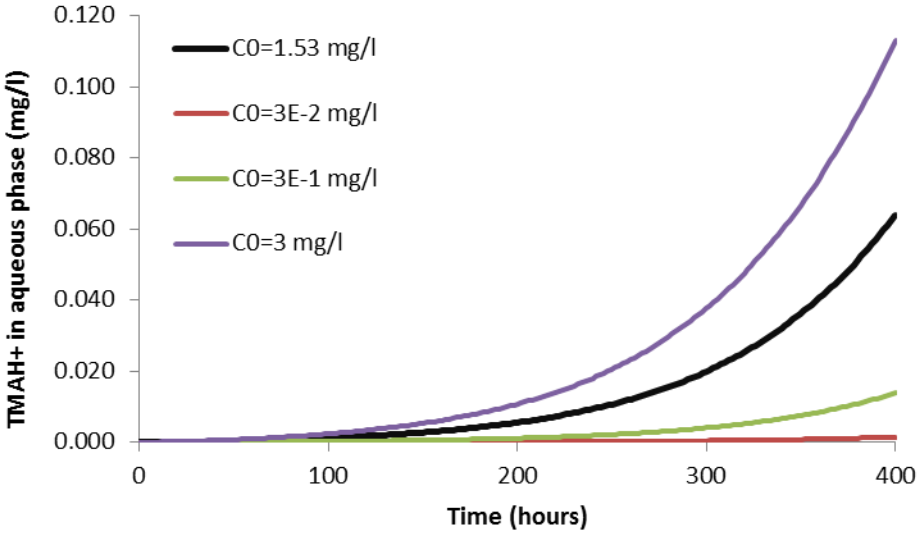

692

693 *Figure 6: Effect of parameter C_0 on the concentration of $TMAH^+$ in the sensing*

694 *aqueous phase from simulations at 0 °C (V_s=0.1 ml; A_s=5.13*10^{-5};V_h=750 ml).*

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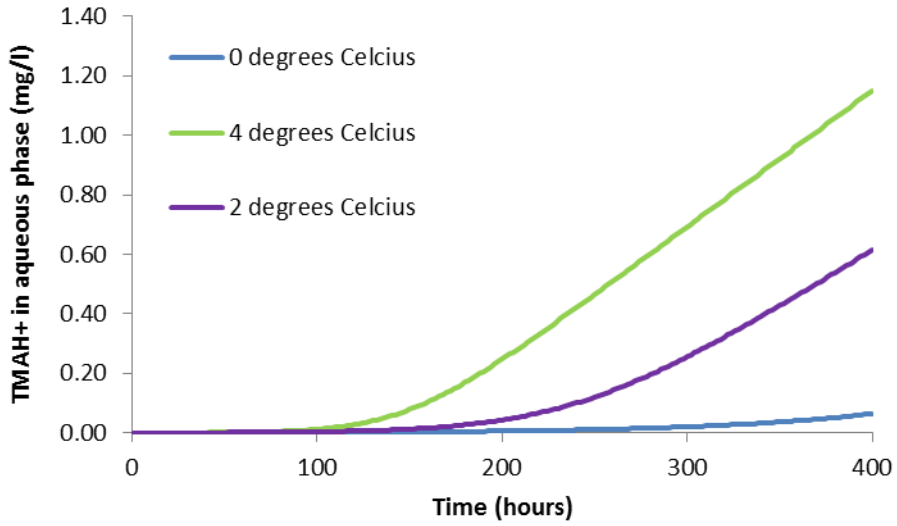
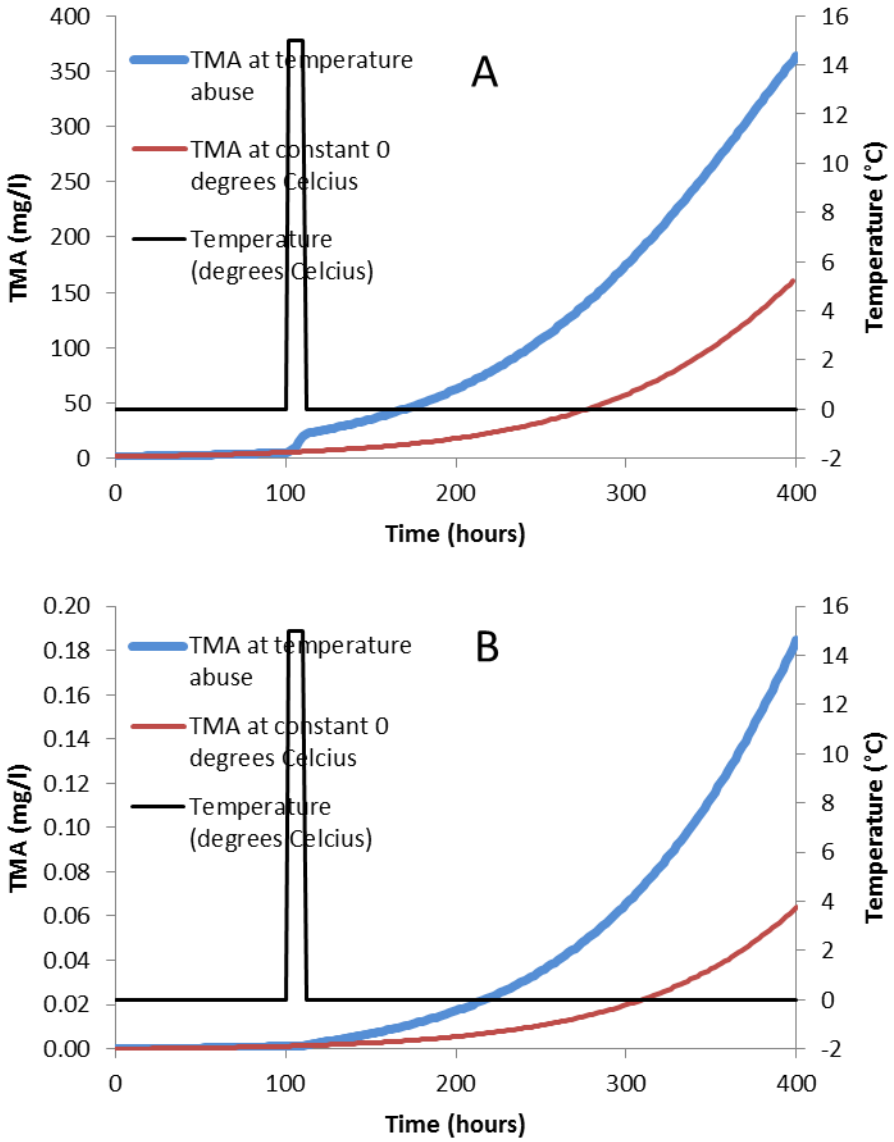


*Figure 7: Effect of storage temperature on the concentration of $TMAH^+$ in the sensing aqueous phase from simulations at 0, 2 and 4 °C (V_s=0.1 ml; A_s=5.13*10^{-5};V_h=750 ml).*



704

705 *Figure 8: Effect of abuse temperature on the concentration of TMA in the packed fish*

706 *(A) and of $TMAH^+$ in the sensing aqueous phase (B) from a simulation at 0 °C except*

707 *for 10 hours at 15 °C compared to a simulations with a constant T of 0 °C (V_s=0.1*

708 *ml; A_s=5.13*10^{-5};V_h=750 ml).*

709

*Table 1: Results of the simulations of the different scenarios, with varying temperature and initial content: Time when fish is spoiled, corresponding content of TMA in packed fish, and corresponding sensor signal $TMAH^+$ in aqueous phase (simulations performed with miniaturized sensor conditions: V_s=0.1 ml; A_s=5.13*10^{-5};V_h=750 ml)*

Simulation T	Time (hours)	TMA in packed fish (mg l^{-1})	$TMAH^+$ in aqueous phase (mg l^{-1})
0 °C constant	387	142.3	0.0552
T abuse	278	142.3	0.0503
	286	153.3	0.0552
4 °C constant	105	142.3	0.015
	269	654.4	0.0552
C_0 = 3 mg l^{-1}	334	144.4	0.0552