



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

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

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25

26 **Abstract**

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

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

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

48

49

50 **Keywords**

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

53

## 54 **1. Introduction**

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

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

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

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

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

94

## 95 **2. Materials and Methods**

96

### 97 **2.1 Data collection**

98

#### 99 *2.1.1 Storage trial of cod fillets*

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

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

110

#### 111 *2.1.2 Non-destructive method*

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

115 Cod fillets (~375 g) sliced into pieces of approximately 30 g, were put in the glass  
116 cell. Each experiment contained randomly mixed pieces from different cod fillets. The  
117 glass cell contained a conductivity electrode (TetraCon 325 conductivity electrode  
118 with inoLab Cond 730 precision conductivity meter, WTW) with the electrode-tip in  
119 65 ml Milli-Q (deionized) water in the beaker. The conductivity electrode was logged  
120 automatically at time-intervals of 15 minutes.

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

123

#### 124 *2.1.3 Destructive TMA analysis*

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

132 The TMA content was determined in duplicate in an extract of the cod fillet according  
133 to the steam distillation method from Malle & Tao (1987) as described in Heising et  
134 al. (2012). 20 ml of ~36% aqueous formaldehyde-solution (Fluka 47630)  
135 (formaldehyde complexes with primary and secondary amines, but not with tertiary  
136 amine TMA) was added to 25 ml of filtrate, followed by 5 ml of 10% (w/v) NaOH.  
137 Steam distillation (Gerhard Vadopest 12-Kjedahl type distillatory) was carried out for  
138 7.3 minutes on the TCA extract. A beaker containing 10 ml of a 4% aqueous boric  
139 acid solution (Merck 1.00165) and 0.04 ml of Mixed indicator 5 for ammonia  
140 titrations (Merck 1.06130) was placed at the end of the condenser. The boric acid  
141 solution turned green when alkalinized by the distilled TMA. The green alkaline  
142 distillate was titrated using a digital burette (Schott type T80 /20) containing an  
143 aqueous 0.1N hydrochloric acid solution (Merck 1.09973). Complete neutralization  
144 was obtained when the colour turned pink on the addition of a further drop of  
145 hydrochloric acid. This procedure was repeated for duplicate analysis.

146

## 147 **2.2 Parameter estimation and simulations**

148

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](http://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  $\text{NH}_3$  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,  $\text{NH}_3$  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



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

178

### 179 3.1.1 Formation of TMA

180 TMA is produced on fresh cod fillets stored at chilled and higher temperatures (in the  
181 range 0-15 °C) by the micro-organisms *Shewanella putrefaciens* and *Photobacterium*  
182 *phosphoreum* and the formation can be described by a dynamic model (Heising et al.,  
183 2014c):

$$184 \quad \frac{dC_{TMA}}{dt} = \mu_{max} C_{TMA} \left( 1 - \left( \frac{C_{TMA}}{C_{max}} \right) \right) \quad (1)$$

185

186 With:

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

188  $t$  time (hours)

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

190  $\mu_{max}$  maximum specific formation rate coefficient (hours<sup>-1</sup>)

191

192 With parameter for initial value in the numerical integration:

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

194

195 The parameter  $C_0$  incorporates the initial freshness status and the effect of natural  
196 variation in the quality that influences the freshness of fish and  $C_{max}$  was estimated to  
197 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  $\mu_{max}$  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<sup>-1</sup>)

210  $c$  additional parameter for fit (°C h<sup>-1</sup>)

211

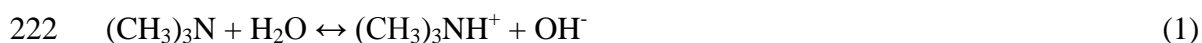
212 The parameter estimate for  $T_{max}$  was 25 °C (taken from Dalgaard (1993)) and was  
213 based on the maximum growth temperature of the bacteria *Photobacterium*  
214 *phosphoreum*. The parameter estimates for the parameters  $T_{min}$ ,  $b$  and  $c$  were -4 °C,  
215 0.029 °C h<sup>-1</sup>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



223

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

228

$$229 \quad F = \frac{[TMA]}{[\Sigma TMA]} = \frac{[TMA]}{[TMA]+[TMAH^+]} \quad (3)$$

230

231 With:

232  $F$  fraction of TMA in  $\Sigma TMA$  of the fish (-)

233  $[TMA]$  concentration of undissociated TMA ( $\text{mg l}^{-1}$ )

234  $[TMAH^+]$  concentration of dissociated TMA ( $\text{mg l}^{-1}$ )

235  $[\Sigma TMA]$  concentration of total TMA from equation X ( $\text{mg l}^{-1}$ )

236

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

$$239 \quad K_a = \frac{[TMA][H^+]}{[TMAH^+]} \quad (4)$$

240

241 With:

242  $K_a$  dissociation constant

243  $[H^+]$  concentration of hydrogen ion ( $\text{mg l}^{-1}$ )

244

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

248 dependence of the  $pK_a$  of  $\text{NH}_3$  (Emerson, Russo, Lund, & Thurston, 1975), assuming  
249 the same temperature coefficient for TMA as was reported for  $\text{NH}_3$  (Howgate, 2010).

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

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  $K_{hf}$ :

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

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

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

269

270 With:

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

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

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

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

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

276  $T$  temperature (K)

277

278 The Henry's Law constant for TMA between water and gas phase at 25 °C is 9.6 ( $\text{mol}$   
279  $\text{L}^{-1} \text{atm}^{-1}$ ) (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):

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

284

285 In integrated form (Equation 9):

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

287

288 With:

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

290  $T$  temperature (K)

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

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

293

294 The value taken for the slope  $\frac{d \ln k}{d \frac{1}{T}}$  was  $4100 \text{ M atm}^{-1}$  (Sander, 1999), assuming the

295 temperature dependence of TMA to be similar to that of  $\text{NH}_3$ . 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 ( $\text{h}^{-1}$ ) is described by equation 10:

$$304 \quad V \frac{dc_{TMA}}{dt} = K_L A_f (c_f - c_h) \quad (10)$$

305

306 With:

307  $c_{TMA}$  concentration of dissolved TMA in fish ( $\text{mg l}^{-1}$ )

308  $V$  volume of fish ( $=M_f/\rho_f$ ) ( $=0.36 \text{ l}$ )

309  $K_L$  mass transfer coefficient ( $\text{mm h}^{-1}$ )

310  $A_f$  surface of fish exposed to headspace ( $= 0.02 \text{ m}^2$ )

311

312 The mass transfer coefficients of TMA were assumed to be similar to the mass  
313 transfer coefficient for  $\text{NH}_3$ . 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  $K_{L,\text{ref}}$ , using the experimental data (Heising et al., 2014a) at a reference temperature of  
320 15 °C (Equation 11).

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

322

323 With:

324  $K_L$  mass transfer coefficient (mm h<sup>-1</sup>)

325  $K_{Lref}$  reference mass transfer coefficient at 15 °C (mm h<sup>-1</sup>)

326  $T$  temperature (K)

327  $T_{ref}$  reference temperature (=288 K)

328

### 329 *3.1.5 Mass transfer of TMA from the headspace to the sensor aqueous phase*

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

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

338

### 339 *3.1.6 Sensor measurement*

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

345 electrode, from which the TMAH<sup>+</sup> 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<sup>+</sup>- 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<sup>-1</sup>)

363 U(2) initial concentration of TMA in headspace at time 0 = 0 (mg l<sup>-1</sup>)

364 U(3) initial concentration of TMA in aqueous phase at time 0 = 0 (mg l<sup>-1</sup>)

365 U(4) initial concentration of TMAH<sup>+</sup> in aqueous phase at time 0 = 0 (mg l<sup>-1</sup>)

366

## 367 **3.2 Model application**

368

### 369 *3.2.1 Fit of the mathematical model on measurements of a fish storage trial*



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

373

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

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

$$385 \quad K_L = \frac{D}{\delta} \quad (12)$$

386

387 With

388  $D$  diffusion coefficient ( $\text{m}^2/\text{s}$ )

389  $\delta$  distance across diffusion occurs (m)

390

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

395 mass transfer coefficients for the release of TMA from the fish is in the order of  $10^{-9}$   
396 m/s, which is in the same order as diffusion coefficients for  $\text{NH}_3$  reported by Frank,  
397 Kuipers, & Van Swaaij (1996), but is expected to be higher because of the low  $\delta$ .

398

399 The conductivity in the aqueous phase is measured by the sensor, but the conductivity  
400 electrode is non-specific and can measure all volatile compounds that dissolve and  
401 dissociate in the aqueous phase. Therefore, also other volatile compounds, e.g.  $\text{NH}_3$ ,  
402  $\text{CO}_2$ , and  $\text{H}_2\text{S}$  that can be formed by the fish can influence the signal that is measured  
403 by the sensor. Furthermore, from reaction 1 it can be seen that  $\text{OH}^-$  ions are formed  
404 together with the  $\text{TMAH}^+$ . Besides, the compounds can interact with each other, e.g.  
405 the carbonic acid from dissolved  $\text{CO}_2$  can react with the hydrogen ions formed from  
406 the dissociation of trimethylamine in the aqueous phase.

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

414

### 415 *3.2.2 Simulations with geometry*

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

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

422

### 423 3.2.2.1 Effect of sensor volume and surface

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

$$433 \quad A_2 = A_1 \left( \frac{V_2}{V_1} \right)^{2/3} \quad (13)$$

434

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

$$440 \quad K_{L2} = K_{L1} \left( \frac{V_2}{V_1} \right)^{1/3} \quad (14)$$

441

442 When the volume of the aqueous phase is reduced, the concentration of TMA in the  
443 aqueous phase increases (Figure 4). This increased TMAH<sup>+</sup> concentration in the

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

446

#### 447 3.2.2.2 Effect of headspace volume

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

459

460

#### 461 *3.2.3 Simulations with variation in initial freshness on the prediction of freshness*

##### 462 *in the supply chain*

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

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

482

483

#### 484 *3.2.4 Simulations with dynamic temperatures on the prediction of freshness in the* 485 *supply chain*

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

490

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

494 after 100 hours, the temperature increased to 15 °C for 10 hours, and then returned to  
495 0 °C. The temperature abuse is clearly seen in a sudden fast increase in the TMA  
496 concentration in the packed fish (Figure 8A). This sudden increase is not seen directly  
497 in the aqueous phase, but after the temperature abuse the concentration of dissociated  
498 TMA in the sensing aqueous phase is considerably higher compared to the simulation  
499 at constant 0 °C (Figure 8B).

500

501

502 *3.2.5 Practical considerations to translate the predicted sensor outcome to a*  
503 *freshness signal*

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

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

518 Simulations where performed with the miniaturized parameter conditions, the  
519 temperature and initial TMA concentration in the fish were varied for the simulations  
520 of different scenarios. The freshness predictions based on the TMA content of the fish  
521 were compared to the model prediction of the content of TMAH<sup>+</sup> in the aqueous  
522 phase.

523 At a constant temperature of 0 °C, the spoilage limit of 15 mg N TMA 100 g<sup>-1</sup> fish  
524 was reached after 387 h. At this time, the TMAH<sup>+</sup> in the sensing aqueous phase was  
525 0.0552 mg l<sup>-1</sup> (Table 1). In the temperature abuse simulation (fish stored at 0 °C, the  
526 temperature increases to 15 °C for 10 hours after 100 hours, then returns back to 0 °C  
527 for remaining time) the fish reached the spoilage limit after 278 hours, which is more  
528 than 100 hours earlier compared to the simulation at a constant temperature of 0 °C.

529 At 278 hours the TMAH<sup>+</sup> concentration in the sensing aqueous phase is 0.0503 mg l<sup>-1</sup>,  
530 the TMAH<sup>+</sup> concentration of 0.0552 mg l<sup>-1</sup> (comparable to TMAH<sup>+</sup> concentration in  
531 aqueous phase at spoilage moment at 0 °C constant) is reached after 286 hours. If one  
532 would base the spoilage limit on 0.055 mg l<sup>-1</sup> in the aqueous phase, this would give a  
533 difference in the remaining shelf life of 8 hours.

534 The sensor should also give accurate predictions with different initial TMA  
535 concentrations  $C_0$ . A higher initial TMA concentration will lead to a shorter remaining  
536 shelf life. When the initial TMA concentration was increased in the simulation from  
537 1.53 mg l<sup>-1</sup> to 3 mg l<sup>-1</sup> the fish reached the spoilage limit of 0.055 mg l<sup>-1</sup> in the  
538 aqueous phase after 334 hours. At this time also the spoilage limit of 15 mg N TMA  
539 100 g<sup>-1</sup> fish in the packed fish was reached. This shows that the sensor is able to give  
540 accurate freshness predictions with a variable initial freshness status.

541

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

549

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

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

557

#### 558 **4. Conclusions**

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

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



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

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

577

### 578 **Acknowledgement**

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582

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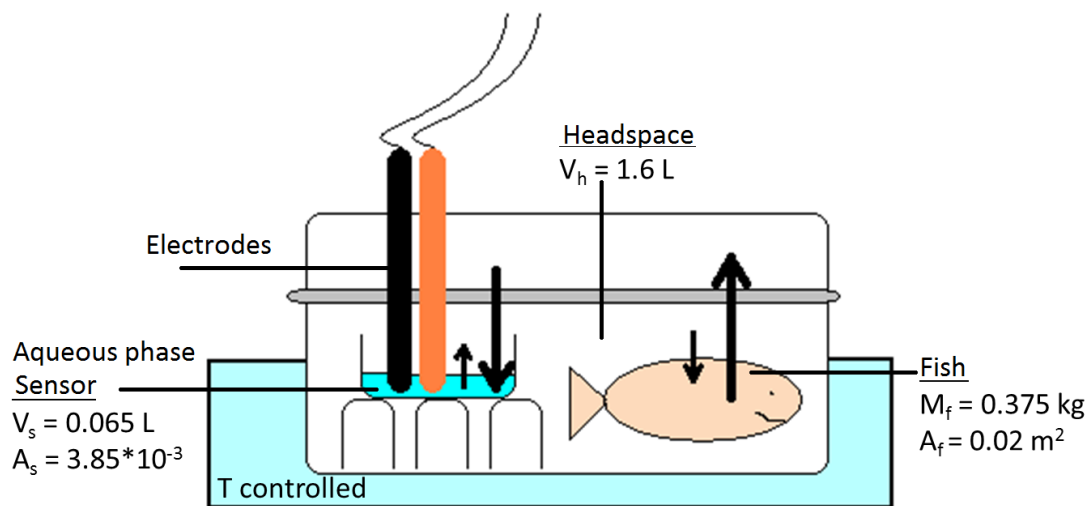
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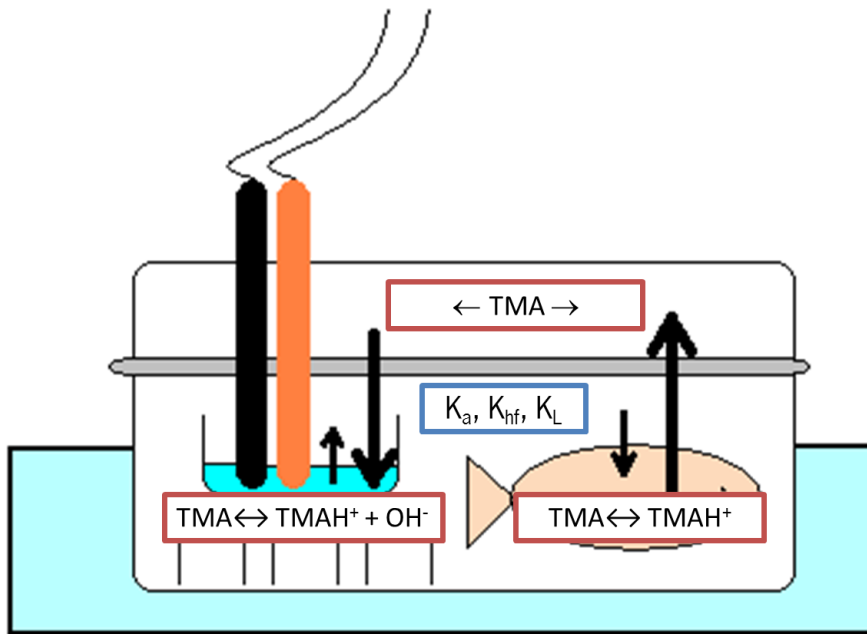
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671

672 *Figure 1: Systematic picture of the measurement set-up with geometric parameter*  
 673 *values ( $M = \text{mass}$ ,  $V = \text{Volume}$ ,  $A = \text{Surface}$ ,  $T = \text{Temperature}$ ) of fish (subscript  $f$ ),*  
 674 *headspace (subscript  $h$ ) and sensor (subscript  $s$ ).*

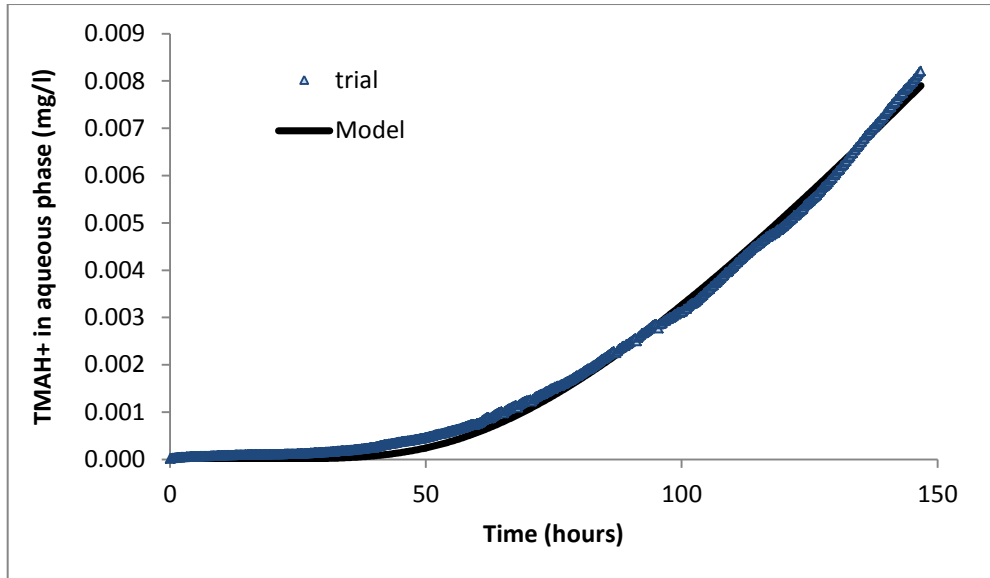
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677 *Figure 2: Schematic picture of mass transfer of TMA in the fish package*

678



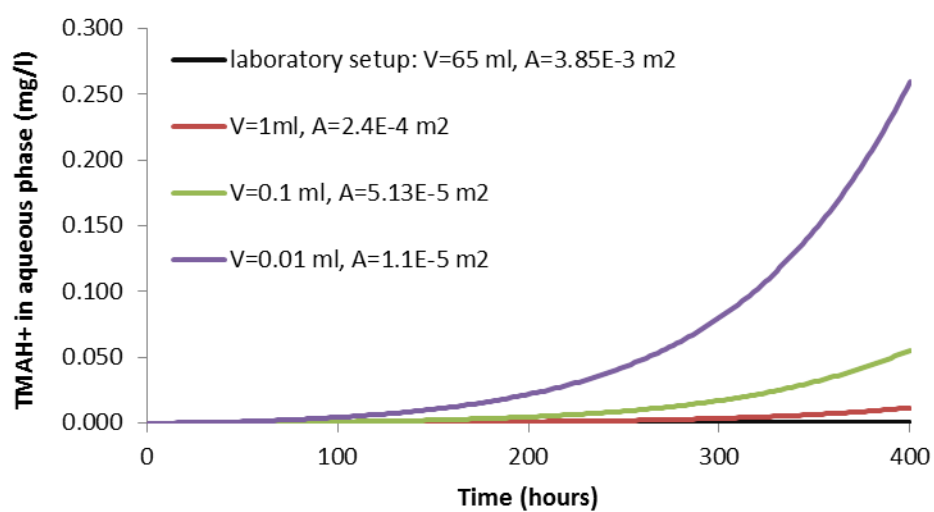
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680 *Figure 3: Fit of the model on sensor measurements of TMAH<sup>+</sup>-concentration from a*

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

682

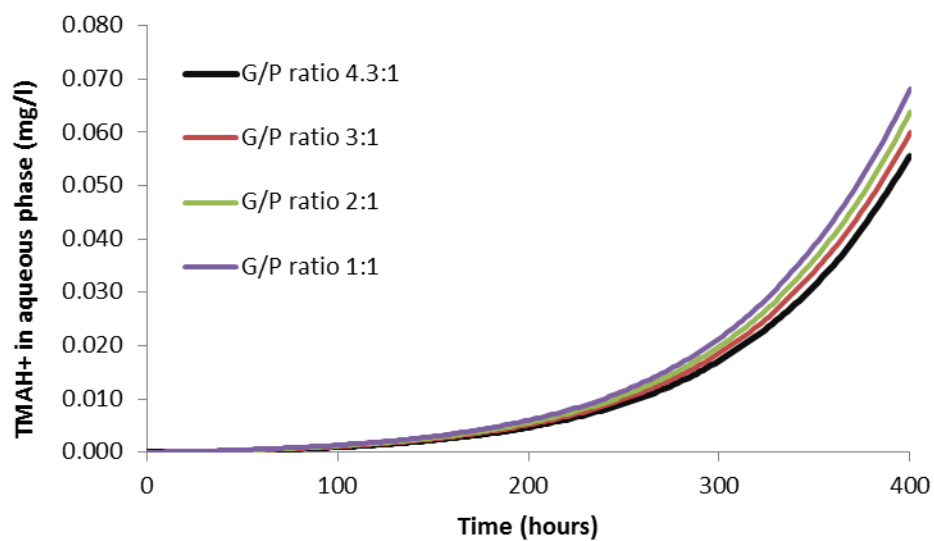




683

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.*

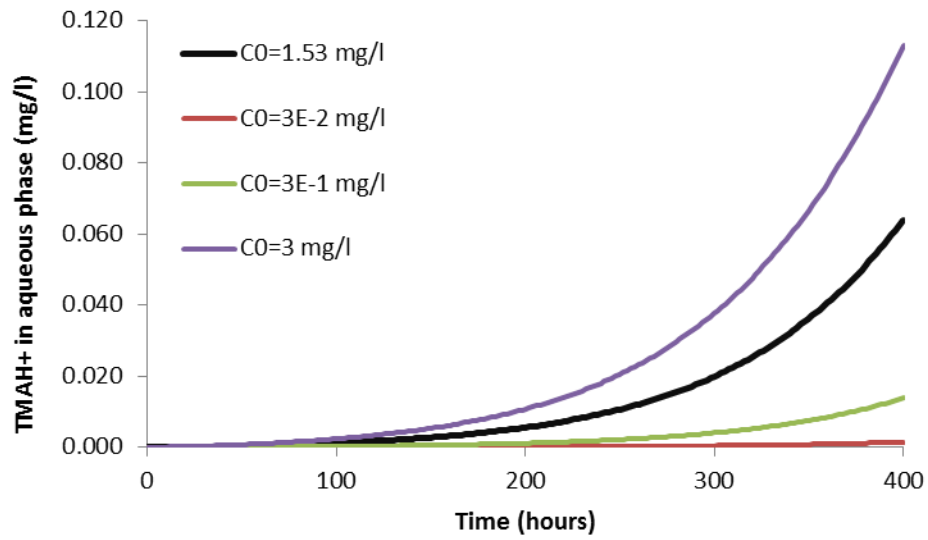
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688

689 *Figure 5: Effect of headspace volume on the concentration of TMAH<sup>+</sup> in the sensing*  
 690 *aqueous phase from simulations at 0 °C ( $V_s=0.1$  ml;  $A_s=5.13 \cdot 10^{-5}$ ).*

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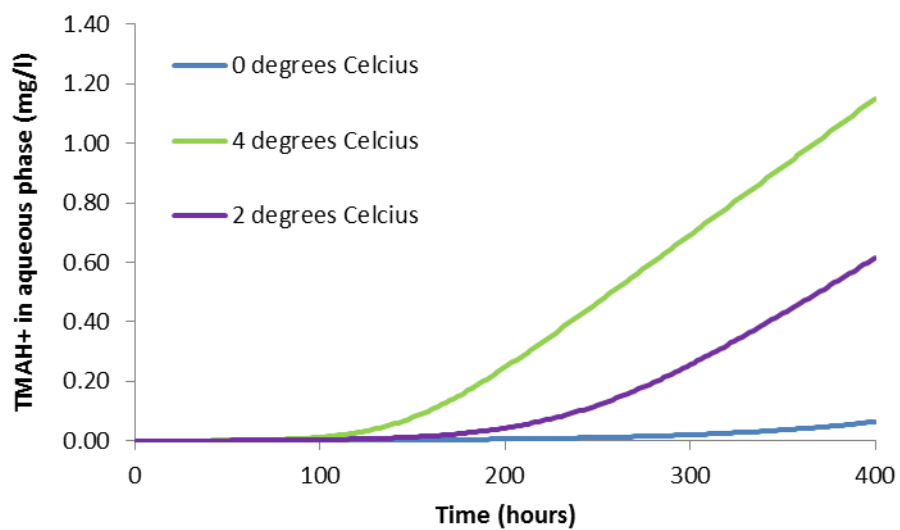


692

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

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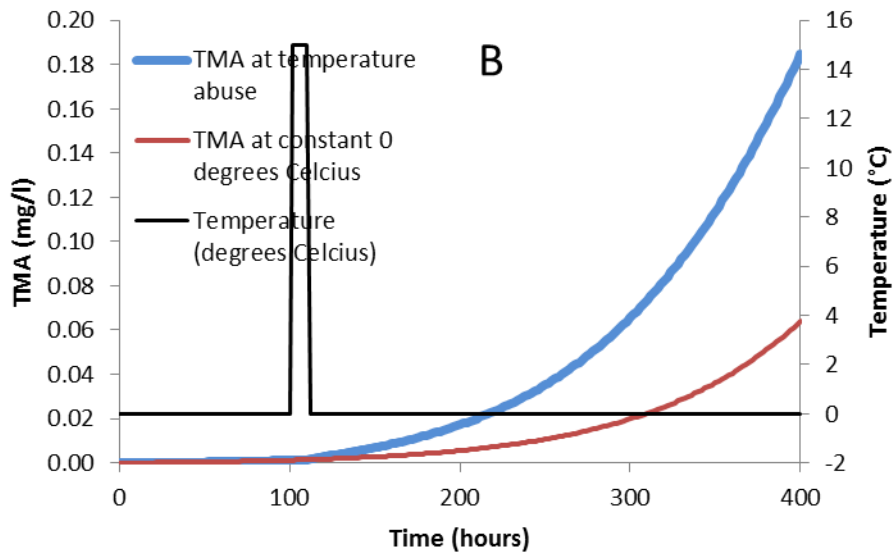
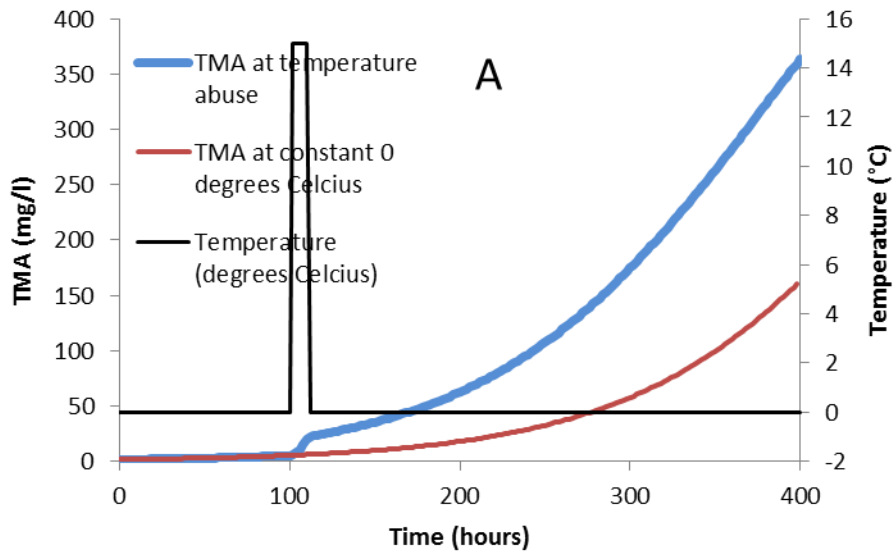
697

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

701

702

703



704

705 *Figure 8: Effect of abuse temperature on the concentration of TMA in the packed fish*  
 706 *(A) and of TMAH<sup>+</sup> 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;  $A_s=5.13 \cdot 10^{-5}$ ;  $V_h=750$  ml).*

709

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

Simulation T	Time (hours)	TMA in packed fish (mg l <sup>-1</sup> )	TMAH <sup>+</sup> in aqueous phase (mg l <sup>-1</sup> )
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 <sup>-1</sup>	334	144.4	0.0552

715

716