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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
The mass transfer of TMA in packed cod fillets was modelled using sets of algebraic and differential equations. Simulations and parameter estimation from numerical integration of the differential equations, including the statistical evaluation of the parameters and performance of the complete model, were obtained by least squares regression with the help of the software package Athena Visual Workbench (Stewart et al., 1992; www.athenavisual.com).

3. Results and Discussion

3.1 Model development

The modelling approach is based on the formation of volatile compounds. In freshly caught cod the volatile NH$_3$ and some other volatile compounds are present. During subsequent storage the content of volatiles increases, mainly due to the formation of TMA. In a later stage, when the fish is already spoiled, NH$_3$ is increasing further. This formation of volatiles can be linked to the freshness and quality of fish (Ólafsdóttir et al., 1997). We realize that quality is a broad concept and the combined analysis of several quality attributes (e.g. protein and fat degradation, microbial growth and sensory aspects) could lead to more accurate description of the quality status. However for the development of a sensor a non-destructive approach is required. Volatiles can be measured non-destructively. The TMA content is a good indicator since it is correlated to the freshness status and also other quality attributes (Burt et al., 1976).
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):

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

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

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

(2)

With:

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

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

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

$c$ additional parameter for fit (°C h$^{-1}$)

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

3.1.2 Dissociation of TMA in fish

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

$$(\text{CH}_3)_3\text{N} + \text{H}_2\text{O} \leftrightarrow (\text{CH}_3)_3\text{NH}^+ + \text{OH}^-$$

(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 $\rho_f$ 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.

$$F = \frac{[TMA]}{[\Sigma TMA]} = \frac{[TMA]}{[TMA]+[TMAH^+]} \tag{3}$$

With:

$F$ fraction of TMA in $\Sigma$TMA 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):

$$K_a = \frac{[TMA][H^+]}{[TMAH^+]} \tag{4}$$

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

\[ pK_a = 0.6516 + 2729.2T^{-1} \]  \hspace{1cm} (5)

With:

- $pK_a$ dissociation constant of TMA
- $T$ temperature (K)

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

3.1.3 Partitioning of TMA between the fish and the headspace

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

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

\[ K_{hf} = \frac{c_f}{c_h} = k_H \ast RT \]  \hspace{1cm} (6)

or rewritten to equation 7 (Sander, 1999):

\[ T \ast k_H = 12.2 \ast K_{hf} \]  \hspace{1cm} (7)

With:

- $K_{hf}$ ratio of concentrations in headspace and fish (-)
$c_h$ concentration of TMA in headspace (mg l$^{-1}$)

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

$k_H$ Henry’s Law constant (mol l$^{-1}$ atm$^{-1}$)

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

$T$ temperature (K)

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

$$\frac{d \ln k}{dT} = -\frac{\Delta H^\ominus}{R}$$ (8)

In integrated form (Equation 9):

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

With:

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

$T$ temperature (K)

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

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

The value taken for the slope $\frac{d \ln k}{dT}$ was 4100 M atm$^{-1}$ (Sander, 1999), assuming the temperature dependence of TMA to be similar to that of NH$_3$. After the Henry’s law
constant has been adjusted for the storage temperature of the fish, equation 6 is used
to calculate the ratio $K_{hf}$ of concentration of TMA in the fish and in the headspace.

3.1.4 Mass transfer coefficient

On the surface of the fish that is exposed to the headspace, the release of TMA will
take place when there is a driving force if the concentrations of undissociated TMA in
the fish and in the headspace are not in equilibrium. The rate of change of TMA
concentration in the fish per unit of time (h$^{-1}$) is described by equation 10:

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

With:

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

$V$ volume of fish ($=M_f/\rho_f$) (=0.36 l)

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

$A_f$ surface of fish exposed to headspace (= 0.02 m$^2$)

The mass transfer coefficients of TMA were assumed to be similar to the mass
transfer coefficient for NH$_3$. Since there is no airflow inside the package, the overall
mass transfer rate is mainly dependent on the diffusion coefficient. The diffusion
coefficient changes proportionally with the temperature change (according to the
Stokes-Einstein equation, the other parameters of the Stokes-Einstein equation were
assumed to remain constant in the sensor for the temperature range of 0-15 °C). In the
model, the the mass transfer coefficient $K_L$ at temperature T (K) was estimated from
$K_{L ref}$, using the experimental data (Heising et al., 2014a) at a reference temperature of
15 °C (Equation 11).
\[ K_L = K_{L,\text{ref}} \times \frac{T}{T_{\text{ref}}} \]  \hspace{1cm} (11)

With:

- \( K_L \): mass transfer coefficient (mm h\(^{-1}\))
- \( K_{L,\text{ref}} \): reference mass transfer coefficient at 15 °C (mm h\(^{-1}\))
- \( T \): temperature (K)
- \( T_{\text{ref}} \): 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...
electrode, from which the TMAH$^+$ concentration in the aqueous phase is calculated. From this signal the freshness stage of the fish is predicted.

3.1.7 Model equations

The formation and mass transfer of TMA from the fish to the aqueous phase of the sensor is described by differential equations, based on the equations described above. The model equations are based on mass balances, for example the TMA content in the fish is based on the formation of TMA from microbial growth minus the release of TMA from the fish to the headspace. The mass balances are described separately for the TMA in the fish, the headspace and the sensing aqueous phase. Finally, a dissociated TMAH$^+$ concentration in the sensing aqueous phase can be calculated at each time $t$ resulting in a conductivity value. This conductivity value represents the freshness status of the fish.

The differential equations are numerically integrated with the software in order to simulate the TMA content in the fish, the headspace and the sensing aqueous phase. The initial conditions for numerical integration of the differential equations are:

- $U(1)$ initial concentration of TMA in fish at time $0 = C_0 \rho_f$ (mg l$^{-1}$)
- $U(2)$ initial concentration of TMA in headspace at time $0 = 0$ (mg l$^{-1}$)
- $U(3)$ initial concentration of TMA in aqueous phase at time $0 = 0$ (mg l$^{-1}$)
- $U(4)$ initial concentration of TMAH$^+$ in aqueous phase at time $0 = 0$ (mg l$^{-1}$)

3.2 Model application

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 $3.81 \times 10^{-3} \pm 2.0 \times 10^{-4}$ mm*h$^{-1}$ and from the uptake of TMA from the headspace into the sensing aqueous phase was $6.93 \times 10^{-3} \pm 2.7 \times 10^{-4}$ 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):

$$K_L = \frac{D}{\delta}$$  \hspace{1cm} (12)

With

$D$ diffusion coefficient (m$^2$/s)

$\delta$ 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$_2$S 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 $\mu_{max}$ 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 $3.85 \times 10^{-3}$ 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.

$$A_2 = A_1 \left(\frac{V_2}{V_1}\right)^{2/3}$$  \hspace{1cm} (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:

$$K_{L2} = K_{L1} \left(\frac{V_2}{V_1}\right)^{1/3}$$  \hspace{1cm} (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 $5.13 \times 10^{-5}$ 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 microorganisms. 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 $5.13\times 10^{-5}$ 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 (Haflíðason, 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.

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Figure 1: Systematic picture of the measurement set-up with geometric parameter values \((M = \text{mass, } V = \text{Volume, } A = \text{Surface, } T = \text{Temperature})\) of fish (subscript \(f\)), headspace (subscript \(h\)) and sensor (subscript \(s\)).
Figure 2: Schematic picture of mass transfer of TMA in the fish package.
Figure 3: Fit of the model on sensor measurements of TMAH$^+$-concentration from a storage trial with cod stored at 15 °C.
Figure 4: Effect of volume and surface of sensor exposed to headspace (with corrected $K_L$) on the concentration of TMA (mg/l) in the sensing aqueous phase from simulations at 0 °C.
Figure 5: Effect of headspace volume on the concentration of TMAH$^+$ in the sensing aqueous phase from simulations at 0 °C ($V_s=0.1$ ml; $A_s=5.13 \times 10^{-5}$).
Figure 6: Effect of parameter $C_0$ on the concentration of $\text{TMAH}^+$ in the sensing aqueous phase from simulations at 0 °C ($V_s=0.1 \text{ ml}; A_s=5.13*10^{-5}; V_b=750 \text{ ml}$).
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).
Figure 8: Effect of abuse temperature on the concentration of TMA in the packed fish (A) and of TMAH$^+$ in the sensing aqueous phase (B) from a simulation at 0 °C except for 10 hours at 15 °C compared to a simulations with a constant T of 0 °C ($V_s=0.1$ ml; $A_s=5.13*10^{-5}$; $V_h=750$ ml).
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: $V_s=0.1 \text{ ml}$; $A_s=5.13 \times 10^{-5}$; $V_h=750 \text{ ml}$)

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