Intelligent packaging for monitoring food quality:

A case study on fresh fish

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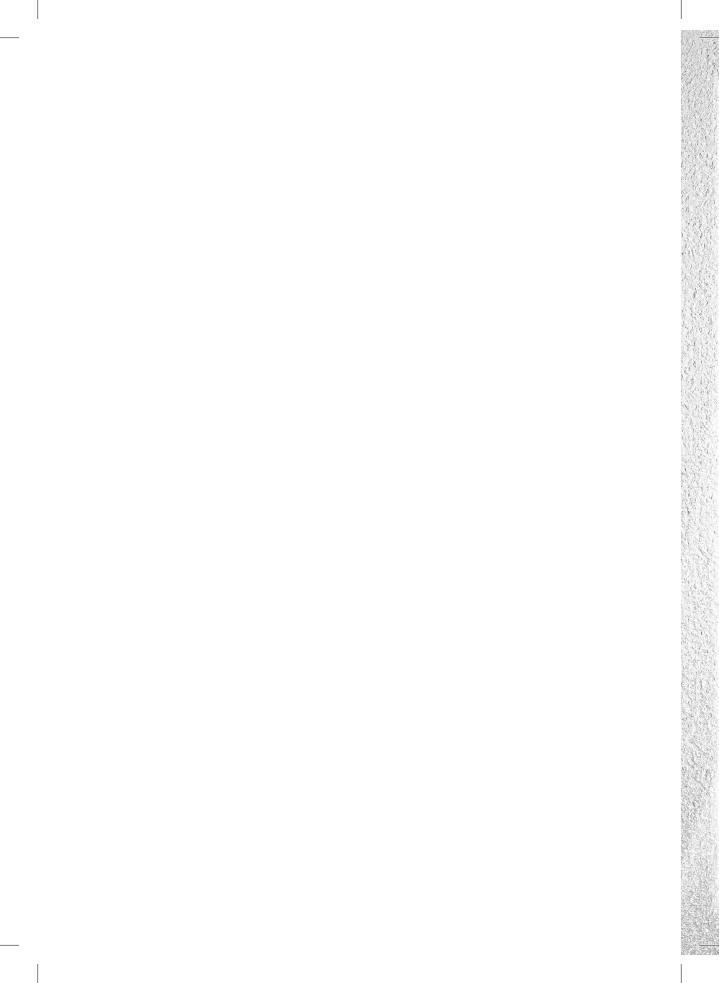
General introduction

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The quality of a perishable food changes as it proceeds from harvest or producer to consumer. Perishable foods are a class of foods that are active biological systems with high reaction rates of spoilage after harvest, processing or slaughter (Van Boekel, 2009). There is a large economic and environmental loss due to expired perishables, because of their limited shelf life. Perishable foods are often not, or only minimally processed and storage conditions strongly influence the quality of foods. Food manufacturers can face difficulties to ensure quality of perishable foods and can experience negative consequences when consumers are disappointed by the quality of their foods. If the quality of foods can be monitored during the entire supply chain, quality assurance can prevent consumer disappointment and retailers don't need to discard food that has reached the printed shelf life date but still has an acceptable quality. Intelligent packaging can be used, in principle, to inform all actors in the chain, like wholesalers, retailers and consumers about the quality status of foods and offers the possibility to take logistic actions based on dynamically estimated shelf life, thereby reducing waste of foods.

The research described in this thesis investigated the possibility to use intelligent packaging for monitoring food quality and it describes, as a case study, an approach for the development of a non-destructive sensor for monitoring quality of packed fish.

1.1. Overview of Intelligent Packaging

A package of foods has 4 basic functions, being containment, protection, convenience and communication (Robertson, 1993). The communication function of the package can be extended by providing dynamic information when sensors or indicators are included in/on the package and then the package becomes an intelligent packaging. Yam et al. (2005) defined intelligent packaging as a packaging system that is capable of carrying out intelligent functions (such as detecting, sensing, recording, tracing, communicating, and applying scientific logic) to facilitate decision making to extend shelf life, enhance safety, improve quality, provide information, and warn about possible problems. Smart packaging is sometimes used interchangeably with intelligent packaging, but it is also used for active packaging. In this thesis we will consider intelligent packaging as packaging systems that monitor the condition of packaged food during its life cycle to communicate (i.e., indicate) information related to the quality or safety of the packaged product.

1.1.1 The mechanism behind intelligent packaging
An intelligent packaging concept comprises an interaction system between the product, the package and the environment. The user should also be included in the final intelligent packaging design, but this is outside the scope of this thesis. An intelligent package can

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contain sensors or indicators monitoring quality indicator compounds of the product or monitoring quality indicator environmental conditions (Figure 1.1). The output of the sensor needs to be translated to a clear message, like a quality or safety status or the remaining shelf life period. Next the signal needs to be communicated to different actors in the supply chain; this can also include the consumer.

Figure 1.1 Schematic illustration of Intelligent Packaging with sensors that monitor environmental conditions, or quality attributes of the product related with overall food quality change.

A Time-Temperature Indicator or Integrator (TTI) is an example of an intelligent packaging concept that monitors an environmental condition that influences the quality of food. A TTI gives a cumulative integration of the time-temperature history to which the product has been exposed (Figure 1.2). Many different time-temperature indicators have been developed and adapted to be applicable to different types of foods and other products (Taoukis, 2006).

Figure 1.2 The Fresh-Check® TTI label (Fresh-Check is a registered brand of the TEMPTIME Corporation, Morris Plains, NJ, USA) of which the colour of the inner circle changes (depending on time and temperature) and needs to be compared to the outer circle in order to establish use-by status.

Another example of an intelligent packaging concept is a colourimetric indicator label that monitors the freshness of a dessert (Figure 1.3). The sensor monitors carbon dioxide in the package as indication for the microbial growth that limits the shelf life of the food (Nopwinyuwong et al., 2010). Although this sensor responds to the formation of a spoilage metabolite, this sensor does not monitor directly a quality attribute. CO_2 gives a good indication for the quality of this food and can be used as indicator compound, but it is not a quality attribute since CO_2 does not cause the bad taste or spoilage itself, the quality loss is caused by micro-organisms.

Figure 1.3 A sensor that monitors carbon dioxide as indication for the freshness of the dessert golden drop (Nopwinyuwong et al., 2010).

An example of an intelligent packaging concept that monitors directly a quality attribute of a food are biosensors that monitor pathogenic bacteria on a food causing food safety problems. An example of such a biosensor is the Food Sentinel System™ (SIRA Technologies, California, USA) that consists of a barcode that contains a membrane with antibodies that can attach to specific pathogens (Kerry et al., 2006). When the pathogenic bacteria are growing during storage, the barcode (partly) changes colour, resulting in a barcode that cannot be scanned anymore (Figure 1.4).

Another example is a sensor that monitors directly a quality attribute of a food via a volatile compound that causes rejection of sensorial quality of foods. Commercial examples of these type of sensors are still limited, most attempts are still in research phases.

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Figure 1.4 Example of a biosensor from Food Sentinel System™ (SIRA Technologies, California, USA) that results in unreadable barcode in case of food safety risk.

The quality status can be communicated visually (e.g., a change in colour on an indicator label that needs to be compared to a reference signal (Figure 1.2 and 1.3)) or by an electronic signal that can be combined with RFID tags to construct wireless sensor networks (Figure 1.5). For optimal supply chain management, the sensor should not only be able to monitor food quality, but there should also be a system that all actors in the supply chain can use the data for decision-making. Therefore the monitoring device, which should contain the actual sensor and a microcontroller with memory, should be placed on the package together with an RFID chip or any other wireless communicator (Hoofman, 2010). These wireless sensor networks offer the most advantages for automatically monitoring of each food product or its conditions in the whole supply chain and optimal supply chain management (Aung et al., 2012; Hafliðason et al., 2012).

Figure 1.5 Schematic representation of RFID-tag with sensor for temperature monitoring that is communicated electronically.

More research on electronic sensors is necessary, because current sensors for perishables monitoring are often insufficient in electrical power consumption (too high), size (too large) or performance (not sensitive or not specific) and most commercial gas sensors operate at elevated temperatures (Hoofman, 2010). However, the developments in this area are quickly taking place, which make them potentially very promising.

1.1.2 Intelligent packaging and food quality assurance

Consumers demand mildly preserved, minimally processed, high quality and safe foods, which requires quality control. Due to the nature of perishable food, its quality can be considered as a dynamic state that decreases continuously until the point when it is unfit for sale or consumption (Wang and Li, 2012). For optimal food supply chain management, perishable food must be sold to consumers before the product spoils to ensure food safety and quality while maximising profit. Only in the UK alone, total food waste in the supply chain and households amounts to 11.3 million tonnes, and total packaging 5.1 million tonnes (WRAP, 2010). The current practices in management of perishable products is far from satisfactory and perishable food loss at grocery retailers can be as high as 15% due to damage and spoilage (Ferguson and Ketzenberg, 2005). The loss in a grocery supply chain is mainly caused by inappropriate quality control or excessive inventories that have to be either priced down before the "sell-by-date" or discarded after it (Wang and Li, 2012). The actual shelf life of foods is often longer than the shelf life date printed on the package. When setting expiry dates, producers have to deal with uncertainty of fluctuations in conditions during transport and storage, but also with variability in initial quality of foods. Therefore, expiry dates calculations are not performed with optimal conditions, certain ranges are used to ensure quality. This uncertainty can be reduced when data are obtained from intelligent packaging sensors and used in the supply chain to make dynamic expiry dates (Figure 1.6).
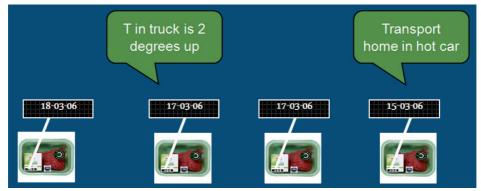

Figure 1.6 Illustration of using a dynamic expiry date as used on the label of a food package, using an electronic matrix display (Bartels et al., 2010).

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Actors in the supply chain use sampling as method for quality assurance. Samples can be taken from a food batch to obtain an estimation of the average food quality of the batch (Luning et al., 2002). Intelligent Packaging contains sensors or indicators that are supposed to monitor the actual quality status of the packaged food or the conditions that influence the quality of the food. Continuously monitoring of all individual food packages makes sampling for doing quality analyses along the supply chain no longer necessary. This improves quality assurance by preventing difficulties in sampling with foods, like with a high variability in the quality of foods.

When the quality is monitored from the moment of harvesting or catch until the moment of consuming by the final consumer, more efficient and practical complex management decisions (e.g. using Quality Controlled Logistics (van der Vorst et al., 2011)) can be taken as a result of which food wastage can be reduced (Hoofman, 2010). If a retailer in the supply chain knows which of the products have the shorter shelf life, he could sell these food products before the ones with the longer shelf life – a concept known as FEFO "First Expire, First Out". With the shelf life data and FEFO strategy, management decisions can be taken by a food distributor to direct shipments to a specific store in the most profitable location (Aung et al., 2012).

For the food industry, intelligent packaging can help to provide a greater assurance of food quality, and it enables quick identification of problems, which helps to reduce the production and distribution of unsafe or poor quality products, which in turn reduces the potential for bad publicity, liability and recalls.

Besides improving logistic actions, wholesalers, retailers and consumers can benefit from a dynamic pricing system where the price can be adjusted, based on the quality of foods. The shelf-life or freshness is taken as quality indicator for perishable foods. The principle is utilising dynamically identified food shelf life information to support the pricing decision when retailers price down food products that are approaching their expiration dates. Customers are usually sensitive to changes in the quality of expiring foods and therefore give them a lower value. In supermarkets, customers will first select the freshest foods instead of expiring ones if the seller charges the same price for them (Ferguson and Ketzenberg, 2005). Dynamic pricing can help to optimise retail chain profits and reduce food wastage (Wang and Li, 2012).

1.1.3 Intelligent packaging for different food types
Food quality deterioration mechanisms are different for each different type of food and it also depends on the storage conditions of the supply chain. Therefore, different intelligent packaging concepts need to be designed for different types of food. Therefore it is necessary to study food quality deterioration in some detail specifically for the food of interest. In food supply chains, deterioration arises mainly on fresh products, because

of their short shelf life and perishability. The critical quality reactions determining shelf life need to be identified and suitable quality indicator compounds need to be selected. Next to that, the influence of other storage conditions, like temperature and modified atmosphere packaging (MAP), on quality reactions and quality indication compounds need to be studied. A compound that is used as quality indicator compound to be monitored by a sensor should indicate the quality accurately under a broad range of storage conditions, like in the case of temperature abuse.

1.2. Modelling to translate sensor data into food quality status

Modelling is crucial in the development of reliable intelligent packaging concepts. The signal of the sensors needs to be translated to a quality status of the packaged food. This is done with the help of mathematical modelling.

Some foods can be monitored by a sensor that monitors the conditions influencing the quality of the foods. The kinetics of the limiting shelf life reactions in the food needs to be related to the kinetics of the changes in the sensors (Taoukis, 2006). For example with time-temperature indicators, the colour or diffusion or enzymatic change of the indicator needs to correlate to the change in the quality of the packaged food, therefore the reaction rate constant and the activation energy should be approximately similar.

For sensors that monitor a quality attribute of the food directly, models need to be developed that translate the signals of the sensors into a quality value that reflects the quality status of the food under a range of conditions. It is necessary to obtain knowledge about the effect of factors that influence the quality of the food. Variables like temperature or relative humidity should be taken into account in the models for the formation/degradation of the quality indicator compound. It is also necessary to evaluate the system and develop different models when other packaging conditions are being used, like modified atmosphere packaging (MAP). All factors that influence the quality of the food or influence the food-package matrix should be taken into account.

1.3. Development of a fish quality sensor

Fresh fish packaging and storage is used as a case study within this thesis. Fresh fish was chosen because this is a highly perishable food, with a relatively high price/kg (therefore high income losses when food is wasted) and a high variation in initial quality that is difficult to control. For the development of a quality sensor for fresh fish, quality and deterioration was studied (including the influence of different storage conditions and packaging), and subsequently conditions or compounds that indicate quality were selected.

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The sensors or indicators needed for intelligent packaging require non-destructive measurements that can monitor the quality status of the food continuously without affecting the food or the package. Many quality analysis methods are destructive, or if they are non-destructive, they are too expensive to apply on individual food packages, or these methods are too complex to be performed outside a factory or laboratory. Therefore a new non-destructive method needed to be developed and studied on its possibilities and limitations to be used as sensor for an intelligent packaging.

1.4. Objectives and Outline of this thesis

The main objective of this thesis is the development of an intelligent packaging concept that can monitor and predict food quality and/or safety within the supply chain. Intelligent packaging systems, based on internal or external sensors that are combined with mathematical models, will be evaluated on their abilities to predict the quality of the packed perishable foods under different conditions that can occur in the supply chain. The development of new non-destructive methods for monitoring changes in the freshness status of packed fresh fish was chosen as a case study for the proof of concept. The approach that is described in this thesis for the development of an intelligent packaging is schematically shown in Figure 1.7. Quality indicator compounds for indicating freshness of foods were selected from literature study and a non-destructive method was developed. The sensors will be tested under different conditions and the results need to be compared with destructive analyses of the packed food, therefore the output of the sensors can be evaluated on their ability to be suitable as quality measurement in the method. This output needs to be translated with mathematical models to a meaningful food quality indicator value that can be communicated to designated actors in the chain that can make decisions based on that value. The focus of this study was on the proof of principle of the application of intelligent packaging for monitoring food quality.

Figure 1.7 Overview of the approach of the development of an intelligent packaging concept for non-destructive monitoring in the headspace of packed foods with fresh fish as case example.

In **chapter 2** our viewpoint on the use of intelligent packaging for perishable foods is given and discussed via a review article. In **chapter 3** we investigated a new method for monitoring fish freshness in a non-destructive way via a specific ammonium electrode, which could be used for development of a direct quality sensor. In **chapter 4** the same method was used as introduced in chapter 3, but the specific ammonium electrodes were replaced by a conductivity electrode and a pH-electrode. In **chapter 5** the formation of the quality indicator compound trimethylamine (TMA) in fish is described by mathematical models, including the effect of temperature on TMA formation. In **chapter 6** the relation between sensor data and food quality and safety status is described by and cast in a

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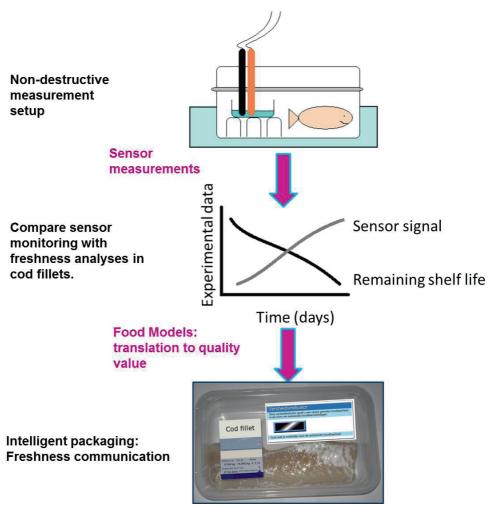mathematical model. **Chapter 7** is the general discussion of this thesis. It contains a critical view on the research on the development of the fish sensor described in this thesis, and concludes with an outlook for future research in intelligent packaging.

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Monitoring the quality of perishable foods: opportunities for intelligent packaging

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Abstract

This review paper discusses opportunities for intelligent packaging for monitoring directly or indirectly quality attributes of perishable packaged foods. The possible roles of intelligent packaging as a tool in supply chain management are discussed as well as the barriers to implement this kind of technology in commercial applications. Cases on pasteurized milk and fresh cod fillets illustrate the application of different intelligent packaging concepts to monitor and estimate quality attributes. Conditions influencing quality (e.g. temperature-time) can be monitored to predict the quality of perishable products when the initial quality is known and rather constant (e.g. pasteurized milk). Products with a highly variable initial quality (e.g. fresh fish) require sensors monitoring compounds correlated with quality.

Keywords: Intelligent packaging, food quality, perishable foods, sensors, indicators, supply chain management

2.1. Introduction

The basic functions of a food package are containment, protection, convenience and communication (Robertson, 1993). The communication function of packages can be greatly extended in the future: Packages can inform consumers about allergy, nutritional preferences or discounts; the package of a ready-to-eat meal can control the microwave; and the freshness of a perishable product can be read-out from the package. This article reviews the state of the art of intelligent packages that give information about the quality of the packaged food within the supply chain. The possible application of intelligent packaging for perishable foods is further illustrated for two cases: pasteurized milk and fresh cod fillets. Suggestions are given to select the type of intelligent packaging for different types of foods.

2.2. Denomination

Intelligent packaging (IP) can be defined in many ways. Yam et al. (2005) defined it as "a packaging system that is capable of carrying out intelligent functions (like detecting, sensing, recording, tracing, communicating, and applying scientific logic) to facilitate decision making, to extend shelf life, enhance safety, improve quality, provide information, and warn about possible problems". In this article IP is considered as packaging that monitors conditions of food during its life cycle to communicate information related to the quality of the product.

IP contains sensors or indicators to estimate and communicate quality of a food product to users. The terms sensors and indicators are often used interchangeably, but we make a distinction: A sensor only measures certain aspects, whilst an indicator integrates measurement and display. Sensors have to be connected to a separate device to transduce the sensor signal to an observable response, while an indicator directly provides qualitative or semi-quantitative information about quality by a visible change. For example, a pH electrode is considered a sensor and pH indicator paper is considered an indicator.

Product quality can be described as "meeting or exceeding customers' expectations" (Luning et al., 2002). Product quality can be described by quality attributes. Product attributes are turned into quality attributes by the perception of a consumer. Intrinsic attributes are directly related to the physical product properties and involve (for consumers) safety, nutritional value, shelf life and sensory properties, convenience and product reliability (Luning et al., 2002).

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2.3. Intelligent packaging types

Several authors have described developments in IP for foods and their principles of operation (Han et al., 2005; Yam et al., 2005; Kerry et al., 2006) and about the legislation (Dainelli et al., 2008). In short there are three types of indicators and sensors used for IP:

Environmental conditions: This type monitors conditions that influence the kinetics of changes in quality attributes of the food, e.g. time-temperature indicators, gas leakage indicators, and relative humidity sensors. These devices might be attached inside or outside the package, depending on the condition monitored. Information on the conditions can be translated with mathematical models to predict quality of foods in the supply chain. However, these predictions can only be reliable when the initial quality (defined as: quality status of the food on the moment right after packaging at the manufacturer) is known and rather constant.

Quality attributes or quality indicator compounds: The second type of devices monitors quality indicator compounds of the product itself that change due to changes in the product. Direct quality sensors, e.g. biosensors and freshness sensors and indicators, measure quality-related compounds formed in the product (or micro-organisms in/on the product) and are good direct indicators of food quality attributes. These devices are usually placed inside the package but some could also monitor certain properties indirectly from the outside e.g. by an optical system.

Data carriers: The third type of devices are data carriers and other information devices that are used to increase the efficiency of information flow and effective communication between the product and the actor in the chain. Devices include barcode labels and radio frequency identification (RFID) tags and other product traceability, anti-theft, anti-counterfeiting and tamperproof devices belong to this group (Yam et al., 2005).

Figure 2.1 illustrates IP concepts containing sensors or indicators from the first and second type, which monitor environmental conditions, or quality attributes of the product related with overall food quality change.
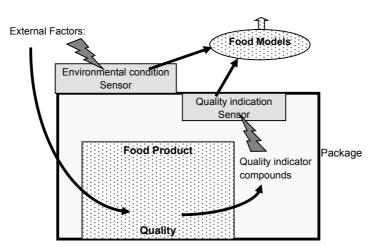
Figure 2.1 Schematic illustration of IP with sensors that monitor environmental conditions, or quality attributes of the product related with overall food quality change.
Note 1: Environmental condition sensor can also be placed inside the package, depending on the measured condition.

2.4. IP monitoring quality attributes

Many intrinsic product attributes of foods can be checked during processing and remain constant in most products during the whole supply chain until consumption (e.g. mineral content). Some intrinsic attributes of highly perishable foods always change after processing. This can result in an increase in the quality performance (e.g. ripening of fruits improves sensory properties up to a certain level), or a decrease in the quality performance (e.g. microbial spoilage). These product attributes are often difficult to estimate by consumers. Besides, the reaction rate of the changes is influenced by several factors (Van Boekel, 2009). Other quality changes only occur with a low frequency (e.g. food safety issues), they are the result of occasional defects in product composition, packaging or environmental conditions. Consumers implicitly expect certain quality attributes, like product safety or packaging integrity, to be obvious (Luning et al., 2002). These product attributes usually cannot be perceived by consumers. When changes happen after processing the quality attributes will normally not be monitored in the supply chain. Consumers, wholesalers or retailers have difficulties in estimating quality, since they cannot estimate certain quality attributes while the food is in the package. IP can be used to give various actors in the chain a tool to estimate quality attributes that are difficult to estimate and thereby IP can assist in assuring good product quality to consumers (Figure 2.2).
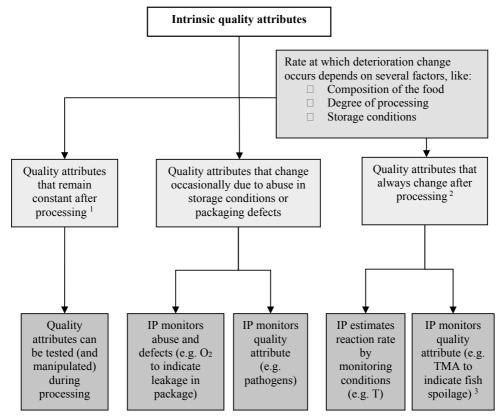
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                    ┌──────────────────────────────┐
                    │  Intrinsic quality attributes │
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Intrinsic quality attributes

Rate at which deterioration change
occurs depends on several factors, like:
   ☐   Composition of the food
   ☐   Degree of processing
   ☐   Storage conditions

Quality attributes
that remain
constant after
processing [1]

Quality attributes that change
occasionally due to abuse in
storage conditions or
packaging defects

Quality attributes that
always change after
processing [2]

Quality
attributes can
be tested (and
manipulated)
during
processing

IP monitors
abuse and
defects (e.g. O_2
to indicate
leakage in
package)

IP monitors
quality
attribute
(e.g.
pathogens)

IP estimates
reaction rate
by
monitoring
conditions
(e.g. T)

IP monitors
quality
attribute (e.g.
TMA to
indicate fish
spoilage) [3]

Figure 2.2 Overview of use of Intelligent Packaging to communicate about quality attributes.

Note 1: Whether a quality attribute changes after processing or not, depends on the food type and the shelf life. For example, the quality attribute 'nutritional value' of fresh fish will not change much during its life cycle, since the sensory changes happen much faster and are rate limiting for the shelf life of the product. Therefore nutritional value is considered stable in fresh fish, although lipid oxidation can become important (rate-limiting) in fish with longer shelf life.

Note 2: Some quality attributes can be estimated well by consumer, e.g. colour of banana indicates ripeness, in this case there is no need to monitor this quality attribute by an intelligent package.

Note 3: To monitor a quality attribute, product properties that can be measured in a non-destructive way in the package must give a good indication of the quality attribute.

2.5. Role of intelligent packaging in supply chain management

The quality of food products during their life cycle changes since foods are perishable by nature. Product properties and the related intrinsic quality attributes of food products are susceptible to various deterioration processes and quality defects may be due to different mechanisms and depend on the type of food product, the package and the conditions in the supply chain. Moreover, agricultural products are often heterogeneous

with respect to various quality parameters, since variation is caused by many variables like seasonal and local differences. On the other hand, consumers are demanding for more convenient, less processed and fresh, constant high quality food products. This complexity necessitates quality control of food products during and after processing. IP can be used as such a tool in supply chain management to monitor the established quality requirements and inform and provide confidence to the next customers in the supply chain and consumers at the end. If IP can be combined with AP in a smart packaging, new possibilities arise to extend the shelf life and improve the quality of foods. Figure 2.3 shows how the package can be used for adaptive quality control: the quality can be monitored by the IP and corrective actions can be taken automatically by the active packaging component (e.g. IP monitored gas composition and AP absorbs/emits gas to maintain optimal gas composition in respiring fruits).

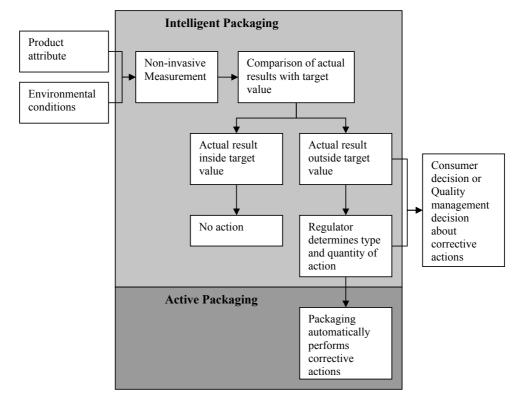**Figure 2.3** Role of intelligent packaging and active packaging in quality control of food products after processing.

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For perishable foods the quality changes fast along the supply chain. QACCP (Quality Analysis Critical Control Points) is a novel tool to support supply chain management to take technological actions in a food chain to realize a desired quality performance (Verkerk et al., 2007). IP can complement quality assurance systems to support managerial decisions along the supply chain. QACCP can be used to identify processes that strongly affect the quality attributes and to efficiently improve the final food quality. Mathematical models can describe and predict changes in quality along the chain by modelling the relationship between environmental conditions and quality attributes (Van Boekel, 2009). These models require input data to make predictions. This is where IP can help quality assurance: IP monitors environmental conditions, or quality attributes of the product related with overall food quality change in the supply chain.

An advantage of IP as a tool in quality assurance is that environmental conditions can be monitored close to the product and the history can be seen directly from the product. Monitoring quality attributes directly has the great advantage of being able to incorporate variation, which is inherent in many foods since raw materials vary in their composition and many environmental conditions change over time. Sensors that measure external conditions, like Time Temperature Indicators (or Integrators) (TTI), have the advantage that they can be applied on many products. TTI are cheap and reliable IP concepts that can be adapted to the temperature sensitivity of the quality changes of specific food products (Shimoni et al., 2001). However, measuring environmental conditions is only related to the change of quality attributes and therefore, if the initial quality of the product is not known and constant this information is of limited value. This is not always described correctly, because temperature indicators are sometimes described as quality indicators, without mentioning the necessary analysis of initial quality.

Mathematical modelling is important in the development of IP concepts to translate the sensor signal in prediction of the quality or the remaining shelf life of the food product. Knowledge of kinetics of reactions that influence the quality of food is necessary to model the relation between sensor data and food quality status. Figure 2.4 displays the approach that is needed to predict the quality of food, dependent on the type of sensor.
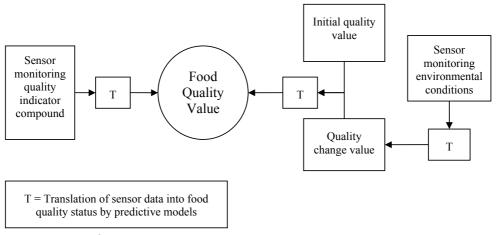
Figure 2.4 Approaches to predict quality, dependent on sensor type.

Despite the potential roles of IP in supply chain management the implementation of the IP in commercial applications is still limited. Although initial concepts are available in Japan from 1970's, commercial uptake in the USA and Europe only started in the mid 1990's. The market for was estimated at $ 1.4 billion in 2008 and is expected to grow to $ 2.3 billion in 2013 (Restuccia et al., 2010). The commercial uptake of IP in the EU was described to be limited due to cost aspects and industry and consumer acceptance of this technology. Especially consumers do not perceive strong benefits of IP and are not prepared to pay extra for this feature (Dainelli et al., 2008). In Table 2.1 an overview is given of intelligent packaging applications for quality control (adapted from Ahvenainen, 2003).

Table 2.1 Overview of intelligent packaging concepts for quality control (adapted from Ahvenainen, 2003)

Indicator	Principle	Information	Application
TTI (external)	Mechanical Chemical Enzymatic	Storage conditions	Chilled and frozen foods
Oxygen (internal)	Dyes (redox, pH) Enzymatic	Storage conditions Leakage	MAP packed foods
CO_2 (internal)	Chemical	Storage conditions Leakage	MAP packed Foods
Microbial growth (internal/external)	Dyes (pH or reacting with volatile or non-volatile metabolites	Spoilage	Meat, fish, poultry, etc.
Pathogens	(Immuno) Chemical methods	Pathogenic safety	Meat, fish, poultry, etc.

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2.6. Application of intelligent packaging to highly perishable food products

IP might be used as a tool in supply chain management. Since foods are complex products, it should be considered per product type whether IP can be worthwhile or not. IP will increase packaging costs (Dainelli et al., 2008), therefore it will be profitable if the income from increased sales and/or reduced wastage will be higher than the increased costs of the package. This makes the price and the shelf life of the food important criteria for applying IP. In Figure 2.2 we already concluded that products with relatively stable intrinsic quality attributes and long shelf life will hardly take advantage of IP. Examples of such products are soft drinks, canned products and sweets.

Foods that will benefit the most from IP are expensive, highly perishable foods, especially if consumers cannot estimate their essential quality attributes. Dada and Thiesse (2008) studied the impact of novel sensor-based policies on product quality in the supply chain of perishables. They found that the classical 'First-In-First-Out' (FIFO) policy is not the best policy for perishables. In most cases the Lowest Quality First Out (LQFO) showed to be the best policy with the lowest percentage of unsold items. Policies that rely on automatically collected expiry dates and product quality bear the potential to improve the quality of items in stores (Dada and Thiesse, 2008).

In the next section we will discuss the application of IP to monitor quality of highly perishable foods. To illustrate this for two very different products in terms of price and control of initial quality pasteurized milk and chilled fresh fish will be taken as case studies. First the product specific quality development and factors influencing quality development are described. Subsequently considerations whether to measure quality attributes or external conditions are given and possible sensors for monitoring quality development are discussed.

We will not discuss IP that monitors food safety. Safety is an essential quality attribute, but consumers demand safe food and will not negotiate with food producers about this quality attribute (Botta, 1995). We will focus on IP monitoring freshness of foods. Freshness is an essential quality attribute of perishable foods and this quality attribute of perishable foods always changes after processing as is discussed in the cases.

2.7. Case 1: Pasteurized milk

2.7.1 Changes in intrinsic quality attributes
The quality of pasteurized milk can be controlled quite well by processing conditions. Pasteurization is used to increase shelf life by destroying enzymes and micro-organisms, but heat resistant spores and recontamination make pasteurized milk still highly perishable. The cold storage of pasteurized milk may result in defects and off-flavours

caused by psychrotrophic micro-organisms after some time. Without contamination, pasteurized milk is generally spoiled by enzymes from *Bacillus* species (Ternström et al., 1993). If the milk is recontaminated after pasteurization, deterioration is generally faster and caused by the formation of bitter peptides and rancidity from enzymes produced by *Pseudomonas* spp. (Ternström et al., 1993). Uncooled recontaminated milk often becomes sour from the production of lactic acid by lactic acid bacteria.

The keeping quality of pasteurized milk is determined by raw milk quality, pasteurization conditions, extent of recontamination, storage temperature, and effect of light. The shelf life of pasteurized milk varies greatly between different countries (Antonelli et al., 2002), but if machinery is working properly and microbial growth and enzymatic action in raw milk is controlled, it can be assumed that the quality of pasteurized milk from the same factory is uniform, due to mixing large amounts of raw milk, standardization and pasteurization. By sampling batches of pasteurized milk this initial quality can be quantified.

2.7.2 Approaches for intelligent packaging for pasteurized milk

2.7.2.1 Temperature sensors and indicators

Increasing the temperature increases the growth-rate of micro-organisms and reduces shelf life of milk, as is the case for most perishable products. In general, every 2 °C increase of storage temperature, reduces the shelf life of pasteurized milk by 50% (Rysstad and Kolstad, 2006). The actual cold chain varies considerably between countries and regions, but also within different steps in the supply chain (Rysstad and Kolstad, 2006). Usually estimates of a weighted average temperature or a worst case temperature exposure are taken for shelf life estimations. Deviations from these estimated temperatures can result in food products with unacceptable quality before the end of shelf life (in case of temperature abuse), or discard of foods of good quality (if too conservative temperature estimates were used). The prediction of quality status and remaining shelf life becomes more accurate if the rate of quality deterioration is estimated from a monitored temperature history of the food during the supply chain. This can be done with temperature-sensitive food quality sensors and indicators. TTI are small tags or labels attached onto a package that show a readable, time- and temperature-dependent irreversible change that reflects the full or partial temperature history of a food product or package from the point of manufacture to the end-consumer (Taoukis and Labuza, 1989). Full history TTI give a continuous temperature-dependent integrating response throughout a product's history and can be used to predict the freshness of food, especially when microbial growth is the major deterioration mode of the food (Shimoni et al., 2001). However, a TTI can only predict a quality or freshness status if the initial quality is known, otherwise only prediction of the quality change is possible, as described earlier in Figure 2.4, but this is the case for

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pasteurized milk. TTI provide visual indications by a colour change, diffusion of a dye along a straight reference line or mechanical deformation. The mechanisms of TTI can be based on a polymerization, diffusion-based or enzymatic reaction (Kerry et al., 2006). The change of the TTI must be irreversible and correlate well to the quality deterioration rate of the food (Taoukis and Labuza, 1989), i.e. the temperature dependency of the TTI and the deterioration reaction should be similar. Fu et al. (1991) studied the application of TTI for monitoring spoilage of dairy products, including milk, and concluded that the application of TTI for dairy products is feasible.

2.7.2.2 Freshness sensors and indicators

Ideally, a quality indicator for packaged food indicates direct spoilage or freshness by measuring compounds that are produced or consumed in reactions that determine the quality of the food. These freshness sensors and indicators have the advantage that a constant initial quality is no longer a requirement for accurate estimation of the quality (de Jong et al., 2005).

Winquist et al. (1998) described an electronic tongue on the basis of voltammetry for monitoring the freshness of milk. The electronic tongue contains 5 electrode wires of different metals that have different selectivity for the target components in milk. The output pattern of the 5 different sensors needs to be analysed with pattern-recognition software to give an analysis of all relevant components, for practical application of this sensor the software needs to be integrated with the package. This device was used to follow the deterioration processes in quality caused by microbial growth in milk at room temperature. Voltammetry can be used, because electroactive species are both consumed and generated during these processes and the pH of the solution will also change (Winquist et al., 1998). Sim et al. (2003) also described a disposable taste sensor based on screen-printed disposable lipid membrane strips. With PCA (Principle Component Analysis) plots based on the sensor signals different stages of the ageing process of milk can be distinguished. Also electronic nose technology can be used for measuring the freshness of milk, by monitoring volatile compounds, direct contact with food not being necessary (Labreche et al., 2005).

Milk can become sour from produced lactic acid or by the formation of free fatty acids by lipolytic enzymes. This souring can be indicated by a pH sensitive indicator dye. pH is a better quality indicator for raw milk than for pasteurized milk, it is however not a very sensitive signal due to the buffering capacity of the milk (Antonelli et al., 2002).

2.7.2.3 Biosensor

A biosensor is a device that is used to analyse the concentration of a specific target component with a biological sensing element. A biosensor converts a biologically

induced recognition event (e.g. based on an antibody, enzyme or microorganism) into a detectable signal, via a transducer and a processor (Terry et al., 2005). A disadvantage of biosensors in IP is that they often cannot continuously monitor the quality of the food. Advantages of using biosensors are their specificity and sensitivity, however some reactions will be induced only at the surface of food in contact with the sensors, which reduces the sensitivity in solid foods.

A disposable microbial based potentiometric biosensor for determination of urea levels is described for quality control in milk (Verma and Singh, 2003). An immobilized microbe, that produces urease in the presence of urea which hydrolyzes urea to ammonium, is coupled to an ammonium selective electrode (Verma and Singh, 2003). Recently, several biosensors for determining the concentration of lactose in milk are described in literature, most based on (multiple) enzymes and electrodes (e.g. Stoica et al., 2006; Marrakchi et al., 2008). However, changes in urea and/or lactose do not reflect quality attributes that influence consumer acceptability of a pasteurized milk product. Both types of biosensors are more interesting for monitoring quality during processing and for management purposes than for application in IP.

Schmidt et al. (1996) described a biosensor for milk based on an oxygen electrode that measures oxygen consumption by respiratory activity of the immobilized micro-organism *Arthrobacter nicotianae* that degrades short chain fatty acids ($C_{4:0}$-$C_{12:0}$) that are produced by lipolysis of milk fat and can be used as a quality index of milk (Antonelli et al., 2002).

Many immunosensors containing specific antibodies against pathogens are developed for food safety applications (Ricci et al., 2007). Examples, applied on milk, are immunosensors to detect *Listeria monocytogenes* (Crowley et al., 1999), *Salmonella typhimurium* (Lakshmanan et al., 2007) and *Staphylococcus aureus* ssp. (Huang et al., 2008). Since this review focuses on food quality rather than safety these biosensors are not discussed further.

2.8. Case 2: Fresh cod fillets

2.8.1 Changes in intrinsic quality attributes

Freshness is an essential quality attribute of fresh fish (Ólafsdóttir et al., 1997). The major changes in the eating quality of iced cod (0 °C) are initially due to the degradation of adenosine triphosphate (ATP) through the following pathway (Venugopal, 2002):

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow inosine \rightarrow hypoxanthine \rightarrow xanthine \rightarrow uric\ acid$$

The formation of inosine is a fast process caused by endogenous enzymes present in the fish muscle (Huss, 1995). The oxidation of hypoxanthine to xanthine and to uric acid proceeds much slower and is catalysed by bacterial enzymes (Venugopal, 2002). A strong

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correlation exists between the nucleotide loss and freshness, since IMP is responsible for the characteristic fresh fish flavour (Huss, 1995).

After a period of neutral flavour, off-flavours and softer flesh from bacterial activity determine the eating quality of fish (Herbert et al., 1971; Huss, 1995). After death, the fish's regulatory mechanism to prevent bacteria invade into the tissue stops and bacteria invade the fish body (Fraser and Sumar, 1998). The initial number of microorganisms 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 and Huss, 1996). Not all micro-organisms initially present on the fish contribute to the spoilage. The specific spoilage organisms (SSO) are the micro-organisms that produce the spoilage metabolites that cause the sensorial rejection of the fish (Dalgaard, 1995). After the fish is gutted and fillets are prepared from it and the fillets are packed, the most important factors influencing the quality of the fresh cod are the storage temperature and the gas composition inside the package (use of MAP) (Table 2.2). *Shewanella putrefaciens* is the SSO of aerobically stored cod at 0 °C and *Photobacterium phosphoreum* is the SSO of MAP cod stored at 0 °C (Dalgaard, 1995; Gram and Huss, 1996; Gram and Dalgaard, 2002). Above 5°C *Aeromonas spp*. becomes important for spoilage for fish packed under normal or modified atmosphere. The most important spoilage compounds produced by the SSO are volatile basic nitrogen compounds (TVB-N), sulphur containing compounds, hypoxanthine, aldehydes and ketones (Fraser and Sumar, 1998). Absence of these components indicates freshness. Volatile sulphur compounds are responsible for the putrid off-odours in aerobically chill-stored spoiled cod (Herbert, Hendrie et al. 1971; Gram and Huss 1996), but H$_2$S is not produced in significant amounts by *Photobacterium Phosphoreum*, the SSO of MAP stored fish (Dalgaard 1995). Under normal chilled storage conditions, the end of shelf life of fresh packed cod is usually reached when spoilage related sensory attributes such as trimethylamine and rotten odour from microbial origin lead to rejection of the fish (Huss, 1995, Gram and Huss, 1996).

Table 2.2 Specific spoilage bacteria of fresh and packed fish stored at 4°C or in ice (Gram and Huss, 1996)

Atmosphere	Specific spoilage organisms of fresh, chilled fish			
	Temperate waters		Tropical waters	
	Marine	Fresh	Marine	Fresh
aerobic	*S. putrefaciens* *Pseudomonas spp.*	*Pseudomonas spp.*	*S. putrefaciens* *Pseudomonas spp.*	*Pseudomonas spp.*
vacuum	*S. putrefaciens* *P. phosphoreum*	Gram-positive bacteria Lactic acid bacteria	Lactic acid bacteria	Lactic acid bacteria
CO_2	*P. phosphoreum*	Lactic acid bacteria	Lactic acid bacteria TMAO reducing bacteria	Lactic acid bacteria TMAO reducing bacteria

In the post-mortem muscle, the activity of micro-organisms results in the accumulation of volatile basic nitrogen (TVBN) compounds due to decarboxylation of amino acids and other nitrogenous compounds (FAO, 1995). Ammonia (NH_3), dimethylamine (DMA) and especially trimethylamine (TMA) contributes most to the TVB-N level. In the first stage of storage small amounts of ammonia are produced in the autolytic breakdown of AMP in chilled fish. Most ammonia is formed in the advanced stage of spoilage by the bacterial degradation/deamination of proteins, and non-protein compounds such as amino- acids and urea, especially by anaerobic spoilers. TMA is formed by *Aeromonas* spp., Enterobacteriaceae, *Shewanella Putrefaciens*, *Photobacterium phosphoreum* and *Vibrio* spp. are from the bacterial reduction of the odourless compound trimethylamine oxide (TMAO) found in the fish muscle of many marine fish species, including cod (Gram and Huss, 1996; Gram and Dalgaard, 2002). If the TMAO supply is depleted in spoiled fish and TMA cannot increase further, TVB-N levels can still rise due to formation of NH_3 and other volatile amines (Huss 1995).

When microbial growth is suppressed due to storage at T<0 °C, texture changes from proteolytic digestion and alterations in smell and taste from enzymatic lipid hydrolysis and oxidation can become the dominant factor for determining the shelf life of fish (Ólafsdóttir, Martinsdóttir et al. 1997; Sivertsvik 2002).

2.8.2 Approaches for intelligent packaging for fresh cod fillets
2.8.2.1 Temperature sensors and indicators
Proper chilling is important to delay autolytic and microbial activity that deteriorate fish freshness and quality. An increase in the temperature from 0 to 3 °C doubles the spoilage of chilled fish, and an increase to 10 °C accelerates the spoilage by a factor of 5-6 (Venugopal, 2002). Taoukis et al. (1999) and Giannakourou et al. (2005) studied application of TTI for fresh tropical fish. Modelling of the temperature dependence of the shelf life

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was done by describing the effect of temperature on growth of the SSO *Pseudomonas* spp. and *S. putrefaciens* and by correlating the bacterial count to the organoleptic quality (Taoukis et al., 1999). A kind of TTI based on the enzymatic conversion of hypoxanthine that closely matches the kinetics for loss of freshness has been developed (Watanabe et al., 2005).

The initial quality of fish, however, is highly variable (Venugopal, 2002). The quality of packed cod leaving the factory will therefore be variable and uncertain. This makes it difficult to predict the spoilage of individual fish products accurately with an indirect quality sensor as the TTI.

A temperature sensor might be used to predict the remaining shelf life if the initial quality of individual packages with cod is known and can be incorporated in the prediction. Seafood Spoilage and Safety Predictor (SSSP) (www.dfu.min.dk/micro.sssp/) is software that contains relative rate of spoilage (RRS) models based on the growth of SSO to predict shelf life at different storage temperatures. The software enables combining temperature profiles from electronic data loggers and analysis of the initial quality attributes from sensory or instrumental methods to predict remaining shelf life (Dalgaard et al., 2002). Variables like CO_2 concentration, initial SSO concentration and T are used by models to describe the effect of storage conditions on the growth of SSO.

A TTI/RFID tag that uses a micro-chip to sense and integrate temperature over time (Yam et al., 2005) gives the potential to access the data remotely and makes optimization of supply chain management and connecting temperature data to initial quality determination more easily. Electronic temperature loggers allow for more flexible translation of the temperature profile into quality by software instead of (bio)chemical reactions of TTI. TTI for fish would only be applicable if the initial quality of the fresh fish can be better controlled.

2.8.2.2 Freshness indicators and sensors

Sensors or indicators for estimating the freshness of fish that are based on volatile compounds are promising. In the post-mortem muscle, the TVB-N level increases in time and off-flavours are responsible for decreasing sensorial quality of fish. TMA is a good indicator for the spoilage of fish, since this compound is related to the microbial spoilage and related to the sensory quality of fish since the compound is responsible for the characteristic 'fishy' off-flavour. The correlation between TMA and eating quality has been reported to be excellent (Huss, 1995). The TMA yield of *P. Phosphoreum* is 30x higher compared to *S. Putrefaciens*, so a smaller bacterial count can obtain higher TMA values and cause spoilage in MAP-packed fish at lower bacterial counts compared to aerobically stored fish (Dalgaard, 1995). When stored aerobically 10^8-10^9 cfu/g of *S. Putrefaciens* are required to cause spoilage of iced fish, in CO_2-packed fish 10^7 *P. Phosphoreum* are required (Dalgaard, 1995; Debevere and Boskou, 1996).

Pacquit et al. (2007) developed an in-package pH colour indicator that monitors spoilage of fish. The indicator colour changes by a pH increase caused by the release of volatile amines, which correlated at room temperature with the changes in total viable count and *Pseudomonas* spp. in cod (Pacquit et al., 2007). However, the indicator changes colour when the fish is spoiled, therefore it cannot be used to predict the remaining shelf life.

The sensory quality of fish largely determines rejection of fish by consumers. Sensory properties can be determined with panels and sensory analysis. Quality Index Method (QIM) is a scoring system for freshness and quality estimation of fishery products (Bonilla et al., 2007). Electronic noses aim to imitate the sensory analyses by simulating the sense smell. (Olafsdottir, 2005) studied the use of electronic nose as a rapid technique to detect volatile compounds like CO, H_2S, NO, SO_2 and NH_3 related to quality changes during chilled storage of different fish species, including cod. The complex data-analysis needs to be integrated with the sensor on the package to enable practical applications.

2.8.2.3 *Biosensors*

The development of biosensors for fish quality was reviewed by (Venugopal, 2002). However, these biosensors use extracts of a fish sample, therefore the required non-destructive analysis inside a package is not possible.

2.8.2.4 *Gas indicator*

Modified atmosphere packaging (MAP) is often used to extend the shelf life of fish (Sivertsvik et al., 2002). Gas indicators in the form of a label or printed on packaging films can monitor changes in the gas composition to indicate integrity or to assure O_2 scavengers function properly (Mills, 2005). Most indicators are based on colour change as a result of a chemical or enzymatic reaction (de Jong et al., 2005), like CO_2-sensitive indicator strips. The colour changes only when the CO_2 is below a thresh-hold. A problem using a CO_2 indicator for a leakage in MAP is that microbial growth can compensate for the CO_2 leakage (Smolander et al., 1997). In future, gas indicators can be integrated with RFID tags to allow distant monitoring (Yam et al., 2005).

2.9. Conclusion and future research

The dynamic information about the quality status of foods supplied by IP can contribute substantially to the optimization of supply chain management. Expensive, highly perishable foods are the most important target group for IP, because the intrinsic quality attributes of highly perishable foods change fast after processing and cause important economic losses. IP will only be profitable if the income from increased sales and/ or reduced wastage exceeds the increased costs of the package. Quality deterioration

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is specific for each product type, therefore IP concepts have to be tailored to different perishable foods. IP concepts that monitor environmental conditions like temperature are most suitable for foods that have a known and constant initial quality. Foods with variable initial quality require IP monitoring quality attributes or compounds correlated with quality attributes. The decision tree for choosing IP is illustrated in Figure 2.5.
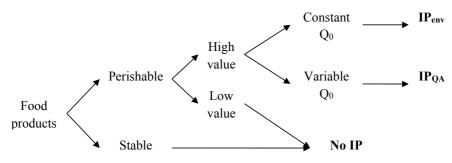
Constant Q_0 ——→ **IP_{env}**

High value

Perishable

Variable Q_0 ——→ **IP_{QA}**

Low value

Food products

Stable ——————————→ **No IP**

Figure 2.5 Decision-tree for choice of IP. Q_0 = initial quality, IP_{env} = IP monitoring environmental conditions, IP_{QA} = IP monitoring quality attributes

For the application of IP in the cases discussed (pasteurized milk and fresh cod), the overall quality and freshness is influenced most by temperature. TTI can give reliable predictions of freshness of pasteurized milk because the quality of the product at the moment of packaging is relatively constant. The kinetics of the TTI should ideally be similar to the kinetics of the main deterioration reactions influencing the eating quality of the milk, which is microbiological activity. An advantage of the use of TTI is that they are relatively cheap. The initial quality of fresh cod fillets, on the other hand, is highly variable at the moment of packaging. Therefore this fish product requires sensors monitoring compounds correlated with quality. Sensors monitoring volatile compounds, like TMA, can be used to predict the spoilage of individual fish products.

Indirect quality indicators, like TTI, can give more reliable estimation of the quality of a food if several IP concepts are combined. A multi-sensor can give more information about complex spoilage changes than sensors or indicators based on monitoring one single quality indicator compound. If IP is combined with AP, further improvements for maintaining high quality of perishable foods could be realized. IP devices could monitor the quality or the conditions of the food and take decisions for corrective actions of the AP device.

Further research is necessary to develop low cost indicators and micro-sensors. Food specific mathematical models need to be developed for translating the measured information with the quality perception of the consumer.

When these issues have been tackled intelligent packaging offers an enormous potential for commercial applications to improve supply chain management and guarantees for product quality for consumers.

Acknowledgement

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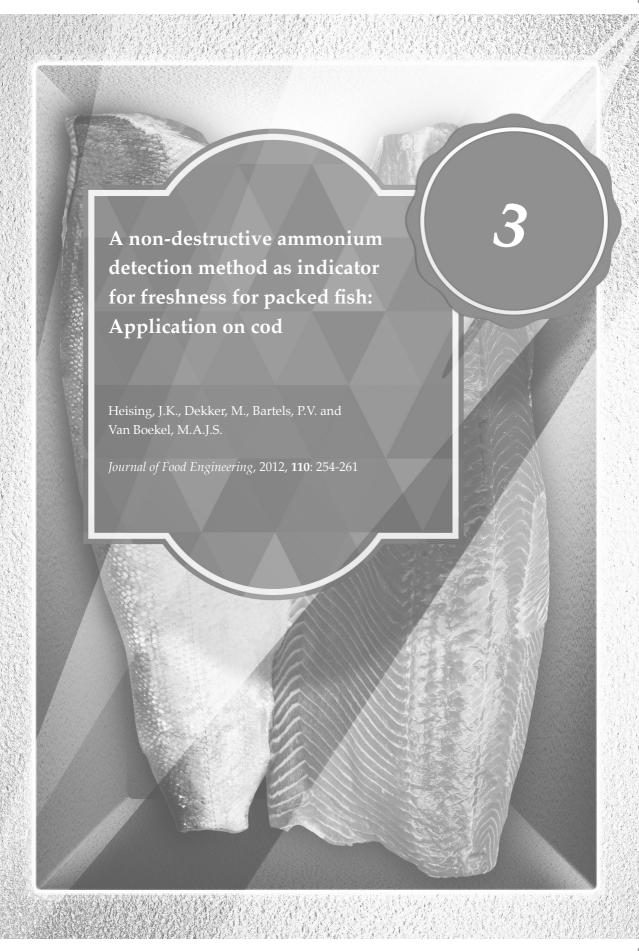R7
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A non-destructive ammonium detection method as indicator for freshness for packed fish: Application on cod

3

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Abstract

This paper introduces a non-destructive method for monitoring headspace ammonium as an indicator for changes in the freshness status of packed fish. Electrodes in an aqueous phase in the package monitor changes in the concentration of ammonia produced in/on the packed fish and released in the headspace. The outputs of an ammonium ion-selective electrode (NH_4^+-ISE) were compared with the volatile amines content of the fish fillets. The method was tested in triplicate with fresh cod fillets stored between -0.5 and 1.9 °C. Changes in the ammonia content of the fish could be monitored in the aqueous phase with the NH_4^+-ISE. The changes in the NH_4^+-ISE signal correlated with the content of volatile amines (TVB-N) in the cod fillets. This non-destructive method might be the basis for the development of an intelligent packaging for monitoring freshness of packed fish.

Keywords: Non-destructive method, NH_4^+-ISE, ammonia, sensor, packed fish, intelligent packaging

3.1. Introduction

All actors in the fishery chain wish to deliver high quality fresh fish to the final consumer. Freshness is the most important quality attribute of fresh fish and is the key element to be implemented in quality control and labelling for all partners in the fishery chain (including consumers) (Jørgensen et al., 2003; Luten, 2003). Quality control requires rapid methods for measuring fish freshness (Jørgensen et al., 2003). An intelligent packaging monitoring fish freshness would be an ideal method to estimate and communicate the freshness of packed fresh fish to all partners in the fish supply chain. Intelligent packages contain sensors or indicators that non-destructively monitor quality attributes of foods.

Several non-destructive methods for fish freshness determination have been developed previously. Electric instruments such as the Fischtester VI measure electric properties (resistance, conductivity and capacitance) of the fish flesh by placing electrodes on the body (Oehlenschläger, 2005). These methods can be useful to monitor fish freshness by sampling batches on several points in the supply chain, except at the consumers level. However, the freshness of packed fish cannot be monitored with these instruments.

Also electronic noses, rapidly detecting volatile compounds such as CO, H_2S, NO, SO_2 and NH_3 related to freshness changes, can be useful for quality control during chilled storage of different fish species, including cod (Ólafsdóttir, 2005). The different sensors of the electronic nose are sensitive to different volatile compounds, and therefore spoilage compounds formed during the later stage of storage as well as typical freshness odours can be monitored, which allows better freshness prediction than monitoring a single compound (Di Natale at al., 2001). However, the complex data-analysis for the sensor output needs to be integrated with the sensor on the package to enable application in the whole supply chain. Electronic nose sensors are too complicated and expensive to allow in-package applications.

Watanabe et al. (2005) described a non-destructive sensor for fish freshness control, based on K_1-value (a simplified K-value). The K-value is a freshness index for fish and is based on the often destructive measurement of the degradation of adenosine triphosphate (ATP). The K-value is the ratio of the total amounts of the ATP-degradation compounds inosine and hypoxanthine relative to the total amount of all ATP-related compounds. The enzymatic conversion of hypoxanthine causes a colour change in the sensor tube that is stored parallel with the fish. However, the enzymatic reaction in the tube is only influenced by temperature and not by the (initial) freshness of the fish. Therefore, this sensor is actually a time-temperature indicator of which the kinetics are validated by the quality attribute K_1-value to calculate remaining shelf life. The sensor cannot indicate freshness of the packed fish without knowledge of the initial freshness status of the fish (Heising et al., 2014).

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The total volatile basic nitrogen (TVB-N) content is often used as an index of fish freshness and the volatile amines are correlated to the sensorial quality of fish (Huss, 1995). Trimethylamine (TMA) is a volatile amine produced in large amounts in fish and is responsible for the characteristic 'fishy' off-flavour. Steam distillation methods are mostly used for measuring TVB-N in fish, but several simple biosensors have been developed for destructive quantification of TMA (Gamati et al., 1991; Mitsubayashi et al., 2004; Wong & Gill, 1987); like most methods for analysing volatile amines, a measurement is performed in an extract of the fish. Besides, these methods analyse volatile amines at one sampling moment, instead of monitoring one fish sample non-destructively continuously from fresh to spoiled.

Pacquit et al. (2007) described a non-destructive method to monitor the freshness of packed fish. The colour of an in-package pH colour indicator changed at room temperature through the release of volatile amines in the headspace. However, the indicator only changed colour when the fish was already spoiled and therefore it cannot be used to predict the remaining shelf life. The sensor was described as a volatile amine sensor (Pacquit et al., 2006), although no correlations with TVB-N measurements from the fish were shown. The sensor signal was only correlated to the total viable count (TVC) and the count of *Pseudomonas* spp., it should be noted however, that TVC has not a good correlation with remaining fresh fish shelf life (Gram & Dalgaard, 2002) and *Pseudomonas* spp. are not able to reduce trimethylamine oxide (TMAO) to TMA (Gram & Huss, 1996), therefore *Pseudomonas* spp. will not be responsible for the major formation of TVB-N compounds.

Although several non-destructive methods exist to determine the freshness of unpacked fresh fish, appropriate non-destructive methods for monitoring the loss of freshness of packed fish are not available. Non-destructive methods are needed for the development of an intelligent packaging for communicating fish freshness throughout the supply chain from the moment of packaging until the day the fish is spoiled.

The purpose of this study was to develop a new non-destructive method to monitor changes in freshness of packed fish. In this new method an aqueous phase is placed inside the fish package. The principle of this method is that in this aqueous phase, changes in the ammonium content can be monitored by an Ammonium Ion-Selective Electrode (NH_4^+-ISE). These changes are related to the often used freshness indicator of the fish, the volatile amine content, and to the ammonia content of the fish. One approach for monitoring freshness of cod fillets with this method is discussed in this article: measuring changes in the potential induced in an NH_4^+-ISE. The NH_4^+-ISE has the advantage of high selectivity to a volatile amine produced by the fish.

3.2. Materials and Methods

3.2.1 Storage trials of cod fillets

Cod (*Gadus morhua*) was bought at "Zeevisgroothandel J. Thiele & ZN" in IJmuiden (NL). The cod was caught in the North Sea off the Netherlands, gutted on board the fishing vessels, stored on ice and brought to IJmuiden. The time between catch and delivery at the auction was maximally 1 day. 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 trials started. Each batch of cod was used for both the non-destructive and destructive analysis during each trial. Trial A was in May 2009; trial B was in May 2008; trial C was in October 2008.

3.2.2. Non-destructive method

The non-destructive measurement setup consisted of a glass-cell with openings in the lid for the electrodes that analysed an aqueous phase in a beaker separate from the fillets (Figure 3.1).
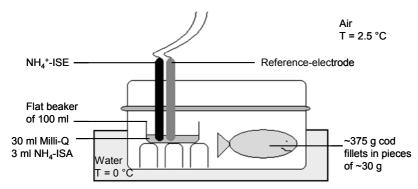

Figure 3.1 Measurement set-up for monitoring changes in aqueous phase with ammonium electrode. The whole set-up was placed in a 2.5 °C room.

~375 g cod fillets, sliced into pieces of approximately 30 g, was put in the glass cells. A glass cell contained pieces from different cod fillets. One glass cell contained the cod fillet pieces, and the NH_4^+-ISE (Orion 93-18) and Reference Electrode (Orion 90-02) in a beaker (diameter of 6.6 cm and height 3 cm) with 30 ml of Milli-Q water (Millipore) and 3 ml of Ionic Strength Adjustment Buffer (NH_4-ISA) for the NH_4^+-ISE (28.7 ml glacial acetic acid (Merck 1.00063) and 53.6 g magnesium acetate (Merck 1.05819) in 1 l Milli-Q water). This NH_4^+-ISE consists of an electrode body and a replaceable sensing module.

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The sensing module contains an internal reference element (Ag/AgCl) and a internal aqueous reference solution in contact with a gelled, organophilic membrane containing an ammonium-selective ionophore (Orion 93-18 instruction manual). The other glass cell contained a pH electrode (SCHOTT instruments type N62) with the electrode-tip in 65 ml Milli-Q water in the beaker. The pH was recorded 2-6 times a day; the NH_4^+-ISE was logged automatically with the ISE-meter (Orion 4-Star Meter) at time-intervals of 30 minutes. The NH_4^+-concentrations measured with the NH_4^+-ISE are expressed in this article as mg N per 100 ml aqueous phase for 100 g fish. The glass cells were placed in cryostats set at 0 °C, filled with water and antifreeze, located in a 2.5 °C room.

For the measurement of the dynamics of the electrode-response, a flat beaker with 95 ml of 50 ppm (mg/kg) aqueous NH_4Cl solution (Merck 1.01145) and 5 ml of 10% NaOH (Merck 1.06498) was put into the glass cell in place of the cod fillet pieces.

3.2.3. Destructive TVB-N and TMA analysis

3.2.3.1. Sample storage

The cod fillet samples for TVB-N and TMA measurements were packed separately in aluminium 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 cod fillet was put into each box with a lid for storage. The boxes were stored in a refrigerator at 0 °C.

3.2.3.2. Sample preparation

At each measurement day, one box was taken out of storage and the sample was immediately prepared for analysis. The content of the basket was ground in a Waring commercial blender for 30 seconds at high speed, resulting in cod fillet pieces of approximately 0.1 to 0.5 cm³. 200 ml of 7.5% aqueous trichloroacetic acid (TCA) (Merck 1.00807) solution was added to 100 g of comminuted cod fillet; this mixture was homogenized for 2 minutes at high speed to make a suspension. The mixture was centrifuged (Beckman Coulter Avanti J26 XP) at 21612 x g for 10 minutes at 4 °C and the supernatant was filtered through a Whatman No.2 filter paper.

3.2.3.3. TVBN-analysis

TVB-N was determined in triplicate in an extract of the cod fillet according to the steam distillation method described by Malle and Tao (1987). Steam distillation (Gerhard Vadopest 12-Kjedahl type distillatory) was carried out for 7.3 minutes on the TCA extract. 25 ml of filtrate were loaded into the distillation tube followed by 5 ml of 10% (w/v) NaOH. 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 TVB-N. 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 triplicate analysis.

3.2.3.4. TMA analysis

TMA was determined in duplicate in an extract of the cod fillet according to the steam distillation method described by Malle and Tao (1987). 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 was performed as for TVB-N determination.

3.2.4. Destructive ammonia analysis

The ammonia content of the cod fillet pieces was determined with an ammonia Assay Kit (Sigma-Aldrich AA0100). This method is based on the reaction of ammonia with α-ketoglutaric acid (KGA) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of L-glutamate dehydrogenase (GDH). This reaction results in the formation of L-glutamate and oxidized nicotinamide adenine dinucleotide phosphate (NADP$^+$). The ammonia concentration is proportional to the decrease in absorbance, due to the oxidation of NADPH.

10 ml of the TCA filtrate of the TVB-N analysis was neutralized to pH 7-8 with 10% (w/v) NaOH. Dilutions were made with Milli-Q. 100 μl of the sample extract was added to ammonia assay reagent (containing KGA and NADPH) and the absorbance of each solution was measured with a spectrophotometer (Varian, Cary 50) at 340 nm. Subsequently 10 μl of GDH was added and the absorbance of the samples was measured again after an incubation of 5 minutes at 25 °C.

3.2.5. Temperature recording

Automatic wireless temperature loggers (DS1921G Thermochron® iButton) with OneWireViewer software were used in the trials to monitor the temperature of the fillets and the storage rooms. The loggers were inserted between the fillets during transport from the wholesaler to the university and during the storage trials between the cod fillet pieces in both glass cells and in randomly selected aluminium boxes as well. Temperature was recorded at 15 minute intervals.

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3.3. Results and Discussion

3.3.1. pH development in aqueous phase during storage and the use of a NH_3-Electrode

For the development of this new non-destructive method, several electrodes were considered. Pivarnik et al. (2001) described a destructive method using an ammonia-selective electrode as a rapid screening method to determine fish freshness directly in a fish extract. The use of the gas-sensitive NH_3-Selective Electrode (Orion 9512) has the advantage that it can measure volatile amines that are produced in fish. The pH is an important factor influencing measurements with the NH_3-Electrode. Therefore, during one of the trials B, the pH was monitored in the aqueous sensor phase with 30 ml Milli-Q water with 0.6 ml NH_3-ISA (Orion 951211) for a NH_3-Selective Electrode (Figure 3.2). This NH_3-ISA increased the pH of the water; the pH at the start of the trial was 12.5. The pK_a of NH_3 and TMA are 9.2 and 9.8 (at room temperature), respectively (Hall, 1957); therefore at pH 12.5 virtually all ammonia is in the volatile NH_3 form and can be measured by the gas-sensitive NH_3-Electrode. During the trial, the pH decreased to a final relatively stable value of 7.1. This pH decrease is likely caused by the production of CO_2, H_2S and other volatile acids from microbial activity. The pH decrease causes a shift in the $NH_3 \leftrightarrow NH_4^+$ equilibrium and influences measurements with the NH_3-Electrode, since the non-volatile NH_4^+ cannot be measured with this NH_3-Electrode. The results of this pH monitoring show that the NH_3-Electrode cannot be used to monitor volatile amines from packed fish with this non-destructive method. This phenomenon implies also that problems can arise when using a simple indicator, like an in-package pH indicator as Pacquit et al. (2007) used, as freshness sensor at 0 °C. Therefore, for the non-destructive method of this article an NH_4^+-ISE was used, which is selective to NH_4^+.
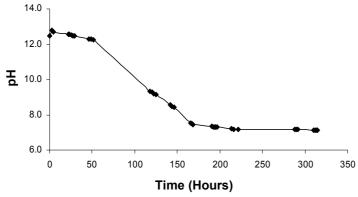

Figure 3.2 pH monitoring in the aqueous phase during trial B at 0 °C.

3.3.2. NH_4^+-electrode: Trials with NH_4Cl-solutions

3.3.2.1. Electrode calibration

The production of volatile compounds in a package containing cod fillets during storage at ~1 °C was monitored in a non-destructive way. Among the volatile compounds ketones and amines are present in the largest amounts in cod at the time of sensory rejection (Olafsdottir, et al., 2005). Ammonia has a major contribution to the TVB-N content of fish (Gill, 1990; Oehlenschläger, 1997). An ammonium-selective electrode was used to measure changes in the electric properties of an aqueous phase in the package. The NH_4^+-ISE was calibrated with NH_4Cl-solutions at ~3 °C. The response of the electrode in the range of 1 to 250 ppm was linear when plotting versus the NH_4Cl concentration on a logarithmic scale. In the low concentration range of 0.01 to 1 ppm, the electrode showed non-linear response below 0.1 ppm.

3.3.2.2. Proof of principle of non-destructive method

The proof of principle and the dynamics of the electrode response in the non-destructive measurement set-up was measured at ~3 °C with a beaker containing 100 ml of a 'source' solution of 50 ppm (= 4.11 mg N/100 ml) NH_3-solution (NH_4Cl-solution + NaOH). The 'source' solution was placed together with a beaker with the 'sensing' aqueous phase (30 ml Milli-Q water and 3 ml NH_4^+-ISA) + NH_4^+-ISE in the glass cell. The response of the NH_4^+-ISE in the 'sensing' aqueous phase increased linearly in time when exposed to the volatile ammonia from the NH_3 solution in the other beaker (Figure 3.3). The concave shape in the beginning of the graph is also seen in a decreasing headspace pressure with other gas/liquid systems when gases dissolve in a liquid phase, and is called the "incubation period" (Sivertsvik et al., 2004; Zhang et al., 2000). After 64 hours, the concentration of ammonium measured in the aqueous phase increased to half of the initial concentration of the 'source' NH_4Cl-solution. This proves that this NH_4^+-ISE in the aqueous phase can be used for this non-destructive method to monitor ammonia that is present/produced elsewhere in the package.
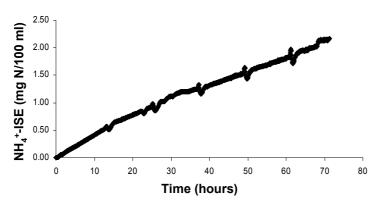
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Figure 3.3 NH_4^+-ISE response (measurement of NH_4^+/33 ml is converted to mg N/100 ml) in the aqueous phase that is placed together with a 50 ppm (as ammonia) NH_4^+-solution (≈4.11 mg N/100 ml) in the glass cell.

The mass transfer in both glasses with liquid is described below (Figure 3.4).

Source NH_4Cl-solution:

$$NH_4^+ + OH^- \Leftrightarrow NH_3$$

NaOH was added to the NH_4Cl-solution to increase the pH for the formation of the NH_3; NH_3 will partition between the 'source' solution and the gas phase in the glass cell and migrate to the 'sensing' aqueous phase by diffusion. The addition of NaOH increased the pH of the NH_4Cl-solution above 12. Since the pK_a of NH_3 in water at 3 °C is ~9.97 (Clegg & Whitfield, 1995), at pH 12 more than 99% of the total ammonia nitrogen will be undissociated dissolved ammonia gas in the NH_4Cl-solution at equilibrium (Srinath & Loehr, 1974).

The volatile NH_3 will partition between the 'source' solution and the headspace. At the interface an equilibrium will exist between the chemical activities of the NH_3, which can be expressed as the partial pressure in the gas phase and the concentration in the aqueous phase of NH_3 according to Henry's law. The NH_3 in the headspace in turn will partition in the 'sensing' aqueous phase and convert into NH_4^+ due to the low pH from the ISA. Therefore, equilibrium will not be reached for this solution and NH_3 will be released from the solution continuously until almost depletion.
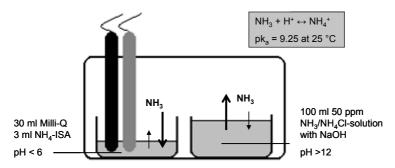
Figure 3.4 Schematic drawing of flow(↑) of ammonia.

If ammonia is desorbed from an alkaline solution and the pH of the solution is not controlled, then the pH will decrease, causing a decrease in the ratio of the undissociated ammonia to the total ammoniacal nitrogen and a lower ammonia desorption rate (Srinath et al., 1974). However, in the above described experiment, the pH was still above 12 after 70 hours ammonia desorption. Therefore the pH changes had little influence on the ammonia transfer.

Sensing aqueous phase:

$$NH_3 + H^+ \Leftrightarrow NH_4^+$$

The NH_3 from the headspace will dissolve and convert into NH_4^+ in the aqueous phase. The pH in this aqueous phase is below 6 due to the addition of pH-lowering ISA for the NH_4^+-ISE. At this pH, the equilibrium lies strongly on the side of NH_4^+ (more than 99.9% of the total ammonia nitrogen will be as dissociated NH_4^+), therefore the NH_4^+-ISE will measure almost all ammonia that is transported.

In the end, almost all ammonia will be transferred from the NH_4Cl-solution to the aqueous phase and converted to NH_4^+. In conclusion, the NH_4^+-ISE measures the NH_3 that is released from the NH_4Cl/NH_3-solution. The rate of ammonium increase in the aqueous phase is restricted by the release of ammonia from the NH_4Cl-solution. The NH_4Cl-solution is not stirred, therefore we assume that diffusion will limit the rate of NH_3 release to the headspace. The diffusion rate in a stagnant layer can be described by Fick's first law (Equation 3.1):

$$J = -D\frac{\Delta C}{\Delta x}$$

(Equation 3.1)

where J is the diffusion flux of ammonia (g/m^2/s), ΔC is the concentration difference of ammonia (g/m^3), x is the position (m), and D is the diffusion coefficient (m^2/s). In our

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experiments, we assume a linear gradient with $\Delta C = 50$ g/m^3 (50 mg/dm^3 in solution and 0 mg/dm^3 on the surface in equilibrium with the gas-phase, assuming a density of 1 kg/dm^3), $\Delta x = 0.02$ m (although liquid in the glass is 3.6 cm high, 0.02 m was taken since the diffusion will be faster in the beginning and the maximum diffusion distance would be 0.036 m), and assuming $D = 10^{-9}$ m^2/s (D of 1.08-1.23 x 10^{-9} m^2/s for aqueous ammonia with mole fractions <0.02 were reported for 4-11 °C in Frank et al. (1996), but D decreases with lower temperature and low NH$_3$ concentrations, and general diffusion coefficients for small molecules in water are in the order of 10^{-9} m^2/s (Walstra, 2003)).

With a surface area of 0.0034 m^2, this would result in a transport rate of 0.031 mg/h. This is in the same range as experimentally observed (0.029 mg/h for 100 ml from the linear trendline that was made from Figure 3.3). This calculation shows that diffusion in the source solution is indeed an important rate-limiting factor in this setup. For the detection of volatiles from fish internal diffusion is expected to be of less importance, since microbial growth and volatiles production mainly takes place at the surface (Huss, 1995).

The diffusion of NH$_4^+$ in the aqueous phase on the rate of NH$_4^+$ increase measured by the NH$_4^+$-ISE is not limiting, because the surface area/volume in the sensing aqueous phase is larger (factor 3) and:

$$\Delta C_{\text{surface}_{\text{NH}_4^+}} \gg \Delta C_{\text{surface}_{\text{NH}_3}}$$

The fluctuations in the linear graph of Figure 3.3 every 12 hours can be explained by temperature fluctuation, since electrode potentials (absolute potential and slope) are affected by changes in temperature. These temperature deviation occurred due to experimental setup, possibly due to the ice defrosting cycle in the cold room: every 12 hours an increase of maximal 5 °C in the temperature of the cold room occurred for a short time (after 30 min the temperature was back to the set point).

3.3.3. NH$_4^+$-electrode: Trials with cod fillet
3.3.3.1. NH$_4^+$ detection during storage trial
After the proof of principle of the method had been shown with NH$_4$Cl-solutions, the method was tested with cod fillets during three storage trials. In the storage trials, the NH$_4^+$-ISE monitored ammonium in a non-destructive way in the aqueous phase. This ammonium is formed from ammonia that is produced by autolytical and microbiological action in the cod fillets. In trial A the signal of the NH$_4$-ISE was compared with the destructive analyses of the ammonia content of the cod fillets (Figure 3.5A). The trend of the NH$_4^+$-ISE during trial A was equal to the ammonia content of the cod fillet, except for the increase and decrease after 50 and 150 hours.

Ammonia is produced the first day after slaughter during the autolytic breakdown of adenosine monophosphate, which is a degradation product of ATP, to inosine monophosphate (Gill, 1990; Huss, 1995). It was probably this autolytic formation that caused an ammonia content of ~12 mg N/100 g cod fillet during the first 200 hours of storage trial A. This value is somewhat higher than the concentration of ammonia (7.34 mg/100 g fish) in muscle tissue of freshly caught cod found by Oehlenschläger (1997), the TVB-N value (12.84 mg N/100 g fish) of Oehlenschläger (1997) was also lower than the TVB-N value (15.9 mg N/100 g fish) from the first analysis of fresh cod fillet in trial A. The microbiological action can explain the increase of the ammonia content in the cod fillet after 200 hours of storage. Spoilage bacteria produce proteolytic enzymes that degrade/ deaminate proteins, peptides and amino acids (Gill, 1990; Huss, 1995) and ammonia is produced from the bacterial degradation of non-protein nitrogen compounds such as urea, which is available in large amounts in elasmobranches (Fraser & Sumar, 1998). Ammonia is also produced together with TMA during the anaerobic reduction of TMAO by *Shewanella putrefaciens* (Huss, 1995).

As the method proved to monitor the changes in the ammonia content of the cod fillet well in this experimental setup, the storage trials with the cod fillet were performed twice to study whether the method is robust and sensitive. In these extra trials the NH_4^+-ISE measured changes in the electric properties of the aqueous phase in the package and these signals were compared with destructive freshness analyses (TVB-N and TMA) of parallel stored cod fillets (Figure 3.5B and C). One has to keep in mind that these experiments cannot be repeated exactly because the quality of fresh fish will be different each time due to biological variation and environmental conditions. So it is difficult to test reproducibility of the method, but the method can be tested with fish of different initial freshness, which is representative for realistic applications.

The results of the NH_4^+ monitoring in the storage trials, with three different batches of cod fillet, were comparable. The trend of the NH_4^+-ISE in the aqueous phase followed the trend of the TVB-N measurements in the cod fillet, especially in trial C. Since TVB-N is a well-known freshness indicator, these findings imply that changes in the freshness status of fish can be monitored with the NH_4^+-ISE in the aqueous phase.

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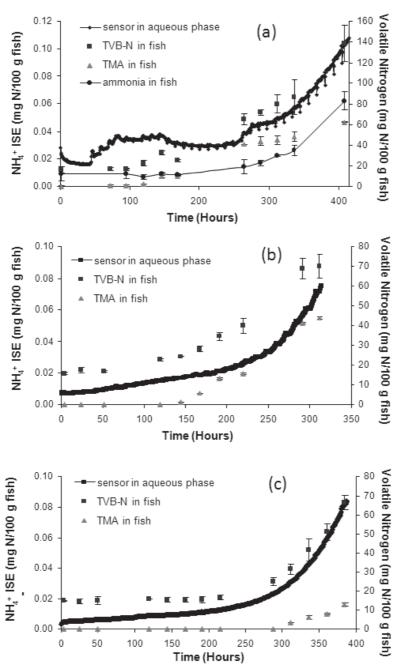R26
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Figure 3.5 NH_4^+ in aqueous phase measured by NH_4^+-ISE and mg N/100 g fish fillets from TVB-N, TMA and ammonia (only trial A) analyses in cod fillets during trial A (a), B (b) and C (c). Average temperatures of: Fillets used for non-destructive measurements: trial A: 1.3 °C, trial B: 1.1 °C, trial C: 0.8 °C; Fillets used for destructive measurements: trial A: 0 °C, trial B: 0 °C, trial C: -0.2 °C.

In the trial B, the increase of the slope of the ammonium monitoring by the electrode in the aqueous phase showed a delay compared to the increase of the values of the freshness indicator compounds TVB-N and TMA in the cod fillet. During trial A the trend of the NH_4^+-ISE in the aqueous phase followed the trend of the TVB-N measurements in the cod fillet, but the absolute TVB-N values were higher compared to the values in trial B.

The graph with the results of the TVB-N and TMA analysis during trial C is comparable to results reported in literature. Botta et al. (1984) described a gradual increase in TVB-N content of cod stored in ice during the initial 10 days, but thereafter the TVB-N content increased rapidly. Ólafsdóttir et al. (2005) described the detection of TMA and increasing TVB-N values for cod fillets after 12 days stored at 0.5 °C, and Di Natale et al. (2001) described that TMA values became considerably different from zero only after 9 days of storage and TVB-N following the behaviour of TMA. However, the graph with the TVB-N and TMA analyses during trial B is different from results in literature for cod stored at 0 °C, because there is not a clear lag-phase and sharp rise between 9-12 days storage. Instead, the TVB-N content rose continuously during the whole storage trial. These results differ also from other analyses of TVB-N during storage of cod at 0 °C (data not shown here), where TVB-N and TMA did not increase until 200 hours of storage. An explanation might be a temperature deviation for the storage of the cod fillet pieces in the aluminium boxes, which were used for the TVB-N and TMA analyses and stored in a refrigerator. The temperature of the pieces of cod fillet in the glass cells was between 0.5 and 1.9 °C. The temperature in the aqueous phase was ~3 °C. The temperature of the parallel stored cod fillets was between -0.5 and 0.5 °C. The storage temperature highly influences the autolytic and microbial activity that causes the formation of volatile compounds in fish. An increase in the temperature from 0 to 3 °C doubles the spoilage of chilled fish, and an increase to 10 °C accelerates the spoilage by a factor 5-6 (Venugopal, 2002). A small temperature difference between the cod fillet in the glass cell and the cod fillet in the aluminium boxes stored in the refrigerator might have caused the differences in the trend of the TVB-N and TMA analyses and the trend of the NH_4^+-electrode in the aqueous phase.

Other factors that might have caused the delay in the increase of the slope of the electrode signal in trial B can be variation in the initial freshness and spoilage rate of different cod fillet pieces, which caused a difference in the lag-phase before the TVB-N rises, or because the migration from amines generated in the cod fillet to the headspace and finally to the aqueous phase is somewhat delayed (mass transport between the cod fillet pieces that are touching each other in the glass and are therefore not all directly exposed to the headspace, although this is expected to be similar for different trials).

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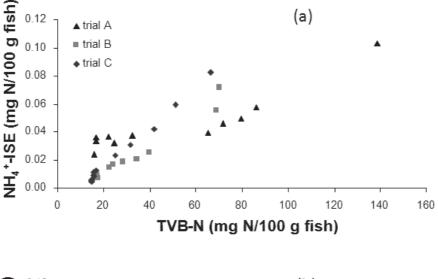R19
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3.3.3.2. Correlation between NH_4^+-ISE and volatile amines development during storage trial

The NH_4^+-ISE trend was similar to the ammonia trend in the cod fillet. The NH_4^+-ISE is selective to the ammonium-ion, not to the other amines present in the aqueous phase. However, there was a positive linear correlation between the TVB-N measurements in the cod fillet and the NH_4^+-measurements in the aqueous phase with the NH_4^+-ISE (Figure 3.6A) because ammonia largely contributes to TVB-N-content. This implies that the NH_4^+-ISE measures an increase in the NH_4^+-content in the aqueous phase if the freshness indicator TVB-N content in the cod fillets increases.

The initial increase in the electrode signal at low TVB-N concentrations can be explained as follows: at low temperatures, the TVB-N hardly increases during the first days, but the NH_4^+-ISE signal rises, because the initial TVB-N content of the cod fillet is already about 15-20 mg N/100 g fish. A sensor monitoring specifically TMA would overcome the problem of the initial increase in the electrode signal, since the TMA content of fresh cod is 0 mg N/100 g fish initially.

As expected, the NH_4^+-content measured by the NH_4^+-ISE in the aqueous phase was positively correlated to the NH_3-content in the cod fillet (Figure 3.6B). There is a factor 1000 in absolute difference between the NH_3 content of the aqueous phase measured by the NH_4^+-ISE and the NH_3 content of the cod fillet. This can be explained by the partition of produced NH_3 in the cod fillet, the headspace of the package and the aqueous phase. This factor 1000 was not seen with the NH_4Cl-solution, but the pH of the NH_4Cl-solution was higher than 12, while the pH of cod fillets is generally between 6 and 7. With a pK_a of 9.97 at 3 °C for NH_3 in water, this means that a large part of the produced NH_3 will be converted to NH_4^+ in the water of the fish flesh and will not be available as free NH_3 for the headspace of the package.
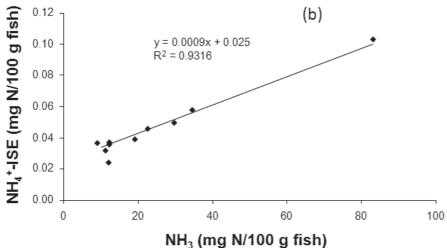
Figure 3.6 (a) Correlation between NH_4^+ in aqueous phase measured by NH_4^+-ISE and mg N/100 g fish from TVB-N analyses in cod fillets from all trials. (b) Correlation between NH_4^+ in aqueous phase measured by NH_4^+-ISE and NH_3 from ammonia analyses in cod fillets from trial A.

3.4. Conclusions

A non-destructive method for monitoring changes in the headspace ammonium as an indicator for the freshness status of packed fish was introduced in this study. The results showed that a NH_4^+-ISE in an aqueous phase can be used to monitor volatile amines in the package, compounds that are generally used as freshness indicator of the packed fish.

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The NH_4^+-ISE measures changes in the aqueous phase when ammonia, from an NH_3-solution or from cod fillets, is released in the headspace of the package. The electrode response correlates with the trend of the generation of the TVB-N in the cod fillet, which is an established freshness indicator for fish in destructive analysis. Therefore the quality decay of fish can be monitored non-destructively by this method. Optimization of the method (reproducibility, accuracy) is still required. Besides, the electrode response should be correlated with the results of freshness evaluated by a sensory panel.

Currently, research is done to study the performance of the method at other temperatures and conditions for storage of the packed cod fillet (MAP). Besides, the use of other (simple) types of electrodes is studied.

This method offers new opportunities for continuously monitoring the loss of freshness of individual packages of fish. Therefore, this non-destructive method might be used for the development of a freshness sensor in an intelligent packaging for fresh fish. This sensor could consist of a chip and minimized electrode in a gel that contains the aqueous sensor phase. Such an intelligent packaging sensor can give an indication of freshness of the packed fish at all points during the supply chain.

Acknowledgement

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4

Non-destructive sensing of the freshness of packed cod fish using conductivity and pH electrodes

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Abstract

The use of pH and conductivity electrodes as non-destructive methods for monitoring changes in the freshness status of packed fish is explored. The electrodes monitor changes in the electrical properties of an aqueous phase positioned in the headspace of the fish package. Volatile compounds produced in/on the packed fish and released in the headspace dissolve into this aqueous phase. Several compounds affect the electrode signals. The signals of the electrodes were compared with the volatile amines content of fresh cod fillets stored at 0-15 °C. The changes in the pH signal were strongly temperature dependent and not suitable as quality indication for practical storage temperatures. The conductivity signal showed a characteristic pattern, that correlated with the increasing volatile amines content at all temperatures. The non-destructive measurement of conductivity establishes the proof of principles for monitoring the freshness of packed fish and paths the way towards new developments of intelligent packaging concepts.

Keywords: Non-destructive method; fish quality; conductivity-electrode; volatile compounds; pH-electrode; intelligent packaging

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4.1. Introduction

Intelligent packaging for fresh fish requires the development of sensors that monitor and communicate freshness from the moment of packaging until the day the fish is spoiled. To develop such sensors, non-destructive analysis methods are needed. Although several non-destructive methods to monitor the quality of unpacked fresh fish have been described, no appropriate methods for use in intelligent packaging are available (Heising et al., 2014).

In a previous study the proof of principle of a non-destructive method to monitor changes in the freshness of packed cod fillets was given (Heising et al., 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. The method was tested using an Ammonium Ion-Selective Electrode (NH_4^+-ISE) to measure changes in the potential of the aqueous phase. The ammonium content of the aqueous phase increased during storage due to the production of ammonia in/on the cod fillets, subsequent diffusion through the headspace of the package and dissolution in the aqueous phase. These changes were related to the volatile amine content of the fish itself, which is an often used freshness indicator.

The NH_4^+-ISE could monitor the changes in NH_3, produced on the fish, well. But the disadvantage of monitoring ammonia is that this compound is not always suitable as quality indicator (Huss, 1995). During storage trials with fresh cod fillets, a clear increase in the ammonia electrode signal was seen just at the moment of spoilage (according to the Total Volatile Basic Nitrogen (TVB-N) level). Therefore it is difficult to predict the changes in freshness during early storage or remaining shelf life by using ammonia as quality indicator. The storage time of the fish fillet can be divided into 4 stages; in the first phase typical freshness compounds disappear due to autolytic reactions. Endogenous enzymes, autoxidation and microbial growth change the aroma of fish fillets by converting and degrading muscle compounds like sarcoplasmic proteins and membrane bound phospholipids. Polyunsaturated fatty acids are enzymatically converted to volatile aroma compounds that are responsible for the fresh fish odour or fresh-plant-like aroma (Ólafsdóttir, 2005). These volatile compounds are present in very low concentrations and the method is not yet sensitive enough to monitor these compounds. Therefore, these volatiles are currently not useful as freshness indicator compounds in our method. In later stages the typical spoilage compounds (volatile amines like trimethylamine (TMA), sulphur compounds) that limit the shelf life are formed, but also other compounds like alcohols (Duflos et al., 2006). These volatile compounds are formed in relatively high concentrations and might be detected by a sensor. However, not all volatile compounds will dissociate in the aqueous phase causing a change in the conductivity.

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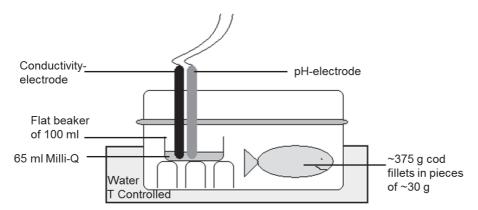R25
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In this article, we investigate the use of two other simpler and cheaper sensors by electrodes in the aqueous phase: i) monitoring the conductivity, ii) monitoring the pH. Although the NH_4^+-ISE has the advantage of high selectivity to a volatile amine produced by the fish, the non-selective conductivity or pH electrode are cheaper and might be able to monitor a broader range of basic or acidic volatile compounds generated by the fish. All electrodes have the advantage of submitting an electrical signal that can be used in electronic intelligent packaging concepts.

The purpose of this study was to see whether unspecific electrodes are suitable to be used in a non-destructive method as proof of principles to monitor the freshness of fish. The changes in the conductivity and pH of the aqueous phase are compared with the changes in the freshness of packed cod fillets as assessed by their TVB-N content.

4.2. Materials and Methods

4.2.1. Storage trials of cod fillets

Cod (*Gadus morhua*) was bought at "Zeevisgroothandel J. Thiele & ZN" in IJmuiden. 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 fish and the cod 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 trials started. Each batch of fish was used for both the non-destructive and destructive analysis during each trial. Trials at 0 °C were in May 2008, in October 2008, and in May 2009; trial at 5 °C was in June 2009; trial at 10 °C was in July 2009; trials at 15 °C were in October 2008.

4.2.2. 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 that analysed an aqueous phase in a beaker separate from the fish (Figure 4.1) (Heising et al., 2012).

~375 g cod fillets, sliced into pieces of approximately 30 g, was put in the glass cell. Each experiment contained randomly mixed pieces from different cod fillets. The glass cell contained both a pH electrode (SCHOTT instruments type N62) and 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 pH was recorded 2-6 times a day; the conductivity electrode was logged automatically at time-intervals of 30 minutes.

The glass cells were placed in a cryostat set at 0, 5, 10 or 15 °C, filled with water and antifreeze, located in a temperature controlled room.

Figure 4.1 Measurement set-up when monitoring changes in aqueous phase with pH- and conductivity electrode.

4.2.3. Destructive Total Volatile Basic Nitrogen (TVB-N) analysis

4.2.3.1. Sample storage

The fish samples for these measurements were packed separately in aluminium 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 0 °C, 5 °C, 10 °C or 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).

4.2.3.2. Sample preparation

Sample preparation was performed as described in Heising et al. (2012). At each measurement day, one box was taken out of storage and the sample was immediately prepared for analysis. TVB-N was determined in triplicate in an extract of the fish according to the steam distillation method of Malle and Tao (1987) and described in Heising et al. (2012).

4.3. Results and Discussion

4.3.1. pH development in aqueous phase during storage trials

To investigate whether a pH-electrode based sensor would be suitable for the non-destructive sensing of freshness related compounds in the headspace of fish packages, pH measurements during storage were performed. The pH was monitored in a cell containing Milli-Q (deionized) water located in the headspace during storage trial at 0 °C. The pH at the start was 6.3, but it dropped fast to values just below <5 during the first hours of the trial. During the remaining time of the trial, the pH decreased more

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gradually to a final value of 4.4 (Figure 4.2 (0 °C)). This pH drop is likely to be caused initially by dissolving of CO_2 that was already present in the headspace of the package (1st hours) and later by the CO_2 produced by micro-organisms on the fish surface. The pH of deionized water will quickly drop from pH 7 to pH 5.7 when the water is exposed to normal atmospheric conditions, air with a CO_2 content of 0.033%, due to dissolving atmospheric carbon dioxide (Light et al., 1995). Furthermore the temperature of fresh Milli-Q water decreased fast from ambient (~25 °C) before the start of the experiment to the cryostat temperature (~1 °C); this decreasing temperature increased the solubility of CO_2 (Light et al., 1995). Dissolved CO_2 forms carbonic acid after reacting with water. Other volatiles produced by the fish that dissolve in the aqueous phase might influence the pH as well, but their contribution is expected to be minor.

This continuous decrease in pH was not seen during storage trials at higher temperatures. After the initial drop the pH fluctuated during a storage trial at 5 °C (Figure 4.2 (5 °C)). A pH increase in the aqueous phase can be expected from the release of volatile basic amines from the fish. Indeed at higher temperatures (10 °C and 15 °C) the pH did increase after the initial drop and the pH increase appears to be correlated with the volatile amine (TVB-N) content (Figure 4.2 (10 °C) and (15 °C)). The contents of these volatile amines are often taken as freshness indications, and besides, these compounds can also dissolve and dissociate in the aqueous phase and cause a change in the conductivity. Ammonia and trimethylamine (TMA) are quantitatively the most important compounds of the TVB-N content. The dissociation reactions are respectively:

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \tag{1}$$
$$(CH_3)_3N + H_2O \leftrightarrow (CH_3)_3NH^+ + OH^- \tag{2}$$

Figure 4.2 pH (◆) change in aqueous phase during storage trials of cod fillets at 0, 5, 10, and 15 °C, compared with TVB-N content (▲) of fish fillets.

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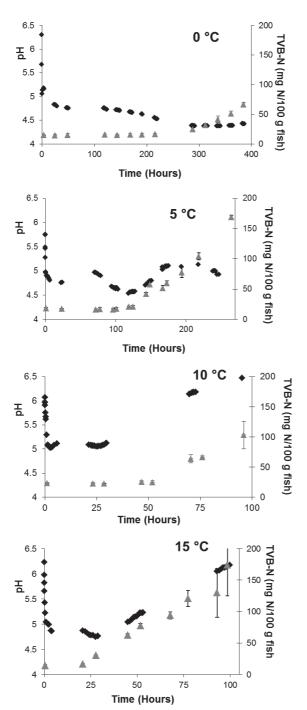R26
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Pacquit et al. (2007) reported a pH increase monitored by a dye indicator within the headspace of an enclosed food package with cod stored at 21 °C, but no pH values were mentioned. Pacquit et al. (2007) developed an in-package pH colour indicator that monitored spoilage of fish. The indicator colour changed by a pH increase caused by the release of volatile amines, which correlated at room temperature with the changes in total viable count and *Pseudomonas* spp. in cod (Pacquit et al., 2007). However, from our results it is clear that the pH in an aqueous phase in the headspace is not suitable as freshness indicator for packed fish under practical conditions, because the pH in the aqueous phase is strongly influenced by the effect of temperature on the production of volatile compounds by the packed fish. The pH value of an aqueous phase in the headspace does indicate freshness well at high temperatures (15 °C), since the pH increase correlates with the formation of the volatile basic amines produced by the packed fish. However, to be useful as an accurate freshness indicator, the pH in the aqueous phase should correlate to the freshness of the fish especially at lower temperatures (0-7 °C) as well. At low temperatures, the pH-lowering effect of the formation of volatile acids like CO_2 apparently overrules the pH-increasing effect of the formation of the volatile amines. A pH electrode is therefore not suitable to monitor the amines at the low temperatures that are characteristic for the fish supply chain (Huss, 1995).

Using a pH signal to monitor the freshness of packed fish was shown to be unreliable due to a large variation in response patterns and correlation with TVB-N content at different temperatures. Therefore, the next step was to study whether a conductivity measurement would be suitable as a non-destructive method.

4.3.2. Conductivity-electrode
4.3.2.1. Conductivity development during storage trial
A conductivity electrode was used to monitor changes in the aqueous phase in a glass cell located in the headspace of fish packages. The results of the conductivity monitoring in three trials using three different cod batches at 0 °C were comparable, both in trend and in absolute value (Figure 4.3).

The signal of the conductivity electrode was compared with the volatile amines content (TVB-N) of the fish fillets. According to the reactions (1) and (2) ions are formed when these amines dissolve in the water cell. These ions cause an increase in the conductivity (molar conductivity of NH_4^+ is 73.5 S-cm^2/mol, that of TMA is 47.2 S-cm^2/mol and that of OH$^-$ is 199.1 S-cm^2/mol) (Coury, 1999).

Figure 4.3 Conductivity monitoring in aqueous phase (●) and mg N/100 g fish fillets from TVB-N analyses (▲) in three different batches of cod fillets during trials A (I), B (II), and C (III) at 0 °C.

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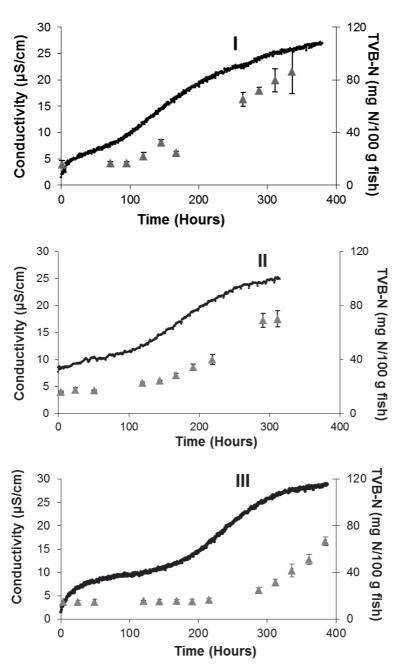R25
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In all trials, the conductivity and TVB-N profiles were unique, probably due to differences in initial quality of the different cod fillet batches; conductivity in the aqueous phase increased strongly before the sharp rise of TVB-N content in the fish. The slope of this conductivity rise seems correlated with the (delayed) slope of the TVB-N rise: in trial C the steeper slope of the conductivity rise matches the steeper slope of the TVB-N slope occurring later, while in trials A and B a more gradual increase in the conductivity rise is followed by a gradual TVB-N rise.

In trial B the initial conductivity was much higher than expected. According to literature and the specifications of the supplier, standard Milli-Q water has a conductivity of $0.055 – 0.294 \ \mu S/cm$ at 25 °C (Francis, 2008). Therefore, the high starting value of $8 \ \mu S/cm$ of the conductivity measured in trial B was possibly caused by pollution of the glass or water. This initial deviation did not mask the visibility of the large increase upon further storage time.

Besides the volatile amines, also other volatile acidic or basic compounds can influence the signal of the conductivity electrode in the aqueous phase. The non-selective nature of the conductivity electrode might be an advantage for sensing the fish freshness status, since the loss of freshness and the spoilage of fresh fish are complex endogenous and microbial processes (Fraser & Sumar, 1998; Jørgensen et al., 1988; Gram & Huss, 1996). A large number of volatile components has been identified in stored fish samples; the main classes of compounds in cod fillets are ketones, amines, alcohols, acids, aldehydes, and esters. Volatile compounds like amines and acids (e.g. CO_2 and H_2S) dissolve and dissociate in the aqueous phase, after which they cause an increase in the conductivity. However, many of the volatile compounds do not increase conductivity, because they do not dissociate. The volatile amines, especially TMA, are the most abundant volatile compounds during advanced stages of storage (Duflos et al., 2006; Ólafsdóttir et al., 2005).

Also during the trials at higher temperatures, an increase in conductivity was seen during the storage trial (Figure 4.4). The conductivity is higher, but the TVB-N values were higher as well (Figure 4.5). In the correlation plots with the data of TVB-N and conductivity from the trials at different temperature a positive correlation is seen. The non-linear behavior at the low concentrations of TVB-N (Figure 4.5(I)) can be explained by adjustment of the equilibrium of the aqueous phase and the headspace from the amount of TVB-N already present in the fish fillets at the start of the experiments, or from a higher sensitivity of the system at low concentrations, which would be an advantage. Just caught cod usually has an initial value of 12-15 mg N/100 g fish, in the first analyses during our storage trials the values ranged from 15, 16, and 16 mg N/100 g fish for 0 degrees, to 18 mg N/100 g fish for 5 °C, 23 mg N/100 g fish for 10 °C, and 14 mg N/100 g fish for 15 °C.
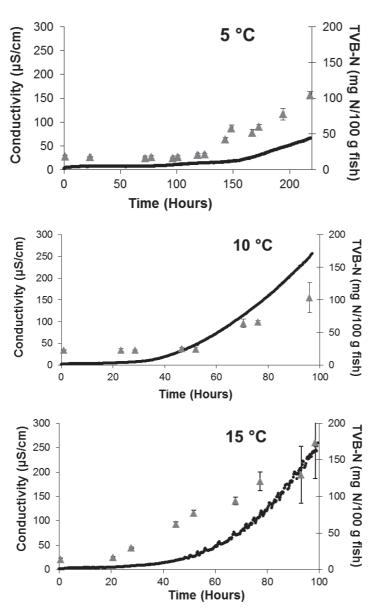
Figure 4.4 Conductivity monitoring in aqueous phase (●) and mg N/100 g fish fillets from TVB-N analyses in fish fillets (▲) during trials at 5, 10, and 15 °C.

4.3.2.2. Correlation between conductivity and TVB-N development during storage trials

In the trials at 0 °C, the trends of the conductivity and TVB-N development were not similar; the conductivity in the aqueous phase increased strongly before the sharp rise of TVB-N content in the fish. The conductivity in the aqueous phase might be

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strongly influenced by other volatile compounds, like carbon dioxide that is produced by microbial growth. This can also be concluded from the correlation plots (Figure 4.5). The conductivity in the aqueous phase and TVB-N development in the fish were positively correlated, but the trend is not linear. The trend is comparable between the three trials, but the absolute values of trial C are different from the others. In trial C higher conductivity values were found for lower TVB-N values compared to the other trials. This might be caused by small temperature differences between the measurements in the aqueous phase and the storage of the aluminium boxes in the refrigerator or in the TVB-N analysis, since the level of these amines was higher overall during the trial A and B. The correlation between the TVB-N content of fish and the conductivity in the aqueous phase at 0 °C and 5 °C was fitted in Figure 4.5(I) by non-linear regression of the empirical model described in Equation 4.1.

$$Y(x) = a\left(1 - e^{-b(x-c)}\right) \qquad \text{(Equation 4.1)}$$

Initial measurements of TVB-N resulted in a value of 15 for c, parameters a and b were estimated to be respectively 25.6 and 0.13. However, we cannot give physical explanations for these values at this time.

The correlation plots have been split up for normal temperature (Figure 4.5(I)) and for temperature abuse conditions (Figure 4.5(II)), since the scales and ratio of conductivity and TVB-N are different for the different temperature ranges. The correlation plot between the conductivity in the aqueous phase and the TVB-N content of the fish stored at different temperatures shows that a higher temperature resulted in a higher conductivity at similar TVB-N values, except for the results from the trial at 10 °C. The correlation between the TVB-N content of fish and the conductivity in the aqueous phase at 10 °C and 15 °C was fitted in Figure 4.5(II) by non-linear regression of the empirical model described in Equation 4.2.

$$Y(x) = c/(1 + ae^{-bx}) \qquad \text{(Equation 4.2)}$$

The parameter estimates for a and b at 10 °C were 70.8 and 0.07 respectively and a and b at 15 °C were 779 and 0.06 respectively with an estimation of 253 for c at both temperatures.

Differences between the change in conductivity and TVB-N at different temperatures can be explained by several factors. The conductivity in the aqueous phase can only be influenced by volatile compounds. TVB-N has quantitatively a large contribution to the total volatile compound content, but at different temperatures different (amounts of) volatile compounds can be formed. Higher conductivity at higher temperatures can be

caused by the formation of volatile compounds that have large influence on conductivity and are not formed at low temperatures. Besides, different compounds have different volatility, solubility and dissociation and these properties are also influenced by the temperature. And there might be an effect of the temperature on the performance of the measurements with the electrode.

Figure 4.5 Correlation between TVB-N analyses in fish fillets (mg N/100 g fish fillets) and conductivity in aqueous phase in 3 trials (A,B and C) at 0 °C and 1 trial at 5 °C (I) and at temperature abuse conditions (10 and 15 °C) (II).

The conductivity in the aqueous phase cannot be related directly to the formation of volatile amines in cod, but the conductivity changes do follow a characteristic trend during storage and might therefore still be useful for monitoring fish freshness. Algorithms can be developed to predict the fish freshness based on the pattern of the conductivity signal, or by a combination of temperature and conductivity signals.

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4.3.3. Perspectives of applications of sensors

To predict the freshness status of a food product with a sensor in an intelligent packaging, changes in a freshness indicator (e.g. concentration of one or more compounds) need to occur during the life cycle of the product. Measurement of these freshness indicators need to give reliable predictions at a broad range of conditions, since fluctuations can occur in the supply chain.

Since different electrode signals are seen at different temperatures, it can be concluded that monitoring the pH in the aqueous phase is not an appropriate approach to monitor the freshness of packed fish with a non-destructive method. The conductivity electrode output of the aqueous phase showed a characteristic response pattern during the storage trials at all temperatures investigated. This is promising to function as indicator in a sensor for an intelligent packaging for packed fish. Since the conductivity pattern did not always correlate with the pattern of TVB-N content of the fish fillets, more research is needed before using this sensor for practical applications. As could be concluded from the correlation plots, temperature influences the correlation of the TVB-N content in the fish and conductivity in the aqueous phase. Algorithms can be developed that take into account delays in response patterns at different temperatures, or to correct for the formation of other volatiles that affect conductivity. Current research is performed on the development of a mathematical model to simulate and predict the sensor response, based on formation kinetics, partitioning, dissociation etc..

Further research is also necessary to study the performance of the conductivity electrode to monitor fish freshness when modified atmosphere packaging is applied.

Currently the sensor is not able to monitor changes in the loss of freshness at the very early stages of the storage in an accurate way. In this first phase typical volatile freshness compounds, present in very low concentrations, disappear due to autolytic reactions (Ólafsdóttir, 2005). Improving the sensitivity of the method can possibly lead to monitoring of the loss of freshness at the beginning of storage and this would lead to more accurate predictions of remaining shelf life.

This article proves that the principle of the new non-destructive method can be used as a general concept in which different electrodes can be used to monitor different volatile compounds. Therefore this method might also be used for the development of intelligent packaging for other (food) products. Selective electrodes can be used to monitor specific compounds, but also general electrodes like conductivity or pH electrodes can be used, depending on the freshness or spoilage compounds of the product (Heising et al., 2014).

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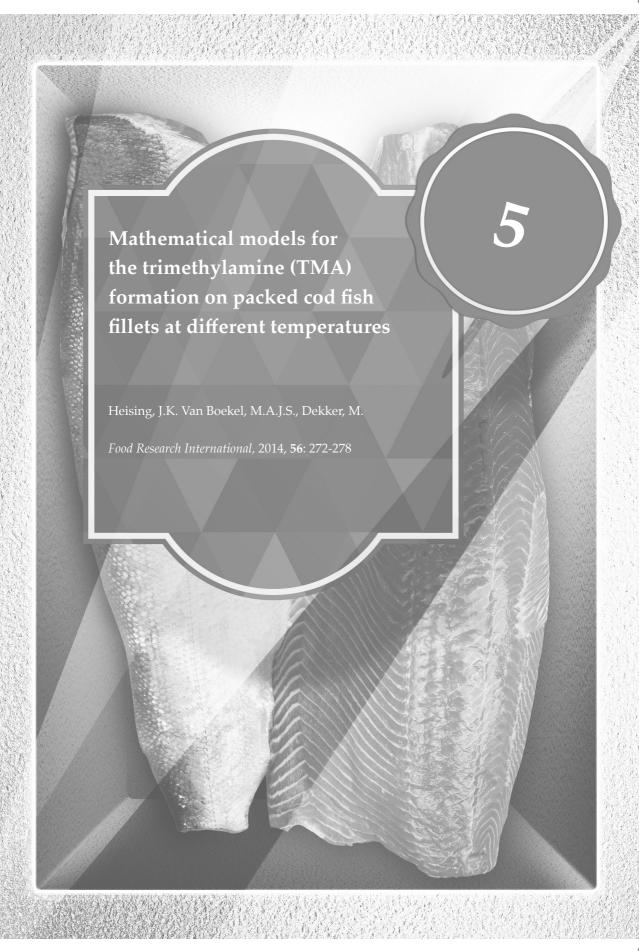R26
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Mathematical models for the trimethylamine (TMA) formation on packed cod fish fillets at different temperatures

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Abstract

The microbial formation of trimethylamine (TMA) can be used as a quality indicator compound to predict the freshness of fish during its shelf life. In a supply chain with fluctuating temperatures, mathematical models will be valuable tools to simulate this formation as a function of temperature and time. These models are essential to link sensor data on the formation of TMA to the actual freshness of fish. Existing models for the formation of TMA in fish needed improvements and secondary models for the effect of temperature on the formation of TMA are lacking in the literature. Three different approaches were evaluated on their ability to simulate the experimental observed TMA formation at 4 different temperatures (0, 5, 10 and 15 °C). In the first approach the existing models were improved and the temperature effect was modelled by an empirical model using four parameters. This model is able to simulate the TMA formation at static temperatures. Since TMA is produced on fresh cod fillets by the micro-organisms *Shewanella putrefaciens* and *Photobacterium phosphoreum* the microbial Baranyi–Roberts model was initially used for modelling the TMA formation, but this model was found to be too complex (too many correlated parameters that could not be estimated). In the third approach it was seen that a simplified Baranyi–Roberts model with only three parameters could be used to predict the TMA formation with equal accuracy. The influence of the temperature on the parameter μ_{max} was modelled using the extended square root model of Ratkowsky and the differences in TMA formation profiles of different batches could be described by the batch specific parameter N_0 representing the initial quality. The presented dynamic model is valuable in predicting the formation of TMA in a fresh fish supply chain with dynamic temperatures. This model has the potential to be used to link sensor data of TMA in the headspace to the actual freshness status of the fish.

Keywords: Mathematical modelling, trimethylamine (TMA), fish freshness, dynamic models, temperature effect, batch effect

5.1. Introduction

Freshness is a very important factor determining the quality of fish and freshness can be evaluated by different approaches (Ólafsdóttir et al., 1997). Sensory analysis is often used to evaluate fish freshness, but fast objective methods are preferred for routine analysis. The contents of Total Volatile Basic Nitrogen (TVB-N) compounds in fish are often used as freshness index and TVB-N limits are specified for different fish species by the European Commission (Council Regulation No. 95/149/EC of 8 March 1995). 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, 2010a). The TMA content is strongly correlated to the sensory quality of cod (Gill, 1990), and the TVB-N trend has proven to be a good indicator for the freshness of many marine fish (Botta et al., 1984).

TMA is formed in fish fillets during storage at chilled conditions by some micro-organisms that can use TMAO in anaerobic respiration (Gram and Dalgaard, 2002). *Aeromonas* spp., psychrotolerant Enterobacteriaceae, *Photobacterium phosphoreum*, *Shewanella putrefaciens*-like organisms and *Vibrio* spp. are the bacteria that are able to reduce TMAO to TMA (Gram and Dalgaard, 2002). Also other volatiles are formed by micro-organisms and by autolytic reactions and the most important variables that influence the formation of volatile compounds that are formed during the storage time are the initial quality (amount of substrate and initial bacterial flora), Modified Atmosphere Packaging (MAP) (the packaging atmosphere will influence the amount of volatiles formed and the composition/type of volatiles), and especially the temperature will have a large influence on the (amount of) volatiles formed.

The content of TMA can be used as variable that is linked to the freshness status at the moment of analysis. To be able to predict the remaining shelf-life from the TMA measurement, predictive models can be used. Not many models were found in literature that describe the formation of TMA in fresh fish. Howgate (2010b) reviewed TVB-N data and proposed some empirical mathematical models to describe the TMA formation. However, the effect of temperature was not mentioned in the models. The effect of temperature on the TMA formation is necessary to predict the fish freshness and the remaining shelf life based on storage conditions with dynamic temperatures, which is inherent to the fish supply chain (Giannakourou et al., 2005). The effect of temperature on the shelf life of cod fillets has been studied with a square-root model for the applicability of time–temperature indicators (TTI) (Mai et al., 2011). The effect of variability in fish quality is not taken into account in predictions of shelf life from the use of a TTI, but square-root models were studied for the temperature effect in the present research. Besides lacking the temperature dependence, some limitations were found in

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the existing models when applying them on our TMA measurements. Therefore adapted and also other mathematical models were evaluated and presented in this article.

It is proposed in the present paper to describe TMA formation starting from microbial models. However, we decided not to analyse the counts of the specific spoilage organisms that produce the TMA as a function of time stored at different temperatures. The pathways for the formation of TMA are too complex and all parameters would need to be estimated for all micro-organisms, and this would result in models with so many details and parameters that it would make them useless. Therefore, it was decided to model the TMA content instead of the microbial count in microbial models.

The objective of this research is to study mathematical models for the formation of TMA in cod fillets. The development of mathematical models is necessary for predicting the freshness of fish from the outcome of sensors that monitor the TMA content and changes therein of packed fish. The models presented in this article describe the formation of TMA at different temperatures and from different batches and these models can be used to predict the remaining shelf life.

5.2. Materials and Methods

5.2.1 Data collection
5.2.1.1 Storage trials of cod fillets
Data for parameter estimation belong to the experimental trials with cod fillets described by Heising et al. (2014) and the data can be found in the present article.

Cod (*Gadus morhua*) was bought at "Zeevisgroothandel J. Thiele & ZN" in IJmuiden (NL). 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 h. Purchase, fillet preparation and transport all took place in the same morning. Immediately after arriving in Wageningen, the fillets were prepared for analysis and storage, and from this moment the storage trials started. Trials at the different temperatures were performed during the period 2008-2009.

Cod fillets were stored in a refrigerator at different temperatures 0 °C, 5 °C, 10 °C and 15 °C, packed in aluminium boxes. The temperature of the fillets and the storage rooms was monitored with automatic wireless temperature loggers as described in Heising et al. (2012).

5.2.1.2 TMA analysis
The TMA content was determined in duplicate in an extract of the cod fillet according to the steam distillation method from Malle and 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 min 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.

5.2.2 Parameter estimation and simulations

The formation of TMA in packed cod fillets was modelled using different models that are all sets of algebraic and differential equations. Numerical integration of the differential equations and parameter estimation, including the statistical evaluation of them and of the performance of the complete models, were obtained by least squares regression with the help of the software package Athena Visual Workbench (www.athenavisual.com).

Models were discriminated using statistical analysis of the residuals and the corrected Akaike criterion, but also by evaluating the practical application to predict the TMA formation from the model in a dynamic supply chain. The corrected Akaike criterion was calculated from Equation 5.1 (van Boekel, 2009):

$$AIC_c = n \ln\left(\frac{SS_r}{n}\right) + 2(p+1) + 2(p+1)\left(\frac{p+2}{n-p}\right) \qquad \text{(Equation 5.1)}$$

With:

AIC_c corrected Akaike criterion
SS_r residual sum of squares
n number of observations
p number of parameters.

5.3. Results and Discussion

The results obtained from the storage trials were used to consider existing TMA-models from literature (approach 1), to consider new models for the TMA formation (approach 2 and 3) and to describe the effect of temperature on the parameters of the mathematical models.

5.3.1 Description of the models

5.3.1.1 Model 1 (Adapted Howgate model)

TMA formation in fish fillets stored in ice or at refrigerated conditions from the moment of catch often starts with a period in which the TMA content does not increase. This phase is followed by another phase in which a rapid increase in the concentration of TMA is seen, followed by a third phase in which the rate of TMA increase diminishes. TMA is formed from the bacterial conversion of TMAO which is available in limited amount, therefore TMAO could be a limiting factor for the growth of the specific spoilage organisms and might be an explanation for the maximum TMA limit seen in the graphs of the TMA formation after the exponential phase. The formation of TMA can be described by a logistic model (Howgate, 2010b) (Equation 5.2):

$$C_{TMA} = \frac{C_{\max} - C_{\min}}{1 + e^{k(t_d - t)}} \qquad \text{(Equation 5.2)}$$

With:

C_{TMA} concentration 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)

C_{min} lower asymptote concentration (mg TMA-N per 100 g fish)

k   growth rate coefficient (hours⁻¹)

t_d inflection point (hours)

C_{min} is a parameter that represents the minimum asymptotic concentration of TMA, which will usually have a low value (<1). Although Howgate (2010b) suggested the logistic model for the TMA formation described in Equation 5.2, he mainly used an exponential model $C_{TMA} = e^{k(t-d)} - 1 + a$ to describe TMA formation in fish stored on ice, with the parameter *d* to represent the dwell (hours) and *a* (mg TMA-N per 100 g fish) to describe a coefficient representing the concentration at *t*=0. This model does not include a parameter for the maximum concentration and therefore the model is increasing exponentially also during advanced spoilage, while the TMA-plots show a decreasing rate of TMA formation. This model is therefore only applicable for the initial stage of TMA formation. Therefore, the 4-parameter logistic model for the TMA formation, with the inclusion of the parameter C_{max}, resulted in a better general trend for our data at higher temperature (5, 10 and 15 °C).

When studying the logistic model described by Howgate (2010b), a problem with the function was noticed and perhaps this was the reason for Howgate (2010b) to use the simpler exponential model, although he did not mention it. In Equation 5.2 the values

for the parameters C_{min} and C_{max} (the lower and upper asymptotes, respectively) are completely correlated to each other, because the function describes the difference of both parameters. This implies that when C_{min} is higher than 0, the C_{max} concentration of TMA can never be reached with the model, because the graph will reach an asymptotic value of $C_{max}-C_{min}$ for high values of t, instead of a value of C_{max}. But also increasing values for the parameter C_{min} will actually lead to lower estimations of concentrations of C_{TMA} at the same t and C_{max}, since the value of the numerator above the fraction bar decreases with increasing C_{min}. These problems might be solved by changing Equation 5.2 to the 'adapted Howgate model' described in Equation 5.3:

$$C_{TMA} = \frac{C_{max} - C_{min}}{1 + e^{k(t_d - t)}} + C_{min}$$

(Equation 5.3)

This 'adapted Howgate model' was used in this research for further investigation on its ability to fit the formation of TMA.

5.3.1.2 Model 2 (Baranyi–Roberts model with yield factor)

The adapted Howgate model is purely empirical. In principle, it is better to strive for mechanistic models. Therefore the mechanism of TMA formation in cod was studied. TMA is produced from the microbial growth of bacteria that use TMAO as substrate. Not all bacteria are able to produce TMA. *Shewanella putrefaciens*, *Photobacterium phosphoreum*, *Vibrionaceae*, and *Enterobacteriacea* are the bacteria that are able to reduce TMAO to TMA during storage of fresh and packed fish (Gram and Huss, 1996). However at chilled (or iced) storage of fresh or packed cod fillets the specific spoilage organisms are *S. putrefaciens* and *P. phosphoreum* with a yield factor of $10^{-9.5}$ mg-N TMA/CFU and $10^{-8.0}$ mg-N TMA/CFU respectively (Dalgaard, 1995b). *P. phosphoreum* is the specific spoilage organism of CO_2 packed fish (Dalgaard, 1995a; Gram and Huss, 1996) and the assumption was made that these bacteria will produce most of the TMA in the air-packed fish studied in this research as well, since O_2 is replaced by CO_2 due to bacterial respiration during storage. So, the formation of TMA might be modelled using microbial growth models of bacteria that produce TMA. The TMA-measurements during storage trials can be used to estimate the count of TMA-producing bacteria by Equation 5.4:

$$\text{TMA - m.o. Count} = \frac{\text{TMA concentration} / 100}{\text{TMA Yield factor}}$$

(Equation 5.4)

With:

TMA-m.o. Count   Count of TMA-producing bacteria (CFU/g)
TMA concentration   TMA concentration in cod (mg N/100 g fish)
TMA Yield factor   TMA Yield factor of *P. phosphoreum* ($10^{-8.0}$ mg-N TMA/CFU).

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However, instead of using the microbial count, the TMA content was used directly, but it was used in microbial models as if it was the microbial count of TMA-producing micro-organisms.

The content of TMA (from bacteria) was modelled using the Baranyi–Roberts model (Equations 5.5 and 5.6) (Baranyi and Roberts, 1994), which can be seen as a semi-mechanistic model (Baranyi et al., 1993). Other empirical sigmoidal models could have been used as well; however the basis of the Baranyi model is also a sigmoidal model and it is not likely that other sigmoidal models would lead to very different results.

The variables for the counts of micro-organisms (*N*) are replaced by variables (*C*) for the TMA content, and the TMA content was used directly in the models, not the logarithmic numbers as is often used in microbiology.

$$\frac{\mathrm{d}C_{TMA}}{\mathrm{d}t} = \mu_{\max} \frac{q(t)}{1+q(t)} C_{TMA} \left(1 - \left(\frac{C_{TMA}}{C_{\max}} \right)^{m} \right) \qquad \text{(Equation 5.5)}$$

$$\frac{\mathrm{d}q}{\mathrm{d}t} = \mu_{\max} \cdot q(t) \qquad \text{(Equation 5.6)}$$

In this equation, the physiological state of the cells that affects the length of the lag period is described by the adjustment function $\alpha(t)$ (Equation 5.7) (Baranyi and Roberts, 1994):

$$\alpha(t) = \frac{q(t)}{1+q(t)} \qquad \text{(Equation 5.7)}$$

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 growth rate coefficient (hours^{-1})

m   curvature parameter to characterize the transition from the exponential to the stationary phase (-)

q(t)   physiological state of the cells (-)

With parameters for initial value in the numerical integration:

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

q(0)   physiological state of the cells at time 0 (-).

The Baranyi–Roberts model is a dynamic model with a differential equation that allows to make predictions with time-varying temperature conditions and the model incorporates

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the effect of changes in the environment (like temperature) on the adaptation of micro-organisms to the new environment (Baranyi and Roberts, 1994).

However, a few disadvantages were noticed when using this model for the TMA formation. First, the model contains a lot of parameters, and the complexity of the model makes it difficult to find reasonable parameter estimates. Different values for the maximum specific growth rate μ_{max} in the growth function and the adjustment function were necessary, while these values were set the same by Baranyi and Roberts (1994). Another main disadvantage was that the parameters N_0 and μ_{max} were strongly correlated. And last, the $q(t)$ values in the adjustment function kept on increasing exponentially, while the adjustment function did not change significantly anymore from a certain level of values for $q(t)$. However, these very high values for $q(t)$ can give difficulties in the fitting process.

5.3.1.3 Model 3 (Simplified Baranyi–Roberts model with yield factor)

The third approach consisted of a simplified exponential microbial model, based on the Baranyi–Roberts model described in Equation 5.5. The Baranyi model could predict the TMA formation well, but a strong correlation was found between several parameters upon estimation. Therefore the amount of parameters was reduced by eliminating parameters that were strongly correlated to other parameters. A model with the least number of parameters as possible is desired according to the principles of mathematical modelling (van Boekel, 2009). Besides, the adjustment function $\alpha(t)$ was removed from the model, since the effect of adjustment of micro-organisms to a new environment can be neglected in storage trials where the micro-organisms already had some time to adapt and grow on the fillets before the storage trials started. The following 'Baranyi simple' model was the result (Equation 5.8):

$$\frac{\mathrm{d}C_{TMA}}{\mathrm{d}t} = \mu_{\max} C_{TMA}\left(1 - \left(\frac{C_{TMA}}{C_{\max}}\right)\right) \qquad \text{(Equation 5.8)}$$

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 growth 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).

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The differential equation for C_{TMA}, which is the TMA concentration at time t, contains the remaining parameters C_0 which is the TMA concentration at $t=0$, and C_{max}, which is the maximum concentration that can be reached, and μ_{max} which is the maximum rate of TMA formation. This model is similar to the Richards equation described in Baranyi (2010) with parameter $m=1$ (value of $m=1$ is suggested by Baranyi (1997)), which is originally a model that describes the growth of plants (Richards, 1959).

5.3.2 Parameter estimations and effect of fish batch and temperature
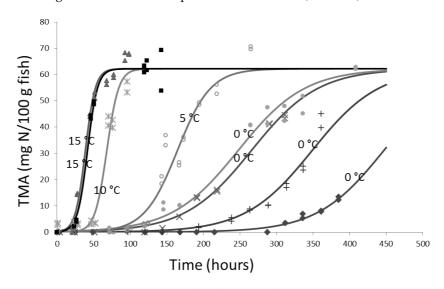
5.3.2.1 Adapted Howgate model (Equation 5.3)

The models were fitted to the data from the TMA measurements (Figure 5.1) and non-linear regression was used for parameter estimation (Table 5.1).

Figure 5.1 Adapted Howgate model (lines) and data (markers) of TMA content from cod stored at 0 °C (●, ✕, ＋, and ◆), 5 °C (○), 10 °C (✳) and 15 °C (▲ and ■).

During advanced storage the TMA content reached a maximum asymptote of C_{max}. The parameters C_{max} and C_{min} were not highly dependent on temperature and batch and the estimations of respectively 62.4 ± 0.82 mg N/100 g fish and -0.665 ± 0.50 mg N/100 g fish for these parameters were therefore fixed for all batches at all temperatures. The negative value for the parameter C_{min} is not a realistic value, but can be estimated since the model is purely empirical.

Table 5.1 Overview of parameter estimates with standard deviation, residual sum of squares (*RSS*), and Akaike criterion (*AIC*) for applied 'adapted Howgate model' (with parameters: growth rate coefficient (*k*), inflection point (t_d), and lower (C_{min}) and upper (C_{min}) asymptote concentration of TMA) and the 'Baranyi simple model' (with parameters: maximum specific growth rate coefficient (μ_{max}), initial concentration at time *t*=0 (C_0), and upper (C_{min}) asymptote concentration of TMA).

	Adapted Howgate Model		Simple Baranyi Model	
k / μ_{max} (h⁻¹)	0 °C	$0.0211 \pm 1.23{*}10^{-3}$	0 °C	$0.0219 \pm 1.16{*}10^{-3}$
	5 °C	$0.0430 \pm 3.38{*}10^{-3}$	5 °C	$0.0440 \pm 3.40{*}10^{-3}$
	10 °C	$0.119 \pm 1.34{*}10^{-2}$	10 °C	$0.123 \pm 1.42{*}10^{-2}$
	15 °C	$0.142 \pm 1.11{*}10^{-2}$	15 °C	$0.146 \pm 1.13{*}10^{-2}$
t_d (hours)	0 °C	265 ± 4.86		
		446 ± 9.12		
		246 ± 5.08		
		348 ± 4.40		
	5 °C	164 ± 1.97		–
	10 °C	67.7 ± 1.04		
	15 °C	38.3 ± 1.04		
		41.2 ± 1.00		
C_{min} / C_0 (mg N/100 g fish)			0 °C	$0.183 \pm 5.65{*}10^{-2}$
				$0.00346 \pm 1.61{*}10^{-3}$
				$0.272 \pm 8.57{*}10^{-2}$
		-0.665 ± 0.50		$0.0297 \pm 1.14{*}10^{-2}$
			5 °C	$0.0449 \pm 2.43{*}10^{-2}$
			10 °C	$0.0146 \pm 1.46{*}10^{-2}$
			15 °C	$0.222 \pm 1.04{*}10^{-1}$
				$0.145 \pm 7.53{*}10^{-2}$
C_{max} (mg N/100 g fish)		62.4 ± 0.8		62.2 ± 0.8
RSS		1975.44		1999.28
AIC		482.8		480.9

There was a high variation in the estimation of C_{max} at 0 °C and this can be explained by not reaching the limit of TMA formation in some trials, making it difficult to make reliable estimation of C_{max} for these trials. The data of all storage trials (from different batches and different temperatures) were used to estimate one value of C_{max}, this explains the high TMA measurements ($>C_{max}$) seen in some trials.

TMAO found in muscle cells of fish fillets can be reduced to TMA or enzymatically transformed to dimethylamine and formaldehyde during storage, but the TMA formation is most significant during chilled storage. The estimated value of 62.4 mg N/100 g fish for C_{max} could be explained by the initial TMAO levels in fish, cod fish generally have TMAO concentration of ~70 mg N/100 g fish (Herland et al., 2009). The TMAO content depends on several factors, like age, size, gender, season, feed, water temperature, wild/farmed etc. (Herland et al., 2009); therefore these factors could influence the C_{max} content as well.

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The rate constant k was strongly dependent on temperature as expected, but not batch dependent. Therefore different k-values were estimated for each temperature, but one k-value was predicted for all the batches at the same temperature.

The temperature-dependence of the growth rate coefficient k was modelled using the Arrhenius equation (Equation 5.9).

$$k = Ae^{-E_A/(RT)}$$
(Equation 5.9)

With:

k growth rate coefficient (hours^{-1})

A pre-exponential factor (hours^{-1})

E_A activation energy (J mol^{-1})

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

T temperature (K)

This resulted in a value of $1.77*10^{15}$ h^{-1} for the pre-exponential factor A and 88.3 kJ mol^{-1} for the activation energy E_A, which are typical values for chemical reaction in foods (van Boekel, 2009). But as was seen from the graph (graph not shown here) k did not increase exponentially in the (small) temperature-range, therefore a secondary microbial model was used to model the temperature dependence of the rate constant k, similar as what was done for the simplified Baranyi model. As can be seen from Table 5.1, the values for k from the Howgate model (Equation 5.3) and μ_{max} of the Baranyi simple model (Equation 5.8) are almost the same, therefore the application and results from the secondary microbial model the extended square root model of Ratkowsky (Ratkowsky et al., 1983) (Equation 5.10) to describe the temperature effect on the rate of TMA formation that is described in section 3.2.2 and in Figure 5 can be applied on the k values for the Howgate model as well.

The parameter t_d was temperature dependent, but also batch dependent due to different lag times. This can be explained by the natural variation in the quality of fish fillets (e.g. season, pollution, water temperature, etc.), and by different history of fish samples before the storage experiment started (e.g., variable temperature, time between catch and arrival at auction, etc.).

The temperature-effect of the parameter t_d was modelled using an empirical exponential trendline (Figure 5.2). A few secondary microbial models for lag time were applied (hyperbola model and extended hyperbola model) as described by van Boekel (2009), but the fits were not well.
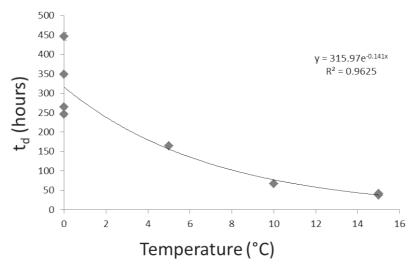
Figure 5.2 Temperature effect on parameter inflection point (t_d) of adapted Howgate model.

One of the reasons to develop mathematical models for the formation of compounds or micro-organisms, is to use the models to predict the formation from early measurements, e.g. from sensor measurements. A disadvantage of the batch dependency and large variation in the inflection point t_d is that this parameter cannot be estimated in advance and needs to be measured to be able to make reliable predictions. However, estimations from measurements for the inflection point can only be made after the increase of the TMA content has passed its maximum rate, making reliable estimation in earlier stages impossible.

5.3.2.2 Simple Baranyi model (Equation 5.8)
The plots for the Baranyi simple model (Equation 5.8) look similar to the plots with the adapted Howgate model (Equation 5.3) and prove that the TMA formation can also be predicted at 0 °C and at 5 °C, 10 °C and 15 °C (Figure 5.3) from a model with 3 parameters. The parameter estimates are given in Table 5.1. To be sure, the Baranyi simple model was not integrated analytically but numerically using the software program Athena Visual Studio.
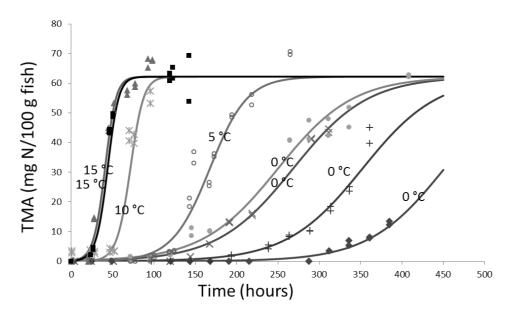
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Figure 5.3 Simple Baranyi model (lines) and data (markers) of TMA content from cod stored at 0 °C (●, ✕, +, and ◆), 5 °C (○), 10 °C (✳) and 15 °C (▲ and ■).

The parameter C_{max} could be predicted by a global estimation for all the trials at the different temperatures and from the different batches, being 62.2 ± 0.811 mg N / 100 g fish. The maximum concentration parameter seems to underestimate the actual maximum concentration in some trials in Figure 5.1 and 5.3, but this is caused by the fact that one maximum concentration was estimated from all the trials.

The value for C_0 was found to be batch dependent, but an effect of temperature was not found (Figure 5.4). Probably, the history (time between catch and auction and environmental conditions) of the fish fillet before the storage experiment started and the natural variation in fish quality will have an influence on these values.
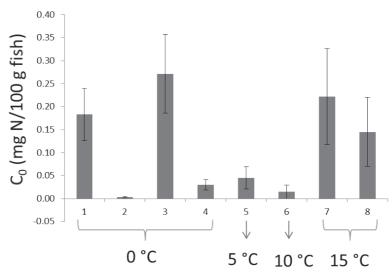
Figure 5.4 Effect of batch and temperature on the parameter C_0 (error bars represent standard deviation) of the Baranyi simple model.

Temperature had a large influence on the maximum growth rate μ_{max} of the formation of TMA (Figure 5.5). The estimates of the parameter μ_{max} of the Baranyi simple model are similar to the estimates for the parameter k of the adapted Howgate model. The estimates of the parameter μ_{max} for the TMA formation was compared with the maximum growth rate of *P. phosphoreum*, since this bacteria is the specific spoilage organisms of packed fish and therefore probably the main producer of TMA. The extended square root model of Ratkowsky (Ratkowsky et al., 1983) was used to model the parameter μ_{max} with the use of the parameter estimation of T_{max} is 25 °C taken from Dalgaard (1993) (Equation 5.10):

$$\mu_{max} = \left(b(T - T_{min})\left(1 - e^{c(T - T_{max})}\right) \right)^2 \qquad \text{(Equation 5.10)}$$

With:

T_{min} = minimum temperature at which the rate of growth is zero (°C)
T_{max} = maximum temperature at which the rate of growth is zero (°C)
b = regression coefficient (°C h^{-1})
c = additional parameter for fit (°C h^{-1})

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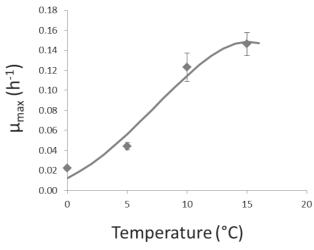R31
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Figure 5.5 Effect of temperature on the parameter maximum specific growth rate coefficient (μ_{max}) of the Baranyi simple model.

The estimated values for the parameters b, c and T_{min} are respectively 0.029 °C h^{-1}, 0.12°C h^{-1} and −4 °C. The shape of the curve for the μ_{max} for the TMA formation and for the maximum specific growth rate of *P. phosphoreum* described by Dalgaard (1993) was similar with a similar predicted T_{max}, but differences were seen in the absolute value for μ_{max}. But Dalgaard (1993) described the growth of *P. phosphoreum* in a medium containing TMAO, which is usually higher than the growth of micro-organisms on fish tissue (Gram and Melchiorsen, 1996).

5.3.3 Comparison of the models

The three approaches all describe a sigmoid curve that can be used to described the TMA formation. The second approach with the Baranyi–Roberts equation had too many parameters and it was decided that this model was too complex to describe the TMA formation. The results of the first and third model approach were compared to each other.

The first approach is purely empirical, the third one is semi-mechanistic based on microbial growth models. Although the 'adapted Howgate model' (with C_{min}) of Equation 5.3 of approach 1 is an empirical model, the model is similar to the integrated form of the 4-parameter logistic model suggested by Dalgaard (1995a) for the growth of micro-organisms on packed cod. The prediction of shelf life is based on the growth of *S. putrefaciens* and *P. phosphoreum* in model substrates and on cod fillets stored at 0 °C. No experiments at higher temperatures were done, therefore the models were not evaluated at higher temperatures as was done in this research.

We plotted the adapted Howgate model on the model predictions of the Baranyi simple model (Equation 5.8) and they describe almost similar sigmoid shape, but the Baranyi simple model only uses 3 parameters, while the Howgate model needs 4 parameters to predict the shape.

The parameter estimation for the values of C_{max} of the adapted Howgate model and the Baranyi simple model and k and μ_{max} of both models are comparable to each other. The adapted Howgate model uses the t_d and C_{min} (the lower asymptote) to describe the moment of increase, while the Baranyi simple approach uses the C_0 for it. The shape of the equations look similar ($R^2=1$) and the residuals between both fits are small, but as can be seen in the plot with the data and both models at 5 °C and 10 °C shown in Figure 5.6 that the highest values for the residuals between the two curves take place around the inflection point of the adapted Howgate model.
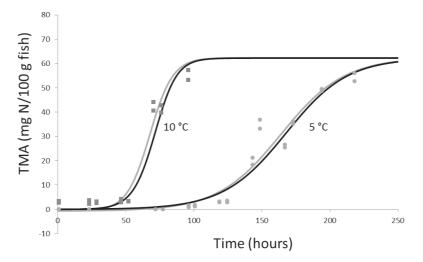

Figure 5.6 Plot with adapted Howgate model (—) and the Baranyi simple model (**—**) and data points at 5 °C (●) and 10 °C (■).

Statistical analysis showed that both models resulted in a residual sum of squares of $2.00 * 10^3$ and an Akaike criterion of 482.8 and 480.9 for the adapted Howgate model and the Baranyi simple model, respectively. On basis of these statistical values model discrimination appears not possible, in other words, both models function equally well. In the "Baranyi simple model" a high correlation (correlation coefficients R of −0.92 to −0.99) was found between C_0 and μ_{max}, but since this function is a differential equation, a value for C_0 is necessary. A disadvantage is that very small values for C_0 are sometimes estimated to give the best fit, which might lead to difficulties with the modelling software, at least this was experienced when using Athena Visual. A big advantage of

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"Baranyi simple model" compared to the adapted Howgate model is that the first one is a differential equation, describing the rate at a certain condition in the fish, while the second one is an algebraic equation. A differential equation makes it easier to make predictions of the freshness status or shelf life with dynamic temperatures, which occur in the supply chain and the history of the food can be taken into account. The adapted Howgate model is an explicit model and explicit models cannot always be converted straightforward to differential equations, without losing necessary parameters, e.g. parameters that describe the lag time (Van Impe et al., 1992). A large disadvantage of the adapted Howgate is the batch-dependent t_d value that cannot be established from early measurements, making practical application of this model to predict the remaining shelf life in the supply chain from early measurements impossible.

5.4. Conclusions

In this research mathematical models for the formation of TMA were studied in order to predict the freshness of cod fillets. Overall, it can be concluded that the dynamic simple Baranyi–Roberts model (Equation 5.8) has the most potential for the simulation of TMA formation in fresh fish in food supply chains where temperature is not always constant, since this type of models can incorporate the effect of temperature changes. The temperature effect, from 0 °C to 15 °C, on the parameters of the Baranyi simple model is known and the model is able to fit the TMA formation at different constant temperatures well, but it needs to be studied and validated with dynamic temperatures in future research since the experiments were carried out only at static temperatures.
When sensors monitor the TMA content during storage of packed fish, accurate models to translate the sensor signal into a freshness status are crucial. This model can be used for the prediction of freshness or remaining shelf life from early measurements of TMA in the fish fillets stored in a supply chain with dynamic temperatures.

Acknowledgement

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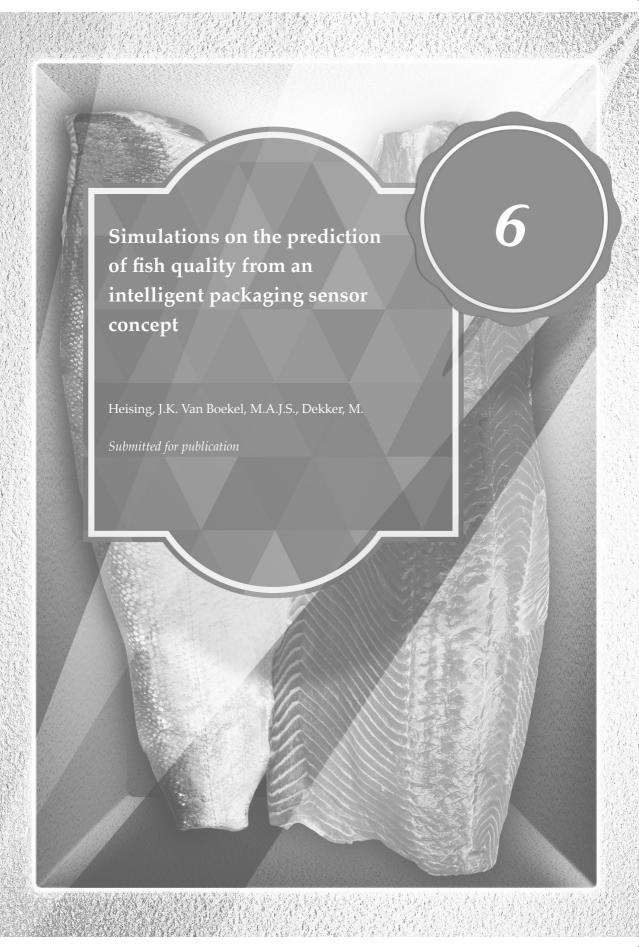R14
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Simulations on the prediction of fish quality from an intelligent packaging sensor concept

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Heising, J.K. Van Boekel, M.A.J.S., Dekker, M.

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Abstract

A non-destructive method that monitors changes in the freshness 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. This article describes the development of a mathematical model to predict the freshness of the packed fish from the sensor signal. The model describes the mass transfer of the freshness related compound trimethylamine (TMA) that is formed on the fish, released in the headspace and finally dissolves and dissociates in the sensing aqueous phase. The model is based on physical and biochemical principles of biological formation, mass transport, partitioning, and dissociation of TMA. The parameters in the model are partly from physical chemical properties, partly 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 sensing aqueous phase comparable with sensor measurements from a storage trial at 15 °C. Model outcomes from simulations with the geometry parameters 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 temporally temperature abuse, but needs a temperature-dependent correction for higher temperatures. 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. When the conductivity-sensor is combined with a temperature sensor this model can 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

6.1. Introduction

Dynamic information about the quality status of foods supplied by intelligent packaging can contribute substantially to the optimization of supply chain management. 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. Foods like fresh fish, with a highly variable quality on the moment of packaging, require sensors monitoring compounds directly correlated with food quality (Heising et al., 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 et al., 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 et al., 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 et al., 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 (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 with 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.

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 quality and to simulate the miniaturized intelligent packaging concept.

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6.2. Materials and Methods

6.2.1 Data collection
6.2.1.1 Storage trial of cod fillets
Data for parameter estimation were collected in the experimental trial with cod fillets described by Heising et al. (2014a) and the data are reported in the present article.

Cod (*Gadus morhua*) was bought at "Zeevisgroothandel J. Thiele & ZN" 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.

6.2.1.2 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 analyse an aqueous phase in a beaker separate from the fish (Figure 6.1) (Heising et al., 2012).

~375 g cod fillets, sliced into pieces of approximately 30 g, was 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 15 °C, filled with water and antifreeze, located in a temperature controlled room.

6.2.1.3 Destructive TMA analysis
The fish samples for the destructive TMA analysis were packed separately in aluminium 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 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 and 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.

6.2.2 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 of the performance of the complete model, were obtained by least squares regression with the help of the software package Athena Visual Workbench (www.athenavisual.com).

6.3. Results and Discussion

6.3.1 Model development

The non-destructive method consists of an aqueous phase in which electrodes measure the changes in the electrical properties of the aqueous phase (Figure 6.1). These changes are caused by volatiles produced by the packed fish fillet, partitioning in the headspace and dissolving in the aqueous phase.
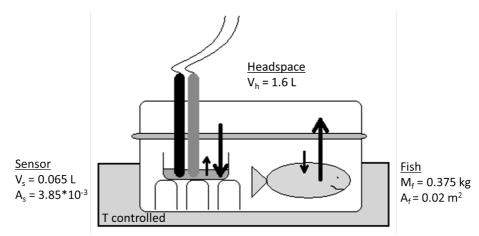
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Figure 6.1 Systematic picture of the measurement set-up with geometric parameter values (M = mass, V = Volume, A = Surface, T=Temperature) of fish (subscript f), headspace (subscript h) and sensor (subscript s).

6.3.1.1 Formation of TMA

TMA is produced on fresh cod fillets stored at chilled temperatures by the micro-organisms *Shewanella putrefaciens* and *Photobacterium phosphoreum* and the formation can be described by a dynamic model (Equation 6.1) (Heising et al., 2014c):

$$\frac{\mathrm{d}C_{TMA}}{\mathrm{d}t} = \mu_{\max} C_{TMA}\left(1 - \left(\frac{C_{TMA}}{C_{\max}}\right)\right)$$ (Equation 6.1)

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 growth 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 quality status and the effect of natural variation in the quality of fish and C_{max} was estimated to be 62.2 mg N/100 g cod (Heising et al., 2014c). The effect of temperature on the the maximum growth rate μ_{max} of the formation of TMA could be described by the extended square root model of Ratkowsky (Ratkowsky et al., 1983) (Equation 6.2):

$$\mu_{max} = \left(b(T - T_{min})\left(1 - e^{c(T - T_{max})}\right)\right)^2$$ (Equation 6.2)

With:
T_{min} minimum temperature at which the rate of growth is zero (°C)
T_{max} maximum temperature at which the rate of growth is zero (°C)
b regression coefficient (°C h^{-1})
c additonal 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).

6.3.1.2 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:

$$(CH_3)_3N + H_2O \leftrightarrow (CH_3)_3NH^+ + OH^- \tag{1}$$

The fraction of the total TMA that is formed (from Equation 6.1, formation model) that remains in the undissociated form can be expressed according to Equation 6.3. 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 6.1 to the unit of mg/l.

$$F = \frac{[TMA]}{[\Sigma TMA]} = \frac{[TMA]}{[TMA] + [TMAH^+]} \tag{Equation 6.3}$$

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})
[ΣTMA] concentration of total TMA from Equation 6.1 (mg l^{-1})

The dissociation equilibrium is described by the dissociation constant, which is expressed as (Equation 6.4):

$$K_a = \frac{[TMA][H^+]}{[TMAH^+]} \tag{Equation 6.4}$$

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

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The dissociation constant pK_a (=-log K_a) for TMA at 25 °C is 9.81, but the dissociation constant depends on the temperature of the fish. Equation 6.5 describes the temperature dependence of the pK_a of TMA and this equation was adapted from the temperature dependence of the pK_a of NH_3 (Emerson et al., 1975), assuming the same temperature coefficient as was reported for NH_3 (Howgate, 2010).

$$pK_a = 0.6516 + 2729.2T^{-1}$$ (Equation 6.5)

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, but in the model a pH of 6.9 for raw cod fillets was used in the simulations (Sivertsvik et al., 2004).

6.3.1.3 *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 and partitioned in the headspace of the fish package. The partitioning 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} (Equation 6.6)

$$K_{hf} = \frac{c_f}{c_h} = k_H * RT$$ (Equation 6.6)

or rewritten to Equation 6.7 (Sander, 1999):

$$T * k_H = 12.2 K_{hf}$$ (Equation 6.7)

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 at 25 °C is 9.6 (mol L^{-1}) atm^{-1} (Sander, 1999) and 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 6.8):

$$\frac{d \ln k}{d \frac{1}{T}} = -\frac{\Delta H^{\ominus}}{R}$$

(Equation 6.8)

In integrated form (Equation 6.9):

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

(Equation 6.9)

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 $\dfrac{d \ln k}{d \frac{1}{T}}$ 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 6.6 is used to calculate the ratio K_{hf} of concentration of TMA in the fish and in the headspace.

6.3.1.4 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 6.10:

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

(Equation 6.10)

With:

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

V volume of fish (=M_f / ρ_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 K_L at temperature T (K) was estimated from K_{Lref}, the mass transfer coefficient that was estimated from the model at reference temperature 15 °C (Equation 6.11).

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$$K_L = K_{Lref} * \frac{T}{T_{ref}}$$

(Equation 6.11)

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)

6.3.1.5 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 6.2.
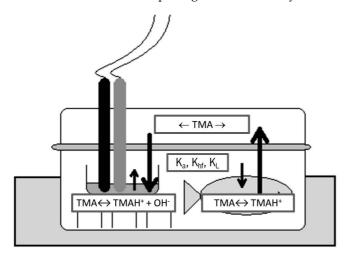

Figure 6.2 Schematic picture of mass transfer of TMA in the fish package.

6.3.1.6 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 this signal the freshness stage of the fish is predicted.

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6.3.1.7 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 $0 = C_0 \cdot \rho_f$ (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})

6.3.2 Model application

6.3.2.1 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 6.3). The fits of the model and the measurements are quite similar, therefore the general trend of the measurements is confirmed by the model.
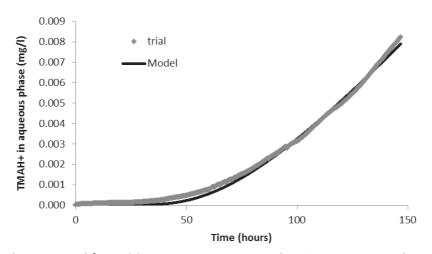

Figure 6.3 Fit of the model on sensor measurements of $TMAH^+$-concentration from a storage trial with cod stored at 15 °C.

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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 6.12):

$$K_L = \frac{D}{\delta}$$ (Equation 6.12)

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 et al., 1943). 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 et al. (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 μ_{max} for the formation of TMA at different temperature is estimated from Equation 6.2. 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.

6.3.2.2 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 6.1), except when other values for geometry parameters are mentioned.

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. 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 6.13 to calculate the surface belonging to the reduced volume.

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

(Equation 6.13)

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 6.14:

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

(Equation 6.14)

When the volume of the aqueous phase is reduced, the concentration of TMA in the aqueous phase increases (Figure 6.4). 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.
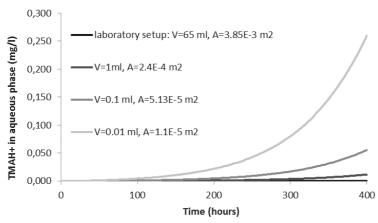
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Figure 6.4 Effect of volume and surface of sensor exposed to headspace (with corrected K_L) on the concentration of TMA (mg/l) in the sensing aqueous phase from simulations at 0 °C.

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 et al., 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 6.5 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.
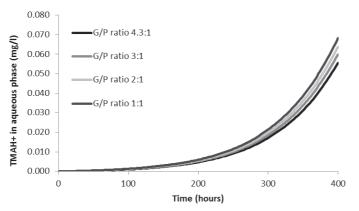

Figure 6.5 Effect of headspace volume on the concentration of $TMAH^+$ in the sensing aqueous phase from simulations at 0 °C (V_s=0.1 ml; A_s=5.13*10^{-5}).

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6.3.2.3 Simulations with variation in initial quality on the prediction of quality 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 and 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 quality 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 6.6), 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 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.
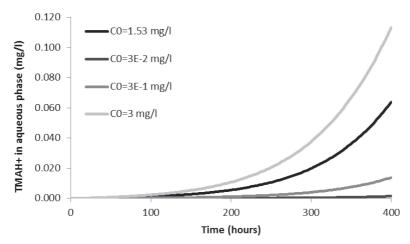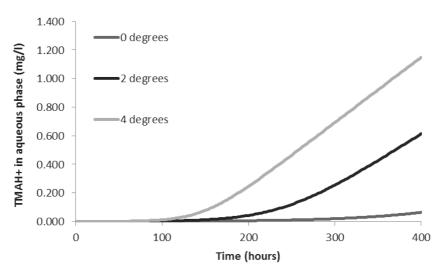

Figure 6.6 Effect of parameter C_0 on the concentration of TMAH$^+$ in the sensing aqueous phase from simulations at 0 °C (V_s=0.1 ml; A_s=5.13*10^{-5};V_h=750 ml).

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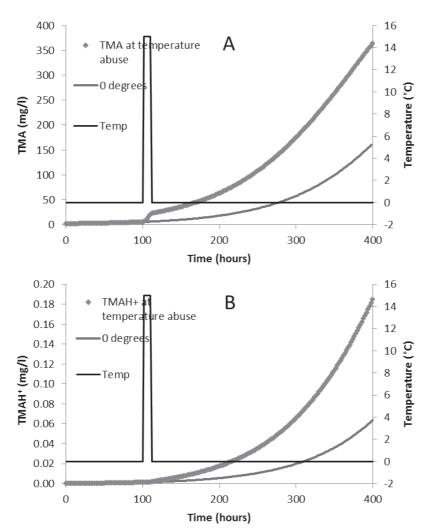R25
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6.3.2.4 Simulations with dynamic temperatures on the prediction of quality in the supply chain

In the simulations above the temperature was set at 0 °C. Figure 6.7 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.

Figure 6.7 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).

However, the temperature fluctuates in the cod supply chain (Hafliðason et al., 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 6.8A). 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 6.8B).

Figure 6.8 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).

6.3.2.5 Practical considerations to translate the predicted sensor outcome to a quality 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 quality 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 usual acceptability limit for chilled cod of 15 mg TMA 100 g^{-1} (Venugopal, 2002) to calculate the moment of spoilage according to

the sensor predictions. Simulations where performed with the miniaturized parameter conditions, but the temperature and initial TMA concentration in the fish was varied for the different simulations.

At a constant temperature of 0 °C the spoilage limit of 15 mg N TMA 100 g^{-1} fish was reached after 387 h and the TMAH$^+$ in the sensing aqueous phase was 0.0552 mg l^{-1} (Table 6.1). 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, so more than 100 hours earlier compared to the constant 0 °C simulation. 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 0.55 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 should lead to a shorter remaining shelf life. When the initial TMA concentration was increased from 1.53 mg l^{-1} to 3 mg l^{-1} the fish reached the spoilage limit of 0.55 mg l^{-1} in the aqueous phase after 334 hours this was also the moment when 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 variable initial quality.

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.

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.

Table 6.1 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

6.4. 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 and taken into account in the model 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. When the conductivity-sensor is combined with a temperature sensor this model can be used in the development of an intelligent packaging to monitor the freshness of fish.

Acknowledgement

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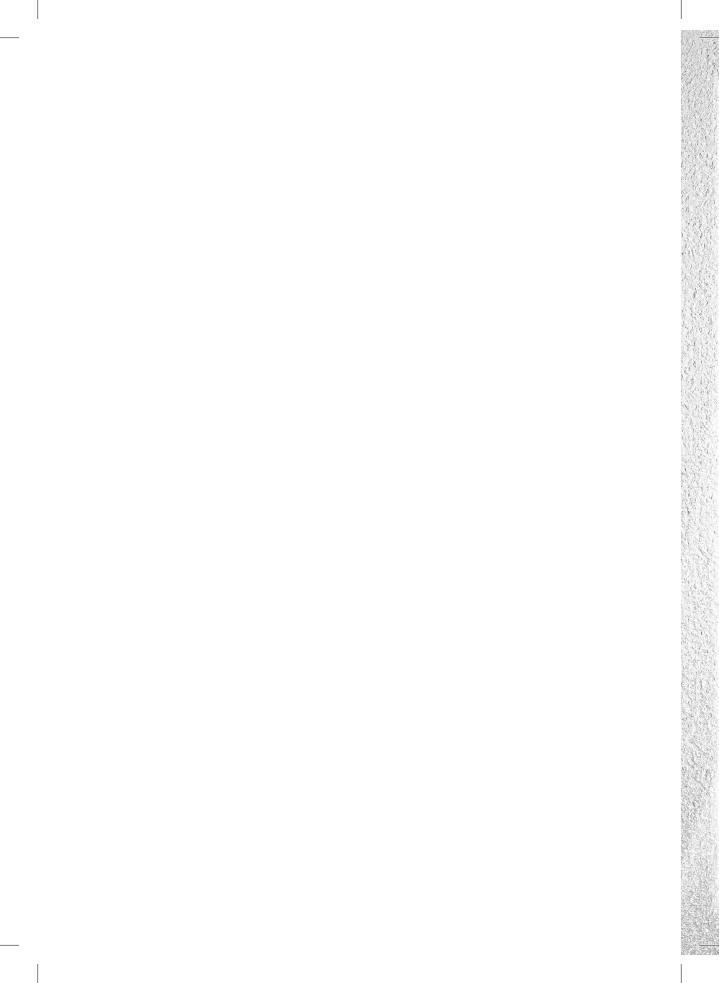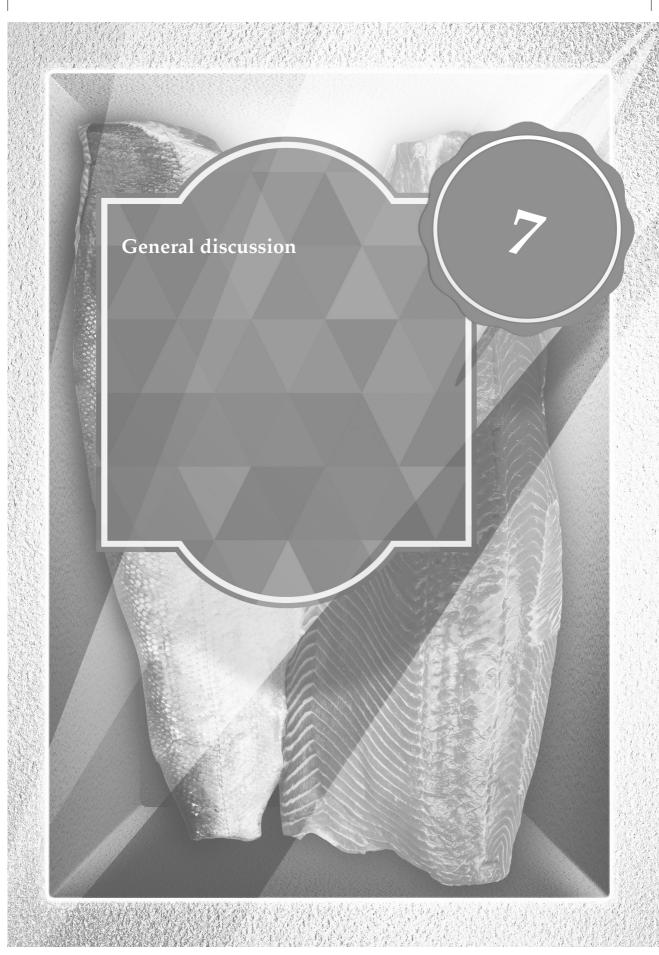
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General discussion

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The general aim of this thesis was to study the possibilities to develop intelligent packaging for monitoring the quality of food. This thesis started with a review article in which our viewpoint on how to approach this was given as well. In the review, we identified opportunities to monitor food quality by intelligent packaging by giving guidelines for which foods this will be most beneficial and what type of intelligent packaging needs to be developed for which type of food from our point of view. In the following chapters the development of an intelligent packaging concept for the selected food, fresh cod fillets, is described (approach shown in Figure 7.1). The present chapter contains the discussion of the results and the discussion of the implementation of a sensor on packed fish and the application of intelligent packaging on foods in general.
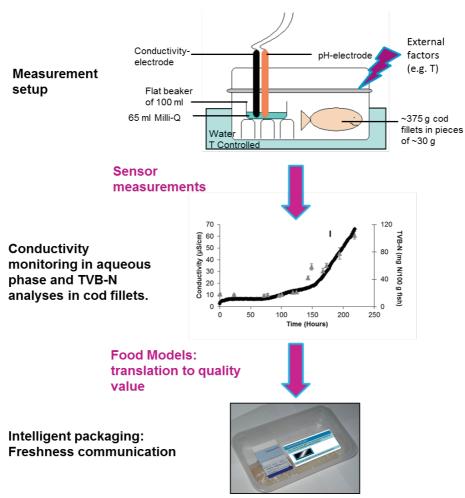

Figure 7.1 Overview of the approach of the development of an intelligent packaging concept for non-destructive monitoring in the headspace of packed foods with fresh fish as case study.

7.1. Main findings

Foods that will benefit the most from intelligent packaging are relatively expensive, highly perishable foods **(chapter 2)**. Foods with known or low variation in initial quality can benefit from sensors that monitor the conditions that influence the quality of the packed foods, like storage time and temperature. Foods with an unknown initial quality, or a high variation in initial quality, need sensors that monitor compounds or properties of the food that are directly linked to quality attributes of the food **(chapter 2)**. The initial quality of fresh fish is highly variable and therefore fresh cod fillets require sensors monitoring compounds correlated with the quality of the fish **(chapter 2)**. Non-destructive methods are needed for the development of an intelligent packaging for communicating fish freshness, the most important quality attribute of fresh fish **(chapter 2)**, throughout the supply chain from the moment of packaging until the day the fish is spoiled **(chapter 3)**.

We succeeded to develop a new non-destructive method with electrodes in an aqueous phase located inside the headspace of the package to monitor volatile amines in the package, compounds that are generally used as freshness indicator of the packed fish **(chapter 3, 4)**. An ammonium ion-selective electrode (NH_4^+-ISE) measures changes in the aqueous phase when ammonia, from an NH_3-solution or from cod fillets, is released in the headspace of the package **(chapter 3)**. The electrode response correlated with the trend of the generation of the TVB-N in the cod fillet **(chapter 3)**. Since very different electrode signals were observed at different temperatures, it was concluded that monitoring the pH in the aqueous phase is not an appropriate approach to monitor the freshness of packed fish with a non-destructive method **(chapter 4)**. The conductivity electrode output of the aqueous phase showed a characteristic response pattern during all the storage trials at 0-15 °C and is the most promising as freshness sensor for intelligent packaging due to the consistent output at different storage conditions **(chapter 4)**.

In **chapter 5** and **6** the signal of the conductivity-electrode was translated to a freshness status (on the basis of the quality indicator compound TMA) with the help of mathematical models. Existing models for the formation of TMA in fish needed improvements and models were needed for the effect of temperature on the formation of TMA **(chapter 5)**. A simplified dynamic Baranyi-Roberts model could describe the TMA formation with 3 parameters **(chapter 5)**. The effect of temperature on the parameter μ_{max} was described by the expanded square root model of Ratkowsky and the effect of different batches could be described by the initial TMA concentration C_0 **(chapter 5)**. This TMA formation model was used for the development of a model that describes the mass transfer of TMA that is formed on the fish, released in the headspace and finally dissolved and dissociated in the sensing aqueous phase **(chapter 6)**. The model predicted

TMA increase in line with actual sensor measurements from a storage trial at 15 °C and can, therefore, be used to predict the freshness of packed fish from sensor measurements **(chapter 6)**. Simulations with the model showed that minimizing the sensing aqueous phase will improve the sensitivity of the sensor to different freshness stages **(chapter 6)**.

7.2. Methodological considerations

7.2.1 Experimental set-up
To study the possibility to monitor the freshness of fish by sensors with electrodes, storage experiments were performed from which the non-destructive electrode signal results were compared with destructive measurements of TVB-N content of fish.

The experiments for the sensor monitoring in chapter 3 and 4 were performed with the measurement setup as shown in Figure 7.2. This setup consists of an air-tight glass cell with fish inside that represents a package for fish commonly used in retail, but important differences might be the different ratio of the volume of the headspace and the fish, and the package material being glass or plastic, respectively. The permeability of the package for volatile compounds depends on the package material used (Siracusa, 2012), but a low permeability for gases must be taken into account in the selection of plastics and due to the relatively short shelf life, the glass set-up was representative for the volatile compounds migration in a plastic fish package. The headspace/fish gas to product volume ratio (*g*/*p*-ratio) in our setup was 4.3:1 and is usually 2:1 in a commercial fish package (Sivertsvik, 2007). Besides from being not comparable to retail conditions, this high ratio in our set-up was not optimal for the sensor signal as it can dilute the signal and slow down the transfer of the volatile compounds from the fish to the aqueous phase where the compounds can be measured by the electrodes. The ratio might be improved, for example by minimizing the electrodes and the aqueous phase, which should be done by electrode developers (e.g. at the Holst Institute in Eindhoven http://www.holstcentre.com). This minimization will improve the sensor signal; the higher concentration of volatile compounds in the headspace and subsequently in the aqueous phase leads to a higher sensor signal that makes a better distinction between different stages of freshness.

Another improvement in the sensor signal will be to optimize the surface/volume ratio of the aqueous phase. The influence of the geometry on the kinetics was mentioned in calculations for the diffusion and partitioning of NH_3 in the aqueous phase, headspace and source solution/fish in chapter 3. Improvements in the sensor signal might be obtained by increasing the sensor surface to accelerate the diffusion or decreasing the amount of water in the aqueous phase to increase the concentration of dissolved compounds. In chapter 6 the influence of the geometry of the setup was studied in model

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simulations and the hypotheses on the effects of the geometry on the sensitivity of the sensor were confirmed. Experiments with a minimized sensor need to prove the effect of the geometry on the sensor signal.
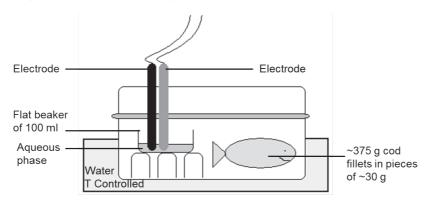

Figure 7.2 General measurement set-up for monitoring changes in aqueous phase with electrodes. The whole set-up was placed in a temperature-controlled room.

The TVB-N content was determined during the storage experiments to determine the freshness status of the fish fillets (chapter 3 and 4). The TVB-N content was measured in the cod fillet according to the steam distillation method described by Malle and Tao (1987). Quantifying TVB-N compounds by steam distillation in a protein-free fish extract was chosen, because it is proven to be a simple, rapid and inexpensive method, and both TMA and TVB-N content could be determined in the same extract (Botta et al., 1984; Malle and Tao, 1987; Howgate, 2010a). Although differences (up to >100%) in actual values do exist between different methods for TVB-N determination (Botta et al., 1984; Howgate, 2010a), this was not a problem in this thesis, since the same method was used for all storage trials and it was only the trend that was important in this research to compare to the trend of the electrode signals.

A difficulty in the experimental setup was found in the use of different fish samples for the non-destructive electrode monitoring and the destructive TVB-N measurements. The storage conditions for the parallel stored fish samples were kept as similar to the storage conditions of the fish in the glass cells as possible, but small differences in temperature were noticed and natural variation between the fish samples existed. The natural variation was minimized by slicing different fish fillets of 1 batch into pieces of 30 g and dividing the fish pieces between the different glass cells and parallel stored samples. The temperature was controlled by using temperature loggers on several places during the storage trials. However, these efforts could not prevent small differences that lead to a variation in the quality measurements and differences in the quality of the destructive and non-destructive fish samples. This is very important when comparing the trend of

these 2 signals, as was done in the thesis, and better results might be obtained when the variation and differences in storage conditions can be further reduced. However, the use of parallel stored fish samples for non-destructive electrode monitoring and destructive TVB-N measurements, while comparing the results of both methods, will always be a critical aspect of the experimental setup. This is a disadvantage of doing experiments with real foods instead of model systems where a lot of variation can be controlled. But the choice of the use of fish fillets for testing the sensor systems is necessary, since fish quality deterioration at different conditions is a complex process that depends on many factors, therefore large differences can occur between model systems and the real foods.

7.2.2 Choice of freshness measurements: Are TVB-N compounds good quality indicators?

Since continuously monitoring requires non-destructive methods (chapter 2), volatile compounds are often chosen as quality indicator compounds for the development of intelligent packaging concepts (Kerry et al., 2006). It needs to be considered per type of food whether volatile compounds can be used as reliable quality indicator compounds. Freshness is a very important factor for the quality of fish and freshness can be evaluated by different approaches (Ólafsdóttir et al., 1997). Sensory analysis is often used to evaluate fish freshness, but fast objective methods were preferred for this thesis. Odour is one of the most important parameters used to evaluate fish freshness (Ólafsdóttir et al., 1997; Ólafsdóttir, 2005). The performances of the electrodes were evaluated by measuring TVB-N in the packed fish. The increase in the TVB-N content is mainly caused by the formation of 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, 2010a). The TMA content is strongly correlated to the sensory quality of cod (Gill, 1990), and the TVB-N trend has proven to be a good indicator for the freshness of many marine fish (Botta et al., 1984). Also in the trials conducted in our research, the curves of TVB-N showed characteristic and repeatable results (chapter 3 and 4). Variation in the dwell period could be explained by variability in initial quality, and small differences in temperature between different trials and between the non-destructive sensors measurements and the destructive TVB-N measurements. Besides, the TVB-N compounds are abundant in large amounts compared to other volatile compounds (Ólafsdóttir, 2005) and these TVB-N volatiles are able to dissociate in the aqueous phase, thereby directly related to the signal of the electrodes.

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7.2.3 Selection of electrodes

In chapter 3, an NH_4^+-selective electrode was used to monitor ammonia that is released from the packed fish fillet. The method functions well, as was demonstrated with the ammonia-solution that was used as ammonia-source in the glass cell instead of the fish. Also when ammonia was measured that was released from the fish fillet, it was monitored well by the NH_4^+-selective electrode. However, ammonia is not a very good freshness indicator compound for chilled cod fillets. Ammonia is formed in autolytic reactions that occur in the first hours after death (Gill, 1990), but after this phase the ammonia content doesn't change substantially until the fish is almost spoiled. In advanced stages of spoilage, ammonia is formed by bacteria (Huss, 1995). Since there are periods when the content of the indicator compound doesn't change, there is not a good correlation between the NH_3 content and the remaining shelf life. Therefore, only using an ammonium-electrode might not give enough information to predict the remaining shelf life of fish during its whole shelf life.

In literature, pH is described as freshness indicator for intelligent packaging, being able to monitor fish freshness by a pH electrode (Pacquit et al., 2006; Pacquit et al., 2007; Kuswandi et al., 2012). We performed pH measurements in the aqueous phase, when fish was stored in the glass cell. The conclusion was that using a pH signal to monitor the freshness of packed fish is unreliable due to a large variation in response patterns and correlation with TVB-N content at different temperatures (chapter 4). These results showed the different spoilage patterns at different temperatures and the importance of conducting measurements at different storage conditions, which is regularly overlooked in literature in the development of sensors for fish when sensors are giving good results at room temperature (Pacquit et al., 2006; Pacquit et al., 2007; Kuswandi et al., 2012). In the framework for the development of sensors for intelligent packages for food presented in this thesis, we emphasize the importance of the effect of storage conditions on the performance of the sensor by selecting it as one of the steps necessary to be taken in the development process of a sensor (see the section later on in this chapter on Guidelines and recommendations for developing an intelligent packaging for monitoring food quality). It also shows the difficulty of selecting a sensor for an intelligent packaging that is able to make reliable predictions under variable circumstances that can occur in the supply chain.

The pH had also influence on the performance of the NH_3-ISE that was studied in chapter 3. Pivarnik et al. (2001) described a destructive method using an ammonia-selective electrode as a rapid screening method to determine fish freshness directly in a fish extract. We used the NH_3-electrode to monitor dissolved volatile amines in the aqueous phase in the fish package, but found that the action of volatile acids (produced during storage) lowered the pH of the aqueous phase that caused a shift in the

$NH_3 \leftrightarrow NH_4^+$ equilibrium and making it impossible to monitor volatile amines from packed fish with an NH_3-ISE in this non-destructive method. This method might perform well when the influence of the acidic compounds can be prevented, making it possible to selectively monitor the volatile amines.

In chapter 4 a more general electrode was chosen, being a conductivity-electrode. This electrode is able to monitor a broad range of compounds, also compounds that are formed in early stages of freshness. Compounds that are formed in the fish can be measured only by the conductivity electrode when they comply with all of the following requirements:

- Compounds need to be volatile (the position of the partition equilibrium needs to be more towards the gas-phase, because compounds needs to partition sufficiently into the headspace) at 0 °C, so being able to be released from the fish into the headspace of the package
- Compounds need to be able to dissolve in the aqueous phase
- Compounds need to be able to dissociate in the aqueous phase or being able to induce a reaction in the aqueous phase causing a measurable change in the signal (e.g. enzymatic conversion)

This limits the amount and type of compounds being able to influence the signal of the conductivity electrode, but the TVB-N compounds satisfy all requirements.

7.2.4 Choice of fish as case example

Fresh cod fillet was chosen as an example food product for the development of an intelligent packaging, for several reasons. In chapter 2, it was concluded that foods for which intelligent packaging is most beneficial are relatively expensive, highly perishable foods, especially if consumers cannot estimate their essential quality attributes.

Fresh fish is a food category with a relatively high price/kg compared to other foods. The waste of expensive foods will result in a large loss of income in the food chain. Food is wasted throughout the whole supply chain, from initial agricultural production down to final household consumption. In medium- and high-income countries, food is to a significant extent wasted at the consumption stage, meaning that it is discarded even if it is still suitable for human consumption. The waste of fish at supermarket retailers and consumer level in Europe and North America is 9% and 11%, and 9% and 33% (Gustavsson et al., 2011). The application of freshness sensors on fish packages might help to reduce this large waste of fish at retailer and consumer level. The inclusion of a sensor on a package will increase the costs of the package and producers, retailers or consumers need to be willing to pay extra money for the extra costs of the package. For expensive foods, these costs are relatively lower than for a cheap food product. The

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extra costs can be balanced by the decrease in waste and thereby reduced loss of income (Kärkkäinen, 2003).

Highly perishable foods are prone to spoilage, which lead to waste of foods. Fish that is stored at chilled temperatures (0-4 °C) is still highly perishable. The shelf life of fresh fish (and other fresh foods) is highly influenced by temperature fluctuations or abuse that can happen in the supply chain (Hafliðason, 2012). A freshness sensor communicates information about the freshness of the packed food product. At present, consumers have to base their decision to consume the food on the 'use by date' printed on the package, because the quality of packed foods is difficult to estimate. For some products like vegetables in transparent packages, consumers can judge the freshness based on the appearance of the product. When the freshness needs to be assessed based on the non-visible properties of the food, people will face difficulties when the product is packed, e.g., consumers cannot evaluate the 'smell' of packed fish, which is an often used quality attribute (Ólafsdóttir, 2005).

Another reason that fresh fish was selected as case study, is because it has a large variation in initial quality. From Figure 2.4 from chapter 2 it was concluded that measuring environmental conditions is only related to the change of quality attributes and, therefore, if the initial quality of the product is not known and constant, a sensor that monitors a quality attribute of the food itself is necessary to give accurate predictions of the actual quality status. The initial quality of wild caught fresh fish is influenced by many factors that cannot be controlled (Ashie et al., 1996), therefore, we conclude indeed that fresh fish is a good example of a food for which a direct quality sensor is necessary. However, it was also found to be a difficult study object due to the high natural variation in quality. In the category fresh fish, cod fillets were chosen, because cod is marine fish that is often studied in scientific studies, which makes comparison and the use of other data more easy. Besides, fresh caught cod is available almost on a daily basis in the Dutch auction in IJmuiden.

From the decision tree developed in chapter 2 (Figure 7.3) it could be concluded that fish is a good case product for the development for an intelligent packaging because it is highly perishable and an expensive food product. The high variability in the initial quality of fish requires a sensor that directly monitors a quality attribute of the fish, otherwise only a quality change can be predicted, as is also the case for other foods with a high variation or unknown initial quality. This is not always described correctly in studies in which a freshness sensor was developed for a food, while actually an environmental condition was monitored and the variability in initial quality was not taken into account, e.g. monitoring the temperature (Watanabe et al., 2005) or monitoring oxygen or carbon

dioxide (Kim et al., 2011). The present research for the development of a quality sensor of fish could serve as an example for other foods and we would like to emphasize the scheme below as starting point for selecting an appropriate sensor for the right food.
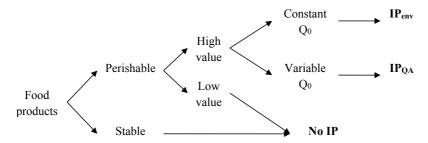

Figure 7.3 Decision-tree for choice of IP. Q_0 = initial quality, IP_{env} = IP monitoring environmental conditions, IP_{QA} = IP monitoring quality attributes

7.3. Modelling the sensor concept

The results obtained from the storage trials (chapter 3 and 4), supplemented with knowledge from literature, were used to develop mathematical models for the formation of TMA in packed fish (chapter 5), and used to develop a model for the mass transport of TMA in the fish, headspace and sensing aqueous phase of the package and to perform simulations with the intelligent packaging concept (chapter 6). Obviously, several assumptions had to be made to develop models for this system and these assumptions are discussed below. Besides, the mathematical models from literature and the ones developed by ourselves are discussed.

7.3.1 Remark on time 0
A remark has to be made on the definition of the time 0 that is used in this thesis. The time 0 of the storage trials, TMA formation models and mass transfer models is the moment when the sensor is applied on the package and the monitoring or storage of samples starts (chapters 3, 4, 5 and 6). Therefore, the time 0 in our models is not the same as the time 0 for the start of the formation of TMA or growth of micro-organisms in fish, which can be assumed to be the moment of slaughter. This can lead to difficulties with modelling, due to differences in the lag time when the period before 'time 0' has a different length (or temperature history).

7.3.2 Mathematical models for TMA formation
For the formation of TMA, several models were considered: Models for the formation of TMA from literature (Howgate, 2010b) and bacterial growth models, since TMA

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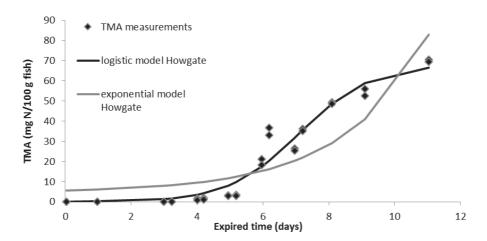R25
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is produced as a substrate and the shape of the graph of the TMA formation at static temperature has the characteristic S-shape of microbial growth.

7.3.3 Models for TMA formation, approach 1

Howgate (2010b) reviewed the kinetics of the formation of the volatile bases and applied the published data of TMA and TVB-N of many studies on several empirical models. According to Howgate (2010b) the formation of TMA can be described by an exponential model:

$$C_t = e^{k(t-d)} - 1 + a \qquad \text{(Equation 7.1)}$$

With:

C_t concentration at time t (mg TMA-N per 100 g fish)

k rate coefficient (days^{-1})

t time (days)

d dwell (days before the exponential phase starts)

a coefficient representing the concentration at time $t = 0$ (mg TMA-N per 100 g fish)

This model (Equation 7.1) does not include a parameter for a maximum concentration or asymptote, which we clearly saw in our data of TMA formation (Figure 7.4) and could be due to a limiting supply of TMAO or a maximum microbial growth limit. Therefore in the present research it was decided to study the use of the logistic model (Equation 5.2) with a parameter for the maximum TMA concentration in chapter 5. Howgate (2010b) mainly used the exponential model without a maximum concentration to describe TMA formation in fish stored on ice, but the inclusion of the parameter C_{max} resulted in more accurate predictions of TMA formation for our data, especially at higher temperature (5, 10 and 15 °C). Since the concentrations of the lower and upper asymptotes (C_{min} and C_{max}, respectively) of this model are fully correlated to each other, an 'adapted' Howgate model (Equation 5.3) was compared to new models for the TMA formation in the present thesis.

Overall we concluded that for the formation of TMA only a few empirical models are described in literature and that these models needed some improvement. Models for the TMA formation in fresh fish stored at 0-15 °C need to incorporate a maximum concentration that can be reached, and a parameter for the initial status.

Figure 7.4 Typical curve for TMA formation at 5 °C (own data) modelled with the logistic models from Howgate (2010b) (Equations 5.2 and 7.1).

7.3.4 Models for TMA formation, approach 2

After considering existing empirical models for the TMA formation from literature, we studied options to model TMA formation based on microbial growth models in chapter 5. The content of TMA was modelled using the microbial model the Baranyi-Roberts model, but we were able to make accurate simulations for the TMA formation in packed fish with a simplified Baranyi-Roberts as well.

We could not discriminate between the adapted Howgate model from Equation 5.3 and the Baranyi simple from Equation 5.8 on the basis of the Akaike criterion and residual sum of squares. But a large disadvantage of the adapted Howgate is the batch-dependent t_d value that cannot be established from early measurements. This makes the practical application of this adapted Howgate model to predict the remaining shelf life in the supply chain from early measurements in an intelligent packaging concept impossible.

A big advantage of "Baranyi simple model" compared to the adapted Howgate model is that the first one is a differential equation, describing the rate at a certain condition in the fish, while the second one is an algebraic equation. A differential equation makes it easier to make predictions of the freshness status or shelf life with dynamic temperatures, which occur in the supply chain and the history of the food can be taken into account. This is needed for the use of the models for the predictions of the freshness with a sensor on a package. Therefore the Baranyi simple model has the most potential to be used for the prediction of freshness or remaining shelf life from early measurements of TMA in the fish fillets stored in a supply chain with dynamic temperatures. This TMA formation model was used as basis for a model to translate the electrode signal into a prediction of the freshness of the packed fish.

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7.3.5 Development of a model to translate sensor signal into a quality status

The model for the mass transfer of TMA from the fish, to the headspace and finally to the aqueous phase is based on physical and biochemical principles. The mass transfer coefficients were estimated from least squares regression of the mass transfer model and a storage trial at 15 °C, since mass transfer coefficients depend on factors like the storage temperature and the air velocity and are system dependent (Ni, 1999). In the sensor system described in this thesis there is no airflow, so the convective mass transfer will be low and the mass transfer coefficient will mainly be influenced by the diffusion of molecules in the aqueous phase. Therefore, the proportional effect of temperature on the diffusion coefficient, according to the Stokes Einstein equation, was used to correct the estimated mass transfer coefficient for the temperature.

It is generally assumed that bacteria responsible for the spoilage changes are normally present and most active on the surface of the fish. Bacterial metabolites like TMA will be accumulated mainly in the surface layer of the fish (Wood et al., 1942), therefore, diffusion of TMA in the bulk of the fish is not taken into account in the models as a rate limiting factor in the release of volatile compounds from the fish. This is in contradiction with chapter 3 where diffusion of TMA in the bulk was taken into account. However, in chapter 3, NH_3 was released from an unstirred solution and it was assumed that the kinetics of the increase of the signal of the sensor was limited due to this release of NH_3 to the headspace due to diffusion of NH_3 in the liquid. The effect of diffusion in the sensing aqueous phase was taken into account in the simulations with changes in the geometry (chapter 6).

Furthermore, the parameter μ_{max} has a large influence on the TMA formation and therefore also on the model simulations from the TMA concentration in the sensor aqueous phase. The parameter μ_{max} at each temperature is estimated from Equation 5.9 from chapter 5. No data from literature could be used for comparison of the values for μ_{max}. A small deviation in the prediction, compared to the actual TMA formation rate, will strongly influence the predicted values of the mass transfer rate of chapter 6.

Currently it is not yet possible to predict the TMA content of the packed fish without information about the temperature history, but more data could lead to more accurate predictions of the parameters μ_{max} and the mass transfer coefficient, which could lead to improvements in the model to predict the TMA concentration of the packed fish without information of the temperature.

Furthermore, different micro-organisms grow at different temperatures and also at different speed (Gram et al., 1987), therefore the composition of volatile compounds inside the fish package will be different in a quantitative and qualitative way when the fish is stored at different temperatures. More information about the exact composition of the aqueous phase could lead to the development of algorithms that take into account

the different signal at different temperatures, or to correct for the formation of other volatiles that affect the conductivity.

7.3.6 Comparison with other freshness models for fish in literature.

In this thesis, the freshness of fish is described by mathematical models that take the effect of time and temperature on the TMA content into account. Not many models for the formation of volatile amines have been described in literature, but attempts were made to describe the temperature dependence on the relative rate of spoilage and shelf life of fish. The shelf life is often based on several measures, including sensorial tests.

The shelf life of fish is strongly influenced by temperature. The shelf life at different storage temperatures (at T °C) can be expressed relative to the storage on ice (at 0 °C), called the relative rate of spoilage (RRS) (McMeekin et al., 1992):

$$Relative\ rate\ of\ spoilage\ at\ T\ (°C) = \frac{keeping\ time\ at\ 0\ °C}{keeping\ time\ at\ T\ °C} \qquad \text{(Equation 7.2)}$$

For packed cod, a shelf life of 14 days at ice, gives a shelf life of 6.0 days at 5 °C with a RRS of 2.3 and a RRS of 4.7 at 10 °C gives a shelf life of only 3 days (Huss, 1995). The relative rate model for the temperature dependence of the spoilage of fish was further developed into a square root model with -10 °C as minimum temperature (Huss, 1995; McMeekin et al., 1992).

$$\sqrt{relative\ rate\ of\ spoilage} = \frac{b(T\ °C - (-10\ °C))}{b(0\ °C - (-10\ °C))} = 0.1T\ °C + 1 \qquad \text{(Equation 7.3)}$$

This model predicts the spoilage rate at T is 10 °C to be 4 times faster compared to 0 °C (McMeekin et al., 1992).

Equations 7.2 and 7.3 are a kind of secondary model, because the spoilage (or freshness) itself is not calculated, only the temperature dependence of the spoilage. These models can be useful when incorporated with a time-temperature integrator. A disadvantage of these kind of models where the fish spoilage rate is compared to the spoilage at other temperatures, is that knowledge of the spoilage of the fish sample at a reference temperature is necessary to calculate the spoilage rate at different temperatures. However, the natural variation of fish quality cannot be incorporated; the spoilage rate of an individual sample cannot be compared to another sample without obtaining a large uncertainty in the prediction.

The model developed in chapter 6 in this thesis does incorporate a parameter C_0 for the initial TMA concentration, which can describe the natural variation in the quality of fish (chapter 5). In the last paragraph of chapter 6 the results of simulations on predictions

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of the shelf life with the model showed that when the parameter C_0 was changed, this was taken into account in the signal and the freshness status of the fish could still be estimated from the sensor signal. This is an important prerequisite for an appropriate model for an intelligent packaging sensor for fish.

7.4. Comparison of monitoring fish quality by direct or indirect quality indicators

The results in this thesis showed that intelligent packaging can be useful to monitor the quality of foods, and an intelligent packaging concept that monitors volatile compounds can be used to predict the freshness of packed fish. In the paragraph below our direct monitoring method is compared with an indirect type of intelligent packaging to monitor food quality, the time-temperature indicator.

Temperature is one of the most important factors influencing the quality of perishable foods. Stable and controlled temperature is therefore very important for the food supply chain to prevent high loss of quality of foods. However, the food supply chain is still prone to temperature fluctuations (Hoang et al., 2012). Many studies on intelligent packaging focus on implementing temperature sensors in the package, such as Time-Temperature integrators or indicators (TTI), to monitor, record and translate the effect of temperature on the freshness of foods, ideally from catch to consumption (chapter 2). Several researchers studied the use of TTI's on fish for the predictions of freshness (Taoukis et al. 1999; Koutsoumanis et al., 2002; Nuin et al., 2008; Tsironi et al., 2011). Although we agree that temperature is the most important factor that influences the freshness degradation of fish, in our view the predictions of quality status of fish using a TTI would be of limited value, since there is no knowledge about the initial quality of the fish. In many food products the initial quality status can be controlled as a result of processing or measured by taking samples that are representative for a whole batch. Then, a TTI can be very useful to monitor the quality decay of food products (chapter 2). This is not the case for fresh fish, of which the initial microflora can vary significantly, depending on a number of environmental factors such as sea water temperature, handling and processing after catch (Huss, 1995; Ashie et al., 1996). A high variability in the initial TMA concentration was also seen during the storage trials in this thesis (chapter 5). The importance of knowledge about the initial quality status of each product unit was described by Koutsoumanis et al. (2002) who studied the variability of the initial population of marine cultured Mediterranean fish species and found a very high interspecies and intraspecies variation, e.g. the initial count of the SSO pseudomonads in 32 gilthead sea bream samples varied between 2.00 log cfu/g and 5.66 log cfu/g (mean 3.80 log cfu/g). These results show that the actual quality status of each product unit can

only be predicted from its time-temperature history when its initial quality is incorporated in the prediction. So when applying a TTI on the package, additional knowledge of the initial quality is required to estimate the quality of fish and therefore it can be concluded that TTI's have to be combined with rapid methods of quality measurement, in order for any predictive procedure to account for that variability. Tsironi et al. (2011) performed a study on the use of TTI's on modified atmosphere packed cultivated gilthead seabream fillets from 1 batch, therefore several factors influencing the initial quality on the moment of packing could be controlled and less variation in initial quality and quality decay can be expected. The initial load (N_0) was taken into account in their shelf life model and it was mentioned that when the initial count of specific spoilage bacteria cannot be measured or reliably estimated, the error due to its variability could be up to 30% (Tsironi et al., 2011).

Taoukis et al. (1999) described a systematic approach for the use of a TTI to predict shelf life of fish by mathematical models. The development and application of reliable TTI systems must be approached based on kinetic principles, because a difference in the temperature sensitivity of the TTI response and food spoilage can result in an accumulating error in the translation of the response to actual quality loss of the food under the variable temperature conditions of the chill chain (Taoukis et al., 1999). This makes the disadvantage of an indirect quality measurement obvious: errors in the prediction can occur due to differences in the response of the measured property (temperature) and the desired property (freshness). To prevent this error, one needs to measure a direct quality attribute, so a compound that determines the organoleptic quality of the food that limits the freshness of fish. The TMA formation model that was developed in chapter 5 incorporated a parameter for the initial TMA concentration on the moment of packaging and the model developed in chapter 6 could take into account this variability accurately in the initial quality in a prediction of the freshness status.

Although TTIs do have some advantages like general applicability and can be useful for some type of foods, it is concluded that the method has some important limitations for monitoring the freshness of fish. A direct quality monitoring method, as developed in this thesis, is preferred for fresh chilled packed fish.

7.5. Implementation of the sensor concept into a (commercial) intelligent packaging

7.5.1 Developments needed for implementation
It is proposed that the research described in this thesis can be used as concept for intelligent packaging. In this thesis large electrodes were used that monitored in an aqueous phase in a glass inside the headspace of the package. Whether this sensor concept

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will be implemented into an intelligent packaging depends mainly on the technological challenges (miniaturization, reliability) and on the cost/unit. The possibilities for intelligent packaging are increasing due to developments in nanotechnology. The technology in which properties of matter with lengths of between 1 and 100 nm are observed and manipulated creates possibilities for the development of new nanosensors for intelligent packaging (Mahalik, 2009). This will solve, most likely, the technological challenges (e.g. power), and give more solutions for minimization and reliability. In recent years the on-going trends of miniaturization and price decline are increasingly allowing for the use of tiny RFID tags and sensors and Wireless Sensor Technologies in supply chain applications (Dada and Thiesse, 2008; Ruiz-Garcia, 2009). For example, developments in 3D printing make it possible to print low-cost disposable electronic sensors on a package (Tan et al., 2007; Leigh et al., 2012). It is expected that these developments lead to the development of sensors that become cheaper and more sophisticated being able to perform more complex tasks and can be applied on individual food products. According to Bartels et al. (2010), the average price of an item level RFID tag will be $0.01 or cheaper when printed on a package and a chip version will be priced about $0.04 or less. A miniaturized sensor could consist of a chip and minimized electrode in a gel that contains the aqueous sensor phase and should be applied inside the package on the moment the fish is packed. The sensor can be integrated with a RFID-tag, in this way the continuous readings from the sensor can be stored in the tag's memory and the data can be assessed by a RF reader at any point in the supply chain (Dada and Thiesse, 2008) and possibly also be assessed by a mobile phone at consumer level (Bartels et al., 2010).

Since the envisaged sensor needs to be placed inside the package it might come into contact with the food and the regulations should be taken into account in the design of the ultimate package. Intelligent packaging has a bright future according to Restuccia et al. (2010) since new EU regulations pose a new basis for the use of intelligent packaging in the industry in the EU. Apart from the costs and technological challenges, which are expected to become less an issue due to continuing developments, consumer acceptance might be an important hurdle to take (Restuccia et al., 2010). However, the most vulnerable part in the chain is still the refrigerator at the consumer's home and therefore an intelligent packaging that is understood and trusted by consumers will be very useful.

7.5.2 Applications on other foods

In this thesis we succeeded to develop a new non-destructive method that can be used as a general concept in which different electrodes can be used to monitor different volatile compounds. The expansion of the concept, of placing an aqueous phase in the food package in which dissolved volatile compounds are monitored, to other food products (such as ready-to-eat meals, fresh meat and meat products, desserts, bakery

products, and fresh-cut fruits and vegetables) is a possible area for future developments of intelligent packaging. Selective electrodes can be used to monitor specific compounds, but also general electrodes like conductivity or pH electrodes can be used, depending on the freshness or spoilage compounds of the product (chapter 2). An important requirement to be able to adapt the method to a food product is the requirement that volatile compounds need to be able to function as good quality indicator. All foods that are relatively expensive, highly perishable, and produce volatile compounds that can be monitored in the aqueous phase by electrodes as a direct indication of food quality or spoilage, are therefore interesting within this concept.

7.6. Application of intelligent packaging for monitoring food quality

7.6.1 Reducing waste
The causes of food losses and waste in medium and high-income countries mainly relate to consumer behaviour as well as to a lack of coordination between different actors in the supply chain (Gustavsson et al., 2011). Unfortunately it is in these steps of the supply chain (consumer and supermarket retailer) where quality monitoring is difficult to achieve, because there are no appropriate non-destructive methods that can be performed at these stages, since most methods are too complicated or require expensive or dangerous equipment or materials.

Van der Vorst et al. (2011) described how real-time information on actual product quality can be combined with logistics decision-support models to improve the performance of AgriFood Supply Chain Networks (AFSCNs). The additional information gained from sensors from intelligent packaging can be incorporated in quality change models during the complete distribution process leading to knowledge of the required product quality at its finally destination and advanced logistics decision-making, a concept called "Quality Controlled Logistics" (van der Vorst et al., 2011). Quality Controlled Logistics (QCL) makes use of variation in product quality, developments in technology, heterogeneous needs of customers, and the possibilities to manage product quality development in the distribution chain. The concept uses variation in quality to match consumer demand for specific products and the price that is paid for the products with the available supply of products with a variation in quality prediction. The use of intelligent packaging makes it possible to implement a dynamic pricing system, in which the price of the food is automatically adapted from the (electronic) signal of the quality sensor. Van der Vorst et al. (2011) identified six basic elements of QCL, two of them being 'product quality measurement and prediction' and 'logging and exchange of information', for which sensors combined with RFID in an intelligent packaging would be the perfect solution.

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Tromp et al. (2012) studied the use of dynamic expiry date (DED) as alternative for a fixed expiry date (FED) that would be possible due to the implementation of intelligent packaging. They performed computer simulations to quantify the effect of DED on product losses and out of stock of pork chops at retail outlets and predicted that opportunity losses can be decreased by almost 80% and product losses were predicted to decrease with more than 90% (Tromp et al., 2012). Dada and Thiesse (2008) also performed computer simulations and found that up to almost 90% reduction in unsold items of perishables can be achieved with a Lowest Quality First Out policy compared to several other issuing policies at a retailer in a perishables supply chain. These simulations show a high potential for reducing waste and a decrease of loss of income when the quality of a food is known at a retailer and correcting actions can be taken (Figure 2.3).

7.6.2 Quality guarantees for the producer

Producing companies are obliged to put an expiry date on a package, but they can't monitor their product anymore when the product leaves the factory. This gives the company a lot of uncertainty in guaranteeing quality to their customers. Storage and transport temperatures until consumption will be uncertain and variable. Until the point of sale, these temperatures are more or less controlled (but variable), but after the point of sale until the moment of consumption, temperature is totally uncontrolled. With intelligent packaging the quality of foods can be monitored in phases in the supply chain that could not be monitored before (Figure 7.5).
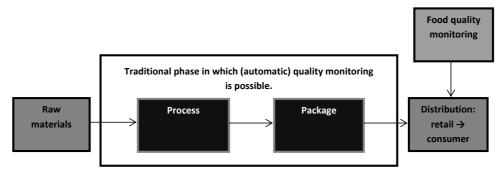

Figure 7.5 Extended possibility for monitoring of food quality during various stages of supply chain of a product.

7.6.3 Other future applications

When intelligent packaging can be combined with active packaging, even more possibilities for food packaging will arise (Figure 2.3). An example is placing a sensor on the package that monitors the gas content in the headspace of a package and takes correcting actions with a gas emitter. In this way the quality of a food cannot only be

monitored and controlled, but even improved or the spoilage rate can be slowed down. The combination of intelligent packaging with active packaging might be called smart packaging, although different definitions exists for the term smart packaging and is sometimes used interchangeably with the term intelligent packaging (Kuswandi et al., 2011). This concept might be very useful to maintain the optimal gas composition in the package of food products, for fish this could help to maintain the optimal O_2 and CO_2 balance to minimize microbial growth and production of TMA and maximize sensory properties of the fish fillets (Sivertsvik, 2007). For other food products, like fruits or other respiring foods, smart packaging can help to control ripening and actively prevent spoilage.

7.7. Guidelines and recommendations for developing an intelligent packaging for monitoring food quality

In this thesis fresh fish was taken as a case study for the development of an intelligent packaging concept and, as mentioned before, the developed method could be adapted for other foods as well. In this section we give some guidelines and recommendations for the development of an intelligent packaging for monitoring the quality of foods in general (Figure 7.6).

The first step in the development of an intelligent packaging is to study the food quality deterioration of the food of interest. The critical quality reactions determining the shelf life of the food need to be determined and one has to decide whether a direct quality sensor needs to be developed or a sensor that monitors environmental conditions that influence the quality of the packaged food can be used. This will depend on knowledge about and variation of the initial quality. A decision tree for the choice of intelligent packaging has been developed in Figure 2.5.

When a sensor that monitors environmental conditions can be applied on a package to monitor the quality of a food product, the kinetics of the sensor should be equal to the kinetics of the quality decay of the food. Mathematical models need to be used to translate the sensor signal into an actual food quality value, but this can only be done when the initial quality value is taken into account in the models (Figure 2.4).

The next step for the development of a direct quality sensor is to identify quality indicator compounds that can be measured without affecting the food or the package. Especially volatile compounds produced by the food are suitable for a non-destructive method. Apart from being non-destructive, a suitable method should also be able to measure continuously and reliably even under a broad range of storage conditions (chapter 4). Tests need to be performed under different storage conditions, including abuse conditions that could happen in the supply chain. The quality indicator compound should correlate under all conditions to the freshness of the food and being able to be measured by the sensor.

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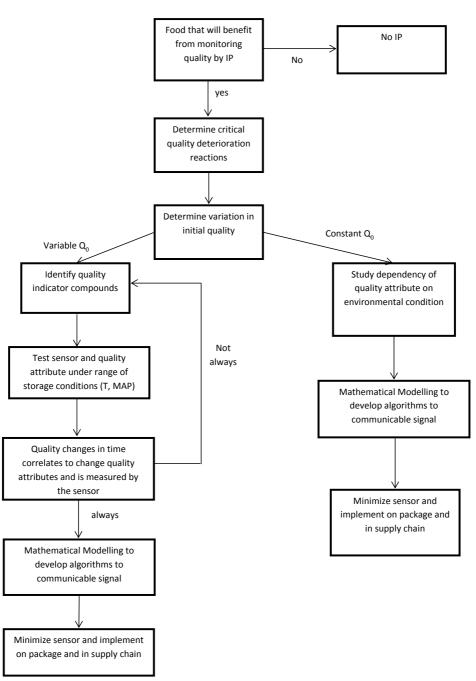R25
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The results of the storage trials need to be used to develop mathematical models to translate the sensor signal into a quality status of the food (chapter 5 and 6). It is necessary to obtain knowledge about the effect of factors that influence the quality of the food or the food-package matrix and important factors (e.g. temperature) should be taken into account as variable in the models. It is also necessary to evaluate the system and develop different models when other packaging conditions are being used, like MAP. These models can lead to algorithms to make quality predictions and communicate to the user. The models can also give information on the optimal geometry of the sensor.

Next, the sensor should be miniaturized and implemented in a food package and used in the supply chain, but this part of the development process is out of scope of food science research and requires cooperation with electronic engineering. However, we can give some recommendations for the final sensor: A miniaturized sensor that is integrated into a package for food units, should be able to be produced on a large scale at low-cost (relative to the value of the food product), accurate and reliable, easy to read and understand by the user, preferably sustainable, and off course safe for food contact.

Figure 7.6 General framework for development of intelligent packaging sensor.

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7.8. Main conclusions and outlook

The objective of this thesis was to study and develop an intelligent packaging concept that can monitor and predict food quality within the supply chain. We succeeded to develop an intelligent packaging concept based on a new non-destructive method that monitors changes in the volatiles, that are often used as a proxy for the freshness of fish, in the package of fish. Such an intelligent packaging sensor can give an indication of freshness of the packed fish at all points during the supply chain, which can help to improve supply chain management and reduce waste. Such a direct quality sensor is necessary for fresh fish because of the high variation in initial quality. A sensor that only monitors environmental conditions that influence the quality of fish is not sufficient to predict the quality of fish. The general concept can also be applied on other food products that produce volatile gas compounds that can function as quality indicator. Mathematical models were developed that can translate the sensor signal in an actual quality status. The model is based on a TMA formation model, that incorporates the effect of temperature and initial TMA content, and a mass transfer model to predict the freshness of the packed fish based on the actual TMA concentration in the sensing aqueous phase calculated from the sensor signal.

The next step is to optimize the sensor signal by designing a more sensitive sensor in a minimized format with a minimized aqueous phase. When the sensor is more sensitive to small changes in the freshness status of the food, the model distinguishes better between different freshness stages and the reliability of the predictions improves.

Furthermore, supply chain management systems might need to be adapted to make the maximum profit of the increased dynamic quality information and the actors in the supply chain and the final consumer need to be informed about the use of the sensor. This might require new policies and can take some time.

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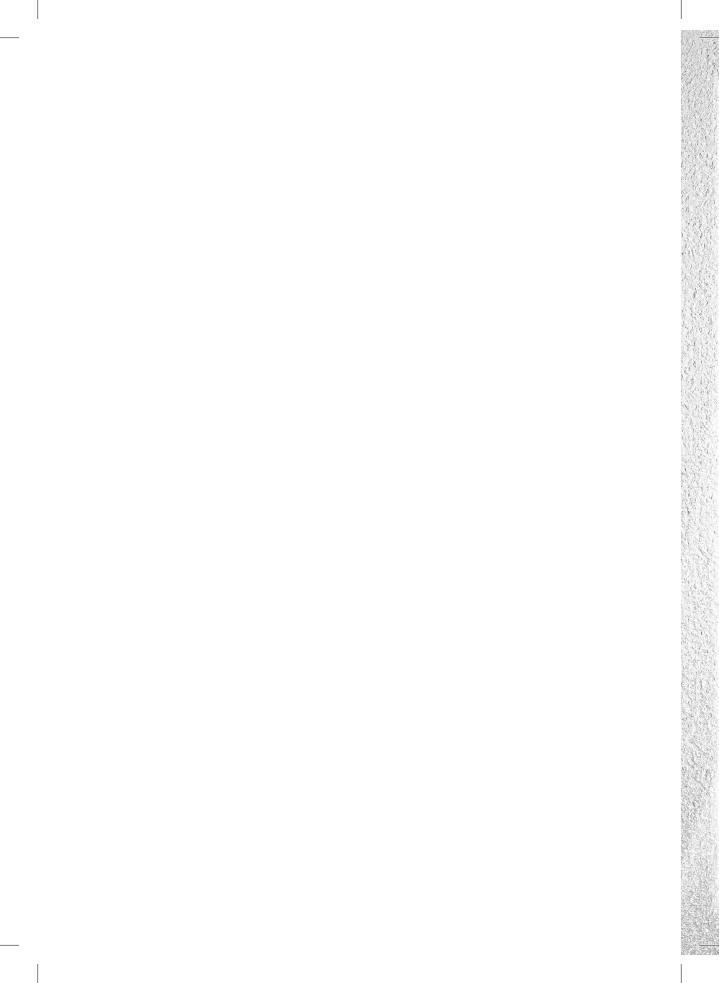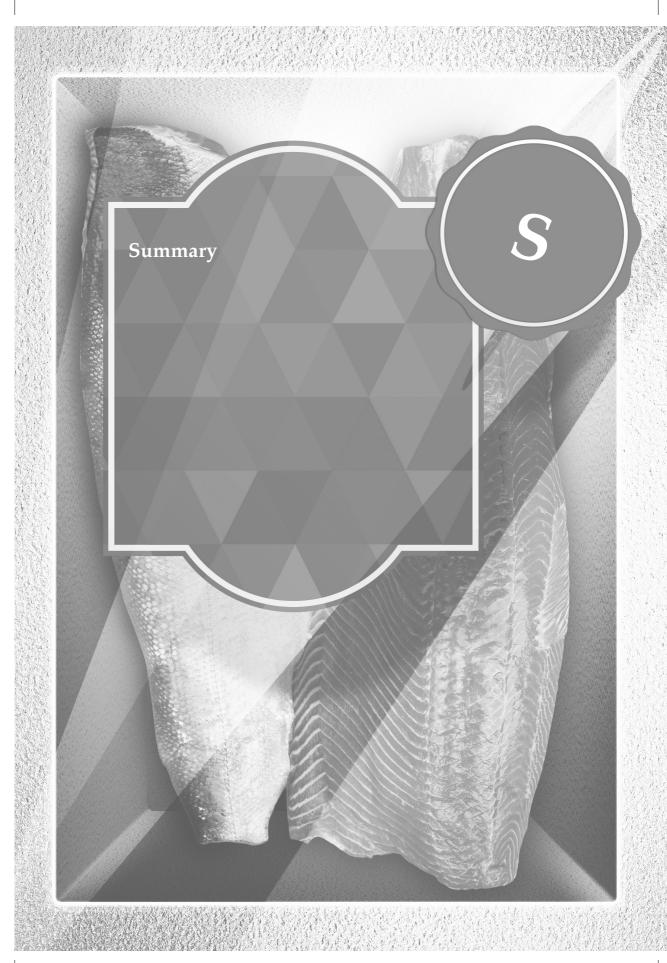
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Summary

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Background

Foods are prone to quality degradation in the whole supply chain, but the possibilities for monitoring the quality of foods inside the package are limited. When sensors of quality indicators are included into the package of a food, the package can become an intelligent packaging that is able to communicate information about the packed food. An intelligent packaging could be used to monitor quality attributes of the food and translate this into a message about the quality of the packed food that is communicated to all actors in the supply chain. Knowledge of the quality status of the food could lead to improvements in quality based logistic decision making in the food chain and reduce food waste. In this thesis the possibilities and pitfalls to use intelligent packaging to monitor the quality of foods were explored.

Aim of this thesis

The objective of this thesis was to study and develop an intelligent packaging concept that can monitor and predict food quality within the supply chain. Intelligent packaging systems, based on a direct or indirect quality monitoring sensor and combined with mathematical models, were evaluated on their abilities to predict the quality of the packed perishable foods under different conditions that can occur in the supply chain. The development of new non-destructive methods for monitoring changes in the freshness status of packed fresh fish was chosen as a case study for the proof of principle of the application of intelligent packaging for monitoring food quality.

Results

First, a literature study was performed on the possibilities for intelligent packaging for monitoring the quality of foods (**chapter 2**). One of the conclusions was that foods that benefit the most from intelligent packaging are expensive, highly perishable foods. Foods with a known or low variation in initial quality can benefit from sensors that monitor the environmental conditions surrounding the food that influence the quality of the packed foods. Foods with an unknown or high variation in the initial quality need sensors that directly monitor a quality attribute of the food itself.

The initial quality of fresh fish is highly variable and therefore fresh cod fillets require sensors monitoring compounds correlated with the quality of the fish. Therefore, fresh cod was chosen as case example for the development of a quality sensor for intelligent packaging. The content of total volatile basic nitrogen (TVB-N) compounds were identified as possible quality indicator compounds to predict the freshness of fish, the most important quality attribute of fresh fish.

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After the quality indicator compounds were identified, suitable methods to monitor the quality indicator compounds were studied. Non-destructive analysis methods are needed for the development of an intelligent packaging for communicating fish freshness throughout the supply chain from the moment of packaging until the day the fish is spoiled. Apart from being non-destructive, a suitable method should also be cheap, simple and able to measure continuously and reliably even under a broad range of storage conditions.

Appropriate non-destructive methods for monitoring the loss of freshness of packed fish were not available; therefore a new non-destructive method was introduced (**chapter 3**). In this new method an aqueous phase is placed inside the fish package. The principle of this method is that changes in this aqueous phase from dissolving and dissociating compounds can be monitored by electrodes. These measured changes in the potential are related to the often used freshness indicator of the fish, the total volatile basic amine content (TVB-N), the trimethylamine (TMA) content and to the ammonia (NH_3) content of the fish. Several electrodes were used, being an NH_4^+-, pH- and conductivity-electrode.

The ammonium ion-selective electrode (NH_4^+-ISE) had the advantage of high selectivity to a volatile amine produced by the fish. The NH_4^+-ISE measured changes in the aqueous phase when ammonia, from an NH_3-solution or from cod fillets, is released in the headspace of the package. The electrode response correlated with the trend of the generation of the TVB-N in the cod fillet, which is an established freshness indicator for fish in destructive analysis. Therefore the quality decay of fish can be monitored non-destructively by this method (**chapter 3**).

In contradiction to what is described in literature, we concluded that monitoring the pH in the aqueous phase is not an appropriate approach to monitor the freshness of packed fish. The pH is not a reliable indicator under different temperature conditions, which leads to unreliable predictions in a supply chain with variable temperatures.

The non-selective conductivity-electrode is able to respond to a broad range of compounds that are formed on the fish during all stages of freshness. The conductivity electrode output of the aqueous phase showed a characteristic response pattern during the storage trials at all temperatures investigated and is therefore promising to function as a sensor for an intelligent packaging for packed fish (**chapter 4**).

The next step after developing a suitable method to monitor the selected quality indicator compounds was to develop mathematical models to translate the sensor data into a message about the quality of the packed food. We developed a mathematical model to translate the signal of the conductivity-electrode into a freshness status on basis of the formation of the quality indicator compound TMA from the fish. The results obtained from the storage trials, supplemented with knowledge from literature, were used to

develop mathematical models for the formation of TMA in packed fish, and used to develop a model for the mass transport of TMA in the fish, headspace and sensing aqueous phase of the package.

Existing models for the formation of TMA on fish were evaluated on their ability to use for the prediction of TMA under dynamic storage temperatures, but at static temperatures the models needed improvements and the models lacked the ability for practical application to predict the TMA concentration under dynamic supply chain conditions with variable temperatures and variable initial quality of the fish.

A simplified dynamic Baranyi-Roberts model could describe the TMA formation with 3 parameters (**chapter 5**). The effect of temperature on the maximum formation rate (μ_{max}) was described by the "expanded square root" model of Ratkowsky and the effect of different batches could be described by the initial TMA concentration (C_0). With this model, predictions of the TMA concentration at each time can be made under dynamic temperature conditions and the variable initial quality is also taken into account.

This TMA formation model was used for the development of a further model that describes the mass transfer of TMA that is formed on the fish, released in the headspace and finally dissolved and dissociated in the sensing aqueous phase (**chapter 6**). The model predicted TMA increase comparable with experimental sensor measurements from a storage trial at 15 °C and can be used to predict the freshness of packed fish from sensor measurements. The model was also used to study the effect of the geometry of the sensor system on the predictions and simulations showed that minimizing the sensing aqueous phase will improve the sensitivity of the sensor to distinguish between different freshness stages. Simulations were performed with variable temperatures which showed a deviation in the sensor signal. Therefore currently, freshness can only be predicted from the sensor signal when the temperature history of the product is monitored as well. Algorithms can be developed that take into account the response patterns at different temperatures, or (when more research is done to the influences of other volatile compounds) to correct for the formation of other volatiles that affect the sensor signal. From simulations with variations in the initial quality it was seen that the model takes this variation into account and predictions of the TMA content in the fish can be made from the sensor signals of packages with fish with variable initial quality.

Conclusions

In this thesis a non-destructive method has been developed from which the freshness of packed fish can be predicted from electrode monitoring in the aqueous phase inside the package. Mathematical models have been developed to translate the sensor signal into a freshness status. This method has potential to be developed into an intelligent packaging

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to communicate about an important quality attribute of fish. Simulations show that the sensor signal will improve in sensitivity when the sensor system will be minimized, which is an essential step for further development into an intelligent package for fish.

The development process of a non-destructive method for an intelligent packaging concept for fish as proof of concept resulted in the setup of guidelines and recommendations for the development and use of intelligent packaging for monitoring the quality of foods in general. Different food products require different approaches in order to benefit the most from the developments in intelligent packaging. Currently, expensive, highly perishable foods will benefit the most when intelligent packages can be used to monitor the quality of the foods.

Samenvatting
(Summary in Dutch)

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Achtergrond

Voedingsmiddelen zijn gevoelig voor kwaliteitsachteruitgang in de hele keten, maar de mogelijkheden om de kwaliteit van voedsel in de verpakking te monitoren zijn beperkt. Wanneer sensoren of kwaliteitsindicatoren in de voedselverpakking worden geïntegreerd, kan de verpakking een intelligente verpakking worden die in staat is om informatie over het verpakte voedsel te communiceren. Een intelligente verpakking kan gebruikt worden om kwaliteitsattributen van het voedsel te monitoren en om dit te vertalen naar een boodschap over de kwaliteit van het verpakte voedsel dat gecommuniceerd kan worden naar alle betrokkenen in de keten. Kennis van de kwaliteitsstatus van het voedingsmiddel kan leiden tot verbeteringen in, op kwaliteit gebaseerde, logistieke besluitvorming in de keten en tot vermindering van voedselverspilling. In deze thesis zijn de mogelijkheden en valkuilen onderzocht om intelligente verpakkingen te gebruiken om de kwaliteit van voedingsmiddelen te kunnen monitoren.

Doel van dit proefschrift

Het doel van deze thesis was om een concept voor een intelligente verpakking te ontwikkelen en te bestuderen, dat gebruikt kan worden om de kwaliteit van voedsel in de keten te monitoren en te voorspellen. Intelligente verpakkingssystemen, gebaseerd op sensoren die direct of indirect de kwaliteit monitoren in combinatie met wiskundige modellen, zijn geëvalueerd op hun mogelijkheden om de kwaliteit van verpakte bederfelijke levensmiddelen te voorspellen onder verschillende condities die in de keten kunnen voorkomen. De ontwikkeling van nieuwe niet-destructieve methodes voor het monitoren van veranderingen in de versheid-status van verpakte verse vis was gekozen als een casestudie om het principe van de toepassing van intelligente verpakkingen voor het monitoren van voedsel-kwaliteit mee aan te tonen.

Resultaten

Eerst is er een literatuuronderzoek gedaan naar de mogelijkheden om met intelligente verpakkingen de kwaliteit van voedsel te monitoren (**hoofdstuk 2**). Eén van de conclusies was dat de duurdere, en erg bederfelijke voedingsmiddelen het meest zullen profiteren van intelligente verpakkingen. Voedingsmiddelen met een bekende of lage variatie in hun beginkwaliteit kunnen profiteren van sensoren die de omgevingscondities, die het voedsel beïnvloeden, monitoren. Voedingsmiddelen met een onbekende of hoge variatie in de oorspronkelijke kwaliteit, hebben sensoren nodig die rechtstreeks een kwaliteitsattribuut van het voedsel zelf monitoren.

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De beginkwaliteit van verse vis is erg variabel en hierdoor hebben bijvoorbeeld verse kabeljauw filets sensoren nodig die stoffen meten die gecorreleerd zijn met de kwaliteit van de vis. Daarom was verse kabeljauw gekozen als voorbeeld product voor de ontwikkeling van een kwaliteitssensor voor een intelligente verpakking. Het totale gehalte aan stikstof in vluchtige basische componenten (TVB-N) wordt gezien als mogelijke indicator om de versheid van vis, het belangrijkste kwaliteitsattribuut van verse vis, te voorspellen.

Nadat de kwaliteitsindicator componenten waren bepaald, is er een studie gedaan naar geschikte methodes om deze componenten te monitoren. Niet-destructieve analyse methodes zijn noodzakelijk voor de ontwikkeling van een intelligente verpakking die informatie over de versheid van vis in de keten kan communiceren vanaf het moment van verpakken tot de dag waarop de vis bedorven is. Behalve dat de methode niet-destructief moet zijn, moet een geschikte methode ook goedkoop en eenvoudig zijn. Ook moet de methode continu en betrouwbaar meten, zelfs onder een breed scala aan opslagcondities.

Geschikte niet-destructieve methodes voor het monitoren van het verlies van de versheid van verpakte vis waren nog niet beschikbaar; daarom werd een nieuwe niet-destructieve methode ontwikkeld (**hoofdstuk 3**). Bij deze nieuwe methode is een waterfase in de visverpakking geplaatst. Het principe van deze methode is dat in deze waterfase veranderingen, door oplossende en dissociërende stoffen, kan worden gemonitord met elektrodes. Deze gemeten potentiaalveranderingen werden gerelateerd aan vaak gebruikte versheidsindicatoren van vis: het totale gehalte aan vluchtige basische amines (TVB-N), het trimethylamine (TMA) gehalte en het ammoniak (NH_3) gehalte van de vis. Verschillende elektrodes werden gebruikt, namelijk een NH_4^+-, pH- en geleidbaarheid-elektrode.

De ammonium ion-selectieve elektrode (NH_4^+-ISE) had het voordeel van een hoge selectiviteit voor een vluchtige amine dat geproduceerd wordt door de vis. De NH_4^+-ISE mat veranderingen in de waterfase wanneer ammonia vrijkwam uit een NH_3-oplossing of uit kabeljauwfilets, in de gasruimte van de verpakking. De respons van de elektrode correleerde met de trend van de TVB-N vorming in de kabeljauwfilet, wat een algemeen geaccepteerde versheidindicator voor vis is in destructieve methodes. Daarom kan de kwaliteitsachteruitgang van vis op een niet-destructieve manier worden gemonitord met deze methode (**hoofdstuk 3**).

In tegenstelling tot wat in literatuur beschreven is, concludeerden wij dat het monitoren van de pH in de waterfase geen geschikte aanpak is om de versheid van verpakte vis te monitoren. De pH respons bleek sterk te variëren onder verschillende temperatuur condities, wat leidt tot onbetrouwbare voorspellingen gezien de variabele temperaturen die voorkomen in de visketen.

De niet-selectieve geleidbaarheid-elektrode is in staat om te reageren op een breed scala aan componenten die gevormd worden op de vis tijdens alle fases van versheid. De respons van de geleidbaarheid-elektrode in de waterfase toonde een karakteristieke respons gedurende de opslagproeven bij alle bestudeerde temperaturen en is daardoor veelbelovend als sensor voor een intelligente verpakking voor verpakte vis (**hoofdstuk 4**).

Na het ontwikkelen van een geschikte methode voor het monitoren van de geselecteerde kwaliteitsindicator componenten, was de volgende stap het ontwikkelen van wiskundige modellen om de sensor data te vertalen in een boodschap over de kwaliteit van het verpakte voedsel. Wij hebben wiskundige modellen ontwikkeld die het signaal van de geleidbaarheid-elektrode omzetten in een versheidstatus op basis van de kwaliteitsindicator component TMA van de vis. De resultaten die verkregen zijn uit de opslagproeven zijn, aangevuld met kennis uit de literatuur, gebruikt voor de ontwikkeling van wiskundige modellen voor de vorming van TMA in verpakte vis, en deze zijn gebruikt voor de ontwikkeling van een model voor de massa transport van TMA in de vis, gasruimte en de waterfase van de sensor van de verpakking.

Bestaande modellen voor de vorming van TMA op vis werden geëvalueerd op hun vermogen om ze te gebruiken voor de voorspelling van TMA onder dynamische bewaartemperaturen, maar bij statische temperaturen hadden deze modellen verbeteringen nodig en de modellen misten de mogelijkheid voor praktische toepassing onder dynamische keten-condities met variabele temperaturen en variabele initiële kwaliteit van de vis.

Een vereenvoudigd dynamisch Baranyi-Roberts model kon de TMA vorming beschrijven met 3 parameters (**hoofdstuk 5**). Het effect van temperatuur op de maximale vormingssnelheid (μ_{max}) kon worden beschreven met het "expanded square root" model van Ratkowsky en het effect van verschillende batches kon worden beschreven door de initiële TMA concentratie (C_0). Met dit model kunnen op elk moment voorspellingen van de TMA concentratie onder dynamische temperatuur condities gedaan worden, waarbij rekening gehouden is met de variabele initiële kwaliteit.

Dit TMA vormingsmodel werd gebruikt voor de ontwikkeling van een uitgebreider model waarbij de vorming is gecombineerd met het massa transport van TMA naar de gasruimte en uiteindelijk met het oplossen en dissociëren in de waterfase van de sensor (**hoofdstuk 6**). Het model voorspelt een TMA toename dat vergelijkbaar is met de experimentele sensor metingen in een bewaarproef bij 15 °C en kan worden gebruikt om de versheid van verpakte vis door sensor metingen te voorspellen. Het model werd ook gebruikt om het effect van de geometrie van het sensor systeem op de voorspellingen te bestuderen en simulaties toonde aan dat het minimaliseren van de waterfase van de

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sensor zal leiden tot een verbetering in de gevoeligheid van de sensor om de verschillende versheid fases te kunnen onderscheiden. De simulaties die werden uitgevoerd met variabele temperaturen toonden een afwijking in het sensor signaal aan. Daarvoor kan met deze methode de versheid alleen vanuit het sensor signaal worden voorspeld als de temperatuur geschiedenis van het product ook gemonitord wordt. Algoritmes kunnen worden ontwikkeld die rekening houden met het respons patroon bij verschillende temperaturen, of die corrigeren voor de vorming van andere vluchtige stoffen die het sensor signaal beïnvloeden (wanneer meer onderzoek wordt gedaan naar de invloed van deze stoffen). Simulaties met een variërende initiële kwaliteit toonde aan dat het model rekening houdt met deze variatie en het TMA-gehalte van de vis voorspeld kan worden uit de sensor signalen van verpakkingen met vis van verschillende initiële kwaliteit.

Conclusies

In dit proefschrift is een niet-destructieve methode ontwikkeld waarmee de versheid van verpakte vis kan worden voorspeld door metingen met een elektrode in een waterfase in de verpakking. Wiskundige modellen zijn ontwikkeld om het sensor signaal te vertalen in een versheid status. Deze methode heeft potentie om te worden ontwikkeld in een intelligente verpakking die communiceert over een belangrijk kwaliteitsattribuut van vis. Simulaties tonen aan dat de gevoeligheid van het sensor signaal zal verbeteren wanneer het sensor systeem wordt geminiaturiseerd, wat een essentiële stap is in de verdere ontwikkeling tot een intelligente verpakking voor vis.

Het ontwikkelingsproces van een niet-destructieve methode voor een intelligente verpakking voor vis resulteerde in een opzet van richtlijnen en aanbevelingen voor de ontwikkeling en het gebruik van een intelligente verpakking voor het monitoren van de kwaliteit van voedingsmiddelen in het algemeen. Verschillende voedsel producten hebben een verschillende aanpak nodig om te kunnen profiteren van de ontwikkeling van intelligente verpakkingen. Momenteel zullen de duurdere, meer bederfelijke voedingsmiddelen het meeste profiteren van de toepassing van intelligente verpakkingen voor het monitoren van de voedselkwaliteit.

**Dankwoord
(Acknowledgements)**

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Mijn proefschrift is af! Ik kan het nog nauwelijks bevatten dat het nu toch zover is. Het is geen gemakkelijk promotie-traject geweest, des te meer ben ik er trots op dat ik dit resultaat nu voor me ligt! Vele mensen hebben op een of andere manier bijgedragen aan het tot stand komen hiervan, maar op deze plek wil ik een aantal mensen hier graag speciaal voor bedanken.

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About the author

A

Curriculum Vitae

Jenneke Heising was born on April 13th, 1983 in IJsselstein, The Netherlands. After completing the gymnasium at Oosterlicht College in Nieuwegein in 2001, Jenneke started her study Food Technology at the Wageningen University. She conducted a bachelor thesis project "Designing Healthy Fries: Technological aspects" in 2004 and conducted her MSc-thesis at the Product Design and Quality Management Group, which had the title: "Kinetic study of thermal treatment in mango and pineapple". After an internship at Masterfoods te Veghel, she graduated in November 2006 for her Master's degree.

Subsequently, she started her PhD-project at the Wageningen University at the Product Design and Quality Management Group. The topic of this PhD-project was Monitoring of Product Quality and Safety by Intelligent Packaging. Besides the research activities and joining the educational programme of the Graduate School of VLAG, she had a special interest for the education of the group and was involved in many teaching activities and supervised many Bachelor and Master students. Besides she organised several social activities for the group and she was a member of the organizing committee of the PhD study tour from the Product Design and Quality Management Group in 2010. This was a two-week tour to Melbourne and Sydney in Australia, where several companies and universities were visited to exchange scientific knowledge.

Currently, Jenneke is still working at the Food Quality and Design Group where she develops new education materials and she is as teacher involved in several courses of the group.

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List of publications

Publications in peer-reviewed journals

Heising, J.K., Dekker, M., Bartels, P.V., and Van Boekel M.A.J.S. (2014). Monitoring the quality of perishable foods: opportunities for intelligent packaging. *Critical Reviews in Food Science and Nutrition* **54**(4), 645-654.

Heising, J.K., Dekker, M., Bartels, P.V., and Van Boekel, M.A.J.S. (2012). A non-destructive ammonium detection method as indicator for freshness for packed fish: Application on cod. *Journal of Food Engineering* **110**, 254-261.

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Heising, J.K., Van Boekel, M.A.J.S., and Dekker, M. (2014). Mathematical models for the trimethylamine (TMA) formation on packed cod fish fillets at different temperatures. *Food Research International* **56**, 272-278.

Submitted publications

Heising, J.K., Van Boekel, M.A.J.S., and Dekker, M. Simulations on the prediction of fish quality from an intelligent packaging concept. *Submitted for publication*

Publications in books

Heising, J.K. and Dekker, M. (2014). Food Packaging, in: *Handbook of Indigenous Foods Involving Alkaline Fermentation*. Edited by Sarkar, P.K. and Nout, M.J.R. CRC Press, 600 p.

Conference abstracts and proceedings

Heising, J.K., Dekker, M., Bartels, P.V., and Van Boekel, M.A.J.S. (2010). Monitoring fish quality by intelligent packaging. *2009 EFFoST Annual Conference: New Challenges in Food Preservation, Processing, Safety and Sustainability. Budapest, Hungary; 11-13 November, 2009* (Poster Presentation)

Heising, J.K., Dekker, M., Bartels, P.V., and Van Boekel, M.A.J.S. (2010). Monitoring food quality and/or safety by intelligent packaging. *17th IAPRA World Conference on Packaging 2010, Tianjin, China; 12-15 October, 2010* (Oral Presentation)

Heising, J.K., Dekker, M., Bartels, P.V., and Van Boekel, M.A.J.S. (2010). Monitoring of food quality and/or safety by intelligent packaging. *First International Conference on Food Innovation 2010 (foodInnova2010), Valencia, Spain; 25-29 October, 2010* (Oral Presentation)

Award

1st Price winner of the Poster presentation. 3rd International Course: Advanced Food
Analysis 2010. 15-19 November 2010. VLAG Graduate School.

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Overview of completed training activities

Courses	Year
Discipline specific courses	
Reaction kinetics in food science	2006
Management of microbial hazards in foods	2007
Digital course of food preservation	2007
Advanced food analysis	2010
International Conferences and meetings	
From soil to healthy people (Wageningen, The Netherlands)	2006
Sustainpack conference 'New Technologies and Applications in Communicative Packaging' (Wageningen, The Netherlands)	2007
EFFoST Annual Conference: New Challenges in Food Preservation, Processing, Safety and Sustainability (Budapest, Hungary)	2009
17th IAPRA World Conference on Packaging (Tianjin, China)	2010
First International Conference on Food Innovation 2010 (Valencia, Spain)	2010
General courses	
Techniques for writing and presenting a scientific paper	2007
Philosophy and ethics of Food Science and Technology	2007
VLAG PhD-week 18th edition	2007
Scientific writing	2009
Teaching and supervising thesis students	2009
Scientific publishing	2009
Career perspectives (individual program)	2013
Project and Time Management	2014
Optional courses and activities	
Writing research proposal	2006
Participating PhD study tour to USA	2007
Organizing and participating PhD study tour to Australia	2010
Research presentations	2007-2013

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