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Assessment of a novel active packaging material to improve the resource efficiency of food production by increasing the safety and shelf life of perishable products

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Meiner ganzen Familie besonders meinen Eltern

Doch Forschung strebt und ringt, ermüdend nie,
Nach dem Gesetz, dem Grund, *Warum* und *Wie*.

Johann Wolfgang von Goethe (1749 - 1832)

Kurzfassung

Bewertung eines neuartigen aktiven Verpackungsmaterials zur Steigerung der Ressourceneffizienz der Lebensmittelproduktion durch eine Erhöhung der Sicherheit und Haltbarkeit leichtverderblicher Produkte

Ziel der Arbeit war die Bewertung neuartiger aktiver Verpackungsmaterialien auf Basis von poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS)) hinsichtlich der Anwendung in Wertschöpfungskette für leichtverderbliche Lebensmittel. Weiterhin wurde im Rahmen der Arbeit eine standardisierte Vorgehensweise entwickelt, um das Potential von aktiven Verpackungen zur Erhöhung der Sicherheit und Haltbarkeit leichtverderblicher Produkte sowie deren Beitrag zur Verbesserung der Ressourceneffizienz zu bewerten.

Für die Bewertung des neuen Polymers wurde der Einfluss von Lebensmittelinhaltsstoffen, Umweltfaktoren und verschiedener Verfahrenstechniken bei der Herstellung der Verpackungsmaterialien auf die antimikrobielle Aktivität analysiert. Insgesamt wurden 714 poly(TBAMS) enthaltende Materialproben und 1032 Referenzen untersucht. Basierend auf den Ergebnissen erfolgte die Herstellung verschiedener Folien und Vliese für die Lagerungstests. 883 Proben wurden in 15 Zeitreihen mit leichtverderblichen Lebensmitteln getestet, um den Effekt von poly(TBAMS) auf produktspezifische Parameter zu bewerten. Die Modellierung des Wachstums verschiedener Bakterien, Hefen und Pilze, und die Ermittlung der Haltbarkeitszeiten erfolgte mit der modifizierten Gompertzfunktion. Auf Grundlage aller Analysen wurde ein Prüfschema zur Bewertung aktiver Materialien im Hinblick auf ihre Eignung, die Haltbarkeit leichtverderblicher Lebensmittel zu erhöhen, entwickelt. Zudem wurde eine Vorgehensweise entwickelt, um die Kosten und Nutzen von aktiven Verpackungen für die unterschiedlichen Stufen der Wertschöpfungskette zu berechnen.

Verschiedene poly(TBAMS) enthaltende Materialen zeigten eine hohe antimikrobielle Aktivität gegen eine Vielzahl Verderbnis erregende und pathogene Bakterien. Einige Lebensmittelinhaltstoffe (insbesondere Proteine), die Temperaturbedingungen und die Compoundierung des Polymers mit verschiedenen Matrixpolymeren beeinflussten die Wirksamkeit. Die Aktivität des Polymers konnte mit der Erhöhung der Konzentration von poly(TBAMS) oder durch eine größere Polymeroberfläche gesteigert werden. So zeigte eine Mehrschichtfolie mit 15 % poly(TBAMS) in der Innenschicht eine Haltbarkeitsverlängerung bei Fleischprodukten und bei proteinarmen Lebensmitteln, wie zum Verzehr fertiges Gemüse.

Das entwickelte Prüfschema sowie die Kosten-Nutzen Analyse ermöglichen es, geeignete Anwendungsgebiete antimikrobieller Polymere schon während der Entwicklungsphase zu identifizieren. Dadurch können die Entwicklungszeiten und -kosten deutlich reduziert werden und durch verlängerte Haltbarkeitszeiten wichtige Ressourcen geschont werden.

Abstract

Assessment of a novel active packaging material to improve the resource efficiency of food production by increasing the safety and shelf life of perishable products

The objective of this thesis was the assessment of novel active packaging materials containing the polymer poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS)) for the application in perishable food supply chains. Furthermore, the potential of active packaging materials to increase the safety and shelf life of perishable products and thus to improve the resource efficiency of food production was assessed.

The influence of food components, environmental factors, and different processing steps was analysed on the antimicrobial activity of 24 different processed materials containing poly(TBAMS). In total 714 samples containing poly(TBAMS) and 1032 references were investigated. Based on the results, certain kinds of packaging prototypes were produced for storage tests. 883 samples were tested in 15 storage trials with perishable products to assess the effect of poly(TBAMS) on product specific microbial, sensory, quality and chemical parameters. The modelling of the growth of spoilage and pathogenic bacteria, yeasts and moulds and the shelf life determination were conducted by using the modified Gompertz function. Based on all results, a standardized evaluation scheme for the assessment of active packaging materials` ability to increase the safety and shelf life of perishable foods were developed. Further an approach to analyse the cost and benefits by implementation of active packagings were developed. Calculations were exemplified for poly(TBAMS) packagings.

Different materials containing poly(TBAMS) showed a high antimicrobial activity against spoilage and pathogenic organisms relevant for perishable products. The food components (especially proteins), temperature, gas atmosphere and the processing of the polymer itself influenced the activity. The activity depended on the availability of functional groups of poly(TBAMS) on the polymeric surface, which increased by increasing the concentration or enlarging the surface. A multilayer foil containing 15 % of poly(TBAMS) in the inner layer increased the microbial safety and shelf life of products with low protein contents, such as RTE vegetables, and of processed meat products. Thus, the novel packaging solutions have high potential to reduce food waste by increasing the safety and shelf life of perishable foods.

The developed evaluation scheme allows the identification of the application area where an active material delivers the highest benefit during the development phase. The cost-benefit analysis represents a new approach for the calculation of the economic and resource-efficient impact by implementation of different active packaging materials. Both can lead to a reduction of time and costs during developing of antimicrobial packaging materials.

Contents

1 Introduction

1.1 Food waste and spoilage process of perishable products

Large quantities of the foods intended for human consumption are wasted during the entire supply chain (Gustavsson et al. 2011; Mena et al. 2011; Gunders 2012; Noleppa and von Witzke 2012; Verghese et al. 2013). Precisely the products that have short shelf lives due to product characteristics, and thereby shortened sellable periods are often discarded prior to sale or consumption (Mena et al. 2011; Kreyenschmidt et al. 2013; Verghese et al. 2013). Per different data sources for example, the amount of wasted meat and meat products varies between 9 and 22 % along the entire supply chain of industrial countries. In meat supply chains, the amount of food waste increases with each link in the chain until the consumer stage (Gustavsson et al. 2011; Gunders 2012; Noleppa and von Witzke 2012; Verghese et al. 2013; Rossaint and Kreyenschmidt 2014). At the consumer stage of fruit and vegetable chains, high amounts (15 – 30 %) of purchases are also wasted (Gustavsson et al. 2011).

The amount of food waste means that, not only are the products themselves lost, but also enormous amount of primary resources. Thus, resources are needed for breeding and fattening of animals, for cultivation of plants, for processing and packaging during production as well as along the entire chain (Gustavsson et al. 2011; Williams et al. 2012; Beretta et al. 2013; Roissant and Kreyenschmidt 2014; Noleppa and Cartsburg 2015).

There are several different causes for wasting perishable products, such as wrong handling, so that the product is spoiled before the use-by-date is reached, or a lack of effective monitoring technologies to determine the real quality and safety of a product. Several products are also thrown away because they were not sold during the short selling time, which means the length of the shelf life of the products has a significant impact on the amount of food waste (Kreyenschmidt et al. 2013; Mena et al. 2014).

Microbial growth and metabolism are mostly responsible for the short shelf life of perishable products, especially for those of fresh meat and fish (Gill 1983; Genigeorgis 1985; Huis in’t Veld 1996). However, chemical, biochemical and/or physical reactions can also lead to deteriorative changes in perishable foods (Hayes 1985; Mead 2004; Singh and Anderson 2004). In several fresh fruits and vegetables for example, spoilage is often not only caused by microbial activity, but the respiration process and physiological reactions also influence the shelf life (Ahvenainen 1997; Barry-Ryan and O’Beirne 1998). But treatment of these products in a minimally technological way, such as peeling, trimming or cutting before commercial distribution, can favour microbiological reactions (Buick et al. 1987; Legnani and Leoni 2004; Kreyenschmidt and Ibald 2012).

Thus, reducing, the microbial contamination and growth as well as prolonging the lag time is important for increasing the shelf life of such products and decreasing the risk of food borne illness (Olsson et al. 2003; Massoni et al. 2012). During processing, microorganisms are transferred to food by cross contamination via surfaces, machines, humans or from the surrounding atmosphere (Quintavalla and Vicini 2002; Skandamis and Nychas 2002; Kleer 2007; Kreyenschmidt and Ibald 2012). Due to the different sources of contamination during processing, the initial microbial flora can vary and consists of several different microorganisms. During storage, the microbial flora of perishable products is not static. Due to interaction between the organisms and supply chain parameters, the initial flora after processing differs from the one at the end of shelf life. In most perishable products some specific bacteria dominate the growth (Borch et al. 1996; Olsson et al. 2003; Nychas et al. 2008; Bruckner 2010). The composition of the flora depends on product characteristics and ingredients, the processing conditions, the environmental parameters like the temperature and packaging conditions (Mossel 1971; Kreyenschmidt and Ibald 2012). Besides temperature the packaging of a product plays an important role in slowing down microbial growth during the supply chain (Han 2005; Zhou et al. 2010). Packaging can also reduce several other spoilage reactions like respiration rates or browning reactions (Ahvenainen 2003). Thus, the application of innovative packaging strategies can deliver a contribution to reducing the amount of food waste (Verghese et al. 2013; SusFoFlex 2015).

1.2 The role of packaging for perishable products

Packaging generally fulfils several different roles. Besides the effect on spoilage reactions, which ensures that products can be transported and distributed over long distances, packaging material should meet specific requirements to protect the perishable product from damage or contamination by microorganisms and air, while also serving as a communication tool to the consumer. Thus, food packaging not only serves protection and marketing purposes but is also important in maintaining the safety and quality of food throughout the distribution chain (Ahvenainen 2003; Zhou et al. 2010). The requirements on the packaging and on the materials, depend on the product and supply chain characteristics (temperature conditions, transport routes and length, mode of transport). For fresh meat for example, a diversity of packaging technologies currently exists: Aerobic packaging is often used for short supply chains such as from a butcher or direct marketers. Modified atmosphere packaging which prolongs the shelf life up to 50 % is often applied at self-service retailers (Borch et al. 1996),

whereas vacuum packaging is often used in international meat supply chains. The developments of novel active packaging solutions to retain food quality and prolong shelf life have steadily increased during the last years (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Kenawy et al. 2007).

1.3 Active packaging for perishable products

Active packaging strategies are based on an integration of devices or antimicrobial components in the package, which interact with the product itself or the internal gaseous atmosphere between the package and the food (Labuza and Breene 1989). These reactions are leading to an increase of food quality, safety and shelf life or to an improve of sensory properties (Vermeiren 1999; Han 2000; Quintavalla and Vicini 2002; Ahvenainen 2003; Suppakul et al. 2003).

The first developments in this field took place in the 1970s. During the last years, several different active packaging solutions have been developed and are now available in the market. The global market for active packaging solutions was represented in 2011 with sales of nearly $ 8.8 billion in the food and beverage industry. The anticipated compounded annual growth rate is 5.2 %, leading to a market value of around $11.9 billion in 2017 (BCC Research Report 2013). Different examples of active packaging materials have appeared in the market in Japan and in the U.S., whereas in Europe these packagings are not widespread (Lavoine 2015). Active packaging technologies need to be approved by regulatory authorities in countries and have to comply with specific regulations in each country (Coma 2008; Prasad and Kochhar 2014). In Europe, the European Commission Regulation 1935/2004/EC and specifically Regulation EC 450/2009 are tackling the legal basis for requirements, correct use, safety and marketing of active materials for the application as food contact materials and articles. The European Food Safety Authority (EFSA) assesses active substance and, if approved, places substances on the positive list ”Community List“. Different threshold limit levels exist for agents regarding the release into the food and environment (EC No 1935/2004; EC No 450/2009).

The spectrum of active packaging solutions includes different fields, such as:

- Absorbers, scavengers of gases, off flavours, moisture, taints, UV light
- Removing of catalysing undesired food components
- Emitters, generators of gases and flavours
- Releasing antioxidant systems
- Antimicrobial systems
- Temperature controlled systems (insulting materials; self-heating or cooling) (Kerry et al. 2006; Brody et al. 2008)

These strategies can be applied in the form of foils, trays, pads, and sachets in packaging. Most of the systems, which are under development or available in the market, are focused on an antimicrobial effect to control or reduce microbial growth (Coma 2008). Here, the antimicrobial effect means against bacteria, yeasts and moulds and can be distinguished from the antibacterial effect means against only bacteria. In this connection however, they are usually considered equivalent, even if a material is only tested against bacteria.
The different antimicrobial strategies are based on absorption systems, release systems and immobilization systems (Han 2003). The different antimicrobial packaging solutions are shown in figure 1.

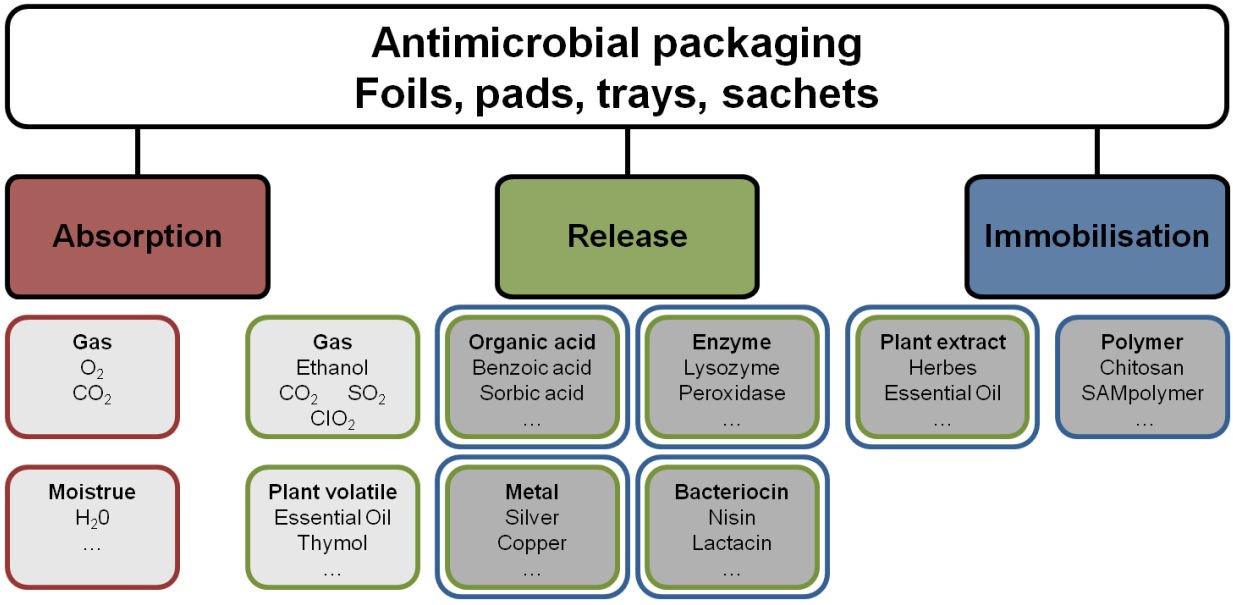


Figure 1 Different antimicrobial packaging systems

Commercially important antimicrobial packaging strategies for perishable foods are oxygen scavengers/ absorbers and moisture absorbers/regulators, carbon dioxide emitters/generators and antimicrobials integrated in packaging materials.

Oxygen scavengers/ absorbers for example bind the residual oxygen within the packaging to prevent oxidation of food constituents (Rooney 1981; Cruz et al. 2007). O_2 scavengers reduce and actively control the levels of oxygen, in some cases to < 0.01% oxygen (Vermeiren et al. 1999). Therefore, the growth of aerobic bacteria such as *Pseudomonas* spp.could be reduced for example in ground chicken meat (Mexis et al. 2012). Most systems are based on enclosing reactive compounds such as iron powder within porous sachets. Based on the presence of moisture in the headspace of food packaging, iron powder will oxidize and so absorb free oxygen (Vermeiren et al. 1999; Appendini and Hotchkiss 2002). Other systems are based on ascorbic acid oxidation catechol oxidation, photosensitive dye oxidation, or enzymatic oxidation (Floros et al. 1997). The first commercially developed oxygen scavenging sachets under the trade name Ageless® by Mitsubishi Gas Chemical Limited in Japan contained ferrous iron oxide which oxidizes to the ferric state (Nakamura et al 1983; Gilberg and Grattan 1994).

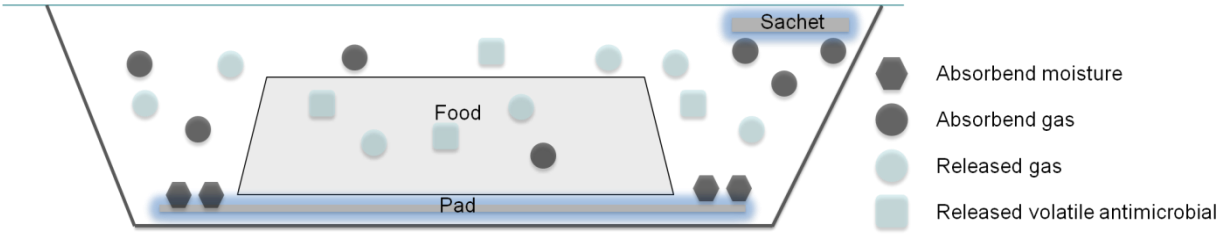
Moisture absorbers control the moisture in the packaging. Different drip-absorbent sheets or pads bind liquids, for example meat juice by packaged meat, poultry, fish or condense water by packaged fresh fruits. Thereby the growth of bacteria in the liquids can be reduced (Vermeiren et al. 1999). These systems are placed under the food and basically consist of a superabsorbent polymer which is incorporated between two layers (Suppakul et al. 2003).

An example for a release effect is the carbon dioxide emitters/ generators, which include additives to produce a steady stream of carbon dioxide inside the package. The production of CO_2 can be based on different chemical reactions, for example the reaction of sodium bicarbonate and citric acid with the moisture of the packaged food (Hansen et al. 2007; Hansen et al. 2009). The moisture can be absorbed for example in a cellulose pad containing the additives. CO_2 pads can be used in fresh meat, poultry and fish packages to increase the shelf life (Hansen et al. 2007; Hansen et al. 2009; Holck et al. 2014). Different modes of action of CO_2 on the microbiological growth are discussed. The effect could be mainly explained by dissolved CO_2 in the water- and fat phase of the product, for example meat, resulting in the formation of carbonic acid, which a directly ionizes, ultimately leading to a decrease of the surface pH (Devlieghere et al. 1998).

A decrease of the microbial spoilage can also be reached by integrating antimicrobials in packaging materials which directly inhibiting microbial growth or killing microorganisms during food storage. Per the origin and chemical properties, the antimicrobials can be divided in different classes, such as organic acids, bactericides, plant extracts, enzymes, metals and active polymers (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Ilg and Kreyenschmidt 2012; Prasad and Kochhar 2014). The mode of action of antimicrobial packaging materials can be differentiated between a release or an immobilization. For the implementation in packaging materials there are three approaches: Either a volatile or non-volatile antimicrobial agent is temporarily trapped within the backbone material and released from the polymer to the environment (time-releasing killing); or a non-volatile antimicrobial agent is permanently immobilised to a polymer backbone, or the whole macromolecule is antimicrobial on its own (contact-active killing) (Appendini and Hotchkkiss 2002; Han 2003; Siedenbiedel and Tiller 2012).

Regarding the time-releasing effect, volatile antimicrobial agents are released through evaporation or diffusion into the headspace or food, or through diffusion into the food surface for non-volatile antimicrobial agents. Volatile antimicrobials can penetrate most of the food matrix without direct contact, whereas non-volatile antimicrobials need direct contact with the food surface to be active (Han 2000; Appendini and Hotchkiss 2002; Ahvenainen 2003; Cooksey 2005). The antimicrobials can be integrated in foils as well as trays and pads. Besides a temporary attachment of antimicrobials to a material, the antimicrobial packaging materials can also consist of polymers with ionic or covalently immobilized antimicrobials or of polymers that are inherently antimicrobial. The integration of antimicrobials in packaging can be carried out by direct incorporation of the antimicrobial into the packaging material or by coating the packaging material with antimicrobials (Han 2000; Appendini and Hotchkkiss 2002; Ahvenainen 2003; Han 2003; Cooksey 2005; Siedenbiedel and Tiller 2012). Figure 2 shows exemplary MA packaging and vacuum packaging, integrating different antimicrobial systems.

Exemplary MA packaging – Antimicrobial pad and sachet



Exemplary vacuum packaging – Antimicrobial foil

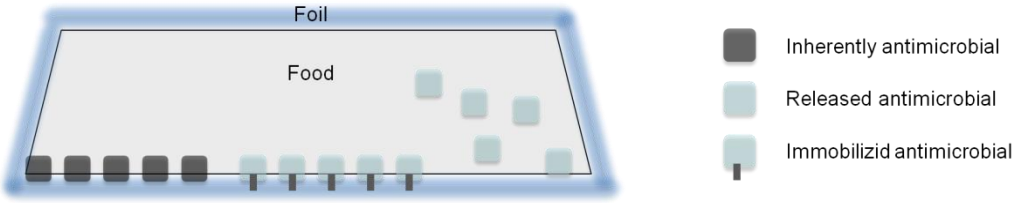


Figure 2 Exemplary MA packaging including an antimicrobial pad and sachet and an exemplary antimicrobial foil as vacuum packaging

Antimicrobial metals such as silver are common migrating antimicrobial additives, due to the high temperature and mechanical stability as well as low volatility (Kumar and Münstedt 2005; Simon et al. 2008; de Azeredo 2013). A commonly used antimicrobial agent incorporated into plastics is Ag-substituted zeolite in Japan (Lavoine 2015). A silver substituted zeolite is also approved by the FDA (FCN No. 773; 2008) for preserving food contact-polymers. The EFSA specifies a maximum level of 0.05 mg silver per kg food (EFSA 2004). Silver leads to structural changes in the cell wall of bacteria, interact with thiol groups in proteins and enzymes, interrupt replication by damaging the DNA and can generate reactive oxygen species (Gupta and Silver 1998; Morones et al. 2005; Dallas et al. 2011). For the antimicrobial effect of silver ions, the rate of silver release from the surface is of vital importance (Ovington 2004). The release of silver depends on the silver compound and on the polymer in which it is integrated (Monteiro et al. 2009). Studies have shown that the release process of different agents is also influenced by factors such as temperature or humidity (MacKeen et al. 1987; Russel and Hugo 1994; Kampmann et al. 2008; Simon et al. 2008; Asharani et al. 2009; Lee et al. 2011; Cushen et al. 2013).

Inherently antimicrobial polymers are contact-active due to their chemical structure (Siedelbiedel and Tiller 2012).

The bioactive polymer chitosan is of interest for antimicrobial packaging because it is naturally occurring and edible. The antimicrobial property of the polymer is possible due to the interaction between the positively charged chitosan molecules and the negatively charged microbial cell components resulting in the leakage of intracellular constituents and its work as chelating agents of essential minerals (Shahidi et al. 1999; Dutta et al. 2009; Higueras et al.

2013; Sung et al. 2013; Dehnad et al. 2014; Soysal et al. 2015; van den Broek et al. 2015). New active polymers are Sustainable Active Microbicidal (SAM®) polymers. They are intrinsic contact antimicrobials due to their chemical structure. The first industrial prototype of SAM® polymers was poly-[2-(tert-butylamino)ethyl methacrylate] (poly(TBAEMA)). The toxicity of SAM® polymers to mammalian cells is low, as the acute toxic level is higher than 2000 mg kg^{-1} and the polymers do not lead to skin irritation (Buranasombop 2005).

Per Thölmann et al. (2003), the antimicrobial activity of these polymers is based on its three-dimensional helical structure with high concentration of protonated functional amino groups. Different possible modes of actions to explain the antimicrobial activity are discussed. Previously conducted studies show strong antimicrobial activity of poly(TBAEMA) against a wide range of typical spoilage and pathogenic bacteria, moulds, yeasts and algae (Thölmann et al. 2003; Hewitt et al. 2004; Buranasompob 2005; Lenoir et al. 2006; Seyfriedsberger et al. 2006; Thomassin et al. 2007; Zuo et al. 2012; Compagnoni et al. 2014). A drawback of poly(TBAEMA) for the application as a packaging material relates to its glass transition temperature (T_G) of 40 °C. Based on this polymer, a new SAM® polymer, poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS)), with a raised T_G of 68 °C was developed (Patent WO2014118339 A1). This polymer shows a high antimicrobial activity at 35 °C against *Staphylococcus aureus* and *Escherichia coli*. Due to the material characteristic and the activity this polymer bears potential to act as packaging material. But up to now it is not known if poly(TBAMS) can be processed as packaging material generating an antimicrobial activity. It is also not clear if the material is still active under conditions which are typical for perishable food supply chains.

Furthermore, even if studies described that the application of innovative active packaging strategies can deliver a contribution to reduce the amount of food waste (Verghese et al. 2013, SusFoFlex 2015), the impact of active packaging application on economic and environmental aspects is not described in the literature. No studies are described the real effect of costs and benefits including the reduction of waste on a resource-efficient food production by an implementation of novel active packaging material.

1.4 Research questions and outline of the thesis

The main objective of this thesis is the assessment of novel active packaging materials containing the polymer poly(TBAMS) for the application in perishable food supply chains. Furthermore, the potential of poly(TBAMS) and active packaging materials to increase the safety and shelf life of perishable products and thus to improve the resource efficiency of food production is assessed.
These objectives lead to the following research questions:

1. How is the antimicrobial activity of poly(TBAMS) influenced by different product-, process and environmental factors typical for perishable food supply chains? (chapter 2, 3)

2. How is the antimicrobial activity of poly(TBAMS) influenced by processing the polymer to different packaging materials? (chapter 3, 4)

3. How is the effect of different materials containing poly(TBAMS) on the shelf life and safety of perishable products and on environmental and economic impact by reducing food waste? (chapter 3, 4, 6)

4. Is it possible to develop a standardized approach for the assessment of active packaging material's ability to increase the safety and shelf life of perishable products and to improve the resource efficiency of perishable food production? (chapter 5, 6)

In the first part (*chapter 2*) of the thesis, the influence of product-, process and environmental factors on the antimicrobial activity of poly(TBAMS) against several microorganisms is determined. The focus is laid on the application of poly(TBAMS) as packaging material for perishable products, especially for chilled meat products, focusing on the influence of specific organisms, e. g. psychrotrophic spoilage bacteria, food components, temperatures, packaging atmosphere and time.

In *chapter 3*, the antimicrobial activity of different packaging materials consisting of different concentrations of the homopolymer poly(TBAMS) and different standard matrix polymers are analysed and compared. In this context, the influence of different processing steps on the

antimicrobial activity of poly(TBAMS) are tested to produce different packaging solutions. The antimicrobial activity against typical spoilage and pathogenic bacteria in fresh meat is determined under dynamic temperature conditions as are usual in perishable food chains.

In *chapter 4* of the thesis, the effect of antimicrobial foils and pads containing poly(TBAMS) on the microbial and sensory spoilage process and chemical parameters of different perishable products is determined. Thereby, different product-, process and environmental influence factors on the activity and thus shelf life increase are analysed. For this purpose, fresh meat, meat products and ready to eat vegetables are packaged in different packagings containing poly(TBAMS) and in reference packagings and the shelf life is calculated by using the modified Gompertz model.

In the next part (*chapter 5*), a comprehensive evaluation scheme is developed to assess the ability of active materials acting as packaging to increase the safety and shelf life of perishable products. Based on all results which are described in chapter 2-4 and on literature data, the most important parameters of the main activity influencing product-, process and environmental factors are integrated into the scheme. The scheme supports researchers and the industry to identify the real antimicrobial activity and suitable application of a material in the food industry in a standardized way.

In the last *chapter 6*, an approach to determine the impact of the implementation of active packaging materials on the resource-efficiency of food production is carried out. The results are used for the establishment of standardized cost-benefit analysis. The approach is performed exemplarily for the foils and pads containing poly(TBAMS) based on the results (chapter 2-5) and expert interviews.

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2 Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products

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2.1 Introduction

In the recent years the interest for novel active packaging solutions to retain food safety and prolong shelf life has steadily increased (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Kenawy et al. 2007). Particularly in the meat supply chain, there is a high demand of active packaging, due to the short shelf life and safety concerns (Quintavalla and Vicini 2002). During slaughtering and processing, the meat surface is contaminated with a variety of microorganisms such as Enterobacteriaceae, *Pseudomonas* spp., *Aeromonas* spp., *Acitenobacte*r spp. and *Staphylococcus* spp. (Olsson et al. 2003). During storage, the growth of the bacteria is influenced by the product and environmental factors, such as: nutritional content, temperature, and packaging conditions (Kreyenschmidt and Ibald 2012). The use of antimicrobial packaging materials, which inhibit the growth of bacteria or even kill them during food storage, is increasingly discussed (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Kenawy et al. 2007). The mode of action of antimicrobial materials can be classified in two basic approaches. Either the antimicrobial agent is temporarily trapped with the polymer backbone (time-releasing killing), or the antimicrobial agent is permanently attached to the polymer backbone or is a part of the polymer (contact-active killing). The antimicrobial characteristic of the release effect is based on active substances, which are released from the material to the food or environment (Siedenbiedel and Tiller 2012). Typical agents of this group are organic acids, bactericides, plant extracts, enzymes, nano-silver as well as metals (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Prasad and Kochhar 2014). A requirement for the application as an antimicrobial packaging material is the controlled release of these antimicrobial agents under low temperature conditions (Quintavalla and Vicini 2002). However, studies have shown, that the antimicrobial action of different agents is often influenced by different factors including temperature (MacKeen et al. 1987; Russel and Hugo 1994; Kampmann et al. 2008; Simon et al. 2008; Asharani et al. 2009; Lee et al. 2011; Cushen et al. 2013). During the last years, the interest in contact-active killing mechanisms for packaging materials has increased. By using this mechanism, the antimicrobial activity is permanently attached to polymer chains or is generated by the whole macromolecule (Siedenbiedel and Tiller 2012). Cationic polymers such as chitosan bear high potential. A possible mode of action could be the interaction between positively charged polymers and negatively charged microbial cell membranes resulting in the leakage of intracellular constituents (Shahidi et al. 1999; Dutta et al. 2009; Higueras et al. 2013; Dehnad et al. 2014; Soysal et al. 2015). Sustainable active microbicidal (SAM®) polymers also belong to the intrinsic contact antimicrobials due to their chemical structure. The first industrial

prototype of SAM® polymers was poly-[2-(tert-butylamino)ethyl methacrylate] (poly(TBAEMA)). This SAM® polymer was developed in 2001 by Creavis Technologies & Innovation of Degussa (Brodkorb et al. 2015).

According to Thölmann et al. (2003), the antimicrobial activity of these polymers is based on the three-dimensional helical structure with a high concentration of protonated functional amino groups. Different modes of action are discussed to explain the antimicrobial activity. Hewitt et al. (2004) described a protonation of the functional amino groups based on the chemical reaction between functional groups on the surface and water molecules. This reaction could lead to an increase of the local pH-value and electrostatic interactions between the positively charged polymer and the negatively charged surface of bacteria in physiological solutions. The cytoplasmic membrane is depolarized resulting in permeability and cell death (Hewitt et al. 2004). Lenoir et al. (2006) assumed that the mode of action of poly(TBAEMA) relies on the exchange of Ca^{2+} and/or Mg^{2+} cations at the outer membrane of gram-negative bacteria through protonated functional amino groups. Thus, the outer membrane is disorganized and disrupted, leading to cell death of bacteria.

Previously conducted studies show strong antimicrobial activity of poly(TBAEMA) against a wide range of bacteria, moulds, yeasts and different algae (Thölmann et al. 2003; Hewitt et al. 2004; Lenoir et al. 2006; Seyfriedsberger et al. 2006; Thomassin et al. 2007; Zuo et al. 2012; Compagnoni et al. 2014). The major drawback of poly(TBAEMA) for the application as a packaging material relates to its glass transition temperature (T_G) of 40 °C. A low T_G leads to a sticky surface. Based on this polymer, a new SAM® polymer, poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS)), with a raised T_G was developed (Brodkorb et al. 2015). Figure 1 shows the chemical structure of poly(TBAEMA) and the new polymer poly(TBAMS).

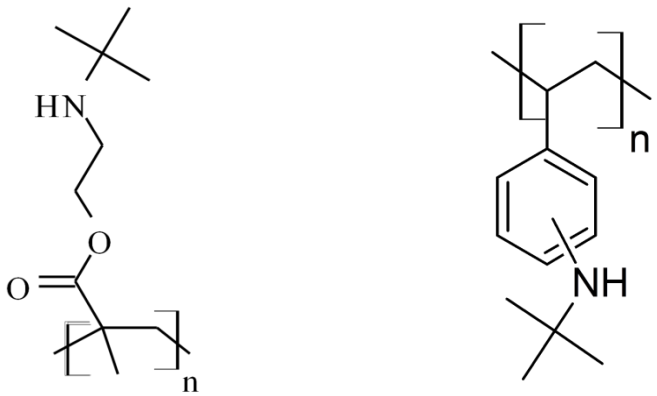

Figure 1 Chemical structure of poly(TBAEMA) (left) and poly(TBAMS) consisting of a mixture of meta- and para-isomers (right)

TBAMS consists of a mixture of meta- and para-isomers. The new polymer poly(TBAMS) has a glass transition temperature (T_G) of 68 °C. Furthermore, a high antimicrobial activity was detected against *Staphylococcus aureus* and *Escherichia coli* at 35 °C after 2 h contact time (Brodkorb et al. 2015). For the implementation as antimicrobial packaging material however, typical conditions of meat supply chains have to be considered.

Thus, the aim of this study is to investigate the antimicrobial potential of poly(TBAMS) for application as packaging material for chilled meat products. Therefore, the influence of environmental and product factors on the antimicrobial activity against several typical microorganisms is investigated.

2.2 Materials and Methods

Film preparation

The antimicrobial activity of poly(TBAMS) was tested for the potential to serve as antimicrobial packaging material. Therefore, poly(TBAMS) was synthesized according to Bordkorb et al. (2015). Petri dishes coated with a thin film of poly(TBAMS) were prepared in the following manner. For each sample, 125 mg of polymer were dissolved at 70 °C in 3 ml of 70 % ethanol. The ethanol poly(TBAMS) solution (41.97 mg ml^{-1}) was then spread as a thin film in polystyrene petri dishes without vents (VWR, Darmstadt, Germany) and then dried in a vacuum drying cabinet at 70 °C and 2 mbar for 1 h. The poly(TBAMS) formed a thin (thickness: around 20 µm), uniform transparent film. Petri dishes without vents were used as reference materials.

To determine the influence of carbon dioxide on the activity of poly(TBAMS), for a few experiments petri dishes with poly(TBAMS) film were stored under a specific gas atmosphere for 7 days at 7 °C before the antimicrobial film test was started. The dishes were placed in polypropylene trays (R. Fearch Plast A/S, Holstebro, Denmark, tray volume 680 ml). Thereafter, the trays were heat-sealed with a polypropylene foil (Suedpack Verpackungen GmbH and Co. KG, Ochsenhausen, Germany; water vapour permeability < 3.5 g/m^2d at 23 °C / 85 % RH; oxygen permeability </=1.5 cm^2/m^2d bar at 23 °C / 35 % RH) for 3 s/175°C using a tray sealer packaging machine (Traysealer T200, Multivac Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany). Gas mixtures were prepared by a four-component gas blender machine (KM 60-4 MEM SO, Witt Gasetechnik, Witten, Germany) to achieve an atmosphere containing 95 % CO_2 and 5 % O_2 or 95 % O_2 and 5 % CO_2. As references petri

dishes were packed under air atmosphere. After the storage period the concentrations of oxygen and carbon dioxide inside the trays, were monitored, using a hand-held gas analyser (Oxybaby V O_2/CO_2, Witt Gasetechnik, Witten, Germany).

Antimicrobial film test

The antimicrobial activity of poly(TBAMS) was analysed by modifying the Japanese Industrial Standard (JIS) Z 2801:2000 in order to test conditions typical for meat products. The JIS is based on a comparison of bacteria counts (*Staph. aureus*, *E. coli*) in saline solution on reference and sample materials after a defined storage temperature and time (35 °C, 24 h). In the present study, the test microorganisms, inoculated solutions and food samples, temperatures, and times were adapted to conditions typical for meat products. The investigations were subdivided into three parts. In the first part, the general antimicrobial activity of poly(TBAMS) samples against different spoilage and pathogenic bacteria was tested after 24 h and 48 h at 7 °C. The second part included the determination of the influence of different temperatures for 24 h. The influence of meat components and time on the antimicrobial activity of poly(TBAMS) were investigated at 7 °C for 24 h and 48 h in the third part.

Bacterial cultures

Staphylococcus aureus, *Listeria monocytogenes*, *Lactobacillus* spp., *Brochothrix thermosphacta*, *Escherichia coli* and *Pseudomonas fluorescens* were chosen as typical test organisms. The strains were delivered by the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany) and were frozen in cryogenic pellets for preservation. The bacterial solutions for the inoculation of the samples and references were prepared by transferring frozen cultures (-18 °C) into 10 ml of nutrient broth (Roth, Karlsruhe, Germany). The broth was then incubated for 24 h at the optimal growth temperature for each microorganism according to the instructions of the DSMZ (table 1).

Preparation of inoculum

At the beginning of each trial, 1 ml of the 24 h culture was diluted in 9 ml of physiological saline solution (8.5 µg ml^{-1}) with 1 µg ml^{-1} tryptone (Oxoid, Hampshire, United Kingdom). The general antimicrobial activity (part I of experiments) and the influence of temperature (part II of experiments) were tested by diluting the inoculum of the pure bacteria in physiological saline solution up to a concentration of 10^5 – 10^6 cfu ml^{-1}. To test the influence

of meat components (part III of experiments) on the activity, different concentrations of meat extract (Merck, Darmstadt, Germany), which was prepared from selected animal tissues, and different concentrations of heat shocked bovine serum albumin (BSA) (Roth, Karlsruhe, Germany) were dissolved in sterile distilled water. Additionally, the influence of pure meat juice, obtained from fresh pork tenderloin, on the antimicrobial activity was analysed. Meat juice, meat extract-, and BSA solution were mixed with the pure bacteria saline solution to a concentration of $10^5 – 10^6$ cfu ml^{-1} (Table 2). The mixed culture inoculum (part I and III of experiments) was prepared by adding *Staph. aureus*, *B. thermosphacta*, *E. coli* and *Ps. fluorescens*, (table 2) to a total concentration of $10^5 – 10^6$ cfu ml^{-1}.

Determination of the antimicrobial activity of poly(TBAMS) film against different microorganisms

A minimum of three test samples (poly(TBAMS)) and six references (without poly(TBAMS)) samples were used for each experiment. In the different trials, each reference and sample was inoculated with 0.4 ml of a $10^5 – 10^6$ cfu ml^{-1} concentrated *Staph. aureus*, *L. monocytogenes*, *Lact. spp.*, *B. thermosphacta*, *E. coli* or *Ps. fluorescens* suspensions as well as with mixed cultures. The bacterial concentrations on three references were determined immediately after inoculation to determine the initial concentration at t = 0. Each reference was washed out with 10 ml of soybean-casein digest broth with lecithin polysorbate (Roth, Karlsruhe, Germany) and the microbial count of the solution was determined. The other references and samples were stored in a high precision incubator (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) at 7° C for 24 h or 48 h. To prevent evaporation and to ensure a standardized contact of the material and the bacteria suspension during storage, each inoculum was covered by sterile poly ethylene film (40mm x 40 mm). After this storage time, the references and test samples were washed in the same manner as described above and the microbial load was analysed. Colony-forming units (cfu) were determined by using the pour plate method with plate count agar (PCA, Merck, Darmstadt, Germany). Petri dishes were incubated 48 h for *Staph. aureus*, *L. monocytogenes*, *E. coli* at 37 °C, for *Lact.* spp. at 30 °C and for *B. thermosphacta* and *Ps. fluorescens* at 25 °C. Determination of total viable counts (TVC) of mixed cultures were enumerated using pour plate technique with plate count agar, where the plates were incubated at 30 °C for 72 h. The individual counts of bacteria in mixed culture were determined with different media and culture techniques. Table 1 gives an overview of the used media, techniques, and the incubation time and temperature.

Table 1 Summary of the used strains, cultivation and enumeration temperature, technique and growth medium

Bacteria	Strain DSMZ	Cultivation and enumeration temperature	Technique	Medium (Brand)
Staphylococcus aureus	799	37 °C	Spread plate	Baird Parker Agar (Oxoid, Cambridge, United Kingdom)
*Listeria monocytogenes**	19094	37 °C	Pour plate	Plate count agar (Merck, Darmstadt, Germany)
*Lactobacillus spp.**	20182	30 °C	Pour plate	Plate count agar (Merck, Darmstadt, Germany)
Brochothrix thermosphacta	20171	25 °C	Drop plate	Streptomycin Inosit Toluylene Red Sheep Blood Agar Base (Oxoid, Cambridge, United Kingdom)
Escherichia coli	787	37 °C	Overlay plate	Violet Red Bile Dextrose Agar (Merck, Darmstadt, Germany)
Pseudomonas fluorescens	50090	25 °C	Spread plate	Pseudomonas agar with Cetrimide-Fucidin-Cephalosporin selective supplement (Oxoid, Cambridge, United Kingdom)

* not tested in mixed culture

Results were expressed as the number of colony forming units per millilitre. Detection limits were determined to be of 1.0 $\log_{10}$ cfu ml^{-1} for pour plate, overlay and spread plate technique and 2.0 $\log_{10}$ cfu ml^{-1} for drop plate technique.

In summary 223 references and 128 poly(TBAMS) samples were tested against different microorganisms in a concentration of 10^5- 10^6 cfu^{-1} ml at 7 °C for 24 h.

Determination of the influence of temperatures and meat components on the antimicrobial activity of poly(TBAMS) film

In the second part of the experiment, the influence of temperatures on the antimicrobial activity was tested. The experiments were conducted in the same way as described above, but references and samples were stored at 2, 4, 7, 25 and 35 °C for 24 h.

In the third part, the influence of meat components on the activity of the polymer were investigated by using different concentration of meat extract- and BSA solutions and meat juice as inoculums. Therefore, the solutions were either inoculated with pure bacterial cultures or mixed cultures to a concentration of 10^5 – 10^6 cfu ml^{-1}. The references and samples were stored for 24 h or 48 h at 7 °C.

Furthermore, chicken breast fillets were inoculated with bacteria suspensions. For the experiments, the surface meat of chicken breast fillets was removed aseptically by using a sterile scalpel. The product samples had measurements of 4 x 4 x 0.5 cm meat surface and had a total weight of about 10 g. The meat surface was inoculated with a bacteria concentration of 10^5-10^6 cfu ml^{-1} in saline solution. The inoculated side was put in sample dishes on the

poly(TBAMS) film and reference dishes and the meat piece was covered with a sterile poly ethylene film. The references and samples were stored for 24 h at 7 °C. For the microbiological analyses, the meat samples were transferred to a filtered, sterile stomacher bag and filled with 90 ml saline tryptone diluent (8.5 µg ml^{-1} with 1 µg ml^{-1} tryptone). Samples were homogenized with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the sample homogenates were prepared in saline tryptone diluents. Total viable count (TVC) was enumerated. Counts of colony forming units (cfu) were expressed as $\log_{10}$ cfu g^{-1} for each medium and sample.

Table 2 gives an overview of the different experiments

Table 2 Times, temperatures and meat components to investigate the antimicrobial activity of poly(TBAMS) against pathogenic and spoilage bacteria

Test conditions	7 °C		24 h					7 °C 48 h	7 °C 24 h					
	Time h		Temperature °C					Meat extract	Meat extract µg ml^{-1}			Meat juice	Poultry fillets	BSA µg ml^{-1}	
Microorganisms	24	48	35	25	7	4	2	18 µg ml^{-1}	18	9	0.18	fillet of pork	pieces	45	4.5
Staphylococcus aureus	+ *	+	+	+	+ *†°	+	+	+	+ *	+	+	+	+	+	+
Listeria monocytogenes	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Lactobacillus spp.	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Brochothrix thermosphacta	+ *	+	+	+	+ *†°	+	+	+	+ *	+	+	+	+	+	+
Escherichia coli	+ *	+	+	+	+ *†°	+	+	+	+ *	+	+	+	+	+	+
Pseudomonas fluorescens	+ *	+	+	+	+*†°	+	+	+	+ *	+	+	+	+	+	+

+ tests with pure cultures * tests with mixed culture - not tested
†further tested: bacteria concentration of 10^3- 10^4 cfu ml^{-1}
°further tested: previous storage of the petri dishes samples under specific gas atmosphere

To investigate the influence of environmental factors 150 references and 129 samples were tested. To test the product factors 300 references and 171 poly(TBAMS) samples were analysed.

Zeta potential and electrophoretic mobility measurement

Furthermore, the zeta potential and electrophoretic mobility of *B. thermosphacta* and *Ps. fluorescens* were measured at different temperatures.The zeta potential and electrophoretic mobility of *B. thermosphacta* and *Ps. fluorescens* were measrured with the Malvern ZEN2600 Zetasizer Nano Z (Malvern Instruments Ltd, Worcestershire, UK). Therefore, pure bacteria in physiological saline solution of a concentration of 10^5 – 10^6 cfu ml^{-1} were stored 24 h at 25 °C or 7 °C temperatures.

Antimicrobial activity analysis

The value of antimicrobial activity was calculated by subtracting the logarithmic value of the calculated viable counts on the poly(TBAMS) material from the viable counts on the reference material after inoculation and incubation, as shown in the following equation:

$$\log_{10}\text{-reduction} = \log_{10} (T_{xRe} / T_{xSa})$$

where $T_{x,Re}$ = bacterial concentration on reference material, x hours after inoculation
and $T_{x,Sa}$ = bacterial concentration on sample material, x hours after inoculation

Adapted to the Japanese Industrial Standard (JIS Z 2801:2000), the material that showed a calculated $\log_{10}$-reduction $\geq$ 2.0 $\log_{10}$ units after 24 h at different temperatures were considered as effective antimicrobial (JIS Z 2801:2000).

Statistical analysis

All data were transformed into $\log_{10}$ values before statistical analysis. The standard deviations in bacterial counts on references and poly(TBAMS) samples were calculated. The differences in bacterial counts on references at t = 0 h and t = 24 h, differences in bacterial counts on poly(TBAMS) samples and references, and differences in the $\log_{10}$-reduction of bacteria in saline solution and bacteria in meat extract solution (18 µg ml^{-1}) were analysed for significance using the Mann-Whitney U test for independent samples. The significance level was defined as a p value of $\leq$ 0.05, respectively the p values of $\leq$ 0.01 and $\leq$ 0.001 as highly significant. For description of data, box plots and bar charts were used. Data analysis was conducted with SPSS Statistics 22 (IBM Corp. 1989, 2013, New York, USA) and OriginPro 8G (OriginLab Corp., Northampton MA, USA).

2.3 Results

Antimicrobial activity of poly(TBAMS) film against different microorganisms

The bacterial concentrations of *Staph. aureus*, *L. monocytogenes*, *Lact. spp.*, *B. thermosphacta*, *E. coli* and *Ps. fluorescens* in saline solution before and after storage for 24 h are shown in figure 2. The bacterial counts of *B. thermosphacta* and *Ps. fluorescens* on reference material at t = 0 h and t = 24 h show the slight differences of 0.21 $\log_{10}$ units ($p \leq 0.01$) and 0.27 $\log_{10}$ units ($p \leq 0.001$). All bacterial counts on the poly(TBAMS) samples (n = 126) are significantly decreased compared with the bacterial counts on the references after 24

h storage at 7 °C ($p \leq 0.001$). The concentrations of *Staph. aureus* is minimized below the detection limit of 1.0 $\log_{10}$ cfu ml^{-1} on all poly(TBAMS) samples (n = 39) after 24 h. The bacterial counts of *Staph. aureus*, *L. monocytogenes*, *Lact. spp.*, *B. thermosphacta* and *E. coli* are reduced more than 4.0 $\log_{10}$ units. A reduction rate of 2.69 $\log_{10}$ units is identified against *Ps. fluorescens* (figure 2). The standard deviations in bacterial counts on the poly(TBAMS) samples are marginal with < 0.6 $\log_{10}$ cfu ml^{-1} except for *Ps. fluorescens.* There is a standard deviation in bacterial counts of 1.09 $\log_{10}$ cfu ml^{-1}.

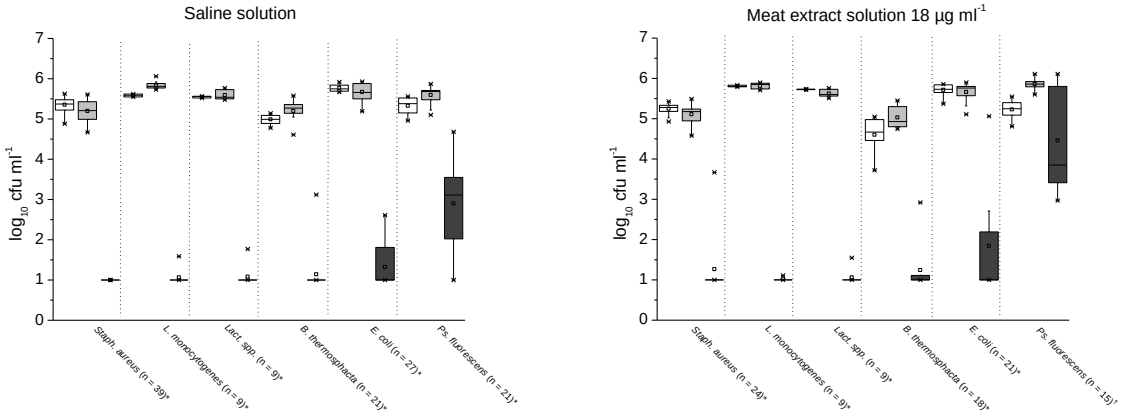

Figure 2 Box plots of viable counts on reference compared with poly(TBAMS) samples after inoculation with various microorganisms in saline solution (left) and meat extract solution 18 µg ml^{-1} (right) after 24 h storage time at 7 °C. * Differences in bacterial counts on poly(TBAMS) samples and references: Level of significance $p \leq 0.001$; † Differences in bacterial counts on poly(TBAMS) samples and references: Level of significance $p \leq 0.01$. Bacterial counts: (▭) Inoculum, (▭) Reference and (▭) Sample

Initial concentrations of around 10^3 $\log_{10}$ cfu ml^{-1} of *Staph. aureus*, *B. thermosphacta*, *E. coli* and *Ps. fluorescens* are reduced under the detection limit after 24 h at 7 °C (data not shown).

Figure 3 shows the TVC of a mixed culture and the individual counts of *Staph. aureus*, *B. thermosphacta*, *E. coli* and *Ps. fluorescens* in saline solution on references and poly(TBAMS) samples directly and after 24 h storage at 7 °C. A reduction rate of 4.45 $\log_{10}$ units of the TVC is observed on the poly(TBAMS) samples after storage. The individual counts of *Staph. aureus, E. coli* and *B. thermosphacta* are reduced lower than the detection limit. *Ps. fluorescens* is decreased to a bacterial concentration of 1.84 ± 0.75 $\log_{10}$ cfu ml^{-1}.

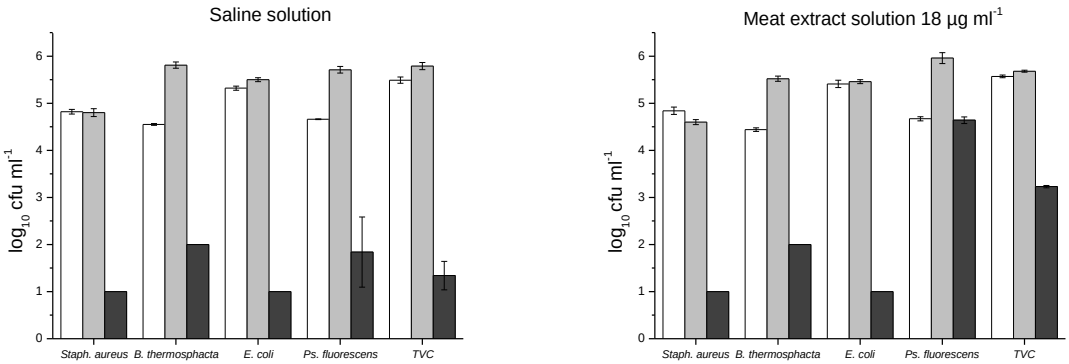

Figure 3 Arithmetic means of bacterial counts of mixed culture in saline solution (left) and in meat extract solution 18 µg mL^{-1} (right) on reference and poly(TBAMS) samples after 24 h at 7 °C. Total viable counts (TVC) of mixed bacteria were determined with plate count agar, the individual counts with different media. Bacterial counts (n = 3): (▭) Inoculum, (▭) Reference and (▭) Sample

The influence of different gas atmospheres was tested. In the experiments with previous storage of the samples for 7 days under 95 % CO_2 until the end of storage, all bacteria are reduced under the detection limit with log_{10}-reduction of more than > 4.3 log_{10} units. The samples stored under high oxygen concentration show comparable results with those stored under the usual air atmosphere (data not shown).

The influence of time on the antimicrobial activity of poly(TBAMS) was tested with two gram-positive and two gram-negative bacteria. The bacterial counts of *Staph. aureus, B. thermosphacta, E. coli* and *Ps. fluorescens,* are reduced lower than the detection limit at 7 °C after 48 h (n = 3, data not shown).

Influence of temperatures on the antimicrobial activity of poly(TBAMS) film

The bacterial concentrations of *Staph. aureus, B. thermosphacta, E. coli* and *Ps. fluorescens* in saline solution on the references and poly(TBAMS) samples after storage at different temperatures are shown in figure 4. At 2 °C and 4 °C none of the tested bacteria show an increase of bacterial counts on the reference material after 24 h. The bacterial counts of *Staph. aureus* and *E. coli* increase more than 2.2 log_{10} units at 25 °C and 35 °C, the counts of *Ps. fluorescens* and *B. thermosphacta* raise at 25 °C. On the poly(TBAMS) samples all bacteria stored at 25 °C and 35 °C are minimized to the detection limit of 1 log_{10} unit after 24 h. The counts of *Staph. aureus* and *E. coli* are reduced > 6.2 log_{10} units at 25 °C and 35 °C, *B. thermosphacta* and *Ps. fluorescens* are reduced > 5.2 log_{10} units at 25 °C after 24 h. At 2 °C and 4 °C poly(TBAMS) shows log_{10}-reductions > 3.1 log_{10} units against the tested bacteria except *Ps. fluorescens* (figure. 4).

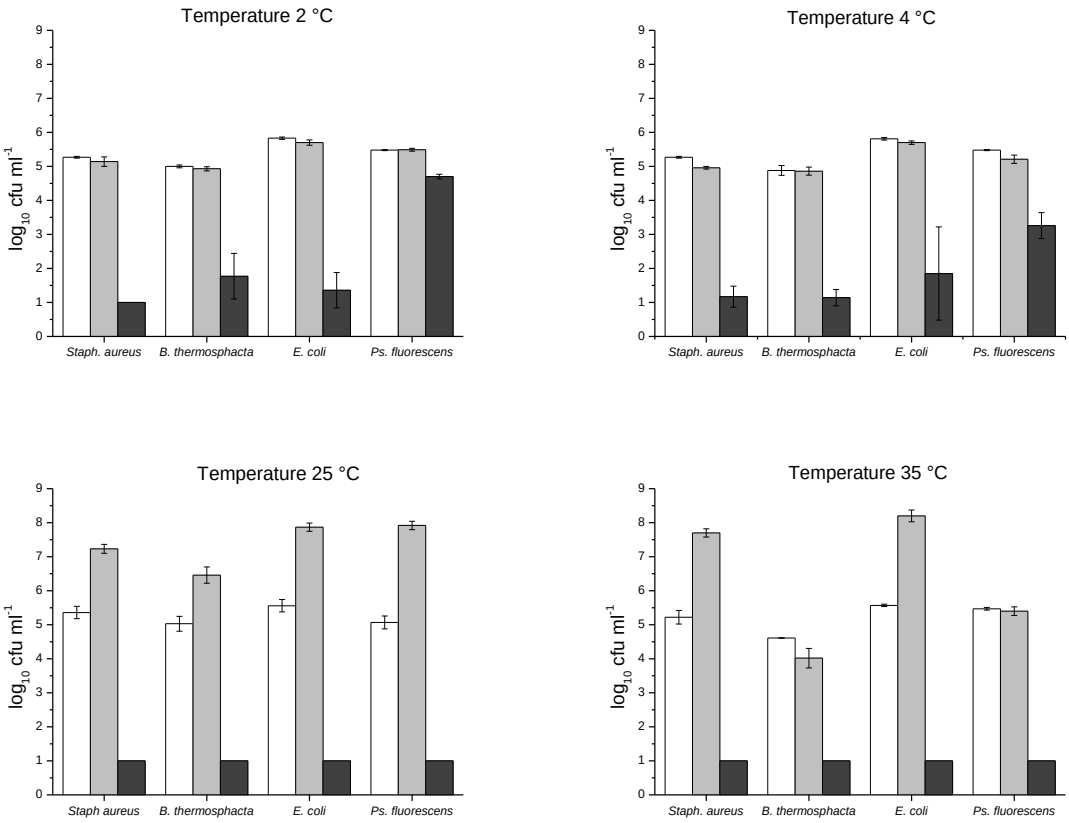

Figure 4 Arithmetic mean of bacterial counts in saline solution at 2 °C (top left), 4 °C (top right), 25 °C (bottom left), and 35 °C (bottom right) on reference and poly(TBAMS) samples after 24 h.
Bacterial counts (n = 3): (▭) Inoculum, (▭) Reference and (▬) Sample

Influence of meat components on the antimicrobial activity of poly(TBAMS) film

For the most part, influence of meat components on the antimicrobial activity of poly(TBAMS) was tested with six different bacteria inoculum containing meat extract (18 µg ml^{-1}). The bacterial counts of *Staph. aureus*, *L. monocytogenes*, *Lact.* spp., *B. thermopshacta*, *E. coli* and *Ps. fluorescens* on the references and poly(TBAMS) samples after 24 h storage at 7 °C are shown in figure 2. Changes of the counts on the references in the presence of meat extract solution are comparable with those in saline solution after 24 h. Slight differences are observed for *B. thermosphacta* with an increase of 0.43 $\log_{10}$ units ($p \leq 0.05$) and *Ps. fluorescens* with an increase of 0.63 $\log_{10}$ units ($p \leq 0.001$) after 24 h storage at 7 °C.

The bacterial concentrations of *Staph. aureus*, *L. monocytogenes*, *Lact.* spp., *B. thermosphacta* and *E. coli* are significantly reduced > 3.7 $\log_{10}$ units ($p \leq 0.001$) on the poly(TBAMS) samples. The reduction in bacterial counts of *Ps. fluorescens* between the references and samples from 5.86 ± 0.12 to 4.46 ± 0.14 is significant with a p value of ≤ 0.01 (figure 2).

Mixed culture experiments with meat extract solution (18 µg ml^{-1}) show the same trends. The bacterial counts of *Ps. fluorescens* are higher on poly(TBAMS) material compared to the other microorganisms (figure 3). Significant differences ($p \leq 0.05$) are demonstrated by comparing log_{10}-reductions of *Staph. aureus*, *B. thermosphacta* and *Ps. fluorescens* in meat extract solution (18 µg ml^{-1}) with log_{10}–reductions in saline solution after 24 h at 7 °C.

The concentrations of *Staph. aureus* and *B. thermosphacta* as well as *E. coli* in meat extract solution (18 µg ml^{-1}) are minimized below the detection limit of 1.0 log_{10} unit on samples by prolonging the storage time to 48 h at 7 °C. For *Ps. fluorescens*, the log_{10}-reduction increases to 3.48 log_{10} units (n = 3, data not shown).

The antimicrobial activity was also investigated with inoculated meat juice. The bacterial counts of *Staph. aureus*, *B. thermosphacta* and *E. coli* are reduced > 3.9 log_{10} units on poly(TBAMS) samples at 7 °C after 24 h. Poly(TBAMS) shows a log_{10}-reduction < 2.0 log_{10} units against *Ps. fluorescens* (n = 3, data not shown). By the experiments with inoculated chicken breast fillets a log_{10}-reduction of 0.9 log_{10} units is achieved against *Ps. flureoscens*. The other tested bacteria are reduced between 1.2 and 1.7 log_{10} units.

Further tests were conducted with lower concentrations of meat extract (9 µg ml^{-1} and 1.8 µg ml^{-1}) and different concentrated BSA solutions (figure 5). For *Ps. fluorescens*, the log_{10}-reduction increases to 2.18 log_{10} units at a meat extract concentration of 9 µg ml^{-1} and to 4.18 log_{10} units at a concentration of 1.8 µg ml^{-1}. All other bacteria are reduced lower than the detection limit of 1.0 log_{10} unit.

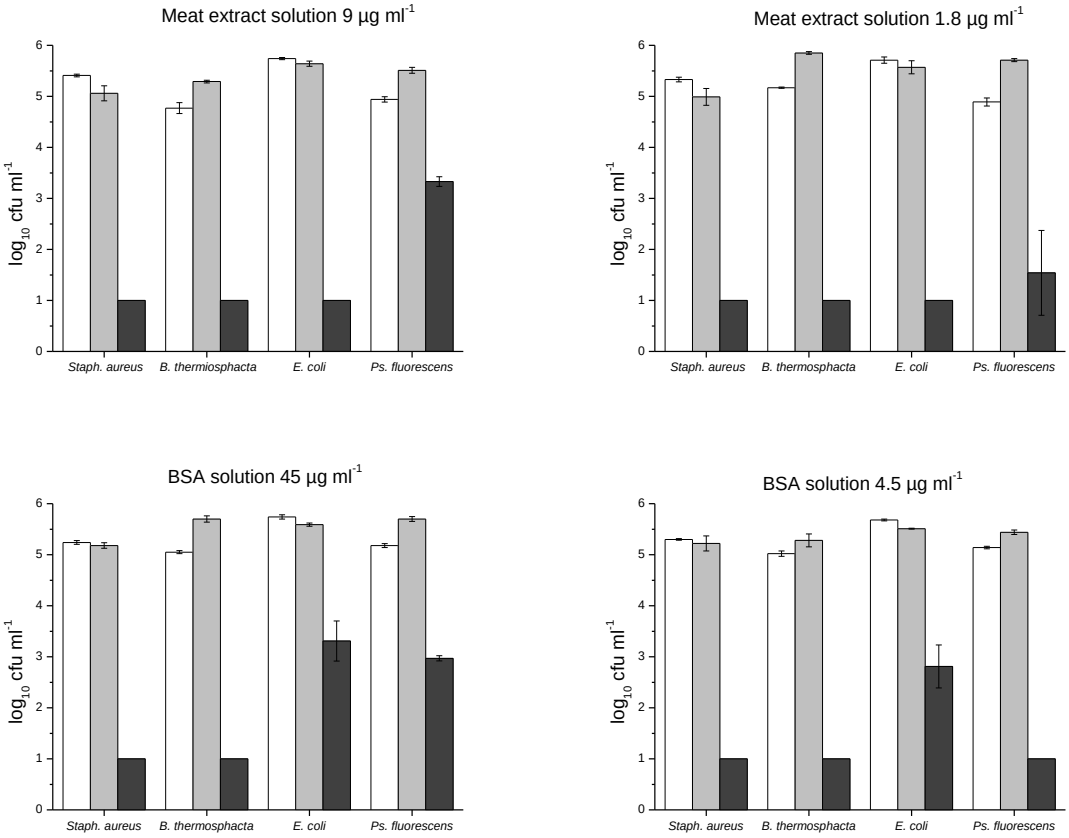

Figure 5 Arithmetic means of bacterial counts in meat extract solution with concentration of 9 µg ml^{-1} (top left) and 1.8 µg ml^{-1} (top right) and BSA with concentration of 45 µg ml^{-1} (bottom left) and 4.5 µg ml^{-1} (bottom right) on reference and poly(TBAMS) samples after 24 h at 7 °C.
Bacterial counts (n = 3): (▭) Inoculum, (▭) Reference and (▬) Sample

The influence of BSA (45 µg ml^{-1} or 4.5 µg ml^{-1}) on the antimicrobial activity of poly(TBAMS) is shown in figure 5 on reference and poly(TBAMS) materials. The bacterial counts of *Staph. aureus* and *B. thermosphacta* are reduced lower than the detection limit in all experiments. For *E. coli*, the $\log_{10}$-reductions are 2.28 $\log_{10}$ units (45 µg ml^{-1}) and 2.70 $\log_{10}$ units (4.5 µg ml^{-1}) on poly(TBAMS) material.

Electrophoretic mobility and zeta potential

The electrophoretic mobility and zeta potential of *B. thermosphacta* and *Ps. fluorescens* after 24 h storage at 7 °C and 25 °C are shown in table 3.

Table 3 Electrophoretic mobility and zeta potential of *B. thermosphacta* and *Ps. fluorescens* after 24 h at 7 °C and 25 °C

	Temperature (°C)	Zeta potential (mV)	Electrophoretic mobility (µmcm/Vs)
B. thermosphacta	25	-14.92	-1.09
	7	-10.81	-0.62
Ps. fluorescens	25	-5.44	-0.40
	7	-4.44	-0.32

The zeta potential and electrophoretic mobility of *B. thermosphacta* is stronger negative that of *Ps. fluorescens*. Decreasing the temperature leads to bacteria with lower negative charge.

2.4 Discussion

The poly(TBAMS) material shows a very high antimicrobial activity against a wide range of individual bacteria cultures as well as a typical mixed culture for meat products after 24 h at 7 °C. The marginal standard deviations in all bacterial counts except of *Ps. fluorescens* on 128 poly(TBAMS) samples show the reproducibility and standardized condition of the experiments and the antimicrobial activity at low temperature. Electrostatic interactions between the positively charged polymer and the negatively charged surface of bacteria lead to depolarization of the cytoplasmic membrane resulting in cell death. The protonation of the functional amino groups on polymer surface is initiated by carbon dioxide which is dissolved in aqueous film forming carbonic acid. In the directly following reaction the basic amino groups are protonated by the carbonic acid. If the samples were stored under high carbon dioxide concentration before the antimicrobial test was started, the activity can be increased. However, it is apparent that the activity of poly(TBAMS) is influenced by the bacterial strains itself. The gram-negative bacteria *E. coli* and *Ps. fluorescens* are less sensitive to the polymer compared with the other tested microorganisms after 24 h at 7 °C. Differences in the level of antimicrobial activity can possibly be explained by the different varying strength of the negative charges that these bacteria exhibit in physiological solutions. These different charges could lead to different electrostatic interactions with the positive charged polymer. In different studies, a higher activity of high molecular weight chitosan against gram-positive bacteria compared with gram-negative bacteria was observed (No et al. 2002; Fernandes et al. 2008; Fernandez-Saiz et al. 2009). Seyfriedsberger et al. (2006) demonstrated that the antimicrobial activity of a compound containing linear low density polyethylene and poly(TBAEMA) and the positive charge of the compound depend on the amount of poly(TBAEMA) on polymer surface´s. For a high antimicrobial activity against *E. coli* a higher percentage of poly(TBAEMA) compared with tests against *Staph. aureus* was necessary. A study by Rawlinson et al. (2011) investigated the antimicrobial activity of a cationic polymer, poly-[2-(dimethylamino ethyl)methacrylate], on the growth of *Staph. epidermis* and *Staph. aureus.* The authors detected a significant correlation between the electrophoretic mobility of the microorganisms and the antimicrobial activity of the cationic polymer. Potter et al. (2005) showed that the electrophoretic mobility of the gram-positive bacteria *B. thermopshacta, Lact. sake, Staph. aureus* and *L. monocytogenes* is higher than that of the gram-negative bacteria *E. coli* and *Ps. fluorescens.* The lowest electrophoretic mobility of -0,037 μm/s/v/cm x 10^9 was evidence of *Ps. fluorescens* (Potter et al. 2005). In the own measurements, a higher negative electrophoretic mobility of *B. thermosphacta* than of *Ps. fluorescens,* is also

observed. This could also explain own results, in which *Ps. fluorescens* is most resistant followed by *E. coli*. In their experiments with *Staph. epidermidis* and *Ps. fluorescens* moreover, Hewitt et al. (2004) observed, a higher sensitivity of gram-positive bacteria to poly(TBAEMA) than gram- negative bacteria. *Ps. fluorescens* treated with EDTA showed a higher sensitivity to poly(TBAEMA) due to a permeability of the outer membrane. Based on the results of their study, they assumed the existence of a protective function of the outer membrane of gram- negative bacteria (Hewitt et al. 2004). Similar findings were described by Chung et al. (2003). They detected a significant increase of the antimicrobial activity of chitosan by adding EDTA to bacterial concentration of *E. coli* (Chung et al. 2003). As stated by Gottenbos et al. (2001) the resistance of *Pseudomonas* spp. compared with *E. coli* against cationic surfaces is originated by a higher production of exopolysaccharides.

The results show that the level of antimicrobial activity of poly(TBAMS) against *E. coli* and *Ps. fluorescens* can be increase by prolonging the storage time to 48 h. Further investigations will be necessary to determine the most likely responsible mechanisms of the longer stability and lower sensitivity of *Ps. fluorescens* and, to a lesser extent, *E. coli* against poly(TBAMS).

If the storage temperatures are near the optimal temperatures of the microorganisms in the experiments, the bacterial counts of the references are higher than the initial counts after 24 h. Microorganisms showed a higher metabolism and growth at their temperature optima (Jay 1992). Psychrotrophic bacteria such as *B. thermosphacta* and *Ps. fluorescens* slowed their growth at 30 °C (Eddy 1960). At 25 °C and 35 °C a high activity of poly(TBAMS) is observed against all tested bacteria. Despite of the microbial growth on references at optimal temperatures, the bacterial counts on samples are reduced lower than the detection limit. As a result, high $\log_{10}$-reductions are achieved. Particularly there is a gain in sensitivity of *Ps. fluorescens*. The results indicate that the antimicrobial activity is influenced by the temperature, especially the activity against gram-negative bacteria. The material shows a high activity against *Staph. aureus*, *B. thermosphacta* and *E. coli* at 7 °C, 4 °C and 2 C. Nonetheless, an effect of temperature can be observed on the activity against *E. coli* and particularly *Ps. fluorescens* at 7° C, 4 °C and 2 °C. Tsai and Su (1999) investigated the influence of different temperatures on the antimicrobial activity of chitosan against *E. coli*. The authors also observed a reduction of antimicrobial activity with decreasing temperature. A possible explanation for the different antimicrobial activity against *E. coli* and *Ps. fluorescens* could be the different fatty acids, especially at cool temperatures, present in the outer membrane of *Ps. fluorescens* and *E. coli* (Cullen et al. 1971; Gill and Suisted 1978). A study by Briandet et al. (1999) showed a lower negatively charged surface of

L. monocytogenes at temperature of 8 °C compared with higher temperature, which is confirmed by own results with *B. thermosphacta* and *Ps. fluorescens*.

If meat components in form of meat extract solution (18 µg ml^{-1}) are present, a decrease of the antimicrobial activity against *Staph. aureus*, *B. thermosphacta* and particularly *Ps. fluorescens* is observed. Nonetheless, the majority of the samples the bacterial counts of *Staph. aureus* and *B. thermosphacta* are reduced lower than the detection limit. Furthermore, like the experiments with saline solutions the activity in the presence of meat extract can be increased by prolonging the time. Mixed culture experiments with meat extract solution show the same trends, with, *Ps. fluorescens* being more resistant to poly(TBAMS) than the other microorganisms. The experiments with meat juice demonstrate similar results to those with meat extract solution (18 µg ml^{-1}). The effect of meat components on the level of antimicrobial activity is probably related to cationic ions like Ca^{2+}, Mg^{2+}, and Fe^{2+} in meat. An investigation by Lenior et al. (2006) showed that ≥ 0.2 M of Ca^{2+} ions inhibit the antimicrobial activity poly(TBAEMA) (0.167 M) against *E. coli*. Lenior et al. (2006) and Roy et al. (2007) explained this effect as an inhibition of the mode of action, which is based on a displacement of Ca^{2+} and/or Mg^{2+} by the protonic amino groups of the SAM® polymer in the outer membrane of gram-negative bacteria. The authors assumed that the antimicrobial activity can be inhibited above a certain concentration of Ca^{2+} ions (Lenior et al. 2006; Roy et al. 2007).

Decreasing the concentration of meat extract from 18 to 9 or 1.8 µg ml^{-1} leads to an increase of the antimicrobial activity against *E. coli* and *Ps. fluorescens*. The conducted tests with lower concentration of meat extract solutions underline the hypothesis that the level of antimicrobial activity of poly(TBAMS) depends on the concentration of meat components. Moreover, Ignatova et al. (2009) described in their experiments with poly(TBAEMA) that proteins like fibrinogen can interact with poly(TBAEMA)´s surface. Thus, proteins could inhibit the interactions between bacteria and polymers. The results of the experiments with BSA (45 µg ml^{-1} and 4.5 µg ml^{-1}) seems like proteins are influencing the activity of poly(TBAMS) against *E. coli* negatively. Noticeable is that *E. coli* is less sensitive than *Ps. fluorescens* under a BSA concentration of 4.5 µg ml^{-1}. In the experiments, BSA has a negative charge due to the pH-value of 6.3 of the solutions and could interact with the polymer surface and occupy the functional amino groups. Shearer et al. (2000) investigated the influence of 0.1 % BSA on the antimicrobial activity of an amine against *Staph. aureus* and *Ps. fluorescens* and observed a complete inhibition of the antimicrobial activity in the presence of the protein. The authors assumed that the effect is based on the negative charge of

BSA in the used medium (Shearer et al. 2000). The electric charge of the bacteria and of the material could be different by using different test solutions (Potter et al. 2005). Seyfriedsberger et al. (2006) reported a dependence of the positive charge of poly(TBAEMA) on the pH-value. In own experiment the charges of the bacteria and of the polymer could be different in meat extract- and BSA solutions compared with in saline solution. As a result, the electrostatic interaction between the polymer and bacteria is inhibited. Further investigations will be necessary to clarify the detailed mechanism of meat components and the reduced antimicrobial activity.

2.5 Conclusion

The results from this study show a high antimicrobial activity of the SAM® polymer poly(TBAMS) against the six tested spoilage and pathogenic organisms typical for meat products after 24 h at 7 °C. The gram-positive bacteria *Staph. aureus*, *L. monocytogenes*, *B. thermosphacta* and *Lact.* spp., are more sensitive to poly(TBAMS) than the gram-negative bacteria *E. coli* and *Ps. fluorescens*. Poly(TBAMS) is also active under storage conditions typical for meat, such as low temperature conditions and in the presence of meat components. Temperatures and meat components influence the effectiveness of poly(TBAMS). If the activity is reduced by both factors after 24 h of storage, the activity can be increased by prolonging the storage time. The findings underline the potential of poly(TBAMS) as a new class of packaging material for the meat industry. Overall, the experiments give a first evaluation of the general antimicrobial activity of poly(TBAMS) under specific conditions in perishable food supply chains, especially meat. In the next step, the antimicrobial activity of the material should be investigated with different meat products under typical storage temperatures and times.

In summary, the results foreground that common test methods like JIS Z 2801 or ISO 22196 for testing antimicrobial activity of materials, are not sufficient to investigate the potential of antimicrobial materials to be used as packaging material for perishable products. The common procedures do not take realistic application conditions into account (*Staph. aureus, E. coli,* 35 °C, 24 h). In future, particularly the influence of meat specific organisms' e. g. psychrotrophic spoilage bacteria, temperatures, times, and meat components have to be considered. Thus, reducing storage temperature, adapting microorganisms and considering food components are important aspects for new test methods.

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3 The influence of the processing of different packaging materials containing poly-[2-(tert-butylamino) methylstyrene] on the antimicrobial activity against spoilage and pathogenic bacteria

3.1 Introduction

Meat safety and the length of shelf life are influenced by several different factors, like the product characteristics and ingredients, hygienic conditions during production and processing, the logistic structures, and especially the temperature conditions in the chain (Kreyenschmidt and Ibald 2012). Besides these factors, the packaging conditions have a significant influence. In the past, huge efforts have been made to develop multilayer foils based on different polymers, such as LLDPE, LDPE, PE, PP, PA, to improve the water and gas barriers and provide optimal mechanical strength properties to prolong shelf life (Scetar 2010; Siracusa et al. 2014).

In recent years, several papers have been published about the use of antimicrobial mechanisms in foils to decrease the spoilage rate by directly inhibiting the microbial growth or killing the microorganisms (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002). Reduction of the initial bacterial count of fresh poultry by one $\log_{10}$ unit results in prolonging the shelf life for several days (Bruckner 2010).

Three general types of antimicrobial polymers can be described: biocide-releasing polymers, polymeric biocides and biocidal polymers. Biocide-releasing polymers contain biocides which are released from polymers to the environment. In polymeric biocides, the biocidal molecules are attached to a polymer backbone. The third group are the biocidal polymers, in which the whole macromolecule is biocidal (Siedenbiedel and Tiller 2012).

Antimicrobials can be integrated into polymers in foils for shrink, skin or vacuum packaging. Most of the antimicrobial polymers are based on a biocide-releasing killing mechanism, meaning that a release of active agents from the polymer into the food product or environment is required. A variety of migrating antimicrobial agents have been developed and investigated for the application in packaging material. Even if several antimicrobials are effective against various organisms under labor conditions, it has proven difficult to develop foils that are adequate antimicrobial in contact with food (Balasubramanian et al. 2009). The presence of proteins effects for example the antimicrobial activity of silver negatively (Liau et al. 1997; Matsumura et al. 2003; Asharani et al. 2009; Ilg and Kreyenschmidt 2011; Martínez-Abad et al. 2012). Furthermore, it has to be considered that the antimicrobial action of different agents is often reduced at cool temperature conditions, which are typical in meat chains (MacKeen et al. 1987; Russel and Hugo 1994; Kampmann et al. 2008; Simon et al. 2008; Asharani et al. 2009; Lee et al. 2011; Cushen et al. 2013).

The effect of polymeric biocides and biocidal polymers is based on a contact-active killing mechanism (Siedenbiedel and Tiller 2012). Example for a biocidal polymer is chitosan. A

possible mode of action could be the interaction between the positively charged polymers and negatively charged microbial cell membranes resulting in the leakage of intracellular constituents (Shahidi et al. 1999; Dutta et al. 2009; Higueras et al. 2013; Dehnad et al. 2014; Soysal et al. 2015). Sustainable active microbicidal (SAM®) polymers are a new class of contact antimicrobials. The antimicrobial activity of these polymers is based on their three-dimensional helical structure with a high concentration of protonated, functional amino groups (Thölmann et al. 2003; Hewitt et al. 2004). The protonation of the functional amino groups leads to electrostatic interactions between the positively charged polymer and the negatively charged surface of bacteria. The cytoplasmic membrane is depolarized resulting in cell death.

The relatively new developed polymer poly(TBAMS) shows very good antimicrobial properties against different pathogenic and spoilage bacteria at cool temperatures and in the presence of different meat components after 24 h (Dohlen et al. 2016). Furthermore, the polymer exhibits good mechanical-chemical properties, like the high glass transition temperature (T_G) and the low water uptake. Depending on the isomer compositions of the TBAMS monomers, the T_G ranges between 68 °C and 91 °C, which can be raised by copolymerization with other monomers from 80 °C to 160 °C. The water uptake is less than 2 % and comparable or better than a lot of polyamides. Due to this adaption of properties by copolymerisation, the polymer can effectively be compounded with several matrix polymers and further be adapted for the demands of packaging solutions such as multilayer foils and pads (Brodkorb et al. 2015). Therefore, SAM® polymer poly(TBAMS) and its copolymers are bearing promising potential for application as a packaging material to increase the food safety and shelf life of different chilled products.

Up to now however, it is not clear if poly(TBAMS) homopolymer is still active after compounding with standard polymers to produce multilayer foils and which concentration of poly(TBAMS) is necessary to decrease microbial growth.

The aim of the study is to investigate the influence of different processing steps of poly(TBAMS) on the activity and the effect of novel antimicrobial packaging materials containing different concentrations of poly(TBAMS) homopolymer on the growth of typical spoilage and pathogenic bacteria present on meat under cool temperature conditions.

3.2 Materials and Methods

Polymer discs production

Poly(TBAMS) was synthesized per Brodkorb et al. (2015). Polymer discs were processed from different LLDPE based polymers [Dowlex 2344 (Dow plastics, Midland, USA), Dowlex SC 2108G (Dow plastics, Midland, USA), Plexar PX 3236 (LyondellBasell, Wesseling, Germany) mixed with 10 % poly(TBAMS) in a coextruder. Further polymer discs were coextruded out of a LLDPE based polymer (Dowlex 2344, Dow plastics, Midland, USA) mixed with different concentrations of poly(TBAMS) (1.5, 3, 5, 10, 12, 15 %). The resulting compounds from the coextrusion process were pressed to polymer discs (3.5 cm in diameter, 2 g) by a fully hydraulic injection moulding machine. Test discs of different base polymers were produced as reference materials. Parts of the resulting compounds consisting of the Dowlex 2344 LLDPE and 10 % poly(TBAMS) were used for the multilayer foil production and fibre spinning.

Foils production

In total, three multilayer blown foils and one flat foil containing poly(TBAMS) were produced. The multilayer polymer foils, consisting out of five layers with different barriers (table 1), were coextruded at a pilot plant scale (Germany). The inner layer of the foils was the one containing poly(TBAMS) with LLDPE. Two of the foils contained 10% poly(TBAMS) as inner layer, with different thicknesses of 10 µm (foil 2) and 5 µm (foil 3). The third foil was produced with an inner layer of 70 % LLDPE/10 % poly(TBAMS) compound and 30 % LLDPE antiblocking agent (thickness: 15 µm). The inner layer of the reference foil consists of 100% LDPE (3026K). Table 1 gives an overview of the polymers used for the multilayer foils. All multilayer blown foils were produced as rolls with a width of 21 cm. Foils were of even thickness and transparent.

Table 1 Summary of the produced multilayer foils with different layers and layer thickness

	Foil 1 (Reference)	Foil 2	Foil 3	Foil 4
Layer 1	80 % Copolymer PP (PPR3221†) / 20 % Homopolymer PP (MR2002†) 15 µm			
Layer 2	Bonding agent LLDPE (PX3226*) 5 µm			
Layer 3	EVOH (EV3201) 5 µm			
Layer 4	Bonding agent LLDPE (PX3226*) 5 µm			
Layer 5	100 % of LDPE (3026K*)	100 % of LLDPE/10 % poly(TBAMS) Compound		70 % of LLDPE/10 %poly(TBAMS) Compound / 30 % LLDPE antiblocking agent (Dowlex2035°)
Thickness layer 5	10µm	10 µm	5 µm	15 µm

* LyondellBasell, Wesseling, Germany
° Dow plastics, Midland, USA
† Total Research & Technology Feluy, Belgium

Furthermore, a flat foil with a coated thin film containing poly(TBAMS) was produced at a packaging producer in Germany. A solution consisting of 2 % poly(TBAMS) and ethylene acetal was prepared. The flexographic printing method was used for applying the solution on a polyethylene foil (40 µm thickness, corona pre-treated). The final thickness of the coating on the PE foil was 0.04 µm. The foil measured 100 cm in with.

For testing the general antimicrobial activity, circular test pieces with a diameter of 95 mm were trimmed. For storage tests with poultry fillets and veal cutlets the multilayer blown foil 2 and 1 was cut in pieces of 21 cm x 21 cm.

Fibre spinning

A part of the LLDPE compounds with 10 % poly(TBAMS) and without poly(TBAMS) as reference was spun into fibres with a diameter of 1 mm. The fibres were cut in 2 cm pieces. To test the general antimicrobial activity, 2 g of the fibres were used. The fibres were got stacked onto each other in order to form a surface of 2 x 2 cm.

Experimental design

The investigations were subdivided into two parts. In the first part, the general antimicrobial activity of all polymer discs and foils was investigated employing the Japanese Industrial Standard 2806:2000 test. In order to evaluate the general antimicrobial activity all materials were first tested with *Staph. aureus* at 7 °C for 24 h. Based on the results, the antimicrobial activity tests of multilayer foil 2, containing 10 % poly(TBAMS) in the inner layer, were

tested against several spoilage and pathogenic bacteria, under different time and temperatures conditions and in the presence of meat components.

In the second part of the study, storage tests with chicken breast fillets and veal cutlets were also conducted with foil 2 and the effect on the microbial growth and sensory quality of the meat was analysed.

Determination of the antimicrobial activity of the polymer discs and foils

Details of the method have been described by Dohlen et al. (2016). Thus, only a short general description of the antimicrobial test method will be provided here.

For all tests a minimum of three samples (poly(TBAMS)) and six references (without poly(TBAMS)) were used for each experiment. In the different trials, each sample and reference was inoculated with 0.4 ml of bacteria suspension.

The bacterial concentrations on three references were determined immediately after inoculation to determine the initial concentration at t = 0. The other references and samples were stored in a high precision incubator (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) at defined storage temperature and time. The storage temperature were monitored by data loggers (ESCORT JUNIOR Internal Temperature Data Logger, Escort, Auckland, New Zealand) every 5 min. After this storage time, the microbial load of the references and test samples was analysed.

At first, the general antimicrobial activity of all polymer discs and foils containing poly(TBAMS) were tested against *Staph. aureus* (10^5 – 10^6 cfu ml^{-1}) suspended in a physiological saline solution (8.5 µg ml^{-1}) with 1 µg ml^{-1} tryptone (Oxoid, Hampshire, United Kingdom) at 7 °C after 24 h (Dohlen et al. 2016).

Further experiments were conducted with multilayer foil 2 containing 10 % poly(TBAMS) in the inner layer. The investigations included the determination of the antimicrobial activity against different spoilage and pathogenic bacteria and a mixed culture (see table 2). Tests were carried out with an initial concentration of 10^5 – 10^6 cfu ml^{-1} in physiological saline solution with a storage time of the inoculated samples of 24 h or 48 h at 7 °C. To investigate the influence of meat components on the activity, various bacteria were added to different concentrated meat extract solutions (1.8, 9, 18 µg ml^{-1}) and were stored for 24 h at 7 °C. In order to simulate typical initial bacterial counts after processing, the activity of the foil in the presence of meat components (18 µg ml^{-1}) was also tested with a lower initial bacterial count of 10^3 – 10^4 cfu ml^{-1}. The antimicrobial activity was analysed after 24 h to 96 h at 7 °C to illustrate the application as a packaging material.

To investigate the influence of non-isothermal temperature conditions, further experiments were conducted employing three different dynamic temperature profiles. The temperature profiles were chosen based on temperature mappings which were conducted under practical conditions. One profile illustrated the variation of lower temperature conditions as are usual from the packaging process of poultry to the retailer (-1 °C – 6 °C for 24 h) followed by a constant temperature of 4 °C. The second temperature scenario simulated variation during storage by the retailer (2 °C – 7 °C for 48 h). The third temperature profile simulated higher temperature variations (5 °C – 11 °C for 96 h) as they can appear after the point of sale and during the storage in household refrigerators.

Table 2 shows an overview of the different experiments conducted with foil 2 and foil 1 as reference.

Table 2 Summary of the experiments with the multilayer foil 2 containing poly(TBAMS) and foil 1 as reference

	$10^5 – 10^6$ cfu ml^{-1}					$10^3 – 10^4$ cfu ml^{-1}			
	Saline Solution		Meat extract solution (µg ml^{-1})						
			18	9	1.8	18			
Microorganisms	24 h	48 h	24 h			24 h	48 h	72 h	96 h
Staph. aureus	+ *°	+°	+ *	+	+	+°	+°	†	†
L. monocytogenes	+	-	+	-	-	-	-	-	-
Lactobacillus spp.	+	-	+	-	-	-	-	-	-
B. thermosphacta	+ *°	+°	+ *	-	-	+°	+°	+†	+†
E. coli	+ *°	+°	+ *	+	+	+°	+°	†	†
Ps. fluorescens	+ *°	+°	+ *	-	-	+°	+°	+†	+†

+ tested with pure cultures at 7°C *tested with mixed culture at 7 °C °tested with three dynamic scenarios †tested with third dynamic scenario -not tested

The bacterial counts on the polymer discs and foils at time zero and after certain defined time intervals were determined by using cultural methods. Total viable counts (TVC) of mixed cultures were determined using the pour plate technique with plate count agar (PCA, Merck, Darmstadt, Germany); the plates were incubated at 30 °C for 72 h. The individual counts of bacteria in mixed culture were determined with different media and culture techniques. Table 3 gives an overview of the used media and techniques, and the incubation time and temperature.

Results were expressed as the number of colony forming units per millilitre. Detection limits were determined to be 1.0 $\log_{10}$ cfu ml^{-1} for pour plate, overlay and spread plate technique and 2.0 $\log_{10}$ cfu ml^{-1} for drop plate technique.

Table 3 Summary of the used strains, cultivation and enumeration temperature, technique and growth medium

Bacteria	Strain DSM No.	Cultivation and enumeration temperature	Technique	Medium (Brand)
Staphylococcus aureus	799	37 °C	Spread plate	Baird Parker Agar (Oxoid, Cambridge, United Kingdom)
Listeria monocytogenes	19094	37 °C	Pour plate	Plate count agar (Merck, Darmstadt, Germany)
*Lactobacillus*spp.	20182	30 °C	Pour plate	De Man, Rogosa, Sharpe Agar (Merck, Darmstadt, Germany)
Brochothrix thermosphacta	20171	25 °C	Drop plate	Streptomycin Inosit Toluylene Red Sheep Blood Agar Base (Oxoid, Cambridge, United Kingdom)
Escherichia coli	787	37 °C	Overlay plate	Violet Red Bile Dextrose Agar (Merck, Darmstadt, Germany)
Enterobacteriaceae*				
Pseudomonas fluorescens	50090	25 °C	Spread plate	Pseudomonas agar with Cetrimide-Fucidin-Cephalosporin selective supplement (Oxoid, Cambridge, United Kingdom)
Pseudomonas spp.*				
Total viable count		30 °C	Pour plate	Plate count agar (Merck, Darmstadt, Germany)

* tested only in product studies

Determination of the microbial growth on fresh meat packaged in foils

Preparation and packaging of meat samples

Storage tests were conducted with chicken breast fillets and veal cutlets. Unsexed, 42-day-old broiler chickens (Ross 308/708) were slaughtered and air-chilled in a poultry processing plant in Germany. The vacuum packaged, skinless, double-breast chicken fillets were transported from the poultry slaughter plant to a wholesaler and further to the retailer within 24 h. From the retailer, the chicken fillets were transported to the laboratory, under temperature-controlled conditions in isolated boxes with cooling packs. In the laboratory the double-breast fillets were divided into single fillets (250 g) using a sterile scalpel. In each experiment, one fillet was vacuum packaged in the multilayer foil 2 with 10 % poly(TBAMS) in the inner layer; the other fillet was vacuum packaged in the reference foil (foil 1). The polymer foils were vacuum heat-sealed for using a vacuum sealing machine (Vacuum C100, Multivac Sepp Haggenmuller GmbH & Co. KG, Wolfertschwenden, Germany). The packaged poultry breast fillets were stored at 4 °C for 288 h in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The microbiological, sensorial and chemical analyses were conducted after certain time intervals.

In a further experiment, the breast fillets were pre-treated with 5 % lactic acid dissolved in sterile distilled water (90 % lactic acid, Merck, Darmstadt, Germany) to decrease the initial bacterial count on meat. Fillets were spread with 2 ml of the solution and dried.

The storage tests with veal cutlets (each 130 g) were performed in the same way as described above: Animals were slaughtered and the meat was air-chilled in a veal processing plant in Germany. The vacuum packaged veal cutlets were transported from the veal processing plant

to the laboratory under temperature-controlled conditions. The cutlets were repacked with the poly(TBAMS) foil respectively the reference foil and stored for 504 h at 2 °C. Samples were analysed after defined time intervals, with the first investigation starting within 24 h for chicken breast fillets and max. 72 h for veal cutlets after slaughter.

Microbiological analysis of meat samples

For the microbiological analysis, the meat was removed from packaged sample aseptically by using a sterile scalpel. The product samples had measurements of 4 x 7 x 0.5 cm meat surface for the poultry breast fillets and 4 x 8 x 1 cm for the veal cutlets to achieve a total weight of about 25 g. The samples were transferred to a filtered sterile stomacher bag and filled up to 250 ml saline tryptone diluent (8.5 µg ml^{-1} with 1 µg ml^{-1} tryptone). Samples were homogenized with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 s. Ten-fold dilutions of the sample homogenates were prepared in saline tryptone diluents. Total viable count (TVC), *Pseudomonas* spp., *B. thermosphacta, Lactobacillus* spp. and Enterobacteriaceae were enumerated. Table 3 gives an overview of all used media, techniques, and the incubation times and temperatures. Counts of colony forming units (cfu) were expressed as $\log_{10}$ cfu g^{-1} for each medium and sample.

pH-measurement

The pH of the meat samples was measured over the entire storage period using a portable pH-meter (Escort Junior EJ-2E-D-16L, Escort, Auckland, New Zealand). Three measurements were performed for each meat sample by placing the electrode onto the meat surface, and an average pH-value was calculated.

Sensory evaluation

Sensory analysis was carried out by trained sensory panellists. During the trials, each sample was evaluated directly after opening the foil. Verbal descriptions of various sensory impressions for different attributes in accordance to a test form were used for the sensory evaluation. Attributes were defined as general appearance, colour, odour, texture and drip loss. A difference evaluation between the meat packaged in the reference and sample foil was performed for each attribute.

Data Analysis

Antimicrobial activity analysis

Antimicrobial activity was determinate by comparing the logarithmic value of the viable counts on the poly(TBAMS) material with the viable counts on the reference material after inoculation and incubation, as shown in the following equation:

$$\log_{10}\text{reduction} = \log_{10}(T_{xRe} / T_{xSa})$$

where $T_{x,Re}$ = bacterial concentration on reference material, x hours after inoculation
and $T_{x,Sa}$ = bacterial concentration on sample material, x hours after inoculation

For materials to count as antimicrobial, the calculated value of antimicrobial activity should reach $\log_{10}$-reduction values ≥ 2.0 $\log_{10}$ units (JIS Z 2801:2000).

Modelling microbial growth

The Gompertz equation was used to model the development of the total viable count, and the growth of individual bacteria counts as a function of time (Gibson et al., 1987).

$$N(t) = A + C * e^{-e^{-B*(t-M)}}$$

with N(t): microbial count [$\log_{10}$ cfu g^{-1}] at any time, A: lower asymptotic line of the growth curve (initial bacterial count), C: difference between upper asymptotic line of the growth curve (Nmax = maximum population level) and the lower asymptotic line, B: relative maximum growth rate at time M [h-1], M: time at which maximum growth rate is obtained (reversal point), and t: time.

Statistical Analysis

All data were transformed into $\log_{10}$ values before statistical analysis. The standard deviations in bacterial counts on reference and poly(TBAMS) materials were calculated. The differences in bacterial counts on poly(TBAMS) and the reference materials, and the differences in the $\log_{10}$-reduction of bacteria in saline solution and bacteria in meat extract solution (18 µg ml^{-1}) were analysed for significance using the Mann-Whitney U test for independent samples. The significance level was defined as a p value of < 0.05. For the description of data, box plots and bar charts were used. Data analysis was conducted with SPSS Statistics 22 (IBM Corp. 1989, 2013, New York, USA) and OriginPro 8G (OriginLab Corp., Northampton MA, USA). The microbiological growth data were fitted using the statistical software program Origin 8.0G.

3.3 Results

Determination of the antimicrobial activity of the polymer discs and foils

The general antimicrobial activity of all materials containing poly(TBAMS) was tested against *Staph. aureus* in saline solution at 7 °C for 24 h. The bacterial concentration of *Staph. aureus* on different LLDPE based polymer discs and sample discs containing 10 % poly(TBAMS) after 24 h storage at 7 °C are listed in table 4.

Table 4 Arithmetic mean values and standard deviation of bacterial counts of *Staph. aureus* in saline solution on different LLDPE reference and LLDPE sample discs containing 10 % poly(TBAMS) after 24 h at 7 °C

Staph. aureus in saline solution	PX 3236	SC 2108 G	DOW 2344
Inoculum $\log_{10}$ cfu ml^{-1}	5.34 ± 0.10 (n = 6)	5.28 ± 0.16 (n = 9)	5.62 ± 0.24 (n = 6)
Reference $\log_{10}$ cfu ml^{-1}	5.26 ± 0.04 (n = 6)	5.21 ± 0.22 (n = 9)	5.16 ± 0.17 (n = 6)
Sample $\log_{10}$ cfu ml^{-1}	4.80 ± 0.23 (n = 6)	4.53 ± 0.51 (n = 9)	3.39 ± 1.24 (n = 6)
$\log_{10}$ reduction	0.46*	0.68*	1.88*

* Significant difference in bacterial counts on poly(TBAMS) discs and reference discs with $p < 0.05$

Significant differences between the bacterial counts on references and samples are detected on all materials. The highest $\log_{10}$-reduction among the LLDPE polymers is 1.88 $\log_{10}$ units, observed on the polymer disc consisting of LLDPE (Dow 2433) with 10 % poly(TBAMS). On the fibres consisting of LLDPE (Dow 2433) with 10 % poly(TBAMS), the bacterial concentration of *Staph. aureus* is reduced lower than the detection limit with a $\log_{10}$-reduction of 3.92 $\log_{10}$ units (n = 3, data not shown).

Figure 1 shows the bacterial counts on LLDPE (Dow 2433) polymer discs and sample discs with different concentrations of poly(TBAMS) after 24 h storage at 7 °C.

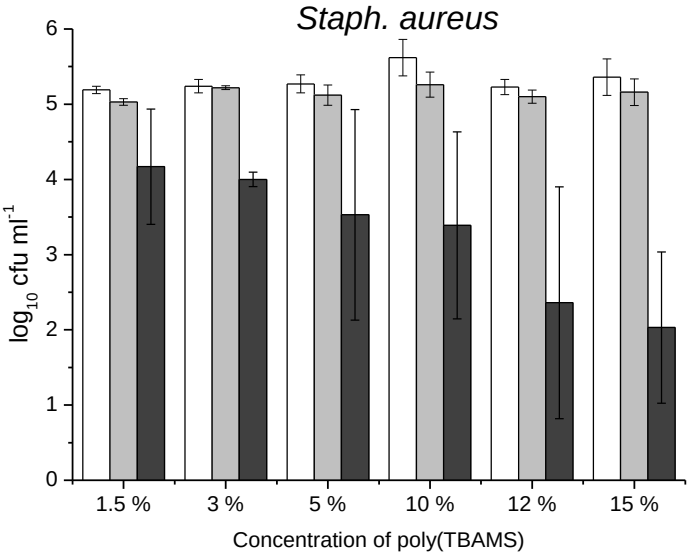

Figure 1 Arithmetic mean of bacterial counts of *Staph. aureus* in saline solution on LLDPE (Dow 2344) reference and LLDPE sample discs containing different percentage of poly(TBAMS) after 24 h at 7 °C.
Bacterial counts: (□) Inoculum, (▒) Reference disc and (■) Sample disc

The bacterial counts of *Staph. aureus* are decreased on all LLDPE discs containing poly(TBAMS). The $\log_{10}$-reductions increase with increasing poly(TBAMS) concentration (1.5 % - 15 %) and range between 0.86 to 3.13 $\log_{10}$ units. The bacterial counts of six sample discs containing 15 % poly(TBAMS) are reduced lower than the detection limit ($n = 18$, $p < 0.05$). The $\log_{10}$-reductions of *Staph. aureus* on the different multilayer blown foils containing poly(TBAMS) vary between 0.81 $\log_{10}$ units ($n = 3$, foil 4) and 3.65 $\log_{10}$ units ($n = 12$, foil 2).

The flat foil with a thin film coating containing 2 % poly(TBAMS) reduces the initial bacterial counts of *Staph. aureus* under the detection limit ($\log_{10}$-reduction: 4.32 $\log_{10}$ units with $p < 0.05$, $n = 12$, data not shown) after 24 h at 7 °C.

Further tests were conducted with the multilayer foil 2. The bacterial concentrations of meat specific spoilage and pathogenic bacteria in saline solution before and after storage on the reference foil and the antimicrobial polymer foil 2 at 7 °C after 24 h are shown in figure 2.

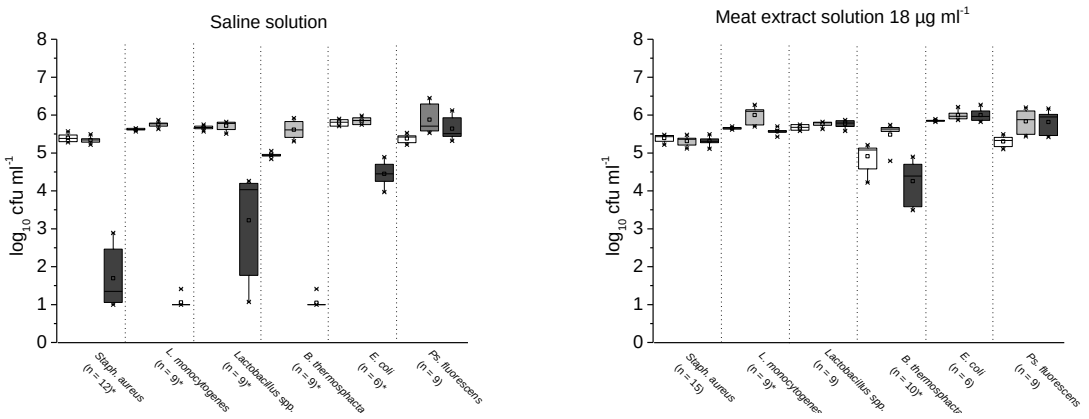

Figure 2 Boxplots of viable counts on reference foil compared with poly(TBAMS) foil after inoculation with bacteria in saline solution (left) and meat extract solution (right) after 24 h at 7 °C. * Significant difference in bacterial counts on poly(TBAMS) foil and reference foil with $p < 0.05$.
Bacterial counts: (▭) Inoculum, (▭) Reference foil and (▭) Sample foil

Expect for *Ps. fluorescens,* the bacterial counts in saline solution are significantly decreased on the poly(TBAMS) foil compared with the bacterial counts on the reference foil ($p < 0.05$). Log_{10}-reductions of more than 4.5 log_{10} units are achieved against *L. monocyt*ogenes (n = 9) and *B. thermosphacta* (n = 9). Bacterial concentrations of *Staph. aureus* are decreased 3.65 log_{10} units (n = 12). A reduction rate of 1.77 log_{10} units is identified against *Lactobacillus* spp. (n = 9) and 1.40 log_{10} units against *E. coli* (n = 6). Of the microorganisms tested, *Ps. fluorescens* shows the lowest log_{10}-reduction of 0.24 log_{10} units when in contact with poly(TBAMS) (figure 2).

Figure 3 shows the TVC and the individual counts of a mixed inoculum of *Staph. aureus*, *B. thermosphacta*, *E. coli* and *Ps. fluorescens* in saline solution on reference and poly(TBAMS) foils directly after inoculation and after 24 h storage at 7 °C. A reduction rate of 1.16 log_{10} units of the TVC on the poly(TBAMS) foil becomes apparent after incubation. The individual count of *B. thermosphacta* is reduced to the detection limit. Reduction rates more than 2.0 log_{10} units are identified against *Staph. aureus* and *E. coli*. *Ps. fluorescens* is marginal decreased on poly(TBAMS) foil (0.32 log_{10} units) (figure 3).

By prolonging the storage time to 48 h the concentrations of *Staph. aureus* and *B. thermosphacta* in saline solution are reduced below the detection limit of 1.0 log_{10} unit on all poly(TBAMS) samples at 7 °C. *E. coli* and *Ps. fluorescens* are reduced < 2.0 log_{10} units on poly(TBAMS) foil.

Several experiments were conducted at three dynamic temperatures scenarios. The results of the experiments with different microorganisms at the three dynamic temperature scenarios show the same trends as those at 7 °C after 24 h and 48 h (data not shown).

Similar to the tests with saline solution (7 °C / 24 h), the antimicrobial activity of the poly(TBAMS) foil was investigated with the six selected bacteria in meat extract solution

(18 µg ml^{-1}) (figure 2). The bacterial concentrations on the poly(TBAMS) foil and on the reference foil show significant differences with a p value of < 0.05 for the microbial load of *L. monocytogenes* (n = 9) and *B. thermosphacta* (n = 10). Highest $\log_{10}$-reduction in bacterial counts of *B. thermosphacta* from 5.47 to 4.26 is significant. Significant differences ($p < 0.05$) are demonstrated by comparing $\log_{10}$-reductions of bacteria, except *Ps. fluorescens,* in meat extract solution (18 µg ml^{-1}) with $\log_{10}$-reductions in saline solution after 24 h at 7 °C.

In the experiments with mixed culture in meat extract solution (18 µg ml^{-1}), the highest $\log_{10}$-reduction with 1.18 $\log_{10}$ units is observed with *B. thermosphacta* (figure 3).

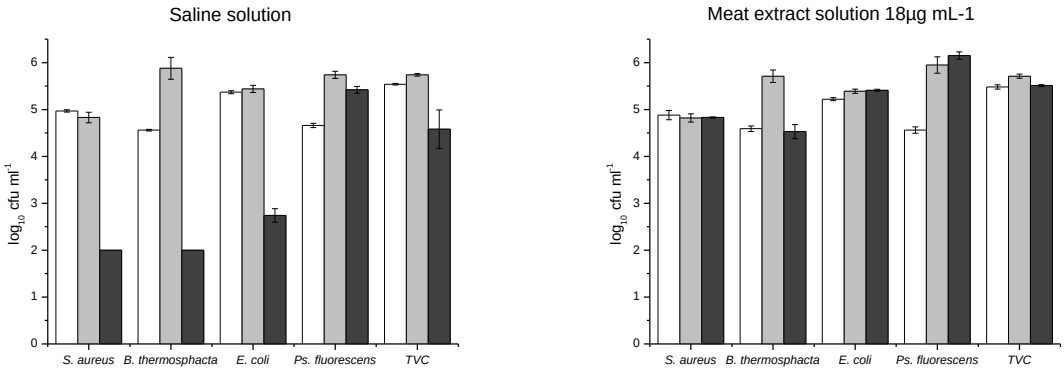

Figure 3 Arithmetic mean of bacterial counts of mixed culture in saline solution (left) and meat extract solution 18 µg mL^{-1} (right) on reference foil and poly(TBAMS) foil after 24 h at 7 °C.
Bacterial counts (n = 3): (▭) Inoculum, (▭) Reference foil and (▬) Sample foil

The antimicrobial activity of the multilayer foil was further tested with *Staph. aureus* and *E. coli* in lower concentrations of meat extract solution (9 µg ml^{-1}; 1.8 µg ml^{-1}). Decreasing the concentration of meat extract from 18 to 1.8 µg ml^{-1} leads to an increase of the $\log_{10}$-reductions for *Staph. aureus* and *E. coli. Staph. aureus* is reduced below the detection limit ($\log_{10}$-reduction: 4.47 $\log_{10}$ units). For *E. coli,* the $\log_{10}$-reduction increases to 1.52 $\log_{10}$ units by reducing the meat extract concentration (data not shown).

The antimicrobial activity of poly(TBAMS) foil 2 was tested with four different bacteria with initial counts of $10^3 – 10^4$ cfu ml^{-1} in meat extract solution (18 µg ml^{-1}) at 7 °C and at three dynamic temperatures scenarios. For *Staph. aureus, E. coli* and *Ps. fluorescens* no differences are detected on poly(TBAMS) foil compared with counts on the reference foil at 7 °C and at the three dynamic temperatures after 24 h. Log_{10}-reductions for *B. thermosphacta* are comparable with the $\log_{10}$-reductions with initial bacteria concentration of $10^5 – 10^6$ cfu ml^{-1}.

The bacterial concentrations of *Staph. aureus, B. thermosphacta, E. coli* and *Ps. fluorescens* on the reference and poly(TBAMS) foils before and after storage for 24 h, 48 h, 72 h and 96 h at the third dynamic temperature scenarios are shown in figure 4.

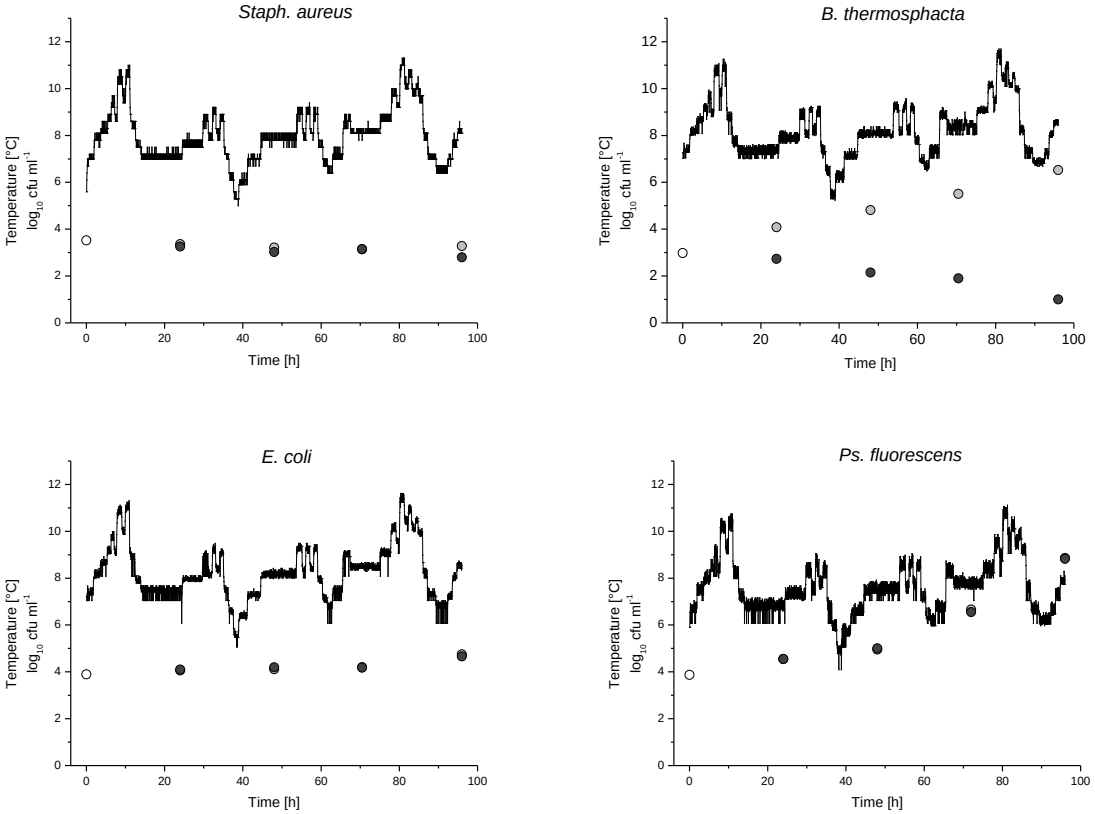

Figure 4 Arithmetic mean of bacterial counts of *Staph. aureus* (top left), *B. thermosphacta* (top right), *E. coli* (bottom right) and *Ps. fluorescens* (bottom left) in meat extract solution (18 µg ml^{-1}) on reference and poly(TBAMS) foils after 24 h, 48 h, 72 h and 96 h at the third dynamic temperature conditions (▬).
Bacterial counts (n = 3): (○) Inoculum, (◎) Reference foil and (●) Sample foil

The concentration of *B. thermosphacta* is reduced below the detection limit of 1 log_{10} cfu ml^{-1} after 96 h at the dynamic temperature conditions and at 7 °C. Over the entire storage, no reductions are detected on the poly(TBAMS) foil for *Staph. aureus*, *E. coli* and *Ps. fluorescens*.

Determination of the effect of multilayer foils on the microbial growth on fresh meat

Figure 5 shows the development of typical spoilage microorganism and total viable count on chicken breast fillets packaged in reference foil and sample foil containing 10 % poly(TBAMS) in the inner layer at a constant temperature of 4 °C.

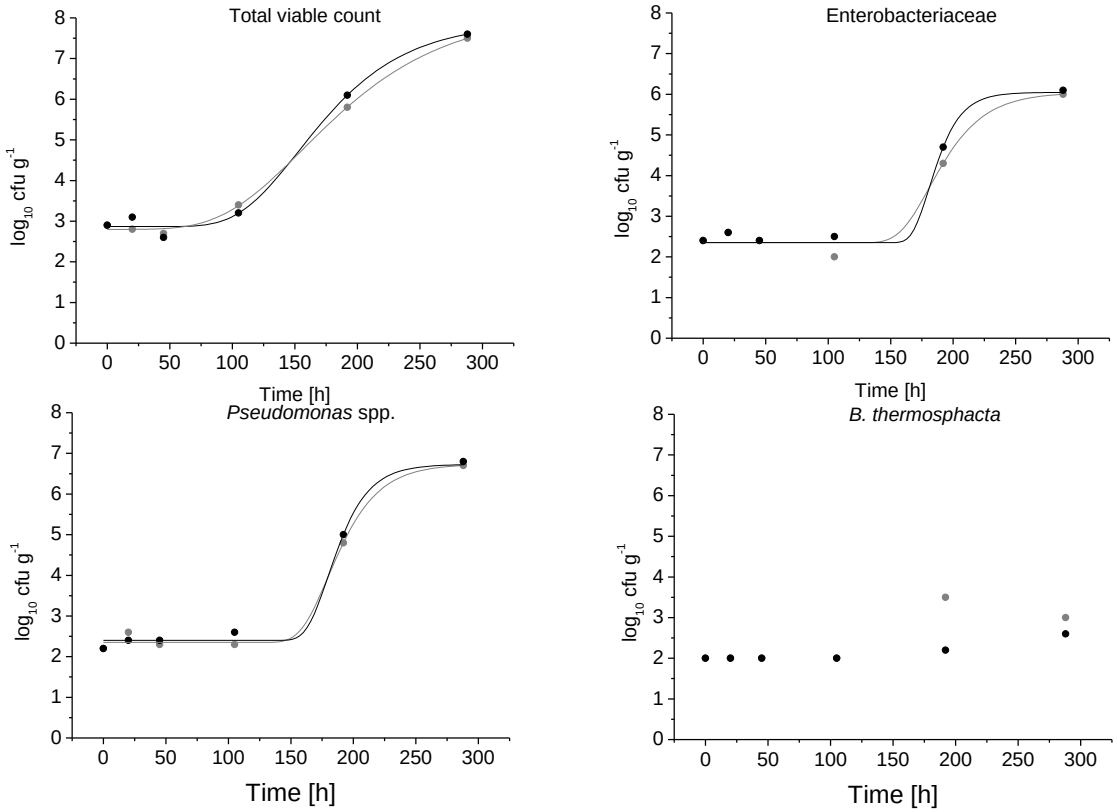

Figure 5 Growth of total viable count and typical spoilage microorganism on chicken breast fillets stored in reference foil and sample foil containing 10 % of poly(TBAMS) at a constant temperature of 4 °C.
Bacterial counts (n = 4): (●) Reference foil and (●) Sample foil

In contact with meat, foil 2 shows no reduction in bacterial counts during the entire storage time, except for *B. thermosphacta*. The highest $\log_{10}$-reduction with 1.48 $\log_{10}$ units on chicken breast fillets packaged in the poly(TBAMS) foil is identified against *B. thermosphacta* after 180 h.

The lactic acid treatment of the chicken breast fillets results in a delay of the growth of Enterobacteriaceae and *Pseudomonas* spp. around 1 $\log_{10}$ unit over the storage. But it does not lead to a higher reduction in bacterial counts on the lactic acid treated chicken breast fillets packaged in poly(TBAMS) foil compared with those in the reference foil (data not shown).

Figure 6 shows the development of total viable count and *Lactobacillus* spp. on veal cutlets packaged in reference foil and the sample foil at a constant temperature of 2 °C under vacuum.

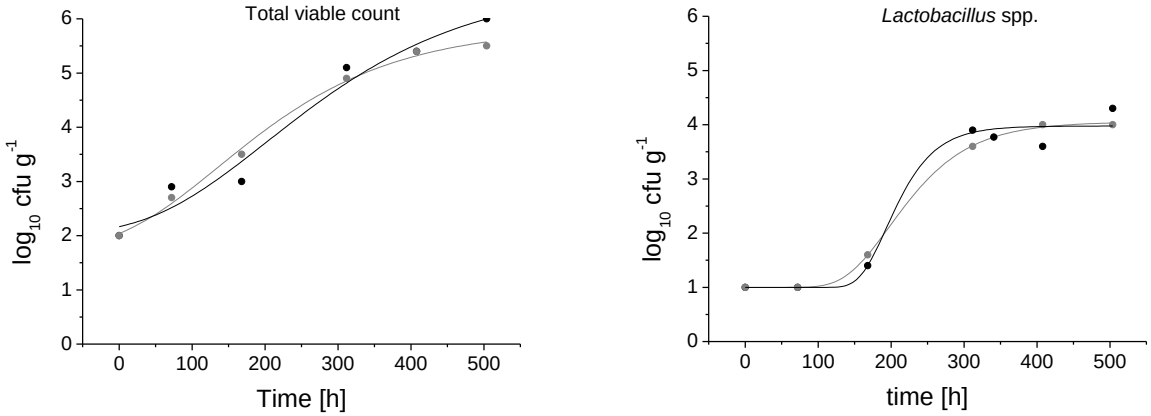

Figure 6 Growth of total viable count and *Lactobacillus* spp. on veal cutlets stored in reference foil and sample foil containing 10 % of poly(TBAMS) at a constant temperature of 2 °C.
Bacterial counts (n = 4): (●) Reference foil and (●) Sample foil

No significant reduction in bacterial counts of TVC and *Lactobacillus* spp. on veal cutlets packaged in the poly(TBAMS) foil is identified over the entire storage.

Regarding the other tested parameters, it becomes obvious that the poly(TBAMS) has no negative effect on the pH-value and the sensory quality. The initial poultry breast pH (24 h after slaughter) varies between 5.72 and 6.05. The initial veal cutlets pH varies between 5.85 and 5.99. There is no difference in pH-value between the samples stored in the different foils over the entire storage. No differences of various sensory impressions (general appearance, meat colour, odour, texture and drip loss) for different attributes are described between the meat packaged in the poly(TBAMS) foil compared with the meat in the reference foil during storage.

3.4 Discussion

The polymer poly(TBAMS) can be compounded with different standard, multilayer, packaging polymers like LLDPE, generating an antimicrobial property in these materials. Multilayer foils combining polymers possess high barriers for water and gases as well as increased mechanical strength (Scetar 2010; Siracusa et al. 2014). The dynamic-mechanical properties of the antimicrobial polymers coextruded with a standard polymer can be adjusted to a level of standard technical polymers in a way that the polymer can be used in normal polymer production machines. The incorporation of poly(TBAMS) in the inner layer of multilayer foils by extrusion turns out to be an excellent possibility to produce antimicrobial packaging. Another possibility is the coating of foils with antimicrobial polymer. A coating with poly(TBAMS) on a foil shows a very high antimicrobial activity, however the adhesion of the coating to the foil is still a challenge.

The activity of poly(TBAMS) is based on electrostatic interactions between the positively charged polymer and the negatively charged surface of bacteria. It is apparent that the antimicrobial activity of poly(TBAMS) is influenced by different factors.

Limited miscibility of two polymers can lead to non-homogeneous compounds (Lenoir et al. 2006; Seyfriedsberger et al. 2006; Roy et al. 2007; Thomassin et al. 2007). Therefore, differences in the level of antimicrobial activity can possibly be explained by the different amount and availability of the active groups on produced polymer discs' surfaces, due to the miscibility properties of the standard polymers and poly(TBAMS). The highest activity is identified on polymer discs based on the LLDPE Dow 2433, which was also used for incorporating the SAM® polymer poly(TBAEMA) in a study by Seyfriedsberger et al. (2006). The authors detected a high antimicrobial activity against a similar initial concentration of *Staph. aureus* with a concentration of 1.5 % poly(TBAEMA). Dohlen et al. (2016) reported that the effectiveness of SAM® polymers was influenced by the storage temperature. At cool temperatures, the activity was reduced compared with the activity at 35 °C after 24 h. This could explain own results, in which 1.5 % poly(TBAMS) shows a marginal antimicrobial activity at a low temperature of 7 °C. This means that for the application of the new material at low temperature, a higher concentration and thus availability of poly(TBAMS) is necessary. The more poly(TBAMS) is integrated, the stronger the antimicrobial activity is. The charge of the antimicrobial material itself is important for the electrostatic interactions with the bacteria and so for the antimicrobial activity. In different studies, this dose-dependent activity of poly(TBAEMA) was observed (Lenoir et al. 2006; Seyfriedsberger et al. 2006; Zuo et al. 2012). The activity of chitosan could also be increased with the concentration of chitosan (Darmaji and Izumimotot 1994; Zheng and Zhu 2003). The effect against *E. coli* of a cationic polymer, poly(2-(dimethylamino)ethyl methacrylate), grafted with quaternary ammonium was increased with the concentration and density of available quaternary ammonium units on the surface (Huang et al. 2008). Furthermore, the results show that an enlargement of the polymeric surface containing poly(TBAMS) due to spinning fibres leads to an increase of the activity around 2 $\log_{10}$ units. Seyfriedsberger et al. (2006) demonstrated that the antimicrobial activity of a compound containing LLDPE and poly(TBAEMA) and the positive charge of the compound depend on the amount of poly(TBAEMA) on the polymeric surface. Accordance to the findings, it can be assumed that the amount and the availability of the active groups on the polymeric surface and thus the charge are raised by increasing the concentration of poly(TBAMS). Concerning this matter, Dohlen et al. (2016) investigated the antimicrobial activity of 100% poly(TBAMS) against a wide range of individual bacteria

cultures as well as a mixed culture typical for meat products at 7 °C after 24 h. The authors detected a very high activity with $\log_{10}$-reductions more than 4 $\log_{10}$ units except for *Ps. fluorescens*. Despite only 10 % poly(TBAMS) in the inner layer, the multilayer foil 2 also shows an antimicrobial activity against different bacteria. The higher level of antimicrobial activity by the previous study could be explained by the different concentrations of poly(TBAMS) and the amount of amino functional groups on the surface as well as the surface charge. Besides the charge of the polymeric surface, the surface charge of bacteria is of vital importance for the antimicrobial activity. The results confirm the results of the previous study, in which the gram-positive bacteria *Staph. aureus*, *L. monocytogenes*, *B. thermosphacta* and *Lactobacillus* spp. are more sensitive to poly(TBAMS) than the gram-negative bacteria *E. coli* and *Ps. fluorescens* (Dohlen et al. 2016). The varying strengths of the negative charges that these bacteria exhibit in physiological solutions could lead to different electrostatic interactions with the positively charged polymer. Therefore, the antimicrobial activity of poly(TBAMS) is influenced by the bacterial strains itself (Dohlen et al. 2016). In a study by Seyfriedsberger et al. (2006), a higher concentration of poly(TBAEMA) was necessary for a high antimicrobial activity against *E. coli* compared with *Staph. aureus*. In different studies, a higher sensitivity of gram-positive bacteria to high molecular chitosan was observed compared with gram-negative bacteria (No et al. 2002; Zheng and Zhu 2003; Fernandes et al. 2008; Fernandez-Saiz et al. 2009).

In this study, it also becomes clear that the activity of the foils decreases with increasing concentration of meat components. The storage tests with chicken breast fillets and veal cutlets demonstrate similar results to those with the meat extract solution. No significant reductions in microbial growth on the meat packaged in the multilayer foil containing poly(TBAMS) can be observed during storage. These findings are similar to those by Park et al. (2010). In their study, the authors investigated the total viable count on fresh bovine meat applied with chitosan-incorporated LDPE films with different concentrations of chitosan (1 %, 4 %, 8 %). No antimicrobial activity was observed in chitosan incorporated films after storage of 10 days at 3 °C. Results by Higueras et al. (2013) however, a film with 1.5 % chitosan in LDPE, achieved surface reductions in the range of 0.47 – 2.96 $\log_{10}$ units on slices of chicken breast fillet, also dependent on time and bacterial group. Soysal et al. (2015) detected a reduction of 1 $\log_{10}$ unit of total viable counts on chicken drumsticks vacuum packaged in a LDPE foil containing 2 % chitosan and stored at 5 °C for six days. Interactions between chitosan and meat components may reduce the amount of chitosan interacting with microorganisms on the meat surface (Devlieghere et al. 2004; Park et al. 2010). Due to its

high density of amino groups, chitosan can bind with negatively charged food substances such as proteins and fatty acids (Devlieghere et al. 2004). Furthermore, cationic ions like Ca^{2+}, Mg^{2+}, Zn^{2+} and Ba^{2+} can influenced the antimicrobial activity of chitosan negatively. The negatively charged bacteria can interact with the cationic ions (Tsai and Su 1999; Chung et al. 2003). Therefore, the effect of meat components on the level of antimicrobial activity of poly(TBAMS) is also probably related to cationic ions like Ca^{2+}, Mg^{2+}, and Fe^{2+} in meat. Lenoir et al. (2006) showed an inhibition of the mode of action of poly(TBAEMA) against *E. coli* in the presence of Ca^{2+} ions, which is based on a displacement of Ca^{2+} and/or Mg^{2+} by the protonic amino groups of the SAM® polymer in the outer membrane of gram-negative bacteria. The authors assumed that the antimicrobial activity can be inhibited above a certain concentration of Ca^{2+} ions (Lenoir et al. 2006). The conducted tests with low concentrations of meat extract solutions (1.8 µg ml^{-1}) underline the hypothesis that the level of antimicrobial activity of poly(TBAMS) depends on the concentration of meat components. Moreover, Ignatova et al. (2009) described in their experiments with poly(TBAEMA) that proteins can interact with poly(TBAEMA)´s surface. Additionally, the results of the previous study showed a negative influence of BSA on the antimicrobial activity against different bacteria (Dohlen et al. 2016). Thus, proteins could inhibit the interactions between bacteria and polymers. In the experiments, the negatively charged meat components could interact with the polymer surface and occupy the functional amino groups. Generally, a higher amount of available functional amino groups on the polymer surface is necessary to raise the activity against different bacteria in the presence of meat at low temperatures. This can be reached by incorporation of a higher concentration of poly(TBAMS) and by changing the structure, resulting in an increased surface area.

3.5 Conclusion

In this study, the polymer poly(TBAMS) could be compounded with standard polymers to produce a broad spectrum of multilayer polymer foils with good antimicrobial activity. It became evident that the standard polymer, the concentration of poly(TBAMS), the bacteria and meat components possess an influence on the activity. The antimicrobial activity is increased with the concentration and amount of poly(TBAMS) in the matrix polymer and with an surface enlargement. A higher antimicrobial activity of a multilayer foil containing 10 % poly(TBAMS) in the inner layer was detected against gram-positive bacteria than against gram-negative bacteria at cool temperatures. The poly(TBAMS) foil showed reduced activity

in contact with meat due to the interaction of the active amino groups on the foil surface with meat components. In the future, further studies are necessary to optimise the packaging material in a way that high concentrations of poly(TBAMS), and thus a high availability and amount of active groups, are on the surface.

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4 The effect of packaging materials containing poly-[2-(tert-butylamino)methylstyrene] on the safety and microbial shelf life of vacuum packaged perishable products

4.1 Introduction

Microbial, chemical, biochemical or physical reactions lead to a deteriorative change of food products that make these unacceptable for consumption (Hayes 1985; Mead 2004; Singh and Anderson 2004). The deterioration reactions are different in different kinds of products. The spoilage process of fresh meat and meat products for example, is mostly caused by bacterial growth and their metabolism (Gill 1983; Genigeorgis 1985; Huis in't Veld 1996). The shelf life of fresh meat products is often determined by the growth of one or a few so called specific spoilage organisms and by the probability of the presence pathogenic bacteria and their potential to grow in the product (Gram and Huss 1996; Gram et al. 2002). Therefore, the most important factor to prolong the shelf life is the reduction of the initial contamination and the microbial growth. However, several fresh fruits and vegetables stay metabolically active after harvest. Therefore, the deteriorative changes are often not only caused by microbial activity, but also the respiration process and physiological reactions influence the shelf life (Ahvenainen 1997; Barry-Ryan and O'Beirne 1998). However, the processing of these products in a minimally technological way, such as peeling, trimming or cutting before commercial distribution, can favour microbiological reactions (Buick et al. 1987; Legnani and Leoni 2004; Kreyenschmidt and Ibald 2012). Thus, the processing steps of perishable products have a significant influence on the length of the shelf life and increase the risk of food borne illness (Olsson et al. 2003; Massoni et al. 2012). After the processing steps, the packaging of a product and temperature conditions play an important role in slowing down the spoilage process and increase the shelf life (Han 2005; Zhou et al. 2010).

A relatively new packaging strategy to prolong shelf life of perishable products is active packaging (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Kenawy et al. 2007). Several different active packaging solutions have been developed in the last years, for example, moisture absorbers and antimicrobial materials. Moisture absorbers control the moisture in the packaging and bind liquids such as meat juice. Antimicrobials can be incorporated for example, into foils or soaker pads to decrease the spoilage rate by directly inhibiting the microbial growth or killing the pathogenic and spoilage bacteria during food storage (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002, Kenawy et al. 2007). Non-volatile antimicrobials are directly temporally or permanently bonded to the material respectively polymer chains and appear to be one of the promising active packaging solutions (Quintavalla and Vicini, 2002). Especially, the interest in inherently antimicrobial polymers as packaging material has increased during the last years (Siedenbiedel and Tiller 2012). For all approaches, a direct contact between the packaging material and the perishable products is

essential to generate the inhibition of microbial growth during the storage, as given in shrink, skin or common vacuum packaging and soaker pads (Han 2000; Appendini and Hotchkiss 2002; Ahvenainen 2003; Cooksey 2005). Inherently antimicrobial is the biopolymer chitosan due to its chemical structure. The novel sustainable active microbicidal (SAM®) polymers are a promising technology for such kinds of packaging material. The antimicrobial activity of the intrinsic SAM® polymers is based on their three-dimensional helical structure, which has a high concentration of protonated functional amino groups, resulting in interactions between the positively charged polymer and the negatively charged bacteria (Thölmann et al 2003; Hewitt et al. 2004; Brodkorb et al. 2015; Dohlen et al 2016). One promising antimicrobial polymer is the relatively new poly(TBAMS). This polymer shows very good antimicrobial properties against different pathogenic and spoilage bacteria after 24 h at cool temperatures under laboratory conditions, however, gram-positive bacteria are more sensitive to the polymer than gram-negative bacteria (Dohlen et al. 2016). Furthermore, it could be considered, that the antimicrobial activity of positively charged polymers including poly(TBAMS) is influenced by food components. These polymers can bind with negatively charged food substances, such as proteins, due to their high density of amino groups. Therefore, interactions between cationic polymers and food components may reduce the interactions with microorganisms on food (Devlieghere et al. 2004; Ignatova et al. 2009; Park et al. 2010; Dohlen et al. 2016). As also shown, the higher the SAM® polymer concentration after compounding with different matrix polymers, the higher the antimicrobial activity (Lenoir et al. 2006; Seyfriedsberger et al. 2006; Zuo et al. 2012; chapter 3).

Consequently, all these factors could have an influence on the antimicrobial activity and thus on the shelf life. But up to know, it is not clear if the antimicrobial activity of different packaging solutions such as multilayer foils and pads containing the SAM® polymer poly(TBAMS) is influenced by these factors and if these materials are able to increase the shelf life of different perishable products. Furthermore, no data are available regarding the possible influence of the polymer on the sensory, physico-chemical parameters of different products.

Thus, the objective of the study is to investigate the influence of novel packaging materials containing the polymer poly(TBAMS) on the microbial and sensory spoilage process, chemical and physical parameters and on the shelf life of fresh meat, meat products and vegetable RTE products.

4.2 Materials and Methods

Multilayer foils and soaker pads production

Poly(TBAMS) was synthesized according to Bordkorb et al. (2015). LLDPE (NG 5056, Dow plastics, Midland, USA) was compounded with 15 % poly(TBAMS) in a coextruder. From the resulting compounds an one-layer foil consisting of the compound with 15 % poly(TBAMS) and a reference foil without poly(TBAMS) were produced in a technical trial. Furthermore, three multilayer blown foils containing poly(TBAMS) consisting of 6 layers with different barriers were developed at a pilot plant scale in Germany. The layers differ in the material properties and thickness (table 1). All multilayer foils had measurements of 100 cm.

Table 1 Summary of the produced multilayer foils with different layers and layer thickness

	Layer 1 12.6 µm	Layer 2 2.4 µm	Layer 3 4.0 µm	Layer 4 4.0 µm	Layer 5 2.4 µm	Layer 6
Foil 1	PE	PE	PA	EVOH	PE	14.6 µm LLDPE
Foil 2	PE	PE	PA	EVOH	PE	8 µm NG5056 with 15% poly(TBAMS)
Foil 3	PE	PE	PA	EVOH	PE	11 µm NG5056 with 15% poly(TBAMS) + adhesion-promoting agent MSA-PE
Foil 4	PE	PE	PA	EVOH	PE	14 µm NG5056 with 15% poly(TBAMS) + adhesion-promoting agent MSA-PE

The mixing density was 0.97 kg/dm^3 and the weight was 38.82 g/m^3 for all foils. Besides the reference multilayer foil 1, a two-layer foil containing PA and PE (thickness 90 µm) was used as a reference to compare the effect of commonly used foil with the produced antimicrobial multilayer foils on microbial growth.

To testing the antimicrobial activity, circular test pieces (95 mm in diameter) of the foils were cut. For storage tests the foils were cut into pieces with different measurements for the perishable products.

Two different soaker pads were produced. polypropylene (PP) (Moplen HP2774) was compounded with 15% poly(TBAMS) in a coextruder. From the resulting compounds, absorbent soaker pads were produced with the meltblown technology at a pilot plant scale in Germany. A cellulose based pad was put under a meltblown pad and was closed with a PP foil on the bottom side. Cellulose pads with a poly ethylene (PE) pouch were used as references. Furthermore, the cellulose core of the pad was taken as a base pad for the second pad. A solution consisting of 2 % poly(TBAMS) and ethylenacetyl was prepared and 2 ml was

spread on each pad. Cellulose core without poly(TBAMS) was used as reference. All pads had measurements around 8.0 x 12.0 cm. For testing the antimicrobial activity, test pieces were trimmed to 4 x 4 cm.

Experimental design

The investigations were subdivided into two parts. In the first part, the general antimicrobial activity of the different pads and foils was tested by modifying the Japanese Industrial Standard 2806:2000 with *Staph. aureus* in saline solution and meat extract solution at 7 °C for 24 h. Based on the results the antimicrobial activity of foil 2 containing 15 % poly(TBAMS) in the inner layer was analysed against several spoilage and pathogenic bacteria in saline solution and meat extract solution at 7 °C for 24 h. Besides this, Raman spectroscopy and scanning electron microscopy were performed with foil 1 as the reference foil and foil 2 as the sample foil.

The investigations of the second part of the study included storage tests with chicken breast fillets, veal cutlets, cooked ham, smoke-cured ham and ready to eat carrots and cabbage. All products were packed in foil 2 and in the reference foil 1. During the storage, the effect on the microbial growth, sensory quality and chemical parameters of the products was analysed. Microbial growth was modelled and the microbial shelf life was calculated based on the results.

Determination of the generally antimicrobial activity of the foils and pads (Part 1)

The general antimicrobial activity was conducted as described by Dohlen et al. (2016).

For all tests a minimum of three samples containing poly(TBAMS) and six references (without poly(TBAMS)) were used for each experiment. In the different trials, each sample and reference was inoculated with 0.4 ml of *Staph. aureus* (DSM No. 799) suspension in a concentration of 10^5-10^6 cfu ml^{-1} consisting of physiological 8.5 µg ml^{-1} saline solution with 1 µg ml^{-1} tryptone (Oxoid, Hampshire, United Kingdom) or meat extract solution (18 µg ml^{-1}, Merck, Darmstadt, Germany). The pads were tested with 1 ml inoculum. Further experiments were conducted with multilayer foil 2 containing 15 % poly(TBAMS) in the inner layer. The investigations included the determination of the antimicrobial activity against *B. thermosphacta* (DSM No. 20171), *Ps. fluorescens* (DSM No. 50090) and *E. coli* (DSM No. 1576). The bacterial concentrations on three references were determined immediately after inoculation to determine the initial concentration at t = 0.

The other references and samples were stored in a high precision incubator (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) at 7 °C for 24 h. After this storage time, the microbial load of the references and test samples were analysed.

Colony-forming units (cfu) were determined by using the pour plate method with plate count agar incubated at 37 °C for 48 h for *Staph. aureus* and *E. coli* and 25 °C for *B. thermosphacta* and *Ps. fluorescens*. Results were expressed as the number of cfu per millilitre. Detection limits were determined to be of 1.0 $\log_{10}$ cfu ml^{-1} for pour plate.

Raman spectroscopy and scanning electron microscopy

Raman spectroscopy was performed of the multilayer foil 2 containing 15 % poly(TBAMS) in the inner layer (layer thickness: 8 µm) to get information about the distribution of TBAMS. An Alpha 300R confocal Raman microscope (WITec GmbH, Germany) was used according to Timma et al. (2016). Imaging Raman scan was carried out on selected scanning frames of 30 µm x 20 µm with 3 spectrum/ µm on foil samples. All spectra were recorded with a 488 nm Ar^{+}-laser (Spectra Physics, USA) on a CCD-Detector in a WITec UHTS 300 Spectrometer (Timma et al. 2016).

Scanning electron microscopy (SEM) images were generated with *B. thermosphacta* on reference multilayer foil and sample multilayer foil containing 15 % in the inner layer for the identification of the antimicrobial mechanism. The foils were inoculated with *B. thermosphacta* (DSM No. 20171) suspension in a concentration of 10^{5}-10^{6} cfu ml^{-1} consisting out of physiological saline solution 8.5 µg ml^{-1} with 1 µg ml^{-1} tryptone (Oxoid, Hampshire, United Kingdom). After 12 h storage at 7 °C and 12 h storage at 25 °C the foils were examined with a SEM. The foils were transferred to a SEM sample holder to characterize exterior morphology of the bacteria so as to analyse the effect of poly(TBAMS) foil on bacterial cells.

Determination of the microbial growth, sensory and chemical parameters of perishable foods packaged with the poly(TBAMS) foil and pads (Part 2)

Preparation and packaging of product samples

Storage tests were conducted with fresh meat (chicken breast fillets, veal cutlets), meat products (cooked ham, smoke-cured ham) and ready to eat vegetables (carrots, white cabbage). The details of the packaging, the weight of the samples, the storage conditions and the investigated parameters are listed in table 2. Vacuum packaging with the reference multilayer foil and multilayer foil containing 15 % poly(TBAMS) in the inner layer with low permeability and a high gas barrier was chosen for the storage tests with fresh meat, meat products and vegetables under specific temperatures. Additionally, absorbent pads were used.

Unsexed 42-days-old-broiler chickens (Ross 308/708) were slaughtered in a poultry processing plant in Germany. After processing at the poultry slaughter plant, one part of single chicken fillets was directly packaged in the multilayer foil with 15 % poly(TBAMS) in the inner layer and in the reference foil. The packed samples and the other skinless double-breast chicken fillets were transported from the poultry slaughter plant to a wholesaler and further to the retailer and forwarded to the laboratory. The other parts of the fillets were vacuum packed using a vacuum sealing machine (Cambermachine, C100, Multivac Sepp Haggenmuller GmbH and Co. KG, Wolfertschwenden, Germany). One single fillet of a double-breast fillet was vacuum packaged in multilayer foil 2 (15 % poly(TBAMS) in the inner layer), the other fillet were vacuum packaged in the reference foil 1. In a further trial, the combination of poly(TBAMS) pads and foils were tested. After packaging all samples were stored in low-temperature high precision incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan). The storage tests with matured veal cutlets were conducted in the same way. Fewer than 8-month-old veal were slaughtered and air-chilled in a veal processing plant in Germany. The veal cutlets were transported from the veal slaughter house to the laboratory under temperature-controlled conditions. Veal cutlets were packed with the poly(TBAMS) foil 2 and the reference foil, a part with additional pads. Cooked ham and smoke-cured ham were produced in a pork processing plant in Germany. The vacuum packaged cooked hams and smoke-cured hams were transported from the processing plant to the laboratory under temperature-controlled conditions. Cooked ham was cut in slices (measurements: around Ø 18 cm, thickness: 1 cm). The slices of cooked ham and the smoke-cured ham (measurements: around of 20 cm x 7 x 4 cm) were cut in two pieces (cooked ham 80 g each; smoke-cured ham 175 g each). The one piece was vacuum packed with the poly(TBAMS) foil, the other one with the reference foil.

Ready to eat (RTE) carrots and white cabbage were sold by a retailer. In the laboratory, three RTE carrots (measurements: around 7 cm Ø 1.5 cm) together weighting 25 g were packaged in the reference and sample foil. The outer leaves of the cabbage were removed, and the cabbage was cut into pieces (measurements: around 2 cm x 2 cm thickness 0.5 cm). Immediately after cutting, the pieces were washed with water. After drying, 6 – 8 pieces were packaged in the foils to a total weight of 25 g. Samples were taken after defined time intervals, with the first investigation starting after arrival of the products in laboratory.

The microbiological, sensory and chemical analyses of the different products were conducted after specific time intervals.

Table 2 Tested products packaged in different packaging solutions and tested parameters

Product and size (g)	Packaging	Number	Time (h)	Temperature (°C)	Quality parameter*
Chicken breast fillet	Foil 1**	13	288	4	*B. thermosphacta*
250	Foil 2**	17			Enterobacteriaceae
	PE/PA foil	4			*Pseudomonas* spp.
	Foil 1+Pad	2			*Lactobacillus* spp.
	Foil 2+Meltblown	2			
Veal cutlet	Foil 1	4	504	2	*B. thermosphacta*
130	Foil 2	4			Enterobacteriaceae
	Foil 1+Core Cellulose	4			*Pseudomonas* spp.
	Foil 2+Core Cellulose/	4			*Lactobacill*us spp.
	2% poly(TBAMS)	4			
Cooked ham	Foil 1	5	504	7	*B. thermosphacta*
80	Foil 2	5			Enterobacteriaceae
					Pseudomonas spp.
					Lactobacillus spp.
					Staph. aureus
					L. monocytogenes
Smoke-cured ham	Foil 1	4	1680	7	*B. thermosphacta*
175	Foil 2	4			Enterobacteriaceae
					Pseudomonas spp.
					Lactobacillus spp.
Carrot	Foil 1	9	504	7	*B. thermosphacta*
25	Foil 2	14			Enterobacteriaceae
					Pseudomonas spp.
					Lactobacillus spp.
					Yeast and moulds
					Staph. aureus
					L. monocytogenes
White Cabbage	Foil 1	4	504	7	*B. thermosphacta*
25	Foil 2	4			Enterobacteriaceae
					Pseudomonas spp.
					Lactobacillus spp.
					Yeast and moulds
					Staph. aureus
					L. monocytogenes

* In each experiment total viable count, sensory, pH, and weight loss analysis were conducted
**Six samples were directly packaged in the processing company

Microbiological analysis

For the microbiological analysis, the product samples were removed aseptically by using a sterile scalpel. Each product sample of the different perishable foods had a total weight of nearly 25 g. For the poultry breast fillets, the product samples had measurements of 4 x 7 x 0.5 cm of meat surface. Additionally, in order to determinate the effect of the pads, the product samples were removed from the underside of the poultry breast. For the veal cutlets, the product samples had measurements of 4 x 8 x 1 cm. For the ham, a sterile, stainless steel punch was used to remove 25 g of meat for the samples. For the vegetables, the whole packaged product (25 g) was used for the samples. The product samples were transferred to a filtered sterile stomacher bag and filled with 225 ml saline diluent (8.5 µg ml^{-1} with 1 µg ml^{-1} tryptone). Samples were blended with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden,

Germany) for 60 s. Ten-fold dilutions of the samples were prepared in saline tryptone diluents.

For microbial analysis of the meat juice in the pads, the pads were weighed before and after storage. Each reference pad and sample pad was transferred to a filtered sterile stomacher bag and washed out with 100 ml of soybean-casein digest broth with lecithin polysorbate (Roth, Karlsruhe, Germany) in a Stomacher 400 for 60 s. Ten-fold dilutions of the solution were prepared with saline tryptone diluents and the microbial count of the solution was determined.

Total viable count (TVC), *Pseudomonas* spp., *B. thermosphacta, Lactobacillus* spp., Enterobacteriaceae and yeast and moulds were enumerated. In experiments with meat products and RTE vegetables *Staph. aureus* and *L. monocytogenes* were determined at the beginning and at the end of the storage. Table 3 gives an overview of all used media, techniques, and the incubation time and temperature. Counts of colony forming units (cfu) were expressed as $\log_{10}$ cfu g^{-1} for each medium and sample.

Table 3 Summary of the determined microorganisms, cultivation and enumeration temperature, technique and growth medium

Bacteria	Cultivation and enumeration temperature	Technique	Medium (Brand)
Total viable count	30 °C	Pour plate	Plate count agar (Merck, Darmstadt, Germany)
Staphylococcus aureus	37 °C	Spread plate	Baird Parker Agar (Oxoid, Cambridge, United Kingdom)
Listeria monocytogenes	37 °C	Spread plate	Listeria selective agar (Oxoid, Cambridge, United Kingdom)
Lactobacillus spp.	30 °C	Pour plate	De Man, Rogosa, Sharpe Agar (Merck, Darmstadt, Germany)
Brochothrix thermosphacta	25 °C	Drop plate	Streptomycin Inosit Toluylene Red Sheep Blood Agar Base (Oxoid, Cambridge, United Kingdom)
Enterobacteriaceae	37 °C	Overlay plate	Violet Red Bile Dextrose Agar (Merck, Darmstadt, Germany)
Pseudomonas spp.	25 °C	Spread plate	Pseudomonas agar with Cetrimide-Fucidin-Cephalosporin selective supplement (Oxoid, Cambridge, United Kingdom)
Yeast and moulds	30 °C	Pour plate	Yeast Glucose Chloramphenicol (Roth, Karlsruhe, Germany)

pH-, a_w- and weight loss- measurement

The pH of the products was measured over the entire storage period, using a portable pH-meter (Escort Junior EJ-2E-D-16L, Escort, Auckland, New Zealand). Three measurements were performed for each product sample, by placing the electrode onto the food surface and an averaging pH-value.

The a_w-value of the products was measured using an aw- LabMaster-Standard (Meintrup DWS, Lähden, Germany). One measurement was performed for each product sample, by placing around 10 g in the plastic dish.

All products were weighed before and after storage to determine the weight loss during the storage. The percentage weight loss was determined per the following equation:

$$\%\, WL\ (t) = \frac{W_0 - W\ (t)}{W_0} \times 100$$

with %WL(*t*) is the percentage weight loss at time *t*, W_0 is the initial sample weight, *W*(*t*) is sample weight at time *t*.

Sensory evaluation

The sensory analysis of all products was conducted by a trained sensory panellist. The developed scheme included the predefined detailed descriptions of the sensory attributes at different stages of spoilage during storage of the foods. The sensory scheme for chicken breast fillets was adapted per Roissant et al. (2014). General appearance (G), odour (O), colour (C), texture (T), drip loss (D) and the difference between products packaged in reference and sample foil were rated. 5-point and 3-point scoring systems for each attribute (1 = highest quality; 3 or 5 = unacceptable quality) were used for fresh meat and processed meat and vegetables, respectively. During the experiments, after opening the packaging, each sample was evaluated directly via the developed sensory scheme. The lower the score, the better the quality and freshness of the product. A weighted sensory quality index (QI) was calculated, parameter more relevant for to the spoilage process were weighted higher.

Chicken breast fillets:

$$\text{QI} = \frac{2G + 2C + T + 2O + D}{8}$$

Veal cutlets:

$$\text{QI} = \frac{2G + 2C + T + 2O + 2D}{9}$$

Ham:

$$\text{QI} = \frac{G + 3C + T + 2O}{7}$$

Vegetables:

$$\text{QI} = \frac{2G + 2C + T + O + D}{7}$$

Data Analysis

Antimicrobial activity analysis

Antimicrobial activity was calculated by subtracting the logarithmic value of the viable counts on the poly(TBAMS) material from the viable counts on the reference material after inoculation and incubation, as shown in the following equation:

$$\log_{10}\text{-reduction} = \log_{10} (T_{xRe} / T_{xSa})$$

where $T_{x,Re}$ = bacterial concentration on reference material, x hours after inoculation
and $T_{x,Sa}$ = bacterial concentration on sample material, x hours after inoculation

For materials to count as antimicrobial, the calculated value of antimicrobial activity should reach $\log_{10}$-reduction values ≥ 2.0 $\log_{10}$ units (JIS Z 2801:2000).

Modelling microbial growth

The Gompertz equation was used to model the development of the total viable count, and the growth of Enterobacteriacea, *Brochothrix thermosphacta*, *Pseudomonas spp.* and *Lactobacillus* spp., yeast and moulds as a function of time (Gibson et al., 1987).

$$N(t) = A + C \times e^{-e^{-B\times(t-M)}}$$

with N(t): microbial count [$\log_{10}$ cfu g^{-1}] at any time, A: lower asymptotic line of the growth curve (initial bacterial count), C: difference between upper asymptotic line of the growth curve (Nmax = maximum population level) and the lower asymptotic line, B: relative maximum growth rate at time M [h-1], M: time at which maximum growth rate is obtained (reversal point), and t: time [h].

Microbial shelf life calculation

To calculate the maximum specific growth rate (μ_{max}), the lag phase (λ t_{lag}) duration and the shelf life of the packed perishable foods, the equation obtained by rearranging the Gompertz equation was used.

Statistical Analysis

All data were transformed into $\log_{10}$ values before statistical analysis. The standard deviations in bacterial counts, pH-values, a_w-values and weight losses were calculated. The microbiological growth data were fitted using the statistical software program Origin 8.0 G (OriginLab Corp., Northampton MA, USA).

4.3 Results

Determination of the generally antimicrobial activity of the pads and multilayer foils

The general antimicrobial activity of all foils and pads was tested against *Staph. aureus* in saline solution and meat extract solution at 7 °C for 24 h. The bacterial counts of *Staph. aureus* in saline solution are decreased on all foils and pads containing poly(TBAMS) after 24 h at 7 °C. The bacterial counts of the foils and the spread with the 2 % poly(TBAMS) cellulose pad are reduced ≥ 4.0 $\log_{10}$ units, below the detection limit of 1.0 $\log_{10}$ unit. On poly(TBAMS) meltblown pads *Staph. aureus* with an initial concentration of 5.28 ± 0.09 $\log_{10}$ cfu ml^{-1} is decreased maximally 1.32 $\log_{10}$ units.

The $\log_{10}$-reductions of *Staph. aureus* in meat extract solution on the different multilayer blown foils containing poly(TBAMS) vary between 0.21 $\log_{10}$ units (n = 3, foil 4) and 1.87 $\log_{10}$ units (n = 6, foil 2). The one-layer foil decreases the bacteria concentration about 2.14 $\log_{10}$ units (n = 3). The highest $\log_{10}$-reduction of 3.88 $\log_{10}$ units is measured on the pad spread with 2 % poly(TBAMS), which reduces the bacterial counts under the detection limit. The poly(TBAMS) meltblown pads show with a $\log_{10}$-reduction of maximal 0.24 $\log_{10}$ units the lowest reductions compared with the other packaging materials.

Further tests were conducted with the multilayer foil 2 (15 % poly(TBAMS)) with *B. thermosphacta*, *E. coli* and *Ps. fluorescens* in saline solution and meat extract solution at 7 °C for 24 h (figure. 1).

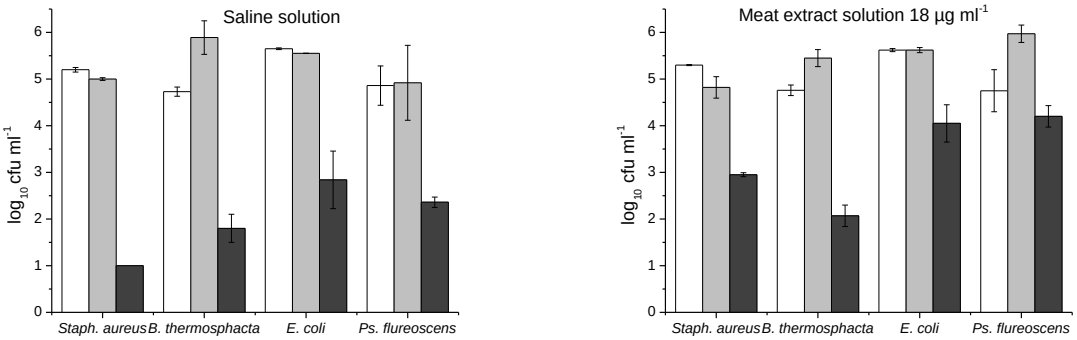

Figure 1 Arithmetic means of bacterial counts in saline solution (left) and meat extract solution (18 µg ml^{-1}) (right) on reference foil and foil containing 15 % poly(TBAMS) in the inner layer after 24 h at 7 °C.
Bacterial counts (n = 3): (▭) Inoculum, (▭) Reference and (▭) Sample

All bacteria counts in saline solution and meat extract solution are decreased on the sample foil compared with the bacterial counts on the reference foil. *Staph. aureus* and *B. thermosphacta* in saline solution are reduced ≥ 4.0 $\log_{10}$ units. Log_{10}-reduction of > 2.5 $\log_{10}$ units are achieved by *E. coli* and *Ps. fluorescens.*

Meat extract influences the activity. The highest $\log_{10}$-reduction among the bacteria is identified against *B. thermosphacta* with more than 3.5 $\log_{10}$ units, followed by *Staph. aureus*. The poly(TBAMS) foil shows with a $\log_{10}$-reduction of 1.57 $\log_{10}$ units the lowest reduction against *E. coli* compared with the other microorganisms (figure 1).

Raman spectroscopy and scanning electron microscopy

Figure 2 shows a Raman spectrum of the multilayer foil containing 15 % poly(TBAMS) in the inner layer (layer thickness: 8 µm).

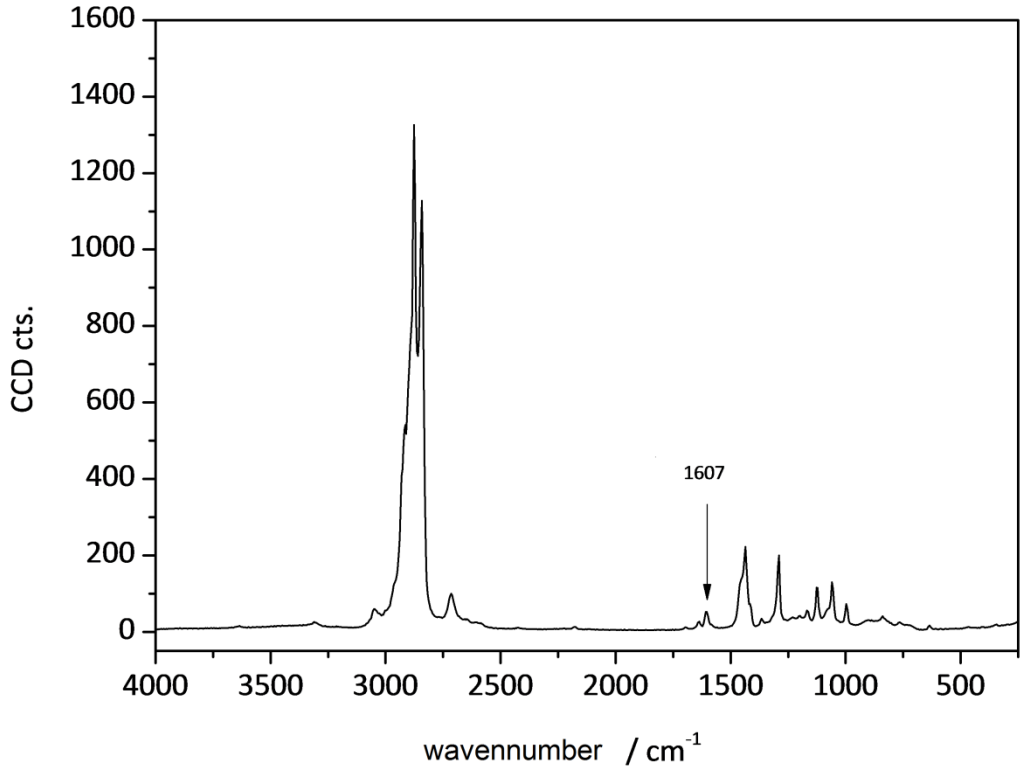

Figure 2 Raman Spectra in the area of 4000–250 cm^{-1} of the inner layer (layer thickness: 8 um) containing 15 % poly(TBAMS) of the multilayer foil

The recorded spectrum shows characteristic peaks of TBAMS. Vibration bands at 1607 cm^{-1} can be attributed to the bond C=C of the benzene ring. To visualize the distribution of TBAMS on the surface of the inner layer, an imaging Raman scan was carried out on selected scanning frames of 30 µm × 20 µm with 3 spectrum/ µm on foil samples. The vibration band of the bond C=C at 1607 cm^{-1} is chosen. The intensity of the observed vibration is calculated from the area under the considered band. A brighter colour generated in the image corresponds to a higher intensity of the observed band. Lower intensities result in darker areas. The performed Raman area scans are shown in figure 3. The CCD-cts. – colour ratio is shown in the illustrated scale bars.

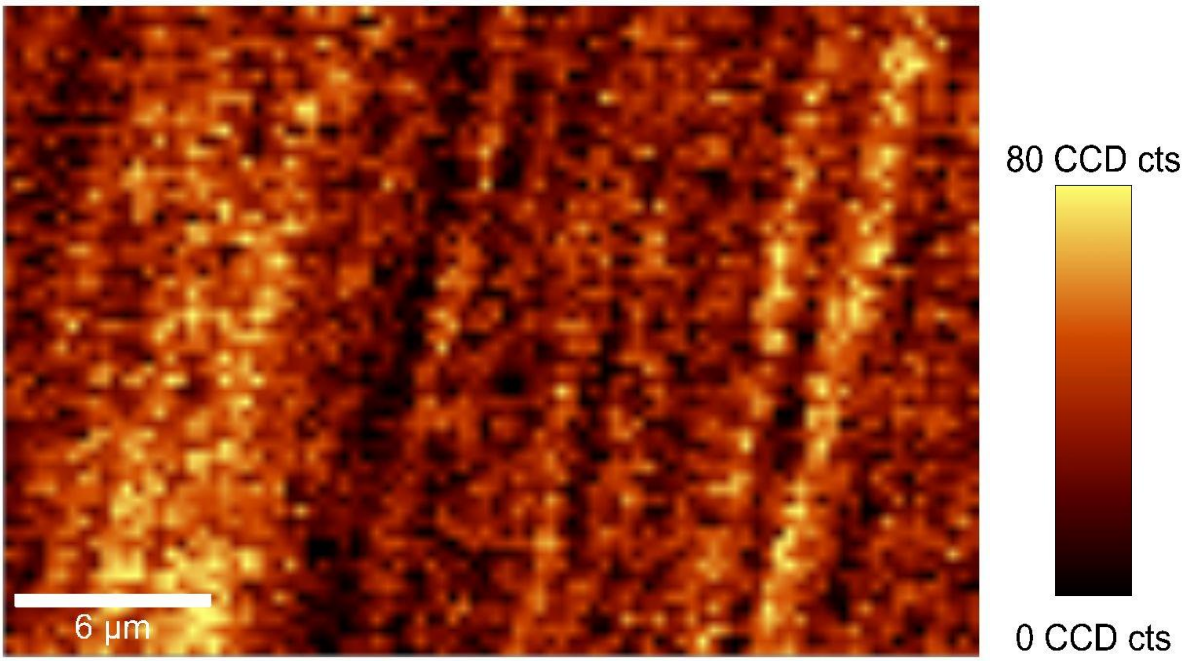

Figure 3 False colour images of the inner layer (layer thickness: 8 um) containing 15 % poly(TBAMS) of the multilayer foil visualize the intensity of the vibration at 1607 cm^{-1}(C=C) of TBAMS in the scanned area

The image shows that the 15 % of poly(TBAMS) are unevenly distributed on the surface of the inner layer.

The SEM images of *B. thermosphacta* on reference and sample foils containing 15 % poly(TBAMS) in the inner layer are shown in figure 4.

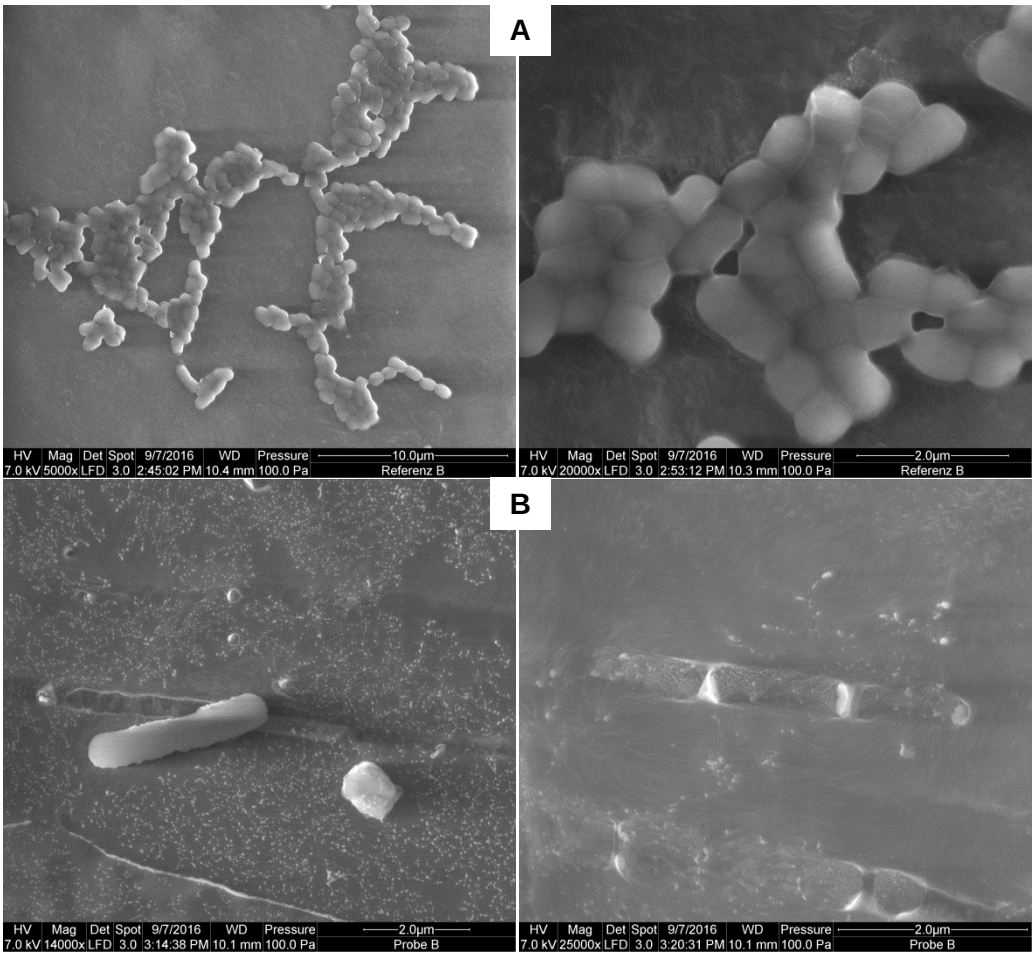

Figure 4 (A) bacterial cells on reference foil after 24 h contact time; (B) bacterial cells on sample foil after 24 h contact time

It becomes visible that the bacterial cells on the reference foil show a common shape of intact bacteria, normal morphology, smooth surface, regular rhabditiform and proliferation. On the sample foil bacteria show no proliferation during 24 h. In the first images, slight rupture, no original regular rhabditiform and single cells occur. In the second image cells show much more rupture in the cell membrane, the bacterial cytoplasm is completely outflowing, cell morphology occurs deformed and dry pieces remain.

Determination of the microbial growth, sensory and chemical/physical parameters of perishable foods packaged with the poly(TBAMS) foil and pads

Chicken breast fillets

The results of the chicken breast fillets packaged in the laboratory in the multilayer foil (15% poly(TBAMS)), the multilayer reference foil and the two-layer reference foil are shown in figure 5.

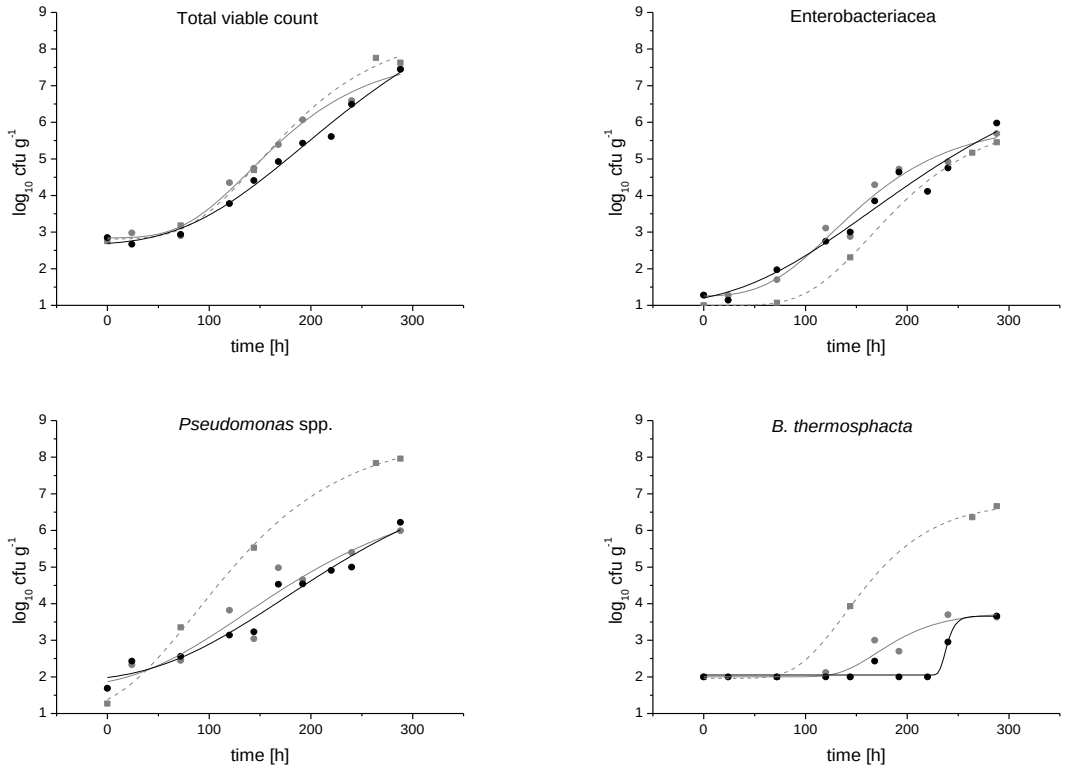

Figure 5 Growth of total viable count and typical spoilage microorganism on chicken breast fillets stored in different reference foils and sample foil containing of poly(TBAMS) at a temperature of 4 °C.
Bacterial counts: (●) Multilayer reference foil (n = 7) and (●) Multilayer Sample foil (n = 10); (■) Two-layer reference foil PE/PA (n = 4)

Enterobacteriaceae and *Pseudomonas* spp. dominate the flora during the entire storage period. *B. thermosphacta* is slightly increased. *Lactobacillus* spp. show no growth (data not shown). There are marginal differences in the development of the TVC, Enterobacteriaceae and *Pseudomonas* spp. packaged in the multilayer reference foil and poly(TBAMS) foil. In contrast, different behaviour is observed for *B. thermosphacta*. The bacterial counts of *B. thermosphacta* are lower on fillets packaged in the sample foil than the ones packaged in the reference foil. Poly(TBAMS) prolongs the lag phase for 98.08 h. *Lactobacillus* spp. are reduced in the poly(TBAMS) foil by maximal 0.91 $\log_{10}$ units after 240 h storage. Comparing the bacteria growth of the meat packaged in the commonly used two-layer foil with the developed multilayer foil, a high increase of the count of *Pseudomonas* spp. and *B. thermosphacta* can be observed, with maximum counts differing more than 2 $\log_{10}$ units (see also table 4).

The results of the poultry samples, which were packed directly in the slaughterhouse after processing, are comparable to the results described above (data not shown).

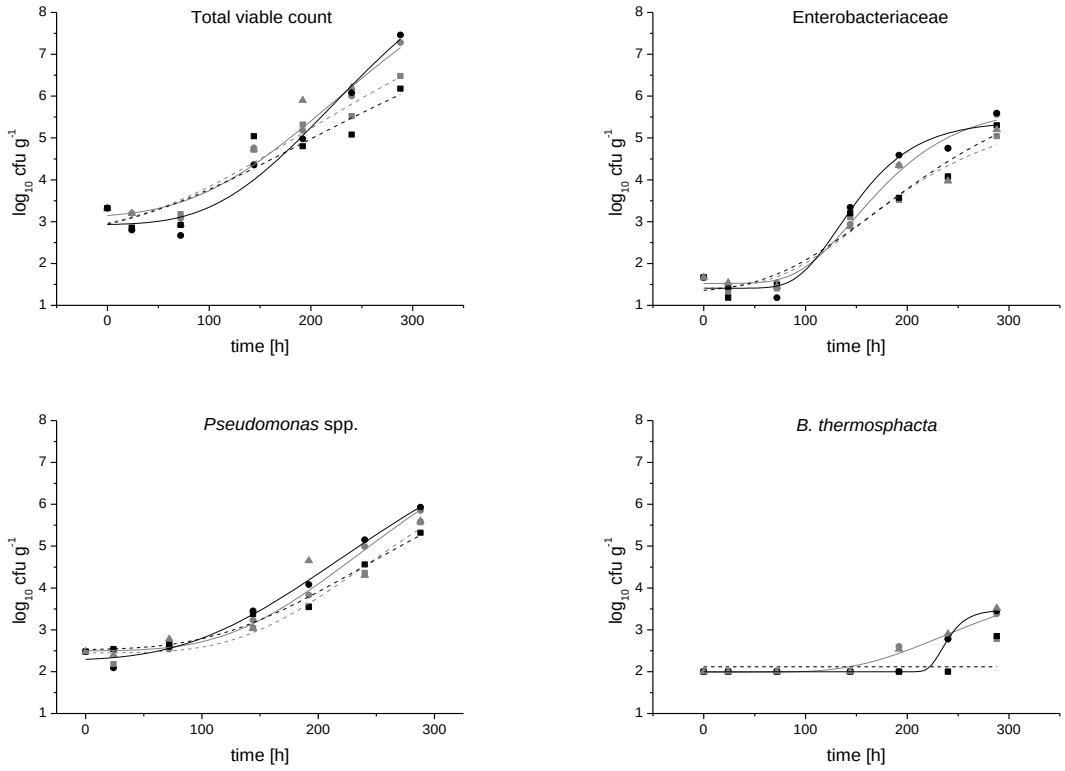

Figure 6 Growth of total viable count and typical spoilage microorganism on chicken breast fillets stored in reference foil and pad and sample foil and pad containing of poly(TBAMS) at a temperature of 4 °C.
Bacterial counts: (●) Multilayer reference foil and (●) multilayer sample foil; (■) Reference foil+pad and (■) Sample foil+pad (n = 2)

It can be inferred from figure 6 and table 4, adding a soaker pad the microbial growth is slightly decreased. The microbial count on the surface of the meat shows no difference between the packaging with and without poly(TBAMS), except for the growth of *B. thermosphacta*, which is reduced by the new polymer until 240 h. Comparing the counts of the bacteria on the fillet side in contact with the pad with the one on the meat surface a slower growth of the bacteria can be observed. There is a marginal delay in the development of the TVC by the poly(TBAMS) containing pad after 192 h. There is no difference between the growth of the specific spoilage organisms. The results of the meat juice investigation, which is absorbed in the pad, show a decrease of the TVC over the entire storage period in poly(TBAMS) meltblown pad compared to the reference pad. The maximal $\log_{10}$-reduction with 0.93 $\log_{10}$ units is identified after 192 h.

The growth parameter calculated with the Gompertz function and the microbial shelf life of chicken breast fillets stored in the different packaging trials are listed in table 4.

Table 4 Development of growth parameter calculated by fitting the data using the modified Gompertz function and microbial shelf life of poultry during storage

	Total viable count		*Pseudomonas* spp.		Enterobacteriaceae		Microbial
	t_{lag} [h]	μ_{max} [1/h]	t_{lag} [h]	μ_{max} [1/h]	t_{lag} [h]	μ_{max} [1/h]	shelf life [h]**
Reference multilayer*	81.95	0.0223	128.15	0.0208	60.41	0.0173	296.5
Sample multilayer*	55.94	0.0194	67.94	0.0125	55.00	0.0173	288.7
Reference PA/PE	79.87	0.0305	7.60	0.0348	100.31	0.0298	229.6
Reference multilayer	73.03	0.0274	26.35	0.0186	60.18	0.0263	254.2
Sample multilayer	75.05	0.0230	48.08	0.0182	34.20	0.0198	268.2
Reference multilayer + reference pad	87.33	0.0204	123.52	0.0207	92.43	0.0278	279.4
Sample multilayer + meltblown pad	107.2	0.0250	89.83	0.0188	91.16	0.0354	271.6
Reference multilayer + reference pad †	29.26	0.0147	132.57	0.0193	71.35	0.0186	212.5***
Sample multilayer+ meltblown pad †	11.37	0.0126	112.27	0.0157	63.41	0.0181	291.4***

* Poultry packaged in slaughter plant
** Microbial shelf life was estimated from time point zero of the laboratory investigations, which means 24 h after slaughtering

† Product sample for the microbial analysis taking from the bottom side

The microbial shelf life of poultry is evaluated by a TVC concentration of 7.0 $\log_{10}$ cfu g^{-1}. As shown in the table 4, a shorter microbial shelf life is detected on poultry packaged in the reference foil PA/ PE compared with the multilayer foil. A slight increase of microbial shelf life is detected in the active packaging system with poly(TBAMS) foils and meltblown pads when considering the bottom side of poultry for microbial analysis.

The results of chemical and sensory parameters are similar in the different trials. The QI increases linearly in the different storage trials. No differences of various sensory impressions for different attributes become visible between the poultry breast packaged in the sample packaging compared with the one in the reference packaging. The pH-values of the meat packaged in poly(TBAMS) foils are comparable to the references. The initial pH-values (24 h after slaughtering) vary between 5.72 ± 0.13 in the samples which were packaged in the processing company and 6.05 ± 0.05 for the fillets packaged in the laboratory. At the end of storage, the pH-values of all samples reached a value of 6.17 ± 0.05 independent of packaging used in the experiments. Table 5 lists the initial pH-values and weight losses after certain time intervals. The values of the pH of the chicken breasts samples are slightly increased during the storage. The weight loss 24 h after slaughtering is around 0.5 % and ranges between 2 % (reference foil) and 1 % (sample foil) after storage.

Table 5 Initial values, values after 72 h storage and values at the end of the storage (312 h) of pH-value and weight loss example of directly packaged fillets

	pH-value			weight loss %		
	0 h	72 h	312 h	0 h	72 h	312 h
Reference multilayer	5.72 ± 0.09	5.99 ± 0.11	6.15 ± 0.06	0.57 ± 0.42	0.92 ± 0.45	2.03 ± 0.88
Sample multilayer	5.72 ± 0.13	6.04 ± 0.10	6.17 ± 0.05	0.48 ± 0.23	1.00 ± 0.56	1.01 ± 0.62

Veal cutlets

Figure 7 shows the development of the total viable count and *Lactobacillus* spp. on veal cutlets packaged in the multilayer reference and poly(TBAMS) foils with or without reference and poly(TBAMS) pads at a temperature of 2 °C.

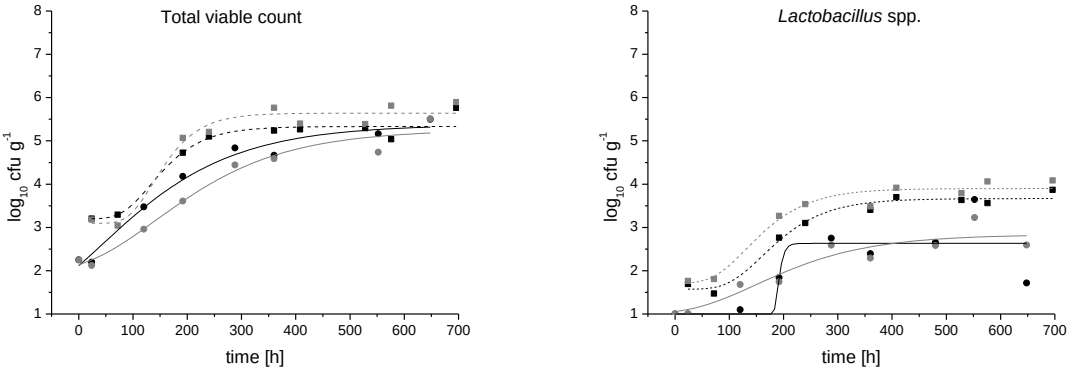

Figure 7 Growth of total viable count and *Lactobacillus* spp. on veal cutlets stored in reference foil with or without reference pad and sample foil with or without sample pad containing poly(TBAMS) at a temperature of 2 °C.
Bacterial counts: (●) Multilayer reference foil and (●) Multilayer sample foil; (■) Reference foil+pad and (■) Sample foil+pad (n = 4)

Lactobacillus spp. show a low initial concentration, but become dominant during the storage at 2 °C in the different packagings. Nearly no growth of *Pseudomonas spp., B. thermosphacta* and Enterobacteriaceae can be identified on veal cutlets stored in reference and sample packaging during the storage period of 700 h. Comparing the development of the TVC on the cutlets packaged in poly(TBAMS) containing foils without pads to references no beneficial effect of poly(TBAMS) can be observed, whereas a slightly positive effect on the growth of *Lactobacillus* spp. is visible. In the trials with the pads inside the package, poly(TBAMS) reduces the bacterial growth by reducing the maximal growth rate (see table 6), even if the initial counts of the cutlets are higher in this trial.

Table 6 Development of growth parameter calculated by fitting the data using the modified Gompertz function and microbial shelf life of packaged veal cutlets during storage (n = 4)

	Total viable count		*Lactobacillus* spp.		Microbial
	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	shelf life* [h]
Reference multilayer	61.71	0.0113	30.63	0.0057	n. a.
Sample multilayer	0	0.0112	183.19	0.0959	n. a.
Reference multilayer + reference pad	91.09	0.0202	68.67	0.0199	n. a
Sample multilayer + sample pad	79.09	0.0147	96.33	0.0127	n. a.

*Microbial shelf life was estimated from time point zero of the laboratory investigations, which means max. 72 h after slaughtering

The sensory evaluation of the veal cutlets is evaluated slightly better in the poly(TBAMS) packed samples without pads. No differences are detected in pH-value among the investigated samples packaged in the sample foil and in the reference foil. The initial pH-value is 5.54 ± 0.05. During the entire period of observation, the highest pH-values are detected after 16 d storage, with 5.74 ± 0.04 for meat in the reference foil and 5.75 ± 0.04 in the sample foil, the lowest at the end of storage (27 d) with 5.35 ± 0.07 for references and 5.34 ± 0.07 for samples.

Cooked ham

The development of the microbial growth on cooked ham packaged in the different foils during storage at 7 °C is shown in figure 8.

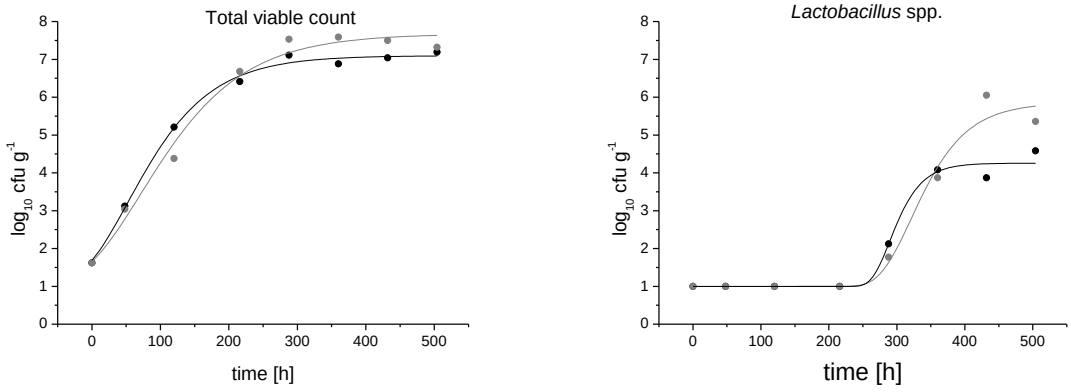

Figure 8 Growth of total viable count and *Lactobacillus* spp. on cooked ham stored in reference foil and sample foil containing of poly(TBAMS) at a temperature of 7 °C.
Bacterial counts: (●) Multilayer reference foil and (●) Multilayer sample foil (n = 5)

Reductions in bacterial counts are detected on ham packaged in poly(TBAMS) foil by *Lactobacillus* spp. after approximately 360 h and until the end of the storage. The sample foil

decreases the bacterial growth by reducing the maximal growth rate and the maximum cell count of *Lactobacillus* spp. (see also table 7), with a maximal difference of 2.18 $\log_{10}$ units around the end of storage. The TVC is slightly decreased after 260 h. No positive effect of poly(TBAMS) containing foils is detected in the growth of *B. thermosphacta*, *Pseudomonas* spp. and Enterobacteriaceae (data not shown). In the experiments, no *Staph. aureus* and *L. monocytogenes* can be detected at the end of the storage. The calculated microbial shelf life indicates a 15 % longer microbial shelf life of the samples packed in the new polymer than the in reference foil packaged ham.

Table 7 Development of growth parameter calculated by fitting the data using the modified Gompertz function and microbial shelf life of packaged cooked ham during storage (n = 5)

	Total viable count		*Pseudomonas* spp.		*B. thermosphacta*		*Lactobacillus* spp.		Microbial
	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	shelf life* [h]
Reference multilayer	0	0.029	0	0.0157	41.72	0.0208	262.98	0.0449	261.5
Sample multilayer	0	0.0330	0	0.0303	37.64	0.0192	274.66	0.0285	334.4

*Microbial shelf life was estimated from time point zero of the laboratory investigations, which means 216 h after processing

The pH-value, weight loss and a_w-value of the ham packaged in the sample foil are in the same range as ham packaged in the reference foil (see table 8).

No differences in sensory evaluation for different attributes are determined between the ham packaged in the sample foil compared with ham in the reference foil. The QI increases linearly during storage.

Table 8 Initial values, values after 216 h storage and values at the end of the storage (504 h) of pH-value, a_w-value and weight loss of packaged cooked ham (n = 5)

	pH-value			a_w-value			weight loss %	
	initial 0 h	after 216 h	end 504 h	initial 0 h	after 216 h	end 504 h	after 216 h	end 504 h
Reference multilayer	5.93 ± 0.03	5.92 ± 0.08	5.81 ± 0.05	0.967	0.970	0.968	2.43±0.32	2.38±0.48
Sample multilayer		5.93 ± 0.07	5.82 ± 0.06		0.972	0.968	2.42±0.37	2.27±0.45

Smoke-cured ham

Figure 9 shows the development of the total viable count and *Lactobacillus* spp. on smoke-cured ham packed with different foils at 7 °C (n = 4).

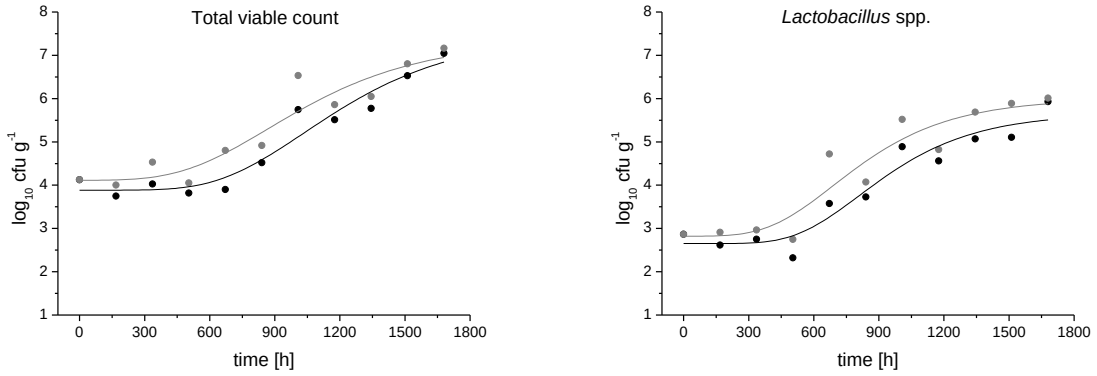

Figure 9 Growth of total viable count and *Lactobacillus* spp. on smoke-cured ham stored in reference foil and sample foil containing of poly(TBAMS) at a temperature of 7 °C.
Bacterial counts: (●) Multilayer reference foil and (●) Multilayer sample foil (n = 4)

The bacterial counts of TVC and *Lactobacillus* spp. are lower on ham stored in the sample foil than on those in the reference foil at each investigation point. The maximum difference in the cell count of *Lactobacillus* spp. between the samples packed in the different foils is 1.14 $\log_{10}$ units after 28 days of storage. The $\log_{10}$-reductions of TVC vary between 0.12 (70 d) and 0.90 $\log_{10}$ (28 d) units during the entire storage period. A prolongation of lag phases of TVC and *Lactobacillus* spp. is observed in the ham packaged in poly(TBAMS) foil (see also table 9). *Pseudomonas* spp. are only slightly increased over the entire storage with maximum counts under 3 $\log_{10}$ cfu g^{-1}. Ham packed with the new material show a decrease in the growth of *Pseudomonas* spp. and a prolonged lag phase. No growth of *B. thermosphacta* and Enterobacteriaceae can be identified during the storage over 70 days at 7 °C (data not shown).

Table 9 Development of growth parameter calculated by fitting the data using the modified Gompertz function and microbial shelf life of packaged smoke-cured ham during storage (n = 4)

	Total viable count		*Lactobacillus spp.*		Microbial
	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	shelf life [h]
Reference multilayer	469.09	0.0030	387.65	0.0039	1735.0
Sample multilayer	637.76	0.0033	513.18	0.0037	1800.4

*Microbial shelf life was estimated from time point zero of the laboratory investigations

The microbial shelf life is increased by around 5 % in ham stored in sample foil. The impressions of the sensory attributes are also slightly better evaluated in the poly(TBAMS) packed samples. The QI increases to around 2.1 at the end of storage (data not shown).
The results show, that the pH-value and weight loss are not influenced by the poly(TBAMS) foil during storage (table 10).

Table 10 Initial values, values after 672 h storage and values at the end of the storage (1680 h) of pH-value and weight loss of packaged smoke-cured ham (n = 4)

	pH-value			**weight loss %**	
	initial 0 h	after 672 h	end1680 h	after 672 h	end 1680 h
Reference multilayer	5.66 ± 0.04	5.55 ± 0.04	5.27 ± 0.02	0.38 ± 0.05	0.62 ± 0.04
Sample multilayer		5.50 ± 0.10	5.28 ± 0.03	0.46 ± 0.06	0.56 ± 0.06

Carrots

Next to meat products the effect of poly(TBAMS) on RTE vegetables was investigated. Figure 10 shows the development of the investigated microorganisms and the TVC on carrots at a temperature of 7 °C.

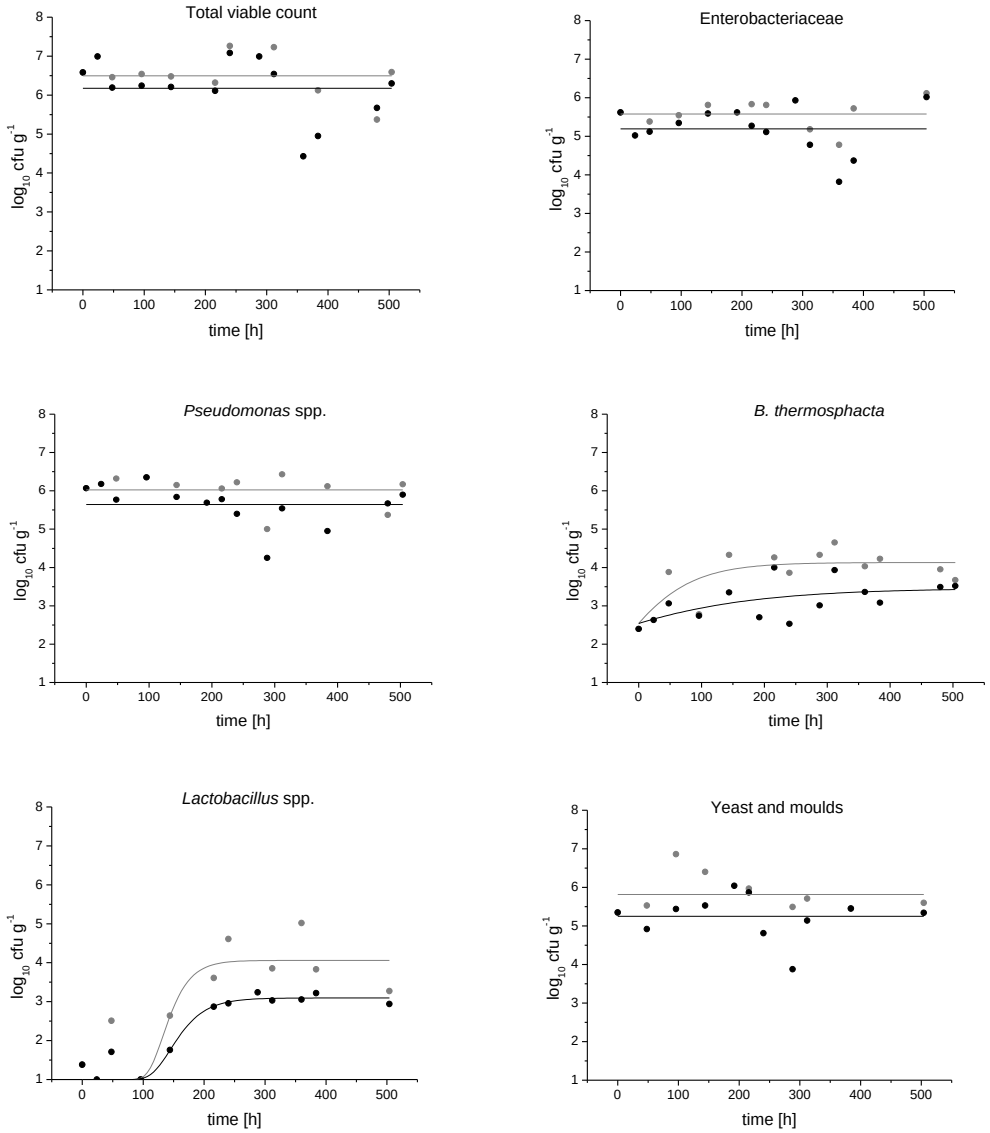

Figure 10 Growth of total viable count and typical spoilage microorganism on carrots stored in reference foil and sample foil containing of poly(TBAMS) at a temperature of 7 °C.
Bacterial counts: (●) Multilayer reference foil (n = 9) and (●) Multilayer sample foil (n =14)

Carrots show a high initial TVC value of of 6.58 ± 0.41 $\log_{10}$ cfu g^{-1}. Additionally, the initial count of Enterobacteriaceae, *Pseudomonas* spp. as well as yeasts and moulds is above 5 $\log_{10}$ cfu g^{-1}. Lower initial counts are observed for *B. thermosphacta* and *Lactobacillus* spp..

In contrast to the packed meat, poly(TBAMS) decreases the counts of all bacteria, and yeasts and moulds. For TVC, the highest $\log_{10}$-reduction of 1.17 $\log_{10}$ units is identified after 384 h. A high decrease of the microbial growth is achieved against *Lactobacillus* spp. and *B. thermosphacta*. Even if the bacterial counts of yeast and moulds, Enterobacteriaceae and *Pseudomonas* spp. of the samples packed in reference material are not increased during the storage period, the novel packaging material is decreased their count during the storage test at nearly all investigation points.

The pH-values of carrots packaged in the sample foil are comparable to those in the reference foil. During storage, the pH-value decreases roughly 2 units to around 4.5 (table 11).

Table 11 The values after 48 h, 216 h storage and values at the end of the storage (504 h) of pH-value and weight loss of packaged carrots

	pH-value			weight loss %		
	48 h	216 h	504 h	48h	216 h	504 h
Reference multilayer	6.20 ± 0.39	6.01 ± 0.21	4.55 ± 0.10	1.01 ± 0.12	3.46 ± 0.70	8.96 ± 2.08
Sample multilayer	6.53 ± 0.15	6.00 ± 0.20	4.56 ± 0.10	2.16 ± 1.43	3.64±1.03	11.46 ± 0.95

The weight loss of carrots increases during storage to an end loss of around 11.46 % ± 0.95 in the sample foils and 8.96 ± 2.08 in the reference foils. No difference can be observed in the description of sensory attributes between the carrots stored in the reference and sample foils.

Cabbage

Figure 11 shows the development of TVC and typical spoilage organisms on white cabbage packed in the reference and sample foils at 7 °C.

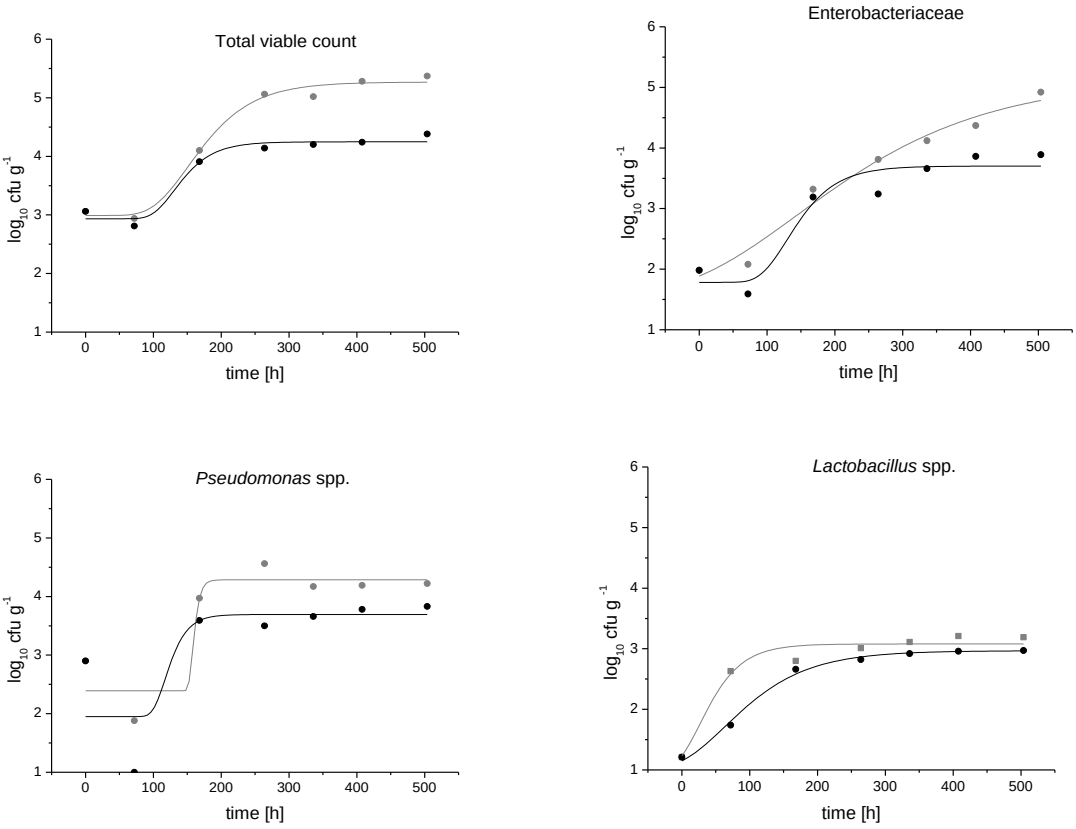

Figure 12 Growth of total viable count and typical spoilage microorganism on white cabbage stored in reference foil and sample foil containing of poly(TBAMS) at a temperature of 7 °C.
Bacterial counts: (●) Multilayer reference foil and (●) Multilayer sample foil (n = 4)

In contrast to the carrot trial, all bacteria are characterized by a typical growth curve, and none of the bacteria stay close to a constant level. Enterobacteriaceae are shown to be the dominant microorganisms, followed by *Pseudomonas* spp.. In the experiments no *Staph. aureus* and *L. monocytogenes* can be detected at the beginning and at the end of the storage. At each point of investigation, TVC and individual counts are reduced on cabbage stored in the sample foil compared with in the reference foil. The highest $\log_{10}$-reduction of 1.17 $\log_{10}$ units is identified for TVC at the end of storage. During storage, the maximal $\log_{10}$-reductions of the individual bacteria vary between 1.06 $\log_{10}$ units for *Pseudomonas* spp. after 264 h, 1.03 $\log_{10}$ units for Enterobacteriaceae after 504 h and 0.89 $\log_{10}$ units for *Lactobacillus* spp. after 72 h. For the cabbage packed in reference foils, the microbial shelf life is reached after 334.8 h, whereas the shelf life of the cabbage packaged in the sample foil is not reached during the storage period (table 12).

Table 12 Development of growth parameter calculated by fitting the data using the modified Gompertz function and microbial shelf life of packaged cabbage during storage (n = 4)

	Total viable count		*Pseudomonas* spp.		Enterobacteriacea		*Lactobacillus* spp.		Microbial
	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	T_{lag} [h]	μ_{max} [1/h]	shelf life* [h]
Reference multilayer	98.83	0.0160	99.20	0.1190	0	0.0082	0	0.0221	334.8**
Sample multilayer	98.93	0.0152	152.16	0.0352	89.97	0.0177	0	0.0106	n. a.

*Microbial shelf life was estimated from time point zero of the laboratory investigations
**Evaluated by count if TVC: End of shelf life: 5.2 $\log_{10}$ cfu g^{-1}

The sensory evaluation of the cabbage stored in the sample foil is comparable to cabbage in the reference foil.

The pH-value in the cabbage in the reference foil decreases from 6.33 ± 0.08 to 5.33 ± 0.04. The cabbage in the sample foil reaches a value of 5.95 ± 0.05 after 504 h storage. The weight loss of the cabbage varies between 3.94 % ± 1.03 in the sample foil and 4.20 % ± 1.52 in the reference foil after 504 h storage.

4.4 Discussion

Generally, the results underline a high potential of poly(TBAMS) for its future use as antimicrobial packaging material. But it is also shown in this study that the activity is influenced by several different factors. The activity of poly(TBAMS) is based on electrostatic interactions between the positively charged polymer and the negatively charged surface of bacteria (Dohlen et al. 2016). One important influence factor on the electrostatic interactions and thus activity is the composition of the different materials containing poly(TBAMS) (charge, concentration, availability, surface structure and the miscibility of the polymer). In previous study, the antimicrobial activity could be increased from $\log_{10}$-reduction of 1.9 $\log_{10}$ units to 3.89 $\log_{10}$ units by spinning fibres with 10 % polymer. In this study however, the meltblown pads show a lower activity, which can be caused by the lower availability of the active amino groups on the produced polymer surfaces. The availability can be influenced by the miscibility properties between the standard polymers and poly(TBAMS) (chapter 3). Limited miscibility of two polymers can lead to non-homogeneous compounds (Lenoir et al. 2006; Seyfriedsberger et al. 2006; Roy et al. 2007; Thomassin et al. 2007). Furthermore, as also shown in previous studies and per experiments with a compound containing LLDPE and poly(TBAEMA) by Seyfriedsberger et al. (2006), the more active polymer there is integrated into the matrix polymer, the stronger the antimicrobial activity (chapter 3). Therefore, the availability of the active groups on the polymeric surface and thus the charge can be raised by increasing the concentration of poly(TBAMS) or enlargement of the polymeric surface area.

The Raman spectroscopy images demonstrate, that by an integration of 15 % poly(TBAMS), TBAMS is not available on the complete surface of the multilayer foil 2. Nevertheless, a high antimicrobial activity with more than 2.5 $\log_{10}$ units is detected against all tested bacteria in saline solution after 24 h at 7 °C. The SEM images show that the sample foil leads to cell membrane rupture and cytoplasm escaping, resulting in bacteria death. However, the results confirm the results of previous study, in which gram-positive bacteria are more sensitive to poly(TBAMS) than gram-negative bacteria *E. coli* and *Ps. fluorescens*. According to the results of a study by Potter et al. (2005) a higher electrophoretic mobility of *B. thermosphacta* with -1.085 µmcm/Vs than of *Ps. fluorescens* with -0.396 µmcm/Vs was evidenced (chapter 2). The different negative charges of bacteria result in varying electrostatic interactions with the positively charged polymer. The electric charge of the bacteria and of an antimicrobial material could be different by using different test solutions (Potter et al. 2005). The results of this study and of previous study show a negative influence of meat extract solution on the antimicrobial activity of poly(TBAMS) (Dohlen et al. 2016; chapter 3). SAM® polymers surface can bind with negatively charged food substances such as proteins (Ignatova et al. 2009; Dohlen et al. 2016), which is also detected with chitosan (Devlieghere et al. 2004; Aider 2010; Park et al. 2010). Thus, proteins could inhibit the interactions between bacteria and polymers. But nonetheless, the results of this study and the one in chapter 3 confirm that the negative influence of proteins on the activity can be partly reduced by a higher concentration of poly(TBAMS).

Consequently, the mentioned factors and the factors mentioned in chapter 2 (temperature, initial bacterial concentration, gas atmosphere) influence the antimicrobial activity and thus the effect on shelf life. This is confirmed in the conducted storage tests. Besides these factors, it also becomes evident that the composition and interaction between these bacteria during the storage can have an effect on the activity. The composition of the flora depends on several different factors, such as the product itself, processing steps, temperature conditions or packaging.

In different publications for example, the anaerobic *Lactobacillus* spp. are described as an important spoilage originator for fresh meat also poultry under anaerobic conditions, whereas the aerobic *Pseudomonas* spp. normally play a minor role in the spoilage process (Lambert et al. 1991; Borch et al. 1996; Huis in't Veld 1996; Garcia-Lopez et al. 1998; Gram et al. 2002; Shaw and Harding 2008). In own experiments with vacuum packaged poultry breast fillets at 4 °C, *Pseudomonas* spp. show growth stability followed by Enterobacteriacea, whereas the number of *Lactobacillus* spp. and *B. thermosphacta* remains relatively constant throughout

the entire storage period. Even though the bacteria are aerobic competitors, *Pseudomonas* spp. can grow in oxygen concentrations of less than 1% (Clark and Burki 1972; Herbert et al. 2013; Roissant et al. 2014). A remaining oxygen concentration of 0.1 – 2 % or even more is usual for vacuum packaging (Vermeiren et al. 2003). Completely anaerobic conditions are rarely achieved, because the commonly used foils have to greater or lesser extent of oxygen permeability (Garcia-Lopez et al. 1998). Furthermore, *Pseudomonas* spp. are often responsible for spoilage at cool temperatures because of its psychrotrophic properties (Hood and Mead 1993), whereas *Lactobacillus* spp. and most of Enterobacteriaceae prefer to grow under mesospheric temperatures (Huis in't Veld 1996; Jay et al. 2005). Under low oxygen concentrations, *Pseudomonas* spp. sequentially metabolize, besides the depletion of glucose, several products such as amino acids (Varnam and Sutherland 1995; Garcia-Lopez et al. 1998; Nychas et al. 2008). This proteolytic activity bears special mentioned when compared with *Lactobacillus* spp. and *B. thermosphacta,* as it gives an advantage towards domination of the microflora (Nychas et al. 2007). Furthermore, *Pseudomonas* spp. can produced siderophores, which can bind iron, and can be used to prevail against other bacteria, especially in low less iron availability conditions (Takase et al. 2000; Nychas et al. 2007; Cornelis and Dingemans 2013). If *Pseudomonas* spp. are main microorganisms on the meat surface directly after slaughtering, these bacteria seem to have a kind of selective advantage due to the mentioned factors. Jimenez et al. (1997) also demonstrated an unattenuated growth of *Pseudomonas* spp. on fresh chicken breast stored at 4°C in vacuum packaging.

The high microbial count of the gram-negative bacteria *Pseudomonas* spp. during the whole storage time and the high concentration of proteins, explain the negative effect on the antimicrobial activity of the foil. Furthermore, the low temperature conditions can reduce the activity, because of the lower negative charge of bacteria (Dohlen et al. 2016). This was also confirmed by Briandet et al. (1999) in experiments with *L. monocytogenes*. Thus, no increase in shelf life of fresh poultry at 4°C can be achieved, even if the growth of the gram-positive bacteria *Lactobacillus* spp. and *B. thermosphacta* is slightly decreased. However, the tests with the meltblown pads indicate that the antimicrobial activity can be increased by increasing the surface area of the material. Generally, it becomes evident that multilayer foils have a positive effect on shelf life in comparison with the commonly used two-layer foil. This is caused by the strong oxygen barrier of the multilayer foil which reduces the growth of *Pseudomonas* spp. and *B. thermosphacta*. In studies higher maximal growth rate and end concentration of *Pseudomonas* spp. were detected on poultry in foils with high permeability

compared in foils with low permeability under cool temperatures conditions (Borch et al. 1996; Roissaint et al. 2014).

In contrast to the specific spoilage organism on poultry fillets, *Lactobacillus* spp. predominate the other organisms on veal cutlets during storage at 2 °C. In different studies, *Lactobacillus* spp. were also identified as the main spoilage originator for fresh beef (Lambert et al. 1991; Borch et al. 1996; Blixt and Botch 2002; Pennacchia et al. 2011). The initial pH-value of 5.5 supports *Lactobacillus* spp. in dominating the flora, because other microorganisms are more acid sensitive than *Lactobacillus* spp. (Garcia-Lopez et al. 1998). The growth of *Lactobacill*us spp. are slightly decreased by poly(TBAMS), but no effect could be observed in the development of the TVC. In a study of Park et al. (2010), the authors investigated the TVC on fresh bovine meat applied with chitosan-incorporated LDPE films with different concentrations of chitosan (1 %, 4 %, 8 %). Here also, no antimicrobial activity of chitosan films was detected against bacteria on meat after storage of 10 days at 3 °C. As with the study of poultry, an increase of the amount of poly(TBAMS), by adding a poly(TBAMS) pad inside the package, leads to an slight increase of the activity.

According to Kreyenschmidt et al. (2010) and Kalschne et al. (2015), *Lactobacillus* spp. are observed as dominant spoilage organisms on cooked ham under low oxygen concentration and temperatures. The late growth of *Lactobacillus* spp. is probably related to the mesophilic character and belonging to a slow-growing group of bacteria (Jay et al. 2005). It can be assumed that the prolongation of the microbial shelf life due to the sample foil is mainly influenced by *Lactobacillus* spp.. Besides acids, *Lactobacillus* spp. can produce CO_2 through the glucose utilization (Madigan and Martinko 2006). A higher amount of functional amino groups of the polymer could be protonated by a higher concentration of carbon dioxide which can lead to an increase in the activity (chapter 2). Within the vacuum packs CO_2 can increase to about 20 % (Garcia-Lopez et al. 1998).

However, a negative effect on the interaction between the bacteria and the polymer occurred through the addition of salt during processing. Devlieghere et al. (2004) observed, that the Cl-ions neutralized the positive charge of chitosan. Furthermore, the Na^+ and cationic ions like Ca^{2+}, Mg^{2+}, Zn^{2+} and Ba^{2+} can influence the antimicrobial activity of Chitosan and SAM® polymers, caused by an interaction with the bacteria (Lenoir et al. 2006; Rafatt et al. 2008). In contrast, the processing step cooking is advantageous for the interactions, because it leads to a denaturation of the proteins and loss of acids groups, which results in a higher isoelectric point and a lower negative charge of the muscle proteins (Hamm and Detherage 1960).

In smoke-cured ham, poly(TBAMS) positively influences the development of the TVC and the growth of *Lactobacillus* spp. during the entire storage period. *Lactobacillus* spp. dominate the flora directly after processing since they are inoculated in the product as protection against the growth of undesirable bacteria (Gram et al. 2002; Weber 2008). *Lactobacillus* spp. can inhibit for example the growth of *Staph. aureus* (Charlier et al. 2009). Furthermore, it can be assumed, that the pH-value of the samples is lower than the isoelectric point of several proteins in the meat. Therefore, these proteins have a positive charge and are not able to interact with the functional amino groups of poly(TBAMS). The dominate *Lactobacillus* spp. and the possible effect of a low pH of around 5.5 on proteins could lead to the increase of microbial shelf life in smoked-cured ham. Studies with chitosan also described the positive effect on the shelf life of meat products (Sagoo et al. 2002; Soultos et al. 2008; Siripatrawan and Noipha 2012; Vasilatos and Savvaidis 2013).

Different studies had also shown, that chitosan had the potential to delay or inhibit the growth of pathogenic and spoilage microorganism in ready to eat carrots and cut broccoli (Dutta, et al. 2009; Moreira et al. 2011; Leceta et. al 2015).

In the present study with RTE vegetables, the microbial count and growth of all tested bacteria, including *Pseudomonas* spp., can be reduced by poly(TBAMS) foil. These results with processed vegetable underline the high influence of food components such as proteins in meat, which can interact with the active amino groups of the polymer. Thus, this multilayer foil containing the novel polymer has a high potential to increase the quality, safety and shelf life of products with low protein contents such as ready to eat vegetables. But it has to be considered, that the respiration process and enzymatic activities of vegetables also can affect the length of shelf life (Barry-Ryan and O'Beirne 1998). However by integrating poly(TBAMS) in foils with specific gas-and water barriers, a significant effect on shelf life of RTE products can be reached, as presented in the results. The overall study shows the complexity of different influence factors on the antimicrobial packaging material. Further investigations could be conducted to clarify if other factors are affecting the antimicrobial mechanism of the novel polymer.

4.5 Conclusion

The conducted experiments considered realistic application conditions in perishable food supply chains and gave a first evaluation for the potential of poly(TBAMS) containing packaging material to increase the safety and shelf life of perishable products. Overall, the

tests with chicken breast fillets and veal cutlets, hams and vegetables demonstrated, that the activity of the poly(TBAMS) and its effect on shelf life are influenced by several factors. It became evident that especially proteins, as there are high concentrated present in fresh meat, influence the activity of the polymer. To increase the shelf life of protein-rich products in the future, a higher amount of available functional amino groups on the polymeric surface is important, as it raises the activity against different bacteria under low temperature conditions. Approaches to increase the availability of active groups on the polymeric surface, such as increasing the concentration, changing the matrix polymer or enlargement of the surface area by electro spinning or three-dimensional structure printing, can be possible solutions to optimize the material.

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5 Development of an evaluation scheme to assess the potential of antimicrobial polymers acting as packaging for perishable products

5.1 Introduction

As mentioned previously, several scientific papers have been published in the field of antimicrobial packaging material. The variety of developed antimicrobial materials ranges from bactericides, organic acids, plant extracts and silver up to active polymers. Most of these studies describe the potential of the material to prolong the storage time in the chain (Appendini and Hotchkiss 2002; Quintavalla and Vicini 2002; Ilg and Kreyenschmidt 2012; Prasad and Kochhar 2014). However, this kind of active packaging has not been widespread in the market. Common used integrated in plastics is for example silver due to the temperature and mechanical stability (Kumar and Münstedt 2005; Simon et al. 2008; de Azeredo 2013; Lavoine 2015).

Several studies and these investigations (chapter 2, 3, 4) had shown, that the antimicrobial action of different agents is often influenced by a diversity of product-, process and environmental factors. This fact makes the predictions of the precise antimicrobial activity in certain practical fields of application to a very complex challenge. While antimicrobials are active against different microorganisms under lab conditions, in most cases it is difficult to achieve sufficient antimicrobial activity in contact with perishable foods under typical conditions of cold supply chains (Balasubramanian et al. 2009). The common test methods for testing antimicrobial activity of materials, like Japanese Industrial Standard (JIS) Z 2801:2000 or ISO 22196:2007, allow a general estimation of the activity under high temperature conditions. The level of antimicrobial activity against the standard test organisms *Staph. aureus* and *E. coli* at 35 °C in saline solution is determined by these methods. But typical influence factors which are relevant for applications in the food industry, such as packaging of food, are not considered in the standards. However, for the implementation of antimicrobial materials in the food industry, for example as packaging material of perishable foods, typical conditions during processing, transport and storage of perishable food supply chains, have to be considered. As shown in studies with the contact-active killing SAM® polymer poly(TBAMS) important aspects for the investigation of antimicrobial polymers are: relevant organisms for the field of application, temperature and time conditions, and food components (chapter 2, 3, 4; Dohlen et al. 2016).

However, no standardized evaluation scheme is currently available that considers the mentioned factors and delivers meaningful information about the level of antimicrobial activity of active packaging materials for certain fields of application and the effect on microbial shelf life.

Thus, the aim of the study is to develop a comprehensive evaluation scheme to assess the potential of active materials acting as packaging to increasing the safety and shelf life of perishable products in a standardized way. The evaluation scheme should be developed based on the experiments conducted with the different poly(TBAMS) materials and literature results.

5.2 Method

An evaluation scheme was developed for the assessment of the capacity of active materials to increase the safety and shelf life of different perishable foods. The following requirements should be met to establish a standardized evaluation scheme for the industry and the research sector:

- Consideration of important influence factors which are relevant for the application in the food industry
- Integration of a minimum number of parameters, which allow a meaningful estimation for the use of the material in a certain field of application
- Expression of results so that there is a comparability to the Japanese Industrial Standard Z 2801:2000 and ISO 22096:2007
- Easy to use and simple to follow structure
- Identification of a product and of alternative application areas

To achieve these requirements, it was necessary to select the most meaningful and relevant parameters and their attributes for the integration in the scheme. In the previous experiments (chapter 2, 3, 4) several different parameters were investigated (figure 1). The conducted studies and data collected from literature formed the basis for the selection of the most relevant parameters und attributes.

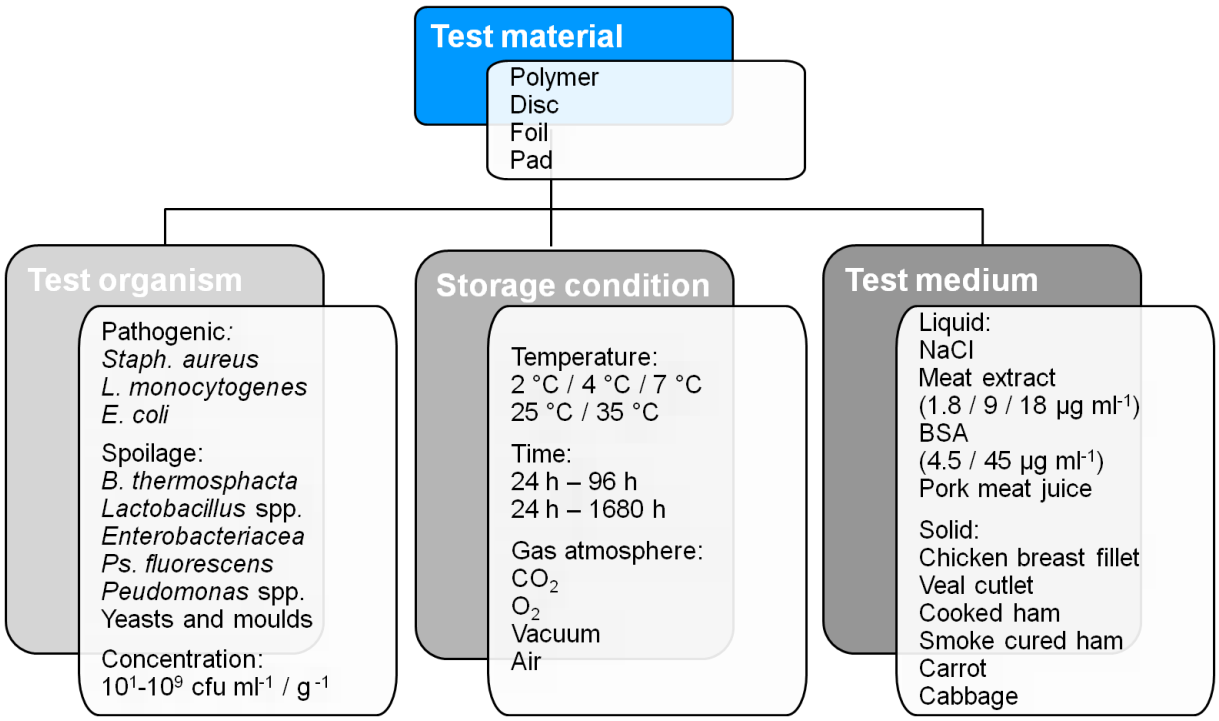

Figure 1 Investigated influence factors on the antimicrobial activity of poly(TBAMS) (chapter 2, 3, 4)

All results were analysed to determine the most relevant influence factors on the antimicrobial activity and the most relevant parameters for the practical application. Simultaneously, a literature study in the fields of antimicrobials, antimicrobial systems and antimicrobial packaging materials was conducted, focusing on the activity influencing factors, the tested organisms, storage conditions and tested mediums.

The test organisms were selected based on their properties, sensitivity (criteria: pathogenic and spoilage, criteria: gram-behaviour), relevance to perishable products and typical concentration in perishable and refrigerated foods. The environmental conditions were chosen based on the application-oriented time and temperature profile for perishable food chains. The test medium was chosen to achieve an adequate standardized medium including several important food components, which are relevant for certain perishable products.

To facilitate comparability of the antimicrobial activity with the existing standards, the general investigation methods were adapted to the JIS Z 2801 and ISO 22196. The JIS is based on a comparison of bacteria counts (*Staph. aureus*, *E. coli*) in saline solution on reference and sample materials after a defined storage temperature and time (35 °C, 24 h). A minimum of three test samples and six reference samples should be used for each experiment.

The expression of the results as a $\log_{10}$-reduction value was also adapted to the common standards.

To assess the antimicrobial activity as a function of the different defined parameters, the log_{10}-reduction was used in the evaluation scheme, like in the JIS:

$$\log_{10}\text{-reduction} = \log_{10} (T_{xRe} / T_{xSa})$$

where $T_{x,Re}$ = bacterial concentration on reference material, after x hours storage
and $T_{x,Sa}$ = bacterial concentration on sample material, after x hours storage

Different log_{10}-reduction values were defined to evaluate the potential of the antimicrobial polymer for certain fields of application and for increasing the shelf life of perishable products.

5.3 Results

The evaluation scheme, based on the conducted experiments and literature data collection, developed for the assessment of the potential of active materials to increase the safety and shelf life of perishable foods is shown in figure 2.

The general scheme is divided in three parts for testing: 1. Antimicrobial activity of the polymer for the food industry, 2. Antimicrobial activity of polymers for packaging of perishable products, 3. Antimicrobial potential of packaging to increase the safety and shelf life of certain products.

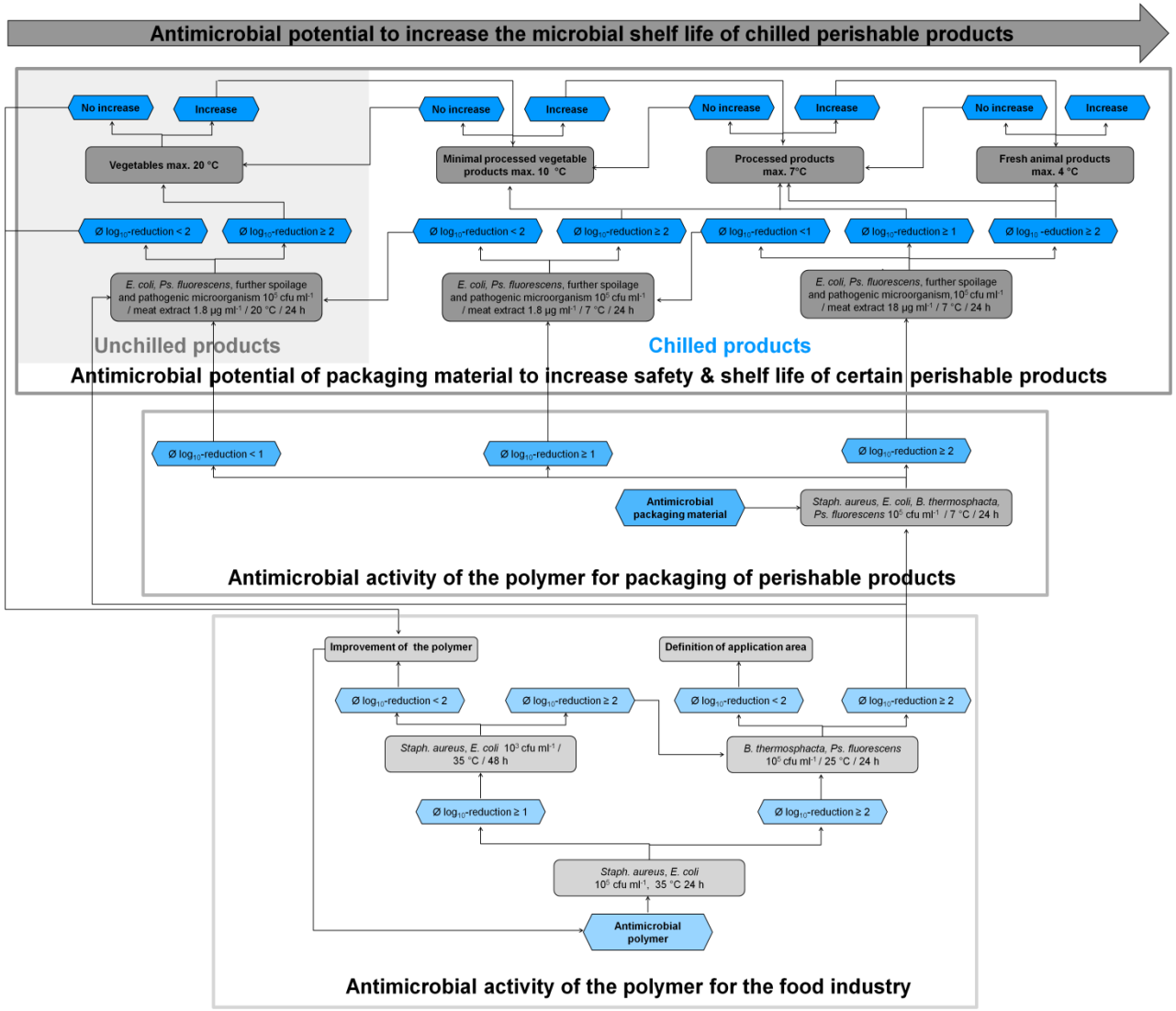

Figure 2 Evaluation scheme for the assessment of the antimicrobial potential of active materials to increase the safety and shelf life of perishable products. Experiment Result

In the first part of the scheme, the usability of an antimicrobial polymer in the food industry is investigated. The scheme starts with the test method JIS Z 2801:2000 for analysing the general antimicrobial activity of materials against *Staph. aureus* and *E. coli* at 35 °C for 24 h in pure culture. Similar to the JIS Z 2801:2000, the polymer has to reach a log_{10}-reduction Ø ≥ 2.0 log_{10} units to count as an antimicrobial polymer. Log_{10}-reductions ≥ 1.0 log_{10} units result in a further experiment with a lower bacterial concentration of 10^3- 10^4 log_{10} cfu ml^{-1} and a prolongation of the storage time to 48 h. If a log_{10}-reduction Ø ≥ 2 log_{10} units is reached, the next step will test the general antimicrobial activity against two typical spoilage organisms. Based on the mentioned criteria, *B. thermosphact*a and *Ps. fluorescens* were selected as test organisms. A log_{10}-reduction Ø ≥ 2 log_{10} units allows for progression to the next step or, if the application is defined as a packaging material for vegetables stored at room temperature, to skip directly to part 3.

In the second step of the scheme, the usability of the material for perishable food supply chains and as a packaging material will be tested. If a packaging material containing an antimicrobial polymer, which was count as antimicrobial effective in previous experiments, was developed, the activity of the new material can be directly proven in the second step. Again, two spoilage and two pathogenic bacteria will be used as test organisms. The test will be conducted at a constant temperature of 7 °C for 24 h. If the results show an arithmetic mean of a log_{10} reduction Ø ≥ 1 log_{10} units, the usability as a packaging material for chilled products will be proven, allowing progression to the next trial. Otherwise, the material can be tested for the application as a packaging material for fruits and vegetables stored under higher temperature conditions.

In the following step the influence of product factors will be analysed. In case the Ø log_{10}-reduction is ≥ 2 $log_{10,}$ highly concentrated meat extract solution (18 µg ml^{-1}) is chosen as the reference media to simulate nutrition-rich fresh animal products. In previous studies, a negative influence of meat extract solution on antimicrobial activity has been described. The effect on activity of meat extract of 18 µg ml^{-1} is comparable to pork meat juice. The trials will be conducted at 7 °C with *Ps. fluorescens*, *E. coli* and one further product specific spoilage organism as well as one further product specific pathogenic bacteria. The organisms should be selected based on the planned field of application. Different microorganisms which can be tested for different products of animal or plant orgin are listed in the previous chapters and by Kreyenschmidt and Ibald (2012). If the application is unclear, the organisms *Staph. aureus* and *B. thermosphacta* can be used again as reference microorganisms for different perishable foods.

In case the log_{10}-reduction of the previous test is ≥ 1 log_{10} unit, a similar test to the described ones will be conducted, but with meat extract solution in a concentration of 1.8 µg ml^{-1}. Such a test delivers information about the potential of the material to act as packaging material for minimally processed vegetables. Furthermore, with a Ø log_{10}-reduction < 1, tests can be performed with higher temperatures to get information about a possible application as packaging for unrefrigerated vegetables foods.

For a defined application of a material as packaging for a liquid food, for example juice or milk, the food itself could be used as the media for the tests. With semisolid products, for example cream cheese, 1 g of the food can be mixed with 10 ml of bacteria (10^5 cfu ml^{-1}) in saline solution. The tests should also be performed with the bacteria *E. coli*, *Ps. fluorescens* and with one further gram-positive pathogenic and spoilage organism depending on the specific products (chapter 2; 3; 4, Kreyenschmidt and Ibald 2012).

All materials that show a calculated arithmetic mean of a Ø log_{10}-reduction ≥ 1.0 log_{10} units after 24 h in the presence of the highly concentrated meat extract solution or food components of animal origin are considered as effective antimicrobial. The results provide information about the antimicrobial potential of the material to increase the safety and shelf life of different perishable products.

If a material achieved a Ø log_{10}-reduction ≥ 2.0 log_{10} units in contact with highly concentrated meat extract, it has a high potential as a packaging material to increase the safety and shelf life of fresh meat and fish, such as beef, poultry, or salmon and for processed products like ham or dairy products and RTE vegetable products. By a determined Ø log_{10}-reduction between 1.0 and 1.9 log_{10} units, the material shows a potential for processed products. Otherwise experiments can be performed with the weakly concentrated meat extract solution. In experiments with food of vegetable origin or with weakly concentrated meat extract solution, the log_{10}-reduction should reach Ø ≥ 2.0 log_{10} units. This indicates a potential to increase the microbial shelf life of processed products and RTE vegetable products. By Ø log_{10}-reductions < 2 of the polymer, the previous tests can be performed with higher temperatures to get information for a possible application as packaging for vegetable foods which are not stored at low temperature conditions.

Last in the evaluation scheme, the prolonging effect of an antimicrobial packaging, such as foil or pad, on the safety and shelf life of certain products will be analysed in storage tests with different perishable products. Product specific storage tests will be conducted to determine the effect on microbial growth, sensory quality and chemical parameters as well as on microbial shelf life. In the trials, the products will be packaged in the novel material and in

the commonly used material. Samples will be stored until the end of shelf life under the typical temperature profile. During storage, the TVC and product specific spoilage and pathogenic bacteria will be analysed. Besides the microbial investigations, the determination of sensory and chemical parameters is necessary to ensure that the material has no negative effect on the perishable product. Investigations will be performed directly after packaging and a minimum of four further investigation points must be conducted over the entire storage time. If a material increases the shelf life of fresh foods from animal origin, it can be assumed that an increased shelf life can also be identified in the other perishable products. If an increased shelf life is not achieved in fresh animal products, the material can be tested with processed products, followed by RTE vegetable products. This process can be performed with the other products.

5.4 Discussion

The developed evaluation scheme allows a standardized assessment of antimicrobial materials regarding their usability as packaging material for certain perishable products. Due to the inclusion of product-, environmental and microbial factors in the scheme, much more information is delivered in comparison to common test procedures. Relevant influence factors on the antimicrobial activity can be identified based on previous experiments and the literature. The most important ones are integrated in this easy to use evaluation scheme.

In the literature and in the previous investigations, it was shown that the sensitivity of each microorganism has an important contribution on the rate of antimicrobial activity. The gram-positive bacteria are often more sensitive than the gram-negative bacteria, such as Enterobacteriaceae and *Pseudomonas* spp.. Differences in the level of antimicrobial activity can be explained by the different structures of the cell membrane/wall and the negative charges of bacteria, which result varyingly electrostatic interactions with positively charged surfaces such as poly(TBAMS) (chapter 2; Dohlen et al. 2016). This is also described in different studies with high molecular weight chitosan (No et al. 2002; Fernandes et al. 2008; Fernandez-Saiz et al. 2009). In contrast, gram-negative bacteria are much more sensitive than gram-positive bacteria to low molecular weight additives, such as silver ions or nano silver particles or low molecular weight chitosan (Shrivastava et al. 2007; Kampmann et al. 2008; Fernandez-Saiz et al. 2009). Since several gram-positive and gram-negative spoilage and pathogenic bacteria are relevant on different perishable foods, it is important to include these different kinds of bacteria in the test conditions.

Furthermore, environmental factors such as temperature and time have a strong impact on the level of activity. Under low temperature conditions, a retarded effect on the activity of poly(TBAMS) can be observed (chapter 2; Dohlen et al. 2016). Tsai and Su (1999) detected a reduction of antimicrobial activity of chitosan with decreasing the temperature. An explanation could be the lower negative charge of bacteria when decreasing the temperature (chapter 2). The antibacterial action of different release agents is also reduced at cool temperatures, caused by a lower release rate (MacKeen et al. 1987; Russel and Hugo 1994; Kampmann et al. 2008; Simon et al. 2008; Asharani et al. 2009; Lee et al. 2011; Cushen et al. 2013). Temperature conditions in perishable food range normally between -1 and 10 °C. A reference temperature of 7 °C was chosen for the scheme, since the statutory temperature of several products is 7 °C (EC No 853/2004).

Besides these influence factors, food components, especially proteins, can strongly inhibit the bactericidal effectiveness of different agents, which is also detected in SAM® polymers (Dohlen et al. 2016; Ignatova et al. 2009) and chitosan (Devlieghere et al. 2004; Aider 2010; Park et al. 2010). The negatively charged food substances can bind on the polymer surfaces and thus inhibit the interactions between bacteria and polymers. The presence of proteins also affects the antimicrobial activity of silver negatively (Liau et al. 1997; Matsumura et al. 2003; Asharani et al. 2009; Ilg and Kreyenschmidt 2011; Martínez-Abad et al. 2012). In the different studies, several kinds of proteins have been tested. Due to the variety of proteins and their different characteristics and concentrations, the described activity of the material is most often not comparable to other published data in the literature. Additionally, experiments with one single protein are often not meaningful regarding to the application for perishable products. Meat extract is a standardized synthetic media, which contains several food components and results can be transferable to several different food products.

The integration of these influence factors allows a rapid identification of the usability of a material in different fields of application.

The storage tests conducted with fresh meat, meat products and RTE vegetables (chapter 4) illustrated that further product factors, such as the availability and content of nutrients, pH-value, water activity and the composition and interaction of bacteria in the product can affect the activity of the material. In literature also reported that product tests, especially with fresh meat, resulted often in lower level of activities than was detected in previous lab experiments. Therefore, standardized laboratory tests as described in the scheme (step 1 and step 2, first part of part 3) deliver important information about the activity for certain fields of application,

but storage tests are still necessary to assess the antimicrobial potential of the material to increase the microbial shelf life and safety of perishable products.

The evaluation scheme is an application-oriented method for use during the development of antimicrobial polymers. It supports researchers and companies in the development or improvement of materials in a structured way, based on an assessment of the antimicrobial potential to increase the safety and shelf life of products. The flexibility of the scheme allows the addition of information which may be useful for the decision of the field of application.

As shown by the previous development of the different materials containing poly(TBAMS), results can be compared via the scheme and therefore, a quick identification of a suitable material can be made. The scheme could contribute to decreasing the product development time and thus, start-up and engineering costs.

5.5 Conclusion

The limited number of selected parameters and attributes in the scheme represents a valid measurement of the influence factors on the antimicrobial activity of a material. Therefore, with the development of a material, the main application-oriented influence factors can be recognized in a very early stage. Through the scheme, the antimicrobial polymer development and the improvement to a packaging material can be outlined step-by-step. This scheme is intended to be used as a basis to determine effective or ineffective materials during development and improvement.

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6 Development of an approach to evaluate the contribution of active packaging solutions on the resource efficiency of food production

6.1 Introduction

During the development of an antimicrobial packaging material, not only the assessment of the materials ability to increase the safety and shelf life of perishable products, but also the costs and benefits of the packaging material in improving the resource efficiency of food production have to be considered. The novel contact-active killing polymer poly(TBAMS), which can be incorporated into different kinds of packaging solutions, has a potential to increase the safety and the shelf life of perishable foods (chapter 2, 3, 4, 5). As described in several studies, the application of innovative active packaging strategies can deliver a contribution in reducing food waste and ensuring a resource-efficient food production by increasing food quality, safety and ultimately shelf life (Verghese et al. 2013; SusFoFlex 2015; Dohlen and Kreyenschmidt 2016a; Dohlen and Kreyenschmidt 2016b). Especially perishable products with short shelf lives, and thus short sellable periods, are often discarded prior to sale or consumption. There are several different causes for product waste, such as improper handling or premature spoilage before the use-by-date is reached. Several products are also thrown away because they are not sold during the short sellable period, which means the length of the products' shelf life has a significant impact on the amount of food waste (Kreyenschmidt et al. 2013; Mena et al. 2014). Per different studies, between 9 and 22 % of meat and meat products are wasted in industrial countries over the chain (Gustavsson et al. 2011; Gunders 2012; Noleppa and von Witzke 2012; Roissant and Kreyenschmidt 2014). Reducing the high amount of waste in perishable food chains is a challenge for food processing and logistics companies, as well as wholesalers and retailers. In meat supply chains, the amount of food waste increases with each link in the chain until the consumer stage (Gustavsson et al. 2011; Gunders 2012; Noleppa and von Witzke 2012; Roissant and Kreyenschmidt 2014). Wasted products in meat chains represent not only the loss of individual products and revenue, but also all the primary energy resources, such as water and feed for breeding and fattening, as well as energy, water and packaging material for meat processing (Gustavsson et al. 2011; Beretta et al. 2013; Roissant and Kreyenschmidt 2014; Noleppa and Cartsburg 2015). However, data are missing which describe the impact of the implementation of novel active packaging materials in a resource-efficient food production based on the costs and benefits, including the reduction of waste in the different links in the chain.

Thus, the aim of the study is to develop an approach to determine the impact of the implementation of active packaging materials on resource-efficient food production. Therefore, a cost-benefit analysis, including a possible reduction of food waste by active

packaging, is established based on the experiments, literature data and expert interviews. An exemplary cost-benefit analysis is performed for the novel poly(TBAMS) foils and pads.

6.2 Method

The cost-benefit analysis, including the definition of the food waste scenario by the application of active packaging, was exemplified carried out for a German poultry supply chain. The method was adapted to the one described by Rossaint and Kreyenschmidt (2014) for intelligent packaging.

In the first instance a large group of articles from peer-reviewed journals were analysed. The papers were identified through computer aided searches of databases of published works and proceedings of conferences. One special focus was the average amount of food waste in each link of the meat chain, the environmental impact of food waste, the main reasons why meat and meat products are thrown away and the impact of active packaging application on the reduction of food waste in all steps of the chain, since this is a key factor for the economic impact on active packaging application. In the frame of the Safe-Pack project (313-06.01-28-1-68.034-10), stakeholder interviews with meat and packaging companies were performed to verify the data and the main reasons why several of the products are thrown away in German meat chains. Data from previous studies done by the CCM Group (University of Bonn) were also used to define the waste scenario (Roissant and Kreyenschmidt 2014).

The definition of the economic and environmental impact of active packaging application depends on different factors, like the length of shelf life of the product, the transport routes and time, the characteristics of the novel packaging material and its effect on shelf life. To represent the economic and resource-efficient impact of active packaging material a specific German poultry supply chain was selected. For this characteristical poultry chain, the percentage of food waste in each link in this typical chain and the reduction potential of novel materials were determined. All calculations were based on the sales volume of 310.843 t/a of fresh poultry meat by German retailers for the private consumer in 2015 (MEG 2016). The sales volume was used to calculate the total amount of food waste (t/a) in the different links in the chain and the reduction of food waste due to the general implementation of the active packaging. For the calculation of the number of animals that are slaughtered, an average weight of chicken breast and boned leg meat of 1.1 kg per animal was assumed per the poultry producer.

The environmental and resource impact categories were focused on the carbon dioxide equivalent and feed and water consumption per animal. The focus was laid on environmental indicators during breeding and fatting, the largest source of emissions along the supply chain. For the breeding of the animals, the amounts of feed (13 kg), water (28 l) consumption and CO_2 (2 kg) emission were chosen per Staudt (2007) and Heissenhuber (2008).

The reduction of food waste by a prolongation of shelf life due to the implementation of an active packaging material was defined for different prolongation times. The definition was based on literature data, interviews and the antimicrobial potential to increase the shelf life of fresh poultry meat of packaging materials containing poly(TBAMS).

All benefits and costs from production to consumption associated with the active packaging implementation were collected and analysed for the cost-benefit analysis. Interviewees from the meat, chemical and packaging industries in the framework of the Safe-Pack project were asked to list all process changes which occur due to the implementation and application of active packaging materials such as multilayer foils and pads containing poly(TBAMS). The process changes were defined for each step of the food chain from production to consumption, the changes were also defined by the packaging and chemical industry. The corresponding costs and benefits these are caused by the effects were defined. All cost and benefits were devided in direct and indirect measurable values.

Based on the data, an Excel Tool, which integrated food waste and waste reduction and the reduction of animals and environmental factors as well as the associated direct and indirect cost and benefit values by the implementation of active packaging solutions, was developed for predicting the resource-efficient impact.

The cost-benefit analysis was exemplified for the implementation of multilayer foils and meltblown pads containing poly(TBAMS). Based on the pilot plant scales, the current costs for foils and pads containing poly(TBAMS) were calculated with support of the packaging- and chemical companies. The average price of fresh poultry (5.99 Euro/kg) was delivered by a poultry producer. Participating companies from the food, packaging and chemical sector were asked to fill out the monetary value of each relevant position (costs/benefits) and not measurable benefit for the implementation of poly(TBAMS) based packaging solutions.

6.3 Results

Based on the collected data in the literature, and in agreement with the interviewed stakeholders, the amount of waste for each step of German poultry supply chain was defined. The total amount of food loss and waste over the entire chain is 15.5 %. The highest level of

wasted meat (6 %) occurs in the consumer´s step, followed by the retailers (5 %). The calculated amount of food loss and waste (15.5 %) based on the amount of packaged poultry breast and boned leg meat are shown in table 1. This percentage equates to a total amount of food loss and waste of 50 million kg/a of fresh poultry in the chain and results in monetary losses of around 300 Million €/a. Around 97 million packages/year are necessary to pack the fresh poultry which never is consumed. For the wasted production, approximately 45.617.586 animals must be bred and fattened without being consumed, which is illustrated in table 1.

Table 1 Food loss and waste in the German poultry supply chain

Poultry supply chain	Processing	Transport	Wholesaler	Transport	Retailer	Consumer	Total
Food waste [%]	0.5	1	2	1	5	6	15.5
Meat [t]	1.712	3.407	6.745	3.305	16.360	18.651	50.179
Animals	1.556.235	3.096.908	6.131.878	3.004.620	14.872.871	16.955.073	45.617.586

For the definition of the economic and resource-efficient impact of the active packaging application, the amount of food waste that can be reduced by the implementation of an active material is of vital importance. To calculate the realistic amount of food waste reduction in a certain supply chain, the main causes for wasting products and the relationship between prolonging the shelf life and waste reduction must be identified.

Together with the food production company and the retailer in the German poultry supply chain, a reduction rate of 15 % was determined when prolonging the shelf life by two days compared with currently used MAP or vacuum packaging. Reduction rates were also determined when prolonging the shelf life one day (7.5 %) and three days (22.5 %). The developed Excel tool automatically calculates the sum of the reduction of food waste due to the implementation of active packaging, based on the shelf life prolongation, in the form of meat/t.

Figure 1 shows the whole amount of waste and the reduction of waste when prolonging the shelf life two days (left side) and three days (right side) due to active packaging for every link in the chain.

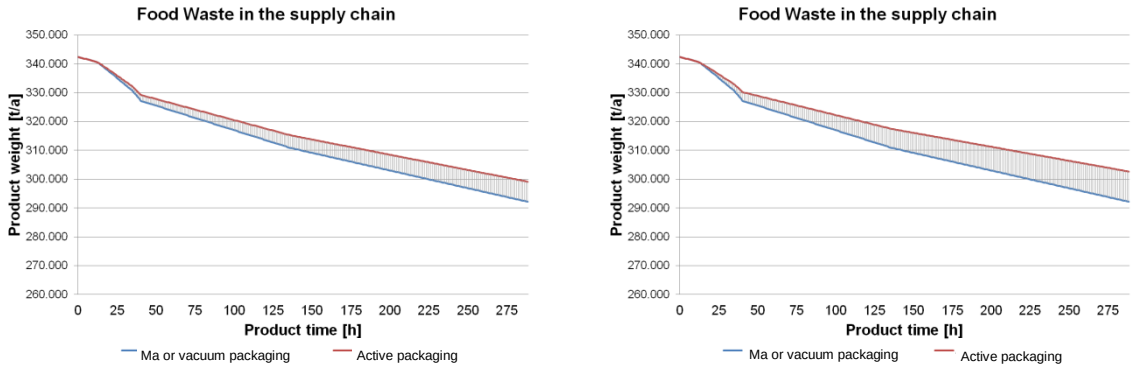

Figure 1 Food waste of fresh poultry meat in the German supply chain and the reduction of food waste by application of active packaging and an increased shelf life of two days (left side) and three days (right side)

The tool can be flexibly adapted to different assumptions of food waste reduction calculations. Taking the assumption that 15.5 % of fresh meat is wasted in the poultry chain, and the amount can be reduced by 15 % due to prolonging the shelf life by two days through the active packaging implementation, this equates to 6.941 t/a (41.576.735 €/a) fresh poultry meat saved in the entire supply chain. The most waste can be saved at the consumer stage (2.575 t/a). The total amount of reduced food waste corresponds to around 6.310.022 animals, which can be saved. Based on the amount of poultry meat and animals, the tool calculates the reduction in consumed environmental resources. Owing to the reduction of animal breeding, the saved animals mean that the amount of feed and water can be reduced by 82.030 t/a (feed) and by 176.681 m^3 (water) respectively, and the carbon dioxide emissions can be reduced by 12.620 t/a for the chosen scenario. Furthermore, the production and disposal of 13 million packages can be saved.

For the costs and benefits of active packaging implementation, the effects on the different processes were defined. Several selected effects of the active packaging implementation on the processes of every stage in the chain are shown in table 2, additional changes are integrated in the Excel tool.

Table 2 Effect of an active packaging implementation on the processes for the different links in the chain

Link in the chain	**Effect on the process**
Packaging Industry	Changes in production process
	Changes in material usage
	Changes in storage system
	Changes in reclamations
	Changes in market distribution
Processing company	Changes in packaging process
	Changes in packaging usage
	Changes in reclamations
	Changes in total market sales
	Changes in market distribution
	Changes in product price
	Changes in product quality, safety and shelf life
	Changes in wasted products
Transport	Changes in transport system
Wholesaler	Changes in product storage system
	Changes in product quality, safety and shelf life
Retailer	Changes in product storage system
	Changes in reclamations
	Changes in market distribution
	Changes in product quality, safety and remaining shelf life
	Changes in sales
	Changes in product price
	Changes in disposal
Consumer	Changes in product price
	Changes in purchasing and handling of the product
	Changes in product quality, safety and remaining shelf life
	Changes in satisfaction
	Changes in recycling
Packaging disposal	Changes in recycling
	Changes in disposal

The associated costs and benefits of the process changes are determined in the following. Changes in the packaging production process for example, can result in additional cost for the material, an increase in investment cost for new equipment, an increase in production time, and an increase in cleaning costs, as well as additional staff costs. Changes in the packaging process at the processing step can lead to additional staff and equipment costs. Changes to the shelf life in the form of an increase can reduce food waste and reclamation costs for the processers and retailers and prices for the consumers. The food waste reduction is listed as a direct, measurable benefit. In the tool, the costs and the calculated monetary benefit of the novel active packaging compared with the currently used packaging are automatically summed up for the specific enterprise. For the ‘indirectly measurable benefits’, the tool includes expected non-quantitative criteria and provides the possibility for every enterprice in

the chain to take different parameters into account by weighting them, ascending from 1 (unimportant) to 5 (very important), depending on the relevance. The indirectly measurable benefits are, beside the improvement of quality and increasing safety and shelf life, customer satisfaction and loyalty as well as an improved public image with special regard to sustainability. Figure 2 illustrates the screenshot of the Excel tool of the determined cost and benefits for meat processing plants.

By application and implementation of different active packagings, the effects on the processes in the steps of chain and therefore the costs and benefits are different. A developed Excel tool demonstrates the overall concepts in the different stages. The tool is generated of several different sheets, including one for every link. The Excel tool can be flexibly used for each participant in the chain to make their own calculation of its specific costs and benefits from the implementation of different active packagings.

Implementation of a multilayer foil and pad containing poly(TBAMS)			
Supply Chain: Fresh poultry for German retailer (Processing step)			
Cost -benefits analysis for the year:			
		Old technology	New technology
	Product: Fresh poultry	**Cost per unit: old packaging**	**Cost per unit: new packaging**
I. Direct costs			
1.1	**One-off costs of procurement**		
1.1.1	Equipment costs		
1.1.2	Staff costs (employee training)		
1.1.3	Marketing campaign		
1.1.4	Lead management		
1.2	**Packaging material**		
1.2.1	Multilayer foil		
1.2.2	Pad		
	Packaging (foil and pad)		
	Amount of direct costs (€) for packaging VE		
	Subtotal €		
II.	**Indirect costs and general investment**		
2.1	**Process adjustment**		
2.1.1	Packaging per minute		
2.1.2	Upkeep, maintenance and cleaning		
3.1.3	Depreciation		
2.1.4	Energy costs		
2.1.5	Other indirect costs (rent, insurance...)		
2.2	**Complaint costs**		
2.2.1	Food quality		
2.2.2	Packaging quality		
	Total indirect costs (€)		
	Total indirect sum for packages (€)		
	Total costs (€)		
III. Directly measurable benefits			
3.1	**Development of new markets**		
3.1.1	New customers		
3.1.2	Development of new market sectors		
3.1.3.	Market advantage (sole supplier)		
3.1.4	Sales increase		
3.2	**Food waste**		
3.2.1	Reduced food loss (€)		
3.2.2	Sales increases		
3.2.3	Storage costs		
3.2.4	Disposal costs		
3.3	**Increase of the sale window**		
	Total directly measurable benefit (€):		
	Subtotal of benefit (€):		
IV. Indirectly measurable benefits			
	1 = unimportant 5 = very important	**Weighting (1-5)**	**Weighting (1-5)**
4	**Image**		
4.1	Improved customer-supplier relationship		
4.2	Improved customer satisfaction		
4.3	Improved quality and safety		
4.4	Higher protection against product return		
4.5	Improved image due to sustainability		
Total non-directly measurable benefits			
	Total cost-benefit		

Figure 2 Exemplified Excel Tool for a meat processing plant

A calculation was conducted, with respect to the calculations of costs and benefits in the exemplary poultry supply chain until the consumer stage, with packaging solutions containing poly(TBAMS).

The direct costs refer to the additional costs for the active foil and the optional soaker pad per package. Per the packaging- and chemical industry, the application of a multilayer foil with 15 % poly(TBAMS) in the inner layer leads to an increase of the common packaging price of around 7 %. Changing the packaging material results in additional costs for the packaging, but not in an increase in investment costs. It has been shown in pilot plant scales at packaging and meat companies and by the interviews, that no new equipment and no additional staff costs for the packaging containing poly(TBAMS) are required. Poly(TBAMS) can be flexibly compounded with different standard polymers, so that it can be produced for different kinds of packaging solutions on standard production machines. The dynamic mechanical and physical properties of these packaging systems are comparable to the commonly used packaging and allow for the application of the packaging machines in meat companies and the same handling in the following chain steps. The reduction of wasted products results in a decrease of costs in the supply chain. Considering the criteria of indirectly measurable benefits, consumer satisfaction is estimated to be the most important criteria for meat producers, with a value of 5.

For the described scenario, owing to different prolongations of the shelf life (1 day, 2 days, or 3 days) by the poly(TBAMS) application, the savings through food waste reduction up until and including the consumer stage are calculated. An average packaging price was used for the calculation. The direct costs given in the sheet refer to the additional costs for a foil containing poly(TBAMS) (0.045 € with 15 % poly(TBAMS) in the inner layer) and a additional soaker pad (0.027 €) per vacuum package of 500 g meat compared with the reference foil (0.042 €; pad 0.025 €). The followed diagram demonstrates the monetary benefit depending on packaging used and reduction of food waste by increasing the shelf life in the German supply chain for fresh poultry.

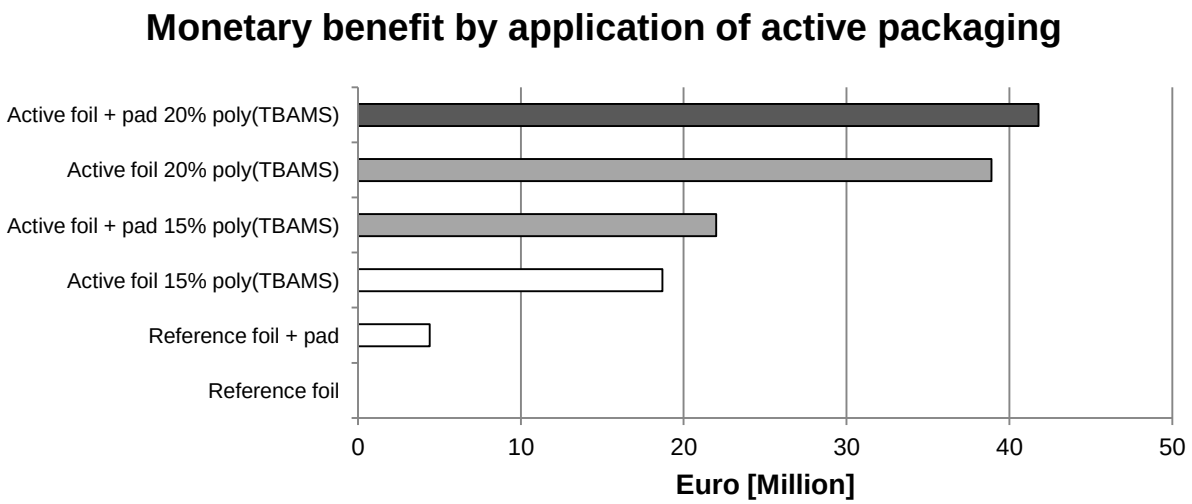

Figure 3 Monetary benefit in the German poultry supply chain based on a reduction of food waste by application of active packaging with increased shelf life of: (▭) 1 day, (▬) 2 days or (▬) 3 days

As shown by the results in chapter 4 with the tested material it is up to now not possible to increase the shelf life of poultry for three days. In the future, to increase the shelf life of poultry by several days, possible approaches to optimizing the material through an increase of the availability of active groups on the polymer surface have to be developed. Furthermore, it can be assumed that by the implementation of poly(TBAMS) containing packaging materials, the price of the polymer could be reduced around 50 % due to the higher production volume.

6.4 Discussion

It has been shown in this work, that the reduction of food waste in the poultry supply chain by prolonging the shelf life delivers an important contribution to an enhanced resource-efficient food production.

Accordingly, this contribution of active packaging strategies to a resource-efficient food production through food waste reduction is described in several papers (Kreyenschmidt et al. 2013; Verghese et al. 2013; SusFoFlex 2015; Dohlen and Kreyenschmidt 2016a; Dohlen and Kreyenschmidt 2016b). However, the results of different studies in the field of innovative packaging strategies are often not transferable due to differences in product and chain specific factors, economic aspects as well as cultural considerations. In this study, a 15% waste reduction, due to an increased shelf life of 2 days by application of active packaging, of fresh poultry sold in German retailer shops means a breeding reduction of 6.310.022 animals per year. Meat and meat products cause the greatest environmental impact within the food sector (Tukker et al. 2006). In this study, 12.620 t carbon dioxide emission, 82.030 t feed and

176.681 m^3 of water could be saved during broiler chicken breeding alone. The environmental impact during the breeding varies for example for the different countries and regions as well as between the conventional and free range breeding (Leinonen et al. 2012; Prudêncio da Silva 2014; Wiedemann et al. 2016). Several factors like energy savings and the impact on water and land use by reducing the cultivation of feed plants and the breeding of animals are not considered. Taking the assumption that the feed includes 25 % wheat, this corresponds to a used volume of around 15 million m^3 water and to an area of 2.400 ha per year of wheat. Furthermore, these factors can result in environmental aspects such as acidification, eutrophication, terrestrial ecotoxicity and cumulative energy (Prudêncio da Silva et al. 2014). However, the savings of environmental factors during breeding and fattening are only a small part of the potential savings during the whole chain. The later the product in the chain is wasted, the higher is the environmental impact. For example, the CO_2 emission increases from breeding until the retailer to more than 3 kg CO_2 per kg poultry meat. Furthermore, others factors such as the energy are not considered. Therefore, the savings of environmental resources and thus environmental and economic benefits by preventing food waste are larger than described.

The results of the study demonstrate that for small, medium and large enterprises producer, the benefits due to the savings from food waste reduction are huge, even if only the sales of fresh meat by the retailers for private consumers are considered. The amount of reduced food waste due to an active packaging application can vary in different chains since it depends on several factors, such as: the structure of the chain, slaughter capacity, product characteristics, the causes for food waste in the different food chains and the active packaging itself. Since the data refer to the national poultry chain in Germany, the data for further perishable foods and for global supply chains are often much higher. Due to continuing globalisation, food chains become more and more complex and transport routes longer. Therefore, the remaining shelf life and selling times of perishable products are of short duration, whereby the amount of food waste often increases (Verghese et al. 2013; Roissant and Kreyenschmidt 2014).

Nevertheless, antimicrobial packaging is not widely used in the market. One reason is the gap between research in the area of novel packagings and practical development. As discovered in the interviews, there is interest from the chemical-, packaging and meat industries and retailer to implement antimicrobial packaging material to prolong shelf life, but the retailers are also sceptical due to the lack of cost-benefit analysis and the declaration of the active agents, especially the agents based on the release mechanism. Concerning this

matter, for the implementation of an active material as food packaging, a key factor is the approval and legislative conditions in different countries, including aspects such as the legitimate maximal migration and release rate and toxicity level, which are defined by the regulatory authorities and agencies of the different countries. For example, European Commission Regulation EC No 450/2009 was developed as a legal basis to tackle the correct use, safety and marketing of active food packaging applications. For agents based on the biocide-releasing killing mechanism different threshold limit levels exist regarding the release into food and the environment. Therefore, despite of the intensive research and development work on packaging materials included antimicrobials, in Europe its use is not widespread in the market.

Furthermore, the current discussion in the field of packaging and the contribution to a resource-efficient food production is sometimes contradictory. Packaging has been accepted as essential for perishable foods due to its important role in maintaining quality and safety for the consumer. A public discussion has begun over the abundance of packaging material, the use of natural resources in their production and the emission of carbon dioxide when disposing of these materials. For example, more than 600 Million packages per year are needed for fresh poultry, which are sold in German retail shops. To reduce the amount of natural resources for the production and the high volumes of greenhouse gases for the disposal of these materials, bio plastic strategies have been developed over the last years. Bio plastics, respectively, bio polymers mean they are fully or partly produced from natural or organic ingredients, biodegradable, or both such as starch blends or PLA (Markarian 2008; Vieira et al. 2011; Imre and Pukánszky 2013). However, the properties of the materials like gas barriers or mechanical stability, which are necessary to ensure quality, safety and long shelf life times of perishable foods such as fresh meat products, are often not comparable to synthetic not biodegradable packaging materials. In the future, a new approach could be to combined the polymer poly(TBAMS) with a bio based packaging material to reduce the use of fossil fuels.

It becomes clear, that it is a challenge to evaluate a novel active packaging regarding to their ability to improve the resource efficiency of food production in a standardised way. The antimicrobial potential of a new active material to increase the safety and shelf life of perishable products can be assessed by the developed evaluation scheme (chapter 5). The study represents an approach for the costs and benefits calculation of the economic and environmental impact including the amount of food waste regarding a resource-efficient food production by an implementation of active packaging. However, for the assessment of the

overall contribution of different active packaging strategies to a resource-efficient food production, it is important to consider all environmental aspects (life-cycle assessment and the reduction of food waste, etc.), social aspects (cultural aspects, acceptance of the food industry and consumer, etc.) and economic aspects (costs and legislation, etc.). Thereby, aspects such as product and chain specific requirements and the use of natural resources for the production and recycling of the material have to be considered, too. As such, a close cooperation within the food chain with packaging-, chemical-, transport-, food-, recycling and reuses industries and the consumer is important. Finally, it should be kept in mind that it will never be one single packaging solution for an all chilled product groups which achieves the overall and the highest level in all aspects.

6.5 Conclusion

By using the developed evaluation scheme in chapter 5, the application area can be defined where the active material deliver the highest benefit for a certain field of application. The cost–benefit analysis represents a new approach for the calculation of the economic and resource-efficient impact. Thus, the use of an active packaging solution can deliver an important contribution to a reduction of food waste and thereby to an improvement the resource-efficient food production by increasing the safety and shelf life of perishable foods. However, for assessing the overall contribution of active packaging solutions to a resource-efficient food production several different aspects have to be considered by a close cooperation with all actors in the chain.

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7 Summary

Reducing the high amount of food waste in perishable food chains is a challenge for food processing and logistics companies, as well as wholesalers and retailers. Precisely those products that have short shelf lives due to product characteristics, and thereby shortened sellable periods, are often discarded prior to consumption. Thus, the length of the shelf life of the products has an impact on the amount of food waste.

There is a broad interest in the food industry on an implementation of active packaging materials to prolong the shelf life of perishable products. Inherently antimicrobial polymers, such as the novel SAM® polymer poly(TBAMS), bear a potential to act as active packaging material.

Until now however, the real effect of poly(TBAMS) and of novel active packaging materials on shelf life and on resource-efficient food production, including a reduction of food waste, was unclear.

Thus, the main objective of this thesis was the assessment of novel active packaging materials containing the polymer poly(TBAMS) for application in perishable food supply chains. Furthermore, the potential of poly(TBAMS) and active packaging materials to increase the safety and shelf life of perishable products and thus to improve the resource efficiency of food production, was assessed.

The first question was aimed at the determination of different product-, process- and environmental influence factors on the antimicrobial activity of materials containing poly(TBAMS), focusing on its suitability to be applied as packaging material in perishable food chains. Therefore, the influence of food components, temperature, time and gas atmosphere on the antimicrobial activity was analysed against different spoilage and pathogenic bacteria by using a modified test method based on the Japanese Industrial Standard 2801:2000. Furthermore, scanning electron microscopy, zeta potential and electrophoretic mobility measurements were made of bacteria stored under different temperatures.

Generally, the results showed a high antimicrobial activity of different materials containing the polymer poly(TBAMS) against several spoilage and pathogenic organisms typical for perishable products. Scanning electron microscopy images of *B. thermosphacta* showed a cell membrane rupture leading to an out flowing of the cytoplasm. Overall, gram-positive bacteria were more sensitive to materials containing poly(TBAMS) than the gram-negative bacteria. A higher negatively electrophoretic mobility of *B. thermosphacta* compared with *Ps. fluorescens* became evident, which explained different electrostatic interactions with the positively

charged polymer. It was also shown by the results that environmental factors influence the activity. In the experiments, a retarding effect on the activity against *E. coli* and particularly *Ps. fluorescens* was observed at cool temperatures (2 °C – 7 °C). However, poly(TBAMS) showed significantly activity against all tested bacteria with $\log_{10}$-reductions more than 2.5 $\log_{10}$ units after 24 h. A stronger protonation of the functional amino groups of poly(TBAMS) due to storage under a high concentration of CO_2 (95 %) led to a higher activity at cool temperatures, so that all bacteria were reduced under the detection limit. A reduction of the antimicrobial activity of poly(TBAMS) was determined if different meat components were present. Results showed a negative influence of negatively charged components, for example BSA, on the antimicrobial activity, due to the interaction of these components with the positively charged polymer surface.

For the second research question the effect of different processing steps to produce different packaging materials containing poly(TBAMS) on the antimicrobial activity was assessd. Different polymer discs, fibres, multilayer foils and meltblown pads, were processed from different LLDPE based polymers melded with different concentrations of poly(TBAMS). The antimicrobial activity of all materials was tested against several bacteria with or without food components under different time and temperatures conditions. Besides this, Raman spectroscopy was performed with a multilayer foil containing poly(TBAMS).
The different experiments with the materials containing poly(TBAMS) made obvious that the composition of the material had a strong effect on the activity. The higher the concentration of poly(TBAMS) (1.5, 3, 5, 10, 12, 15 %) that was integrated into the matrix polymer, the stronger the antimicrobial activity was. The multilayer foil containing 15 % poly(TBAMS) in the inner layer showed higher $\log_{10}$-reductions (> 2.5 $\log_{10}$ units) against different bacteria compared with that containing 10 % poly(TBAMS). The miscibility properties between the matrix polymer and poly(TBAMS) also affected the amount and availability of poly(TBAMS) on the polymeric surface and thus the activity. An increased rate of activity around 2 $\log_{10}$ units was achieved by an enlargement of the surface through its spinning into a fibre.

The third question was focused on the determination of the effect on the shelf life of perishable products packaged in packaging solutions containing poly(TBAMS) and on the cost and benefits of these materials. Storage tests with fresh meat, hams and RTE vegetables were performed under typical conditions in cold supply chains. During the storage the effect on the microbial growth, sensory quality and chemical parameters of the products was analysed and assessed. Typical spoilage microorganisms and pathogenic bacteria as well as

yeast and moulds were investigated. Microbial growth was modelled using the modified Gompertz function.

The costs and benefits including a food waste reduction associated with the application of packaging containing poly(TBAMS), were analysed in accordance to expert interviews and literature data. A food waste reduction scenario by using active packaging material was determined for a German poultry supply chain.

The conducted storage tests confirmed that the above-mentioned factors influenced the antimicrobial activity and the effect of poly(TBAMS) on shelf life. The effect of different packaging materials containing poly(TBAMS) on shelf life was different for different kinds of food. Nearly no prolongation in shelf life was achieved in protein rich food, like fresh meat. Whereas the multilayer foil containing 15 % of poly(TBAMS) increased the microbial safety and shelf life of products with low protein contents such as ready to eat vegetables. All bacteria, yeasts and moulds were reduced during storage at 7 °C. However, in fresh meat and meat products an activity was reached against *B. thermosphaca* and *Lactobacillus* spp. during storage. The effect on *Lactobacillus* spp. led to an increased shelf life of hams.

The amount of food waste of 15.5 % was determined for fresh poultry sold in Germany. The reduction of food waste through an active packaging implementation was analysed at 15 % by prolonging the shelf life by two days compared with currently used MAP or vacuum packaging. The total amount of reduced food waste of 6.941 t/a corresponded to approximately 6.310.022 animals, which are bred and fatten without being consumed.

The results from the cost-benefits analysis revealed that the application of the multilayer foil with 15 % poly(TBAMS) in the inner layer led to an increase of the common packaging price of around 7 %. For the described scenario including the consumer stage, the savings owing to the poly(TBAMS) application was calculated for maximal 38 million Euro through food waste reduction by an increased shelf life of 2 days.

The final research question was aimed at the development of a standardized evaluation scheme for the assessment of active packaging material to increase the safety and shelf life of perishable products and of an approach to analyse the cost and benefits by an implementation of active packagings regarding a resource-efficient food production.

For the development of the scheme the main influence factors on antimicrobial activity of poly(TBAMS) and active materials described in the literature were determinate regarding for an application for perishable products.

The mentioned-above cost-benefits analysis including the calculation of food waste reduction was expended for all process changes these occur during the implementation of different active packaging solutions.

The developed standardized evaluation scheme allows the identification of the application area where active materials deliver the highest benefit already during the development phase of active packaging solutions. The cost–benefit analysis represents a new approach for the calculation of the economic and resource-efficient impact over the whole food supply chain by implementation of different active packaging materials. In future, the scheme supports researchers and companies by the development of antimicrobial polymers in an application-oriented structured way. Both approaches deliver a contribution to reduce the time and costs during the development of materials.

The overall results of this thesis showed the complexity of different influence factors on the antimicrobial activity of packaging materials containing poly(TBAMS). These new materials have a high potential to act as packaging material and to increase the safety and shelf life of products, especially with low protein contents such as ready to eat vegetables. In future, to increase the shelf life of protein-rich products, a higher amount of available functional amino groups of poly(TBAMS) on the polymeric surface is important, as it raises the activity against different bacteria under low temperature conditions. Approaches to increase the availability of active groups on the polymer surface, such as increasing the concentration, changing the matrix polymer or the enlargement of the surface area can be possible solutions to optimize the material. Furthermore, the approval and legislative conditions in the different countries are a key factor for the implementation of material containing poly(TBAMS) as food packaging. Further clarification is necessary regarding this issue.

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List of publications

2016

Dohlen, S., Braun, C., Brodkorb, F. Fischer, B., Ilg, Y., Kalbfleisch, K., Kreyenschmidt, M., Lorenz, R., Robers, O., and Kreyenschmidt, J. (2016) Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products. *J Appl Microbiol*, **4**, 1059-1070.

Dohlen, S. and Kreyenschmidt, J. (2016) Sustainable Packaging solution to Improve Cold Chain Management in fresh Meat supply Chains. In: China Cold-chain Logistics Development Report.

Dohlen, S., Albrecht, A., Braun, C., Brodkorb, F. Fischer, B., Ilg, Y., Kalbfleisch, K., Kreyenschmidt, M., Lorenz, R., Robers, O., and Kreyenschmidt, J. (2016**)** Entwicklung eines neuartigen kationischen Polymers zur Verzögerung des Frischeverlustes von leicht verderblichen Lebensmitteln. Oral Presentation. GDL-Fachtagung Fortschritte bei konservierenden und antioxidativ wirkenden Systemen, 14. - 15. Juni 2016, Bonn**,** Germany.

Dohlen, S. and Kreyenschmidt, J. (2016) Packaging strategies for chilled meat products – a contribution to a sustainable food production? Oral Presentation, Proceedings of the 6th Cold Chain Management Conference, 6. -7. Juni 2016, Bonn, Germany. 123-127, ISBN-978-3-9812345-2-7.

Dohlen, S.. (2016) Packaging strategies for chilled meat products – a contribution to a sustainable food production? of the 6th International Conference Cold Chain Management

Dohlen, S. and Kreyenschmidt, J. (2016): Frischeverlust verzögern – Aktive und intelligente Verpackungen reduzieren Ausschüsse in Fleisch erzeugenden Ketten. Fleischwirtschaft 5, 56-70.

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2015

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Braun, C., **Dohlen, S.**, Brodkorb, F., Fischer, B., Ilg, Y., Kalbfleisch, K., Robers. O., Kreyenschmidt, M., Lorenz, R., Kreyenschmidt, J. (2015) Einsatz von Poly(tert.-Butylaminomethylstyrol) zur Verbesserung der Betriebshygiene in lebensmittelverarbeitenden

Unternehmen- Poster Presentation: 11. ThGOT, 15.-16.09.2015, Zeulenroda-Triebes, Germany. Tagungsband: ISBN 978-3-00-046056-2

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Braun C., Baske A., Brodkorb F., **Dohlen S.**, Fischer B., Ilg Y., Kalbfleisch K., Kreyenschmidt M., Lorenz R., Robers O., Witte A.-L., Kreyenschmidt J.(2014): Bewertung des Einsatzes von SAM-Polymeren zur Reduzierung von Kreuzkontaminationen in der Fleischindustrie. Vortag im Rahmen des GDL Kongress Lebensmitteltechnologie 2014, 17.10.2014, Frankfurt-Rodgau

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