Institut für Tierwissenschaften

Intrinsically antimicrobial active polymers to improve the hygienic conditions during processing and preparation of fresh meat

Dissertation
zur
Erlangung des Grades

Doktor der Ingenieurwissenschaften
(Dr.-Ing.)

der
Landwirtschaftlichen Fakultät
der
Rheinischen Friedrich-Wilhelms-Universität Bonn

von

Carina Hüwe

aus
Hemer

Bonn 2018

Referentin:	PD Dr. Judith Kreyenschmidt
1. Koreferentin:	Prof. Dr. Margit Schulze
2. Koreferentin:	Prof. Dr. Brigitte Petersen
Tag der mündlichen Prüfung:	19.10.2018
Erscheinungsjahr:	2018

Angefertigt mit Genehmigung der Landwirtschaftlichen Fakultät der Universität Bonn

Mit dem Wissen wächst der Zweifel.

Johann Wolfgang von Goethe (1749 - 1832)

Abstract

Intrinsically antimicrobial active polymers to improve the hygienic conditions during processing and preparation of fresh meat

The objective of this thesis was the investigation of the potential of intrinsically antimicrobial active polymers to improve the hygienic conditions in all steps of the processing and preparation of fresh meat, from the food industry to the domestic kitchen.

The antimicrobial activity of the homopolymer poly(TBAMS) and the copolymer poly (TBAMS:acrylonitrile) [1:1] was screened against pathogenic and spoilage bacteria present on meat processing and preparation equipment. Further, the influence of conditions typical during the processing and preparation of fresh meat on the activity was analyzed. For these aspects, 837 samples containing poly(TBAMS) and 1587 references were investigated. To analyze the long-term activity 646 samples of three poly(TBAMS) containing materials (poly(TBAMS), poly(TBAMS:acrylonitrile), poly(TBAMS:4-vinylpyridine) were stored under three environmental conditions over a period of three years. The antimicrobial activity was screened at fixed intervals and the long-term activity of the polymers was modelled. Also, the activity of poly(TBAMS) incorporated into LLDPE was determined and compared via antimicrobial screening of two other antimicrobially treated boards (poly(TBAEMA), Microban®). In three different scenarios typical during the preparation of meals in domestic kitchens, the effect on the transfer of pathogens between the cutting boards and food was compared between cutting boards with poly(TBAMS) and untreated material. The ability of the new polymer to reduce the colonization of surfaces was examined via comparing biofilms on 200 samples as well as references. Based on the overall results, the potential of intrinsically antimicrobial active materials to improve the hygienic conditions during the processing and preparation of fresh meat was assessed.

The antimicrobial screenings showed the general potential of poly(TBAMS)-containing materials to improve the hygienic condition in the food chain. Good antimicrobial activity was proven against various bacteria. The activity was decreased marginally over a period of three years. Screenings identified different environmental and processing factors influencing the activity, which was differently pronounced for the individual bacteria strains. Thus, no effect on the activity against *L. monocytogenes* was proven while the effect against *P. fluorescens* was evident. However, a retarding effect, e.g. of lowering temperature, could be counteracted by prolonging the contact time. The LLDPE-board with 10 % poly (TBAMS) showed, in comparison to other antimicrobial treated polymeric boards, the greatest antimicrobial profile. However, the effect on cross-contamination of pathogenic bacteria was limited. Still, the same material suppressed or delayed respectively the formation of biofilms. Considering all results, the application of poly(TBAMS) in food contact materials bears the potential to improve the hygienic conditions during the processing and preparation of fresh meat, especially via affecting the persistence of bacteria on surfaces. For an effective application of poly(TBAMS) as food contact material further developments and legitimate validation is necessary.

Kurzfassung

Intrinsisch antimikrobielle Polymere zur Verbesserung der hygienischen Bedingungen bei der Verarbeitung und Zubereitung von frischem Fleisch

Ziel der Arbeit war es, das Potential intrinsisch antimikrobiell wirksamer Polymere zur Verbesserung der hygienischen Bedingungen in allen Stufen während der Verarbeitungs- und Zubereitungsprozesse in der fleischerzeugenden Kette zu untersuchen.
Die antimikrobielle Aktivität des Homopolymers poly(TBAMS) und des Copolymers poly(TBAMS:Acrylnitril) wurde gegen verschiedene fleischspezifische Bakterien erfasst. Zudem wurde der Einfluss von relevanten Faktoren auf die Wirksamkeit analysiert. Hierfür wurden 837 Proben mit poly(TBAMS) und 1587 Referenzen ohne poly(TBAMS) getestet. Um die Langzeitaktivität zu untersuchen wurden 646 Proben von drei unterschiedliche Materialien (Poly(TBAMS), Poly(TBAMS:Acrylnitril), Poly(TBAMS:4-Vinylpyridin) bei verschiedenen Umweltbedingungen über einen Zeitraum von drei Jahren gelagert. Die Aktivität wurde in regelmäßigen Abständen untersucht und die Langzeitstabilität der Werkstoffe modelliert. In weiteren Untersuchungen wurde die Aktivität von Schneidebrettern mit poly(TBAMS) analysiert und mit der von anderen antimikrobiell Oberflächen (poly (TBAEMA), Microban®) verglichen. In drei typischen Szenarien der häuslichen Zubereitung von frischem Fleisch wurde der Effekt von poly(TBAMS) auf den Transfer von pathogenen Keimen vom Schneidebrett auf das Lebensmittel untersucht. Der Einfluss von poly(TBAMS) auf die Biofilmbildung von Bakterien in Mono- und Mischkulturen wurde durch den Vergleich von Biofilmen auf 200 Proben sowie Referenzen geprüft. Basierend auf allen Ergebnissen wurde das Potential intrinsisch antimikrobiell aktiver Materialien zur Verbesserung der hygienischen Bedingungen bei der Verarbeitung und Zubereitung von Frischfleisch bewertet.
Die antimikrobiellen Tests weisen ein Potential von poly(TBAMS)-haltigen Materialien auf die hygienischen Bedingungen währen der Verarbeitung und Zubereitung von Fleisch zu verbessern. Eine gute Aktivität wurde gegen verschiedene fleischspezifische Bakterien nachgewiesen. Die Aktivität nahm über einen Zeitraum von drei Jahren nur marginal ab. Das Screening identifizierte jedoch verschiedenen Faktoren, die die antimikrobielle Aktivität beeinflussen. So zeigte sich ein deutlicher Effekt auf die Aktivität gegen *P. fluorescens*, während bei *L. monocytogenes* keine Beeinflussung festgestellt wurde. Der negative Einfluss von niedrigeren Temperaturen konnte durch die Verlängerung der Kontaktzeit kompensiert werden. Obwohl die LLDPE-Schneidebretter mit 10 % poly(TBAMS) im Vergleich zu den anderen antimikrobiell ausgerüsteten Brettern das beste Aktivitätsprofil aufwiesen, war der Effekt auf die Kreuzkontamination von pathogenen Bakterien limitiert. Das gleiche Material unterdrückte bzw. verzögerte allerdings die Biofilmbildung. Poly(TBAMS) weist das Potential auf als Lebensmittelkontaktfläche die hygienischen Bedingungen bei der Verarbeitung von Fleisch zu verbessern, insbesondere durch die Wirkung auf die Persistenz der Bakterien auf Oberflächen. Eine weitere Entwicklung der Materialien sowie eine rechtliche Beurteilung sind für den effektiven Einsatz als Lebensmittelkontaktfläche erforderlich.

Contents

1 General introduction 1
1.1 Impact of hygienic conditions during processing and preparation of fresh meat 1
1.2 Antimicrobial material to improve the hygienic status of food contact surfaces 7
1.3 Research questions and outline of the thesis 14
1.4 References 16
2 Antimicrobial activity of intrinsic antimicrobial polymers based on poly((tert-butyl-amino)-methyl-styrene) against selected pathogenic and spoilage microorganisms relevant in meat processing facilities 24
2.1 Abstract 24
2.2 Introduction 25
2.3 Materials and Methods 26
2.4 Results and Discussion 30
2.5 Conclusion 38
2.6 References 39
3 Long-term antimicrobial activity of poly(TBAMS)-containing films and activity under conditions typical during the processing and preparation of meat 42
3.1 Abstract 42
3.2 Introduction 43
3.3 Materials and Methods 44
3.4 Results 49
3.5 Discussion 59
3.6 Conclusion 63
3.7 References 65
4 Effect of antimicrobial treated cutting-boards on cross-contamination of pathogens during preparation of meat and ready-to-eat food 68
4.1 Abstract 68
4.2 Introduction 69
4.3 Materials and Methods 70
4.4 Results and Discussion 75
4.5 Conclusion 81

4.6 References ... 82
5 Potential of antimicrobial treatment of LLDPE with poly((tert-butyl-amino)-methyl-styrene) to reduce biofilm formation in food industry ... 86
5.1 Abstract ... 86
5.2 Introduction ... 87
5.3 Materials and Methods ... 88
5.4 Results ... 90
5.5 Discussion ... 93
5.6 Conclusion ... 96
5.7 References ... 98
6 Summary ... 102
List of figures ... 107
List of tables ... 109
List of publications ... 110
Acknowledgment ... 114

1 General introduction

1.1 Impact of hygienic conditions during processing and preparation of fresh meat

During the processing and preparation of industrially sourced fresh meat by wholesalers, retailers as well as in professional and domestic kitchens, the products encounter numerous different food contact surfaces like conveyer belts, boxes for storage and transport, counters, packaging materials or cutting boards [1–3]. Due to the automation of processing, the number of those contacts are progressively increasing [4]. One major problem is that every contact increases the risk of cross-contamination [3, 4]. Cross-contamination is defined as the direct or indirect transfer of bacteria or viruses from a contaminated object to a non-contaminated product [5–7]. Thus, even if fresh meat from healthy animals is sterile, the meat surface will be contaminated with spoilage and pathogenic bacteria due to these transfers during processing and preparation [8–10]. Studies observed that final meat products were more contaminated than the raw material at the beginning of the process, furthermore, the bacterial spectrum on the products changed during the process [11–17]. Recent reports indicated that cross-contamination occurs at any stage between the meat processing plant to the final consumer's home [18, 19]. Hence, the hygienic status of food contact surfaces is of major concern, because contamination of fresh meat leads to accelerated spoilage and to reduced food safety [4, 20–23].

1.1.1 Microbial contamination of food contact surfaces

The microbial contamination of food contact surfaces occurs mainly from three sources: contact with food workers, cross-over from raw materials and transfer via environmental sources like water, dust, soil or aerosols (Figure 1.1) [24–29].

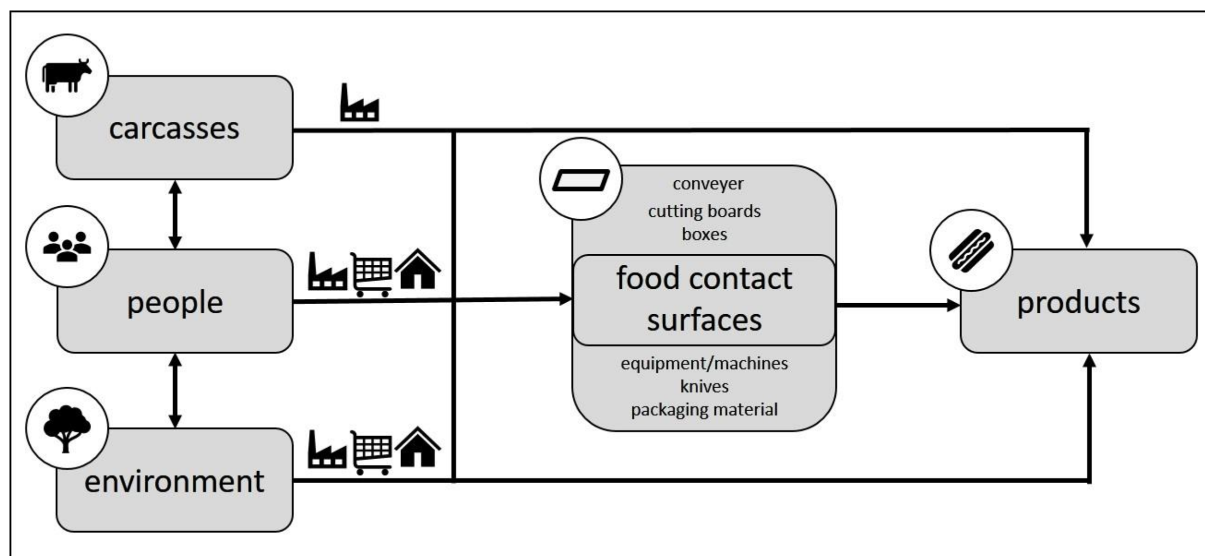

Figure 1.1 Microbial contamination routes during processing and preparation of fresh meat

The ability of microorganism to attach, grow and persist on inert surfaces after transfer depends on several factors: the characteristics of the microorganism itself (hydrophobicity, electronic

charge, flagellation, motility as well as the growth phase), the physicochemical properties of the surface (hydrophobicity, electronic charge, roughness) and the environmental conditions (temperature, pH, humidity, availability of nutrients) [29–32]. Several studies showed that microorganisms can attach to all materials commonly found during food processing, preparation and storage, such as stainless steel, glass, wood as well as different kinds of polymers. The attachment occurs despite different surface properties and under almost all environmental conditions prevalent during processing and preparation of fresh meat [23, 33]. The colonization of food contact surfaces is supported by high levels of organic material remaining on the surfaces [34–38].

Hence, a broad spectrum of microorganism is present on meat contact surfaces. The incidence of bacteria on surfaces in meat processing and preparation was studied by several authors. The results indicate that conveyer belts, other transport equipment, cutting machines, floors, drains, gasket materials, work tables and cutting boards are most often contaminated with high bacterial counts, furthermore, door handles, gloves of personnel and cleaning equipment often tested positive for bacteria. [4, 9, 11, 14, 26, 39–44]. In general, the flora is a mixture of many species [45]. Accordingly, in a study by Roder et al. [46] more than 680 bacterial strains were detected in seven different locations (cutting boards, foil packer, meat chopper and air samples) in a meat processing environment. The flora involves mainly spoilage bacteria, non-pathogenic bacteria as well as pathogenic bacteria [17, 45]. Spoilage flora is dominated by bacteria of the genus *Brochothrix*, *Lactobacillus* and *Pseudomonas* [36, 47, 48]. Gounadaki et al. [49] investigated the microbial ecology of food contact surfaces of small-scale facilities producing traditional sausage. They found out that most sample sites were highly (> 4 $\log_{10}$ cfu cm^{-2}) contaminated with spoilage flora. The study of Roder et al. [46] detected that *Pseudomonas* spp. accounted for the largest proportion (nearly 70 %) of the flora on a cutting board used during meat processing. This is because this gram-negative bacteria species grows well even at the low temperature prevalent in those environments and has generally low growth requirements [9, 45]. Comparably, the human pathogen *Listeria monoytogenes* grows under harsh environmental conditions and is therefore one of the pathogens often isolated from food contact surfaces of meat production and processing [1, 28, 38, 50, 51]. While *L. monocytogenes* has a high prevalence in processing environments of ready-to-eat meat and poultry products, the pathogens *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., which are also of concern in the context of meat, are associated with raw meat and poultry products [29, 38, 52, 53]. In the small-scale facilities producing traditional pork and beef sausages, up to 26.4 % of the samples were contaminated with *Salmonella* spp, *S. aureus* and *L. monocytogenes* [49].

All the mentioned bacteria species can survive and proliferate on the surfaces of utensils and equipment for hours or days; in the case of *L. monocytogenes*, even a persistence over a period longer than one year in ham producing facility was proven [13, 17, 23, 37, 40, 54–61]. Larsen et al. [17] reviewed the persistence of foodborne pathogens in food production chains. The

authors point out that various factors cause this persistence, including disinfection and desiccation resistance, differences in gene expression and biofilm formation.

Biofilms are assemblages of microorganisms, which interact with each other and are embedded in self-produced extracellular polymeric substances (EPS) adhering to surfaces [62, 63]. Mixed-species biofilms are the target form of bacteria to colonize surfaces, because the EPS film protects the embedded bacteria against environmental stress (e.g. cleaning and disinfection measurements, drying). Furthermore, interactions between the different species support the survival of the individual species [7, 17, 64, 65]. Many surfaces during the processing of meat are almost permanently wet, and meat processing equipment such as conveyer belts, pipelines, tanks or packaging equipment are difficult to clean and disinfect; both support biofilm formation [66–68]. *Pseudomona*s spp., as a great biofilm producer, is known as a pioneering species in surface colonization, and its presence was proven to facilitate the attachment of less adhesive species, particularly of pathogenic bacteria [21, 64, 67, 69–74].

Biofilms have the potential to act as long-term reservoirs for bacteria, and the retention of bacteria on food contact surfaces increases the risk of transfer of bacteria to food [7, 37, 44, 48, 75, 76]. The risk of this cross-contamination is not only dependent on the contamination of surfaces with bacteria, but also on the probability of transfer to food surfaces [77]. Similar to the adherence process of bacteria to food contact surfaces, the transfer from these surfaces to meat surfaces is a multifactorial process, which depends on the bacterial species, surface types (source and recipient) and contact time [32]. For example, studies showed that the content of moisture and fat on the recipient influences the transfer, thus the moisture content of cucumber surfaces might positively affect the retrieval of pathogens from stainless steel [32, 37]. Certainly, it was identified that even after contact times of a few seconds, significant counts of *S. aureus* could be transferred [78, 79].

1.1.2 Consequence of contamination of food contact surfaces

Contamination of food contact surfaces and the possible subsequent cross-contamination to products are of concern in all stages of processing and preparation of fresh meat, because they lead to various consequences for public health, the environment and the economy (Figure 1.2) [80, 81].

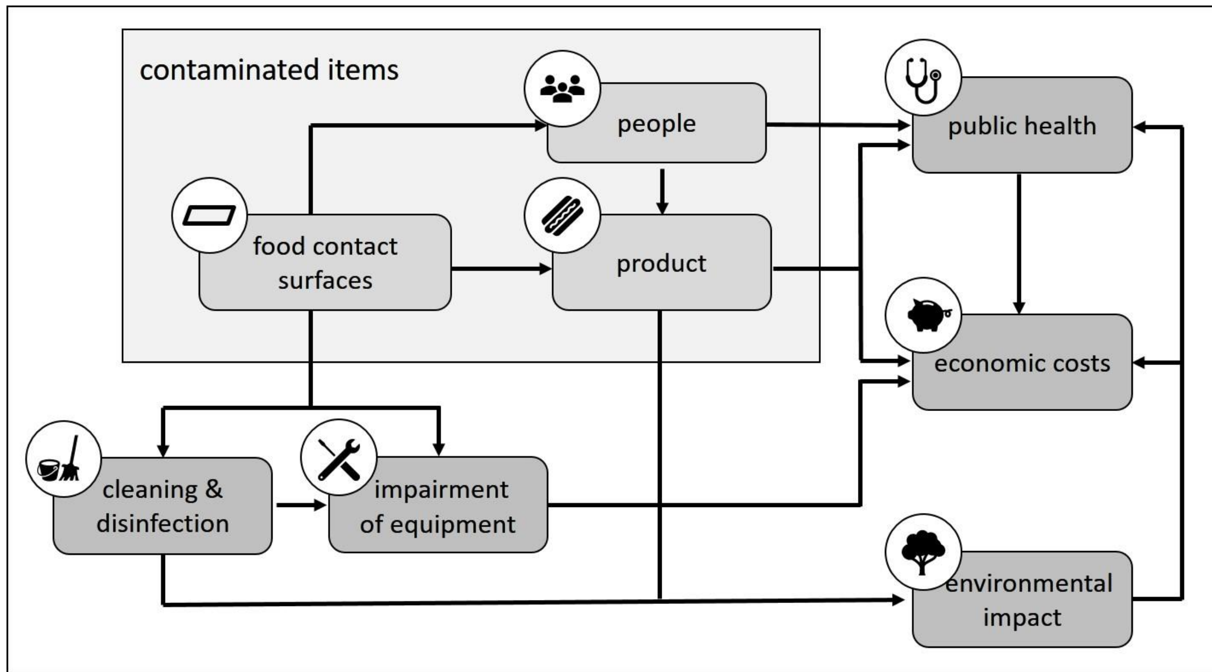

Figure 1.2 Consequences of microbial contamination of food contact surfaces

The cross-contamination of bacteria to food influences the quality and safety of the products, which is of concern to public health and results in economic losses for the public and industry, respectively [33, 66, 67, 82]. The consumption of contaminated food causes a variety of food poisoning. In the EU in 2011, 5,648 food-borne outbreaks were reported, resulting in 69,553 human cases, 7,125 hospitalizations and 93 deaths [83]. Epidemiological investigations demonstrated that many outbreaks, 25 % of outbreaks according to a report by the WHO [84], are associated with cross-contamination scenarios involving deficient hygiene practices, contaminated equipment, contamination via food handlers, processing, or inadequate storage [3, 6, 18, 57, 84, 85]. An evaluation of the reports by the Centers for Disease Control and Prevention (CDC, USA) observed that 12 % of all outbreaks from 1998 to 2016 are linked in some way to surface cross-contamination, which therefore presents the 6th most contributing factor of 32 [86]. Referring to a French survey, ca. 60 % of food-borne infections occurred by microbial transfer from food contact surfaces to processed foods [33].

The economic burden of foodborne diseases is high due to medical care expenditures associated with diagnosis, treatment and management of a disease in an individual as well as productivity losses due to illness and death [87]. The data reported in literature varies widely because of variable data collection [17]. To name same data, Scott et al. [88] estimated the total cost of potential food-borne infectious diseases in New Zealand to be $ 88.8 million in 2000. For the US, a report by the CDC suggested costs of $ 77.7 billion annually [89]. A ranking of the disease burden of 14 pathogens in the US observed that poultry, pork and beef belong to the top five categories of estimated annual disease burden [90].

Next to costs associated with public health, outbreaks generate costs for the food industry including costs for rejection or recalling of the products, inspections of the plants with extensive

decontamination procedures and possible production stops [87, 91]. Indeed, every outbreak is concomitant with loss of consumer trust resulting in inestimable costs [29, 87]. Furthermore, not only the contamination with pathogens, but also the transfer of spoilage bacteria to products, leads to an economic burden for the food industry due to reduced shelf life. The contamination of meat with spoilage bacteria correlates with reducing shelf life. Thus, an increase in the starting concentration of *Pseudomonas* spp. from 1 $\log_{10}$ cfu cm^{-2} to 3.6 $\log_{10}$ cfu cm^{-2} results in a reduction of shelf life of about 2 days [92, 93]. The shelf life has significant impact on the amount of food waste, because products are thrown away if they were not sold during the short selling time [94]. Furthermore, high amounts of food are wasted when contaminated with pathogenic bacteria. For instance, a listeriosis outbreak in the US in 2000 lead to a recall of 7.3 million kilograms of processed turkey and chicken meat [95]. Not only the final products themselves, but also huge amounts of primary resources used for the breeding and feeding of animals or the production of products are lost [96]. Dohlen [97] predicted that an increased shelf life of 2 days lead to a 15 % waste reduction of poultry sold by German retailers. This means a breeding reduction of more than 6 million animals per year, which would produce nearly 13,000 t carbon dioxide and consume 200,000 m^3 of water and about 80,000 t of feed [97]. Thus, next to the public health issues, the economic losses and the environmental impact associated with those processes should not be underestimated.

Further environmental problems are caused by using various sanitizers in great quantities. For example, biocide induced mutations of water organisms were observed [98]. Furthermore, bacteria embedded in biofilms are known to be more resistant to sanitizers [20, 38, 99, 100]. Thus, the use of biocides induces resistance development in bacteria against those biocides as well as against antibiotics, which poses problems in the therapy of human infections with foodborne or environmental pathogens [27, 100, 101].

Lastly, the colonization of surfaces can result in biofouling, for example, caused by the acidic conditions in biofilm environments. Additionally, the strong forces required to remove biofilms abrades surfaces. Both lead to economic losses, because the corrosion and equipment impairment adversely affects the function of specific interfaces, while also requiring more frequent maintenance and replacement of the equipment [3, 20, 34, 35, 45, 102, 103].

1.1.3 Activities to reduce bacterial count on food contact surfaces

Considering these far-reaching consequences to public health, the environment and the economy, the hygienic status of food contact surfaces is of great concern during meat processing and preparation [9, 38, 80]. In this context, hygienic design of equipment as well as cleaning and disinfection are established, and in the case of the food industry, even statutory [104].

Hygienic design is aimed at high cleanability, e.g. by open design of processing equipment and by selection of materials used for food contact surfaces. A hygienic surface should be inert and easy to clean. Thus, during industrial processing, predominantly stainless steel is used as a food-

contact material. However, for many applications, like conveyers or gaskets, the use of polymeric materials, which are more problematic in terms of hygienic status, is unavoidable [1, 25, 105]. Also, plastic surfaces are often used during the domestic preparation of fresh meat.

Frequent, adequate cleaning and disinfection are essential steps for the prevention of colonization of food contact surfaces by bacteria [104]. These hygienic operations are generally performed in the food industry, but it seems that they are often not completely effective [17, 28, 106, 107]. Thus, several studies detected contaminations of food contact surfaces in meat processing environments after routine cleaning and disinfection, with bacterial densities of higher than 10^5 cfu cm^{-2} [9, 14, 45, 53, 63, 106, 108–111]. The detected flora was dominated by *Pseudomonas* spp., but the food contact surfaces also tested positive for the pathogenic bacteria *L. monocytogenes*, *S. aureus*, *E. coli*, *Bacillus* spp. as well as *Salmonella* spp. The highest bacterial levels were found in niches, small spaces or narrow openings of equipment, which are difficult to access and therefore difficult to clean, for example conveyers or drains [9, 18, 112]. Bacteria, which adhere to surfaces and survive the hygienic operations, and may form a biofilm, hence, they are more resistant against sanitizers and are difficult to remove [67, 100, 112, 113]. In addition, not all surfaces in the food industry are cleaned daily. Thus, surfaces like walls or ceiling can act as a reservoir of bacteria [67, 114]. During industrial processing and preparation, cleaning and disinfection are routinely (every night after one day of processing) practiced, maintaining and improving the hygienic status of food contact surfaces. But it is well known that inadequate cleaning and handling of food and preparation equipment is performed in restaurants and in domestic kitchens [32, 115–117]. Hence, according to different studies, up to 81 % of consumers use the same kitchen equipment, such as knives and cutting boards, for raw meat and ready-to-eat products like vegetables without intermediate cleaning [7, 116–119].

But even if cleaning and disinfection operations are undertaken effectively, the sterile state of the surfaces do not last for a long time and will be soiled by the first contact with a contaminated product [4, 30]. Hence, the count of bacteria on the surfaces will increase during processing. A cleaning interval of 2-hours, which was proven to be expedient to reduce the adherence of bacteria cells, is unrealistic in the meat processing industry [30, 99]. Long production stops during the sanitizing procedure lead to high economic losses, in addition to the high environmental impact of excessive sanitizer use.

The relevance of efficient and frequently performed cleaning and disinfection measures as well as the hygienic design of food processing equipment is unquestioned [35, 56, 76, 99]. But between the cleaning and disinfection operations, the food contact surfaces exhibit no defense against the colonization by spoilage and pathogenic bacteria [3]. Therefore, the protection of food contacts surfaces against bacterial colonization was the focus of research in the last decades [4, 120].

1.2 Antimicrobial material to improve the hygienic status of food contact surfaces

The protection of food contact surfaces against bacterial colonization was a focus of research in the last decades [4, 120]. For maintenance and improvement of food quality and security, the hygienic status of food contact surfaces should be improved during food processing and preparation even between the sanitation cycles. In this context, the application of antimicrobial materials as food contact surfaces is more and more discussed. In some food contact surfaces, e.g. conveyer belts, cutting boards, refrigerators, countertops and storage boxes, antimicrobial materials are already used [2, 4, 17, 38, 53, 56, 76, 80, 100, 118, 121–124].

1.2.1 Materials used as antimicrobial surfaces

Antimicrobial surfaces are defined as surfaces of any material or agent that prevent or limit the growth and proliferation of bacteria [103, 125]. This includes not only bactericidal surfaces but also bacteriostatic and antibiofouling surfaces [103, 126]. Therefore, antimicrobial surfaces are distinguished regarding their mode of action. In the literature, next to the classification into active/passive, bactericidal/bacteriostatic or antifouling, the division in killing and repelling surfaces is widely-used [76, 100, 103, 125, 127]. Figure 1.3 gives an overview over the general principles of antimicrobial surfaces classified by repelling and killing mechanisms.

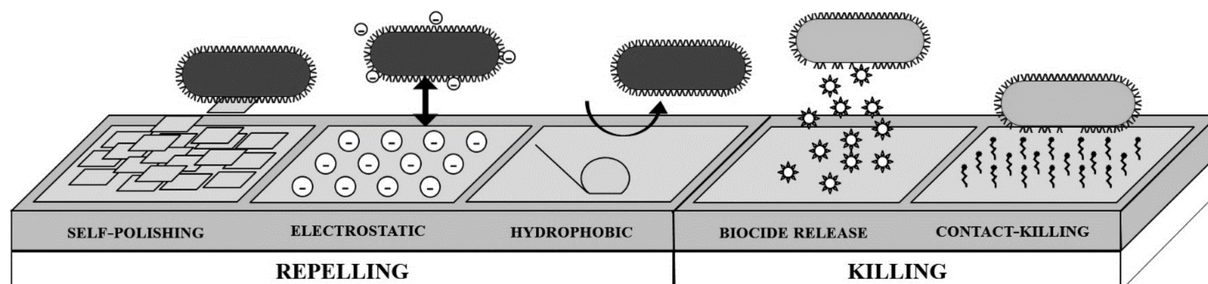

Figure 1.3 Principles of antimicrobial surfaces (mod. [100, 127])

In case of repelling antimicrobial materials, surface characteristics including self-polishing, negative charge, micro-structure or hydrophobicity are responsible for the prevention of fouling and colonization [127, 128]. The contact killing principle is subdivided into biocide releasing surfaces and contact killing surfaces [8, 22, 76, 129]. In contrast to the killing surfaces, the repelled microorganisms remain viable. Thus, only the attachment of microorganism and of pollutants as food debris is inhibited, but the bacteria are still in the food environment and can contaminate other surfaces or the food directly. To reduce cross-contamination during meat processing and preparation, the use of surfaces which kill the bacteria are more effective.

The approach of biocide releasing surfaces can be based on materials which generate biocides themselves or on materials which are treated with antimicrobial agents. First, materials generate and subsequently release an antimicrobial agent induced by a stimulus. For example, surfaces with photocatalytic titanium dioxide generate and release reactive oxygen species when they are exposed to light of a specific wavelength [4, 100, 127]. For the second approach

antimicrobial agents are embedded directly into the material or absorbed as well as coated onto the material [8]. A wide spectrum of antimicrobial agents are currently used or discussed for use as biocide releasing materials, which can be classified regarding their chemical properties and their origins in metals (silver, titan, copper), plant extracts (essential oils like thymol, linalool), enzymes (lactoperoxidase, lactoferrin, lysozyme), bacteriocines (nisin, pediocin) and organic acids (sorbic acid, benzoic acid) [4, 8, 129–131]. Indeed, the choice of agent is dependent of the field of application. For example, plant extracts are particularly used in packaging material, whereas mostly metals are used in food contact surfaces such as conveyers.

Contact-killing surfaces can be prepared by immobilization of antimicrobials on the surface [8, 132]. Thus, QACs, antimicrobial peptides, cationic polymers or enzymes were applied to surfaces via functional groups on polymer and antimicrobial agent or via spacer in different techniques like grafting or cross-linking [4, 8, 127, 128, 133, 134]. The other approach for contact-killing surfaces is the use of inherently antimicrobial active materials. Cationic polymers such as chitosan and poly-L-lysine exhibit antimicrobial and film-forming properties [8, 122, 132, 135]

For the use as antimicrobial food contact surfaces numerous agents were developed, but just a few like silver or titan have been introduced into the market. Most of mechanisms which were discussed in literature, could not fulfill the extensive requirements for food contact surfaces. Furthermore, the integration of antimicrobials in food contact surfaces has a lot of challenges.

1.2.2 Requirements on antimicrobial food contact surfaces and challenges in material processing

Antimicrobial surfaces for food contact must fulfill different requirements which are partly statutory and partly reasonably inferred from conditions in the application field.

In the European Union, different regulations on antimicrobial food contact materials exist. In general, the material must comply with all regulatory requirements for materials intended to come in contact with food which are regulated in Regulation (EC) No 1935/2004. This regulation sets out the general principles of safety and inertness for all food contact materials. These principles require that the release of material constituents into food is not at levels harmful to human health. Furthermore, food composition, taste and odor must not be changed in unacceptable ways due to the material. Moreover, this framework regulation provides special rules for active and intelligent materials and powers to enact additional measures for specific materials. Furthermore, principles of good manufacturing practices (Regulation (EC) No 2023/2006) and of hygienic design (i. a. Regulation (EC) No 852/2004, EC Directive 2006/42/EC) must be observed. According to that machinery intended for use with foodstuffs must be designed and conducted in such a way as to avoid any risk of infection, sickness or contagion. This regulation includes requirements on the construction and surface properties. For example, food contact surfaces must be smooth, have neither ridges nor crevices and must be easy to clean and disinfect. Regulation (EU) No 10/2011 (and corresponding amendments)

stipulates rules on the composition of plastic food contact surfaces and establishes a Union List of substances which are permitted for manufacturing plastic food contact materials. In particular, migration limits are defined to ensure the safety of plastic materials. Requirements on active materials, which include antimicrobial materials, are regulated in Regulation (EC) No 450/2009. Like for plastic materials a Union list of substances permitted for manufacturing active materials is provided. More general, principles regarding the use of biocidal agents are regulated in the Regulation on Biocidal products (EU) No. 528/2012. Active substances used in biocidal material must be approved for the relevant product type and mentioned in the positive list of the regulation.

Besides the legal aspects, requirements dealing with the antimicrobial activity of treated materials in consideration of the conditions in the application field are also important. These include:

- broad antimicrobial activity in adequate time,
- long-lasting antimicrobial activity,
- no resistance development of the microorganism against the biocidal agent,
- activity under relevant environmental conditions like temperature and humidity,
- effectiveness under different processing factors, like presence of food components and acid or alkaline cleaning and sanitization. [4, 22, 76, 80, 120, 136, 137]

Per these multifactorial requirements, many aspects must be considered during the development of antimicrobial active food contact surfaces regarding their intended use. During the processing and preparation of fresh meat and down the chain to retailers, restaurants and consumers, the conditions vary in the separate stages of the food industry, hence making various demands on the material [4, 80]. As mentioned before, a variety of antimicrobial surfaces were investigated, and good activity were proven in vitro. In tests under practical conditions however, the effectiveness is often inhibited [4, 22, 76, 80, 138]. This is caused by the microorganism and test conditions used in antimicrobial screening, which deviate from the practical conditions during food processing and preparation.

For an effective implementation of antimicrobial agents in food contact surfaces the agents must offer a broad spectrum of activity. The activity of many antimicrobial agents is differently pronounced against gram-negative and gram-positive bacteria. For instance, silver, triclosan and chitosan are more active against gram-negative than gram-positive bacteria, which limits their use during food processing and preparation, because a variety of bacteria is present [76, 80, 136, 139, 140]. A further problem of many in vitro studies regarding antimicrobial activity of new materials surfaces is the use of mono-species cultures as test solutions, while bacteria occur in mixed-species in nature and often in the form of biofilms. Different tests show that even if silver or triclosan-containing materials offer good activity against different bacteria in mono-cultures, the ability to reduce biofilms do not exist [1, 141, 142]. Tabak et al. [142] also showed a dependence of the activity of triclosan on the different growth phases of

S. thypimurium. Altogether, this meant higher activity against log-phase cells compared to stationary and biofilm-associated cells.

Not only the bacteria, but also several of the mentioned environmental and processing factors are not considered in in vitro screenings. A major factor leading to a decrease or even inhibition of antimicrobial activity is the presence of food or food components like proteins, fat or mineral nutrients, which was proven for different materials [22, 76, 80, 112, 118, 140, 143–147]. The food matrix can protect microorganisms from the biocidal agents, with proteins interacting with and bind on the active groups of the antimicrobial surfaces, thus inactivating them; and furthermore, the presence of nutrients can stabilize bacterial membranes and facilitate bacterial growth [112, 138, 144–146].

One environmental factor influencing the antimicrobial activity is the temperature. Hence, a decrease in temperature leads to a decrease in activity, which was shown especially for releasing systems with silver or copper components [56, 136, 145, 146, 148–150]. This is due to the slower release of the biocidal agents out of the material. The effectiveness of the inherently antimicrobial active chitosan also showed a dependence on temperature, which was shown in a study by Chang et al. [151]. This can be explained by comparatively weak interactions between antimicrobial surfaces and microorganisms due to changes in bacterial surface characteristics. Prolonging the contact time can compensate for the effect of reduced temperature [136]. Further factors proven to influence the antimicrobial activity of different materials are the pH-value and the humidity. For humidity, it was shown that the activity of copper and silver releasing systems was higher at higher relative humidity [147, 150]. The activity of chitosan increased with decreased pH-value, contrarily the activity of tertiary amines bonded to polystyrene fiber was inhibited by lowering the pH [140, 143, 151].

Even if an antimicrobial agent is active under conditions relevant for the intended application field, the implementation as a food contact material is still a challenge; the active components must be incorporated into approved food contact materials in a way that facilitates an effective antimicrobial activity over a long time-period, meanwhile, the material properties must not be affected and processability and machinability must be assured.

For releasing materials, it is necessary that the antimicrobial agents are incorporated into food contact materials in a way that they are released at constant rates. However, the ability of antimicrobial agents to be incorporated into or to be homogeneously distributed in common polymers used as food contact materials varies, and the release of agent could be inhibited due to incorporation in other materials [8, 129]. For example, the antimicrobial properties of silver treated materials is dependent on the matrix polymer [152, 153]. A fast or inconsistent release of biocides leads to loss of antimicrobial effect. In general, a major drawback of releasing systems is the terminated antimicrobial activity, because the amount of antimicrobial agent in the material is limited [76, 80, 99, 124, 127, 132, 134, 141]. Additionally, users of those antimicrobial material cannot distinguish if the material is still active, which may give a false

impression of protection [80]. In addition, the carryover of biocides into the environment supports the building of biocide-resistance in microbial strains [99, 127].

For contact-active materials, the direct contact of a bacterium and a food contact surface must be given. Therefore, the accumulation of active groups on the interface must be achieved via adjustment of material processing. Due to no release of the active groups, the long-term stability of contact-active materials is more promising than for biocide releasing material. However, chemical rearrangement of the material or abrasion of active components on the material surface could lead to a loss of antimicrobial activity [4, 80, 134].

A further challenge is that the antimicrobial treatment of materials affects the general physical and mechanical properties as well as the processability or machinability of a material [22, 129, 154, 155]. For example, silver-zinc zeolites decreased the flexural as well as impact strength of acrylic resins [156]. At the same time, processing parameters like temperature, pressure or shear forces can affect the activity of antimicrobial agents. Thus, enzymes cannot be used in polymer film processing via extrusion due to the high temperature [4, 22, 80, 122, 129, 132].

An approach to maintaining the physical properties of the base material as well as the antimicrobial properties of the antimicrobial agent is to coat commonly used materials with the antimicrobial material [76, 80, 132]. Due to these advantages and the lower cost in comparison to using bulk antimicrobial material, coatings are the favored method for antimicrobial treatment of a surface [22, 76]. In general, the economic aspect is not to be underestimated. Platinum and gold, which show significant promise as bactericidal agents, are prohibitively expensive to be used on industrial scale [132].

1.2.3 Sustainable Active Microbiocidal (SAM)-Polymers® as contact-active antimicrobial polymers

As described for the successful implication of antimicrobial materials as food contact materials, antimicrobial surfaces must fulfill several requirements. SAM-Polymers® belong to intrinsically contact-active antimicrobial materials and offer a great potential for implementation due to their antimicrobial mechanism and processing abilities.

The first SAM-Polymer® poly(tert-butyl-amino-ethyl)-methacrylate (poly(TBAEMA)) was introduced into the market by the Creavis Technologies and Innovation of the Degussa (Marl, Germany) in 2001 [157]. Figure 1.4 shows the chemical structure of the polymer with the carbon-based backbone that bears a high density of amino functionalized side chains [133].

The antimicrobial action of poly(TBAEMA) is not fully understood, but it seems to be evident that the amino functionalized groups, located on the surface due to the three-dimensional structure of the polymers, are responsible for the antimicrobial activity. The main difference to other antimicrobial polymers is that the constituent monomers do not exhibit any biocidal activity; the antimicrobial activity is attributed only to the final polymer itself [133, 158, 159]. Hewitt et al. [160] clarify that physical interactions, chemical reactions or a combination of

both are required for the antimicrobial activity. The exposure of bacteria to the polymers initially leads to a depolarization of the cytoplasmic membrane, resulting in permeability which initiates cell death through a release of fibrous and cellular material [160, 161]. Lenoir et al. [161] assumed that the charged amino groups replace divalent cations of the outer membrane, which leads to membrane disorganization. A certain degree of protonation of the amino groups is necessary for the antimicrobial activity [158]. The resulting localized pH-gradient and additional electrostatic interactions between the positively charged surface of the polymer and the negatively charged bacteria membrane are responsible for the antimicrobial activity. This mechanism of action leads to a good antimicrobial activity against a wide range of microorganism, while exhibiting only a low toxicity in mammals [133, 158, 160–164]. In addition, the non-specific mechanism of action offers little risk of the development of resistant microorganisms [162]. This fact and the contact-activity with no transfer into the environment and thus no loss of activity promises a long-lasting antimicrobial effect of the material [133, 161]. Furthermore, no contamination of food can be expected. All these facts fulfill the requirements for a successful and sustainable application of antimicrobial surfaces as food contact material.

Also, the processability of poly(TBAEMA) allows for use as a food contact material. Poly(TBAEMA) can be prepared from commercially available materials, has film-building properties and can be manufactured in thermoplastic processes. Thus, it is feasible to compound poly(TBAEMA) with other polymeric materials, offering the possibility to manufacture a broad range of polymeric materials with antimicrobial surfaces [159, 162–164]. However, the mechanical properties of the material deteriorated if it was compounded with poly(TBAEMA). In general, a major drawback of poly(TBAEMA) is the low glass transition temperature (TG) of 40 °C which limits the application, as the surface become sticky at elevated temperatures, as well as the high water uptake and a tendency for hydrolysis [157].

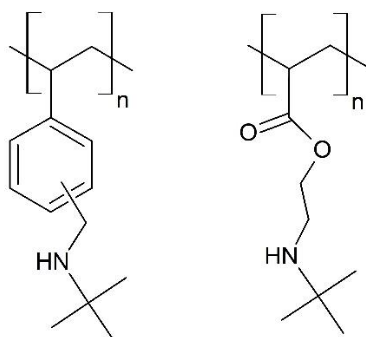

Figure 1.4 Structure of the SAM-Polymers® poly-[2-(tert-butylamino) ethyl methacrylate] (poly(TBAEMA) (right) and poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS) (left) (mod. [157])

A new monomer 2-(tert-butylamino) methylstyrene (TBAMS) (Figure 1.4), which can be polymerized to the corresponding intrinsically antimicrobial active polymer poly(TBAMS), was developed by Brodkorb et al. [157]. Just like poly(TBAEMA), this polymer can be

prepared from commercially available monomers and form transparent, colorless and uniform polymeric films, but the basic material characteristics are enhanced compared to poly(TBAEMA). Thus, the TG of poly(TBAMS) is about 68 °C and can be further increased by copolymerization. In addition, the water uptake is low, and the heat resistance is reasonable [165].

Regarding these improved properties in comparison to poly(TBAEMA), surfaces based on the new monomer TBAMS show a great potential for use as food contact materials. A good antimicrobial activity of poly(TBAMS) was shown against *E. coli* and *S. aureus* at 35°C [157], but until now there are no results regarding the antimicrobial activity and long-term stability under conditions prevalent during meat processing and preparation. Furthermore, no data about the reduction of cross-contamination and biofilm formation are available.

1.3 Research questions and outline of the thesis

The main objective of this thesis is the investigation of the potential of intrinsically antimicrobial active polymers to improve the hygienic conditions during processing and preparation of fresh meat. For this purpose, the following research questions are proposed:

- Are different kinds of poly(TBAMS)-containing surfaces able to reduce the microbial count of single and mixed species of spoilage and pathogenic bacteria present on meat processing and preparation equipment? (chapter 2, 3, 4)
- How is the antimicrobial activity of poly(TBAMS) influenced by conditions typical during the processing and preparation of fresh meat? (chapter 3, 4)
- Are different environmental conditions effecting the long-term activity of poly(TBAMS)-containing surfaces? (chapter 3)
- Are surfaces containing poly(TBAMS) able to improve hygienic conditions through the reduction of biofilms and cross-contamination during the processing and preparation of fresh meat? (chapter 4, 5)

In the first part of this thesis (chapter 2), the antimicrobial activity of poly(TBAMS) as a homopolymer and copolymer with acrylonitrile [1:1] is screened against various pathogenic and spoilage bacteria prevalent in meat processing facilities. Activity against pure and mixed cultures as well as moderate and high initial bacteria counts are analyzed.

In chapter 3, the activity under conditions typical during the processing and preparation of meat and the long-term antimicrobial activity of poly(TBAMS)-containing films is investigated. Therefore, the test method is modified concerning the contact time between microorganisms and surfaces as well as environmental and processing conditions, represented as temperature, pH-value, air humidity and presence of food components. The long-term effectiveness of three different poly(TBAMS)-materials is tested by storing the materials under adverse conditions and monitoring the activity over a period of three years. The trend of activity is modelled to predict the long-term stability.

In the next part (chapter 4), the antimicrobial activity of poly(TBAMS) incorporated in LLDPE is determined against relevant pathogens. The activity is compared between antimicrobial screenings of poly(TBAEMA)-containing LLDPE and a commercially available PP-cutting board with Microban®. In three different scenarios typical during the preparation of meals in domestic kitchens, the effect of a cutting board with poly(TBAMS) in comparison with untreated LLDPE material on the transfer of pathogens between the cutting boards and food is investigated.

In the last chapter (5), the formation of mono and multi-species biofilms on polymers with and without poly(TBAMS) is studied. Biofilm cells adhering to the materials are determined and compared. The effect of poly(TBAMS) treatment of LLDPE on the hygienic status of food contact surfaces is evaluated.

In the last chapter of this thesis, the potential of intrinsically antimicrobial active materials to improve the hygienic conditions during the processing and preparation of fresh meat is assessed. Therefore, the results of the antimicrobial screenings are compared with the requirements on antimicrobial food contact materials. Furthermore, the effect on hygienic conditions is evaluated. In this regard, the results of biofilm formation and cross-contamination are surveyed. In a last step, potential improvement of the poly(TBAMS)-containing material is suggested.

1.4 References

1. Berrang ME, Frank JF, Meinersmann RJ (2010) Listeria monocytogenes biofilm formation on silver ion impregnated cutting boards. Food Prot Trends 30(3), 168–171.
2. Llorens A, Lloret E, Picouet PA, Trbojevich R, Fernandez A (2012) Metallic-based micro and nanocomposites in food contact materials and active food packaging. Trends Food Sci Technol 24(1), 19–29.
3. Griffith A, Neethirajan S, Warriner K (2015) Development and Evaluation of Silver Zeolite Antifouling Coatings on Stainless Steel for Food Contact Surfaces. J Food Saf 35(3), 345–354.
4. Bastarrachea LJ, Denis-Rohr A, Goddard JM (2015) Antimicrobial Food Equipment Coatings: Applications and Challenges. Annu Rev Food Sci Technol 6(1), 97–118.
5. Pérez-Rodríguez F, Valero A, Carrasco E, García RM, Zurera G (2008) Understanding and modelling bacterial transfer to foods: a review. Trends Food Sci Technol 19(3), 131–144.
6. Teixeira P, Silva SC, Araújo F, Azeredo J, Oliveira R (2015) Bacterial adhesion to food contacting surfaces, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 13–20. Spain: Formatex Research Center.
7. Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by Salmonella in foods: A review. Food Res Int 45(2), 545–556.
8. Appendini P, Hotchkiss JH (2002) Review of antimicrobial food packaging. Innovative Food Sci Emerging Technol 3(2), 113–126.
9. Møretrø T, Moen B, Heir E, Hansen AA, Langsrud S (2016) Contamination of salmon fillets and processing plants with spoilage bacteria. Int J Food Microbiol 237, 98–108.
10. Rivera-Betancourt M, Shackelford SD, Arthur TM, Westmoreland KE, Bellinger G, Rossman M, Reagan JO, Koohmaraie M (2004) Prevalence of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella in Two Geographically Distant Commercial Beef Processing Plants in the United States. J Food Prot 67(2), 295–302.
11. Lawrence LM, Gilmour A (1994) Incidence of Listeria spp. and Listeria monocytogenes in a Poultry Processing Environment and in Poultry Products and Their Rapid Confirmation by Multiplex PCR. Appl Environ Microbiol 60(12), 4600–4604.
12. Ojeniyi B, Christensen J, Bisgaard M (2000) Comparative investigations of Listeria monocytogenes isolated from a turkey processing plant, turkey products, and from human cases of listeriosis in Denmark. Epidemiol Infect 125(2), 303–308.
13. Senczek D, Stephan R, Untermann F (2000) Pulsed-field gel electrophoresis (PFGE) typing of Listeria strains isolated from a meat processing plant over a 2-year period. Int J Food Microbiol 62(1-2), 155–159.
14. Gudbjörnsdóttira B, Suihko M-L, Gustavsson P, Thorkelsson G, Salo S, Sjöberg A-M, Niclasen O, Bredholt S (2004) The incidence of Listeria monocytogenes in meat, poultry and seafood plants in the Nordic countries. Food Microbiol 21(2), 217–225.
15. Thevenot D, Dernburg A, Vernozy-Rozand C (2006) An updated review of Listeria monocytogenes in the pork meat industry and its products. J Appl Microbiol 101(1), 7–17.
16. Barros MA, Nero LA, Silva LC, d'Ovidio L, Monteiro FA, Tamanini R, Fagnani R, Hofer E, Beloti V (2007) Listeria monocytogenes: Occurrence in beef and identification of the main contamination points in processing plants. Meat Sci 76(4), 591–596.
17. Larsen MH, Dalmasso M, Ingmer H, Langsrud S, Malakauskas M, Mader A, Møretrø T, Smole Možina S, Rychli K, Wagner M, John Wallace R, Zentek J, Jordan K (2014) Persistence of foodborne pathogens and their control in primary and secondary food production chains. Food control 44, 92–109.
18. Reij MW, Den Aantrekker ED (2004) Recontamination as a source of pathogens in processed foods. Int J Food Microbiol 91(1), 1–11.
19. Yemmireddy VK, Hung Y-C (2017) Using Photocatalyst Metal Oxides as Antimicrobial Surface Coatings to Ensure Food Safety—Opportunities and Challenges. Comprehensive Reviews in Food Science and Food Safety 16(4), 617–631.
20. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42(1–2), 9–27.
21. Frank JF (2001) Microbial attachment to food and food contact surfaces, in: Jeya Henry (ed.) 74. Advances in Food and Nutrition Research, pp. 319–370: Elsevier.

22. Quintavalla S, Vicini L (2002) Antimicrobial food packaging in meat industry. Meat Sci 62(3), 373–380.
23. Patrignani F, Siroli L, Gardini F, Lanciotti R (2016) Contribution of Two Different Packaging Material to Microbial Contamination of Peaches: Implications in Their Microbiological Quality. Front Microbiol 7, 938.
24. den Aantrekker ED, Boom RM, Zwietering MH, van Schothorst M (2003) Quantifying recontamination through factory environments—a review. Int J Food Microbiol 80(2), 117–130.
25. Verran J, Airey P, Packer A, Whitehead KA (2008) 8 Microbial Retention on Open Food Contact Surfaces and Implications for Food Contamination. Adv Appl Microbiol 64(64), 223–246.
26. Todd ECD, Greig JD, Bartleson CA, Michaels BS (2009) Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 6. Transmission and Survival of Pathogens in the Food Processing and Preparation Environment. J Food Prot 72(1), 202–219.
27. Verraes C, van Boxstael S, van Meervenne E, van Coillie E, Butaye P, Catry B, Schaetzen M-A de, van Huffel X, Imberechts H, Dierick K, Daube G, Saegerman C, Block J de, Dewulf J, Herman L (2013) Antimicrobial Resistance in the Food Chain: A Review 10(7).
28. Møretrø T, Langsrud S, Heir E (2013) Bacteria on Meat Abattoir Process Surfaces after Sanitation: Characterisation of Survival Properties of Listeria monocytogenes and the Commensal Bacterial Flora. Adv Microbiol 03(03), 255–264.
29. Lelieveld H (2014) Sources of contamination, in: Lelieveld HLM, Holah J, Napper D (eds.) Hygiene in food processing: principles and practice, 2nd edn., pp. 21–50. Cambridge, UK: Woodhead Publishing Limited.
30. Pompermayer DMC, Gaylarde CC (2000) The influence of temperature on the adhesion of mixed cultures of Staphylococcus aureus and Escherichia coli to polypropylene. Food Microbiol 17(4), 361–365.
31. Bremer PJ, Monk I, Osborne CM (2001) Survival of Listeria monocytogenes Attached to Stainless Steel Surfaces in the Presence or Absence of Flavobacterium spp. J Food Prot 64(9), 1369–1376.
32. Gkana E, Chorianopoulos N, Grounta A, Koutsoumanis K, Nychas G-JE (2017) Effect of inoculum size, bacterial species, type of surfaces and contact time to the transfer of foodborne pathogens from inoculated to non-inoculated beef fillets via food processing surfaces. Food Microbiol 62, 51–57.
33. Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard J-C, Naitali M, Briandet R (2015) Biofilm-associated persistence of food-borne pathogens. Food Microbiol 45(Pt B), 167–178.
34. Genigeorgis C (1995) Biofilm: Their significance to cleaning in the meat sector, in: Burt SA, Bauer F (eds.) New Challenges in Meat Hygiene: Specific Problems in Cleaning and Disinfection, pp. 29–47. Utrecht: Ecceamst.
35. Bower CK, McGuire J, Daeschel MA (1996) The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci Technol 7(5), 152–157.
36. Hood SK, Zottola EA (1997) Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Food Microbiol 37(2-3), 145–153.
37. Kusumaningrum HD, Riboldi G, Hazeleger WC, Beumer RR (2003) Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int J Food Microbiol 85(3), 227–236.
38. Sofos JN, Geornaras I (2010) Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of Escherichia coli O157:H7 in nonintact, and Listeria monocytogenes in ready-to-eat, meat products. Meat Sci 86(1), 2–14.
39. Ak NO, Cliver DO, Kaspari CW (1994) Cutting Boards of Plastic and Wood Contaminated Experimentally with Bacteria. J Food Prot 57(1), 16–22.
40. Lawrence LM, Gilmour A (1995) Characterization of Listeria monocytogenes isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl Environ Microbiol 61(6), 2139–2144.
41. Carpentier B (1997) Sanitary quality of meat chopping board surfaces: a bibliographical study. Food Microbiol 14(1), 31–37.
42. Aarnisalo K, Sheen S, Raaska L, Tamplin M (2007) Modelling transfer of Listeria monocytogenes during slicing of 'gravad' salmon. Int J Food Microbiol 118(1), 69–78.

43. Papadopoulou OS, Chorianopoulos NG, Gkana EN, Grounta AV, Koutsoumanis KP, Nychas G-JE (2012) Transfer of foodborne pathogenic bacteria to non-inoculated beef fillets through meat mincing machine. Meat Sci 90(3), 865–869.
44. Cabarkapa I, Levic J, Djuragic O (2015) Biofilm, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 42–51. Spain: Formatex Research Center.
45. Bagge-Ravn D, Ng Y, Hjelm M, Christiansen JN, Johansen C, Gram L (2003) The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. Int J Food Microbiol 87(3), 239–250.
46. Roder HL, Raghupathi PK, Herschend J, Brejnrod A, Knochel S, Sorensen SJ, Burmolle M (2015) Interspecies interactions result in enhanced biofilm formation by co-cultures of bacteria isolated from a food processing environment. Food Microbiol 51, 18–24.
47. Russo F, Ercolini D, Mauriello G, Villani F (2006) Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups. Food Microbiol 23(8), 797–802.
48. Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib N-E (2014) Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Arch Microbiol 196(7), 453–472.
49. Gounadaki AS, Skandamis PN, Drosinos EH, Nychas G-JE (2008) Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. Food Microbiol 25(2), 313–323.
50. Chasseignaux E, Gérault P, Toquin M-T, Salvat G, Colin P, Ermel G (2002) Ecology of Listeria monocytogenes in the environment of raw poultry meat and raw pork meat processing plants. FEMS Microbiol. Lett. 210(2), 271–275.
51. Gandhi M, Chikindas ML (2007) Listeria: A foodborne pathogen that knows how to survive. Int J Food Microbiol 113(1), 1–15.
52. Williams SK, Roof S, Boyle EA, Burson D, Thippareddi H, Geornaras I, Sofos JN, Wiedmann M, Nightingale K (2011) Molecular ecology of Listeria monocytogenes and other Listeria species in small and very small ready-to-eat meat processing plants. J Food Prot 74(1), 63–77.
53. Gutierrez D, Delgado S, Vazquez-Sanchez D, Martinez B, Cabo ML, Rodriguez A, Herrera JJ, Garcia P (2012) Incidence of Staphylococcus aureus and Analysis of Associated Bacterial Communities on Food Industry Surfaces. Appl Environ Microbiol 78(24), 8547–8554.
54. Giovannacci I, Ragimbeau C, Queguiner S, Salvat G, Vendeuvre J-L, Carlier V, Ermel G (1999) Listeria monocytogenes in pork slaughtering and cutting plants. Int J Food Microbiol 53(2-3), 127–140.
55. Berrang ME, Meinersmann RJ, Frank JF, Smith DP, Genzlinger LL (2005) Distribution of Listeria monocytogenes Subtypes within a Poultry Further Processing Plant. J Food Prot 68(5), 980–985.
56. Wilks SA, Michels H, Keevil CW (2005) The survival of Escherichia coli O157 on a range of metal surfaces. Int J Food Microbiol 105(3), 445–454.
57. Wilks SA, Michels HT, Keevil CW (2006) Survival of Listeria monocytogenes Scott A on metal surfaces: implications for cross-contamination. Int J Food Microbiol 111(2), 93–98.
58. da Silva Meira QG, Medeiros Barbosa I de, Alves Aguiar Athayde AJ, Siqueira-Júnior JP de, Souza EL de (2012) Influence of temperature and surface kind on biofilm formation by Staphylococcus aureus from food-contact surfaces and sensitivity to sanitizers. Food control 25(2), 469–475.
59. Martinon A, Cronin UP, Quealy J, Stapleton A, Wilkinson MG (2012) Swab sample preparation and viable real-time PCR methodologies for the recovery of Escherichia coli, Staphylococcus aureus or Listeria monocytogenes from artificially contaminated food processing surfaces. Food control 24(1–2), 86–94.
60. Ferreira V, Wiedmann M, Teixeira P, Stasiewicz MJ (2014) Listeria monocytogenes Persistence in Food-Associated Environments: Epidemiology, Strain Characteristics, and Implications for Public Health. J Food Prot 77(1), 150–170.
61. Di Ciccio P, Vergara A, Festino AR, Paludi D, Zanardi E, Ghidini S, Ianieri A (2015) Biofilm formation by Staphylococcus aureus on food contact surfaces: Relationship with temperature and cell surface hydrophobicity. Food control 50, 930–936.
62. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerging Infect. Dis. 8(9), 881–890.
63. Jessen B, Lammert L (2003) Biofilm and disinfection in meat processing plants. Int Biodeterior Biodegradation 51(4), 265–269.

64. Klayman BJ, Volden PA, Stewart PS, Camper AK (2009) Escherichia coli O157: H7 requires colonizing partner to adhere and persist in a capillary flow cell. Environ Sci Technol 43(6), 2105–2111.
65. Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, Doulgeraki A, Nychas G-J, Kačániová M, Czaczyk K, Ölmez H, Simões M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6, 841.
66. Sinde E, Carballo J (2000) Attachment of Salmonella spp. and Listeria monocytogenes to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol 17(4), 439–447.
67. Chmielewski R, Frank J (2003) Biofilm Formation and Control in Food Processing Facilities. Comp Rev Food Sci Food Safety 2(1), 22–32.
68. Lin C-M, Takeuchi K, Zhang L, Dohm CB, Meyer JD, Hall PA, Doyle MP (2006) Cross-Contamination between Processing Equipment and Deli Meats by Listeria monocytogenes. J Food Prot 69(1), 71–79.
69. Sasahara KC, Zottola EA (1993) Biofilm Formation by Listeria monocytogenes Utilizes a Primary Colonizing Microorganism in Flowing Systems. J Food Prot 56(12), 1022–1028.
70. Hassan AN, Birt DM, Frank JF (2004) Behavior of Listeria monocytogenes in a Pseudomonas putida Biofilm on a Condensate-Forming Surface. J Food Prot 67(2), 322–327.
71. Carpentier B, Chassaing D (2004) Interactions in biofilms between Listeria monocytogenes and resident microorganisms from food industry premises. Int J Food Microbiol 97(2), 111–122.
72. Castonguay M-H, van der Schaaf S, Koester W, Krooneman J, van der Meer W, Harmsen H, Landini P (2006) Biofilm formation by Escherichia coli is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. Res Microbiol 157(5), 471–478.
73. Giaouris ED, Nychas G-JE (2006) The adherence of Salmonella Enteritidis PT4 to stainless steel: The importance of the air–liquid interface and nutrient availability. Food Microbiol 23(8), 747–752.
74. Dourou D, Ammor MS, Skandamis PN, Nychas G-JE (2011) Growth of Salmonella enteritidis and Salmonella typhimurium in the presence of quorum sensing signalling compounds produced by spoilage and pathogenic bacteria. Food Microbiol 28(5), 1011–1018.
75. Dawson P, Han I, Cox M, Black C, Simmons L (2007) Residence time and food contact time effects on transfer of Salmonella Typhimurium from tile, wood and carpet: testing the five-second rule. J Appl Microbiol 102(4), 945–953.
76. Moerman F (2014) Antimicrobial materials, coatings and biomimetic surfaces with modified microtography to control microbial fouling of product contact surfaces within food processing equipment: Legislation, requirements, effectiveness and challenges. Journal of Hygienic Engineering and Design 7, 8–29.
77. Bloomfield SF, Scott E (1997) Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. J Appl Microbiol 83(1), 1–9.
78. Wachtel MR, McEvoy JL, Luo Y, Williams-Campbell AM, Solomon MB (2003) Cross-Contamination of Lettuce (Lactuca sativa L.) with Escherichia coli O157: H7 via Contaminated Ground Beef. J Food Prot 66(7), 1176–1183.
79. da Silva Malheiros P, dos Passos CT, Casarin LS, Serraglio L, Tondo EC (2010) Evaluation of growth and transfer of Staphylococcus aureus from poultry meat to surfaces of stainless steel and polyethylene and their disinfection. Food control 21(3), 298–301.
80. Møretrø T, Langsrud S (2011) Effects of Materials Containing Antimicrobial Compounds on Food Hygiene. J Food Prot 74(7), 1200–1211.
81. Jensen DA, Friedrich LM, Harris LJ, Danyluk MD, Schaffner DW (2013) Quantifying transfer rates of Salmonella and Escherichia coli O157:H7 between fresh-cut produce and common kitchen surfaces. J Food Prot 76(9), 1530–1538.
82. Al-Adawi AS, Gaylarde CC, Sunner J, Beech IB (2016) Transfer of bacteria between stainless steel and chicken meat: A CLSM and DGGE study of biofilms. AIMS Microbiol. 2(3), 340–358.
83. EFSA E (2013) The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011. EFSA J. 11(4), 3129.
84. Schmidt K (1995) WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe: 6th report 1990-1992. Berlin.

85. Srey S, Jahid IK, Ha S-D (2013) Biofilm formation in food industries: A food safety concern. Food control 31(2), 572–585.
86. Miranda RC, Schaffner DW (2016) Longer Contact Times Increase Cross-Contamination of Enterobacter aerogenes from Surfaces to Food. Appl Environ Microbiol 82(21), 6490–6496.
87. McLinden T, Sargeant JM, Thomas MK, Papadopoulos A, Fazil A (2014) Component costs of foodborne illness: a scoping review. BMC Public Health 14, 509.
88. Scott WG, Scott HM, Lake RJ, Baker MG (2000) Economic cost to New Zealand of foodborne infectious disease. N Z Med J 113(1113), 281–284.
89. Scharff RL (2012) Economic burden from health losses due to foodborne illness in the United States. J Food Prot 75(1), 123–131.
90. Batz MB, Hoffmann S, Morris JG, JR (2012) Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. J Food Prot 75(7), 1278–1291.
91. Jemmi T, Stephan R (2006) Listeria monocytogenes: food-bornepathogen and hygiene indicator. Rev Sci Tech 25(2), 571–580.
92. Bruckner S (2010) Predictive shelf life model for the improvement of quality management in meat chains. Dissertation. Bonn.
93. Kampmann Y (2010) Assessment of sustainable antimicrobial methods with regard to their ability to reduce airborne and surface bacteria in the food supply chain. Dissertation. Bonn.
94. Kreyenschmidt J, Albrecht A, Braun C, Herbert U, Mack M, Roissant S, Ritter G, Teitscheid P, Ilg Y (2013) Food Waste in der Fleisch verarbetenden Kette: Um Lebensmittetverluste zu minimieren, sind Handlungen entlang der Kette Fleisch notwendig. Fleischwirtschaft 93, 57–63.
95. Olsen SJ, Patrick M, Hunter SB, Reddy V, Kornstein L, MacKenzie WR, Lane K, Bidol S, Stoltman GA, Frye DM, Lee I, Hurd S, Jones TF, LaPorte TN, Dewitt W, Graves L, Wiedmann M, Schoonmaker-Bopp DJ, Huang AJ, Vincent C, Bugenhagen A, Corby J, Carloni ER, Holcomb ME, Woron RF, Zansky SM, Dowdle G, Smith F, Ahrabi-Fard S, Ong AR, Tucker N, Hynes NA, Mead P (2005) Multistate Outbreak of Listeria monocytogenes Infection Linked to Delicatessen Turkey Meat. Clin. Infect. Dis. 40(7), 962–967.
96. Rossaint S, Kreyenschmidt J (2015) Intelligent label – a new way to support food waste reduction. Proceedings of the Institution of Civil Engineers - Waste and Resource Management 168(2), 63–71.
97. Dohlen S (2016) 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. Dissertation. Bonn.
98. Langston W) (1995) Tributyl tin in the marine environment: a review of past and present risks.
99. Meyer B (2003) Approaches to prevention, removal and killing of biofilms. Int Biodeterior Biodegradation 51(4), 249–253.
100. Siedenbiedel F, Tiller JC (2012) Antimicrobial Polymers in Solution and on Surfaces: Overview and Functional Principles. Polymers (Basel) 4(4), 46–71.
101. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F (2011) Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27(9), 1017–1032.
102. Herald PJ, Zottola EA (1988) Attachment of Listeria monocytogenes to Stainless Steel Surfaces at Various Temperatures and pH Values. J Food Sci 53(5), 1549–1562.
103. Hasan J, Crawford RJ, Ivanova EP (2013) Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol 31(5), 295–304.
104. Gram L, Bagge-Ravn D, Ng YY, Gymoese P, Vogel BF (2007) Influence of food soiling matrix on cleaning and disinfection efficiency on surface attached Listeria monocytogenes. Food control 18(10), 1165–1171.
105. Sofos JN (2009) Biofilms: Our constant enemies. Food Saf. Mag. 38(February/March), 40–41.
106. Mettler E, Carpentier B (1998) Variations over Time of Microbial Load and Physicochemical Properties of Floor Materials after Cleaning in Food Industry Premises. J Food Prot 61(1), 57–65.
107. Clayborn J, Adams J, Baker CA, Ricke SC (2015) Assessment of Salmonella spp. Attachment to Reusable Plastic Containers Based on Scanning Electron Microscopy and BAX® PCR. J. Food Res. 4(2), 166.
108. Bizzaro S, Deneuve L, Vendeuvre JL (1990) Etude de la contamination microbienne des surfaces en entreprise. Viandes et Produits Carnés 11, 220.

109. Frank JF, Koffi RA (1990) Surface-adherent Growth of Listeria monocytogenes is Associated with Increased Resistance to Surfactant Sanitizers and Heat. J Food Prot 53(7), 550–554.
110. Holah J, Bird J, Hall K (2004) The microbial ecology of high-risk, chilled food factories; evidence for persistent Listeria spp. and Escherichia coli strains. J Appl Microbiol 97(1), 68–77.
111. Marouani-Gadri N, Augier G, Carpentier B (2009) Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection — Influence of isolated strains on biofilm formation by Sakaï and EDL 933 E. coli O157:H7. Int J Food Microbiol 133(1–2), 62–67.
112. Chaitiemwong N, Hazeleger WC, Beumer RR (2010) Survival of Listeria monocytogenes on a conveyor belt material with or without antimicrobial additives. Int J Food Microbiol 142(1–2), 260–263.
113. Zottola EA, Sasahara KC (1994) Microbial biofilms in the food processing industry—Should they be a concern? Int J Food Microbiol 23(2), 125–148.
114. Gibson H, Taylor JH, Hall KE, Holah JT (1999) Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. J Appl Microbiol 87(1), 41–48.
115. Lynch MF, Tauxe RV, Hedberg CW (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137(3), 307–315.
116. Redmond EC, Griffith CJ (2003) Consumer Food Handling in the Home: A Review of Food Safety Studies. J Food Prot 66(1), 130–161.
117. Gkana E, Lianou A, Nychas G-JE (2016) Transfer of Salmonella enterica Serovar Typhimurium from Beef to Tomato through Kitchen Equipment and the Efficacy of Intermediate Decontamination Procedures. J Food Prot 79(7), 1252–1258.
118. Møretrø T, Høiby-Pettersen GS, Habimana O, Heir E, Langsrud S (2011) Assessment of the antibacterial activity of a triclosan-containing cutting board. Int J Food Microbiol 146(2), 157–162.
119. Zhu J, Bai Y, Wang Y, Song X, Cui S, Xu H, Jiao X, Li F (2017) A risk assessment of salmonellosis linked to chicken meals prepared in households of China. Food control 79, 279–287.
120. Kenawy E-R, Worley SD, Broughton R (2007) The Chemistry and Applications of Antimicrobial Polymers: A State-of-the-Art Review. Biomacromolecules 8(5), 1359–1384.
121. Gundelley R, Youm GW, Kwon YM (2007) Survival of bacterial pathogens on antimicrobial conveyer belts. J Rapid Meth Aut Mic 15(3), 259–266.
122. Karam L, Jama C, Dhulster P, Chihib N-E (2013) Study of surface interactions between peptides, materials and bacteria for setting up antimicrobial surfaces and active food packaging. J Mater Environ Sci 4(5), 798–821.
123. Rocha M, Ferreira FA, Souza MM, Prentice C (2015) Antimicrobial films: a review, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 23–31. Spain: Formatex Research Center.
124. Williams K, Valencia L, Gokulan K, Trbojevich R, Khare S (2017) Assessment of antimicrobial effects of food contact materials containing silver on growth of Salmonella Typhimurium. Food Chem. Toxicol. 100, 197–206.
125. Mucha H (2005) Wirksamkeitsnachweise erhöhen Sicherheit. KGK, Kautsch. Gummi Kunstst. 58(12), 624–625.
126. Webb HK, Crawford RJ, Ivanova EP (2015) Introduction to Antibacterial Surfaces, in: Ivanova E, Crawford R (eds.) Antibacterial Surfaces, pp. 1–8. Cham: Springer International Publishing.
127. Tiller JC (2011) Antimicrobial Surfaces, in: Börner HG, Lutz J-F (eds.) Bioactive Surfaces, pp. 193–217: Springer Berlin Heidelberg.
128. Thebault P, Jouenne T (2015) Antibacterial coatings, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 483–489. Spain: Formatex Research Center.
129. Suppakul P, Miltz J, Sonneveld K, Bigger S (2003) Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications. J Food Sci 68(2), 408–420.
130. Irkin R, Esmer OK (2015) Novel food packaging systems with natural antimicrobial agents. J Food Sci Technol 52(10), 6095–6111.
131. Mauriello G (2016) Control of microbial activity using antimicrobial packaging, in: Barros-Velazquez J (ed.) Antimicrobial Food Packaging, pp. 141–152: Elsevier.

132. Park E-S (2015) Antimicrobial polymeric materials for packaging applications: A review, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 500–511. Spain: Formatex Research Center.
133. Thölmann D, Kossmann B, Sosna F (2003) Polymers with antimicrobial properties. EC Journal(1-2), 16–33.
134. Madkour AE, Dabkowski JM, Nusslein K, Tew GN (2009) Fast disinfecting antimicrobial surfaces. Langmuir 25(2), 1060–1067.
135. Coma V (2008) Bioactive packaging technologies for extended shelf life of meat-based products. Meat Sci 78(1–2), 90–103.
136. Kampmann Y, Clerck E de, Kohn S, Patchala DK, Langerock R, Kreyenschmidt J (2008) Study on the antimicrobial effect of silver-containing inner liners in refrigerators. J Appl Microbiol 104(6), 1808–1814.
137. Warnes SL, Keevil CW (2011) Mechanism of Copper Surface Toxicity in Vancomycin-Resistant Enterococci following Wet or Dry Surface Contact. Appl Environ Microbiol 77(17), 6049–6059.
138. Balasubramanian A, Rosenberg LE, Yam K, Chikindas ML (2009) Antimicrobial packaging: potential vs. reality—a review. J Appl Pack Res 3(4), 193–221.
139. Chung Y-c, Su Y-p, Chen C-c, Jia G, Wang H-l, Wu JCG, Lin J-g (2004) Relationship between antibacterial activityof chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 25(7), 932–936.
140. Devlieghere F, Vermeulen A, Debevere J (2004) Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol 21(6), 703–714.
141. Junker LM, Hay AG (2004) Effects of triclosan incorporation into ABS plastic on biofilm communities. J Antimicrob Chemother 53(6), 989–996.
142. Tabak M, Scher K, Hartog E, Romling U, Matthews KR, Chikindas ML, Yaron S (2007) Effect of triclosan on Salmonella typhimurium at different growth stages and in biofilms. FEMS Microbiol. Lett. 267(2), 200–206.
143. Endo Y, Tani T, Kodama M (1987) Antimicrobial activity of tertiary amine covalently bonded to a polystyrene fiber. Appl Environ Microbiol 53(9), 2050–2055.
144. Cutter CN (1999) Effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J Food Prot 62(5), 474–479.
145. Noyce JO, Michels H, Keevil CW (2006) Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing. Appl Environ Microbiol 72(6), 4239–4244.
146. Ilg Y, Kreyenschmidt J (2011) Effects of food components on the antimicrobial activity of polypropylene surfaces containing silver ions (Ag+). Int J Food Sci Technol 46(7), 1469–1476.
147. Møretrø T, Høiby-Pettersen GS, Halvorsen CK, Langsrud S (2012) Antibacterial activity of cutting boards containing silver. Food control 28(1), 118–121.
148. Braid JJ, Wale MCJ (2002) The antibacterial activity of triclosan-impregnated storage boxes against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Shewanella putrefaciens in conditions simulating domestic use. J Antimicrob Chemother 49(1), 87–94.
149. Faúndez G, Troncoso M, Paola N, Figueroa G (2004) Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni. BMC Microbiol 19(4).
150. Michels H, Noyce J, Keevil C (2009) Effects of temperature and humidity on the efficacy of methicillin-resistant Staphylococcus aureus challenged antimicrobial materials containing silver and copper. Lett Appl Microbiol 49(2), 191–195.
151. Chang S-H, Lin H-TV, Wu G-J, Tsai GJ (2015) pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. Carbohydr Polym 134, 74–81.
152. Radheshkumar C, Münstedt H (2006) Antimicrobial polymers from polypropylene/silver composites—Ag+ release measured by anode stripping voltammetry. Reactive and Functional Polymers 66(7), 780–788.
153. Damm C, Münstedt H (2008) Kinetic aspects of the silver ion release from antimicrobial polyamide/silver nanocomposites. Applied Physics A 91(3), 479–486.

154. Perez-Perez C, Regalado-González C, Rodríguez-Rodríguez CA, Barbosa-Rodríguez, JR, Villaseñor-Ortega F (2006) Incorporation of antimicrobial agents in food packaging films and coatings. Adv Agric Food Biotechnol, 193–216.
155. Bastarrachea L, Dhawan S, Sablani SS (2011) Engineering properties of polymeric-based antimicrobial films for food packaging: a review. Food Engineering Reviews 3(2), 79–93.
156. Casemiro LA, Martins CHG, Pires-de-Souza FdC, Panzeri H (2008) Antimicrobial and mechanical properties of acrylic resins with incorporated silver–zinc zeolite–part I. Gerodontology 25(3), 187–194.
157. Brodkorb F, Fischer B, Kalbfleisch K, Robers O, Braun C, Dohlen S, Kreyenschmidt J, Lorenz R, Kreyenschmidt M (2015) Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. Int J Mol Sci 16(8), 20050–20066.
158. Buranasompob A (2005) Kinetics of the inactivation of microorganisms by water insoluble polymers with antimicrobial activity. Dissertation. Berlin.
159. Zuo H, Wu D, Fu R (2012) Preparation of antibacterial poly(methyl methacrylate) by solution blending with water-insoluble antibacterial agent poly[(tert-buty1amino) ethyl methacrylate]. J Appl Polym Sci 125(5), 3537–3544.
160. Hewitt CJ, Franke R, Marx A, Kossmann B, Ottersbach P (2004) A study into the anti-microbial properties of an amino functionalised polymer using multi-parameter flow cytometry. Biotechnol Lett 26(7), 549–557.
161. Lenoir S, Pagnoulle C, Galleni M, Compère P, Jérôme R, Detrembleur C (2006) Polyolefin Matrixes with Permanent Antibacterial Activity: Preparation, Antibacterial Activity, and Action Mode of the Active Species. Biomacromolecules 7(8), 2291–2296.
162. Seyfriedsberger G, Rametsteiner K, Kern W (2006) Polyethylene compounds with antimicrobial surface properties. Eur Polym J 42(12), 3383–3389.
163. Marra J, Paleari AG, Rodriguez LS, Leite ARP, Pero AC, Compagnoni MA (2012) Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. J Appl Oral Sci 20, 643–648.
164. Paleari AG, Marra J, Pero AC, Rodriguez LS, Ruvolo-Filho A, Compagnoni MA (2011) Effect of incorporation of 2-tert-butylaminoethyl methacrylate on flexural strength of a denture base acrylic resin. J Appl Oral Sci 19, 195–199.
165. Kreyenschmidt M, Lorenz R, Fischer B, Kreyenschmidt J, Brodkorb F, Kalbfleisch K, Blang T, Geschwentner A (2014) Anti-microbial polymer: WO2014118339 A1.

2 Antimicrobial activity of intrinsic antimicrobial polymers based on poly((tert-butyl-amino)-methyl-styrene) against selected pathogenic and spoilage microorganisms relevant in meat processing facilities

2.1 Abstract

Objective: Antimicrobial materials are used as a possible approach to improve hygienic conditions in the food industry. The aim of this study was the investigation of the antimicrobial activity of the homopolymer of poly((tert-butyl-amino)-methyl-styrene) (poly(TBAMS)) and of the copolymer poly(TBAMS:acrylonitrile) [1:1] against microorganism present on meat processing equipment.

Method: Antimicrobial polymers were characterized by Fourier transform infrared spectroscopy and differential scanning calorimetry analysis. The antimicrobial activity against various pathogenic and spoilage bacteria (*S. aureus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp., *Pseudomonas* spp., *B. thermosphacta*) was determined using a modified test method based on the Japanese Industrial Standard JIS Z 2801: 2000. Furthermore, the influence of high initial bacterial counts (up to 8.9 $\log_{10}$ cfu ml^{-1}) as well as the exposure of bacteria in mixed cultures on the antimicrobial activity was evaluated.

Results: Spectroscopy identified the homopolymer poly(TBAMS) as well as a successful copolymerization with acrylonitrile. Results of antimicrobials tests showed significant reductions of bacterial counts on both polymers compared with the reference material of microorganisms in pure culture after 2 h at 35 °C. *L. monocytogenes*, *E. coli* and *S. aureus* were reduced to the detection limit (>4.2 $\log_{10}$-units). *P. fluorescens* was less sensitive to poly(TBAMS)-based films, especially to the copolymer. The homopolymer offers slightly higher activity than the copolymer, but glass transition temperature was lower. Tests with mixed cultures affirmed the dependency of activity on bacteria species. A tendency of higher antimicrobial activity against gram-positive was observed, if high initial counts were used; however, significant reduction of gram-negative were still determined.

Conclusion: Poly(TBAMS)-films show excellent antimicrobial properties against microorganisms relevant in meat processing facilities, and the implementation of those surfaces could contribute to improving the hygienic conditions during production and processing.

PUBLISHED IN THE JOURNAL OF ANTIMICROBIAL AGENTS SEE LIST OF PUBLICATIONS (BRAUN ET AL. 2016)

2.2 Introduction

In the meat processing industry, a mixture of spoilage bacteria like *Pseudomonas* spp. or *Brochothrix thermosphacta* as well as pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes,* is continuously prevalent [1–3]. The bacteria attach, grow and form multi-species biofilms on surfaces and consequently present a source of microbial contamination [3, 4]. A crossover of bacteria from the contaminated food contact surfaces to meat could lead to deteriorative changes in the quality and safety of the products and to a decreased shelf life [2, 5–9].

Therefore, the control and improvement of hygienic conditions during meat processing is of high importance. A promising procedure to improve the hygienic status of food contact surfaces is the application of antimicrobial materials in the food industry [10–13]. Due to the self-sterilizing effect of such surfaces, the bacterial contamination is reduced even between cleaning and disinfection steps and furthermore the treated surfaces are protected against biodegradation [5, 11, 13, 14]. In last decades, a wide spectrum of antimicrobial agents, ranging from plant extracts [15, 16], enzymes [17], antimicrobial peptides [18] and metals [19–21] to bioactive polymers [22, 23], were researched for the application in food contact materials [12, 24, 25]. Generally, the application can be conducted via integration of the agents in the material of food contact surfaces themselves or by coating existing surfaces.

Depending on the mode of biocidal action, the resulting surfaces are classified as biocide releasing or contact-active surfaces [26]. A new class of non-leaching, contact-active surfaces are SAM-Polymers® (sustainable active microbiocidal) [22]. Poly(tert-butyl-amino-ethyl)-methacrylate (poly(TBAEMA)) is the most comprehensively investigated agent in this polymer group. It has a good antimicrobial activity against a wide range of microorganisms, while exhibiting only a low toxicity in mammals [22]. It seems to be evident that the amino functionalized groups, located on the surface due to the three-dimensional structure of the polymers, are responsible for the antimicrobial activity. Hewitt et al. [27] clarify that physical interactions, chemical reactions or a combination of both are required for the antimicrobial activity. The exposure of bacteria to the polymers initially leads to a depolarization of the cytoplasmic membrane resulting in permeability which initiates cell death through a release of fibrous and cellular material [27, 28]. Lenoir et al. [28] assumed that the charged amino groups replace divalent cations of the outer membrane, which leads to membrane disorganization. A certain degree of protonation of the amino groups is necessary for the antimicrobial activity [29]. The resulting localized pH-gradient and additional electrostatic interactions between the positively charged surface of the polymer and the negatively charged bacteria membrane are responsible for the antimicrobial activity.

In addition to the antimicrobial activity, the material properties are relevant for the implementation of antimicrobial surfaces in the food industry. According to Thölmann et al. [22], poly(TBAEMA) is insoluble in water, possesses a glass transition temperature T_G of about

40 °C and temperature stability up to 180 °C. But this low T_G is, next to its high water uptake, a weak point of poly(TBAEMA) [30], because it reduces the processability and usability of these polymers. Brodkorb et al. [30] developed and characterized a new monomer (tert-butyl-amino)-methyl-styrene (TBAMS). Poly(TBAMS), the corresponding intrinsically antimicrobial active polymer offers improved properties in comparison to poly(TBAEMA). Hence, poly(TBAMS) shows a T_G of about 68 °C, which can be further increased by copolymerization [30]. In addition, the water uptake of poly(TBAMS) is low and the heat resistance is reasonable [30, 31].

Due to these material properties, polymer films based on TBAMS are potentially suited for the use as food contact material. Up to now, it is not clear if these polymers are active over the broad microbial spectrum which is typical in meat production and processing.

The aim of this study is the investigation of the antimicrobial activity of two films based on poly((tert-butyl-amino)-methyl-styrene) against various pathogenic and spoilage bacteria relevant in meat processing facilities. In the first step, therefore, two films with different poly(TBAMS)-concentrations were characterized via Fourier transform infrared (FTIR) spectroscopy, and differential scanning calorimetry (DSC) analyses were done to determine the T_G as one important parameter for material usability as a food contact surface. Subsequently, the antimicrobial activity of both films was screened against various pure bacteria cultures. In the next step, the effect of increased initial counts of bacteria on the antimicrobial activity of poly(TBAMS) films was tested. In the third antimicrobial test series, mixed cultures containing *Pseudomonas* spp., a typical biofilm former, were used to simulate processing conditions and to investigate whether interactions between the bacteria species or different electrostatic interactions between bacterium and antimicrobial surfaces influence the antimicrobial activity against individual bacteria species.

2.3 Materials and Methods

2.3.1 Antimicrobial test material

Two polymer, the homopolymer poly(TBAMS) and the copolymer poly(TBAMS:acrylonitrile) [1:1], were investigated. Figure 2.1 shows the chemical structure of the polymers used. For each homopolymer sample, 125 mg of purified polymer was dissolved in 3 ml ethanol under stirring. The solution was then cast in a petri dish without vents (polystyrene, VWR, Germany) and dried in a vacuum drying cabinet at 70 °C and 2 mbar for 1 h, resulting in colourless and transparent polymer films. For activity tests of the copolymer, a polyethylene film (40 µm, corona pre-treated) was coated with a poly(TBAMS:acrylonitrile) [1:1] solution in ethylacetate, resulting in a 0.08 µm thick layer of the copolymer, and trimmed into circular test pieces (94 mm in diameter). The reference material used depended on the sample material. Clear petri dishes of the same size (diameter: 94 mm, without vents) and petri dishes with trimmed pieces of PE-film were used as references for the homopolymer and for the copolymer respectively.

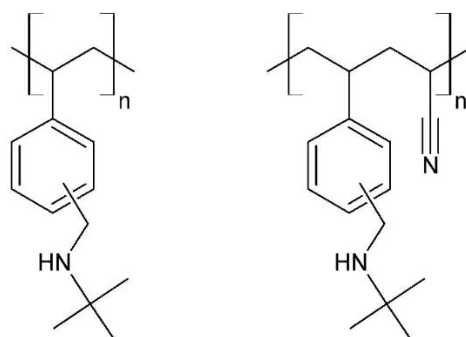

Figure 2.1 Chemical structure of poly(TBAMS) (left) and poly(TBAMS:acrylonitrile) (right) consisting of a mixture of meta- and para-isomers.

2.3.2 Characterization of polymers

Infrared spectra were recorded via a Spectrum two FT-IR spectrometer (Perkin Elmer, Waltham, USA) with UATR two technique. The method was attenuated total reflection (ATR) in the range of 450 cm^{-1} to 4000 cm^{-1}. Differential scanning calorimetry (DSC) analysis was carried out on a DSC 821e (Mettler Toledo, Greifensee, Swiss) system.

2.3.3 Bacterial strains

To test the antimicrobial activity nine typical pathogenic and spoilage microorganisms were chosen as test organisms (Table 2.1). For both categories, at least one gram-negative as well as one gram-positive representative bacterium was tested.

2.3.4 Preparation of inoculum

All bacteria strains were stored at -18 °C in a CRYOBANK^{TM} system (Mast, Reinfeld, Germany). The inoculum was prepared by transferring a frozen culture to 10 ml nutrient broth (Merck KGaA, Darmstadt, Germany). Afterwards the broth was incubated overnight at cultivating temperatures (Table 1). At the beginning of each trial, the overnight culture was diluted in physiological saline solution with tryptone (1 g l^{-1}) (Oxoid, Hampshire, United Kingdom) to a final concentration of 10^5 cfu ml^{-1}. In addition to the pure cultures, mixed cultures were used for the antimicrobial activity tests. In a first step the different gram-negative *Pseudomonas* spp. (*P. aeruginosa*, *P. fluorescens*, *P. putida*) were mixed with the gram-positive bacteria *B. thermosphacta*. In a second step, based on the results, *P. fluorescens* was mixed with the two pathogens *E. coli* or *L. monocytogenes,* which differ in gram reaction. For the mixed inocula, each culture was initially prepared and diluted separately, and the two different cultures were mixed in the final dilution step. For the tests with high initial concentrations (second experiment series) 0.1 ml of the overnight cultures in nutrient broth were transferred in 10 ml saline solution with tryptone and were incubated another night leading to concentrations of 6.6-8.9 log_{10} cfu ml^{-1}. These solutions were used as inocula in the test trials.

Table 2.1 Summary of tested bacteria, cultivating temperature, and used selective media in mixed cultures.

Bacteria	Strain	Cultivating temperature	Selective medium
Brochothrix thermosphacta	ATCC 20171	25 °C	Streptomycin inosit toluylene red agar (SIN agar) referring to the method of Hechelmann [32] (Sheep Blood Agar Base, Oxoid, Cambridge, United Kingdom)
Listeria monocytogenes	ATCC 19111	37 °C	*Listeria* agar according to Ottaviani and Agosti (ALOA, Oxoid, Cambridge, United Kingdom)
Staphylococcus aureus	ATCC 6538	37 °C	Baird Parker agar (Oxoid, Cambridge, United Kingdom)
Escherichia coli	ATCC 8739	37 °C	Violet red bile dextrose agar (VRBD, Merck, Darmstadt, Germany)
Pseudomonas aeruginosa	ATCC 15442	30 °C	*Pseudomonas* agar with cetrimide sodium nalidixate (CN) selective supplement (Oxoid, Cambridge, United Kingdom)
Pseudomonas fluorescens	ATCC 13525	25 °C	*Pseudomonas* agar with cetrimide fucidin cephaloridine (CFC) selective supplement
Pseudomonas putida	ATCC 12633	25 °C	*Pseudomonas* agar with cetrimide sodium nalidixate (CN) selective supplement
Salmonella enterica subsp. *enterica* serovar Enteritidis *9:g,m:-*	DSM 14221	37 °C	*
Salmonella enterica subsp. *enterica* serovar Typhimurium	ATCC 14028	37 °C	*

*Not tested in mixed culture

2.3.5 Test performance

Tests were conducted on the basis of the Japanese Industrial Standard (JIS) Z 2801:2000, a quantitative method to investigate the antibacterial effectiveness of plastic surfaces treated with an antibacterial agent. The antibacterial effectiveness is determined by the value of antibacterial activity, which is defined as the difference of the logarithm (log_{10}-reduction) of the bacterial count on untreated materials (reference) and treated materials (sample) after inoculation with microorganisms and incubation under defined conditions.

Per test standard a minimum of three samples and six references were tested in every trial for each bacteria inocula in the experiment series. The total number of separate samples per bacteria strain varied between 3 and 33. Most separate samples were tested of the homopolymer against *S. aureus* to prove the reproducibility of the material's effect.

In all experimental series, the materials were inoculated with 0.4 ml of bacteria solution. To prevent evaporation and to standardize the contact area, sterile PE films (40x40 mm²) covered

the inocula loosely. Three references were washed out immediately after inoculation (t=0 h) by rinsing via pipette with 10 ml soybean-casein digest broth with lecithin polysorbat (Roth, Karlsruhe, Germany) to determine the initial concentration. Sample surfaces and remaining references were incubated at 35 °C and high relative humidity (80 -90 %) for 2 h. Data loggers (Testo 174H, Testo AG, Lenzkirchen, Germany) monitored the temperature and humidity in five-minute intervals. Afterwards (t=2 h), they were washed out in a similar manner. Viable counts of the pure culture tests of the first two test series were determined by counting the colonies on plate count agar (Roth, Karlsruhe, Germany) using the pour plate technique. Plates were incubated for 48 h at the appropriate cultivating temperature of the bacteria (table 1). The tests of the first series were conducted with both types of films. For the experiments with high initial counts, the copolymer poly(TBAMS:acrylonitrile) [1:1] were used. In the third experimental series, the antimicrobial activity of the copolymer was tested against mixed cultures. The total viable counts (TVC) of the mixed culture were also enumerated on plate count agar (pour plate technique); to determine the individual bacterial counts, different selective media (drop plate technique), table 1, were used in addition to the plate count agar. Mentioned optimal cultivating temperatures were used for the selective media in the mixed culture test series, while the plate count agar plates were incubated at 30 °C for the determination of total viable counts when the cultivating temperature of the two bacteria varied. Detection limits for all tests were determined to be 1.0 $\log_{10}$ cfu ml^{-1} for pour plate technique and 2.0 $\log_{10}$ cfu ml^{-1} for drop plate technique.

2.3.6 Analysis

Reduction or growth on material after 2 h incubation was calculated by subtracting the logarithmic average value of bacterial concentration on reference material immediately after inoculation ($N_{t=0}$) from the average value of bacterial concentration on the reference (Ref) and sample (SAM) material after 2 h incubation ($N_{t=2}$) (Eq. 2.1)

$$f(N_{Ref,\ t=0}, N_{t=2}) = \log_{10}(N_{Ref,\ t=0}) - \log_{10}(N_{t=2}). \tag{2.1}$$

Standard errors (df) were calculated following the Gaussian propagation of uncertainty (Eq. 2.2)

$$df = \sqrt{\left(\frac{1}{N_{Ref,t=0} \times \ln 10} \times dN_{t=0}\right)^2 + \left(\frac{-1}{N_{t=2} \times \ln 10} \times dN_{t=2}\right)^2} \tag{2.2}$$

where $N_{Ref,t=0}$= average bacterial concentration on the reference material immediately after inoculation; d=standard error, and $N_{t=2}$= average bacterial concentration on the reference respectively sample material after 2 h incubation, ln= natural logarithm base e.

The value of the antimicrobial activity was calculated by subtracting the logarithmic value of the viable counts on the sample material from the logarithmic value of the reference material after inoculation and incubation (Eq. 2.3):

$$\log_{10}\text{-reduction} = \log_{10}\left(N_{Ref,t=2}/N_{SAM,t=2}\right) \quad (2.3)$$

with $N_{Ref,\,t=2}$= average of bacterial concentration on reference material, and $N_{SAM,\,t=2}$= average of bacterial concentration on sample material both after 2 h incubation.

According to the JIS Z 2801:2000 a material can be characterized as antimicrobial if the calculated $\log_{10}$-reduction is ≥ 2.0 after 24 h at 35 °C.

Statistical significance (n>3) in reduction-levels was tested using the Mann–Whitney U test in SPSS 22 (IBM®SPSS®Statictics). Significance was defined as p≤0.05. Figures of antimicrobial activity were generated with the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, USA).

2.4 Results and Discussion

Both polymer films were characterized by FTIR and the spectra are depicted in figure 2.2. For the homopolymer poly(TBAMS) the secondary amine (R-NH-R) is observed at 3310 cm^{-1}. The aromatic hydrogen (Ar-H) is located at 3017 cm^{-1}. The aromatic structure of poly(TBAMS) can be explained due to following bands: 1605 cm^{-1}, 1510 cm^{-1} and 1443 cm^{-1}. The aromatic system is meta (704 cm^{-1} and 793 cm^{-1}) and para (819 cm^{-1}) substituted. 2961 cm^{-1}, 2925 cm^{-1} and 2866 cm^{-1} indicate symmetric and asymmetrical stretching vibrations of $-CH_3$ and $-CH_2$ groups. The tertiary butyl group belongs to 1360 cm^{-1} and 1386 cm^{-1}. Both wave numbers 1089 cm^{-1} as well as 1019 cm^{-1} cannot be assigned to functional groups in poly(TBAMS) but they are characteristic. Results confirm with analyses of Brodkorb et al. [30] and identified the used material as the newly described SAM-Polymer®. For the copolymer used, the nitrile group (R-CN) is observed at 2238 cm^{-1}, whereas the other bands show almost the same wave numbers and characteristics compared with poly(TBAMS), proving a successful copolymerization.

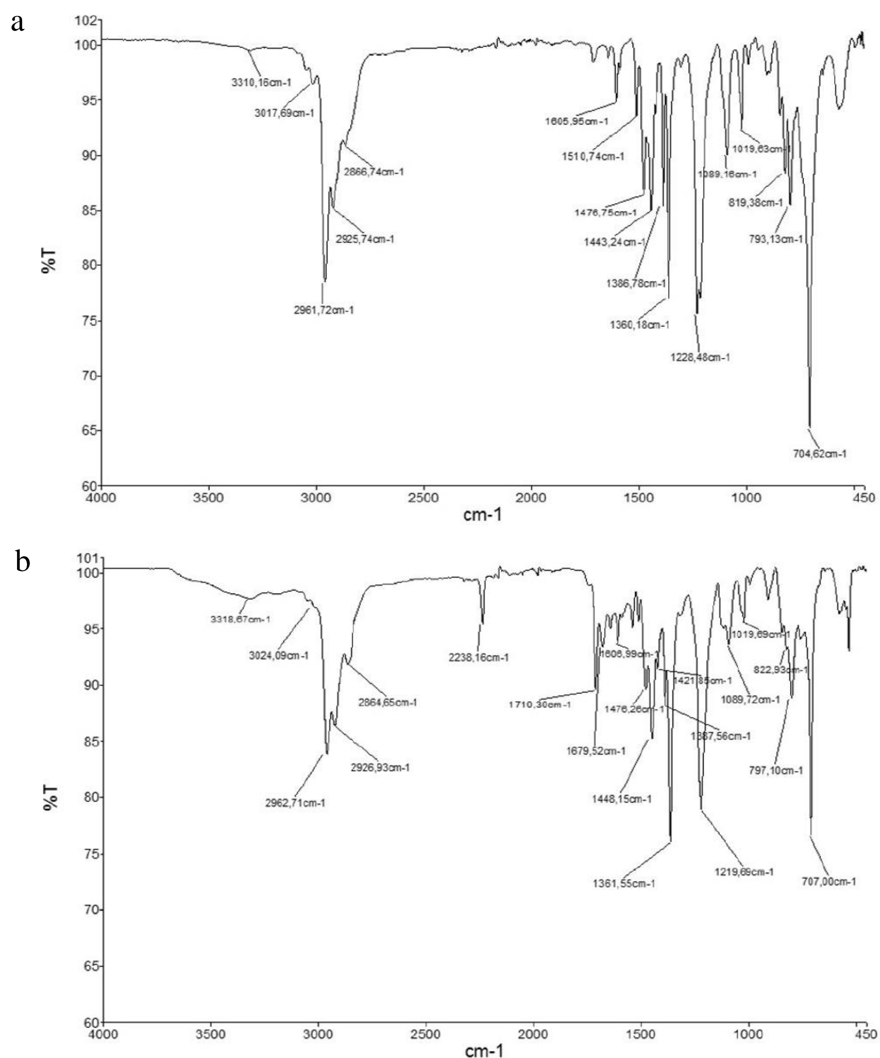

Figure 2.2 FTIR-ATR spectrum of the homopolymer poly(TBAMS) (a) and of the copolymer poly(TBAMS:acrylonitrile) (b).

The copolymerization increased the T_G from 68 °C for the homopolymer, to 103 °C for the copolymer (Figure 2.3). The T_G is one important parameter for the processability and usability of the polymers as a food contact material. Thus, the copolymer offers better material properties (higher T_G and lower water uptake) than the homopolymer, but copolymerization can possibly influence the antimicrobial activity. Thus, both materials were screened for antimicrobial activity.

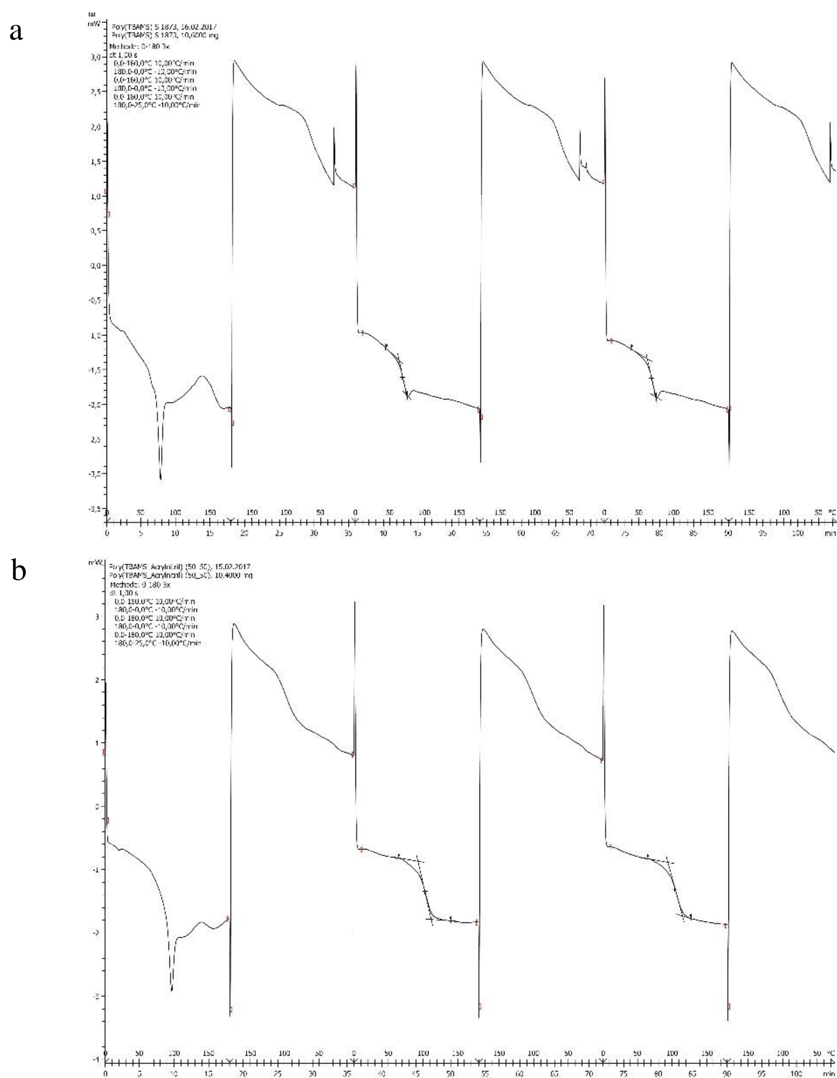

Figure 2.3 DSC analysis of poly(TBAMS) (a) and poly(TBAMS:acrylonitrile) (b).

The antimicrobial tests revealed good antimicrobial properties against various bacteria relevant in meat processing facilities for both tested intrinsically antimicrobial polymers based on poly((tert-butyl-amino)-methyl-styrene). Figure 2.4a shows the reduction of bacterial count observed on the reference material and the homopolymer poly(TBAMS) after 2 h contact at 35 °C. Comparing the reductions of all bacteria, significantly more bacteria were reduced on poly(TBAMS) then on the reference material ($p<0.001$). The highest reduction was determined for *E. coli*, which was decreased from an initial average concentration of 5.73±0.01 $\log_{10}$ cfu ml^{-1} down to the detection limit (1.0 $\log_{10}$ cfu ml^{-1}) on all samples (n= 12). At the same time, *E. coli* showed the second highest growth on the reference material during two hours; the high increase of bacterial count on the reference material, in conjunction with the high initial count, results in the observation of the highest $\log_{10}$-reduction of 5.6 $\log_{10}$-steps. The bacterial counts of gram-positive *L. monocytogenes*, *S. aureus* and gram-negative *P. aeruginosa* were reduced to detection limit. The $\log_{10}$-reductions of the tested bacteria vary among 2.4 and 5.6 $\log_{10}$-steps, which classify the material as antimicrobial according to the JIS already after 2 h at 35 °C. The gram-negative bacteria *S. enterica* (Serovar Enteritidis) was the less sensitive

bacteria, however, the bacterial count on poly(TBAMS) was reduced 2.4 $\log_{10}$-steps in comparison to reference material.

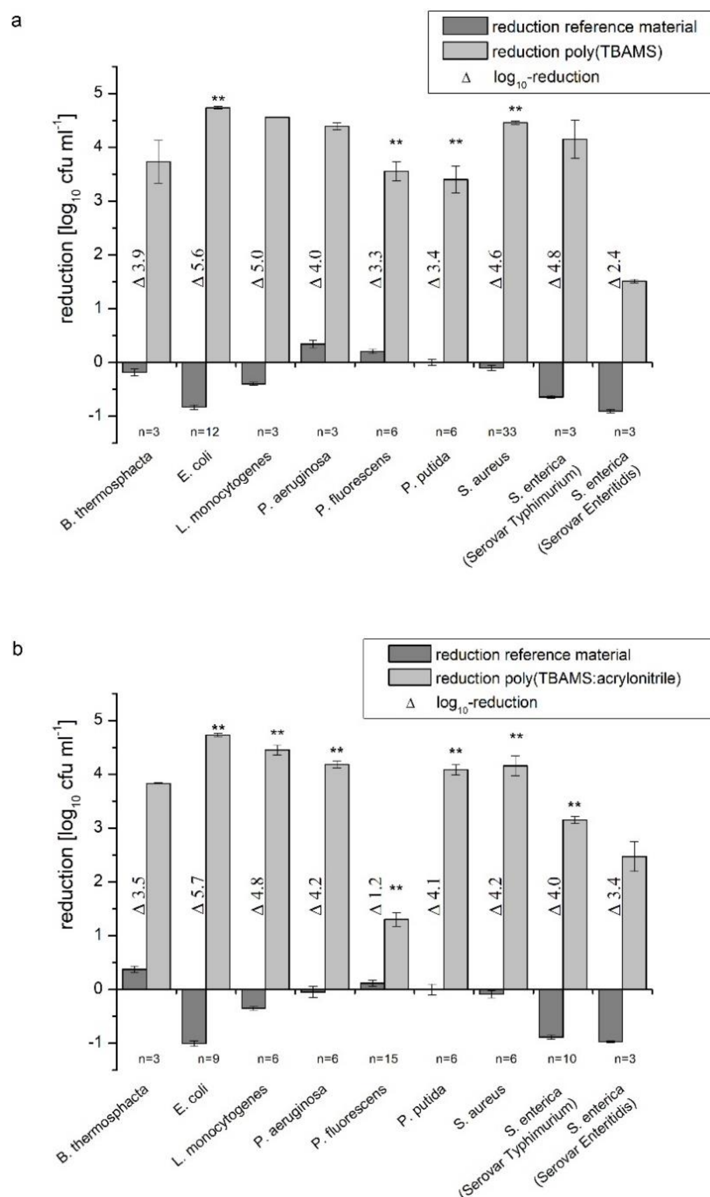

Figure 2.4 Reduction [$\log_{10}$ cfu ml^{-1}] of bacteria after 2 h incubation at 35 °C applied on reference material (dark grey bars) or sample material (light grey bars): (a) homopolymer poly(TBAMS) or (b) copolymer poly(TBAMS:acrylonitrile). The values are changes from initial concentration (a: 4.9- 5.9 $\log_{10}$ cfu ml^{-1}, b: 4.8- 5.7 $\log_{10}$ cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on sample material after incubation ($\log_{10}$-reduction). Asterisks indicate significant differences (** $p \leq 0.005$, * $p \leq 0.05$) between sample and reference material (n>3).

33 samples of the homopolymer poly(TBAMS) were tested with *S. aureus* to investigate the reproducibility of the material. The low standard error (4.5±0.03 $\log_{10}$ cfu ml^{-1}) of the bacterial reduction of the sample material shows the high reproducibility of the antimicrobial activity of the poly(TBAMS).

To test if copolymerization influences the antimicrobial activity of poly(TBAMS), the copolymer with acrylonitrile [1:1] applied as a coating was also tested against the mentioned

bacteria. Thin coatings are the most common concept to add antimicrobials on the outside of materials, because this form enables the subsequent equipment of established food contact surfaces with antimicrobial properties [13], while the good properties of the used materials are not affected by integration of antimicrobial agents in the materials themselves [33]. Results of the copolymer poly(TBAMS:acrylonitrile) are comparable to those of the homopolymer. Also, a significant difference ($p<0.001$) between the reference and the sample material could be observed (figure 4b), but the comparison of the reductions of all bacteria together shows a trend of a better activity of the homopolymer. A reduced activity against *P. fluorescens* is mainly responsible for this trend (log_{10}-reduction: 1.2). For remaining bacteria, high log_{10}-reductions (3.4–5.7), which characterize the material as antimicrobial active according to JIS Z 2801, could be detected. For the pathogenic bacteria *E. coli*, *L. monocytogenes* and *S. aureus*, as well as for spoilage bacteria *B. thermosphacta*, *P. aeruginosa* and *P. putida* a reduction down or close to detection limit were proven.

General, the charge of the antimicrobial surface plays an important role in electrostatic interactions between polymer surface and bacteria, and so for the antimicrobial activity. The more active groups are present on the surface, the higher the antimicrobial activity is expected to be [34], because the number and availability of active groups determines the charge of the polymer. Thus, the homopolymer poly(TBAMS) shows a higher reduction ($p=0.001$) when comparing counts of all tested bacteria together then the copolymer, which features less positive surface charge. Also, Potter et al. [35] detected decreased antimicrobial activity for modified cationic antimicrobial peptides with decreased electrophoretic mobility. The dose-dependent activity of poly(TBAMS) conforms to the investigations on poly(TBAEMA) [28, 34, 36, 37]. Zuo et al. [37] determined a correlation between the dosage of poly(TBAEMA) and the molecular weight of the macromolecules to the antimicrobial activity. The authors explain that a higher molecular weight results in a higher local congregation of active groups with a resulting increase in charge density and electrostatic attraction. Seyfriedsberger et al. [34] proved a correlation between the physio-chemical surface properties and the relative amount of poly(TBAEMA) in a compound with LDPE. In the study, the antimicrobial activity against *E. coli* increased with an increasing relative amount of poly(TBAEMA). Interestingly, for *S. aureus* there was no difference in activity between the concentrations; *S. aureus* was reduced to zero independently of the poly(TBAEMA)-concentration [34]. Furthermore, Zuo et al. [37] showed that, in general, *S. aureus* is more susceptive to poly(TBAEMA) then *E. coli*, but, particularly at lower local concentrations of active groups, the effect of molecular weight is different between *E. coli* and *S. aureus*. A relationship between dose-dependence and bacteria species is in accordance with the present study. Particularly the antimicrobial effect on the gram-negative *P. fluorescens* is notably lower using the copolymer with only 50 % poly(TBAMS) in comparison to the homopolymer. Otherwise, the antimicrobial activity against *S. aureus* was not affected by reducing the percentage of poly(TBAMS) in a poly(TBAMS:acrylonitrile) copolymer down to 20 % (data not shown).

Other authors also proved good antimicrobial properties of SAM-Polymers® against gram-positive (*S. aureus*, *L. innocua*, *L. monocytogens*, *Lactobacillus* spp., *S. mutans, S. epidermidis, B. thermosphacta*) and gram-negative (*E. coli*, *P. aeruginosa, P. fluorescens*) bacteria [22, 28, 29, 36–39]. Nevertheless, some studies observed that gram-positive species are more sensitive than gram-negative bacteria [27, 34, 39]. In the study of Hewitt et al. [27], almost all cells of *S. epidermidis* exhibit depolarized, permeablised, cytoplasmic membrane potential after 30 min exposure to 0.1 % poly(TBAEMA) suspension; whereas after 5.5 h, only 59 % of *P. fluorescens* cells showed the same status. In contrast, Buranasompob [29] detected a higher reduction of the gram-negative *P. aeruginosa* in comparison to *L. innocua*. In the present study for the biofilm former *P. aeruginosa,* high antimicrobial activity of both tested materials made from TBAMS was also detected. A general trend of a dependence of antimicrobial activity on gram reactivity was not observed at initial counts around 10^5 cfu ml^{-1}. At higher initial counts (6.6–8.9 $\log_{10}$ cfu ml^{-1}) however, a trend of higher reduction of gram-positive bacteria than gram-negative bacteria became visible.

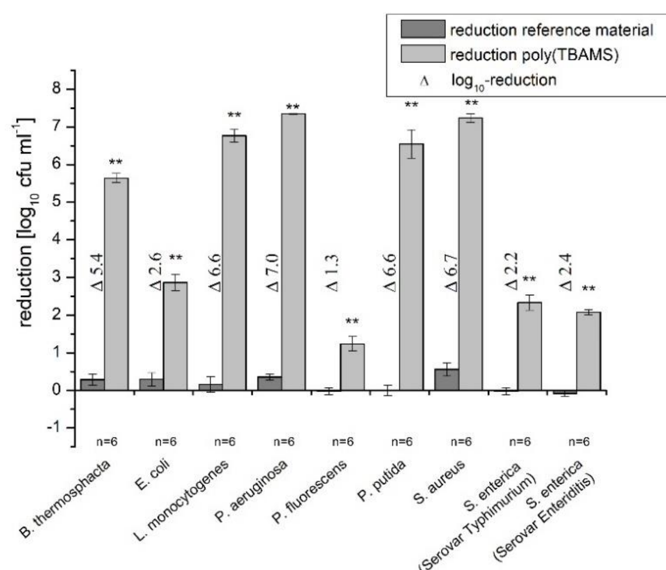

Figure 2.5 Reduction [$\log_{10}$ cfu ml^{-1}] of overnight cultures of bacteria in saline solution with tryptone applied on reference material (dark grey bars) or poly(TBAMS) (light grey bars) incubated at 35 °C for 2 h. The values are changes from initial concentration (6.6-8.9 $\log_{10}$ cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on poly(TBAMS) after incubation ($\log_{10}$-reduction). Asterisks indicate significant differences (** $p \leq 0.005$) between poly(TBAMS) and reference material.

Figure 2.5 shows the reduction values of the high initial bacterial counts, which are typical during industrial processing. Analysing the reduction of all bacteria together, the reduction on the sample material is significantly higher than on the reference material ($p<0.001$). For the homopolymer film, the decrease of the initial bacterial count is highly significant for all bacteria ($p \leq 0.001$) with reductions between 1.2 and 7.3 $\log_{10}$ cfu ml^{-1}. The three tested gram-positive bacteria were reduced down to the detection limit. Very high $\log_{10}$-reductions (7.0 and 6.7 $\log_{10}$ cfu ml^{-1}) were identified for the gram-negative bacteria *P. aeruginosa* and *P. putida* also. These results are comparable with the results of moderate initial counts, but, for the remaining tested

gram-negative bacteria, the antimicrobial activity was decreased. Particularly noticeable is the low reduction of *E. coli* (2.6 $\log_{10}$ cfu ml^{-1}), which was reduced in higher values during 2 h if it was exposed in moderate initial concentration. Also, Zuo et al. [37] showed a higher antimicrobial activity of poly(TBAEMA) film for gram-positive *S. aureus* than for gram-negative *E. coli* at high initial bacterial load. The higher resistance of selected gram-negative bacteria, which became visible at high initial counts, can be charge of the outer membrane. Hewitt et al. [27] showed that the *P. fluorescens* cells become more sensitive to the poly(TBAEMA) suspension if the outer membrane of is permeabilised with EDTA.

The surface charge of bacteria itself plays, next to the charge of the polymeric surface, an important role for antimicrobial activity. In general, most bacteria carry a net negative surface charge under most physiological conditions [40]. According to Potter et al. [35], the electrophoretic mobility of the, for the present study, relevant gram-positive bacteria (*B. thermosphacta*, *S. aureus*, *L. monocytogenes*) was more negative than for tested gram-negative bacteria (*P. fluorescens*, *S. enterica*, *E. coli*). The authors proved a correlation between the electrophoretic mobility and the antimicrobial efficiency of a cationic antimicrobial peptide. Thus, the nearly neutral charge of *P. fluorescens* could cause the decreased activity of TBAMS-based films compared to the more negative charged bacteria. Furthermore, Kurinčič et al. [41] showed a high electrophoretic mobility comparability between the *P. aeruginosa* strain used in this study and *Listeria* spp., which could explain the differences in activity against the three *Pseudomonas* spp. used. The effect of the electrophoretic mobilities of the bacteria is more distinctive at high than at moderate initial concentration. Next to the electrostatic interactions, the availability of active groups is also proportional to the number of bacterial count. Lenoir et al. [28] revealed that killed cells do not remain on the surface, potentially allowing an extension of contact time to achieve successive killing of bacteria, leading to comparable results in moderate initial concentrations. Likewise, longer contact could compensate the lower number of active groups in the copolymer and lead to comparable results as detected for the homopolymer.

Under practical conditions, bacteria colonize surfaces not as pure cultures, but rather as mixed bacteria populations and mostly in the form of biofilms. *Pseudomonas* spp., as ubiquitous spoilage organisms and great biofilm formers [42] were used in all cultures of the last experiments, because it is known, that their presence promotes the attachment and survival of pathogens, like *L. monocytogenes,* on surfaces [43, 44].

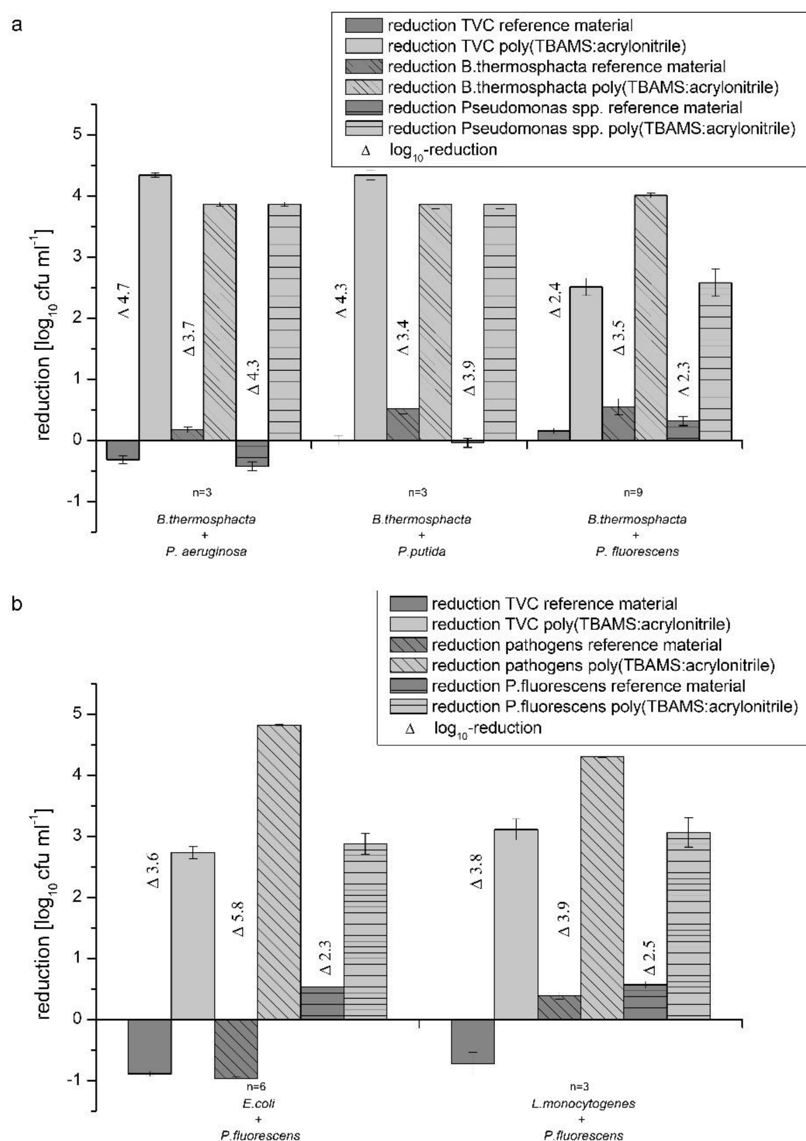

Figure 2.6 Reduction [$\log_{10}$ cfu ml^{-1}] of mixed cultured bacteria: (a) *B. thermosphacta* with *Pseudomonas* spp., (b) *P. fluorescens* with *E. coli* or *L. monocytogenes* applied on reference material (dark grey bars) on reference material or poly(TBAMS:acrylonitrile) (light grey bars) incubated at 35 °C for 2 h. The values of the plain bars are the changes from initial concentration of the total viable count (TVC); the patterned bars are the changes of the individual bacteria counts. The delta values are the differences between the surface counts on reference material and on poly(TBAMS:acrylonitrile) after incubation ($\log_{10}$-reduction)

When *Pseudomonas* spp. and *B. thermosphacta*, another dominant spoilage bacteria of fresh meat [45], were inoculated together, it became evident that the antimicrobial activity of poly(TBAMS:acrylonitrile) against the individual bacteria in the mixed culture is comparable to the results of the pure culture test series (Figure 2.6a). The copolymer showed maximum reduction to the detection limit for *B. thermosphacta* as well as against *P. putida* and *P. aeruginosa*, and decreased activity against *P. fluorescens*. The TVC of the mixed culture of *B. thermosphacta* and *P. fluorescens* were reduced 2.4 $\log_{10}$-steps, while the results on selective media show obviously, that only *P. fluorescens* survived on poly(TBAMS:acrylonitrile). Furthermore, it was investigated if this resilience of *P. fluorescens* protects cohabitating pathogens against the antimicrobial action of poly(TBAMS)-containing films. Analogous to

pure cultures tests and to mixed tests with the spoilage bacteria *B. thermosphacta*, the counts of the pathogens were reduced down to the detection limit within 2 h at 35 °C and *P. fluorescens* represents the remaining TVC (Figure 2.6b). Investigations with the homopolymer indicate that the presence of *P. fluorescens* do not have an impact on the activity against the pathogens *S. aureus* and *S. enterica,* too (data not shown). Thus, the less sensitivity of *P. fluorescens* does not affect the good antimicrobial activity of poly(TBAMS)-based films against the different microorganisms. The electrostatic interactions between the bacteria and the polymer surface seem to differ between the individual bacteria species, but have no effect on the individual antimicrobial activities in mixed bacteria cultures. The reduction of *Pseudomonas* spp., which are known members of biofilms in the food industry, promises a potential reduction of biofilm formation and should be investigated further. Especially the fact that main pathogens associated with meat are significantly reduced on poly(TBAMS)-containing films has a considerable impact for enhanced food security.

2.5 Conclusion

Regarding the two poly(TBAMS)-containing films tested, it can be summarized that a good antimicrobial activity exists against a wide range of pathogenic and spoilage bacteria relevant in meat processing facilities, both in pure or in mixed bacteria cultures. Effects on antimicrobial activity due to copolymerization, with the resulting concentration reduction of poly(TBAMS), or higher initial bacterial counts were mainly dependent on the sensitivity of the bacteria itself. The surface counts of pathogenic bacteria with high relevance in food-associated diseases were reduced in comparison to the reference material, whereas the spoilage bacterium *P. fluorescens* was not that susceptible. Due to these results and the improved material properties, the application of SAM-Polymer®-surfaces based on poly((tert-butyl-amino)-methyl-styrene) could be an additional hurdle for bacterial growth on food contact surfaces such as cutting boards or conveyer belts and thus could counteract cross-contamination. Further development and characterisation of the material is required before application. In future, the antimicrobial activity of the material, with due consideration of environmental factors existing in the food industry, should be investigated to check the efficiency of poly(TBAMS) films under real conditions. Furthermore, the effect of poly(TBAMS) films on biofilm formation should be studied.

2.6 References

1. Bagge-Ravn D, Ng Y, Hjelm M, Christiansen JN, Johansen C, Gram L (2003) The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. Int J Food Microbiol 87(3), 239–250.
2. Gounadaki AS, Skandamis PN, Drosinos EH, Nychas G-JE (2008) Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. Food Microbiol 25(2), 313–323.
3. Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, Doulgeraki A, Nychas G-J, Kačániová M, Czaczyk K, Ölmez H, Simões M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6, 841.
4. Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by Salmonella in foods: A review. Food Res Int 45(2), 545–556.
5. Bower CK, McGuire J, Daeschel MA (1996) The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci Technol 7(5), 152–157.
6. Brooks JD, Flint SH (2008) Biofilms in the food industry: problems and potential solutions. Int J Food Sci Technol 43(12), 2163–2176.
7. Giaouris E, Chorianopoulos N, Skandamis P, Nychas G-J (2012) Attachment and biofilm formation by Salmonella in food processing environments, in: Mahmoud DBSM (ed.) Salmonella - A Dangerous Foodborne Pathogen, pp. 157–180: InTech.
8. Møretrø T, Langsrud S, Heir E (2013) Bacteria on Meat Abattoir Process Surfaces after Sanitation: Characterisation of Survival Properties of Listeria monocytogenes and the Commensal Bacterial Flora. Adv Microbiol 03(03), 255–264.
9. Giaouris E, Heir E, Hébraud M, Chorianopoulos N, Langsrud S, Møretrø T, Habimana O, Desvaux M, Renier S, Nychas G-J (2014) Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. Meat Sci 97(3), 298–309.
10. Gundelley R, Youm GW, Kwon YM (2007) Survival of bacterial pathogens on antimicrobial conveyer belts. J Rapid Meth Aut Mic 15(3), 259–266.
11. Møretrø T, Langsrud S (2011) Effects of Materials Containing Antimicrobial Compounds on Food Hygiene. J Food Prot 74(7), 1200–1211.
12. Ilg Y, Kreyenschmidt J (2012) Review: Benefits and risks of the use of antimicrobial components in the food chain. J Food Saf Food Qual 63(2), 28–34.
13. Moerman F (2014) Antimicrobial materials, coatings and biomimetic surfaces with modified microtography to control microbial fouling of product contact surfaces within food processing equipment: Legislation, requirements, effectiveness and challenges. Journal of Hygienic Engineering and Design 7, 8–29.
14. Appendini P, Hotchkiss JH (2002) Review of antimicrobial food packaging. Innovative Food Sci Emerging Technol 3(2), 113–126.
15. Gutierrez J, Barry-Ryan C, Bourke P (2008) The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int J Food Microbiol 124(1), 91–97.
16. Ramos M, Jiménez A, Peltzer M, Garrigós MC (2012) Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. J Food Eng 109(3), 513–519.
17. Barbiroli A, Bonomi F, Capretti G, Iametti S, Manzoni M, Piergiovanni L, Rollini M (2012) Antimicrobial activity of lysozyme and lactoferrin incorporated in cellulose-based food packaging. Food control 26(2), 387–392.
18. Héquet A, Humblot V, Berjeaud J-M, Pradier C-M (2011) Optimized grafting of antimicrobial peptides on stainless steel surface and biofilm resistance tests. Colloids Surf., B 84(2), 301–309.
19. Noyce JO, Michels H, Keevil CW (2006) Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing. Appl Environ Microbiol 72(6), 4239–4244.
20. Ilg Y, Kreyenschmidt J (2011) Effects of food components on the antimicrobial activity of polypropylene surfaces containing silver ions (Ag+). Int J Food Sci Technol 46(7), 1469–1476.
21. Kursunlu AN, Guler E, Sevgi F, Ozkalp B (2013) Synthesis, spectroscopic characterization and antimicrobial studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with Schiff bases derived from 5-bromo-salicylaldehyde. J Mol Struct 1048, 476–481.

22. Thölmann D, Kossmann B, Sosna F (2003) Polymers with antimicrobial properties. EC Journal(1-2), 16–33.
23. Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114(4), 1173–1182.
24. Bastarrachea LJ, Denis-Rohr A, Goddard JM (2015) Antimicrobial Food Equipment Coatings: Applications and Challenges. Annu Rev Food Sci Technol 6(1), 97–118.
25. Mauriello G (2016) Control of microbial activity using antimicrobial packaging, in: Barros-Velazquez J (ed.) Antimicrobial Food Packaging, pp. 141–152: Elsevier.
26. Tiller JC (2011) Antimicrobial Surfaces, in: Börner HG, Lutz J-F (eds.) Bioactive Surfaces, pp. 193–217: Springer Berlin Heidelberg.
27. Hewitt CJ, Franke R, Marx A, Kossmann B, Ottersbach P (2004) A study into the anti-microbial properties of an amino functionalised polymer using multi-parameter flow cytometry. Biotechnol Lett 26(7), 549–557.
28. Lenoir S, Pagnoulle C, Galleni M, Compère P, Jérôme R, Detrembleur C (2006) Polyolefin Matrixes with Permanent Antibacterial Activity: Preparation, Antibacterial Activity, and Action Mode of the Active Species. Biomacromolecules 7(8), 2291–2296.
29. Buranasompob A (2005) Kinetics of the inactivation of microorganisms by water insoluble polymers with antimicrobial activity. Dissertation. Berlin.
30. Brodkorb F, Fischer B, Kalbfleisch K, Robers O, Braun C, Dohlen S, Kreyenschmidt J, Lorenz R, Kreyenschmidt M (2015) Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. Int J Mol Sci 16(8), 20050–20066.
31. Kreyenschmidt M, Lorenz R, Fischer B, Kreyenschmidt J, Brodkorb F, Kalbfleisch K, Blang T, Geschwentner A (2014) Anti-microbial polymer: Google Patents(WO2014118339 A1). http://www.google.com/patents/WO2014118339A1?cl=en.
32. Hechelmann H (1981) Vorkommen und Bedeutung von Brochothrix thermosphacta bei Kühllagerung von Fleisch und Fleischerzeugnissen. Mitteilungsblatt BAFF, 4435–4437.
33. Suppakul P, Miltz J, Sonneveld K, Bigger S (2003) Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications. J Food Sci 68(2), 408–420.
34. Seyfriedsberger G, Rametsteiner K, Kern W (2006) Polyethylene compounds with antimicrobial surface properties. Eur Polym J 42(12), 3383–3389.
35. Potter R, Truelstruphansen L, Gill T (2005) Inhibition of foodborne bacteria by native and modified protamine: Importance of electrostatic interactions. Int J Food Microbiol 103(1), 23–34.
36. Marra J, Paleari AG, Rodriguez LS, Leite ARP, Pero AC, Compagnoni MA (2012) Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. J Appl Oral Sci 20, 643–648.
37. Zuo H, Wu D, Fu R (2012) Preparation of antibacterial poly(methyl methacrylate) by solution blending with water-insoluble antibacterial agent poly[(tert-buty1amino) ethyl methacrylate]. J Appl Polym Sci 125(5), 3537–3544.
38. Thomassin J-M, Lenoir S, Riga J, Jérôme R, Detrembleur C (2007) Grafting of Poly[2-(tert-butylamino)ethyl methacrylate] onto Polypropylene by Reactive Blending and Antibacterial Activity of the Copolymer. Biomacromolecules 8(4), 1171–1177.
39. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Robers O, Kreyenschmidt M, Kreyenschmidt J (2016) Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products. J Appl Microbiol 121(4), 1059-70.
40. Jucker BA, Harms H, Zehnder AJ (1996) Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and Teflon. J Bacteriol 178(18), 5472–5479.
41. Kurincic M, Jersek B, Klancnik A, Mozina SS, Fink R, Drazic G, Raspor P, Bohinc K (2016) Effects of natural antimicrobials on bacterial cell hydrophobicity, adhesion, and zeta potential. Arh Hig Rada Toksikol 67(1), 39–45.
42. Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT - Food Sci Technol 43(4), 573–583.
43. Sasahara KC, Zottola EA (1993) Biofilm Formation by Listeria monocytogenes Utilizes a Primary Colonizing Microorganism in Flowing Systems. J Food Prot 56(12), 1022–1028.

44. Hassan AN, Frank JF (2004) Attachment of Escherichia coli O157: H7 grown in tryptic soy broth and nutrient broth to apple and lettuce surfaces as related to cell hydrophobicity, surface charge, and capsule production. Int J Food Microbiol 96(1), 103–109.
45. Russo F, Ercolini D, Mauriello G, Villani F (2006) Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups. Food Microbiol 23(8), 797–802.

3 Long-term antimicrobial activity of poly(TBAMS)-containing films and activity under conditions typical during the processing and preparation of meat

3.1 Abstract

For the effective implementation of antimicrobial food contact materials, the materials must be active over a long period of use and under conditions typical for food processing and preparation. Thus, the aim of this study was to investigate the long term activity of the homopolymer poly(TBAMS) and two copolymers (poly(TBAMS:acrylonitrile) [1:1], poly(TBAMS:4-vinylpyridine) [1:1]) as well as the activity under conditions typical during the processing and preparation of food. To test the long-term activity, the samples were stored three years under different temperature and humidity conditions and activity was investigated in defined time intervals according to the test method JIS Z 2801:2000. The activity against several microorganisms under typical processing and preparation conditions were investigated under the influence of food components and at different pH-values, temperatures and contact times. Therefore, the JIS Z 2801:2000 was adapted. The materials showed antimicrobial stability (log_{10}-reduction: 1.9-5.6 log_{10} cfu ml^{-1}, $p<0.005$) over three years under all storage conditions. Linear modelling of the relative log_{10}-reduction over the period of 3 years showed that, for the homopolymer poly(TBAMS)-film, a decrease of maximal 0.5 % a-1 could be expected (97.5 % quartile). The practical conditions tests generally showed an influence on the antimicrobial activity by temperature, air humidity, pH-value, high initial counts or the presence of food ingredients. The materials exhibit a strong antimicrobial profile against *L. monocytogenes*, *P. fluorescens*, *S. aureus* and *S. enterica*. However, the general influence of the factors is different between the different strains of bacteria. The activity against the pathogenic gram-positive bacteria *L. monocytogenes* and *S. aureus* is less effected than against the tested gram-negative bacteria. The results confirm the potential of poly(TBAMS)-containing materials to act as antimicrobial food contact surfaces during food processing and preparation. The long-term stability and the antimicrobial action profile, in consideration of relevant practical conditions, represent a potential of poly(TBAMS) as food contact materials used for meat processing and preparation.

3.2 Introduction

Microbial contamination of food is a public health problem. Due to improper handling and cross-contamination during the production, processing and preparation of food, pathogens and spoilage microorganisms can spread into the environment and to different surfaces, leading to a decrease of product quality and safety [1–5]. Common sources of cross-contamination are the food contact surfaces of equipment, e.g. conveyer belts or cutting boards, which is used during processing and preparation [2, 3, 6].

The integration of antimicrobial surfaces in food contact surfaces is one possibility to improve hygiene by reduction of microbial counts or biofilms and therefore cross-contamination [7–9]. This leads to beneficial effects on food safety and quality and prevents the material itself against biodegrading [8, 9]. During recent years, many different antimicrobial active materials have been established. In general, there are two different principles of active materials: biocide releasing and contact-killing [10]. In the first systems, antimicrobial agents are integrated in the material or parts of the material and the active substances are released into the environment. For example, different metals (e.g. copper, silver), organic acids (e.g. benzoic, lactic), bacteriocins (e.g. nisin, magainin), essential oils (e.g. linalool, thymol) are used [8, 9, 11, 12]. The principle of contact killing surfaces means that the material itself has antimicrobial properties (e.g. cationic polymers such as chitosan) or antimicrobial agents are immobilized on the surface (e.g. QACs, N-halamine) [8, 9, 13]. Hence, food contact surfaces can feature antimicrobial activity either if the bulk material is intrinsic antimicrobial or antimicrobial agents are incorporated in the bulk material. An alternative concept is to coat typical bulk material for food contact surfaces with an antimicrobial coating.

The results of the previous chapters show a very good antimicrobial activity of poly(TBAMS)-containing films against a broad spectrum of bacteria in mono as well as mixed cultures under standard test conditions. This offers the potential of these films as antimicrobial material during the production and processing of meat. For the application of an antimicrobial surface however, it is also important to investigate if the material has a long-term benefit on the surface hygiene and to examine the activity under relevant processing and preparation conditions like temperature, humidity and the presence of food. These factors can vary in the different stages of processing and preparation. For example, temperature conditions range between cold temperature in industry and ambient conditions during domestic use. Furthermore, it must be taken in account that the materials are exposed to extreme conditions routinely over prolonged periods. Further on, the cleaning and disinfection agents used in industrial sanitation processes produce extreme pH-values and air humidity during this process is very high [9].

All the mentioned factors can have an influence on the activity of antimicrobial surfaces [7, 14]. The correlation between decreased activity and decreased temperature is well documented for different antimicrobial agents: silver [15], chitosan [16, 17], copper [1, 18], triclosan [19]. For silver a considerable increased reduction of *S. aureus* was indicated at lower humidity level

(20-35 % rH) in comparison to higher rates of 70 or 93 % rH [20, 21]. In addition to mentioned factors, the presence of food and their respective ingredients often limit the effect of antimicrobial surfaces. The good antimicrobial effect of a silver treated conveyer was neutralized in the presence of a 10 % suspension of food debris, especially of meat and fish [22]. Proteins especially affect the activity of antimicrobials such as silver due to the interaction of amino acids with the functional groups of the biocidal agents [21, 23–27]. Other studies have also shown that, due to a stabilization of bacterial membranes in presence of mineral nutrients (divalent cations of calcium or magnesium), the effect of antimicrobial active cationic polymers was decreased [28, 29].

Another requirement which must be fulfilled by active materials is the long-term antimicrobial activity under harsh conditions [7, 9]. The mechanism of releasing systems implies that the activity of such materials is time-limited, because the antimicrobial agents are released, partially uncontrolled, into the environment and the agents are no more available in the surface [9, 30]. Møretrø [26] showed, that triclosan-containing cutting boards have a reduced antimicrobial activity after repeated washing. Furthermore, the release into environment is conducive to the development of bacterial resistance which additionally limits the long-term activity. Another problem is that the time where the material is no longer active is vague. Hence the user cannot distinguish if the material is still active or not which may give a false impression of hygiene [26].

In this context contact active materials might be a more promising technology [8], because the function of such kinds of material is not based on a migrating effect. An example of such kind of material are SAM-Polymers® (Thölmann 2003). Antimicrobial activity of the SAM-Polymer® poly(2-tert-butylaminoethyl) methacrylate (poly(TBAEMA)) was observed against a broad spectrum of microorganisms [31]. The newer agent of this material class is poly((tert.-butyl-amino)-methyl-styrene) (poly(TBAMS) [32, 33] which showed excellent antimicrobial activity [33–35]. Up to now however, there are no results about the long-term stability and activity under the practical conditions of processing and preparation in the meat industry as well as in homes.

The aim of this study was to investigate the long term antimicrobial activity of the homopolymer poly(TBAMS) and two copolymers (poly(TBAMS:acrylonitrile) [1:1], poly(TBAMS:4-vinylpyridine) [1:1]) as well as the activity under conditions typical during the processing and preparation of food.

3.3 Materials and Methods

The general antimicrobial activity of different polymer surfaces based on poly((tert.-butyl-amino)-methyl-styrene) (poly(TBAMS)) was tested on the basis of the Japanese Test Standard JIS Z 2801: 2000. The antibacterial effectiveness was determined by the value of antibacterial activity, which is defined as the difference of the logarithm of the bacterial count on untreated

materials (reference) and treated materials (sample) after inoculation with microorganisms and incubation under defined conditions.

In the first part of the study, the long-term activity was investigated. Therefore, three different films were stored under different temperature and humidity conditions up to three years and the antimicrobial activity was tested in intervals of 6 and 12 months respectively. The activity was investigated against *S. aureus* and *E. coli*.

In the second part of the study the effect of conditions typical during food processing and preparation on the activity of poly(TBAMS)-containing films was tested. For this, the standard test method JIS Z2801:2000 was adjusted in the following way: next to *S. aureus* and *E. coli*, mentioned in the standard, the activity of the material against further meat associated bacteria (*Listeria monocytogenes*, *Salmonella enterica* and *Pseudomonas fluorescens*) was analyzed. The activity tests against the different bacteria were conducted under refrigerated and ambient temperature conditions and activity was measured after different time intervals. Furthermore, the effect of extreme air humidity and pH-values was investigated. The influence of food ingredients was tested in additional experiments.

3.3.1 Antibacterial test material

In this study, different polymer samples based on poly(TBAMS) were tested for their antimicrobial activity: the homopolymer poly(TBAMS) and the two copolymers poly(TBAMS:acrylonitrile) [1:1], poly(TBAMS:4-vinylpyridine) [1:1]. The used copolymers possess generally better material properties, like higher glass transition temperatures (T_G) and a lower water uptake. The T_G of the homopolymer is about 68 °C, of poly(TBAMS:acrylonitrile) [1:1] is about 80 °C and of poly(TBAMS:4-vinylpyridine) [1:1] about 136 °C. However, the amounts of antimicrobial active groups of the copolymers are lower than for the homopolymer, which can potentially lead to reduced activity. The polymers were tested as colorless and transparent films in petri dishes (diameter 94 mm, polystyrene, VWR, Germany) or as a coating on a PE-layer, which was cut in circular pieces (about 98 mm) and put inside petri dishes. As reference material, clear petri dishes without vents or uncoated PE-layer were used.

3.3.2 Bacterial strains

The following test organism, which are associated with meat, were chosen: *Listeria monocytogenes* (ATCC 19111), *Staphylococcus aureus* subsp. *aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas fluorescens* (ATCC 13525), and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028).

All bacteria strains were stored at -18 °C in a CRYOBANKTM system (Mast, Reinfeld, Germany). The inoculum was prepared by transferring a frozen culture to 10 ml nutrient broth (Merck KGaA, Darmstadt, Germany). Afterwards the broth was incubated overnight at cultivating temperatures (*P. fluorescens* 25 °C, all others at 37 °C). At the beginning of each

trial the overnight culture was diluted in physiological saline solution with tryptone (1 g l^{-1}) (Oxoid, Hampshire, UK).

3.3.3 Test performance

The antimicrobial activity of the films was tested based on JIS Z 2801: 2000 which is described in Braun et al. [35]. For each test, a minimum of three samples and six references were used. Samples and references were inoculated with 0.4 ml of the prepared bacteria solution (composition is dependent on experimental series). To prevent evaporation and to standardize the contact area, sterile PE films (40x40 mm²) covered the inoculum loosely. Three references were washed immediately after inoculation (t=0 h) with 10 ml soybean-casein digest broth with lecithin polysorbate (Roth, Karlsruhe, Germany) via pipette to determine the initial concentration. The sample surfaces and the remaining references were incubated (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) at defined conditions depending on the experimental series (mentioned below). Data loggers (Testo 174H, Testo AG, Lenzkirchen, Germany) monitored the temperature and humidity at five-minute intervals.

After incubation they were washed in an equivalent manner. Viable counts were determined by counting the colonies on plate count agar (Roth, Karlsruhe, Germany) using drop plate technique and pour plate technique for the lowest dilutions. Agar plates were incubated for 24 h (drop plate technique) or 48 h (pour plate technique) using the respective temperature optima of each bacteria: 37 °C (*S. aureus*, *L. monocytogenes*, *E. coli*, *S. enterica*), or 25 °C (*P. fluorescens*). Detection limits were determinate at 1.0 $\log_{10}$ cfu ml^{-1} for pour plate technique and 2.0 $\log_{10}$ cfu ml^{-1} for drop plate technique.

Test to analyze the antimicrobial long-term stability

To determine the effect of long-term storage on the antimicrobial properties of poly(TBAMS), poly(TBAMS:acrylonitrile) [1:1] and poly(TBAMS:4-vinylpyridine) [1:1], the prepared sample petri dishes were stored under three conditions (Table 3.1) over a period of up to 36 month. The conditions used are typical in food industries of perishable food products and in domestic households. Antimicrobial activity tests with *E. coli* and *S. aureus* were performed after 0, 6, 12, 18, 24, 30 and 36 months of storage at cold (5°C) as well as ambient temperature (office cupboard) and after 0, 12, 24 and 36 months of storage at freezing conditions (-20 °C). The temperature and humidity during storage was monitored by data loggers (Testo 174H, Testo AG, Lenzkirchen, GermanyI at two-hour intervals.

Table 3.1 Survey of long-term storage conditions.

storage condition	temperature ± SD [°C]	relative air humidity ± SD [% RH]
freezing temperature	-20.7 ± 1.9	61.4 ± 7.5
cold temperature	4.8 ± 1.3	56.7 ± 3.7
ambient temperature	22.0 ± 1.6	43.9 ± 7.6

At each of the mentioned time intervals, samples were analyzed according to described test performance. At the beginning of each trial, the overnight culture was diluted in physiological saline solution with tryptone (1 g l^{-1}) (Oxoid, Hampshire, United Kingdom) to a final concentration of 10^5 cfu ml^{-1}. The contact conditions during antimicrobial tests were 35 °C and 2 h.

Test to analyze the antimicrobial activity during processing and preparation of food

The test performance to analyze the antimicrobial activity during the processing and preparation of food was adjusted regarding the inocula (media, pH-value, bacterial concentration) and/or the environmental conditions (temperature, air humidity, contact time) during contact of material with bacteria.

To investigate the influence of temperature, contact time and air humidity, a bacteria solution with a concentration of 10^5 cfu ml^{-1} in sodium chloride with tryptone was used as inoculum. To test the influence of storage temperature and the effect of different contact times on the activity of the homopolymer and the copolymer poly(TBAMS:acrylonitrile), the experiments were conducted with various contact times (1 h, 2 h, 6h ,24 h) over a temperature spectrum (4 °C, 7 °C, 20 °C, 35 °C). The effect of air humidity was investigated in two relative air humidity scenarios (high humidity: 98.3 ± 1.3 % rH, low humidity: 23.1 ± 4.3 % rH) at 7 °C with a contact time of 24 h. Air humidity was adjusted by placing a bowl with water (high humidity) or silica gel (low humidity) next to the homopolymer-samples.

The effect of a range of different pH-values (5-9) typical for cleaning and disinfection solutions was investigated (contact conditions: 35 °C, 2 h). For this test series Sorenson's buffer were inoculated with bacteria and used as test inocula.

The effect of food ingredients was tested in two sections. In the first section the influence of different mineral nutrients on the activity of the copolymer poly(TBAMS):acrylnonitrile [1:1] as a coating on PE-layer was investigated. These tests were conducted to check if the findings of Lenoir [29] and [28], which observe an effect of calcium and magnesium ions on the antimicrobial activity of poly(TBAEMA) and poly(N,N-dimethylaminomethylstyrene), also apply to poly(TBAMS). The authors trace this influence back to a membrane stabilization by the divalent cations and, consequently, an interference of the presumed antimicrobial action mode. The inocula of *S. aureus* and *E. coli* in sodium chloride with tryptone (10^5 cfu ml^{-1}) were added with stock solutions of calcium chloride dihydrate or magnesium chloride hexahydrate ($CaCl_2$*2 H_2O, $MgCl_2$*6 H_2O, Carl Roth, Karlsruhe, Germany) to an end concentration of 4-40 mmol l^{-1} of the divalent mineral ions.

In the food industry, the food contact surfaces are often contaminated with high counts of bacteria and food ingredients are present. Thus, in the second section, the homopolymer was inoculated with high initial bacterial counts (9.0 ± 0.5 log_{10} cfu ml^{-1}) in a nutrient rich media. Media nutrient broth was used, which is a good standard media to simulate many food

components, especially in the context of meat due to its composition (5 g l^{-1} peptone from meat and 3 g l^{-1} meat extract). In both experimental sections, 35 °C for 2 h was used as contact conditions.

3.3.4 Analysis

The reduction on the sample material after incubation was calculated by subtracting the logarithmic average value of bacterial concentration on the reference material immediately after inoculation ($N_{t=0}$) from the average value of bacterial concentration on the sample material after x h incubation ($N_{t=x}$) (Eq. 3.1)

$$f(N_{Ref,t=0}, N_{t=x}) = \log_{10}(N_{Ref,t=0}) - \log_{10}(N_{t=x}). \tag{3.1}$$

Standard errors ($df_{R,G}$) were calculated following the Gaussian propagation of uncertainty (Eq. 3.2)

$$df_{R,G} = \sqrt{\left(\frac{1}{N_{Ref,t=0} \times \ln 10} \times dN_{t=0}\right)^2 + \left(\frac{-1}{N_{t=x} \times \ln 10} \times dN_{t=2}\right)^2} \tag{3.2}$$

where $N_{Ref,\ t=0}$=average of bacterial concentration on reference material immediately after inoculation; d=standard error, and $N_{t=x}$=average of bacterial concentration on reference respectively sample material after x h incubation, ln= natural logarithm base *e*.

The value of the antimicrobial activity was calculated by subtracting the logarithmic value of the viable counts on the sample material from the logarithmic value of the reference material after inoculation and incubation (Eq. 3.3):

$$\log_{10} - \text{reduction} = \log_{10}\left(N_{Ref,t=x}/N_{SAM,t=x}\right) \tag{3.3}$$

with $N_{Ref,\ t=x}$= average of bacterial concentration on reference material, and $N_{SAM,\ t=x}$= average of bacterial concentration on sample material both after x h incubation.

According to the JIS Z 2801:2000 a material can be characterized as antimicrobial if the calculated $\log_{10}$-reduction is ≥ 2.0 after 24 h at 35 °C.

Statistical significance (n>3) in reduction-levels between reference and sample material was tested using Mann–Whitney U test in SPSS 22 (IBM®SPSS®Statictics). Significance was defined as p≤0.05 and highly significant as p≤0.005.

Trends relating to changes in antimicrobial activity due to long-term storage were identified by linear regression of relative $\log_{10}$-reductions (Eq. 3.4).

$$\text{rel.}\log_{10} - \text{reduction} = \log_{10} - \text{reduction}/\log_{10}(N_{Ref,t=2h}/N_{min}) \tag{3.4}$$

with $N_{Ref,\ t=2}$= average of bacterial concentration on reference material, and $N_{SAM,\ t=x}$= average of bacterial concentration on sample material both after 2 h incubation, N_{min}= bacterial count at detection limit.

In case of a reduction to the detection limit, the relative log_{10}-reduction becomes 1. This definition is used to adjust the log_{10}-reduction value of fluctuations in the count on the references, which could influence the assessment of the long-term stability of poly(TBAMS)-containing material. Based on the linear regressions of the relative log_{10}-reductions, the 97.5 %-quartile for the slopes of the regressions were estimated. The value of the 97.5 % quantile determines the upper bound of the slope, which will not be exceeded with a probability of 97.5 %.

Figures and linear fits were generated with the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, MA).

3.4 Results

3.4.1 Effect of long-term-storage under various environmental conditions on antimicrobial activity

Figures 3.1-3.3 show the relative log_{10}-reduction against *S. aureus* and *E. coli* of poly(TBAMS) films, poly(TBAMS:acrylonitrile) [1:1] films and poly(TBAMS:4-vinylopyridin) [1:1] films over a period up to 36 month. The results of linear regression are summarized in table 3.2. All films show good antimicrobial activity against both bacteria over the total investigation period with log_{10}-reductions of 1.9-5.6 log_{10} cfu ml^{-1}. However, the long-term stability of the materials is dependent on the storage condition and the material. In summary, storage at freezing temperature conditions had the lowest and storage at ambient temperature conditions had the highest influence on the antimicrobial activity. Deterioration of activity was only measured for the copolymer with acrylonitrile, although the activity against *S. aureus* stayed constant longer than for *E. coli*.

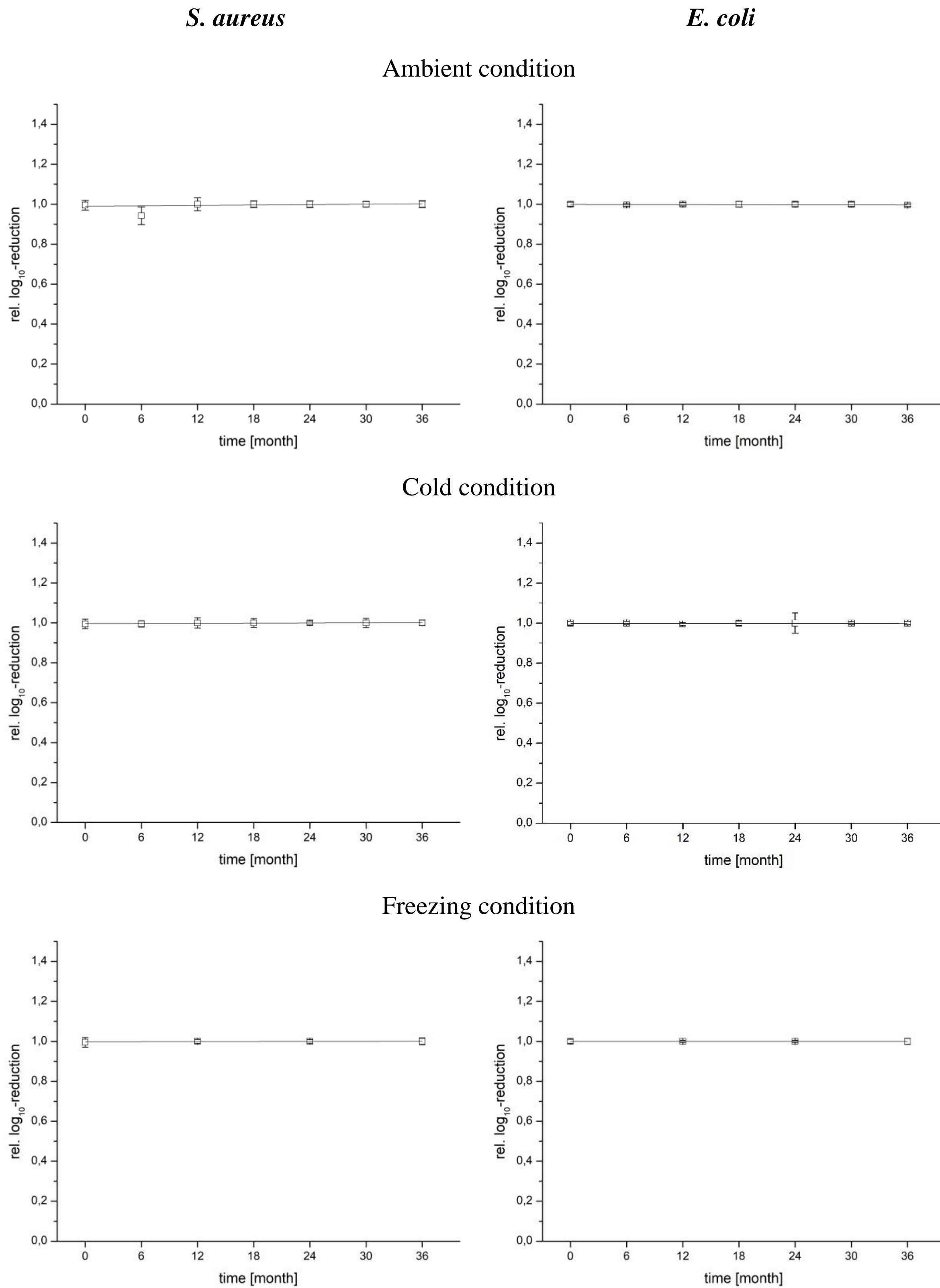

Figure 3.1 Linear regression of the rel. $\log_{10}$-reduction of poly(TBAMS) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions.

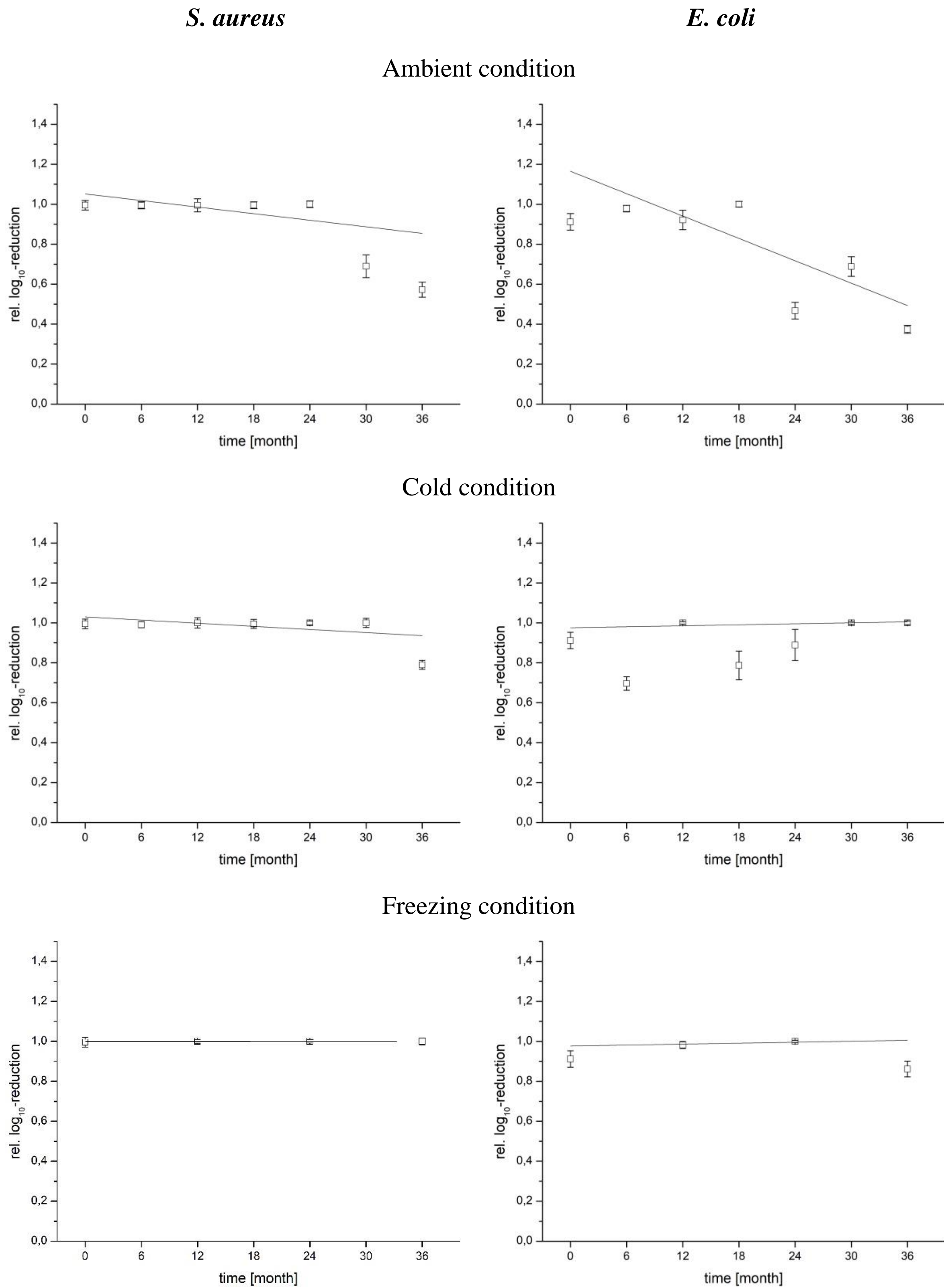

Figure 3.2 Linear regression of the rel. $\log_{10}$-reduction of poly(TBAMS:acrylonitrile) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions.

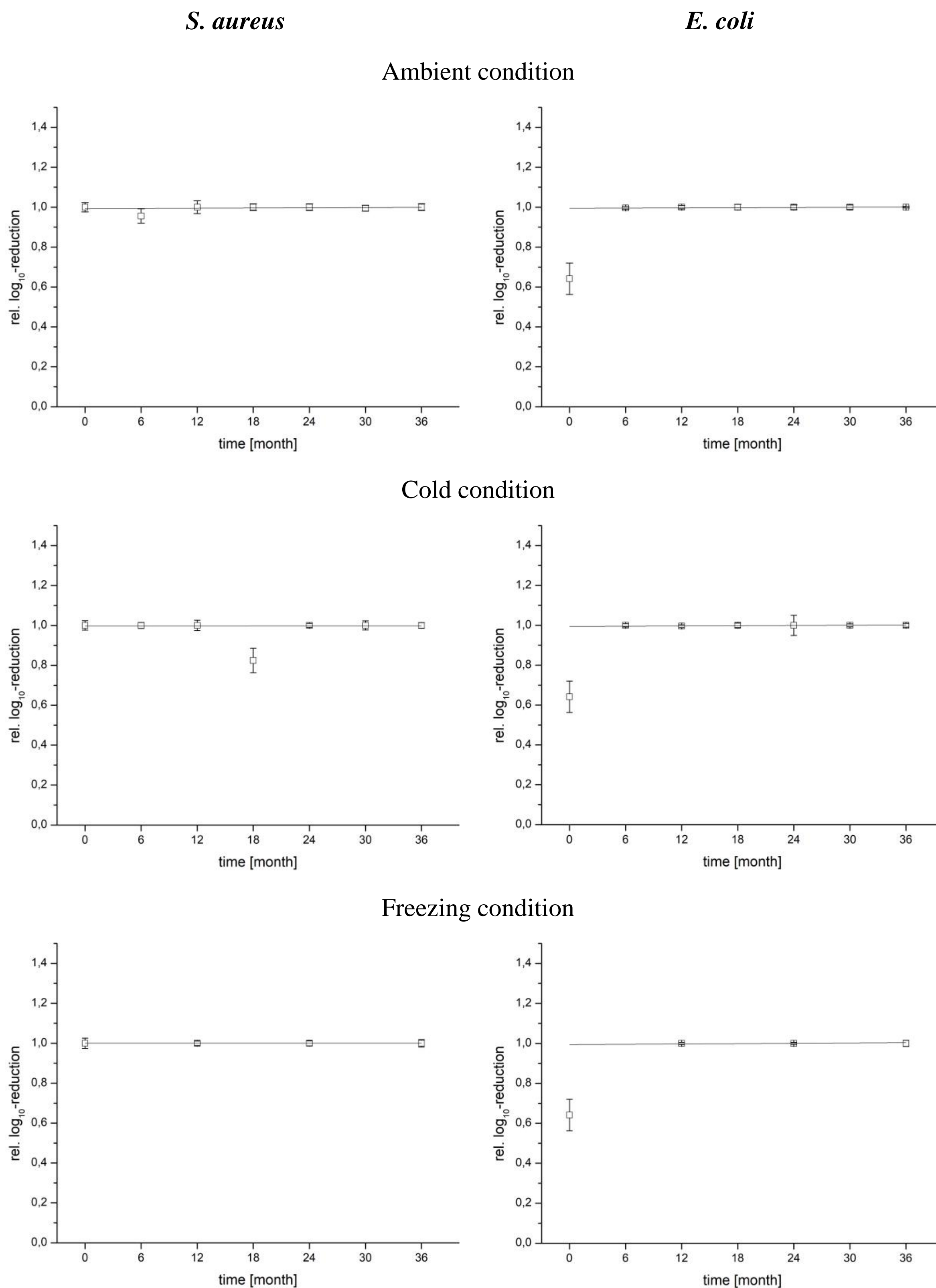

Figure 3.3 Linear regression of the rel. log_{10}-reduction of poly(TBAMS:vinylopyridin) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions.

Table 3.2 Fit parameter of the linear regression of relative $\log_{10}$-reduction of *S. aureus* and *E. coli* on poly(TBAMS)-containing films over a storage period of 36 month under different environmental conditions

microorganism	material	ambient condition			cold condition			freezing conditions		
		y-intercept	gradient [% month^{-1}]	97.5%-quartile of gradient [% month^{-1}]	y-intercept	gradient [% month^{-1}]	97.5%-quartile of gradient [% month^{-1}]	y-intercept	gradient [% month^{-1}]	97.5%-quartile of gradient [% month^{-1}]
E. coli	poly(TBAMS:acrylonitrile)	1.165 ± 0.122	-1.867 ± 0.579	-3.002	0.976 ± 0.040	0.082 ± 0.165	-0.241	0.977 ± 0.071	0.079 ± 0.307	-0.522
	poly(TBAMS:vinylopyridin)	0.995 ± 0.013	0.017 ± 0.047	-0.075	0.994 ± 0.015	0.020 ± 0.069	-0.115	0.994 ± 0.033	0.028 ± 0.161	-0.288
	poly(TBAMS)	0.999 ± 0.002	-0.006 ± 0.007	-0.020	0.998 ± 0.001	0.003 ± 0.006	-0.008	1.000 ± 0.000	0.000 ± 0.000	0.000
S. aureus	poly(TBAMS:acrylonitrile)	1.052 ± 0.072	-0.549 ± 0.393	-1.319	1.030 ± 0.050	-0.262 ± 0.226	-0.705	0.999 ± 0.001	0.005 ± 0.006	-0.006
	poly(TBAMS:vinylopyridin)	0.993 ± 0.009	0.018 ± 0.035	-0.050	0.997 ± 0.017	0.004 ± 0.071	-0.134	1.000 ± 0.000	0.000 ± 0.000	0.000
	poly(TBAMS)	0.990 ± 0.009	0.033 ± 0.037	-0.038	0.996 ± 0.001	0.014 ± 0.004	0.006	0.998 ± 0.002	0.007 ± 0.007	-0.008

For the materials which were stored at freezing conditions, no significant effect on the antimicrobial activity over a period up to 36 months could be observed (Table 3.2). The *S. aureus* count was reduced to or near the detection limit at all investigation points on all poly(TBAMS)-containing materials which was stored at freezing conditions ($\log_{10}$-reduction≥4.0 $\log_{10}$ cfu ml^{-1}; rel. $\log_{10}$-reduction>0.99, 97.5 %-quartile of gradient<-0.1% a^{-1}) (Table 3.2 and 3.3). Also, a high antimicrobial activity ($\log_{10}$-reduction≥4.6 $\log_{10}$ cfu ml^{-1}; rel. $\log_{10}$-reduction>0.86) was detected for *E. coli* after 12, 24 and 36 months. The expected decrease of antimicrobial activity (97.5 %-quartile) against *E. coli* is maximally 6.3% a^{-1} for the copolymer poly(TBAMS:acrylonitrile), 3.5 % a^{-1} for the copolymer poly(TBAMS:acrylonitrile) and 0 % a^{-1} for the homopolymer.

At cold and ambient temperature conditions, the effect of material and bacterial strain was clearer. The bacterial counts on poly(TBAMS) and poly(TBAMS:vinylopyridin) was reduced down to the detection limit with few exceptions independent of the storage conditions and bacterial strain ($\log_{10}$-reduction≥3.4 $\log_{10}$ cfu ml^{-1}; rel. $\log_{10}$-reduction>0.82, 97.5 %-quartile of gradient<-0.2 % month^{-1}) (Table 3.3). Thus, no significant trend in rel. $\log_{10}$-reduction, with a maximal decrease (97.5 %-quartile) in antimicrobial activity in the period of 3 years of 1.6 % a^{-1}, of these materials were observed (Table 3.2). The copolymer with acrylonitrile showed a negative trend in antimicrobial activity against *S. aureus*, which is more pronounced under ambient than cold conditions, but this trend is insignificant over the investigation period (Table 3.2). A decreased activity at different investigation points (18, 24 months) was detected for *E. coli* on poly(TBAMS:acrylonitrile) stored under cold conditions, but because of the standard error at these points and reductions down to the detection limit after 30 and 36 months, there is no significant change in antimicrobial activity during storage at cold temperature conditions (Table 3.2 and 3.4). When the copolymer with acrylonitrile was stored at ambient conditions, a significant negative trend (gradient: -1.9±0.6 % month^{-1}) in antimicrobial activity against *E. coli* was detected during the investigation period (Table 3.2). After 2 years of storage the *E. coli* count was not reduced to the detection limit for the first time, however, with a $\log_{10}$-reduction of 1.9 after 2 h contact at 35 °C, the reduction in comparison to the reference material under all conditions is highly significant ($p<0.005$) (Table 3.2) as well as for all other investigation points.

Table 3.3 Bacterial counts on reference material and log_{10}-reductions of *E. coli* and *S. aureus* at the different investigation points during storage at the three different conditions.

	E. coli				*S. aureus*			
time	reference	poly(TBAMS : acrylonitrile)	poly(TBAMS: vinylopyridin)	poly(TBAMS)	reference	poly(TBAMS : acrylonitrile)	poly(TBAMS/ vinylopyridin)	poly(TBAMS)
[month]	bacterial count [log_{10} cfu ml^{-1}]	log_{10}-reduction [log_{10} cfu ml^{-1}]			bacterial count [log_{10} cfu ml^{-1}]	log_{10}-reduction [log_{10} cfu ml^{-1}]		
ambient conditions								
0	6.5	5.0 ± 0.2	3.5 ± 0.4	5.5 ± 0.1	5.5	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
6	6.6	5.5 ± 0.1	5.5 ± 0.0	5.5 ± 0.0	5.2	4.2 ± 0.1	4.0 ± 0.2	4.0 ± 0.2
12	6.4	5.0 ± 0.3	5.4 ± 0.0	5.4 ± 0.0	4.8	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1
18	6.4	5.4 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.4	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
24	6.6	2.6 ± 0.2	5.6 ± 0.1	5.6 ± 0.1	5.3	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
30	6.2	3.6 ± 0.3	5.2 ± 0.1	5.2 ± 0.1	5.2	2.9 ± 0.2	4.2 ± 0.1	4.2 ± 0.1
36	6.1	1.9 ± 0.1	5.1 ± 0.0	5.1 ± 0.0	5.1	2.3 ± 0.2	4.1 ± 0.1	4.1 ± 0.1
cold conditions								
0	6.5	5.0 ± 0.2	3.5 ± 0.4	5.5 ± 0.1	5.5	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
6	6.5	3.9 ± 0.2	5.5 ± 0.0	5.5 ± 0.0	5.7	4.7 ± 0.1	4.7 ± 0.1	4.7 ± 0.1
12	6.5	5.5 ± 0.0	5.5 ± 0.0	5.5 ± 0.0	5.2	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
18	6.6	4.4 ± 0.4	5.6 ± 0.1	5.6 ± 0.1	5.2	4.1 ± 0.1	3.4 ± 0.3	4.2 ± 0.1
24	6.5	4.9 ± 0.4	5.5 ± 0.3	5.5 ± 0.3	5.4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0
30	6.4	5.4 ± 0.0	5.4 ± 0.0	5.4 ± 0.0	5.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
36	6.3	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	4.9	3.1 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
freezing conditions								
0	6.5	5.0 ± 0.2	3.5 ± 0.4	3.5 ± 0.4	5.5	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.1
12	6.6	5.5 ± 0.1	5.6 ± 0.0	5.6 ± 0.0	5.2	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0
24	6.5	5.5 ± 0.0	5.5 ± 0.0	5.5 ± 0.0	5.4	4.4 ± 0.0	4.4 ± 0.0	4.4 ± 0.0
36	6.3	4.6 ± 0.2	5.3 ± 0.1	5.3 ± 0.1	5.0	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.1

3.4.2 Effect of conditions during processing and preparation of food on antimicrobial activity

The reductions of different bacteria on the reference material and on poly(TBAMS) films as a function of exposure time at 7 °C and 35 °C are shown in figure 3.4. *L. monocytogenes* was reduced to the detection limit on poly(TBAMS) films under all temperature conditions. Already after a contact time of 1 h at cold temperature, a difference between the sample and the reference of 4.5 log_{10} cfu ml^{-1} in bacterial load was reached. Thus, for *L. monocytogenes* no influence of temperature or contact time was detected. An effect of environmental conditions on the antimicrobial activity of poly(TBAMS) against *P. fluorescens*, *S. aureus* and *S. enterica* was shown. *S. aureus* was reduced to the detection limit on the sample material after 1 h contact at 35 °C. Due to growth on the reference material, log_{10}-reduction increases from 4.5 after 1 h to 6.9 log_{10} cfu ml^{-1} after 24 h. The log_{10}-reduction at 7 °C also shows a positive trend with prolonging contact time, but this trend is caused by increased reduction on poly(TBAMS) films. After 1 h, the bacterial count is reduced 1.2 log_{10} cfu ml^{-1}, after 2 h 1.9 log_{10} cfu ml^{-1} and after 6 h, the detection limit is almost reached with a reduction of 4.7 log_{10} cfu ml^{-1}. The temperature as well as the contact time has an influence on the antimicrobial activity on *S. aureus*. The same effect was observed for *P. fluorescens* and *S. enterica.* At both temperatures the reduction on poly(TBAMS) increases with contact time. The reduction at 35 °C is always higher than at 7 °C.

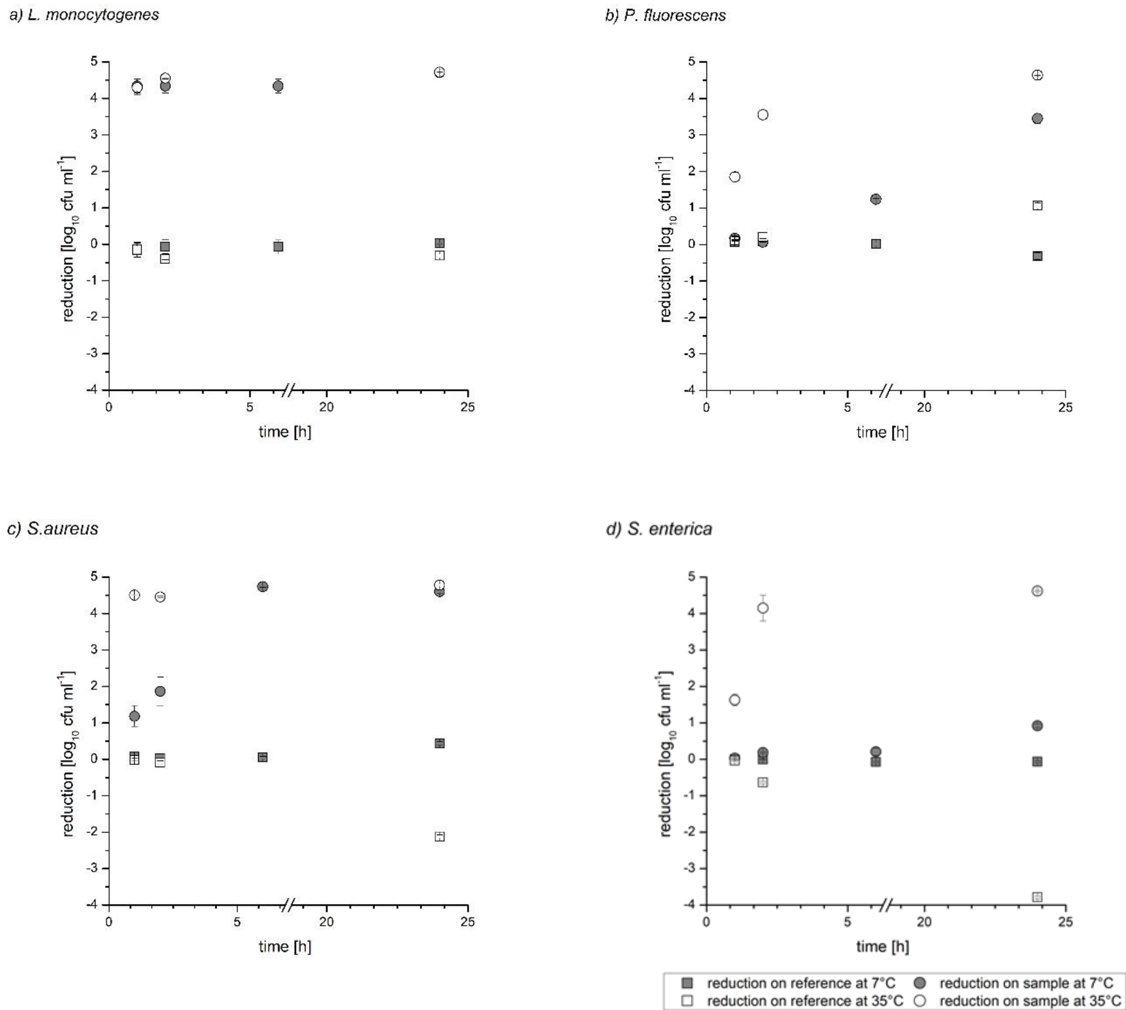

Figure 3.4 Reduction [$\log_{10}$ cfu ml^{-1}] of different bacteria (*L. monocytogenes* (a), *P. fluorescens* (b), *S. aureus* (c), *S. enterica* (d)) applied on reference material (squares) or the sample material (homopolymer poly(TBAMS)) (circles) after 1-24 h contact at 7 °C (grey symbols) or 35 °C (white symbols). The values are changes from initial concentration.

Table 3.5 summarized the results for various bacteria after 2 h contact with poly(TBAMS:acrynonitrile) films at three different temperatures. An increased antimicrobial activity with increased temperature was shown for *S. aureus* and *S. enterica* on poly(TBAMS:acrylnonitrile) films, too. For *S. enterica* the reduction increased similarly, with initial counts from 0.3 $\log_{10}$cfu ml^{-1} at 4 °C to 1.4 $\log_{10}$ cfu ml^{-1} at 20 °C to 3.2 $\log_{10}$cfu ml^{-1} at 35 °C. Prolonging contact time to 24 h at 4 °C led to enhanced $\log_{10}$-reduction as well (data not shown). No effect on the antimicrobial activity by temperature was observed for *E. coli* and *L. monocytogenes*. At all temperatures the bacterial count was reduced to or near the detection limit within 2 h. The $\log_{10}$-reduction for *P. fluorescens* is 1.2-1.3 $\log_{10}$ cfu ml^{-1}. A lower reduction on sample material at 35 °C in comparison to the lower temperature is caused by lower initial bacterial count.

Table 3.4 Reduction [log_{10} cfu ml^{-1}] of different bacteria applied on reference material (Red_R) or on poly(TBAMS:acrylonitrile) sample material (Red_S) after 2 h contact at various temperatures. The values are changes from initial concentration.

			2 h		
		microorganism	n	Red_R [log_{10} cfu ml^{-1}]	Red_S [log_{10} cfu ml^{-1}]
temperature	4°C	*E.coli*	n=3	-0.01±0.04	4.61±0.16
		L. monocytogenes	n=3	-0.04±0.06	4.56±0.02
		P. fluorescens	n=3	0.95±0.13	2.32±0.19
		S. aureus	n=3	0.45±0.07	1.68±0.27
		S. enterica	n=3	-0.03±0.02	0.25±0.05
	20°C	*E.coli*	n=3	-0.05±0.04	4.33±0.27
		L. monocytogenes	n=3	-0.22±0.03	4.56±0.02
		P. fluorescens	n=3	0.97±0.12	2.22±0.17
		S. aureus	n=3	0.48±0.04	3.84±0.21
		S. enterica	n=3	-0.03±0.04	1.39±0.03
	35°C	*E.coli*	n=9	-1±0.05	4.73±0.03
		L. monocytogenes	n=6	-0.35±0.03	4.45±0.09
		P. fluorescens	n=15	0.12±0.06	1.3±0.13
		S. aureus	n=6	-0.09±0.07	4.16±0.18
		S. enterica	n=10	-0.88±0.03	3.15±0.07

Next to temperature and contact time, the effect of air humidity was investigated. For *E. coli*, *L. monocytogenes* and *S. enterica* no effect of air humidity was observed. At both humidity conditions (98.3 ± 1.33 % rH and 23.1 ± 4.3 % rH) during the 24 h contact at 7 °C, the bacterial counts were reduced to or near the detection limit on poly(TBAMS) film and growth on reference material was observed (log_{10}-reductions: 4.5-4.8 log_{10} cfu ml^{-1}). At lower air humidity, the maximal log_{10}-reduction on poly(TBAMS), due to reduction to the detection limit, was reached for *S. aureus* (4.2 log_{10} cfu ml^{-1}) and *P. fluorescens* (4.9 log_{10} cfu ml^{-1}). But incubation at higher humidity conditions results in a decreased reduction for both bacteria, the behavior on reference material stayed comparable to low humidity. Consequently, the log_{10}-reduction for *P. fluorescens* is reduced to 1.5 log_{10} cfu ml^{-1} and for *S. aureus* to 3.0 log_{10} cfu ml^{-1}.

The reductions of *S. aureus* and *E. coli* on poly(TBAMS) films at different pH-values are shown in figure 3.5. Both bacteria were reduced under the detection limit within 2 h at 35 °C under acidic and neutral conditions on the poly(TBAMS) film resulting in log_{10}-reductions of 4.8-5.8. While the antimicrobial activity of the material against *S. aureus* was not affected at pH-values of 8 or 9, the reduction of *E. coli* decreased at these alkaline conditions in comparison to pH-values of 5-7. *E. coli* was reduced about 2.2 log_{10}-units at a pH-value of 8 and 2.3 log_{10}-units at a pH-value of 9; thus, antimicrobial activity according to test standard was also achieved at these conditions after 2 h contact.

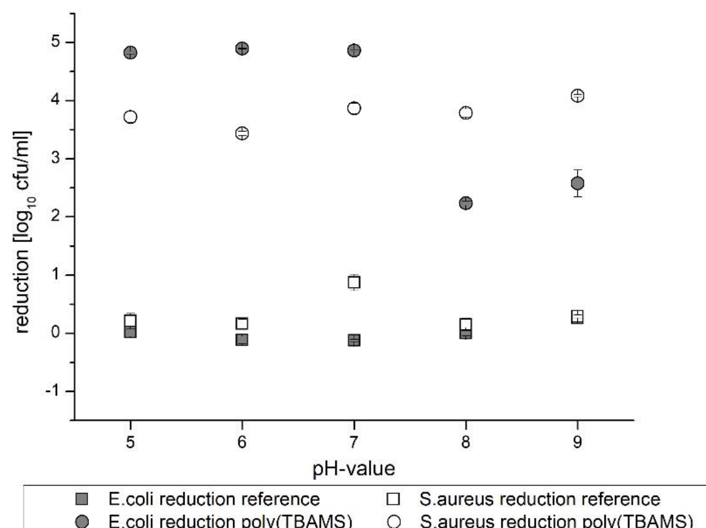

Figure 3.5 Reduction [$\log_{10}$ cfu ml^{-1}] of *S. aureus* (white symbols) and *E. coli* (grey symbols) applied on reference material (squares) or poly(TBAMS) (circles) after 2 h contact at 35 °C as a function of pH-value. The values are changes from initial concentration.

The effect of the presence of food ingredients are shown in figures 3 and 4. It became evident that with increasing concentration of the mineral nutrient calcium the antimicrobial activity of poly(TBAMS) films decreases for both tested bacteria (Figure 3.6). The maximal reduction to detection limit was only reached for *S. aureus* at a calcium concentration of 4 mmol l^{-1}, but at all magnesium concentrations. No effect of magnesium on the antimicrobial activity of poly(TBAMS) could be observed for *S. aureus*. For *E. coli* the reduction at a concentration of 20 mmol l^{-1} was lower than at 4 mmol l^{-1}. All reductions, even at high concentrations of mineral nutrients, were highly significant.

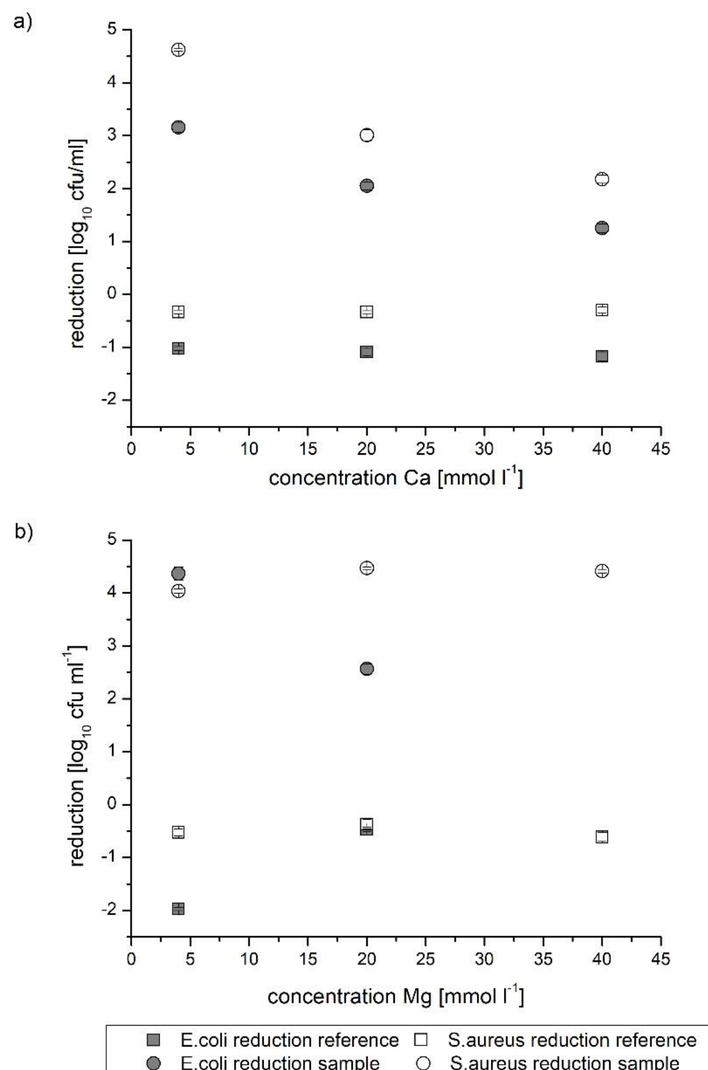

Figure 3.6 Reduction [$\log_{10}$ cfu ml^{-1}] of *S. aureus* (white symbols) and *E. coli* (grey symbols) applied on reference material (squares) or sample material (circles) after 2 h contact at 35 °C as a function of concentration of mineral nutrients (a: calcium, b: magnesium). The values are changes from initial concentration.

Figure 3.7 shows the antimicrobial activity of poly(TBAMS) if high counts of bacteria are present on the material. Even if high counts of bacteria are present, gram-positive bacteria were reduced to the detection limit within 2 h at 35 °C, resulting in $\log_{10}$-reduction of 7.8 for *L. monocytogenes* and 7.5 $\log_{10}$ cfu ml^{-1} for *S. aureus*. The differences in bacterial counts on poly(TBAMS) and reference material of *E. coli* (p=0.002), *P. fluorescens* (p=0.034) and *S. enterica* (p=0.002) were also significant. However, the antimicrobial activity of poly(TBAMS) films was reduced if the gram-negative bacteria were inoculated with high initial counts in nutrient rich media. Thus, *S. enterica* were reduced 0.6 $\log_{10}$ cfu ml^{-1} on the sample, while on the reference material a growth of 0.4 $\log_{10}$ cfu ml^{-1} was observed. For *P. fluorescens* a slight $\log_{10}$-reduction of 0.3 $\log_{10}$ cfu ml^{-1} was reached. The initial count of *E. coli* was the highest (9.4 $\log_{10}$ cfu ml^{-1}) and the reduction was 3.0 log cfu ml^{-1} within 2 h at 35 °C.

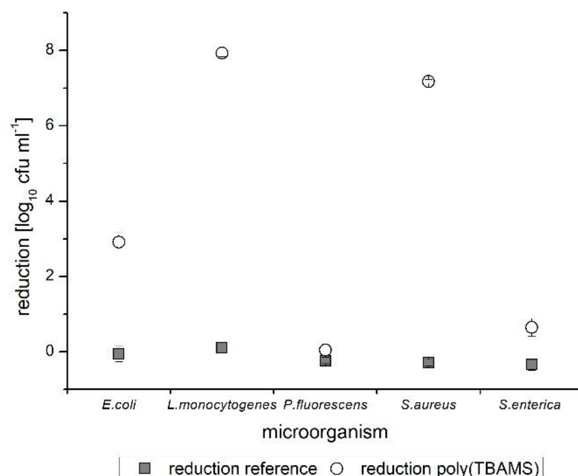

Figure 3.7 Reduction [$\log_{10}$ cfu ml^{-1}] of overnight cultures of bacteria in nutrient broth applied on reference material (dark grey bars) or poly(TBAMS) (light grey bars) incubated at 35 °C for 2 h. The values are changes from initial concentration (8.1-9.4 $\log_{10}$ cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on poly(TBAMS) after incubation ($\log_{10}$-reduction). Asterisks indicate significant differences (** $p \leq 0.005$, * $p \leq 0.05$) between poly(TBAMS) and reference material.

3.5 Discussion

Various antimicrobial systems were introduced to improve the hygienic status of food contact materials, but the activity proven under standard test conditions often cannot withstand the practical conditions during food processing and preparation [7–9]. Moreover, for a sustainable and effective use as food contact material, the activity of the materials must be long-lasting. Therefore, this study focused on the long-term stability of poly(TBAMS)-containing materials as well as their activity under conditions typical during the processing and preparation of meat.

Warnes and Keevil [36] emphasize a constant killing of microorganisms for a long period and under changing environmental conditions as a major requirement for active surfaces. This aspect is especially important for food contact surfaces which should be used over longer periods, like preparation equipment (e.g. cutting boards, handles of knives), storage boxes or machine surfaces. It must mention however, that long-term studies regarding this are mostly missing. A reason could be that long-term studies are time intensive and that materials are developed for application fields in which long-term stability over years often is not necessary, e.g. packaging material. The antimicrobial activity of the tested poly(TBAMS)-films retained a high antimicrobial activity over a period of 3 years. Highly significant differences between the reference and test material were detected independently of environmental storage conditions. This emphasizes the advantage of a contact-killing system over biocide-releasing systems [8, 9]. For example, a study of Møretrø [26] showed a reduced activity of a triclosan-containing cutting board after regular washing in a dish washer, due to release of active agents.

The long-term study of poly(TBAMS)-containing materials observed that the composition of the material can define the potential application. Modeling the relative $\log_{10}$-reductions showed

that copolymerization as well as the kind of copolymer led to more pronounced changes in activity. Environmental conditions during the long-term storage had no significant effect on the activity of the homopolymer and the copolymer with vinylopyridin. Hence, the materials could be used in cold environments like those prevalent in the meat industry as well as in domestic use with ambient room temperature conditions around 22 °C. Models of relative log_{10}-reduction of the copolymer poly(TBAMS):acrylonitrile [1:1] revealed that the lower the storage temperature (-20, 5, 22 °C on average) the more stable the antimicrobial activity was. Madkour et al. [37] showed for a facially amphiphilic, cationic polymer a complete loss of antimicrobial activity after heating at 80 °C for 4 days, but a stability in activity if stored at low temperature (-20 °C). At higher temperatures, chemical reactions are faster; hence, the results might be caused due to chemical modification or rearrangement of the polymer [37]. Additional investigations (data not shown) suggested that internal rearrangement between poly(TBAMS) and the copolymer component lead to a decrease of antimicrobial activity assumedly due to regression of active groups on the surface over time. Thus, after one year of storage under ambient conditions, a copolymer, poly(TBAMS:acrylonitrile) with an increased amount of acrylonitrile up to 70 % showed, no changes in antimicrobial activity was detected; a decrease in activity was detected if the amount of acrylonitrile was 90 %.

A further possible explanation is the humid conditions during the storage, which were lowest at ambient conditions. Additional experiments (data not shown) revealed a slightly better activity against gram-negative *P. fluorescens* if the material was stored under high humidity (96.7 ± 3.8 % rel. rh, 24 h) before antimicrobial tests were conducted. It can be hypothesized that, due to the higher relative air humidity, more carbon dioxide was dissolved in the aqueous film on the polymer, which led to protonation of the functional amino groups of poly(TBAMS)-film.

The results of the antimicrobial tests with adjusted air humidity during the contact between bacteria and antimicrobial materials showed a reduction of all bacteria by poly(TBAMS) at high relative air humidity (98.3 ± 1.3 % rH) as well as low humidity conditions (23.1 ± 4.3 % rH) after 24 h contact at 7 °C. Thus, an antimicrobial effect of poly(TBAMS) films is ensured both during processing or cleaning with high humidity conditions and during drying/production stop, when humidity decreases. However, the activity against *P. fluorescens* and *S. aureus* was reduced with high relative air humidity during contact time. Also, a study of Møretrø et al. [26] detected a higher activity at 70 % rH than at 100 % rH on a triclosan-containing cutting board when the initial count was 6.7 to 7.0 cfu ml^{-1}. The authors assumed that the dry conditions generate osmotic stress which sensitized the bacteria for the antimicrobial agent.

The results of humidity tests already showed good antimicrobial activity on the different bacteria under cold conditions after 24 h. The temperature was often identified to affect the antimicrobial activity of different materials [1, 15–19], but despite the possible use for

refrigerated products, most tests for antimicrobial activity are conducted at 35°C like prescribed in the standard test method. This study showed a temperature dependent activity of poly(TBAMS) for *S. aureus*, *S. enterica* and *P. fluorescens* with a reduced activity at cold temperatures. For migrating systems, the effect is caused by the slower release of active components into the environment at lower temperatures [38]. The lower activity of contact active materials like poly(TBAMS) can be explained by changes in bacteria cell structure due to adaptation in gene regulation depending on environmental conditions [39, 40]. Cell surface structures have a direct influence on the interactions between bacteria and surfaces. This is traced back to changes in the hydrophobicity or electrophoretic mobility of bacteria cells [39, 41, 42]. Bonaventura et al. [39] showed a positive correlation between cell surface hydrophobicity and temperature. Furthermore, Briandet et al. [41] described that the electrophoretic mobility of *L. monocytogenes* decreased if the growth temperature was reduced from 15 to 8 °C due to more carboxyl groups and the presence of flagella at 15 °C. Studies of Dohlen et al. [34] and Braun et al. [35] showed that the electrophoretic mobility of the bacteria surfaces is a key factor for the antimicrobial activity of poly(TBAMS). This was also confirmed by the results of this study: the antimicrobial activity of both poly(TBAMS)-films is higher for the comparatively more negative bacteria (*E. coli, L. monocytogenes, S. aureus*) than for bacteria with lower electrophoretic mobility (*P. fluorescens, S. enterica*) [43–46]. Thus, even after 1 h contact at 35 °C, *S. aureus* and *L. monocytogenes* is reduced to the detection limit on both materials, resulting in a log_{10}-reduction of around 4.5 log_{10} cfu ml^{-1}, whereas the log_{10}-reduction of *P. fluorescens* and *S. enterica* is 1.7 log_{10} cfu ml^{-1}.

The antimicrobial activity of releasing systems can be improved by prolonging the contact time. Similar results were mentioned for contact-active materials [34, 47]. A reason could be that, over time, even weak interactions could be generated. Moreover, during bacterial growth, the composition, and accordingly the charge, of bacterial surface changes. For example, the hydrophobicity of bacteria surfaces increases during the exponential phase of growth [48]. For *S. enterica* it was noticeable that the effectiveness of the homopolymer increased parallel to growth on the reference material.

The effect of bacterial surface charge also became evident in the tests with high initial counts in nutrient rich media. The more negatively charged gram-positive bacteria *L. monocytogenes* and *S. aureus* were maximally reduced while the less negatively charged gram-negative bacteria were less effected. Both the high initial counts and the nutrient rich media might contribute to the more distinct effect on activity in comparison to previous results in antimicrobial screening. Former investigations with low nutrient inoculum also showed an effect of high initial bacterial concentration on the antimicrobial activity of poly(TBAMS) for some gram-negative bacteria [35]. A lower activity of triclosan-containing cutting boards against higher initial counts was also described by Møretrø et al. [26]. Studies with cast copper alloys showed faster killing and better activity if the inoculum concentration was reduced [18].

The influence of nutrient rich media can be explained in different ways. Møretrø et al. [21] assumed that the osmotic stress for bacterial cells in media with low ionic strength is perhaps higher than for cells in nutrient rich media. In the studies of Noyce et al. [49] and Cutter [50] fatty acids seem to provide a protective matrix for microorganisms, leading to reduced activity of cast copper alloys as well as triclosan-incorporated plastics. Studies of silver-containing materials showed that the antimicrobial activity is inhibited in the presence of proteins [21, 23, 24]. Dohlen et al. [34] concluded that the presence of proteins is the limiting factor for antimicrobial activity of poly(TBAMS). In their study, a negative effect of meat extract and BSH on the antimicrobial activity of poly(TBAMS), especially on gram-negative bacteria, was shown. Proteins can interact with the antimicrobial agents and occupy the functional amino groups. Furthermore, in the presence of nutrients, the electrical charge of the bacteria is affected [34]. Additionally, for different cationic antibiotics and cationic polymers, whose modes of action are related to membrane disruption, it was shown that those divalent cations inhibit the antibacterial activity [28, 29, 51, 52]. In the study of Lenoir et al. [29], the antimicrobial activity of poly(TBAEMA) was completely inhibited by adding an excess of calcium ions compared to the secondary amino groups of poly(TBAEMA) in LLDPE. In the experiments, an effect of calcium ions on the antimicrobial activity of poly(TBAMS:acrylonitrile) was detected. The effect decreased with rising calcium concentration for *E. coli* and *S. aureus*. A negative effect on the activity against *E. coli* was also measured for magnesium ions, but not for *S. aureus*. Divalent calcium and magnesium ions are structural elements of bacteria membranes and of numerous foods. One announced thesis for the mode of action is that SAM®-Polymers displace these divalent cations resulting in a disruption of the membrane [29]. If divalent cations are represented in bacteria solutions, the membrane is stabilized, or rather the mineral cations are in competition with protonated amino side chains of the polymer and are preferentially integrated into the membrane. This effect was more pronounced for calcium, because it is a more frequent membrane component than magnesium [52]. Due to the additional outer membrane, magnesium protected the gram-negative bacteria *E. coli* against poly(TBAMS) while antimicrobial activity against *S. aureus* persisted. However, an antimicrobial activity of the copolymer poly(TBAMS:acrylonitrile) is maintenance even under concentrations of mineral nutrients which exceed concentrations in meat.

The presence of organic ingredients determines the characteristics of bacterial cell surfaces. The hydrophobicity and electrophoretic mobility changes subject to organic content of growth media of bacteria. For example, *E. coli* grown under minimal media conditions had a lower zeta potential and hence were more negative compared to those grown in rich media [45, 53]. Van Loosdrecht et al. [48] assumed an increase of electrophoretic mobility with decreasing salt concentration. Furthermore, *E. coli* grown in nutrient broth was more hydrophobic than in TSB [53]. These surface changes led, like mentioned before, to reduced interaction between antimicrobial surfaces and bacteria, but the effect is dependent on bacterial strain.

In additional to bacteria surface charge, the charge of the polymer surface affects the antimicrobial activity. During processing as well as cleaning and disinfection, food contact surfaces are exposed to a variety of pH-values [9], which influence the charge of both surfaces. The activity of the homopolymer of poly(TBAMS) was reduced against *E. coli* at alkaline conditions. This conforms with the investigations of poly(TBAEMA) by Buranasompob [47]. A possible explanation was mentioned by Seyfriedsberger et al. [54] who showed a decreasing zeta potential of compounds of LLDPE and TBAEMA at increasing pH-value. Also, Chang et al. [17] substantiated a negative correlation of pH and zeta potential and a positive correlation of zeta potential and antimicrobial activity of chitosan. For SAM-Polymers® a certain degree of protonation of the amino groups is essential for activity [47]. Comparing the results of the homo and the copolymer showed a slightly better antimicrobial activity for the homopolymer, caused by the higher number of active groups and consequently more protonation on the surface. This also explains the higher activity under acidic conditions. At pH-values of 8 and 9, the surface of poly(TBAMS) is presumably mostly neutrally charged. Caused by the more negative charge of *S. aureus,* the interactions between *S. aureus* and poly(TBAMS) surface is more pronounced than for *E. coli,* resulting in no significant changes in activity even under acidic test conditions [43, 46]. The same effects were shown in the long-term studies. Due to the assumed rearrangements, the surface charge of the copolymer poly(TBAMS:acrylonitrile) is reduced, and the difference in bacterial surface charge lead to a previously mentioned decrease in activity against *E. coli*.

3.6 Conclusion

The tested polymer films based on poly((tert.-butyl-amino)-methyl-styrene) show a good antimicrobial activity under certain processing conditions and a very promising long-term effectiveness. The long-term stability under various environmental conditions is advantageous, especially over migrating systems. The extensive investigations used several factors typical during the processing and preparation of meat which influence the bacteria or the polymer itself, leading to changed interactions and hence to divergent antimicrobial activity.

Subjected to the individual bacterial strain, a decrease in surrounding temperature leads to a decrease in antimicrobial activity of poly(TBAMS)-material, which can be improved by prolonging the contact time. Furthermore, the presence of food ingredients effects the antimicrobial activity. In comparison to many antimicrobial agents, the tested SAM-Polymers® also show a good antimicrobial activity against a wide range of relevant bacteria under conditions predominant in meat processing and preparation companies. In addition, factors like extreme values of pH occuring during sanitation, or of air humidity have marginal effects on the polymeric activity.

Thus, poly(TBAMS) has a great potential to be used as an antimicrobial agent in food contact materials, facilitating a reduction of surface contamination and therefore improvement of the hygienic status of food contact surfaces, consequently reducing cross-contamination. In a next

step, the direct impact of poly(TBAMS)-containing materials on bacterial loads during cross-contamination scenarios will be studied.

3.7 References

1. Faúndez G, Troncoso M, Paola N, Figueroa G (2004) Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni. BMC Microbiol 19(4).
2. Reij MW, Den Aantrekker ED (2004) Recontamination as a source of pathogens in processed foods. Int J Food Microbiol 91(1), 1–11.
3. Gundelley R, Youm GW, Kwon YM (2007) Survival of bacterial pathogens on antimicrobial conveyer belts. J Rapid Meth Aut Mic 15(3), 259–266.
4. Todd ECD, Greig JD, Bartleson CA, Michaels BS (2009) Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 6. Transmission and Survival of Pathogens in the Food Processing and Preparation Environment. J Food Prot 72(1), 202–219.
5. Giaouris E, Chorianopoulos N, Doulgeraki A, Nychas G-J, Webber MA (2013) Co-Culture with Listeria monocytogenes within a Dual-Species Biofilm Community Strongly Increases Resistance of Pseudomonas putida to Benzalkonium Chloride. PLOS ONE 8(10), e77276.
6. Giaouris E (2015) Ability of Foodborne Bacterial Pathogens to Attach to Meat and Meat Contact Surfaces, in: Pometto AL, Demirci A (eds.) Biofilms in the Food Environment: Second Edition, pp. 145–175. Chichester, UK: John Wiley & Sons, Ltd.
7. Møretrø T, Langsrud S (2011) Effects of Materials Containing Antimicrobial Compounds on Food Hygiene. J Food Prot 74(7), 1200–1211.
8. Moerman F (2014) Antimicrobial materials, coatings and biomimetic surfaces with modified microtography to control microbial fouling of product contact surfaces within food processing equipment: Legislation, requirements, effectiveness and challenges. Journal of Hygienic Engineering and Design 7, 8–29.
9. Bastarrachea LJ, Denis-Rohr A, Goddard JM (2015) Antimicrobial Food Equipment Coatings: Applications and Challenges. Annu Rev Food Sci Technol 6(1), 97–118.
10. Tiller JC (2011) Antimicrobial Surfaces, in: Börner HG, Lutz J-F (eds.) Bioactive Surfaces, pp. 193–217: Springer Berlin Heidelberg.
11. Ilg Y, Kreyenschmidt J (2012) Review: Benefits and risks of the use of antimicrobial components in the food chain. J Food Saf Food Qual 63(2), 28–34.
12. Rocha M, Ferreira FA, Souza MM, Prentice C (2015) Antimicrobial films: a review, in: Méndez-Vilas A (ed.) Microbial pathogens and strategies for combating them: science, technology and education, 2nd edn., pp. 23–31. Spain: Formatex Research Center.
13. Appendini P, Hotchkiss JH (2002) Review of antimicrobial food packaging. Innovative Food Sci Emerging Technol 3(2), 113–126.
14. Balasubramanian A, Rosenberg LE, Yam K, Chikindas ML (2009) Antimicrobial packaging: potential vs. reality—a review. J Appl Pack Res 3(4), 193–221.
15. Kampmann Y, Clerck E de, Kohn S, Patchala DK, Langerock R, Kreyenschmidt J (2008) Study on the antimicrobial effect of silver-containing inner liners in refrigerators. J Appl Microbiol 104(6), 1808–1814.
16. Tsai GJ, Su WH (1999) Antibacterial activity of shrimp chitosan against Escherichia coli. J Food Prot 62(3), 239–243.
17. Chang S-H, Lin H-TV, Wu G-J, Tsai GJ (2015) pH Effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. Carbohydr Polym 134, 74–81.
18. Noyce JO, Michels H, Keevil CW (2006) Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant Staphylococcus aureus in the healthcare environment. J Hosp Infect 63(3), 289–297.
19. Braid JJ, Wale MCJ (2002) The antibacterial activity of triclosan-impregnated storage boxes against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Shewanella putrefaciens in conditions simulating domestic use. J Antimicrob Chemother 49(1), 87–94.
20. Michels H, Noyce J, Keevil C (2009) Effects of temperature and humidity on the efficacy of methicillin-resistant Staphylococcus aureus challenged antimicrobial materials containing silver and copper. Lett Appl Microbiol 49(2), 191–195.
21. Møretrø T, Høiby-Pettersen GS, Halvorsen CK, Langsrud S (2012) Antibacterial activity of cutting boards containing silver. Food control 28(1), 118–121.

22. Chaitiemwong N, Hazeleger WC, Beumer RR (2010) Survival of Listeria monocytogenes on a conveyor belt material with or without antimicrobial additives. Int J Food Microbiol 142(1–2), 260–263.
23. Berrang ME, Frank JF, Meinersmann RJ (2010) Listeria monocytogenes biofilm formation on silver ion impregnated cutting boards. Food Prot Trends 30(3), 168–171.
24. Ilg Y, Kreyenschmidt J (2011) Effects of food components on the antimicrobial activity of polypropylene surfaces containing silver ions (Ag+). Int J Food Sci Technol 46(7), 1469–1476.
25. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD (1997) Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. Letters in Applied Microbiology 25(4), 279–283.
26. Møretrø T, Høiby-Pettersen GS, Habimana O, Heir E, Langsrud S (2011) Assessment of the antibacterial activity of a triclosan-containing cutting board. Int J Food Microbiol 146(2), 157–162.
27. Larsen MH, Dalmasso M, Ingmer H, Langsrud S, Malakauskas M, Mader A, Møretrø T, Smole Možina S, Rychli K, Wagner M, John Wallace R, Zentek J, Jordan K (2014) Persistence of foodborne pathogens and their control in primary and secondary food production chains. Food control 44, 92–109.
28. Endo Y, Tani T, Kodama M (1987) Antimicrobial activity of tertiary amine covalently bonded to a polystyrene fiber. Appl Environ Microbiol 53(9), 2050–2055.
29. Lenoir S, Pagnoulle C, Galleni M, Compère P, Jérôme R, Detrembleur C (2006) Polyolefin Matrixes with Permanent Antibacterial Activity: Preparation, Antibacterial Activity, and Action Mode of the Active Species. Biomacromolecules 7(8), 2291–2296.
30. Kalyon BD, Olgun U (2001) Antibacterial efficacy of triclosan-incorporated polymers. Am J Infect Control 29(2), 124–125.
31. Thölmann D, Kossmann B, Sosna F (2003) Polymers with antimicrobial properties. EC Journal(1-2), 16–33.
32. Kreyenschmidt M, Lorenz R, Fischer B, Kreyenschmidt J, Brodkorb F, Kalbfleisch K, Blang T, Geschwentner A (2014) Anti-microbial polymer: Google Patents(WO2014118339 A1). http://www.google.com/patents/WO2014118339A1?cl=en.
33. Brodkorb F, Fischer B, Kalbfleisch K, Robers O, Braun C, Dohlen S, Kreyenschmidt J, Lorenz R, Kreyenschmidt M (2015) Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. Int J Mol Sci 16(8), 20050–20066.
34. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Robers O, Kreyenschmidt M, Kreyenschmidt J (2016) Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products. J Appl Microbiol 121(4), 1059-70.
35. Braun C, Dohlen S, Ilg Y, Brodkorb F, Fischer B, Heindirk P, Kalbfleisch K, Richter T, Robers O, Kreyenschmidt M, Lorenz R, Kreyenschmidt J (2017) Antimicrobial Activity of Intrinsic Antimicrobial Polymers Based on Poly((tertbutyl-amino)-methyl-styrene) Against Selected Pathogenic and Spoilage Microorganisms Relevant in Meat Processing Facilities. J Antimicrob Agents 03(01).
36. Warnes SL, Keevil CW (2011) Mechanism of Copper Surface Toxicity in Vancomycin-Resistant Enterococci following Wet or Dry Surface Contact. Appl Environ Microbiol 77(17), 6049–6059.
37. Madkour AE, Dabkowski JM, Nusslein K, Tew GN (2009) Fast disinfecting antimicrobial surfaces. Langmuir 25(2), 1060–1067.
38. Quintavalla S, Vicini L (2002) Antimicrobial food packaging in meat industry. Meat Sci 62(3), 373–380.
39. Di Bonaventura G, Piccolomini R, Paludi D, D'Orio V, Vergara A, Conter M, Ianieri A (2008) Influence of temperature on biofilm formation by Listeria monocytogenes on various food-contact surfaces: Relationship with motility and cell surface hydrophobicity. J Appl Microbiol 104(6), 1552–1561.
40. Liu S, Graham JE, Bigelow L, Morse PD, Wilkinson BJ (2002) Identification of Listeria monocytogenes genes expressed in response to growth at low temperature. Appl Environ Microbiol 68(4), 1697–1705.
41. Briandet R, Meylheuc T, Maher C, Bellon-Fontaine MN (1999) Listeria monocytogenes Scott A: Cell Surface Charge, Hydrophobicity, and Electron Donor and Acceptor Characteristics under Different Environmental Growth Conditions. Appl Environ Microbiol 65(1), 5328–5333.

42. Giovannacci I, Ermel G, Salvat G, Vendeuvre JL, Bellon-Fontaine MN (2000) Physicochemical surface properties of five Listeria monocytogenes strains from a pork-processing environment in relation to serotypes, genotypes and growth temperature. J Appl Microbiol 88(6), 992–1000.
43. Potter R, Truelstruphansen L, Gill T (2005) Inhibition of foodborne bacteria by native and modified protamine: Importance of electrostatic interactions. Int J Food Microbiol 103(1), 23–34.
44. Gottenbos B (2001) Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. J Antimicrob Chemother 48(1), 7–13.
45. Soni KA, Balasubramanian AK, Beskok A, Pillai SD (2008) Zeta Potential of Selected Bacteria in Drinking Water When Dead, Starved, or Exposed to Minimal and Rich Culture Media. Curr. Microbiol. 56(1), 93–97.
46. Mitik-Dineva N, Wang J, Truong VK, Stoddart P, Malherbe F, Crawford RJ, Ivanova EP (2009) Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus attachment patterns on glass surfaces with nanoscale roughness. Curr. Microbiol. 58(3), 268–273.
47. Buranasompob A (2005) Kinetics of the inactivation of microorganisms by water insoluble polymers with antimicrobial activity. Dissertation. Berlin.
48. van Loosdrecht MC, Lyklema J, Norde W, Schraa G, Zehnder AJ (1987) Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 53(8), 1898–1901.
49. Noyce JO, Michels H, Keevil CW (2006) Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing. Appl Environ Microbiol 72(6), 4239–4244.
50. Cutter CN (1999) Effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J Food Prot 62(5), 474–479.
51. Chen CZ, Cooper SL (2002) Interactions between dendrimer biocides and bacterial membranes. Biomaterials 23(16), 3359–3368.
52. Sahalan AZ, Aziz AHA, Hing HL, Ghani MKA (2013) Divalent cations (Mg2+, Ca2+) protect bacterial outer membrane damage by polymyxin B. Sains Malays 42(3), 301–306.
53. Hassan AN, Frank JF (2004) Attachment of Escherichia coli O157: H7 grown in tryptic soy broth and nutrient broth to apple and lettuce surfaces as related to cell hydrophobicity, surface charge, and capsule production. Int J Food Microbiol 96(1), 103–109.
54. Seyfriedsberger G, Rametsteiner K, Kern W (2006) Polyethylene compounds with antimicrobial surface properties. Eur Polym J 42(12), 3383–3389.

4 Effect of antimicrobial treated cutting-boards on cross-contamination of pathogens during preparation of meat and ready-to-eat food

4.1 Abstract

The objective of this study was to evaluate the effect of antimicrobially treated cutting-boards on the bacterial transfer during food preparation.

The activity of one biocide-releasing (polypropylene with Microban®) and two contact-active (LLDPE with poly(tert-butyl-amino-ethyl)-methacrylate (poly(TBAEMA)) and poly((tert-butyl-amino)-methyl-styrene) (poly(TBAMS)) cutting-boards against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica* serovar Thypimurium was investigated at different temperatures and contact times. Transfer rates were determined in cross-contamination scenarios: inoculated pork to board (1), inoculated board to cucumber (2), inoculated pork via board to cucumber (3). The effect of poly(TBAMS) on cross-contamination were determined by comparing transfer rates between sample boards with and reference boards without antimicrobial treatment.

Just the contact-active boards showed antimicrobial activity, which is dependent on the material, bacterium and decreases with decreasing temperature and time. Bacterial transfer and effect of poly(TBAMS) varies depending on bacterium and food component. *Listeria* crossed over in higher rates than *Staphylococcus.* Transfer rates from reference boards to cucumber are higher than from pork to board. Poly(TBAMS)-boards could reduce transfer of *Listeria*, but no significant effect on *Staphylococcus*-transfer could be identified. The highest effect was measured in scenario 2 with a reduction of transfer rates from 59.82±3.05 % on the reference to 0.49±0.09 % on the sample. Effects were lower in scenarios with pork.

Poly(TBAMS) offered the most promising antimicrobial profile, but the effect on pathogenic transfer is restricted.

4.2 Introduction

Microorganisms can adhere to and persist on food contact surfaces for long times [1–5]. This often leads to food safety and quality problems caused by cross-contamination in all stages of the food chain [4, 6–14]. Several studies determined high transfer rates of bacteria from food to food contact surfaces and vice versa even after very short contact times of 5 sec and independent of the applied pressure of food on the surfaces [3, 15–19]. Therefore, the hygiene of food contact surfaces (e.g. conveyers, cutting boards, containers) is necessary to reduce the risk of transfer of pathogens. However, unsafe and risky handling of food often facilitates cross-contaminations during meal preparation in domestic kitchens as well as in restaurants [10, 20]. According to a study of Josephson et al. [21], which investigated the prevalence of bacteria in kitchens in the U.S., cutting boards belong to the top five sites most contaminated with heterotrophic bacteria and therefore are perceived as fomites in cross-contamination of foodstuff with pathogens [12, 13, 17, 22–26]. Redmond and Griffith [20] reviewed several studies of consumer food handling in Europe, the U.S., Australia and New Zealand. They indicated that 30-71 % of the respondents reported to using the same equipment (utensils, cutting boards, surfaces) to prepare raw meats and other products. Also, 40 % of interviewed restaurant food manager declared that they do not designate certain cutting boards for raw chicken consistently [27]. Besides, even if cutting boards are rinsed with water after use, bacteria are often not significantly removed; only via scrubbing with detergent and hot water sufficient reduction in bacterial count be reached [17, 28]. In the food industry, bacterial load on food contact surfaces is reduced by extensive cleaning and disinfection treatments after processing. But during the processing step of food, the contamination increases significantly on food contact surfaces and leads to transfer of bacteria to food [29, 30]. And often the treatments are insufficient, thus, investigations in a salmon fillet processing plant detected spoilage bacteria on 75 % of tested equipment surfaces after cleaning [14], and pathogens like *S. aureus*, *S. enterica* and *L. monocytogenes* were recovered from food contact surfaces being persistent for several days [3, 31, 32].

To reduce cross-contamination, the application of antimicrobial food contact surfaces is gaining more interest [4, 33, 34]. In the last decade, different antimicrobial materials have been developed, especially, commonly used polymers like polyethylene and metallic materials like stainless steel were antimicrobially treated [35]. In general, these materials can be classified according to their bactericidal mechanism in biocide releasing and contact-active materials. The most frequently used antimicrobial agents in biocide releasing food contact material are silver components as well as triclosan [24, 35–37]. The use of those systems is critically discussed, because biocides are released uncontrolled into the environment as well as into the food products, and furthermore, the effectivity of such materials is limited over a short time period [14]. Contact-active materials are favored regarding these topics. As a new class of those materials, sustainable active microbiocidal polymers (SAM-Polymers®) were introduced into the market. Compounding of the SAM-Polymers® poly(tert-butyl-amino-ethyl)-methacrylate

(poly(TBAEMA)) and poly((tert-butyl-amino)-methyl-styrene) (poly(TBAMS)) with established polymers is a new approach to produce surfaces combining sustainable antimicrobial activity and low costs as well as good mechanical properties due to the contingent of commonly used polymers [33, 38–40].

The general antimicrobial activity of both mentioned approaches in food contact materials were proven in numerous studies. The general antimicrobial activity of the different polymers is dependent on several factors like humidity, temperature, contact time, presence of food components and the amount of antimicrobial active agent [24, 40–45]. The influence of the different named parameters depends on the material itself, thus, especially for biocide releasing systems, a decreased activity was proven at low temperatures and in the presence of food components, which limits the potential to reduce cross-contamination [42, 46–48]. Even if the general antimicrobial activity of different materials has been studied, limited research is available about the effect of antimicrobial treated polymers on bacterial cross-contamination

Hence, the objective of this study was to investigate the potential of three kinds of cutting boards to reduce pathogenic cross-contamination during food processing and preparation. One of the cutting boards was treated with Microban®, in which activity is based on the biocide-releasing effect, while the two other boards were contact-active due to incorporation of two different SAM-Polymers®.

4.3 Materials and Methods

To evaluate the potential of antimicrobially treated cutting boards to reduce cross-contamination, two experimental series were conducted. In the first one, the antimicrobial activity of one biocide releasing material (polypropylene with Microban®) and two contact-active materials (LLDPE with poly(TBAEMA) or poly(TBAMS)) were screened and compared. Therefore, the materials were tested on the basis of the Japanese standard (JIS) 2801:2000 against selected pathogens (*Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium) under defined conditions, which were different in temperature and time to consider the unequal environmental conditions in the different stages of food processing and preparation as well as the varying value of contact between food and antimicrobial surface. A high antimicrobial activity is an important requirement for the reduction of cross-contamination. Therefore, in the second experimental series, only the material which showed the most promising antimicrobial activity was selected for the detailed experiment studying the effect on pathogenic cross-contamination during preparation of meat and ready-to-eat food. Three cross-contamination scenarios with pork filets and cucumber slices were simulated: in the first scenario pork was contaminated with pathogens and placed on the cutting-board; in the second one the cutting boards was inoculated with pathogens and the transfer to a cucumber was investigated; in the last scenario the transfer from inoculated pork via cutting-board to cucumber was tested. In all scenarios the viable counts on

food stuffs and cutting boards with or without poly(TBAMS) were determined and finally the transfer rates were assessed.

4.3.1 Antibacterial test material

Three different test materials were used in the first experimental series of the study. The silver releasing board is commercially available and was bought in a local discounter. According to the manufacturer, the polypropylene (PP) material was treated with silver phosphate glass (Microban®). The contact-active boards with SAM-Polymers® were prepared by the University of Applied Sciences Münster. Boards were made on a press out of the two different compounds of the base polymer, linear-low density polyethylene (LLDPE) (Dowlex 2433, DOW, Edegem, Belgium) combined with 10 % poly(TBAMS) and poly(TBAEMA) respectively. PP and LLDPE boards were used as reference materials. All boards were cut into square cuboids (4 x 4 cm) and decontaminated by dipping in boiling water for 15 sec prior to use. After sterilization, the materials were transferred into petri dishes.

4.3.2 Bacterial strains

Listeria monocytogenes (ATCC 19111), *Staphylococcus aureus* subsp. *aureus* (ATCC 6538), and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 14028), as typical pathogens involved in cross-contamination during meat processing, were used in the test series. All strains, delivered by the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany), were stored at -18 °C in a CRYOBANKTM system (Mast, Reinfeld, Germany). Before starting this trial, a frozen culture was transferred to 10 ml nutrient broth (Merck KGaA, Darmstadt, Germany) and incubated overnight at 37 °C. The inocula were prepared via dilution in physiological saline solution with tryptone (1 g l^{-1}) (Oxoid, Hampshire, UK) to a final concentration of 10^5 cfu ml^{-1}.

4.3.3 Food products

Food products used in the second experimental series of the study were pork filet and cucumber. Both were purchased at a local retail shop. Cucumbers were washed with water and wiped with tissue paper soaked with ethanol, after air drying they were sliced into pieces with an approximate surface area per side of 12.5 cm² and a weight of around 25 g. To attain comparable surface areas of meat and cucumber, the filets of pork were wrapped in plastic wrap and tin foil forming filets with diameters similar to those of the cucumbers and were deep-frozen for 24 h. Afterwards they were cut in approximately equal thick pieces of about 15 g. Pieces were defrosted overnight in the refrigerator.

4.3.4 Test performance

Screening of antimicrobial activity of treated cutting boards

Antimicrobial screening was done based on the test standard JIS 2801:2000. To test the antimicrobial activity in each single trial, the surfaces of 3 treated boards (samples) and 6 untreated boards (references) were inoculated with 400 µl of *S. aureus*, *L. monocytogenes* or

S. Thypimurium solutions applied in 8 drops of 50 µl which were spread over the surface with a pipette tip. Afterwards the 3 samples and 3 references were transferred to incubators (Sanyo model MIR 153, Sanyo Electric Co., Ora-Gun, Gumma, Japan) and exposed to different temperature conditions (7 °C, 20 °C, 35 °C) for different time intervals (2 min, 2 h, 24 h). The initial count of inoculum was determined by washing out the remaining 3 references immediately after inoculation with soybean-casein digest broth with lecithin polysorbate (Roth, Karlsruhe, Germany) via pipetting. After exposure, the samples and references were handled in the same way. Viable counts were determined by counting the colonies on plate count agar (Merck KGaA, Darmstadt, Germany) using drop plate technique; to lower the detection limit, the lowest dilution of samples were also determined by pour plate technique. Agar plates were incubated for 24 h (drop plate technique) or 48 h (pour plate technique) at 37 °C. Detection limits were determined to be 1.0 $\log_{10}$ cfu ml^{-1} for pour plate technique and 2.0 $\log_{10}$ cfu ml^{-1} for drop plate technique. All experiments were conducted two-fold for each type of cutting board.

Reduction or growth on material after incubation was calculated by subtracting the logarithmic average value of bacterial concentration on the reference material immediately after inoculation (N_i) from the average value of bacterial concentration on the material after incubation (N_c) (Eq. 4.1)

$$f(N_i, N_c) = \log_{10}(N_i) - \log_{10}(N_c). \quad (4.1)$$

Standard errors ($df_{R,G}$) were calculated following the Gaussian propagation of uncertainty (Eq. 4.2)

$$df_{R,G} = \sqrt{\left(\frac{1}{N_i \times \ln 10} \times dN_i\right)^2 + \left(\frac{1}{N_c \times \ln 10} \times dN_c\right)^2} \quad (4.2)$$

where N_i=average of bacterial concentration on reference material immediately after inoculation; d=standard error, and N_c=average of bacterial concentration on reference, respectively sample material, after incubation, ln= natural logarithm base *e*.

The value of the antimicrobial activity was calculated by subtracting the logarithmic value of the viable counts on the sample material from the logarithmic value of the reference material after inoculation and incubation (Eq. 4.3):

$$\log_{10} - \text{reduction} = \log_{10}(N_{CR}/N_{CS}) \quad (4.3)$$

with N_{CR}= average of bacterial concentration on reference material, and N_{CS}= average of bacterial concentration on sample material both after 2 h incubation.

Simulation of cross-contamination scenarios

Three different scenarios of cross-contamination were simulated using the poly(TBAMS) containing LLDPE-board (Figure 4.1). For each pathogenic bacterium, each scenario was

conducted in at least two replicates with bacterial counts determined on at least two cutting boards.

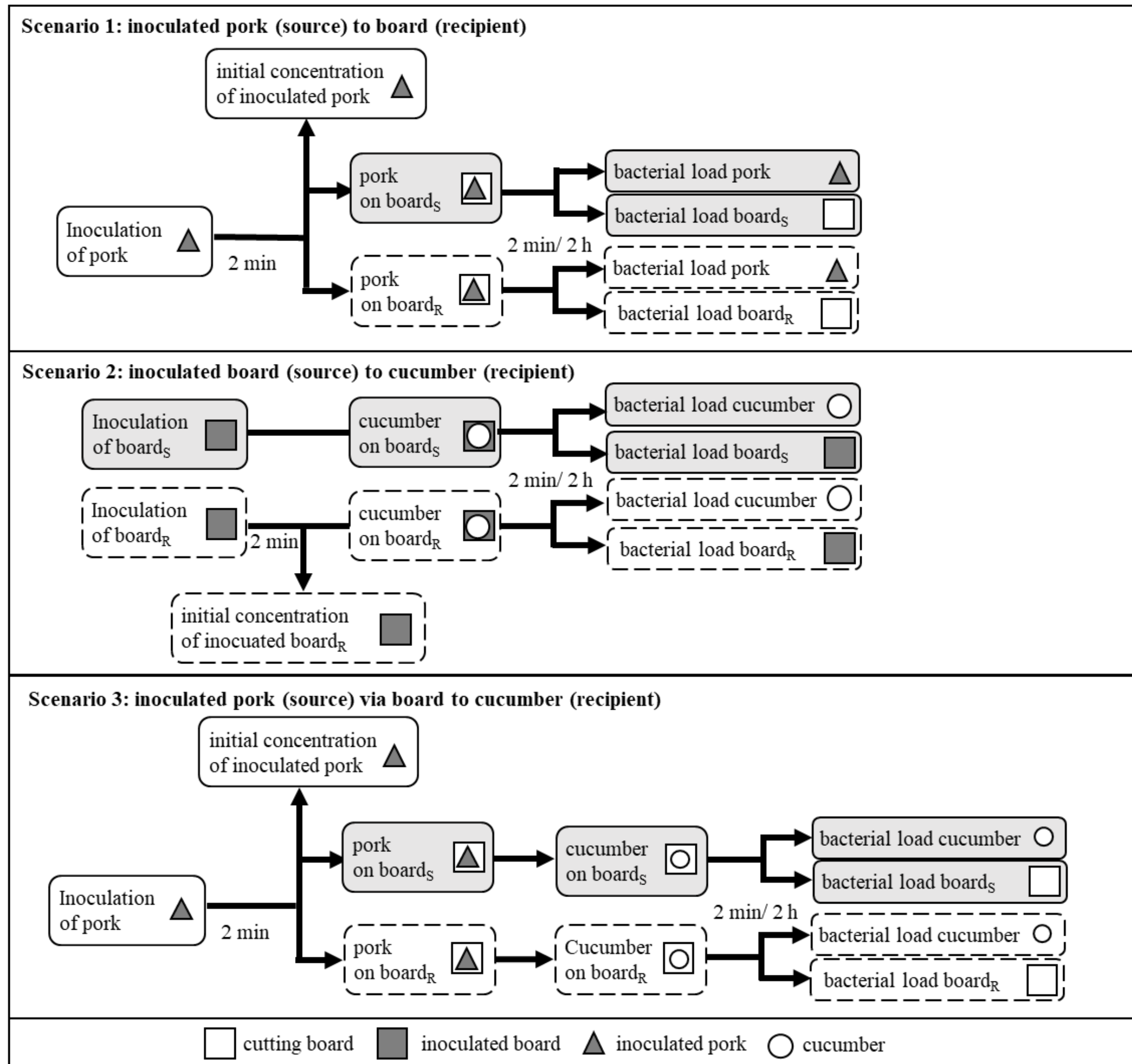

Figure 4.1 Shematic representation of cross-contamination the three scenarios. Processes with poly(TBAMS)-treated cutting boards (S) are presented in light grey and processes with untreated LLDPE (R) are presented in white and with dashed lines.

In scenario 1, each of the 14 pieces of pork was inoculated with a total of 200 µl (4 drops of 50 µl each) of bacterial solution (*L. monocytogenes* or *S. aureus*) to reach a final bacterial concentration on the meat of 10^3 cfu cm^{-2}. After spreading the inoculum with a pipette tip, the filet pieces were stored for 2 minutes to let the bacteria attach to the surface. To control the initial concentration 2 filet pieces were sampled immediately. The remaining 12 pieces were placed with the inoculated surfaces half on treated and half on untreated cutting boards. To standardize contact area between cutting board and pork fillet, the gap between the pork and the lid of the petri dishes were filed with circular sterile LLDPE disks. On top of the petri lids, weights (350 g) were placed to generate similar pressure. After 2 min and 2 h contact at 20 °C the total viable counts, which consist of the natural flora and the inoculated bacteria, as well as

individual bacterial counts of the inoculated pathogens of filet pieces and the surface count of cutting boards were determined.

In the second part of the cross-contamination study (scenario 2), the transfer from a contaminated cutting board to cucumber was simulated. Cutting boards were inoculated with 400 µl (8x50 µl) of the bacterial solutions, which was spread via pipette tip, resulting in a concentration of 10^3 cfu cm^{-2}. After 2 minutes, 2 boards were used to determine the initial bacterial level. With the remaining boards (3 treated and 3 untreated boards for each pathogen and contact time), a slice of cucumber was placed on each. Like scenario 1, contact between the boards and food was induced. The bacterial counts of the cutting boards and the cucumber slices were enumerated after 2 h at 20 °C for *L. monocytogenes* and for *S. aureus* after 2 min and 2 h at 20 °C.

In the last scenario, the two prior scenarios were combined into a two-stage cross-contamination. The inoculated filet pieces were prepared analogous to scenario 1. They were stored on cutting boards for 2 minutes at 20 °C and then replaced by cucumber slices. After following incubation at 20 °C for 2 h, the bacterial counts on treated and untreated cutting boards and on the cucumber slices were determined.

To determine the viable counts on the cutting boards, the boards were initially rinsed with 10 ml sodium chloride with tryptone and washed several times via pipette. The washing solution was collected carefully and transferred to sterile test tubes. Afterwards, the cutting boards were swabbed with moistened cotton swabs. The swabs were allowed to stand in the collected washing solution for 1 minute and mixed by vortexing for one further minute.

The filet pieces and the cucumber were transferred to a filtered, sterile stomacher bag and filled with sodium chloride solution with tryptone (135 ml for filets of pork and 225 ml for cucumber respectively). Samples were homogenized with a Stomacher 400 (Kleinfeld Labortechnik, Gehrden, Germany) for 60 seconds.

Total viable counts of the homogenate of food and of the washing solution of the cutting boards were enumerated by drop plate technique and pour plate technique on plate count agar (Merck KGaA, Darmstadt, Germany). Both were incubated at 37 °C for 24 h for dropped plates and 48 h for pour plates. Individual counts of *L. monocytogenes* were counted on ALOA-plates (Merck KGaA, Darmstadt, Germany) and *S. aureus* on Baird Parker-plates (BDH Prolabo® VWR Chemicals, Leuven, Belgium) by drop plate technique. All petri dishes were incubated for 48 h at 37 °C.

The detection limit for cutting boards (cb) was defined as 0.7 log_{10} cfu cb^{-1} for total viable count and 2.0 log_{10} cfu cb^{-1} for individual counts. For pieces (p) of meat and cucumber the detection limits were 2.0 log_{10} cfu p^{-1} for total viable counts and 3.0 log_{10} cfu p^{-1} for individual counts.

The transfer rates of bacteria were assessed for the three scenarios: Inoculated filet of pork to cutting board; inoculated cutting board to cucumber, inoculated filet of pork to cucumber via cutting board.

The transfer rates were calculated as follows (Eq. 4.4):

$$Transfer\ rate\ [\%] = \frac{count\ on\ recipient\ [cfu]}{(count\ on\ source\ [cfu] + count\ on\ recipient\ [cfu])} \times 100 \qquad (4.4)$$

Statistical analysis

Statistical significance (n>3) in reduction-levels was tested using Mann–Whitney U test in SPSS 22 (IBM Corp. 1989, 2013, New York, USA). Significance was defined as $p \leq 0.05$ and highly significant as $p \leq 0.005$. Figures were generated with the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, USA).

4.4 Results and Discussion

4.4.1 Antimicrobial activity

The changes of bacteria count on the different reference materials (PP, LLDPE) and sample materials (poly(TBAMS)/LLDPE, poly(TBAEMA)/LLDPE, Microban®/PP) within 2 h at 20 °C are shown in figure 4.2. On both reference boards, just marginal growth of the three bacteria was detected. Changes of the bacterial counts on antimicrobial boards are dependent on the bacterial strain and the type of antimicrobial agent. In general, *L. monocytogenes* was most sensitive to the three different treated boards. Counts of *L. monocytogenes* were decreased under the detection limit on both SAM-Polymer®-boards, resulting in a $\log_{10}$-reduction of 4.5 $\log_{10}$ cfu ml^{-1}. The Microban®-board led to a difference between reference and sample material of 0.3 $\log_{10}$ cfu ml^{-1}. Bacterial counts of *S. aureus* and *S.* Thypimurium do not differ on Microban®-containing and untreated polypropylene boards. On poly(TBAMS)-containing boards, bacterial counts of both microorganisms were reduced significantly ($p \leq 0.001$), although higher $\log_{10}$-reduction for *S. aureus* (2.7 $\log_{10}$-steps) than for *S.* Thypimurium (0.4 $\log_{10}$-steps) was detected. Reduction of *S. aureus* on LLDPE-boards with poly(TBAEMA) was comparable with reduction on poly(TBAMS)-containing boards (2.4 $\log_{10}$-steps), but the bacterial count of *S.* Thypimurium was unchanged.

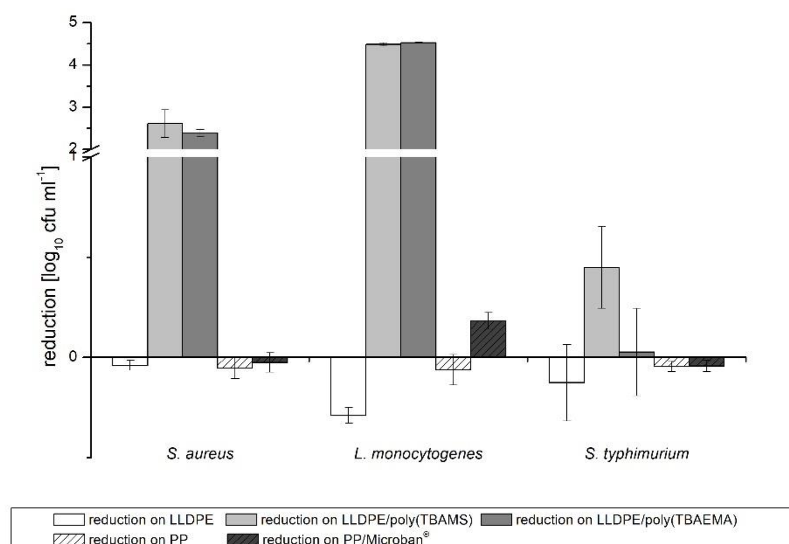

Figure 4.2 Reduction [$\log_{10}$ cfu ml^{-1}] of bacterial counts after 2 h incubation at 20 °C applied on LLDPE and PE as reference cutting boards and antimicrobial treated sample cutting boards: LLDPE/poly(TBAMS) (n=18), LLDPE/poly(TBAEMA) (n=15), PP/Microban® (n=3).

Contrary to the biocide releasing board the contact-active boards showed, after 2 h contact at 20 °C, an antimicrobial activity, but the value of reduction was variably pronounced regarding pathogen used. These findings are confirmed in various studies, which also identified bacterial strain dependent antimicrobial activity of poly(TBAEMA) and poly(TBAMS) [38, 39, 43, 44, 49, 50]. Dohlen et al. [43] argued that the electrophoretic mobility of gram-positive bacteria is more negative than in gram-negative bacteria resulting in more intensive interactions with antimicrobial surface for gram-positive bacteria than for gram-negative ones. *S*. Thypimurium exhibits a considerably more neutral electrophoretic mobility than *L. monocytogenes* or *S. aureus* [51]. The Microban®-board did not influence the bacterial count of the pathogens. The temperature as well as the contact time are known factors affecting the activity of antimicrobial materials [52]. In the case of releasing systems, this is caused by slower release of antimicrobial agents with a decrease of the temperature [46, 47]. But neither the decreased temperature nor the shortened contact time when compared with the required conditions of the test standard JIS 2801 was responsible for the missing antimicrobial activity of the silver-releasing board. Even at the JIS required temperature of 35 °C and contact time of 24 h no significant differences in *S. aureus* count on the reference and sample materials ($p=0.171$) were detected. On references as well as samples, the bacterial count increased (reference: 2.4 ± 0.05 $\log_{10}$ cfu ml^{-1}, sample: 2.3 ± 0.16 $\log_{10}$ cfu ml^{-1}). Thus, the tested Microban®-board did not show a potential to reduce cross-contamination during meal preparation.

The antimicrobial screening of the SAM-Polymer®-containing boards was continued to evaluate if these boards also show an antimicrobial activity under cold temperature conditions, which are present in different steps of food production and processing. The results of the antimicrobial activity tests at 7 °C are presented in figure 4.3. As detected by Dohlen et al. [43], the extent of temperature effect on the antimicrobial activity is dependent on the bacterial strain.

The antimicrobial activity of both materials against *L. monocytogenes* is not influenced by temperature. The activity at 7 °C against *S. aureus* is reduced compared with 20 °C. However, log_{10}-reductions of 1.2 log_{10} cfu ml^{-1} for boards with poly(TBAMS) and 1.5 log_{10} cfu ml^{-1} for poly(TBAEMA)-boards were measured. An antimicrobial effect of the treated boards against *S. typhimurium* was lacking on both boards. The reduced activity could be attributed to a lower bacterial surface charge at lower temperatures [43].

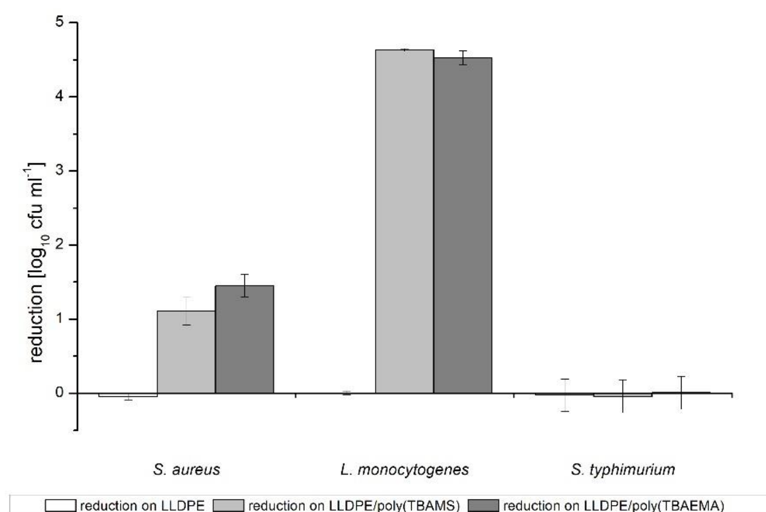

Figure 4.3 Reduction [log_{10} cfu ml^{-1}] of bacterial counts after 2 h incubation at 7 °C applied on reference cutting boards (LLDPE) and antimicrobial treated sample cutting boards (LLDPE/poly(TBAMS), LLDPE/poly(TBAEMA), n=6).

Since the contact time during processing is normally very short, the influence of such short contact time was tested with the poly(TBAMS)-containing board. After a 2 minutes contact with the cutting boards at ambient temperature conditions (20-22 °C) a reduction of 0.7 log_{10}-steps for *L. monocytogenes* was detected. Bacterial counts of *S. aureus* were just marginally reduced.

The antimicrobial action of SAM-Polymers® is traced back to chemical reactions of the amino functionalized groups leading to local changes in pH-value and physical interactions between the positively charged polymer surface and the negatively charged surfaces of the bacteria [49]. To enhance the antimicrobial effect of the SAM-Polymer®-boards for a broader spectrum of microorganisms and for short contact times, the number of active groups on the surface of the polymer has to be increased [38, 53]. Possible ways are to increase the amount of poly(TBAMS) and poly(TBAEMA), respectively, in the bulk composition or the improvement of material elaboration leading to accumulation of SAM-Polymers® at the interface.

From the results, it became evident that poly(TBAMS) showed the highest potential to reduce pathogen transfer during food preparation, but, in this material composition, only under ambient conditions with temperatures around 20°C.

4.4.2 Cross-contamination scenarios

In the second part of the study three different test scenarios were conducted to assess the effect of the poly(TBAMS)-containing cutting boards on cross-contamination of *L. monocytogenes* and *S. aureus* during food preparation. Table 4.1 summarized the total viable counts and transfer rates in the different scenarios after 2 h contact time.

The transfer of bacteria in the different scenarios ranges between 4.02 and 59.82 % on reference LLDPE boards. This confirms the results from other studies, where similar rates were found [3, 15, 19, 54–56]. This wide range clarifies that cross-contamination is a multifactorial process, which is influenced by different factors like contact time, bacterial strain, bacterial load, food type as well as surface material and its properties [57–59]. In our experiments, a clear influence of the bacterial strain as well as the kind of food was observed, but no significant effect of contact time (2 h, 2 min) was observed.

Table 4.1 Transfer of bacteria (total viable counts) after 2 h in different scenarios with untreated LLDPE material and LLDPE with 10 % poly(TBAMS). Different superscript letters in one line indicate significant differences in total viable count on LLDPE and LLDPE+10 % poly(TBAMS) ($p<0.05$), capital letters mark highly significant differences ($p<0.005$).

Scenario	Organism	Total Viable Counts [log_{10} cfu surface^{-1}]									Transfer rate [%]	
		Source			Recipient							
					LLDPE			LLDPE+TBAMS			LLDPE	LLDPE+TBAMS
		min	max	mean ± SE	min	max	mean ± SE	min	max	mean ± SE	mean ± SE	mean ± SE
(1) inoculated meat to cutting board	*L. monocytogenes*	5.02	6.31	5.92 ± 0.00	4.34	4.52	4.43 ± 0.05 [a]	4.00	4.37	4.21 ± 0.11 [a]	17.06 ± 3.42	10.93 ± 3.15
	S.aureus	4.47	5.05	4.73 ± 0.13	3.49	4.21	3.80 ± 0.13 [a]	3.52	4.06	3.87 ± 0.08 [a]	10.44 ± 4.96	12.05 ± 4.20
(2) inoculated cutting board to cucumber	*L. monocytogenes*	5.19	5.26	5.22 ± 0.01	5.16	5.55	5.39 ± 0.06 [a]	2.69	2.69	2.91 ± 0.07 [B]	59.82 ± 3.05	0.49 ± 0.09
	S.aureus	4.88	4.95	4.91 ± 0.02	4.42	4.76	4.64 ± 0.06 [a]	4.27	4.76	4.56 ± 0.08 [a]	34.84 ± 2.88	31.18 ± 4.04
(3) inoculated meat via cutting board to cucumber	*L. monocytogenes*	5.85	6.31	6.00 ± 0.08	4.34	5.40	4.84 ± 0.19 [a]	3.48	5.29	4.63 ± 0.36 [b]	6.51 ± 2.45	4.14 ± 2.38
	S.aureus	4.62	7.21	6.87 ± 0.71	5.01	6.01	5.49 ± 0.14 [a]	4.65	6.34	5.69 ± 0.31 [a]	4.02 ± 2.87	6.19 ± 6.04

Table 4.2 summarizes the total viable counts and the individual counts of the inoculated *S. aureus* as well as the determined transfer rates after 2 min contact time between bacterial source and recipient. The transfer rates on reference boards range between 2.01 and 50.65 % for TVC. In comparison, the transfer rates of TVC after 2 h are 4.02-34.84 %. The rates for individual counts reached values between 0.27 and 34.20 % after 2 min and 1.55 % and 36.8 % after 2h. The results of the Mann–Whitney U test determined no significant effect of prolonging the contact time from 2 min to 2 h on the transfer of *S. aureus*. This was also confirmed in other studies: Gkana et al. [59] tested the transfer of different inoculum levels of *L. monocytogenes* from beef filet to polyethylene boards after 1 and 15 min and determined no significant difference in transfer rates. Dawson et al. [60] could not detect a significant effect of food contact time on contaminated surfaces, but the residence time of bacteria on the surface prior to food contact affected the transfer to food. Thus, the prolonged rest time of bacteria on meat in the study of Gkana et al. [26] can explain the lower bacterial transfer from inoculated meat to a cutting board in comparison to our results.

Table 4.2 Transfer of bacteria (total viable counts and individual counts of *S. aureus*) after 2 min in different scenarios with untreated LLDPE material and LLDPE with 10 % poly(TBAMS). Different superscript letters in one line indicate significant differences in total viable count on LLDPE and LLDPE+10 % poly(TBAMS) ($p<0.05$), capital letters mark highly significant differences ($p<0.005$).

Scenario		Bacterial Counts [log_{10} cfu surface^{-1}]									Transfer rate [%]	
		Source			Recipient							
					LLDPE			LLDPE+TBAMS			LLDPE	LLDPE+TBAMS
		min	max	mean ± SE	min	max	mean ± SE	min	max	mean ± SE	mean ± SE	mean ± SE
(1) inoculated meat to cutting board	TVC	4.47	5.05	4.73 ± 0.09	3.26	4.02	3.73 ± 0.07 ^{a}	3.40	4.00	3.76 ± 0.06 ^{a}	8.97 ± 2.25	9.66 ± 2.30
	Individual count	4.56	5.16	4.86 ± 0.10	3.18	4.24	3.84 ± 0.09 ^{a}	3.36	3.98	3.75 ± 0.05 ^{a}	8.88 ± 2.55	7.33 ± 1.84
(2) inoculated cutting board to cucumber	TVC	4.88	4.95	4.91 ± 0.02	4.49	5.43	4.92 ± 0.18 ^{a}	4.07	4.79	4.52 ± 0.13 ^{a}	50.65 ± 14.05	29.27 ± 5.69
	Individual count	4.81	4.93	4.85 ± 0.03	4.45	4.69	4.57 ± 0.05 ^{a}	4.10	4.41	4.32 ± 0.06 ^{b}	34.20 ± 3.09	22.69 ± 2.32
(3) inoculated meat via cutting board to cucumber	TVC	4.62	7.21	6.87 ± 0.71	4.68	5.55	5.18 ± 0.12 ^{a}	4.41	5.59	5.10 ± 0.20 ^{a}	2.01 ± 1.27	1.68 ± 1.29
	Individual count	4.72	6.92	6.60 ± 0.63	3.30	4.36	4.03 ± 0.24 ^{a}	3.30	4.18	3.74 ± 0.15 ^{a}	0.27 ± 0.20	0.14 ± 0.10

While the contact time was insignificant, differences in the transfer rates between the two pathogens as well as between the two food types were apparent. In all 2 h scenarios with the reference boards, the transfer rates of *S. aureus* are always lower than for *L. monocytogenes* (Table 4.1). Regarding the food participation, it was shown that the transfer rates of both bacteria from inoculated cutting board to cucumber (scenario 2: *L. monocytogenes*: 59.82±3.05 %, *S. aureus*: 34.84±2.88 %) were clearly higher than from inoculated meat to cutting board (scenario 1: *L. monocytogenes*: 17.06±3.42 %, *S. aureus*: 10.44±4.96 %).

These findings corroborate what other studies have observed with those of other authors. It can be explained by the surface characteristics of the bacteria as well as of the food or plastic respectively, which influenced the attachment strength of bacteria to the surfaces and consequently the probability of transfer to other surfaces [6, 16, 26, 58, 61–63]. Gkana et al. [59] and Zilelidou et al. [64] detected that the transfer rates of *L. monocytogenes* are higher in comparison to those of *S. typhimurium* and *E. coli.* A study of Dickson [62] showed that *L. monocytogenes* as well as *S. aureus* attached more preferably to fatty muscle tissue than to lean surfaces. Studies observed that bacteria on meat surfaces are not only attached but also trapped between muscle fibers and collagen bundles and are therefore difficult to remove. Next to the beneficial surface topography, the moisture content on the cucumber slices positively affects the transfer of bacteria [3, 58]. An additional experiment (data not shown), determining the transfer of Listeria from an inoculated cucumber slice to a cutting board, confirmed this explanation, because the measured transfer rate of 35.05±3.96 % is higher than the mentioned transfer from inoculated meat to cutting board (scenario 1). The high transfer rates of pathogens to fresh vegetables, like tomatoes or cucumber, were also observed by other authors [3, 17, 26, 28, 55, 58].

The effect of antimicrobial surfaces with poly(TBAMS) on the transfer rates of the pathogens is pronounced variably and depends on bacterial strain, food stuff and contact time between source and recipient, with the pathogenic strain showing the most apparent influence. As expected, based on the antimicrobial survey in the first experimental series, the effect on transfer of *L. monocytogenes* in cross-contamination scenarios was higher than for *S. aureus*.

Contrary to antimicrobial screening, prolonging contact time did not clearly lead to reduced transfer to poly(TBAMS)-containing boards in comparison with the reference boards.

For all scenarios with *S. aureus*, no significant effect of the antimicrobial cutting board on the total viable count after 2 h (Table 4.1) as well as after 2 min (Table 4.2) could be determined. After just 2 min contact of the inoculated cutting board and the cucumber (scenario 2) the individual *S. aureus* count was significantly (p=0.008) lower on antimicrobially treated (4.32±0.06 log_{10} cfu p^{-1}) than on untreated boards (4.57 ± 0.05 log_{10} cfu p^{-1}). The total viable count was not significantly affected. A possible explanation could be a variable accompanying natural flora of the cucumber, which is reflected in the high standard error of total viable counts on the cucumber after the transfer. A significant but marginal reduction of *S. aureus* counts after 2 min contact was also detected in antimicrobial assays mentioned before. It could be, that the reduced transfer of *S. aureus* (LLDPE: 34.2±3.09 %, LLDPE/poly(TBAMS): 22.69±2.32 %) is caused by stronger interactions between bacteria and the poly(TBAMS)-containing board, which inhibited the transfer. This is supported by the fact that the remaining bacterial counts on the cutting boards after the contact with the cucumber did not significantly differ. The highest positive effect on pathogen transfer was mentioned in scenario 2, during the transfer of *L. monocytogenes* from an inoculated cutting board to a cucumber slice. The antimicrobial polymer reduces the cross-contamination highly significantly (p=0.004) about 2.5 log_{10}-steps leading to a reduction of transfer rate from about 60 % for untreated board to 0.5 % for the treated board. A comparable effect on cross-contamination was determined if an inoculated cucumber was in contact with a cutting board (data not shown).

The investigations with *L. monocytogenes* showed the influence of the presence and the nutritional composition of food on the transfer rate in general, but also on the activity of antimicrobial treated surface. Many studies demonstrate a decrease or even an absence of antimicrobial activity in the presence of food debris [24, 42, 43, 65–67]. This is caused due to two effects: first the external preconditions for survival of bacteria are better, and second, the food ingredients, especially proteins, interact with the antimicrobial agents [67]. Therefore, the presence of pork filet enhances the survival of bacteria to a greater extent than cucumber does. Thus, in scenario 3, the TVC on cucumber, after contact with cutting board which was contaminated via contact with inoculated meat, only differ about 0.2 log-steps (p=0.046), leading to a reduction in transfer rate of 2 %. And the difference in transfer of *L. monocytogenes* from inoculated meat to cutting board (scenario 1) was not even significant after 2 h. Interestingly, after 2 min contact, total viable counts determined on poly(TBAMS)-containing cutting boards were significantly lower (1.3±0.28 log_{10} cfu cb^{-1}, p=0.005) as populations retrieved from reference boards (4.51±0.16 log_{10} cfu cb^{-1}). These findings are not explainable with our investigations. Maybe the transfer to the antimicrobial cutting board in the first 2 min is low because of electrostatic interactions and the bacteria are not killed but rather are attached on the meat. Therefore in further experiments, the remaining bacterial count on the filet piece should be determined.

The antimicrobial screening of the antimicrobial treated cutting boards showed, that the activity is affected by the factors bacterial strain, contact time and contact temperature. The SAM-Polymer® poly(TBAMS) was identified to have the highest potential to reduce cross-contaminations during food preparation. However, the cross-contamination scenarios observed that, despite the good antimicrobial properties of poly(TBAMS), the effect on the transfer of the pathogens is highly limited and varying in dimension, and dependent on involved microorganisms, food stuffs and environmental conditions. But the integration of antimicrobial food contact surfaces can have an indirect positive effect on cross-contamination, due to improvement of the hygienic status of the surfaces. During food processing, particularly when cleaning is insufficient, the bacterial load on contact surfaces increases; this could be inhibited or deferred via antimicrobial materials. In a study by Dohlen et al [43], it was shown that prolonging the contact time lead to a very high reduction of bacteria, even in the presence of meat extract and under low temperature conditions. Thus, poly(TBAMS) may support the inactivation of pathogens until the next contact with food. But it is unquestioned that the use of these antimicrobial food contact surfaces cannot replace hygienic measures.

4.5 Conclusion

The antimicrobial screening of the investigated cutting boards confirmed the varying activity of different kinds of active materials. The lowest activity was shown for the commercially available silver phosphate glass containing cutting board, and the highest activity was reached with LLDPE containing 10 % poly(TBAMS). It became evident that even if a material shows a high antimicrobial activity, the potential to reduce cross contamination is influenced by several other factors, like bacterial strain and type of food stuff. The study shows, that the reduction of transfer of bacteria during direct contact of food and food contact surfaces is restricted, but the use of those surfaces could contribute to the hygienic status of the food contact surfaces, leading to a lower risk of cross-contamination of products. Further studies are necessary to investigate if an optimization of the boards, for example by higher content of poly(TBAMS), can enhance the antimicrobial activity, leading to a higher effect on cross-contamination.

4.6 References

1. Mafu AA, Roy D, Goulet J, Magny P (1990) Attachment of Listeria monocytogenes to Stainless Steel, Glass, Polypropylene, and Rubber Surfaces After Short Contact Times. J Food Prot 53(9), 742–746.
2. Barnes LM, Lo MF, Adams MR, Chamberlain AH (1999) Effect of milk proteins on adhesion of bacteria to stainless steel surfaces. Appl Environ Microbiol 65(10), 4543–4548.
3. Kusumaningrum HD, Riboldi G, Hazeleger WC, Beumer RR (2003) Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int J Food Microbiol 85(3), 227–236.
4. Wilks SA, Michels H, Keevil CW (2005) The survival of Escherichia coli O157 on a range of metal surfaces. Int J Food Microbiol 105(3), 445–454.
5. Martinon A, Cronin UP, Quealy J, Stapleton A, Wilkinson MG (2012) Swab sample preparation and viable real-time PCR methodologies for the recovery of Escherichia coli, Staphylococcus aureus or Listeria monocytogenes from artificially contaminated food processing surfaces. Food control 24(1–2), 86–94.
6. Frank JF (2001) Microbial attachment to food and food contact surfaces, in: Jeya Henry (ed.) 74. Advances in Food and Nutrition Research, pp. 319–370: Elsevier.
7. Vogel BF, Huss HH, Ojeniyi B, Ahrens P, Gram L (2001) Elucidation of Listeria monocytogenesContamination Routes in Cold-Smoked Salmon Processing Plants Detected by DNA-Based Typing Methods. Appl Environ Microbiol 67(6), 2586–2595.
8. Bagge-Ravn D, Ng Y, Hjelm M, Christiansen JN, Johansen C, Gram L (2003) The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. Int J Food Microbiol 87(3), 239–250.
9. Rørvik LM, Aase B, Alvestad T, Caugant DA (2003) Molecular epidemiological survey of Listeria monocytogenes in broilers and poultry products. J Appl Microbiol 94(4), 633–640.
10. Reij MW, Den Aantrekker ED (2004) Recontamination as a source of pathogens in processed foods. Int J Food Microbiol 91(1), 1–11.
11. Barros MA, Nero LA, Silva LC, d'Ovidio L, Monteiro FA, Tamanini R, Fagnani R, Hofer E, Beloti V (2007) Listeria monocytogenes: Occurrence in beef and identification of the main contamination points in processing plants. Meat Sci 76(4), 591–596.
12. Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by Salmonella in foods: A review. Food Res Int 45(2), 545–556.
13. Goh SG, Leili A-H, Kuan CH, Loo YY, Lye YL, Chang WS, Soopna P, Najwa MS, Tang JYH, Yaya R (2014) Transmission of< i> Listeria monocytogenes from raw chicken meat to cooked chicken meat through cutting boards. Food control 37, 51–55.
14. Møretrø T, Moen B, Heir E, Hansen AA, Langsrud S (2016) Contamination of salmon fillets and processing plants with spoilage bacteria. Int J Food Microbiol 237, 98–108.
15. Chen Y, Jackson KM, Chea FP, Schaffner DW (2001) Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. J Food Prot 64(1), 72–80.
16. Midelet G, Carpentier B (2002) Transfer of Microorganisms, Including Listeria monocytogenes, from Various Materials to Beef. Appl Environ Microbiol 68(8), 4015–4024.
17. Wachtel MR, McEvoy JL, Luo Y, Williams-Campbell AM, Solomon MB (2003) Cross-Contamination of Lettuce (Lactuca sativa L.) with Escherichia coli O157: H7 via Contaminated Ground Beef. J Food Prot 66(7), 1176–1183.
18. da Silva Malheiros P, dos Passos CT, Casarin LS, Serraglio L, Tondo EC (2010) Evaluation of growth and transfer of Staphylococcus aureus from poultry meat to surfaces of stainless steel and polyethylene and their disinfection. Food control 21(3), 298–301.
19. Jeyaletchumi P, Tunung R, Selina PM, Chai LC, Radu S, Farinazleen MG, Cheah YK, Mitsuaki N, Yoshitsugu N, Kumar MP (2012) Assessment of Listeria monocytogenes in salad vegetables through kitchen simulation study. J trop agric and fd sc 40(1), 55–62.
20. Redmond EC, Griffith CJ (2003) Consumer Food Handling in the Home: A Review of Food Safety Studies. J Food Prot 66(1), 130–161.
21. Josephson KL, Rubino JR, Pepper IL (1997) Characterization and quantification of bacterial pathogens and indicator organisms inhousehold kitchens with and without the use of a disinfectant cleaner. J Appl Microbiol 83(6), 737–750.

22. Carpentier B (1997) Sanitary quality of meat chopping board surfaces: a bibliographical study. Food Microbiol 14(1), 31–37.
23. Todd ECD, Greig JD, Bartleson CA, Michaels BS (2009) Outbreaks Where Food Workers Have Been Implicated in the Spread of Foodborne Disease. Part 6. Transmission and Survival of Pathogens in the Food Processing and Preparation Environment. J Food Prot 72(1), 202–219.
24. Møretrø T, Høiby-Pettersen GS, Habimana O, Heir E, Langsrud S (2011) Assessment of the antibacterial activity of a triclosan-containing cutting board. Int J Food Microbiol 146(2), 157–162.
25. Faour-Klingbeil D, Kuri V, Todd E (2016) The transfer rate of Salmonella Typhimurium from contaminated parsley to other consecutively chopped batches via cutting boards under different food handling scenarios. Food Res Int 89, Part 1, 495–503.
26. Gkana E, Lianou A, Nychas G-JE (2016) Transfer of Salmonella enterica Serovar Typhimurium from Beef to Tomato through Kitchen Equipment and the Efficacy of Intermediate Decontamination Procedures. J Food Prot 79(7), 1252–1258.
27. Green Brown L, Khargonekar S, Bushnell L (2013) Frequency of inadequate chicken cross-contamination prevention and cooking practices in restaurants. J Food Prot 76(12), 2141–2145.
28. Ravishankar S, Zhu L, Jaroni D (2010) Assessing the cross contamination and transfer rates of Salmonella enterica from chicken to lettuce under different food-handling scenarios. Food Microbiol 27(6), 791–794.
29. Pompermayer DMC, Gaylarde CC (2000) The influence of temperature on the adhesion of mixed cultures of Staphylococcus aureus and Escherichia coli to polypropylene. Food Microbiol 17(4), 361–365.
30. Bastarrachea LJ, Denis-Rohr A, Goddard JM (2015) Antimicrobial Food Equipment Coatings: Applications and Challenges. Annu Rev Food Sci Technol 6(1), 97–118.
31. Boer ED, Hahné M (1990) Cross-contamination with Campylobacter jejuni and Salmonella spp. from Raw Chicken Products During Food Preparation. J Food Prot 53(12), 1067–1068.
32. Pricope L, Nicolau A, Wagner M, Rychli K (2013) The effect of sublethal concentrations of benzalkonium chloride on invasiveness and intracellular proliferation of Listeria monocytogenes. Food control 31(1), 230–235.
33. Moerman F (2014) Antimicrobial materials, coatings and biomimetic surfaces with modified microtography to control microbial fouling of product contact surfaces within food processing equipment: Legislation, requirements, effectiveness and challenges. Journal of Hygienic Engineering and Design 7, 8–29.
34. Yemmireddy VK, Hung Y-C (2017) Using Photocatalyst Metal Oxides as Antimicrobial Surface Coatings to Ensure Food Safety—Opportunities and Challenges. Comprehensive Reviews in Food Science and Food Safety 16(4), 617–631.
35. Møretrø T, Langsrud S (2011) Effects of Materials Containing Antimicrobial Compounds on Food Hygiene. J Food Prot 74(7), 1200–1211.
36. Berrang ME, Frank JF, Meinersmann RJ (2010) Listeria monocytogenes biofilm formation on silver ion impregnated cutting boards. Food Prot Trends 30(3), 168–171.
37. Llorens A, Lloret E, Picouet PA, Trbojevich R, Fernandez A (2012) Metallic-based micro and nanocomposites in food contact materials and active food packaging. Trends Food Sci Technol 24(1), 19–29.
38. Seyfriedsberger G, Rametsteiner K, Kern W (2006) Polyethylene compounds with antimicrobial surface properties. Eur Polym J 42(12), 3383–3389.
39. Marra J, Paleari AG, Rodriguez LS, Leite ARP, Pero AC, Compagnoni MA (2012) Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. J Appl Oral Sci 20, 643–648.
40. Brodkorb F, Fischer B, Kalbfleisch K, Robers O, Braun C, Dohlen S, Kreyenschmidt J, Lorenz R, Kreyenschmidt M (2015) Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. Int J Mol Sci 16(8), 20050–20066.
41. Thölmann D, Kossmann B, Sosna F (2003) Polymers with antimicrobial properties. EC Journal(1-2), 16–33.
42. Møretrø T, Høiby-Pettersen GS, Halvorsen CK, Langsrud S (2012) Antibacterial activity of cutting boards containing silver. Food control 28(1), 118–121.
43. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Robers O, Kreyenschmidt M, Kreyenschmidt J (2016) Potential of the polymer poly-[2-(tert-butylamino)

methylstyrene] as antimicrobial packaging material for meat products. J Appl Microbiol 121(4), 1059-70.
44. Braun C, Dohlen S, Ilg Y, Brodkorb F, Fischer B, Heindirk P, Kalbfleisch K, Richter T, Robers O, Kreyenschmidt M, Lorenz R, Kreyenschmidt J (2017) Antimicrobial Activity of Intrinsic Antimicrobial Polymers Based on Poly((tertbutyl-amino)-methyl-styrene) Against Selected Pathogenic and Spoilage Microorganisms Relevant in Meat Processing Facilities. J Antimicrob Agents 03(01).
45. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Kreyenschmidt M, Kreyenschmidt J (2017) Effect of different packaging materials containing poly-[2-(tert-butylamino) methylstyrene] on the growth of spoilage and pathogenic bacteria on fresh meat. Int J Food Microbiol 257, 91–100.
46. Kampmann Y, Clerck E de, Kohn S, Patchala DK, Langerock R, Kreyenschmidt J (2008) Study on the antimicrobial effect of silver-containing inner liners in refrigerators. J Appl Microbiol 104(6), 1808–1814.
47. Michels H, Noyce J, Keevil C (2009) Effects of temperature and humidity on the efficacy of methicillin-resistant Staphylococcus aureus challenged antimicrobial materials containing silver and copper. Lett Appl Microbiol 49(2), 191–195.
48. Ilg Y, Kreyenschmidt J (2011) Effects of food components on the antimicrobial activity of polypropylene surfaces containing silver ions (Ag+). Int J Food Sci Technol 46(7), 1469–1476.
49. Hewitt CJ, Franke R, Marx A, Kossmann B, Ottersbach P (2004) A study into the anti-microbial properties of an amino functionalised polymer using multi-parameter flow cytometry. Biotechnol Lett 26(7), 549–557.
50. Buranasompob A (2005) Kinetics of the inactivation of microorganisms by water insoluble polymers with antimicrobial activity. Dissertation. Berlin.
51. Potter R, Truelstruphansen L, Gill T (2005) Inhibition of foodborne bacteria by native and modified protamine: Importance of electrostatic interactions. Int J Food Microbiol 103(1), 23–34.
52. Ilg Y, Kreyenschmidt J (2012) Review: Benefits and risks of the use of antimicrobial components in the food chain. J Food Saf Food Qual 63(2), 28–34.
53. Dohlen S (2016) 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. Dissertation. Bonn.
54. Zhao P, Zhao T, Doyle MP, Rubino, JR, Meng J (1998) Development of a model for evaluation of microbial cross-contamination in the kitchen. J Food Prot 61(8), 960–963.
55. Soares VM, Pereira JG, Viana C, Izidoro TB, Bersot LdS, Pinto JPdAN (2012) Transfer of Salmonella Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes. Food Microbiol 30(2), 453–456.
56. Kuan CH, Lim LWK, Ting TW, Rukayadi Y, Ahmad SH, Wan Mohamed Radzi CWJ, Thung TY, Ramzi OB, Chang WS, Loo YY, Kuan CS, Yeo S-K, Radu S (2017) Simulation of decontamination and transmission of Escherichia coli O157: H7, Salmonella Enteritidis, and Listeria monocytogenes during handling of raw vegetables in domestic kitchens. Food control 80, 395–400.
57. Pérez-Rodríguez F, Valero A, Carrasco E, García RM, Zurera G (2008) Understanding and modelling bacterial transfer to foods: a review. Trends Food Sci Technol 19(3), 131–144.
58. Miranda RC, Schaffner DW (2016) Longer Contact Times Increase Cross-Contamination of Enterobacter aerogenes from Surfaces to Food. Appl Environ Microbiol 82(21), 6490–6496.
59. Gkana E, Chorianopoulos N, Grounta A, Koutsoumanis K, Nychas G-JE (2017) Effect of inoculum size, bacterial species, type of surfaces and contact time to the transfer of foodborne pathogens from inoculated to non-inoculated beef fillets via food processing surfaces. Food Microbiol 62, 51–57.
60. Dawson P, Han I, Cox M, Black C, Simmons L (2007) Residence time and food contact time effects on transfer of Salmonella Typhimurium from tile, wood and carpet: testing the five-second rule. J Appl Microbiol 102(4), 945–953.
61. Bloomfield SF, Scott E (1997) Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. J Appl Microbiol 83(1), 1–9.
62. Dickson JS, Koomaraie M (1989) Cell Surface Charge Characteristics and Their Relationship to Bacterial Attachment to Meat Surfaces. Appl Environ Microbiol 55(4), 832–836.

63. Jensen DA, Friedrich LM, Harris LJ, Danyluk MD, Schaffner DW (2013) Quantifying transfer rates of Salmonella and Escherichia coli O157:H7 between fresh-cut produce and common kitchen surfaces. J Food Prot 76(9), 1530–1538.
64. Zilelidou EA, Tsourou V, Poimenidou S, Loukou A, Skandamis PN (2015) Modeling transfer of Escherichia coli O157:H7 and Listeria monocytogenes during preparation of fresh-cut salads: impact of cutting and shredding practices. Food Microbiol 45(Pt B), 254–265.
65. Cutter CN (1999) Effectiveness of triclosan-incorporated plastic against bacteria on beef surfaces. J Food Prot 62(5), 474–479.
66. Noyce JO, Michels H, Keevil CW (2006) Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing. Appl Environ Microbiol 72(6), 4239–4244.
67. Chaitiemwong N, Hazeleger WC, Beumer RR (2010) Survival of Listeria monocytogenes on a conveyor belt material with or without antimicrobial additives. Int J Food Microbiol 142(1–2), 260–263.

5 Potential of antimicrobial treatment of LLDPE with poly((tert-butyl-amino)-methyl-styrene) to reduce biofilm formation in food industry

5.1 Abstract

Antimicrobial surfaces are one approach to prevent biofilm formation in the food industry. The aim of this study was to investigate the effect of poly((tert-butyl-amino)-methyl-styrene) (poly(TBAMS)) incorporated into linear low-density polyethylene (LLDPE) on the formation of mono and mixed-species biofilms. The biofilm count on untreated and treated LLDPE was determined after 48 and 168 h. The comparison of the results indicated that the ability of the pathogen *Listeria monocytogenes* to form biofilms was completely suppressed by poly(TBAMS) ($\Delta_{168\,h}$ 3.2 log_{10} cfu cm^{-2}) and colonization of the pathogens *Staphylococcus aureus* and *Escherichia coli* was significantly delayed, but no effect on biofilms built by *Pseudomonas fluorescens* could be observed. Results of dual-species biofilms showed the complex interactions between the microorganisms, but comparable effects on the individual bacteria by poly(TBAMS) were identified. Antimicrobial treatment with poly(TBAMS) showed great potential to prevent biofilm formation of polymeric surfaces in the food industry. However, a further development of the material is necessary to reduce the colonization of strong biofilm formers like *Pseudomonas fluorescens*.

PUBLISHED IN BIOFOULING- THE JOURNAL OF BIOADHESION AND BIOFILM RESEARCH SEE LIST OF PUBLICATIONS (HÜWE ET AL. 2018)

5.2 Introduction

In food processing environments, bacteria attach to food contact surfaces, where they can survive for long periods, grow and form biofilms, especially when supported by the presence of food debris [1–8]. Biofilms are defined as assemblages of microorganisms, which interact with each other and are embedded in self-produced, extracellular, polymeric substances (EPS) and adhere to surfaces [9]. Most bacteria reside in multispecies biofilms, because this sessile form offers advantageous over the planktonic form like protection against environmental stress [2, 8, 10, 11]. Hence, it is widely accepted that sessile forms of bacteria exhibit an up to 500-fold increased resistance to antimicrobial treatment in comparison to planktonic individuals [6, 7, 12]. Furthermore, interactions in multispecies biofilms were shown to enhance the colonization and persistence of pathogens on food contact surfaces [8, 13–19]. Spoilage bacteria like *Pseudomonas* spp., known as good biofilm formers, as well as pathogens like *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*, which are often involved in outbreaks of foodborne diseases, were proven as members of biofilm communities in the food industry [4, 6, 8, 20–26]. These biofilms present a permanent source of microbial contamination of food leading to accelerated spoilage and reduced safety [1, 11, 21, 27, 28]. Furthermore, biofilms contribute to biofouling of work surfaces, which can adversely affect the function of the interface [12, 29]. In addition, the removal of biofilms is more demanding than planktonic cells due to the increased tolerance, up to resistance, against sanitizers of bacteria embedded in biofilms, and stronger physical force is also necessary [3, 5, 30–32].

For these reasons, the prevention of biofilm formation is of great concern in the food industry. To improve the effectiveness of cleaning and disinfection, three approaches are discussed to inhibit the adhesion of bacteria and the subsequent formation of a biofilm [29]: optimizing equipment design, altering surface chemistry and treating materials with antimicrobial agents [2, 7, 32–34]. The first one can hinder bacterial attachment and improve sanitation measures; the second approach includes different concepts, such as hydrophilicity, to modify surface characteristics that lead to repelling of microorganisms from the interface. Finally, the treatment of materials with antimicrobial agents leads to killing of microorganisms, either near surfaces due to the release of biocides from the material or due to proximity to contact-active biocidal materials [35]. The application of biocide releasing systems is limited in the food industry, because of possible carry over of biocides into food and only temporary effectiveness [31]. Contact-active surfaces are superior concerning these facts. Thus, various technologies were explored to immobilize different antimicrobial active agents such as essential oils, enzymes, antimicrobial peptides and quaternary ammonium on surfaces [34, 36, 37]. Although good anti-biofilm properties of some approaches were shown [38], the durability and stability of the immobilization are often still a challenge. Thus, intrinsically antimicrobial active materials offer great potential for the use as food contact surfaces, which are exposed to strong forces especially during cleaning processes. Sustainable Active Microbiocidal (SAM)-Polymers® belong to this class of antimicrobial materials. The SAM-Polymer®, poly((tert-butyl-amino)-

methyl-styrene) (poly(TBAMS)) has good antimicrobial activity against a broad spectrum of bacteria, even at low temperature and in the presence of food components [39–42]. Hence, poly(TBAMS) shows potential for application in food contact surfaces, but antimicrobially active surfaces do not necessarily inhibit biofilms [43, 44].

Up to now, no information about the ability of poly(TBAMS) to inhibit biofilm formation are available. Thus, the aim of this study was to determine the effects of poly(TBAMS), which was incorporated into LLDPE, on the formation of mono and mixed-species biofilms of different microorganisms relevant to the food industry.

5.3 Materials and Methods

The potential of poly(TBAMS) to reduce biofilm formation on polymeric food contact surfaces was investigated in two experimental series. In the first experimental series, the cell count in mono-species biofilms of *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* subsp. *aureus* and *Pseudomonas fluorescens* on neat LLDPE surfaces and LLDPE surfaces with incorporated poly(TBAMS) were determined and compared after 48 and 168 hours of biofilm formation. The second experimental series was conducted with heterogeneous cultures, composed of two bacterial strains each in all possible combinations of the four bacterial strains used in mono-culture experiments.

5.3.1 Bacterial strains

The mentioned bacteria are typical members of biofilm communities in the food industry and were therefore chosen as test organisms for the two different trials. All strains, delivered by the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany), were frozen in cryogenic pellets for preservation. A stock solution was prepared by transferring a frozen culture to 10 ml of nutrient broth (Merck KGaA, Darmstadt, Germany) and afterwards incubated overnight at the optimal growth temperature for each bacterium per the instructions of DSMZ (Table 5.1). For the inoculum, the stock solutions were diluted in physical saline solution with tryptone (1 g l^{-1}) (Oxoid, Hampshire, United Kingdom) to an end concentration of 10^2 cfu ml^{-1}. For experiments with the heterogeneous inoculum, each bacteria culture was initially prepared and diluted separately, and the two different cultures were mixed in the final dilution step to the desired concentration.

Table 5.1 Summary of used bacteria, cultivating as well as enumeration temperature and selective media in mixed cultures

Bacteria	Strain	Temperature	Selective medium
Listeria monocytogenes	ATCC 19111	37 °C	*Listeria* agar according to Ottaviani and Agosti (ALOA, Oxoid, Cambridge, United Kingdom)
Staphylococcus aureus	ATCC 6538	37 °C	Baird Parker agar (Oxoid, Cambridge, United Kingdom)
Escherichia coli	ATCC 8739	37 °C	Violet red bile dextrose agar (VRBD, Merck, Darmstadt, Germany)
Pseudomonas fluorescens	ATCC 13525	25 °C	*Pseudomonas* agar with cetrimide fucidin cephaloridine (CFC) selective supplement (Oxoid, Cambridge, United Kingdom)

5.3.2 Test material

Materials were prepared by the University of Applied Sciences Münster. Poly(TBAMS) was synthesized as described in Brodkorb et al. [39]. Test surfaces were processed from the linear-low density polyethylene (LLDPE) (Dowlex 2433, Dow plastics, Midland, USA) mixed with 10 % poly(TBAMS) in a double screw extruder. The resulting compounds from the extrusion process were pressed to polymer discs (diameter: 4.0 cm, thickness: 0.2 cm) by a fully hydraulic injection moulding machine (Babyplast Typ 6/10 PT, CHRISTMANN Kunststofftechnik GmbH, Kierspe, Germany). Discs of raw LLDPE were produced identically as a reference material. The discs were divided in half and decontaminated by dipping in boiling water for 15 s prior to use.

5.3.3 Biofilm development

The prepared polymer surfaces were placed individually in Falcon® tubes (50 ml, polypropylene, Corning, NY, USA) and 20 ml of the bacteria solution were added. Each experiment was conducted with 6 references and 6 sample test pieces respectively, and additional blank tests with the reference material and pure sodium chloride were done to assure sterility. For the development of biofilms, the tubes were incubated at 15 °C for preassigned time intervals (48 h, 168 h). In the case of 168 h, the growth medium was renewed after 48 and 120 h to support biofilm formation, which was proven in pre-tests. For this purpose, the present bacterial solution and the test pieces were aseptically removed. The polymer slices were washed with 10 x 1 ml sodium chloride with tryptone via pipette on each side to remove unattached cells and were afterwards reinsert into the falcon tube with 20 ml fresh sodium chloride with tryptone.

5.3.4 Enumeration of biofilm

To enumerate biofilm cells after incubation the bead vortexing method, described by Lindsay and Holy [45] as well as by Dorou et al. [22], was used. After 48 h and 168 h the slices were washed in the same way mentioned before and placed in a new Falcon® tube with 20 ml sodium chloride with tryptone and 10 glass beads (3 mm, Paul Marienfeld GmbH & Co KG, Lauda-

Königshofen, Germany). To remove the biofilm cells from surfaces the tubes were vortexed for 2 min (2700 rpm). Immediately after this procedure the polymer slices were removed and the bacterial counts of the solutions were enumerated. Plate count agar was used for enumeration of biofilm cells in experiments with mono-cultures of bacteria and for total viable counts (TVC) of mixed-species biofilms. To determine the individual counts of the two bacteria involved in mixed-cultures, selective media were used (Table 5.1). Plate count agar plates in mono-culture experiments as well as selective media for individual counts in mixed-cultures tests were incubated at the cultivating temperatures mentioned in table 1 for 48 hours; plate count agar plates for TVC in mixed-species experiments were incubated at 30 °C for 48 hours.

5.3.5 Statistical analyzing

Statistical significance in the difference of viable counts of biofilm cells on reference and sample material was tested using the Mann–Whitney U test in SPSS 22 (IBM Corp. 1989, 2013, New York, USA). Significance was defined as $p \leq 0.05$ and highly significant as $p \leq 0.005$. Figures were generated with the statistical software program Origin 8.0G (OriginLab Corporation, Northampton, USA).

5.4 Results

5.4.1 Mono-species biofilms

After 48 h incubation, all four tested bacteria formed a biofilm on the untreated LLDPE surfaces. The counts ranged between 2.0 and 6.1 $\log_{10}$ cfu cm^{-2}; the lowest count was detected for *S. aureus*, and *P. fluorescens* showed the highest density of cells. Figure 4.1 illustrates the distribution of viable counts of biofilm cells of the various microorganisms on the reference and the sample material. Except for *P. fluorescens,* significantly less biofilm ($p<0.005$) was formed by the three remaining bacteria on the poly(TBAMS)-containing LLDPE surfaces after 48 h than on untreated LLDPE. The highest difference (Δ 2 $\log_{10}$-steps) in mean biofilm cell counts was shown for *L. monocytogenes*. In contrast to untreated LLDPE, nearly no *S. aureus* cells adhere to the poly(TBAMS) samples (Δ 0.9 $\log_{10}$-steps).

The biofilms of all microorganisms increased during the following 5 days of incubation on untreated LLDPE. Thus, after 168 h more than 6 $\log_{10}$ cfu cm^{-2} were detected for *E. coli* and *P. fluorescens* (Figure 5.1b). Likewise, growth of the biofilm on poly(TBAMS)-containing surfaces except for *L. monocytogenes* was proven. No *L. monocytogenes* cells could be recovered from the poly(TBAMS) surfaces, while a biofilm of 4.2 $\log_{10}$ cfu cm^{-2} formed on the reference material ($p=0.002$). Also, the highly significant difference of *E. coli* ($p=0.002$; Δ 3.6 $\log_{10}$-steps) indicates an inhibition of biofilm formation due to poly(TBAMS). No significant difference after 168 h could be determined for *S. aureus* and for *P. fluorescens*.

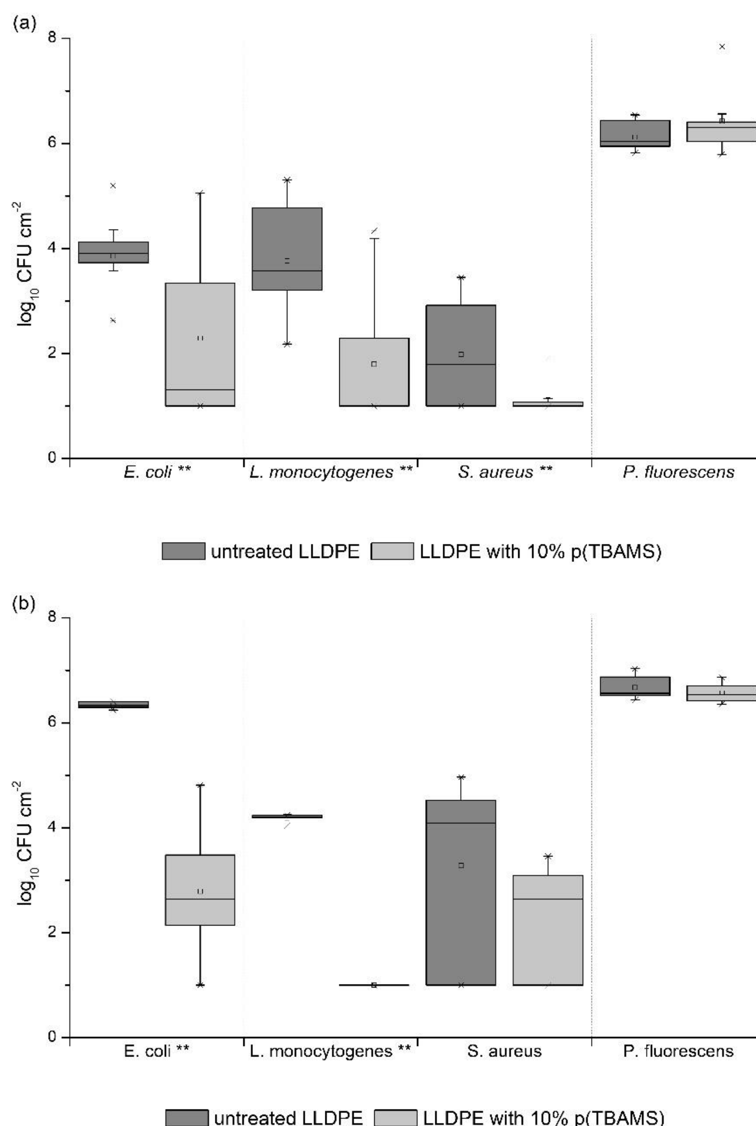

Figure 5.1 Box plots of viable counts of biofilm cells on untreated LLDPE compared to poly(TBAMS)-containing LLDPE after 48 h (a) and 168 h (b) incubation in solution of various microorganisms in mono-culture. Asterisk indicate significance of biofilm counts between the two used materials ($p \leq 0.005$).

5.4.2 Mixed-species biofilms

The results of total viable counts of biofilm formed by heterogeneous cultures on LLDPE material with and without poly(TBAMS) are shown in figure 5.2, the individual counts of the two bacteria used are summarized in table 4.2. In general, results are comparable with those of the mono-species biofilms, with higher biofilm counts of the gram-negative bacteria than of the gram-positive one. Most biofilms were formed by heterogeneous cultures in which *P. fluorescens* was present, with around 6 $\log_{10}$ cfu cm^{-2} after 48 h and 6.5 $\log_{10}$ cfu cm^{-2} after 168 h on both materials, corresponding with the count of monoculture biofilm of *P. fluorescens* after both time intervals (Figure 5.1). Also, the individual counts of two microorganisms indicate that the count of the heterogeneous biofilm is based on the density of *Pseudomonas* cells. No *S. aureus* cells could be detected in the presence of *P. fluorescens* after both incubation periods on the reference and sample material. Similarly, *L. monocytogenes* was not

present after 48 h on either material, but after 168 h, the biofilm consisted of both species on untreated LLDPE (*P. fluorescens* 6.49 $\log_{10}$ cfu cm^{-2}, *L. monocytogenes* 4.19 $\log_{10}$ cfu cm^{-2}) whereas still no *Listeria* were proven on poly(TBAMS)-containing surfaces. The mixture of *E. coli* and *P. fluorescens* formed a heterogeneous biofilm, with higher counts of *P. fluorescens* both on the reference material (*P. fluorescens*: 6.66 $\log_{10}$ cfu cm^{-2}, *E. coli*: 5.39 $\log_{10}$ cfu cm^{-2}) and sample material (*P. fluorescens* 6.54 $\log_{10}$ cfu cm^{-2}, *E. coli* 2.31 $\log_{10}$ cfu cm^{-2}). The difference of *E. coli* counts between both types of material is significant ($p \leq 0.005$) after 48 h as well as 168 h. Also, a significant decrease (48 h: $p=0.002$, 168 h: $p=0.01$) of biofilm formation on LLDPE with poly(TBAMS) was detected for *E. coli* in the presence of *L. monocytogenes*. In addition, due to the absence or minimal count of *L. monocytogenes* on the sample material, the difference of total viable counts is also significant after both times ($p=0.002$). As in the case of mono-culture biofilm, the biofilm cell counts of *S. aureus* cells were low compared with the other microorganisms. If the material was incubated with the mixture of *E. coli* and *S. aureus,* the detected biofilm was formed only by *E. coli*, no *S. aureus* could be proven on either material after either incubation period. Unlike the mono-culture results of *E. coli*, the difference in cell counts after the long incubation period were still not significant. The highest difference (Δ 4.7 $\log_{10}$ cfu cm^{-2}) in mean total viable count between the two materials was detected for the biofilm formed by *S.°aureus* and *L. monocytogenes* after 168 h. On LLDPE with poly(TBAMS), no cells of the pathogens could be measured, whereas both pathogens were components of the biofilm formed on untreated LLDPE.

Table 5.2 Quartiles and mean value of individual counts in heterogeneous biofilms on LLDPE and LLDPE with 10 % poly(TBAMS) after 48 and 168 h incubation. Asterisks indicate significance of biofilm counts between the two used materials (*: p≤0.05; **: p≤0.005).

	Individual microorganism count in biofilm [$\log_{10}$ CFU cm^{-2}]															
	48 h								**168 h**							
	Q1	median	Q3	mean	Q1	median	Q3	mean	Q1	median	Q3	mean	Q1	median	Q3	mean
microorganisms	*E. coli***				+ *P. fluorescens**				*E. coli***				+ *P. fluorescens*			
neat LLDPE	4.31	4.42	4.81	4.52	5.86	5.95	6.03	5.93	5.23	5.24	5.39	5.34	6.52	6.69	6.81	6.66
LLDPE/poly(TBAMS)	1.00	1.00	1.33	1.31	5.73	5.75	5.80	5.75	2.03	2.33	2.61	2.31	6.49	6.52	6.52	6.54
microorganisms	*E. coli***				+ *L. monocytogenes***				*E. coli**				+ *L. monocytogenes***			
neat LLDPE	3.65	3.78	3.90	3.78	1.98	2.18	2.48	2.17	5.87	6.02	6.18	6.04	4.11	4.37	4.46	4.29
LLDPE/poly(TBAMS)	1.00	1.00	1.82	1.44	1.00	1.00	1.00	1.00	1.63	2.32	2.76	2.69	1.13	1.18	1.22	1.16
microorganisms	*P. fluorescens*				+ *S. aureus*				*P. fluorescens*				+ *S. aureus*			
neat LLDPE	5.85	6.00	6.32	6.05	1.00	1.00	1.00	1.00	6.30	6.47	6.49	6.41	1.00	1.00	1.00	1.00
LLDPE/poly(TBAMS)	5.84	5.93	6.00	5.92	1.00	1.00	1.00	1.00	6.50	6.55	6.64	6.59	1.00	1.00	1.00	1.00
microorganisms	*P. fluorescens*				+ *L. monocytogenes*				*P. fluorescens*				+ *L. monocytogenes***			
neat LLDPE	5.80	5.90	6.10	5.88	1.00	1.00	1.00	1.00	6.52	6.52	6.54	6.49	4.07	4.23	4.28	4.19
LLDPE/poly(TBAMS)	5.76	5.91	6.07	5.92	1.00	1.00	1.00	1.00	6.45	6.47	6.50	6.48	1.00	1.00	1.00	1.00
microorganisms	*S. aureus*				+ *E. coli***				*S. aureus*				+ *E. coli*			
neat LLDPE	1.00	1.00	1.00	1.00	3.23	3.28	3.36	3.27	1.00	1.00	1.00	1.00	6.08	6.27	6.34	6.16
LLDPE/poly(TBAMS)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	5.94	6.05	6.18	6.07
microorganisms	*S. aureus*				+ *L. monocytogenes*				*S. aureus***				+ *L. monocytogenes***			
neat LLDPE	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.14	3.33	3.46	3.57	3.47	3.12	3.18	3.39	3.23
LLDPE/poly(TBAMS)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Asterisks indicate significance of biofilm counts between the two used materials (*: p≤0.05; **: p≤0.005)

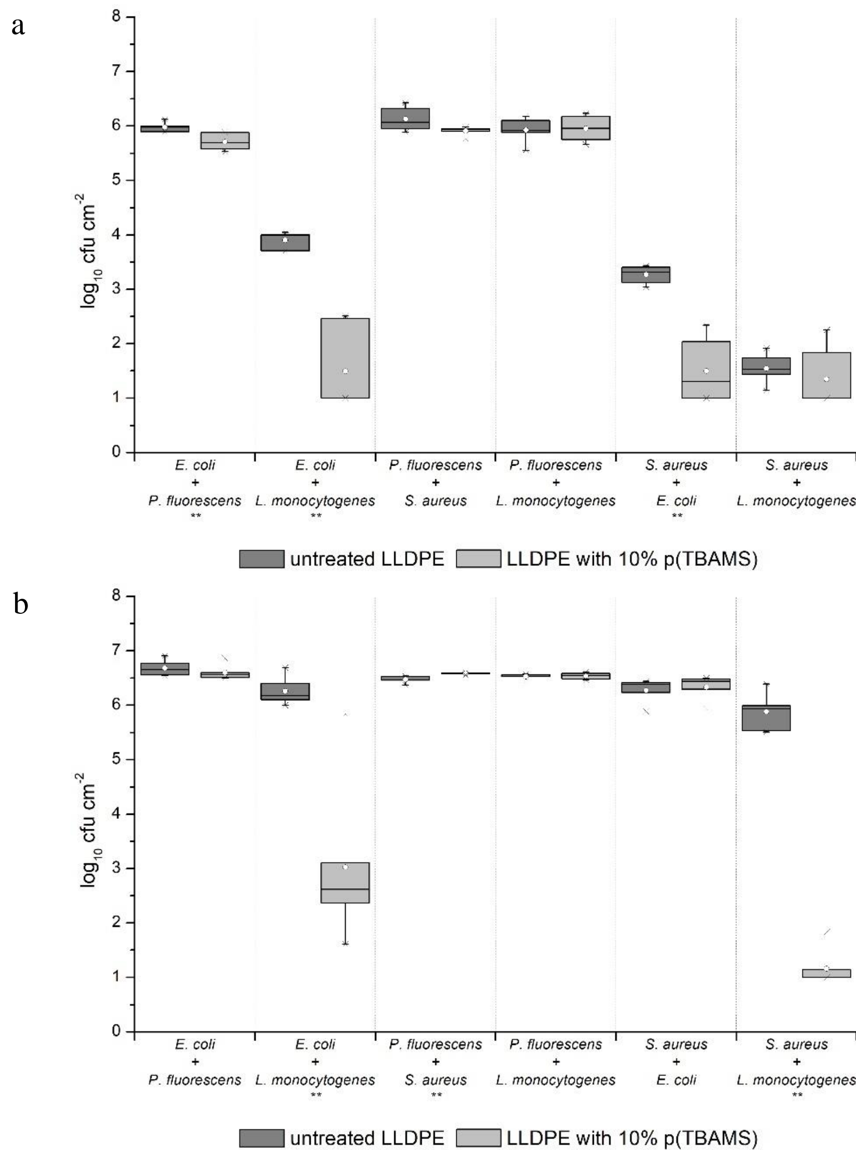

Figure 5.2 Box plots of total viable counts of biofilm cells on untreated LLDPE compared to poly(TBAMS)-containing LLDPE after 48 h(a) and 168 (b) incubation in solution of various microorganisms in mixed-culture. Asterisks indicate significance of biofilm counts between the two used materials ($p \leq 0.005$).

5.5 Discussion

It is established, that biofilms, which built a reservoir of bacteria, are a major source of contamination of food products [34]. Hence, the inhibition of biofilm formation is of concern in the food industry. The results of this study show that poly(TBAMS) compounded with LLDPE can reduce and delay the formation of biofilms of the pathogens *S. aureus*, *L. monocytogenes* and *E. coli*, but no effect on biofilm formation of the spoilage bacterium *P. fluorescens* could be detected. The study confirms on the one hand the general antimicrobial profile of poly(TBAMS), and on the other hand, the complex process of biofilm formation, which is depended on the general ability of the individual bacteria strains to form biofilms, and furthermore the interactions between bacteria in heterogeneous cultures [40, 41, 46, 47].

When biofilms were formed by mono-cultures, most biofilm cells were recovered from *P. fluorescens* from both materials after both incubation periods. Also, Sommer et al. [48] detected that a stable level of *P. fluorescens* was reached even after short contact times. The higher biofilm formation of gram-negative compared to gram-positive bacteria can be attributed to the surface charge, hydrophobicity and higher propensity to produce EPS [46, 48–50]. This EPS production and the general antimicrobial activity of poly(TBAMS) could be the cause of the different effects of the poly(TBAMS)-treated surfaces on the biofilm formation of the different bacteria. Studies regarding the antimicrobial profile of poly(TBAMS) detected a lower activity against the gram-negative bacteria *E. coli* and especially *Pseudomonas* spp. than against the gram-positive bacteria *S. aureus* and *L. monocytogenes*, which is attributed to the different surface charges of the bacteria species, leading to varying interaction strength [40, 41]. If cells survive on poly(TBAMS)-containing surfaces, they can produce exopolymer matrix films, which afterwards protect further bacteria from interaction with the antimicrobial active groups of poly(TBAMS). This results in no significant difference in biofilm formation on reference and sample material for *P. fluorescens,* because the activity of poly(TBAMS) is time dependent and the production of EPS by this fast biofilm former already starts 15 minutes after first contact with inert surfaces [13, 40, 51, 52]. As previously mentioned, the activity of poly(TBAMS) is higher against *E. coli* than against *P. fluorescens*. This led to significantly lower *E. coli* biofilm counts on the sample material than on untreated LLDPE, but due to EPS production of the surviving cells, the biofilm grew between the two investigation points. However, the treated surface led to a delay in biofilm formation by *E. coli*. The gram-positive *S. aureus* showed the poorest biofilm formation, which likewise was delayed on the antimicrobial surfaces. The highest effect of poly(TBAMS) on biofilm formation, with no detectable cells on 50-100 % of treated surfaces, was detected for *L. monocytogens*. This is caused primary by the high antimicrobial activity of poly(TBAMS) against *L. monocytogens* under various conditions as well as the short contact time and poor EPS production of *L. monocytogens* [40, 41, 47].

In nature, biofilms are most often a community of different bacteria species. Studies proved that mixed-species biofilms are often thicker and more stable than mono-species biofilms and that interactions between the biofilm community members influence the growth and survival of individual bacteria species [8, 10]. These facts could also have an influence on the effect of poly(TBAMS) on biofilm formation. In previous studies [41] we investigated the general antimicrobial activity of poly(TBAMS)-containing surfaces against bacteria in mono and mixed-culture after 2 h contact. The antimicrobial activity against the individual strains in mixed-culture showed the same behavior as in mono-culture. Thus, interactions between the bacteria strains or different electrostatic interactions between bacterium and antimicrobial surfaces seems to have no effect on the antimicrobial activity against the individual bacteria species. Although the results on the reference surfaces showed interactions between the bacteria species during biofilm formation of mixed-cultures, the tendential effect of poly(TBAMS) on

the colonization of the individual bacterial strains was not affected by the mixed-culture colonization.

The good antimicrobial activity of poly(TBAMS) against *L. monocytogenes* and *S. aureus* led to no biofilm formation when these two gram-negative bacteria were co-cultured on the sample material, while a biofilm was detected on the reference material. But in comparison to the results of mono-cultures, the biofilm formation on untreated LLDPE was also clearly delayed. Hence, it could be possible, that the concurrence between the bacteria stresses the individual cells. Moreover, this can explain the higher effect on *S. aureus* after 168 h incubation compared to mono-culture results, because it is known that the effect of antimicrobial systems is often enhanced when no optimal growth conditions leading to osmotic stress are present [53]. If the gram-positive bacteria were co-cultured with one of the gram-negative bacteria, a general suppression of the gram-positive bacteria was detected. On both materials, no *S. aureus* cells could be recovered and therefore no additional effect of the antimicrobial material could be achieved. The biofilm colonization of *L. monocytogenes* was delayed on the untreated LLDPE, but on the poly(TBAMS)-material it was nearly completely inhibited.

A dominance of *E. coli* over *S. aureus* in biofilms was also proven in various studies [49, 50, 54, 55], and an *E. coli* secreted, biofilm-associated, anti-adhesive polysaccharide, which hinders *S. aureus* cells in integrating in multispecies biofilms, was identified by Rendueles et al. [56]. Also, the clear dominance of *Pseudomonas spp.* over co-cultured species in biofilms, which was obvious on both materials, conforms with results of other authors. Thus, biofilms consist of up to 98 % *Pseudomonas* spp., while *Salmonella enterica*, *L. monocytogenes* or *E. coli* were less represented [17, 50, 57]. But our results do not confirm the findings that *Pseudomonas* spp. is a pioneering species in surface colonization, facilitating the attachment of less adhesive species [13, 16, 17, 52, 58–62]. Furthermore, the high biofilm production of *P. fluorescens* on both materials had no negative effect on the inhibition of colonization of the co-cultured bacterium on the antimicrobial surface.

This could be originated not only by the suppression of co-cultured bacteria, but also by the time dependence and strength of interaction of the bacteria species with the active groups of poly(TBAMS) [40, 41]. Within the first 48 h, *P. fluorescens* suppressed the colonization of *L. monocytogenes* on both materials, but after 168 h, *L. monocytogenes* adapted to the stress factors that come along with co-culturing, leading to a clear presence of *Listeria* on the reference material. In contrast, on the antimicrobial treated surface, the fast and strong interaction led to a complete suppression of *L. monocytogenes* before the EPS formed by *P. fluorescens* could protect cells from the contact with poly(TBAMS). In addition, this electro-negativity of the cells is also mentioned as one factor, next to growth rate and exopolymer production, which affects the structure of biofilms [49, 54, 63]. In a study by Almeida et al. [54], *E. coli* was found in the top layer of a biofilm, while *L. monocytogenes* was found close to the material surface. The author reasoned that the bacteria on the bottom are discriminated

against concerning nutrients and oxygen, leading to lower growth and explaining the reduced formation of a biofilm by *L. monocytogenes* in the presence of *E. coli* after 48 h on untreated LLDPE. Combined, the stronger interaction of *S. aureus* with poly(TBAMS), the assumed presence of *E. coli* in the top layer of the biofilm and the production of EPS could explain the higher biofilm production of *E. coli* on antimicrobial surface in co-culture with *S. aureus*, when compared with the results of mono-species culture. Similar results of a negative effect of the SAM-Polymer® poly(TBAEMA) on the biofilm formation of *S. aureus*, *S. mutans* as well as against an undefined mixed culture of river organisms were found by Seyfriedsberger et al. [43] and Marra et al. [64]. For releasing systems, the results are more inconsistent. For silver-ion impregnated PE-material, no difference in *L. monocytogenes* and *P. putida* biofilm formation was detected [65]. In contrast, a study of Roe et al. [66] showed that silver-coated plastic catheters inhibited the biofilm formation of different bacteria over a defined period of 72 h. Chaw et al. [67] identified that the activity of silver is limited on the peripheral areas of the biofilm, due to constricted penetration in these areas. The exposure of bacteria to sublethal concentrations of migrated biocides in those areas, as well as the irregular release of biocides, is supposed to contribute to resistance development and therefore, the use of releasing systems is critically discussed [12, 66, 68]. In contrast, no resistance development of bacteria is expected for contact-active materials [35]. A further advantage over releasing systems is the long-term activity of poly(TBAMS)-containing surfaces, whereas the activity of releasing systems is time-limited.

The results indicated that an implementation of poly(TBAMS) in food contact surfaces is a promising approach to reduce biofilm formation in the food industry. Especially the fact that the colonization of the pathogens in biofilms is inhibited or deferred, emphasizes the potential to improve the hygienic status of food contact surfaces.

The influence of cleaning and disinfection treatments of the surfaces was not part of this study. Thus, further investigation should clarify if the removing of condition films and frequent interruption of biofilms could maintain the effect of the contact-active antimicrobial surface. Furthermore, the integration of higher amounts of poly(TBAMS) should be studied to find out if an increase of active groups enhances the effect on biofilm formation, especially of strong EPS formers.

5.6 Conclusion

Biofilms represent a chronic source of microbial contamination in the food industry. Thus, the inhibition of biofilm formation is a matter of importance. A treatment of LLDPE with 10 % of the antimicrobial SAM-Polymer® poly(TBAMS) showed negative effects on biofilm formation of food related pathogens both in mono and mixed-species cultures. The tested material showed no effect on the colonization of *P. fluorescens*, but a further improvement of poly(TBAMS) containing materials could lead to an effectiveness even against such strong biofilm formers. The implementation of poly(TBAMS) treated materials can extend the time frame until

pathogens colonize food contact surfaces. Hence, the materials contribute to the reduction of pathogenic cross-contamination during food production and processing, leading to improved food safety. The use of antimicrobial surfaces does not substitute efficient cleaning and disinfection treatments but can improve the hygienic status of food contact surfaces.

5.7 References

1. Genigeorgis C (1995) Biofilm: Their significance to cleaning in the meat sector, in: Burt SA, Bauer F (eds.) New Challenges in Meat Hygiene: Specific Problems in Cleaning and Disinfection, pp. 29–47. Utrecht: Ecceamst.
2. Bower CK, McGuire J, Daeschel MA (1996) The adhesion and detachment of bacteria and spores on food-contact surfaces. Trends Food Sci Technol 7(5), 152–157.
3. Chmielewski R, Frank J (2003) Biofilm Formation and Control in Food Processing Facilities. Comp Rev Food Sci Food Safety 2(1), 22–32.
4. Berrang ME, Frank JF, Meinersmann RJ (2010) Listeria monocytogenes biofilm formation on silver ion impregnated cutting boards. Food Prot Trends 30(3), 168–171.
5. Simões M, Simões LC, Vieira MJ (2010) A review of current and emergent biofilm control strategies. LWT - Food Sci Technol 43(4), 573–583.
6. Sofos JN, Geornaras I (2010) Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of Escherichia coli O157:H7 in nonintact, and Listeria monocytogenes in ready-to-eat, meat products. Meat Sci 86(1), 2–14.
7. Siedenbiedel F, Tiller JC (2012) Antimicrobial Polymers in Solution and on Surfaces: Overview and Functional Principles. Polymers (Basel) 4(4), 46–71.
8. Giaouris E, Heir E, Desvaux M, Hébraud M, Møretrø T, Langsrud S, Doulgeraki A, Nychas G-J, Kačániová M, Czaczyk K, Ölmez H, Simões M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6, 841.
9. Donlan RM (2002) Biofilms: microbial life on surfaces. Emerging Infect. Dis. 8(9), 881–890.
10. James GA, Beaudette L, Costerton JW (1995) Interspecies bacterial interactions in biofilms. J Ind Microbiol 15(4), 257–262.
11. Reuter M, Mallett A, Pearson BM, van Vliet AHM (2010) Biofilm Formation by Campylobacter jejuni Is Increased under Aerobic Conditions. Appl Environ Microbiol 76(7), 2122–2128.
12. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42(1–2), 9–27.
13. Sasahara KC, Zottola EA (1993) Biofilm Formation by Listeria monocytogenes Utilizes a Primary Colonizing Microorganism in Flowing Systems. J Food Prot 56(12), 1022–1028.
14. Bremer PJ, Monk I, Osborne CM (2001) Survival of Listeria monocytogenes Attached to Stainless Steel Surfaces in the Presence or Absence of Flavobacterium spp. J Food Prot 64(9), 1369–1376.
15. Brooks JD, Flint SH (2008) Biofilms in the food industry: problems and potential solutions. Int J Food Sci Technol 43(12), 2163–2176.
16. Hassan AN, Birt DM, Frank JF (2004) Behavior of Listeria monocytogenes in a Pseudomonas putida Biofilm on a Condensate-Forming Surface. J Food Prot 67(2), 322–327.
17. Klayman BJ, Volden PA, Stewart PS, Camper AK (2009) Escherichia coli O157: H7 requires colonizing partner to adhere and persist in a capillary flow cell. Environ Sci Technol 43(6), 2105–2111.
18. Marouani-Gadri N, Augier G, Carpentier B (2009) Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection — Influence of isolated strains on biofilm formation by Sakaï and EDL 933 E. coli O157:H7. Int J Food Microbiol 133(1–2), 62–67.
19. Roder HL, Raghupathi PK, Herschend J, Brejnrod A, Knochel S, Sorensen SJ, Burmolle M (2015) Interspecies interactions result in enhanced biofilm formation by co-cultures of bacteria isolated from a food processing environment. Food Microbiol 51, 18–24.
20. Bagge-Ravn D, Ng Y, Hjelm M, Christiansen JN, Johansen C, Gram L (2003) The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. Int J Food Microbiol 87(3), 239–250.
21. Di Bonaventura G, Piccolomini R, Paludi D, D'Orio V, Vergara A, Conter M, Ianieri A (2008) Influence of temperature on biofilm formation by Listeria monocytogenes on various food-contact surfaces: Relationship with motility and cell surface hydrophobicity. J Appl Microbiol 104(6), 1552–1561.
22. Dourou D, Beauchamp CS, Yoon Y, Geornaras I, Belk KE, Smith GC, Nychas G-JE, Sofos JN (2011) Attachment and biofilm formation by Escherichia coli O157:H7 at different temperatures,

on various food-contact surfaces encountered in beef processing. Int J Food Microbiol 149(3), 262–268.

23. Gutierrez D, Delgado S, Vazquez-Sanchez D, Martinez B, Cabo ML, Rodriguez A, Herrera JJ, Garcia P (2012) Incidence of Staphylococcus aureus and Analysis of Associated Bacterial Communities on Food Industry Surfaces. Appl Environ Microbiol 78(24), 8547–8554.
24. Bridier A, Sanchez-Vizuete P, Guilbaud M, Piard J-C, Naitali M, Briandet R (2015) Biofilm-associated persistence of food-borne pathogens. Food Microbiol 45(Pt B), 167–178.
25. Čabarkapa I, Škrinjar M, Lević J, Kokić B, Blagojev N, Milanov D, Suvajdžić L (2015) Biofilm Forming Ability Of Salmonella Enteritidis In Vitro. Acta Vet (Belgrade) 65(3).
26. Di Ciccio P, Vergara A, Festino AR, Paludi D, Zanardi E, Ghidini S, Ianieri A (2015) Biofilm formation by Staphylococcus aureus on food contact surfaces: Relationship with temperature and cell surface hydrophobicity. Food control 50, 930–936.
27. Carrasco E, Morales-Rueda A, García-Gimeno RM (2012) Cross-contamination and recontamination by Salmonella in foods: A review. Food Res Int 45(2), 545–556.
28. Fisher L, Ostovapour S, Kelly P, Whitehead KA, Cooke K, Storgards E, Verran J (2014) Molybdenum doped titanium dioxide photocatalytic coatings for use as hygienic surfaces: the effect of soiling on antimicrobial activity. Biofouling 30(8), 911–919.
29. Hasan J, Crawford RJ, Ivanova EP (2013) Antibacterial surfaces: the quest for a new generation of biomaterials. Trends Biotechnol 31(5), 295–304.
30. Hood SK, Zottola EA (1997) Adherence to stainless steel by foodborne microorganisms during growth in model food systems. Int J Food Microbiol 37(2-3), 145–153.
31. Meyer B (2003) Approaches to prevention, removal and killing of biofilms. Int Biodeterior Biodegradation 51(4), 249–253.
32. Jorge P, Lourenco A, Pereira MO (2012) New trends in peptide-based anti-biofilm strategies: a review of recent achievements and bioinformatic approaches. Biofouling 28(10), 1033–1061.
33. Srey S, Jahid IK, Ha S-D (2013) Biofilm formation in food industries: A food safety concern. Food control 31(2), 572–585.
34. Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib N-E (2014) Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Arch Microbiol 196(7), 453–472.
35. Moerman F (2014) Antimicrobial materials, coatings and biomimetic surfaces with modified microtography to control microbial fouling of product contact surfaces within food processing equipment: Legislation, requirements, effectiveness and challenges. Journal of Hygienic Engineering and Design 7, 8–29.
36. Glinel K, Thebault P, Humblot V, Pradier C-M, Jouenne T (2012) Antibacterial surfaces developed from bio-inspired approaches. Acta biomaterialia 8(5), 1670–1684.
37. Karam L, Jama C, Dhulster P, Chihib N-E (2013) Study of surface interactions between peptides, materials and bacteria for setting up antimicrobial surfaces and active food packaging. J Mater Environ Sci 4(5), 798–821.
38. Elchinger P-H, Delattre C, Faure S, Roy O, Badel S, Bernardi T, Taillefumier C, Michaud P (2015) Immobilization of proteases on chitosan for the development of films with anti-biofilm properties. Int J Biol Macromol 72(Supplement C), 1063–1068.
39. Brodkorb F, Fischer B, Kalbfleisch K, Robers O, Braun C, Dohlen S, Kreyenschmidt J, Lorenz R, Kreyenschmidt M (2015) Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. Int J Mol Sci 16(8), 20050–20066.
40. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Robers O, Kreyenschmidt M, Kreyenschmidt J (2016) Potential of the polymer poly-[2-(tert-butylamino) methylstyrene] as antimicrobial packaging material for meat products. J Appl Microbiol 121(4), 1059-70.
41. Braun C, Dohlen S, Ilg Y, Brodkorb F, Fischer B, Heindirk P, Kalbfleisch K, Richter T, Robers O, Kreyenschmidt M, Lorenz R, Kreyenschmidt J (2017) Antimicrobial Activity of Intrinsic Antimicrobial Polymers Based on Poly((tertbutyl-amino)-methyl-styrene) Against Selected Pathogenic and Spoilage Microorganisms Relevant in Meat Processing Facilities. J Antimicrob Agents 03(01).
42. Dohlen S, Braun C, Brodkorb F, Fischer B, Ilg Y, Kalbfleisch K, Lorenz R, Kreyenschmidt M, Kreyenschmidt J (2017) Effect of different packaging materials containing poly-[2-(tert-

butylamino) methylstyrene] on the growth of spoilage and pathogenic bacteria on fresh meat. Int J Food Microbiol 257, 91–100.

43. Seyfriedsberger G, Rametsteiner K, Kern W (2006) Polyethylene compounds with antimicrobial surface properties. Eur Polym J 42(12), 3383–3389.
44. Tabak M, Scher K, Hartog E, Romling U, Matthews KR, Chikindas ML, Yaron S (2007) Effect of triclosan on Salmonella typhimurium at different growth stages and in biofilms. FEMS Microbiol. Lett. 267(2), 200–206.
45. Lindsay D, Holy A von (1997) Evaluation of dislodging methods for laboratory-grown bacterial biofilms. Food Microbiol 14(4), 383–390.
46. Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56, 187–209.
47. Renier S, Hébraud M, Desvaux M (2011) Molecular biology of surface colonization by Listeria monocytogenes: an additional facet of an opportunistic Gram-positive foodborne pathogen. Environ Microbiol 13(4), 835–850.
48. Sommer P, Martin-Rouas C, Mettler E (1999) Influence of the adherent population level on biofilm population, structure and resistance to chlorination. Food Microbiol 16(5), 503–515.
49. Pompermayer DMC, Gaylarde CC (2000) The influence of temperature on the adhesion of mixed cultures of Staphylococcus aureus and Escherichia coli to polypropylene. Food Microbiol 17(4), 361–365.
50. Al-Adawi AS, Gaylarde CC, Sunner J, Beech IB (2016) Transfer of bacteria between stainless steel and chicken meat: A CLSM and DGGE study of biofilms. AIMS Microbiol. 2(3), 340–358.
51. Davies DG, Geesey GG (1995) Regulation of the alginate biosynthesis gene algC in Pseudomonas aeruginosa during biofilm development in continuous culture. Appl Environ Microbiol 61(3), 860–867.
52. Giaouris ED, Nychas G-JE (2006) The adherence of Salmonella Enteritidis PT4 to stainless steel: The importance of the air–liquid interface and nutrient availability. Food Microbiol 23(8), 747–752.
53. Møretrø T, Høiby-Pettersen GS, Halvorsen CK, Langsrud S (2012) Antibacterial activity of cutting boards containing silver. Food control 28(1), 118–121.
54. Almeida C, Azevedo NF, Santos S, Keevil CW, Vieira MJ (2011) Discriminating Multi-Species Populations in Biofilms with Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH). PLOS ONE 6(3), e14786.
55. Millezi FM, Pereira MO, Batista NN, Camargos N, Auad I, Cardoso M, Piccoli RH (2012) Susceptibility of monospecies and dual-species biofilms of Staphylococcus aureus and Escherichia coli to essential oils. J Food Saf 32(3), 351–359.
56. Rendueles O, Travier L, Latour-Lambert P, Fontaine T, Magnus J, Denamur E, Ghigo J-M (2011) Screening of Escherichia coli Species Biodiversity Reveals New Biofilm-Associated Antiadhesion Polysaccharides. mBio 2(3).
57. Chorianopoulos NG, Giaouris ED, Skandamis PN, Haroutounian SA, Nychas G-JE (2008) Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of Satureja thymbra and comparison with standard acid–base sanitizers. J Appl Microbiol 104(6), 1586–1596.
58. Frank JF (2001) Microbial attachment to food and food contact surfaces, in: Jeya Henry (ed.) 74. Advances in Food and Nutrition Research, pp. 319–370: Elsevier.
59. Carpentier B, Chassaing D (2004) Interactions in biofilms between Listeria monocytogenes and resident microorganisms from food industry premises. Int J Food Microbiol 97(2), 111–122.
60. Castonguay M-H, van der Schaaf S, Koester W, Krooneman J, van der Meer W, Harmsen H, Landini P (2006) Biofilm formation by Escherichia coli is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. Res Microbiol 157(5), 471–478.
61. Simões M, Simões LC, Vieira MJ (2008) Physiology and behavior of Pseudomonas fluorescens single and dual strain biofilms under diverse hydrodynamics stresses. Int J Food Microbiol 128(2), 309–316.
62. Dourou D, Ammor MS, Skandamis PN, Nychas G-JE (2011) Growth of Salmonella enteritidis and Salmonella typhimurium in the presence of quorum sensing signalling compounds produced by spoilage and pathogenic bacteria. Food Microbiol 28(5), 1011–1018.

63. Banks MK, Bryers JD (1991) Bacterial species dominance within a binary culture biofilm. Appl Environ Microbiol 57(7), 1974–1979.
64. Marra J, Paleari AG, Rodriguez LS, Leite ARP, Pero AC, Compagnoni MA (2012) Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation. J Appl Oral Sci 20, 643–648.
65. Berrang ME, Meinersmann RJ, Frank JF, Smith DP, Genzlinger LL (2005) Distribution of Listeria monocytogenes Subtypes within a Poultry Further Processing Plant. J Food Prot 68(5), 980–985.
66. Roe D, Karandikar B, Bonn-Savage N, Gibbins B, Roullet J-B (2008) Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. J Antimicrob Chemother 61(4), 869–876.
67. Chaw KC, Manimaran M, Tay FEH (2005) Role of Silver Ions in Destabilization of Intermolecular Adhesion Forces Measured by Atomic Force Microscopy in Staphylococcus epidermidis Biofilms. Antimicrob Agents Chemother 49(12), 4853–4859.
68. Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F (2011) Resistance of bacterial biofilms to disinfectants: a review. Biofouling 27(9), 1017–1032.

6 Summary

Colonization of food contact surfaces with spoilage and pathogenic bacteria is of great concern in all steps of the processing and preparation of fresh meat from the food industry to the domestic kitchen, because bacteria can cross-over from contaminated surfaces to food products. Such cross-contamination can have far-reaching consequences for the environment, public health and causes extensive economic losses. Cleaning and disinfection measures are performed to achieve a hygienic status of food contact surfaces. But those hygienic operations are often not fully effective in removing all bacteria from food contact surfaces, allowing remaining bacteria to form biofilms, which present a long-term reservoir of bacteria. In addition, the measures are only performed in defined intervals, in the interim the surfaces are unprotected against the colonization with bacteria.

The application of antimicrobial materials can be a further hurdle for bacteria to attach to food contact surfaces. Several antimicrobial systems were developed, but most systems are unsuitable for use during the processing and preparation of fresh meat, because the antimicrobial activity is considerably reduced or even lacking under the prevalent environmental and processing conditions. Furthermore, material properties are often inadequate and release of biocides out of materials is seen critical. The intrinsically antimicrobial active SAM-Polymer® poly(TBAMS) bears a great potential as contact-active antimicrobial material, however until this thesis no results regarding the antimicrobial activity and long-term activity under conditions prevalent during meat processing and preparation; furthermore, no data about the reduction of cross-contamination and biofilm formation were available.

Thus, the main objective of this thesis was the investigation of the potential of intrinsic antimicrobial active polymers to improve the hygienic conditions during the processing and preparation of fresh meat. The antimicrobial activity and the long-term stability was assessed under relevant conditions and the effect of poly(TBAMS) on cross-contamination and biofilm formation was investigated. Therefore, five research questions were proposed.

The first research question was focused on the antimicrobial spectrum of poly(TBAMS) as a homopolymer as well as a copolymer (poly(TBAMS):acrylonitrile [1:1]) which offered improved material properties. The antimicrobial activity against spoilage and pathogenic bacteria (*S. aureus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp., *Pseudomonas* spp., *B. thermosphacta*) typically present on processing and preparation surfaces of fresh meat was determined based on the Japanese Industrial Standard (JIS) 2801:2000. The tests were conducted with mono-species cultures in moderate as well as high bacterial counts. Furthermore, tests were conducted with mixed-species cultures considering natural incidence of bacterial flora. The results indicated a good antimicrobial activity of both poly(TBAMS)-containing films against the mentioned bacteria at 35 °C after 2 h, the copolymer showed a slightly lower activity. Especially tests with high bacterial counts revealed a higher sensitivity

of gram-positive bacteria than of gram-negative on the materials. This can be attributed to the more neutral charge of the surface of gram-negative bacteria, which leads to weaker interactions with the positive charged polymer surface in comparison to the more negatively charged gram-positive bacteria. The tests with mixed species confirmed this tendency, but the reduced antimicrobial action against the gram-negative *P. fluorescens* did not affect the activity against the other co-cultured pathogens *L. monocytogenes*, *E. coli*, *S. aureus* and *S. enterica*.

The second research question aimed at the detection of effects of different environmental and processing factors on the antimicrobial activity of poly(TBAMS)-containing materials. This question focused on the ability of the application as antimicrobial material during the processing and preparation of fresh meat. Therefore, the test method of the JIS was modified regarding the contact time, temperature, air humidity, pH-value and presence of food components. The effect of investigated parameters was differently pronounced for the individual bacterial strains, with higher impairment for gram-negative bacteria. This was particularly evident if high bacterial counts in nutrient rich solutions were in contact with the material. In this test series the counts of gram-positive bacteria were reduced to the detection limit within 2 h at 35 °C, while the activity in comparison to the results of the first part of the thesis was decreased for gram-negative bacteria. Mineral nutrients seem to stabilize the outer membrane of bacteria, thus, higher concentrations of calcium led to a decrease of the reduction rate, but even at high calcium concentrations, irrelevant to contact with meat, the log_{10}-reductions were higher than 2.4 log_{10}-reductions after 2 h at 35 °C. A retarding effect of low temperature on the antimicrobial activity was proven, thus, lowering the temperature led to a decrease in the reduction of bacteria, but this could be counteracted by prolonging the contact time. An exception was the activity against *L. monocytogenes*. After 1 h at low temperature of 4 °C the maximal reduction of *L. monocytogenes* to the detection limit was reached. This maximal reduction was proven for all bacteria under low humidity conditions at 7°C after 24 h, but under high humidity conditions, the activity against *S. aureus* and *P. fluorescens* was reduced compared to standard conditions. The variance of the pH-value during the contact with the pathogens *S. aureus* and *E. coli* from neutral tending toward acidic conditions had no effect on the reduction at 35 °C after 2 h. Under alkaline conditions, the poly(TBAMS)-containing surface is more neutrally charged, leading to decreased activity. However, significant reductions of *E. coli* were still determined.

The third research question was focused on the long-term stability of different poly(TBAMS)-containing surfaces. The materials were stored under different environmental conditions and the activity was proven over a period up to 3 years. The relative log_{10}-reduction were modeled via linear regression to predict the expected changes in activity during use solely due to application in food contact materials under those conditions. The investigations showed that the lower the storage temperature (-20, 5, 22 °C on average), the more stable the antimicrobial activity was and that copolymerization as well as the kind of copolymer led to more pronounced changes in activity. Linear modelling of the relative log_{10}-reduction over the period of 3 years showed that, for the homopolymer poly(TBAMS)-film, a decrease of maximal 0.5 % a^{-1} could

be expected (97.5% quartile). The highest loss of activity was determined for the copolymer with acrylonitrile which was stored at ambient conditions. The previous antimicrobial tests revealed the general potential as antimicrobial active food contact material during processing and preparation of fresh meat.

The remaining research question was focused on the application as food contact material to improve the hygienic status of food contact materials and to reduce cross-contaminations. As a typical food contact material, LLDPE was co-extruded with poly(TBAMS). To check if this process caused an impairment of antimicrobial activity, the material was tested according to the standard test method. This antimicrobial screening showed a highly significant reduction of pathogenic counts of *S. aureus*, *L. monocytogenes* and *S. enterica* at ambient conditions after 2 h contact. The previously detected outstanding sensitivity of *L. monocytogenes* against poly(TBAMS) was confirmed, after 2 minutes contact at ambient temperature conditions the counts were reduced about 0.7 log_{10}-units. Due to reduced activity of LLDPE with 10 % poly(TBAMS) at cold temperature, the material was preferentially qualified for the application as food contact surfaces used in the domestic environment.

The comparison with other antimicrobially treated cutting boards indicated the superiority of poly(TBAMS)-containing LLDPE over the other poly(TBAEMA)-containing LLDPE as well as a commercially available PP-board with Microban®. For the last board, no reduction of bacterial load was detected even under the standard tests conditions of the JIS (24 h, 35 °C).

However, the cross-contamination scenarios using the poly(TBAMS)-containing boards detected that the effect on the transfer of bacteria during the preparation of food was highly limited and varied in dimension dependent on microorganism and food stuff. For scenarios with *S. aureus* no significant effects could be detected, while for *L. monocytogenes* the impact was dependent on the food stuff. A highly negative influence on cross-contamination of *L. monocytogenes* was measured for the transfer from an inoculated cutting board to a cucumber slice. The transfer rate from the untreated board to the cucumber was nearly 60 %, whereas less than 1 % of bacterial count was transferred from the poly(TBAMS)-containing board. However, the involvement of fresh meat inhibited this effect. Hence, the transfer rate of *L. monocytogenes* from an inoculated pork filet slice to a cucumber via a cutting board only exhibited a difference of 2 % between the reference and the antimicrobial material.

The last research question was also focused on the ability of poly(TBAMS) to reduce the colonization of food contact surfaces with bacteria in the form of biofilms. Biofilms were allowed to grow on LLDPE with and without 10 % poly(TBAMS) by culturing up to 7 days in a bacterial suspension. The suspension consisted in a first experimental series of one bacterial species and in a second series of mixtures of two bacteria each in all possible combinations. The reduction of biofilm formation on the poly(TBAMS)-containing material was regulated by the antimicrobial activity of the material against the individual bacteria strains. Thus, nearly no *Listeria*-biofilm was formed on the antimicrobial material caused by the effective killing of

bacteria by the material. For *S. aureus* and *E. coli* the antimicrobial activity was not as pronounced as for *L. monocytogenes*. Hence, over time bacteria attached to the surface and built a biofilm, the produced exopolymer substances presumably protect the bacteria from the necessary contact with the active groups of the antimicrobial polymer, resulting in growth of biofilm. However, the treatment of LLDPE with 10 % poly(TBAMS) delayed the formation of biofilms of *S. aureus* and *E. coli*. No effect on biofilm formation of *P. fluorescens* was detected, which could also be explained by the minor antimicrobial activity of poly(TBAMS) against this bacterium and furthermore, *Pseudomonas* is known to produce a high amount of exopolymer material even after a short time. The investigation with mixed cultures emphasized the complexity of formation as well as of interactions in mixed biofilms. Thus, interactions between the two bacterial strains used led to changed behavior of individual strains in colonization of the untreated surfaces, like suppression of *S. aureus* colonization by gram-negative bacteria. The effect of poly(TBAMS) on the colonization of bacteria co-cultured in mixed-species on the LLDPE surface was comparable to mono-species results. The colonization with *L. monocytogenes* and of *S. aureus* was nearly completely suppressed on the antimicrobial material, although *P. fluorescens* formed strong biofilms.

The overall results of this thesis revealed the complexity of a sustainable application of antimicrobial contact surfaces with the aim to improve the hygienic conditions during the processing and preparation of fresh meat. Poly(TBAMS) fulfill a lot of requirements on antimicrobial food contact surfaces, like long-term effectiveness under various conditions and a broad antimicrobial profile under standard test conditions. Furthermore, due to the contact-activity, the development of resistances as well as a harmful effect on humans and the environment is minimized. However various environmental and processing factors were identified that influence the antimicrobial activity. But in comparison to other antimicrobial materials, poly(TBAMS) reduces the bacterial counts more effectively under conditions typical during processing and preparation of fresh meat.

The processing of poly(TBAMS) as a copolymer or a compound led to a loss of activity. Thus, the effect of material with a poly(TBAMS) content of 10 % is certainly limited for the use in contact with fresh meat, but by increasing the availability of active groups on the surface, e.g. by increasing the amount of poly(TBAMS) in bulk material, or the use of other basis materials, the effect could potentially be increased. In addition, other fields of the food processing and preparation industry (e.g. vegetables, juice) could be expected to be favored as application fields because of the environmental and processing factors like higher temperature and less proteins.

Despite the limitation due to the low content of poly(TBAMS) it was proven that the colonization of surfaces was delayed for pathogens on the material. Thus, the application of poly(TBAMS) in food contact materials has the potential to improve the hygienic conditions of the surfaces, especially during holding times, and could simplify as well as enhance the effectiveness of cleaning and disinfection methods. This would affect the persistence of

pathogens in the food industry and consequently lead to a reduction of cross-contamination, even if the direct transfer of pathogens during typical preparation scenarios in domestic kitchens was only marginal effected. But for a sustainable and effective application of the SAM-Polymer® poly(TBAMS) as a food contact material further development of the material as well the legitimate validation is necessary.

List of figures

Figure 1.1 Microbial contamination routes during processing and preparation of fresh meat 1

Figure 1.2 Consequences of microbial contamination of food contact surfaces 4

Figure 1.3 Principles of antimicrobial surfaces (mod. [100, 127]) 7

Figure 1.4 Structure of the SAM-Polymers® poly-[2-(tert-butylamino) ethyl methacrylate] (poly(TBAEMA) (left) and poly-[2-(tert-butylamino) methylstyrene] (poly(TBAMS) (right) (mod. [157])... 12

Figure 2.1 Chemical structure of poly(TBAMS) (left) and poly(TBAMS:acrylonitrile) (right) consisting of a mixture of meta- and para-isomers. 27

Figure 2.2 FTIR-ATR spectrum of the homopolymer poly(TBAMS) (a) and of the copolymer poly(TBAMS:acrylonitrile) (b).. 31

Figure 2.3 DSC analysis of poly(TBAMS) (a) and poly(TBAMS:acrylonitrile) (b). 32

Figure 2.4 Reduction [$\log_{10}$ cfu ml^{-1}] of bacteria after 2 h incubation at 35 °C applied on reference material (dark grey bars) or sample material (light grey bars): (a) homopolymer poly(TBAMS) or (b) copolymer poly(TBAMS:acrylonitrile). The values are changes from initial concentration (a: 4.9- 5.9 $\log_{10}$ cfu ml^{-1}, b: 4.8- 5.7 $\log_{10}$ cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on sample material after incubation ($\log_{10}$-reduction). Asterisks indicate significant differences (** $p \leq 0.005$, * $p \leq 0.05$) between sample and reference material (n>3). 33

Figure 2.5 Reduction [$\log_{10}$ cfu ml^{-1}] of overnight cultures of bacteria in saline solution with tryptone applied on reference material (dark grey bars) or poly(TBAMS) (light grey bars) incubated at 35 °C for 2 h. The values are changes from initial concentration (6.6-8.9 $\log_{10}$ cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on poly(TBAMS) after incubation ($\log_{10}$-reduction). Asterisks indicate significant differences (** $p \leq 0.005$) between poly(TBAMS) and reference material. 35

Figure 2.6 Reduction [$\log_{10}$ cfu ml^{-1}] of mixed cultured bacteria: (a) *B. thermosphacta* with *Pseudomonas* spp., (b) *P. fluorescens* with *E. coli* or *L. monocytogenes* applied on reference material (dark grey bars) on reference material or poly(TBAMS:acrylonitrile) (light grey bars) incubated at 35 °C for 2 h. The values of the plain bars are the changes from initial concentration of the total viable count (TVC); the patterned bars are the changes of the individual bacteria counts. The delta values are the differences between the surface counts on reference material and on poly(TBAMS:acrylonitrile) after incubation ($\log_{10}$-reduction) 37

Figure 3.1 Linear regression of the rel. $\log_{10}$-reduction of poly(TBAMS) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions... 50

Figure 3.2 Linear regression of the rel. $\log_{10}$-reduction of poly(TBAMS:acrylonitrile) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions. 51

Figure 3.3 Linear regression of the rel. $\log_{10}$-reduction of poly(TBAMS:vinylopyridin) against *E. coli* (right) and *S. aureus* (left) as a function of storage time under various temperature and humidity conditions. 52

Figure 3.4 Reduction [$\log_{10}$ cfu ml^{-1}] of different bacteria (*L. monocytogenes* (a), *P. fluorescens* (b), *S. aureus* (c), *S. enterica* (d)) applied on reference material (squares) or the sample material (homopolymer poly(TBAMS)) (circles) after 1-24 h contact at 7 °C (grey symbols) or 35 °C (white symbols). The values are changes from initial concentration..................... 55

Figure 3.5 Reduction [$\log_{10}$ cfu ml^{-1}] of *S. aureus* (white symbols) and *E. coli* (grey symbols) applied on reference material (squares) or poly(TBAMS) (circles) after 2 h contact at 35 °C as a function of pH-value. The values are changes from initial concentration. 57

Figure 3.6 Reduction [$\log_{10}$ cfu ml^{-1}] of *S. aureus* (white symbols) and *E. coli* (grey symbols) applied on reference material (squares) or sample material (circles) after 2 h contact at 35 °C as a

function of concentration of mineral nutrients (a: calcium, b: magnesium). The values are changes from initial concentration. ... 58

Figure 3.7 Reduction [log_{10} cfu ml^{-1}] of overnight cultures of bacteria in nutrient broth applied on reference material (dark grey bars) or poly(TBAMS) (light grey bars) incubated at 35 °C for 2 h. The values are changes from initial concentration (8.1-9.4 log_{10} cfu ml^{-1}). The delta values are the differences between the surface counts on reference material and on poly(TBAMS) after incubation (log_{10}-reduction). Asterisks indicate significant differences (** $p \leq 0.005$, * $p \leq 0.05$) between poly(TBAMS) and reference material. ... 59

Figure 4.1 Shematic representation of cross-contamination the three scenarios. Processes with poly(TBAMS)-treated cutting boards (S) are presented in light grey and processes with untreated LLDPE (R) are presented in white and with dashed lines. ... 73

Figure 4.2 Reduction [log_{10} cfu ml^{-1}] of bacterial counts after 2 h incubation at 20 °C applied on LLDPE and PE as reference cutting boards and antimicrobial treated sample cutting boards: LLDPE/poly(TBAMS) (n=18), LLDPE/poly(TBAEMA) (n=15), PP/Microban® (n=3). 76

Figure 4.3 Reduction [log_{10} cfu ml^{-1}] of bacterial counts after 2 h incubation at 7 °C applied on reference cutting boards (LLDPE) and antimicrobial treated sample cutting boards (LLDPE/poly(TBAMS), LLDPE/poly(TBAEMA), n=6). ... 77

Figure 5.1 Box plots of viable counts of biofilm cells on untreated LLDPE compared to poly(TBAMS)-containing LLDPE after 48 h (a) and 168 h (b) incubation in solution of various microorganisms in mono-culture. Asterisk indicate significance of biofilm counts between the two used materials ($p \leq 0.005$). ... 91

Figure 5.2 Box plots of total viable counts of biofilm cells on untreated LLDPE compared to poly(TBAMS)-containing LLDPE after 48 h(a) and 168 (b) incubation in solution of various microorganisms in mixed-culture. Asterisks indicate significance of biofilm counts between the two used materials ($p \leq 0.005$). ... 93

List of tables

Table 2.1 Summary of tested bacteria, cultivating temperature, and used selective media in mixed cultures. 28

Table 3.1 Survey of long-term storage conditions. 46

Table 3.2 Fit parameter of the linear regression of relative log_{10}-reduction of *S. aureus* and *E. coli* on poly(TBAMS)-containing films over a storage period of 36 month under different environmental conditions 53

Table 3.3 Bacterial counts on reference material and log_{10}-reductions of *E. coli* and *S. aureus* at the different investigation points during storage at the three different conditions. 54

Table 3.5 Reduction [log_{10} cfu ml^{-1}] of different bacteria applied on reference material (Red_R) or on poly(TBAMS:acrylonitrile) sample material (Red_S) after 2 h contact at various temperatures. The values are changes from initial concentration. 56

Table 4.1 Transfer of bacteria (total viable counts) after 2 h in different scenarios with untreated LLDPE material and LLDPE with 10 % poly(TBAMS). Different superscript letters in one line indicate significant differences in total viable count on LLDPE and LLDPE+10 % poly(TBAMS) ($p<0.05$), capital letters mark highly significant differences ($p<0.005$). 78

Table 4.2 Transfer of bacteria (total viable counts and individual counts of *S. aureus*) after 2 min in different scenarios with untreated LLDPE material and LLDPE with 10 % poly(TBAMS). Different superscript letters in one line indicate significant differences in total viable count on LLDPE and LLDPE+10 % poly(TBAMS) ($p<0.05$), capital letters mark highly significant differences ($p<0.005$). 79

Table 5.1 Summary of used bacteria, cultivating as well as enumeration temperature and selective media in mixed cultures 89

Table 5.2 Quartiles and mean value of individual counts in heterogeneous biofilms on LLDPE and LLDPE with 10 % poly(TBAMS) after 48 and 168 h incubation. Asterisks indicate significance of biofilm counts between the two used materials (*: $p\leq0.05$; **: $p\leq0.005$). 92

List of publications

Hüwe C., Schmeichel J., Brodkorb F., Dohlen S., Kalbfleisch K., Kreyenschmidt M., Lorenz R., Kreyenschmidt J. (2018): Potential of antimicrobial treatment of linear low-density polyethylene with poly((tert-butyl-amino)-methyl-styrene) to reduce biofilm formation in food industry**.** Biofouling, 34(4), 378-387.

Schulze M., El Khaldi-Hansen B., Dreier T., Alzagameem A., Kamm, B., **Braun C.**, Kreyenschmidt J. (2017): Environmentally Benign Antioxidants: Lignin-based Materials for Food Packaging Applications. Poster, GDCh-Wissenschaftsforum Chemie 2017, 10.-14.09.2017, Berlin, Germany.

Dohlen S., **Braun C.**, Brodkorb F., Fischer B., Ilg Y., Kalbfleisch K., Lorenz R., Kreyenschmidt M., Kreyenschmidt J. (2017): Effect of different packaging materials containing poly-[2-(tert-butylamino) methylstyrene] on the growth of spoilage and pathogenic bacteria on fresh meat. International Journal of Food Microbiology, 257, 91-100.

Alzagameem A., El Khaldi-Hansen B., Dreier T., Schulze M., **Braun C.**, Kreyenschmidt J., Kamm, B. (2017): Lignocellulose-rich biomass: renewable sources for sustainable lignin-based antioxidants to be used in packaging and biomedicine. Poster, European Polymer Federation Congress 02.-07.07.2017, Lyon, France.

Braun C., Dohlen S., Ilg Y., Brodkorb F., Fischer B., Heindirk P., Kalbfleisch K., Richter T., Robers O., Kreyenschmidt M., Lorenz R., Kreyenschmidt J. (2017): Antimicrobial activity of intrinsic antimicrobial polymers based on poly((tert-butyl-amino)-methyl-styrene) against selected pathogenic and spoilage microorganisms relevant in meat processing facilities. Journal of Antimicrobial Agents, 3:136.

Albrecht A., Herbert U., Miskel D, Heinemann C., **Braun C.**, Dohlen S., Zeitz J., Eder K, Saremi B., Kreyenschmidt J. (2017): Effect of methionine supplementation in chicken feed on the quality and shelf life of fresh poultry meat. Poultry Science *96* (8): 2853-2861.

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

Lorenz R., Brodkorb F., Fischer B., Kalbfleisch K., Kreyenschmidt M., Kreyenschmidt J., **Braun C.**, Dohlen S. (2016): Multiresistent aus eigenem Antrieb- Breite antimkrobielle Aktivität als intrinsische Eigenschaft thermoplastischer und duromerer Kunststoffe. Kunststoffe, 4/2016.

Alzagameem A., Hansen B., Kamm B., Schulze M., Witzleben S., **Braun C.**, Kreyenschmidt J. (2016): Lignocellulose-rich biomass: renewable source for sustainable lignin-based

antioxidants to be used in packaging and biomedicine. Poster, 10th European Wood Panel Conference, 05.-07.10.2016, Hamburg, Germany.

Albrecht A., Herbert U., Heinemann C., Miskel D., **Braun C.**, Hüwe S., Zorn K., Dohlen S., Zeitz J.O., Eder K., Saremi B., Kreyenschmidt J. (2016): Effect of varying levels of dietary methionine sources on the quality and shelf life of fresh broiler meat. Proceedings, World Poultry Congress, 05.-09.09.2016, Beijing, China.

Dohlen S., Albrecht A., **Braun C.**, Brodkorb F. Fischer B., Ilg Y., Kalbfleisch K., Kreyenschmidt M., Lorenz R., Robers O., 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.-15.06.2016, Bonn, Germany.

Alzagameem A., Neuwald T., Hansen B., **Braun C.**, Kreyenschmidt J., Witzleben S., Schulze M. (2016): Sustainable Packaging materials developed on lignin-based antioxidants. Poster presentation: 6th International Cold Chain Management Conference, 06.06.-07.06.2016, Bonn, Germany.

Brodkorb F., Fischer B., Kalbfleisch K., Robers O., **Braun C.**, Dohlen S., Kreyenschmidt J., Lorenz R., Kreyenschmidt M. (2015): Development of a New Monomer for the Synthesis of Intrinsic Antimicrobial Polymers with Enhanced Material Properties. International Journal of Molecular Science, Special Issue Antimicrobial Polymers 2015.

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, Proceedings: 11. ThGOT, 15.-16.09.2015, Zeulenroda-Triebes, Germany. ISBN 978-3-00-046056-2

Brodkorb F., Robers O., Fischer B., Kalbfleisch K., **Braun C.**, Dohlen S., Kreyenschmidt J., Lorenz R., Kreyenschmidt M (2015): Eine neue Stoffklasse intrinsisch-antimikrobieller Kunststoffe auf Basis von tert.-Butylaminomethylstyrol. Oral presentation, Proceedings, 11. ThGOT, 15.-16.09.2015, Zeulenroda-Triebes, Germany. ISBN 978-3-00-046056-2

Dohlen S., **Braun. C.**, Brodkorb F., Fischer B., Ilg Y., Kalbfleisch K., Kreyenschmidt M., Lorenz R., Kreyenschmidt J. (2015): The action profile of a new class of antimicrobial packaging material to improve meat safety and shelf life. Oral presentation, Innovations in Food Packaging, Shel Life and Food Safety, 15.-17.09.2015, Erding, Germany.

Alzagameem A., **Braun C.**, Kreyenschmidt J., Buechner D., Fine D., Witzleben S., Schulze M. (2015): Isolation, Purification and Antioxidant Activity Studies of Lignin. Poster presentation: GDCh Wissenschaftsforum 2015, 30.08.-02.09.2015, Dresden, Germany.

Dohlen S., Ilg Y., **Braun C.**, Brodkorb F., Fischer B., Kalbfleisch K., Lorenz R., Kreyenschmidt M., Robers O., Kreyenschmidt, J. (2015): The action profile of a new class of antimicrobial packaging material for meat. Poster presentation, International Journal of Food Science and Technology 50th Volume Celebration Conference, 17.-19.02.2015, Lincoln University, New Zealand.

Dohlen S., Kalbfleisch K., **Braun C.**, Brodkorb F., Fischer B., Robers O., Ilg Y., Kreyenschmidt M., Lorenz R., Kreyenschmidt J. (2014): Neuartige antimikrobiell wirkende Polymere als Verpackungsmaterial von Fleischwaren zur Verlängerung der Haltbarkeit. Poster presentation, Tagung "Von der Verschwendung zur Wertschätzung der Lebensmittel - Wissenschaftliche Erkenntnisse und praktische Umsetzung", 21.11.2014, Münster, Germany.

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. Oral presentation, Proceedings presentation, GDL Kongress Lebensmitteltechnologie 2014, 17.10.2014, Frankfurt-Rodgau, Germany.

Alzagameem A., **Braun C.**, Kreyenschmidt J., Witzleben S., Schulze M. (2014): Extraction, Purification, and Bioactivity of Kraft Lignin. Poster presentation, PhD-Exhibition "Opening of the academic year an der Hochschule Bonn-Rhein-Sieg", 29.09.2014, Rheinbach, Germany and 10.10.2014, Sankt Augustin, Germany.

Robers O., Brodkorb F., Fischer B., Kalbfleisch K., **Braun C.**, Dohlen S., Kreyenschmidt J., Kreyenschmidt, M., Lorenz R. (2014): Entwicklung polymerer Kontaktbiozide für Lebensmittelverpackungen (Forschungsvorhaben Safe-Pack). Oral presentation, Food Science Dialog, 19.09.2014, Hamburg, Germany.

Braun C., Ilg Y., Kreyenschmidt J (2013): Improvement of food quality and safety by intrinsic antimicrobial food contact surfaces. Oral presentation, Proceedings, 5th International Workshop Cold Chain Management, Workshop, 10.-11.06.2013, Bonn, Germany.

Kreyenschmidt J., **Braun C.**, Dohlen S., Ilg Y., Lorenz R., Kreyenschmidt M. (2014): Active packaging solutions to improve food quality and safety of meat products. Oral presentation, Interpack “Fusion Platform Meeting”, 09.05.2014, Düsseldorf, Germany.

Kreyenschmidt J., Albrecht A., **Braun C.**, Herbert U., Mack M., Rossaint S., Ritter G., Teitscheid P., Ilg Y. (2013): Food Waste in der Fleisch verarbeitenden Kette. Fleischwirtschaft 10, 57-63.

Kreyenschmidt J., **Braun C.**, Herbert U., Mack M., Rossaint S. (2013): Cold Chain Optimisation and Predictive Tools and Technology. Oral presentation, Cold Supply System & solution, CSS Symposium, 06- 08.02.2013, Brüssel, Belgium.

Kreyenschmidt J., **Braun C.**, Ilg Y. (2012): SmartSurf - Entwicklung antimikrobiell wirkender Kunststoffe für die Lebensmittel- und Haushaltswarenindustrie als wichtiger Beitrag zur Verbesserung der Lebensmittelqualität und -sicherheit: Schlussbericht zum Teilvorhaben: Smart Surf; Laufzeit des Vorhabens: 05.2008 - 06.2012.

Acknowledgment

The project was partly financed by a postgraduate scholarship of the Heinrich-Stockmeyer-Foundation and was supported by funds of the Federal Ministry of Economics and Technology within the framework InnoNet (contract number 16INO640; Verbund-Nr. 01063175) and the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme (contract number 313-06.01-28-1-68.034-10) (Safe-Pack).

Thanks to the companies for their cooperation and support of the study.