TECHNISCHE UNIVERSITÄT MÜNCHEN
TUM School of Life Sciences

Developing Antimicrobial PET Beverage Containers by Incorporating a Silver Additive

Sabrina Braun

Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung des akademischen Grades einer

Doktorin der Ingenieurwissenschaften (Dr.-Ing.)

genehmigten Dissertation.

Vorsitzende: apl. Prof. Dr. Petra Först

Prüfende/-r der Dissertation:

1. Prof. Dr. Horst-Christian Langowski

2. Prof. Dr. Vladimir Ilberg

3. Prof. Dr. Petra Mela

Die Dissertation wurde am 15.09.2021 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 01.06.2022 angenommen.

Acknowledgement

The present work was conducted at the Hochschule Weihenstephan-Triesdorf and in the course of a project funded by the Federal Ministry for Economic Affairs and Energy.

First of all, I would like to express special gratitude to my supervisor Prof. Dr. Horst-Christian Langowski for supervising the dissertation, reviewing my manuscripts, and giving useful and important input. A deep acknowledgment is owed to Prof. Dr.-Ing. Vladimir Ilberg for supervising the work, giving useful suggestions and making the dissertation possible. Particular thanks to Prof. Dr. Mela who has agreed to act as an additional examiner.

My special appreciation is offered to Dr. Uwe Blum, as a very competent contact person for my chemical questions, and for giving me support with chemical analyses.

My sincere thanks goes to my mentor Dr. Ute Weisz for supporting me in all of my questions around the dissertation and also for supporting me by networking with people who were helpful in getting useful information.

A big thank you to Josef Gleixner from Inotech for agreeing to the dissertation and for producing the containers.

I acknowledge my supervised students Teresa Schwind, Julia Neher, and Susannah Lindermeier for laboratory work and literature research.

I would also like to thank the staff from the Institute of Food Technology at the Hochschule-Weihenstephan and from Fraunhofer IVV for helping me with laboratory or technique questions.

Finally, my deepest gratitude goes to my family and friends for supporting me and making me laugh and helping me enjoy life.

Table of contents

Index of tables

Index of illustrations

Abbreviations

AAS	Atomic Absorption Spectrometry
Ag	Silver
Ag^+	Silver ion
$AgNO_3$	Silver nitrate
Ag_3PO_4	Silver phosphate
AgNPs	Silver Nanoparticles
CFU	Colony-Forming Units
EC	European Commission
EFSA	European Food Safety Authority
EVA	Ethylene Vinyl Acetate
EVOH	Ethylene Vinyl Alcohol Copolymer
FCM	Food Contact Material
FDA	Food and Drug Administration
GF-AAS	Graphite Furnace Atomic Absorption Spectrometry
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LAE	Lauramide Arginine Ethyl Ester
LOD	Limit Of Detection
MRSA	Methicillin-resistant *Staphylococcus aureus*
NOAEL	No Observed Adverse Effect Level

P105	95% TiO_2 + 5 % metal nanosilver with a particle diameter of about 10 nm
PE	Polyethylene
PE-HD	High-Density Polyethylene
PE-LD	Low-Density Polyethylene
PET	Polyethylene Terephthalate
PLA	Polylactic acid
PP	Polypropylene
SN-ICP-MS	Solution Nebulization Inductively Coupled Mass Spectrometry
TiN	Titanium nitride
WHO	World Health Organization

Summary

In the food industry, constantly increasing demands are being placed on food packaging and packed products. The protection of food against pathogenic or spoilage microorganisms is becoming increasingly important. Antimicrobial packaging, a form of active packaging, could be an option to fulfill this demand. Since silver is antimicrobially effective against a wide range of microorganisms and stable at the high temperatures required for processing polymers into packaging, it is increasingly being researched for incorporating into food contact polymers. Especially for beverages, antimicrobial packaging has not often been used and the question arises whether an antimicrobial effect in the product can be achieved with a silver concentration leading to a concentration in the food equal to or below the migration limit of 0.05 mg Ag/kg food currently discussed in the European Union.

In this dissertation, antimicrobial experiments were first performed in milk, iced tea, and distilled water with the targeted addition of silver ions but without using antimicrobial packaging. For milk, required concentrations were far above 0.05 mg Ag/kg food, in the range of 5 mg/L, due to the interactions of silver ions with milk constituents. Experiments in iced tea were performed using *Z. bailii* (<10 cfu/mL) and those in distilled water using *E. coli* (approx. 10^6 cfu/mL). Silver concentrations of 0.015 and 0.05 mg/L Ag were added. 0.05 mg/L Ag reduced *Z. bailii* in iced tea within 1 h; however, cells started to grow again within 14 d. *E. coli* in distilled water was reduced by a factor of 10^5 cfu/mL within 48 h using 0.05 mg/L Ag and by a factor of 10^3 cfu/mL using 0.015 mg/L Ag.

Subsequently, PET bottles were produced containing a bi-layer structure created by injection and stretch blow molding. The inner layer incorporated silver phosphate (Ag_3PO_4) glass with a mass fraction of 470 or 1600 mg Ag/kg PET or silver nanoparticles (AgNPs) with a mass fraction of 650 mg Ag/kg PET, which were added as a masterbatch (AgPURE®). The release of silver from these bottles into distilled water, and into 0.5 and 3% (w/w) acetic acid, was investigated and quantified by means of inductively coupled plasma mass spectrometry (ICP-MS). Silver amounts released from the bottles containing silver phosphate glass ranged from 2 to 72 µg/L after 10 d at either 21 or 43 °C. The bottles containing AgNPs showed no silver-release at all. Release was dependent on the type and percentage of antimicrobial additive in the polymer,

temperature, and acidic strength of the filled liquid. The difference in the amount of silver released into 0.5 and 3% acetic acid was not significant. The diffusion coefficients for the migration of Ag in PET ranged from $3.2x10^{-17}$ to $1.7x10^{-15}$ cm^2/s. The amount of silver released into 0.5 or 3% acetic acid within 10 d at a temperature of 43 °C exceeded 0.05 mg Ag/kg food for the bottles containing 1600 mg Ag/kg PET , but not for the bottles containing 470 mg Ag/kg PET.

The bottles merely filled with distilled water showed an antimicrobial effect, but not those filled with iced tea. The antimicrobial effect occurred time-delayed compared to direct addition of silver and at a higher silver concentration. This phenomenon is very possibly due to interactions of silver ions with residual monomers or other production aids released from PET, which can lead to complexation of the first released Ag ions. If such an application comes to the market, it would be best suited to preserve water. This could especially be useful for astronauts in space, where water should be sterile over a long time; for emergency supplies kept on ships at sea; or for soldiers during a war mission. Furthermore, points such as choosing the appropriate combination of initial silver concentration in the polymer, the required concentration for antimicrobial effectiveness in the intended use medium, and the intended storage temperature have to be kept in mind.

Zusammenfassung (Summary in German)

In der Lebensmittelindustrie werden stetig steigende Anforderungen an Lebensmittelverpackungen und verpackte Produkte gestellt. Insbesondere der Schutz von Lebensmitteln vor Pathogenen oder Mikroorganismen, die einen Verderb herbeiführen können, wird immer wichtiger. Antimikrobielle Verpackungen, eine Form von aktiven Verpackungen, könnten eine Möglichkeit sein, diese Forderung zu erfüllen. Da Silber ein antimikrobielles Mittel ist, das gegen eine Vielzahl von Mikroorganismen wirksam ist und bei hohen Temperaturen, die für die Verarbeitung von Polymeren zu Verpackungen erforderlich sind, stabil ist, wird zunehmend die Einarbeitung von Silber in Polymere mit Lebensmittelkontakt erforscht. Insbesondere für Getränke werden antimikrobielle Verpackungen bisher selten genutzt und es stellt sich die Frage, ob eine antimikrobielle Wirkung im Produkt mit einer Silberkonzentration erzielt werden kann, die zu einer Konzentration im Lebensmittel führt, die dem derzeit in der Europäischen Union diskutierten Migrationsgrenzwert von 0,05 mg Ag/kg Lebensmittel entspricht oder darunter liegt.

In dieser Arbeit wurden zuerst antimikrobielle Experimente in Milch, Eistee und destilliertem Wasser mit gezielter Zugabe von Silberionen ohne Verwendung einer antimikrobiellen Verpackung durchgeführt. Bei Milch lagen die geforderten Konzentrationen weit über 0,05 mg Ag/kg Lebensmittel, im Bereich von 5 mg/L, bedingt durch Wechselwirkungen der Silberionen mit Milchbestandteilen. Die Experimente in Eistee wurden mit *Z. bailii* (< 10 KbE/ml) und die in destilliertem Wasser mit *E. coli* (ca. 10^6 KbE/ml) durchgeführt. Es wurden Silberkonzentrationen von 0,015 und 0,05 mg/l Ag zugesetzt. 0,05 mg/l Ag reduzierten *Z. bailii* in Eistee innerhalb von 1 h, die Zellen begannen jedoch innerhalb von 14 d wieder zu wachsen. *E. coli* wurde in destilliertem Wasser mit 0,05 mg/l innerhalb von 48 h um einen Faktor von 10^5 KbE/ml und mit 0,015 mg/l Ag um einen Faktor von 10^3 KbE/ml reduziert.

Nachfolgend wurden PET-Flaschen mit einer Zweischichtstruktur durch Spritzgießen und Streckblasen hergestellt. Die Innenschicht enthielt Silberphosphat (Ag_3PO_4)-Glas mit einem Massenanteil von 470 oder 1600 mg Ag/kg PET bzw. Silber-Nanopartikel (AgNPs) mit einem Massenanteil von 650 mg Ag/kg PET, die als Masterbatch (AgPURE®) zugegeben wurden. Die Freisetzung von Silber aus diesen Flaschen in

destilliertes Wasser, sowie in 0,5 und 3 % (w/w) Essigsäure wurde untersucht und mittels induktiv gekoppelter Plasma-Massenspektrometrie (ICP-MS) quantifiziert. Die freigesetzte Silbermenge für die Flaschen, die Silberphosphatglas enthielten, reichte von 2 bis 72 µg/l nach 10 d bei 21 bzw. 43 °C. Die Flaschen mit AgNPs zeigten keinerlei Silberfreisetzung. Die Freisetzung war abhängig von der Art und dem Anteil des antimikrobiellen Additivs im Polymer, der Temperatur sowie der Säurestärke des abgefüllten Produktes. Der Unterschied der freigesetzten Silbermenge in 0,5 und 3 % Essigsäure war nicht signifikant. Die Diffusionskoeffizienten für die Migration von Ag in PET reichten von $3{,}2 \times 10^{-17}$ bis $1{,}7 \times 10^{-15}$ cm^2/s. Die freigesetzte Silbermenge der Flaschen mit 1600 mg Ag/kg PET in 0,5 bzw. 3 % (w/w) Essigsäure innerhalb von 10 d bei einer Temperatur von 43 °C überschritt 0,05 mg Ag/kg Lebensmittel, aber nicht die freigesetzte Silbermenge der Flaschen, die 470 mg Ag/kg PET enthielten.

Die Flaschen, die nur mit destilliertem Wasser gefüllt waren zeigten einen antimikrobiellen Effekt, aber nicht die Flaschen, die mit Eistee gefüllt waren. Der antimikrobielle Effekt trat zeitverzögert im Vergleich zur Direktzugabe von Silber ein und bei einer höheren Konzentration. Dieses Phänomen tritt mit hoher Wahrscheinlichkeit aufgrund von Wechselwirkungen der Silberionen mit freigesetzten Restmonomeren oder anderen Produktionshilfsmitteln aus PET auf, die zu einer Komplexierung der ersten freigesetzten Ag-Ionen führen können. Falls eine solche Anwendung auf den Markt kommen sollte, wäre sie am besten für die Konservierung von Wasser geeignet. Dies könnte vor allem für Astronauten im Weltraum, wo das Wasser über lange Zeit steril sein soll, für Notvorräte, die auf Schiffen auf See aufbewahrt werden oder für Soldaten während eines Kriegseinsatzes sinnvoll sein. Darüber hinaus müssen Punkte wie die Auswahl einer geeigneten Kombination aus anfänglicher Silberkonzentration im Polymer, die erforderliche Konzentration für die antimikrobielle Wirksamkeit im vorgesehenen Einsatzmedium sowie die vorgesehene Lagertemperatur beachtet werden.

1. Introduction

In the food industry, constantly increasing demands are being placed on food packaging and packed products. In addition to conventional demands for functional properties e.g. protection against mechanical influences during transportation, consumer demands for healthy, fresh, and high-quality products have been attracting more and more attention. Furthermore, safety demands, i.e., protecting the food against possible contamination, are gaining importance. Antimicrobial packaging could fulfill these demands by inhibiting the growth of microorganisms as well as minimizing chemical additives in food (Appendini & Hotchkiss, 2002; Balasubramanian et al., 2009; Muriel-Galet et al., 2012). Therefore, more and more studies focus on the development of antimicrobial food packaging. For polymer food packaging, particularly silver is often the object of research due to its good antimicrobial effectiveness and its stability at the higher temperatures (Llorens at al., 2012) required to produce the packaging. However, studies in this area of research are partly incomplete, so it is unknown whether it is possible to develop antimicrobial food packaging containing silver in compliance with the maximum proposed European migration limit of 0.05 mg Ag/kg food (EFSA, 2006 & EFSA, 2011). Furthermore, there are no studies regarding the possibility of incorporating silver into PET, although PET is the polymer of choice for bottles. Therefore, PET bottles containing silver in different forms (silver phosphate glass and silver nanoparticles) were developed and their antimicrobial effectiveness, as well as their rate of silver-release into food simulants, was investigated.

1.1 Active packaging

Active packaging is “designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food” (European Parliament & Council of the European Union, 2004; European Commission, 2009). The aims of active packaging are prolonging shelf life of the product, increasing its quality, and guaranteeing its health safety for the consumer. This is achieved by adding active substances or introducing them into the packaging (Appendini & Hotchkiss, 2002; Balasubramanian et al., 2009). The purpose of active

packaging is not to mask a possibly spoiled product, as this would be deceiving (European Commission, 2009). Specifically, the purpose of antimicrobial active packaging is to inhibit or slow down the growth of microorganisms or prevent the degradation of the product, thereby reducing the risk of foodborne illness through food consumption (Han et al., 2018).

Active packaging belongs to the class of non-thermal food technologies (Gavara et al., 2015). In general, active packaging can be divided into two systems. On the one hand are the so-called absorbent systems for oxygen, water vapor, CO_2, ethylene or volatile compounds. On the other hand are the so-called releasing systems containing CO_2, ethylene, ethanol, or antimicrobial substances (Pant, 2016).

1.1.1 Antimicrobial packaging

Antimicrobial packaging is a form of active packaging (Appendini & Hotchkiss, 2002) and could contribute especially to the improvement of food safety by inhibiting spoilage and the growth of pathogenic microorganisms (Sofi et al. 2018). Antimicrobial packaging can be advantageous over the direct addition of preservatives to the food (Balasubramanian et al., 2009). In most antimicrobial films, the antimicrobial substance is incorporated into a coating and migrates to its surface. Through direct contact with the product, an antimicrobial effect is achieved. This continuous and controlled effect of the antimicrobial substance can also be present in a polymer carrying the antimicrobial substance (Muriel-Galet et al., 2012). However, the concentration of the antimicrobial substance decreases over time, e.g. by interaction with food constituents, and becomes ineffective. As a result, microorganisms that have merely been inhibited slightly continue to grow and the possibility of resistant mutants arises.

This problem of possible resistance can be avoided if the release rate is high enough to allow for a sufficiently high concentration of the active substance (Balasubramanian et al., 2009). The slow release of the antimicrobial substance leads to a prolongation of the lag phase in the growth of the microorganisms and accordingly, the shelf life of the product can be extended. This is a big step forward in terms of safety (Muriel-Galet et al., 2012). However, the difficulty with antimicrobial packaging lies in

finding the appropriate materials and methods to ensure this continuous release of antimicrobial substances into the product over time (Balasubramanian et al., 2009).

Appendini & Hotchkiss (2002) presented five different possibilities for introducing antimicrobial substances into the packaging (Table 1).

Table 1: Overview of antimicrobial packaging types (according to Appendini & Hotchkiss, 2002)

Antimicrobial packaging types	Forms, examples or prerequisites	Applications
1. Addition of volatile antimicrobial substances contained in bags or "pads" into packaging (enclosed loose or attached)	Ethanol vapor generators → retard molds	Bakery, dried fish products
2. Incorporation of antimicrobial substances into the polymer matrix	Metals such as silver or copper, antimicrobial enzymes or peptides, natural phenols, fatty acid esters, antibiotics, antimicrobial vapors or gases	Biomedical devices, textiles, household goods, some food applications
3. Coating or adsorbing of the antimicrobial substances on the surface of the polymer	For temperature-sensitive antimicrobial substances	Fruits, vegetables, poultry
4. Immobilization of the antimicrobial substance by means of ionic or covalent bonds	Prerequisite: Functional groups on the antimicrobial substance and the polymer e.g. peptides, enzymes, polyamines, organic acids	Medical devices
5. Intrinsically antimicrobial polymers	e.g. Chitosan, poly-L-lysine	Fruits, vegetables

In addition to the types of antimicrobial packaging, a distinction is also made between the types of antimicrobial agents. According to Gavara et al. (2015), the antimicrobial agents used for antimicrobial packaging can be classified into eight categories: Enzymes, bacteriocins, surfactants, bacteriophages, plant extracts, polysaccharides, organic acids, and inorganic substances.

A more general categorization of antimicrobial agents is the distinction between organic and inorganic (Hoseinnejad et al., 2018). The great advantage of inorganic substances is that these are stable at high temperatures (Lee et al., 2010a) that occur, for

example, during injection molding, at which the antimicrobial substance can be integrated into the polymer. An inorganic antimicrobial substance often used in food packaging applications is silver, especially nanosilver.

1.2 Silver additives for plastics

Silver is well-known as an antimicrobial agent. Archaeological findings indicate that silver had already been used by Egyptians in 3000 B.C. or even earlier. They used silver vessels for keeping water safe (Varner et al., 2010) and even nowadays NASA uses the antimicrobial effect of silver ions to keep drinking water on spaceships clean (Birmele et al., 2011).

Before the discovery of antibiotics, the effect of silver was often used to disinfect wounds and purify drinking water, but the use of silver still continues today. A decisive advantage of silver ions over antibiotics is the long shelf life of silver and the possibility of simply integrating the metal into solids, such as wound dressings or packaging (Duncan, 2011). Furthermore, there is no scientific proof of bacteria becoming resistant to silver as they do to antibiotics, despite bacteria having been exposed to sub-inhibitory Ag^+ concentrations over billions of years (Percival et al., 2005).

The antimicrobial effect of silver is based on three mechanisms that can occur (Duncan, 2011; Figure 1 from Liao et al., 2019):

1. Silver ions reacting with functional groups of cell membrane proteins or enzymes, resulting in protein denaturation (Percival et al., 2005)
2. Oxygen radicals being formed with the help of silver particles (He et al., 2011), leading to oxidative stress (Duncan, 2011)
3. DNA replication being inhibited (Yakabe et al., 1980)

Silver ions act against many microorganisms such as gram-positive and gram-negative bacteria, viruses, fungi and even methicillin-resistant *Staphylococcus aureus* (MRSA). Gram-negative bacteria, such as *E. coli*, are more susceptible to cell destruction due to their thinner membrane than gram-positive bacteria with their thicker peptidoglycan-rich membrane (Duncan, 2011; Feng et al., 2000).

Recently, nanosilver is increasingly being used in food packaging research (Bumbudsanpharoke & Ko, 2015; Chaudhry et al., 2008; Chaudhry & Castle, 2011; Rai & Bai, 2018). "'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm" (European Commission, 2011a). Compared to larger silver particles (>100 nm), the small-size particles release more silver ions due to an increase of active surface area, thus resulting in better antimicrobial effectiveness by requiring less material. Silver ion release is dependent on the size and shape of the silver nanoparticles (AgNPs) (Dallas et al., 2011).

Due to the continuous formation of new silver ions from AgNPs, a depot of silver ions develops, which leads to the good antimicrobial effectiveness of nanosilver. The effect of nanosilver on human health and the environment is the object of current research. It is discussed whether nanonsilver can accumulate in the human body, and whether the higher surface-to-volume ratio might possibly result in additional mechanisms of action (Federal Institute for Risk Assessment, 2009). There are indications that nanosilver could potentially accumulate in the spleen, liver, and testes, lead to changes in the activity of the immune system, and/or lead to genotoxicity (European Commission & Directorate General for Health & Consumers, 2014). However, this is irrelevant for the types of active packaging in which nanoparticles are incorporated into polymers and only the ions migrate (Bott et al., 2012).

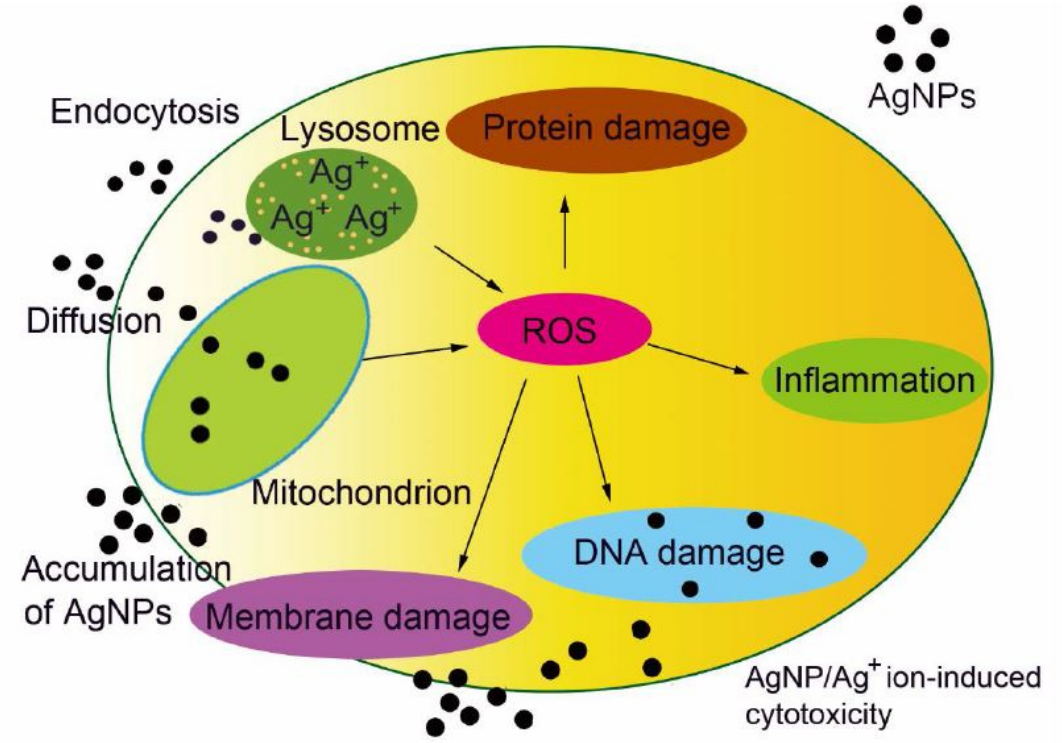

Figure 1: Mode of action of silver and nanosilver (from Liao et al., 2019)

1.3 Antimicrobial packaging for beverages

Table 2 shows an overview of current research for antimicrobial beverage packaging. Media investigated were milk and different kind of juices. Antimicrobial beverage packaging using PET is rare, although Jin et al. (2014) achieved good results by coating PET with potassium sorbate and sodium benzoate.

Antimicrobial packaging containing silver was separately regarded. Table 3 shows an overview of current research for antimicrobial beverage packaging containing silver. Media investigated were milk, different kinds of juices, and tea. Most polymers used were PE-LD and PE. No scientists used PET as packaging material and most of them used nanosilver. Until now not many commercial applications have been available (Gavara et al., 2015) and most of them exist in the Asian region (Lee & Han, 2010b).

Table 2: Overview of current research for antimicrobial beverage packaging

Beverage treatment and storage conditions	Packaging material	Antimicrobial substance	Production method	Microorganisms	Maximum achieved antimicrobial effect	Reference
Infant milk formula; 6 d at 4 °C	EVOH film	10% (w/w) flavonoid-rich cocoa	Solution casting	*Listeria monocytogenes*	i of growth: 1.5 log within 1 d and 0.52 log within 6 d	Calatayud et al., 2013
		20% (w/w) flavonoid-rich cocoa			i of growth: 1.5 log within 1 d and 0.76 log within 6 d	
Pasteurized apple juice; 200 h at 44 °C	Polyvinylalcohol solution casting	Lysozyme	Immobilization	*Alicyclobacillus acidoterrestris* spores	r: approx.1.5 log	Conte et al., 2006
Freshly squeezed orange juice; 56 d at 4 °C	PE-LD	0.25% (w/w) nano-ZnO	Melt-mixing	Aerobic bacteria	r: 0.63 log	Emamifar et al., 2010
				Yeasts and molds	r: 0.17 log	
Sterilized orange juice; 56 d at 4 °C	PE-LD	0.25% (w/w) nano-ZnO	Melt mixing	*Lactobacillus plantarum*	r: 0.26 log	Emamifar et al., 2011
		1% (w/w) nano-ZnO			r: 0.09 log	
Pasteurized milk, treatment: 95 °C for 10 min; However, no control sample for the heat treatment is evident; 12 d at 4 °C	Chitosan	Cinnamaldehyde	Imino-covalent bonding	*Listeria monocytogenes*	r: 4.15±0.02 log within 3 d;	Higueras et al., 2015

Pasteurized Skim milk; 21 d at 4 °C and 10 °C	Glass coated with PLA	250 or 500 mg nisin / g PLA	Coating	*Listeria monocytogenes*	Complete r after 3 d, control reached 8 log after 10 d at 10 °C and after 21 d at 4 °C	Jin, 2010
Pomegranate juice; 56 d at 4 °C	PET	(900 µg Potassiumsorbate + 1500 mg sodiumbenzoate) / g PLA	Coating	Total aerobic bacteria Yeasts and molds	Shelf life for untreated juice < 1 week and in antimicrobial bottles 56 d	Jin et al., 2014
Freshly squeezed orange juice and pasteurized milk; 16 d at 3 and 10 °C	Paperboard coated with EVA	3% (w/w) Nisin or 3% (w/w) Chitosan or 3% (w/w) (Nisin + Chitosan)	Coating	Yeasts Aerobic bacteria	i of microbial growth and lower max. growth level; at 3 °C extension of lag-phase compared to control; at 20°C only marginal antimicrobial effect	Lee et al., 2004
Milk (raw, pasteurized and UHT); 7 d at 4 °C	PE-LD	Nisin	Coating	*Micrococcus luteus*	i of microbial growth and lower max. growth levels; data non quantified	Mauriello et al., 2005
Infant formula milk; 6 d at 4 °C	EVOH-29 copolymer (29 % ethylene molar content)	10% (w/w) LAE	Solution casting	*Listeria monocytogenes*	r: 4 log	Muriel-Galet et al., 2012
				Salmonella enterica	r: 3.95 log	
	EVOH-44 copolymer (44 % ethylene molar content)			*Listeria monocytogenes*	r: 4 log (EVOH-44)	
				Salmonella enterica	r: 3.27 log (EVOH-44)	

Freshly squeezed lemon juice; 30 d at 4 °C	PP	5% (w/w) ZnO	Blown film method	Total aerobic bacteria	r: 0.21 log	Polat et al., 2018
				Yeasts and molds	r: 0.28 log	

Abbreviations and meanings: EVA: Ethylene-vinyl Acetate; EVOH: Ethylene Vinyl Alcohol Copolymer; i: Inhibition – means that microbial growth is reduced compared to a reference without antimicrobial substance; LAE: Lauramide Arginine Ethyl Ester; PLA: Polylactic Acid; r: Reduction – means that microbial population is reduced relative to its original population

Table 3: Overview of current research for antimicrobial beverage packaging containing silver

Beverage and storage conditions	Packaging material	Silver	Production method	Microorganisms	Maximum achieved antimicrobial effect	Reference
Pasteurized milk; 14 d at 4 °C	PE-LD modified by corona air plasma treatment	AgNPs*	Direct coating from solution	Total bacterial count	Microbial criteria for the shelf life of milk was defined as the time until Hungary's regulatory limit reaches 100°000 cfu/mL: This was reached after 5 d and for treated packages after about 14 d	Bayani Bandpey et al., 2017
Chinese Oolong Tea; 48 h at 25 °C	PE	1-3%* Ag zeolite	Presumably extrusion	Total count	r: 4.71 log	Brody et al., 2001
				Escherichia coli	r from $3.6x10^5$ to <10 i.e. r. by 4.56 log	
				Streptococcus pneumoniae	No effect: r by 3.41 log, (control 3.76 log)	
				Pseudomonas aeruginosa	No effect: r from $9.2x10^5$ to <10 (i.e. r. by 4.96 log, but also for control sample)	

Apple juice; 30 h at 44 °C	PE	7%* Ag inorganic coating	Plasma polymerization	*Alicyclobacillus acidoterrestris*	i: No growth with treated film within about 200 h, without film growth after 30 h	Cannarsi et al., 2003; Del Nobile et al., 2004
Orange Juice; approx. 60 h at 44 °C					No effect	
Cane Juice; up to 75 d at 5 °C	PE-HD / TiO_2	0.3%* Ag/ZnO	Extrusion	Aerobic mesophiles, Aerobic psychrotrophs, Molds and yeasts	No significant effect	Da Costa Ribeiro et al., 2019
Freshly squeezed orange juice; 56 d at 4 °C	PE-LD	5% (w/w) P105	Melt mixing	Total aerobic bacteria	r: 0.69 log	Emamifar et al., 2010
				Yeasts and molds	r: 0.45 log	
Sterilized orange juice; 56 d at 4 °C	PE-LD	1.5% (w/w) P105	Melt mixing	*Lactobacillus plantarum*	r: 0.34 log	Emamifar et al., 2011
		5% (w/w) P105			r: 0.59 log	
Freshly squeezed lemon juice; 30 d at 4 °C	PP	5% (w/w) P105	Blown film method	Total aerobic bacteria	r: 0.21 log	Polat et al., 2018
				Yeasts and molds	r: 0.36 log	

Abbreviations: i: Inhibition – means that microbial growth is reduced compared to a reference without antimicrobial substance; P105: 95% TiO_2 + 5 % metal nanosilver with a particle diameter of about 10 nm; r: Reduction – means that microbial population is reduced relative to its original population
*Concentration statements are incomplete, because these were not quantified in the corresponding publication

In most studies, the silver amounts released into the medium investigated remained unclear or were questionable. Bayani Bandpey et al. (2017) provided unclear data on the quantities deposited onto the PE-LD films used and the quantity of Ag^+ released into water. However, based on their data provided in the unit ppm/area, it can be deduced that up to 1.18 µg of silver per cm² of film area was applied, of which up to 60 ng (about 5% of the applied silver) of Ag^+ per cm² of film area was released in water. This apparently leads to concentrations of Ag^+ of just under 0.05 mg/kg water, i.e., just below the proposed European migration limit of 0.05 mg Ag/kg food (European Food Safety Authority (EFSA), 2006 & EFSA, 2011). The actual concentration of Ag^+ in the milk, however, cannot be deduced from their findings.

Emamifar et al. (2010) provided the amounts of Ag ions released from nanocomposite PE-LD films containing 5 % P105 into orange juice. These were 0.1 ± 0.003 µg/L after 28 d. Based on this dissertation's investigations, it is highly questionable that this low silver amount in the medium is able to cause an antimicrobial effect in beverages. The real quantity of silver in orange juice was presumably higher. Possibly, only the free Ag^+ in the medium was detected and not the total silver content, including Ag which reacted with the constituents of the orange juice.

The maximum silver amount released by Del Nobile et al. (2004) was 0.25 mg/kg at 44 °C after 5 d in apple juice, which is higher than the proposed European migration limit of 0.05 mg/L for Ag (EFSA, 2006 & EFSA, 2011). Furthermore, they used no specific packaging form. They only immersed the active film in the medium investigated. Polat et al. (2018) provided silver amounts of 20.46 ± 0.16 µg/kg food simulant released into 3% acetic acid after 10 d at 40 °C. They achieved an antimicrobial effect in lemon juice with bags made of polypropylene containing P105, but only a marginal one (max. 0.21 log for total aerobic bacteria and 0.36 log for yeasts and molds).

Brody et al. (2001) and Da Costa Ribeiro et al. (2019) provided no data at all regarding silver-release rates.

1.4 Production of PET containers

The basic material of PET bottles is polyethylene terephthalate (PET), which is thermoplastic. PET is the polymer of choice for bottles due to its low weight, transparency, recyclability, and due to its resistance to breakage (Thielen et al., 2020, pp. 150-151).

PET bottles are commonly produced in two process steps:

- Production of PET preforms by injection molding (scheme see Figure 2)
- Production of PET bottles from the preforms by stretch blow molding (scheme see Figure 3; image from Nayak, 2015; Thielen et al., 2020, pp. 140-150)

To produce lower quantities of hollow bodies, a one-step process can also be carried out directly (injection stretch blow molding) (Michaeli & Hopmann, 2017, p. 143). An example used in this dissertation is shown in Figure 4.

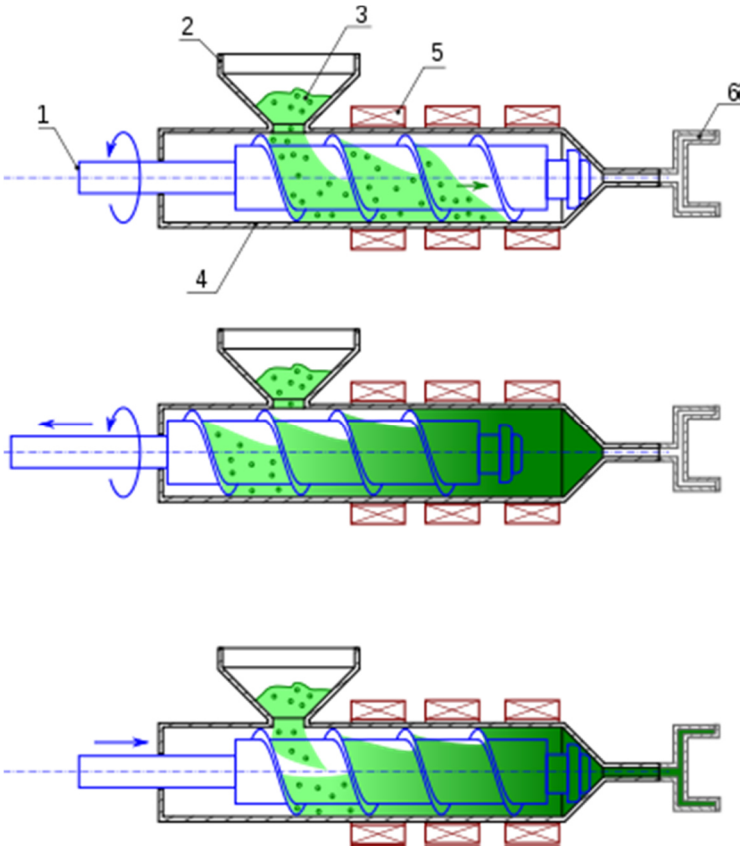

Figure 2: Principle of injection molding with 1: reciprocating screw; 2: hopper; 3: granules; 4: barrel; 5: heaters; 6: mold (from Cdang & Rockey, 2010)

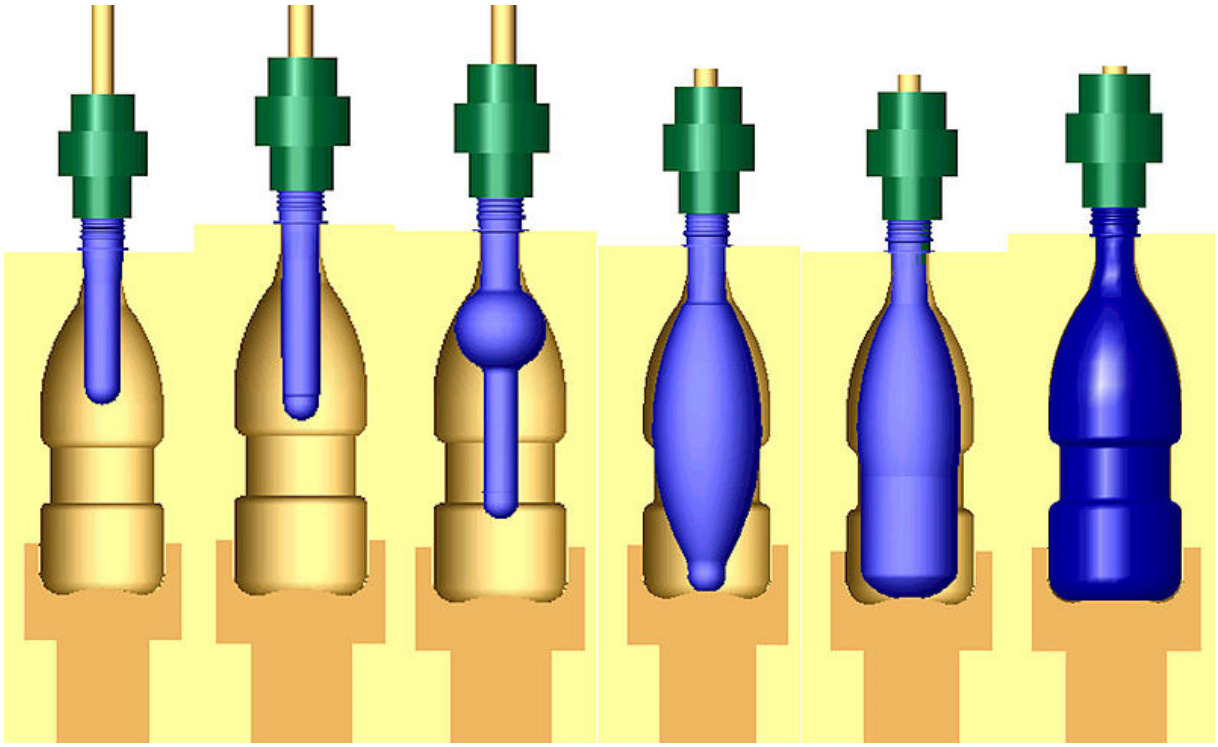

Figure 3: Stretch blow molding from preforms to bottles (from Nayak, 2015)

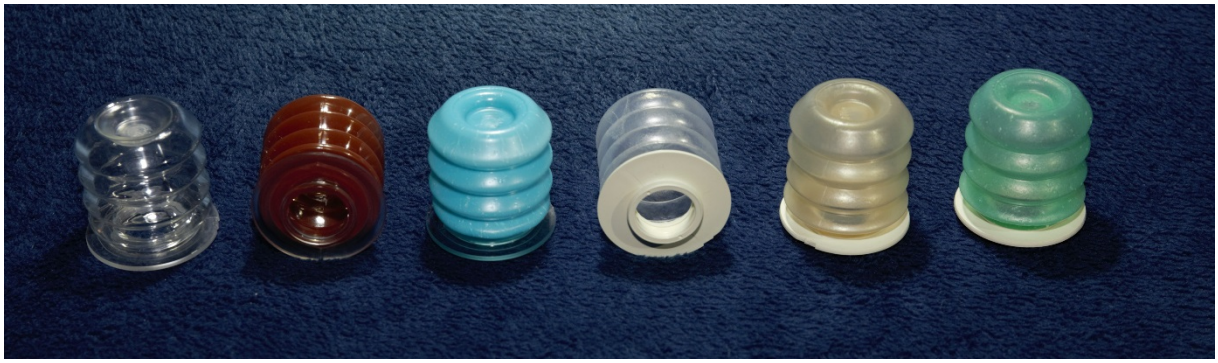

Figure 4: Developed bellows (from left): made of PET, made of PET and containing Ag-PURE, made of PET and containing silver phosphate glass, made of PP, made of PP and containing AgPURE, made of PP and containing silver phosphate glass

In order to achieve antimicrobial properties, bi-layer bottles could be produced, where antimicrobial substances or masterbatches could be integrated into the inner layer and additionally colour additives for better light protection or simply optical reasons could be integrated into the outer layer. The integration of the antimicrobial substance or masterbatch and the colour additives takes place in the PET granulate before the production of the preform. Manufactured bottles used in the experiments for this dissertation are shown in Figure 5. The manufacturing procedure can be found in publication 2 (under 3.2).

Figure 5: Developed silver doped preforms and bottles made of PET

1.5 Conformity of packaging with food contact regulations

1.5.1 Migration

Migration in the context of food means the transport of a substance from a package into the foodstuff. The active force of migration is diffusion during which molecular transport of particles occurs due to thermal energy. Particles or molecules may move in non-determinable directions due to a simultaneous movement of the diffusing substance and the neighboring polymer chains (Figure 6 from Brandt, 1959). This leads to collisions of the particles or molecules and accordingly, to a movement of the particles in another direction. As the change of direction is random, it is therefore called “random walk”. Despite the random movement of the single particles, overall particle transport occurs due to statistical reasons along a concentration gradient, from areas with high concentration to areas with low ones (Crank, 1975).

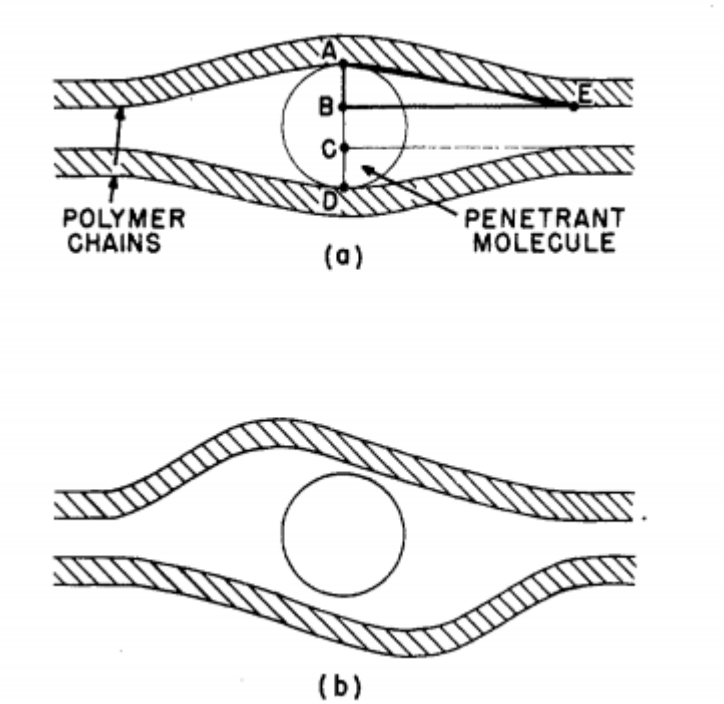

Figure 6: Diffusion of a molecule in non-determinable directions occurs due to thermal energy which is assumed to consist of (a) an intermolecular term due to the repulsion of the polymer chains from their neighbors and (b) an intramolecular term due to their resistance to bending (Brandt, 1959)

In food packaging which is in direct contact with food, there exists a concentration gradient between the additives used in the packaging (high concentration) and the food (low concentration). Diffusion along the concentration gradient occurs until concentration differences are balanced. When the number of particles migrating from the packaging material equals the number of particles reabsorbed by the packaging material, we speak of a state of equilibrium.

The net substance transfer per unit time and unit area, *J*, is vertical to the packaging surface in the direction of decreasing substance concentration *c*, named as x-direction, and corresponds to Fick´s first and second law of diffusion (Eq. 1a and 1b).

$J = -D\frac{\partial c}{\partial x}$ (1a)	$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$ (1b)

c: Concentration of the diffusing substance
t: Time
x: Space coordinate in vertical direction from the corresponding area along the gradient
D: Diffusion coefficient

For polymer applications, the diffusion coefficient can be determined by Eq. 2, the approximate solution of Eq. 1b, valid under the condition that the reservoir of the migrating substance does not entirely decrease within the measurement time (semi-infinite substrate) (Piringer, 2007). The diffusion coefficient is the time-determining constant for migration. The higher the diffusion coefficient, the faster the migration of a substance.

$$\frac{m}{A} = 2c_{p,0}\rho_P\sqrt{\frac{Dt}{\pi}} \quad (2)$$

m: Mass of the migrated substance into the simulant after time t
A: Surface area
$c_{p,o}$: Initial concentration in the packaging wall at $t = 0$
ρ_P: Density of the polymer
D: Diffusion coefficient
t: Time

The diffusion coefficient describes the mobility of a substance in a polymer matrix under the influence of different aspects. The mobility of a substance is dependent on the surrounding polymer matrix. For example, particles can move more easily through a polymer with many cavities between its polymer chains ("free-volume model") (Vrentas et al., 1985; Vrentas & Duda, 1976). Therefore, the structure of a polymer influences the diffusion of a migrating substance. More branches of the polymer chains means greater elasticity, since such a polymer is less compact than a linear one. This phenomenon can be described by the crystallinity of a polymer. Crystalline areas are formed by long molecular chains, which show no or only a few short and symmetrically linked side chains. Molecular chains can thereby accumulate in parallel and adopt a more compact, denser structure. In amorphous areas, however, a polymer branches into many side chains that interact with each other. Therefore, no ordered crystal structure can be built. In these areas, the polymer has a loose structure and a lower density.

The diffusion process is temperature dependent. Higher temperature leads, on the one hand, to a better mobility of the particles and, on the other hand, to a lower viscosity of the polymer, resulting in higher flexibility of the polymer chains. A critical parameter in this context is the glass transition temperature. By exceeding the glass-transition temperature T_g, which is specific for the polymer used, the polymer chains remain flexible and the whole structure acquires rubber-like properties. Below T_g on the other hand, polymers have brittle or less flexible properties, since the polymer chains are relatively rigid (Brydson, 1995).

The diffusion coefficients of larger molecules are lower than those of smaller ones or single ions. For example, silver nanoparticles have much lower diffusion coefficients than Ag^+ ions. For food packaging applications, this means that only Ag^+ ions may migrate into the food (Bott et al., 2012). Modeling results from Bott et al. (2014a) showed that particles up to merely about 3.5 nm in diameter can cause measurable migration (with detection limits of 0.09-0.11 µg/kg for 95% ethanol and iso-octane and 0.24 µg/kg for 3% acetic acid), if their concentration in the polymer is high. Larger particles have no potential to migrate, since their mobility in the polymer decreases exponentially with their size. Usually, nanoparticles in plastic nanocomposites for food contact materials (FCMs) have a diameter starting at 10-20 nm and form much larger aggregated structures and agglomerates, up to 1000 nm and more. Bott et al. (2014a) further used TiN nanoparticles, in concentrations up to 1000 mg/kg, which showed no migration at all into 95% ethanol or 3% acetic acid at 60 °C for 10 d. In a later study, Bott & Franz (2019) even showed that under thermal, chemical, and mechanical stress conditions, no release of nanoparticles occurs. For chemical substances in PET, size dependent mobility has been investigated by Welle (2013).

The distribution of the migrating substance between polymer and food in equilibrium, i.e. after infinite time, can be described with the partition coefficient $K_{P,F}$ (Eq. 3):

$$K_{P,F} = \frac{c_P \rho_P}{c_F \rho_F} \quad (3)$$

$K_{P,F}$: Partition coefficient of the substance between polymer and food (dimensionless)
c_P: Concentration of the substance in the polymer (e.g. mg/kg)
ρ_P: Density of the polymer (e.g. kg/m³)
c_F: Concentration of the substance in the food (e.g. mg/kg)
ρ_F: Density of the food (e.g. kg/m³)

$K_{P,F}$ is dependent on the physical-chemical properties of the polymer, the food and the migrating substance. Therefore, the migration of a non-polar substance from a non-polar polymer into a non-polar food results in a transfer of a larger amount of the migrating substance compared to the migration into a polar food. Hence, $K_{P,F}$ is ≤1. In contrast, $K_{P,F}$ is higher for the migration of non-polar substances from non-polar polymers into more polar foods. For example, pure water has a very low solubility for non-polar substances, leading to values of $K_{P,F}$ >>1000 for many polymers (Piringer, 2007). Conversely, a polar food should have a good solubility for aqueous silver ions migrating from PET. However, for migration of substances from PET, $K_{P,F}$ plays a minor role, because,

due to the generally low diffusion coefficient in PET, equilibrium is not reached during a typical shelf-life of beverages. Overall, aside from solubility in food, the migrated amount is dependent on temperature, time, initial concentration, and type of substance in the polymer (Welle & Franz 2011).

1.5.2 Regulation of silver for use in food contact materials made of plastics

In the European Union, the general use of materials and articles intended to come into contact with food is regulated by Regulation (EC) No 1935/2004 of the European Parliament and of the council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC (European Parliament & Council of the European Union, 2004). The use of active and intelligent materials and articles intended to come into contact with food is regulated by Commission Regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. The application of materials in food contact materials made of plastics is explicitly regulated by European Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food . Regarding nanomaterials, Article 9 of the latter regulation says that "Substances in nanoform shall only be used if explicitly authorized and mentioned in the specifications in Annex I". For example, the plastic additives titanium nitride (TiN), silicon dioxide (SiO_2) and zinc oxide (ZnO) are approved in nanoform and listed with some specifications and restrictions (European Commission, 2011b).

Before a specific substance is authorized to be used in food contact materials (FCMs) and is included in a positive list, the European Food Safety Authority (EFSA) evaluates its safety. For glass that contains silver (silver-magnesium-aluminium-phosphate-silicate) and for silver zeolite (silver doped zinc sodium ammonium aluminium silicate), the European Food Safety Authority (EFSA, 2006 & EFSA, 2011) proposed a specific migration limit of 0.05 mg Ag/kg food. This value corresponds to less than 13% of the No Observed Adverse Effect Level (NOAEL) for silver, which is about 10 g of oral silver intake over the entire lifetime of a person (World Health Organization (WHO), 2003). According to the WHO (2003), a concentration of 0.1 mg Ag per liter of drinking water over a period of 70 years is harmless to health. The best described adverse effect of silver consumption is

Argyria that causes a permanent bluish-grey discoloration of the skin or eyes, but is considered relatively harmless (European Commission & Directorate General for Health & Consumers, 2014).

Apart from FCMs, silver is authorized as food additive E174 for coatings of confectionery, decoration of pralines and coloring of liqueurs without maximum use levels in Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (European Parliament & Council of the European Union, 2008). The European Food Safety Authority (EFSA, 2016) re-evaluated the use of silver (E174) in its elemental form regarding human safety. However, data were not enough to assess the safety of silver as a food additive. When antimicrobial substances are released in the food by active packaging, these become part of the food and therefore, should be approved as food additives for their intended use (European Commission, 2009).

The Food and Drug Administration (FDA, 2009) modified the food additive regulations and permitted the direct addition of silver nitrate ($AgNO_3$) into commercially bottled water at concentrations not to exceed 17 µg/kg. For the use of nanomaterials in FCMs, the FDA has not established specific regulations (Störmer et al., 2017).

1.5.3 Studies on the release of silver from plastics

An overview of literature for migration studies from silver into food contact polymers is given in Table 4. Overall, PE, PE-LD and PP were used. Most authors used AgNPs and simulant media such as 3% acetic acid. As the shelf life of many products is longer than a reasonable time frame of migration tests, the EU Regulations give testing conditions that accelerate the migration processes. According to No 10/2011 EU, 3% acetic acid (w/v) is a typical simulant for food that has a pH below 4.5 (European Commission, 2011b). In some studies the proposed migration limit of 0.05 mg Ag/kg food is exceeded, but in most not and some authors used units, which were not comparable with this limit.

Table 4: Release of silver from silver doped polymers (from Braun et al., 2020b)

Type of silver	Polymer	Test material	Manufacturing method of the test material	Analyzing method	Migration simulant or test medium	Test conditions	Silver content incorporated in polymer	Silver content released at final time point*[1]	Reference
AgNPs	PE-LD	Film	Coating by immersion of corona modified films	AAS	Deionized water	14 d 4 °C	1.17 "ppm/cm^{2}"*	1.5 "ppm/m^{2}"*[2]	Bayani Bandpey et al., 2017
AgNPs	PE-LD	Film	Extrusion	ICP-MS	3% acetic acid	10 d 60 °C	250 mg/kg	10.1 ng/cm^{2}	Bott, 2017
AgNPs	PE	Film	Extrusion	ICP-MS	Chicken breast	3.1 d 21.8 °C	5000 mg/kg	0.039 mg/kg	Cushen et al., 2014a
AgIon	PE	Film	Extrusion	ICP-AES	Distilled water	10 d 40 °C	not specified	6.07 ± 1 µg/L	Cushen et al., 2014b
					3% acetic acid			15 ± 10 µg/L	
AgNPs					Distilled water		3.62*10^{-4} mg/L	160 µg/L	
					3% acetic acid			520 µg/L	
AgNPs	PP	Food container (Kinetic Go Green Nano Silver Basic)	Probably extrusion/injection molding	ICP-MS	3% acetic acid	10 d 40 °C	0.39 mg/cm^{2}	31.46 ng/cm^{2}	Echegoyen & Nerín, 2013
	PE-LD	Bag					0.02 mg/cm^{2}	3.76 ng/cm^{2}	

AgNPs P 105	PE-LD	Film	Extrusion	GF-AAS	Orange juice	28 d 4 °C	5000 mg/kg	0.1 ± 0.003 µg/L	Emamifar et al., 2010) and (Emamifar et al., 2012
silver zeolite	PLA	Film	Solution-casting/solvent evaporation	GF-AAS	Distilled water	24 h 20 °C	413.2 ng/cm^2	0.043 mg/kg	Fernández et al., 2010c
					3% acetic acid			0.71 mg/kg	
					Tryptone soy broth (TSB)			0.35 mg/kg	
			Melt-mixing/compression molding		3% acetic acid			approx. 0.04 mg/kg	
AgNPs	PP	Food container (Kinetic Go Green Nano Silver Basic)	Probably extrusion/injection molding	SN-ICP-MS	Distilled water	10 d 20 °C	11.9 ± 2.4 mg/kg	5.8 ng/cm^2	von Goetz et al., 2013
					3% acetic acid			9.5 ng/cm^2	
	PE	Bag (Fresher-Longer^TM, Sharper Image Corporation, USA)	Extrusion		Distilled water		37.1 ± 1.2 mg/kg	< 0.5 ng/cm^2	
					3% acetic acid			0.5 ng/cm^2	

AgNPs	PE	Bag/film	Extrusion	AAS	Ultrapure water	10 d 25 °C	100 mg/kg	7 ng/cm^2	Huang et al., 2011
					4% acetic acid			8 ng/cm^2	
					Ultrapure water	10 d 40 °C		25 ng/cm^2	
					4% acetic acid			28 ng/cm^2	
AgNPs	PE	Film	melt blending	AAS	Distilled water	30 d 40 °C	22.64 mg/kg	0.896 ± 0.8 mg/kg	Jokar & Rahman, 2014
					3% acetic acid			1.034 ± 0.04 mg/kg	
					Apple juice			0.926 ± 0.11 mg/kg	
			Layer-by-layer deposition		Distilled water		16.28 mg/kg	1.107 ± 0.02 mg/kg	
					3% acetic acid			1.434 ± 0.04 mg/kg	
					Apple juice			1.084 ± 0.02 mg/kg	
AgNPs	PE	Food container (The Original Always Fresh Container, Gourmet Trends, USA)	Probably extrusion/injection molding	ICP-MS	Milli-Q water	10 d 40 °C	11.9 ± 3.3 mg/kg	< 0.9 µg/L	Mackevica et al., 2016
					3% acetic acid			8.2 ± 0.5 µg/L	
AgNPs P 105	PP	Film	Blown film extrusion	AAS	3% acetic acid	10 d 40 °C	5 wt%	0.02046 ± 0.00016 mg/kg	Polat et al., 2018

Abbreviations: AAS: Atomic Absorption Spectrometry; AgIon: Commercial silver ion filler with a mean particle size of 3 µm and a maximum particle size of 15 µm; AgNPs: Silver nanoparticles; GF-AAS: Graphite Furnace Atomic Absorption Spectrometry; ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometry; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; P105: 95% TiO_2 + 5 % metal nanosilver with a particle diameter of about 10 nm; SN-ICP-MS: Solution Nebulization Inductively Coupled Mass Spectrometry

*[1]limit according to EU-directive 10/2011 0.05 mg Ag/kg food

*[2]Unfortunately, this unit is not comprehensible

2. Motivation and aims of the dissertation

In the area of antimicrobial food packaging, there is already a great deal of research, especially with silver incorporated into different polymers. Reasons for choosing silver are: its great antimicrobial potential in various applications even outside the food sector, its stability at high temperatures that is needed for the production of antimicrobial food packaging, for example by injection molding, and lastly that it is not harmful to health in certain concentrations. There is a quantity of studies investigating the release of silver ions from food contact polymers such as polyethylene, low density polyethylene, polypropylene, polyamide or polylactic acid, and most times nanosilver is used for incorporation. However, to the best of the author's knowledge, there are neither studies regarding the diffusion from silver out of PET nor studies about antimicrobial PET bottles containing silver, although PET being the polymer of choice for bottles. Within the European Union, the main reason that those applications are not on the market is the unfinished risk assessment for silver. In other countries, mainly in the Asian region, such applications already exist on the market (Lee & Han, 2010b). Here, nevertheless, the question arises whether these can be antimicrobially effective with a silver concentration of 0.1 mg/L, considered as harmless to health for drinking water by the WHO, and within the proposed European migration limit of 0.05 mg/L. Studies so far were contradictory or questionable, as reported above, in order to evaluate whether an antimicrobial effect can be achieved with those silver concentrations.

The main aim of this dissertation is therefore, to find out whether it is theoretically possible to develop an antimicrobially effective PET bottle containing silver quantities that do not pose a health risk. This would have great market potential, since there are many applications where beverages should be protected from spoilage germs or pathogenic microorganisms, and common methods for enhancing shelf life are often not sufficient. For example, a big market would be milk applications, especially in subtropical or developing countries, where milk spoils fast due to high temperatures, unhygienic production conditions, contamination of filling containers, and interrupted or even absent cooling chains. Another extreme application with a high initial cell count could be to use the bottle for filling up fresh raw milk at a farm. However, there are also other applications, where the initial cell count of microorganisms is much lower and where such a bottle would be beneficial. For example, a sterile bottle for the filling up process

or conditions where people are longer on the road. This can range from longer trips of individuals to soldiers during a war mission, emergency supply at sea or astronauts in space.

Important questions to be answered in this dissertation can be divided into scientific questions and questions relevant for applications in practice.

The scientific questions (no. 1 - 5) are listed as follows.

1. Can silver achieve an antimicrobial effect in milk?
   Few studies (Bayani Bandpey et al., 2017; Yildiz & Pala, 2012; Kalaiselvi et al., 2013) suggest that this is possible. However, these studies are incomplete or questionable regarding required silver concentrations. In this dissertation, it should be evaluated if silver can cause an antimicrobial effect in milk with concentrations that are below the proposed use levels. This can be done by performing antimicrobial experiments with silver in milk.
2. Do silver based additives incorporated into PET release silver ions in measurable quantities?
   There is a quantity of studies investigating the release of silver ions from food contact polymers such as polyethylene, low density polyethylene, polypropylene, polyamide or polylactic acid, and in most cases nanosilver is used for incorporation (see Table 4). For PET, on the other hand, data are lacking, although PET being the most polymer material used for beverage packaging. PET is known to be a less diffusive polymer, but does it nevertheless release silver ions in food packaging applications within a detection limit of 0.1 µg Ag/L of food? To answer this question, silver-release investigations should be performed with silver doped PET bottles produced by injection and stretch blow molding under the addition of silver phosphate (Ag_3PO_4) glass or silver nanoparticles, and the release thereof into food simulants should be evaluated by ICP-MS. Investigations from Bott et al. (2014a; 2014b & 2019) already showed that only silver ions and not silver nanoparticles migrate from FCMs. Therefore, ICP-MS, which does not differentiate between silver ions or silver nanoparticles, but measures the total silver content, here with a detection limit of 0.1 µg Ag/L of food, is adequate for the investigations.
3. What would be the active principle of an appropriate container and on what parameters does the release of silver ions depend?

It is expected that the main principle of action is based on a diffusion of the antimicrobial substance into the foodstuff. Parameters influencing this mass transfer are dependent on factors such as temperature, time, amount of substance in the polymer, type of substance and solubility in food (Welle & Franz, 2011). This should be tested and evaluated for the bottles by investigating the release of silver from the bottles into food simulants.

4. Does the quantity of silver released from PET have an antimicrobial effect in beverages?

   As shown in section 1.3, studies with other polymers have already shown antimicrobial effectiveness. Whether this is also possible with PET should be answered by combining the silver-release investigations with the antimicrobial experiments.

5. Can an antimicrobial effect in beverages be achieved within recommended use levels for silver in drinking water given by the WHO and within the proposed migration limit for food given by the EFSA?

   From Polat et al. (2018) it can be concluded that this could be possible. Using polypropylene bags containing P105 (i.e., 95% TiO_2 + 5 % metal nanosilver with a particle diameter of about 10 nm), they achieved antimicrobial activity in lemon juice, albeit marginally. Silver amounts released into 3% acetic acid after 10 d at 40 °C were 0.02046 ± 0.00016 mg/kg food and thereby below the proposed migration limit from the EFSA of 0.05 mg Ag/kg food and below the recommended use level of 0.1 mg/L given by the WHO. However, other studies on this topic were incomplete (Brody et al., 2001; Da Costa Ribeiro et al., 2019), questionable (Emamifar et al., 2010; Bayani Bandpey et al., 2017) or concentrations above this levels were needed (Del Nobile et al., 2004) as reported above. To answer this question, silver at a concentration of 0.05 mg/L and one below should be directly added into a beverage containing a target organism, and it should be evaluated if an antimicrobial effect is achievable.

The following questions (no. 6 – 8) are relevant for applications in practice:

6. What problems have to be regarded for practical applications?
   Antimicrobial experiments with the bottles containing silver should be performed and thereof possible limitations for practical applications should be evaluated.
7. Can the silver doped bottles be reused?
   This should be tested by comparing the silver amount released from the bottles at first usage with the silver amount released from those after second usage, using the same experimental conditions.
8. What is the prognosis for applicability?
   Finally, a prognosis for practical applicability should be drawn by combining the recognitions gained by the experiments.

3. Results - publications

3.1 Nanosilver in dairy applications – Antimicrobial effects on *Streptococcus thermophilus* and chemical interactions

Publication 1 describes the antimicrobial and chemical effects of silver in milk applications. Special focus was laid on milk spoilage in subtropical or developing countries, where milk spoils fast due to unhygienic production conditions, contamination of filling containers and interrupted or even missing cooling chains. Therefore, high temperatures (43, 33 and 23 °C) were chosen for the experiments. *Streptococcus thermophilus* was selected as representative of the spoilage flora under tropical conditions and as an example germ that can survive short-term heating.

Antimicrobial experiments were performed by establishing a correlation between acidification (pH decrease) of milk and cell count. Acidification, which is dependent on temperature and concentration, served as indicator for bacterial activity. Chemical interactions between silver ions and milk constituents were investigated by using potentiometric measurements. These revealed interactions of Ag^+ with milk constituents, reducing the antimicrobial activity of the silver ions. Therefore, high silver concentrations (100 mg/L for nanosilver and at least 5 mg/L when adding $AgNO_3$) were required for obtaining antimicrobial effectiveness.

Temperature contributed less to antimicrobial effectiveness than silver nanoparticle (AgNP) concentration. The minimum inhibiting silver concentration for all microorganisms will be at least 50 mg/L for AgNPs and 5 mg/L for Ag, since the experiments suggested that an antimicrobial effect in milk first occurs when milk proteins have become saturated with Ag^+. Below these concentrations, merely a small number of free Ag^+ will be available, while these are in equilibrium with proteins and relatively few could interact with bacterial proteins. The results showed that silver ions or nanosilver can prevent milk spoilage at all temperatures investigated.

However, the concentrations required for antimicrobial effectiveness are higher than the level recommended by the WHO for drinking water (0.1 mg/L Ag). Therefore, practical use of silver for dairy applications is not expected, neither by direct addition nor by incorporation of silver into polymer packaging.

CRediT authorship contribution statement:

Sabrina Braun: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization, Supervision, Project administration.

Vladimir Ilberg: Writing - review & editing, Supervision.

Uwe Blum: Methodology, Investigation.

Horst-Christian Langowski: Writing - review & editing, Supervision.

International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.12686

ORIGINAL RESEARCH

Nanosilver in dairy applications – Antimicrobial effects on *Streptococcus thermophilus* and chemical interactions

SABRINA BRAUN,[1,2]* VLADIMIR ILBERG,[2] UWE BLUM[3] and HORST-CHRISTIAN LANGOWSKI[1,4]

[1]*TUM School of Life Sciences Weihenstephan, Technical University of Munich, Alte Akademie 8, Freising 85354, Germany,* [2]*Institute of Food Technology, University of Applied Sciences Weihenstephan-Triesdorf, Am Staudengarten 11, Freising 85354, Germany,* [3]*Bavarian State Institute of Forestry, Hans-Carl-von-Carlowitz-Platz 1, Freising 85354, Germany, and* [4]*Fraunhofer Institute for Process Engineering and Packaging (IVV), Giggenhauser Strasse 35, Freising 85354, Germany*

Milk spoilage continues to be a major problem. Incorporation of silver into milk packaging might solve this problem. We evaluated the antimicrobial and chemical effects of silver in milk. Antimicrobial experiments were performed by measuring milk acidification by Streptococcus thermophilus *at temperatures of 43, 33 and 23 °C and concentrations of silver nanoparticles at 10, 50, 100 and 200 mg/L. Chemical interactions were investigated using potentiometric measurements.* Ag^+ *interacted with milk constituents. Nanosilver was antimicrobially effective at all temperatures and at 100 mg/L. At least 5 mg/L are required by using* $AgNO_3$*. Practical use of silver for dairy applications is not expected, due to the required high silver concentrations.*

Keywords Acidification, Active packaging, Antibacterial activity, Antimicrobial activity, Shelf life, *Streptococcus thermophilus*.

INTRODUCTION

Ineffective control of microorganisms in milk continues to be a major problem, mainly in subtropical and developing countries. Typical problems include unhygienic production conditions, contamination of filling containers and interrupted or even absent cooling chains, which can all lead to faster milk spoilage. These issues result in economic losses to dairy farmers (Lingathurai and Vellathurai 2013). In countries like Uganda, smallholder farmers usually have no possibility to cool their milk during storage after milking, or during transportation on foot, bicycle or public transport, due to the lack of grid electricity (Kisaalita 2010). Therefore, bacteria are afforded the opportunity to grow, and the increase in bacterial counts leads to a decrease in milk quality. Similar situations exist in India, where milk is often stored in huge vessels after milking without being processed until it is delivered to local dairies or to the final consumer; this often leads to quality problems, which have been discussed in several reports (Kumar and Prasad 2010; Lingathurai and Vellathurai 2013). Furthermore, the demand for dairy products in India is increasing due to rising incomes and the growth of the middle and upper classes (Bundesministerium für Ernährung und Landwirtschaft 2016). To accommodate this growing demand, it would be advantageous to minimise waste of this valuable resource.

Raw milk is generally pasteurised before consumption to kill pathogenic bacteria and to reduce spoilage bacteria. However, even when it is pasteurised, the problems of surviving spoilage microorganisms or recontamination (i.e. contamination after pasteurisation) remain. For instance, thermoduric bacteria such as *Streptococcus thermophilus* are capable of surviving the short-term heating of milk (Riemelt *et al.* 2003). *Streptococcus thermophilus*, as well as other lactic acid bacteria, can indeed inhibit the growth of many pathogenic bacteria and also act as starter organisms to ferment milk for the production of dairy products such as yoghurt or

*Author for correspondence. E-mail: Sabrina.Braun@tum.de

cheese (Lu *et al*. 2013). However, they can also spoil milk by producing lactic acid that precipitates milk proteins and results in milk thickening (Niamsiri and Batt 2009). Furthermore, *S. thermophilus* can be regarded as a surrogate for pathogenic *Streptococcus* species, such as *Streptococcus agalactiae*, that can be found in raw milk; this is one of the most common mastitis-causing pathogens of cattle and is able to trigger various infectious diseases (Zangerl 2006).

The application of silver by, for example its incorporation into milk cans or antimicrobial milk packaging for storage or transportation, could be a solution to the problem of the spoilage of pasteurised or even raw milk. Silver is well known as an antimicrobial agent, and the use of inorganic antibacterial agents has demonstrated its worth due to their stability at high temperatures (Lee *et al*. 2010). High temperatures occur, for example, during heating of the milk or during the injection moulding process in which the antimicrobial substance can be added to the polymer from which plastic milk packaging is fabricated. Recently, nanosilver is increasingly being used in food packaging applications (Chaudhry *et al*. 2008; Chaudhry and Castle 2011; Bumbudsanpharoke and Ko 2015; Rai and Bai 2018). In general, nanomaterials are defined as natural or manufactured materials that contain aggregates, agglomerates or unbound particles, of which least 50% have at least one external dimension between 1 and 100 nm (European Commission 2011). The use of silver nanoparticles (AgNPs) is advantageous because of the relatively large surface area of the small-sized particles, which facilitates the release of more silver ions compared to larger silver particles (>100 nm) and thus increases antimicrobial efficacy while requiring less material. The release of the silver ions also depends on the size and shape of the AgNPs (Dallas *et al*. 2011).

Studies have shown that silver is antimicrobially active in its ionised form (Ag^+), which interacts with bases of deoxyribonucleic acid (DNA) and accordingly inhibits replication (Yakabe *et al*. 1980; Feng *et al*. 2000). Furthermore, Ag^+ can react with nucleophilic amino acid residues in proteins and attach to sulphydryl, amino, imidazole, phosphate and carboxyl groups, resulting in protein denaturation and eventually loss of functionality and bacteria damage (Percival *et al*. 2005). Moreover, bacteria are killed by silver particles because these particles help produce reactive oxygen species from air or water, which are toxic to the microbes (He *et al*. 2011). While the toxicity mechanisms of Ag^+ are well understood, there is a lack of knowledge regarding the molecular mechanisms of AgNP toxicity in bacteria. Durán *et al*. (2016) suggested that the same mechanisms responsible for the toxicity of Ag^+ are also the main root causes of antimicrobial action of AgNPs due to their release of silver ions. However, for nanoparticles below 10 nm the activity of the particles themselves is predominant.

The no-observed-adverse-effect level (NOAEL) for silver is about 10 g over an entire lifetime oral intake for humans. On this basis, the World Health Organization (2003) considers 0.1 mg/L silver in drinking water as not being a risk to human health. This means that drinking 2 L of water per day over 70 years results in a lifetime silver intake of approximately 5 g, corresponding to half of the NOAEL. There is also increasing concern that bacterial silver resistance may emerge from the use of silver for consumer products. However, in contrast to antibiotics, no widespread resistance to Ag^+ has yet been observed, despite bacteria having been exposed to sub-inhibitory Ag^+ concentrations for over four billion years (Percival *et al*. 2005).

A few studies have considered the antimicrobial effects of silver in milk applications. Kalaiselvi *et al*. (2013) investigated the antimicrobial effect of free AgNPs and of AgNPs applied to aluminium foil on *Trichosporan asahii* and *Lactobacillus casei* obtained from raw milk samples. Their results indicated that bacteria were partially inhibited in culture medium supplemented with AgNPs compared to growth without AgNPs; however, AgNPs applied to aluminium foil exhibited no significantly enhanced antimicrobial effect than aluminium foil itself. Yildiz and Pala (2012) tested the antimicrobial effects of AgNPs with a diameter of 6–8 nm on the total aerobic count of bovine milk. The aim of their investigation was to keep bacterial growth in milk low until thermal processing, for example by coating automated milking systems or items such as milk cans, bulk tanks and milk pipes with AgNPs. They used AgNPs produced by terminated gas condensation and varying voltages (0, 100 or 200 V) for coating metal braids, which produced different coatings with respect to the amount of AgNPs stuck to the braids. Milk was treated with AgNP-coated braids for different times (1 s, 30 s, 10 min, 1 h and 10 h) and temperatures (10, 18 and 22 °C). Their findings indicated that AgNPs were antimicrobially effective at all treatment times, but started losing effectiveness after 10 h. Treatment was most effective at the highest temperature where cell count was 6.86 ± 0.01 log cfu/mL compared to the control without treatment where it was 7.14 ± 0.01 log cfu/mL. Mikiciuk *et al*. (2016) determined the MIC_{90} of different AgNPs (34, 100 and 150 nm) against *S. thermophilus*, which was isolated from fermented milk products. This was 0.05 µg/mL.

The objective of this study was to investigate the antimicrobial and chemical effects of silver in milk. *Streptococcus thermophilus* was selected as a representative of the spoilage flora under tropical conditions and further as a representative that can survive short-term heating. The study was designed to evaluate the antimicrobial effects of silver on *S. thermophilus* with respect to the different temperature conditions that could occur because of interrupted or absent cooling chains. In this study, a correlation between acidification of milk (pH decrease) and cell count was established and acidification was regarded as a measure for bacterial activity. Silver concentrations were higher than the recommended use levels, and these were then gradually reduced to determine the minimum amount of silver needed for

antimicrobial effectiveness in milk. The chemical mode of action of Ag^+ or charged nanoparticles in milk was investigated using potentiometric measurements. By combining the antimicrobial and chemical results, the present investigation allows us to draw conclusions about the silver content in milk required to produce significant antimicrobial effectiveness.

MATERIALS AND METHODS

Medium, bacterial strain and materials

Milk was prepared from spray whole milk powder obtained from Lactoland (Dülmen, Germany). Milk powder was used since it is stable in terms of microbiology, and thereof, standardised milk can be produced for all the experiments. Milk was produced in a 1:9 ratio with demineralised water by stirring at 90 °C and 750 rpm for 1 h until the milk powder was dissolved completely. This was controlled by visual inspection for any undissolved residues at the bottom of the bottle. In preliminary experiments, the homogeneity of the milk samples with respect to fat, lactose and protein content was tested by Lactoflash (Funke-Gerber, Berlin, Germany) using an integrated method for whole milk analysis in order to ensure constant starting conditions for the experiments. The fat content was 2.7 ± 0.2, the lactose content was 3.8 ± 0.2, and the protein content was 2.5 ± 0.1% determined out of three milk productions in fivefold measurement, respectively. These correspond to typical constituents of milk powder which are 26% fat, 38% lactose and 25% protein (Töpel 2016). Milk was autoclaved at 121 °C for 15 min to ensure sterile initial conditions for the experiments and then cooled until use.

S. thermophilus Lyofast ST 055 (UC 5; Unita Cento) was obtained from Sacco (Cadorago, Italy). The stock culture was prepared by transferring Lyofast ST 055 at a dose of 5 Unita Cento (UC) to 500 mL of milk using a funnel followed by stirring at 1400 rpm for 40 min until the Lyofast powder had completely dissolved. This was also controlled by visual inspection for any undissolved residues at the bottom of the bottle. Aliquots of the inoculated milk were frozen and stored at −18 °C until use. Cell number of the thawed stock culture was determined by the spread plate technique on M 17 agar as proposed by Terzaghi and Sandine (1975). The plates were anaerobically incubated for 24 h at 42 °C. M 17 agar was obtained from VWR, and Anaerocult A was obtained from Merck (Darmstadt, Germany). The mean initial cell count in the prepared whole milk was $9.2 \times 10^5 \pm 1.1 \times 10^5$ cfu/mL for 27 samples.

Nanosilver AgPURE® W10 from RAS AG (Regensburg, Germany) was used since this has been selected as the world standard reference material for industrially available nanosilver by the Organisation for Economic Co-operation and Development (OECD) and corresponds to the OECD reference material NM-300. It is a colloidal dispersion with 10 % w/w silver. Silver particles in AgPURE have an average diameter of about 15 nm, and 99% of the particles are smaller than 20 nm (Klein *et al.* 2011; RAS AG 2017). Silver phosphate glass ION PURE WPA from ISHIZUKA Glass (Iwakura City, Aichi, Japan) was chosen because it releases Ag^+ in the presence of liquid medium and, like AgNPs, it could be used for integration into polymers. For experiments on chemical interactions, 0.01 and 0.4 M $AgNO_3$ dissolved in water from Applicam and NaCl from Merck Millipore were used.

Determination of the antimicrobial effectiveness of AgNPs in milk

The spread plate technique was used to determine cell numbers using M 17 agar plates anaerobically incubated for 24 h at 42 °C according to the recommendation in the technical data sheet from VWR. Milk acidification through bacterial growth of *S. thermophilus* was investigated by pH measurements using a portable meter MultiLine® Multi 3630 IDS (WTW, Weilheim, Germany) and related electrodes SenTix® 980 (WTW). A correlation between pH and the cfus was created using TableCurve 2D software (Systat Software Inc., Richmond, VA, USA). Tracking of the pH proved the better and more accurate method, since an easily accessible value measurable with high accuracy was available for every time point, and therefore, acidification of milk and indirectly bacterial growth could be tracked in real time. For the purpose of this study, acidification was regarded as a measure of bacterial activity and the beginning of milk acidification corresponds to the point at which the pH drops by 0.1 unit relative to the initial pH.

Bacterial growth in milk was investigated at 43 ± 1, 33 ± 1 and 23 ± 2 °C to study the effectiveness of the nanoparticles with respect to temperature. Furthermore, these temperatures cover different climate zones. The milk was inoculated with *S. thermophilus* and stirred at 750 rpm for 2 min. Subsequently, the desired concentration of AgNPs (10, 50, 100 or 200 mg/L) or silver phosphate glass (8 or 32 mg/L) was added to the milk, which was again stirred at the same conditions. The silver content in the silver phosphate glass powder was 1.6%, according to the manufacturer. To obtain 8 mg/L of total Ag in a suspension consisting of glass matrix and milk, 250 mg silver phosphate glass powder was added into 500 mL milk. Finally, the pH of the inoculated milk was monitored over time at the defined temperature and the experiments were carried out under aseptic conditions to prevent any contamination. Non-inoculated milk served as negative control, non-inoculated milk with AgNPs as blank and inoculated milk lacking AgNPs or silver phosphate glass as positive control. The negative control ensured that pH decrease was not a result of contamination during the experimental procedure nor a consequence of the different temperature conditions and the blank ensured that no pH decrease occurred due to the

presence of AgNPs or silver phosphate glass. Blank, positive control, negative control and treatment sample were incubated and kept under the same conditions.

Potentiometric measurements

Potentiometric measurements were conducted to investigate the chemical interactions of Ag^+ or charged AgNPs with milk constituents. Free silver ions (Ag^+) are known to be antimicrobially effective. These were detected using potentiometric measurements, since the potential is proportional to the free Ag^+ concentration in the medium. The experiments were carried out using a Metrohm 605 pH metre. The potential was measured with an Ag electrode against an Ag/AgCl standard reference electrode from Metrohm (Herisau, Switzerland). Stock solutions of different Ag content were prepared from 0.01 and 0.4 M $AgNO_3$ and stepwise pipetted into the milk in order to obtain Ag concentrations between 0.1 and 10 000 mg/L in milk. Samples were stirred continuously during the addition of Ag. For comparison, experiments were also conducted in distilled water containing Ag concentrations between 0.1 and 10 000 µg/L. For determining whether Ag^+ reacts with Cl^- in milk, the milk was supplemented with a final concentration of 5 g/L chloride, which was added in the form of sodium chloride.

Experiments with AgPURE® W 10 nanosilver were conducted in the same way but using AgNP concentrations between 0.1 and 1000 mg/L in milk or water.

Statistical analysis

Antimicrobial experiments were performed three times for the different concentrations of AgNPs as well as for silver phosphate glass, and five times for the positive controls and the different temperatures. All experiments on chemical interactions were conducted in triplicate. Data points are illustrated as confidence intervals for the means calculated using a *t*-distribution ($\bar{x} \pm \frac{t*SD}{\sqrt{N}}$). Significance was expressed at the 5% level.

RESULTS

Correlation between acidification of milk (pH) and colony-forming units (cfu)

The bacterial growth curve of *S. thermophilus* in milk obtained by spread plate technique exhibited the typical phases of bacterial growth. This corresponded with the pH curve obtained from pH measurements over time, indicating that pH serves as a surrogate marker for bacterial growth (Figure 1). A correlation between pH and the cfus was created. The selected function fitted the data with an $R^2 > 99.9\%$, and its course was similar to a typical bacterial growth curve. Initially, the bacteria were in lag phase where the number of cells was low and the pH did not change. Following the lag phase, the exponential phase began, where the pH began to decrease, which was accompanied by an increase in the number of cells as they metabolised lactose to lactate. The maximum number of cells was not yet reached until the investigated pH of 4.9. However, this was not decisive, since merely the beginning of acidification was evaluated. Furthermore, the death phase plays no role in this application and was not considered. For the purpose of this study, acidification was regarded as a measure of bacterial activity and the beginning of milk acidification corresponds to the point at which the pH drops by 0.1 unit relative to the initial pH.

Antimicrobial effectiveness of AgNPs in milk

As to be seen in Figure 2, the acidification of milk by *S. thermophilus* could be slowed by the addition of AgNPs at a concentration of 100 mg/L. Increasing the AgNP concentration slowed milk acidification further. At 50 mg/L

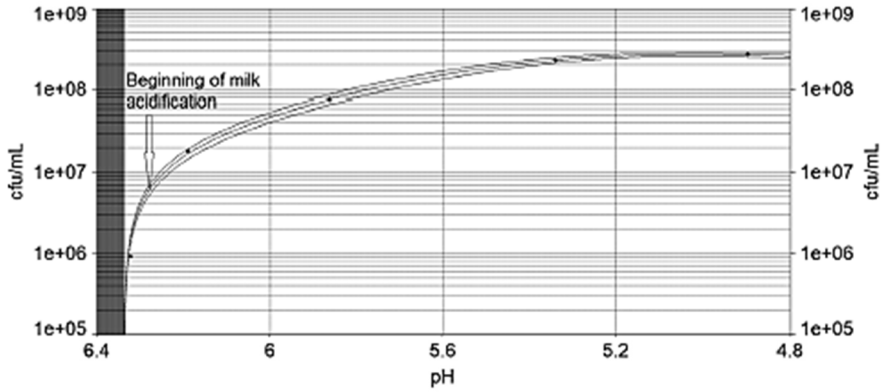

Figure 1 Correlation between acidification of milk (pH) and colony-forming units (cfu) of *Streptococcus thermophilus* in whole milk at 43 ± 1 °C. Beginning of milk acidification was defined as the point at which the pH had decreased by 0.1 units from the starting value.

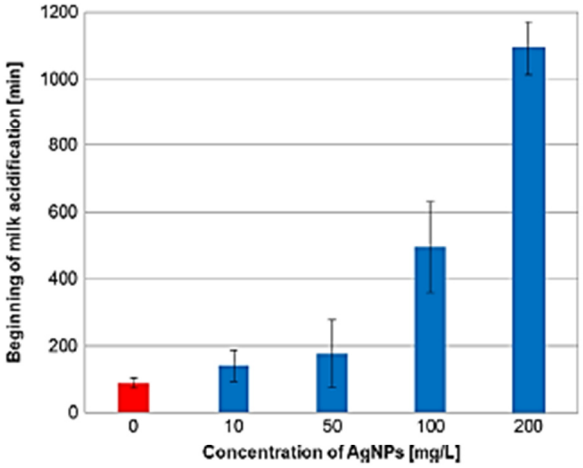

Figure 2 Acidification of whole milk by *Streptococcus thermophilus* at 43 ± 1 °C in the presence of 10, 50, 100 or 200 mg/L AgNPs compared to a control lacking AgNPs. Beginning of milk acidification corresponds to the time at which the pH dropped by 0.1 unit relative to the initial pH. [Colour figure can be viewed at wileyonlinelibrary.com]

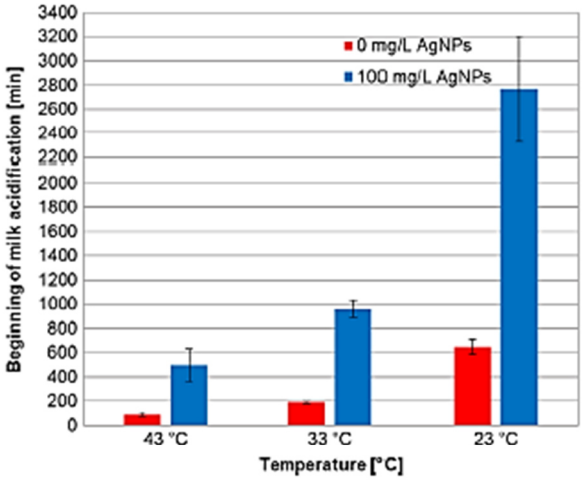

Figure 3 Acidification of whole milk by *Streptococcus thermophilus* at 43 ± 1, 33 ± 1 or 23 ± 2 °C in the presence of 100 mg/L AgNPs compared to controls lacking AgNPs. Beginning of milk acidification corresponds to the time at which the pH dropped by 0.1 unit relative to the initial pH. [Colour figure can be viewed at wileyonlinelibrary.com]

AgNPs, milk acidification began at 176 ± 100 min; however, this difference was not significant at $P < 0.05$ compared to the positive control lacking AgNPs (89 ± 15 min). Lower concentrations also exhibited no significant effect, while uninoculated blank and negative control exhibited no pH decrease at all.

The influence of temperature on the effectiveness of the AgNPs was investigated at a concentration of 100 mg/L and showed a significant difference ($P < 0.05$) at all temperatures (43, 33 and 23 °C) with respect to antimicrobial activity compared with the positive control without AgNPs (Figure 3). Blank and negative control samples exhibited no pH decrease at all. As expected, lower temperatures slowed down milk acidification in the absence of AgNPs as well as in their presence. The start of acidification after adding 100 mg/L AgNPs was delayed by a factor of four to five in relation to the positive control lacking AgNPs at all temperatures. Therefore, temperature was less effective in preventing growth of *S. thermophilus* than AgNPs themselves. However, the combination of low temperature and the use of silver contribute to even longer shelf life of the milk.

Antimicrobial effectiveness of silver phosphate glass in milk

Differences regarding antimicrobial activity of *S. thermophilus* between AgNPs and silver phosphate glass were also investigated. Silver phosphate glass was added to the milk which was then investigated at 43 °C. At 8 mg/L of total Ag in the suspension consisting of glass matrix and milk, milk acidification was slowed by a factor of 1.9 compared to the positive control without Ag (Table 1). Preliminary results showed that the release of the silver ions from the glass matrix occurred gradually and that the total 8 mg/L of Ag was not released within the duration of the antimicrobial experiments. Therefore, even Ag concentrations below 8 mg/L are antimicrobially effective. No acidification at all was observed at 32 mg/L and higher concentrations of total Ag within a 7-day time course of the experiments.

Chemical interactions of silver with milk

Figure 4 shows the electrochemical potential increase as $AgNO_3$ is added to water, milk and milk supplemented with 5 g/L chloride in the form of sodium chloride; the increase of $AgNO_3$ led to an increase in potential; that is as more

Table 1 Beginning acidification time points in whole milk containing *Streptococcus thermophilus* at 43 ± 1 °C in the absence or presence of 8 or 32 mg/L of total Ag in a suspension in the form of silver phosphate glass. Beginning of milk acidification corresponds to the time at which the pH has dropped by 0.1 unit relative to the initial pH

Total Ag concentration [mg/L]	*Mean beginning of milk acidification ± t × SD/√N [min]*
0	98 ± 13
8	187 ± 27
32	No acidification

free silver ions became available. In water, the potential began to rise at a concentration of 0.5 mg/L, whereas in milk, this occurred between 5 and 15 mg/L. Thus, free Ag^+ in milk became available at higher added concentrations than in water, obviously because of the reaction of Ag^+ with milk constituents. Visible precipitation of milk was observed at around 1000 mg/L of total added Ag^+, which is an indication of AgCl precipitation. We postulated that Ag^+ reacts with Cl^- in milk, and this was confirmed when the milk was supplemented with 5 g/L chloride and the potential curve shifted to the right. This occurred because more Cl^- was available to react with Ag^+ to form insoluble AgCl, thereby decreasing the amount of free Ag^+ in solution. Moreover, the potential curve in milk was not as steep as in water, which will be discussed later. These results are consistent with those on the antimicrobial effectiveness of silver phosphate glass in milk and further suggest that 5 mg/L of total Ag is least required for antimicrobial effectiveness in milk.

Figure 5 shows the potential increase upon the addition of AgNPs to water and milk. Increasing AgNP concentration led to an increase in potential, that is as free silver ions or charged AgNPs became available. In water, the potential began to rise at 5 mg/L of added AgNPs, whereas in milk, the rise began between 50 and 100 mg/L. The results for AgNPs in water were not as easily reproducible as the other results. The linear range for the increase in silver ions or charged nanoparticles in water was between 50 and 450 mg/L, above the potential plateaued, that is the addition of more nanoparticles did not produce more free silver ions or charged nanoparticles. In comparison with the results with $AgNO_3$ in water, free silver ions were first available at the 10-fold concentration, which indicates that only about one in ten silver atoms is liberated from nanoparticles and becomes free silver ions. Furthermore, the results for the chemical interactions are consistent with those for the antimicrobial effectiveness of AgNPs in milk, showing that concentrations between 50 and 100 mg/L AgNPs are required to produce free Ag^+ or charged AgNPs and so confer antimicrobial properties to milk.

DISCUSSION

The present study investigated the antimicrobial and chemical effects of silver in milk. Milk acidification by *S. thermophilus* could be slowed down by the application of AgNPs at a concentration of 100 mg/L, and by the addition of $AgNO_3$, at least 5 mg/L are required for antimicrobial effectiveness. AgNPs were active at all temperatures (43, 33 and 23 °C), and temperature contributed less to antimicrobial effectiveness than the AgNP concentration used, with higher concentrations leading to greater effectiveness. Silver phosphate glass exhibited antimicrobial effectiveness in milk at 8 mg/L of total Ag in a suspension consisting of glass matrix and milk.

Our measurements revealed that considerably more $AgNO_3$ has to be added to milk for free Ag^+ to become available than in water, since Ag^+ reacts with milk

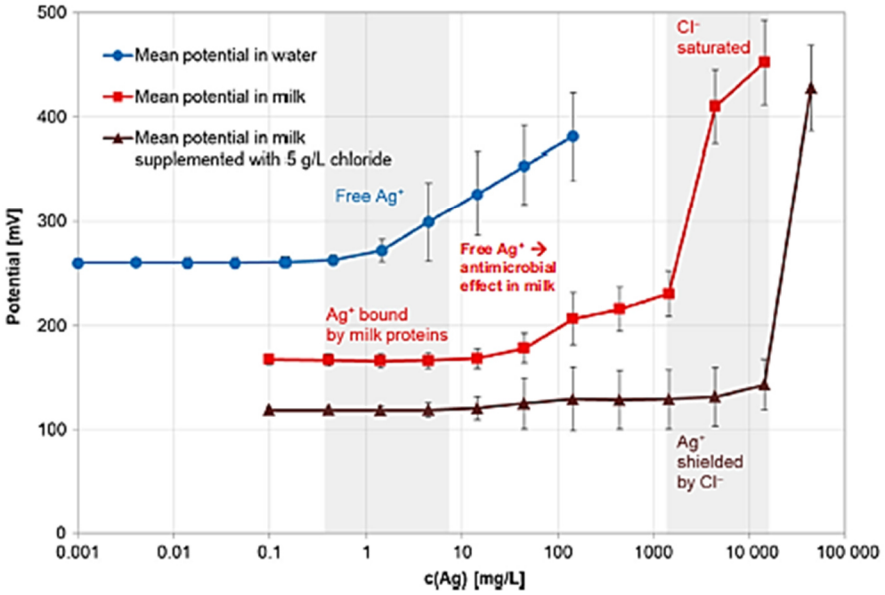

Figure 4 Dependence of potential on total Ag concentration in water + $AgNO_3$, milk + $AgNO_3$ and milk + $AgNO_3$ + chloride. Potential was measured with an Ag electrode against an Ag/AgCl standard reference electrode. [Colour figure can be viewed at wileyonlinelibrary.com]

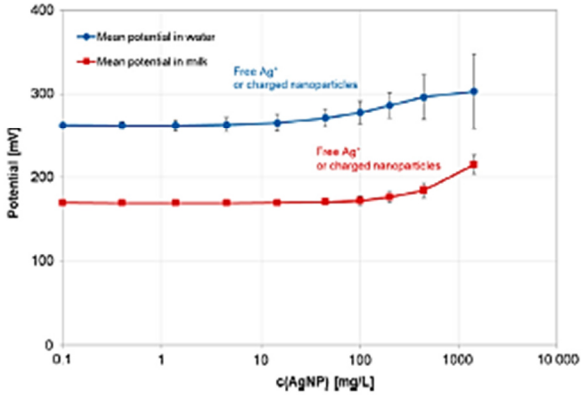

Figure 5 Dependence of potential on total Ag concentration in water + AgNP and milk + AgNP. Potential was measured with an Ag electrode against an Ag/AgCl standard reference electrode. [Colour figure can be viewed at wileyonlinelibrary.com]

constituents. Chloride is the most abundant anion in bovine milk and is present at a concentration of 1 g/L (Töpel 2016). The end point of chloride saturation in the produced milk that was similar to bovine milk was found to be at around 2000 mg/L of total Ag^+ (Figure 4). This is the equivalent of 19 mM silver, and on a 1:1 basis, this corresponds to 0.7 g/L of chloride. This is in the same range as the 1 g/L chloride content of bovine milk. Other silver-binding species such as bromide, iodide thiosulfate or free ammonia are present only in trace amounts or entirely absent in milk, so these would not contribute to the potential curves. Hence, only chloride and proteins remain as candidates for the binding of Ag^+. Silver is also known to react with sulphur-containing compounds, and the sulphur-containing amino acids cysteine and methionine are present at 0.9% and 2.7% of the total protein content in bovine milk, respectively (Töpel 2016). Thus, Ag^+ may also react with methionine and cysteine in milk. The solubility products of Ag_2S and AgCl are 5.5×10^{-51} mol^3/L^3 and 1.7×10^{-10} mol^2/L^2 at 25 °C, respectively (Dickerson *et al.* 1988). By comparison, the binding affinity of silver to sulphide groups in proteins is much higher than to chloride, so as long as free sulphide groups are available in solution, no AgCl will precipitate. After these groups are saturated with Ag^+, silver ions will be free to react with chloride. Therefore, the initial change in the slope of the curve in Figure 4 could be due to the saturation of proteins, followed by the saturation of chloride ions. The silver bound to proteins, chloride and other milk constituents is unavailable for contributing to antimicrobial effectiveness. Brody *et al.* (2001) suggested that silver ions react with sulphur-containing compounds like cysteine and other constituents of food, and therefore, not all are antimicrobially active. This appears to also be the case with milk.

The results of the antimicrobial experiments were consistent with those of the potentiometric measurements. They showed that significant antimicrobial effectiveness of AgNPs occurs at 100 mg/L, while the potentiometric measurements showed that free Ag^+ or charged AgNPs first became available between 50 and 100 mg/L of added AgNPs (Figure 5). The experiments also agreed well in the investigations using silver phosphate glass, which exhibited antimicrobial effectiveness at 8 mg/L of total Ag in a suspension consisting of glass matrix and milk, while the experiments with $AgNO_3$ in milk revealed that free Ag^+ was available between 5 and 15 mg/L of added $AgNO_3$. Considering that silver phosphate glass releases Ag^+ over time, and that within the time period of the antimicrobial experiments the entire 8 mg/L of total Ag was not released, 5 mg/L of Ag can be regarded as the minimum concentration required for antimicrobial effectiveness in milk. The minimum inhibiting silver concentration is also dependent on the microorganism species. However, it is likely that the minimal required silver concentration for other microorganisms in milk will be at least 50 mg/L for AgNPs and 5 mg/L for Ag, due to the chemical interactions of Ag^+ with milk constituents. Our potentiometric measurements suggest that the minimum concentration for antimicrobial effectiveness in milk occurs once proteins have become saturated. Below this point, only a small number of free silver ions will be available while they are in equilibrium with protein, and so relatively few would be able to interact with bacterial proteins to produce an antimicrobial effect.

The difference in the antimicrobially effective concentrations between $AgNO_3$ and AgNPs was likely due to the fact that every silver ion is free upon the addition of $AgNO_3$, whereas only a small percentage of silver atoms is liberated from nanoparticles and becomes free silver ions upon the addition of AgNPs.

Our study indicates that the minimal silver concentration for significant antimicrobial effectiveness in milk is at least 5 mg/L. This is substantially higher than the WHO-recommended concentration limit of 0.1 mg/L silver for drinking water, and therefore, it appears that the use of silver for extending shelf life of milk is not possible.

Studies published so far on migration of silver from packaging polymers to food suggest that the release of silver is very low, that is in the range of some µg/L (Goetz *et al.* 2013). Therefore, usable antimicrobial effects of polymer packaging materials doped with different silver species for milk applications are not expected.

CONCLUSION

The results of this study indicate that silver ions or nanosilver can prevent milk spoilage at all elevated temperatures. However, the concentrations required are too high based on the NOAEL for silver. Therefore, practical use of silver to

extend milk shelf life appears to be unfeasible by direct addition as well as by incorporation of silver into polymer packaging materials.

ACKNOWLEDGEMENTS

The Federal Ministry for Economic Affairs and Energy financially supported this research project on the basis of a resolution of the German Bundestag and RAS AG provided AgPURE. The authors would like to thank their student Julia Neher for her contribution to this work. TUM proofreading support is gratefully acknowledged.

REFERENCES

Brody A L, Strupinsky E R and Kline L R (2001) *Active Packaging for Food Applications*, 1st edn, pp. 138–141. Lancaster: Technomic Publishing Company.

Bumbudsanpharoke N and Ko S (2015) Nano-food packaging: an overview of market, migration research, and safety regulations. *Journal of Food Science* **80** R910–R923.

Bundesministerium für Ernährung und Landwirtschaft (2016) LänderberichtIndien. [Internet document] URL https://www.agrarexportfoerderung.de/fileadmin/SITE_MASTER/content/files/Laenderberichte 2016/Laenderbericht_Indien_2016.pdf. Accessed 05/03/2019.

Chaudhry Q and Castle L (2011) Food applications of nanotechnologies: an overview of opportunities and challenges for developing countries. *Trends in Food Science & Technology* **22** 595–603.

Chaudhry Q, Scotter M, Blackburn J, Ross B, Boxall A, Castle L, Aitken R and Watkins R (2008) Applications and implications of nanotechnologies for the food sector. *Food Additives & Contaminants: Part A* **25** 241–258.

Dallas P, Sharma V K and Zboril R (2011) Silver polymeric nanocomposites as advanced antimicrobial agents: classification, synthetic paths, applications, and perspectives. *Advances in Colloid and Interface Science* **166** 119–135.

Dickerson R E, Gray H B, Darensbourg M Y and Darensbourg D J (1988) *Prinzipien der Chemie*, 2nd edn, pp. 249–256. New York: de Gruyter.

Durán N, Durán M, de Jesus M B, Seabra A B, Fávaro W J and Nakazato G (2016) Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine* **12** 789–799.

European Commission (2011) Commission recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union L275/38.

Feng Q L, Wu J, Chen G Q, Cui F Z, Kim T N and Kim J O (2000) A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *Journal of Biomedical Materials Research* **52** 662–668.

Goetz N, Fabricius L, Glaus R, Weitbrecht V, Günther D and Hungerbühler K (2013) Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. *Food Additives & Contaminants: Part A* **30** 612–620.

He D, Jones A M, Garg S, Pham A N and Waite T D (2011) Silver nanoparticle–reactive oxygen species interactions: application of a charging–discharging model. *The Journal of Physical Chemistry C* **115** 5461–5468.

Kalaiselvi A, Gantz S and Ramalingam C (2013) Effects of silver nanoparticles on storage stability of raw milk. *International Journal of Pharmacy and Pharmaceutical Sciences* **5** 274–277.

Kisaalita W S (2010) Milk in the cold chain. *IDM International Dairy Magazine* **3** 11–14.

Klein C L, Comero S, Stahlmecke B *et al.* (2011) *NM-300 Silver Characterisation, Stability, Homogeneity*. Luxembourg: Publications Office of the European Union.

Kumar R and Prasad A (2010) Detection of Ecoli and Staphylococcus in milk and milk products in and around Pantnagar. *Veterinary World* **3** 495–496.

Lee S M, Lee B S, Byun T G and Song K C (2010) Preparation and antibacterial activity of silver-doped organic–inorganic hybridcoatings on glass substrates. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **355** 167–171.

Lingathurai S and Vellathurai P (2013) Bacteriological quality and safety of raw cow milk in Madurai, South India. *Bangladesh Journal of Scientific and Industrial Research* **48** 109–114.

Lu M, Shiau Y, Wong J *et al.* (2013) Milk spoilage: methods and practices of detecting milk quality. *Food and Nutrition Sciences* **4** 113–123.

Mikiciuk J, Mikiciuk E, Wrońska A and Szterk A (2016) Antimicrobial potential of commercial silver nanoparticles and the characterization of their physical properties toward probiotic bacteria isolated from fermented milk products. *Journal of Environmental Science and Health. Part B* **51** 222–229.

Niamsiri N and Batt C A (2009) Applied microbiology: agro/food. Dairy products. In *Encyclopedia of Microbiology*, 3rd edn, Vol. **1**, pp. 34–44. Schaechter M, ed. San Diego: Academic Press.

Percival S L, Bowler P G and Russell D (2005) Bacterial resistance to silver in wound care. *Journal of Hospital Infection* **60** 1–7.

Rai V and Bai J, ed. (2018) *Nanotechnology Applications in the Food Industry*, 1st edn. Boca Raton: CRC Press.

RAS AG (2017) Technical Information agpure®. [Internet document] URL https://ras-ag.com/wp-content/downloads/TIs/TI_agpure.pdf. Accessed 05/03/2019.

Riemelt I, Bartel B and Malczan M (2003) *Milchwirtschaftliche Mikrobiologie*, 2nd edn, pp. 112–116. Hamburg: Behr's.

Terzaghi B E and Sandine W E (1975) Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology* **29** 807–813.

Töpel A (2016) *Chemie und Physik der Milch: Naturstoff, Rohstoff, Lebensmittel*, 4th edn, pp. 512–513. Hamburg: Behr's.

World Health Organization (2003) Silver in drinking-water. [Internet document] URL https://www.who.int/water_sanitation_health/dwq/chemicals/silver.pdf. Accessed 05/03/2019.

Yakabe Y, Sano T, Ushio H and Yasunaga T (1980) Kinetic studies of the interactions between silver ion and deoxyribonucleic acid. *Chemistry Letters* **9** 373–376.

Yildiz N and Pala A (2012) Effects of small-diameter silver nanoparticles on microbial load in cow milk. *Journal of Dairy Science* **95** 1119–1127.

Zangerl P (2006) Mikrobiologie der Produkte. In *Kurzes Lehrbuch Milchkunde und Milchhygiene*, pp. 156–179. Krömker V, ed. Stuttgart: Parey.

3.2 Release of silver from silver doped PET bottles

Publication 2 describes the incorporation of silver into PET bottles and the release thereof. The development of active packaging based on silver incorporated into polymers, for enhancing shelf life, has gained increasing interest. Studies are known in which silver is incorporated into polymers such as low density polyethylene, polyethylene, polypropylene, polyamide or lactic acid. However, studies are lacking in which silver is incorporated into polyethylene terephthalate (PET), although it is the most common material used for bottles. Data on the release of silver from PET would be important for health safety policy and food packaging industry to ensure human health. Furthermore, the mechanism of action of the bottles was investigated in terms of the parameters: time, temperature, medium (acidity strength), percentage of filler in the polymer, and type of antimicrobial filler. Moreover, diffusion coefficients of silver ion in PET and the influence of the geometry were investigated.

Injection and stretch blow molded PET bottles contained silver phosphate glass at 3 and 10% (w/w) and AgPURE masterbatch at 10% (w/w) which in turn contains 6500 ppm (w/w) silver nanoparticles (AgNPs). Silver released from these bottles into distilled water and into aqueous solutions of 0.5 and 3% (w/w) acetic acid ranged from 2 to 72 µg/L after 10 d at either 21 or 43 °C. This was quantified by inductively coupled plasma mass spectrometry (ICP-MS). The bottles containing AgNPs showed no release within a detection limit (LOD) of 0.1 µg/L. The parameters time, temperature, acidic strength, and antimicrobial filler influenced the release as expected. Diffusion coefficients of silver ions in PET lay between 3.2×10^{-17} and 1.7×10^{-15} cm^2/s. The proposed European migration limit of 0.05 mg Ag/L was partly exceeded by the bottles containing 10% (w/w), but not by the bottles containing 3% (w/w) silver phosphate glass.

CRediT authorship contribution statement:

Sabrina Braun: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Supervision, Project administration.

Vladimir Ilberg: Writing - review & editing, Supervision.

Uwe Blum: Methodology, Investigation.

Horst-Christian Langowski: Writing - review & editing, Supervision.

Food Packaging and Shelf Life 25 (2020) 100517

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Release of silver from silver doped PET bottles

Sabrina Braun[a,b,*], Vladimir Ilberg[b], Uwe Blum[c], Horst-Christian Langowski[a,d]

[a] Technical University of Munich, TUM School of Life Sciences Weihenstephan (WZW), Weihenstephaner Steig 22, 85354, Freising, Germany
[b] University of Applied Sciences Weihenstephan-Triesdorf, Institute of Food Technology, Am Staudengarten 11, 85354, Freising, Germany
[c] Bavarian State Institute of Forestry, Hans-Carl-von-Carlowitz-Platz 1, 85354, Freising, Germany
[d] Fraunhofer Institute for Process Engineering and Packaging (IVV), Giggenhauser Strasse 35, 85354, Freising, Germany

ARTICLE INFO

Keywords:
Migration
Silver release
Diffusion coefficient
Active packaging
PET bottle
Bellow
Container

ABSTRACT

Development of active packaging based on polymers doped with silver has gained increasing interest. PET bottles were produced by injection and stretch blow molding under addition of silver phosphate (Ag_3PO_4) glass at 3 % and 10 % (w/w) and of AgPURE masterbatch at 10 % (w/w) which contained 6500 ppm silver nanoparticles (AgNPs). These bottles underwent release tests. Silver released into distilled water, 0.5 % and 3 % (w/w) acetic acid was quantified using inductively coupled plasma mass spectrometry (ICP-MS). The amount of silver released ranged from 2 $\mu g L^{-1}$ to 72 $\mu g L^{-1}$ after 10 days at either 21 °C or 43 °C. For PET bottles containing AgNPs no release of silver was detected. Release was dependent on percentage of antimicrobial filler in the polymer, temperature, and acidic strength. Diffusion coefficients of silver ion (Ag^+) in PET were observed to be between 3.2×10^{-17} and 1.7×10^{-15} cm^2/s. Bottles containing 3% (w/w) did not exceed the proposed specific migration level of 0.05 mg Ag per kg food, whereas bottles containing 10 % (w/w) did.

1. Introduction

Active packaging aim to increase the shelf life of packed products as well to maintain and improve its quality. Its function is based on interactions between the filled product and the packaging material; while substances are principally released or removed from the packaging material to the filled product or to the headspace (Pant, 2016). A form of active packaging- antimicrobial packaging, intends to reduce or inhibit growth of microorganisms in the packaging material or in the packed food. There are several forms of antimicrobial packaging. A special form is the incorporation of antimicrobial agents into polymers (Appendini & Hotchkiss, 2002).

Among metallic cations, silver ion (Ag^+) is known to have the best antimicrobial effect against a huge range of microorganisms. Thermal polymer processing methods like injection molding is suitable for silver since it can withstand very high temperatures (up to 800 °C). Thus, active packaging based on release of silver ions has gained increasing (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012). The most widely used polymer additives for food applications are silver substituted zeolites (Appendini & Hotchkiss, 2002). Moreover, silver nanomaterials have attracted increasing attention (see Table 1).

Migration, i.e., the mass transfer of a substance from a package into the foodstuff, is one of the most important safety concerns in food packaging with regard to the consumer. Migration tests should be performed in a worst case scenario, i.e., migration level should be equal or higher than those expected during real food storage (Begley et al., 2005). European Commission (EC) Regulation No 10/2011 on plastic materials and articles intended to come in contact with food sets out conditions of migration testing for the introduction of new packaging material (European Commission Regulation, 2011). The European Food Safety Authority (European Food Safety Authority (EFSA), 2011) released a positive opinion concerning the use of a certain silver (Ag) zeolite in food contact surfaces with a general specific migration limit of 0.05 mg Ag per kg food. Therefore, the maximum oral intake was limited to less than 13 % of the No Observed Adverse Effect Level (NOAEL), which is about 10 g over a total lifetime intake for humans (World Health Organization (WHO) (2003)). Mass transfer of substances from plastic into food simulants usually follows Fick´s law of diffusion (Begley et al., 2005) which is dependent on factors such as temperature, time, amount of substance in the polymer, type of substance and solubility in food (Welle & Franz, 2011).

Migration of silver from food contact materials (FCMs) has gained

Abbreviations: AgNPs, Silver nanoparticles; EC, European Comission; EFSA, European food safety authority; FCMs, food contact materials; LOD, limit of detection; NOAEL, no observed adverse effect level; WHO, World Health Organization

* Corresponding author at: University of Applied Sciences Weihenstephan-Triesdorf, Institute of Food Technology, Am Staudengarten 11, 85354, Freising, Germany.
E-mail address: Sabrina.Braun@tum.de (S. Braun).

https://doi.org/10.1016/j.fpsl.2020.100517
Received 18 February 2020; Received in revised form 23 April 2020; Accepted 11 May 2020

Table 1
Overview: Release of silver from food contact polymers.

Type of silver	Polymer	Test material	Manufacturing method of the test material	Analyzing method	Migration simulant or test medium	Test conditions	Silver content incorporated in polymer	Released silver content at final time point (limit according to EU-directive 10/2011 0.05 mg/kg Ag)	Reference
AgNPs	PE-LD	film	coating by immersion of corona modified films in Ag solution	AAS	deionized water	14 d 4 °C	1.17 ppm/cm²	1.5 ppm/m²	Bayani Bandpey, Aroujalian, Raisi, and Fazel (2017)
AgNPs	PE-LD	film	extrusion	ICP-MS	3 % acetic acid	10 d 60 °C	250 mg/kg	10.109 ng/cm²	Bott (2017)
AgNPs	PE	nanocomposites	extrusion	ICP-MS	chicken breast	3.1 d 21.8 °C	5000 mg/kg	0.039 mg/kg	Cushen, Kerry, Morris, Cruz-Romero, and Cummins (2014)
Aglon	PE	film	extrusion	ICP-AES	distilled water	10 d 40 °C	$6.07*10^{-3}$ mg/L	6.07 ± 1 µg/L	Cushen et al. (2014a)
					3 % acetic acid			15 ± 10 µg/L	
AgNPs					distilled water		$3.62*10^{-4}$ mg/L	160 µg/L	
					3 % acetic acid			520 µg/L	
AgNPs	PP	food container (Kinetic Go Green Nano Silver Basic)	probably extrusion/ injection molding	ICP-MS	3 % acetic acid	10 d 40 °C	0.39 mg/cm²	31.46 ng/cm²	Echegoyen and Nerín (2013)
	PE-LD	bag					0.02 mg/cm²	3.76 ng/cm²	
AgNPs P 105	PE-LD	film	extrusion	GFAAS	orange juice	28 d 4 °C	5000 mg/kg	0.1 ± 0.003 µg/l	Emamifar, Kadivar, Shahedi, and Soleimanian-Zad (2010) and Emamifar, Kadivar, Shahedi, and Solimanian-Zad (2012)
silver zeolite	PLA	film	solution-casting/solvent evaporation	GFAAS	distilled water	24 h 20 °C	413.2 ng/cm²	0.043 mg/kg	Fernández et al. (2010)
					3 % acetic acid			0.71 mg/kg	
					Tryptone soy broth (TSB)			0.35 mg/kg	
			melt-mixing/ compression molding		3 % acetic acid			appr. 0.04 mg/kg	
AgNPs	PP	food container (Kinetic Go Green Nano Silver Basic)	probably extrusion/ injection molding	SN-ICP-MS	distilled water	10 d 20 °C	11.9 ± 2.4 mg/kg	5.8 ng/cm²	von Goetz et al. (2013)
					3 % acetic acid			9.5 ng/cm²	
	PE	bag (FresherLonger™, Sharper Image Corporation, USA)	probably extrusion		distilled water		37.1 ± 1.2 mg/kg	< 0.5 ng/cm²	
					3 % acetic acid			0.5 ng/cm²	
AgNPs	PE	bag/film	probably extrusion	AAS	ultrapure water	10 d 25 °C	100 mg/kg	7 ng/cm²	Huang et al. (2011)
					4 % acetic acid			8 ng/cm²	
					ultrapure water	10 d 40 °C		25 ng/cm²	
					4 % acetic acid			28 ng/cm²	
AgNPs	PE	nanocomposites	melt blending	AAS	distilled water	30 d 40 °C	22.64 mg/kg	0.896 ± 0.8 mg/kg	Jokar and Abdul Rahman (2014)
					3 % acetic acid			1.034 ± 0.04 mg/kg	
					apple juice			0.926 ± 0.11 mg/kg	
			layer-by-layer deposition		distilled water		16.28 mg/kg	1.107 ± 0.02 mg/kg	
					3 % acetic acid			1.434 ± 0.04 mg/kg	
					apple juice			1.084 ± 0.02 mg/kg	
AgNPs	PE	food container (The Original Always Fresh Container, Gourmet Trends, USA)	probably extrusion/ injection molding	ICP-MS	Milli-Q water	10 d 40 °C	11.9 ± 3.3 mg/kg	< 0.9 µg/L	Mackevica, Olsson, and Hansen (2016)
					3 % acetic acid			8.2 ± 0.5 µg/L	
AgNPs P 105	PP	film	blown film method	AAS	3 % acetic acid	10 d 40 °C	5 wt%	0.02046 ± 0.00016 mg/kg	Polat, Fenercioglu, Unal Turhan, and Guclu (2018)

increasing interest. Table 1 shows a literature overview of the release of silver from FCMs. In the following, two examples were selected. Von Goetz et al. (2013) studied the migration of silver from commercially available polypropylene silver doped food containers and a polyethylene plastic bag into food simulants. The food storage container with an initial silver content of 11.9 ± 2.4 µg g^{-1} plastic released 9.5 ng cm^{-2} Ag into 3 % (w/v) acetic acid at 20 °C after 10 d. Migration into distilled water was approximately half as much. The migration from the plastic bag with an initial silver content of 37.1 ± 1.2 µg g^{-1} plastic was 0.5 ng cm^{-2} after 10 d. Bott (2017) showed that the amount that migrated from PE-LD nanocomposites containing 250 mg kg^{-1} silver was 1010.9 ng dm^{-2}, into 3 % (v/v) acetic acid after 10 d at 60 °C. He found out that the silver that migrated was only present in the form of silver ions and not nanoparticular. Moreover, it became clear that targeted analytics tailored to the nanomaterial were decisive for the identification of the silver species.

Antimicrobial agents based on silver like silver zeolites or silver nanoparticles have been incorporated into polymers such as polyethylene, low density polyethylene, polypropylene, polyamide and polylactic acid (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2014; Damm & Münstedt, 2008; Fernández, Soriano, Hernández-Muñoz, & Gavara, 2010; Jokar & Abdul Rahman, 2014; Pehlivan, Balköse, Ülkü, & Tihminlioğlu, 2005). However, data regarding silver incorporated in polyethylene terephthalate (PET) are lacking, despite PET is one of the most common packaging material for beverages. Data on the release of silver from PET would be beneficial for health safety policies and food packaging industry.

The objectives of this study were to evaluate the release of silver from silver doped PET bottles into food simulants and to investigate the parameters such as time, temperature, medium (in terms of acidity strength), percentage of filler in the polymer and type of antimicrobial filler on the release of silver and its diffusion coefficients. Furthermore, the influence of the geometry was investigated and the mechanism of action on the release of silver ions from silver phosphate glass out of the bottle was postulated. To the best of the author´s knowledge, no publication describing the incorporation of silver into PET bottles, nor the migration of silver from them existed.

2. Materials and methods

2.1. Manufacturing of the silver doped bottles and bellows

The preforms were made of PET resin (Selenis MASTER 10, Ribeira de Nisa Portalegre, Portugal) with an intrinsic viscosity of 0.86 ± 0.02 dl/g. IONPURE WPA < 10 µ (Ishizuka Glass Co., Ltd, Iwakura City, Aichi, Japan) containing silver phosphate (Ag_3PO_4) glass as active substance was incorporated into the PET in powder form at 3 and 10 % (w/w). According to the manufacturer, the silver content in the silver phosphate glass powder was 1.6 % (w/w). From the obtained mixture, the preforms were produced by an Allrounder 470 S injection molding machine from Arburg (Loßburg, Germany). The weight of the preform that contains the antimicrobial filler was 16.9 g. All preforms were injection molded under the same processing conditions.

The obtained preforms were stretch blown in a laboratory machine (Vema, Aldachen, Germany) to produce bottles with a volume of 500 mL and a height of 208 mm at a blowing pressure of 15 bar. The wall thickness of the bottles was 0.27 mm in average. The geometry and sizes of the bottle are shown in Fig. 1.

Bottles and preforms containing silver nanoparticles (agpure®PBT6500 from RAS AG, Regensburg, Germany) were manufactured in the same way as mentioned above. According to the manufacturer, the silver content in agpure®PBT6500 is 6500 ppm and 10 % (w/w) agpure®PBT6500 was added to the PET. AgPURE corresponds to the OECD reference material NM-300. Silver particles in AgPURE have an average diameter of about 15 nm and a primary size distribution of D99 < 20 nm (Klein et al., 2011; RAS AG, 2015). The weight of the preform was 16.2 g. Bottles without antimicrobial filler were also produced in the same manner and served as control.

It was investigated if a higher silver amount released could be achieved by changing the ratio of filled volume to container surface. For this purpose, bellows with a larger surface area than the bottles were produced. The bellows were made of the same PET resin and IONPURE, hereinafter referred to as silver phosphate glass, was incorporated in powder form into the PET at 10 % (w/w). About 10 % (w/w) of the masterbatch agpure®PBT6500 was added for the bellows containing nanosilver. From the obtained mixture, the bellows were produced by a SUMA one step injection blow molding machine (Taichung City, Taiwan). The weight of the bellows was 5 g for PET/silver phosphate glass and 4.8 g for PET/nanosilver. The wall thickness of the bellows was 0.28 mm in average. Fig. 1 shows the dimensions of the bellow.

2.2. Experimental procedure

For every test condition, three to five silver doped bottles and one bottle without antimicrobial filler were used. The bottle without antimicrobial filler served as control sample. The bottles were filled with either 500 mL of pure distilled water or a solution of 0.5 or 3 % (w/w) acetic acid (stock solution ≥ 99 %, Supra quality, Roth, Karlsruhe, Germany) in distilled water and stored at 43 or 21 °C. According to No 10/2011 EU, 3 % acetic acid is an official food simulant. For the release studies, 43 °C was chosen as a temperature suitable for the growth of thermophilic microorganisms. These organisms can cause problems in beverages since they can survive heat treatment. Room temperature (21 °C–23 °C) was chosen as a typical storage temperature for beverages. Samples were taken after defined times (0 h, 1 h, 6 h, 24 h, 3 d, 7 d, 14 d, 28 d). Before taking samples, the bottles were always shaken in the same manner ten times per hand. At every point in time, 10 mL simulant sample was pipetted twice from each bottle into PP tubes (Cellstar, Greiner Bio-One, Frickenhausen, Germany) for further analysis. The tubes were washed with 2 % nitric acid and distilled water before use and the pipette tip was changed for every sample taken to ensure that there was no contamination. For stabilization, 100 µL nitric acid (p. a. 65 %, Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany; specially purified by sub boiled distillation) was added to each sample and these were stored cool in the tubes packed in aluminum foil until measuring. After taking samples, 20 mL water or 0.5 or 3 % acetic acid was refilled in each bottle to have the same contact area with the bottle wall at every point in time. The resulting dilution was considered in the measuring values.

The bellows were filled with 50 mL of pure distilled water and then stored at 43 °C. Samples were taken after 14 d and treated in the same manner as mentioned above.

Experiments with pure silver phosphate glass powder not integrated into the polymer were conducted by weighing 0.5 g silver phosphate glass powder into 500 mL distilled water and stored at 43 °C. Another approach contained additionally 0.8 g NaCl (Merck Millipore, Darmstadt, Germany). Samples of 15 mL each were taken at the defined times, filtered over a syringe filter and 100 µL of 65 % nitric acid was added.

2.3. Determination of the silver content in the silver doped bottles

The total silver content in the simulants was determined according to DIN EN ISO 17294-2 (01/2017) by using a Nexion 2000 ICP-MS (Perkin Elmer, Waltham, USA). The setup of the ICP-MS is summarized in Table 2. The ICP-MS was calibrated using Ag multi-element ICP-MS calibration standard (Roth, Karlsruhe, Germany) in 2 % HNO_3. Rhodium and rhenium served as internal control standard. The method allowed the detection of Ag with a 0.1 µg/L limit of detection (LOD).

After preparing the samples according to DIN EN 16711-1 (04/2014), the actual silver content in the manufactured bottle was determined with the same method of analysis. Samples (3 × 3 cm) were

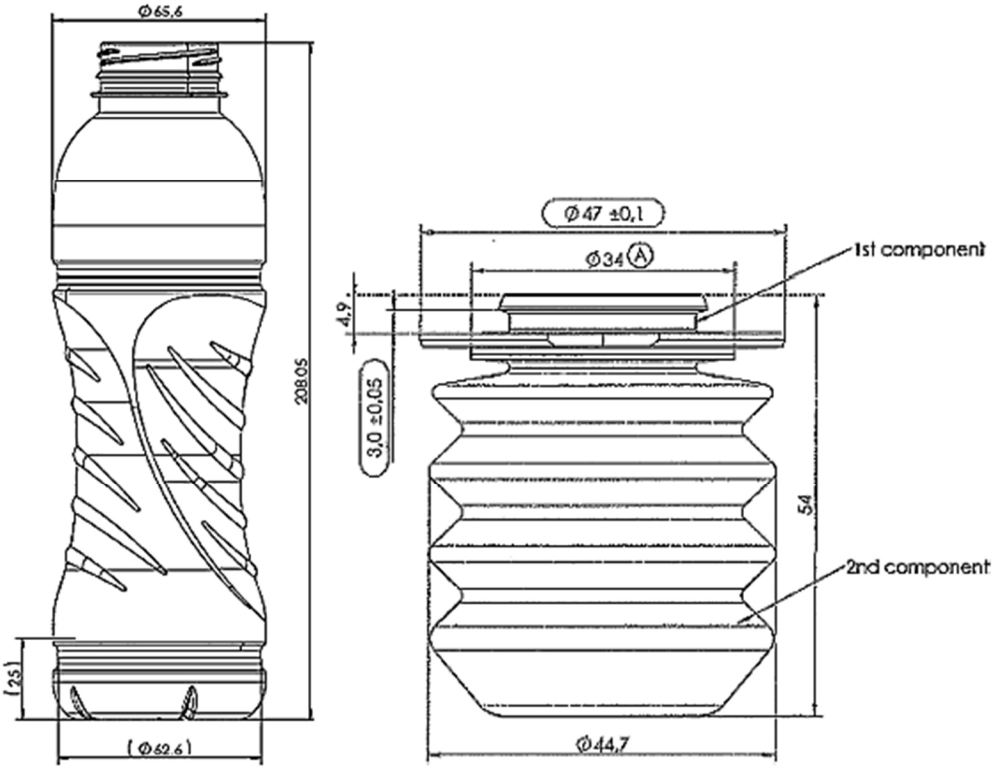

Fig. 1. Dimensions of the bottle and bellow.

Table 2
ICP-MS setting.

Settings	
RF generator power	1250 W
Plasma gas	Argon, 15 L/min
Flow rates:	
Nebulization gas	1.04 L/min
Auxiliary gas	1.2 L/min
Sample	1 mL/min
Nebulizer	Meinhard nebulizer
Spray chamber	Meinhard cyclonic spray chamber
Monitored masses	^{107}Ag and ^{109}Ag
Dwell time	1 s with 3 repeats

taken at ten different parts of the bottle in order to avoid possible fluctuations due to an inhomogeneous distribution of the silver material dispersed therein. These were crushed manually and processed into a homogeneous sample in an analysis mill from IKA (Staufen im Breisgau, Germany) at 25 000 rpm. The digestion took place in a microwave (multiwave PRO) from Anton Paar (Graz, Austria) at 250 °C for 45 min. For the digestion, 250 mg of the crushed material was used and 6 mL concentrated nitric acid (HNO_3) served as digestion reagent.

The silver concentration in the bottle wall ($c_{p,o,\ measured}$) was determined to be $\bar{x}$ = 300 mg kg^{-1} with x_{min} = 120 mg kg^{-1} and x_{max} = 460 mg kg^{-1} for 3 % silver phosphate glass addition, $\bar{x}$ = 1420 mg kg^{-1} with x_{min} = 1225 mg kg^{-1} and x_{max} = 1545 mg kg^{-1} for the bottles containing 10 % silver phosphate glass and $\bar{x}$ = 440 mg kg^{-1} with x_{min} = 350 mg kg^{-1} and x_{max} = 530 mg kg^{-1} for the bottle containing nanosilver. The values calculated from the manufacturer's data ($c_{p,o,\ calculated}$) were 470, 1600 and 650 mg kg^{-1} PET. Recovery rates were calculated by Eq. (1). These were 64, 89 and 68 %. The variations of the silver content in the bottle wall are due to an inhomogeneous distribution of the antimicrobial substance during the production process. Furthermore, there was a loss of the antimicrobial substance, since the powder stuck to the container wall used for weighing the powder before the injection molding process. Consequently, this leads to lower recovery rates.

$$Revovery\ rate = \frac{c_{p,0,\ measured}}{c_{p,0,calculated}} \times 100 \quad (1)$$

$c_{p,o,\ measured}$: Concentration in the bottle wall measured by ICP-MS
$c_{p,o,\ calculated}$: Concentration in the bottle wall calculated from the manufacturer´s data

2.4. Determination of diffusion coefficients

The concentrations of silver in the simulants at a defined time were plotted against its square root of time and from this correlation, the diffusion coefficient of silver ions in PET was determined according to Eq. (2) (Piringer, 2007).

$$\frac{m}{A} = 2c_{p,0}\rho_{PET}\sqrt{\frac{Dt}{\pi}} \quad (2)$$

m: Mass of the migrated Ag in the simulant after time t
A: Surface area which is 401.96 cm^2
$c_{p,o}$: Concentration in the bottle wall which is 300 or 1420 mg kg^{-1}
ρ_{PET}: Density of polyethylene which is 0.00138 kg/cm^3
D: Diffusion coefficient
t: Time

2.5. Statistical analysis of the migration experiments

All experiments were carried out in triplicate. Measured Ag^+ concentrations are illustrated with their confidence intervals for the average values calculated using a t-distribution ($\bar{x} \pm \frac{t^* SD}{\sqrt{N}}$). Significance was expressed at the 5 % level.

2.6. Calculation of the surface-specific release rate

The surface-specific release rate (SSRR) was calculated according to Eq. (3).

$$SSRR = \frac{c_{rs} \times V}{A}$$

c_{rs}: Concentration of the released silver

V: Volume

A: Surface area which is 401.96 cm^2 for the bottles and 87.79 cm^2 for the bellows.

3. Results and discussion

3.1. Release of silver from silver phosphate glass powder into distilled water and distilled water supplemented with 0.028 M NaCl

It was ensured that the pure silver phosphate glass powder in general releases silver ions before incorporating it into the polymer (Fig. 2). The experiments showed that Ag^+ was released over time out of the silver phosphate glass matrix. After 24 h at 43 °C, 10.3 ± 1 mg/L or 5.15 mg Ag^+ was released from 0.5 g of silver phosphate glass into 0.5 L distilled water. The total concentration of silver in the dispersed powder corresponded to 16 mg/L that is 8 mg in 0.5 L in total. This means that 64 % of the initial silver content was released from the glass powder into the free solution within 24 h.

For simulating chloride containing media like milk, NaCl was added to the simulant in a concentration of 0.028 M. The silver concentration remained constant at approximately 0.067 mg/L, since Ag^+ was immediately precipitated by chloride ions and therefore, would no longer have antimicrobial effect. In a previous study, Braun, Ilberg, Blum, and Langowski (2020) demonstrated the chemical interactions of Ag^+ with milk constituents. The recognition that ionic silver may react with constituents in packed products has to be kept in mind while developing antimicrobial packaging on the basis of silver ions.

3.2. Release of silver from silver doped PET bottles into distilled water in dependency of different percentages of antimicrobial filler in the polymer and temperature

Fig. 3 shows the concentration of silver released into the bottle filled with distilled water over time. The released amount of silver increases with time. High temperature as well as concentration of antimicrobial additive in the polymer led to high silver content in the liquid medium.

According to European Food Safety Authority (EFSA) (2011), the proposed migration limit of 0.05 mg/L Ag was not exceeded in distilled water within 10 d at temperatures > 40 °C.

Compared to the previous results with pure glass powder, merely 0.03 % silver in relation to the initial silver content was released within 24 h at 43 °C into distilled water. In all the studies reviewed by Westerband and Hicks (2018) 1.6 % or less of the initial Ag content was found to migrate within 10 d at 40 °C into acetic acid. The migrated amount of silver was in the ionic form (Bott, Störmer, & Franz, 2014).

3.3. Release of silver from silver doped PET bottles into 0.5 and 3 % (w/w) acetic acid at different temperatures (21, 43 °C)

Furthermore, the influence of acidic strength on the release of the silver ions into the medium was investigated. These results can be used for migration appraisals of different acidic beverages such as fruit juice or iced tea. Fig. 4 shows the release of silver dependent on acidic strength differences. At 22 °C, by adding 3 % acetic acid, the pH was 2.7 and by adding 0.5 % acetic acid, it was 3.3. The results suggest that at 43 °C as well as at 21 °C, the acidic strength has no significant effect on the release of the silver ions. Therefore, acidic strength differences in the range of 0.5 units in the medium can be neglected. However, bigger differences have to be kept in mind; when compared to the results in pure water, differences in the released silver concentrations at 43 °C as well as at 21 °C were observed.

The release of more silver ions under more acidic conditions can be explained by a high rate of water diffusion into the polymer film in acidic conditions, since the water sorption capacity of PET exposed to acidic solutions was higher than that observed for pure water (Jokar & Abdul Rahman, 2014 from Kyser, 1993 and Dinh & Kubouchi, 2010). Moreover, a high release rate of Ag^+ can be expected under more acidic

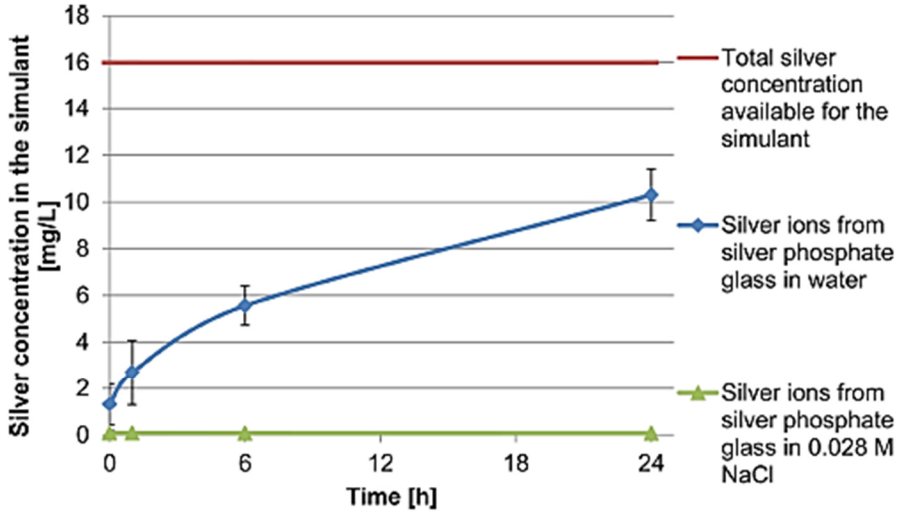

Fig. 2. Release of silver from silver phosphate glass powder into distilled water (curve with diamonds) and into a 0.028 M NaCl solution (curve with triangles) compared to the total concentration of silver contained in the powder (single line).

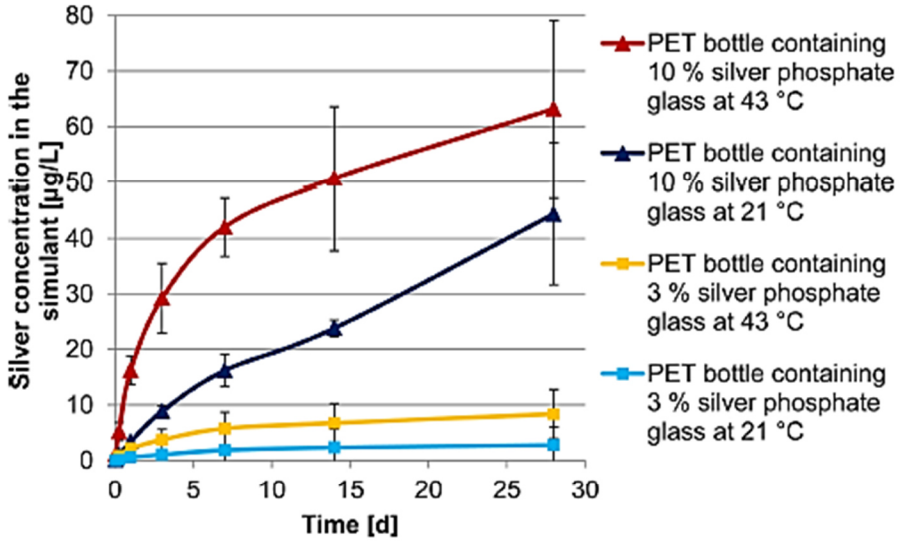

Fig. 3. Release of silver from silver doped PET bottles into distilled water in dependency of different silver concentrations in the polymer (3, 10 % w/w) and temperature (43, 21 °C).

conditions due to an increased dissolution (Simbine et al., 2019).

According to European Food Safety Authority (EFSA) (2011), the proposed migration limit of 0.05 mg/L Ag within 10 d at temperatures > 40 °C is now exceeded. For acidic media, the percentage of additive in the bottle should be less than 1600 mg kg^{-1} PET, if this would be put in the market.

3.4. Determination of diffusion coefficients of Ag^+ in PET

The amount of silver that migrated into the simulant plotted against the square root of time, showed approximately a linear correlation for all tested conditions within a certain time frame, which indicates Fickian migration behavior (see Figs. 5 and 6). The values of the diffusion coefficients given in Table 3 are calculated by Eq. (2). The masses used for calculation are obtained from the concentrations, which were related to the volume, ascertained from the different linear ranges marked in Figs. 5 and 6.

The release of silver in this application could occur as follows:

- Penetration of water into the PET surface (approximately within one day)
- Simulant begins to dissolve the silver phosphate glass to form

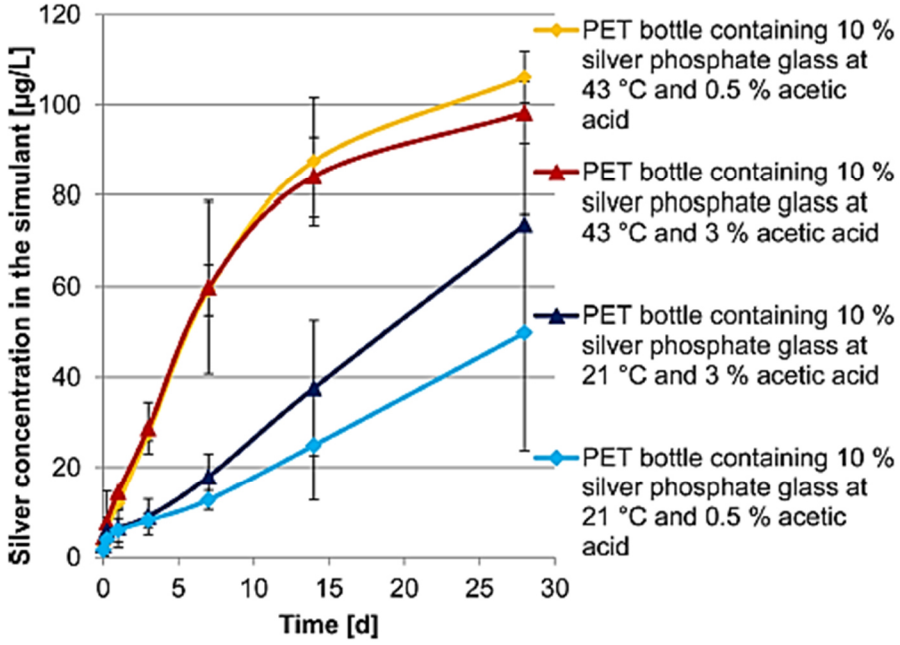

Fig. 4. Release of silver from silver doped PET bottles into acetic acid (0.5, 3 % w/w) at different temperatures (43, 21 °C).

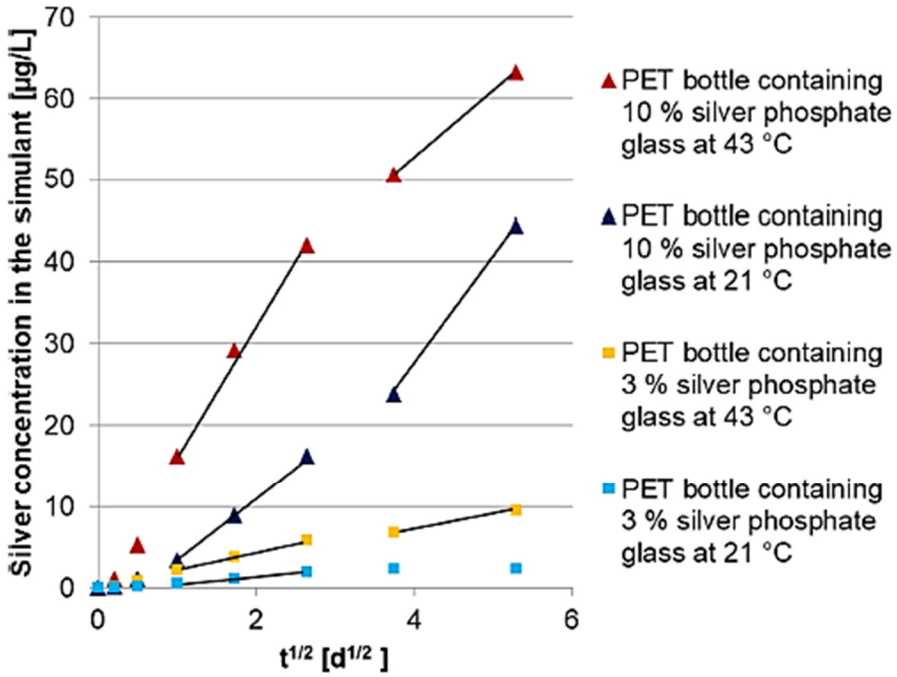

Fig. 5. Released silver concentration from silver doped PET bottles with different silver concentrations in the polymer (3, 10 % w/w) into distilled water at different temperatures (43, 21 °C) plotted over $t^{1/2}$ for determining Fickian behavior.

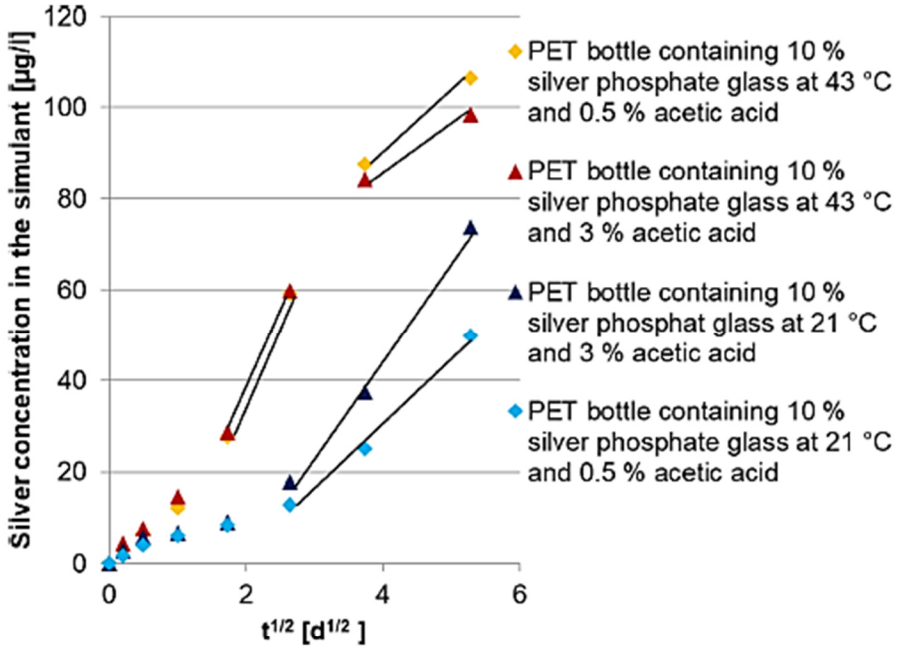

Fig. 6. Released silver concentration from silver doped PET bottles into acetic acid (0.5, 3 % w/w) at different temperatures (43, 21 °C) plotted over $t^{1/2}$ for determining Fickian behavior.

hydrated Ag ions
- Ag ions pass from silver phosphate glass into the plastic
- Ag ions diffuse through the PET to the surface. This is the time-determining step for which only Fickian diffusion behavior can be expected
- Diffusion of Ag ions from the polymer surface into the contact medium

Table 3
Diffusion coefficients estimated for the release of silver from silver doped PET bottles (3, 10 % w/w silver phosphate glass) into distilled water or acetic acid (0.5, 3 % w/w).

Condition	Diffusion coefficient [cm^2/s]
43 °C, 3 % (w/w) filler	$2.7...3.8 \times 10^{-16}$
43 °C, 10 % (w/w) filler	$7...9.8 \times 10^{-16}$
21 °C, 3 % (w/w) filler	3.2×10^{-17}
21 °C, 10 % (w/w) filler	$1...2 \times 10^{-16}$
43 °C, 10 % (w/w) filler, 0.5 % acetic acid	$1.4...1.7 \times 10^{-15}$
43 °C, 10 % (w/w) filler, 3 % acetic acid	$1.4...1.6 \times 10^{-15}$
21 °C, 10 % (w/w) filler, 0.5 % acetic acid	1.9×10^{-16}
21 °C, 10 % (w/w) filler, 3 % acetic acid	4.2×10^{-16}

Diffusion coefficients increased with concentration of filler, with temperature and in the presence of acetic acid compared to distilled water (Table 3). Effective diffusion coefficients from silver phosphate glass in PET filled with distilled water at 43 °C (2.7×10^{-16} - $9.8\ 10^{-16} cm^2/s$) lay in a similar range as reported diffusion coefficient of antimony from PET bottles ($1.4 \times 10^{-16} cm^2/s$) at 45 °C (Alt, Haldimann, & Dudler, 2008).

3.5. Release of silver from nanosilver doped PET bottles into distilled water

Contrary to silver phosphate glass powder, nanosilver incorporated into PET showed a release that was below the detection limit of < 0.1 µg/L for Ag within the examined time range of 14 d, although the total silver content of nanosilver in the bottle was about 440 mg Ag/kg PET, to be compared with the total silver content in the bottle containing 3 % (w/w) silver phosphate glass which was about 300 mg Ag/kg PET.

In IONPURE, the silver phosphate glass is embedded in a special glass matrix which can retain Ag^+. In the presence of water or moisture, the silver ions are gradually released from the glass matrix (Ishizuka Glass Co, 2020). In nanosilver like AgPURE, the Ag^+ ions should be released from the silver nanoparticles (AgNPs) while the nanoparticles should be firmly embedded in the plastic (Störmer, Bott, Kemmer, & Franz, 2017). After the diffusion of water into the polymer, elemental silver particles (Ag^0) are oxidized and formed Ag^+ ions diffuse from the bulk material to the surface (Damm & Münstedt, 2008). This process seems to progress very slowly, if at all, so that no detection of the Ag^+ ions could be determined within the investigated time period.The previous study (Braun et al., 2020) showed that pure nanosilver released ions slower with time compared to silver phosphate glass powder. This is in line with this finding, as the detection limit for Ag was not reached. This finding is further in line with the conclusions from Störmer et al. (2017). They stated that nanoparticles which are completely covered or encapsulated by the host polymer matrix do not penetrate from it and therefore, do not migrate into food.

For nanosilver AgPURE no or only neglected substance transport into liquid food can be expected when incorporated in PET. As long as no specific detection limits have been established for certain substances or groups of substances, a LOD of 0.01 mg/kg shall apply (EC, 2011). The LOD in this application was 0.1 µg/L. Therefore, it would not be a problem for registration of AgPURE, but antimicrobial effectiveness in beverages cannot be expected.

3.6. Release of silver from blow molded PET bellows into distilled water

The ratio of surface to filling volume of the bellows was 17.6 dm^{-1}, whereas this ratio was 8 dm^{-1} for the bottles (Table 4). According to this, about twice the ratio could be achieved with the bellows. The results in terms of volume of the bottle or bellow showed that the silver content released by the bellows was about twice as high as that released by the bottles at 43 °C after 14 d. Thus, it is possible to achieve a higher silver content in the solution by changing the bottle geometry and thus, possibly produce a better antimicrobial effect.

The surface-specific release rate calculated according to Eq. (3) was 63 ng/cm^2 for the PET bottles and 65 ng/cm^2 for the PET bellows. Thus it depends, as to be assumed, only on the plastic and not on the geometry.

Silver release from PET bellows containing AgPURE nanosilver was not detected (< 0.1 µg/L) like from the bottles.

3.7. Options for reuse and refill

The possibility of reusing silver doped bottles was also tested. For this purpose, migration experiments were performed on bottles reused after one year. Fig. 7 shows the released silver concentration from the bottle into distilled water at 43 °C compared to that released into distilled water at the same temperature after one year. The curves showed that by reusing the bottle, the released silver concentration was less. Accordingly, antimicrobial effectiveness is not as good as at the first usage. This has to be kept in mind if reuse is planned. It further indicates that particles near the surface are much more active and the silver ions in the polymer do not move within the dry polymer in a way that more particles would be available after a longer storage time. Thus, costs could be saved by incorporating the silver only on the surface during the manufacturing process. Compared to the initial silver content of 1420 mg kg^{-1} in the bottle, only a small part of silver was released.

4. Conclusions

The experiments showed that the release of silver from silver doped PET bottles depends on the percentage of antimicrobial filler in the polymer, temperature, and acidic strength of the simulants. The type of antimicrobial filler was also a decisive parameter. However, the amount of silver released partly exceeded proposed migration limits from EFSA. For PET bottles containing nanosilver no release was detected. Further study will show if migrated silver concentrations can lead to antimicrobial effectiveness in water or acidic beverages.

CRediT authorship contribution statement

Sabrina Braun: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Supervision, Project administration. **Vladimir Ilberg:** Writing - review & editing, Supervision. **Uwe Blum:** Methodology, Investigation. **Horst-Christian Langowski:** Writing - review & editing, Supervision.

Table 4
Comparison of silver release from bottle and bellow with regard to changed ratio of surface to volume.

Condition	Interior wall surface [cm^2]	Volume [L]	Ratio of surface to volume [1/dm]	Released silver concentration [µg/L]	Surface-specific release [ng/cm^2]
PET bottle	401.96	0.5	8.0	51	63
PET bellow	87.79	0.05	17.6	115	65

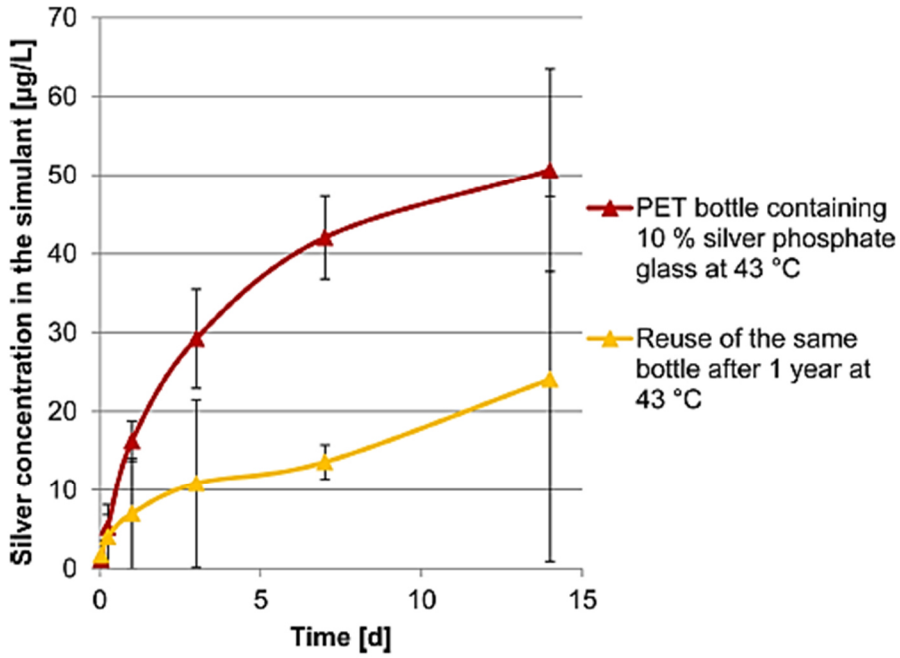

Fig. 7. Release of silver from silver doped PET bottles into distilled water at 43 °C compared to the released silver concentration when the same bottle is reused after one year.

Declaration of Competing Interest

None.

Acknowledgments

The Federal Ministry for Economic Affairs and Energy financially supported this research project on the basis of a resolution of the German Bundestag and RAS AG provided AgPURE. The authors would like to thank Josef Gleixner from Inotech for producing the bottles and bellows and Indicator GmbH for ICP-MS analysis. TUM proofreading support is gratefully acknowledged.

References

Alt, A., Haldimann, M., & Dudler, V. (2008). Diffusion coefficient of antimony catalysts in polyethylene terephthalate (PET) materials [poster presentation]. November 19 *4th International Symposium on Food Packaging - Scientific Developments Supporting Safety and Quality*http://www.akts.ch/dwlds/SML-Software-Diffusion-of-antimony-catalysts-in-PET.pdf.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies, 3*(2), 113–126. https://doi.org/10.1016/S1466-8564(02)00012-7.

Bayani Bandpey, N., Aroujalian, A., Raisi, A., & Fazel, S. (2017). Surface coating of silver nanoparticles on polyethylene for fabrication of antimicrobial milk packaging films. *International Journal of Dairy Technology, 70*, 204–211. https://doi.org/10.1111/1471-0307.12320.

Begley, T., Castle, L., Feigenbaum, A., Franz, R., Hinrichs, K., Lickly, T., ... Piringer, O. (2005). Evaluation of migration models that might be used in support of regulations for food-contact plastics. *Food Additives and Contaminants, 22*(1), 73–90. https://doi.org/10.1080/02652030400028035.

Bott, J. W. (2017). *Untersuchungen zum Migrationspotential von Nanomaterialien aus Kunststoff-Lebensmittelverpackungen.* Technische Universität München.

Bott, J., Störmer, A., & Franz, R. (2014). A comprehensive study into the migration potential of nano silver particles from food contact polyolefins. In M. A. Benvenuto, S. Ahuja, T. V. Duncan, G. O. Noonan, & E. S. Roberts-Kirchhoff (Vol. Eds.), *Chemistry of food, food supplements, and food contact materials: From production to plate: Vol. 1159*, (pp. 51–70). American Chemical Society. https://doi.org/10.1021/bk-2014-1159.ch005.

Braun, S., Ilberg, V., Blum, U., & Langowski, H. (2020). Nanosilver in dairy applications – Antimicrobial effects on *Streptococcus thermophilus* and chemical interactions. *International Journal of Dairy Technology, 73*(2), 376–383. https://doi.org/10.1111/1471-0307.12686.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., & Cummins, E. (2014a). Silver migration from nanosilver and a commercially available zeolite filler polyethylene composites to food simulants. *Food Additives & Contaminants: Part A, 31*(6), 1132–1140. https://doi.org/10.1080/19440049.2014.905874.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., & Cummins, E. (2014b). Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment. *Journal of Agricultural and Food Chemistry, 62*, 1403–1411. https://doi.org/10.1021/jf404038y.

Damm, C., & Münstedt, H. (2008). Kinetic aspects of the silver ion release from antimicrobial polyamide/silver nanocomposites. *Applied Physics A, 91*(3), 479–486. https://doi.org/10.1007/s00339-008-4434-1.

Dinh, C. V., & Kubouchi, M. (2010). Diffusion behaviour of corrosive solution environments in carbon black filled modified polyethylene linings. *VNU Journal of Science Mathematics - Physics, 26*, 71–81.

Echegoyen, Y., & Nerín, C. (2013). Nanoparticle release from nano-silver antimicrobial food containers. *Food and Chemical Toxicology, 62*, 16–22. https://doi.org/10.1016/j.fct.2013.08.014.

Emamifar, A., Kadivar, M., Shahedi, M., & Soleimanian-Zad, S. (2010). Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice. *Innovative Food Science & Emerging Technologies, 11*, 742–748. https://doi.org/10.1016/j.ifset.2010.06.003.

Emamifar, A., Kadivar, M., Shahedi, M., & Solimanian-Zad, S. (2012). Effect of nanocomposite packaging containing Ag and Zno on reducing pasteurization temperature of orange juice: Nanopackaging on pasteurization of orange juice. *Journal of Food Processing and Preservation, 36*, 104–112. https://doi.org/10.1111/j.1745-4549.2011.00558.x.

European Commission Regulation (2011). *European Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.* https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02011R0010-20190829.

European Food Safety Authority (EFSA) (2011). Scientific Opinion on the safety evaluation of the substance, silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2–5 %, for use in food contact materials. *EFSA Journal, 9*(2), 1999. https://doi.org/10.2903/j.efsa.2011.1999.

Fernández, A., Soriano, E., Hernández-Muñoz, P., & Gavara, R. (2010). Migration of antimicrobial silver from composites of polylactide with silver zeolites. *Journal of Food Science, 75*(3), E186–E193. https://doi.org/10.1111/j.1750-3841.2010.01549.x.

Huang, Y., Chen, S., Bing, X., Gao, C., Wang, T., & Yuan, B. (2011). Nanosilver migrated into food-simulating solutions from commercially available food fresh containers. *Packaging Technology and Science, 24*, 291–297. https://doi.org/10.1002/pts.938.

Ishizuka Glass Co (2020). *Ishizuka glass Co.* (Accessed on 15.04.2020) https://www.ishizuka.co.jp/english/material/antimicrobial/.

Jokar, M., & Abdul Rahman, R. (2014). Study of silver ion migration from melt-blended

and layered-deposited silver polyethylene nanocomposite into food simulants and apple juice. *Food Additives & Contaminants Part A, 31*(4), 734–742. https://doi.org/10.1080/19440049.2013.878812.

Klein, C. L., Stahlmecke, B., Romazanov, J., Kuhlbusch, T. A. J., Van Doren, E., De Temmerman, P.-J., Mast, J., Wick, P., Krug, H., Locoro, G., Hund-Rinke, K., Kördel, W., Friedrichs, S., Maier, G., Werner, J., Linsinger, T., Gawlik, B. M., Comero, S., Institute for Health and Consumer Protection, ... Institute for Reference Materials and Measurements (2011). *NM-Series of representative manufactured nanomaterials: NM-300 silver characterisation, stability, homogeneity*. Publications Office.http://dx.publications.europa.eu/10.2788/23079.

Kyser, E. A. (1993). *Acid solution absorption of extruded polyethylene foam (WSRC-TR-93-011, 6622519; pp. WSRC-TR-93-011, 6622519)*. https://doi.org/10.2172/6622519.

Llorens, A., Lloret, E., Picouet, P. A., Trbojevich, R., & Fernandez, A. (2012). Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends in Food Science & Technology, 24*(1), 19–29. https://doi.org/10.1016/j.tifs.2011.10.001.

Mackevica, A., Olsson, M. E., & Hansen, S. F. (2016). Silver nanoparticle release from commercially available plastic food containers into food simulants. *Journal of Nanoparticle Research, 18*. https://doi.org/10.1007/s11051-015-3313-x.

Pant, A. (2016). *Einsatz aktiver & intelligenter Materialien in Verpackungen – Status Quo. Recyclingfähigkeit von aktiven und intelligenten Verpackungen. Expert talk in the federal press office*. October Berlin, Germany.

Pehlivan, H., Balköse, D., Ülkü, S., & Tihminlioğlu, F. (2005). Characterization of pure and silver exchanged natural zeolite filled polypropylene composite films. *Composites Science and Technology, 65*(13), 2049–2058. https://doi.org/10.1016/j.compscitech.2005.04.011.

Piringer, O. (2007). *Mathematical modelling of chemical migration from food contact materials. Chemical migration and food contact materials*. Elsevier180–202. https://doi.org/10.1533/9781845692094.2.180 S.

Polat, S., Fenercioglu, H., Unal Turhan, E., & Guclu, M. (2018). Effects of nanoparticle ratio on structural, migration properties of polypropylene films and preservation quality of lemon juice. *Journal of Food Processing and Preservation, 42*, e13541. https://doi.org/10.1111/jfpp.13541.

RAS AG (2015). *AgPURE - technical information*.

Simbine, E. O., Rodrigues, L. da C., Lapa-Guimarães, J., Kamimura, E. S., Corassin, C. H., ... de Oliveira, C. A. F. (2019). Application of silver nanoparticles in food packages: A review. *Food Science and Technology, 39*(4), 793–802. https://doi.org/10.1590/fst.36318.

Störmer, A., Bott, J., Kemmer, D., & Franz, R. (2017). Critical review of the migration potential of nanoparticles in food contact plastics. *Trends in Food Science & Technology, 63*, 39–50. https://doi.org/10.1016/j.tifs.2017.01.011.

von Goetz, N., Fabricius, L., Glaus, R., Weitbrecht, V., Günther, D., & Hungerbühler, K. (2013). Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. *Food Additives & Contaminants Part A, 30*(3), 612–620. https://doi.org/10.1080/19440049.2012.762693.

Welle, F., & Franz, R. (2011). Migration of antimony from PET bottles into beverages: Determination of the activation energy of diffusion and migration modelling compared with literature data. *Food Additives & Contaminants Part A, 28*(1), 115–126. https://doi.org/10.1080/19440049.2010.530296.

Westerband, E. L., & Hicks, A. L. (2018). Life cycle impact of nanosilver polymer-food storage containers as a case study informed by literature review. *Environmental Science Nano, 5*(4), 933–945. https://doi.org/10.1039/C7EN01043E.

World Health Organization (WHO) (2003). *Silver in drinking-water*. WHO. accessed on 15.04.20 https://www.who.int/water_sanitation_health/dwq/chemicals/silver.pdf.

3.3 Antimicrobial effectiveness of beverage containers made from silver doped PET

Publication 3 describes the antimicrobial effectiveness of silver incorporated into and released from PET bottles. Despite the fact that active packaging has gained increasing interest, applications for beverages are rare. Furthermore, the question remains what amount of silver is required for those applications and if this is beyond the proposed European migration limit of 0.05 mg Ag/kg food.

The experiments were performed in distilled water and iced tea using media with no or few constituents, since results from publication 1 showed that those applications are not useful for a medium such as milk. The experiments in distilled water were performed with *Escherichia coli* as a typical indicator for hygienic conditions. For the experiments in iced tea, *Zygosaccharomyces bailii* was chosen as a typical representative for a spoilage yeast in the fruit juice industry. Experiments were performed using silver doped PET bottles and by adding $AgNO_3$ to the liquid model products. Tested $AgNO_3$ concentrations were 0.015 and 0.05 mg/L Ag.

Results showed that it is possible to develop effective antimicrobial packaging for beverages with those concentrations, thereby being below the proposed European migration limit. However, this strongly depends on the medium used due to the interactions of media constituents with Ag^+. For beverages with constituents that can react with Ag^+, silver concentrations need to be close to the proposed European migration limit or even higher. For those applications, there could be an initial silver concentration close to the proposed limit of 0.05 mg Ag/kg food, for example in the form of a silver reservoir to reduce the initial number of bacteria. Subsequent to this action, the bottle could prevent them from growing by continuously delivering Ag^+. However, proposed migration limits for food applications should be kept in mind. Therefore, another antimicrobial substance with a higher proposed migration limit other than silver could be used. Furthermore, those bottles could be used in other sectors such as cosmetics, lacquers, or medicine.

The best potential for beverage applications is seen for water. In water applications, even a high concentration of *E. coli* (10^5 cfu/mL) can be reduced by concentrations of 0.05 mg Ag/kg food. Therefore, such bottles can be useful for astronauts in space for

keeping water sterile over longer periods of time, for soldiers during a war or for emergency supply at sea.

CRediT authorship contribution statement:

Sabrina Braun: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization, Supervision, Project administration.

Vladimir Ilberg: Supervision.

Horst-Christian Langowski: Writing - review & editing, Supervision.

Journal of Food Engineering 300 (2021) 110526

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: http://www.elsevier.com/locate/jfoodeng

Antimicrobial effectiveness of beverage containers made of silver doped PET

Sabrina Braun [a,b,*], Vladimir Ilberg [b], Horst-Christian Langowski [a,c]

[a] Technical University of Munich, TUM School of Life Sciences Weihenstephan (WZW), Weihenstephaner Steig 22, 85354, Freising, Germany
[b] University of Applied Sciences Weihenstephan-Triesdorf, Institute of Food Technology, Am Staudengarten 11, 85354, Freising, Germany
[c] Fraunhofer Institute for Process Engineering and Packaging (IVV), Giggenhauser Strasse 35, 85354, Freising, Germany

ARTICLE INFO

Keywords:
Active packaging
Silver
E. coli
Z. bailii
Distilled water
Iced tea

ABSTRACT

Active packaging based on a release of silver ions (Ag^+) has become a focus of increasing interest. However, applications are rare for beverages in particular, and the question remains whether the amount for antimicrobial effectiveness is below the migration limit of 0.05 mg Ag/kg food proposed by the EFSA. Experiments were performed using *E. coli* (10^6 cfu/mL) in distilled water and *Z. bailii* (<10 cfu/mL) in iced tea by directly adding $AgNO_3$ at concentrations of 0.015 and 0.05 mg/L Ag, and by using manufactured PET bottles containing silver phosphate glass enabling these concentrations to be reached by a release of Ag^+ into the medium. The results showed that these Ag concentrations can inhibit bacteria in distilled water, as well as in iced tea. Ag concentrations of 0.05 mg/L reduced *E. coli* in distilled water by 10^5 cfu/mL and 0.015 mg/L by 10^2 cfu/mL. *Z. bailii* population was significantly reduced by 0.05 mg/L Ag, but cells started to grow again.

1. Introduction

Active packaging is realized by incorporating components that release or absorb substances into or from the packed product or its surrounding environment (European Parliament & Council of the European Union, 2004). The aim thereby is to both increase the shelf life of packed products as well as maintain and improve the quality thereof. Antimicrobial packaging is a form of active packaging which can be realized by incorporating antimicrobial substances into polymers in order to reduce or inhibit the growth of microorganisms in the packaging material or in the packed food (Appendini and Hotchkiss, 2002).

Inorganic antimicrobial agents are best suited for use in the injection molding process with which polyethylene terephthalate (PET) bottles are produced because they can withstand very high temperatures. Silver, for example, can withstand temperatures of up to 800 °C and is known for having a good antimicrobial effect against a wide range of microorganisms. Therefore, active packaging based on the release of silver ions has attracted increasing attention (Llorens et al., 2012). Regarding food applications, silver substituted zeolites are the most widely used of silver-based polymer additives (Appendini and Hotchkiss, 2002). Furthermore, silver nanomaterials have also gained increasing interest (see Bayani Bandpey et al., 2017; Emamifar et al., 2010; Polat et al., 2018). Among metallic cations, the silver ion (Ag^+) is known for having the best antimicrobial effect against a wide range of microorganisms (Llorens et al., 2012).

Most applications seek to prevent surface growth in food, e.g., meat spoilage at the surface, given the assumption that the interior of the food contains very few microorganisms (Appendini and Hotchkiss, 2002). However, applications also exist with regard to liquid products. The literature regarding beverage applications is contradictory. In one respect, positive results have been reported (Bayani Bandpey et al., 2017; Cannarsi et al., 2003; Emamifar et al., 2010; Polat et al., 2018). Otherwise, there are indications that real food applications have failed (Balasubramanian et al., 2009) and that high silver concentrations are required (Lee and Han, 2010). Furthermore, in the studies that reported positive results, the migrated silver amount in the medium often remained unclear or was questionable, as reported below.

Emamifar et al. (2010) prepared nanocomposite low density polyethylene (LDPE) films containing P105 powder (a combination of 95% TiO_2 powder and 5% (w/w) metal nanosilver with a particle diameter of about 10 nm) by means of the melt mixing method. They packed fresh orange juice in packages (similar to the Doypack packaging commonly used for fruit packaging) containing these films and stored them at 4 °C. The results after 28 d showed $2.7 \times 10^5 \pm 1.2$ cfu/mL for yeast and molds compared to $1.8 \times 10^6 \pm 1$ cfu/mL for the reference with pure LDPE, and $3.4 \times 10^4 \pm 1.1$ cfu/mL for total aerobic bacteria compared to the reference, which had $1.9 \times 10^5 \pm 1.1$ cfu/mL. The shelf life of orange

* Corresponding author. University of Applied Sciences Weihenstephan-Triesdorf, Institute of Food Technology, Am Staudengarten 11, 85354, Freising, Germany.
E-mail address: Sabrina.Braun@tum.de (S. Braun).

https://doi.org/10.1016/j.jfoodeng.2021.110526
Received 30 August 2020; Received in revised form 28 November 2020; Accepted 30 January 2021
Available online 4 February 2021

Table of abbreviations

CFU	Colony-Forming Units
EFSA	European Food Safety Authority
LDPE	Low Density Polyethylene
LOD	Limit Of Detection
PE	Polyethylene
PET	Polyethylene Terephthalate
PP	Polypropylene

juice was defined as the time until 10^6 cfu/mL microbial load was reached. The released quantity of Ag ions from nanocomposite LDPE films containing 5% P105 into orange juice was 100 ± 3 ng/L after 28 d. On the basis of our investigations to date, it is highly questionable that this low silver concentration in the medium is able to cause an antimicrobial effect in beverages. Presumably, the real quantity of silver in orange juice was higher. It is possible that only free silver ions in the medium were detected and not the total silver content, including Ag which reacted with constituents of the orange juice.

Cannarsi et al. (2003) investigated the effectiveness of polyethyleneoxide-like coatings containing 7% Ag deposited via plasma on polyethylene on the growth of *Alicyclobacillus acidoterrestris* in malt extract broth, orange juice and apple juice. This film was effective in the case of malt extract broth, achieving a reduction of approximately 10^2 after inoculation. In apple juice, growth occurred after about 30 h without the active film, whereas, in the presence of the active film, no growth occurred during the investigated period of almost 200 h. No antimicrobial effect at all was recognizable in orange juice. No information was provided regarding the testing temperature and the silver concentration released. However, practically the same results for malt extract broth and apple juice were published by Del Nobile et al. (2004), in which case the indicated temperature was 44 °C, and maximum silver concentration released after 5 d was 0.38 ppm into malt extract broth, and 0.25 ppm into apple juice.

Bayani Bandpey et al. (2017) deposited Ag nanoparticles by means of immersion onto previously corona-treated PE-LD films and used these films to prepare antimicrobial pouch packages for pasteurized milk. The pouches were stored at 4 °C, and the shelf life of milk was defined as the time until Hungary's regulatory total plate count limit of 10^5 cfu/mL in pasteurized milk was reached. For milk stored in pouches without Ag nanoparticles, the limit was exceeded after 5 d, whereas, for the packages treated at a corona power of 800 W, it was exceeded after 14 d. Unfortunately, the authors provided unclear data on the quantities deposited onto the PE-LD films and the quantity of silver ions released in water. However, based on the data provided in the unusual unit ppm/area, it can be deduced that up to 1.18 µg of silver per cm^2 of film area was applied, of which up to 60 ng (about 5% of the applied silver) of Ag^+ per cm^2 of film area was released in water. This apparently led to concentrations of Ag^+ of just under 0.05 mg kg^{-1} water, i.e., just below the limit currently being discussed in the EU for food contact applications. What definitely cannot be deduced from their findings is the actual concentration of Ag^+ in the milk. Since the constituents of milk are able to immobilize significant amounts of Ag^+ (Braun et al., 2020a), thus leading to a constant depletion of Ag^+ in the liquid product, it is very likely that the total silver concentration in the milk samples was much higher than the amount measured in distilled water.

Polat et al. (2018) investigated polypropylene (PP)-based P105 nanocomposite films obtained by the blown film method on packaged lemon juice during storage for 30 d at 4 °C. The films demonstrated a significant difference between control and total aerobic bacteria or total yeast-mold. However, the difference was maximum a factor of 2.29. The silver concentration released from these in 3% acetic acid after 10 d at 40 °C was 20.46 ± 0.16 µg/kg food simulant.

(An et al., 1998, cited from Lee and Han, 2010) investigated the growth of *Escherichia coli* (*E. coli*) and the total microbial count in Oolong tea packed in silver-zeolite impregnated PE stored at 25 °C. After 24 h, the *E. coli* was reduced by approximately 3×10^4 cfu/g, and the control by 3×10^1 cfu/g. The total microbial count after 24 h was reduced by approximately 10^3 cfu/g, and the control by 3×10^1 cfu/g.

Braun et al. (2020a) determined the minimal required silver concentration for significant antimicrobial effectiveness in milk as at least 5 mg/L.

Llorens et al. (2012) claimed that in water or low-buffered systems, around 50–100 µg Ag^+/kg food are required for antimicrobial effectiveness. However, in the presence of proteins, higher concentrations were necessary; i.e., about 10–100 mg Ag^+/kg food for realistic food applications. For example, 60 mg Ag^+/kg food led to a reduction of spoilage in beef (Fernández et al., 2010b). A higher reduction of microbial population at the same concentration was found for melon pieces (Fernández et al., 2010a).

The European Food Safety Authority (EFSA, 2006 & 2011) released a positive opinion concerning the use of silver containing glass and the use of a certain silver (Ag) zeolite in food contact surfaces with a general specific migration limit of 0.05 mg Ag/kg food.

The objective of this study was to discover whether silver can bring about an antimicrobial effect in beverages within the proposed European migration limit. Studies so far were contradictory or questionable as reported above. This study will help industry and research by deciding whether it is meaningful to invest in such an application and brings them more clarity about the limitations of it. In milk, no antimicrobial effect within this proposed limit of 0.05 mg Ag/kg food is to be expected given the chemical interactions of the silver ions with the milk proteins or the chloride of the milk (Braun et al., 2020a). Therefore, further tests were performed using a medium containing fewer proteins in order to be able to determine whether any antimicrobial effect whatsoever can be achieved in beverages using an application of this kind within the proposed migration limit. Considering the results regarding the improved release of Ag^+ from a bottle into a medium with low pH (Braun et al., 2020b), it was also advisable to choose an acidified product, which would be expected to provide better antimicrobial efficacy. For this reason, and that of working with a medium with few constituents, iced tea was chosen. For the experiments in iced tea, *Zygosaccharomyces bailii* (*Z. bailii*) was chosen as a typical representative for spoilage in fruit juice concentrates. *Z. bailii* is the most common and dangerous yeast in fruit juice concentrates and is most frequently found in citrus concentrates or concentrated apple juice. *Z. bailii* produces CO_2 in addition to alcohol, which can lead to overpressure and, in severe cases, even to the bursting of a sealed container. Furthermore, it can produce undesired odors and flavors (Back, 2000). Moreover, *Z. bailii* is found in iced tea (Stratford et al., 2013). Tests were also performed in distilled water in order to achieve a result entirely independent of the interactions between silver ions and media constituents. For the experiments in distilled water, *E. coli* was chosen as a typical indicator of poor hygienic conditions (Baumgart et al., 2016). Guidelines or limit values exist for *E. coli* respecting nearly all foodstuffs, especially drinking water (Eisgruber and Stolle, 2004). For example, the German drinking water regulation (TrinkwV, 2001) says that no *E. coli* may be detected in 100 mL of drinking water intended for human consumption. The limit is even stricter for water that is additionally bottled and distributed: No *E. coli* at all may be found in 250 mL of water.

2. Materials and methods

2.1. Medium, bacterial strain, and materials

Peach iced tea (pH = 2.95) was prepared from a granulate (Krüger GmbH & Co. KG, Bergisch Gladbach, Germany). A granulate was used given its stability in terms of microbiology, so a standardized iced tea was able to be produced therefrom for all the experiments. The iced tea

was produced by weighing 35 g of granulate and adding 500 mL of distilled water, resulting in a sugar content of 16.6 g/L. This was mixed by shaking per hand and autoclaved at 121 °C for 15 min.

Z. bailii was purchased from DSMZ (DSM No.: 70492). A culture was produced in universal medium for yeasts (YM, DSMZ medium 186). The cultures were plated on malt extract agar (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) which was acidified to pH < 4 by adding 0.5% acetic acid (stock solution ≥ 99%, Supra quality, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) over a syringe filter into the autoclaved liquid agar according to Pitt and Hocking (2009). The plates were incubated aerobically for 5 d at 25 °C.

E. coli was purchased from DSMZ (DSM No.: 423). An overnight culture was produced in caso bouillon from Carl Roth GmbH + Co. KG (Karlsruhe, Germany) on a shaker at 37 °C and 119 rpm. The cultures were plated on VRBD agar (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and incubated for 24 h at 37 °C.

Silver nitrate (0.1 M concentrated liquid solution, Titrisol, Supelco) and NaCL from Merck KGaA (Darmstadt, Germany) were used.

2.2. Manufacturing of the bottles made from silver doped PET

The silver doped bottles were manufactured as described in Braun et al. (2020b). PET resin (Selenis MASTER 10, Ribeira de Nisa Portalegre, Portugal) with an intrinsic viscosity of 0.86 ± 0.02 dL/g served as the raw material for the preforms. IONPURE WPA < 10 μ (Ishizuka Glass Co., Ltd, Iwakura City, Aichi, Japan) with silver phosphate glass as the active component, and a silver content of 1.6% (w/w) according to the manufacturer, was incorporated in powder form into the PET at 10% (w/w). Based on this mixture, the preforms were produced by an Allrounder 470 S injection molding machine from Arburg (Loßburg, Germany). The preforms containing the antimicrobial agent weighed 16.9 g. The calculated total Ag content in the bottle was therefore 0.027 g.

Bottles were produced by a stretch blow laboratory machine (Vema, Altlachen, Germany) from the manufactured preforms at a blowing pressure of 15 bar. The volume of the bottles was 500 mL, the height was 208 mm, and the wall thickness was 0.27 mm on average. Control bottles with no antimicrobial filler served as a reference in the experiments and were produced in the same manner.

2.3. Determining antimicrobial effectiveness

500 mL of iced tea or distilled water was filled into glass bottles for the experiments in sections 3.1 and 3.3, and into silver doped or plain PET bottles for the experiments in sections 3.2 and 3.4. The media and glass bottles used were autoclaved before the experiments at 121 °C for 15 min, and the PET bottles were irradiated with gamma radiation at 25.8 kGy by Synergy Health (Allershausen, Germany) before use.

The desired silver concentration in water or iced tea was added in the experiments in sections 3.1 and 3.3. Tested silver concentrations were 0.05 mg/L of final contents, based on the migration limit proposed by the European Food Safety Authority (EFSA, 2006 & 2011) for silver-zinc-sodium-ammonium-alumino-silicate, and 0.015 mg/L of final contents, based on the US Food and Drug Administration (2009) that permits the direct addition of silver nitrate ($AgNO_3$) into commercially bottled water at concentrations up to 17 μg/kg.

500 μL of produced *E. coli* suspension and 5 μL of produced *Z. bailii* suspension were added to obtain an initial cell count of approximately 10^6 cfu/mL for *E. coli* and < 10 cfu/mL for *Z. bailii*. The initial cell count chosen for *Z. bailii* was intentionally low due to the expected interactions of Ag^+ with food constituents and the lower antimicrobial effectiveness expected thereby. Moreover, the initial cell count of bottled iced tea was expected to be low. The initial cell count chosen for *E. coli* was intentionally high in order to simulate highly contaminated drinking water. Furthermore, no growth of *E. coli* was expected in distilled water. Therefore, the mortality kinetics of *E. coli* could be better evaluated by choosing a high initial cell count.

For every test condition, a zero sample containing the medium used or the medium used plus added silver, both without inoculation, served as a control used for ensuring sterile conditions. These samples were treated in the same manner as the inoculated samples. No contamination was found for the zero samples in any of the experiments.

Antimicrobial effectiveness was evaluated by means of the spread plate method using malt extract agar for the enumeration of *Z. bailii*, and VRBD agar for the enumeration of *E. coli*. Iced tea or water samples were taken at certain times, and decimal dilutions were prepared with a sterile saline solution (0.9% NaCl). Volumes of dilution samples (0.1 mL) were then used. Serial dilutions of bacterial suspensions were performed in order to select the optimal dilution of inocula. Bacteria from the selected solutions were transferred onto the plates. The results were expressed as colony-forming units (cfu) per milliliter of beverage.

2.4. Determining the silver amount in the bottles made of silver doped PET and that released from the bottles into the simulant

The release of silver from the bottles into the simulant was determined as described in Braun et al. (2020b). For every test condition, a control bottle containing no antimicrobial substance was used which was treated in the same manner as the others. 500 mL of pure distilled water, or a solution of 0.5 or 3% (w/w) acetic acid (stock solution ≥ 99%, Supra quality, Roth, Karlsruhe, Germany) in distilled water was filled into the bottles. These were stored at 43 or 21 °C. According to EU No. 10/2011 (European Commission, 2011), 3% acetic acid is an official food simulant. 10 mL sample was taken twice after shaking the bottle ten times per hand, always in the same manner at defined time points and pipetted into PP tubes (Cellstar, Greiner Bio-One, Frickenhausen, Germany) for further analysis. The pipette tip was changed for every sample withdrawal to ensure zero silver contamination, and the tubes were washed with 2% nitric acid and distilled water before use. 100 μL nitric acid (p.a. 65%, Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany; specially purified by subboiled distillation) was added for stabilization to each sample. The samples were packed in aluminium foil and stored cool until measuring. An identical contact area with the bottle wall was ensured by refilling 20 mL water, or 0.5 or 3% acetic acid, after every sample withdrawal. The measured values were adjusted to the dilution resulting from the repeated refilling of 20 mL.

The total silver content in the simulants was analyzed according to DIN EN ISO 17294-2 (01/2017) using a Nexion 2000 ICP-MS (PerkinElmer, Waltham, USA) and the setup shown in Table 1. An Ag multi-element ICP-MS calibration standard (Roth, Karlsruhe, Germany) in 2% HNO_3 served for calibration, and rhodium and rhenium were used as internal control standards. The limit of detection (LOD) of the method was 0.1 μg/L for Ag.

The actual silver content in the bottle wall was determined using the same method of analysis, and the samples were prepared according to

Table 1
ICP-MS settings (modified from Braun et al., 2020b).

RF generator power	1250 W
Plasma gas	Argon, 15 L/min
Flow rates:	
Nebulization gas	1.04 L/min
Auxiliary gas	1.2 L/min
Sample	1 mL/min
Nebulizer	Meinhard nebulizer
Spray chamber	Meinhard cyclonic spray chamber
Monitored masses	^{107}Ag and ^{109}Ag
Dwell time	1 s with 3 repeats

DIN EN 16711-1 (04/2014). Samples (3 × 3 cm) were taken at ten different parts in order to avoid possible fluctuations due to an inhomogeneous distribution of the silver material dispersed in the bottle. The samples were crushed manually and processed into one homogeneous sample in an analysis mill from IKA (Staufen im Breisgau, Germany) at 25,000 min^{-1}. The digestion was performed with 250 mg of the crushed material and 6 mL of concentrated nitric acid (HNO_3) in a microwave (multiwave PRO) from Anton Paar (Graz, Austria) at 250 °C for 45 min.

2.5. Statistical analysis of the experiments

All experiments were performed in triplicate, with the exception of the *Z. bailii* count in iced tea filled in PET bottles (duplicate). The cell counts determined and the Ag^+ concentrations measured are illustrated along with their confidence intervals for the average values calculated using a *t*-distribution ($\overline{x} \pm \frac{t^*SD}{\sqrt{N}}$). Significance was expressed at the 5% level.

3. Results and discussion

3.1. Antimicrobial effectiveness of silver on Z. bailii in iced tea

Fig. 1 shows the development of *Z. bailii* count in iced tea in the presence of 0.015 mg/L (point) and 0.05 mg/L silver (square), compared to the reference without silver (triangle) at 25 °C. The reference without silver shows the highest cell count, followed by the development of *Z. bailii* in iced tea containing 0.015 mg/L silver. In the iced tea containing 0.05 mg/L silver, the cell count decreased within 24 h. However, cells started to grow after 3 d, since these were not completely eliminated by 0.05 mg/L Ag. Nevertheless, the total cell count was lower than the reference over the investigated time period of 28 d.

Therefore, it can be concluded that an antimicrobial effect is able to be obtained within the proposed migration limit of 0.05 mg/L (EFSA, 2006 & 2011).

3.2. Antimicrobial effectiveness of silver doped PET bottles on Z. bailii in iced tea

Fig. 2 shows the development of *Z. bailii* in iced tea filled into PET bottles containing silver phosphate glass compared to plain PET bottles at 27 °C. The cell count increased within 3 d by a factor of 10^6. There was no difference regarding antimicrobial effectiveness between the plain PET bottles and the bottles containing silver phosphate glass over a time period of 28 d. Compared to Fig. 1, the difference in the maximum cell count can be explained by temperature differences.

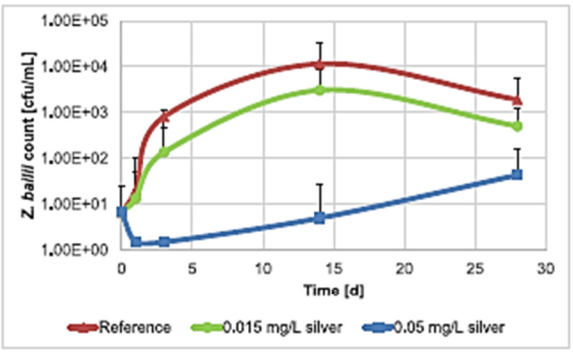

Fig. 1. Development of *Z. bailii* count in iced tea in the presence of 0.015 mg/L (point) and 0.05 mg/L (square) silver compared to the reference without silver (triangle) at 25 °C.

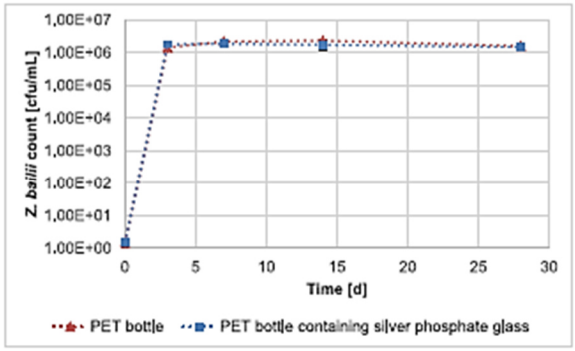

Fig. 2. Development of *Z. bailii* count in iced tea filled in PET bottles containing silver phosphate glass (square) compared to pure PET bottles (triangle) at 27 °C.

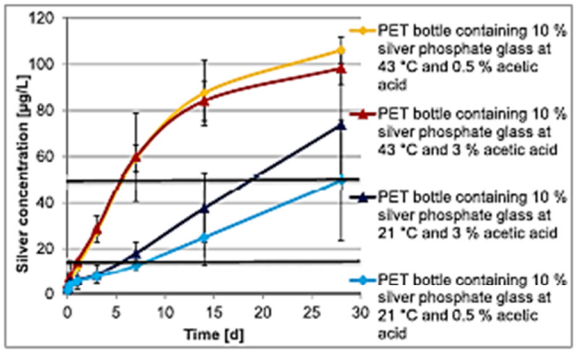

Fig. 3. Release of silver from silver doped PET bottles into acetic acid (0.5 and 3%) at 21 and 43 °C. The investigated Ag concentrations of 0.015 and 0.05 mg/L were drawn in with a line (modified from Braun et al., 2020b).

Fig. 3 shows the release of silver from silver doped PET bottles into acetic acid (0.5 and 3%) at 21 and 43 °C. The investigated Ag concentrations of 0.015 and 0.05 mg/L were drawn in with a line. As expected, the plain bottles showed no silver release at all (not drawn in Fig. 3). Determined silver concentration in the bottle wall was $\overline{x} = 1420\ mg\ kg^{-1}$ with $x_{min} = 1225\ mg\ kg^{-1}$ and $x_{max} = 1545\ mg\ kg^{-1}$.

The problem in this application is that, at the beginning, insufficient Ag^+ was released from the bottle for achieving antimicrobial effectiveness. The silver release into iced tea can be estimated from Fig. 3 by using the mean value from the observed release into 0.05% acetic acid (pH 3.3) and 3% acetic acid (pH 2.7), since the pH in iced tea was 2.95. The corresponding values estimated for iced tea were about 0.015 mg/L after 7 d at 21 °C, and above 0.05 mg/L after 28 d. Within 28 d, enough silver should be available for antimicrobial effectiveness, but the cell count was then already too high to observe an effect. This is in line with Lee and Han (2010). They indicated that antimicrobial packaging works best at a low microbial contamination. This application could probably work when cells lack the optimum conditions for growth, or when 0.05 mg/L Ag is added at the beginning, with the bottle then ensuring silver delivery such that no growth can occur. However, the problem for silver in food applications is that the proposed migration limit of 0.05 mg/L Ag is then exceeded. This could be useful for applications outside the food sector, however.

3.3. Antimicrobial effectiveness of silver on E. coli in distilled water

Fig. 4 illustrates the development of *E. coli* count in distilled water in the presence of 0.015 mg/L silver (point) and 0.05 mg/L silver (square) compared to the reference, which lacked silver (triangle). The cell count decreased by approximately a factor of 10^2 in the presence of 0.015 mg/L, and by a factor of 10^4 in the presence of 0.05 mg/L within 24 h, and by 10^5 within 48 h. After 28 d, a further sample was taken, in which case the cell count was similar to that after 48 h by adding 0.05 mg/L silver. Accordingly, one advantage of the bottle could be that the continous delivery of Ag^+ eliminates the entire population of *E. coli*.

As assumed, it can be concluded that the antimicrobial effect is better in distilled water than in iced tea, since no disturbing media components are present that can react with Ag^+ ions. By adding 0.05 mg/L Ag, *E. coli* can be reduced by a factor of 10^4. Given a lower initial cell count, which is more realistic in practical applications, 0.05 mg/L Ag or less is sufficient for eliminating *E. coli* in such an application. As an example, the limit value for *E.coli* for good quality bathing water, which could be used as drinking water, is 10 cfu/mL (Directive, 2006/7/EC of the European Parliament and of the council, 2006).

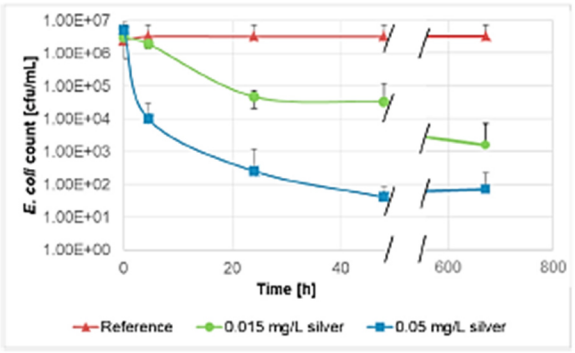

Fig. 4. Development of *E. coli* count in distilled water in the presence of 0.015 mg/L (point) and 0.05 mg/L silver (square) compared to the reference without silver (triangle) at 21 °C.

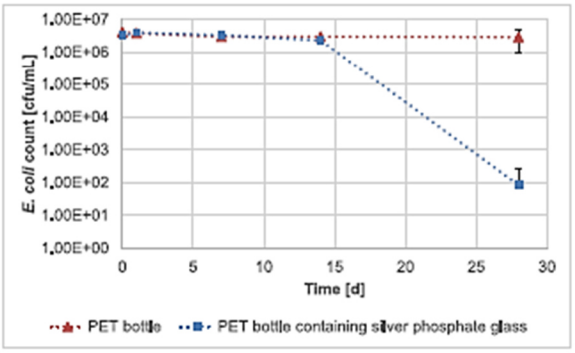

Fig. 5. Development of *E. coli* count in distilled water in a PET bottle containing silver phosphate glass (square) compared to a plain PET bottle (triangle) at 21 °C.

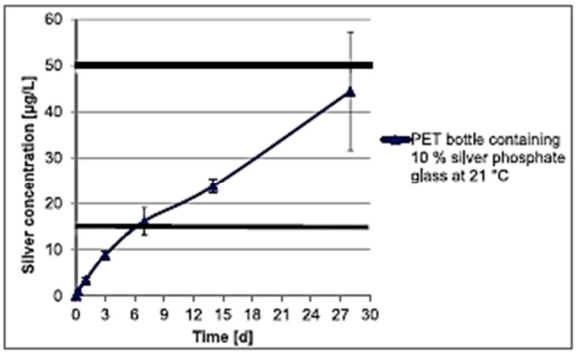

Fig. 6. Release of silver from a silver doped PET bottle into distilled water at 21 °C (modified from Braun et al., 2020b).

3.4. Antimicrobial effectiveness of silver doped PET bottles on E. coli in distilled water

Fig. 5 shows the development of *E. coli* count in distilled water in a PET bottle containing silver phosphate glass compared to a plain PET bottle at 21 °C. The cell count remained approximately constant over a time period of 14 d. After 28 d, the cell count in the bottle containing silver phosphate glass had decreased by approximately 3×10^4, whereas the cell count in the plain PET bottle remained constant. After 28 d, approximately 0.05 mg/L of silver was released into distilled water (Fig. 6). The results fit with those in 3.3, in which case the *E. coli* was reduced by 10^4 in the presence of 0.05 mg/L. According to the silver release from the bottle in Fig. 6, it would be expected that an antimicrobial effect would have already been observed between days 7 and 14, when 15 µg/L Ag was exceeded. However, the effect was delayed, occurring between day 14 and day 28. One possible explanation might be that, in addition to the Ag ions, the freshly produced PET might also release residual monomers or other production aids into the surrounding water. These substances themselves could then act as complexing agents and lead to an additional complexation of the first released Ag ions, so that the antimicrobial effective concentration of 15 µg/L by free Ag ions is only exceeded when these complexing agents are completely saturated. Typical substances in PET bottles are e.g. the monomers ethylene glycol and terephthalic acid or the catalyst antimony trioxide which are also subjected to migration limits (30, 7.5 and 0.04 mg/L) (Welle, 2016).

3.5. Final discussion

The results suggest that it is indeed possible to develop effective antimicrobial packaging for beverages based on silver having a release rate below the proposed migration limit of 0.05 mg Ag per kg food (EFSA, 2006 & 2011). However, the concentrations need to be near this proposed limit. Above all, it should also be considered that bacteria can grow again if they are merely inhibited and not completely reduced. Before putting such food packaging on the market, there would have to be certainty about the minimal inhibitory concentration (MIC) for the target organism in the medium used as well as how the appropriate silver release rate can be guaranteed by choosing an appropriate initial silver concentration in the polymer and regarding typical storage temperature of the intended used medium. In this context, it is important to ensure that the proposed silver migration level in the medium is not exceeded. Furthermore, the type of polymer plays an important role

with respect to different diffusion coefficients. In this study PET was chosen, since it is the polymer of choice for bottles. Nevertheless, for future application polymers having higher diffusion coefficients than PET such as polyethylene (PE) are also conceivable, whereby higher silver release rates are expected within a shorter time. Using PE lower initial silver concentrations in the polymer can be used for not exceeding the proposed migration limit. The antimicrobial effectiveness is also dependent on the type of used silver additive (Braun et al. 2020a, 2020b). Successes with different silver additives incorporated into PE were e.g. achieved by Marchetti et al. (2016) or Cerrillo et al. (2020). Cerrillo et al. (2020) further demonstrated that the type of zeolitic structure as well as the zeolite Si/Al ratio strongly influences antibacterial effectiveness. For creating the ideal packaging, all these aspects have to be kept in mind.

Braun et al. (2020b) suggested that multiple applications weaken the antimicrobial effectiveness, since the released silver concentration is less. Therefore, if reuse of such a bottle is planned, it has to be determined how often it is possible to reuse the bottle again for still achieving an antimicrobial effect. This study further demonstrates that it is possible to eliminate a very high concentration (10^5 cfu/mL) of *E. coli*, which can also be pathogenic, with silver concentrations of 0.05 mg/L Ag. Moreover, media with constituents that can react with silver ions are more problematic for practical applications, whereas water is unproblematic. Therefore, the best potential is seen for water applications. Possible applications for such a bottle filled with water could be for astronauts in space, where water should be sterile over a long period of time, e.g., as a portable alternative to potable water systems in spacecraft in which silver ions are sometimes used (Birmele et al., 2011). Further applications could be for an emergency supply at sea, or for soldiers during a mission as an alternative for their field bottle if an additional outer wall over the bottle is created for more stability, or simply as outdoor equipment for a hiking tour. For other beverage applications, there could be a silver concentration near the proposed limit of 0.05 mg/L Ag at the outset, for example in the form of a silver reservoir used to reduce the bacteria from the beginning and prevent them from growing by continuously delivering Ag^+. However, the migration limit proposed for food applications must also be kept in mind. Therefore, another antimicrobial substance in which the proposed limit is higher could be more profitable for beverage or food applications. Moreover, another industry sector, e.g., cosmetics, medicine, or lacquer, might conceivably benefit from such a bottle.

4. Conclusion

The results show that silver can cause an antimicrobial effect in distilled water as well as in iced tea within the proposed European migration limit of 0.05 mg Ag per kg food. Therefore, silver could be useful for beverage packaging purposes. However, its suitability strongly depends on the medium used, due to the interactions between the filled product and the packaging. Therefore, the best potential is seen for water applications like for astronauts in space, where water should be sterile over a long period of time, for an emergency supply at sea, for soldiers during a mission, or simply as outdoor equipment for a hiking tour. For market applications several aspects have to be kept in mind like choosing the appropriate combinations of initial silver content in the bottle wall, the required concentration for antimicrobial effectiveness in the intended used medium as well as its intended storage temperature.

CRediT author contribution statement

Sabrina Braun: Conceptualization, Methodology, Validation, Investigation, Writing - original draft, Visualization, Supervision, Project administration. Vladimir Ilberg: Supervision. Horst-Christian Langowski: Writing - review & editing, Supervision.

Declaration of competing interest

None.

Acknowledgments

The Federal Ministry for Economic Affairs and Energy financially supported this research project on the basis of a resolution of the German Bundestag. The authors would like to thank Josef Gleixner from Inotech for producing the bottles, Synergy Health for irradiating the PET bottles, the Research Center Weihenstephan for Brewing and Food Quality as well as our student Teresa Schwind for their contribution to this work. TUM proofreading support is gratefully acknowledged.

References

An, D.S., Hwang, Y.I., Cho, S.H., Lee, D.S., 1998. Packaging of fresh curled lettuce and cucumber by using low density polyethylene films impregnated with antimicrobial agents. Journal of Korean Society of Food Science and Nutrition 4 (27), 675–681.

Appendini, P., Hotchkiss, J.H., 2002. Review of antimicrobial food packaging. Innovat. Food Sci. Emerg. Technol. 3 (2), 113–126. https://doi.org/10.1016/S1466-8564(02)00012-7.

Back, W., 2000. *Fruchtsaft- und Limonadenbetriebe, Wasser, Betriebshygiene, Milch und Milchprodukte, Begleitorganismen der Getränkeindustrie*. Nuremberg. Fachverlag Hans Carl.

Balasubramanian, A., Rosenberg, L.E., Yam, K.L., Chikindas, M.L., 2009. Antimicrobial packaging: potential vs. Reality—A Review 3 (4).

Baumgart, J., Becker, B., Stephan Hrsg., R., 2016. Mikrobiologische Untersuchung von Lebensmitteln: Ein Leitfaden für das Studium, sixth ed. (Behr).

Bayani Bandpey, N., Aroujalian, A., Raisi, A., Fazel, S., 2017. Surface coating of silver nanoparticles on polyethylene for fabrication of antimicrobial milk packaging films. International Journal of Dairy Technology 70 (2), 204–211. https://doi.org/10.1111/1471-0307.12320.

Birmele, M., Roberts, M., McCoy, L., 2011. Disinfection of spacecraft potable water systems by passivation with ionic silver. In: 41st International Conference on Environmental Systems. 41st International Conference on Environmental Systems, Portland, Oregon. https://doi.org/10.2514/6.2011-5278.

Braun, S., Ilberg, V., Blum, U., Langowski, H., 2020a. Nanosilver in dairy applications – antimicrobial effects on *Streptococcus thermophilus* and chemical interactions. International Journal of Dairy Technology 73 (2), 376–383. https://doi.org/10.1111/1471-0307.12686.

Braun, S., Ilberg, V., Blum, U., Langowski, H.-C., 2020b. Release of silver from silver doped PET bottles. Food Packaging and Shelf Life 25, 100517. https://doi.org/10.1016/j.fpsl.2020.100517.

Cannarsi, M., Altieri, C., Del Nobile, M.A., Favia, P., Iacoviello, G., D'Agostino, R., 2003. Study and development of an antimicrobial packaging system based on the release of silver ions. Ital. J. Food Sci. 15, 166–172.

Cerrillo, J.L., Palomares, A.E., Rey, F., 2020. Silver exchanged zeolites as bactericidal additives in polymeric materials. Microporous Mesoporous Mater. 305, 110367. https://doi.org/10.1016/j.micromeso.2020.110367.

Del Nobile, M.A., Cannarsi, M., Altieri, C., Sinigaglia, M., Favia, P., Iacoviello, G., D'Agostino, R., 2004. Effect of Ag-containing nano-composite active packaging system on survival of Alicyclobacillus acidoterrestris. J. Food Sci. 69 (8), E379–E383. https://doi.org/10.1111/j.1365-2621.2004.tb09899.x.

Eisgruber, H., Stolle Hrsg., A., 2004. *Mikrobiologische Kriterien für Lebensmittel: Zusammenstellung aktueller Vorschriften aus Deutschland und der Schweiz* (first revised reprint) (Behr).

Emamifar, A., Kadivar, M., Shahedi, M., Soleimanian-Zad, S., 2010. Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice. Innovat. Food Sci. Emerg. Technol. 11 (4), 742–748. https://doi.org/10.1016/j.ifset.2010.06.003.

European Commission, 2011. Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. Official Journal of the European Union L 12, 1–89.

European Food Safety Authority (EFSA), 2006. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to the 12th list of substances for food contact materials. EFSA Journal 395–401.

European Food Safety Authority (EFSA), 2011. Scientific Opinion on the safety evaluation of the substance, silver zeolite A (silver zinc sodium ammonium alumino silicate), silver content 2 – 5 %, for use in food contact materials. EFSA Journal 9 (2). https://doi.org/10.2903/j.efsa.2011.1999, 1999.

European Parliament & Council of the European Union, 2004. Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official Journal of the European Union L338, 4–17.

European Parliament & Council of the European Union, 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Official Journal of the European Union*. L 64, 37–51.

Fernández, A., Picouet, P., Lloret, E., 2010a. Cellulose-silver nanoparticle hybrid materials to control spoilage-related microflora in absorbent pads located in trays of fresh-cut melon. Int. J. Food Microbiol. 142 (1–2), 222–228. https://doi.org/10.1016/j.ijfoodmicro.2010.07.001.

Fernández, A., Picouet, P., Lloret, E., 2010b. Reduction of the spoilage-related microflora in absorbent pads by silver nanotechnology during modified atmosphere packaging of beef meat. J. Food Protect. 73 (12), 2263–2269. https://doi.org/10.4315/0362-028X-73.12.2263.

Food and Drug Administration, 2009. Food additives permitted for direct addition to food for human consumption. Silver Nitrate and Hydrogen Peroxide 74, 11476–11478. FR 11476. https://www.federalregister.gov/documents/2009/03/18/E9-5852/food-additives-permitted-for-direct-addition-to-food-for-human-consumption-silver-nitrate-and.

Lee, D.S., Han, J.H., 2010. In: Zhang, H.Q., Barbosa-Cánovas, G.V., Balasubramaniam, V. M., Dunne, C.P., Farkas, D.F., Yuan Hrsg, J.T.C. (Eds.), Nonthermal Processing Technologies for Food: Zhang/Nonthermal Processing Technologies for Food. Wiley-Blackwell. https://doi.org/10.1002/9780470958360.

Llorens, A., Lloret, E., Picouet, P.A., 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. https://doi.org/10.1016/j.tifs.2011.10.001.

Marchetti, F., Palmucci, J., Pettinari, C., Pettinari, R., Marangoni, M., Ferraro, S., Giovannetti, R., Scuri, S., Grappasonni, I., Cocchioni, M., Maldonado Hodar, F.J., Gunnella, R., 2016. Preparation of polyethylene composites containing silver(I) acylpyrazolonato additives and SAR investigation of their antibacterial activity. ACS Appl. Mater. Interfaces 8 (43), 29676–29687. https://doi.org/10.1021/acsami.6b09742.

Pitt, J.I., Hocking, A.D., 2009. Fungi and Food Spoilage. Springer.

Polat, S., Fenercioglu, H., Unal Turhan, E., Guclu, M., 2018. Effects of nanoparticle ratio on structural, migration properties of polypropylene films and preservation quality of lemon juice. J. Food Process. Preserv. 42 (4), e13541 https://doi.org/10.1111/jfpp.13541.

Stratford, M., Steels, H., Nebe-von-Caron, G., Novodvorska, M., Hayer, K., Archer, D.B., 2013. Extreme resistance to weak-acid preservatives in the spoilage yeast Zygosaccharomyces bailii. Int. J. Food Microbiol. 166 (1), 126–134. https://doi.org/10.1016/j.ijfoodmicro.2013.06.025.

Trinkwasserverordnung - TrinkwV. https://www.gesetze-im-internet.de/trinkwv_2001/BJNR095910001.html, 2001.

Welle, F., 2016. Verpackungsmaterial aus Polyethylenterephthalat (PET). DLG-Expertenwissen 4, 6.

4. Discussion

4.1. Scientific questions

The discussion is based on the following questions.

4.1.1 Can silver achieve an antimicrobial effect in milk?

Publication 1 showed that silver ions can achieve an antimicrobial effect in milk at all investigated temperatures (23, 33 and 43 °C). However, the concentrations required were high with at least 5 mg/L for Ag and 50 mg/L for AgNPs and therefore, above recommended use levels for Ag. The reason is the interaction of Ag^+ with milk constituents. Results suggested that antimicrobial effectiveness does not occur before milk proteins are saturated. Below this point only a very small number of free silver ions are available while these are in equilibrium with the proteins. Therefore, relatively few would be able to interact with bacterial proteins to produce an antimicrobial effect. Differences between Ag and AgNPs can be explained by the fact that every silver ion is set free upon the addition of $AgNO_3$, whereas only about one in ten silver atoms is liberated from nanoparticles to become a free silver ion. Based on these investigations, the use of silver for dairy applications is not recommended.

Few studies showed that an antimicrobial effect in milk can be achieved with AgNPs. Yildiz & Pala (2012) tested the antimicrobial effect of AgNP coated metal braids in cow milk. They achieved a microbial reduction of 0.71 log cfu/mL after 1 h at 22 °C with a silver amount released of 6.1 ± 0.4 µg/L. Based on the results from publication 1, this silver amount seems to be very low for achieving an antimicrobial effect in milk. The authors, however, did not describe how the sample was prepared for ICP-MS analysis. Possibly, they only measured the concentration of free Ag^+ in milk and not the part which reacted with milk constituents. Bayani Bandpey et al. (2017) achieved a shelf life extension of about 9 d for pasteurized milk by pouches made of corona-treated PE-LD and deposited with AgNPs. They measured a silver amount released into distilled water of approximately just under 0.05 mg Ag/kg water as described in 1.3. However, the silver concentration actually released into milk remained unclear. Taking into account that the constituents of milk are able to immobilize significant amounts of Ag^+ (Braun et al., 2020a), thus leading to a constant depletion of Ag^+ in the liquid medium,

it is very likely that the total silver concentration in milk was much higher than the amount measured in distilled water.

4.1.2 Do silver based additives incorporated into PET release silver ions in measurable quantities?

There is a quantity of studies investigating the release of silver ions from food contact polymers such as polyethylene, low density polyethylene, polypropylene, polyamide or polylactic acid and most times nanosilver is used for incorporation. For PET data are lacking, although PET being the most polymer material used for beverage packaging. Those data would be helpful for food packaging industry and further for health safety policies. To answer the question if silver compounds incorporated into PET release silver ions in measurable quantities, silver phosphate glass and nanosilver were incorporated into PET bottles and the release of silver ions into food simulants was detected by ICP-MS.

Results from publication 2 showed that within a detection limit of 0.1 µg Ag/L of food simulant, silver ions were released from bottles where silver phosphate glass was incorporated, but not from bottles where nanosilver was incorporated. This was observed even though the total Ag content in the bottles containing nanosilver (650 mg Ag/kg PET) was higher than in the bottles containing silver phosphate glass (470 mg Ag/kg PET). The difference between the two materials is that the silver phosphate glass was embedded in a special glass matrix, which can retain Ag^+ and releases it in the presence of water. Contrarily, the diffusion of water into polymers containing AgNPs, first, has to achieve the oxidation of elemental silver particles (Ag^0) from AgNPs to Ag^+ ions. Then the Ag^+ ions should diffuse from the bulk material to the surface (Damm & Münstedt, 2008), while nanoparticles should remain in the plastic (Störmer et al., 2017). This process seems to be very slow. This is further underlined by the results shown in publication 1, where nanosilver was less effective due to slower or no release of silver ions from AgNPs. If silver is released from silver nanoparticles, this occurs in the form of silver ions as investigations from Bott et al. (2012) showed.

4.1.3 What would be the active principle of an appropriate container and on what parameters does the release of silver ions depend?

The release partly indicated Fickian diffusion behavior as shown in publication 2 (Figures 5 and 6) and diffusion coefficients of silver ions in PET lay between 3.2×10^{-17} and 1.7×10^{-15} cm^2/s. The effective diffusion coefficients obtained from Ag in PET bottles filled with distilled water at 43 °C (2.7×10^{-16} … 9.8×10^{-16} cm^2/s) lay in a similar range as those from antimony in PET bottles (1.4×10^{-16} cm^2/s) at 45 °C (Alt et al., 2018).

The mechanism of action of the bottles is discussed as follows:

1. The water penetrates into the PET surface
2. The simulant accumulates around the silver phosphate glass particles and forms hydrated Ag ions
3. These Ag ions diffuse through the PET to the surface, which is the time-determining step. Only in this step Fickian diffusion behavior is assumed
4. The Ag ions diffuse from the polymer surface into the contact medium

It was further investigated whether a higher release rate can be achieved by changing the geometry. This was done by producing bellows with a ratio of surface to filling volume about twice as high as for the bottles. As expected, the release rate was about twice as high. This should be kept in mind for future applications, as it could lead to better antimicrobial effects. In contrast, the surface-specific release rate was as expected about the same for the bottles and the bellows, since this depends not on the geometry but only on the plastic.

Welle & Franz (2011) reported that the amount of migrated substance is dependent on temperature, time, amount, and type of substance in the polymer and its solubility in food. This can be confirmed for the PET bottles containing silver. The release of silver from the bottles was dependent on the percentage of antimicrobial filler and temperature. Higher concentrations, as well as higher temperatures, resulted in higher Ag^+ release rates. The concentrations tested were 3 and 10%, corresponding to 470 and 1600 mg Ag/kg PET, and the temperatures tested were 21 and 43 °C. Another influence parameter was the acidic strength of the simulant. Rates released into 0.5 or 3% acetic acid, as the simulant for acidic beverages such as fruit juice or iced tea, were higher than those into distilled water. The reason is a higher rate of diffusion into the polymer film in acidic conditions, since the water sorption capacity of PET exposed to

acidic solutions is higher than for pure water (Jokar & Rahman, 2014). Furthermore, a higher release rate is expected due to an increased dissolution of ions (Simbine et al., 2019). However, the difference between 0.5 and 3% acetic acid was not significant. As discussed in question 4.1.2, of course the type of silver material used (silver compounds, silver nanoparticles) also plays a role.

4.1.4 Does the quantity of silver released from PET have an antimicrobial effect in beverages?

For milk applications, the silver content released from PET is too low. As shown in publication 1, at least 5 mg/L Ag are required for antimicrobial effectiveness in milk due to the interactions of Ag with milk constituents. The amount of silver released from silver doped PET bottles was far below 5 mg/L, in the range of some µg/L to approximately hundred µg/L. Besides the concentration and type of antimicrobial filler, the release of silver from PET was dependent on time, temperature, and food simulant. The amount of silver released increased with time until an equilibrium was reached. Thereafter the release stagnated. Higher temperatures, as well as higher concentrations in the polymer, led to higher silver-release rates. Furthermore, acidic strength and therefore, different kinds of food have an impact on the release of silver ions. More acidic conditions result in a higher release rate. Small differences of 0.5 pH units in acidic strength had no significant influence on the release rate.

With the knowledge gained from publication 1 and 2, media with few and no constituents were chosen for further investigations. Iced tea was chosen as the medium with few constituents and low pH, since the silver-release rate of the bottle is higher at low pH. Distilled water was chosen for being totally independent of any disturbing media constituents that could react with Ag. Results showed that silver concentrations released from silver doped PET bottles are sufficient to produce an antimicrobial effect in distilled water. However, for iced tea only the experiments in which Ag ions were manually added showed an antimicrobial effect and not those in which Ag ions were released from the bottles. *E.coli* in distilled water was reduced by the release of silver from silver doped PET bottles, but the reduction was time-delayed compared to direct addition of $AgNO_3$ into distilled water. The bottles require some time to release the sufficient silver concentration, since the ions firstly need to go into solution and then

diffuse. Furthermore, it was assumed that Ag ions possibly react with residual monomers or other production aids released from the freshly produced PET, since the experiments in which $AgNO_3$ was manually added showed an antimicrobial effect at a concentration of 0.15 mg/L, while this concentration had already been exceeded for some time through the bottles' release, until an antimicrobial effect was observable. This is supported by Appendini & Hotchkiss (2002). They reported that additives used in polymer processing can reduce the activity of antimicrobial substances in polymers by directly interacting with them or by changing the conformation, thus altering the diffusion.

4.1.5 Can an antimicrobial effect in beverages be achieved within recommended use levels for silver in drinking water given by the WHO and within the proposed migration limit for food given by the EFSA?

It was further investigated whether an antimicrobial effect could be achieved within recommended use levels of silver for drinking water given by the WHO and the proposed migration limit for food given by the EFSA, thereby estimating if such a bottle could have market application. Results showed that within the recommended use level of 0.1 mg/L Ag for drinking water proposed by the WHO and even within the proposed European migration limit of 0.05 mg Ag/L food, an antimicrobial effect can be achieved in distilled water and in iced tea. This was shown by adding $AgNO_3$ (0.015, 0.05 mg/L) directly into water or iced tea. However, one has to be sure not to exceed this limit by using antimicrobial packaging where a continuous release of Ag^+ ions occurs. Finding a suitable setting for the initial silver concentration in the polymer to achieve silver-release rates high enough for antimicrobial efficacy in the appropriate medium, while not exceeding the proposed migration limit, remains a challenge.

4.2 Questions relevant for applications in practice:

4.2.1 What problems have to be regarded for practical applications?

The experiments in which silver ions were released from PET bottles into distilled water showed antimicrobial effectiveness, while the experiments with iced tea demonstrated this only when Ag ions were manually added. The problems in using such a bottle system are, on the one hand, that not enough Ag^+ is initially released from the bottle for antimicrobial effectiveness and, on the other hand, cells often grow faster than the bottle can be effective. This has to be optimized for the intended application. Before using such a bottle in a defined application, it is necessary to know what the minimum inhibitory concentration is for the target organism in the medium used, what the expected initial contamination is and how the corresponding silver-release rate can be guaranteed in a time before these microorganisms start to grow. However, since diffusion can hardly be accelerated, such a bottle system is limited to media with few food constituents. For further studies, multi-layer bottles, where the inner layer contains a higher concentration of antimicrobial substance, could be used. Since the bottle needs to be rinsed anyway, these could be treated with a hot, acidic solution and therefore more silver ions would be initially available. However, for beverage and food applications, it further has to be kept in mind that Ag^+ release rates should not exceed proposed migration limits for ensuring human health.

4.2.2 Can the silver doped bottles be reused?

The results from publication 2 showed that the bottles can be reused. A silver-release rate is also observed upon reuse. However, this is lower than at first usage. Therefore, it is expected that antimicrobial effectiveness is reduced when the bottles are used several times. The experiments also indicated that particles near the surface are much more active and silver ions in the dry polymer do not move in a way that these would be available after a longer period of dry storage. Therefore, costs could be saved in the manufacturing process by incorporating Ag particles only in a surface layer or using another technique such as coating. Theoretically, as a silver depot, nanosilver could be more suitable for reuse, since it releases silver ions gradually over a long period of time. However, as results from this dissertation showed, the silver ions must first be released, but this process is presumably too slow for such an application.

4.2.3 What is the prognosis for applicability?

In the beverage sector, the best market potential is seen for water applications. Results showed that even high concentrations of *E. coli* in distilled water can be eliminated with such a bottle. The experiments showed that by adding 0.05 mg/L Ag directly into distilled water, the population of *E. coli* was reduced by 10^5 cfu/mL and the experiments with the silver released from the bottles showed that *E. coli* was reduced by approximately $3*10^4$ cfu/mL. This corresponds to highly contaminated water, since typical bathing water with good quality, which could be used as drinking water and filled in such a bottle, merely contains 10 cfu/mL (Directive 2006/7/EC of the European Parliament and of the council, 2006). Therefore, such a bottle could be useful for soldiers during a war mission, for emergency supply at sea or for astronauts in space, where water should be sterile for a long period of time. For other beverages, market application is moderate, since required concentrations for antimicrobial effectiveness needs to be higher due to interactions with media constituents and therefore, the proposed limit of 0.05 mg Ag/L food can be exceeded quickly.

Good market application is seen in sectors outside food and beverage, where silver concentrations released can be higher, for example in the lacquer industry to protect colors, in medicine for implants or in the cosmetics industry. For cosmetics, the application of the technique in the form of the bellows tested could be interesting, such as for the preservation of soaps.

Furthermore, the use of other polymers such as PE-LD could also be a possibility in order to incorporate less silver in the polymer, since PE-LD is a more diffusive polymer generally leading to higher releasing rates.

5. Conclusions

The dissertation showed that it is possible to develop PET containers containing silver in quantities that do not pose a health risk when released to the filled product. Concentrations of silver required for antimicrobial effectiveness were below the currently discussed limit of 0.05 mg Ag/kg food proposed by the EFSA and far below the tolerable limit of 0.1 mg/L for drinking water recommended by the WHO. However, antimicrobial effectiveness strongly depends on the food constituents in the medium used. Protein-rich media and media with many constituents afford much higher silver concentrations due to chemical interactions with silver ions and therefore are not suitable for those applications. For example, antimicrobial effective silver concentrations found for milk lay in the range of 5 mg/L.

Nanosilver showed lower antimicrobial effectiveness than silver salts, which is very likely due to a slower release of Ag ions. Furthermore, the silver-release investigations showed no silver-release from bottles containing nanosilver. The results further showed that the release of silver from silver doped beverage containers depends on the type and percentage of antimicrobial filler in the polymer, temperature, and acidic strength of the medium.

Developing an effective antimicrobial beverage container made of silver doped PET, however, still remains a challenge. For further studies, multi-layer bottles, where the inner layer contains a higher concentration of antimicrobial substance, could eventually be used. Since the bottle needs to be rinsed anyway, these could be treated with a hot, acidic solution and thereby more silver ions would be available at the beginning. Furthermore, for beverage and food applications, it has to be kept in mind that Ag^+ release rates should not exceed proposed migration limits for ensuring human health.

This dissertation helps industries and researchers in the areas of food and beverage packaging to see what might be possible and also reveals more clarity about the limitations of PET bottles containing silver. Furthermore, the results are beneficial as guidance for crafting government health safety policy.

6. References

Alt, A., Haldimann, M., & Dudler, V. (2008, November 19). *Diffusion coefficient of antimony catalysts in polyethylene terephthalate (PET) materials* [poster session presentation]. 4th International Symposium on Food Packaging - Scientific Developments Supporting Safety and Quality, Prague, Czech Republic. http://www.akts.ch/dwlds/SML-Software-Diffusion-of-antimony-catalysts-in-PET.pdf

An, D. S., Hwang, Y. I., Cho, S. H., & Lee, D. S. (1998). Packaging of fresh curled lettuce and cucumber by using low density polyethylene films impregnated with antimicrobial agents. *Journal of Korean Society of Food Science and Nutrition*, *4*(27), 675–681.

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*(2), 113–126. https://doi.org/10.1016/S1466-8564(02)00012-7

Back, W. (2000). *Farbatlas und Handbuch der Getränkebiologie Teil 2: Fruchtsaft- und Limonadenbetriebe, Wasser, Betriebshygiene, Milch und Molkereiprodukte, Begleitorganismen der Getränkeindustrie* (1st ed.). Nuremberg: Hans Carl.

Balasubramanian, A., Rosenberg, L. E., Yam, K. L., & Chikindas, M. L. (2009). *Antimicrobial Packaging: Potential vs. Reality—A Review*. *3*(4), 193-221.

Baumgart, J., Becker, B., & Stephan, R. (Eds.). (2016). *Mikrobiologische Untersuchung von Lebensmitteln: Ein Leitfaden für das Studium*. (6th ed.). Hamburg: Behr's.

Bayani Bandpey, N., Aroujalian, A., Raisi, A., & Fazel, S. (2017). Surface coating of silver nanoparticles on polyethylene for fabrication of antimicrobial milk packaging films. *International Journal of Dairy Technology*, *70*(2), 204–211. https://doi.org/10.1111/1471-0307.12320

Begley, T., Castle, L., Feigenbaum, A., Franz, R., Hinrichs, K., Lickly, T., Mercea, P., Milana, M., O'Brien, A., Rebre, S., Rijk, R., & Piringer, O. (2005). Evaluation of migration models that might be used in support of regulations for food-contact

plastics. *Food Additives and Contaminants*, *22*(1), 73–90. https://doi.org/10.1080/02652030400028035

Birmele, M., Roberts, M., & McCoy, L. (2011, July 17). *Disinfection of Spacecraft Potable Water Systems by Passivation with Ionic Silver*. 41st International Conference on Environmental Systems, Portland, Oregon. https://doi.org/10.2514/6.2011-5278

Bott, J., Störmer, A., Wolz, G., & Franz, R. (2012, November 14-16). *Migration potential of nanoscale silver particles in food contact polyolefins*. 5th international Symposium on Food Packaging, Berlin, Germany.

Bott, J., Störmer, A., & Franz, R. (2014a). A model study into the migration potential of nanoparticles from plastics nanocomposites for food contact. *Food Packaging and Shelf Life*, *2*(2), 73–80. https://doi.org/10.1016/j.fpsl.2014.08.001

Bott, J., Störmer, A., & Franz, R. (2014b). A Comprehensive Study into the Migration Potential of Nano Silver Particles from Food Contact Polyolefins. In M. A. Benvenuto, S. Ahuja, T. V. Duncan, G. O. Noonan, & E. S. Roberts-Kirchhoff (Eds.), *Chemistry of Food, Food Supplements, and Food Contact Materials: From Production to Plate* (pp. 51–70). American Chemical Society. https://doi.org/10.1021/bk-2014-1159.ch005

Bott, J. W. (2017). *Untersuchungen zum Migrationspotential von Nanomaterialien aus Kunststoff-Lebensmittelverpackungen*. Technische Universität München.

Bott, J., & Franz, R. (2019). Investigations into the Potential Abrasive Release of Nanomaterials due to Material Stress Conditions-Part A: Carbon Black Nano-Particulates in Plastic and Rubber Composites. *Applied Sciences*, *9*(2), 214. https://doi.org/10.3390/app9020214

Brandt, W. W. (1959). Model Calculation of the Temperature Dependence of Small Molecule Diffusion in High Polymers**.** *The Journal of Physical Chemistry*, *63*(7), 1080-1085. https://doi.org/10.1021/j150577a012

Braun, S., Ilberg, V., Blum, U., & Langowski, H.-C. (2020a). Nanosilver in dairy applications – Antimicrobial effects on *Streptococcus thermophilus* and chemical

interactions. *International Journal of Dairy Technology*, *73*(2), 376–383. https://doi.org/10.1111/1471-0307.12686

Braun, S., Ilberg, V., Blum, U., & Langowski, H.-C. (2020b). Release of silver from silver doped PET bottles. *Food Packaging and Shelf Life*, *25*, 100517. https://doi.org/10.1016/j.fpsl.2020.100517

Braun, S., Ilberg, V., & Langowski, H.-C. (2021). Antimicrobial effectiveness of beverage containers made of silver doped PET. *Journal of Food Engineering*, *300*, 110526. https://doi.org/10.1016/j.jfoodeng.2021.110526

Brody, A. L., Strupinsky, E. R., & Kline, L. R. (2001). *Active packaging for food applications* (1st ed.). Lancaster: Technomic Publishing Company, (Chapter 10).

Brydson, J. A. (1995). *Plastic materials* (6th ed.). Oxford: Butterworth-Heinemann.

Bumbudsanpharoke, N., & Ko, S. (2015). Nano-Food Packaging: An Overview of Market, Migration Research, and Safety Regulations. *Journal of Food Science*, *80*(5), R910–R923. https://doi.org/10.1111/1750-3841.12861

Bundesministerium für Ernährung und Landwirtschaft (2016). *Länderbericht Indien*. https://www.agrarexportfoerderung.de/fileadmin/SITE_MASTER/content/files/Laenderberichte2016/Laenderbericht_Indien_2016.pdf/ Accessed 05 March 2019.

Calatayud, M., López-de-Dicastillo, C., López-Carballo, G., Vélez, D., Hernández Muñoz, P., & Gavara, R. (2013). Active films based on cocoa extract with antioxidant, antimicrobial and biological applications. *Food Chemistry*, *139*(1–4), 51–58. https://doi.org/10.1016/j.foodchem.2013.01.097

Cannarsi, M., Altieri, C., Del Nobile, M. A., Favia, P., Iacoviello, G., & D'Agostino, R. (2003). Study and development of an antimicrobial packaging system based on the release of silver ions. *Italian Journal of Food Science*, *15*, 166–172.

Cdang, & Rockey, B. (2010). *Principe moulage injection polymere*. https://commons.wikimedia.org/w/index.php?curid=11478077

Cerrillo, J. L., Palomares, A. E., & Rey, F. (2020). Silver exchanged zeolites as bactericidal additives in polymeric materials. *Microporous and Mesoporous Materials*, *305*, 110367. https://doi.org/10.1016/j.micromeso.2020.110367

Chaudhry, Q., & Castle, L. (2011). Food applications of nanotechnologies: An overview of opportunities and challenges for developing countries. *Trends in Food Science & Technology*, *22*(11), 595–603. https://doi.org/10.1016/j.tifs.2011.01.001

Chaudhry, Q., Scotter, M., Blackburn, J., Ross, B., Boxall, A., Castle, L., Aitken, R., & Watkins, R. (2008). Applications and implications of nanotechnologies for the food sector. *Food Additives & Contaminants: Part A*, *25*(3), 241–258. https://doi.org/10.1080/02652030701744538

Conte, A., Sinigaglia, M., & Nobile, M. A. D. (2006). Antimicrobial Effectiveness of Lysozyme Immobilized on Polyvinylalcohol-Based Film against Alicyclobacillus acidoterrestris. *Journal of Food Protection*, *69*(4), 861–865. https://doi.org/10.4315/0362-028X-69.4.861

Crank, J. (1975). *The mathematics of diffusion* (2nd ed.). Oxford: Clarendon Press.

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., & Cummins, E. (2014a). Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment. *Journal of Agricultural and Food Chemistry*, *62*, 1403–1411. https://doi.org/10.1021/jf404038y

Cushen, M., Kerry, J., Morris, M., Cruz-Romero, M., & Cummins, E. (2014b). Silver migration from nanosilver and a commercially available zeolite filler polyethylene composites to food simulants. *Food Additives & Contaminants: Part A*, *31*(6), 1132–1140. https://doi.org/10.1080/19440049.2014.905874

Da Costa Ribeiro, A. S., de Lima Gomes, J., Mitsui Kushida, M. (2019). Sugarcane juice stability in plastic bottle treated with silver and zinc oxide. *Packaging Technology and Science*, *32*(3), 155-161. https://doi.org/ 10.1002/pts.2423.

Dallas, P., Sharma, V. K., & Zboril, R. (2011). Silver polymeric nanocomposites as advanced antimicrobial agents: Classification, synthetic paths, applications,

and perspectives. *Advances in Colloid and Interface Science*, *166*(1–2), 119–135. https://doi.org/10.1016/j.cis.2011.05.008

Damm, C., & Münstedt, H. (2008). Kinetic aspects of the silver ion release from antimicrobial polyamide/silver nanocomposites. *Applied Physics A*, *91*(3), 479–486. https://doi.org/10.1007/s00339-008-4434-1

Del Nobile, M. A., Cannarsi, M., Altieri, C., Sinigaglia, M., Favia, P., Iacoviello, G., & D'Agostino, R. (2004). Effect of Ag-containing Nano-composite Active Packaging System on Survival of Alicyclobacillus acidoterrestris. *Journal of Food Science*, *69*(8), E379–E383. https://doi.org/10.1111/j.1365-2621.2004.tb09899.x

Dickerson, R. E., Gray, H. B., Darensbourg, M. Y. & Darensbourg, D. J. (1988). *Prinzipien der Chemie* (2nd ed.). New York: de Gruyter.

Dinh, C. V., & Kubouchi, M. (2010). Diffusion behaviour of corrosive solution environments in carbon black filled modified polyethylene linings. *VNU Journal of Science, Mathematics - Physics, 26*, 71-81.

Duncan, T. V. (2011). Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors. *Journal of Colloid and Interface Science*, *363*(1), 1–24. https://doi.org/10.1016/j.jcis.2011.07.017

Durán, N., Durán, M., de Jesus, M. B., Seabra, A. B., Fávaro, W. J. & Nakazato, G. (2016). Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine*, *12*(3), 789–799.

Echegoyen, Y., & Nerín, C. (2013). Nanoparticle release from nano-silver antimicrobial food containers. *Food and Chemical Toxicology*, *62*, 16–22. https://doi.org/10.1016/j.fct.2013.08.014

Eisgruber, H., & Stolle, A. (Hrsg.). (2004). *Mikrobiologische Kriterien für Lebensmittel: Zusammenstellung aktueller Vorschriften aus Deutschland und der Schweiz* (1st revised reprint). Hamburg: Behr's.

Emamifar, A., Kadivar, M., Shahedi, M., & Soleimanian-Zad, S. (2010). Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange

juice. *Innovative Food Science & Emerging Technologies*, *11*(4), 742–748. https://doi.org/10.1016/j.ifset.2010.06.003

Emamifar, A., Kadivar, M., Shahedi, M., & Soleimanian-Zad, S. (2011). Effect of nanocomposite packaging containing Ag and ZnO on inactivation of Lactobacillus plantarum in orange juice. *Food Control*, *22*(3–4), 408–413. https://doi.org/10.1016/j.foodcont.2010.09.011

Emamifar, A., Kadivar, M., Shahedi, M., & Solimanian-Zad, S. (2012). Effect Of nanocomposite packaging containing Ag and Zno on reducing pasteurization temperature of orange juice: nanopackaging on pasteurization of orange juice. *Journal of Food Processing and Preservation*, *36*(2), 104–112. https://doi.org/10.1111/j.1745-4549.2011.00558.x

European Commission. (2009). Commission regulation (EC) No 450/2009 of 29 May 2009 on active and intelligent materials and articles intended to come into contact with food. *Official Journal of the European Union*. L 135, 3-11.

European Commission. (2011a). Commission recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). *Official Journal of the European Union*, *L275 (June 2010)*, 38–40. https://doi.org/10.2777/13162

European Commission. (2011b). Comission regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union*. L 12, 1-89.

European Commission & Directorate General for Health & Consumers. (2014). *Opinion on nanosilver: Safety, health and environmental effects and role in antimicrobial resistance.* http://dx.publications.europa.eu/10.2772/76851

European Food Safety Authority (EFSA). (2006). Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to the 12th list of substances for food contact materials. *EFSA Journal*, *10*(4), 395–401.

European Food Safety Authority (EFSA). (2011). Scientific Opinion on the safety evaluation of the substance, silver zeolite A (silver zinc sodium ammonium

alumino silicate), silver content 2 – 5 %, for use in food contact materials. *EFSA Journal*, *9*(2), 1999. https://doi.org/10.2903/j.efsa.2011.1999

European Food Safety Authority (EFSA). (2016). Scientific opinion on the re-evaluation of silver (E 174) as food additive. *EFSA Journal*, *14*(1), 4364.

European Parliament & Council of the European Union. (2004). Regulation (EC) No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. *Official Journal of the European Union*. L 338, 4-17.

European Parliament & Council of the European Union. (2006). Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. *Official Journal of the European Union*. L 64, 37-51.

European Parliament & Council of the European Union. (2008). Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. *Official Journal of the European Union*. L 354, 16-33.

Federal Institute for Risk Assessment. (2009). *BfR rät von Nanosilber in Lebensmitteln und Produkten des täglichen Bedarfs ab* (Statement Nr. 024/2010). https://www.bfr.bund.de/cm/343/bfr_raet_von_nanosilber_in_lebensmitteln_und_produkten_des_taeglichen_bedarfs_ab.pdf

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., & Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of Biomedical Materials Research*, *52*(4), 662–668.

Fernández, A., Picouet, P., & Lloret, E. (2010a). Cellulose-silver nanoparticle hybrid materials to control spoilage-related microflora in absorbent pads located in trays of fresh-cut melon. *International Journal of Food Microbiology*, *142*(1–2), 222–228. https://doi.org/10.1016/j.ijfoodmicro.2010.07.001

Fernández, A., Picouet, P., & Lloret, E. (2010b). Reduction of the Spoilage-Related Microflora in Absorbent Pads by Silver Nanotechnology during Modified Atmosphere Packaging of Beef Meat. *Journal of Food Protection*, *73*(12), 2263–2269. https://doi.org/10.4315/0362-028X-73.12.2263

Fernández, A., Soriano, E., Hernández-Muñoz, P., & Gavara, R. (2010c). Migration of Antimicrobial Silver from Composites of Polylactide with Silver Zeolites. *Journal of Food Science*, *75*(3), E186–E193. https://doi.org/10.1111/j.1750-3841.2010.01549.x

Fick, A. (1855). Über Diffusion. *Annalen der Physik und Chemie*, *170*(1), 59–86. https://doi.org/10.1002/andp.18551700105

Food and Drug Administration (FDA). (2009). *Food Additives Permitted for Direct Addition to Food for Human Consumption; Silver Nitrate and Hydrogen Peroxide*. 74 FR 11476. 11476-11478. https://www.federalregister.gov/documents/2009/03/18/E9-5852/food-additives-permitted-for-direct-addition-to-food-for-human-consumption-silver-nitrate-and

Gavara, R., López-Carballo, G., Hernández-Muñoz, P., Catalá, R., Muriel-Galet, V., Cerisuelo, J. P., & Domínguez, I. (2015). *Practical guide to antimicrobial active packaging*. Shropshire: Smithers Rapra.

Han, J.-W., Ruiz-Garcia, L., Qian, J.-P., & Yang, X.-T. (2018). Food Packaging: A Comprehensive Review and Future Trends: Food packaging: Review and future trends. *Comprehensive Reviews in Food Science and Food Safety*, *17*(4), 860–877. https://doi.org/10.1111/1541-4337.12343

He, D., Jones, A. M., Garg, S., Pham, A. N., & Waite, T. D. (2011). Silver Nanoparticle−Reactive Oxygen Species Interactions: Application of a Charging−Discharging Model. *The Journal of Physical Chemistry C*, *115*(13), 5461–5468. https://doi.org/10.1021/jp111275a

Higueras, L., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2015). Reversible Covalent Immobilization of Cinnamaldehyde on Chitosan Films via Schiff Base Formation and Their Application in Active Food Packaging. *Food*

and Bioprocess Technology, *8*(3), 526–538. https://doi.org/10.1007/s11947-014-1421-8

Hoseinnejad, M., Jafari, S. M., & Katouzian, I. (2018). Inorganic and metal nanoparticles and their antimicrobial activity in food packaging applications. *Critical Reviews in Microbiology*, *44*(2), 161–181. https://doi.org/10.1080/1040841X.2017.1332001

Huang, Y., Chen, S., Bing, X., Gao, C., Wang, T., & Yuan, B. (2011). Nanosilver migrated into food-simulating solutions from commercially available food fresh containers. *Packaging Technology and Science*, *24*, 291–297. https://doi.org/10.1002/pts.938

Ishizuka Glass Co. https://www.ishizuka.co.jp/english/material/antimicrobial/ Accessed 15 April 2020.

Jin, T. (2010). Inactivation of *Listeria monocytogenes* in Skim Milk and Liquid Egg White by Antimicrobial Bottle Coating with Polylactic Acid and Nisin. *Journal of Food Science*, *75*(2), M83–M88. https://doi.org/10.1111/j.1750-3841.2009.01480.x

Jin, T. Z., Guo, M., & Yang, R. (2014). Combination of pulsed electric field processing and antimicrobial bottle for extending microbiological shelf-life of pomegranate juice. *Innovative Food Science & Emerging Technologies*, *26*, 153–158. https://doi.org/10.1016/j.ifset.2014.07.011

Jokar, M., & Rahman, R., A. (2014). Study of silver ion migration from melt-blended and layered-deposited silver polyethylene nanocomposite into food simulants and apple juice. *Food Additives & Contaminants: Part A*, *31*(4), 734–742. https://doi.org/10.1080/19440049.2013.878812

Kalaiselvi, A., Gantz, S. & Ramalingam, C. (2013). Effects of silver nanoparticles on storage stability of raw milk. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), 274–277.

Kisaalita, W. S. (2010). Milk in the cold chain. *IDM International Dairy Magazine*, *3*, 11–14.

Klein, C. L., Stahlmecke, B., Romazanov, J., Kuhlbusch, T. A. J., Van Doren, E., De Temmerman, P.-J., Mast, J., Wick, P., Krug, H., Locoro, G., Hund-Rinke, K., Kördel, W., Friedrichs, S., Maier, G., Werner, J., Linsinger, T., Gawlik, B. M., Comero, S., Institute for Health and Consumer Protection, … Institute for Reference Materials and Measurements. (2011). *NM-Series of representative manufactured nanomaterials: NM-300 silver characterisation, stability, homogeneity.* Luxembourg: Publications Office. http://dx.publications.europa.eu/10.2788/23079

Kumar, R. & Prasad, A. (2010). Detection of *E.coli* and *Staphylococcus* in Milk and Milk Products in and around Pantnagar. *Veterinary World*, *3*, 495–496.

Kyser, E. A. (1993). *Acid solution absorption of extruded polyethylene foam* (WSRC-TR-93-011). Westinghouse Savannah River Company. https://doi.org/10.2172/6622519

Lee, C. H., Park, H. J., & Lee, D. S. (2004). Influence of antimicrobial packaging on kinetics of spoilage microbial growth in milk and orange juice. *Journal of Food Engineering*, *65*(4), 527–531. https://doi.org/10.1016/j.jfoodeng.2004.02.016

Lee, D. S., & Han, J. H. (2010b). Antimicrobial Packaging. In H. Q. Zhang, G. V. Barbosa-Cánovas, V. M. Balasubramaniam, C. P. Dunne, D. F. Farkas, & J. T. C. Yuan (Eds.), *Nonthermal Processing Technologies for Food* (pp. 462-471). Oxford: Wiley-Blackwell. https://doi.org/10.1002/9780470958360

Lee, S. M., Lee, B. S., Byun, T. G., & Song, K. C. (2010a). Preparation and antibacterial activity of silver-doped organic–inorganic hybrid coatings on glass substrates. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *355*(1–3), 167–171. https://doi.org/10.1016/j.colsurfa.2009.12.010

Liao, C., Li, Y., & Tjong, S. (2019). Bactericidal and Cytotoxic Properties of Silver Nanoparticles. *International Journal of Molecular Sciences*, *20*(2), 449. https://doi.org/10.3390/ijms20020449

Lingathurai, S. & Vellathurai, P. (2013). Bacteriological quality and safety of raw cow milk in Madurai, South India. *Bangladesh Journal of Scientific and Industrial Research*, *48*(2), 109–114.

Llorens, A., Lloret, E., Picouet, P. A., Trbojevich, R., & Fernandez, A. (2012). Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends in Food Science & Technology*, *24*(1), 19–29. https://doi.org/10.1016/j.tifs.2011.10.001

Lu, M., Shiau, Y., Wong, J., Lin, R., Kravis, H., Blackmon, T., Pakzad, T., Jen, T., Cheng, A., Chang, J., Ong, E., Sarfaraz, N., & Wang, N. S. (2013). Milk Spoilage: Methods and Practices of Detecting Milk Quality. *Food and Nutrition Sciences*, *04*(07), 113–123. https://doi.org/10.4236/fns.2013.47A014

Marchetti, F., Palmucci, J., Pettinari, C., Pettinari, R., Marangoni, M., Ferraro, S., Giovannetti, R., Scuri, S., Grappasonni, I., Cocchioni, M., Maldonado Hodar, F. J., & Gunnella, R. (2016). Preparation of Polyethylene Composites Containing Silver(I) Acylpyrazolonato Additives and SAR Investigation of their Antibacterial Activity. *ACS Applied Materials & Interfaces*, *8*(43), 29676–29687. https://doi.org/10.1021/acsami.6b09742

Mackevica, A., Olsson, M. E., & Hansen, S. F. (2016). Silver nanoparticle release from commercially available plastic food containers into food simulants. *Journal of Nanoparticle Research*, *18*. https://doi.org/10.1007/s11051-015-3313-x

Mauriello, G., De Luca, E., La Storia, A., Villani, F., & Ercolini, D. (2005). Antimicrobial activity of a nisin-activated plastic film for food packaging. *Letters in Applied Microbiology*, *41*(6), 464–469. https://doi.org/10.1111/j.1472-765X.2005.01796.x

Michaeli, W. & Hopmann, C. (2017). *Einführung in die Kunststoffverarbeitung* (8th ed.). Munich: Hanser.

Mikiciuk, J., Mikiciuk, E., Wrońska, A. & Szterk, A. (2016). Antimicrobial potential of commercial silver nanoparticles and the characterization of their physical properties toward probiotic bacteria isolated from fermented milk products. *Journal of Environmental Science and Health. Part B*, *51*(4), 222-229.

Muriel-Galet, V., López-Carballo, G., Gavara, R., & Hernández-Muñoz, P. (2012). Antimicrobial food packaging film based on the release of LAE from EVOH. *International Journal of Food Microbiology*, *157*(2), 239–244. https://doi.org/10.1016/j.ijfoodmicro.2012.05.009

Nayak, B. (2015). *Blow molding process*. https://commons.wikimedia.org/wiki/File:Blow_molding_process.jpg

Niamsiri, N. & Batt, C. A. (2009). Applied Microbiology: Agro/Food. Dairy Products. In M. Schaechter (Ed.), *Encyclopedia of microbiology* (pp. 34-44). San Diego: Academic Press.

Pant, A. (2016, October). Einsatz aktiver & intelligenter Materialien in Verpackungen – Status Quo. Recyclingfähigkeit von aktiven und intelligenten Verpackungen. Expert talk in the federal press office, Berlin, Germany.

Pehlivan, H., Balköse, D., Ülkü, S., & Tihminliogˇlu, F. (2005). Characterization of pure and silver exchanged natural zeolite filled polypropylene composite films. *Composites Science and Technology*, *65*(13), 2049–2058. https://doi.org/10.1016/j.compscitech.2005.04.011

Percival, S. L., Bowler, P. G., & Russell, D. (2005). Bacterial resistance to silver in wound care. *Journal of Hospital Infection*, *60*(1), 1–7. https://doi.org/10.1016/j.jhin.2004.11.014

Piringer, O. (2007). Mathematical modelling of chemical migration from food contact materials. In K. A. Barnes, C. R. Sinclair, D. H. Watson (Eds.), *Chemical Migration and Food Contact Materials* (pp. 180–202). Elsevier. https://doi.org/10.1533/9781845692094.2.180

Pitt, J. I., & Hocking, A. D. (2009). *Fungi and Food Spoilage*. Springer.

Polat, S., Fenercioglu, H., Unal Turhan, E., & Guclu, M. (2018). Effects of nanoparticle ratio on structural, migration properties of polypropylene films and preservation quality of lemon juice. *Journal of Food Processing and Preservation*, *42*(4), e13541. https://doi.org/10.1111/jfpp.13541

Rai, V., & Bai, J., (Eds.) (2018). *Nanotechnology Applications in the Food Industry*. (1st ed.). Boca Raton: CRC Press.

RAS AG. (2015). AgPURE - technical information. https://ras-ag.com/wp-content/downloads/TIs/TI_agpure.pdf/ Accessed 05 March 2019.

Riemelt, I., (Ed.), Bartel, B. & Malczan, M. (2003). *Milchwirtschaftliche Mikrobiologie*. (2nd ed.), pp. 112-116. Hamburg: Behr's.

Simbine, E. O., Rodrigues, L. da C., Lapa-Guimarães, J., Kamimura, E. S., Corassin, C. H., & de Oliveira, C. A. F. (2019). Application of silver nanoparticles in food packages: A review. *Food Science and Technology*, *39*(4), 793–802. https://doi.org/10.1590/fst.36318

Sofi, S. A., Singh, J., Rafiq, S., Ashraf, U., Dar, B. N. & Nayik, G. A. (2018). A comprehensive review on antimicrobial packaging and its use in food packaging. *Current Nutrition & Food Science*, *14*(4), 305–312. https://doi.org/10.2174/1573401313666170609095732

Störmer, A., Bott, J., Kemmer, D., & Franz, R. (2017). Critical review of the migration potential of nanoparticles in food contact plastics. *Trends in Food Science & Technology*, *63*, 39–50. https://doi.org/10.1016/j.tifs.2017.01.011

Stratford, M., Steels, H., Nebe-von-Caron, G., Novodvorska, M., Hayer, K., & Archer, D. B. (2013). Extreme resistance to weak-acid preservatives in the spoilage yeast Zygosaccharomyces bailii. *International Journal of Food Microbiology*, *166*(1), 126–134. https://doi.org/10.1016/j.ijfoodmicro.2013.06.025

Terzaghi, B. E. & Sandine, W. E. (1975). Improved medium for lactic streptococci and their bacteriophages. *Applied Microbiology*, *29*(6), 807–813.

Thielen, M., Hartwig, K., & Gust, P. (2020). *Blasformen: Von Kunststoffhohlkörpern*. (2nd ed.). Munich, Germany: Carl Hanser.

Töpel, A. (2016). *Chemie und Physik der Milch: Naturstoff, Rohstoff, Lebensmittel* (4th ed.). Hamburg: Behr's.

Trinkwasserverordnung - TrinkwV, (2001). https://www.gesetze-im-internet.de/trinkwv_2001/BJNR095910001.html

Varner, K. E., El-Badawy, A., Feldhake, D., & Venkatapathy, R. (2010). *State of the Science Literature Review: Everything Nanosilver and More* (EPA/600/R-10/084, 2010). U.S. Environmental Protection Agency Office of Research and Development.

von Goetz, N., Fabricius, L., Glaus, R., Weitbrecht, V., Günther, D., & Hungerbühler, K. (2013). Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. *Food Additives & Contaminants: Part A*, *30*(3), 612–620. https://doi.org/10.1080/19440049.2012.762693

Vrentas, J. S., & Duda, J. L. (1976). Diffusion of Small Molecules in Amorphous Polymers. *Macromolecules*, *9*(5), 785–790. https://doi.org/10.1021/ma60053a019

Vrentas, J. S., Duda, J. L., Ling, H.-C., & Hou, A.-C. (1985). Free-volume theories for self-diffusion in polymer–solvent systems. II. Predictive capabilities. *Journal of Polymer Science: Polymer Physics Edition*, *23*(2), 289–304. https://doi.org/10.1002/pol.1985.180230205

Welle, F., & Franz, R. (2011). Migration of antimony from PET bottles into beverages: Determination of the activation energy of diffusion and migration modelling compared with literature data. *Food Additives & Contaminants: Part A*, *28*(1), 115–126. https://doi.org/10.1080/19440049.2010.530296

Welle, F. (2013). A new method for the prediction of diffusion coefficients in poly(ethylene terephthalate). *Journal of Applied Polymer Science*, *129*(4), 1845–1851. https://doi.org/10.1002/app.38885

Welle, F. (2016). Verpackungsmaterial aus Polyethylenterephthalat (PET). *DLG-Expertenwissen*, *4*, 6.

Westerband, E. I., & Hicks, A. L. (2018). Life cycle impact of nanosilver polymer-food storage containers as a case study informed by literature review. *Environmental Science: Nano*, *5*(4), 933–945. https://doi.org/10.1039/C7EN01043E

World Health Organization (WHO). (2003). *Silver in Drinking-water*. https://www.who.int/water_sanitation_health/dwq/chemicals/silver.pdf/ Accessed 15 April 2020.

Yakabe, Y., Sano, T., Ushio, H., & Yasunaga, T. (1980). Kinetic studies of the interactions between silver ion and deoxyribonucleic acid. *Chemistry Letters*, *9*(4), 373–376. https://doi.org/10.1246/cl.1980.373

Yildiz, N. & Pala, A. (2012). Effects of small-diameter silver nanoparticles on microbial load in cow milk. *Journal of Dairy Science*, *95*(3), 1119–1127.

Zangerl, P. (2006). Mikrobiologie der Produkte. In V. Krömker (Ed.), *Kurzes Lehrbuch Milchkunde und Milchhygiene* (pp. 156-179). Stuttgart: Parey.